GENETICS

Effects of Genetic Variations in the Dopaminergic System on Fatigue in Humans: Gender Aspects

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Changes in the functional status under the effect of intense mental exercise were studied in carriers of different variants of *DAT1, DRD2*, and *COMT* genes. The volunteers (n=140) performed 3-h monotonous mental work (information processing and logical problem solving). The degree of fatigue was evaluated before and after exercise by the HAM (Health status–Activity–Moods) and AMF (Acute Mental Fatigue) questionnaires. A significant relationship between the *DAT1, DRD2*, and *COMT* gene polymorphism and changes in the mental sphere status were revealed. The effects of these polymorphisms were the most pronounced in girls. The results are discussed within the framework of hypothesis on the effects of changes in the phasic/tonic dopamine proportion on the studied functions.

Key Words: *dopamine transporter; dopamine-2 receptor; catechol-o-methyltransferase; mental fatigue; phasic/tonic dopamine*

The knowledge of the neurotransmitter mechanisms regulating functional status will make it possible to regulate and reduce the development of negative conditions, such as fatigue, strain, various forms of mental stress, *etc*. Polymorphisms of the key genes of the dopaminergic system served as the genetic markers in our study: dopamine-1 transporter (*DAT1*, *SLC6A3*), dopamine-2 receptor (*DRD2*), and catechol-o-methyltransferase (*COMT)* [6,10,12]. Changes in the parameters of functional status under the effect of long mental exercise were studied in carriers of *DAT1, DRD2*, and *COMT* gene variants.

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MATERIALS AND METHODS

The study was carried out in 140 students (70 boys and 70 girls, mean age 20±1 year). All volunteers gave informed consent to the use of their DNA and results of psychological tests for the study. All genetic and psychological studies were approved by the Ethic Committee of Institute of Physical Culture. All experiments started at 14.00. During 3 h the volunteers were exposed to monotonous information loading: high-volume psychological tests on a computer, some of these involving the solution of logical problems. Fatigue was evaluated using HAM (Health status–Activity–Moods) and AMF (Acute Mental Fatigue) questionnaires [1,3,4]. Venous blood specimens served as the material for genetic studied. The *DAT1, DRD2*, and *COMT* gene polymorphisms were evaluated by the PCR by identifying the differences in the length

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TABLE 1. PCR Primers and Programs for Detection of *DRD2*, *DAT1,* and *COMT* Polymorphisms

Gene	Primers	Program	Detection	Reference
DAT1	5'-CCGTCGACCCTTCCT GAGTGTCATCA-3' 5'-CCGTCGACGGCTG-GCCAAGTTGTCTA-3'	1. 94°C, 4 min (1 cycle) 2. 94°C, 30 sec, 58°C, 30 sec, 72°C, 30 sec (total 35 cycles) 3. 72°C, 5 min (1 cycle) 4. Storage: 10°C	TaqI restrictase (5 U) was added to 7.5 μl sample and restriction of the amplified products was carried out for 22 h at 65°C in a dry air thermostat. Mutant A1 allele did not cleave, while wild type A2 allele yields 130 and 180 b. p. products detected by electrophoresis in 3% agarose gel	[12]
DRD2	5'-GGT GTA GGG AAC GGC CTG AGA G-3' 5'-CTT CCT GGA GGT CAC GGC TCA AGG-3'	1. 95°C, 5 min (1 cycle) 2. 94°C, 30 sec, 62°C, 30 sec, 72°C, 30 sec (total 35 cycles) 3. 72°C, 5 min (1 cycle) 4. Storage: 10°C	After PCR, the products were detected by electrophoresis in 3% agarose gel	[6]
COMT	5'-TCG TGG ACG CCG TGA TTC AGG-3' 5'-AGG TCT GAC AAC GGG TCA GGC-3'	1. 95°C, 5 min (1 cycle) 2. 94°C, 30 sec, 55°C, 30 sec, 72°C, 1 min (total 35 cycles) 3. 72°C, 10 min (1 cycle) 4. Storage: 10°C	NlaIII restrictase (1 U) was added to 7.5 μl each sample and restriction was carried out overnight at 37°C. Cleavage of wild type allele containing Val yields 136 and 81 b. p. products and cleavage of mutant allele containing Met yielded 96, 81, and 40 b. p. products. The products were detected by electrophoresis in 10% polyacrylamide gel	[10]

of PCR products for each allele (Table 1). The relationships between the genotype and human functional status and the psychological questionnaire score were evaluated by analysis of dispersions, in which the genotype was a categorial independent factor consisting of 3 levels. Multiple paired comparison (Newman–Keuls' test, Fisher's test) for detecting the factor underlying the detected differences was carried out *a posteriori*. The two samplings differing by the *DAT1, DRD2*, and *COMT* genotypes were also compared using Student's *t* test for independent samplings.

RESULTS

One hundred and forty humans were genotyped by the *DAT1, DRD2*, and *COMT* polymorphisms. Based on published data on the similarity of phenotypical manifestations, all carriers of *DAT1* allele with 9 repeats (9/9 and 9/10) were united in one group (9+) and compared to carriers of 10/10 (9-) homozygotes; the carriers of *DRD2* genotypes A1A1 and A1A2 were united in the group of A1+ carriers and compared to the A2A2 (A1-) group; and the carriers of *COMT* VV and VM were united in the V (V+) group and compared to MM (V-) carriers [7-9]. The distribution of all variants is presented in Table 2.

Differential self-estimation fatigue test proposed by V. A. Doskin is based on preliminary distinguishing of the main components of the functional status: health status, activity, and moods (HAM) [1]. On the whole, it is noteworthy that *DAT1, DRD2*, and *COMT* polymorphisms were essential for HAM in girls (Fig. 1, *a-c*). A higher score corresponded to better health status. The most pronounced differences were observed in girls: worse health status, activity, and moods before and after exercise were observed in A1+ carriers in comparison with A1- ones and in (9+) in comparison with (9-) (Fig. 1). The A1+ and 9+ variants are as-

sociated with high dopamine content in the striatum. In boys, this relationship was less manifest; a slight inverted effect for the impact of the *DRD2* polymorphism for HAM was observed: A1- carriers had worse health status before and after mental exercise. Analysis of combinations of polymorphisms for several molecular genetic markers provides more details about the mechanisms of the effect of the dopaminergic system on the functional status. Analysis of combinations of the gene variants showed that female carriers of 9+ variant differently responded to mental exercise, depending on the combination with the *COMT* variant: carriers of low active V- (MM) form in combination with (9+) felt the greatest fatigue after exercise, while girls with the combination with active COMT form were most resistant to it (Fig. 2, *c*). This splitting of the sign was also detected in female (9+) carriers with different *DRD2* forms: female carriers of 9+A1+ combination had lower initial health status, which did not change after exercise, while 9+A1- carriers exhibited a significant reduction of the health status after exercise (Fig. 2, *a*). In this case, the relationship between the fatigue degree and dopamine level was described by an U-shaped curve: 9+A1- and 9-A1+ carriers were similar by the initial and resultant levels of health status.

The similarity of these variants consisted in optimal proportion of dopamine transporter/dopamine-2 receptor: 9+A1- variant corresponded to high dopamine content in the striatum (determined by low content of DAT1) compensated for by sufficient level of *DRD2*, while 9-A1+ variant was characterized by intense capture of dopamine and low reception of *DRD2*. Female carriers of V-, who had a generally worse health status and activity (Fig. 1, *c*), demonstrated different decrease in activity and health status depending on the combinations with *DAT* forms. The differences became more pronounced in combinations with the low-active form of the transporter, and female carriers of V-9+ variant exhibited the highest sensitivity to mental exercise and the greatest deterioration of the health status (Fig. 2, *c*). By contrast, the health status and activity of V-9- carriers approached the levels in V+ carriers. The DRD2 effect on COMT was virtually

TABLE 2. Description of the Sample, Numbers and Incidence of *DAT1, DRD2*, and *COMT* Genotypes

Genotype	Girls						Boys					
	DAT 9 (*N*=24)			DAT 10/10 (*N*=46)			DAT 9 (*N*=33)			DAT 10/10 (*N*=36)		
	N	actual	expected	N	actual	expected	N	actual	expected	N	actual	expected
DRD2 A2A2	14	10.07	10.93	30	21.58	20.50	18	12.95	13.37	20	14.39	14.16
DRD2 A1	10	7.19	6.21	16	10.79	11.65	16	11.51	11.26	16	11.51	11.92
COMT V	18	12.95	12.92	34	24.46	24.76	25	17.99	18.10	28	20.14	19.75
COMT MM	6	4.32	4.47	12	8.63	8.57	9	5.76	5.47	8	5.76	5.96
	Girls						Boys					
	COMT M- (*N*=50)			COMT M+ (*N*=18)			COMT M- (*N*=54)			COMT M+ (*N*=16)		
	N	actual	expected	N	actual	expected	N	actual	expected	N	actual	expected
	50			18			54			16		
DRD2 A2A2	30	21.17	22.57	13	9.42	8.12	32	23.18	22.57	6	43.47	63.852
DRD2 A1	20	14.49	13.12	5	3.62	4.72	22	15.94	18.14	10	63.85	53.77
	Girls						Boys					
	DRD2 A2A2 (*N*=43)			DRD2 A1 (*N*=25)			DRD2 A2A2 (*N*=38)			DRD2 A1 (*N*=32)		

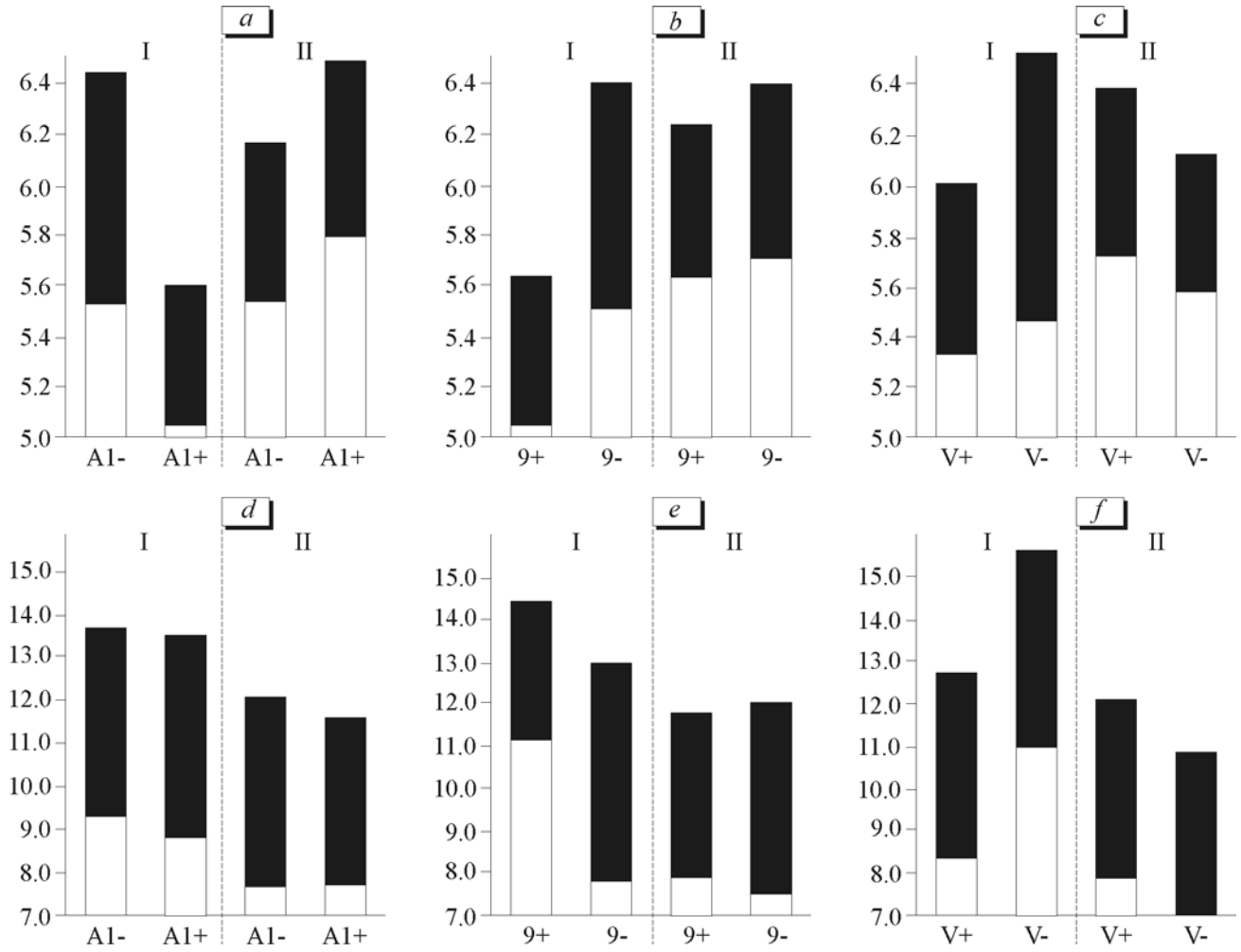

Fig. 1. Associations of *DRD2*, *DAT1*, and *COMT* polymorphisms with changes in the health status (*a-c*, respectively) and degree of mental fatigue (*d-f*, respectively). I – girls, II – boys. For *a-c*: ordinate: health status according to HAM score. Lesser values correspond to worse health status. Dark portions of bars: changes in the health status score after mental exercise. For *d-f*: ordinate: mental fatigue index. Higher values correspond to more intense mental fatigue. Dark portions of bars: changes in AMF after mental exercise.

undetectable (Fig. 2, *e*).

Changes in the functional status detected as sensation of mental fatigue are presented (Fig. 1, *d-f*). This method is intended for evaluation of the degree of mental fatigue developing over the working day in subjects whose work involves processing of great information flows [3,4]. The method contains assumptions characterizing various manifestations of mental fatigue by signs of reduction of common working capacity. The comprehensive index, mental fatigue index (MFI), is calculated from different scores. A higher value corresponds to greater fatigue. MFI in girls largely depended on the variations in the *DAT* and *COMT* genes (Fig. 1, *d-f*). The fatigue sensation in female carriers of low active form of the transporter (9+) was high before and after mental exercise (p=0.015). A similar situation was observed in women with *COMT* variants: high level of fatigue before and after exercise in V- carriers (p=0.010). Hence, the sensation of fatigue is higher in women with dopamine excess in the striatum and prefrontal cortex. This is in agreement with the HAM score. The most demonstrative differences in self-evaluation of fatigue were observed in carriers of combinations of certain genetic variations (Fig. 3). The (9+) female carriers formed two groups by the phenotypical manifestations: those with a combination with V+ are resistant to exercise, while girls with combinations with V- have high initial and resultant MFI values (Fig. 3, *c*). Carriers of (9+) variant also differed depending on the combinations with *DRD2*: carriers of 9+A1- exhibited signs of fatigue even before exercise, while after it the MFI was the maximum for the entire sample of girls (Fig. 3, *a*). Volunteers with lower reception of *DRD2* (9+A1- variant) were resistant to mental strain. Comparing these results with HAM data, we should pay special attention to the U-shaped curves of *DAT1* and *DRD2* impact after exercise. This can be explained by changes in the

distance of dopamine diffusion and efficiency of its reception [2]. Other important factors are presumably the proportion of free dopamine concentration to the receptor number: at the optimal proportion, when all receptors are occupied by dopamine (9+A1- and 09-A1+ variants), fatigue develops, while in the presence of excess of one component of *DRD2* (9-A1- variant) or dopamine (9+A1+ variant) the fatigue sensation is reduced. One more important fact is the MFI relationship with *DAT1* and *DRD2* polymorphisms in men. Carriers of (9+A1-) felt more tired after work than (9+A1+) carriers, which were better resistant to it (Fig. 3, *b*). An opposite situation was observed for (9-) carriers: those with its combination with A1+ felt more tired after mental exercise than those carrying (9+A1-). The *DAT1* and *DRD2* gene combinations were essential for the results of self-evaluation of the functional status in girls and boys. Moreover, the relationship between fatigue sensation after mental exercise and dopamine level was U-shaped: the 9+A1- and 9-A1+ levels were associated with high similar levels, while 9-A1- and 9+A1+ phenotypes were associated with similar low levels. Presumably, these differences are explained by the phasic/tonic dopamine proportion and

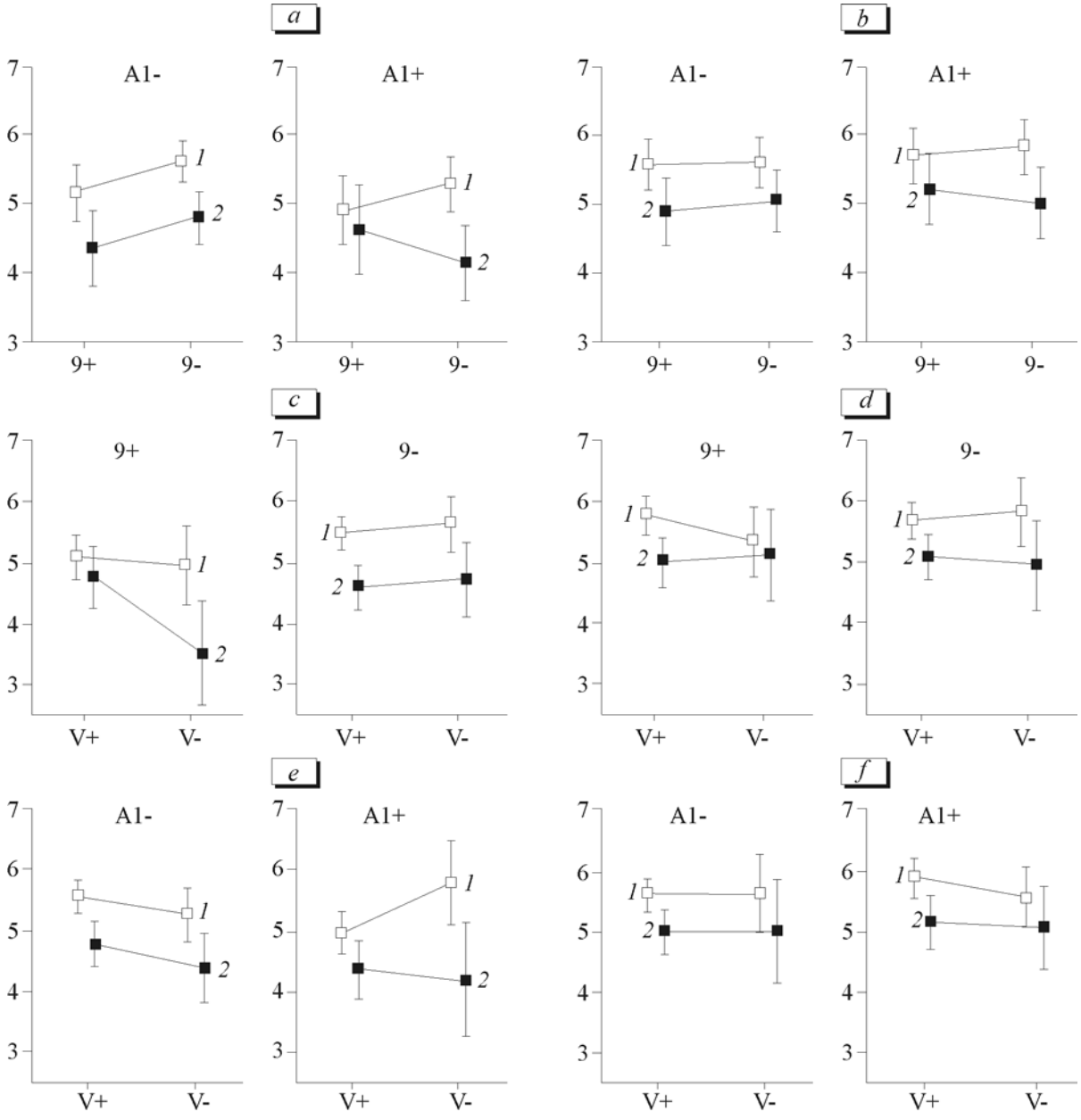

Fig. 2. Analysis of the impact of paired combinations of genes for changes in health status of volunteers. Here and in Fig. 3: *1*) results before mental exercise; *2*) results after exercise. *a*, *c*, *e*: girls; *b*, *d*, *f*: boys. *a*, *b*) combination of *DAT1* and *DRD2* polymorphisms; *c*, *d*) *COMT* and *DAT1*; *e*, *f*) *COMT* and DRD2.

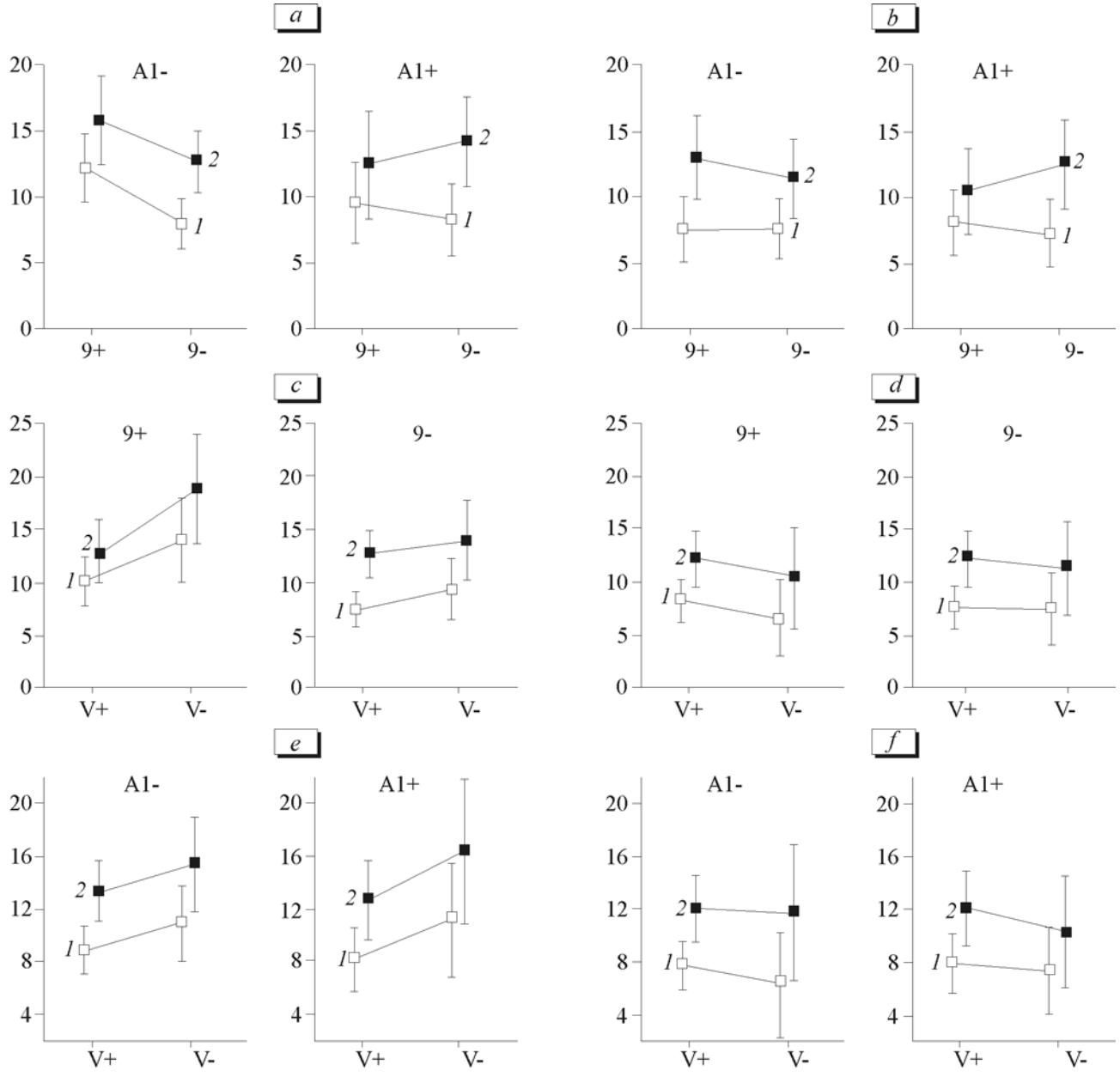

Fig. 3. Analysis of the impact of paired gene combinations for the degree of mental fatigue.

reception level. The tonic/phasic dopamine proportion is essential for plasticity of CNS functioning, excitation degree, and maintenance of working memory and attention [13]. Polymorphisms linked with elevation of phasic dopamine (in our case the V+ and (9-) variant) lead to reduction of tonic dopamine content and hence, reduce the stability of nervous network and facilitate the mobility of behavioral programs. By contrast, reduction of phasic dopamine level increases the tonic dopamine transmission (V- and (9+) variants), which leads to improvement of nervous network stability and improvement of the working memory. However, this impedes the behavioral program switch-over and renewal. In addition, our study revealed a significant gender-associated relationship between dopaminergic system gene variant carriership and mental fatigue resistance.

The effects are more pronounced in girls. Previous studies have detected the impact of sex hormones for the dopaminergic system functioning [5,11]. It was shown that dopamine content in the striatum is elevated during the first (estrogen) phase of the menstrual cycle. Estrogen receptors have been detected in various brain compartments, including the dopamine-rich caudate nuclei and the shell. Hence, there are good grounds to suggest that sex hormones modulate the

effects of genetic changes in the dopaminergic system on the development of mental fatigue.

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