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Expression study of Wnt/β-catenin signaling pathway associated lncRNAs in schizophrenia

Fatemeh Manafzadeh[1,2], Behzad Baradaran[2], Seyed Gholamreza Noor Azar[3], Kamran Javidi Aghdam[1,2], Reza Dabbaghipour[4], Asghar Shayannia[5*] and Soudeh Ghafouri-Fard[6*]

Abstract

Schizophrenia is one of the most debilitating mental illnesses affecting any age group. The mechanism and etiology of schizophrenia are extremely complex and multiple signaling pathways recruit genes implicated in the etiology of this disease. While the role of Wnt/β-catenin signaling in this disorder has been verified, the impact of long noncoding RNAs (lncRNAs) associated with this pathway has not been studied in schizophrenia. The objective of this study was to examine the expression levels of Wnt/β-catenin-related lncRNAs, namely *CCAT2*, *SNHG5*, *PTCSC3*, and *DANCR*, as well as the *CTNNB1* gene encoding beta-catenin protein in two groups of schizophrenia patients (drug-naïve and medicated) compared with healthy individuals. This study included 50 medicated patients in the remission phase of the disease, 25 drug-naive patients in the acute phase, and 50 control subjects. There was no significant difference in *CTNNB1* gene expression in the medicated patients compared to controls (P value = 0.9754). However, the expression of this gene was significantly decreased in drug-naïve first-episode patients compared with controls (P value < 0.001). In contrast, expression of *DANCR*, *PTCSC3*, *SNHG5*, and *CCAT2* genes was significantly higher in medicated (P values < 0.001, < 0.001, = 0.01, < 0.001, respectively) and drug-naive first-episode patients (P value < 0.001) compared to control subjects. ROC curve analysis revealed that *DANCR*, *PTCSC3*, *SNHG5*, and *CCAT2* genes had diagnostic power with specificity and sensitivity of 80% and above in separation between study subgroups. In brief, our data demonstrated dysregulation of Wnt/β pathway related genes and lncRNAs in the peripheral blood of patients with schizophrenia and their potential as biomarkers for this disorder.

Keywords Schizophrenia, Antipsychotic drugs, Wnt/β-catenin pathway, B-catenin, lncRNA

*Correspondence:
Asghar Shayannia
a.shayannia@gmail.com
Soudeh Ghafouri-Fard
s.ghafourifard@sbmu.ac.ir
[1]Student Research Committee, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran
[2]Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[3]Research Center of Psychiatry and Behavioral Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
[4]Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[5]Department of Medical Biotechnology, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran
[6]Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Introduction

Schizophrenia (SCZ) is a destructive mental disorder with a typical onset in early adulthood. This disorder is characterized by a spectrum of symptoms including positive symptoms (delusions and hallucinations), negative symptoms (reduced motivation and apathy), and cognitive deficits (impaired attention and executive function) [1, 2]. With a global prevalence of approximately 1%, SCZ imposes substantial socioeconomic burden and significantly impacts patients' families [3]. Understanding the mechanisms of the pathogenesis of SCZ and identifying effective therapeutic approaches for SCZ patients has always been an important objective of researchers [4].

Despite extensive research, the precise etiology remains elusive. However, evidence suggests that dysregulation of gene expression and protein synthesis in brain tissue may play a critical role in this disorder [5, 6]. Currently, clinical diagnosis relies heavily on symptomatology due to the absence of reliable biomarkers.

A comparative computer-based analysis has proposed shared and distinctive differentially expressed genes in the brains of SCZ exosome-recipient mice and SCZ patients, providing indications for changed prefrontal-hippocampal functions in SCZ [7]. Dysregulated genes in SCZ models have been found to be enriched in pathways related with chemical synaptic transmission, cognition, and inflammatory responses [8].

Long non-coding RNAs (lncRNAs), a subgroup of RNA transcripts over 200 nucleotides in length without protein-coding capacity, have emerged as key regulators of gene expression through interactions with signaling pathways [9]. These transcripts act as sponges for miRNAs, another group of regulatory RNAs that have essential roles in SCZ pathophysiology and regarded as promising biomarkers for SCZ [10]. Recent studies have shown that lncRNAs can influence gene expression by interacting with various signaling pathways, and alterations in their expression may contribute to disease mechanisms [11, 12]. These molecules are expressed not only in various brain tissues but also detectable in peripheral blood, implicating their potential role in neuropsychiatric disorders, including SCZ [13, 14]. SCZ has been shown to be associated with abnormal expression of Wnt gene and related proteins in the plasma. Gene expression assays have shown dysregulation of Wnt-related genes in favor of attenuation of canonical (β-catenin-dependent) signaling [15]. The Wnt/β-catenin signaling pathway, essential for cellular processes such as proliferation and polarity, has been implicated in numerous diseases. Recent research highlights that lncRNA-mediated dysregulation of this pathway could play a significant role in disease pathophysiology [16, 17]. While the role of Wnt/β-catenin signaling in this disorder has been verified [18], the impact of lncRNAs associated with this pathway has not been studied in SCZ. In addition, the precise mechanisms through which lncRNAs interact with the Wnt/β-catenin pathway remain unclear. Since studies suggest that interactions between lncRNAs and Wnt/β-catenin pathway influence the course of different disorders [19], we aim to explicate the role of specific lncRNAs associated with the Wnt/β-catenin pathway in the context of SCZ. Specifically, we assessed the expression profiles of four lncRNAs (colon cancer-associated transcript 2 [*CCAT2*], papillary thyroid carcinoma susceptibility candidate 3 [*PTCSC3*], differentiation antagonizing non-protein coding RNA [*DANCR*], and small nucleolar RNA host gene 5 [*SNHG5*]) as well as expression of β-catenin in the peripheral blood of SCZ patients compared to healthy subjects. These lncRNAs have been selected based on the existing literature on their role in the regulation of Wnt/β-catenin pathway. For instance, *CCAT2* has been shown to activate the Wnt/β-catenin signaling pathway *via* induction of nuclear β-catenin [20]. Similarly, *DANCR* activates this signaling pathway and its silencing leads to reduction of β-catenin signaling and protein expression [21]. Moreover, *SNHG5* has been found to be a robust activator of Wnt/β-catenin pathway [22]. On the other hand, *PTCSC3* has a suppressive role on the activity of Wnt/β-catenin pathway through targeting the active β-catenin as well as other targets [23].

Although the role of these lncRNAs in the pathogenesis of SCZ has not been clarified, based on their role in the modulation of activity of Wnt/β-catenin pathway, we hypothesized that they are dysregulated in the blood samples of SCZ patients. By identifying these expression profiles, we hope to contribute to the understanding of the molecular mechanism underlying SCZ and potentially pave the way for new biomarkers or therapeutic targets. This study is novel in its comprehensive approach to linking specific lncRNAs with the Wnt/β-catenin pathway in SCZ. Unlike previous research that has primarily focused on brain tissue, our investigation utilizes peripheral blood samples, offering a less invasive method to study these molecular interactions. Additionally, this research could provide the first detailed insights into how these specific lncRNAs may influence the Wnt/β-catenin pathway in SCZ, potentially leading to innovative diagnostic and therapeutic strategies.

Materials and methods

Study participants

The current study was conducted on blood samples from 50 medicated SCZ patients in the remission phase, 25 first-episode SCZ patients who had not taken antipsychotic drugs, and 25 healthy subjects. All patients were evaluated according to the 5th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V). Cases were recruited from Razi Hospital (Tabriz, Iran) in 2021. Patients who had no history of cigarette smoking or substance abuse were included in the study. Control subjects were evaluated by a structured psychiatric interview to rule out the presence of psychiatric disorders. The research protocol was approved by the Ethical Committee of Shahroud University of Medical Sciences (IR.SHMU.REC.1401.092). All methods and experiments were performed according to the relevant guidelines and regulations. All participants signed the informed written consent forms.

Expression analysis

The total RNA of whole venous blood samples from study participants was extracted using the Hybrid-R blood RNA extraction kit (Gene All, Seoul, South Korea). The quantity of RNA was evaluated by NanoDrop equipment (Thermo Scientific, USA), and the quality of RNA was verified by 1% agarose gel electrophoresis. In order to remove any genomic DNA contamination, samples were treated with DNase I (Thermo Scientific, Germany). Complementary DNA (cDNA) was synthesized using the FIREScript RT cDNA Synthesis Kit (Solis BioDyne, Estonia). Four lncRNAs, namely *CCAT2*, *SNHG5*, *PTCSC3*, and *DANCR* were selected based on the existing literature on their role in the regulation of Wnt/β-catenin pathway. Relative expressions of lncRNAs as well as *CTNNB1* were evaluated in controls and SCZ patients by utilizing RealQ Plus 2 × PCR Master Mix cyber-Green high ROX PCR Master Mix (Amplicon, Odense, Denmark) in Step One Plus Real-Time PCR equipment (Applied Biosystems, Foster City, CA, USA). The YWHAZ was used as standardized internal references for normalization based on its stable expression in the peripheral blood of SCZ patients. The primer sequences are illustrated in Table 1.

Statistical analysis

Each reaction was independently repeated three times. The relative transcript levels of lncRNAs were assessed in all samples. YWHAZ expression level was considered normalizers in the Ln [Efficiency ^ΔΔCT] method. Expression levels of lncRNAs were compared between SCZ patients and normal controls using the ANOVA and Tukey tests after evaluation of the normality of the data by the Kolmogorov-Smirnov test. Correlations between lncRNAs were assessed by calculating the Spearman's correlation rank (SPSS, Chicago, IL). All the obtained data were analyzed using the R v.4 software. Also, the diagnostic power of the expression levels of genes and lncRNAs was evaluated by the receiver operating characteristic (ROC) curve.

Result

Characteristics of participants

A total of 50 medicated patients with SCZ, 25 first-episode patients without the use of any antipsychotic drug, and 50 healthy controls were recruited for the current study. The medicated patients used a standard dose of antipsychotic drugs such as risperidone, biperiden, olanzapine, tranqupine, and clozapine. The demographic data of the participants in the study is summarized in Table 2.

Expression assay

Expression levels of mentioned genes were compared between first-episode patients vs. controls and medicated patients vs. controls (Fig. 1).

There was a significant difference in the expression level of *CTNNB1*, *DANCR*, *SNHG5*, *CCAT2*, and *PTCSC3* between the first-episode patient and healthy controls. The expression level of *CTNNB1* was very low in the first-episode patient compared with the control (Expression difference= -7.210, *P* value < 0.0001).

On the other hand, the expression levels of *CCAT2*, *SNHG5*, *PTCSC3*, and *DANCR* were higher in the first-episode patients compared with the controls (Expression difference = 46.237, *P* value < 0.0001; Expression difference = 3.896, *P*-value < 0.0001; Expression difference = 7.061, *P*-value < 0.0001; Expression difference = 5.140, *P*-value < 0.0001; respectively). The expression of mRNA/lncRNA genes was changed in medicated patients compared to healthy controls, but these expression changes were less than in first-episode patients. Table 3 shows the relative expression of lncRNAs in medicated patients and first-episode patients compared with healthy controls. While expression of CTNNB1 was lower in first episode patients compared with medicated patients (Expression difference=-6.868, *P*-value < 0.0001), expression of all mentioned lncRNAs was higher in first episode patients compared with medicated patients (*P*-values = 0.0008, < 0.0001, = 0.00062 and

Table 1 Primer sequences

Gene	Sequence (5′–3′)	Primer length (base pairs)
CCAT2	F: AGAGGGAGGTATCAACAGAGAC	22
	R: TCATTTGGACGACGCCTTCA	20
DANCR	F: GCCACTATGTAGCGGGTTT	19
	R: GCTTGTGCCTGTAGTTGTCA	20
PTCSC3	F: GGCTTGAACAATCTTCCCACCTT	23
	R: TTTGGCAACACCCTCACAGACAC	23
SNHG5	F: TCTGGGCGGGTGGTAGGAA	19
	R: GCTACTCGTCCACACTCAGAAC	22
CTNNB1	F: ACAGCAGCAATTTGTGGAGG	20
	R: AGCAAGTTCACAGAGGACCC	20
YWHAZ	F: ACTTTTGGTACATTGTGGCTTC	22
	R: CCGCCAGGACAAACCAGTA	19

Table 2 Demographic data of patients and controls

Study groups	Parameters		Values
Drug-naïve patients	Gender (number, %)	Male	25 (100%)
		Female	0
	Age (Years, mean ± SD)	Male	27.1 ± 7.33
		Female	0
	Family History (number, %)	Yes	15 (60%)
		No	10 (40%)
	Education (%)	Illiterate	40%
		School	32%
		High School	24%
		University	4%
Drug –in use Patients	Gender (number, %)	Male	47 (94%)
		Female	3 (6%)
	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	35 (70%)
		No	15 (30%)
	Education (%)	Illiterate	20%
		School	30%
		High School	44%
		University	6%
Controls	Gender (number, %)	Male	45 (90%)
		Female	5 (10%)
	Age (Years, mean ± SD)	Male	(39.5 ± 8.8)
		Female	(41 ± 9.51)
	Education (%)	Illiterate	0
		School	10%
		High School	48%
		University	42%

= 0.0291, for SNHG5, CCAT2, PTCSC3 and DANCR, respectively).

Also, we evaluated the diagnostic power of transcript quantities of *CCAT2*, *SNHG5*, *PTCSC3*, and *DANCR* in identifying the first-episode patients and controls by depicting a ROC curve (Table 4). Based on AUC values, *CCAT2* had the highest diagnostic power in SCZ patients (Fig. 2).

Evaluation of the pairwise correlation between lncRNAs revealed that *CCAT2* expression levels were correlated with the expression of *PTCSC3* and *DANCR* in both first-episode patients and controls. The correlations between *SNHG5* and *CTNNB1* expression levels were significant in first-episode patients (Correlation coefficient = 0.626, *P*-value = 0.001) (Figure S1).

Such correlations were also identified between other sets of lncRNAs, including *PTCSC3* and *DANCR*, *DANCR* and *CTNNB1* (Figure S2). Table 5 shows partial correlation results in the first-episode patients with SCZ and controls.

Finally, we evaluated correlation between expression of genes and age of disease onset in drug naïve patients (Table 6) and medicated patients (Table 7).

Notably, expression of SNHG5 was correlated with age of disease onset in the medicated patients.

Discussion

SCZ has a complex genetic and neurobiological background that affects brain development, particularly in its early phases [24]. Wnt/β-catenin pathway is critical in the central nervous system [25, 26]. Notably, dysregulation of this pathway is associated with the neuroinflammation in SCZ [18]. Meanwhile, dysregulation of lncRNAs plays a vital role in the progression of various diseases through modulation of Wnt/β-catenin signaling pathway. Additionally, interactions between lncRNAs and the Wnt/β-catenin signaling pathways may be regarded as a novel avenue for identification of biomarkers.

In this study, we selected four lncRNAs, namely *CCAT2*, *SNHG5*, *PTCSC3*, and *DANCR* based on the

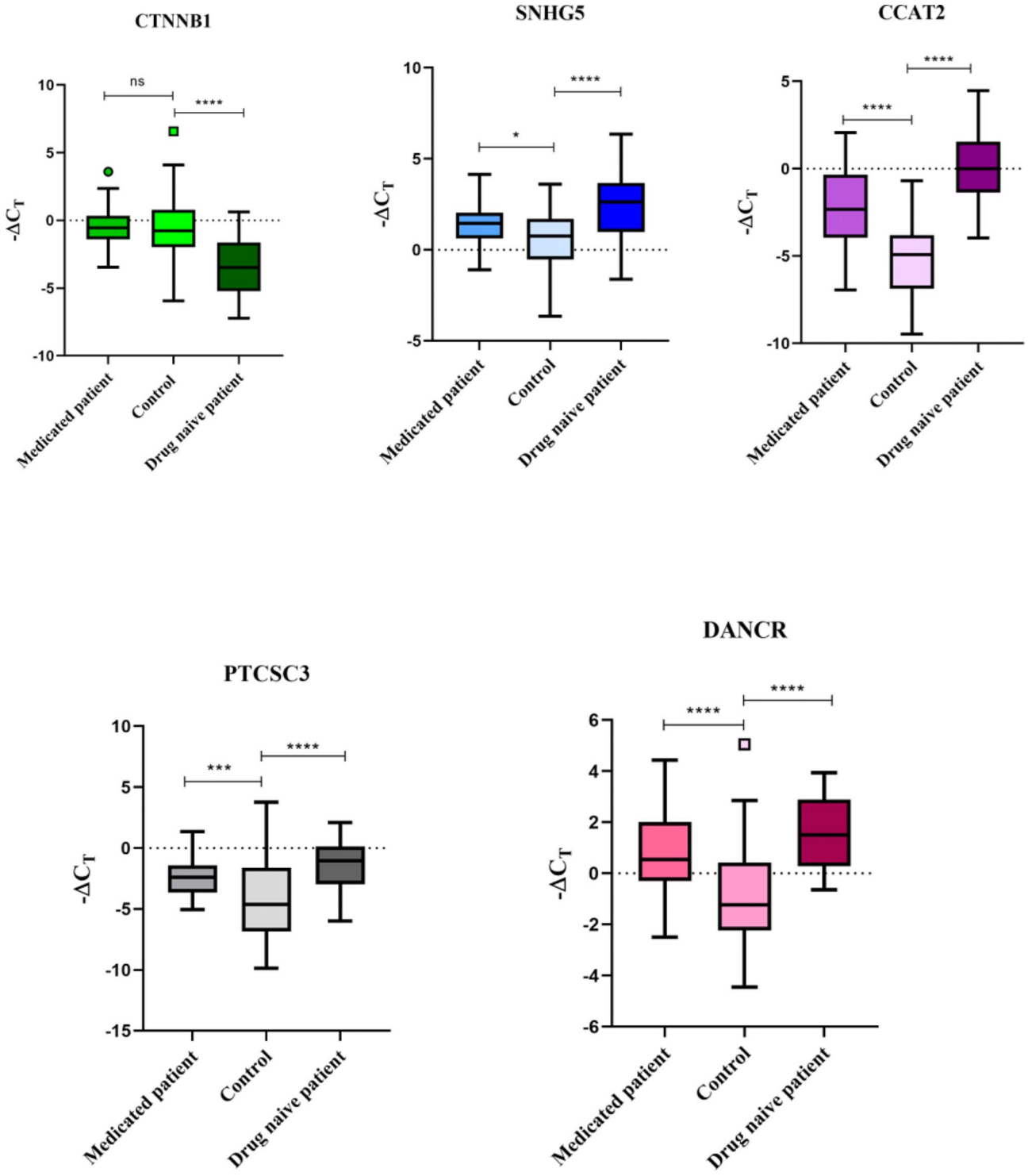

Fig. 1 Validation of relative expressions of *CTNNB1* mRNA-coding gene and lncRNAs by qRT-PCR analysis in the peripheral blood of medicated schizophrenia patients ($n=50$), healthy controls ($n=50$), and first-episode drug-naïve schizophrenia patients ($n=25$). The columns were constructed by using GraphPad Prism 8 software. *$P<0.05$; ***P value <0.001; ****P value <0.0001 in the annotations

existing literature on their role in the regulation of Wnt/β-catenin pathway. Additionally, we evaluated expression of the protein-coding gene, namely *CTNNB1* in the peripheral blood of SCZ patients in both the acute phase (first-episode and drug-naive) and remission phase (medicated) compared to healthy controls. Our findings highlighted significant differences in the expression of these lncRNAs, suggesting their potential roles

Table 3 Difference in the expression levels of mRNA/lncRNAs in first-episode patients, patients with a history of antipsychotic drug use and healthy controls (*shows significance). For multiple gene comparisons, we used Bonferroni test and *P* values remained significant after correction

Gene		Medicated patients vs. controls (50 vs. 50)	First-episode patients vs. controls (25 vs. 50)	First-episode patients vs. medicated patients (25 vs. 50)
CTNNB1	Expression difference	-1.053	-7.210	-6.868
	P-value	0.9754	< 0.0001*	< 0.0001*
SNHG5	Expression difference	1.785	3.896	2.181
	P-value	0.0106*	< 0.0001*	0.0008*
CCAT2	Expression difference	7.674	46.237	6.025
	P-value	< 0.0001*	< 0.0001*	< 0.0001*
PTCSC3	Expression difference	3.271	7.061	2.158
	P-value	0.0008*	< 0.0001*	0.0062*
DANCR	Expression difference	2.885	5.140	1.778
	P-value	< 0.0001*	< 0.0001*	0.0291*

Table 4 The results of ROC curve analysis between drug-naive patients and controls (a: Youden index, significance level *P* (area = 0.5), estimate criterion: optimal cut-off point for gene expression, *shows significance)

Gene	Estimate criterion	Specificity	Sensitivity	AUC	J[a]	*P*-value
PTCSC3	< 3.83	0.56	0.92	0.77	0.48	< 0.0001*
CCAT2	< 2.06	0.96	0.88	0.97	0.84	< 0.0001*
DANCR	< 0.27	0.70	0.92	0.85	0.62	< 0.0001*
SNHG5	< -1.92	0.84	0.64	0.78	0.48	< 0.0001*

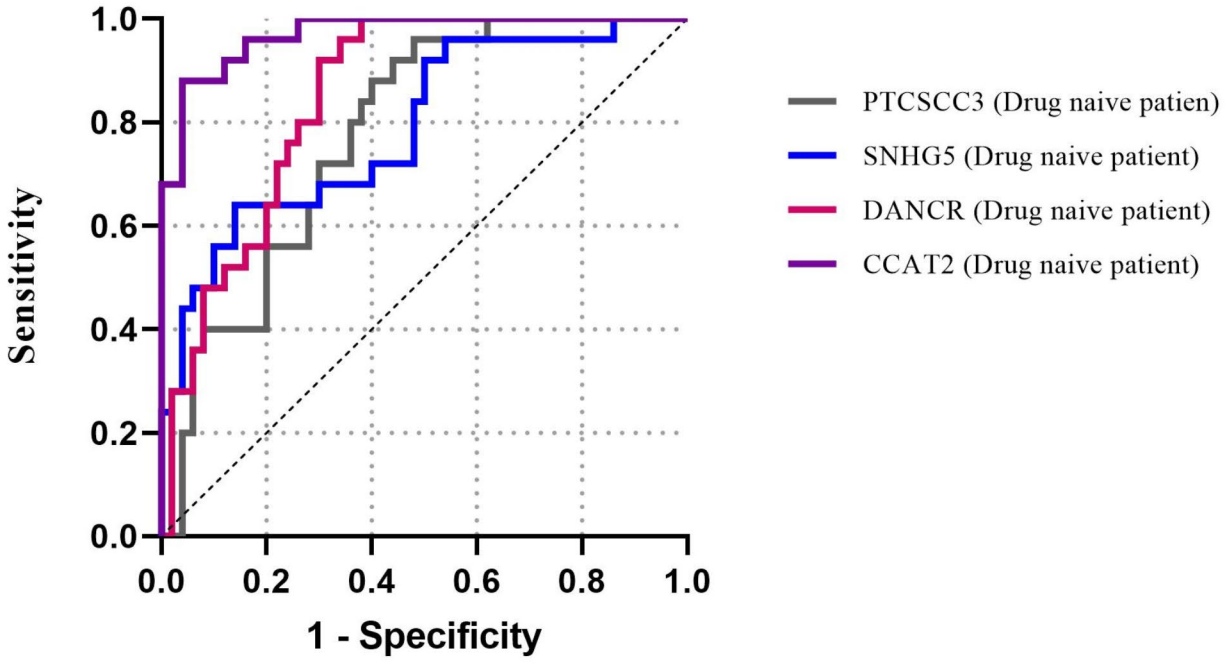

Fig. 2 The diagnostic power of transcript quantities of *CCAT2*, *PTCSC3*, *SNHG5*, and *DANCR* in identifying patients and controls

Table 5 Correlation coefficient and *P*-value of genes in drug-naive patients and controls (* shows significance)

			CTNNB1	PTCSC3	SNHG5	DANCR	CCAT2
CTNNB1	Drug naive patients	R	1	0.335	0.626	0.334	0.382
		P-value	0	0.101	0.001	0.103	0.060
	Controls	R	1	0.242	-0.068	0.483	0.277
		P-value	0	0.090	0.638	< 0.001*	0.052
PTCSC3	Drug naive patients	R	0.335	1	0.269	0.249	0.523
		P-value	0.101	0	0.194	0.231	0.007*
	Controls	R	0.242	1	0.265	0.460	0.433
		P-value	0.090	0	0.063	0.001*	0.002*
SNHG5	Drug naive patients	R	0.626	0.269	1	0.307	0.254
		P-value	0.001*	0.194	0	0.136	0.221
	Controls	R	-0.068	0.265	1	0.215	0.341
		P-value	0.638	0.063	0	0.133	0.015
DANCR	Drug naive patients	R	0.334	0.249	0.307	1	0.560
		P-value	0.103	0.231	0.136	0	0.004*
	Controls	R	0.483	0.460	0.215	1	0.674
		P-value	< 0.001*	0.001*	0.133	0	< 0.001*
CCAT2	Drug naive patients	R	0.382	0.523	0.254	0.560	1
		P-value	0.060	0.007*	0.221	0.004*	0
	Controls	R	0.277	0.433	0.341	0.674	1
		P-value	0.052	0.002*	0.015*	< 0.001*	0

Table 6 Correlation between age of disease onset and expression of genes in drug-naive patients

Parameters	CTNNB1	PTCSC3	SNHG5	DANCR	CCAT2
R	0.04789	0.1267	0.1448	-0.1344	0.2397
P value	0.8202	0.5462	0.4898	0.5219	0.2485
P value summary	ns	ns	ns	ns	ns
Significant (alpha = 0.05)	No	No	No	No	No
Number of XY Pairs	25	25	25	25	25

ns: not significant

Table 7 Correlation between age of disease onset and expression of genes in medicated patients

Parameters	CTNNB1	PTCSC3	SNHG5	DANCR	CCAT2
R	0.05385	-0.1659	0.2839	0.1472	0.08401
P value	0.7103	0.2496	0.0457	0.3075	0.5619
P value summary	ns	ns	*	ns	ns
Significant (alpha = 0.05)	No	No	Yes	No	No
Number of XY Pairs	50	50	50	50	50

ns: not significant

in the pathophysiology of SCZ. Notably, expression of all mentioned lncRNAs was higher in first episode patients compared with medicated patients, showing the possible effect of medications on amendment of the alterations of these lncRNAs.

CTNNB1 has a dual function inside cells. It plays a crucial role in the Wnt/ β-catenin pathway and is essential for cellular junctions, where it attaches cadherins to the cytoskeleton, thus having fundamental role in the cell adhesion [27]. It has been implicated in neurological diseases such as autism, Alzheimer's disease, and SCZ [28–30]. In this study, the expression of *CTNNB1* was not significantly different between medicated SCZ patients and controls, possibly due to the effects antipsychotic treatment. However, in drug-naive patients, *CTNNB1* expression was significantly decreased compared to controls. Our results align with previous studies, such as Beasley et al.'s study that found no significant difference in β-catenin expression between SCZ patients and controls [31], and Alimohamad et al., who reported increased β-catenin levels following antipsychotic treatment [32]. To be more specific, total levels of β-catenin protein were shown to significantly increased after administration of clozapine, haloperidol or risperidone in rat models [32]. In fact, as a common feature, antipsychotics were found to affect levels of β-catenin protein regardless of their drug class. These effects are possibly mediated by D2 dopamine receptors [32]. It is also worth mentioning that

disruption in β-catenin expression, whether down-regulated or up-regulated, can result in abnormal neuronal proliferation and differentiation [33]. Moreover, certain SCZ-like phenotypes have been shown to be associated with *CTNNB1* mutations [34]. Further studies are needed to show whether these mutations change the expression of *CTNNB1* at mRNA level or solely impair protein function.

PTCSC3 is located on chromosome 14q13.3. This tumor-suppressive lncRNA plays a crucial role in various disorders, including cancer and non-cancerous conditions, by regulating apoptosis, cell proliferation, and migration, and also influencing the Wnt/β-catenin signaling pathway [23]. PTCSC3 expression was significantly increased in both medicated and drug-naive patients compared to controls, with more pronounced changes in drug-naive patients. LRP6, as a receptor of the Wnt/β-catenin pathway, was found to be targeted by PTCSC3 [35]. While some members of LRP family have been found to be altered in the context of schizophrenia, LRP6 exhibited no change [36]. It is possible that changes in the expression of PTCSC3 acts as an alternative route in the pathoetiology of SCZ instead of LRP6 dysregulation. PTCSC3 was also shown to absorb miR-574-5p [37], a miRNA that is regarded as a biomarker for SCZ [38]. Thus, PTCSC3/miR-574-5p is another putative axis in the pathogenesis of SCZ. ROC curve analysis for PTCSC3 yielded specificity and sensitivity of 0.56 and 0.92, respectively. Overexpression of this lncRNA may deactivate LRP6, affecting the Wnt signaling pathway by destabilizing the APC/Axin/GSK/β-catenin destruction complex [39–41].

DANCR is located on chromosome 4q12, and is implicated in various cancers [42]. Our results demonstrated significant overexpression of this lncRNA in both medicated and drug-naïve SCZ patients compared to controls. ROC curve analysis for DANCR showed specificity and sensitivity of 0.70 and 0.92, respectively. Wang et al. demonstrated DANCR role in regulating osteogenic differentiation by interacting with CTNNB1 and miR-320a. The results showed that DANCR and miR-320a functioned independently from each other and both suppressed CTNNB1. The inhibitory impact was additive when miR-320a and DANCR were overexpressed together [43]. It is worth mentioning that changes in the expression of miR-320 family members were regarded to have diagnostic value in SCZ patients [44]. Thus, DANCR/miR-320a might be a functional axis in the pathoetiology of SCZ. Further research is needed to fully understand how DANCR modulates this pathway and its potential implications for brain disorders.

Researchers have found that the lncRNA SNHG5 is abnormally expressed in various cancers and influences cellular processes like growth, cell cycle, autophagy, and apoptosis by interacting with miRNAs, signaling pathways, and other proteins [45]. In our study, SNHG5 expression was significantly increased in both medicated and drug-naive SCZ patients compared to controls, with greater changes in drug-naive patients. In addition, expression of SNHG5 was correlated with age of disease onset in the medicated patients. This finding possibly shows the effects of medication on the expression of this lncRNA. SNHG5 has an established role in the regulation of inflammatory responses and oxidative damage [46], two processes that are fundamentally involved in the pathoetiology of SCZ [47, 48]. ROC curve analysis for SNHG5 showed specificity and sensitivity of 0.84 and 0.64, respectively. Previous studies indicate that SCZ is associated with a reduction in GSK3β, a key kinase in the Wnt signaling pathway [31]. Both β-catenin and GSK3β are crucial in this pathway. Others have shown that lncRNA SNHG5 inhibits GSK3β expression; and increased SNHG5 expression leads to decreased GSK3β [45, 49, 50]. Glatt et al. (2011) found increased lncRNA SNHG5 expression in SCZ patients compared to controls [51].

CCAT2 was significantly overexpressed in both medicated and drug-naive SCZ patients compared to controls. ROC curve analysis for CCAT2 showed specificity and sensitivity of 0.96 and 0.88, respectively. This lncRNA is known for its role in promoting tumor growth and metastasis, and its expression is significantly upregulated in various cancers. This upregulation is associated with a decrease in GSK3β expression [52, 53]. Given the inhibitory role of CCAT2 on GSK3β, an increase in CCAT2 expression in SCZ patients is expected to result in decreased GSK3β levels.

Our results align with previous research showing the regulatory roles of lncRNAs in various diseases. The notable changes in lncRNA expression seen in SCZ patients highlight their potential as biomarkers and therapeutic targets. To explore a potential correlation between selected lncRNAs and CTNNB1, we suggest conduction of knockdown and overexpression experiments in the appropriate cell lines. Moreover, the results should be confirmed in larger samples sizes of patients and controls.

Conclusion

In summary, we investigated the expression of lncRNAs related to the Wnt/β-catenin pathway in the peripheral blood of SCZ patients (medicated and non-medicated patients). Dysregulation of lncRNAs and their interactions with this pathway suggest complex mechanisms contributing to the pathogenesis of SCZ. PTCSC3 might affect the stability of the β-catenin destruction complex, while DANCR and SNHG5 could modulate gene expression through interactions with miRNAs and signaling

pathways. The observed difference in the expression of genes between treated and untreated patients might be due to the effect of antipsychotic medication. Moving forward, it is essential to validate these findings in larger patient cohorts and explore in greater detail how lncRNAs affect the Wnt/β-catenin pathway in SCZ. Identifying specific lncRNA expression profiles in SCZ patients could pave the way for diagnostic methods using blood samples and development of targeted therapies that modulate lncRNA activity. However, it is worth mentioning that changes in the expression of these lncRNAs are not specific to SCZ and might be observed in a variety of malignant and non-malignant conditions. Thus, it is necessary to measure baseline levels of these lncRNAs to use them as possible markers in the follow-up of patients. Moreover, assessment of other conditions that might affect their expression is a necessary step in this process.

Our study has some limitations. First, the results of expression assays in the clinical samples should be verified in functional studies to unravel the mechanistical points beyond the findings. Second, larger sample sizes of both medicated and drug-naïve patients are needed to confirm the applicability of mentioned lncRNAs in diagnostic approaches.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12991-025-00545-1.

Supplementary Material 1

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Author contributions
FM performed the experiments and wrote the paper. BB contributed to the study design. SGNA, KJA, and RD contributed to the data analyses. SG-F contributed to the paper writing and revision. AS supervised the study and contributed to the concept and study design. All authors contributed equally and are fully aware of the submission.

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Data availability
The datasets generated and/or analysed during the current study are available in the NCBI repository: CCAT2 (NC_000008.11), DANCR (NC_000004.12), PTCSC3 (NC_000014.9), SNHG5 (NC_000006.12), CTNNB1 (NC_000003.12).

Declarations

Ethics approval and consent to participate
This study protocol was approved by the ethical committee of Shahroud University of Medical Sciences. Informed consent forms were obtained from all study participants.

Competing interests
The authors declare no competing interests.

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References

1. Arlington V, Association. AP Diagnostic and Statistical Manual of Mental disorders. Am Psychiatr Assoc. 2013;5:612–3.
2. Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry. 2003;60(12):1187–92.
3. Knapp M, Mangalore R, Simon J. The global costs of schizophrenia. Schizophr Bull. 2004;30(2):279–93.
4. Fan H-m, Sun X-y, Niu W, Zhao L, Zhang Q-L, Li W-s, et al. Altered microRNA expression in peripheral blood mononuclear cells from young patients with schizophrenia. J Mol Neurosci. 2015;56:562–71.
5. Narayan S, Tang B, Head SR, Gilmartin TJ, Sutcliffe JG, Dean B, et al. Molecular profiles of schizophrenia in the CNS at different stages of illness. Brain Res. 2008;1239:235–48.
6. Ramaker RC, Bowling KM, Lasseigne BN, Hagenauer MH, Hardigan AA, Davis NS, et al. Post-mortem molecular profiling of three psychiatric disorders. Genome Med. 2017;9:1–12.
7. Du Y, Tan WL, Chen L, Yang ZM, Li XS, Xue X, et al. Schizophr Bull. 2021;47(5):1288–99. PubMed PMID: 33837780. Pubmed Central PMCID: PMC8379541. Epub 2021/04/11. eng. Exosome Transplantation From Patients With Schizophrenia Causes Schizophrenia-Relevant Behaviors in Mice: An Integrative Multi-omics Data Analysis.
8. Du Y, Li XS, Chen L, Chen GY, Cheng Y. A Network Analysis of Epigenetic and transcriptional regulation in a neurodevelopmental rat model of Schizophrenia with implications for Translational Research. Schizophr Bull. 2020;46(3):612–22. PubMed PMID: 31738422. Pubmed Central PMCID: PMC7147587. Epub 2019/11/19. eng.
9. Iyer MK, Niknafs YS, Malik R, Singhal U, Sahu A, Hosono Y, et al. The landscape of long noncoding RNAs in the human transcriptome. Nat Genet. 2015;47(3):199–208.
10. Du Y, Yu Y, Hu Y, Li X-W, Wei Z-X, Pan R-Y, et al. Genome-Wide, Integrative Analysis implicates exosome-derived MicroRNA dysregulation in Schizophrenia. Schizophr Bull. 2019;10(24):45:1257–66.
11. Gibbons A, Udawela M, Dean B. Non-coding RNA as novel players in the pathophysiology of schizophrenia. Non-coding RNA. 2018;4(2):11.
12. Liu B, Sun L, Liu Q, Gong C, Yao Y, Lv X, et al. A cytoplasmic NF-κB interacting long noncoding RNA blocks IκB phosphorylation and suppresses breast cancer metastasis. Cancer Cell. 2015;27(3):370–81.
13. Kumarswamy R, Bauters C, Volkmann I, Maury F, Fetisch J, Holzmann A, et al. Circulating long noncoding RNA, LIPCAR, predicts survival in patients with heart failure. Circul Res. 2014;114(10):1569–75.
14. Policarpo R, Sierksma A, De Strooper B, d'Ydewalle C. From junk to function: LncRNAs in CNS health and disease. Front Mol Neurosci. 2021;14:714768.
15. Hoseth EZ, Krull F, Dieset I, Mørch RH, Hope S, Gardsjord ES, et al. Exploring the wnt signaling pathway in schizophrenia and bipolar disorder. Translational Psychiatry. 2018;8(1):55. 2018/03/06.
16. Liu C, Kato Y, Zhang Z, Do VM, Yankner BA, He X. β-Trcp couples β-catenin phosphorylation-degradation and regulates Xenopus axis formation. Proceedings of the National Academy of Sciences. 1999;96(11):6273-8.
17. Hayat R, Manzoor M, Hussain A. Wnt signaling pathway: a comprehensive review. Cell Biol Int. 2022;46(6):863–77.
18. Vallée A. Neuroinflammation in schizophrenia: the key role of the WNT/β-catenin pathway. Int J Mol Sci. 2022;23(5):2810.
19. Astaneh M, Ghafouri-Fard S. Long non-coding RNAs as regulators of Wnt/β catenin pathway. Gene Rep. 2019;16:100404.
20. Wang D, Li Z, Yin H. Long non-coding RNA CCAT2 activates RAB14 and acts as an Oncogene in Colorectal Cancer. Front Oncol. 2021;11:751903. PubMed

PMID: 34868956. Pubmed Central PMCID: PMC8639683. Epub 2021/12/07. eng.
21. Yu JE, Ju JA, Musacchio N, Mathias TJ, Vitolo MI. Long noncoding RNA DANCR activates Wnt/β-Catenin signaling through MiR-216a inhibition in Non-small Cell Lung Cancer. Biomolecules. 2020;10(12). PubMed PMID: 33302540. Pubmed Central PMCID: PMC7764320. Epub 2020/12/12. eng.
22. Li Y, Hu J, Guo D, Ma W, Zhang X, Zhang Z et al. LncRNA SNHG5 promotes the proliferation and cancer stem cell-like properties of HCC by regulating UPF1 and wnt-signaling pathway. Cancer Gene Ther. 2022;29(10):1373–83.
23. Ghasemian M, Poodineh J. A review on the biological roles of LncRNA PTCSC3 in cancerous and non-cancerous disorders. Cancer Cell Int. 2023;23(1):184. 2023/08/29.
24. Kahn RS, Sommer IE, Murray RM, Meyer-Lindenberg A, Weinberger DR, Cannon TD et al. Schizophrenia (primer). Nat Reviews: Disease Primers. 2015;1(1).
25. Cadigan KM, Nusse R. Wnt signaling: a common theme in animal development. Genes Dev. 1997;11(24):3286–305.
26. Miller JR, Moon RT. Signal transduction through beta-catenin and specification of cell fate during embryogenesis. Genes Dev. 1996;10(20):2527–39.
27. van der Wal T, van Amerongen R. Walking the tight wire between cell adhesion and WNT signalling: a balancing act for β-catenin. Open Biology. 2020;10(12):200267.
28. Dong F, Jiang J, McSweeney C, Zou D, Liu L, Mao Y. Deletion of CTNNB1 in inhibitory circuitry contributes to autism-associated behavioral defects. Hum Mol Genet. 2016;25(13):2738–51.
29. Guo X, Yang J, Huang J, Chen Z, Wu X, Zhu L, et al. Influence of CTNNB1 rs2953 polymorphism on schizophrenia susceptibility in Chinese Han population through modifying miR-485 binding to CTNNB1. Genes. Brain Behav. 2019;18(4):e12524.
30. Smith A, Bourdeau I, Wang J, Bondy C. Expression of Catenin family members CTNNA1, CTNNA2, CTNNB1 and JUP in the primate prefrontal cortex and hippocampus. Mol Brain Res. 2005;135(1–2):225–31.
31. Beasley C, Cotter D, Khan N, Pollard C, Sheppard P, Varndell I, et al. Glycogen synthase kinase-3β immunoreactivity is reduced in the prefrontal cortex in schizophrenia. Neurosci Lett. 2001;302(2–3):117–20.
32. Alimohamad H, Rajakumar N, Seah Y-H, Rushlow W. Antipsychotics alter the protein expression levels of β-catenin and GSK-3 in the rat medial prefrontal cortex and striatum. Biol Psychiatry. 2005;57(5):533–42.
33. Zhuang W, Ye T, Wang W, Song W, Tan T. CTNNB1 in neurodevelopmental disorders. Front Psychiatry. 2023;14:1143328.
34. Levchenko A, Davtian S, Freylichman O, Zagrivnaya M, Kostareva A, Malashichev Y. Beta-catenin in schizophrenia: possibly deleterious novel mutation. Psychiatry Res. 2015;228(3):843–8.
35. Xia S, Ji R, Zhan W. Long noncoding RNA papillary thyroid carcinoma susceptibility candidate 3 (PTCSC3) inhibits proliferation and invasion of glioma cells by suppressing the Wnt/β-catenin signaling pathway. BMC Neurol. 2017;17(1):30. PubMed PMID: 28187755. Pubmed Central PMCID: PMC5303216. Epub 2017/02/12. eng.
36. Gibbons AS, Thomas EA, Scarr E, Dean B. Low density lipoprotein receptor-related protein and apolipoprotein E expression is altered in Schizophrenia. Front Psychiatry. 2010;1:19. PubMed PMID: 21423430. Pubmed Central PMCID: PMC3059617. Epub 2010/01/01. eng.
37. Wang X, Lu X, Geng Z, Yang G, Shi Y. LncRNA PTCSC3/miR-574-5p governs Cell Proliferation and Migration of Papillary thyroid carcinoma via Wnt/β-Catenin signaling. J Cell Biochem. 2017;118(12):4745–52. PubMed PMID: 28513866. Epub 2017/05/18. eng.
38. Davarinejad O, Najafi S, Zhaleh H, Golmohammadi F, Radmehr F, Alikhani M, et al. MiR-574-5P, miR-1827, and miR-4429 as potential biomarkers for Schizophrenia. J Mol Neuroscience: MN. 2022;72(2):226–38. PubMed PMID: 34811713. Epub 2021/11/24. eng.
39. Behrens J, Jerchow B-A, Würtele M, Grimm J, Asbrand C, Wirtz R, et al. Functional interaction of an axin homolog, conductin, with β-catenin, APC, and GSK3β. Science. 1998;280(5363):596–9.
40. Schmitz Y, Rateitschak K, Wolkenhauer O. Analysing the impact of nucleocytoplasmic shuttling of β-catenin and its antagonists APC, Axin and GSK3 on Wnt/β-catenin signalling. Cell Signal. 2013;25(11):2210–21.
41. Xia S, Ji R, Zhan W. Long noncoding RNA papillary thyroid carcinoma susceptibility candidate 3 (PTCSC3) inhibits proliferation and invasion of glioma cells by suppressing the Wnt/β-catenin signaling pathway. BMC Neurol. 2017;17:1–11.
42. Thin KZ, Liu X, Feng X, Raveendran S, Tu JC. LncRNA-DANCR: a valuable cancer related long non-coding RNA for human cancers. Pathology-Research Pract. 2018;214(6):801–5.
43. Wang C-G, Hu Y-H, Su S-L, Zhong D. LncRNA DANCR and miR-320a suppressed osteogenic differentiation in osteoporosis by directly inhibiting the Wnt/β-catenin signaling pathway. Exp Mol Med. 2020;52(8):1310–25.
44. Vachev T, Popov N, Stoyanova V, Ivanov H, Minchev D. Alterations of miR-320 family members as a novel diagnostic biomarkers in peripheral blood of schizophrenia patients. Int J Curr Microbiol Appl Sci. 2016;5(3):865–75.
45. Han W, Shi J, Cao J, Dong B, Guan W. Latest advances of long non-coding RNA SNHG5 in human cancers. OncoTargets Therapy. 2020;13:6393.
46. Cai Q, Wang C, Huang L, Wu C, Yan B, Chen T et al. Long non-coding RNA small nucleolar RNA host gene 5 (SNHG5) regulates renal tubular damage in Diabetic Nephropathy via Targeting MiR-26a-5p. Hormone and metabolic research = Hormon- Und Stoffwechselforschung = Hormones et metabolisme. 2021;53(12):818–24. PubMed PMID: 34891212. Epub 2021/12/11. eng.
47. Bitanihirwe BK, Woo TU. Oxidative stress in schizophrenia: an integrated approach. Neurosci Biobehav Rev. 2011;35(3):878–93. PubMed PMID: 20974172. Pubmed Central PMCID: PMC3021756. Epub 2010/10/27. eng.
48. Müller N, Weidinger E, Leitner B, Schwarz MJ. The role of inflammation in schizophrenia. Front Neurosci. 2015;9:372. PubMed PMID: 26539073. Pubmed Central PMCID: PMC4612505. Epub 2015/11/06. eng.
49. Hu X, Hong Y, Shang C. Knockdown of long non-coding RNA SNHG5 inhibits malignant cellular phenotypes of glioma via Wnt/CTNNB1 signaling pathway. J Cancer. 2019;10(5):1333.
50. Alfhili MA, Alsughayyir J, McCubrey JA, Akula SM. GSK-3-associated signaling is crucial to virus infection of cells. Biochim et Biophys Acta (BBA)-Molecular Cell Res. 2020;1867(10):118767.
51. Glatt SJ, Stone WS, Nossova N, Liew CC, Seidman LJ, Tsuang MT. Similarities and differences in peripheral blood gene-expression signatures of individuals with schizophrenia and their first-degree biological relatives. Am J Med Genet Part B: Neuropsychiatric Genet. 2011;156(8):869–87.
52. Ruan R, Zhao X. LncRNA CCAT2 enhances cell proliferation via GSK3beta/beta-catenin signaling pathway in human osteosarcoma. Eur Rev Med Pharmacol Sci. 2018;22(10):2978–84.
53. Huang J-l, Liao Y, Qiu M-x, Li J, An Y. Long non-coding RNA CCAT2 promotes cell proliferation and invasion through regulating Wnt/β-catenin signaling pathway in clear cell renal cell carcinoma. Tumor Biology. 2017;39(7):1010428317711314.

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