International Journal of Medicine and Medical Sciences ISSN: 2167-0404 Vol. 2 (12), pp. 284-289, December, 2012.
Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Genetic markers for trait anxiety as the risk factors for cardiovascular diseases (WHO-MONICA Program and MONICA-Psychosocial Subprogram)

Valery V. Gafarov,[1,2]*, Mikhail I. Voevoda,[2,3], Elena A. Gromova,[1,2], Vladimir N. Maksimov,[2,3], Igor V. Gagulin,[1,2], Nikolay S. Yudin,[3], Almira V. Gafarova,[1,2] and Tatiana M. Mishakova[3]

[1]Federal State Budgetary Institution the Research Institute of Internal Medicine of Siberian Branch of the Russian Academy of Medical Sciences, Novosibirsk, Russia.
[2]Federal State Budgetary Institution the Interdepartmental Laboratory of Cardiovascular Disease Epidemiology of Siberian Branch of the Russian Academy of Medical Sciences, Novosibirsk, Russia.
[3]Cytology and Genetics Institute of Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia.

Received 18 September, 2012; Accepted 01 December, 2012

Anxiety is considered an independent risk factor for cardiovascular diseases (CVD). The objectives of the study were to determine trait anxiety prevalence; to find associations between trait anxiety and VNTR polymorphisms in the DRD4 and DAT genes; and to calculate relative risks (RR) for developing arterial hypertension (AH), myocardial infarction (MI), and stroke. Representative sample of 25 to 64-year-old males (*n* = 2149) was examined in three screening studies in a framework of the WHO MONICA program and MONICA-psychosocial subprogram in Novosibirsk in 1984, 1988, and 1994. All first time MI, AH, and stroke events were registered. Genotyping of VNTR polymorphism was performed for DRD4 and DAT genes. Anxiety levels were evaluated by Spielberger's test. Stratified cox proportional regression model was used for RR estimation. High level of anxiety (HLA) was 50.9%. The DRD4 genotype 4/6 and DAT genotype 9/9 were significantly associated with HLA increasing CVD risk. Stroke and AH risks were maximal during the first five years, whereas maximal MI risk was found for 10-year period. Prevalence of HLA in Novosibirsk was high. Rates of HLA were significantly associated with certain VNTR polymorphisms in the DRD4 and DAT genes. High levels of anxiety were associated with increased CVD risk.

Key words: Trait anxiety, DRD4 gene, DAT gene, cardiovascular disease risk, cardiovascular disease.

INTRODUCTION

Solely adverse environmental factors can hardly be fully responsible for the development of elevated levels of trait anxiety (Spielberger, 1972; Raffety, 1997; Akzhigitov, 2002; Comer, 2006; Kolutskaya, 2006; Lapina and Borovkov, 2008). The study of 8 to 16-year-old twins from Great Britain showed genetic correlation between anxiety and depression (Bouchard and Loehlin, 2001). The genetic correlation coefficient was as high as 80%, whereas factors of general environment accounted for the rest 20% (Bouchard and Loehlin, 2001). Anxiety can be caused by the abnormal dopamine synthesis (Roy-Byrne, 1986; Heninger and Charney, 1998; Nutt and Laurence, 1992), although the study regarding the relationships between anxiety traits and variable number of tandem repeats (VNTR) polymorphisms in the dopamine D4 receptor (DRD4) and the dopamine transporter (DAT) genes gained controversial results (Eley, 1997; Benjamin, 1998; de Brettes, 1998; Noble, 1998). Great interest in studying anxiety is driven also by the fact that anxiety is considered as an independent risk factor for cardiovascular morbidity and mortality (Gafarov and Gagulin, 1993; Gafarov, 2000a, b; Roest, 2010). To our knowledge, no available prospective epidemiologic study in the literature describes data similar to our results based on the World Health Organization (WHO)

*Corresponding author. E-mail: valery.gafarov@gmail.com. Tel: +73832642516. Fax: +7 383 264 2516.

Table 1. Genotype and allele frequencies of variable number of tandem repeat (VNTR) polymorphisms in the dopamine D4 receptor gene in 25 to 64-year-old male population in Novosibirsk.

Genotypes	Population	
	n	%
22	26	6.1
23	1	0.2
24	53	12.5
25	2	0.5
26	10	2.4
27	1	0.2
33	8	1.9
34	24	5.6
36	3	0.7
37	2	0.5
44	246	57.9
45	4	0.9
46	18	4.2
47	9	2.1
48	1	0.2
55	3	0.7
56	2	0.5
66	9	2.1
77	3	0.7
Alleles		
2	119	14
3	46	5.4
4	601	70.7
5	14	1.6
6	51	6.0
7	18	2.1
8	1	0.1

programs.

The objectives of our study were to determine trait anxiety levels in an open population of 25 to 64-year-old males; to carry out an association analysis of trait anxiety and VNTR polymorphisms in the DRD4 and DAT genes; and to calculate relative risks (RR) for developing arterial hypertension (AH), myocardial infarction (MI), and stroke, depending on the anxiety levels over a 24-year period of the study.

MATERIALS AND METHODS

Three screening studies were conducted in a framework of the World Health Organization Multinational Monitoring of Trends and Determinants in Cardiovascular Disease program (MONICA) (Roy-Byrne, 1986; Heninger and Charney, 1988; Nutt and Laurence, 1992) and MONICA-psychosocial subprogram (World Health Organization, 1988) in 1984, 1988, and 1994, respectively. A total of 2149 males aged 25 to 64 years, residents of one district of the city of Novosibirsk, were examined. The response rate was 82.1%. Anxiety levels were evaluated by using the Spielberger's test (the trait anxiety subscale) (Spielberger, 1972). The Spielberger's test scores of less than 30, ranging from 31 to 44, and more than 45 were interpreted as indicators of low level of anxiety (LLA), moderate level of anxiety (MLA), and high level of anxiety (HLA), respectively. The Spielberger's inventories were filled out by each participant individually. The methods were strictly standardized to comply with the requirements of the MONICA project protocol. Processing of data was carried out in Helsinki (Finland). Quality control was performed in quality control centers for MONICA in Dundee (Scotland), Prague (Czech Republic), and Budapest (Hungary) where the results were found satisfactory (WHO MONICA, 1990).

Genotyping of the gene polymorphism was performed in the Molecular Genetics Laboratory of the Research Institute of Internal Medicine by using the methods described in detail elsewhere (Maniatis, 1984; Smith, 1988; Nanko, 1993; Mitchell, 2000). Briefly, Genomic deoxyribonucleic acid (DNA) was isolated from whole blood and analyzed by polymerase chain reaction (PCR) with oligodeoxynucleotide primers specific for part of the DRD4 sequence: 5'-AGGTG-GCACG-TCGCG-CCAAG-CTGCA-3' sense; 5'-TCTGC-GGTGG-AGTCT-GGGGT-GGGAG -3' antisense. Reactions were carried out using the Eppendorf Mastercycler gradient. PCR amplification was done in 25 µL reaction buffer containing 20 mmol/L NH_4SO_4, 75 mmol/L Tris-HCl (pH 9), 1.5 mmol/L $MgCl_2$, 0.01% Tween-20, and 10% dimethylsulphoxide. The reaction contained 0.5–1 µg genomic DNA, 0.4 µmol/L of each primer, 1.25 unit Taq polymerase, and 200 µmol/L each of dNTP. PCR conditions were initial denaturing at 95°C for 4 min, followed by 35 cycles (95°C, 1 min; 65°C, 1 min; 72°C, 1 min). Genotyping of VNTR polymorphism in the DAT gene was performed with oligodeoxynucleotide primers specific for part of the DAT sequence: 5'-TGTGG-TGTAG-GGAAC-GGCCT-GAG-3' sense; 5'-CTTCC-TGGAG-GTCAC-GGCTC-AAGG-3' antisense. PCR amplification was done in 25 µL reaction buffer containing 67 mmol/L Tris-HCl (pH 8.8), 2 mmol/L $MgCl_2$, 0.01% Tween-20, and 98 mmol/L beta-mercaptoethanol. The reaction contained 0.5–1 µg genomic DNA, 0.4 µmol/L of each primer, 1.25 unit Taq polymerase, and 200 µmol/L each of dNTP. PCR conditions were initial denaturing at 95°C for 4 min, followed by 35 cycles (95°C, 1 min; 66°C, 1 min; 72°C, 1 min). Gel PCR product was subjected to 4% polyacrylamide gel electrophoresis. Amplified DNA was stained with ethidium bromide after electrophoresis. The 100 bp DNA ladder for molecular weight identification was purchased from SibEnzyme (Russia).

Frequency distribution of the variable number of tandem repeats (VNTR) polymorphisms in the DRD4 and DAT genes in a population of 25 to 64-year-old males is shown in Tables 1 and 2. All male participants diagnosed

Table 2. Genotype and allele frequencies of variable number of tandem repeats (VNTR) polymorphisms in the DAT gene in 25 to 64-year-old male population in Novosibirsk.

Genotypes	Population	
	n	%
8/8	4	1
9/9	15	3.7
6/10	3	0.7
8/10	1	0.2
9/10	149	36.6
10/10	223	54.8
10/11	4	1.0
10/12	1	0.2
11/11	7	1.7
Alleles		
6	3	0.4
8	9	1.1
9	179	22
10	604	74.2
11	18	2.2
12	1	0.1

with ischemic heart disease, cerebrovascular pathology, arterial hypertension (AH), myocardial infarction (MI), diabetes, and confirmed psychiatric illnesses were screened out from the cohort for prospective study (n = 1423). During the entire 24 years of the study from 1984 to 2008, all first time MI events (n = 104) were registered by using the WHO acute myocardial infarction register program, whereas the arterial hypertension (n = 162) and stroke (n = 76) events were documented in the process of the yearly observations on the cohort.

Statistical analysis of data was performed by using the statistical package for social sciences (SPSS) software package version 11.5. Chi square (χ^2) statistic was used to investigate whether distributions of categorical variables differed from one another in between the groups. The stratified Cox proportional regression model was used for determination of the relative risk (RR) adjusted for different data collection dates. A value of $p < 0.05$ was considered statistically significant (Cox, 1972; Glants, 1998).

RESULTS

Prevalence of trait anxiety in the population of 25 to 64-year-old males was as high as 97.5%. Moderate level of anxiety (MLA) and high level of anxiety (HLA) were found in 46.6 and 50.9% of participants, respectively. Carriers of the DRD4 genotype 4/4 comprised 59.8 and 54.8% of

Table 3. Distribution of genotype and allele frequencies of the dopamine D4 receptor gene and prevalence of trait anxiety.

Genotypes	LLA		MLA		HLA	
	n	%	n	%	n	%
22	0	0	18	7	8	4.8
23	0	0	0	0	1	0.6
24	0	0	37	14.5	16	9.6
25	0	0	1	0.4	1	0.6
26	0	0	5	2	5	3
27	0	0	1	0.4	0	0
33	0	0	3	1.2	5	3.0
34	0	0	12	4.7	12	7.2
36	1	33.3	1	0.4	1	0.6
37	0	0	1	0.4	1	0.6
44	2	66.7	153	59.8***	91	54.8
45	0	0	4	1.6	0	0
46	0	0	5	2	13	7.8**
47	0	0	5	2	4	2.4
48	0	0	0	0	1	0.6
55	0	0	0	0	1	0.6
56	0	0	1	0.4	1	0.6
66	0	0	6	2.3	3	1.8
77	0	0	2	0.8	1	0.6
$X^2 = 69.569$, $df = 36$, $p = 0.001$						
Alleles	**n**	**%**	**n**	**%**	**n**	**%**
2	0	0	80	15.6	39	11.7
3	1	16.7	20	3.9	25	7.5
4	4	66.7	369	72.1	228	68.7
5	0	0	8	1.6	6	1.8
6	1	16.7	24	4.7	26	7.8
7	0	0	11	2.1	7	2.1
8	0	0	0	0	1	0.3
$\chi^2 = 15.980$, $df = 12$, $p = 0.192$						

LLA: low level of anxiety; MLA: moderate level of anxiety; HLA: high level of anxiety. **p < 0.01; ***p < 0.001.

males in MLA and HLA groups, respectively. Individuals with the DRD4 genotype 2/4 were found significantly more often in MLA group (14.5%) than in HLA group (9.6%). In contrast, carriers of the DRD4 genotype 4/6 were found more often in HLA group (7.8%) than in MLA group (2%) (χ^2 = 69.569, df = 36, p = 0.001) (Table 3). Carriers of the alleles 2 and 4 prevailed in MLA group (15.6 and 72.1%, respectively), whereas the occurrence rates for these alleles in HLA group were 11.7 and 68.7%, respectively. The allele 6 was found in 7.8 and 4.7% of HLA group and MLA group, respectively (χ^2 = 15.980, df = 12, p = 0.192) (Table 3).

Carriers of the DAT genotype 10/10 comprised 58.4% of MLA group and 50.6% of HLA group.

Table 4. Distribution of genotype and allele frequencies of the DAT gene and prevalence of trait anxiety.

Genotypes	LLA		MLA		HLA	
	n	%	n	%	n	%
8/8	0	0	2	0.8	2	1.3
9/9	1	25	4	1.6	10	6.3
6/10	1	25	0	0	2	1.3
8/10	0	0	1	0.4	0	0
9/10	2	50	85	35	62	38.8
10/10	0	0	142	58.4	81	50.6
10/11	0	0	3	1.2	1	0.6
10/12	0	0	1	0.4	0	0
11/11	0	0	5	2.1	2	1.3
$\chi^2 = 51.105$, $df = 16$, $p = 0.0001$						
Alleles	**n**	**%**	**n**	**%**	**n**	**%**
6	1	12,5	0	0	2	0,6
8	0	0	5	1,0	4	1,3
9	4	50	93	19,1	82	25,6
10	3	37,5	374	77	227	70,9
11	0	0	13	2,7	5	1,6
12	0	0	1	0,2	0	0
$\chi^2 = 45.402$, $df = 10$, $p = 0.0001$						

LLA: low level of anxiety; MLA: moderate level of anxiety; HLA: high level of anxiety.

The heterozygote DAT genotype 9/10 was found in 35% of MLA group and in 38.8% of HLA group. The distribution among men, carriers of the DAT genotype 9/9, was the opposite, namely: 6.3% of participants had HLA, whereas 1.6% of them had MLA ($\chi^2 = 51.105$, $df = 16$, $p = 0.0001$) (Table 4). Carriers of the allele 9 prevailed in HLA group (25.6%) in comparison with MLA group (19.1%). In contrast, carriers of the allele 10 were found more often in MLA group (77%) than in HLA group (70.9%) ($\chi^2 = 45.402$, $df = 10$, $p = 0.0001$) (Table 4).

Over the entire 24-year period of our study, 5.9, 4.2 and 16.9% of males suffered from MI, stroke, and newly diagnosed AH, respectively. Prevalence rates of HLA in a cohort of males with newly diagnosed cardiovascular diseases (CVD) were 57.4% ($\chi^2 = 8.515$, $df = 1$, $p < 0.001$), 58.7% ($\chi^2 = 23.185$, $df = 1$, $p < 0.0001$), and 68.7% ($\chi^2 = 40.355$, $df = 1$, $p < 0.0001$) in participants with AH, MI, and stroke, respectively. Within the first five years of observation, RR rates for developing AH, MI, and stroke in HLA group were 6.8-fold higher (95% C = 3.24–14.18, $p < 0.05$), 2.5-fold higher (95% CI = 1.63–4.62, $p < 0.001$), and 6.4-fold higher (95% CI = 3.08–13.3, $p < 0.05$) than in MLA group, respectively. A ten-year period of the study showed that RR rates for developing AH, MI, and stroke in HLA group were 5-fold higher (95% CI = 2.89–11.76), 3.1-fold higher (95% CI = 1.48 – 5.61; $p < 0.001$), and 3.8-fold higher (95% CI = 1.67–8.75; $p < 0.05$) than in MLA group, respectively. A twenty-year period of observation revealed that RR rates for developing AH, MI, and stroke in HLA group were 1.8-fold higher (95% CI = 1.087–3.24, $p < 0.05$), 2.7-fold higher (95% CI = 1.27–5.71, $p < 0.05$), and 1.6-fold higher (95% CI = 1.026–2.965, $p < 0.05$), respectively, in comparison with MLA group. We found a tendency towards an increase in RR for developing CVD in HLA group over the entire 24-year period of the study (Figure 1).

DISCUSSION

The study showed that more than half of 25 to 64-year-old males in the study population had HLA. Prevalence of HLA in Russian population was found significantly higher than in Europe and the USA (Alonso et al., 2004; Jacobi et al., 2004; Kessler et al., 2005; Lépine et al., 2005). Unfortunately, we could not compare our results with data acquired in other Russian regions because such studies either do not exist or their data have not been published yet. Such a high prevalence of HLA in our study may be explained by the fact that anxiety was a marker of social tension in the population that experienced major overhaul of the social and psychological bases during the years of the study (Gafarov et al., 2000; 2002).

Carriers of the DRD4 genotypes 4/4 and 2/4 were found more often in MLA group, whereas males with the genotype 4/6 were found more often in HLA group. We observed similar frequency distribution pattern for the DRD4 alleles. Among the possible causes of higher frequency of long DRD4 gene allele variants in males with HLA is the fact that, according to present-day evidence, dopaminergic neuron system is associated with reinforcement or reward pathways. Indeed, long DRD4 gene allele variant is associated with the reduced dopamine receptor sensitivity. Due to the fact that long DRD4 gene alleles encode less sensitive variants of the dopamine receptor, the long allele homozygous individuals require stronger external stimulation to feel comfortable. These people need higher dopamine doses for receptor activation (Jonsson et al., 1997; Alonso et al., 2004; Jacobi et al., 2004; Kessler et al., 2005; Lépine et al., 2005).

Carriers of the DAT genotype 10/10 were found more often in MLA group than in HLA group. A pattern of frequency distribution among the carriers of the genotypes 9/10 and 9/9 was the opposite, namely: these genotypes were found more often in HLA group than in MLA group. The other genotypes in males with various levels of trait anxiety were found significantly rarer with the prevalence rates ranging from 2 to 5%. The ratios of the alleles 9 and 10 in males with trait anxiety were similar to the ratios of the corresponding genotypes. Individuals with short DRD4 gene allele variants in genome possibly have altered dopamine reuptake

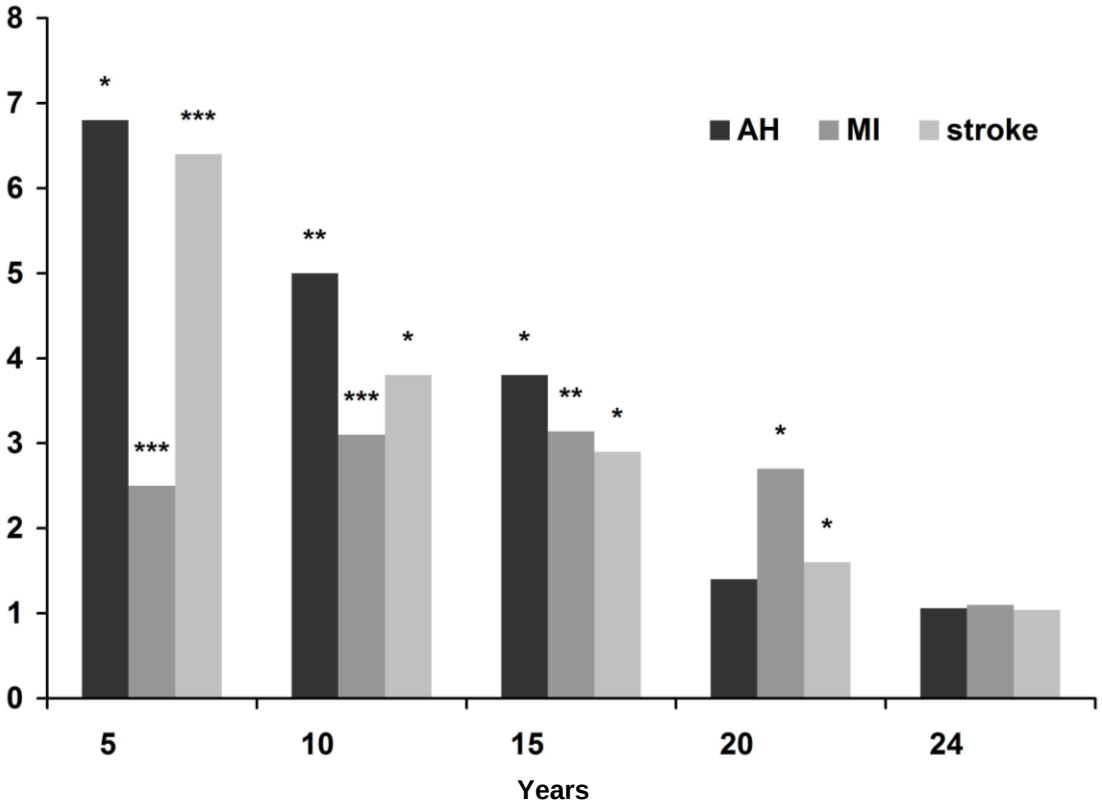

Figure 1. Relative risk of developing CVD among the study participants with high level of anxiety during 24-year period. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. AH: arterial hypertension; MI: myocardial infarction.

resulting in reduced need for dopamine system activation due to smoking and heroin addiction. These individuals also have higher prevalence of posttraumatic stress disorder (Segman et al., 2002; Gerra et al., 2005).

Our data provided evidence that trait anxiety significantly increased the risk of developing CVD. Maximal risks for developing AH (RR_{AH} = 6.8) and stroke (RR_S = 6.4) were observed in males with HLA as early as within the first five years of the study compared to MLA group. Relative risks for developing AH and stroke in HLA group decreased with the course of time (for ten-year period: RR_{AH} = 5 and RR_S = 3.8; for 20-year period: RR_{AH} = 5 and RR_S = 3.8). At the same time, RR for developing MI showed a different pattern, namely: maximal risk for developing MI was found within 10 years of the study (RR = 3.1); 20-year period revealed some downward trend in MI RR (RR = 2.7); both 10-year and 20-year period indices exceeded the RR rates for developing MI within the first five years of the study (RR = 2.5).

The differently directed RR trends in developing AH and stroke versus MI can be explained by the fact that HLA, as a cause of AH and stroke, was found more often in the older groups. Further decrease in RR for 10-year and 20-year periods was caused by reduction in a cohort size due to adverse outcomes in these groups. At the same time, HLA, as a cause of MI development, was found more often in the younger age groups, obviously resulting in a different RR trend pattern, namely: maximal RR was registered for 10-year period, whereas minimal RR was found for the first five years (Gafarov, 2004, 2007, 2008). The results of our study are consistent with data obtained by other authors. Meta-analysis of 20 studies, conducted from 1980 to 2009, showed that anxiety in originally healthy individuals increased risk for developing coronary artery disease (RR = 1.26, 95% CI = 1.15–1.38, p<0.0001) independently of demographic factors, biological risk factors, and lifestyle (Roest, 2010).

Conclusions

1. Prevalence of high level of anxiety in 25 to 64-year-old male population of Western Siberia metropolis (the city of Novosibirsk) was as high as 50.9%.
2. High level of anxiety in 25 to 64-year-old male population was associated with the DRD4 genotype 4/6 and the DAT genotype 9/9.
3. High level of anxiety in 25 to 64-year-old male population caused maximal risk of developing arterial hypertension and stroke within the first five years of observation.
4. High level of anxiety in 25 to 64-year-old male

population resulted in maximal risk of developing myocardial infarction within 10-year period, whereas risk of myocardial infarction events during the first five years of observation was minimal.

REFERENCES

Akzhigitov RG (2002). Current trends in understanding and treatment of anxiety disorders. Rossiysky Meditsinsky Zhurnal, 1: 43–45.

Alonso J, Angermeyer MC, Bernert S, Bruffaerts R, Brugha TS, Bryson H, de Girolamo G, Graaf R, Demyttenaere K, Gasquet I, Haro JM, Katz SJ, Kessler RC, Kovess V, Lépine JP, Ormel J, Polidori G, Russo LJ, Vilagut G, Almansa J, Arbabzadeh-Bouchez S, Autonell J, Bernal M, Buist-Bouwman MA, Codony M, Domingo-Salvany A, Ferrer M, Joo SS, Martínez-Alonso M, Matschinger H, Mazzi F, Morgan Z, Morosini P, Palacín C, Romera B, Taub N, Vollebergh WA (2004). ESEMeD/MHEDEA 2000 Investigators, European Study of the Epidemiology of Mental Disorders (ESEMeD) Project (2004). Prevalence of mental disorders in Europe: results from the European Study of the Epidemiology of Mental Disorders (ESEMeD) project. Acta. Psychiatr. Scand. Suppl., (420): 21-27.

Benjamin J, Osher Y, Belmaker RH, Ebstein R (1998). No significant associations between two dopamine receptor polymorphisms and normal temperament. Hum. Psychopharmacol., 13(1): 11–15.

Bouchard TJ, Loehlin JC (2001). Genes, Evolution, and Personality. Behav Genet., 31(3): 243-273.

Comer R (2007). Fundamentals of Abnormal Psychology. Prime-Euroznak Publishing House.

Cox DR (1972). Regression Models and Life Tables. Journal of the Royal Statistical Society Series B., 34: 187–220.

de Brettes B, Berlin I, Laurent C, Lépine J, Mallet J, Puech A (1998). The dopamine D2 receptor gene TaqI A polymorphism is not associated with novelty seeking, harm avoidance and re-ward dependence in healthy subjects. Eur. Psychiatry, 13: 427–430.

Eley TC, Ball D, E J, Freeman B, Riemann R, Spinath FM, Angleitner A, Plomin R (1997). Association study of extreme high and low neuroticism, with genetic markers for the dopaminergic system. Am. J. Med. Genet., 81(6): 487.

Gafarov VV, Gagulin IV (1993). Epidemiologic approach to the study of trait anxiety. Bulletin of Siberian Branch of the Russian. Acad. Med. Sci., 3: 77-81.

Gafarov VV, Gromova EA, Gagulin IV, Pilipenko PI (2004). World Health Organization MONICA-psychosocial program: psychosocial factors and risk of developing stroke (epidemiologic study). Korsakov Journal of Neurology and Psychiatry. Suppl. Stroke., 12: 40-45.

Gafarov VV, Gromova EA, Kabanov YuN, Gagulin IV (2008). Personality and personal interaction with social environment: the untrodden road. Novosibirsk.

Gafarov VV, Gromova HA, Gagulin IV, Ekimova YC, Santrapinskiy DK (2007). Arterial hypertension, myocardial infarction and stroke: risk of development and psychosocial factors. Alaska Med. 49(2 Suppl): pp. 117-119.

Gafarov VV, Pak VA, Gagulin IV, Gafarova AV (2000a). Psychology of population health in Russia. Siberian Branch of the Russian Academy of Sciences Publishing House. Novosibirsk.

Gafarov VV, Pak VA, Gagulin IV, Gafarova AV (2000b). Epidemiology and prevention of chronic noncommunicable diseases in Russia during two decades of social and economic crisis. Siberian Branch of the Russian Academy of Sciences Publishing House. Novosibirsk.

Gerra G, Garofano L, Pellegrini C, Bosari S, Zaimovic A, Moi G, Avanzini P, Talarico E, Gardini F, Donnini C (2005). Allelic association of a dopamine transporter gene polymorphism with antisocial behaviour in heroin-dependent patients. Addict Biol., 10(3): 275-281.

Glants C (1998). Biomedical statistics. Translated from English. Moscow. Practika.

Heninger GR, Charney DS (1988). Monoamine receptor systems and anxiety disorders. Psychiatr. Clin. North Am., 11: 309–326.

Jacobi F, Wittchen HU, Holting C, Höfler M, Pfister H, Müller N, Lieb R (2004). Prevalence, co-morbidity and correlates of mental disorders in the general population: results from the German Health Interview and Examination Survey (GHS). Psychol. Med., 34(4): 597-611.

Jönsson EG, Nöthen MM, Gustavsson JP, Neidt H, Brené S, Tylec A, Propping P, Sedvall GC (1997). Lack of evidence for allelic association between personality traits and the dopamine D4 receptor gene polymorphisms. Am. J. Psychiatry., 154(5): 697-699.

Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry. Jun;62(6):593-602. Erratum in: Arch Gen Psychiatry, 62(7): 768.

Kolutskaya EV (2006). Anxiety disorders: diagnosis and treatment. Health Ukraine J., 3: 17.

Kuulasmaa K, Pajak A, Pekkanen J, Ruokokoski E, Tunstall-Pedoe H, Madai A, Rajakangas A-M, Akkila J, Moltchanov V (1990). Coronary event registration data book 1980-1986. MONICA Memo 179A; p. 74.

Lapina NS, Borovkov NN (2008). Anxious depressive conditions in patients with gastroesophageal reflux disease. Klin Med (Moscow). 86(2): 59-62.

Lépine JP, Gasquet I, Kovess V, Arbabzadeh-Bouchez S, Nègre-Pagès L, Nachbaur G, Gaudin AF (2005). Prevalence and comorbidity of psychiatric disorders in

the French general population. Encephale. Mar-Apr, 31(2): 182-194.
Maniatis T, Fritsch EF, Sambrook J (1984). Molecular cloning: a laboratory manual. Translation from English. Moscow. Mir Publishers.
Mitchell RJ, Howlett S, Earl L, White NG, McComb J, Schanfield MS, Briceno I, Papiha SS, Osipova L, Livshits G, Leonard WR, Crawford MH (2000). Distribution of the 3' VNTR polymorphism in the human dopamine transporter gene in world populations. Human Biol., 72(2): 295-304.
Nanko S, Hattori M, Ikeda K, Sasaki T, Kazamatsuri H, Kuwata S (1993). Dopamine D4 receptor polymorphism and schizophrenia. Lancet. 341: 689-690.
Noble EP, Ozkaragoz TZ, Ritchie TL, Zhang X, Belin TR, Sparkes RS (1998). D2 and D4 dopamine receptor polymorphisms and personality. Am. J. Med. Genet., 81: 257–267.
Nutt D, Laurence C (1992). Panic attacks: a neurochemical overview of models and mechanisms. Br J Psychiatry, 160: 165–178.
Raffety B, Smith R, Ptacek J (1997). Facilitating and debilitating trait anxiety, situational anxiety and coping with an anticipated stressor: a process analysis. J. Pers. Soc. Psychol., 72(4): 892-906.
Roest AM, Martens EJ, de Jonge P, Denollet J (2010). Anxiety and risk of incident coronary heart disease: a meta-analysis. J Am Coll Cardiol., 56(1): 38-46.
Roy-Byrne PP, Uhde TW, Sack DA, Linnoila M, Post RM (1986). Plasma HVA and anxiety in patients with panic disorder. Biol. Psychiatry, 21: 849–853.
Segman RH, Cooper-Kazaz R, Macciardi F, Goltser T, Halfon Y, Dobroborski T, Shalev AY (2002). Association between the dopamine transporter gene and posttraumatic stress disorder. Mol. Psychiatry, 7(8): 903-907.
Smith CL, Klco SR, Cantor CR (1988). Pulsed Field Gel Electrophoresis and the Technology of Large DNA Molecules. In Davies K, editor. Genome Analysis: A Practical Approach. Translation from English. Moscow. Mir Publishers, 1990: 58-94.
Spielberger CD (1972). Anxiety as an emotional state. In Spielberger CD, editor. Anxiety: Current trends in theory and research. New York: Academic Press. 1: 24-49.
Spielberger CD (1972). Conceptual and methodological issues in anxiety research. In Spielberger CD, editor. Anxiety: current trends in theory and research, NY: Academic Press. 2: 481-493.
World Health Organization (1985). Proposal for the Multinational Monitoring of Trends in cardiovascular disease. Geneva.
World Health Organization (1988). MONICA Psychosocial Optional Study. Suggested Measurement Instruments. Copenhagen: WHO Regional Office for Europe.