GENETICS

Genetic Markers of Predisposition to Increased Anxiety

M. A. Kulikova, E. V. Trushkin, M. A. Timofeeva, V. A. Shlepzova, J. V. Schegolkova, N. V. Maluchenko, and A. G. Tonevitsky

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 146, No. 12, pp. 674-678, December, 2008
Original article submitted July 31, 2008

Associations of functional polymorphism in genes of dopamine receptor DRD2, dopamine transporter DAT, and dopamine degrading enzyme COMT with variations in anxiety sensitivity threshold were studied. On the basis of genetic and psychological analysis, an attempt was undertaken to evaluate the relationship of DRD2, DAT and COMT genotypes with values obtained using different anxiety scales. It was found that carriers of VA1+9+ genotype exhibit increased anxiety and significantly differed from individuals with other genotypes. The results were illustrated with model of dopamine diffusion in the extracellular space of the striatum in carriers of different genotypes.

Key Words: *anxiety; dopamine; striatum; dopamine receptor DRD2; dopamine transporter DAT*

Individual differences in human temperament and character have a complex structure based on both psychosocial and biological factors. During recent decade, numerous reports were published where associations between human personality and genetic variations in the neurotransmitter systems responsible for nervous impulse propagation were demonstrated. Anxiety is one of the most studied psychological traits, because it is associated with numerous psychiatric disorders and is to a large degree genetically determined. Twin studies showed that anxiety inheritance reaches 0.45; which attests to great contribution of both environmental and genetic factors [13]. Genetic mechanisms can act through the increase in the sensitivity to enhanced anxiety state under conditions of long-term stress or other environmental factors.

In positron emission tomography (PET) studies, significant specific interaction between central dopaminergic activity markers and anxiety was demonstrated [10].

Low rate of dopamine re-uptake in the striatum is associated with increased anxiety and irritability [7]. It can be hypothesized that high anxiety in humans is associated with increased dopamine content in the extracellular space of the striatum. The rate of dopamine circulation in the striatum is regulated by two processes: high-amplitude transmission (dopamine release from dopaminergic neurons in the form of short series of intense bursts, phasic release) and persistent weak spontaneous (tonic) activity forming background (basal) level of the neurotransmitter.

In the striatum, dopamine transporter DAT is located in synaptic endings and along axons of dopamine neurons. DAT determines the distance of dopamine diffusion in the extracellular space [5]. 3'-Untranslated region of exon 15 of DAT contains a fragment with variable number of DNA repeats (3-13, 40 b. p. monomer length) [15]. Alleles with 9 and 10 repeats constitute about 98% population. The

All-Russian Research Institute of Sports and Physical Education, Moscow, Russia

density of DAT in subjects with 9 repeats is approximately 25% lower than carriers of 10 repeats.

Dopamine autoreceptors belong to D2 receptor family (DRD2, DRD3, DRD4) and similarly to DAT are located on the presynaptic membrane and modulate activity of ion channels. DRD2 receptors are expressed primarily in the striatum and to a lesser extent in the substatia nigra and in ventral tegmental area [12]. Point TaqIA polymorphism is located in the 3'-untranslated gene region. Its minor allele A1 is associated with reduced DRD2 receptor density.

Catechol-O-methyltransferase (COMT) plays the key role in dopamine catabolism in the prefrontal cortex. Point mutation V158M in the 4th exon of COMT gene leads to a decrease in enzyme activity by 40% in carriers of M allele [4].

The objective of this study was to find associations of functional polymorphisms of DRD2 genes (D2S, short high-affinity form playing the role of autoreceptor) and DAT expressed primarily in the striatum, and COMT enzyme prevailing in the prefrontal cortex with differences in sensitivity threshold to anxiety states.

MATERIALS AND METHODS

Genetic analysis included 156 subjects (74 men, 82 women, mean age 20±5 years). All participants gave informed consent for the use of their DNA and results of psychological tests. DNA was extracted from 100 μl whole blood as described previously [2]. TaqIA DRD2 polymorphism [9], DAT 40 b. p. VNTR genotype [11] and COMT Va1158Met genotype [6] were determined.

All individuals carrying the allele containing 9 repeats comprised a group of DAT 9 carriers (9+) and were compared with homozygotes 10/10 (9-) [8]. The same approach was employed to TaqIA DRD2 genetic typing results. In European population, the incidence of A1A1 genotype is 3%, therefore individuals with A1A1 and A1A2 genotypes comprised an A1+ group and were compared with A2A2 group (A1-).

Diagnosis of anxiety was carried out using three questionnaires. Determination of human personality was performed with Cattell's sixteen personality factor test questionnaire (16PF) including 16 scale factors, one of which is O-factor assessing anxiety level. Emotional susceptibility, irritability, and increased anxiety were evaluated using the neuroticism scale of the Eysenck Personality Inventory. Taylor personal scale of anxiety manifestations adapted by Nemchinov was also used [1].

The relationship between genotype and scale values was evaluated using Mann—Whitney test. This test was used to check the shift hypothesis, because the results of the performed psychological tests represent changes in ordinal scale. Coinciding observations were referred as mean ranks. Probability values were corrected using Holm correction. Calculations were carried out in R medium (Coin software).

The compliance of genotype distribution to Hardy—Weinberg law was assessed using Fisher's exact test ($p>0.9$)

For evaluation of the effects of various DAT and DRD2 concentrations, a mathematical model was developed. According to [5], the distribution of a single portion of released dopamine over a short time can be simulated by equation (1), which describes neurotransmitter diffusion from a point source and its reuptake. In this case, the transporter is uniformly distributed in the volume, while dopamine re-uptake is proportional to its concentration.

$$C(r,t)=\frac{\frac{Q}{NA}}{\alpha(4dtp)^{3/2}}\exp\left(\frac{-r^2}{4dt}\right)\exp\left(\frac{-tV_{max}}{K_m}\right) \quad \textbf{(1)},$$

where $C(r,t)$ is molar concentration of dopamine on a spherical surface with radius r and center in point source (synaptic cleft), at moment t after dopamine release. Diffusion of neurotransmitter molecules in extracellular space if determined by adjusted diffusion constant $d=D/\lambda^2$, where D is diffusion coefficient and λ is extracellular space tortuosity coefficient. Constant α determines a part of volume occupied by extracellular space. In the striatum, $\alpha=0.21$, $\lambda=1.54$, $D=7.63\times10^{-6}$ cm^2/sec for dopamine at 37°C [5]. Dopamine uptake is described by a linear relation $V=V_{max}C/(C+K_m)$; in the striatum $V_{max}=4/1$ μmol/sec and $K_m=0/21$ μmol [5].

Molar concentration of D2 receptors bound with dopamine can be assessed using known relations (2), describing equilibrium concentration.

$$k=\frac{[D2DA]}{[D2_f][DA_f]} \quad \textbf{(2)},$$

where [D2DA] is the concentration of the receptor bound with dopamine, $[D2_f]$ and $[DA_f]$ are concentrations of free dopamine receptor and free dopamine, respectively, and k is equilibrium constant. Expressing baseline concentrations via the concentration of bound receptor, free receptor $[D2_f]$ and free dopamine $[DA_f]$ concentrations, one will get **(3)**:

$$k=\frac{[D2DA]}{([D2_f]—[D2DA])([DA_f]—D2DA])} \quad \textbf{(3)}.$$

Solution of this equation for [D2DA] yields:

$$b([D2_f])=\frac{([D2_f]+[DA_f]+k-\sqrt{([D2_f]+DA_f]+k)^2—4[D2^f][DA^f]}}{2} \quad \textbf{(4)}.$$

Substitution of **(1)** into **(4)**, volume integration, and multiplication by Avogadro number yields equation **(5)** for evaluation of the amount of bound receptor at moment t:

$$D2_b(t)=NA\alpha\iiint_{V\to\infty} b(C(r,t))dx\,dy\,dz=4NA\alpha\pi+\int_0^\infty r^2 b(C(r,t))dr \quad \textbf{(5)}.$$

If $C=EC_{50}$, equation **(1)** can be easily transformed into equation **(6)** describing the radius of sphere, where dopamine concentration exceed EC_{50} threshold:

$$R_{EC_{50}}(t)=\sqrt{4d\ t\left(\left(\ln\left(\frac{Q}{NA\ \ EC_{50}\alpha(4dt\pi)^{3/2}}\right)-t\ \frac{V_{max}}{Km}\right)\right)} \quad \textbf{(6)}.$$

Model assumptions: 1) neurotransmitter loss due to binding with receptors, in particular not present in the model, is not taken into account; 2) Michaelis—Menten ratio was replaced by linear ratio, which led to some inaccuracy during estimation of dopamine concentration in immediate proximity to the synapse; 3) equilibrium concentration of compounds are used; 4) effects of enzymes degrading dopamine were not taken into account and the role of basal dopamine level was considered to be negligible.

RESULTS

MM homozygotes by V158M polymorphism of COMT gene were excluded from the study, because this genotype is associated with increased phasic activity of dopamine neuron, which leads to inhibition of tonic activity in the striatum and, consequently, determined less pronounced effects of DAT and DRD2 on the basal level of dopamine.

During genetic and psychological investigations, an association of DAT, DRD2 (subjects with CIMT allele V) genotypes with anxiety tests values was revealed (Fig. 1).

Significant differences of subjects with genotype VA1+9+ from subjects with genotypes VA1-9+, VA1+9- and VA1-9- ($p<0.1$) were found (Table 1). Individuals with VA1+9+ genotype were characterized by increased anxiety level.

The density of DAT determines the distance of spreading of dopamine and its lifetime in extracellular space. DRD2-autoreceptor produces various effects on the dopamine system. DRD2 activation by dopamine reduces the frequency of tonic contraction. Some studies showed, that activation of DRD2 receptors increases the efficiency of reuptake [3] (Fig. 2).

Thus, DRD2 serves as a feed-back allowing neurons to maintain basal dopamine concentration produced by discrete tonic releases. Moreover, autoreceptors of neurons influence both their own dopamine and dopamine from adjacent neurons.

According to Taq1A DRD2 polymorphism and 40 b. p. DAT repeats, four genotypes were presented. Allele 9+ (Fig. 2, *a*, *b*) differs from 9- (Fig.

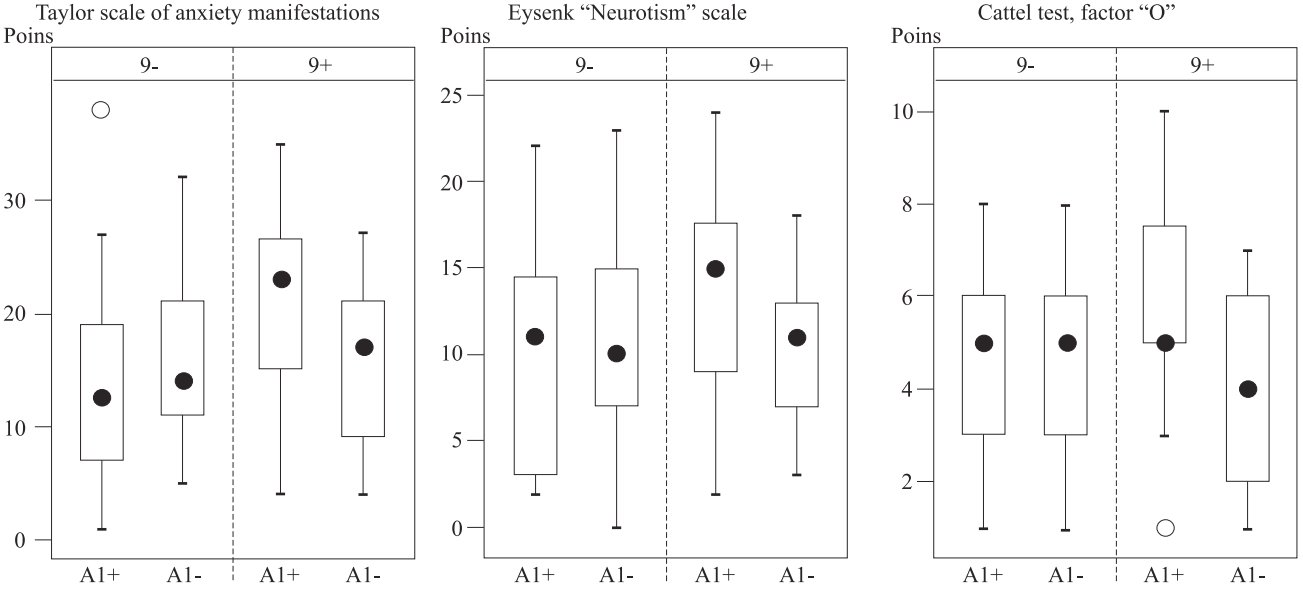

Fig. 1. Distribution of anxiety values over genotypes VA1+9-. VA1-9-, VA1-9+ and VA1+9+.

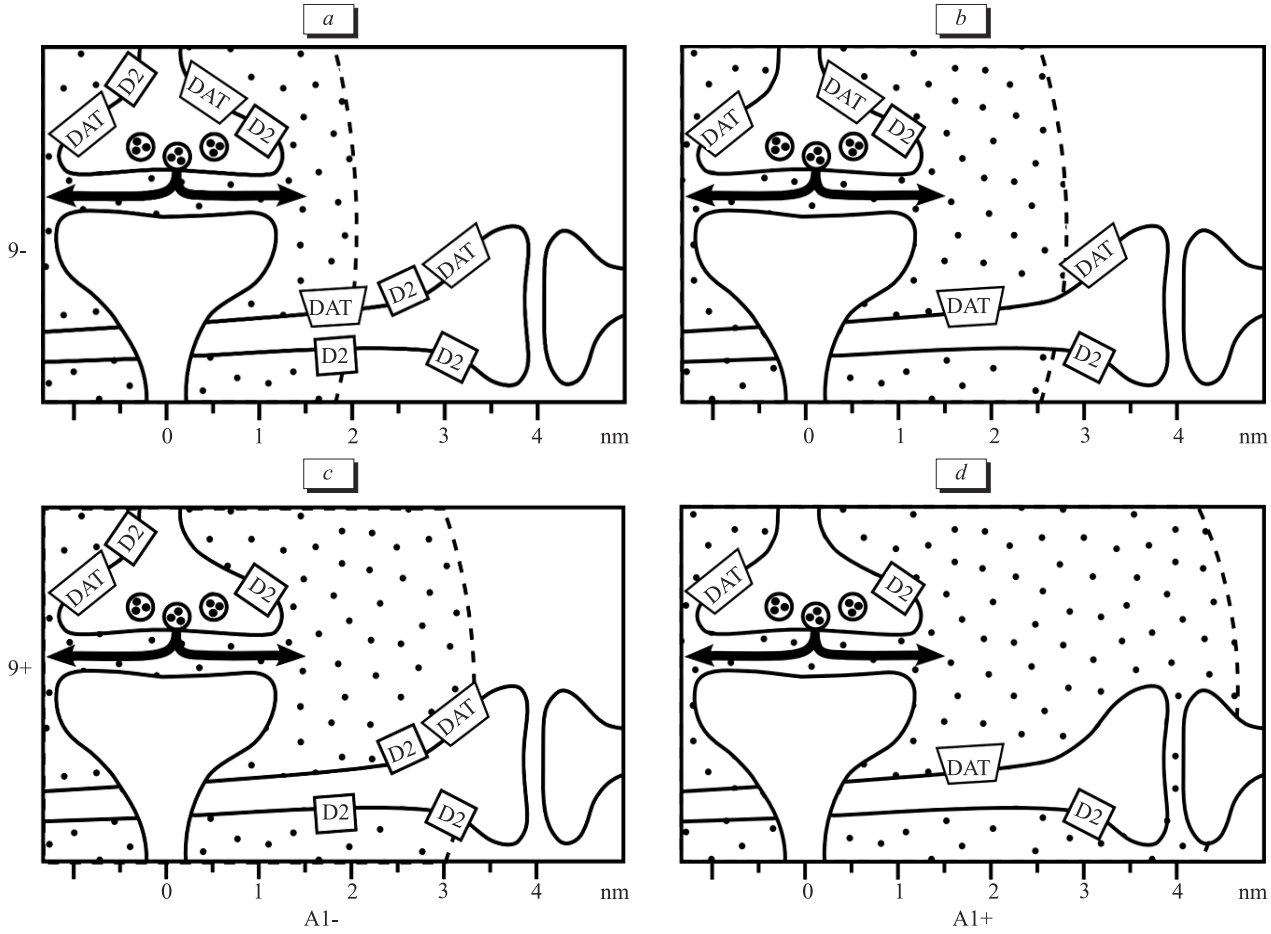

Fig. 2. Diffusion of dopamine in extracellular space of the striatum in subjects with genotypes A1-9- (*a*), A1+9- (*b*), A1+9- (*c*), A1+9+ (*d*).

2, *c*, *d*) by reduced expression of DAT; hence, dopamine diffusion area and lifetime in the extrasynaptic space are greater in allele 9+ carriers. In carriers of A1- allele (*a*, *c*), higher number of autoreceptors D2 are expressed compared to A1+ allele (*b*, *d*), which first, leads to more active functioning of DAT reducing dopamine diffusion area, and second, to lower frequency of tonic releases (Fig. 2).

The dependence of the number of activated D2 receptor on the time elapsed after dopamine quant release (Q=3000 molecules) into the synaptic cleft is shown (Fig. 3, *a*). Curves are obtained by numerical integration by formula (5) with different combinations of V_{max} and [D2] values corresponding to different concentrations of dopamine transporter and receptor. Molar mass concentrations of the receptor in A1+ and A1- genotypes are ~22 and 34 fmol/mg [14]. V_{max} constant is 4.1 μmol/sec and 3.1 μmol/sec in 9- and 9+, respectively.

The dependence of sphere of influence (EC_{50}= 10 nM) of the same dopamine quantum for carriers of alleles 9+ and 9- was shown (Fig 3, *b*).

The number of receptors bound with dopamine in individuals with A1+ is significantly lower than in subjects with A1-, therefore, subsequent tonic release in this region will happen earlier in A1+ (Fig. 3, *a*). Bearing in mind the differences in the number of activated receptors in A1+ and A1-, we

TABLE. 1. Comparative Analysis of Results Obtained from Carriers of Different Genes

Compared groups	Cattel test, factor "O"	Taylor scale of anxiety manifestations	Eysenk "Neuroticism" scale
VA1+9- *vs.* VA1+9+	0.013 (0.004)	0.086 (0.029)	0.080 (0.026)
VA1-9+ *vs.* VA1-9+	0.060 (0.042)	0.086 (0.033)	0.093 (0.046)
VA1-9- *vs.* VA1+9+	0.060 (0.030)	0.086 (0.043)	0.093 (0.051)

Note: Uncorrected *p* values are presented in parentheses.

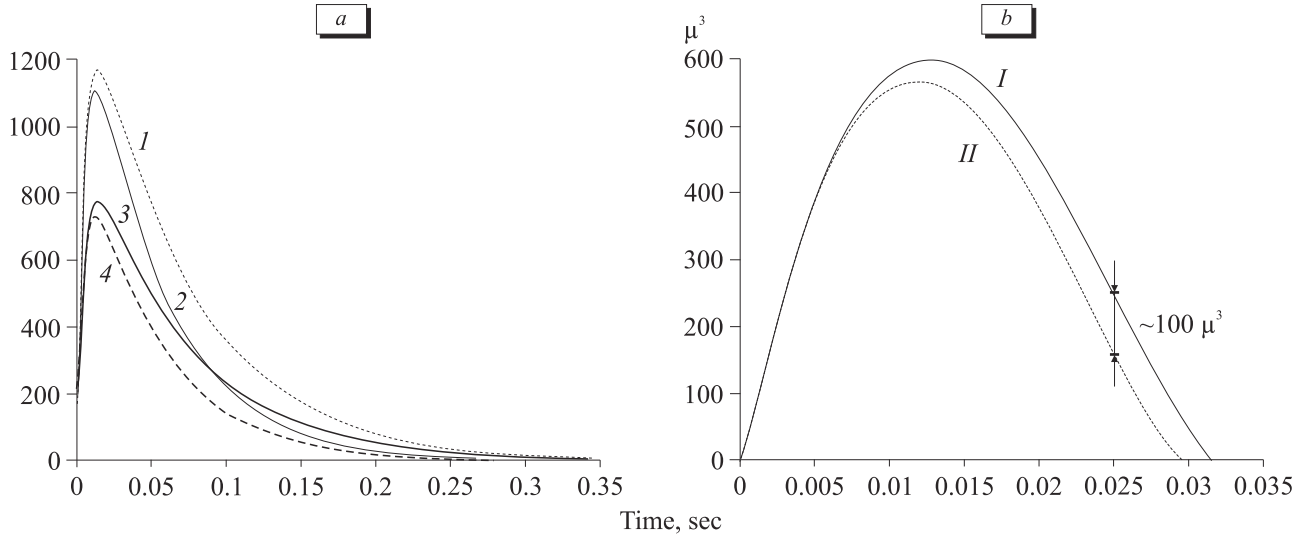

Fig. 3. Time dependence of the number of activated autoreceptors DRD2 (*a*) and the volume of dopamine influence sphere (at EC_{50}=10 nM; *b*). *1*) 9+A1-; *2*) 9-A1-; *3*) 9+A1+; *4*) 9-A1+; *I*: 9+; *II*: 9-

can hypothesize that the difference in the number of activated receptors in subjects with 9+ and 9- does not influence essentially the frequency of tonic dopamine release. It should be noted that dopamine lifetime in the extracellular space and diffusion area are higher in A1+9+ than in A1+9-, which supports the hypothesis on increased level of tonic dopamine in carriers of A1+9+ genotype (Fig. 2).

Thus, carriers of VA1+9+ genotype are characterized by the highest basal dopamine concentration in the striatum, which probably determines increased anxiety of these individuals.

REFERENCES

1. *Diagnostics of Emotional-Ethical Development,* Ed. I. B. Dermanova [in Russian], St. Petersburg (2002), P. 126-128.
2. M. A. Kulikova, N.V. Maluchenko, M.A. Timofeeva, *et al., Bull. Eksp. Biol. Med.,* **145**, No. 1, 68-90 (2008).
3. M. Beniot-Marand, E. Borrelli, and F. Gonon, *J. Neurosci.*, **21**, No. 23, 9134-9141 (2001).
4. J. Chen, B. K. Lipska, N. Halim, *et al., Am. J. Hum. Genet.*, **75**, No. 5, 807-821 (2004).
5. S. J. Cragg and M. E. Rice, *Trends Neurosci.*, **27**, No. 5, 270-277 (2004).
6. C. C. Hong, H. J. Thompson, and C. Jiang, *et al., Cancer Epidemiol. Biomarkers Prev.*, **12**, No. 9, 838-847 (2003).
7. A. Laakso, H. Vilkman, J. Kajander, *et al., Am. J. Psychiatry,* **157**, No. 2, 290-292 (2000).
8. D. Martinez, J. Gelernter, A. Abi-Dargham, *et al., Neuropsychopharmacology,* **24**, No. 5, 553-560 (2001).
9. E. P. Noble, R. E. Noble, T. Ritchie, *et al., Int. J. Eat. Disord.*, **15**, No. 3, 205-217 (1994).
10. A. Reif and K. P. Lesch, *Behav. Brain. Res.*, **139**, Nos. 1-2, 1-20 (2003).
11. A. Sano, K. Kondoh, Y. Kakimoto, and I. Kondo, *Hum. Genet.*, **91**, No. 4, 405-406 (1993).
12. S. R. Sesack, C. Aoki, and V. M. Pickel, *J. Neurosci.*, **14**, No. 1, 88-106 (1994).
13. M. B. Stein, K. L. Jang, and W. J. Livesley, *Am. J. Psychiatr.*, **156**, No. 2, 246-251 (1999).
14. J. Thompson, N. Thomas, A. Singleton, *et al., Pharmacogenetics*, **7**, No. 6, 479-484 (1997).
15. D. J. Vandenbergh, A. M. Persico, A.L. Hawkins, *et al.*, *Genomics*, **14**, No. 4, 1104-1106 (1992).