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ABCA1 gene promoter methylation and sudden cardiac death

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ABSTRACT

Aim. To study the association of the methylation of the promoter of the *ABCA1* gene with sudden cardiac death (SCD).

Materials and methods. The study design is based on the case-control principle. The SCD group included 150 men (mean age (46.7 ± 9.2) years) who died of sudden cardiac death according to forensic medical examination data (the main pathological diagnoses are acute circulatory failure, acute coronary insufficiency). The control group included 150 men (mean age (42.6 ± 1.2) years) who died suddenly, but not due to cardiovascular pathology. DNA was isolated by phenol-chloroform extraction from myocardial tissue. The methylation status of the *ABCA1* gene promoter was assessed by methyl-specific polymerase chain reaction. The results obtained were statistically processed in SPSS 16.0 using Pearson's test and Fisher's test with Yates' correction for continuity. $P < 0.05$ was used as a level of significance.

Results. Comparing the groups revealed statistically significant differences in the methylation status of the gene promoter ($p = 0.015$). In the SCD group, the proportion of individuals whose *ABCA1* gene promoter is methylated is statistically significantly higher compared to the control group ($p = 0.020$; OR = 5.86; 95% CI (1.28–26.89)).

Conclusion. Methylation of the promoter of the *ABCA1* gene is associated with sudden cardiac death.

Key words: sudden cardiac death, methylation, *ABCA1*, promoter.

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Метилирование промотора гена *ABCA1* и внезапная сердечная смерть

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РЕЗЮМЕ

Цель. Исследование ассоциации метилирования промотора гена *ABCA1* с внезапной сердечной смертью (ВСС).

Материалы и методы. Дизайн исследования построен по принципу «случай – контроль». Группа ВСС включала 150 мужчин (средний возраст (46,7 ± 9,2) года), умерших внезапной сердечной смертью согласно данным судебно-медицинской экспертизы (основные патологоанатомические диагнозы – острая недостаточность кровообращения, острая коронарная недостаточность). Контрольная группа включает 150 мужчин (средний возраст (42,6 ± 1,2) года), умерших внезапно, но не вследствие сердечно-сосудистой патологии. ДНК выделена методом фенол-хлороформной экстракции из ткани миокарда. Оценка статуса метилирования промотора гена *ABCA1* проведена методом метил-специфической полимеразной цепной реакции. Полученные результаты статистически обработаны в SPSS 16.0 с применением критерия Пирсона, критерия Фишера с поправкой Йетса на непрерывность. В качестве уровня значимости использован $p < 0,05$.

Результаты. При сравнении групп выявлены статистически значимые различия по статусу метилирования промотора гена *ABCA1* между группами ($p = 0,015$). В группе ВСС доля лиц, у которых промотор гена *ABCA1* метилирован, статистически значимо больше по сравнению с контрольной группой ($p = 0,020$; ОШ = 5,86; 95%-й доверительный интервал (1,28–26,89)).

Заключение. Метилирование промотора гена *ABCA1* ассоциировано с внезапной сердечной смертью.

Ключевые слова: внезапная сердечная смерть, метилирование, *ABCA1*, промотор.

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INTRODUCTION

The term “sudden cardiac death” (SCD), according to the latest recommendations of the European Society of Cardiology, is used when a fatal outcome has developed within 1 hour from the onset of acute symptoms in the case of a known fatal cardiac pathology/found on autopsy fatal cardiac pathology/not found on autopsy of the causes of sudden death (sudden arrhythmic death). In the absence of witnesses to death, the time criterion for SCD is increased to 24 hours.

It is believed that the etiology of SCD at a young age is dominated by cardiac arrhythmias, cardiomyopathies, myocarditis and other more rare diseases, the contribution of which to the occurrence of SCD at the population level is small. At an older age, ischemic heart disease, also called coronary heart disease (CHD), heart failure, valvular defects, and secondary cardiac arrhythmias come out on top. At a young age, even after a high-quality forensic study, the cause of sudden death may remain unknown [1].

Currently, mortality due to cardiovascular diseases (CVD) occupies a leading position in the structure of mortality in the Russian population (583.1 people per 100 thousand of the population per year according to the Federal State Statistics Service of 2018) [2]. In almost half of cases, SCD develops in a provisionally healthy person who did not have any previous manifestations of cardiovascular pathology. The survival rate of patients after an episode of SCD, even while in a hospital, is still low [1].

In this regard, the identification of biomarkers that will help determine the predisposition to the development of SCD, especially in a patient without symptoms of cardiac pathology, is an important task of modern healthcare, since the existing clinical and diagnostic criteria for stratification of SCD risk play a significant role only for patients with already identified heart pathology, previous myocardial infarction and a history of sudden cardiac death. Thus, new biomarkers are essential for early primary prevention of SCD development.

SCD is a multifactorial nosology, the contribution to the development of which is made by genetic and environmental factors. To date, a huge number of polymorphisms and gene mutations associated with SCD have been identified. However, it is still unclear how genes with a critical role in the pathogenesis of SCD interact at the cellular level. It is not yet possible to use individual single nucleotide variants of genes as diagnostic markers of SCD. The data obtained during the study of DNA methylation can help to understand the mechanisms of the implementation of genetic information in the pathogenesis of SCD.

A number of studies have shown that the assessment of methylation of individual genes, as well as the results of genome-wide methylation, can be used as diagnostic markers of the risk of developing a disease (for example, atherosclerosis, CHD), the severity of symptoms, and the prognosis of the course. If epigenetic studies of this kind are carried out for many CVDs, then, according to the world literature data, DNA methylation studies of SCD have not yet been carried out.

The *ABCA1* gene (ATP binding cassette subfamily A member 1, 9q31.1) encodes a protein that is required for the removal of cholesterol from peripheral tissues. It has been shown that gene inactivation by methylation of its promoter is associated with the development of coronary artery disease, which is the most common SCD substrate among middle-aged and older people [3].

Thus, the aim of the project is to study the association of methylation of the *ABCA1* gene promoter with SCD.

MATERIALS AND METHODS

The study design is based on the case-control principle. The SCD group included 150 men (mean age (46.7 ± 9.2) years) who died of sudden cardiac death according to forensic medical examination data (the main pathological diagnoses were acute circulatory failure, acute coronary insufficiency). Criteria for exclusion from the group of sudden cardiac death were the presence of morphological changes in the heart tissue characteristic of myocardial infarction and cardiomyopathies. In addition, persons who were in a state of alcoholic and drug intoxication were excluded from the group. The control group included 150 men (mean age (42.6 ± 1.2) years) who died suddenly (1: 1 matching method), but not due to cardiovascular pathology. DNA was isolated by phenol-chloroform extraction from myocardial tissue in the group of sudden cardiac death and in the control group.

The selection of a control group from the DNA bank of people who died suddenly, and the use of DNA isolated from myocardial tissue, both in the SCD group and in the control group, were dictated by the proven tissue-specificity of DNA methylation.

The methylation status of the *ABCA1* gene promoter was assessed by methyl-specific polymerase chain reaction (PCR) on bisulfite-converted DNA. EZ DNA Methylation Kit (Zymo Research, USA) was used for bisulfite conversion of DNA samples. Methyl-specific PCR was carried out in two tubes: with primers specific for the methylated and unmethylated allele, according to the methods described in the study by H. Ghaznavi et al. [3].

The results obtained were statistically processed in SPSS 16.0 using Pearson's test, Fisher's test with Yates' correction for continuity; $p < 0.05$ was used as a level of statistical significance.

RESULTS

In the SCD group, 22% (33/150) of the *ABCA1* gene promoter is fully methylated (MM); 1.3% (2/150) is completely unmethylated (UU); 76.7% (115/150) had both methylated and unmethylated gene promoter (MU). In the control group, 27.3% (41/150) of the ABCA1 gene promoter is completely methylated; in 7.4% (11/150) it is completely unmethylated; 65.3% (98/150) had both methylated and unmethylated gene promoters. When comparing the groups, statistically significant differences in the methylation status of the *ABCA1* gene promoter were revealed between the groups ($p = 0.015$). In the SCD group, the proportion of individuals in whom the *ABCA1* gene promoter is methylated is statistically significantly greater than in the control group (MM + MU vs. UU: $p = 0.020$; OR = 5.86; 95% confidence interval (1.28–26.89)).

DISCUSSION

Epigenetic changes form the boundary between genotype and environment. It is believed that epigenetic variability may significantly contribute to the development of CVD. Over the past two decades, many studies have been conducted to find the link between DNA methylation and cardiovascular disease. DNA methylation is usually viewed in the context of CpG dinucleotide sequence (CpG sites). In mammalian somatic cells, most of the CpG sites are methylated. But CpG sites in regions of increased CpG density (CpG islands) are usually described as sites with reduced methylation. DNA methylation of the gene promoter is an important factor for the regulation of transcription [4]. It is known that hypomethylation of a gene promoter increases its expression, while hypermethylation decreases it. It is important to note that the level of methylation of individual genes is tissue-specific [5]. The accumulated knowledge suggests that epigenetic changes, such as DNA methylation abnormalities, may help to find an alternative explanation for the pathophysiology of CVD [6].

A variety of loci have been studied for methylation of individual genes for each cardiovascular phenotype. Studies of the methylation level in SCD according to the available world literature have not been carried out. However, studies have been conducted to study methylation in other CVDs that underlie SCD or have a similar pathophysiological basis. The most studied nosology in relation to methylation is coronary heart disease and its varieties.

The *ABCA1* gene (ATP binding cassette subfamily A member 1, 9q31.1) encodes a transporter of molecules across extra- and intracellular membranes. Using cholesterol as a substrate, protein functions as an efflux pump for the reverse transport of lipids in cells. Gene mutations are associated with the development of familial alpha-lipoprotein deficiency (hypoalphalipoproteinemia) and familial high-density lipoprotein deficiency [7]. Expression of the *ABCA1* gene has been identified as an independent predictor of the development of ischemic heart disease, atherosclerotic plaques, including uncalcified ones [8]. Methylation of the *ABCA1* gene promoter was identified as a significant risk factor for the development, but not the severity of the ischemic heart disease: the frequency of methylation of the promoter is higher in the group of people with ischemic heart disease compared to the control group, as well as in the older age group [3]. The methylation level of the *ABCA1* gene promoter negatively correlates with the concentration of high-density lipoprotein cholesterol in individuals with familial hypercholesterolemia. Additionally, methylation of the *ABCA1* gene is associated with a history of ischemic heart disease [7].

In a pilot study, it was shown that methylation of the *ABCA1* gene promoter can be used as a significant biomarker for early diagnosis of atherosclerosis [9]. In women in Japan, an inverse relationship between methylation of the gene promoter and the level of high-density lipoprotein cholesterol was confirmed, and a relationship was found between the methylation level of the *ABCA1* gene promoter and diet. In women with a diet enriched with vegetables and vitamins, the level of gene promoter methylation is significantly lower [10, 11]. Polymorphisms of the *ABCA1* gene are associated with the level of a number of lipid metabolism indicators: rs363717, rs2230806, rs4149313, rs9282541 – with the risk of coronary heart disease, rs2230808 – with the level of total cholesterol in the blood, rs363717, rs4149339, rs4149338 – with the increased levels of triglycerides in the blood. [9, 12, 13]. The rs2230806 polymorphism is associated with triglyceride levels in patients with severe dyslipidemia [14]. According to the meta-analysis, the level of high-density lipoprotein cholesterol is associated with the rs2246293 polymorphism [15].

According to our data, methylation of the *ABCA1* gene promoter is associated with the risk of SCD. In the SCD group, the proportion of individuals in whom the *ABCA1* gene promoter is methylated is statistically significantly higher than in the control group. According to the data of the world scientific literature, studies on the search for the relationship between

methylation of the *ABCA1* gene promoter and SCD have not been carried out. Nevertheless, foreign studies have shown that methylation of the gene promoter is associated with the risk of ischemic heart disease and impaired lipid homeostasis. Since ischemic heart disease is the most common substrate for the development of SCD in the older age group, our results are consistent. However, due to the small number of groups included in the study, additional verification of the identified association in groups with a larger size is required, with the inclusion of women in the study.

CONCLUSION

Methylation of the *ABCA1* gene promoter is statistically significantly associated with sudden cardiac death.

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Authors contribution

Ivanova A.A. – conception and design development, analysis and interpretation of the data. Gurazheva A.A. – carrying out of bisulfite conversion of DNA. Akinshina E.I., Maksimova S.V. – carrying out of methyl-specific PCR. Malyutina S.K. – conception and design development. Novoselov V.P., Rodina I.A., Khamovich O.V. – carrying out of the forensic medical stage of the study. Maximov V.N. – conception and design development, final approval of the article for publication.

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