Role of DNA Methylation in Development of Cardiovascular Diseases, Resulting in a Sudden Cardiac Death (Review)

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An effective system to diagnose predisposition to development of sudden cardiac death (SCD) is required in order to determine the risk of developing a sudden fatal outcome well in advance of the onset thereof, including in people with asymptomatic cardiovascular disease, as well as to implement early preventive measures that can result in a decrease in the population mortality from cardiovascular diseases. Thus, the search for SCD risk markers becomes a topical issue for modern health care.

According to recent studies, epigenetic mechanisms of heredity, and DNA methylation above all, play an important role in development of many diseases. The review provides the results of recent foreign and Russian studies on identification of a link between DNA methylation and development of cardiovascular diseases being the basis for SCD (IHD, cardiomyopathies, heart rhythm disturbances). The major part of the review is dedicated to studying DNA methylation in IHD, which is the most epigenetically explored nosology at the moment. Attention is also paid to studies of the DNA methylation role in development of acute coronary syndrome and myocardial infarction, which have development mechanisms similar to those of SCD. There were only few studies on identification of a link between DNA methylation and cardiomyopathies and cardiac arrhythmias conducted, however, an association of specific genes methylation with the explored nosologies was revealed. The review also provides pathogenetic substantiations of the possibilities to use epigenetic markers of cardiovascular diseases as SCD markers.

Thus, it has been established that study of genes the methylation of which is associated with IHD (*CTH*, *PLCB1*, *PTX3*, *MMP9*, *FN1*, *F2RL3*, *ABCB1*, *FOXP3*, *GDF15*, *IL6*, *CASR*), with lipid metabolism disorders and atherosclerosis (*CETP*, *CCL2*, *SREBF2*, *TIMP1*), as well as with heart rhythm disturbances (*SCN5A* and *KCNQ1*), may be most promising in relation to SCD.

Key words: sudden cardiac death; DNA methylation; ischemic heart disease; cardiomyopathy; myocardial infarction; acute coronary syndrome; heart rhythm disturbances.

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Introduction

Despite a recent significant progress in prevention of ischemic heart disease (IHD) and cardiac failure, the problem of high mortality from cardiovascular diseases has not yet been solved [1, 2]. A large share (25 to 50%) in the cardiovascular mortality breakdown is taken by the sudden cardiac death (SCD) [1, 3]. According to recommendations of the European Society of Cardiology, the "sudden cardiac death" term should be used in case of a sudden lethal outcome (non-traumatic, unexpected death within 1 h after the onset of symptoms in an assumed healthy person or within 24 h from the moment when the deceased was last seen alive if the death was unwitnessed), considering the following: whether the past history of the deceased contains an indication of a congenital or acquired potentially lethal heart disease; whether the autopsy revealed a cardiac or vascular anomaly which might have caused death; whether autopsy revealed no extracardiac causes of death (in this case, arrhythmia is the most probable cause of the lethal outcome) [1].

The dominant SCD cause in adults is chronic degenerative diseases (IHD, valvular heart diseases, heart failure), of which IHD is the first (75%), whereas heart rhythm disturbances and cardiomyopathy are seen less often (15%) [1, 4, 5]. In younger people and children, SCD development is promoted by

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cardiomyopathy, congenital heart rhythm disturbances, myocarditis, myocardial ischemia due to coronary vessel pathologies or heart failure.

Pathophysiologically, SCD may have a predisposing substrate (e.g., an anatomical substrate being regions of collagen mixed with viable cardiomyocytes after myocardial infarction, or a functional substrate represented by channelopathies in long QT syndrome), when there is a trigger, which develops ventricular tachycardia or fibrillation, less often bradyarrhythmia, asystole, or complete atrioventricular block [2, 6, 7]. It is not always possible to identify the factor that caused the heart rhythm disturbance even after a forensic medical examination of the deceased from SCD and a post-mortem molecular genetic study (according to different sources, it is possible only in 2–54% of all SCD instances) [1, 2, 7]. At that, almost 50% of people with SCD were not diagnosed with cardiovascular diseases during their lives [1].

The most promising measure in SCD prevention is stratification of individual risk of its development, including with the help of genetic markers [1]. Genetic and environmental factors contribute to the development of multifactorial SCD nosology. A large number of polymorphisms and gene mutations associated with SCD have been identified by the present day [8]. However, it is still unclear how significant genes interact at the cellular level in the SCD pathogenesis. The discovery of DNA methylation provided for studying the relationship between the genetic information embedded in the DNA sequence and the disease phenotype. Studies on DNA methylation can help explaining the mechanism of genetic information presentation in a disease pathogenesis.

DNA methylation

Epigenetic changes, including DNA methylation, form an important mechanism by which the environment can influence the genome. Epigenetic modifications are not associated with a change in the DNA nucleotide sequence, but they can affect gene expression and contribute to diseases development. During the past two decades, a lot of research has been conducted to find the connection between DNA methylation and cardiovascular diseases.

DNA methylation is usually considered in the context of a CpG dinucleotide sequence (CpG sites) and entails addition of a methyl group to a cytosine in this cytosine phosphate guanine dinucleotide [9]. In somatic mammalian cells, most CpG sites are methylated (70–90%) [10]. But CpG sites in the regions of the increased CpG density (CpG islands) are generally described as sites of the decreased methylation level. DNA methylation of a gene promoter is an important factor in regulation of gene transcription [11]. It is known that hypomethylation of a gene promoter increases its expression, while hypermethylation decreases it [12]. DNA methylation stabilizes chromatin structure during transcription and can vary greatly in different tissues and throughout human life [9]. Moreover, DNA methylation depends on age, gender, and ethnicity; this process is also affected by nicotine consumption [13]. In their systematic review, Asllanaj et al. [14] showed that the level of DNA methylation depends on gender, including in case of lipid metabolism disorders and cerebrovascular accidents; gender-dependent differences were also found in the methylation of individual genes in cardiovascular pathologies. According to the authors, this may explain the different frequency of cardiovascular pathologies development risks in men and women.

DNA methylation is involved in the following processes: X-chromosome inactivation; activity of mobile retro-elements; cellular differentiation; programming, survival, fatality, and imprinting of parental genes; activity of the immune system [15]. Methyltransferases, including DNMT1, DNMT2, DNMT3a, and DNMT3b are responsible for DNA methylation. DNMT3a and DNMT3b are responsible for *de novo* DNA methylation. DNMT1 methyltransferase is required during DNA replication to copy information about the methylation pattern from a parent strand to a daughter strand. Passive change in methylation status is possible only by silencing DNMT1 methyltransferase function. Active change in the methylation pattern can be achieved differently. Deamination converts 5-methylated cystine to thymine, which is corrected into unmethylated cystine in case of repair synthesis. Another path involves TET1, TET2, TET3 enzymes, which can add a hydroxyl group to a methyl group, converting 5-methylcytosine to 5-hydroxymethylcytosine, cleaved by thymine DNA glycosylase. Regardless of the existing mechanisms, DNA methylation of CpG sites is stable for the majority of tissues [16]. In case of methylation, gene expression can be changed differently. The first way to block gene expression involves inability of transcription factors and DNA to interact in case of methylated CpG sites [15]. The second way provides for interaction with the methyl binding domain proteins methylated by CpG sites, including MCP2, MBD1, MBD2, MBD4, which transcribe CpG site methylation and thus stop or suppress transcription [10].

There are several major approaches to studying DNA methylation: measuring the global level of DNA methylation; study of methylation of specific candidate genes; epigenome-wide analysis of DNA methylation, including epigenome-wide association studies (EWAS) [17]. The accumulated knowledge suggests that epigenetic alterations, such as DNA methylation pathologies, may help reveal an alternative explanation for the pathophysiology of a cardiovascular disease [18]. In addition to methylation of specific genes associated with development of a particular disease, the level of genome-wide methylation is also explored, including with the help of modern next-generation sequencing technologies [19]. Genome-wide association studies

identified many single nucleotide polymorphisms localized in non-coding segments, but still associated with diseases. It is assumed that epigenetic mechanisms can explain some of the mentioned results [20].

There were many studies related to DNA methylation for each cardiovascular phenotype. In a number of instances (for example, in case of atherosclerosis, IHD), diagnostic markers of the disease development, severity, and prognosis were determined. There were no articles on studying DNA methylation in SCD found in the available world literature, except for our pilot study [21], which showed that methylation of the *ABCA1* gene promoter is associated with SCD. It is known that the *ABCA1* (ATP binding cassette subfamily A member 1) gene encodes the protein related to cholesterol transportation. Gene inactivation by the gene promoter methylation is associated with the IHD development being the most frequent SCD substrate for the middle-aged and the elder [22].

DNA methylation and ischemic heart disease

The most explored nosology in relation to DNA methylation is ischemic heart disease [5]. The association of the disease with the global DNA hypermethylation is established. The researchers found a large number of genes the methylation of which is associated with IHD, including myocardial infarction and acute coronary syndrome (ACS). Some of these genes have been recently found (see Appendix 1). For instance, Sharma et al. [23] identified 72 hypermethylated regions in individuals with IHD as well as 6 CpG sites, including the intronic region of the *C1QL4* gene, control elements of the *CCDC47* and *TGFBR3* genes, the methylation of which is associated with this disease. A genome-wide methylation in patients with IHD study identified critical genes (*ABCA1, DDAH2*) and sequences (LINE-1 and Alu), the methylation of which is also associated with the risk of this pathology development. LINE-1 and Alu are large, high-copy retrotransposons of the human genome. The level of these elements' methylation differs significantly in patients with IHD and people from the control group [24, 25].

A case-controlled study in a group of patients with IHD (178 people) and a control group (156 people) demonstrated the association of the examined nosology with the *CTH* gene promoter methylation [26]. The *CTH* (cystathionine gamma-lyase) gene encodes a cytoplasmic enzyme that converts cystathionine to cysteine. The rs113044851 insertion-deletion polymorphism of this gene was found to reduce the risk of SCD [27]. Therefore, the *CTH* gene can be considered as a candidate gene for susceptibility to SCD. Guo et al. [28] showed that the level of *PTX3* (pentraxin 3) gene methylation (this gene plays a role in the development of inflammation and atherogenesis) is significantly lower in the IHD group compared to the control group. The *PTX3* gene encodes a protein the expression of which is induced by inflammatory cytokines in response to inflammation; this protein is also involved in angiogenesis and tissue remodeling. Several studies demonstrated a rapid increase in the concentration of the PTX3 protein in the blood plasma in patients with ACS. For instance, Tajo et al. [29] measured the level of PTX3 in individuals who died from a fatal ACS and in those who died differently. It turned out that the PTX3 concentration was higher in the ACS group with coronary thrombosis compared with the control group and the ACS groups with coronary stenosis and the heart tissue alterations typical of myocardial infarction. During a three-year follow-up [30], it was shown that the level of PTX3 in the blood of patients with the chronic heart failure is also higher compared to that of healthy individuals and correlates with the severity of the heart failure according to NYHA. Moreover, it was found that in patients with the developed endpoints (cardiac death, repeated hospitalization, higher severity of the disease) the level of PTX3 in blood plasma is higher than in individuals without such endpoints [30]. Thus, studying of the *PTX3* gene methylation in SCD may be a promising region of research when getting a positive result is highly probable as this gene is involved in angiogenesis and tissue remodeling, the *PTX3* promoter methylation association with IHD was shown, and there is information of a link between the level of its protein with ACS (including fatal ACS) and chronic heart failure (including its outcomes resulted in cardiac death).

According to the case-controlled study [31], hypomethylation of the *COMT* gene promoter is associated with an increased risk of IHD in men. The risk of IHD development, and especially acute myocardial infarction, also increases with hypomethylation of the *IL6* gene promoter [32]. Methylation of the *GCK*, *GALNT2*, *TNNT1*, *PLA2G7*, *MMP9*, *FOXP3*, *ANGPTL2*, and *ABCG1* genes is also associated with IHD [19, 33–37]. The *MMP9* gene (matrix metallopeptidase 9) is of interest from the point of view of studying methylation in SCD. The level of MMP-9 protein is associated with myocardial fibrosis in hypertrophic cardiomyopathy and the related cardiac events in women (syncope, ventricular tachycardia) [38]. Hou et al. [39] found that in the group of people with IHD, in whom according to coronary angiography the coronary artery stenosis did not exceed 50%, the MMP-9 concentration was higher than in the control group and correlated with the Framingham risk score. In this regard, the authors suggest that MMP-9 levels may be helpful in identification of patients at risk related to myocardial infarction and SCD. There is also data [40] on MMP-9 association with atherosclerosis and atherosclerotic plaque instability. Thus, the MMP-9 protein can be a SCD marker, which makes the *MMP9* gene promising for studying methylation in case of SCD.

In 2017, a systematic review on DNA methylation in patients with IHD was published [17]. Based on the analysis of scientific articles the authors concluded that the contribution of global DNA methylation to the

IHD development is uncertain. At the same time, the association of some candidate genes methylation with IHD can be considered as confirmed (hypermethylation of the *ESRα*, *ABCG1*, *FOXP3* genes, hypomethylation of the *IL6* gene). The analysis of epigenome-wide association studies identified 84 genes that are differentially methylated in case of IHD (a third part of these genes are markers of obesity).

In 2019, an epigenome-wide association study [41] identified 52 CpG sites associated with IHD, some of which are localized in calcium regulation genes (*ATP2B2*, *CASR*, *GUCA1B*, *HPCAL1*) and are associated with atherosclerotic plaque calcification (*PTPRN2*) and kidney functioning (*CDH23*, *HPCAL1*). From the point of view of SCD, the study of the *CASR* (calcium-sensing receptor) gene methylation related to calcium metabolism is of interest. For instance, a meta-analysis of data from exome-wide studies on chips [42] provides information about several new polymorphisms associated with the QT interval, one of which is rs1801725 of the *CASR* gene. Long QT syndrome, in turn, is also a risk factor for the SCD development. According to the results of a pilot epigenome-wide association study [18], there were 429 differentially methylated regions (222 hypomethylated and 207 hypermethylated) identified in patients with IHD and individuals from the control group; there was also a panel of loci with the most different methylation status created, it included mainly the genes of the HLA system and inflammation. Using the method of methyl-specific PCR, it was found that the level of methylation of the *ABCA1* gene promoter is statistically significantly higher in patients with angiographically confirmed IHD (n=110) compared to the control group (n=110) [22]. In a large Russian study, the methylation status of the promoter regions of the *TXNRD1*, *GSTP1*, *GCLM* genes, 4–6th exons of the *MPO* gene in IHD, arterial hypertension, and acute cerebrovascular accident was studied. An insignificant decrease in the level of the *GCLM* and *MPO* genes methylation in case of IHD was found in comparison with the control group. In the event of combination of arterial hypertension and IHD, a decrease in the level of methylation was noted for all the studied genes, whereas in the event of combination of arterial hypertension, IHD, and acute cerebrovascular accident — for all genes except *TXNRD1* [43]. Miao et al. [44] reported 11 differentially methylated loci localized in the *BDNF*, *BTRC*, *CDH5*, *CXCL12*, *EGFR*, *IL6*, *ITGB1*, *PDGFRB*, *PIK3R1*, *PLCB1*, and *PTPRC* genes in case of IHD. Only *PLCB1* (phospholipase C beta 1) was studied out of these 11 genes in terms of association with SCD. For instance, our study [45] indicates an association of the single nucleotide polymorphism rs16994849 of the *PLCB1* gene with SCD: the GG polymorphism genotype is a SCD risk genotype for persons under 50 years and has a protective effect for people over 50 years; AA polymorphism genotype has a protective effect in terms of SCD for persons under 50 years. It is known that an increase in *PLCB1* gene expression is associated with hypertrophy of cardiomyocytes. Lin et al. [46] established an association of *PLCB1* gene polymorphisms with the concentration of apolipoprotein B, total cholesterol, and high-density lipoprotein cholesterol in blood. Zhang et al. in their recent study [47] identified potential IHD biomarkers (*FN1*, *PTEN*, *POLR3A*), the expression of which is associated with the level of DNA methylation and the risk of IHD. In people with SCD, type 2 diabetes mellitus, and preserved ejection fraction, the expression of the *FN1* gene (fibronectin 1) is higher than in those who died from other causes. The *FN1* gene is known to be a candidate gene for IHD [48].

The KAROLA prospective cohort study [49] demonstrated an association of *F2RL3* gene methylation (F2R like thrombin or trypsin receptor 3) with mortality in people with IHD. The *F2RL3* gene encodes a proteinase-activated receptor which is involved into coagulation, inflammation, and pain response. 1206 study participants (patients who had myocardial infarction, ACS, or coronary artery surgery) were followed up for 8 years. During this period, there were 64 cardiovascular deaths and 50 deaths from other causes. Representatives of the lowest quartile of the *F2RL3* gene methylation showed the adjusted odds ratio for cardiovascular death of 2.32 compared to representatives of the highest quartile, but the 95% confidence interval (0.97–5.58) was not statistically significant, whereas the odds ratio and 95% confidence interval for non-cardiovascular death and death from all causes were statistically reliable.

In a more recent study [50] of *F2RL3* gene methylation (3588 patients; 10.1 years of follow-up) conducted on the basis of the ESTHER project, the odds ratio for cardiovascular death was 2.45; the 95% confidence interval (1.28–4.68) was statistically significant. The identified association was more significant for men than for women. It was also shown that the *F2RL3* gene methylation is associated with smoking, which is a risk factor for cardiovascular events [50]. Moreover, an increase in the *F2RL3* gene expression in case of IHD was reported [51]. Thus, the *F2RL3* gene, which is related to coagulation, can be considered as a candidate for future studies of methylation in case of SCD, as methylation of this gene is associated with IHD (the main cause of SCD in the adult population), cardiovascular death, and smoking, which is also a risk factor for SCD.

Another gene of interest in relation to SCD is *ABCB1* (ATP binding cassette subfamily B member 1), the hypomethylation of which is associated with less aspirin absorption, higher platelet activity, and an increased risk of ischemic events (vascular death, repeated ischemic stroke, myocardial infarction, or transient ischemic attack) in people with intracranial stenosis [52].

The most severe forms of IHD are ACS and myocardial infarction. Arrhythmias after myocardial infarction and ACS often result in SCD, which in most cases occurs when a patient has ACS at the hospital stage [53]. Soares et al. [54] found that in patients with

ACS (190 persons) the level of global DNA methylation is higher compared to healthy people (75 persons) of the same gender and age. At that, in patients with a low result on the TIMI score, the level of DNA methylation is higher compared to patients at high and medium risk.

In the study of genome-wide methylation in ACS, 19 hypermethylated loci and 17 hypomethylated genes that may be markers of ACS were identified, however, the association of methylation with nosology in the case-control study using methyl-specific PCR was confirmed only for the *SMAD3* locus [55]. Another epigenome-wide study using the whole blood of 102 patients with ACS and 101 people in the control group identified 47 CpG sites associated with ACS. 26 of them correlate with the level of expression of the corresponding genes, including the *IL6R*, *FASLG*, and *CCL18* genes [56]. It has been demonstrated [34] that in patients with ACS, the *ANGPTL2* gene, which encodes a circulating pro-inflammatory protein, is hypomethylated, whereas the level of protein in blood is increased compared to healthy persons of the same gender and age.

The increased methylation of the highly conserved region of the *FOXP3* gene (FOXP3-TSDR), which determines regulatory T cells functioning, is associated with an increased risk of adverse outcomes (cardiovascular death, myocardial infarction, repeated coronary surgery) in patients with ACS and the severity of atherosclerosis (a decrease in regulatory T cells functioning and number results in its advance) [57]. Decreased expression and hypermethylation of the *FOXP3* gene are seen in case of IHD [17, 58]. Thus, taking into account the *FOXP3* gene involvement in development of IHD, ACS, adverse outcomes of ACS (including cardiovascular death), and atherosclerosis, it is possible to find a positive relation between methylation of this gene, in particular its highly conserved FOXP3-TSDR region, and SCD.

According to ICD-10, death from myocardial infarction (I21-I22) is not related to SCD (I46.1) [59], however, SCD is a frequent outcome of a previous infarction due to its recurrence or heart rhythm disturbances [53]. With regard to myocardial infarction, DNA methylation of both the genome and specific genes was studied. In an epigenome-wide association study of cardiovascular pathology conducted in Sweden [60], 211 differentially methylated CpG sites were identified in patients with myocardial infarction (196 genes, 42 of them are associated with heart function), including the *RYR2* and *KCNN1* genes involved in ion transportation, and cardiogenesis genes (including the *GDF15* gene). In a large multistage study based on the KORA, NAS, and InCHIANTI projects, 9 CpG sites altered after myocardial infarction were identified; these were localized in the *DHCR24*, *KCNN1*, *ALKBH1*, and *LRP8* genes [61]. The *LRP8* (low-density lipoprotein receptor-related protein 8) gene encodes a low-density lipoprotein receptor that also functions as a receptor for the ApoE protein. Some single nucleotide polymorphisms of the *LRP8* gene are associated with myocardial infarction, IHD, and early unifamilial IHD [62, 63]. In this regard, the *LRP8* gene may also be of interest when studying methylation in case of SCD. In an epigenome-wide association study conducted in Japan [13], the researchers found that the cg07786668 site of the *ZFHX3* gene and the cg17218495 site of the *SMARCA4* gene are statistically significant in association with myocardial infarction. Single nucleotide polymorphisms of the *ZFHX3* (zinc finger homeobox 3) gene are associated with atrial fibrillation, which can cause SCD [64].

It was demonstrated that methylation of the *ALDH2* gene promoter plays an important role in protection of the myocardium from ischemia; an association of myocardial infarction with methylation of the *GDF15* gene was identified [65]. The *GDF15* (growth differentiation factor 15) gene encodes the protein involved in the cellular stress response to an injury. A higher level of the GDF-15 protein increases the risk of SCD within 24 h after myocardial infarction [66] as well as after ACS [67]. Furthermore, GDF-15 is associated with fatal arrhythmic events and all-cause mortality in case of dilated cardiomyopathy [68].

Association of myocardial infarction with methylation of the *GNAS-AS1* gene in men and women and of the *INS-IGF2* gene in women was also demonstrated [69]. In an epigenome-wide case-controlled study [70] (206 persons with myocardial infarction and 206 persons in the control group) based on the EPICOR project, three differentially methylated loci (the *TCN2* gene promoter, the 5'UTR region of the *CBS* gene, the *AMT* gene) were identified in men and two (*PON1* gene, 5'UTR region of the *CBS* gene) — in women, whose methylation was reduced due to myocardial infarction. Hypomethylation of the *IL6* gene promoter is also associated with this nosology [32]. The study of 27-year-old monozygotic male twins discordant for myocardial infarction showed hypomethylation of the *LDAH*, *APOB*, *ACSM2A*, *ACSM5*, *ACSF3*, *CES1*, *CES1P1*, *AFG3L2*, *ISCU*, *SEC14L2*, *MTTP* genes in a twin without myocardial infarction (twins have the same work and have no risk factors associated with pathology) [71].

Thus, it is possible to identify a number of promising candidate genes to study methylation thereof in SCD from the considered genes, the methylation of which is associated with IHD, myocardial infarction, and ACS:

the *CTH* and *PLCB1* genes, polymorphic variants of which are associated with SCD;

the *PTX3* gene, as the level of the PTX3 protein in the blood is associated with fatal ACS related to coronary thrombosis, or cardiac death related to a chronic heart failure;

the *MMP9* gene, as the protein encoded by the gene is considered as a potential risk marker for SCD;

the *FN1* gene, the expression of which is associated with SCD, type 2 diabetes mellitus, and preserved left ventricular ejection fraction;

the *F2RL3* gene, the methylation of which is associated with cardiovascular death in case of IHD;

the *ABCB1* gene, the methylation of which is mentioned in relation to a cardiac death in case of intracranial stenosis;

the *FOXP3* gene, the methylation of which is associated with a cardiovascular death after ACS;

the *GDF15* gene, as an increased level of GDF-15 raises up the risk of SCD after ACS, myocardial infarction and in dilated cardiomyopathy;

the *CASR* gene, the methylation of which is associated with IHD (see Appendix 2).

The *IL6* gene (interleukin 6), the methylation of which is associated with SCD and myocardial infarction, can also be a possible candidate gene to study methylation in case of SCD [17, 44]. Based on the Cardiovascular Health Study, more than 5000 people had their IL-6 level measured. A 17-year follow-up of participants showed that the IL-6 level is associated with the risk of SCD [72]. The same result was received earlier on the basis of the PRIME study after a 10-year follow-up of participants [73].

DNA methylation and atherosclerosis

Atherosclerosis of the coronary vessels is a direct substrate of IHD, which is the most frequent cause of SCD. According to the Russian National Guidelines for Determination of the Risk and Prevention of Sudden Cardiac Death, high cholesterol is a minor risk factor for SCD, whereas prescription of statins is a preventive measure for SCD in patients with IHD [3]. Therefore, searching global scientific studies on DNA methylation in atherosclerosis and lipid metabolism disorders can be useful to find candidate genes, the methylation of which is associated with SCD.

The epigenome-wide association study by Hedman et al. [20] provided for identification of 33 CpG sites associated with the level of lipids (of which 25 were new, the methylation of which had not been previously associated with the lipid spectrum). One of the sites belongs to the *SREBF2* (sterol regulatory element binding transcription factor 2) gene, the methylation of which is associated with the level of total cholesterol. According to the data available, the rs2228314 polymorphism of the *SREBF2* gene is associated with the risk of SCD [74].

Yamada et al. [75] studied postmortem DNA methylation in patients with atherosclerosis (n=128). The level of methylation was measured in pairs in atherosclerotic and healthy tissues of the aorta of the same person. There were 16 CpG sites identified which were located in genes not previously associated with atherosclerosis (*FHIT*, *WNT8B*, *HOXA10*, *HOXC-AS2*, *ZNF609*, *HOXA-AS3*, *GDF6*, *TBX20*, *HOXA6*, *TUBA4A/TUBA4B*, *CCDC62*, *MYOM2*, *RNASE6*).

Another epigenome-wide study [76] revealed that methylation of the CpG site of the cg06500161 locus of the *ABCG1* gene is associated with the levels of high-density lipoprotein cholesterol and triglycerides. The level of methylation of this locus is higher in persons who had myocardial infarction in history compared to healthy people. In addition to the *ABCG1* gene locus, there were several CpG sites belonging to the genes associated with the level of triglycerides (*TXNIP, SREBF1, CPT1A, MIR33B/SREBF1, APOA5*) and low-density lipoprotein cholesterol (*TNIP1*) identified [76]. In atherosclerosis, triglycerides level is also associated with hypomethylation of the *CCL2* (C-C motif chemokine ligand 2) gene promoter, which encodes a cytokine with chemotactic activity to monocytes and basophils as well as is involved in development of atherosclerosis [19]. It is known that an increase in *CCL2* gene expression in atherosclerotic plaques is closely correlated with SCD [77]. It was demonstrated that the *SMAD7* gene methylation can be a new predictive marker and therapeutic target in case of atherosclerosis because the gene promoter is hypermethylated both in atherosclerotic plaques and in the blood of patients with atherosclerosis, which positively correlates with the level of homocysteine and the degree of atherosclerotic plaque progression [78].

There was a series of studies conducted in Russia to study DNA methylation in atherosclerosis. For instance, it was identified [79] that the level of LINE-1 methylation is significantly reduced in peripheral blood leukocytes in patients with clinically apparent atherosclerosis compared to healthy persons, and this indicator is even lower in carotid artery cells affected by atherosclerosis. Arterial wall cells from the region of atherosclerotic plaques of the coronary arteries are characterized by higher levels of methylation in the promoter region of the *PNPLA2* gene compared to the unaffected wall of the internal thoracic arteries [80]. The level of methylation of the *MIR10B* and *MIR21* genes in leukocytes of patients with atherosclerosis is higher than in leukocytes of the control group [81].

Some studies revealed an association of the *SLAMF7*, *MIR10B*, and *ABCA1* genes methylation with atherosclerosis [82–84]. The *LPL* gene methylation is associated with high-density lipoprotein cholesterol level, whereas of the *CETP* gene — with the level of low-density lipoprotein cholesterol in men and women, and with the level of high-density lipoprotein cholesterol and triglycerides only in men [85]. There was a study conducted based on the DIABHYCAR project with participation of 3124 patients with type 2 diabetes mellitus and high cardiovascular risk. It was identified that the TaqIB polymorphism of the *CETP* gene is associated with SCD in patients with type 2 diabetes mellitus: the B1B1 homozygotes have a higher risk of SCD than carriers of the B2 allele. The *CETP* (cholesteryl ester transfer protein) gene encodes a plasma protein involved in the transportation of cholesterol from high-density lipoproteins to other lipoproteins [86].

It was demonstrated that the *ABCA1*, *ACAT1*, and *TIMP1* genes have high specificity and sensitivity for early diagnosis of atherosclerosis [24]. It is accepted that the level of the TIMP-1 protein may be a marker of mortality in patients with cardiac failure undergoing cardiac resynchronization therapy [87].

As can be seen from the above, the study of methylation of the *CETP*, *CCL2*, and *SREBF2* genes may be of most interest in relation to SCD, as it is associated with lipid metabolism imbalances and atherosclerosis, whereas polymorphisms of the *CETP*, *SREBF2* genes and *CCL2* expression are associated with SCD. The *TIMP1* gene is also of interest because the protein encoded by this gene is accepted as a marker of death in case of a chronic heart failure.

DNA methylation and heart rhythm disturbances

In about 40% of cases, sudden death in people under 35 years of age remains unexplained after a forensic examination. Heart rhythm disturbances, primarily long QT syndrome, Brugada syndrome, and catecholaminergic polymorphic ventricular tachycardia are considered to be the most likely causes of a sudden death. The SCD development in case of rhythm disturbances may be related to dysfunction of ion channels (impaired opening, closing, and functioning thereof), intracellular concentration of such ions as calcium, which, on the background of certain predisposing factors, causes rapid development of tachycardia or ventricular fibrillation and death [88].

DNA methylation studies were conducted for some heart rhythm disturbance syndromes. For instance, methylation of the *KCNQ1* gene was studied in a cohort of patients with a prolonged QT interval [89].

In case of the H558R (rs1805124) polymorphism of the *SCN5A* gene in persons with Brugada syndrome, the level of *SCN5A* gene expression is higher, and its methylation level is lower compared to people without the H558R polymorphism; DNA for analysis was extricated from the right atrium tissue (n=30) [90]. Polymorphisms of the *KCNQ1* and *SCN5A* genes, according to some researches, are related to development of SCD. The *KCNQ1* (potassium voltage-gated channel subfamily Q member 1) gene encodes a voltage-gated potassium channel responsible for the repolarization phase of the cardiac action potential. Mutations in the gene are associated with development of the type 1 long QT syndrome or unifamilial atrial fibrillation. Single nucleotide polymorphisms of the gene (rs10798, rs8234) are associated with an increased risk of SCD in patients with the long QT syndrome [91]. Liu et al. [92] in their meta-analysis demonstrated that single nucleotide polymorphisms rs12296050 and rs2283222 of the *KCNQ1* gene and rs11720524 of the *SCN5A* gene are associated with SCD. The *SCN5A* (sodium voltage-gated channel alpha subunit 5) gene encodes an integral membrane protein being a sodium channel subunit. Gene mutations result in development of the type 3 long QT syndrome. According to Lahtinen et al. [93], the rs41312391 polymorphism of the *SCN5A* gene is associated with SCD.

A genome-wide study of DNA methylation [94] provided for identification of differentially methylated genes in patients with atrial fibrillation compared with persons with the sinus heart rhythm (primarily genes associated with inflammation, ion transportation, fibrosis, and lipid metabolism). Another epigenome-wide association study [9] found 7 CpG sites associated with atrial fibrillation located near the *WFIKKN2*, *STRN*, *SSU72*, *BLCAP*, *DPYSL4*, *RBBP5*, and *WDR37* genes. The overall level of DNA methylation was significantly higher in the atrial fibrillation group compared to the sinus heart rhythm group. In case of atrial fibrillation, the promoter of the *NPRA* gene (natriuretic peptide receptor gene) is hypermethylated, whereas the expression of the gene is reduced [95]. With atrial fibrillation, the *LINC00472* (long intergenic non-protein coding RNA 472) gene is also hypermethylated. It is accepted that its expression is associated with the expression of miR-24 RNA, which in turn regulates the expression of the *JPH2* (junctophilin 2) gene that affects the expression of the *RYR2* (ryanodine receptor 2) gene involved in the pathogenesis of atrial fibrillation. In addition to an increased level of *LINC00472* methylation, one can see an increase in the miR-24 expression level and a decrease in the expression of *LINC00472* [96]. Hypermethylation of the *PITX2* (paired like homeodomain 2) gene is associated with atrial fibrillation [97]. The analysis of researches revealed that the number of group participants is low, DNA samples were extricated from the myocardium of the right [95] or left atrium [94, 96, 97], or venous blood [9].

With this regard, the study of methylation of the *SCN5A* and *KCNQ1* genes (related to the long QT syndrome, a frequent cause of unexplained sudden death), polymorphisms of which are associated with SCD, will be the most promising approach for SCD.

DNA methylation and cardiomyopathies

The most frequent forms of cardiomyopathies leading to SCD include hypertrophic and dilated cardiomyopathies; arrhythmogenic right ventricular and restriction cardiomyopathies are less common. Pathogenetically, in case of cardiomyopathy, SCD can occur due to a mechanical underlying cause (obstruction of the outflow tract of the left ventricle in hypertrophic cardiomyopathy) or development of malignant arrhythmia (dilated cardiomyopathy). The “ischemic cardiomyopathy” term is also used to describe myocardial dysfunction caused by severe IHD [4].

One of the early studies [98] identified 51 hypermethylated promoters and 6 hypomethylated promoters of genes associated with dilated cardiomyopathy and expression of these genes,

including the *AURKB*, *BTNL9*, *CLDN5*, and *TK1* genes, which were not previously described as involved in dilated cardiomyopathy. An epigenomic association study provided for revealing 59 loci, the methylation of which was significantly associated with dilated cardiomyopathy [99].

In case of ischemic cardiomyopathy, hypermethylation of the *ASB1* gene was established; the gene methylation status is associated with the left ventricular ejection fraction, stroke volume, as well as with end-systolic and end-diastolic size of the left ventricle [100]. Li et al. in their study [101] identified three more genes (*SLC2A1*, *MPV17L*, *PLEC*) with different methylation status were in ischemic cardiomyopathy.

Targeted bisulfite sequencing in patients with the heart failure related to an ischemic, dilated and hypertrophic cardiomyopathy revealed 195 unique differentially methylated regions (5 for hypertrophic obstructive cardiomyopathy, 151 for dilated cardiomyopathy, 55 for ischemic cardiomyopathy). Subsequent analysis of expression revealed 6 genes (*HEY2*, *MSR1*, *MYOM3*, *COX17*, *CTGF*, *MMP2*), the expression of which is associated with their methylation pattern and heart failure [102].

Conclusion

Knowledge of gene polymorphisms and mutations only is not enough to understand their role in development of multifactorial diseases, as it does not provide for understanding of the ways in which changes in the DNA structure manifest themselves in the pathogenesis of the disease.

DNA methylation is an important form of epigenetic modification that can impact gene expression without changing the DNA nucleotide sequence. At that, DNA methylation is influenced by environmental factors, it depends on gender, age, and other phenotype characteristics, as well as lifestyle. The genes methylation status differs in various body tissues. DNA methylation is not only involved in the regular cell activities but may also be significant in pathogenesis of diseases. Therefore, epigenetic studies are extremely important for studying the genetic basis of diseases with a hereditary predisposition.

There were few studies of DNA methylation in sudden cardiac death conducted, but DNA methylation was studied for underlying diseases (IHD, cardiomyopathy, heart rhythm disturbances). There were papers on measuring the overall level of DNA methylation published, epigenome-wide studies, and studies of methylation of individual genes conducted. Analysis of their results provides for identification of the most promising genes in relation to sudden cardiac death, the methylation of which is associated with IHD (*CTH*, *PLCB1*, *PTX3*, *MMP9*, *FN1*, *F2RL3*, *ABCB1*, *FOXP3*, *GDF15*, *IL6*, and *CASR*), with lipid metabolism disorders and atherosclerosis (*CETP*, *CCL2*, *SREBF2*, and *TIMP1*), with heart rhythm disturbances (*SCN5A* and *KCNQ1*).

Epigenome-wide association studies aimed at identification of unique differentially methylated loci for sudden cardiac death, the methylation of which was not previously associated with cardiovascular diseases, may also become significant. Studying the level of global DNA methylation will also expand scientific knowledge about epigenetic changes in case of a sudden cardiac death. The data obtained during the study of DNA methylation in case of a sudden cardiac death will permit to advance deeper in understanding of the mechanisms of SCD development, including the relations with genes, their polymorphic variants and mutations. Moreover, studying of DNA methylation is required for development of systems for diagnosis, prevention, and treatment of cardiovascular diseases leading to a sudden cardiac death.

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Appendix 1

Genes with the IHD-associated methylation

Gene; localization	Gene product function	Nosology	References
C1QL4 — complement C1q like 4; 12q13.12	—	IHD	[23]
CCDC47 — coiled-coil domain containing 47; 17q23.3	—	IHD	
TGFBR3 — transforming growth factor beta receptor 3; 1p22.1	Co-receptor for other TGF-β receptors	IHD	
ABCA1 — ATP binding cassette subfamily A member 1; 9q31.1	Transportation of molecules through membranes, part of the cholesterol efflux pump	IHD, SCD, ATS	[21, 19, 22, 84]
DDAH2 — dimethylarginine dimethylaminohydrolase 2; 6p21.33	Regulation of methylarginine concentration (nitric oxide synthesis)	IHD	[19]
CTH — cystathionine gamma-lyase; 1p31.1	Conversion of cystathionine derived from methionine to cysteine	IHD	[26]
PTX3 — pentraxin 3; 3q25.32	Regulation of inflammation, complement activation, fibrocyte differentiation	IHD	[28]
IL6 — interleukin 6; 7p15.3	Involvement into inflammation	IHD, MI	[17, 32, 44]
GCK — glucokinase; 7p13	Involvement into glucose metabolism		[19, 33–37]
GALNT2 — polypeptide N-acetylgalactosaminyltransferase 2; 1q42.13	Glycosylation of peptides in the Golgi apparatus		[37]
TNNT1 — troponin T1, slow skeletal type; 19q13.42	Troponin subunit		[33]
PLA2G7 — phospholipase A2 group VII; 6p12.3	Degradation of platelet activating factor		[35]
MMP9 — matrix metallopeptidase 9; 20q13.12	Degradation of collagen of IV and V types		[24]
FOXP3 — forkhead box P3; Xp11.23	Transcription regulation	IHD, ATS	[17, 19, 58, 57]
ANGPTL2 — angiopoietin like 2; 9q33.3	Blood vessel growth stimulating factor	IHD, ACS	[24, 34, 36]
ABCG1 — ATP binding cassette subfamily G member 1; 21q22.3	Transportation of cholesterol and phospholipids by macrophages	IHD, MI, L	[17, 37, 76]
ATP2B2 — ATPase plasma membrane Ca2+ transporting 2; 3p25.3	Intracellular calcium homeostasis support	IHD	[41]
CASR — calcium sensing receptor; 3q13.33-q21.1	Calcium homeostasis support	IHD	
GUCA1B — guanylate cyclase activator 1B; 6p21.1	Activates photoreceptor guanylate cyclases	IHD	
HPCAL1 — hippocalcin like 1; 2p25.1	Calcium-binding protein of the retina and the brain	IHD	
PTPRN2 — protein tyrosine phosphatase receptor type N2; 7q36.3	Disputable	IHD	
CDH23 — cadherin related 23; 10q22.1	Organization of stereocilia and hair tufts	IHD	
ABCB1 — ATP binding cassette subfamily B member 1; 7q21.12	Medication transportation	MI	[52]
GCLM — glutamate-cysteine ligase modifier subunit; 1p22.1	Synthesis of glutathione	IHD + AH	[43]
MPO — myeloperoxidase; 17q22	Component of azurophilic granules of neutrophils	IHD + AH	
TXNRD1 — thioredoxin reductase 1; 12q23.3	Reduction of thioredoxins	IHD + AH	
GSTP1 — glutathione S-transferase pi 1; 11q13.2	Metabolism of xenobiotics	IHD + AH	
PLCB1 — phospholipase C beta 1; 20p12.3	Intracellular signal transduction	IHD	[44]
BDNF — brain derived neurotrophic factor; 11p14.1	Nerve growth factor	IHD	
BTRC — beta-transducin repeat containing E3 ubiquitin protein ligase; 10q24.32	Ubiquitination	IHD	
CDH5 — cadherin 5; 16q21	Assembly and support of endothelial adhesive junctions	IHD	
CXCL12 — C-X-C motif chemokine ligand 12; 10q11.21	Receptor ligand	IHD	
EGFR — epidermal growth factor receptor; 7p11.2	Epidermal growth factor receptor	IHD	
ITGB1 — integrin subunit beta 1; 10p11.22	Membrane receptor	IHD	
PDGFRB — platelet derived growth factor receptor beta; 5q32	Platelet growth factor receptor	IHD	

PIK3R1 — phosphoinositide-3-kinase regulatory subunit 1; 5q13.1	Insulin metabolism	IHD	
PTPRC — protein tyrosine phosphatase receptor type C; 1q31.3-q32.1	Regulation of T cell and B cell receptor signaling	IHD	
FN1 — fibronectin 1; 2q35	Cell adhesion, migration	IHD	[47]
PTEN — phosphatase and tensin homolog; 10q23.31	Tumor suppressor	IHD	
POLR3A — RNA polymerase III subunit A; 10q22.3	RNA synthesis	IHD	
F2RL3 — F2R like thrombin or trypsin receptor 3; 19p13.11	The receptor is involved in the process of blood coagulation, inflammation, response to pain	IHD	[49, 50, 51]
SMAD3 — SMAD family member 3; 15q22.33	Signal-to-nucleus transduction, tumor suppressor	ACS	[55]
IL6R — interleukin 6 receptor; 1q21.3	IL-6 receptor	ACS	[56]
FASLG — Fas ligand; 1q24.3	Apoptosis induction	ACS	
CCL18 — C-C motif chemokine ligand 18; 17q12	Cytokine	ACS	
RYR2 — ryanodine receptor 2; 1q43	Component of the calcium channel of cardiomyocytes	MI	[60]
KCNN1 — potassium calcium-activated channel subfamily N member 1; 19p13.11	Voltage-gated calcium-activated channel	MI	[60, 61]
GDF15 — growth differentiation factor 15; 19p13.11	TGF-β ligand	MI	[60, 65]
DHCR24 — 24-dehydrocholesterol reductase; 1p32.3	Involvement into cholesterol synthesis	MI	[61]
ALKBH1 — alkB homolog 1, histone H2A dioxygenase; 14q24.3	Participation in the DNA damage repair by alkylation	MI	
LRP8 LDL — receptor related protein 8; 1p32.3	Low-density lipoprotein receptor	MI	
ZFHX3 — zinc finger homeobox 3; 16q22.2-q22.3	Transcription factor, regulation of myogenic and neuronal differentiation	MI	[13]
SMARCA4 — SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily A, member 4; 19p13.2	Transcriptional activation of genes	MI	
ALDH2 — aldehyde dehydrogenase 2 family member; 12q24.12	Alcohol metabolism	MI	[65]
GNAS-AS1 — GNAS antisense RNA 1; 20q13.32	GNAS locus regulation	MI	[71]
INS-IGF2 — INS-IGF2 readthrough; 11p15.5	Disputable	MI	
TCN2 — transcobalamin 2; 22q12.2	B_{12} vitamin binding and its transportation into cells	MI	
CBS — cystathionine beta-synthase; 21q22.3	Participation in transsulfuration	MI	
AMT — aminomethyltransferase; 3p21.31	Component of the glycine cleavage system	MI	
PON1 — paraoxonase 1; 7q21.3	Hydrolysis of thiolactones and xenobiotics	MI	
LDAH — lipid droplet associated hydrolase; 2p24.1	—	MI	
APOB — apolipoprotein B; 2p24.1	Apolipoprotein of chylomicrons and low-density lipoproteins	MI	
ACSM2A — acyl-CoA synthetase medium chain family member 2A; 16p12.3	Involvement in fatty acid metabolism	MI	
ACSM5 — acyl-CoA synthetase medium chain family member 5; 16p12.3	—	MI	
ACSF3 — acyl-CoA synthetase family member 3; 16q24.3	Fatty acid activation	MI	
CES1 — carboxylesterase 1; 16q12.2	Hydrolysis or re-esterification of xenobiotics	MI	
CES1P1 — carboxylesterase 1 pseudogene 1; 16q12.2	*CES1* pseudogene	MI	
AFG3L2 — AFG3 like matrix AAA peptidase subunit 2; 18p11.21	Disputable	MI	
ISCU — iron-sulfur cluster assembly enzyme; 12q23.3	Co-factor with functions of various enzymes	MI	
SEC14L2 — SEC14 like lipid binding 2; 22q12.2	Involvement in cholesterol synthesis	MI	
MTTP — microsomal triglyceride transfer protein; 4q23	Triglyceride transport protein subunit	MI	

N o t e. Information about genes is given in accordance with https://www.ncbi.nlm.nih.gov/gene/; MI — myocardial infarction; ACS — acute coronary syndrome; IHD — ischemic heart disease; SCD — sudden cardiac death; ATS — atherosclerosis; L — lipid metabolic imbalance; AH — arterial hypertension.

Appendix 2

Candidate genes with IHD-associated methylation to study methylation in case of SCD

Gene; localization	Association of gene methylation with IHD	Justification of a possible association with SCD
CTH — cystathionine gamma-lyase; 1p31.1	Gene promoter hypermethylation is associated with an increased risk of IHD [26]	rs113044851 of the gene reduces the risk of SCD [27]
PLCB1 — phospholipase C beta 1; 20p12.3	Methylation of the CpG site of the cg27178677 of the gene is associated with IHD [44]	rs16994849 of the gene is associated with SCD [45]
PTX3 — pentraxin 3; 3q25.32	Hypomethylation of the gene promoter is associated with IHD, higher levels of PTX3 protein in the blood plasma [28]	The level of PTX3 protein in the blood plasma is higher in the group with fatal ACS related to coronary thrombosis as compared with the control group [29] as well as in persons with advanced endpoints (including cardiac death) related to the chronic heart failure [30]
MMP9 — matrix metallopeptidase 9; 20q13.12	Gene methylation is associated with the risk of IHD, age when it started developing, IHD risk factors [24]	The level of MMP-9 in the blood plasma is considered as a risk marker for SCD [39]
FN1 — fibronectin 1; 2q35	Gene promoter hypermethylation is associated with IHD [47]	Gene expression is higher in people with SCD, type 2 diabetes mellitus, and preserved left ventricular ejection fraction as compared with persons who died from another cause [48]
F2RL3 — F2R like thrombin or trypsin receptor 3; 19p13.11	Gene methylation is associated with cardiovascular mortality in case of IHD [50]	
ABCB1 — ATP binding cassette subfamily B member 1; 7q21.12	Gene promoter methylation is associated with the risk of ischemic events (including cardiac death, myocardial infarction, ischemic stroke) in case of intracranial stenosis [52]	
FOXP3 — forkhead box P3; Xp11.23	Gene hypermethylation is associated with IHD [17]	Hypermethylation of the conserved region of the FOXP3-TSDR gene is associated with a risk of cardiovascular death after ACS [57]
GDF15 — growth differentiation factor 15; 19p13.11	Gene methylation is associated with the risk of myocardial infarction [65, 60]	The increased GDF-15 level scales up the risk of SCD after ACS, myocardial infarction, as well as in case of dilated cardiomyopathy [66, 67, 68]
IL6 — interleukin 6; 7p15.3	Gene promoter hypomethylation is associated with the risk of IHD, myocardial infarction [32]	IL-6 level is associated with the risk of SCD [72]
CASR — calcium sensing receptor; 3q13.33-q21.1	Gene methylation is associated with IHD [41]	rs1801725 of the gene is associated with QT interval length [42]

N o t e. Information about genes is given in accordance with the data from https://www.ncbi.nlm.nih.gov/gene/; ACS — acute coronary syndrome; IHD — ischemic heart disease; SCD — sudden cardiac death.