High Level of α-Synuclein mRNA in Peripheral Lymphocytes of Patients with Alcohol Dependence Syndrome

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The content of mRNA for α-synuclein (a key protein of the dopaminergic system) was elevated in the peripheral lymphocytes of patients with alcohol dependence syndrome. Increased level of α-synuclein mRNA was not associated with changes in the expression of *NR4A2* gene encoding Nurrl, one of the main transcription factors of dopaminergic neurons.

Key Words: *alcohol dependence syndrome; gene expression; α-synuclein; α-synuclein gene; NR4A2 gene*

Chronic alcohol intake leads to restructuring of the neurotransmitter systems, primarily the dopaminergic system (DES), which is considered to be the key system involved in the formation of alcohol dependence syndrome (ADS) [9]. α-Synuclein (SNCA), one of the key DES proteins, attracted special attention after discovery of an association between the locus of chromosome 4 containing *SNCA* gene and high predisposition to alcohol dependence phenotype [2,3,11-13]. However, further studies revealed no association between genetic variants of *SNCA* and alcoholism [5,7]. On the other hand, an association of some *SNCA* haplotypes with the phenotype characterized by high craving for alcohol was revealed [7].

Increased level of *SNCA* mRNA and SNCA protein content in the peripheral lymphocytes was also associated with the phenotype linked with high degree of alcohol craving [2]. Similar increase in *SNCA* mRNA content was also observed during chronic alcoholization in rats and monkeys [4]. Hence, these data indicate that high expression of *SNCA* gene is characteristic of at least part of phenotypes linked with the development of ADS.

It therefore seemed to be extremely important to see whether increased concentration of *SNCA* mRNA in the brain and blood was a common phenomenon for all stages of ADS development and which DES genes are involved in the regulation of *SNCA* gene expression. In this context transcription factor Nurrl attracts special attention, because it could be involved in the regulation of *SNCA* gene transcription and maintenance of functional activity of dopamine-synthesizing neurons. This factor belongs to the class of orphan nuclear receptors (*NR4A2*) regulating activities of a wide spectrum of genes in dopaminergic neurons [10,14,15]. For example, the expression of *Nurrl* gene directly correlated with the content of tyrosine hydroxylase, the key protein of dopamine synthesis, in neurons and lymphocytes [4,10,14].

We evaluated the expression of *NR4A2* and *SNCA* genes in the peripheral lymphocytes of patients with ADS and healthy individuals.

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MATERIALS AND METHODS

We examined 26 men (mean age 46±17 years) with ADS (diagnosed on the basis of IDC-10 criteria) hospitalized at the Bechterew Center, Federal Agency for Health and Social Development. The participants belonged to the Slavic population, were permanent residents of St. Petersburg, and were not relatives. The control group consisted of 20 healthy men (mean age 48±20 years) of the same ethnic group, without history of mental diseases and drug and alcohol abuse.

Peripheral blood lymphocytes were chosen for measurements of mRNA because of high correlation between DES genes expression in the brain and peripheral lymphocytes [2,6]. Isolation of lymphocyte mRNA and reverse transcription reaction were carried out using Quiagen kits.

The levels of *NR4A2* and *SNCA* mRNA were measured by real-time PCR using ABI7000 system (PE Biosystems). The results were standardized by the content of GNB2L1 (guanine nucleotide binding protein) mRNA content. PCR for the studied and reference genes was carried out in 96-well plates in one well using fluorogenic labels (FAM and RG6): 15 sec at 94°C, 60 sec at 60°C; 45 cycles. Each sample was studied in 3 independent measurements. Table 1 presents the structure of primers and probes for the analysis of *NR4A2*, *SNCA*, and reference *GNB2L1* genes expression developed using Primer Express TM software (Applied Biosystems).

The data were statistically processed using one-way ANOVA (SPSS12 software).

RESULTS

SNCA is a negative regulator of dopaminergic neurotransmission. Alteration of its intracellular level can underlie many neurodegenerative diseases and addictions [12,13]. Many studies indicate the existence of certain phenotypes associated with predisposition to alcoholism characterized by individual differences in the level of *SNCA* gene expression [2].

On the other hand, the role of SNCA in modification of dopaminergic neurotransmission and development of ADS remains little studied. Presumably, along with *SNCA*, changes in the expression of other DES genes can lead to modification of dopamine metabolism and contribute to the development of ADS. Our study is the first attempt at evaluation of ADS liability phenotypes, which includes evaluation of the expression of not only *SNCA* gene, but of other DES proteins, for example, Nurrl (the main transcription factor of dopaminergic neurons.

The level of *SNCA* mRNA in ADS patients differed significantly from that in controls (1.20±0.53 *vs.* 0.8±0.4 in the control; p=0.006; Fig. 1, *a*). A previous study [2] showed an increase in *SNCA* mRNA content only for phenotype associated with high craving for alcohol (a common syndrome, but not obligatory for ADS development), while our experiments demonstrated an increase in the level of *SNCA* mRNA in ADS.

On the other hand, *NR4A2* mRNA level was virtually the same in patients with ADS and controls (3.2± 3.2 and 1.9±1.7, respectively; p=0.09; Fig. 1, *b*). Hence, our results indicate that the development of ADS is not associated with an increase in *NR4A2* mRNA level.

However, it is noteworthy that these changes in the proportion of *SNCA* and *NR4A2* expression can lead to changes in dopamine metabolism, because both proteins directly regulate activity and expression of tyrosine hydroxylase, the key enzyme in dopamine synthesis [4,10,13,14]. On the other hand, high individual variability of *NR4A2* mRNA level (the minimum value in the control group 0.4, the maximum 5.2 *vs.* 0.4 and 11.5 in the ADS group) can mask the relationship between *NR4A2* gene expression and ADS development. Interestingly, a significant association between *NR4A2* haplotypes and ADS was detected in the residents of Japan [8].

TABLE 1. Primers and Probes Used for Evaluation of DES Genes Expression

DES gene	Primers and probes	Sequence (5'-3')
GNB2L1	**For**	GAA TAC CCT GGG TGT GTG CAA
GNB2L1	**Proba**	**RG6**-TAC ACT GTC CCA GGA TGA GA -RTQ1
GNB2L1	**Rev**	GGA CAG AAG ACA CCC ACT TCG A
NR4A2	**For**	GGC TGT TGG GAT GGT CAA AG
NR4A2	**Proba**	**FAM**-TAA AAG GCC GGA GAG GTC GTT TGC C-RTQ1
NR4A2	**Rev**	TGG GCT CTT CGG TTT CGA
SNCA	**For**	GCC AAG GAG GGA GTT GTG GCT GC
SNCA	**Proba**	**FAM-**ACC AAA CAG GGT GTG GCA GAA GCA GCA-RTQ1
SNCA	**Rev**	TG TTG CCA CAC CAT GCA CCA CTC

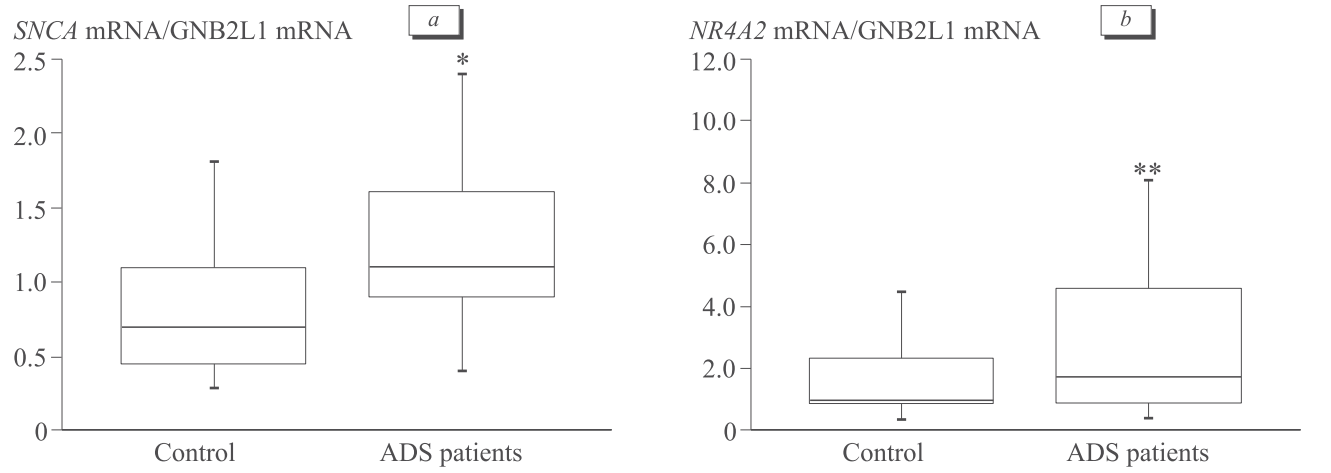

Fig. 1. Content of *SNCA* mRNA (*a*) and *NR4A2* mRNA (*b*) in the peripheral lymphocytes of patients with ADS and controls. **p*=0.006, ***p*=0.09 compared to the control.

Later we confirmed that homozygotic carriership of *NR4A2* gene C3C3 genotype contributes to the formation of ADS in European men, increasing the risk of alcoholism development by 1.8 times [1]. Hence, analysis of *NR4A2* gene expression is to be continued, similarly as analysis of other DES genes, primarily of dopamine receptor genes.

Thus, changes in *SNCA* and *NR4A2* genes expression can reflect the total neuroadaptation process at the cellular level and characterize the main phenotypes associated with liability to ADS. Characterization of these phenotypes seems to be one of the main tasks in studies of the genetic base of alcoholism, practically important for the development of new approaches to this disease prevention.

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