Psychiatr. Pol. ONLINE FIRST Nr 126: 1–11
Published ahead of print 7 January 2019
www.psychiatriapolska.pl
ISSN 0033-2674 (PRINT), ISSN 2391-5854 (ONLINE)
DOI: https://doi.org/10.12740/PP/OnlineFirst/99971

Analysis of the expression of *BAX, BCL2, BIRC6, CASP3, CASP9* apoptosis genes during the first episode of schizophrenia

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Summary

Aim. The aim of the study was the analysis of (1) the level of *BAX*, *BCL2*, *BIRC6*, *CASP3*, *CASP9* apoptosis genes expression in schizophrenic patients and in the control group, and (2) the relationships between the genes expression level and the morphological and biochemical parameters, as well as the severity of psychopathological symptoms.

Method. The study included 21 patients diagnosed with schizophrenia according to ICD-10 and 26 healthy subjects. The following parameters were assessed: genes expression in peripheral blood lymphocytes, laboratory parameters and severity of psychopathological symptoms (using the PANSS).

Results. The expression of the *BCL2* gene and the *CASP3* gene was significantly higher in schizophrenic patients compared to the controls. A significant positive correlation was found between the *BAX* gene expression level and neutrophil, lymphocyte and monocyte counts as well as the severity of negative symptoms (PANSS-N), between *BCL2* and *CASP9* genes expression and the monocyte count, and between the *CASP3* gene expression and the lymphocyte count.

Conclusions. (1) Significantly higher expression of *BCL2* and *CASP3* genes in schizophrenic patients compared to the controls proves the intensification of the apoptosis process, fitting in the theory of increased apoptosis in the pathophysiology of schizophrenia. (2) Significant correlations between the *BAX* gene expression and differential blood count parameters (leucocyte, neutrophil, lymphocyte, monocyte count) in the group of schizophrenic patients suggest a relationship with immune dysregulation and confirm the presence of apoptosis in peripheral blood lymphocytes. (3) The *BAX* gene expression may be indicative of the severity of negative symptoms in schizophrenia. (4) The analysis of the intercorrelation of studied

genes expression indicates that *BAX* and *CASP3* genes were the most active in the apoptosis process in the study group.

Key words: schizophrenia, apoptosis, gene expression

Introduction

Schizophrenia is a severe, chronic mental disorder that primarily affects people in adolescence and early adulthood, the etiology and pathogenesis of which remains unclear [1]. Among many theories concerning the etiopathogenesis of this disorder, there is a neurodevelopmental hypothesis of schizophrenia, formulated in the 1980s [2–4], with subsequent modifications and updates [5, 6]. It assumes that already in the period of fetal and early childhood development, some abnormal changes occur in the central nervous system of people affected by this disorder [7]. In the process of the central nervous system maturation, there is a critical moment of synaptic pruning, which may proceed excessively and lead to a reduction in brain plasticity [8]. It is accompanied by the elimination of some neurons in the apoptosis process, which is a genetically programmed sequence of biochemical processes induced by extrinsic or intrinsic initiator factors [9].

There are reports that this process may also be observed and evaluated outside the CNS, e.g., in peripheral blood lymphocytes [10]. In the intrinsic pathway, the programmed cell death signal is regulated by anti – (Bcl-2, Bcl-XL) and pro-apoptotic (Bax, Bad, Bid) protein factors [11]. Pro-apoptotic proteins, embedding in the inner membrane of the mitochondrion, impair the action of the respiratory chain and, under the influence of calcium ions from the mitochondrion, cytochrome C is released to the cytoplasm. In the final stage, cytochrome C combined with the Apaf-1 protein, combines with caspase-9 (initiator caspase) to form apoptosome. The executive caspases 3, 6 and 7 cause the disintegration of proteins and lead to disintegration of the cell [12]. The decision-making process in the cell as to proceeding to the apoptosis path is the gene activation process [13], the polymorphism of which has been the subject of many studies [14–16]. The available literature is also a source of information on the levels of pro – and anti-apoptotic proteins in patients suffering from schizophrenia: low Bcl-2, increased Bax/Bcl-2 ratio or increased caspase 3 activity [17–20]. In spite of that, there are reports in which reduced activity of pro-apoptotic genes and proteins is observed [21, 22]. The BIRC 6 protein (BRUCE) is anti-apoptotic, inhibiting the activation of caspases [23]. There are few studies on the activity of the *BIRC6* gene in schizophrenia, one of which reports overlapping genes in disorders of autism spectrum and in schizophrenia [24]. Increased apoptosis seems to be a key process also in the neurodegenerative theory and is a link between this theory and the above-described neurodevelopmental hypothesis [19].

Aim of the study

The aim of the study was to evaluate (1) the level of *BAX, BCL2, BIRC6, CASP3, CASP9* apoptosis genes expression in schizophrenic patients and in the control group

and (2) the relationships between the gene expression levels and morphological and biochemical parameters of peripheral blood, as well as the severity of psychopathological symptoms.

Material

The study included 21 patients (18 men and 3 women) during the first episode of schizophrenia, diagnosed according to ICD-10 criteria (F20), 17–35 years old (mean age = 23 years; *SD* = 4.8) (SCH group). The study subjects were patients hospitalized in the I Department of Psychiatry, Psychotherapy and Early Intervention in Lublin and outpatients of Hospital Mental Health Clinic at the Children's University Hospital in Lublin.

The control group (C) included 26 healthy subjects (20 men and 6 women) aged 18–32 years (mean age = 22; *SD* = 4.1) with no family history of mental disorders, matched for gender and age.

The inclusion criteria for the study was the declaration of informed and written consent to participate in the study, no active or past neurological, inflammatory and autoimmune diseases, absence of past or current alcohol or other addictive substances dependence, absence of intellectual impairment assessed on the basis of psychiatric examination. There were no somatic diseases that would constitute an absolute exclusion criterion. In addition, in the control group, the inclusion criteria was a lack of any psychiatric disorder at the present or in the past. The experienced psychiatrist (KS) performed an assessment of the mental state aimed at excluding the occurrence of mental disorders in the control group. The patients were taking atypical antipsychotics.

The study was approved by the Bioethics Committee of the Medical University of Lublin (KE-0254/77/2012).

Methods

The following methods were used in the study.

Determination of *BAX, BCL2, BIRC6, CASP3,* and *CASP9* genes expression from peripheral blood lymphocytes. Total cellular RNA was isolated by the modified method of Chomczyński and Sacchi [25]. Next, a cDNA synthesis reaction was performed using High-Capacity cDNA Transcription Kits with RNAse inhibitor (Applied Biosystems). 1 µg of RNA dissolved in 10 µl of ultrapure water and 10 µl of reagent mix (which consistent of: 2 µl –10xRT Buffer; 0.8 µl – 10xdNTPs (100 mM); 2 µl – 10xRT Random Primer; 1 µl – RNasin 20 U/µl; 1 µl – Reverse transcriptase 50 U/µl, and 3.2 µl – ultrapure water). cDNA was amplified in a real-time PCR reaction in the StepOnePlus system (Applied Biosystems). The reaction was carried out using a TaqMan-type probe specific for the gene being tested Hs00180269_m1 for the *BAX* gene, Hs00608023_m1 for the *BCL2* gene, Hs00212288_m1 for the *BIRC6* gene, Hs00234387_m1 for the *CASP3* gene, Hs00154261_m1 for the *CASP9* gene, and Hs01066071_m1 for the *TRPM2* gene (Applied Biosystems). The *GAPDH* was used as the reference gene. There are reports of a significant role of this gene in cytotoxicity

and apoptotic mechanisms [26]. The expression of the gene in the tested samples was stable. After completion of the reaction, the RQ (relative quantification) value of the genes tested in peripheral blood cells of the patients from the study group relative to the control group was calculated using the following formula $RQ = 2^{-\Delta\Delta}CT$ [27].

Laboratory tests were performed simultaneously (blood count).

The severity of psychopathological symptoms of schizophrenia was assessed using *the Positive and Negative Symptoms Scale for Schizophrenia* (PANSS) [28].

Analysis of the results of expression and statistical analysis was carried out using Expression Suit Software Version 1.0.3 (Life Technologies) and Statistica 10 for Windows. Statistical data between the study and control groups were compared using the Student's *t*-test. The value $p < 0.05$ was considered statistically significant. Correlations between genes and clinical variables were assessed using the Spearman's rank correlation coefficient.

Results

Table 1. **Demographic and clinical features of the studied groups**

	SCH (n = 21)	C (n = 26)	p
Age (x, SD, years)	23 (4.8)	22 (4.1)	0.42
Gender (%, male)	85.71	76.92	0.93
Duration of illness (x, SD, months)	4.71 (2.17)	-	
Equivalent of chlorpromazine – mg (x, SD)	294 (115)	-	
PANSS total (x, SD)	97.05 (21.62)	-	
PANSS-P (x, SD)	22.23 (7.91)	-	
PANSS-N (x, SD)	26.23 (7.17)	-	
PANSS-G (x, SD)	48.59 (13.18)	-	

p – level of significance; SD – standard deviation; PANSS total – Positive and Negative Symptoms Scale for Schizophrenia – total score; PANSS-P –Positive Scale; PANSS-N – Negative Scale; PANSS-G – General Psychopathology Scale

As a result of the performed tests of *BAX, BCL2, BIRC6, CASP3, CASP9* genes expression using qPCR method, it was confirmed that these genes have upregulated expression at mRNA level in peripheral blood lymphocytes in patients with schizophrenia compared to healthy subjects (Figure 1).

Based on the performed studies in peripheral blood lymphocytes in schizophrenic patients, more than 5-fold significantly higher *CASP3* gene expression ($p = 0.005$) was found, as well as over 4-fold significantly higher *BCL2* gene expression ($p = 0.000001$) in comparison to controls (Figure 1).

In peripheral blood lymphocytes, in schizophrenic patients, statistically significant intercorrelations between the studied genes were found (Table 2). The most active genes in the apoptosis process in the study group appear to be *BAX* and *CASP3*

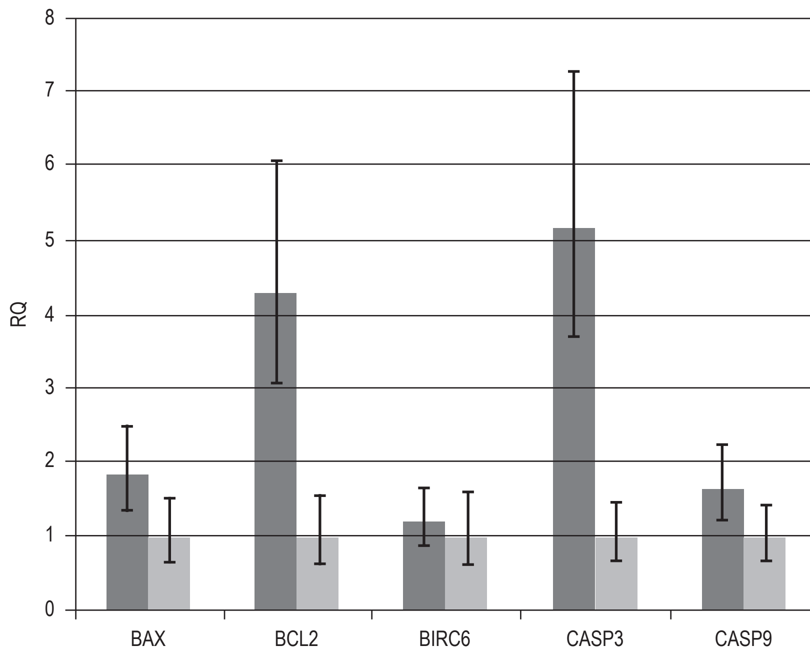

Figure 1. **The differences in the mean level of relative expression RQ±SEM of BAX, BCL2, BIRC6, CASP3, CASP9 genes in peripheral blood lymphocytes between the SCH group and the controls**

** $p < 0.01$; *** $p < 0.001$

genes, the expression value of which correlated with the expression values of all other genes.

Table 2. **Spearman's R correlations between the studied genes**

	logRQ BAX	logRQ BCL2	logRQ BIRC6	logRQ CASP3	logRQ CASP9
logRQ BAX	1.000	0.678*	0.644*	0.856*	0.698*
logRQ BCL2	0.678*	1.000	0.403	0.677*	0.377
logRQ BIRC6	0.644*	0.403	1.000	0.729*	0.621*
logRQ CASP3	0.856*	0.677*	0.729*	1.000	0.565*
logRQ CASP9	0.698*	0.377	0.621*	0.565*	1.000

* $p < 0.05$

The dependence of *BAX, BCL2, BIRC6, CASP3, CASP9* genes expression level on age, sex, length of the illness was assessed and no statistically significant differences were found depending on the mentioned parameters.

The mean total score on the PANSS was 92.0±21.7, the result in the positive symptom subscale was 21.96±6.57, negative symptom subscale – 25±7.3. A significant

positive correlation was found between the severity of clinical symptoms measured with the PANSS-N and the *BAX* gene expression in the group of patients with schizophrenia (Table 3).

Table 3. **Spearman's rank-order correlation between genes expression and the PANSS results**

	PANSS-P	PANSS-N	PANSS-G	PANSS-T
logRQ BAX	-0.122	0.443*	0.064	0.188
logRQ BCL2	-0.184	0.089	-0.107	-0.026
logRQ BIRC6	0.006	0.521	0.075	0.225
logRQ CASP3	-0.058	0.236	0.186	0.209
logRQ CASP9	-0.474	0.334	-0.361	-0.245

* p < 00.5; PANSS-P – Positive Scale; PANSS-N – Negative Scale; PANSS-G – General Psychopathology Scale; PANSS-T – total score

In schizophrenic patients the analysis of the dependence of apoptosis gene expression on blood count and biochemical parameters of the blood showed the following relationships: a significant positive correlation of the *BAX* gene expression with the neutrophil, lymphocyte and monocyte count, of the *BCL2* with monocyte count, of the *CASP3* with lymphocyte count, and of the *CASP9* with monocyte count. These results are presented in Table 4.

Table 4. **Spearman's rank-order correlation between genes expression and blood count and biochemical parameters**

	WBC	NEU	LYM	MON
logRQ BAX	0.350	0.762*	0.810*	0.778*
logRQ BCL2	0.045	0.619	0.595	0.766*
logRQ BIRC6	0.363	0.600	0.771	0.319
logRQ CASP3	0.285	0.690	0.762*	0.467
logRQ CASP9	0.182	0.464	0.500	0.821*

* p <0.05; WBC – leucocyte count; NEU – neutrophil count; LYM – lymphocyte count; MON – monocyte count

Discussion

The results of the study showed a significant increase in the expression of *BCL2* and *CASP3* genes in schizophrenic patients compared to the control group of healthy people. The obtained results indicate that in the studied patients there may be changes in the implementation of the genetic program and disturbances of systems controlling programmed cell death, which are observed in schizophrenia, associated with increased elimination of synapses called pruning [8, 29]. This increased pro-apoptotic activity in the group of schizophrenic patients may confirm

the theory of induced apoptosis in the pathophysiology of schizophrenia [8, 18], having its morphotic exponents in the form of reduced volume of brain structures in schizophrenia [30]. The results of our research correspond with the studies of other authors suggesting the involvement of the Bcl-2 protein in the pathophysiology of schizophrenia [17, 18, 20]. Jarskog [31] in his work suggests that apoptotic activity may be reduced in the chronic phase of the illness. Our results also correspond with reports that showed that altered *BCL2* family genes expression resulted in increased susceptibility to apoptosis [9, 11]. Jarskog et al. [17, 18] and Tsai et al. [20] showed significant relationships between the used antipsychotic treatment and the increase in the Bcl-2 protein, they also underline the neuroprotective and anti-apoptotic effects of atypical antipsychotics. In the light of these reports, the increased expression of the *BCL2* gene, which belongs to the apoptosis inhibitors, in the studied patients with schizophrenia can be explained by antipsychotic treatment. In the study of Bai et al.[32], olanzapine and clozapine were shown to upregulate mRNA Bcl-2 levels in the rat brain by 30–50%.

Another interesting result of our research is the statistically significantly increased expression of the *CASP3* gene. The results obtained in our work correspond with the results of other studies indicating the altered activity of caspase 3 in schizophrenia [33]. Batalla et al. [30] demonstrated statistically significant relationships between caspase 3 activity and neurometabolites (glutamate, N-acetylaspartate) and the volume of brain structure of patients suffering from schizophrenia. Increased expression of *CASP3* and *CASP9* genes in schizophrenic patients may be responsible for the formation of a 'pro-apoptotic environment', which contributes to an increased risk of this illness [8]. There are reports on polymorphism of apoptosis genes [15, 16] as well as the MMP-9 (matrix metalloproteinase 9 gene), ANKK1 (Ankyrin Repeat and Kinase Domain containing 1 gene), DRD2 (dopamine receptor 2 gene) [34, 35]. The results obtained in this work correspond with the results of these studies. The increased level of apoptosis gene expression in our research may result from perinatal damage (hypoxia), prenatal infection [36, 37] or/and genetic predisposition present from the fetal period. The results, in the light of this interpretation, may be a confirmation for neurodevelopmental theory of schizophrenia [3, 38].

The results concerning the correlation between the expression of the examined genes and the differential blood count parameters in the schizophrenia group turned out to be interesting. These results indicate intensified apoptosis in peripheral blood lymphocytes and disorders of the specific immune response, and suggest the activation of the immune system in schizophrenia, supporting the neuroimmunological hypothesis of schizophrenia [39]. Many studies indicate the role of impaired balance between the pro-inflammatory and anti-inflammatory cytokines [40], TH1 cell response and TH2 humoral response [41] in the pathogenesis of schizophrenia. The results obtained in this study suggest significant relationships between the altered expression of the studied genes and the dysregulation of the immune system in the studied group of schizophrenic patients, which is also confirmed by the studies of Li et al. [42], who observed a significant relationship between the *Interferon regulatory factor 3* gene (*IRF3*) and increased schizophrenia morbidity.

In summary, these reports provide important information on pathophysiological processes taking place in the brain, based on the observed changes in parameters measured in the peripheral blood [20, 33, 40–42]. The limitation of our study is the lack of data on the diet and its supplements, alcohol consumption, smoking and the level of steroid hormones that may affect the level of expression of the examined genes. In the next planned study, it will be important to assess the intensity of *BAX, BCL2, CASP3, CASP9* apoptosis genes expression also in the cerebrospinal fluid, depending on the above-mentioned factors, the length of treatment, stage of the illness or perinatal factors, with volumetric assessment of the brain, including larger population of patients. The choice of a different reference gene will be also considered.

Conclusions

1. The results of the performed research indicate a significantly higher expression of *BCL2* and *CASP3* genes in peripheral blood lymphocytes of schizophrenic patients compared to the control group of healthy people, which proves the increased intensity of apoptosis, fitting into the theory of increased apoptosis in the pathophysiology of schizophrenia.
2. The research results showed the most significant correlations between the *BAX* gene expression and differential blood count parameters (leucocyte, neutrophil, lymphocyte, and monocyte count) in the schizophrenic group, suggesting a relationship with immune dysregulation and confirming the presence of apoptosis in peripheral blood lymphocytes.
3. A significant positive correlation was found between the severity of negative symptoms measured with the PANSS-N scale and the expression of the *BAX* gene in patients with schizophrenia. This result suggests that the determination of the *BAX* gene level and the *BAX* gene expression in the peripheral blood may be used as a predictor of the treatment effectiveness regarding the reduction of negative symptoms in schizophrenia.
4. Analysis of the intercorrelation of the studied genes suggests *BAX* and *CASP3* genes to be the most active genes in the apoptosis process in the studied group of patients. The expression value of these genes correlates with the expression values of all other genes.

References

1. Kos MZ, Carless MA, Peralta J, Curran JE, Quillen EE, Almeida M et al. *Exome sequences of multiplex, multigenerational families reveal schizophrenia risk loci with potential implications for neurocognitive performance*. Am. J. Med. Genet. B Neuropsychiatr. Genet. 2017; 174(8): 817–827.
2. Feinberg I. *Schizophrenia: Caused by a fault in programmed synaptic elimination during adolescence?* J. Psychiatr. Res. 1982; 17(4): 319–334.

3. Weinberger DR. *Implications of normal brain development for the pathogenesis of schizophrenia*. Arch. Gen. Psychiatry. 1987; 44(7): 660–669.
4. Murray RM, Lewis SW. *Is schizophrenia a neurodevelopmental disorder?* Br. Med. J. (Clin. Res. Ed.). 1987; 295(6600): 681–682.
5. Murray RM, Bhavsar V, Tripoli G, Howes O. *30 years on: How the neurodevelopmental hypothesis of schizophrenia morphed into the developmental risk factor model of psychosis*. Schizophr. Bull. 2017; 43(6): 1190–1196.
6. Weinberger DR. *Future of days past: Neurodevelopment and schizophrenia*. Schizophr. Bull. 2017; 43(6): 1164–1168.
7. Debnath M, Venkatasubramanian G, Berk M. *Fetal programming of schizophrenia: Select mechanisms*. Neurosci. Biobehav. Rev. 2015; 49: 90–104.
8. Sekar A, Bialas AR, Rivera de H, Davis A, Hammond TR, Kamitaki N et al. *Schizophrenia risk from complex variation of complement component 4*. Nature. 2016; 530(7589): 177–183.
9. Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P et al. *Molecular mechanisms of cell death: Recommendations of the Nomenclature Committee on Cell Death 2018*. Cell. Death Differ. 2018; 25(3): 486–541.
10. Ershova ES, Jestkova EM, Chestkov IV, Porokhovnik LN, Izevskaya VL, Kutsev SI et al. *Quantification of cell-free DNA in blood plasma and DNA damage degree in lymphocytes to evaluate dysregulation of apoptosis in schizophrenia patients*. J. Psychiatr. Res. 2017; 87: 15–22.
11. Wang X. *The expanding role of mitochondria in apoptosis*. Genes. Dev. 2001; 15(22): 2922–2933.
12. Elmore S. *Apoptosis: A review of programmed cell death*. Toxicol. Pathol. 2007; 35(4): 495–516.
13. Kerns D, Vong GS, Barley K, Dracheva S, Katsel P, Casaccia P et al. *Gene expression abnormalities and oligodendrocyte deficits in the internal capsule in schizophrenia*. Schizophr. Res. 2010; 120(1–3): 150–158.
14. Yang Y, Xiao Z, Chen W, Sang H, Guan Y, Peng Y et al. *Tumor suppressor gene TP53 is genetically associated with schizophrenia in the Chinese population*. Neurosci. Lett. 2004; 369(2): 126–131.
15. Chen X, Sun C, Chen Q, O'Neill FA, Walsh D, Fanous AH et al. *Apoptotic engulfment pathway and schizophrenia*. PLoS One. 2009; 4(9): e6875.
16. Benedetti F, Poletti S, Radaelli D, Bernasconi A, Cavallaro R, Falini A et al. *Temporal lobe grey matter volume in schizophrenia is associated with a genetic polymorphism influencing glycogen synthase kinase 3-β activity*. Genes Brain Behav. 2010; 9(4): 365–371.
17. Jarskog LF, Gilmore JH, Selinger ES, Lieberman JA. *Cortical bcl-2 protein expression and apoptotic regulation in schizophrenia*. Biol. Psychiatry. 2000; 48(7): 641–650.
18. Jarskog LF, Selinger ES, Lieberman JA, Gilmore JH. *Apoptotic proteins in the temporal cortex in schizophrenia: High Bax/Bcl-2 ratio without caspase-3 activation*. Am. J. Psychiatry. 2004; 161(1): 109–115.
19. Gassó P, Mas S, Molina O, Lafuente A, Bernardo M, Parellada E. *Increased susceptibility to apoptosis in cultured fibroblasts from antipsychotic-naïve first-episode schizophrenia patients*. J. Psychiatr. Res. 2014; 48(1): 94–101.
20. Tsai MC, Liou CW, Lin TK, Lin IM, Huang TL. *Bcl-2 associated with positive symptoms of schizophrenic patients in an acute phase*. Psychiatry Res. 2013; 210(3): 735–738.
21. Benes FM, Matzilevich D, Burke RE, Walsh J. *The expression of proapoptosis genes is increased in bipolar disorder, but not in schizophrenia*. Mol. Psychiatry. 2006; 11(3): 241–251.

22. Catts VS, Catts SV, McGrath JJ, Féron F, McLean D, Coulson EJ et al. *Apoptosis and schizophrenia: A pilot study based on dermal fibroblast cell lines*. Schizophr. Res. 2006; 84(1): 20–28.
23. Rupniewska Z, Bojarska-Junak A. [*Apoptosis: Mitochondrial membrane permeabilization and the role played by Bcl-2 family proteins*]. Postepy Hig. Med. Dosw. (Online). 2004; 58: 538–547.
24. Habela CW, Song H, Ming GL. *Modeling synaptogenesis in schizophrenia and autism using human iPSC derived neurons*. Mol. Cell Neurosci. 2016; 73: 52–62.
25. Chomczynski P, Sacchi N. *Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction*. Anal. Biochem. 1987; 162(1): 156–159.
26. Hara MR, Agrawal N, Kim SF, Cascio MB, Fujimuro M, Ozeki Y et al. *S-nitrosylated GAPDH initiates apoptotic cell death by nuclear translocation following Siah1 binding*. Nat. Cell Biol. 2005; 7(7): 665–674.
27. Livak KJ, Schmittgen TD. *Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method*. Methods. 2001; 25(4): 402–408.
28. Kay SR, Fiszbein A, Opler LA. *The positive and negative syndrome scale (PANSS) for schizophrenia*. Schizophr. Bull. 1987; 13(2): 261–276.
29. Gabryelska MM, Szymański M, Barciszewski J. [*DNA: From Miescher to Venter and beyond*]. Postepy Biochem. 2009; 55(3): 342–354.
30. Batalla A, Bargalló N, Gassó P, Molina O, Pareto D, Mas S et al. *Apoptotic markers in cultured fibroblasts correlate with brain metabolites and regional brain volume in antipsychotic-naive first-episode schizophrenia and healthy controls*. Transl. Psychiatry. 2015; 5: e626.
31. Jarskog LF. *Apoptosis in schizophrenia: Pathophysiologic and therapeutic considerations*. Curr. Opin. Psychiatry. 2006; 19(3): 307–312.
32. Bai O, Zhang H, Li XM. *Antipsychotic drugs clozapine and olanzapine upregulate bcl-2 mRNA and protein in rat frontal cortex and hippocampus*. Brain Res. 2004; 1010(1–2): 81–6.
33. Djordjević VV, Ristić T, Lazarević D, Cosić V, Vlahović P, Djordjević VB. *Schizophrenia is associated with increased levels of serum Fas and FasL*. Clin. Chem. Lab. Med. 2012; 50(6): 1049–1054.
34. Natarajan G, Shankaran S, McDonald SA, DAS A, Stoll BJ, Higgins RD et al. *Circulating beta chemokine and MMP 9 as markers of oxidative injury in extremely low birth weight infants*. Pediatr. Res. 2010; 67(1): 77–82.
35. Chen SF, Shen YC, Chen CH. *Effects of the DRD3 Ser9Gly polymorphism on aripiprazole efficacy in schizophrenic patients as modified by clinical factors*. Prog. Neuropsychopharmacol. Biol. Psychiatry. 2009; 33(3): 470–474.
36. Ashdown H, Dumont Y, Ng M, Poole S, Boksa P, Luheshi GN. *The role of cytokines in mediating effects of prenatal infection on the fetus: Implications for schizophrenia*. Mol. Psychiatry. 2006; 11(1): 47–55.
37. Pedersen MG, Stevens H, Pedersen CB, Nørgaard-Pedersen B, Mortensen PB. *Toxoplasma infection and later development of schizophrenia in mothers*. Am. J. Psychiatry. 2011; 168(8): 814–821.
38. Davis J, Eyre H, Jacka FN, Dodd S, Dean O, McEwen S et al. *A review of vulnerability and risks for schizophrenia: Beyond the two hit hypothesis*. Neurosci. Biobehav. Rev. 2016; 65: 185–194.
39. García-Bueno B, Bioque M, Mac-Dowell KS, Barcones MF, Martínez-Cengotitabengoa M, Pina-Camacho L et al. *Pro-/anti-inflammatory dysregulation in patients with first episode of psychosis: Toward an integrative inflammatory hypothesis of schizophrenia*. Schizophr. Bull. 2014; 40(2): 376–387.

40. Szymona K, Zdzisińska B, Karakuła-Juchnowicz H, Kocki T, Kandefer-Szerszeń M, Flis M et al. *Correlations of kynurenic acid, 3-hydroxykynurenine, sIL-2R, IFN-α, and IL-4 with clinical symptoms during acute relapse of schizophrenia*. Neurotox. Res. 2017; 32(1): 17–26.

41. Müller N, Weidinger E, Leitner B, Schwarz MJ. *The role of inflammation in schizophrenia*. Front. Neurosci. 2015; 9: 372.

42. Li X, Zhang W, Lencz T, Darvasi A, Alkelai A, Lerer B et al. *Common variants of IRF3 conferring risk of schizophrenia*. J. Psychiatr. Res. 2015; 64: 67–73.

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