ASSOCIATION OF PSYCHOSOCIAL FACTORS WITH DOPAMINE RECEPTOR D4 (DRD4), DAT GENE POLYMORPHISM AND CARDIOVASCULAR INCIDENCE RISK

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Anxiety is considered as an independent risk factor for cardiovascular diseases (CVD). Relationships between genetic markers of anxiety and risk of developing CVD remain unknown.

Aim. 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 Hazard retio (HR) for developing arterial hypertension (AH), myocardial infarction (MI), and stroke.

Material and methods. Representative sample of 25-64-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 from 1984 to 2008. Genotyping of VNTR polymorphism was performed for DRD4 and DAT genes. Anxiety levels were evaluated by using the Spielberger's test. Stratified Cox proportional regression model was used for Hazard ratio (HR) estimation.

Results. High level of anxiety (HLA) in an open male population was 50,9%. The DRD4 genotype 4/6 and DAT genotype 9/9 were significantly associated with HLA. HLA increased CVD risk. HR for developing AH and stroke was maximal during the first five years of the study, whereas maximal risk of developing MI was found for 10-year period.

Conclusion. Prevalence of HLA in an open 25-64-year-old male population in Novosibirsk was high. Rates of HLA were significantly associated with certain VNTR polymorphisms in the DRD4 and DAT genes. HLA were associated with increased risk of developing CVD.

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Key words: cardiology; arterial hypertension, myocardial infarction, stroke, trait anxiety, DRD4 gene, DAT gene, risk of developing disease, cardiovascular disease.

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AH — arterial hypertension, DAT — the dopamine transporter, DRD4 — the dopamine D4 receptor, HLA — high level of anxiety, MI — myocardial infarction, MLA — moderate level of anxiety, MONICA — Multinational Monitoring of Trends and Determinants in Cardiovascular Disease program, VNTR — variable number of tandem repeat.

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АССОЦИАЦИЯ ПСИХОСОЦИАЛЬНЫХ ФАКТОРОВ С ПОЛИМОРФИЗМОМ ГЕНОВ РЕЦЕПТОРА ДОПАМИНА D4 (DRD4), ТРАНСПОРТЕРА ДОПАМИНА (DAT) И РИСК РАЗВИТИЯ СЕРДЕЧНО-СОСУДИСТОЙ ПАТОЛОГИИ

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Тревожность рассматривается как независимый фактор риска сердечно-сосудистых заболеваний (ССЗ). Ассоциации между генетическими маркерами тревоги и риском развития ССЗ остаются неизвестными.

Цель. Определить распространенность личностной тревожности в популяции, найти ассоциативные связи между личностной тревожностью и VNTR-полиморфизмом генов DRD4, DAT; рассчитать риск развития (HR) артериальной гипертензии (АГ), инфаркта миокарда (ИМ) и инсульта.

Материал и методы. Репрезентативна выборка мужчин в возрасте 25-64 лет (n=2149) была обследована на трех скринингах в рамках программы ВОЗ "МОНИКА-психосоциальная" в Новосибирске в 1984, 1988, 1994гг. Впервые возникшие случаи ИМ, АГ и инсульта после скрининга в когорте были зарегистрированы с 1984 по 2008гг. Генотипирование VNTR-полиморфизма было выполнено для генов DRD4 и DAT. Уровень тревожности оценивали с помощью теста Спилбергера. Стратифицированная Кокс-пропорциональная регрессионная модель использовалась для оценки риска развития (Hazard ratio — HR).

Результаты. Высокий уровень тревожности (ВУТ) в открытой мужской популяции составил 50,9%. Генотип 4/6 DRD4 и генотип DAT 9/9 были четко связаны с ВУТ. ВУТ достоверно повышал HR ССЗ. Риск развития АГ и инсульта был максимальным в течение первых пяти лет изучения, в то время как максимальный HR ИМ был в течение 10 лет.

Заключение. Распространенность ВУТ в открытой популяции мужчин 25-64-лет в Новосибирске была высокой. Уровни ВУТ имели ассоциации с определенным VNTR — полиморфизмом в генах DRD4 и DAT. ВУТ связан с повышенным риском развития ССЗ.

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Solely adverse environmental factors can hardly be fully responsible for the development of elevated levels of trait anxiety [1-6]. The study of 8-16-year-old twins from Great Britain showed genetic correlation between anxiety and depression [7]. The genetic correlation coefficient was as high as 80%, whereas factors of general environment accounted for the rest 20% [7]. Anxiety can be caused by the abnormal dopamine synthesis, [8–10] although the study regarding the relationships between anxiety traits and variable number of tandem repeat (VNTR) polymorphisms in the dopamine D4 receptor (DRD4) and the dopamine transporter (DAT) genes gained controversial results [11-14]. Great interest in studying anxiety is driven also by the fact that anxiety is considered as an independ-

Table 1

Genotype and allele frequencies of variable number of tandem repeat (VNTR) polymorphisms in the dopamine D4 receptor gene in 25–64-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

Table 2

Genotype and allele frequencies of variable number of tandem repeat (VNTR) polymorphisms in the DAT gene in 25–64-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

ent risk factor for cardiovascular morbidity and mortality [15-18]. To our knowledge, no available prospective population-based study in the literature describes similar data obtained by using the World Health Organization (WHO) programs.

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

Material 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) [8-10] and MONICA-psychosocial subprogram [21] in 1984, 1988, and 1994, respectively. A total of 2149 males aged 25-64 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 [22]. Spielberger's inventories were filled out by each participant individually. 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 [23-26]. Frequency distribution of the variable number of tandem repeat (VNTR) polymorphisms in the DRD4 and DAT genes in a population of 25-64-year-old males is shown in Table 1 and Table 2. The cohort for prospective study (n=1423) screened out all male participants diagnosed with ischemic heart disease, cerebrovascular pathology, arterial hypertension (AH), myocardial infarction (MI), and diabetes. 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 SPSS (Statistical Package for Social Sciences) 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 HR adjusted for different data collection dates. A value of $p<0,05$ was considered statistically significant [27-28].

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
χ^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
χ^2=15,980, df=12, p=0,192						

Annotation: ** — p<0,01; *** — p<0,001.

Abbreviations: LLA — low level of anxiety, MLA — moderate level of anxiety, HLA — high level of anxiety.

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
χ^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
χ^2=45,402, df=10, p=0,0001						

Abbreviations: LLA — low level of anxiety, MLA — moderate level of anxiety, HLA — high level of anxiety.

Results

Prevalence of trait anxiety in the population of 25–64-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 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. The heterozygote DAT genotype 9/10 was found in 35% of MLA group and in 38,8% of HLA group. The situation 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 (χ^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%) (χ^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% (χ^2=8,515, df=1, p<0,001), 58,7% (χ^2=23,185, df=1, p<0,0001), and 68,7% (χ^2=40,355, df=1, p<0,0001) in participants with AH, MI, and stroke, respectively. Within the first five years of observation, HR for developing AH, MI, and stroke in HLA group were 6,8-fold higher (95% CI=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 HR 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 HR 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 HR for developing CVD in HLA group over the entire 24-year period of the study (Figure 1).

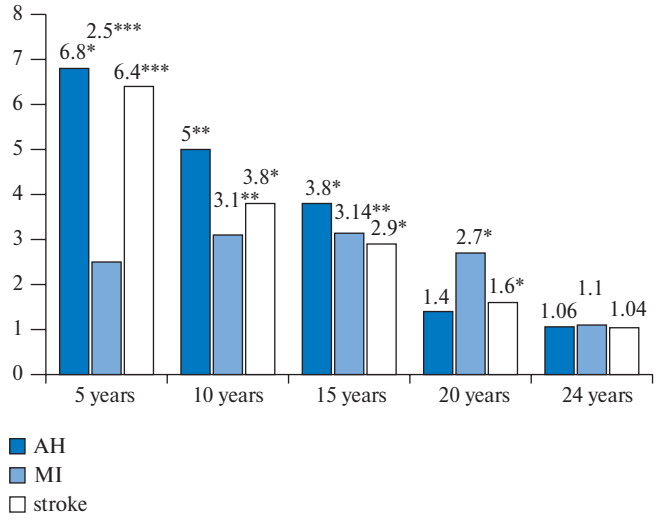

Figure 1. Comparative analysis of relative risk of developing CVD among the study participants with high level of anxiety (HLA) during 24-year period.
Annotation: *– p<0,05, ** — p<0,01, *** — p<0,001.

Discussion

More than half of 25-64-year-old males in the study population had HLA. 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.

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.

Our data provided evidence that trait anxiety significantly increased the risk of developing CVD. Maximal risks for developing AH (HR_{AH}=6,8) and stroke (HR_{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: HR_{AH}=5 and HR_{S}=3,8; for 20-year period: HR_{AH}=5 and HR_{S}=3,8). At the same time, HR for developing MI showed a different pattern, namely: maximal risk for developing MI was found within 10 years of the study (HR=3,1); 20-year period revealed some downward trend in MI HR (HR=2,7); both 10-year and 20-year period indices exceeded the HR rates for developing MI within the first five years of the study (HR=2,5).

The differently directed HR 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 HR 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 HR trend pattern, namely: maximal HR was registered for 10-year period, whereas minimal HR was found for the first five years [29-31]. The results of our study are consistent with data obtained by other authors [15]. Meta analysis of 20 studies, conducted from 1980 to 2009, showed that anxiety in originally healthy individuals increased risk for developing coronary artery disease (HR=1,26, 95% CI=1,15–1,38, p<0,0001) independently of demographic factors, biological risk factors, and lifestyle.

Conclusion

1. Prevalence of high level of anxiety in 25–64-year-old male population of Western Siberia metropolis (the city of Novosibirsk) was as high as 50,9%.

2. High level of anxiety in 25-64-year-old male population was associated with the DRD4 genotype 4/6 and the DAT genotype 9/9.

3. High level of anxiety in 25-64-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 25-64-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

1. Akzhigitov RG. Current trends in understanding and treatment of anxiety disorders. Rossiysky Meditsinsky Zhurnal. 2002; 1:43–5.
2. Kolutskaya EV. Anxiety disorders: diagnosis and treatment. Health of Ukraine Journal. 2006; 3:17.
3. Lapina NS, Borovkov NN. Anxious depressive conditions in patients with gastroesophageal reflux disease. Klin Med (Mosk). 2008; 86(2):59-62.
4. Raffety B, Smith R, Ptacek J. Facilitating and debilitating trait anxiety, situational anxiety and coping with an anticipated stressor: a process analysis. J Pers Soc Psychol. 1997 Apr;72(4):892-906.
5. Spielberger CD. Conceptual and methodological issues in anxiety research. In Spielberger CD, editor. Anxiety: current trends in theory and research (Vol. 2), NY: Academic Press; 1972. 481-93.
6. Comer R. Fundamentals of Abnormal Psychology. Prime-Euroznak Publishing House. 2007.
7. Bouchard TJ, Loehlin JC. Genes, Evolution, and Personality. Behav Genet. 2001 May; 31(3):243-73.
8. Heninger GR, Charney DS. Monoamine receptor systems and anxiety disorders. Psychiatr Clin North Am. 1988; 11: 309–26.
9. Nutt D, Laurence C. Panic attacks: a neurochemical overview of models and mechanisms. Br J Psychiatry. 1992; 160:165–78.
10. Roy-Byrne PP, Uhde TW, Sack DA, et al. Plasma HVA and anxiety in patients with panic disorder. Biol Psychiatry. 1986; 21:849–53.
11. Benjamin J, Osher Y, Belmaker RH, Ebstein R. No significant associations between two dopamine receptor polymorphisms and normal temperament. Hum Psychopharmacol. 1998; 13:(1)11–5.
12. de Brettes B, Berlin I, Laurent C, et al. 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. 1998; 13:427–30.
13. Eley TC, Ball DEJ, Freeman B, et al. Association study of extreme high and low neuroticism, with genetic markers for the dopaminergic system. Am J Med Genet. 1997;81: (6) 487.
14. Noble EP, Ozkaragoz TZ, Ritchie TL, et al. D2 and D4 dopamine receptor polymorphisms and personality. Am J Med Genet. 1998; 81: 257–67.
15. Roest AM, Martens EJ, de Jonge P, Denollet J. Anxiety and risk of incident coronary heart disease: a meta-analysis. J Am Coll Cardiol. 2010 Jun 29;56(1):38-46.
16. Gafarov VV, Pak VA, Gagulin IV, Gafarova AV. Psychology of health in Russia. Siberian Branch of the Russian Academy of Sciences Publishing House. Novosibirsk. 2002.
17. Gafarov VV, Pak VA, Gagulin IV, Gafarova AV. 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. 2000.
18. Gafarov VV, Gagulin IV. Epidemiologic approach to the study of trait anxiety. Bulletin of Siberian Branch of the Russian Academy of Medical Sciences. 1993; 3: 77-81.
19. World Health Organization Proposal for the Multinational Monitoring of Trends in cardiovascular disease. Geneva; 1985.
20. World Health Organization. MONICA Psychosocial Optional Study. Suggested Measurement Instruments. Copenhagen: WHO Regional Office for Europe; 1988.
21. World Health Organization. MONICA Psychosocial Optional Study. Suggested Measurement Instruments. Copenhagen: WHO Regional Office for Europe, 1988.
22. Spielberger CD. Anxiety as an emotional state. In Spielberger CD, editor. Anxiety: Current trends in theory and research. New York: Academic Press. 1972; 1:24-49.
23. 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.
24. Maniatis T, Fritsch EF, Sambrook J. Molecular cloning: a laboratory manual. Translation from English. Moscow. Mir Publishers. 1984.
25. Nanko S, Hattori M, Ikeda K, et al. Dopamine D4 receptor polymorphism and schizophrenia. Lancet. 1993; 341: 689-90.
26. Mitchell RJ, Howlett S, Earl L, et al. Distribution of the 3' VNTR polymorphism in the human dopamine transporter gene in world populations. Human Biology. 2000; 72(2): 295-304.
27. Cox DR. Regression Models and Life Tables. Journal of the Royal Statistical Society Series B. 1972; 34:187-220.
28. Glants C. Biomedical statistics. Translated from English. Moscow. Practika; 1998.
29. Gafarov VV, Gromova EA, Kabanov YuN, Gagulin IV. Personality and personal interaction with social environment: the untrodden road. Novosibirsk; 2008.
30. Gafarov VV, Gromova EA, Gagulin IV, Pilipenko PI. World Health Organization MONICA-psychosocial program: psychosocial factors and risk of developing stroke (epidemiologic study). Korsakov Journal of Neurology and Psychiatry. Suppl. Stroke. 2004; 12: 40-5.
31. Gafarov VV, Gromova HA, Gagulin IV, Ekimova YC, Santrapinskiy DK. Arterial hypertension, myocardial infarction and stroke: risk of development and psychosocial factors. Alaska Med. 2007; 49(2 Suppl):117-9.