

Genetic variations associated with brain disorders: Focus on synaptic plasticity and apoptosis regulatory genes in schizophrenia, posttraumatic stress disorder and ischemic stroke

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Abstract: Epidemiologic, clinical and experimental data indicates that a majority of brain disorders including schizophrenia (SCZ), posttraumatic stress disorder (PTSD), and ischemic stroke (IS) are multifactorial disorders with strong and complex genetic component. Identification of all genetic variations associated with these disorders may sufficiently contribute to understanding of their basic pathomechanisms and encourage development of new innovative approaches to their early diagnosis and treatment. The aim of this review article is to provide overview of our recent studies on evaluation of potential association of SCZ, PTSD and IS with functional single nucleotide polymorphisms (SNPs) of synaptic plasticity and apoptosis regulatory genes in Armenian population. Here, our attention was focused on genes encoding netrin G1 (*NTNG1*), brain-derived neurotrophic factor (*BDNF*), complexin-2 (*CPLX2*), nerve growth factor (*NGF*) and its receptor (*NGFR*), annexin family proteins - annexin A5 and annexin A11 (*ANXAV*, *ANXA11*), and B-cell lymphoma 2 (Bcl-2) family proteins - Bcl-2 proper and Bcl-2-associated X protein (*BCL2*, *BAX*). Genomic DNA samples of diseased and healthy individuals were genotyped for a number of SNPs of the mentioned genes using polymerase chain reaction with sequence-specific primers (PCR-SSP). 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. P-values less than 0.05 were considered statistically significant. Significant associations were found between: (1) SCZ and *BDNF* rs6265, *CPLX2* rs1366116, rs3892909, *NGF* rs6330, rs4839435, *NGFR* rs734194, rs11466155, rs2072446, *ANXAV* rs11575945, *BAX* rs1057369 SNPs; (2) PTSD and *CPLX2* rs1366116, *BCL2* rs956572 SNPs; (3) IS and *NTNG1* rs628117, *CPLX2* rs1366116, *ANXAV* rs11575945 SNPs. The obtained results indicated the involvement of genetically determined alterations in synaptic plasticity and apoptosis in pathomechanisms of SCZ, PTSD and IS. The minor T allele of the *CPLX2* gene rs1366116 polymorphism represents risk factor for all studied diseased conditions indicating important functional significance of this genetic variation in maintenance of synaptic plasticity. Another important conclusion of these studies is that minor alleles of some polymorphic variants of genes, encoding synaptic plasticity and apoptosis regulatory proteins, may play a protective role relative to SCZ decreasing the risk for development of this disorder. In summary, our studies emphasize the important contribution of changes in synaptic plasticity and apoptosis regulation to pathomechanisms of SCZ, PTSD, and IS as well as significant input of genetic factors to these changes.

Keywords: Schizophrenia, Posttraumatic Stress Disorder, Ischemic Stroke, Synaptic Plasticity, Apoptosis, Regulatory Genes, Single Nucleotide Polymorphisms, Genotyping, Association

1. Introduction

Epidemiologic, clinical and experimental data indicates that a majority of brain disorders are multifactorial disorders with strong and complex genetic component [1-3]. Among those disorders are schizophrenia (SCZ) [4], posttraumatic stress disorder (PTSD) [5], and ischemic stroke (IS) [6]. All three disorders are severe diseased conditions and contribute significantly to disability and mortality of the human population worldwide [7-9]. Molecular pathomechanisms responsible for generation, progression and unfavorable clinical outcome of SCZ, PTSD and IS are only beginning to be understood, and currently available prognostic, diagnostic and treatment measures related to these disorders are not very efficient. Identification of all genetic variations associated with these disorders may sufficiently contribute to understanding of their basic pathomechanisms and encourage development of new innovative approaches to their early diagnosis and treatment. Several studies, including

our own, suggest implication of alterations in synaptic neuronal plasticity and apoptosis to pathophysiology of SCZ, PTSD, and IS [10-19]. This review provides overview of our recent studies (including published [20-33] and unpublished data) on evaluation of potential association of SCZ, PTSD and IS with functional single nucleotide polymorphisms (SNPs) of synaptic plasticity and apoptosis regulatory genes. Here, our attention was focused on genes encoding netrin G1 (*NTNG1*), brain-derived neurotrophic factor (*BDNF*), complexin-2 (*CPLX2*), nerve growth factor (*NGF*) and its receptor (*NGFR*), annexin family proteins - annexin A5 and annexin A11 (*ANXAV*, *ANXA11*), and B-cell lymphoma 2 (Bcl-2) family proteins - Bcl-2 proper and Bcl-2-associated X protein (*BCL2*, *BAX*). Genomic DNA samples of diseased and healthy individuals were genotyped for a number of SNPs of the mentioned genes using polymerase chain reaction with sequence-specific primers (PCR-SSP). Brief characteristics of selected genes and SNPs are given in Table 1.

Table 1. Brief characteristics of selected genes and SNPs.

Gene		SNP				
Name	ID	Location	ID	Substitution ⁺	Position	Location (type)
<i>NTNG1</i>	22854	1p13.3	rs628117	A>G	107997106	intronic
<i>BDNF</i>	627	11p13	rs6265	G>A	27679916	exonic (missense)
<i>CPLX2</i>	10814	5q35.2	rs1366116	C>T	175297531	intronic (5' near gene)
			rs3892909	C>T	175305591	intronic
NGF	4803	1p13.1	rs6330	G>A	115829313	exonic (missense)
			rs4839435	G>A	115858104	intronic
			rs11466155	C>T	44942998	exonic (synonymous)
<i>NGFR</i>	4804	17q21-q22	rs2072446	C>T	47587819	missense
			rs734194	T>G	47591609	3'-UTR
			rs11575945	-1C>T	122617745	Kozak sequence
<i>ANXAV</i>	308	4q27	rs11575945	-1C>T	122617745	Kozak sequence
<i>ANXA11</i>	311	10q21-23	rs1049550	G>A	81916682	missense
<i>BAX</i>	581	19q13.33	rs1057369	A>G	49464866	intronic
<i>BCL2</i>	596	18q21.33	rs956572	G>A	60820571	intronic
			rs1801018	A>G	60985879	exonic (synonymous)

⁺ on forward strand.

2. Methodology

2.1. Study Population

Study subjects were chronic patients with paranoid form of SCZ and with PTSD of combat origin, patients with acute IS, and healthy individuals (HI) with no family or past history of any mental, cerebrovascular or cardiovascular disorders, as well as disorders characterized by alterations in apoptosis and synaptic plasticity. All subjects were Armenians born and living in Armenia. Both, the informed consents from all study subjects and the approval of the Ethics Committee of the Institute of Molecular Biology (IRB #00004079) were received for these studies.

2.2. Methods

About 5 ml of peripheral venous blood was collected

from each study subject and transferred to EDTA-containing tubes. Genomic DNA samples were isolated from fresh blood according to a standard phenol-chloroform method [34] and stored at -30°C until use. Using PCR-SSP method [35] DNA samples were genotyped for *NTNG1* rs628117, *BDNF* rs6265, *CPLX2* rs1366116, rs3892909, *NGF* rs6330, rs4839435, *NGFR* rs11466155, rs2072446, rs734194, *ANXAV* rs11575945, *ANXA11* rs1049550, *BAX* rs1057369, *BCL2* rs956572, rs1801018 functional SNPs. The SNPs were selected based on either their functionality according to the National Center of Biotechnology Information (NCBI) databases (<http://www.ncbi.nlm.nih.gov/>) or tagging results obtained using the International HapMap Project database (<http://hapmap.ncbi.nlm.nih.gov/>). All primers for PCR-SSP were designed using the genomic sequences in the GenBank nucleotide sequence database (<https://www.ncbi.nlm.nih.gov/genbank/>) and are indicated

in Table 2. 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 (10% of total) were genotyped twice.

2.3. Data Analysis

The 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 SCZ, their genotype and allele frequencies and minor allele carriage rates in patients and HI were compared. The significance of differences in genotype and allele frequencies and minor allele carriage between patients and HI was determined using Pearson's Chi-square test. P-values less than 0.05 were considered statistically significant. P-values adjusted by Bonferroni multiple comparison correction are further indicated as $P_{corrected}$, and those not adjusted - as $P_{nominal}$.

Table 2. Primers designed for the selected SNPs.

SNP	Nucleotide sequence of primers
rs628117	standard allele: 5' ATCCTTGAATGAAAGCCCA minor allele: 5'- ATCCTTGAATGAAAGCCCG constant: 5'-TCACTGCCCTCTGTGTGCACTG
rs6265	standard allele: 5'- GGCTGACACTTTCGAACACG minor allele: 5'- GGCTGACACTTTCGAACACA constant: 5'- GTTACCCACTCACTAATACTG
rs1366116	standard allele: 5'-ATGTGTAGGAAAATGGCTTCG minor allele: 5'-ATGTGTAGGAAAATGGCTTCA constant: 5'- CAATGGCCTCTGACTGGTG
rs3892909	standard allele: 5'- GGTGAGGCTGCTGTCTGC minor allele: 5'-GGTGAGGCTGCTGTCTGT

SNP	Nucleotide sequence of primers
	constant: 5'-CTGCTTCATGACGAAGTCCA
rs6330	standard allele: 5'-GCATCTTGTCTGTGCAGAT minor allele: 5'-GACACACCATCCCCAAGC constant: 5'-GACACACCATCCCCAAGT
rs4839435	standard allele: 5'-TGGGTGCCAAAAAGCTTGGC minor allele: 5'-TGGGTGCCAAAAAGCTTGGT constant: 5'-GCAGCTCCTGCAATTATCCA
rs11466155	standard allele: 5'-AGGCTATGTAGGCCACAAGG minor allele: 5'-AGGCTATGTAGGCCACAAGA constant: 5'-CAGAGGGCTCGACAGCACA
rs2072446	standard allele: 5'-GTCCACACCCCCAGAGGGCTC minor allele: 5'-GTCCACACCCCCAGAGGGCTT constant: 5'-AGCAGCCAGGATGGAGCAAT
rs734194	standard allele: 5'-GCTGGAGCTGGCGTCTGTCT minor allele: 5'-GCTGGAGCTGGCGTCTGTCTG constant: 5'-CTAGAGCTGGGAGAAATCCC
rs11575945	standard allele: 5'-CCTGACCTGAGTAGTCGC C minor allele: 5'-CCTGACCTGAGTAGTCGCT constant: 5'-GCCACGTACCAGCTGTTGC
rs1049550	standard allele: 5'-CTGCCGCTGCTTGTGGAGCG minor allele: 5'-CTGCCGCTGCTTGTGGAGCA constant: 5'-CACCTCCAGGATGCCCTCATAT
rs1057369	standard allele: 5'-ATCTTCTCCAGATGGTGAGT minor allele: 5'-ATCTTCTCCAGATGGTGAGC constant: 5'-TTACAGGTGTGAGCCACCATG
rs956572	standard allele: 5'-AGAGGGAGTCATGACTGAATC minor allele: 5'-AGAGGGAGTCATGACTGAATT constant: 5'-CAGATCTGTGCTTGAACCTCA
rs1801018	standard allele: 5'ATCTCCCGTTATCGTACCCT minor allele: 5'-ATCTCCCGTTATCGTACCCT constant: 5'-GATCCGAAAGGAATTGGAATA

3. Results and Discussion

The results of genotyping are presented separately for each diseased condition in Tables 3-5.

Table 3. Genotype, allele, and minor allele carriage frequencies of the selected SNPs in patients with SCZ and HI.

SNP ID	Genotypes			Alleles		Carriage	Ref.
rs628117	AA	AG	GG	A	G	G	
SCZ (n=103)	32	50	18	57	43	68	
HI (n=105)	34	41	25	55	45	66	[20,22, 24]
$P_{corrected}$					0.68 ^a	0.73 ^b	
rs6265	GG	GA	AA	G	A	A	
SCZ (n=103)	44	50	6	69	31	55	
HI (n=105)	65	33	2	8	19	35	[20-22,24, 28-30]
$P_{corrected}$					0.004 ^a	0.003 ^b	
rs1366116	CC	CT	TT	C	T	T	
SCZ (n=260)	26	50	24	51	49	74	
HI (n=260)	53	37	10	71	29	47	[26,30]
$P_{corrected}$					1E-49 ^a	2E-19 ^b	
rs3892909	CC	CT	TT	C	T	T	
SCZ (n=260)	33	51	16	58	42	67	
HI (n=260)	23	50	27	48	52	77	[26,30]
$P_{corrected}$					0.00 ^a	0.01 ^b	
rs6330	GG	GA	AA	G	A	A	
SCZ (n=200)	43	46	11	66	34	57	
HI (n=250)	65	29	6	80	20	35	[27,33]
$P_{corrected}$					0.00 ^a	0.00 ^b	
rs4839435	GG	GA	AA	G	A	A	
SCZ (n=225)	24	56	20	52	48	76	
HI (n=225)	58	37	5	76	24	42	[27,33]

SNP ID	Genotypes			Alleles		Carriage	Ref.
IS (n=172)	41	36	23	59	41	59	
HI (n=225)	53	38	9	72	28	47	
$P_{corrected}^{\text{=}}$					0.00 ^a	0.03 ^b	
rs3892909	CC	CT	TT	C	T	T	
IS (n=172)	23	50	27	46	54	77	
HI (n=225)	20	52	28	47	53	80	unpublished
$P_{corrected}^{\text{=}}$					1.4 ^a	1.0 ^b	
rs11575945	CC	CT	TT	C	T	T	
IS (n=94)	48	42	10	69	31	52	
HI (n=110)	75	25	0	88	12	25	unpublished
$P_{nominal}^{\text{=}}$					0.00 ^a	0.00 ^b	

^a Comparison of minor allele frequency between IS and HI.

^b Comparison of minor allele carriage between IS and HI.

The distribution of genotypes for all selected SNPs in all study groups complied with H-W equilibrium.

Significant associations (either positive or negative) were found between: (1) SCZ and *BDNF* rs6265, *CPLX2* rs1366116, *CPLX2* rs3892909, *NGF* rs6330, *NGF* rs4839435, *NGFR* rs734194, *NGFR* rs11466155, *NGFR* rs2072446, *ANXAV* rs11575945, *BAX* rs1057369 SNPs; (2) PTSD and *CPLX2* rs1366116, *BCL2* rs956572 SNPs; (3) IS and *NTNG1* rs628117, *CPLX2* rs1366116, *ANXAV* rs11575945 SNPs.

Based on character of association (positive or negative), minor alleles of the *BDNF* gene rs6265 SNP, *CPLX2* rs1366116 SNP, *NGF* gene rs6330 and rs4839435 SNPs, *NGFR* gene rs2072446 SNP, and *ANXAV* gene rs11575945 SNP, can be considered risk factors of SCZ, whereas the presence of minor alleles of the *CPLX2* gene rs3892909, and *NGFR* gene rs734194 and rs11466155 SNPs, and *BAX* gene rs1057369 SNP decreases the risk for development of this disorder.

In case of PTSD, the *CPLX2* gene rs1366116 SNP and *BCL2* gene rs956572 SNP were identified as risk factors for this diseased condition.

Finally, data obtained in case of IS demonstrated that minor alleles of the *NTNG1* gene rs628117 SNP, *CPLX2* gene rs1366116 SNP and the *ANXAV* gene rs11575945 SNP increase risk for development of stroke.

Brain-derived neurotrophic factor promotes several functions of neurons, modulates neurotransmission, contributes to neuronal growth, survival, and differentiation, and regulates synaptic transmission and cognitive processes [36]. The missense rs6265 SNP of the *BDNF* gene affects activity-dependent secretion of this neurotrophic factor, memory, and hippocampus functions [37]. Association of the *BDNF* gene rs6265 SNP with human cortical morphology and bilateral reductions of hippocampus gray matter volumes in the rs6265*A minor allele carriers compared with the standard rs6265G allele homozygotes was demonstrated [38], and a crucial role of this SNP in neuroplasticity alterations in SCZ was revealed [39]. In addition, a relationship of the rs6265 genotypes with age of onset and the psychotic symptoms of SCZ was observed [40]. Moreover, the *BDNF* gene rs6265*A minor allele was found to associate with increased aggressive behavior in SCZ-affected subjects [41]. However, the results from the

meta-analyses of association of the *BDNF* gene rs6265 SNP with SCZ are controversial, which may reflect the ethnic differences between studied groups [42,43]. According to our data, in Armenian population the rs6265*A minor allele frequency was significantly higher in patients with SCZ than in HI, and the carriers of this allele were overrepresented in patients compared with HI [20-22,24,28-30]. Our results are in concordance with the recent meta-analysis data [42]. We also demonstrated that SCZ is characterized by decreased blood levels of brain-derived neurotrophic factor and that this parameter is lower in the *BDNF* gene rs6265*A minor allele carriers compared to standard rs6265G allele homozygotes [30].

Neuroprotein netrin G1 is an important promoter of neurite outgrowth, regulator of synapse formation and functional activity [44]. Changes in expression levels of the *NTNG1* gene were found in a number of diseases characterized by altered synaptic plasticity including SCZ, bipolar disorder, temporal lobe epilepsy, and Rett syndrome [45-49]. Nevertheless, only few studies have evaluated possible association between the *NTNG1* gene polymorphisms and diseased conditions. Among those are genome wide association study shown no association between common SNPs of the *NTNG1* gene and anorexia nervosa in Europeans [50], study of Zhu et al [51] demonstrated positive association of the *NTNG1* gene rs4132604 SNP and the haplotype of rs4132604, rs2218404, and rs1373336 SNPs of this gene with SCZ. In addition, analyzes of 21 SNPs of the *NTNG1* gene in 124 Japanese schizophrenic pedigrees revealed association of SCZ with specific haplotypes encompassing alternatively spliced exons, SNPs, and haplotypes clustered in the 5'-region of the *NTNG1* gene [45]. Further, Ohtsuki et al investigated 56 tag SNPs of the *NTNG1* gene and found association between SCZ and the rs628117 SNP located in intron 9 in the same haplotype block [48]. However, our own data presented in this review demonstrated no association between the rs628117 SNP and SCZ in Armenian population [20,22,24]. This discrepancy between our results and those reported by Ohtsuki et al most probably reflects population/ethnic differences between the study groups. Our study for the first time demonstrated the presence of positive association between the rs628117 SNP of the *NTNG1* gene and IS [23]. Association between any functional SNP of the *NTNG1* gene

and IS has not been assessed before in any population.

Complexin-2 is a member of complexin family of presynaptic regulatory proteins expressed mainly by excitatory neurons [52]. It has been shown that downregulation of complexin-2 might lead to changes in neurotransmitter release and deficit in synaptic transmission causing significant cognitive and behavioral abnormalities and is implicated in pathogenesis and progression of many diseased conditions characterized by altered cognitive function and behavior [53,54]. In particular, decreased expression levels of this protein in the prefrontal cortex, cerebellum, and hippocampus of patients with SCZ were observed [55], and our own studies revealed decreased production of complexin-2 protein in the blood of SCZ [30] and PTSD [14,15,18] affected subjects. The results obtained in our study with the *CPLX2* gene indicated that the rs1366116*T minor allele of this gene was overrepresented in SCZ, PTSD, and IS patients when compared to HI suggesting a positive association of this polymorphism with these diseased conditions [26,30]. While no association between the *CPLX2* rs3892909 SNP and PTSD [26,31] or IS [unpublished data] was found, rs3892909*T minor allele of this SNP was more frequent in controls than in SCZ patients [26,30] indicating protective role of this allele against SCZ. It should be noted that these are the first studies exploring association of the *CPLX2* gene rs1366116 and rs3892909 SNPs in PTSD and IS in any population. Regarding SCZ, earlier it was shown that current cognitive performance in SCZ patients is modified by a number of *CPLX2* variants modulating posttranscriptional gene expression, and that a haplotype covering six SNPs, including rs1366116 and rs3892909, showed high association with this disease [56].

Nerve growth factor and its receptor are other essential mediators of synaptic and morphological plasticity, neuronal growth, survival, and differentiation, especially in the developing brain [57,58]. The mature form of nerve growth factor derives from a precursor, which was recently discovered to exert crucial brain functions responsible for mood and cognitive activities [59]. It was reported that in generalized anxiety disorder the blood levels of nerve growth factor increase after successful cognitive behavioral therapy [60]. Moreover, decreased blood levels of this protein in patients with SCZ were observed [61,62]. Also, it has been shown that chronic cannabis abuse raises NGF serum concentrations in drug-naive patients with SCZ compared to HI [63]. The results of our study demonstrate a positive association between SCZ and the rs6330 SNP of the *NGF* gene as well as the rs11466155 and rs2072446 SNPs of the *NGFR* gene [27,28,33]. Also, a negative association between this disorder and rs4839435 SNP of the *NGF* gene as well as the rs734194 SNP of the *NGFR* gene was found [27,28,33]. A non-synonymous rs6330 SNP of the *NGF* gene is thought to affect intracellular processing and secretion of the NGF protein [64]. Earlier it was shown that the rs6330 SNP is associated with executive dysfunction in patients with Alzheimer's disease, anxiety related traits and affective disorders [65]. However, study of association

between SCZ and the rs6330 SNP of the *NGF* gene has been performed for the first time by us [27,28,33]. The same applies to the rs4839435 SNP of the *NGF* gene [27,33]. Another polymorphism of the *NGF* gene, the rs12760036 SNP was shown to associate with susceptibility to SCZ in Korean population, and significant differences in the AG and CA haplotype frequencies in the linkage disequilibrium block within the rs12760036 and rs4839435 SNPs between SCZ patients and HI were found indicating the rs12760036*C minor allele as a risk factor for SCZ in Koreans [66]. Regarding the *NGFR* gene SNPs, our study for the first time demonstrated association of the rs2072446, rs11466155 and rs734194 SNPs of this gene with SCZ [27,28,33]. Notably, the rs11466155 synonymous SNP of the *NGFR* gene was not studied before in any diseased condition. It has to be also mentioned that no data relative to functional state of nerve growth factor and its receptor in SCZ either at protein or genetic levels were reported before. The rs2072446 SNP of the *NGFR* gene and the haplotype containing the rs734194 SNP in the 3'-UTR region of the *NGFR* gene were recently found to be associated with an increased risk of Alzheimer's disease in Chinese [67].

Annexin-A5 protein is able in the presence of Ca^{+2} to bind to negatively charged phospholipids, which from the early stages of apoptosis already transfers from the inner to the outer membrane of a cell undergoing apoptosis [68-69]. Annexin-A5 is an important modulator of the process of phagocytosis of apoptotic cells and inflammatory reactions directed to removal of dying cells. Increased levels of this protein induce development of inflammatory reactions [70-72], which are characteristic features of SCZ [22], PTSD [14], and IS [73]. The increased serum levels of this protein were found by us in IS [19] and SCZ [25] and by other research group in SCZ [74], and we also detected the decreased levels of this protein in PTSD [15,18]. The rs11575945 SNP (-1C/T) in the Kozak consensus sequence of the regulatory part of the *ANXA5* gene plays a key role in the initiation of transcription [75], and the rs1157945*T minor allele of this gene correlates with the higher level of synthesis of this protein in the blood [75]. All mentioned above initiated our interest to study this SNP in SCZ, PTSD and IS. In case of IS, the rs1157945 SNP represents a special interest, since earlier it was shown that the rs1157594 SNP of the *ANXA5* gene positively associates with cardiovascular disorders [75] and that rs1157594*T minor allele of this SNP increases the risk for development of venous thromboses [76]. While our results on genotyping of the rs1157945 SNP of the *ANXA5* gene suggest no association between this SNP and PTSD [26,31], we revealed positive association between the rs1157945 SNP and SCZ [25,26,30] as well as the rs1157945 SNP and IS [unpublished data]. These results enable to consider the T minor allele of the rs1157945 SNP as a risk factor for both SCZ and IS. It has to be mentioned that in the earlier published study the association between SCZ and functional SNP of another representative of the annexin family, the *ANXA7* gene, was shown [77]. Also, in our study presented here we found no association between

the *ANXA11* gene functional rs1049550 SNP and SCZ [unpublished], whereas results of our previous investigations indicated increased expression levels of this gene in SCZ [16].

A family of Bcl-2 proteins constitutes one of the most biologically relevant classes of apoptosis regulators acting at the effector 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 [78,79]. Bcl-2 proper and Bax are members of the Bcl-2 family proteins [80]. Bcl-2 proper is an integral, membrane-associated protein with anti-apoptotic and antioxidative effects [80,81]. Bcl-2 is a major anti-apoptotic protein that inhibits apoptotic and necrotic cell death induced by a diverse set of adverse conditions [82]. Bcl-2 also plays critical roles in neuronal morphogenesis and synaptic plasticity [83,84], and reduced Bcl-2 function is hypothesized to contribute to the impairment of cellular plasticity and resilience in patients with mood disorders [82]. BAX is pro-apoptotic member of the family of Bcl-2-related proteins, which has an extensive amino acid homology with Bcl-2 [85, 86]. 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 [78-80]. Postmortem studies demonstrated the increased expression level of BAX encoding gene [87] as well as high BAX/Bcl-2 proteins ratio in the temporal cortex of patients with SCZ [88]. However, it is yet unclear whether these pathologic alterations are genetically determined or caused by other factors. The potential association between SCZ and functional SNPs of genes encoding Bcl-2 family proteins, *BAX* rs1057369 and *BCL2* rs956572, rs1801018, was assessed for the first time by us [32], and no study has yet explored these SNPs in SCZ in any other population. The same applies to our study on evaluation of association between the *BCL2* gene rs956572 SNP and PTSD [unpublished data]. The *BAX* gene rs1057369 SNP was found by us negatively associated with SCZ: the presence of *BAX* rs1057369*G minor allele, especially in homozygous form, was associated with decreased risk of developing SCZ [32]. It has to be mentioned that no data on association of this SNP with any diseased condition has been reported before. Opposite to the *BAX* gene rs1057369 SNP, the *BCL2* gene rs1801018 and rs956572 SNPs have been intensively studied in different conditions. The rs1801018 SNP was mostly found to be associated with oncological disorders [89-92] as well as with poorer clinical outcomes and mortality in patients with traumatic brain injury [93]. The rs956572 SNP 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 [94]. 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 [94]. 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 [95]. Also, it was demonstrated that the 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 [96]. In addition, it was shown that the rs956572 SNP associates with increased anterior cingulate cortical glutamate [97] and disrupted intracellular calcium homeostasis in bipolar I disorder [98] and that abnormal *BCL2* gene expression in the AA genotype of the rs956572 SNP contributes to dysfunctional Ca²⁺ homeostasis [99]. Our study revealed no association between SCZ and the *BCL2* gene rs956572 and rs1801018 SNPs [32] and indicated positive association between the *BCL2* gene rs956572 SNP and PTSD [unpublished data].

In summary, with regard to the genes encoding the synaptic plasticity regulatory proteins, we found positive associations between SCZ and the *BDNF* gene rs6265 SNP, the *NGF* gene rs6330 and rs4839435 SNPs, as well as the *NGFR* gene rs2072446 SNP, whereas negative association with this disorder was found for the *CPLX2* gene rs3892909 SNP as well as the *NGFR* gene rs734194 and rs11466155 SNPs. For the *CPLX2* gene rs1366116 SNP positive association with all three diseases was found, and the same association was detected between IS and the *NTNG1* gene rs628117 SNP.

In case of the genes encoding the apoptosis regulatory proteins, we found positive associations of the *ANXA11* gene rs11575945 SNP with both SCZ and IS as well as of the *BCL2* gene rs956572 SNP with PTSD. Also, we demonstrated that proapoptotic *BAX* gene rs1057369 SNP is negatively associated with SCZ.

Since our studies refer to one distinct population (Armenians), the obtained results, particularly those reported by us for the first time, should be replicated in other populations. Not all genes assessed in SCZ have been evaluated by us in PTSD and IS. This should be done in the future studies and more functional SNPs of the selected genes should be investigated in each disorder to have a complete view on the role of genetic predisposition and gene-environment interactions in pathomechanisms of SCZ, PTSD, and IS.

4. Conclusions

On the basis of the obtained results we conclude that genetically determined alterations in synaptic plasticity and apoptosis are involved in pathomechanisms of SCZ, PTSD, and IS. The minor T allele of the *CPLX2* gene rs1366116 polymorphism represents risk factor for all studied diseased conditions indicating important functional significance of this genetic variation in maintenance of synaptic plasticity. Another important conclusion of these studies is that minor alleles of some polymorphic variants of genes, encoding

synaptic plasticity and apoptosis regulatory proteins, may play a protective role relative to SCZ decreasing the risk for development of this disorder. In summary, our studies emphasize the important contribution of changes in synaptic plasticity and apoptosis regulation to pathomechanisms of SCZ, PTSD, and IS as well as significant input of genetic factors to these changes.

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Competing interests

The authors declare no conflict of interests.

References

- [1] Tsuang MT, Bar JL, Stone WS, Faraone SV. Gene-environment interactions in mental disorders. *World Psychiatry* 2004;3(2):73-83.
- [2] Dauncey MJ. Genomic and epigenomic insights into nutrition and brain disorders. *Nutrients* 2013;5(3):887-914.
- [3] McCarroll SA, Hyman SE. Progress in the genetics of polygenic brain disorders: significant new challenges for neurobiology. *Neuron* 2013;80(3):578-87.
- [4] Gejman PV, Sanders AR, Kendler KS. Genetics of schizophrenia: new findings and challenges. *Annu Rev Genomics Hum Genet* 2011;12:121-44.
- [5] Segman RH, Shalev AY. Genetics of Posttraumatic Stress Disorder. *CNS Spectr* 2003;8(9):693-98.
- [6] Hassan A, Markus HS. Genetics and ischaemic stroke. *Brain* 2000;123(9):1784-812.
- [7] Barbato A. Schizophrenia and Public Health. World Health Organization, Geneva, 1998.
- [8] Zohar J, Fostick L. Comparison of mortality rates between Israeli veterans with and without post traumatic stress disorder. *Eur Neuropsychopharmacol* 2014;24:117-24.
- [9] Di Carlo A. Human and economic burden of stroke. *Age Ageing* 2009;38(1):4-5.
- [10] Broughton BRS, Reutens DC, Sobey CG. Apoptotic mechanisms after cerebral ischemia. *Stroke* 2009; 40:e331-9.
- [11] Font MA, Arboix A, Krupinski J. Angiogenesis, neurogenesis and neuroplasticity in ischemic stroke. *Curr Cardiol Rev* 2010;6(3):238-44
- [12] Sumiyoshi T (ed.) Schizophrenia Research: Recent Advances, Nova Science Publishers Inc., USA, 2012.
- [13] Chavushyan AS. Annexin-A5 as a marker of apoptotic hyperfunction in schizophrenia. *Biol J Armenia* 2012;64(4): 91-3.
- [14] Boyajyan A, Mkrtychyan G, Hovhannisyanyan L, Avetyan D. Chapter 5. Alterations in the immune response, apoptosis and synaptic plasticity in posttraumatic stress disorder: molecular indicators and relation to clinical symptoms. In: Durbano F (ed.) *New Insights Into Anxiety Disorders*, InTech, Croatia, 2013, pp.105-33.
- [15] Mkrtychyan GM, Boyajyan AS, Avetyan DG, Sukiasyan SG. The involvement of anomalous apoptosis in disturbance of synaptic plasticity in posttraumatic stress disorder. *Zh Nevrol Psikhiatr Im S S Korsakova*; 2013;113(1):26-9.
- [16] Ghazaryan H. Annexin 11 expression pattern in schizophrenia. *Electronic J Natural Sciences (NAS RA)* 2013;2(21): 74-6.
- [17] Hakobjanyan A, Boyajyan A, Hovsepian L, Petrek M. Changes of ANXA11 expression level and apoptosis during aging and brain ischemic stroke. *Biol J Armenia* 2013;65(suppl.1):66-7.
- [18] Mkrtychyan GM, Boyadzhyanyan AS, Avetyan DG, Sukiasyan SG. Involvement of anomalous apoptosis in impairments to synaptic plasticity in post-traumatic stress disorder. *Neurosci Behav Physiol* 2014;44(4):442-6.
- [19] Boyajyan A, Tsakanova G, Sim R. Collectins, C3 complement protein, annexin V and C-reactive protein in acute ischemic stroke: interrelation and implication to upregulated apoptosis and inflammation. *Inflamm Cell Signal* 2014;1(2): 44-50.
- [20] Zakharyan R, Boyajyan A, Arakelyan A, Gevorgyan A, Mrazek F, Petrek M. Functional variants of the genes involved in neurodevelopment and susceptibility to schizophrenia in an Armenian population. *Human Immunol* 2011;72 (9):746-8.
- [21] Zakharyan R, Boyajyan A, Arakelyan A, Mrazek F, Petrek M. The BDNF genetic variant as a risk factor for schizophrenia in Armenian population. *Tissue Antigens* 2011;77:493.
- [22] Boyajyan A., Zakharyan R., Khoyetsyan A. Chapter 11. Molecular and genetic indicators of aberrant immunity and apoptosis in schizophrenia. In: Sumiyoshi T (ed.) *Schizophrenia Research: Recent Advances*, Nova Science Publishers Inc., USA, 2012, pp.183-240.
- [23] Stepanyan A, Zakharyan R, Boyajyan A. The netrin G1 gene rs628117 polymorphism is associated with ischemic stroke. *Neuroscience Lett* 2013;549:74-7.
- [24] Zakharyan RV, Boyajyan AS. Neurotrophin family gene as potential target for schizophrenia. *Electronic J Natural Sciences (NAS RA)* 2013;2(21):82-6.
- [25] Boyajyan AS, Chavushyan AS, Zakharyan RV, Mkrtychyan GM. Markers of apoptotic dysfunctions in schizophrenia. *Mol Biol (Moscow)* 2013;47(4):587-91.
- [26] Boyajyan A, Avetyan D, Mkrtychyan G, Sukiasyan S, Zakharyan R, Atshemyan S, Gevorgyan A, Melkumova M, Torosyan S. Population genomics study of molecular pathomechanisms responsible for apoptotic and synaptic plasticity dysfunction in posttraumatic stress disorder and schizophrenia. *Armenian J Mental Health* 2013;4(Suppl. 1): 66-7.

- [27] Zakharyan R, Atshemyan S, Gevorgyan A, Boyajyan A. Synaptic plasticity regulating genes in schizophrenia. *Biol J Armenia* 2013;65(Suppl. 1):150-2.
- [28] Zakharyan R, Gevorgyan A, Atshemyan S, Torosyan S, Boyajyan A. Genetic risk factors for schizophrenia among synaptogenesis regulating proteins. *Armenian J Mental Health* 2013;4 (Suppl. 1):91-2.
- [29] Zakharyan R, Atshemyan S, Boyajyan A. The role of neuroprotein in schizophrenia pathogenesis. *J Neurolog Sci* 2013;333(Suppl. 1):e335-6.
- [30] Boyajyan A, Chavushyan A, Zakharyan R, Mkrtchyan G, Atshemyan S. Implication of genetic polymorphisms and changes in expression levels of proteins regulating neuronal plasticity and apoptosis in schizophrenia disorder. *Eur Psychiatry* 2013;28(Suppl. 1):43.
- [31] Avetyan DA. Changes in apoptotic rate and synaptic plasticity in patients with posttraumatic stress disorder. *Electronic J Natural Sciences (NAS RA)* 2013;2(21):70-3.
- [32] Pirumyan K, Boyajyan A. Study of association between schizophrenia and functional polymorphisms of genes encoding Bcl-2 family proteins. *International Journal of Biological Sciences and Applications*, 2014;1(1):28-34.
- [33] Zakharyan R, Atshemyan S, Gevorgyan A, Boyajyan A. Nerve growth factor and its receptor in schizophrenia. *BBA Clinical*, 2014 (in press).
- [34] Sambrook J, Russell DW. *Molecular Cloning: A Laboratory Manual* (3rd edition). Cold Spring Harbor Laboratory Press, USA, 2001.
- [35] Bunce M, O'Neil CM, Barnado MC, Krausa P, Browning MJ, Morris PJ, Welsh KI. 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.
- [36] Cunha C, Brambilla R, Thomas KL. A simple role for BDNF in learning and memory? *Front J Mol Neurosci* 2010;3:1.
- [37] Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR. The BDNF Val66Met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* 2003;112(2): 257-69.
- [38] Pezawas L, Verchinski BA, Mattay VS, Callicott JH, Kolachana BS, Straub RE, Egan MF, Meyer-Lindenberg A, Weinberger DR. The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. *J Neurosci* 2004;24(45):10099-102.
- [39] Buckley PF, Pillai A, Howell KR. Brain-derived neurotrophic factor: findings in schizophrenia. *Curr Opin Psychiatry* 2011; 24(2):122-7.
- [40] Numata S, Ueno S, Iga J, Yamauchi K, Yamauchi K, Hongwei S, Ohta K, Kinouchi S, Shibuya-Tayoshi S, Tayoshi S, Aono M, Kameoka N, Sumitani S, Tomotake M, Kaneda Y, Taniguchi T, Ishimoto Y, Ohmori T. Brain-derived neurotrophic factor (BDNF) Val66Met polymorphism in schizophrenia is associated with age at onset and symptoms. *Neurosci Lett* 2006;401(1-2):1-5.
- [41] Spalletta G, Morris DW, Angelucci F, Rubino IA, Spoletini I, Bria P, Martinotti G, Siracusano A, Bonaviri G, Bernardini S, Caltagirone C, Bossù P, Donohoe G, Gill M, Corvin AP. BDNF Val66Met polymorphism is associated with aggressive behavior in schizophrenia. *Eur Psychiatry* 2010; 25(6):311-3.
- [42] Gratacòs M, González JR, Mercader JM, de Cid R, Urretavizcaya M, Estivill X. Brain-derived neurotrophic factor Val66Met and psychiatric disorders: meta-analysis of case-control studies confirm association to substance-related disorders, eating disorders, and schizophrenia. *Biol Psychiatry* 2007;61(7):911-22.
- [43] Kanazawa T, Glatt SJ, Kia-Keating B, Yoneda H, Tsuang MT. Meta-analysis reveals no association of the Val66Met polymorphism of brain-derived neurotrophic factor with either schizophrenia or bipolar disorder. *Psychiatr Genet* 2007;17(3):165-70.
- [44] Moore SW, Tessier-Lavigne M, Kennedy TE. Netrins and their receptors. *Adv Exp Med Biol* 2007;621:17-31.
- [45] Aoki-Suzuki M, Yamada K, Meerabux J, Iwayama-Shigeno Y, Ohba H, Iwamoto K, Takao H, Toyota T, Suto Y, Nakatani N, Dean B, Nishimura S, Seki K, Kato T, Itoharu S, Nishikawa T, Yoshikawa T. A family-based association study and gene expression analyses of netrin-G1 and -G2 genes in schizophrenia. *Biol Psychiatry* 2005;57(4):382-93.
- [46] Borg I, Freude K, Kübart S, Hoffmann K, Menzel C, Laccone F, Firth H, Ferguson-Smith MA, Tommerup N, Ropers HH, Sargan D, Kalscheuer VM. Disruption of Netrin G1 by a balanced chromosome translocation in a girl with Rett syndrome. *Eur J Hum Genet* 2005;13(8):921-7.
- [47] Eastwood SL, Harrison PJ. Decreased mRNA expression of netrin-G1 and netrin-G2 in the temporal lobe in schizophrenia and bipolar disorder. *Neuropsychopharmacology* 2008;33(4): 933-45.
- [48] Ohtsuki T, Horiuchi Y, Koga M, Ishiguro H, Inada T, Iwata N, Ozaki N, Ujike H, Watanabe Y, Someya T, Arinami T. Association of polymorphisms in the haplotype block spanning the alternatively spliced exons of the NTNG1 gene at 1p13.3 with schizophrenia in Japanese populations. *Neurosci Lett* 2008;435(3):194-7.
- [49] Pan Y, Liu G, Fang M, Shen L, Wang L, Han Y, Shen D, Wang X. Abnormal expression of netrin-G2 in temporal lobe epilepsy neurons in humans and a rat model. *Exp Neurol* 2010; 224:340-6.
- [50] Wang K, Zhang H, Bloss CS, Duvvuri V, Kaye W, Schork NJ, Berrettini W, Hakonarson H. A genome-wide association study on common SNPs and rare CNVs in anorexia nervosa. *Mol Psychiatry* 2011;16(9):949-59.
- [51] Zhu Y, Yang H, Bi Y, Zhang Y, Zhen C, Xie S, Qin H, He J, Liu L, Liu Y. Positive association between NTNG1 and schizophrenia in Chinese Han population. *J Genet* 2011;90(3): 499-502.
- [52] Harrison PJ, Eastwood SL. Preferential involvement of excitatory neurons in medial temporal lobe in schizophrenia. *Lancet* 1998;352(9141):1669-73.
- [53] Brose N. Altered complexin expression in psychiatric and neurological disorders: cause or consequence? *Mol Cells* 2008;25(1):7-19.

- [54] Glynn D, Gibson HE, Harte MK, Reim K, Jones S, Reynolds GP, Morton AJ. Clorgyline-mediated reversal of neurological deficits in a Complexin 2 knockout mouse. *Hum Mol Genet* 2010;19(17):3402-12.
- [55] Eastwood SL, Harrison PJ. Hippocampal synaptic pathology in schizophrenia, bipolar disorder and major depression: a study of complexin mRNAs. *Mol Psychiatry* 2000;5:425-32.
- [56] Begemann M, Grube S, Papiol S, Malzahn D, Krampe H, Ribbe K, Friedrichs H, Radyushkin KA, El-Kordi A, Benseler F, Hannke K, Sperling S, Schwerdtfeger D, Thanhäuser I, Gerchen MF, Ghorbani M, Gutwinski S, Hilmes C, Leppert R, Ronnenberg A, Sowislo J, Stawicki S, Stödtke M, Szaszies C, Reim K, Riggert J, Eckstein F, Falkai P, Bickeböller H, Nave KA, Brose N, Ehrenreich H. Modification of cognitive performance in schizophrenia by complexin 2 gene polymorphisms. *Arch Gen Psychiatry* 2010;67(9):879-88.
- [57] McAllister AK, Katz LC, Lo DC. Neurotrophins and synaptic plasticity. *Annu Rev Neurosci* 1999;22:295-318.
- [58] Buckley PF, Mahadik S, Pillai A, Terry JrA. Neurotrophins and schizophrenia. *Schizophr Res* 2007;94:1-11.
- [59] Stanisz AM, Stanisz JA. Nerve growth factor and neuroimmune interactions in inflammatory diseases. *Ann N Y Acad Sci* 2000;917:268-72.
- [60] Jockers-Scherubl MC, Zubraegel D, Baer T, Linden M, Danker-Hopfe H, Schulte-Herbruggen O, Neu P, Hellweg R. Nerve growth factor serum concentrations rise after successful cognitive-behavioural therapy of generalized anxiety disorder. *Prog Neuropsychopharmacol Biol Psychiatry* 2007;31(1):200-4.
- [61] Xiong P, Zeng Y, Zhu Z, Tan D, Xu F, Lu J, Wan J, Ma M. Reduced NGF serum levels and abnormal P300 event-related potential in first episode schizophrenia. *Schizophr Res* 2010;119(1-3):34-9.
- [62] Xiong P, Zeng Y, J Wan J, Xiaohan DH, Tan D, Lu J, Xu F, Li HY, Zhu Z, Ma M. The role of NGF and IL-2 serum level in assisting the diagnosis in first episode schizophrenia. *Psychiatry Res* 2011;189(1):72-6.
- [63] Jockers-Scherubl MC, Matthies U, Danker-Hopfe H, Lang UE, Mahlberg R, Hellweg R. Chronic cannabis abuse raises nerve growth factor serum concentrations in drug-naive schizophrenic patients. *J Psychopharmacol* 2003;17(4):439-45.
- [64] Syed Z, Dudbridge F, Kent L. An investigation of the neurotrophic factor genes GDNF, NGF, and NT3 in susceptibility to ADHD. *Am J Med Genet B* 2007;144B(3):375-8.
- [65] Di Maria E, Giorgio E, Uliana V, Bonvicini C, Faravelli F, Cammarata S, Novello MC, Galimberti D, Scarpini E, Zanetti O, Gennarelli M, Tabaton M. Possible influence of a non-synonymous polymorphism located in the NGF precursor on susceptibility to late-onset Alzheimer's disease and mild cognitive impairment. *J Alzheimers Dis* 2012;29(3):699-705.
- [66] Park JK, Lee SM, Kang WS, Kim SK, Cho AR. NGF polymorphisms and haplotypes are associated with schizophrenia in Korean population. *Mol Cell Toxicol* 2011;7:375-80.
- [67] Cheng HC, Sun Y, Lai LC, Chen SY, Lee WC, Chen JH, Chen TF, Chen HH, Wen LL, Yip PK, Chu YM, Chen WJ, Chen YC. Genetic polymorphisms of nerve growth factor receptor (NGFR) and the risk of Alzheimer's disease. *J Negat Results Biomed* 2012;11:5.
- [68] Gerke V, Moss SE. Annexins: from structure to function. *Physiol Rev* 2002;82(2):331-71.
- [69] Boersma HH, Kietselaer BL, Stolk LM, Bennaghmouch A, Hofstra L, Narula J, Heidendal GA, Reutelingsperger CP. Past, present, and future of annexin A5: from protein discovery to clinical applications. *J Nucl Med* 2005;46(12):2035-50.
- [70] Munoz L, Frey B, Pausch F, Baum W, Mueller R, Brachvogel B, Poschl E, Rodel F, von der Mark K, Herrmann M, Gaipf U. The role of annexin A5 in the modulation of the immune response against dying and dead cells. *Curr Med Chem* 2007;14(3):271-7.
- [71] Gaipf US, Munoz LE, Rodel F, Pausch F, Frey B, Brachvogel B, von der Mark K, Poschl E. Modulation of the immune system by dying cells and the phosphatidylserine-ligand annexin A5. *Autoimmunity* 2007;40(4):254-9.
- [72] Martin M, Leffler J, Blom AM. Annexin A2 and A5 serve as new ligands for C1Q on apoptotic cells. *J Biol Chem* 2012;287(40):33733-44.
- [73] Di Napoli M, Arakelyan A, Boyajyan A, Godoy A, Papa F. Chapter 2. The acute phase inflammatory response in stroke: systemic inflammation and neuroinflammation. In: Pitzer JA (ed.) *Progress in Inflammation Research*, Nova Science Publishers Inc., USA, 2005, pp.95-145.
- [74] Francesconi LP, Ceresér KM, Mascarenhas R, Stertz L, Gama CS, Belmonte-de-Abreu P. Increased annexin-V and decreased TNF-alpha serum levels in chronic-medicated patients with schizophrenia. *Neurosci Lett* 2011;502(3):143-6.
- [75] González-Conejero R, Corral J, Roldán V, Martínez C, Marín F, Rivera J, Iniesta JA, Lozano ML, Marco P, Vicente V. A common polymorphism in the annexin V Kozak sequence (-1C>T) increases translation efficiency and plasma levels of annexin V, and decreases the risk of myocardial infarction in young patients. *Blood* 2002;100(6):2081-6.
- [76] Van Heerde WL, DeGroot PG, Reutelingsperger CPM. The complexity of the phospholipid binding protein Annexin V. *Thromb Haemost* 1995;73(2):172-9.
- [77] Liu CM, Fann CS, Chen CY, Liu YL, Oyang YJ, Yang WC, Chang CC, Wen CC, Chen WJ, Hwang TJ, Hsieh MH, Liu CC, Faraone SV, Tsuang MT, Hwu HG. ANXA7, PPP3CB, DNAJC9, and ZMYND17 genes at chromosome 10q22 associated with the subgroup of schizophrenia with deficits in attention and executive function. *Biol Psychiatry* 2011;70(1):51-8.
- [78] 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.
- [79] 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.
- [80] 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.

- [81] Yang J, Liu X, Bhalla K, Kim CN, Ibrado AM, Cai J, Peng TI, Jones DP, Wang X. Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. *Science* 1997;275(5303):1129-32.
- [82] Chen G, Manji HK. The extracellular signal-regulated kinase pathway: An emerging promising target for mood stabilizers. *Curr Opin Psychiatry* 2006;19:313-23.
- [83] Chen DF, Schneider GE, Martinou JC, Tonegawa S. Bcl-2 promotes regeneration of severed axons in mammalian CNS. *Nature* 1997;385:435-9.
- [84] Jonas E. Bcl-x1 regulates synaptic plasticity. *Mol Interv* 2006;6:208-22.
- [85] Oltvai ZN, Millman CL, Korsmeyer SJ. Bcl2 heterodimers in vivo with a conserved homolog Bax, that accelerates programmed cell death. *Cell* 1993;74:609-19.
- [86] Surhone LM, Tennoe MT, Henssonow SF. Bcl-2-associated X Protein. Beau Bassin: Betascript Publishing, 2011.
- [87] Jarskog LF, Selinger ES, Lieberman JA, Gilmore JH. Cortical Bcl2 protein expression and apoptotic regulation in schizophrenia. *Biol Psychiatry* 2000;48:641-50.
- [88] 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.
- [89] Milani L, Gupta M, Andersen M, Dhar S, Fryknäs M, Isaksson A, Larsson R, Syvänen AC. 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.
- [90] Kim DH, Xu W, Ma C, Liu X, Siminovitch K, Messner HA, Lipton JH. Genetic variants in the candidate genes of the apoptosis pathway and susceptibility to chronic myeloid leukemia. *Blood* 2009;113(11):2517-25.
- [91] Eun YG, Hong IK, Kim SK, Park HK, Kwon S, Chung DH, Kwon KH. Polymorphism (rs1801018, Thr7Thr) of BCL2 is associated with papillary thyroid cancer in Korean population. *Clin Exp Otorhinolaryngol* 2011;4(3):149-54.
- [92] Gu S, Wu Q, Zhao X, Wu W, Gao Z, Tan X, Qian J, Chen H, Xie Y, Jin L, Han B, Lu D. 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.
- [93] Hoh NZ, Wagner AK, Alexander SA, Clark RB, Beers SR, Okonkwo DO, Ren D, Conley YP. BCL2 genotypes: functional and neurobehavioral outcomes after severe traumatic brain injury. *J Neurotrauma* 2010;27(8):1413-27.
- [94] 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.
- [95] Yuan P, Baum AE, Zhou R, Wang Y, Laje G, McMahon FJ. 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.
- [96] Salvatore G, Nugent AC, Chen G, Akula N, Yuan P, Cannon DM, Zarate CA Jr, McMahon FJ, Manji HK, Drevets WC. Bcl-2 polymorphism influences gray matter volume in the ventral striatum in healthy humans. *Biol Psychiatry* 2009;66(8):804-7.
- [97] Liu ME, Huang CC, Hwang JP, Yang AC, Tu PC, Yeh HL, Hong CJ, Liou YJ, Chen JF, Lin CP, Tsai SJ. 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.
- [98] Soeiro-de-Souza MG, Salvatore G, Moreno RA, Otaduy MC, Chaim KT, Gattaz WF, Zarate CA Jr, Machado-Vieira R. Bcl-2 rs956572 polymorphism is associated with increased anterior cingulate cortical glutamate in euthymic bipolar I disorder. *Neuropsychopharmacology* 2013;38(3):468-75.
- [99] 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.