Chapter

Association of Personal Anxiety with Dopamine Receptor D4 (DRD4), DAT Genes Polymorphism

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Abstract

Modern studies in the world have attached high priority to the role of genetics in human psychosocial stress. People who have strong biochemical responses to stress are more inclined to develop acute and posttraumatic stress disorders. Why do such unusually strong biological reactions occur in certain people? Psychogenetics focuses on many aspects: personality traits that can affect human behavior directly. Their individual variability has been found to be a genetic trait. At present we already know a number of genes, certain allelic variants and genotypes associated with some neuropsychological characters. Among these are genes encoding intracellular and plasma protein neurotransmitter transporters and their receptors; to date, there are only several dozen genes. Of particular interest are dopaminergic system genes. However, information about the polymorphism of known genes associated with personality traits is quite limited and contradictory for open population. Under these circumstances, the chapter is devoted to the association of polymorphisms of candidate genes of the dopaminergic system with anxiety in the open population.

Keywords: DRD4 gene, DAT gene, anxiety, psychosocial factors, open population

1. Introduction

An individual's unique pattern of behaviors, feelings and thoughts is his or her personality expression, which is a strong predictor of the physical, mental and social aspects of current and future health across the lifespan [1]. The psychobiological model of personality Cloninger C.R. (1987) became the prerequisite for the genetic basis search of personality and temperament where temperament traits are correlated with certain biochemical systems in the brain. Cloninger C.R. identified four dimensions for temperament: 'harm avoidance', 'novelty seeking', 'reward dependence' and 'persistence'.

People with high grades on the 'novelty seeking' scale are impulsive, irritable, and tend to break rules blocking access to what they believe will bring satisfaction or allow them to shake themselves. On the other hand, there is conventionality, adherence to rules. Cloninger C.R. connected the 'novelty seeking' - with the

dopamine system [2]. Dopamine is a neurotransmitter that provides neurochemical transmission in the nigrostriatal, mesolimbic, mesocortical and tuberoinfundibular dopaminergic systems in mammals. These brain structures play a vital part in the implementation of psychomotor, cognitive, neuroendocrine functions [3]. Relationship between 'novelty seeking' and dopamine system has been found: an association of temperament with genetic polymorphism encoding the fourth type dopamine receptor (DRD4) was established [4].

The dopamine D4 receptor gene is located on chromosome 11 (11p 15.5) [5]. The human D4 gene has the regions homologous to the regions of the human D1 and D2 genes and other G-proteins and contains 7 transmembrane domains [6]. Unlike the D2 gene containing 7 exons, the D4 gene contains 5 exons. The encoded polypeptide has a molecular weight of 41,000 Da and consists of 387 amino acids. Currently, four polymorphic regions have been identified in the dopamine D4 receptor gene. Three of these polymorphic alleles in humans are not widely distributed. The widespread polymorphism of variable numbers of tandem repeats 48 bp (VNTR) in exon 3 of the D4 gene is most important. The third exon of the dopamine D4 receptor gene contains alleles with a variable number (2–10) of imperfect DNA repeats 48 bp long encoding the region in the third cytoplasmic loop of a 16 amino acid with a general consensus PXAPXLPXXPXGXXCA [7]. A different number of amino acids in the cytoplasmic loop affects the conformation of transmembrane domains and changes the characteristics of ligand binding. Sequenced at least 19 monomers of nucleotide sequences of imperfect repeats in 48 bp and 25 variations of a polymorphic region containing from 2 to 10 repeats [6]. The study on the global frequency distribution of allelic variations of the D4 gene [8] showed that in the healthy population the most common is 4-repeat allete (D4.4) (global frequency in the world is 64.3%). The D4.4 allele is found in all populations with a frequency of 16–96%. The second most frequent variant was 7 repetitions (D4.7) (global frequency in the world 20.6%), which is quite frequent in the American population (average frequency 48.3%) and is rarely represented in populations of East and South Asia (average frequency 1.9%). 2-repeat allete (D4.2) is the third most frequent (global frequency in the world is 8.2%). More often this allele is found in populations of East and South Asia (average frequency 18.1%) and is almost absent in American and African populations (average frequency 2.9% and 1.7%, respectively). The universality of the polymorphism (three repeat-number alleles) indicates that this polymorphism arose before the global dispersion of modern humans [9].

Unused dopamine is moved back to the presynaptic neuron or oxidized by enzymes in the synaptic cleft. Protein dopamine transporter (DAT) provides a reuptake of the mediator in the synaptic cleft. The gene for the DAT1 protein (dopamine transporter) is localized to chromosome 5p15.3, consists of 64 thousand nucleotide pairs, contains 15 exons and 14 introns. Analysis of the 3′untranslated region revealed the presence of the polymorphic locus in it associated with a different number of repeats of 40-nucleotide sequence, repeated from 3 to 11 times [10].

VNTR allele frequencies of the DAT gene in representatives of different ethnic groups differ significantly. The 10-repeat allele has been indicated as the most represented one in all studied populations. Its frequency ranges from 60% (Italians) to 93% (Japanese). The frequency of the 9-repeat allele, which is the second most frequent allele, varies from 4.2% (Japanese) to 39% (Italians). The remaining alleles are present in all populations, but the frequency is less than 3%. The shortest variation with 3 repeats has been found with a low-frequency only in white Americans and African Americans [11].

As in the case of the D4 gene, the DAT gene polymorphism can be associated with some pathological conditions in the pathogenesis, which play the main role in disorders of dopamine metabolism. However, the results of the study on the association between DAT and the 'novelty seeking' are inconsistent so far [12].

Anxiety may be due to neurotransmitter disorders: impaired dopamine synthesis. Nevertheless, the search for a relationship between disturbing traits and the DRD2, DRD3, DRD4, DAT1 genes has yielded conflicting results at present [13].

There is a wide range of convincing clinical studies in laboratory animals that indicate a disorder in the dopaminergic system in depression [14]. López León et al. (2005) performed a meta-analysis of 12 studies of VNTR of the DRD4 gene polymorphism with depression. According to the results of the study, it turned out that the 'short' allele 2 is associated with depression [15].

Most people believe that a state of vital exhaustion arises from long-term psychosocial problems they are not able to solve [16]. As has been shown, dopamine is involved in certain responses to surrounding stressful events [17], and some dopamine reuptake inhibitors have an antidepressant effect [18]. It is still open to question whether the development of vital exhaustion, as a variant of minor depression, is due to certain changes in the dopaminergic system.

We note that at present, predominantly molecular genetics "leads" psychological research to find associations of personality traits, as well as affective disorders. Given these circumstances, the chapter is devoted to the association of candidate gene polymorphisms in dopaminergic system with psychosocial risk factors.

2. Materials and methods

The research of the association of candidate gene polymorphisms with psychosocial factors was carried out on the basis of a large-scale epidemiological study performed as part of the III screening of the WHO MONICA program (Multinational Monitoring of Trends and Determinants of Cardiovascular Disease) in 1994 [19]. We examined men 25–64 years of age, residents of a district in Novosibirsk. The representative sample was generated according to the requirements of the protocol of the MONICA program [19] on the basis of electoral lists using a random number table. 657 men were examined (average age 44.3 ± 0.4 years). The response was –82.1%.

3. Psychosocial testing

The Spielberger test was used to assess anxiety level. The result was interpreted as follows: up to 30 points – mild anxiety level; 34–45 points – moderate anxiety level, 46 and above – severe anxiety level [20]. To evaluate depression, we used the form of the depression scale - the MOPSY test (Depression Scale), consisting of 15 questions. For each question there are 2 answers given: 'agree', 'disagree'. The severity of depression was assessed as no depression (No D), moderate (Mod. D), major (Major D). To assess vital exhaustion, the MOPSY test [19], consisting of 14 statements, was used. For each question there are three answers given: 'yes', 'no', 'I don't know'. The level of vital exhaustion was regarded as: no vital exhaustion, vital exhaustion (average, high). Questionnaires were filled out by the participants. The methods were strictly standardized and complied with the requirements of the protocol of the MONICA project. Material processing performed in

Helsinki (Finland). Quality control was carried out in MONICA quality control centers: Dundee (Scotland), Prague (Czech Republic), Budapest (Hungary). The presented results were found to be satisfactory [19].

4. Molecular genetic analysis

Genotyping of the studied polymorphisms of the DRD4, DAT genes was performed in the Laboratory of Molecular Genetic Studies of Therapeutic Diseases Research Institute of Internal and Preventive Medicine - Branch of the Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia. DNA preparation was conducted there as well. The phenol-chloroform extraction method was used [21, 22]. 5–6 volumes of buffer A (10 mM Tris–HCl, pH = 7.5; 10 mM NaCl; 3 mM MgCl2) were added to a blood sample (~ 10 ml) and hemodialysis was performed by grinding clots in a Potter homogenizer. The precipitates were obtained after centrifugation at 2500 g and washed twice with buffer A, then resuspended in 0.5 ml of buffer B (10 mM EDTA; 100 mM NaCl; 50 mM Tris–HCl, pH = 8.5). After adding SDS to 0.5% and proteinase K to 200 μg / ml, the mixture was incubated for 2 hours at 65° C, or overnight at 37° C. Deproteinization was carried out sequentially with water-saturated phenol, a mixture of phenol-chloroform (1: 1) and chloroform. DNA was precipitated by the addition of NH4Ac to 2.5 M and 2.5 V ethanol. The precipitate obtained by centrifugation in an Eppendorf microcentrifuge for 10 minutes was washed with 70% ethanol and dissolved DNA in water to a concentration of 0.5 μg / μl.

5. Genotyping of VNTR DRD4 gene polymorphism

Genotyping was performed according to a modified technique of Nanko et al. [23]. A DNA fragment of the DRD4 gene (GenBank identification number L12398) containing a DNA region with a variable size of 96–384 bp was amplified using primers: 5′-AGGTG-GCACG-TCGCG-CCAAG-CTGCA-3 ‘straight, pos. 668–692; 5’-TCTGC-GGTGG-AGTCT-GGGGT-GGGAG-3 ‘reverse, pos. 1129–1105. The amplification reaction mixture contained 0.5–1 μg of genomic DNA, direct and reverse primers at a concentration of 0.4 μM each, dNTP at a concentration of 0.1 mm each, 1.5 mm MgCl2, 10% dimethyl sulfoxide (DMSO), 0.01% by volume Tween-20, 20 mm (NH4) SO4, 75 mM TrisHCl (pH 9.0) and 1.25 units of Taq polymerase. The total volume of the mixture was 25 μl. PCR was performed using a Mastercycler gradient (Eppendorf Scientific Inc., USA). The amplification conditions were as follows: 95°C for 4 min, then 35 cycles: 95°C for 1 min, 65°C for 1 min, 72°C for 1 min. PCR products were analyzed using polyacrylamide gel electrophoresis (4%), in a buffer containing 90 mM Tris-borate (pH 8.0) and 2 mM EDTA, followed by staining with ethidium bromide. DNA markers of 100 bp were used as a molecular weight marker. (Sibenzyme, Russia).

6. Genotyping of VNTR DAT gene polymorphism

For genotyping, we used a modified method of Mitchell et al. [24]. The DNA fragment of the DAT gene (identification number in GenBank M95167), containing a DNA region with a variable size of 240–480 bp, was amplified using primers: 5′-TGTGG-TGTAG- GGAAC-GGCCT-GAG-3 ‘straight, pos. 2718–2740; 5’-CTTCC-TGGAG-GTCAC-GGCTC-AAGG-3 ‘reverse, pos. 3201–3178. The reaction mixture

with a volume of 25 μl contained 0.5–1 μg of genomic DNA, forward and reverse primers at a concentration of 0.4 μM each, dNTP at a concentration of 0.4 mm each, 2 mm MgCl2, 0.01% by volume Tween-20, 98 mm beta mercaptoethanol, 67 mm Tris HCl (pH 8.8) and 1.25 units of Taq polymerase. Each of the 35 amplification cycles consisted of denaturation (95°C, 0.5 min), annealing (66°C, 5 min) and synthesis (72°C, 1.5 min). PCR products were analyzed using polyacrylamide gel electrophoresis (4%), followed by staining with ethidium bromide. DNA markers of 100 bp were used as a molecular weight marker. (Sibenzyme, Russia).

Statistical analysis was performed using the SPSS-19 software package [25]. The distribution of attributes and their numerical characteristics were analyzed. The analysis of simple relationships between variables (contingency tables) was carried out. Using the method of constructing contingency tables, we conducted the hypothesis of factors A and B independence or the homogeneity of factor B with respect to the levels of factor A. The reliability of the factor independence was evaluated using χ^2criterion [26].

7. Results

In the open population among men aged 25–64 years, the frequency of 4/4 homozygous genotype of the D4 subtype of the dopamine receptor (DRD4) was 57.9%, 2/2 genotype was found to be less frequent - 6.1%, 2/4 genotype - 12, 5% and 3/4 genotype - 5.6%; even less frequent: 4/6 genotype - 4.2%, 2/6 genotype, 4/7 and 6/6 genotypes were found in the identical proportions of 2.1%. The frequency distribution of alleles showed that the 4 allele predominates - 70.7%, the 2 allele was found at 14%, the 6 allele was at 6%. The other alleles make up 0.8% - 5.4% (**Table 1**).

The distribution of carriers of VNTR genotypes of DRD4 gene polymorphism by anxiety level is presented in **Table 1**. Associative analysis revealed that carriers of the DRD4 genotype 4/4 are much more likely to be found in the group with moderate anxiety (59.8%) and mild anxiety (66.7%) than in the group with severe anxiety (54.8%). Carriers of the 2/4 genotype were much more frequent in the group with moderate anxiety (14.5%) than with severe anxiety where the level was 9.6% ($\chi2 = 69.569$ υ = 36 p = 0.001). On the contrary, carriers of the 4/6 genotype were more frequently found in the group with a severe level of anxiety - 7.8%, versus with a moderate level of anxiety - 2%. Moreover, the occurrence of carriers of the 4/6 genotype in the group with severe anxiety was more frequent than in the group with moderate anxiety, in comparison with carriers of all other genotypes OR = 4.2 (95% CI 1.4–12.1); ($\chi2 = 8.521$ υ = 1 p < 0.01), 2/2 genotype ($\chi2 = 7.326$ υ = 1 p = 0.007), 2/4 genotype ($\chi2 = 9.825$ υ = 1 p = 0.002), 4/4 genotype ($\chi2 = 8.543$ υ = 1 p = 0.003). The similar situation can be seen in groups of anxiety and carriage of alleles 2, 3, 4 and 6. Carriers of alleles 2 and 4 prevailed in the group with moderate anxiety - 15.6% and 72.1%, respectively, whereas, in the group with severe anxiety they were represented - 11.7% and 68.7%, respectively ($\chi2 = 15.980$ υ = 12 p > 0.05). Carriers of allele 3 in the group with severe anxiety were found in 7.5%, and with moderate anxiety –3.9%, with severe anxiety found in them 2 times (95% CI 1–3.6) more often than carriers of all other alleles ($\chi2 = 5240$ υ = 1 p = 0,022), carriers of allele 2 ($\chi2 = 7122$ υ = 1 p < 0,01) and allele 4 OR = 2 (95% CI 1–3,7); ($\chi2 = 5.284$ υ = 1 p < 0.05). Similarly, carriers of the allele 6 in the group with severe anxiety were found in 7.8%, and in the group with moderate anxiety - 4.7%, and the frequency of severe levels of anxiety was higher than in the carriers of all other alleles OR = 1, 7 (95% CI 0.9–3; $\chi2 = 3.5$ υ = 1, p < 0.05), carriers of allele 2 ($\chi2 = 5.499$ υ = 1 p < 0.01), carriers of allele 4 ($\chi2 = 3689$ υ = 1 p < 0,05).

Genotype	Population		Anxiety						Depression				Vital exhaustion					
			Mild		Moderate		Severe		No D		Depression		None		Moderate		Severe	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
2/2	26	6.1	0	0	18	7	8	4.8	19	6.4	7	5.4	8	6.3	17	7.7	1	1.3
2/3	1	0.2	0	0	0	0	1	0.6	1	0.3	0	0	0	0	1	0.5	0	0
2/4	53	12.5	0	0	37	14.5	16	9.6	43	14.5*	10	7.8	20	15.6	23	10.4	10	13.2
2/5	2	0.5	0	0	1	0.4	1	0.6	0	0	2	1.6	1	0.8	1	0.5	0	0
2/6	10	2.4	0	0	5	2	5	3	6	2.0	4	3.1	4	3.1	6	2.7	0	0
2/7	1	0.2	0	0	1	0.4	0	0	1	0.3	0	0	1	0.8	0	0	0	0
3/3	8	1.9	0	0	3	1.2	5	3.0	6	2.0	2	1.6	1	0.8	4	1.8	3	3.9
3/4	24	5.6	0	0	12	4.7	12	7.2	16	5.4	8	6.2	8	6.3	9	4.1	7	9.2
3/6	3	0.7	1	33.3	1	0.4	1	0.6	1	0.3	2	1.6	1	0.8	1	0.5	1	1.3
3/7	2	0.5	0	0	1	0.4	1	0.6	2	0.7	0	0	0	0	2	0.9	0	0
4/4	246	57.9	2	66.7	153	59.8***	91	54.8	179	60.5	67	51.9	69	53.9	133	60.2	44	57.9
4/5	4	0.9	0	0	4	1.6	0	0	3	1.0	1	0.8	1	0.8	1	0.5	2	2.6
4/6	18	4.2	0	0	5	2	13	7.8**	6	2.0	12	9.3**	7	5.5	8	3.6	3	3.9
4/7	9	2.1	0	0	5	2	4	2.4	5	1.7	4	3.1	2	1.6	6	2.7	1	1.3
4/8	1	0.2	0	0	0	0	1	0.6	0	0	1	0.8	0	0	0	0	1	1.3
5/5	3	0.7	0	0	0	0	1	0.6	2	0.7	1	0.8	1	0.8	2	0.9	0	0
5/6	2	0.5	0	0	1	0.4	1	0.6	1	0.3	1	0.8	1	0.8	0	0	1	1.3
6/6	9	2.1	0	0	6	2.3	3	1.8	4	0.3	5	3.9	3	2.3	6	2.7	0	0
7/7	3	0.7	0	0	2	0.8	1	0.6	1	1.4	2	1.6	0	0	1	0.5	2	2.6
					$\chi^2 = 69.569$ $\upsilon = 36$ $p = 0.001$					$\chi^2 = 32.811$ $\upsilon = 18$ $p = 0.018$					$\chi^2 = 39.186$ $\upsilon = 36$ $p = 0.329$			

Allele	Population		Anxiety						Depression				Vital exhaustion					
			Mild		Moderate		Severe		No D		Depression		None		Moderate		Severe	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
2	26	6.1	0	0	80	15.6	39	11.7	89	15	30	11.6	42	16.4	65	14.7	12	7.9
3	9	2.1	1	16.7	20	3.9	25	7.5	32	5.4	14	5.4	11	4.3	21	4.8	14	9.2
4	323	76.0	4	66.7	369	72.1	228	68.7	431	72.8	170	65.9	176	68.8	313	70.8	112	73.7
5	9	2.1	0	0	8	1.6	6	1.8	8	1.4	6	2.3	5	2	6	1.4	3	2
6	42	9.9	1	16.7	24	4.7	26	7.8	22	3.7	29	11.2	19	7.4	27	6.1	5	3.3
7	15	3.5	0	0	11	2.1	7	2.1	10	1.7	8	3.1	3	1.2	10	2.3	5	3.3
8	1	0.2	0	0	0	0	1	0.3	0	0	1	0.4	0	0	0	0	1	0.7
			$\chi^2 = 15.980$ υ = 12 p = 0.192						$\chi^2 = 24.678$ υ = 6 p = 0.00001				$\chi^2 = 20.495$ υ = 12 p = 0.058					

$^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Table 1.
Frequencies of VNTR genotypes and alleles of DRD4 gene polymorphism in a population and their association with psychosocial factors.

The distribution of carriers of VNTR genotypes of DRD4 gene polymorphism by depression level is shown in **Table 1**. Carriers of the DRD4 genotype 4/4 and 2/4 were most frequently found in the group where there was no depression (60.5% and 14.5%, respectively) than in the group with depression (51.9% and 7.8%, respectively) ($\chi 2 = 32.811$ $\upsilon = 18$ $p < 0.05$). In contrast, carriers of the 4/6 genotype were more likely to be found in the group with depression (9.3%) than in the group without depression (2%), and compared with carriers of all other genotypes, OR = 4.9 (95% CI 1.8–13.5); ($\chi 2 = 11.725$ $\upsilon = 1$ $p < 0.001$), carriers of the 2/2 genotype ($\chi 2 = 6.848$ $\upsilon = 1$ $p < 0.01$), 2/4 genotype ($\chi 2 = 14.356$ $\upsilon = 1$ $p < 0.0001$), 3/4 genotype ($\chi 2 = 4582$ $\upsilon = 1$ $p < 0,05$) and 4/4 genotype ($\chi 2 = 12,436$ $\upsilon = 1$ $p = 0,00001$). Carriers of 6/6 homozygous genotype were also more frequently found in the group with depression (3.9%) than without depression (0.3), compared with carriers of the 2/4 genotype (without depression –14.5%, with depression –7, 8%) ($\chi 2 = 5645$ $\upsilon = 1$ $p = 0,017$). The similar situation can be seen in the groups with and without depression in the carriage of long and short alleles of the DRD4 gene (**Table 1**). Carriers of allele 2 and 4 are more frequent in the group without depression (15% and 72.8%, respectively) than in the group with depression (11.6% and 65.9%, respectively) ($\chi 2 = 24.678$ $\upsilon = 6$ $p < 0.00001$). Carriers of the long allele 6, on the contrary, are more frequently found in the group with depression (11.2%) than in the group without depression (3.7%), and compared with carriers of all other alleles, OR = 3.2 (95% CI 1 8–5.8); ($\chi 2 = 18.036$ $\upsilon = 1$ $p < 0.0001$), carriers of allele 2 ($\chi 2 = 15.784$ $\upsilon = 1$ $p < 0.0001$), allele 3 ($\chi 2 = 6.845$ $\upsilon = 1$ $p < 0.01$) and allele 4 ($\chi 2 = 18.103$ $\upsilon = 1$ $p < 0.0001$). DRD4 genotype 4/4, the most widely represented in the male population, was most frequently found in the group with a moderate level of vital exhaustion (60.2%). Carriers of the second most common genotype in the population: 2/4 genotype were more often found in the group where there was no vital exhaustion (15.6%). Carriers of the 3/3 and 3/4 genotype were more frequently found in the group with a severe level of vital exhaustion (3.9% and 9.2%, respectively) than in the group with moderate vital exhaustion (1.8% and 4, 1%, respectively). Carriers of the 4/5 and 7/7 genotype are more likely to be found in the group with a severe level of vital exhaustion (2.6%, respectively) than in other groups. Carriers of the 2/6 genotype (3.1%) and 4/6 genotype (5.5%) were more frequently found in the group with no vital exhaustion. Carriers of the 4/7 genotype (2.7%) and 6/6 genotype (2.7%) were most often found in the group with moderate vital exhaustion ($\chi 2 = 39.186$ $\upsilon = 36$ $p > 0.05$). Carriers of the 2/2 genotype were more likely to be found in the group with a moderate level of vital exhaustion (7.7%) than in the group with a severe level of vital exhaustion (1.3%) in comparison with: representatives of all other DRD4 genotypes ($\chi 2 = 4.039$ $\upsilon = 1$ $p < 0.05$), carriers of the 2/4 genotype ($\chi 2 = 4.217$ $\upsilon = 1$ $p < 0.05$); 3/3 genotype ($\chi 2 = 5.218$ $\upsilon = 1$ $p < 0.05$); 3/4 genotype ($\chi 2 = 6.868$ $\upsilon = 1$ $p < 0.01$); 3/6 genotype ($\chi 2 = 3951$ $\upsilon = 1$ $p < 0,05$); 4/5 genotype ($\chi 2 = 7.843$ $\upsilon = 1$ $p < 0.01$); 7/7 genotype ($\chi 2 = 7.843$ $\upsilon = 1$ $p < 0.01$). Furthermore, carriers of the 2/2 genotype were more frequently found in the group with no vital exhaustion (6.3%) than in the group with severe vital exhaustion, compared with carriers of the DRD4 genotype 3/3 ($\chi 2 = 5306$ $\upsilon = 1$ $p < 0.05$).

The distribution of the other carriers of the DRD4 genotype does not exceed 1.3% (**Table 1**). The different picture can be seen in men with vital exhaustion in the carriage of long and short alleles of the DRD4 gene. Carriers of the DRD4 gene allele 2 are more frequently represented in the male group without vital exhaustion (16.4%) than in the group with a severe level of vital exhaustion (7.9%), moreover, in comparison with: carriers of all other DRD4 gene alleles ($\chi 2 = 6.017$ $\upsilon = 1$ $p < 0.01$); carriers of allele 3 ($\chi 2 = 8.830$ $\upsilon = 1$ $p < 0.01$); carriers of allele 4 ($\chi 2 = 5.466$ $\upsilon = 1$ $p < 0.01$); allele 7 ($\chi 2 = 5680$ $\upsilon = 1$ $p < 0,01$). Also, carriers of allele 2 were more often found in the group with a moderate level of vital exhaustion (14.7%) than in the group with a severe level (7.9%) as compared with carriers of

all other alleles ($\chi2$ = 4.651 df = 1 p = 0.031); carriers of allele 3 ($\chi2$ = 8.047 df = 1 p = 0.005); allele 4 ($\chi2$ = 4.064 df = 1 p = 0.044). Carriers of the allele 3 are more frequently represented in the group with severe vital exhaustion (9.2%) than in the group where there was no vital exhaustion (4.3%) compared with carriers of all other alleles of the DRD4 gene OR = 2.26 (95% CI 0.9–5.1); ($\chi2$ = 4.003 υ = 1 p < 0.05); carriers of the allele 6 OR = 4.83 (95% CI 1.3–17); ($\chi2$ = 6.379 υ = 1 p < 0.01). Carriers of the allele 3 were also more often found in the group with moderate vital exhaustion (4.8%) than in the group with severe vital exhaustion (9.2%), compared with the carriers of all other alleles OR = 2 (95% CI1–4.1); ($\chi2$ = 4.056 υ = 1 p < 0.05); carriers of the allele 6 OR = 3.6 (95% CI 1.1–11.5); ($\chi2$ = 4.889 υ = 1 p < 0.05). Carriers of allele 7 were more frequently found in the group with severe vital exhaustion (3.3%) than in the group where there is no vital exhaustion (1.2%), compared with carriers of allele 6, which are more often represented in the group of men without vital exhaustion (7.4%) ($\chi2$ = 4.848 υ = 1 p < 0.05). Carriers of the allele 4 of the DRD4 gene are represented at approximately the same frequency in all groups with and without vital exhaustion (68.8%, 70.8%, and 73.7%, respectively). Carrier of all other alleles of the DRD4 gene does not exceed 2% and is presented in **Table 1**. ($\chi2$ = 20.495 υ = 12 p < 0.05).

We determined that the 10/10 homozygous genotype is found more frequently (54.8%), and the heterozygous 9/10 genotype is more rare - 36.6% in the frequency distribution of the VNTR genotypes of DAT gene polymorphism in the population among of men aged 25–64 years. 9/9 genotype was observed in 3.7%. The prevalence of the other genotypes was from 1.7% and lower. The similar situation in the population and in the carriage of alleles is 9–22% and 10–74.2%, which were more common than carriers of all other alleles (**Table 2**).

The distribution of carriers of VNTR genotypes of DAT gene polymorphism by the level of anxiety is presented in **Table 2**. Men, carriers of the 10/10 genotype in the group with moderate anxiety were found in 58.4%, and in the group with a severe level of anxiety - 50.6%, carriers of the heterozygous genotype 9/10 were respectively in the group with the moderate level of anxiety - 35% and in the group with a severe level of anxiety - 38.8% ($\chi2$ = 51.105 υ = 16 p < 0.0001). Carriers of the 9/9 genotype were found much more frequently in the group with a severe level of anxiety (6.3%) than in the group with a moderate level of anxiety (1.6%), moreover, in comparison with representatives of all other genotypes, OR = 3.9 (95% CI 1.2–12.9); ($\chi2$ = 6.098 υ = 1 p < 0.01), carriers of the 9/10 genotype OR = 3.4 (95% CI 1–11.1); ($\chi2$ = 4.424 υ = 1, p < 0.05), and carriers of the 10/10 genotype OR = 4.3 (95% CI 1.3–14.4); ($\chi2$ = 6.863 υ = 1, p < 0.01).

The distribution of carriers of VNTR genotypes of DAT gene polymorphism by the level of anxiety is presented in **Table 2**. Men, carriers of the 10/10 genotype in the group with moderate anxiety, were found in 58.4%, and in the group with severe anxiety - 50.6%, carriers of the heterozygous 9/10 genotype were respectively in the group with moderate anxiety - 35% and in the group with severe anxiety - 38.8% ($\chi2$ = 51.105 υ = 16 p < 0.0001). Carriers of the 9/9 genotype were found much more frequently in the group with a severe level of anxiety (6.3%) than in the group with a moderate level of anxiety (1.6%), moreover, in comparison with representatives of all other genotypes, OR = 3.9 (95% CI 1.2–12.9); ($\chi2$ = 6.098 υ = 1 p < 0.01), carriers of the 9/10 genotype OR = 3.4 (95% CI 1–11.1); ($\chi2$ = 4.424 υ = 1, p < 0.05), and carriers of the 10/10 genotype OR = 4.3 (95% CI 1.3–14.4); ($\chi2$ = 6.863 υ = 1, p < 0.01).

The distribution of carriers of VNTR genotypes of DAT gene polymorphism by depression level is presented in **Table 2**. Carriers of the 10/10 and 9/10 genotypes were found approximately equally in the group with depression (57.7%, 37.9%, respectively) and in the group without depression (56% and 36.1%, respectively)

Genotype	Population		Anxiety						Depression				Vital exhaustion					
			Mild		Moderate		Severe		No D		Depression		None		Moderate		Severe	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
8/8	4	1	0	0	2	0.8	2	1.3	4	1.4	0	0	2	1.6	2	0.9	0	0
9/9	15	3.7	1	25*	4	1.6	10	6.3	6	2.1	9	7.8	0	0	5	2.3	10	15.2***
6/10	3	0.7	1	25	0	0	2	1.3	3	1.0	0	0	1	0.8	1	0.5	1	1.5
8/10	1	0.2	0	0	1	0.4	0	0	1	0.3	0	0	1	0.8	0	0	0	0
9/10	149	36.6	2	50***	85	35**	62	38.8*	105	36.1	44	37.9	49	38.3	79	37.1	21	31.8
10/10	223	54.8	0	0	142	58.4**	81	50.6	163	56	60	51.7	73*	57	118	55.4	32	48.5
10/11	4	1.0	0	0	3	1.2	1	0.6	4	1.4	0	0	1	0.8	3	1.4	0	0
10/12	1	0.2	0	0	1	0.4	0	0	1	0.3	0	0	1	0.8	0	0	0	0
11/11	7	1.7	0	0	5	2.1	2	1.3	4	1.4	3	2.6	0	0	5	2.3	2	3.0
			$\chi^2 = 51.105$ υ = 16 p = 0.0001						$\chi^2 = 13.549$ υ = 8 p = 0.094				$\chi^2 = 41.076$ υ = 16 p = 0.001					
Allele	**Population**		**Anxiety**						**Depression**				**Vital exhaustion**					
			Mild		**Moderate**		**Severe**		**No D**		**Depression**		**None**		**Moderate**		**Severe**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
6	3	0.4	1	12.5*	0	0	2	0.6	3	0.5	0	0	1	0.4	1	0.2	1	0.8
8	9	1.1	0	0	5	1.0	4	1.3	9	1.5	0	0	5	2	4	0.9	0	0
9	179	22	4	50***	93	19.1**	82	25.6*	117	20.1	62	26.7	49	19.1	89	20.9	41	31.1**
10	604	74.2	3	37.5**	374	77***	227	70.9	440	75.6	164	70.7	199	77.7	319	74.9	86	65.2
11	18	2.2	0	0	13	2.7	5	1.6	12	2.1	6	2.6	1	0.4*	13	3.1	4	3
12	1	0.1	0	0	1	0.2	0	0	1	0.2	0	0	1	0.4	0	0	0	0
			$\chi^2 = 45.402$ υ = 10 p = 0.0001						$\chi^2 = 9.235$ υ = 5 p = 0.1				$\chi^2 = 19.792$ υ = 10 p = 0.031					

$^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Table 2.
Frequencies of VNTR genotypes and alleles of DAT gene polymorphism in a population and their association with psychosocial factors.

($\chi2$ = 13.549 υ = 8 p > 0.05). Carriers of the 9/9 genotype were more common in the group with depression (7.8%) than in the group without depression (2.1%), moreover, in comparison with representatives of all other genotypes, OR = 3.9 (95% CI 1.3–11.4); ($\chi2$ = 7.583 υ = 1 p < 0.001) and carriers of the 10/10 genotype OR = 4 (95% CI 1.3–11.9); ($\chi2$ = 7.477 υ = 1, p = 0.006). The ratio of the frequency of alleles 9 and 10 among men in groups with depression and without depression is similar to the distribution of these genotypes ($\chi2$ = 9.235 υ = 5 p < 0.05) (**Table 2**). Carriers of allele 9 and allele 10 were found in the group with depression (26.7% and 70.7%, respectively) and in the group without depression (20.1% and 75.6%, respectively). Moreover, carriers of allele 9 were more frequently found in the group with depression than without it, in comparison with carriers of all other alleles OR = 1.4 (95% CI 1–2); ($\chi2$ = 4.390 υ = 1, p < 0.05).

The distribution of carriers of VNTR genotypes of DAT gene polymorphism by level of vital exhaustion is presented in **Table 2**. Carriers of the DAT gene 9/10 and 10/10 genotypes were more common in the group where there was no vital exhaustion (38.3% and 57%, respectively); either in a group with a moderate level of vital exhaustion (37.1% and 55.4%, respectively); and in the group with a severe level, they were less common (31.8% and 48.5%, respectively) ($\chi2$ = 41.076 υ = 16 p < 0.001). Men, carriers of the DAT gene 9/9 genotype, were significantly more likely to be found in the group with a severe level of vital exhaustion (15.2%) than in the group with a moderate level of vital exhaustion (2.3%) in comparison with carriers of other genotypes OR = 7.4 (95% CI 2.4–22.6); ($\chi2$ = 16.238 υ = 1 p < 0.0001), carriers of the 9/10 genotype OR = 7.5 (95% CI 2.3–24.3); ($\chi2$ = 13.815 υ = 1 p < 0.0001), carriers of the 10/10 genotype OR = 7.3 (95% CI 2.3–23.11); ($\chi2$ = 14.769 df = 1 p = 0.0001). The ratio of the frequencies of alleles 9 and 10 of the DAT gene in men in different groups of vital exhaustion is similar to the distribution of these genotypes ($\chi2$ = 19.792 υ = 10 p < 0.05). Carriers of allele 9 were more frequently found in the group with a severe level of vital exhaustion (31.1%) than in the group with a moderate level (20.9%), in comparison with carriers of all alleles of the DAT gene OR = 1.7 (95% CI 1, 1–2.6); ($\chi2$ = 5.831 υ = 1 p < 0.01), carriers of the allele 10 OR = 1.7 (95% CI 1.1–2.6); ($\chi2$ = 5.772 υ = 1 p < 0.01) and than in the group where there was no vital exhaustion (19.1%) in comparison with carriers of all alleles of the gene DAT OR = 1.9 (95% CI 1.1–3); ($\chi2$ = 6.946 υ = 1 p < 0.01), carriers of the 10 allele of the OR gene = 1.9 (95% CI 1.1–3.1); ($\chi2$ = 7.224 υ = 1 p < 0.01). In contrast, carriers of the 10 allele of the DAT gene, compared with carriers of all other alleles of the DAT gene, were more likely to have a moderate level of vital exhaustion (74.9%) ($\chi2$ = 4.795 υ = 1 p < 0.05) or there was no vital exhaustion (77,7%) ($\chi2$ = 7.072 υ = 1 p < 0.01), than a high level of vital exhaustion was observed (62.2%) (**Table 2**).

8. Discussion

In this study, we made an attempt to analyze the relationship between the DRD4 and DAT genes, since dopamine is involved in many cognitive and motivational processes; dopaminergic neurons are located in several parts in the midbrain; and dopaminergic axons extend to several regions of the striatum, hippocampus, tonsil, thalamus and cortex, and psychosocial factors, because the coordinated work of mediators and brain modulators underlies the emotional state and behavior of animals and humans [27].

The most frequent VNTR polymorphism of the DRD4 gene in the male population was the 4/4 homozygous genotype (57.9%), which is generally characteristic of Caucasoid populations. In second place we can see carriers of genotypes with short allele 2 of the DRD4 gene from 6 to 12% in frequency of occurrence in our

population. This allele is more characteristic of Central Asian populations [28]. The frequency of carriage of longer alleles 6 and higher of the DRD4 gene did not exceed 6% among the participants. In the world the variant with 7 repetitions of DRD4 (20.6%) is more frequently found, and more often we can see this genotype in the US population [28].

In this study, male carriers of the 4/6 genotype of the DRD4 gene were more likely to be found in the group with a severe level of anxiety and depression. We have established a certain trend among men with different levels of vital exhaustion: with an increase in the number of tandem turns of the VNTR of the DRD4 gene polymorphism, the level of vital exhaustion increased. Carriers of the DRD4 allele 6 were more common among men with depression. Severe levels of vital exhaustion were more common among carriers of the allele 7. According to modern concepts of dopamine biosynthesis, it is known to take part in the so-called adaptation process.

Lack of dopamine results in depletion of the nervous system, and its increased level causes bipolar disorders [4, 27]. It has been shown that in people with the long form of the DRD4 gene (the number of repeats is six or more), the affinity of dopamine for the receptor is reduced and the number of receptors is reduced. These individuals are less sensitive to dopamine. So, they need more stimulation than people with a short form of the gene to get the same reaction [29]. Probably this is the reason for the high frequency of occurrence of genotypes with long allele of the DRD4 gene in men with anxiety, depression, and vital exhaustion.

As in the case of the DRD4 gene, VNTR polymorphic variants of the DAT gene can be associated with some pathological conditions in the pathogenesis, which play the main role in dopamine metabolism disorders [30]. In the studied population, the homozygous 10/10 genotype of the DAT gene prevailed - more than 50%, less often the 9/10 genotype was found slightly more than 36%, and finally, the third place was occupied by the 9/9 genotype - 3.7%. The incidence of the other genotypes was below 1.7%. According to literature data, the most represented was the allele with 10 repeats (60% - 93%) in all studied populations. The frequency of the allele with 9 repeats, which is the second most common, varies from 4.2–39%. The other alleles are present in all populations with a frequency of less than 3% [31]. Carriers of VNTR polymorphism of the 9/9 genotype of the DAT gene were more common among men with a severe level of anxiety, depression, and vital exhaustion. Similarly, carriage of allele 9 increased the chance of falling into the groups mentioned above.

Although studies on the association between anxiety, depression, life exhaustion, and VNTR polymorphism in the dopamine transporter gene are not available in the world literature, it may be associated with some pathological conditions in a number of cases; in the pathogenesis of which disorders in the dopaminergic system of the brain play the main role. It is known that individuals having a short version of the DAT gene in the genome, often develop post-traumatic stress disorder [32], which to some extent explains the results.

In summary, we should note that the genetic features found in the open male population may be responsible for the pathophysiological changes in the functioning and compensatory abilities of the dopaminergic system and are a background predisposing to the development of psychological and social risk factors for cardiovascular diseases (arterial hypertension, myocardial infarction, stroke).

9. Summary

In the population among men aged 25–64 years, the 4/4 homozygous genotype of the dopamine receptor 4-subtype DRD4 gene (57.9%) is the most represented.

2/2 genotype - 6.1%, 2/4 genotype - 12.5%, and 3/4 genotype - 5.6% are less frequently found; and more rarely - 4/6 genotype (4.2%), 2/6 genotype, 4/7 genotypes and 6/6 genotype were present in equal proportions of 2.1%.The frequency distribution of alleles showed that the 4 allele predominates - 70.7%, the 2 allele was found at 14%, the 6 allele was at 6%. The other alleles make up 0.8% - 5.4%.

We have found that the 10/10 homozygous genotype is more common (54.8%), and the heterozygous 9/10 genotype is more rare (36.6%) with the frequency distribution of the VNTR genotypes of the DAT gene polymorphism. The 9/9 genotype was observed in 3.7%. The prevalence of the other genotypes was from 1.7% and lower. The frequency distribution of alleles showed that the alleles were 9–22%, 10–74.2%, which were more common than carriers of all other alleles. The 4/6 genotype of the DRD4 gene is strongly associated with mild anxiety, depression. The 7 allele of the DRD4 gene is strongly associated with a severe level of vital exhaustion. The 9/9 genotype of the DAT gene is strongly associated with severe anxiety, depression, and vital exhaustion.

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