ABCA1 gene promoter methylation and sudden cardiac death

Ivanova A.A.1, Gurazheva A.A.1, Akinshina E.I.1, Maksimova S.V.2, Malyutina S.K.1, Novoselov V.P.3, Rodina I.A.3, Khamovich O.V.3, Maksimov V.N.1

1 Institution of Internal and Preventive Medicine, Branch of the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences
175/1, B. Bogatkova Str., Novosibirsk, 630089, Russian Federation
2 Novosibirsk State Medical University
52, Krasny Av., Novosibirsk, 630091, Russian Federation
3 Novosibirsk Regional Office of Forensic Medical Examination
134, Nemirovicha-Danchenko Str., Novosibirsk, 630087, Russian Federation

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 (\( p = 0.020; \text{OR} = 5.86; 95\% \text{CI} (1.28–26.89) \)).

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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Ivanova Anastasia A., e-mail: ivanova_a_a@mail.ru.
Метилирование промотора гена ABCA1 и внезапная сердечная смерть

Иванова А.А.1, Гуражева А.А.1, Акиншина Е.И.1, Максимова С.В.2, Малютина С.К.1, Новоселов В.П.2, Родина И.А.2, Хамович О.В.2, Максимов В.Н.1

1 Научно-исследовательский институт терапии и профилактической медицины (НИИТПМ) – филиал Федерального исследовательского центра «Институт цитологии и генетики» Сибирского отделения Российской академии наук (ФИЦ ИЦиГ СО РАН) Россия, 630089, г. Новосибирск, ул. Б. Богаткова, 175/1
2 Новосибирский государственный медицинский университет (НГМУ) Россия, 630091, г. Новосибирск, Красный проспект, 52
3 Новосибирское областное клиническое бюро судебно-медицинской экспертизы (НОКБСМЭ) Россия, 630087, г. Новосибирск, ул. Немировича-Данченко, 134

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-медицинскими экспертизами (основные патологоанатомические диагнозы – острая недостача 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 р < 0,05.

Результаты. При сравнении групп выявлены статистически значимые различия по статусу метилирования промотора гена ABCA1 между группами (р = 0,015). В группе ВСС доля лиц, у которых промотор гена ABCA1 метилирован, статистически значимо больше по сравнению с контрольной группой (р = 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 symp-
toms 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.

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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. Ghaemzavi 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 (hypalphalipoproteinemia) 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 \textit{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 \textit{ABCA1} gene promoter is statistically significantly associated with sudden cardiac death.

REFERENCES

1. Priori S.G., Blomström-Lundqvist C., Mazzanti A., Blom N., Borggrefe M., Camm J., Elliott P.M., Fitzsimons D., Hatala R., Hindricks G., Kirchhof P., Kjeldsen K., Kuck K.H., Hernandez-Madrid A., Nikolaou N., Norekvål T.M., Spaulding C., van Veldhuisen D.J. The task force for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death of the European Society of Cardiology (ESC). \textit{G. Ital. Cardiol.} 2016; 17 (2): 108–170. DOI: 10.1714/2174.23496.

2. Federal State Statistics Service. Information and analytical materials. Natural movement of the population (in Russ.). URL: https://gks.ru/compendium/document/13269.

3. Ghaznavi H., Mahmoodi K., Soltanpour M.S. A preliminary study of the association between the \textit{ABCA1} gene promoter \textit{DNA methylation} and coronary artery disease risk. \textit{Mol. Biol. Res. Commun.} 2018; 7 (2): 59–65. DOI: 10.22099/mbrc.2018.28910.1312.

4. Nazarenko M.S., Markov A.V., Lebedev I.N., Freidin M.B., Sleptcov A.A., Koroleva I.A., Frolov A.V., Popov V.A., Barbaraš O.L., Puzyrev V.P. A comparison of genome-wide DNA methylation patterns between different vascular tissues from patients with coronary heart disease. \textit{PLoS One.} 2015; 10 (4): e0122601. DOI: 10.1371/journal.pone.0122601.

5. Wang X., Liu A.H., Jia Z.W., Pu K., Chen K.Y., Guo H. Genome-wide DNA methylation patterns in coronary heart disease. \textit{Herz.} 2018; 43 (7): 656–662. DOI: 10.1007/s00059-017-4616-8.

6. Banerjee S., Pondé C.K., Rajani R.M., Ashbaugh T.F. Differential methylation pattern in patients with coronary artery disease: pilot study. \textit{Mol. Biol. Rep.} 2019; 46 (1): 541–550. DOI: 10.1007/s11033-018-4507-y.

7. Guay S.P., Légaré C., Brisson D., Mathieu P., Bossé Y., Gaudet D., Bouchard L. Epigenetic and genetic variations at the \textit{TNNT1} gene locus are associated with HDL-C levels and coronary artery disease. \textit{Epiigenomics.} 2016; 8 (3): 359–371. DOI: 10.2217/epi.15.120.

8. Infante T., Forte E., Schiano C., Punzo B., Cademartiri F., Cavaliere C., Salvatore M., Napoli C. Evidence of association of circulating epigenetic-sensitive biomarkers with suspected coronary heart disease evaluated by cardiac computed tomography. \textit{PLoS One.} 2019; 14 (1): e0210909. DOI: 10.1371/journal.pone.0210909.

9. Ma S.C., Zhang H.P., Kong F.Q., Zhang H., Yang C., He Y.Y., Wang Y.H., Yang A.N., Tian J., Yang X.L., Zhang M.H., Xu H., Jiang Y.D., Yu Z. Integration of gene expression and DNA methylation profiles provides a molecular subtype for risk assessment in atherosclerosis. \textit{Mol. Med. Rep.} 2016; 13 (6): 4791–4799. DOI: 10.3892/mmr.2016.5120.

10. Fujii R., Yamada H., Munetsuna E., Yamazaki M., Mizuno G., Tsuibo Y., Ohashi K., Ishikawa H., Ando Y., Hagiwara C., Maeda K., Hashimoto S., Hamajima N., Suzuki K. Dietary vegetable intake \textit{A1} (ABCA1) \textit{DNA methylation} levels among Japanese women. \textit{Nutrition.} 2019; 65: 1–5. DOI: 10.1016/j.nut.2019.02.010.

11. Fujii R., Yamada H., Munetsuna E., Yamazaki M., Ando Y., Mizuno G., Tsuibo Y., Ohashi K., Ishikawa H., Hagiwara C., Maeda K., Hashimoto S., Suzuki K. Associations between dietary vitamin intake, \textit{ABCA1} gene promoter DNA methylation, and lipid profiles in a Japanese population. \textit{Am. J. Clin. Nutr.} 2019; 110 (5): 1213–1219. DOI: 10.1093/ajcn/nqz181.

12. Lu Y., Liu Y., Li Y., Zhang H., Mu Y., Kanu J.S., Qiao Y., Tang Y., Zhen Q., Cheng Y. Association of ATP-binding cassette transporter \textit{A1} gene polymorphisms with plasma lipid variability and coronary heart disease risk. \textit{Int. J. Clin. Exp. Pathol.} 2015; 8 (10): 13441–13449.

13. Wang F., Ji Y., Chen X., Song Y., Huang S., Zhou C., Huang C., Chen Z., Zhang L., Ge J. \textit{ABCA1} variants rs2230806 (R219K), rs4149313 (M8831I), and rs9282541 (R230C) are associated with susceptibility to coronary heart disease. \textit{J. Clin. Lab. Anal.} 2019; 33 (6): e22896. DOI: 10.1002/jcla.22896.

14. Smirnov G.P., Malyshnev P.P., Rozhkova T.A., Zubareva M.Y., Shuvalova V.A., Rebrikov D.V., Titov V.N. The effect of \textit{ABCA1} rs2230806 common gene variant on plasma lipid levels in patients with dyslipidemia. \textit{Klin. Lab. Diagn.} 2018; 63 (7): 410–413. DOI: 10.18821/0869-2084-2018-63-7-410-413.

15. Ma Y., Follis J.L., Smith C.E., Tanaka T., Manichaikul A.W., Chu A.Y., Samieri C., Zhou X., Guan W., Wang L., Biggs M.L., Chen Y.D., Hernandez D.G., Borecki I., Chasman D., Rich S.S., Ferrucci L., Irvin M.R., Aslibekyan S., Zhi D., Tiwari H.K., Claas S.A., Sha J., Kabagambe E.K., Lal C.Q., Parnell L.D., Lee Y.C., Amouyel P., Lambert J.C., Psaty B.M., King I.B., Mozaffarian D., McKnight B., Bandinelli S., Hernandez-Madrid A., Nikolaou N., Freidin M.B., Sleptcov A.A., Frolov A.V., Popov V.A., Barbaraš O.L., Puzyrev V.P. A comparison of genome-wide DNA methylation patterns between different vascular tissues from patients with coronary heart disease. \textit{PLoS One.} 2015; 10 (4): e0122601. DOI: 10.1371/journal.pone.0122601.

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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.

Authors information

Ivanova Anastasia A., Cand. Sci. (Med.), Senior Researcher, Laboratory of Molecular Genetic Investigation of Internal Diseases, Institution of Internal and Preventive Medicine, Novosibirsk, Russian Federation. ORCID 0000-0002-9460-6294.

Gurazheva Anna A., Junior Researcher, Laboratory of Molecular Genetic Investigation of Internal Diseases, Institution of Internal and Preventive Medicine, Novosibirsk, Russian Federation. ORCID 0000-0003-1547-624X.

Akinshina Elena I., Junior Researcher, Laboratory of Molecular Genetic Investigation of Internal Diseases, Institution of Internal and Preventive Medicine, Novosibirsk, Russian Federation. ORCID 0000-0002-9224-9147.

Maksimova Sofya V., 3rd-Year Student, Department of Pediatrics, Novosibirsk State Medical University, Novosibirsk, Russian Federation. ORCID 0000-0002-2472-181X.

Malyutina Sofia K., Dr. Sci. (Med.), Professor, Head of the Laboratory of Etiopathogenesis and Clinical Features of Internal Diseases, Institution of Internal and Preventive Medicine, Novosibirsk, Russian Federation. ORCID 0000-0001-6539-0466.

Novoselov Vladimir P., Dr. Sci. (Med.), Professor, Head of Novosibirsk Regional Office of Forensic Medical Examination, Novosibirsk, Russian Federations. ORCID 0000-0002-6312-5543.

Rodina Irina A., Cand. Sci. (Med.), Forensic Expert Physician, Novosibirsk Regional Office of Forensic Medical Examination, Novosibirsk, Russian Federation. ORCID 0000-0003-2799-0756.

Khamovich Olesya V., Cand. Sci. (Med.), Forensic Expert Physician, Novosibirsk Regional Office of Forensic Medical Examination, Novosibirsk, Russian Federation. ORCID 0000-0002-960-193X.

Maksimov Vladimir N., Dr. Sci. (Med.), Associate Professor, Head of the Laboratory of Molecular Genetic Investigation of Internal Diseases, Institution of Internal and Preventive Medicine, Novosibirsk, Russian Federation. ORCID 0000-0002-7165-4496.

(✉) Ivanova Anastasia A., e-mail: ivanova_a_a@mail.ru.

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