GENETICS

Effect of *BDNF Val66Met* Polymorphism on Normal Variability of Executive Functions

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Associations of *BDNF Val66Met* polymorphism with such components of executive functions as verbal fluency, working memory, attention set shifting, and response inhibition were evaluated. A total of 401 healthy volunteers were genotyped. The effect of polymorphism on working memory during the counting test was detected. The test performance in heterozygotic carriers was much worse than in homozygotic ones. Individuals with the *MetMet* genotype demonstrated the best results, presumably due to molecular mechanisms compensating for the neuropeptide secretion deficiency.

Key Words: *BDNF gene; working memory; verbal fluency; attention set shifting; Stroop effect*

The executive functions are a complex of processes including initiation and planning of behavior, inhibition of irrelevant reactions, and other aspects of information processing regulation; they are mediated by the prefrontal cortex with its cortical and subcortical relations. Executive function abnormalities are regarded as the key cognitive pathology in some mental diseases, *e.g.* schizophrenia, autism, and the attention deficiency hyperactivity syndrome [8], and it is therefore essential to detect their molecular mechanisms.

Studies of the executive functions were initially focused on genes of the monoaminergic systems modulating the status of the prefrontal cortex [8]. Recent studies of the cognitive (including the executive) functions have been focused on the gene encoding brain-derived neurotrophic factor (BDNF) located at 11p14.1; this factor plays an important role in the neurodevelopment and adult brain plasticity. A single nucleotide polymorphism (*rs6265* or *Val66Met*) leading to valine sustitution for methionine in codone 66 of BDNF precursor protein has been detected in the gene. *Met* allele is essential for neuropeptide transport inside the cell; that is why depolarization-dependent secretion of BDNF decreases in the presence of *Met* allele [4]. Disorders in the structure and patterns of the hippocampus activation and of memory traces consolidation depending on this brain structure have been found in the *Met* allele carriers [4]. The data on the BDNF relationships with various components of the executive functions are contradictory. On the one hand, the presence of *Met* allele is associated with changes in prefrontal cortex activation during performance of tasks addressed to the executive functions [2,15]. The influence of *Val66Met* polymorphism on some aspects of executive functions in mental patients and normal subjects has been noted [3,7,13], including that in combination with other marker genotypes [1,11]. On the other hand, negative results have been obtained for many components of the executive functions [7,10,15].

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We continued studies of *BDNF Val66Met* polymorphism relationships with variations in the executive functions in the common population.

MATERIALS AND METHODS

A total of 401 subjects were genotyped by the *BDNF Val66Met* marker. The sample included subjects without mental and neurological diseases at the age of 16-65 years, Russians, residents of the Moscow region (30.4±10.6 years), 60% women and 60% individuals with higher education or students. All volunteers gave informed consent to participation in the study. Psychological data including at least partial information about certain aspects of executive functions have been obtained for each volunteer. The following aspects of executive functions were evaluated: semantic verbal fluency, working memory, attention set shifting, and irrelevant reactions inhibition. The verbal fluency test consisted in generation of words belonging to the animal and fruit categories. The index was the number of correctly found words converted to T-score with consideration for the education level. The working memory was evaluated by continuous subtraction of 2 and 5 from 200. The index was the number of correctly performed operations per min. The attention set shifting was evaluated by the Trail Making Test, Part B (TMT-B). The task was to connect the figures and letters (1-A-2-B...) written on a sheet of paper as rapidly as possible. The irrelevant reactions inhibition was evaluated by Stroop test in which the volunteer was to: 1) read the names of colors; 2) say the color of the ink in which the letters X were printed; 3) say the color of the ink in which the names of colors were printed (the colors and sense of words did not coincide). Each series was performed during 40 sec. The interference coefficient or Stroop effect served as the index. It reflected the efficiency of problem solving inhibition under conditions of the color—word meaning conflict and was calculated by the formula: $SE=(1-2n_3/(n_1+n_2)\times 100\%$, where n_1, n_2, and n_3 were numbers of correctly read words/named colors in the corresponding series.

DNA was isolated from venous blood by the phenol-chloroform method. Genotyping was carried out using PCR by a previously described method [12]. After one "ejection" was discarded from the TMT-B results, all signs fit the normal distribution according to the Kolmogorov–Smirnov test. Their associations with the polymorphism were evaluated by one-way ANOVA using Statistica 6.0 software.

RESULTS

The incidence of *Val* allele in the studied cohort was 0.81, that of genotypes *ValVal*, *ValMet*, and *MetMet* was 0.64, 0.33, and 0.03, respectively. This did not differ from the expected Hardy–Weinberg distribution ($\chi^2=0.10$, $p>0.1$) and corresponded to the incidence in europeoid populations [4,10,11].

Comparison of men and women by Student's test showed no differences between them by the cognitive signs. The TMT-B and Stroop test correlated with age ($r=0.31$, $p=0.001$ and $r=0.35$, $p=0.001$). For subsequent ANOVA, the age was introduced as a covariate for these variables. The education level was essential for the working memory, TMT-B, and Stroop test values ($t=4.62$, $p<0.00$; $t=3.58$, $p<0.00$; $t=2.27$, $p=0.03$, respectively). Additional analysis with the genotype, education, and relationship between these signs as independent factors was carried out for these signs. Carriers of three genotypes were compared by verbal fluency and working memory. For evaluation of the relationship between the genetic marker and parameters of TMT-B and Stroop test the heterozygotic and homozygotic carriers of *Met* allele were united into one group because of little number of *MetMet* genotype carriers in sub-samples in which these tests were carried out (TMT-B and Stroop test were carried out in 4 and 2 individuals of 122 and 83 examinees, respectively). Groups with different genotypes were similar by demographic characteristics.

Significant differences in the relationship with the genotype were found only for working memory (Table 1). They did not disappear in the analysis with consideration for education: $F=2.95$, $p=0.05$. The genotype explained 1.5% dispersion of the cognitive index. The heterozygotic carriers performed the task significantly worse than the homozygotic ones. Individuals with the *MetMet* genotype demonstrated the best results. A trend to superiority of *ValVal* homozygotes ($F=2.66$, $p=0.10$) was seen in the group of hetero- and homozygotic carriers of *Met* allele. It was more manifest among the subjects with higher education (post-hoc LSD: $p=0.05$).

Our results were in line with other data indicating that *BDNF Val66Met* polymorphism was inessential for such processes of executive functions as word generation, attention set shifting, and irrelevant reactions inhibition [7,10,15]. The available data on working memory are somewhat contradictory. Some authors noted the impact of *BDNF* polymorphism for the working memory [3,7,11,13], while others, who carried out studies on hundreds of normal subjects, failed to detect it [9,15]. However, the effect of *BDNF* on brain stimulation during the working memory tests was recorded in cases when carriers of different genotypes did not differ by the performance efficiency in these tasks [2,4]. Our results showed worse working memory of heterozygotic carriers of *Met* allele in comparison with *ValVal* homozygotes, which was in line with the majority of other reports. On the other

TABLE 1. Cognitive Sign Values in Carriers of Different Genotypes of *BDNF Val66Met* Gene Marker

Sign	Genotype			*F*	*p*
	ValVal	ValMet	MetMet		
Verbal fluency (T score)	50.98±10.80 (*N*=246)	49.22±10.84 (*N*=125)	51.50±6.93 (*N*=12)	1.17	0.31
Working memory* (number of operations/min)	17.82±6.83 (*N*=193)	16.05±6.32 (*N*=95)	20.27±5.55 (*N*=11)	3.33	0.04
	ValVal	ValMet+MetMet			
TMT-B (time of performance, sec)	106±42 (*N*=79)	96±34 (*N*=42)		1.66	0.20
Stroop effect, %	43±12 (*N*=53)	42±16 (*N*=30)		0.01	0.93

Note. *Post-hoc LSD test for paired comparison revealed significant differences in working memory of heterozygotes in comparison with *ValVal* ($p<0.05$) and *MetMet* ($p<0.04$) carriers.

hand, we revealed superiority of *MetMet* homozygotes in this test. The data on the *MetMet* monozygotes are scanty because of low incidence of *Met* allele. Some authors noted their lagging behind in the mnestic tests in comparison with carriers of other genotypes [4,15]. On the other hand, other authors noted a certain cognitive superiority of *Met* allele carriers, especially of *MetMet* homozygotes [5,6,10]. Carriers of *Met* allele suffering from parkinsonism exhibited higher values for such an aspect of executive functions as planning [6]. *MetMet* homozygotes in the elderly cohorts were superior in nonverbal intellect [10]. K. I. Erickson *et al.* [5] noted the protective effect of *Met* allele on age-associated deceleration of prefrontal task performance in elderly people. Disagreement of the results concerning the cognitive effects of *Met* allele can be due to not only age, but also significant effects of gene-gene and allele-environment interactions on the extent and type of these effects. The inhibition of responses in solution of the prefrontal problems was the greatest for the *ValVal COMT+Met BDNF* diplotype [11]. According to some data [14], the impact of *BDNF Val66Met* polymorphism for memory is modulated by the severity of mental injury inflicted by sexual violence act in childhood. The advantage of the *MetMet* homozygotes can be also due to the compensatory mechanisms. Presumably, after a certain threshold of BDNF precursor protein shortage is attained, the number of extracellular molecules responsible for its transformation into mature neuropeptide forms [5] or the number of BDNF binding receptors increases.

The specificity of associations of *BDNF Val66Met* polymorphism with cognitive functions is an important problem. Some authors think that its effects are confined to long-term episodic memory and are mediated by changes in the molecular processes underlying long-term potentiation in the hippocampus [4,15]. However, the data on *BDNF* relationship with other information processing processes, for example, with the executive function complex, contradict this opinion. A sort of specificity is observed within this cognitive domain: the *BDNF Val66Met* polymorphism is essential for some aspects of working memory and attention and does not influence the generation and interference processes. The results suggest that *Met* allele is more likely essential for the velocity of prefrontal tasks performance than for the executive function mechanisms [1,5,11,15]. Our results are in line with the hypothesis on the specificity of *BDNF Val66Met* effect on the working memory, but do not show whether this effect is due to the effects exclusively on the task performance velocity or on the working memory contents updating processes, the requirements to which are so great in the test used in our study.

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