UDC 575.167

DOI: 10.15372/KhUR20160417

Role of Environmental and Genetic Factors in the Development of Hemodynamically Significant Congenital Heart Diseases in a Coal Mining Region

A. V. SHABALDIN[1], S. A. SHMULEVICH[2], E. S. ANIKEEVA[1], A. V. TSEPOKINA[1], V. P. POTAPOV[3] and E. L. SCHASTLIVTSEV[3]

[1]*Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo, Russia*

E-mail: weit2007@yandex.ru

[2]*Kemerovo Cardiology Dispensary, Kemerovo, Russia*

[3]*Kemerovo Branch of the Institute of Computational Technologies, Siberian Branch, Russian Academy of Sciences, Kemerovo, Russia*

Abstract

A cross-sectional retrospective passive epidemiological study for pediatric population aged 0–17 was performed using stratified random sampling. Children with hemodynamically significant congenital heart disease (CHD) in the period from 2005 to 2012 were identified using the official statistical database of the Department of Public Health in the Kemerovo region. In addition, a prospective study of 188 children with hemodynamically significant CHD admitted to the Department of Pediatric Cardiology at the Kemerovo Cardiology Dispensary was conducted. The average incidence of hemodynamically significant CHD in the large cities with ^{137}Cs contaminated soil surface layers was significantly higher compared to that rate in the cities of the Kemerovo region, where the surface layers of intact soils are not contaminated with radioactive cesium (T_{gr} (av.) = +13.24 %; 95 % CI [9.25, 17.23] *vs.* T_{gr} (av.) = +5.31 %; 95 % CI [1.54, 9.07]; $p < 0.05$). Positive associations have been found between the risk of CHD in the next generation and the HLA-DRB1*11 and HLA-DRB1*03 allele in the female, the HLA-DRB1*07 allele in the male and a combination of HLA-DRB1*11 in the female and HLA-DRB1*01 in the male.

Key words: congenital heart diseases, radioactive cesium ^{137}Cs, HLA-DRB*

INTRODUCTION

The number of children with congenital heart disease (CHD) is increasing in the Russian Federation, as well as throughout the world [1]. CHD is the most common cause of early mortality or disability in young children [2]. However, complex hemodynamically significant heart defects prevail among wide spectrum of CHD anomalies. All advances in medical care, particularly high quality of diagnosis and neonatal cardiac surgery, allow providing medical care to all children with CHD and enlarge number of patients for regularly checkups. The rapid rise in CHD burden is caused by negative environmental factors that affect the development of a fetus [3]. Moreover, their number increases every year and results in high incidence of CHD in the population. Almost 90 % of all CHD have multifactorial nature and their formation is caused by a combination of genetic and environmental influences (including radioactive pollution). The critical period when it is known that CHD is forming in the embryo/fetus is rather short, compared with the corresponding periods in the origin of other organs and body systems. Therefore, CHD prevails among other congenital disease and fetal abnormalities around the world. On the other hand, genetic variability to teratogenic response may explain the differentiated formation of fetal CHD in a number of pregnant women living in the same area.

This article presents the results of two studies focused on the role of environmental and genetic factors in the formation of CHD during embryogenesis.

EXPERIMENTAL

A cross-sectional retrospective passive epidemiological study for pediatric population aged 0–17 was performed using stratified random sampling. The study sample covered the territory of the Kemerovo region. 33 communities were included, 17 urban and 16 rural communities. The data of 27 102 children with CHD were analyzed. Children with hemodynamically significant CHD in the period from 2005 to 2012 were identified using the official statistical database of the Department of Public Health in the Kemerovo region. The profile forms for pediatric cardiology service were developed to collect information according to the criteria listed in the ICD-10 and local regulations (the decree of the Department of Public Health No. 24, issued January 1, 2000). The profile form included demographic data, the number of children and adolescents with hemodynamically significant CHD, who required regular check-ups, the number of first registered cases, the structure of defects, and the methods of rehabilitation in patients with CHD. The profile forms for pediatric cardiology service were filed by senior pediatric cardiologist or pediatricians.

RESULTS AND DISCUSSION

The obtained results showed a significant increase in the number of CHD in the Kemerovo region during the analyzed period (Table 1). The long-term average rate of CHD prevalence for urban areas was 322.35 $^{0}/_{0000}$ (95 % CI [315.75, 348.95]), with a marked tendency to increase (T_{gr} (av.) = +10.54 %). Thus, urban areas demonstrated a positive tendency for both, boys and girls (see Table 1). However, the growth rate for girls was higher than for boys. The rural areas of the Kemerovo Region also had a positive tendency in the birth of children with CHD. The growth rate was much higher for boys than that for girls in the urban areas (see Table 1).

The role of contaminated intact soil surface layers with radioactive cesium (^{137}Cs) was studied to assess a radioactive macro environment factor. Figure 1 (an ecological map) presents the accumulation of active cesium (^{137}Cs) in soil surface layers. The United Institute of Geology, Geophysics and Mineralogy of the SB RAS provided their data obtained in the expeditions to depict the ecological system encompassing the region [4].

The assessment of the average growth rates of hemodynamically significant CHD in the urban areas of the Kemerovo region, depending on their location in the areas with ^{137}Cs contaminated surface layers or without it suggested specific patterns, presented in Table 2. According to the data presented in Table 2, the average growth rate of hemodynamically signifi-

TABLE 1

Indicators dynamics of the prevalence of "hemodynamically significant" congenital heart diseases for children aged 0–17 years in the Kemerovo Region depending on the patients gender (2006–2012; per 100 000)

Areas	Gender	2006	2007	2008	2009	2010	2011	2012	Lt. (av.)	T_{gr} (av.), %
Region	Male	182.0	261.5	288.0	305.3	359.4	369.6	390.9	308.1	10.59
	Female	221.28	292.63	332.33	361.47	419.33	423.19	477.00	341.71	11.03
	Both	201.28	276.77	309.71	332.76	388.68	395.87	435.23	334.33	10.88
Urban communities	Male	172.79	261.43	282.51	289.48	344.56	346.39	376.16	296.19	9.99
	Female	213.77	294.83	329.56	351.74	415.3	412.03	467.32	354.94	10.88
	Both	192.88	277.78	305.52	319.91	379.11	378.51	420.64	324.91	10.54
Rural communities	Male	227.64	262.13	315.79	384.38	433.01	492.81	496.32	373.15	13.25
	Female	258.26	281.74	346.14	409.94	439.25	482.09	507.75	389.31	11.42
	Both	242.65	271.73	330.65	396.88	436.06	487.56	511.58	382.45	12.55

Note. Lt. (av.) is long-term average rate; T_{gr} (av.) is average growth rate.

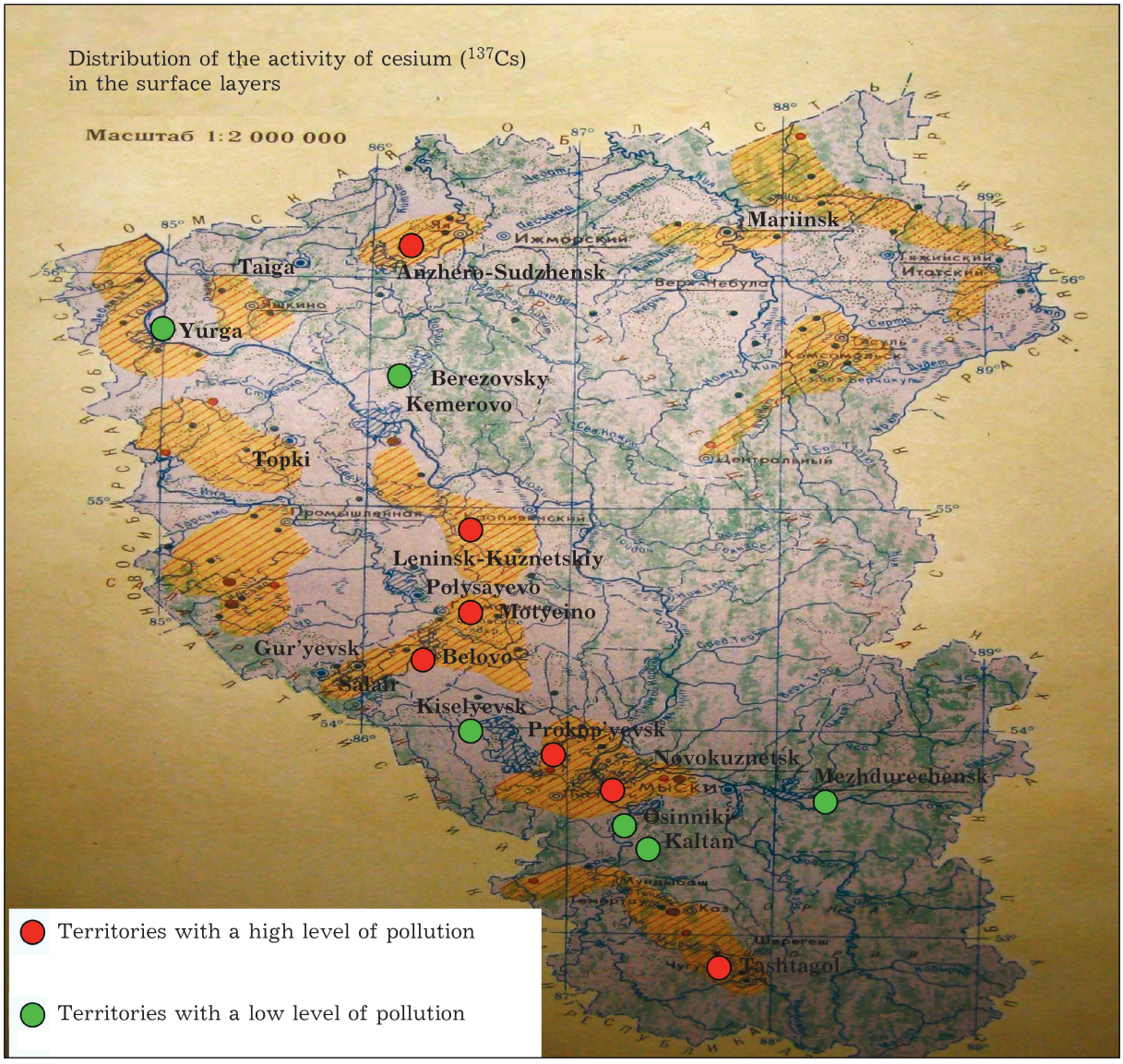

Fig. 1. Ecological map of the Kemerovo Region depicting the accumulation of active cesium (^{137}Cs) in the surface layer of the intact soils.

cant CHD in the large cities with ^{137}Cs contaminated soil surface layers was significantly higher compared to that rate in the cities of the Kemerovo region, where the surface layers of intact soils are not contaminated with radioactive cesium (T_{gr} (av.) = +13.24 %; 95 % CI [9.25, 17.23] *vs.* T_{gr} (av.) = +5.31 %; 95 % CI [1.54, 9.07]; $p < 0.05$). The obtained data suggested the negative effects of contaminated soil surface layers on the development of hemodynamically significant CHD. It should be noted that the highest growth rate of hemodynamically significant CHD was found in the cities of Anzhero-Sudzhensk (T_{gr} (av.) = +17.78 %), Tashtagol (T_{gr} (av.) = +17.51 %) and Leninsk-Kuznetskiy (T_{gr} (av.) = +15.08 %).

The lowest growth rates of CHD were in Polysaevo city (T_{gr} (av.) = +8.32 %) and in Belovo city (T_{gr} (av.) = +9.79 %).

Among the urban areas located on ^{137}Cs uncontaminated soils, the highest growth rate of hemodynamically significant CHD was found in the cities of Kiselyevsk (T_{gr} (av.) = +9.43 %) and in Yurga (T_{gr} (av.) = +8.54 %). The lowest growth rate in the positive tendency of the birth of children with CHD was observed in

TABLE 2

Growth rate of CHD in the urban areas of the Kemerovo Region, depending on their location in the areas with accumulated ^{137}Cs in intact soil surface layers and without radioactive accumulation

Cities	T_{gr} (av.) , %	Cities	T_{gr} (av.), %
Prokop′yevsk	14.02	Berezovskiy	5.90
Leninsk-Kuznetskiy	15.08	Kiselevsk	9.43
Polysaevo	8.32	Kaltan	4.87
Belovo	9.79	Osinniki	3.41
Anzhero-Sudzhensk	17.78	Mezhdurechensk	4.93
Tashtagol	17.51	Yurga	8.54
Mariinsk	11.09	Kemerovo	0.08
Myski	14.26		
Novokuznetsk	11.28		
Average rate for cities 95 % CI [LQ; UQ] 95 % CI [9.25, 17.23]	13.24;	$p < 0.05$	5.31; 95 % CI [1.54, 9.07]

the following cities: Kemerovo (T_{gr} (av.) = +0.08 %) and Osinniki (T_{gr} (av.) = +3.41 %).

The current study allowed determining a positive association between the radioactive macro environmental factor and the growth rate of children with hemodynamically significant CHD.

The prospective study was focused on the impact of genetic factors on the risk of the formation of CHD in the embryo. Children with hemodynamically significant CHD admitted to the Department of Pediatric Cardiology at the Kemerovo Cardiology Dispensary in the period from 2013 to 2015 were included in the study. A total of 188 children with hemodynamically significant CHD were recruited. The self-reporting questionnaires were designed and developed for parents as well as the plan of medical examination for both, parents and children. The self-reporting questionnaire included the following sections: social life of the parents (education, bad habits, occupational hazards, the storey number during pregnancy, the residence address during pregnancy, *etc.*), health issues (chronic diseases and conditions), family history of CHD and other diseases, obstetric and gynecological history, present pregnancy history, maternal history during the prenatal and early postnatal periods. All parents and their children underwent HLA-DRB1* typing. This particular gene was not chosen by chance, since CHD covers over 180 cardiovascular anomalies and the search of specific genetic markers for each CHD type seems to be difficult. The HLA locus is directly involved in the pregnancy gestation. Moreover, the immune interaction between mother's microenvironment and the embryo is determined by HLA suggesting whether the fetus is protected from teratogens or not. The HLA locus is known to be one of the most polymorphic among other human genes, and the alleles are distributed uniformly in the population. Moreover, positive associations have already been determined between the alleles and genotypes of HLA-DRB1* and reproductive losses, pathologies of the fetus and the newborn [5].

The control group included 54 couples who have more than one child without CHD, birth defects and developmental abnormalities. The control group also underwent HLA-DRB1* typing.

According to the results of the comparative analysis, there were no significant differences found in the genotyping frequencies which were identified in the study and control groups of women and the predicted frequencies using the Hardy–Weinberg equilibrium. The obtained data satisfied the Hardy–Weinberg equilibrium in the study sample. Therefore, we can compare the alleles and genotypic frequencies between the study group of women who have children with CHD and the control group of women who have healthy children.

Women who have children with CHD had higher prevalence of HLA-DRB1*03 (p = 0.0007) and HLA-DRB1*11 (p = 0.0036) of the

TABLE 3

Comparative characteristics of the frequencies of the HLA-DRB1 alleles in women

Alleles	Groups				p	OR (CI 95 %)
	Study n = 194		Control n = 108			
	Abs.	Share	Abs.	Share		
HLA-DRB1*01	20	0.10	17	0.16	0.231	0.61(0.24–1.57)
HLA-DRB1*03	22	0.11	2	0.02	0.001	5.56 (2.16–14.24)
HLA-DRB1*04	23	0.12	21	0.19	0.104	0.56(0.21–1.43)
HLA-DRB1*07	18	0.09	11	0.10	1	0.89 (0.34–2.27)
HLA-DRB1*08	6	0.04	9	0.08	0.083	0.36 (0.13–0.90)
HLA-DRB1*09	2	0.01	1	0.01	0.603	0.93 (0.36–2.38)
HLA-DRB1*10	5	0.03	0	0	0.22	6.30 (2.45–16.14)
HLA-DRB1*11	30	0.15	4	0.04	0.0036	4.31 (1.67–11.03)
HLA-DRB1*12	4	0.02	8	0.07	0.048	0.28 (0.10–0.71)
HLA-DRB1*13	30	0.15	14	0.13	0.671	1.21 (0.47–3.09)
HLA-DRB1*14	3	0.02	0	0	0.488	3.97 (1.54–10.16)
HLA-DRB1*15	26	0.13	18	0.17	0.54	0.77 (0.30–1.97)
HLA-DRB1*16	5	0.03	3	0.03	0.79	0.87 (0.34–2.24)

HLA-DRB1 gene (Table 3) compared to women in the control group. The odds ratio (OR) for the HLA-DRB1*11 allele was 4.31 (CI 95 % 1.67–11.03, $p < 0.01$), and for the HLA-DRB1*03 allele – 5.56 (CI 95 % 2.16–14.24, $p < 0.001$). Therefore, the obtained data suggest these alleles to be predisposing towards the determination of the CHD formation.

The HLA-DRB1*12 allele in the female with the odds ratio 0.28 (95 % CI 0.10–0.71) was found to have a protective effect against the risk of the CHD formation in the offspring.

It should be noted that the HLA-DRB1*11 and HLA-DRB1 12 alleles encode the antigenic determinant defined by serological typing of HLA, as HLA-DR5 [6]. But the discrete determination of the risk for CHD in the offspring by the HLA-DRB1*11 and HLA-DRB1*12 states the presence of particular immunogenetic mechanisms with the different impact on the teratogenesis in the embryonic period of the ontogenesis.

It is well known that the functional activity of HLA molecules is co-dominant. Therefore, the incidence of these genotypes in women with CHD children and in the control group of women with healthy children was assessed. It was found that only one genotype in the female was negatively associated with the development of CHD in their children. Thus, the frequency of HLA-DRB1*01.12 genotype in the control group of women with healthy children was 7.41 %, while in the study group this genotype has not been found (OR = 0.06, CI 95 % 0.022–0.148, $p < 0.01$). As one may see, this genotype includes the protective HLA-DRB1*12 allele.

According to the results of the comparative analysis, there were no significant differences found in the genotyping frequencies which were identified in men from the study and the control groups and the frequencies predicted using the Hardy–Weinberg equilibrium. The obtained data satisfied the Hardy–Weinberg equilibrium in the study sample. Therefore, we can compare the alleles and genotypic frequencies between the study group of men who have children with CHD and the control group of men who have healthy children.

One HLA-DRB1*07 allele was more frequently found in men from the study group, compared to the control group of men (15.2 % *vs.* 6 %, respectively, $p < 0.05$; OR = 4.53 CI 95 % [1.744, 11.755]). Both groups of men were comparable in the frequencies of other alleles and genotypes.

The analysis of family alleles and genotypes of HLA-DRB1* reported a high prevalence of

TABLE 4

Logistic regression of medical, social, ecological and genetic factors for the binary variable (CHD^+/CHD^-)

Model: Logistic regression Number 0: 49 1: 184 : Experiment − 1 control, 0 Failure: Maximum success Total failure: 96.804512482 chi2(7) = 46.080 p = .00000

	Maternal family history of CHD	^{137}Cs contaminated soils	HLA-DRB1*11 in the female potentiating	HLA-DRB1*03 in the female potentiating	HLA-DRB1*12 in the female protective	Prenatal growth rate	Maternal alcoholism
Score	1.7397	0.488209	1.19574	0.743103	−1.18534	−1.17024	31.72439
OR (units)	5.6958	1.629396	3.30601	2.102449	0.30564	0.31029	
OR (scale)	184.7822	2.654931	10.92968	9.293434	0.09342	0.31029	

the combinations of the HLA-DRB1*11 allele in the female and the HLA-DRB1*01 allele in the male from the families who have children with CHD. This combination has been found in 19.49 % of couples who have children with CHD and only in 1.53 % of families with healthy children ($p < 0.001$; OR = 13.6 CI 95 % [2.254, 35.205]). The second significant combination for families who have children with CHD, that has been found during the analysis, was a combination of the HLA-DRB1*03 allele in the female and the HLA-DRB1*07 allele in the male. The frequency of this combination of the alleles in the study group of families who have children with CHD was 8.47 %, whereas in the control group − 0.51 % ($p < 0.001$; OR = 12.61 CI 95 % [4.871, 32.640]).

As can be seen from the data presented, human HLA-DRB1* alleles and the combination of alleles in couples have a significant impact on the formation of the CHD.

The prospective study for children with CHD allowed searching the predictors of CHD using the logistic regression analysis. All factors that were mentioned by the parents of children with CHD and healthy subjects in the self-reporting questionnaires were used in the analysis (social, health, environmental and genetic). The regression analysis was aimed at developing an equation able to determine the risk of CHD. This equation may be considered as a software prototype able to determine the risk of CHD in couples who are planning pregnancy, *i. e.* in the preconception period.

Therefore, the factor analysis was applied. Twelve parameters out of 137 social, medical and genetic predictors of the risk of CHD were determined because of the closest relationship with the latent variables associated up to 66.8 % with a binary variable (CHD or no CHD).

Then, the logistic regression was performed using those 12 parameters. The findings of the logistic regression are presented in Table 4. The nonlinear dependence of the binary variable (CHD or no CHD) has been determined only for a few variables relevant to the influences of genetic, ecological, medical and social factors on the risk of CHD in the next generation.

A significant association has been found between these predictors and CHD. The quasi-Newton method was used during the assessment.

The following equation of the risk of CHD was derived based on the results of the logistic regression:

$$Y = (\mathrm{EXP}\ (Z)/(1 + \mathrm{EXP}\ (Z)) \times 100\ \%$$

$$Z = (X_1 \times 1.74) + (X_2 \times 0.49) + (X_3 \times 1.19) + (X_4 \times 0.74) + (X_5 \times (1.18)) + (X_6 \times (-1.17)) + (X_7 \times 31.72)$$

where Y is the probability of CHD in the next generation (%); X_1 is maternal family history of CHD (scores: 0 − no, 1 − yes); X_2 is the contamination of the surface layers of intact soils with radioactive cesium ^{137}Cs (scores: 0 − no pollution, 1 − moderate pollution, 2 − severe pollution); X_3 is HLA-DRB1*11 in the female, potentiating in (scores: 0 − no allele, 1 − in the heterozygote, 2 − in the homozygote); X_4 is HLA-DRB1*03 in the female, potentiating in (scores: 0 − no allele, 1 − in the heterozygote, 2 − in the homozygote); X_5 is HLA-DRB1*12 in females, protective (in points: 0 − no allele, 1 − in the heterozygote, 2 − in homozygote); X_6 is the normal growth in the prenatal period (scores: 0 − no, 1 − yes); X_7 is alcoholism of the mother (scores: 0 − no, 1 − yes).

The logarithmic nature of the equation for calculating the risk probability of CHD limits the range of the indicators from 0 to 1 (the closer

the indicator to 1 (or 100 %), the more likely the formation of CHD in the next generation). Therefore, the current equation clearly defines the risk of developing CHD in the next generation. Moreover, it can be used as an analogue to the calculator of the risk of CHD in the preconception period. According to the equation, female alcoholism is considered as one of the most significant factors contributing to the formation of CHD. The coefficient of the scores multiplying (0 – no alcohol, 1 – alcoholism) is 31.72, which appears to be 7 times higher than the burden of other predictors, including maternal family history of CHD. Another important predictor is the residence of the parents during pregnancy in the areas contaminated with radioactive cesium. A positive significance of the HLA-DRB1*11 and HLA-DRB1*03 alleles in the female has been found, but not in the male or family combinations.

The sensitivity and the specificity of each CHD predictor determined in the logistic regression were calculated, as well as the sensitivity and the specificity of the equation. The diagnostic efficiency of the equation was 78.3 %.

CONCLUSION

Thus, the current studies reported the significant impact of genetic and macro environmental factors on the risk of developing hemodynamically significant CHD.

Since 2015 the CHD Registry was initiated in the Municipal Budgetary Healthcare Institution "Kemerovo Cardiology Dispensary" in order to support all epidemiological, genetic, medical and environmental studies. The registry contains clinical and demographic data of parents and children with CHD, the results of genetic testing, and macroecological data of the residency area. The main goal of the register is to collect all necessary data regarding all CHD cases in the Kemerovo region.

REFERENCES

1 Bokeriya L. A., Gudkova R. G., Serdechno-Sosudistaya Khirurgiya. Bolezni i Vrozhdennye Anomalii Sistemy Krovoobrashcheniya, Bakoulev SCCS of RAMS, Moscow, 2014.
2 Mutaf'yan O. A., Vrozhdennye Poroki Serdtsa u Detey, vol 331, Nevskiy Dialekt, St. Petersburg, 2002.
3 Bespalova E. D., *Detskiye Bolezni Serdtsa i Sosudov*, 3 (2007) 29.
4 Ignatova A. Yu., Ekologiya (Methodological instructions), KuzGTU, Kemerovo, 2014.
5 Kiseleva A. N., Zajceva G. A., Isaeva N. V., Butina E. V., *Vestn. Akushera-Ginekologa*, 3 (2014) 16.
6 Jin B., Luo X. P., Ni H. C., Shen W., Shi H. M., Li Y., *Molec. Biol. Rep.*, 39, 1 (2012) 221.

УДК 575.167

DOI: 10.15372/KhUR20160417

Роль экологических и генетических факторов в формировании гемодинамически значимых врожденных пороков сердца в угледобывающем регионе

А. В. ШАБАЛДИН[1], С. А. ШМУЛЕВИЧ[2], Е. С. АНИКЕЕВА[1], А. В. ЦЕПОКИНА[1], В. П. ПОТАПОВ[3], Е. Л. СЧАСТЛИВЦЕВ[3]

Научно-исследовательский институт комплексных проблем сердечно-сосудистых заболеваний, Кемерово, Россия

E-mail: weit2007@yandex.ru

Кемеровский кардиологический диспансер, Кемерово, Россия

Кемеровский филиал Института вычислительных технологий Сибирского отделения РАН, Кемерово, Россия

Аннотация

Проведено одномоментное ретроспективное пассивное эпидемиологическое исследование детской популяции 0–17 лет методом стратифицированной рандомизации. Были использованы данные официальной медицинской статистики Департамента здравоохранения Кемеровской области за период 2005-2012 гг. о случаях рождения или выявления детей с гемодинамически значимыми врожденными пороками сердца (ВПС). Дополнительно проведено проспективное наблюдение за 188 детьми с гемодинамически значимыми ВПС, находившимся на лечении в детском отделении Кемеровского кардиологического диспансера. Выявлено, что на территориях с загрязнением поверхностных слоев ненарушенных почв ^{137}Cs средний темп прироста гемодинамически значимых ВПС по всем городам был достоверно выше, чем этот показатель в городах находящихся в районах Кемеровской области, где поверхностные слои неповрежденных почв не были загрязнены радиоактивным цезием ($T_{пр}$ (ср.) = +13.24 %; 95 % CI [9.25, 17.23] против $T_{пр}$ (ср.) = +5.31 %; 95 % CI [1.54, 9.07]; $p < 0.05$). Показана положительная ассоциация: женских аллелей HLA-DRB1*11 и HLA-DRB1*03, мужского HLA-DRB1*07 и сочетания в семейной паре женского HLA-DRB1*11 с мужским HLA-DRB1*01 – с риском формирования ВПС в последующем поколении.

Ключевые слова: врожденные пороки седца, радиоактивный цезий ^{137}Cs, HLA-DRB1*