Exploring the mRNA Expression Level of RELN in Peripheral Blood of Schizophrenia Patients Before and After Antipsychotic Treatment

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Research

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Abstract

Background: The Reelin *(RELN)* gene encodes the protein reelin, which is a large extracellular matrix glycoprotein that plays a key role in brain development. Additionally, this protein may be involved in memory formation, neurotransmission, and synaptic plasticity, which have been shown to be disrupted in schizophrenia (SCZ). A decreasing trend in the expression of *RELN* mRNA in the brain and peripheral blood of SCZ patients has been observed. There is a need to determine whether changes in *RELN* mRNA expression in SCZ patients are the result of long-term antipsychotic treatment rather than the etiological characteristics of schizophrenia. The expression levels of *RELN* mRNA in the peripheral blood of 48 healthy controls and 30 SCZ patients before and after 12-weeks of treatment were measured using quantitative real-time PCR.

Results: The expression levels of *RELN* mRNA in the SCZ group were significantly lower than that of healthy controls; however, after 12-weeks of antipsychotic treatment, *RELN* mRNA levels were significantly increased in the SCZ group.

Conclusion: The up-regulation of *RELN* mRNA expression was concurrent with the improvement of symptoms in SCZ patients after antipsychotic treatment, suggesting that the changes in *RELN* mRNA expression were related to the effect of the antipsychotic treatment.

Background

Schizophrenia (SCZ) is a common and severe mental disorder, with a lifetime prevalence estimate of 4.0 (1.6–12.1) per 1,000 persons and a median incidence of 15.2 per 100,000 individuals [1, 2]. The disease is characterized by positive and negative symptoms, including abnormalities in cognitive function and personality, such as language, thought, perception, emotion, and self-awareness. Despite the wide prevalence of SCZ and many decades of major progress, the underlying etiology of this disease remains elusive.

In contemporary practice, clinicians still diagnose clinical symptoms and evaluate progress and treatment responses based on clinical symptoms. The development of objective tests for the diagnosis of SCZ or monitoring of drug reactions is essential to providing early intervention for patients, which is beneficial for improving prognosis. In addition, several studies have shown that gene expression causes changes in different tissues of patients with SCZ [3–8]. Since the detection of mRNA in peripheral blood mononuclear cells (PBMCs) is accessible and non-invasive, the pattern of gene expression serves as a potential biomarker for SCZ diagnosis and therapeutic monitoring [9].

The *RELN* gene is located on chromosome 7q22, and it encodes the protein reelin, a large extracellular matrix glycoprotein that plays a key role in brain development from neuronal migration to dendritic spine formation and synaptic transmission [10–12]. Moreover, it may be involved in memory formation, neurotransmission, and synaptic plasticity [13, 14], as well as SCZ [15]. The earliest direct evidence was based on the postmortem studies of patients with SCZ, which revealed that *RELN* mRNA was reduced up

to 50% in several regions of the brain [16, 17]. Additionally, it has been shown that the expression of *RELN* in schizophrenia was found to be decreased in the blood [18, 19]. The role of *RELN* was associated with SCZ in an earlier large genome-wide association study (GWAS), which found that the polymorphism rs7341475 accounted for a 1.4-fold increase in the risk of the disease in women [20]. Moreover, this gene variant was associated with working memory, episodic memory, and executive functioning in the nuclear families of one member with SCZ [21]. Subsequently, a growing number of studies have reported that a number of single nucleotide polymorphisms (SNPs) in the *RELN* gene were associated with the pathogenesis and/or severity of clinical symptoms of SCZ [22–29]. Hence, based on the low level of *RELN* in SCZ patients and the relationship between its genetic variation and SCZ, *RELN* may play a pathogenic role in SCZ [30]. This view was further supported by heterozygous reelin mouse model. Although the mice had fewer neuroanatomical defects, they had cognitive abnormalities of some common psychotic disorders [31].

In studies on SCZ, the healthy controls were not treated with antipsychotic drugs, whereas patients with SCZ were often treated with these drugs. It is important to determine whether changes in *RELN* gene expression in these patients are the result of long-term antipsychotic treatment or due to etiological characteristics of SCZ. Suzuki et al. reported that the level of the RELN receptor VLDLR in the peripheral blood lymphocytes of patients with SCZ was decreased. After six months of antipsychotic treatment, the gene expression increased [32]. Another study showed that protracted treatment with olanzapine resulted in the upregulation of *RELN* expression in the frontal cortex of rats [33, 34]. However, a previous postmortem study showed no correlation between the levels of *RELN* mRNA and reelin protein and lifetime doses of antipsychotics drugs [17]. Overall, the regulation of *RELN* in patients with SCZ requires broader research including other typical or atypical antipsychotic treatments.

In this study, we investigated the expression of *RELN* mRNA in PBMCs before and after antipsychotic medication in patients with SCZ to explore the therapeutic value of *RELN* as a biomarker for SCZ.

Methods And Methods

Subjects

All participants were unrelated and of the ethnic Han group from the Jiangsu Province of China. The SCZ patients were recruited from the Wuxi Mental Health Center and independently diagnosed by at least two experienced psychiatrists according to the Diagnosis and Statistical Manual of Mental Disorders, 4th ed. (DSM-IV).

A total of 30 patients in the acute stage of SCZ (16 women and 14 men; mean age = 35.87 years, SD = 10.21) were chosen for this study and included 5 first-episode, 18 drug-naive SCZ patients or recurrent, 7 drug-free SCZ patients with self-withdrawn antipsychotic drugs at least one month before enrollment (Table 1). All patients participating in the study were treated with one of the oral second generation or atypical antipsychotics (SGA) and tracked continuous medication treatment for 12 weeks after baseline

assessments. The psychopathology of all patients was assessed through an oral case history interview and psychiatric interview using the Positive and Negative Syndrome Scale (PANSS) [35] at baseline and 12 weeks. Among the 30 patients, 20 were treated with risperidone, 6 with olanzapine, 2 with clozapine, 1 with quetiapine, and 1 with aripiprazole. All patients were administered one of the five SGAs once daily, starting with an initial dose and increasing to a curative dose over the subsequent 2–4 weeks. All patients demonstrated clinical improvement as evidenced by more than a 25% reduction in the PANSS score. A total of 48 healthy controls (HCs) (31 women and 17 men; mean age = 31.67 years, SD = 6.75) without a history of mental health disorders or neurological diseases were recruited from local communities by advertisement (Table 1).

Table 1
Demographic characteristics of the study participants

	Control	SCZ	P value
Sample size	48	30	
Age (mean ± SD)	31.67 ± 6.75	35.87 ± 10.21	0.066
Gender (Men : woman)	17:31	14:16	0.323
Notes:			
Abbreviations: Control: healthy control; SCZ: untreated SCZ patients.			

There were no significant differences in gender and age between the SCZ and HC groups. The Ethics Committees of the Wuxi Mental Health Center approved this study, and the study was conducted according to the principles of the Declaration of Helsinki 1975. Written informed consent was obtained from all participants prior to participation in the study.

Analysis of RELN expression by the real-time quantitative PCR (RT-qPCR)

Whole-blood samples were collected in PAXgene blood RNA tubes (PreAnalytiX QIAGEN/BD, Hombrechtikon, Switzerland) and total RNA was extracted from PBMCs using TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA USA) with on-column DNase I treatment (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. Reverse transcription was performed using 200 ng of total RNA and the High Capacity RNA-to-cDNA Kit (Thermo Fisher Scientific, Waltham, MA, USA). Real-time quantitative PCR was performed using the primers for *RELN*, namely, CATGGTTGCAAGTGTGACCC (forward) and AAACCAGGGCCTTACCACTG (reverse) and a SYBR®Select Master Mix (Thermo Fisher Scientific, Waltham, MA, USA). The reactions were performed in triplicate for each of the three independent samples using the ViiA 7 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) under the following conditions: 95 °C for 10 min and 40 cycles each of 95 °C for 10 s and 60 °C for 60 s. The relative expression level of *RELN* for each individual after normalization to *ACTB* was calculated using the comparative Ct ($2^{-\Delta\Delta Ct}$) method [36].

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) software version 21.0 (SPSS Inc., Chicago, IL, USA) for statistical analyses. The Student's t-test was used to compare age and education between the SCZ group and control subjects. Gender differences were measured using Pearson's chi-square test. PANSS scores before and after treatment were compared using the paired t-test.

The differences of *RELN* mRNA levels between the SCZ group and the healthy control group were analyzed using Mann-Whitney U test, and the changes in *RELN* expression between the SCZ group before and after treatment with antipsychotic medication were analyzed using paired Wilcoxon signed-rank test. All of the *P* values less than 0.05 were considered statistically significant. Spearman's correlation analysis was used to analyze the relationship between the *RELN* mRNA levels and the PANSS scores in SCZ patients before and after treatment.

Results

The expression levels of *RELN* mRNA in each subject were validated in the RT-qPCR shown in Fig. 1. The expression levels of *RELN* mRNA in the PBMCs of 30 SCZ patients was significantly lower than that of the 48 healthy controls (fold change (FC) = 0.68; *P* = 0.0476, Table 2). After 12 weeks of antipsychotic treatment, the expression levels of *RELN* mRNA in the SCZ patients were significantly increased (FC = 1.55; *P* = 0.0384, Table 2).

Table 2
Comparison of *RELN* expression levels in controls, and SCZ patients before and after treatment (log2 transformed)

Control (n = 48)	SCZ (n = 30)	FC	P	SCZ (n = 30)	SCZ_12W (n = 30)	FC	P
6.39E-3 ± 0.68E-3	4.37E-3 ± 0.47E-3	0.68	0.0476	4.37E-3 ± 0.47E-3	6.75E-3 ± 0.77E-3	1.55	0.0384
Notes:							
Abbreviations: Control: healthy control; SCZ: untreated SCZ patients; SCZ_12w: SCZ patients after 12 weeks of antipsychotic treatment; FC: fold change.							

As shown in Table 3, in the SCZ patients before and after treatment, no significant relationship was found between the *RELN* mRNA levels and the positive symptom points, negative points, general pathological symptom points, or the total PANSS scores.

Table 3
Correlation analysis between *RELN* mRNA levels and PANSS scores in SCZ patients before and after treatment (r value)

PANSS	*RELN* mRNA	
	SCZ (n = 30)	SCZ_12W (n = 30)
Total PANSS scores	-0.021	-0.196
Positive symptom points	-0.114	-0.177
Negative symptom points	-0.041	-0.174
General pathological symptom points	0.131	-0.246

Discussion

In this study, we found decreased expression of *RELN* mRNA in the PBMCs of the untreated SCZ patients. This finding is consistent with previous studies in which *RELN* gene expression was down-regulated in the brain and peripheral blood of SCZ patients [16–19]. In addition, the study revealed that the *RELN* mRNA expression levels were significantly upregulated in SCZ patients after 12 weeks of antipsychotic treatment. Fatemi and his colleagues reached a similar conclusion in the frontal cortex of rats [33, 34]. Meanwhile, a similar conclusion was reached in the study of *RELN*-related genes in SCZ patients [32]. Hence, the up-regulation of *RELN* mRNA expression was concurrent with the improvement in symptoms in SCZ patients after treatment, suggesting that the changes in *RELN* mRNA expression were attributed to the antipsychotic treatment.

It is worth explicitly noting that the main limitation of this study was the limited sample size, which may reduce the statistical power to compare the level of *RELN* mRNA expression between different groups. Furthermore, we only measured *RELN* mRNA expression in peripheral blood; therefore, extrapolating these results to the brain should be treated with caution. These findings will need to be confirmed in future studies that involve a larger sample size.

Conclusions

Taken together, these results demonstrate that the expression of *RELN* is down-regulated in the peripheral blood of untreated SCZ patients, whereas its expression level is significantly up-regulated after 12 weeks of antipsychotic treatment. These findings suggest that changes in *RELN* expression are associated with antipsychotic treatment and may provide new clues for understanding the pathogenesis and treatment of SCZ.

Abbreviations

SCZ

Schizophrenia; PBMCs:Peripheral blood mononuclear cells; GWAS:Genome-wide association study; SNPs:Single nucleotide polymorphisms; FC:Fold change; DSM-IV:Diagnosis and Statistical Manual of Mental Disorders, 4th ed.; SGA:The oral second generation or atypical antipsychotics; PANSS:Positive and Negative Syndrome Scale; HCs:Healthy controls; RT-qPCR:Real-time quantitative PCR; $2^{-\Delta\Delta Ct}$:Comparative Ct; SPSS:The Statistical Package for the Social Sciences.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committees of Wuxi Mental Health Center. Written informed consent was obtained from all participants prior to participation in the study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Figures

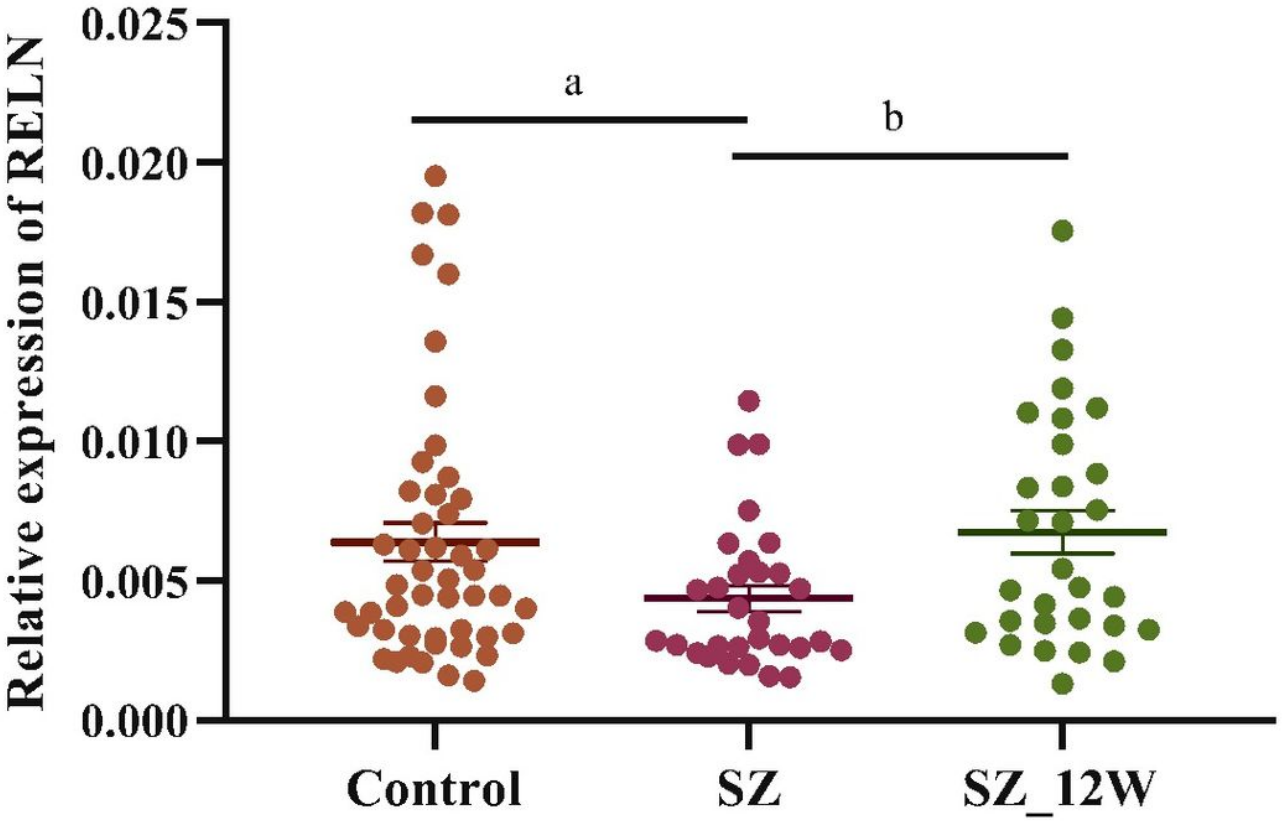

Figure 1

Expression levels of RELN in controls and SCZ patients before and after treatment (log2 transformed) a: The expression of RELN mRNA in the PBMCs of control subjects was higher than that in SCZ patients (Mann-Whitney U test). b: The expression of RELN mRNA in the PBMCs of SCZ_12w patients was higher than that in SCZ patients (paired Wilcoxon signed-rank test). Control: healthy control; SCZ: untreated SCZ patients; SCZ_12w: SCZ patients after 12 weeks of antipsychotic treatment. The horizontal bar represents standard error of the mean.