International Journal of Biological Sciences and Applications
2014; 1(1): 28-34
Published online April 20, 2014 (http://www.aascit.org/journal/ijbsa)

Keywords
Schizophrenia,
Apoptosis,
BAX,
BCL2,
Single Nucleotide Polymorphisms,
Genotyping

Received: March 26, 2014
Revised: April 12, 2014
Accepted: April 13, 2014

Study of association between schizophrenia and functional polymorphisms of genes encoding Bcl-2 family proteins

Kristina Pirumyan, Anna Boyajyan*

Department of Molecular and Applied Biology, Institute of Molecular Biology, National Academy of Sciences of the Republic of Armenia (NAS RA), Yerevan, Armenia

Email address
aboyajyan@sci.am (A. Boyajyan)

Citation
Kristina Pirumyan, Anna Boyajyan. Study of Association between Schizophrenia and Functional Polymorphisms of Genes Encoding Bcl-2 Family Proteins. *International Journal of Biological Sciences and Applications.* Vol. 1, No. 1, 2014, pp. 28-34

Abstract
It is proposed that both pre- and postnatal as well as genetically determined abnormalities of the apoptotic processes are among factors responsible for the development of schizophrenia. The ultimate vulnerability of cells to diverse apoptotic stimuli is determined by the relative ratio of various pro-apoptotic and anti-apoptotic members of the B-cell lymphoma 2 (Bcl-2) family proteins among which Bcl-2 proper is a major anti-apoptotic protein, and Bcl-2-associated X protein (Bax) is pro-apoptotic death promoter. Postmortem studies demonstrated the increased expression level of Bax encoding gene and high Bax/Bcl-2 proteins ratio in schizophrenia. However, it is yet unclear whether these alterations are genetically determined or caused by other factors. In the present study we, for the first, time evaluated the association of single nucleotide polymorphisms (SNPs) rs1057369 (A>G) and rs956572 (G>A), rs1801018 (A>G) of Bax and Bcl-2 encoding genes, *BAX* and *BCL2*, respectively, with schizophrenia in Armenian population using polymerase chain reaction with sequence-specific primers. *BAX* rs1057369 SNP was found negatively associated with this disorder; the presence of *BAX* rs1057369*G minor allele, especially in homozygous form, was associated with decreased risk of developing schizophrenia, whereas no association between schizophrenia and *BCL2* rs956572 and rs1801018 polymorphisms was revealed. Based on the obtained results we concluded that the rs1057369*G minor allele of *BAX* may have a protective effect relative to schizophrenia, and that this effect is most pronounced in individuals with GG homozygous genotype.

1. Introduction

Schizophrenia is one of the most severe and disabling among mental disorders affecting up to 1% of the world population (1) and is characterized by cognitive impairments (2) linked to behavioral changes (3). It is considered a multifactorial disease, likely to be caused by alterations in genome, both acquired (originated during prenatal development) and inherent, combined with postnatal environmental factors (4, 5).

Apoptosis is a mechanism of cell death that operates in normal neurodevelopment and is increasingly recognized for its pathological features in

diverse neuropathological conditions including mental disorders (6, 7). Activation of apoptosis can lead to elimination of neurons and glia in the central nervous system (8, 9). Evidence of progressive clinical deterioration and subtle neurostructural changes following the onset of psychosis, shorter dendrites, reduced neutrophil count, reduction in neuronal and glial cell number, especially synaptic elements (10, 11), together with brain imaging data of schizophrenia-affected subjects (12) indicate that apoptosis may significantly contribute to the pathophysiology of schizophrenia. Moreover, alterations of the apoptotic processes in schizophrenia are detected at the levels of the brain (13, 14) and peripheral blood (15-17). Furthermore, one of the contemporary hypotheses of etiology and pathogenesis of schizophrenia ("neurodevelopmental hypothesis") proposes that in schizophrenia apoptotic processes and their genetic regulation are altered starting from the early stages of neural development (18). It is proposed that both pre- and postnatal as well as genetically determined abnormalities of the apoptotic processes are among factors responsible for the development of schizophrenia (10) and that non-lethal apoptosis may contribute to schizophrenia-associated defects of synaptic plasticity (19), neurodegenerative alterations (20), and immune system dysfunctions, particularly, autoimmune and inflammatory reactions (16, 21, 22) directly linked to apoptosis (23, 24). All together these interrelated anomalies have a significant impact on cognitive function (25-29) and might be responsible for cognitive impairments in schizophrenia.

A family of B-cell lymphoma 2 (Bcl-2) proteins constitutes one of the most biologically relevant classes of apoptosis regulators acting at the effecter stage of apoptosis, with some members functioning as suppressors of apoptosis and others as promoters of apoptosis. The ultimate vulnerability of cells to diverse apoptotic stimuli is determined by the relative ratio of various pro-apoptotic and anti-apoptotic members of the Bcl-2 family (30, 31). Bcl-2 proper and Bcl-2-associated X protein are members of the Bcl-2 family proteins (32). Bcl-2 proper is an integral, membrane-associated protein with anti-apoptotic and antioxidative effects (32, 33). Bcl-2 is a major anti-apoptotic protein that inhibits apoptotic and necrotic cell death induced by a diverse set of adverse conditions (34). Bcl-2 also plays critical roles in neuronal morphogenesis and synaptic plasticity (35, 36), and reduced Bcl-2 function is hypothesized to contribute to the impairment of cellular plasticity and resilience in patients with mood disorders (34).

Bcl-2-associated X protein (Bax*)* is pro-apoptotic member of the family of Bcl-2-related proteins, which has an extensive amino acid homology with Bcl-2 (37, 38). Whether the cell will live or die may depend on the level of either protein; while Bcl-2 prevents death, Bax is a death promoter (30-32).

Postmortem studies of Jarskog et all demonstrated the increased expression level of Bax encoding gene (39) as well as high Bax/Bcl-2 proteins ratio in the temporal cortex of patients with schizophrenia (40). However, it is yet unclear whether these pathologic alterations are genetically determined or caused by other factors.

In the present study we evaluated the association of single nucleotide polymorphisms (SNPs) rs1057369 and rs956572, rs1801018 of Bax and Bcl-2 encoding genes (*BAX* and *BCL2*, respectively) with schizophrenia using polymerase chain reaction with sequence-specific primers (PCR-SSP). Information about functionality of these SNPs was obtained from the databases of the International Haplotype Map Project (HapMap; http://hapmap.ncbi.nlm.nih.gov/cgiperl/gbrowse/hapmap 27_B36/) and the National Institute of Environmental Health Sciences (NIEHS; http://snpinfo.niehs.nih.gov/ snpinfo/snpt ag.htm). To our knowledge this is a first study evaluating potential implication of *BAX* (chromosome 19, locus 19q13.33) and *BCL2* (chromosome 18, locus 18q21.33) polymorphisms in etiopathomechanisms of schizophrenia.

2. Subjects and Methods Study Population

In total 654 unrelated Caucasian individuals of Armenian nationality born and living in Armenia (330 chronic patients with schizophrenia and 326 healthy control subjects) were enrolled in this study. Schizophrenia-affected subjects (female/male: 106/224, mean age ± SD: 48.6 ± 10.58 years, age at the first-onset of illness: 29.16 ± 6.36 years, duration of illness: 17.57 ± 10.81 years) were recruited from the clinics of the Psychiatric Medical Center of the Ministry of Health (MH) RA. All patients were diagnosed as paranoid schizophrenics (ICD-10 code: F20.0, DSM-IV-TR code: 295.30 (41, 42)) by psychiatrists according to the presence of the relevant symptoms and the results of the Structured Clinical Interview for DSM-IV-TR (42). All patients were treated with typical neuroleptic haloperidol (1mg 3 times daily, *per os)*. Age- and sex-matched healthy control subjects (female/male: 118/208, mean age ± SD: 41.2 ± 13.46 years) with no family, past or present history of any mental disorder as determined by the non-patient version of the Structured Clinical Interview for DSM-IV-TR Axis I Disorders (43) were recruited among the blood donors of the Erebouni Medical Center MH RA. Exclusion criteria for all study subjects included any serious neurological, endocrine, oncological, inflammatory, autoimmune, cerebrovascular, heart, or metabolic disorder. All subjects gave their informed consents to participate in the study, which was further approved by the Ethical Committee of the Institute of Molecular Biology NAS RA (IRB #00004079).

Collection of Blood Samples and Extraction of Genomic DNA

About 5 ml of peripheral blood was collected from each study subject by venipuncture and transferred to EDTA-containing tubes. Genomic DNA samples were isolated from fresh blood according to a standard phenol-chloroform method (44) and stored at -30°C until use.

Genotyping of *BAX* Rs1057369 and *BCL2* Rs956572, Rs1801018 SNPs

All DNA samples were genotyped for *BAX* rs1057369 (A>G; chromosome 19: 49464866; intron variant) and *BCL2* rs956572 (G>A; chromosome 18: 60820571, intron variant), rs1801018 (A>G; chromosome 18: 60985879, synonymous codon) SNPs using PCR-SSP as described earlier (45). All primers for PCR-SSP were designed using the genomic sequences in the GenBank database (http://www.ncbi.nlm.nih.gov, Gene IDs: 581 and 596 for *BAX* and *BCL2*, respectively). The primer sequences for three mentioned SNPs were as follows: 1) rs956572: allele A reverse 5'-AGAGGGAGTCATGACTGAATT, allele G reverse 5'-AGAGGGAGTCATGACTGAATC, constant forward 5'-CAGATCTGTGCTTGAACCTCA, 2) rs1801018: allele A reverse 5'-ATCTCCCGGTTATCGTACCCT, allele G reverse 5'-ATCTCCCGGTTATCGTACCCC, constant forward 5'-GATCCGAAAGGAATTGGAATA, 3) rs1057369: allele A reverse 5'- ATCTTCTTCCAGATGGTGAGT, allele G reverse 5'-ATCTTCTTCCAGATGGTGAGC, constant forward 5'-TTACAGGTGTGAGCCACCATG.

The presence/absence of allele-specific amplicons in the PCR products was visualized in 2% agarose gel stained with ethidium bromide fluorescent dye using DNA molecular weight markers as a reference. To check the reproducibility of results, randomly selected DNA samples of study subjects (10% of total) were genotyped twice.

Statistical Analysis

Distributions of genotypes for all investigated SNPs were checked for correspondence to the Hardy-Weinberg (H-W) equilibrium. In order to find potential relevance of the selected SNPs to schizophrenia, their genotype and allele frequencies and minor allele carriage rates in patients and healthy control subjects were compared. The calculations of genotype and allele frequencies were based on the observed number of genotypes. The significance of differences in genotype and allele frequencies and minor allele carriage between patients and healthy control subjects was determined using Pearson's Chi-square test. The odds ratio (OR), 95% confidence interval (CI), and Pearson's p-value were calculated. P-values were adjusted by Bonferroni multiple correction approach (46), and those less than 0.05 were considered statistically significant. Statistical powers of the present study were calculated according to the protocol described elsewhere (47). The data was evaluated using "SPSS-13.0" (SPSS Inc, USA) software.

3. Results

The distribution of genotypes for the selected SNPs in both study groups complied with H-W equilibrium - no significant differences were detected between the observed and expected genotype frequencies ($p>0.05$). The genotyping success rate was 100%, and rescreening of randomly selected DNA samples of study subjects (10% of total) gave 100% identical results. Minor allele frequency in healthy control subjects for all studied polymorphisms was close to that reported for Central European Population (HapMap, NIEHS). Distribution of *BAX* rs1057369 and *BCL2* rs956572, rs1801018 variants in study groups is shown in table 1.

No significant difference was observed between the patients and controls regarding the genotype and allele frequencies and minor allele carriage of the *BCL2* rs956572 and rs1801018 SNPs ($p>0.05$). Thus, the results obtained indicated that there is no significant difference in minor allele frequency of *BCL2* rs956572 and rs1801018 SNPs between patients and controls (rs956572*A patients vs. controls: 40% vs. 41%, $p_{corrected}=1.809$, OR=1.06, 95% CI: 0.851-1.322; rs1801018*G patients vs. controls: 46% vs. 51%, $p_{corrected}=0.372$, OR=0.84, 95% CI: 0.679-1.048, respectively). The same applies to the carriers of the *BCL2* rs956572*A and rs1801018*G minor alleles (rs956572*A patients vs. controls: 66% vs. 64%, $p_{corrected}=1.632$, OR=1.105, 95% CI: 0.8-1.525; rs1801018*G patients vs. controls: 74% vs. 79%, $p_{corrected}=0.348$, OR=1.34, 95% CI: 0.93-1.923). Statistical powers of this part of the study, indicating the differences in the carriage of the *BCL2* rs956572*A and rs1801018*G minor alleles between patients and controls, were 7.52% and 15.11%, respectively. By contrast, significant difference in the genotype and allele frequencies, and minor allele carriage rates of the *BAX* rs1057369 SNP between patients and controls was found ($p<0.05$), and the negative association between schizophrenia and rs1057369 SNP was detected. Thus, minor allele frequency of *BAX* rs1057369 SNP in patients was lower than in controls (rs1057369*G patients vs. controls: 45% vs. 55%, $p_{corrected}=0.00185$, OR=0.68, 95% CI: 0.551-0.851). The same tendency was detected when comparing a number of the rs1057369*G minor allele carriers in patients and controls (rs956572*G patients vs. controls: 73% vs. 82%, $p_{corrected}=0.0206$, OR=0.6, 95% CI: 0.415-0.871).

The obtained results demonstrated that *BAX* rs1057369*G minor allele is overrepresented in controls as compared to patients. Moreover, as it is shown in Table 1, a number of homozygous carriers of minor allele (GG) among controls was significantly higher than among patients (18% vs 29%, $p<0.05$). Statistical power of this part of the study, indicating the difference in the carriage of

the *BAX* rs1057369*G minor allele between patients and controls, was 38.99%.

Table 1. *Genotype and allele frequencies and minor allele carriage of BCL2 rs956572, rs1801018 and BAX rs1057369 SNPs in patients with schizophrenia (SCH) and controls.*

***BCL2* rs956572 (G>A)**		**SCH (n=330)**	**Controls (n=326)**	**$P_{nominal}$**	**$P_{corrected}$**
	GG	110(0.34)	116(0.36)		
Genotypes	AG	176(0.53)	150(0.46)		
	AA	44(0.13)	60(0.18)	0.075	0.225
Alleles	G	396(0.60)	382(0.59)		
	A	264(0.40)	270(0.41)	0.603	1.809
Carriage	A	220(0.66)	210(0.64)	0.544	1.632
BCL2 rs1801018 (A>G)					
	AA	86(0.26)	68(0.21)		
Genotypes	AG	182(0.55)	186(0.57)		
	GG	62(0.19)	72(0.22)	0.295	0.885
Alleles	A	354(0.54)	322(0.49)		
	G	306(0.46)	330(0.51)	0.124	0.372
Carriage	G	244(0.74)	258(0.79)	0.116	0.348
BAX rs1057369 (A>G)					
	AA	90(0.27)	60(0.18)		
Genotypes	AG	180(0.55)	174(0.53)		
	GG	60(0.18)	92(0.29)	0.0023	0.0069
Alleles	A	360 (0.55)	294(0.45)		
	G	300(0.45)	358(0.55)	0.000617	0.0019
Carriage	G	240(0.73)	266(0.82)	0.00685	0.0206

Data is presented as absolute number (proportion).

4. Discussion

In the present study, for the first time, the potential association between schizophrenia disorder and functional polymorphisms of Bcl-2 family proteins encoding genes, *BAX* rs1057369 and *BCL2* rs956572, rs1801018, was assessed.

BAX rs1057369 SNP was found negatively associated with schizophrenia: the presence of *BAX* rs1057369*G minor allele, especially in homozygous form, was associated with decreased risk of developing schizophrenia. It has to be mentioned that no data on association of this polymorphism with any diseased condition has been reported before.

Opposite to *BAX* rs1057369 SNP, *BCL2* rs1801018 and rs956572 polymorphisms have been intensively studied in different diseased and other conditions. *BCL2* rs1801018 SNP was mostly found to be associated with oncological disorders (48-51) as well as with poorer clinical outcomes and mortality in patients with traumatic brain injury (52).

BCL2 rs956572 polymorphism was shown to associate with the risk for developing bipolar disorder and was nominated as modulator of the expression of Bcl-2 protein and cellular vulnerability to apoptosis (53). Preclinical studies show that this SNP exerts functional effects on Bcl-2 expression, as the A homozygous genotype is associated with significantly lower Bcl-2 mRNA expression, 50% lower Bcl-2 protein levels, and greater cellular sensitivity to stress-induced apoptosis (53). This SNP was reported to affect gray matter volume in areas known to play key roles in the neurobiology of reward processes and emotion regulation and in the pathophysiology of mood disorders (54). Also, it was demonstrated that rs956572 SNP may modulate cognitive function and regional gray matter volume in non-demented elderly men, and affect language performance through its effect on the right middle temporal gyrus (55). In addition, it was shown that rs956572 associates with increased anterior cingulate cortical glutamate (56) and disrupted intracellular calcium homeostasis in bipolar I disorder (57) and that abnormal *BCL2* gene expression in the AA genotype of rs956572 contributes to dysfunctional Ca(2+) homeostasis through inositol 1,4,5-trisphosphate receptor-dependent mechanism (58).

Regarding *BCL2* rs956572 and rs1801018 polymorphisms, this study revealed no association between schizophrenia and these SNPs. Further studies with implication of other *BAX* and *BCL2* SNPs will finally reveal whether alterations in *BAX* and *BCL2* genes may contribute to changes in to Bax/Bcl-2 expression levels (39, 40), and, in general, to defects in apoptosis in schizophrenia (10).

5. Limitation of Study

The limitation of this study is a relatively small sample size of study subjects. Also, because all subjects involved in the present study belonged to Armenian population, further investigations in other populations are necessary to replicate the present findings.

6. Conclusion

In conclusion, our results, for the first time, revealed significant negative association between schizophrenia and rs1057369 polymorphism of *BAX* gene encoding pro-apoptotic Bax protein. According to these results the rs1057369*G minor allele of *BAX* may have a protective effect relative to schizophrenia at least in Armenian population. In addition, it was demonstrated that this effect is most pronounced in individuals with GG homozygous genotype. Our study also indicated no significant association between schizophrenia and rs956572, and rs1801018 polymorphisms of *BCL2* gene encoding anti-apoptotic bcl-2 proper protein.

Acknowledgement

We express our sincere thanks to the administration and medical staff of the Psychiatric Medical Center and Erebouni Medical Center MH RA for selection of patients and healthy control subjects for this study.

Declaration of Interest

The authors report no conflicts of interest.

Authors Contribution

Conception and design: AB; genotyping and statistical analysis: KP; interpretation of data and drafting the manuscript: AB+KP; finalizing the manuscript for submission: AB.

References

[1] Perälä J, Suvisaari J, Saarni SI, Kuoppasalmi K, Isometsä E, Pirkola S, et al. Tuulio-Henriksson A, Hintikka J, Kieseppä T, Härkänen T, Koskinen S, Lönnqvist J, Lifetime prevalence of psychotic and bipolar I disorders in a general population. Arch Gen Psychiatry.2007;64:19-28.

[2] Lublin H, Cognitive dysfunction in schizophrenia, Acta Psychiatr Scand Suppl.2001;104(408):5-9.

[3] Matza LS, Buchanan R, Purdon S, Brewster-Jordan J, Zhao Y, Revicki DA, Measuring changes in functional status among patients with schizophrenia: the link with cognitive impairment. Schizophr Bull.2006;32(4):666-78.

[4] van Os J, Rutten BP, Poulton R, Gene-environment interactions in schizophrenia: review of epidemiological findings and future directions. Schizophr Bull.2008;34(6):1066-82.

[5] Zahir FR, Brown CJ, Epigenetic impacts on neurodevelopment: pathophysiological mechanisms and genetic modes of action. Pediatr Res.2011;69(5 Pt 2):92R-100R.

[6] Holcik M, Apoptosis in Health and Disease: Clinical and Therapeutic Aspects. Cambridge: Cambridge University Press,;2005.

[7] Olney JW, Excitotoxicity, apoptosis and neuropsychiatric disorders. Curr Opin Pharmacol.2003;3(1):101-9.

[8] Burek MJ, Oppenheim RW, Programmed cell death in the developing nervous system. Brain Pathol.1996;6(4):427-46.

[9] Mattson MP, Keller JN, Begley JG, Evidence for synaptic apoptosis. Exp Neurol.1998;153(1):35-48.

[10] Jarskog LF, Glantz LA, Gilmore JH, Lieberman JA, Apoptotic mechanisms in the pathophysiology of schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry.2005;29(5):846-58.

[11] Jarskog LF, Apoptosis in schizophrenia: pathophysiologic and therapeutic considerations. Curr Opin Psychiatry.2006;19(3):307-12.

[12] Pantelis C, Yücel M, Wood SJ, Velakoulis D, Sun D, Berger G, et al. Structural brain imaging evidence for multiple pathological processes at different stages of brain development in schizophrenia. Schizophr Bull.2005;31(3):672-96.

[13] Benes FM, Walsh J, Bhattacharyya S, Sheth A, Berretta S, DNA fragmentation decreased in schizophrenia but not in bipolar disorder. Arch Gen Psychiatry.2003;60(4):359-64.

[14] Glantz LA, Gilmore JH, Overstreet DH, Salimi K, Lieberman JA, Jarskog LF, Pro-apoptotic Par-4 and dopamine D2 receptor in temporal cortex in schizophrenia, bipolar disorder and major depression. Schizophr Res.2010;118(1-3):292-9.

[15] 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):1049-54.

[16] Boyajyan A, Zakharyan R, Khoyetsyan A, Chapter XI. Molecular and genetic indicators of aberrant immunity and apoptosis in schizophrenia. In: Sumiyoshi T editor: Schizophrenia Research: Recent Advances. New York: Nova Science Publishers Inc;2012:p.183-240.

[17] Boyajyan AS, Chavushyan AS, Zakharyan RV, Mkrtchyan GM, Markers of apoptotic dysfunctions in schizophrenia. Molecular Biology (Moscow).2013;47(4):587-91.

[18] Fatemi SH, Folsom TD, The neurodevelopmental hypothesis of schizophrenia, revisited. Schizophr Bull.2009;35(3):528-48.

[19] Glantz LA, Gilmore JH, Lieberman JA, Jarskog LF, Apoptotic mechanisms and the synaptic pathology of schizophrenia. Schizophr Res.2006;81(1):47-63.

[20] Pérez-Neri I, Ramírez-Bermúdez J, Montes S, Ríos C, Possible mechanisms of neurodegeneration in schizophrenia. Neurochem Res.2006;31(10):1279-94.

[21] Rothermundt M, Arolt V, Bayer TA, Review of immunological and immunopathological findings in schizophrenia. Brain Behav Immun.2001;15(4):319-39.

[22] Müller N, Schwarz MJ, Immune system and schizophrenia,. Curr Immunol Rev.2010;6(3):213-20.

[23] Haanen C, Vermes I, Apoptosis and inflammation. Mediators Inflamm.1995;4(1):5-15.

[24] Mahoney JA, Rosen A, Apoptosis and autoimmunity. Curr Opin Immunol.2005;17(6):583-8.

[25] Otani S, Prefrontal cortex: from synaptic plasticity to cognition. Kluwer Academic Publishers Group, Dordrecht.2004.

[26] Cunningham C, Campion S, Lunnon K, Murray CL, Woods JF, Deacon RJ, et al. Systemic inflammation induces acute behavioral and cognitive changes and accelerates neurodegenerative disease. Biol Psychiatry.2009;65(4):304-12.

[27] Oliva CA, Vargas JY, Inestrosa NC, Wnts in adult brain: from synaptic plasticity to cognitive deficiencies. Front Cell Neurosci.2013;7:224.

[28] Yasuda T, Nakata Y, Choong CJ, Mochizuki H, Neurodegenerative changes initiated by presynaptic dysfunction. Transl Neurodegener.2013;2(1):16.

[29] Lim L, Lippe S, Silverman E, Effect of autoimmune diseases on cognitive function. Handb Clin Neurol.2013;112:1275-83.

[30] Roset R, Ortet L, Gil-Gomez G, Role of Bcl-2 family members on apoptosis: what we have learned from knock-out mice. Front Biosci.2007;12:4722-30.

[31] Youle RJ, Strasser A, The BCL-2 protein family: opposing activities that mediate cell death. Nat Rev Mol Cell Biol. 2008;9(1):47-59.

[32] Korsmeyer SJ, Shutter JR, Veis DJ, Merry DE, Oltvai ZN, Bcl2/Bax: a rheostat that regulates an anti-oxidant pathway and cell death. Semin Cancer Biol.1993;4(6):327-32.

[33] Yang J, Liu X, Bhalla K, Kim CN, Ibrado AM, Cai J, et al. Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. Science.1997;275(5303):1129-32.

[34] Chen G, Manji HK, The extracellular signal-regulated kinase pathway: An emerging promising target for mood stabilizers. Curr Opin Psychiatry.2006;19:313-23.

[35] Chen DF, Schneider GE, Martinou JC, Tonegawa S, Bcl-2 promotes regeneration of severed axons in mammalian CNS. Nature.1997;385:435-9.

[36] Jonas E, Bcl-xl regulates synaptic plasticity. Mol Interv.2006;6:208-22.

[37] Oltwai ZN, Milliman CL, Korsemeyer SJ, Bcl2 heterodimers in vivo with a conserved homolog Bax, that accelerates programmed cell death. Cell.1993;74:609-19.

[38] Surhone LM, Tennoe MT, Henssonow SF, Bcl-2-associated X Protein. Beau Bassin: Betascript Publishing;2011.

[39] Jarskog LF, Selinger ES, Lieberman JA, Gilmore JH, Cortical Bcl2 protein expression and apoptotic regulation in schizophrenia. Biol Psychiatry.2000;48:641-50.

[40] Jarskog LF, Selinger ES, Lieberman JA, Gilmore JH, Apoptotic proteins in the temporal cortex in schizophrenia: high Bax/Bcl2 ratio without caspase-3 activation. Am J Psychiatry.2004;161:109-15.

[41] World Health Organization, The International Statistical Classification of Diseases and Related Health Problems (10th edition). Geneva:World Health Organization;1992.

[42] American Psychiatric Association Diagnostic and Statistical Manual of mental disorders, (4th edition, text revised), Arlington (VA):American Psychiatric Publishing;2000.

[43] First MB, Spitzer RL, Gibbon M, Williams JB, SCID-I/NP: Structured Clinical Interview for DSM-IV-TR Axis I Disorders (research version, non-patient edition). New York: Biometrics Research, New York State Psychiatric Institute;2002.

[44] Sambrook J, Russell DW, Molecular Cloning: A Laboratory Manual (3rd edition). New York: Cold Spring Harbor Laboratory Press;2001.

[45] Bunce M, O'Neil CM, Barnado MC, Krausa P, Browning MJ, Morris PJ, et al. Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). Tissue Antigens.1995;46:355-67.

[46] Rice TK, Schork NJ, Rao DC, Methods for handling multiple testing. Adv Genet.2008;60:293-308.

[47] Lalouel JM, Rohrwasser A, Power and replication in case-control studies. Am J Hypertens.2002;15(2):201-5.

[48] Milani L, Gupta M, Andersen M, Dhar S, Fryknäs M, Isaksson A, et al. Allelic imbalance in gene expression as a guide to cis-acting regulatory single nucleotide polymorphisms in cancer cells. Nucleic Acids Res.2007;35(5):34.

[49] Kim DH, Xu W, Ma C, Liu X, Siminovitch K, Messner HA, et al. Genetic variants in the candidate genes of the apoptosis pathway and susceptibility to chronic myeloid leukemia. Blood.2009;113(11):2517-25.

[50] Eun YG, Hong IK, Kim SK, Park HK, Kwon S, Chung DH, et al. Polymorphism (rs1801018, Thr7Thr) of BCL2 is associated with papillary thyroid cancer in Korean population. Clin Exp Otorhinolaryngol.2011;4(3):149-54.

[51] Gu S, Wu Q, Zhao X, Wu W, Gao Z, Tan X, et al. Association of CASP3 polymorphism with hematologic toxicity in patients with advanced non-small-cell lung carcinoma treated with platinum-based chemotherapy. Cancer Sci.2012;103(8):1451-9.

[52] Hoh NZ, Wagner AK, Alexander SA, Clark RB, Beers SR, Okonkwo DO, et al. BCL2 genotypes: functional and neurobehavioral outcomes after severe traumatic brain injury. J Neurotrauma.2010;27(8):1413-27.

[53] Manji HK, Bcl-2: A key regulator of affective resilience in the pathophysiology and treatment of severe mood disorders. Biol Psychiatry.2008;63(suppl 1):243S.

[54] Yuan P, Baum AE, Zhou R, Wang Y, Laje G, McMahon FJ, et al. Bcl-2 polymorphisms associated with mood disorders and antidepressant-responsiveness regulate Bcl-2 gene expression and cellular resilience in human lymphoblastoid cell lines. Biol Psychiatry.2008;63(suppl 1):63S.

[55] Salvadore G, Nugent AC, Chen G, Akula N, Yuan P, Cannon DM, et al. Bcl-2 polymorphism influences gray matter volume in the ventral striatum in healthy humans. Biol Psychiatry.2009;66(8):804-7.

[56] Liu ME, Huang CC, Hwang JP, Yang AC, Tu PC, Yeh HL, et al. Effect of Bcl-2 rs956572 SNP on regional gray matter volumes and cognitive function in elderly males without dementia. Age (Dordr).2013;35(2):343-52.

[57] Soeiro-de-Souza MG, Salvadore G, Moreno RA, Otaduy MC, Chaim KT, Gattaz WF, et al. Bcl-2 rs956572 polymorphism is associated with increased anterior cingulate cortical glutamate in euthymic bipolar I disorder. Neuropsychopharmacology.2013;38(3):468-75.

[58] Uemura T, Green M, Corson TW, Perova T, Li PP, Warsh JJ, Bcl-2 SNP rs956572 associates with disrupted intracellular calcium homeostasis in bipolar I disorder. Bipolar Disord.2011;13(1):41-51.

[59] Machado-Vieira R, Pivovarova NB, Stanika RI, Yuan P, Wang Y, Zhou R, et al. The Bcl-2 gene polymorphism rs956572AA increases inositol 1,4,5-trisphosphate receptor-mediated endoplasmic reticulum calcium release in subjects with bipolar disorder. Biol Psychiatry.2011;69(4):344-52.