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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, *ANXA*V 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)**
NTNG1	22854	1p13.3	rs628117	A>G	107997106	intronic
BDNF	627	11p13	rs6265	G>A	27679916	exonic (missense)
CPLX2	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)
NGFR	4804	17q21-q22	rs2072446	C>T	47587819	missense
			rs734194	T>G	47591609	3'-UTR
ANXAV	308	4q27	rs11575945	-1C>T	122617745	Kozak sequence
ANXA11	311	10q21-23	rs1049550	G>A	81916682	missense
BAX	581	19q13.33	rs1057369	A>G	49464866	intronic
BCL2	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′ ATCCTTGGAATGAAAGCCCA minor allele: 5′- ATCCTTGGAATGAAAGCCCG constant: 5′-TCACTGCCCTCTGTGTGCAGTG
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 constant: 5′-CTGCTTCATGACGAAGTCCA
rs6330	standard allele: 5′-GCATCTTGCTCTGTGCAGAT minor allele: 5′-GACACACCATCCCCCAAGC constant: 5′-GACACACCATCCCCCAAGT
rs4839435	standard allele: 5′-TGGGTGCCAAAAAGCTTGGC minor allele: 5′-TGGGTGCCAAAAAGCTTGGT constant: 5′-GCAGCTCCTGCAATTATCCA
rs11466155	standard allele: 5′-AGGCTATGTAGGCCACAAGG minor allele: 5′-AGGCTATGTAGGCCACAAGA constant: 5′-CAGAGGGCTCGGACAGCACA
rs2072446	standard allele: 5′-GTCCACACCCCCAGAGGGCTC minor allele: 5′-GTCCACACCCCCAGAGGGCTT constant: 5′-AGCAGCCAGGATGGAGCAAT
rs734194	standard allele: 5′-GCTGGAGCTGGCGTCTGTCT minor allele: 5′-GCTGGAGCTGGCGTCTGTCG constant: 5′-CTAGAGCTGGGAGAAATCCC
rs11575945	standard allele: 5′-CCTGACCTGAGTAGTCGC C minor allele: 5′-CCTGACCTGAGTAGTCGCT constant: 5′-GCCACGTCACCAGCTGTTGC
rs1049550	standard allele: 5'-CTGCCGCTGCTTGTTGGAGCG minor allele: 5'-CTGCCGCTGCTTGTTGGAGCA constant: 5'-CACCCTCCAGGATGCCCTCATAT
rs1057369	standard allele: 5'-ATCTTCTTCCAGATGGTGAGT minor allele: 5'-ATCTTCTTCCAGATGGTGAGC constant: 5'-TTACAGGTGTGAGCCACCATG
rs956572	standard allele: 5'-AGAGGGAGTCATGACTGAATC minor allele: 5'-AGAGGGAGTCATGACTGAATT constant: 5'-CAGATCTGTGCTTGAACCTCA
rs1801018	standard allele: 5'ATCTCCCGGTTATCGTACCCT minor allele: 5'-ATCTCCCGGTTATCGTACCCC 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	[20,22, 24]
HI (n=105)	34	41	25	55	45	66	
$P_{corrected}$=					0.68[a]	0.73[b]	
rs6265	GG	GA	A A	G	A	A	
SCZ (n=103)	44	50	6	69	31	55	[20-22,24, 28-30]
HI (n=105)	65	33	2	8	19	35	
$P_{corrected}$=					0.004 [a]	0.003[b]	
rs1366116	CC	CT	TT	C	T	T	
SCZ (n=260)	26	50	24	51	49	74	[26,30]
HI (n=260)	53	37	10	71	29	47	
$P_{corrected}$=					1E-49[a]	2E-19[b]	
rs3892909	CC	CT	TT	C	T	T	
SCZ (n=260)	33	51	16	58	42	67	[26,30]
HI (n=260)	23	50	27	48	52	77	
$P_{corrected}$=					0.00[a]	0.01[b]	
rs6330	GG	GA	AA	G	A	A	
SCZ (n=200)	43	46	11	66	34	57	[27,33]
HI (n=250)	65	29	6	80	20	35	
$P_{corrected}$=					0.00[a]	0.00[b]	
rs4839435	GG	GA	AA	G	A	A	
SCZ (n=225)	24	56	20	52	48	76	[27,33]
HI (n=225)	58	37	5	76	24	42	

SNP ID	Genotypes			Alleles		Carriage	Ref.
$P_{corrected}$=					0.00[a]	0.00[b]	
rs11466155	CC	CT	TT	C	T	T	
SCZ (n=200)	39.5	44	16.5	62	38	60.5	[27,28, 33]
HI (n=225)	20	52	28	46	54	80	
$P_{corrected}$=					0.00[a]	0.00[b]	
rs2072446	CC	CT	TT	C	T	T	
SCZ (n=200)	34	53	13	61	39	66	[27,28, 33]
HI (n=250)	56	33	11	89	29	47	
$P_{corrected}$=					0.00[a]	0.00[b]	
rs734194	TT	TG	GG	T	G	G	
SCZ (n=200)	70	27	4	83	17	31	[27,28, 33]
HI (n=250)	54	37	9	73	27	46	
$P_{corrected}$=					0.01[a]	0.02[b]	
rs11575945	CC	CT	TT	C	T	T	
SCZ (n=225)	24	56	20	52	48	76	[25,26, 30]
HI (n=225)	58.3	36.4	5.3	76	24	42	
$P_{nominal}$=					0.00[a]	0.00[b]	
rs1049550	GG	GA	AA	G	A	A	
SCZ (n=225)	30	53	17	56	44	70	unpublished
HI (n=225)	34	48	18	58	42	66	
$P_{nominal}$=					0.64[a]	0.42[b]	
rs1057369	AA	AG	GG	A	G	G	
SCZ (n=330)	27	55	18	55	45	73	[32]
HI (n=326)	18	53	29	45	55	82	
$P_{corrected}$=					0.00[a]	0.02[b]	
rs956572	GG	GA	AA	G	A	A	
SCZ (n=330)	34	53	13	60	40	66	[32]
HI (n=326)	36	46	18	59	41	64	
$P_{corrected}$=					1.81[a]	1.63[b]	
rs1801018	AA	AG	GG	A	G	G	
SCZ (n=330)	26	55	19	54	46	74	[32]
HI (n=326)	21	57	22	49	51	79	
$P_{corrected}$=					0.37[a]	0.35[b]	

[a] Comparison of minor allele frequency between SCZ and HI.
[b] Comparison of minor allele carriage between SCZ and HI.

Table 4. *Genotype, allele, and minor allele carriage frequencies of the selected SNPs in patients with PTSD and HI.*

SNP ID	Genotypes			Alleles		Carriage	Ref.
rs1366116	CC	CT	TT	C	T	T	
PTSD (n=87)	39	41	20	60	40	61	[26,31]
HI (n=75)	60	32	8	76	24	40	
$P_{corrected}$=					0.01[a]	0.02[b]	
rs3892909	CC	CT	TT	C	T	T	
PTSD (n=87)	18	52	30	44	56	82	[26,31]
HI (n=75)	20	55	25	47	53	80	
$P_{corrected}$=					1.7[a]	2.4[b]	
rs11575945	CC	CT	TT	C	T	T	
PTSD (n=80)	79	17	4	87.5	12.5	21	[26,31]
HI (n=75)	71	28	1	85	15	29	
$P_{corrected}$=					1.4[a]	0.75[b]	
rs956572	GG	GA	AA	G	A	A	
PTSD (n=132)	14	46	40	37	63	86	upublished
HI (n=131)	35	47	18	59	41	65	
$P_{nominal}$=					0.00[a]	0.00[b]	

[a] Comparison of minor allele frequency between PTSD and HI;
[b] Comparison of minor allele carriage between PTSD and HI.

Table 5. *Genotype, allele, and minor allele carriage frequencies of the selected SNPs in patients with IS and HI.*

SNP ID	Genotypes			Alleles		Carriage	Ref.
rs628117	AA	AG	GG	A	G	G	
IS (n=127)	17	47	36	41	59	83	[23]
HI (n=128)	37.5	40	22.5	57	43	62.5	
$P_{corrected}$=					0.00[a]	0.00[b]	
rs1366116	CC	CT	TT	C	T	T	unpublished

SNP ID	Genotypes			Alleles		Carriage	Ref.
IS (n=172)	41	36	23	59	41	59	
HI (n=225)	53	38	9	72	28	47	
$P_{corrected}$=					0.00[a]	0.03[b]	
rs3892909	CC	CT	TT	C	T	T	
IS (n=172)	23	50	27	46	54	77	unpublished
HI (n=225)	20	52	28	47	53	80	
$P_{corrected}$=					1.4[a]	1.0[b]	
rs11575945	CC	CT	TT	C	T	T	
IS (n=94)	48	42	10	69	31	52	unpublished
HI (n=110)	75	25	0	88	12	25	
$P_{nominal}$=					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 *NTGN1* 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 *ANXAV* 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 *ANXAV* 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 *ANXAV* 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 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 [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 Ca^{2+} 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 *ANXAV* 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.

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