BBA Clinical 1 (2014) 24–29

Contents lists available at ScienceDirect

BBA Clinical

journal homepage: http://www.journals.elsevier.com/bba-clinical/

# Nerve growth factor and its receptor in schizophrenia

Roksana Zakharyan [a,*], Sofi Atshemyan [a], Anaida Gevorgyan [b,1], Anna Boyajyan [a]

[a] Institute of Molecular Biology, National Academy of Sciences of the Republic of Armenia (NAS RA), 7 Hasratyan St., 0014 Yerevan, Armenia
[b] Nork Clinic attached to the Psychiatric Medical Center of the Ministry of the Health of the Republic of Armenia, 2a Hovsepyan St., 0047 Yerevan, Armenia

ARTICLE INFO

*Article history:*
Received 1 February 2014
Received in revised form 3 May 2014
Accepted 8 May 2014
Available online 20 May 2014

*Keywords:*
Schizophrenia
Nerve growth factor
Nerve growth factor receptor
Genetic polymorphism
Blood levels
PCR-SSP

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

* Corresponding author. Tel.: +374 10281626; fax: +374 10281540.
*E-mail addresses:* r_zakharyan@mb.sci.am (R. Zakharyan), s_atshemyan@mb.sci.am (S. Atshemyan), anaida_gevorgyan@yahoo.com (A. Gevorgyan), aboyajyan@sci.am (A. Boyajyan).
[1] Tel.: +374 10650832.

http://dx.doi.org/10.1016/j.bbacli.2014.05.001

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′ |