EXPERIMENTAL GENETICS

Association of 5-HTTLPR Serotonin Transporter Gene Polymorphism and Val66Met Brain-Derived Neurotrophic Factor Gene Polymorphism with Auditory N100 Evoked Potential Amplitude in Patients with Endogenous Psychoses

V. E. Golimbet, I. S. Lebedeva, G. I. Korovaitseva, T. V. Lezheiko, P. E. Yumatova

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We studied the relationship between genes of serotonin transporter and brain-derived neurotrophic factor and parameters of AEP N100 wave to a non-significant stimulus in patients with endogenous mental diseases. In patients with endogenous psychoses, a significant effect of BDNF Val66Met marker on N100 wave amplitude was revealed: the mean N100 amplitude was higher in carriers of Val/Val genotype compared to Val/Met genotype carriers. The effect of the 5-HTTLPR marker on the wave amplitude was less pronounced (tendency): the SS genotype was associated with higher N100 amplitude.

Key Words: *schizophreni; gene; serotonin transporter; neurotrophic factor; acoustic evoked potentials*

Changes in the parameters of auditory evoked potentials (AEP) in schizophrenia and affective disorders were reported in numerous studies. Specifically, lengthened latency and reduced amplitude of early and late waves were revealed in mentally diseased patients. The possibility of using these indices as markers of genetic predisposition to endogenous mental diseases, pathophysiological markers, and as indices of the severity of cognitive disorders is discussed [14]. At the same time, little is known about their molecular genetic correlates. An association of polymorphic markers of some genes [4,6,10] with parameters of late component P300 was revealed in schizophrenic patients and their first-degree relatives. However, no reports about genes associated with early waves of AEP appeared until present.

AEP wave to a non-significant stimulus with a peak latency of about 100 msec reflects processes of nonspecific activation of attention, analysis of information on physical characteristics of the sound, formation of memory trace with oscillators in auditory cortex, prefrontal cortex, hippocampus, anterior cingulate cortex. The serotoninergic system plays an important role in the modulation of the amount of external stimuli and one can anticipate that its activity affects this AEP wave. Expression of serotonin transporter gene, one of the best studied genes of this system, determines the rate of neurotransmitter reuptake and transfer to the presynaptic neuron, and finally the level of serotonin. BDNF is active in different types of neurons, *e.g.*

Research Center for Mental Health, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** golimbet@mail.ru. V. E. Golimbet

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it modulated the expression of serotonin transporter gene in lymphoblastoid cultures [11]. Association of this gene with morphological characteristics of the brain and cognitive function was reported.

Here we studied possible association between serotonin transporter and brain-derived neurotrophic factor (BDNF) genes and parameters of AEP N100 wave to a non-significant stimulus in patients with endogenous mental diseases.

METHODS

The study included 106 patients of clinical departments of Research Center of Mental Health, Russian Academy of Sciences: 48 (45.2%) men and 58 (54.8%) women, age 29.7±11.3 years, age at the disease onset 23.5±6.9 years. Schizophrenia (F20) according to diagnostic criteria ICD-10 was diagnosed in 71 patients, schizoaffective disorder (F25) in 18 patients, and affective disorders (F3) in 17 patients. The severity of clinical state was quantitatively assessed using international Positive and Negative Syndrome Scale (PANSS). All patients received psychotropic drugs at the time of investigation. Control group consisted of 75 patients: 31 (41.3%) men, 44 (58.7%) women, mean age 44.0± 12.6 years). It included 60 mentally healthy relatives and 15 individuals without familial history of mental diseases. All participants gave their informed consent for their blood to be drawn for DNA extraction with consequent genetic typing and for electrophysiological examination. Inclusion criteria for the latter were right-handedness, absence of organic CNS lesions, complaints on hearing impairment and alcohol abuse. AEP registration was conducted in the standard oddball paradigm (paradigm P300) described elsewhere [4]. N100 component was isolated as a dominant negative wave in the interval 80-160 msec in AEP to a non-significant stimulus. Its peak latency and amplitude were defined in leads F3, F4, F7, F8, T3, T4, C3, C_z, C4, P3, P_z and P4.

Molecular and genetic study involved venous blood sampling, DNA extraction, and genotyping using PCR on polymorphic marker 5-HTTLPR determined by different number of DNA sequences in the region adjacent to the promoter. This marker allows to distinguish two allelic variants of the gene, which are designated as long (L) and short (S) depending on the number of sequences. For the BDNF gene, Val66Met polymorphism presented by to allelic variants Val and Met was studied. Genotyping was performed using previously described techniques [3,12]. 5-HTTLPR genotypes were obtained for 104 patients and 74 healthy individuals and BDNF Val66Met for 101 and 71 individuals, respectively. 5-HTTLPR genotype distribution in the control group was as follows: LL 31 (41.9%), LS 32 (43.2%), and SS 11 (14.9%); this distribution in patients was as follows: LL 38 (36.5%), LS 50 (48.1%), and SS 16 (15.4%). For BDNF marker Val66Met genotype frequency in the control group was: Val/Val 56 (78.8%), Val/Met 15 (21.2%), Met/Met 0; in patients: Val/Val 69 (68.3%), Val/Met 32 (31.7%), Met/Met 0.

Statistical analysis was performed using generalized linear model of multivariate analysis of covariance. At the first stage, a composite index including wave N100 latencies and amplitudes in all leads was regarded as an dependent variable and the genotype for one of the markers was an independent variable. The effect of the group (patients, mentally healthy relatives of patients, mentally healthy individuals without familial cases of endogenous psychoses) was also taken into consideration. If significant effect of the genotype was revealed, a series of analyses was conducted using composite index including wave N100 latencies or amplitudes separately in frontal (F), central (C), temporal (T), and parietal (P) leads. In case of association, a posteriori analysis was conducted to define the relationship between the specified genotype and wave characteristics. At all stages, sex, age, and severity of clinical symptoms were introduced into the model as covariants.

RESULTS

Significant effect of the group was revealed for N100 wave amplitude ($F=1.6$, $df=12$, $p=0.03$), while no differences between the group of mentally healthy individuals and group of patients' relatives were observed. No association between polymorphic markers 5-HTTLPR and BDNF Val66Met and wave amplitude was found ($F=1.1$, $p=0.4$) in the pooled group of relatives and mentally insane people. A significant effect of BDNF marker Val66Met on N100 wave amplitude was found in patients with endogenous psychoses ($F=1.8$, $p=0.02$). The effect of the 5-HTTLPR marker on the wave amplitude was revealed on a trend level ($F=1.5$, $df=24$, $p=0.07$). There was no association between these markers and latent period ($F=1.0$, $p=0.43$, and $F=1.1$, $p=0.37$ respectively). In further analysis we determined the localization of brain area, where the association between polymorphic marker and amplitude occurred. BDNF Val66Met marker was significantly associated ($F=4.9$, $p=0.001$) only with the composite index including amplitudes in the frontal area (leads F3, F4, F7, F8). Analysis of the data for each lead revealed an association between BDNF Val66Met

marker and amplitude in lead F7 (p=0.04). In lead T3 (temporal zone), a trend for association was observed (p=0.07). The mean amplitude was higher in carriers of Val/Val genotype (3.4±1.7 μV in F7 and 3.6±1.8 μV in T3) than in Val/Met carriers (2.6±1.6 and 2.8±1.6 μV, respectively) in both specified leads. For marker 5-HTTLPR, no association with the specified area of electrode position was observed. Analysis performed for each lead demonstrated that the mean amplitude in genotype SS carriers was higher than in allele L carriers (Fig. 1). However, only a trend to difference was observed in leads F4 (p=0.10), F8 (p=0.07), T3 (p=0.13), T4 (p=0.15). No additive effect of BDNF gene and serotonin transporter gene on wave N100 amplitude was observed (F=0.6, p=0.80).

We found no published reports about genes associated with early AEP waves, *i.e.* genes analyzed by us are the first genes for which the association with wave N100 parameters was demonstrated. The observed changes in electrophysiological activity of the brain depending on the BDNF genotype can be analyzed in light of association of this polymorphic marker with neuromorphological and neuropsychological indices. Thus, decreased volumes of the dorsolateral prefrontal cortex and the hippocampus and reduced amount of the gray matter with simultaneous dilatation of the lateral ventricles were found in carriers of Met allele compared to carriers of Val/Val genotype [7,13]. These differences can be explained by the fact that Val66Met polymorphism regulates BDNF secretion

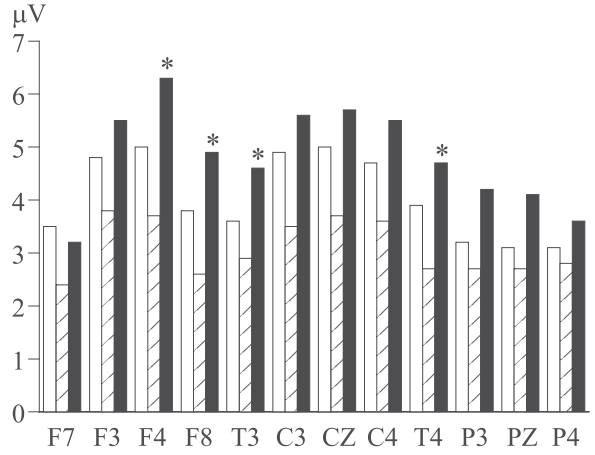

Fig. 1. Mean amplitude of AEP N100 wave to a non-significant stimulus in patients with endogenous mental diseases depending on serotonin transporter genotype (5-HTTLPR). Light bars: LL, hatched bars: LS, dark bars: SS. Abscissa: electrode application points (frontal leads: F3, F4, F7, F8; temporal leads: T3, T4; central leads: C3, CZ, C4, parietal leads: P3, PZ, P4); ordinate: N100 amplitude. *Tendency for significance of differences between the amplitudes for genotypes LL and SS (lead F4: p=0.10, F8: p=0.07, T3: p=0.13, T4: p=0.15).

in neurons via modulation of intracellular transport and packing of pro-BDNF [2]; it should be noted that they are observed only in patients with endogenous psychosis. In neuropsychological studies, schizophrenic patients carrying at least one copy of the Met allele in the genome demonstrated worse episodic memory [8] and selective visual attention results [1]. The same regularities were found in patients with bipolar affective disorder [12]. It is important to note that in patients with schizophrenia, the decrease of AEP N100 wave amplitude to a non-significant stimulus was one of the most pronounced neurophysiologic abnormalities correlating with attention impairment. Therefore, the hypothesis that genetic variant of BDNF is linked with neurophysiologic processes of nonspecific activation of attention and primary memory trace formation is quite reasonable.

The tendency to amplitude increase in carriers of SS genotype seems to be most interesting in the context of accumulating data on more pronounced activation of brain structures involved in processing of emotionally significant stimuli in the presence of allele S [5]. These data were obtained using neurovisualization methods, while electrophysiological investigation showed that during performance of visual attention cognitive task the amplitude of negative wave Ne/ERN generated upon feedback on the number of errors was higher in carriers of SS genotype than in carriers of other genotypes [3]. Higher values of N100/P200 index denoted as loudness dependence (which according to some researchers reflects the degree of serotoninergic system dysfunction in schizophrenic patients) were detected in the presence of SS genotype [9]. The observed differences are attributed to changes in gene function depending on allele variant, in particular in case of allele L gene expression is higher than in the presence of the short variant S.

Thus, our results show the perspective for further investigation of association between serotonin transporter and BDNF genes and indices of early AEP waves in patients with endogenous psychosis.

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