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Nerve growth factor and its receptor in schizophrenia

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ABSTRACT

Promising studies suggest that defects in synaptic plasticity detected in schizophrenia may be linked to neurodevelopmental and neurodegenerative abnormalities and contribute to disease-associated cognitive impairment. We aimed to clarify the role of the synaptic plasticity regulatory proteins, nerve growth factor (NGF) and its receptor (NGFR) in the pathogenesis of schizophrenia by comparative analysis of their blood levels and functional single nucleotide polymorphisms (SNPs) in genes encoding these proteins (*NGF* and *NGFR*) in schizophrenia-affected and healthy subjects. Relationships between the selected SNPs' genotypes and NGF and NGFR plasma levels were also assessed. Our results demonstrated a positive association between schizophrenia and the *NGF* rs6330 as well as the *NGFR* rs11466155 and rs2072446 SNPs. Also, a negative association between this disorder and *NGF* rs4839435 as well as *NGFR* rs734194 was found. In both, haloperidol-treated and antipsychotic-free patients decreased blood levels of the NGF and NGFR were found, and a positive interrelation between rs6330 and rs2072446 carriage and decreased NGF and NGFR levels, respectively, was revealed. In conclusion, our results demonstrate association of schizophrenia with the rs6330, rs4839435 and rs734194, rs11466155, rs2072446 as well as with the decreased blood levels of corresponding proteins. Our findings indicate the implication of alterations in *NGFR* and *NGFR* genes in schizophrenia, particularly, in defects of synaptic plasticity. Furthermore, the data obtained suggests that at least in Armenian population the *NGF* rs6330*T and *NGFR* rs11466155*T, rs2072446*T alleles might be nominated as risk factors, whereas the *NGF* rs4839435*A and *NGFR* rs734194*G alleles might be protective against developing schizophrenia.

1. Introduction

Schizophrenia is a chronic, severe, and disabling mental disorder with a high heritability (approximately 80%) [1,2]. This complex disorder with still unclear etiology and molecular pathomechanisms is characterized by both neurodevelopmental [3] and neurodegenerative abnormalities [4–6] and cognitive impairments [7] linked to behavioral changes [8].

Promising studies suggest that defects in synaptic plasticity detected in schizophrenia [9] may be linked to neurodevelopmental and neurodegenerative abnormalities [10–13] and contribute to cognitive impairment associated with this disease [14–17]. Therefore, study of synaptic plasticity regulatory genes in schizophrenia represents a special interest, as it can provide insight into molecular mechanisms of schizophrenia-associated cognitive dysfunction and sufficiently contribute to development of target-oriented therapy for this disorder. Here, genes encoding neurotrophins might be considered as the most attractive candidates, because these proteins and their receptors are expressed in the neuronal populations of the brain undergoing synaptic plasticity and also participate in neuronal development, synaptogenesis, and response to stress/anxious stimuli [18]. In addition, neurotrophins play an important role in the immune response [19], which is upregulated in schizophrenia [20,21].

In our recent study we demonstrated implication of genetic variation of brain-derived neurotropic factor, modulators of brain plasticity in cognitive processes [15], in pathogenesis of schizophrenia [12]. Other important members of neurotrophin family are nerve growth factor (NGF) and its receptor (NGFR), the essential mediators of synaptic and morphological plasticity, neuronal growth, survival, and differentiation, especially in the developing brain [18,22]. The mature form of nerve growth factor (NGF) derives from a precursor, proNGF, which was recently discovered to exert crucial brain functions responsible for mood and cognitive activities [23]. Jockers-Scherubl et al. reported that in generalized anxiety disorder the NGF serum level increases in response to positive environments, namely, after successful cognitive behavioral therapy [24]. Moreover, decreased blood levels of NGF among first-episode schizophrenia patients compared to healthy subjects have been observed [25,26]. Interestingly, it has been shown that

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chronic cannabis abuse raises NGF serum concentrations in drug-naive patients with schizophrenia compared to healthy control subjects [27]. The potential implication of NGFR in schizophrenia either at protein or genetic levels has not been studied yet.

This study was aimed to clarify the role of the NGF and NGFR proteins in the pathogenesis of schizophrenia by comparative analysis of their blood levels and functional single nucleotide polymorphisms (SNPs) in genes encoding these proteins (*NGF* and *NGFR*) in schizophrenia-affected and healthy subjects. Relationships between the selected SNPs' genotypes and NGF and NGFR plasma levels were also assessed.

2. Materials and methods

2.1. Study population

A total of 475 unrelated Caucasian individuals of Armenian nationality living in Armenia (200 chronic schizophrenia patients, 25 first-episode schizophrenia patients and 250 healthy subjects) were enrolled in this study. All chronic patients (female/male: 62/138, mean age ± SD: 42.4 ± 8.2 years, age at the first-onset of disease: 25.2 ± 9.1 years, duration of disease: 17.2 ± 7.2 years, patients with/without family history of psychiatric disorders: 84/116) and first-episode patients (female/male: 12/13, mean age ± SD: 25.3 ± 9.2 years, patients with/without family history of psychiatric disorders: 10/15) were recruited from clinics of the Psychiatric Medical Center MH RA. They were diagnosed as paranoid schizophrenics (ICD-10 code: F20.0, DSM-IV-TR code: 295.30 [28,29]) by two independent experienced psychiatrists according to the presence of the relevant symptoms and the results of the Structured Clinical Interview for DSM-IV-TR [29]. Chronic patients were treated with haloperidol and first-episode patients were antipsychotic-free. Age- and sex-matched healthy volunteers (female/male: 77/173, mean age ± SD: 43.6 ± 9.1 years) were recruited among the staff and blood donors of the Erebouni Medical Center MH RA and served as a reference control population (controls). They passed a special examination by two independent experienced psychiatrists to establish no personal or family history of mental disorders. Any medical condition or treatment known to affect the brain, or meeting DSM-IV criteria for mental retardation as determined from the non-patient version of the Structured Clinical Interview for DSM-IV-TR Axis I Disorders [30]. Also, the healthy subjects were free of any medication for at least 1 month prior to blood sampling. Exclusion criteria for all study participants included any serious neurological, endocrine or metabolic disorder, acute or chronic infections, autoimmune, inflammatory or autoinflammatory diseases, malignancies, and any surgical interventions within the previous 12 months. Fifty-two schizophrenia-affected subjects and sixty-seven healthy subjects were nicotine-dependent (tobacco cigarette smokers).

All subjects gave their informed consents to provide 10 ml of venous blood for the purposes of this study. The study was approved by the Ethical Committee of the Institute of Molecular Biology of the National Academy of Sciences (NAS) RA (IRB #00004079).

2.2. Collection of blood samples and separation of plasma

10 ml of the morning fasting venous blood was collected from each study subject using EDTA as anticoagulant. The plasma was isolated by centrifugation (1500 g × 10 min, 4 °C) and kept at −30 °C until further use.

2.3. Genomic DNA extraction

Genomic DNA was isolated from the fresh blood samples according to the standard phenol–chloroform method [31] and stored at −30 °C until further use.

2.4. Selection of SNPs for NGF and NGFR genes

In total, five SNPs within the *NGF* and *NGFR* genes 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 [32].

2.5. Genotyping of NGF and NGF SNPs

DNA samples of all patients with chronic schizophrenia and controls were genotyped for *NGF* rs6330, rs4839435 and *NGFR* rs734194, rs11466155, rs2072446 SNPs using polymerase chain reaction with sequence-specific primers (PCR-SSP) [33]. The sequences of specific primers were designed based on relevant DNA sequences available in the NCBI GenBank database (http://www.ncbi.nlm.nih.gov/genbank/; Gene IDs: 4803, 4804). Nucleotide sequences of the primers used for genotyping of the *NGF* and *NGFR* SNPs are presented in Table 1.

The presence/absence of allele-specific amplicons was visualized by electrophoresis in 2% agarose gel stained with ethidium bromide fluorescent dye. To check the reproducibility of results, randomly selected DNA samples of study subjects (10% of total) were genotyped twice.

2.6. Determination of the NGF and NGFR levels in the blood plasma

The levels of the NGF and NGFR proteins in the blood plasma samples of study subjects were measured with a solid-phase enzyme-linked immunosorbent assay (ELISA) using commercial kits (Human NGF/NGFR ELISA kit, Boster Biological Technology Co., Inc., USA and Human NGFR ELISA kit, RayBiotech, Inc., USA) according to manufacturers' instructions. Each sample, standard, and blank control (zero standard) were run in duplicates on the same microplate. Also, duplicates of the same cases and controls (three of each) were run in each assay/on each microplate. The calculated overall intra-assay coefficient of variation (CV) was 5%, and the calculated overall inter-assay coefficient of variation was 8%. Standard curves were reproducible with CV < 4%. Detection limit of the NGF (beta subunit) and NGFR assays was 1 pg/ml and 80 pg/ml, respectively. Concentration of proteins was expressed in pg/ml of plasma.

From 200 chronic patients with schizophrenia and 250 controls enrolled in the genotyping experiments a total of 240 plasma samples (120 of patients and 120 of controls) were subjected to ELISA. Additionally, in order to check the effect of antipsychotic treatment, plasma samples of a small group of antipsychotic-naive first-episode schizophrenia patients were also analyzed.

Table 1
Primer nucleotide sequences for *NGF* and *NGFR* genes for PCR-SSP.

Gene, SNP	Sequence
NGF, rs6330	5′-GAC-ACA-CCA-TCC-CCC-AAG-C-3′
	5′-GAC-ACA-CCA-TCC-CCC-AAG-T-3′
	5′-GCA-TCT-TGC-TCT-GTG-CAG-AT-3′
NGF, rs4839435	5′-TGG-GTG-CCA-AAA-AGC-TTG-GC-3′
	5′-TGG-GTG-CCA-AAA-AGC-TTG-GT-3′
	5′-GCA-GCT-CCT-GCA-ATT-ATC-CA-3′
NGFR, rs734194	5′-GCT-GGA-GCT-GGC-GTC-TGT-CT-3′
	5′-GCT-GGA-GCT-GGC-GTC-TGT-CG-3′
	5′-CTA-GAG-CTG-GGA-GAA-ATC-CC-3′
NGFR, rs11466155	5′-AGG-CTA-TGT-AGG-CCA-CAA-GG-3′
	5′-AGG-CTA-TGT-AGG-CCA-CAA-GA-3′
	5′-CAG-AGG-GCT-CGG-ACA-GCA-CA-3′
NGFR, rs2072446	5′-GTC-CAC-ACC-CCC-AGA-GGG-CTC-3′
	5′-GTC-CAC-ACC-CCC-AGA-GGG-CTT-3′
	5′-AGC-AGC-CAG-GAT-GGA-GCA-AT-3′

2.7. Statistical analysis

Distributions of genotypes for investigated SNPs were checked for correspondence to the Hardy–Weinberg equilibrium (H–W). To reveal a potential association of these SNPs with schizophrenia, their genotype, allele (gene) and phenotype frequencies (carriage rates) in patients and controls were compared. The significance of differences between allele and phenotype frequencies in study groups 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 [34], and those less than 0.05 were considered statistically significant. Statistical power of the present study was estimated according to the earlier described protocol [35]. Overall descriptive statistics and the Mann–Whitney *U* test were used for evaluation of intergroup differences in the blood plasma levels of the NGF and NGFR proteins. Group statistics, otherwise specified, was presented as median [interquartile range]. p-Values less than 0.05 were considered significant. The data were evaluated using GraphPad Prism 3.03 software (GraphPad Software Inc., USA).

3. Results

3.1. Distribution of the NGF rs6330, rs4839435 and NGFR rs734194, rs11466155, rs2072446 polymorphisms in patients with schizophrenia and controls

Distribution of *NGF* rs6330, rs4839435 and *NGFR* rs734194, rs11466155, rs2072446 alleles and genotypes in the groups of schizophrenia patients and healthy subjects were in compliance with H–W equilibrium. Statistical power of the present study, the difference in the carriage of the rs6330*T and rs4839435*A alleles of the *NGF* gene and rs734194*G, rs11466155*T, rs2072446*T alleles of the *NGFR* gene between the patients and healthy subjects, reached 99.8%, 100%, and 94.7%, 95.9%, 99,2%, respectively.

The allele and phenotype frequencies of the studied genetic variant in schizophrenia-affected and healthy subjects are shown in Table 2.

According to the data obtained, the rs6330*T allele of the *NGF* gene was more frequent in patients than in controls (patients vs. controls, 0.34 vs. 0.20, $p_{nominal} = 4.0E-6$, OR = 2.01, 95%CI: 1.24–1.66). Also, the carriers of rs6330*T minor allele were overrepresented in the group of patients compared to controls (0.57 vs. 0.35, $p_{nominal} = 3.0E-6$, OR = 2.48, 95%CI: 1.33–2.03). In contrast, the rs4839435*A minor allele of the *NGF* gene was more frequent among controls compared to patients (0.33 vs. 0.22, $p_{nominal} = 0.00016$, OR = 0.56, 95%CI: 0.59–0.86). Also, the carriers of this allele were more in the group of controls compared to patients (0.58 vs. 0.38, $p_{nominal} = 2.2E-5$, OR = 0.44, 95%CI: 0.51–0.79). Further, we found that the rs11466155*T minor allele of the *NGFR* gene was overrepresented in patients with schizophrenia compared to healthy subjects (0.38 vs. 0.26, $p_{nominal} = 0.0001$, OR = 1.77, 95%CI: 1.16–1.55). Also, the carriers of the rs11466155*T minor allele (CT + TT) were more frequent in patients than in controls (0.61 vs. 0.45, $p_{nominal} = 0.0012$, OR = 1.86, 95%CI: 1.14–1.75). On the contrary, the frequency (0.27 vs. 0.17, $p_{nominal} = 0.0004$, OR = 0.56, 95%CI: 0.57–0.87) and carriers (0.46 vs. 0.31, $p_{nominal} = 0.0011$, OR = 0.52, 95%CI: 0.55–0.87) of the rs734194*G allele of *NGFR* gene were higher in controls than in schizophrenia-affected subjects. The *NGFR* rs2072446*T minor allele frequency again was higher in patients than in controls (0.39 vs. 0.29, $p_{nominal} = 0.0009$, OR = 1.59, 95%CI: 1.13–1.64). The same applies to the carriers of the *NGFR* rs2072446*T allele (0.66 vs. 0.47, $p_{nominal} = 6.7E-05$, OR = 2.17, 95%CI: 1.24–1.95). After Bonferroni correction for the number of tested loci (n = 5), difference in allele frequency between the patient and control groups for the rs6330*T: ($p_{corrected} = 2.00E-05$), rs4839435*A ($p_{corrected} = 0.0008$), rs11466155*T ($p_{corrected} = 0.006$), rs734194*G ($p_{corrected} = 0.006$), and rs2072446*T ($p_{corrected} = 0.005$) minor alleles remained significant.

3.2. Levels of the NGF and NGFR proteins in the blood plasma

The NGF and NGFR plasma levels in 120 haloperidol-treated, 25 antipsychotic-free schizophrenia patients and 120 controls were compared.

According to the data obtained, reduced median levels of NGF in haloperidol-treated patients compared to controls were detected (patients vs. controls: 4.21 [4.1, 5.67] pg/ml vs. 7.01 [6.89, 7.14] pg/ml, $p < 0.0001$). The NGF median levels in antipsychotic-free patients were also lower compared to controls (patients vs. controls: 4.66 [3.79, 4.73] pg/ml vs. 7.01 [6.89, 7.14] pg/ml, $p < 0.0001$), while no significant difference in this parameter between two groups of patients was found ($p = 0.96$).

Concerning NGFR, haloperidol-treated patients with schizophrenia had significantly lower median levels of this protein than controls (patients vs. controls: 624.2 [617.4, 631.2] pg/ml vs. 636.8 [628.3, 640.0] pg/ml, $p < 0.0001$). The same applies to antipsychotic-free patients (patients vs. controls: 624.8 [615.5, 629.2] pg/ml vs. 636.8

Table 2
Distribution of genotypes, alleles and carriage of mutant alleles of *NGF* rs6330, rs4839435 and *NGFR* rs734194, rs11466155, rs2072446 SNPs in patients with schizophrenia (SCZ; n = 200) and controls (n = 250). The data are presented as absolute numbers with proportions (%) in parentheses.

Gene, SNP	Genotypes			Alleles		Carriage
NGF rs6330	CC	CT	TT	C	T	T
SCZ	86 (0.43)	92 (0.46)	22 (0.11)	264 (0.66)	136 (0.34)	114 (0.57)
Controls	163 (0.65)	72 (0.29)	15 (0.06)	398 (0.80)	102 (0.20)	87 (0.35)
p					4.00E−06	3.00E−06
NGF rs4839435	GG	GA	AA	G	A	A
SCZ	125 (0.63)	62 (0.31)	13 (0.06)	312 (0.78)	88 (0.22)	75 (0.38)
Controls	106 (0.42)	121 (0.48)	23 (0.1)	333 (0.67)	167 (0.33)	144 (0.58)
p					0.00016[a]	2.2E−05[b]
NGFR rs734194	TT	TG	GG	T	G	G
SCZ	139 (0.70)	53 (0.27)	8 (0.03)	331 (0.83)	69 (0.17)	61 (0.31)
Controls	136 (0.54)	92 (0.37)	22 (0.09)	364 (0.73)	136 (0.27)	114 (0.46)
p					0.0004[a]	0.0011[b]
NGFR rs11466155	CC	CT	TT	C	T	T
SCZ	79 (0.40)	88 (0.44)	33 (0.16)	246 (0.62)	154 (0.38)	121 (0.61)
Controls	137 (0.55)	94 (0.38)	19 (0.07)	368 (0.74)	132 (0.26)	113 (0.45)
p					0.0001[a]	0.0012[b]
NGFR rs2072446	CC	CT	TT	C	T	T
SCZ	68 (0.34)	106 (0.53)	26 (0.13)	242 (0.61)	158 (0.39)	132 (0.66)
Controls	132 (0.56)	91 (0.33)	27 (0.11)	355 (0.89)	145 (0.29)	118 (0.47)
p					0.0009[a]	6.7E−05[b]

[a] $p_{nominal}$ values for comparison of minor allele frequency between SCZ and controls.
[b] $p_{nominal}$ values for comparison of minor allele carriage between SCZ and controls.

[628.3, 640.0] pg/ml, $p < 0.0001$). Also, as was detected in the case of NGF, we found no difference in the median levels of NGFR between schizophrenia patients non-treated and treated with antipsychotics (non-treated patients vs. treated patients: 624.8 [615.5, 629.2] pg/ml vs. 624.2 [617.4, 631.2] pg/ml, $p = 0.79$).

3.3. Relationships between the selected SNPs' genotypes of the NGF and NGFR genes and the blood plasma levels of NGF and NGFR proteins

The relationships between the genotypes of the *NGF* gene rs6330 and rs4839435 SNPs and the NGF protein plasma levels as well as between the genotypes of the *NGFR* gene rs734194, rs11466155 and rs2072446 SNPs and the NGFR protein plasma levels in schizophrenia patients and controls were evaluated.

Relevant intergroup analysis revealed significantly higher NGF median plasma level in *NGF* rs6330 CC homozygotes than in rs6330*T minor allele carriers (CT + TT) both in patients (CC vs. CT + TT: 5.63 [4.18, 5.75] pg/ml vs. 4.17 [4.09, 4.24] pg/ml, $p < 0.0001$) and controls (CC vs. CT + TT: 7.05 [6.94, 7.28] pg/ml vs. 6.89 [5.66, 7.02] pg/ml, $p < 0.0001$). Concerning NGFR median plasma level of this protein was found significantly increased in rs2072446 CC homozygotes compared to rs2072446*T minor allele carriers (CT + TT) both in patients (CC vs. CT + TT: 628 [619, 634.3] pg/ml vs. 623.6 [614.6, 628] pg/ml, $p = 0.038$) and controls (CC vs. CT + TT: 639.4 [638.7, 656.2] pg/ml vs. 628 [621.7, 631.1] pg/ml, $p < 0.0001$). The results are presented in Figs. 1 and 2.

4. Discussion

The results of the present study demonstrated a positive association between schizophrenia and the rs6330 SNP of the *NGF* gene as well as the rs11466155 and rs2072446 SNPs of the *NGFR* gene. 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. In both, haloperidol-treated and antipsychotic-free patients with schizophrenia decreased blood plasma levels of the NGF and NGFR proteins were found, and a positive interrelation between carriage of the minor alleles of the rs6330 and rs2072446 SNPs and decreased plasma levels of the NGF and NGFR proteins, respectively, was revealed.

A non-synonymous rs6330 (104C>T) SNP of the *NGF* gene produces an alanine to valine substitution at amino acid position 35, and is thought to affect intracellular processing and secretion of the NGF protein [36], and our present study demonstrating relationship between the rs6330 genotypes and NGF plasma levels provides further evidence to support this suggestion. It has been also shown that the rs6330 SNP is associated with executive dysfunction in patients with Alzheimer's disease, anxiety-related traits and affective disorders [37]. However, study of association between schizophrenia and the rs6330 SNP of the NGF gene has been for the first time performed in the present study. The

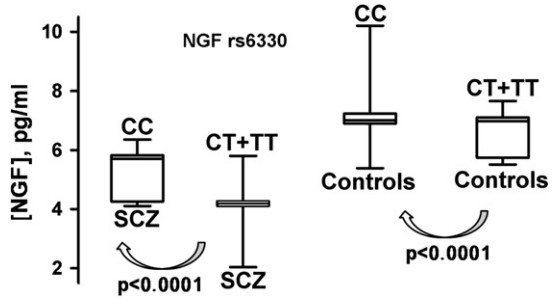

Fig. 1. The NGF protein plasma levels [median (interquartile range)] in patients with schizophrenia (SCZ, n = 120) and controls (n = 120). The data are expressed as whisker box plots; the box represents the 25th–75th percentiles, the median is indicated by a bar across the box, the whiskers on each box represent the minimum and maximum levels.

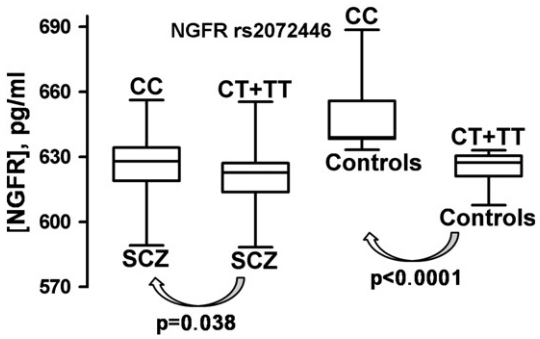

Fig. 2. The NGFR protein plasma levels [median (interquartile range)] in patients with schizophrenia (SCZ, n = 120) and controls (n = 120). The data are expressed as whisker box plots; the box represents the 25th–75th percentiles, the median is indicated by a bar across the box, the whiskers on each box represent the minimum and maximum levels.

same applies to the rs4839435 SNP of the *NGF* gene. It has to be mentioned that in schizophrenia another polymorphism of the *NGF* gene, the rs12760036 SNP was studied by Park et al. in Korean population. The results of their study suggested an association of the mentioned SNP with susceptibility to schizophrenia. Also, significant differences in the AG and CA haplotype frequencies within the linkage disequilibrium block between the rs12760036 and rs4839435 SNPs between schizophrenia patients and controls were found indicating the rs12760036*C minor allele as a risk factor for schizophrenia in Koreans [38].

Concerning blood levels of NGF, our results are in agreement with previous report of Xiong et al. that demonstrated lower blood levels of this protein among first-episode schizophrenia patients compared to healthy subjects [25,26]. Increased blood levels of NGF were found in several inflammatory and autoimmune states [39–41]. Moreover, in both animal and human studies a correlation between some psychopathological conditions (diabetes mellitus, allergic diseases and asthma) and blood levels of NGF was demonstrated [42–44].

It has been shown that both pre- and postnatal injections of NGF lead to production of massive transformation of chromaffin cells in sympathetic nerve cells of the rat adrenal medulla [45]. Moreover, Aloe and Levi-Montalcini reported that NGF treatment after birth results in only a partial replacement of chromaffin cells with nerve cells [45]. Study of Chen et al. in newborn mice revealed the ability of NGF to prevent destructive effects of vinblastine on sympathetic ganglia [46]. Further, experiments with daily treatment of newborn rats revealed that NGF induces increased volume and enhanced synthesis of tyrosine hydroxylase in chemically axotomized sympathetic ganglia [47]. Some earlier studies also showed that mouse submandibular glands synthesize and release into the saliva large quantities of NGF and that synthesis of this protein is controlled by testosterone and thyroxine [reviewed in 48]. Later, Levi-Montalcini and Aloe found that systemic murine NGF injections lead to growth and differentiation of sensory and sympathetic nerve cells as well as ~~on~~ several populations of cells in the central nervous system of *Xenopus laevis* tadpoles [49]. Using animal model of aggression Spillantini et al. detected increased NGF mRNA and protein production in the hypothalamus [50]. Aloe et al. found that intraspecific fighting causes release of NGF from salivary gland into the bloodstream in mice and that the amount of circulating NGF depends on the number of fighting episodes [51]. Furthermore, Alleva and Francia showed that intermale aggression in mice, representing a psychosocial stressful condition, markedly alters NGF levels both in plasma and selected brain areas, including the hypothalamus and hippocampus [42]. Also, it was shown that murine NGF can be used successfully for the treatment of such human diseases as corneal and pressure ulcers [52–54], vasculite [55], and crush syndrome [56]. Moreover, therapeutic efficiency of recombinant human NGF (rhNGF) in rodent and primate models of experimental allergic Alzheimer's disease [57,58] and encephalomyelitis [59] was demonstrated. Also, recently, an important role of NGF in embryo chicken development, namely, in the regulation of somite survival

and axial rotation was detected [60]. These observation together with our present data, suggested the potential pharmacological role of NGF as a useful therapeutic agent in many pathological conditions.

In the case of the NGFR, our study for the first time demonstrated association of the *NGFR* gene rs2072446, rs11466155 and rs734194 SNPs with schizophrenia as well as decreased plasma levels of this protein in schizophrenia-affected subjects. Notably, the rs11466155 synonymous SNP of the *NGFR* gene was not studied before in any diseased condition. The rs2072446 SNP of the *NGFR* gene leading to substitution of serine to leucine at 205 amino acid position of the NGFR protein polypeptide chain and the haplotype containing rs734194 SNP in the three prime untranslated region (3′-UTR) of this gene were recently found to be associated with an increased risk of Alzheimer's disease in Chinese [61].

5. Conclusions

In summary, our results demonstrate association of schizophrenia with the rs6330, rs4839435 and rs734194, rs11466155, rs2072446 functional SNPs of genes encoding NGF and NGFR, respectively, as well as with the decreased blood levels of these proteins. Our findings indicate the implication of alterations in genes encoding NGF and its receptor in pathogenesis of schizophrenia, particularly, in defects of synaptic plasticity detected in this disorder. Furthermore, the data obtained suggests that at least in Armenian population the rs6330*T and rs11466155*T, rs2072446*T alleles of the *NGF* and *NGFR* genes, respectively, might be nominated as risk factors for schizophrenia, whereas the *NGF* rs4839435*A and the *NGFR* rs734194*G alleles might be protective against developing schizophrenia.

Conflict of interest

The authors declare that they have no conflict of interests.

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