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REVIEWS

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miRNA Regulome in Different Atherosclerosis Phenotypes

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Abstract—Dysregulation of microRNA (miRNA) expression is associated with a susceptibility to many diseases, including atherosclerotic lesions of the coronary and carotid arteries and the development of clinical complications such as coronary heart disease, myocardial infarction, chronic cerebral ischemia, ischemic stroke. Recently, more and more studies analyze the miRNA regulome including a network of regulatory elements for the expression of miRNAs themselves and targets under their control. The review summarizes the data from articles concerned miRNA expression and changes in DNA methylation in the miRNA genes in human atherosclerotic arteries, as well as with the analysis of the association between single nucleotide polymorphisms and copy number variations in the miRNA genes with clinical complications of atherosclerosis.

Keywords: microRNA regulome, expression, DNA methylation, single nucleotide variants, copy number variations, atherosclerosis, ischemic heart disease, myocardial infarction, ischemic stroke

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INTRODUCTION

Atherosclerosis is a complex, chronic inflammatory disease with a progressive course. It characterizes by remodeling of the arteries, atherosclerotic plaques formation with luminal stenosis of the arteries and the development of ischemia, thrombosis or embolism of target organs, in particular the heart, brain, etc. Pathological changes in the arteries include endothelial cell dysfunction, oxidized lipoproteins deposition and arterial wall infiltration by monocytes/macrophages with their transformation into foam cells, as well as intimal thickening, smooth muscle cells (SMCs) migration and proliferation, trans-differentiation of the cell phenotype, calcification, angiogenesis, formation of a fibrous plaque with a lipid-necrotic core and its subsequent destabilization [1–3].

Almost all stages of atherosclerosis development—from early endothelial dysfunction to erosion or rupture of an unstable atherosclerotic plaque—involve microRNAs (miRNAs) that are small regulatory evolutionarily conserved non-coding RNAs of 16–27 nucleotides in length [4–8]. The expression of miRNAs in leukocytes and arterial wall cells at each stage of atherosclerosis development is controlled by various stimuli, and miRNAs themselves regulate multiple signaling pathways involved in atherosclerotic plaque formation and destabilization [5, 6].

The mechanism of miRNA action mainly involves the negative regulation of gene expression through mRNA degradation or repression of target mRNA translation in cell cytoplasm [9–11]. However, in the cell nucleus, individual miRNAs can activate gene transcription, acting as enhancer triggers [11].

According to miRBase v. 22.1 online database (updated in October 2018), more than 2,600 miRNAs have been found in human tissues [12], and these miRNAs can regulate the expression of more than 60% of all protein-coding genes, thereby participating in all major biological processes [9, 12, 13]. The transcription of miRNA genes localized in the intergenic space is controlled by their own regulatory elements, while miRNAs located in the region of protein-coding genes often share a common promoter with the host gene [14, 15]. Nevertheless, approximately 50% of intragenic miRNAs have their own promoter [16].

It is believed that miRNA genes correspond to about 1–5% of the nucleotide sequence of all human genes [11, 17]. MiRNA genes can be located either in the region of protein-coding genes (exon or intron), or in intergenic regions [14, 18]. According to the miRIAD database [19], among 1881 miRNAs, 169 (9%) miRNAs are exonic, 988 (52.5%) miRNAs are intronic, and 724 (38.5%) miRNAs are intergenic.

The Human microRNA Disease Database (HMDD v3.2, updated March 27, 2019) [20] contains information on 116 different experimentally validated miRNAs associated with atherosclerosis, 26 miRNAs associated with carotid atherosclerosis and 67 miRNAs associated with ischemic stroke, as well as 85 miRNAs associated with coronary artery disease and 60 miRNAs

associated with acute coronary syndrome and myocardial infarction.

The aim of this review is to summarize the results of the study of the miRNA regulome, which includes a network of regulatory elements of the expression of miRNAs themselves and target molecules under miRNA control in atherosclerosis of carotid and coronary arteries.

This review focuses on the studies of the molecular mechanisms of atherosclerosis through miRNA dysregulation in cells and organs that are the target of the disease in humans, i.e. atherosclerotic plaques, in contrast to reviews, that characterized the patients with acute vascular events and searched for biomarkers through the detection of miRNA expression in plasma or blood serum [21].

The association of miRNAs with atherosclerosis is reviewed at several levels: miRNAs expression, changes in DNA methylation of miRNA genes, and DNA polymorphisms in the miRNA genes. Comparison of the data obtained will characterize the miRNA regulome in different phenotypes of atherosclerosis in humans (coronary artery disease, myocardial infarction, chronic cerebral ischemia, ischemic stroke), which will help in more accurate planning of experiments with cell cultures and model animals aimed at understanding the function of miRNAs and molecular mechanisms of regulation of their functional activity.

EXPRESSION OF miRNAs IN CORONARY AND CAROTID ARTERIES IN ATHEROSCLEROTIC LESIONS

To date, there are several dozen studies that analyzed changes in miRNA expression in whole human arteries damaged by atherosclerosis using various groups of comparison and various methods. Most studies analyzed the expression of candidate miRNAs in the arteries using real-time PCR [22–33]. Moreover, the expression of miRNA was compared between atherosclerotic plaques of coronary or carotid arteries and intact vessels using microarrays [34–40].

In particular, the pioneering microarray study of Raitoharju E. et al. (2011) revealed 58 miRNAs that differentially expressed between atherosclerotic plaques of various arteries and non-atherosclerotic left internal thoracic arteries [34]. The up-regulated expression of five miRNAs (miR-21, -34a, -146a, -146b-5p, and -210) in atherosclerotic plaques was confirmed on the larger samples of the arteries using real-time PCR. The expression of 187 mRNAs of these miRNA target genes was down-regulated in atherosclerotic plaques. The protein products of these genes are involved in signal transduction, the regulation of transcription and vesicular transport.

The Table 1 shows 31 miRNAs with statistically significantly expression changes in coronary and carotid atherosclerotic plaques compared with intact vascular tissues, or between unstable (symptomatic) and stable (asymptomatic) atherosclerotic plaques in two or more studies [22–24, 26–41].

In total, in coronary and carotid atherosclerotic plaques expression changes of 31 miRNAs were registered (Table 1). The miRNAs up-regulated in atherosclerotic plaques compared to controls (miR-19b, -22, -34a, -100, -125a, -127, -133a, -133b, -146a, -155) were prevalent in the carotid arteries. The expression of let-7f, miR-1, and miR-92a was down-regulated in carotid atherosclerotic plaques. Conflicting results have been reported in different studies for miR-21, -29b, -143, -145, -221.

In contrast, coronary arteries were characterized by a large number of down-regulated miRNAs (let-7f, miR-1, -19a, -22, -24, -34a, -106b, -125a, -133b, -143, -145) and multidirectional changes of miRNA expression across studies (miR-9, -10a, -10b, -16, -19b, -21, -25, -29b, -29c, -92a, -100, -155, -486, -497). In coronary atherosclerotic plaques miR-146a, -150, -221, -223 were up-regulated.

In the coronary and carotid arteries, unidirectional expression changes were revealed for miR-146a (up-regulation), miR-1, and let-7f (down-regulation), while the miR-21 and miR-29b in different studies were either up- or down-regulated.

In the carotid arteries, the expression of miR-21, -100, -125a, -127, -133a/b, -143/-145, -221 was associated with atherosclerotic plaque instability [22, 27, 29, 33, 38, 42]. Unstable plaques are characterized by up-regulation of most miRNAs (miR-100, -127, -133a/b, -145) and down-regulation of miR-21 and miR-143. Conflicting results were obtained for miR-221. In the coronary arteries, the down-regulation of miR-24 is associated with atherosclerotic plaque instability [26].

The expression of miRNA was evaluated in biopsy specimens of carotid atherosclerotic plaques obtained during endarterectomy. Most studies used the clinical criteria of atherosclerotic plaque instability; patients with symptomatic carotid artery stenosis who had an acute cerebrovascular event (transient ischemic attacks/ipsilateral stroke) less than 6 months before surgery considered as having an unstable plaque. However, in some studies, the time from an acute cerebrovascular event to surgery varied from less than 5 days to 3 months [27, 33, 38]. Only in limited studies miRNA expression was determined in macrophages taken from areas of histologically unstable (low-calcified) plaques or in plaques with thinned (<200 μm) and ruptured fibrous cap [33].

The association of miRNA expression in carotid atherosclerotic plaques with atherosclerosis risk factors (arterial hypertension and diabetes mellitus type 2) has been shown for miR-145 and the let-7 family [25, 43].

A systematic review by Sharma et al. (2013) revealed that 155 miRNAs detected in the human blood serum or plasma are associated with coronary

Table 1. The expression of miRNAs in coronary and carotid atherosclerotic plaques

miR	Carotid arteries	Coronary arteries	References
	Atherosclerotic plaque vs. intact vascular tissues/unstable vs. stable atherosclerotic plaque	Atherosclerotic plaque vs. intact vascular tissues/unstable vs. stable atherosclerotic plaque	
-1†	↓	↓	[37–39]
let-7f†	↓	↓	[38, 39]
-9	ND	↑	[37]
	ND	↓	[39]
-10a	ND	↑	[37]
	ND	↓	[40]
-10b	ND	↑	[37]
	ND	↓	[40]
-16†	ND	↑	[37]
	ND	↓	[40]
-19a	ND	↓	[37, 40]
-19b	↑	↑	[37–39]
	ND	↓	[40]
-21†	↑	↑	[28, 34, 38, 39]
	↓ C (≤6 weeks)	↓	[37, 38]
	↓ C (>14 days), H (thin fibrous cap, rupture)	ND	[33]
-22	↑	↓	[37–39]
-24†	ND	↓ H	[26]
	ND	↓	[37]
-25	ND	↑	[37]
	ND	↓	[40]
-29b†	↑	↑	[34, 39]
	↓	↓	[37, 38]
-29c	ND	↓	[37]
	ND	↑	[36]
-34a	↑	↓	[34, 37]
-92a	↓	↓	[34, 37]
	ND	↑	[39]
-100	↑ C	↑	[22, 29, 36]
	ND	↓	[37]
-106b†	ND	↓	[37, 40]
-125a	↑ C	↓	[29, 41]
-127	↑ C	ND	[22, 29]
-133a†	↑ C	ND	[22, 29]
-133b	↑ C	↓	[22, 37]
	↑ H (low-calcified plaques)	ND	[42]

Table 1. (Contd.)

miR	Carotid arteries	Coronary arteries	References
	Atherosclerotic plaque vs. intact vascular tissues/unstable vs. stable atherosclerotic plaque	Atherosclerotic plaque vs. intact vascular tissues/unstable vs. stable atherosclerotic plaque	
-143	↓	↓	[34, 37, 39]
	↑	ND	[38]
	↓ C (≤6 weeks)	ND	[38]
-145†	↓	↓	[23, 37]
	↑ C	ND	[22, 29]
-146a†	↑	↑	[28, 34, 37]
-150	ND	↑	[32, 37]
-155†	↑	↑	[24, 34, 36]
	ND	↓	[41]
-221	↑ C	↑	[24, 29, 31, 36, 37]
	↓ C (<5 days)	ND	[24]
-223†	ND	↑	[37, 39]
-486†	ND	↑	[37]
	ND	↓	[40]
-497	ND	↑	[36]
	ND	↓	[37]

Unstable atherosclerotic plaque: clinically (C) or histologically (H). ND, no data. † miRNAs detected in the blood serum/plasma of individuals with CAD, acute coronary syndrome, myocardial infarction, ischemic and hemorrhagic stroke (according to [21]).

artery disease, acute coronary syndrome, myocardial infarction, ischemic and hemorrhagic strokes [21]. Of these, 33 miRNAs have been found in more than one study. Comparison of these miRNAs in blood serum/plasma and in tissues of coronary/carotid atherosclerotic plaques revealed 13 shared miRNAs (let-7f, miR-1, -16, -21, -24, -29b, -106b, -133a, -145, -146a, -155, -223, -486; Table 1). It appears that these

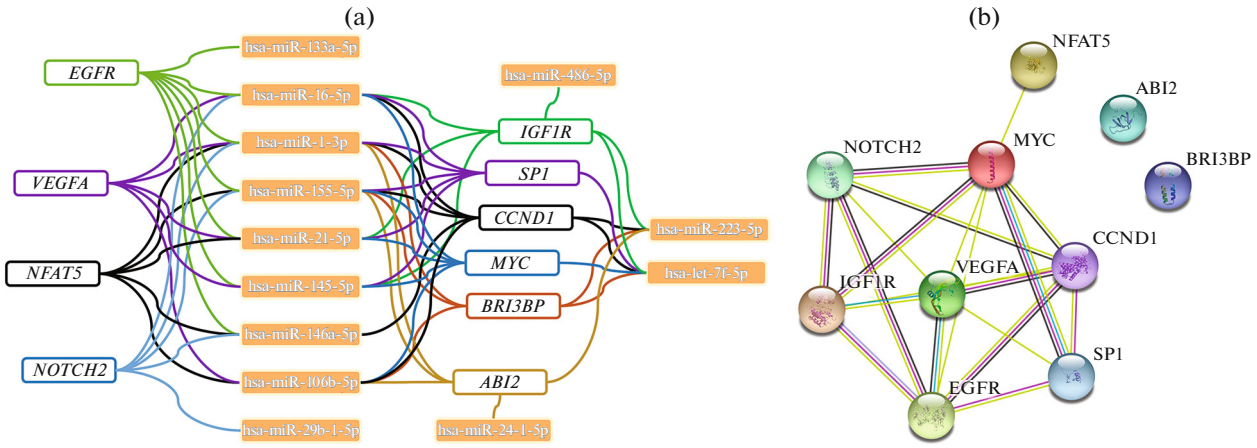

Fig. 1. Interaction between miRNAs and their target genes in coronary and carotid atherosclerosis. (a) miRNAs and target genes, which are regulated by five or more miRNAs; (b) interaction between the protein products of miRNA target genes. The STRING v. 11.0 tool was used for visualization [48].

miRNAs seem to be of particular interest not only as biomarkers of the complicated course of atherosclerosis, but also as therapeutic targets of this disease.

As a rule, individual miRNA is analyzed separately in expression profiling studies of human coronary and carotid atherosclerotic plaques. Taking into account the complex and long-term process of atherosclerotic plaque development, that characterized by high cellular heterogeneity, we can assume that different miRNAs may change their expression together and form coexpression modules during the development of atherosclerosis or changes in the cellular composition of arteries. However, many aspects of the relationship between these molecules and the regulation of mRNAs of their target genes remain poorly understood. Bioinformatic approaches, tools and databases that allow functional annotation of miRNAs and their target genes provide a significant help in solving this problem [44, 45].

For example, the characterization of the 13 aforementioned differentially expressed miRNAs (let-7f, miR-1, -16, -21, -24, -29b, -106b, -133a, -145, -146a, -155, -223, -486) in the coronary/carotid plaques and detected in the blood serum/plasma of individuals with clinical complications of atherosclerosis, using the miRTargetLink 2.0 [44] and miEAA 2.0 [46] tools and the miRTarBase database [47] allows us to identify 10 target genes (*EGFR*, *ABI2*, *IGF1R*, *NFAT5*, *BRI3BP*, *VEGFA*, *CCND1*, *SP1*, *MYC*, *NOTCH2*), which are regulated by five miRNAs or more (Fig. 1a). The *EGFR* and *CCND1* genes are targets for seven miRNAs, and their protein products interact with IGF1R, VEGFA, SP1, MYC, NOTCH2 and control angiogenesis (Fig. 1b). Activation of the immune response is associated with the protein product of the *NFAT5* gene, migration and cell adhesion is associated with the ABI2 and apoptosis is associated with the *BRI3BP*.

It should be noted that methodological approaches of microarray and real-time PCR analysis of miRNA expression in arterial tissues damaged by atherosclerosis are associated with certain disadvantages, as researchers had analyzed only already known miRNAs. In addition, there are difficulties in comparing the results obtained in different studies, due to the incomparability of different microarray platforms or groups of comparison. Massively parallel sequencing of miRNA (miRNA-seq) is free from such disadvantages, but at the moment this approach has not been used to study the whole human arteries or atherosclerotic plaques; it has been used only to analyze miRNA expression in plasma or in whole blood of patients with CAD [49].

DNA METHYLATION OF miRNA GENE REGIONS IN CORONARY AND CAROTID ARTERIES IN ATHEROSCLEROTIC LESIONS

Despite the fact that miRNAs are involved in the epigenetic regulation of the functional activity of many genes, these molecules can be targets for epigenetic modifications themselves. The expression of miRNA is controlled, among other things, by such epigenetic mechanisms as methylation of CpG islands (clusters of CpG dinucleotides) overlapping the promoter regions of genes or located near to the miRNA transcription start site [15, 50]. CpG islands are usually hypomethylated, and their excessive hypermethylation suppresses gene transcription and hence down-regulated the expression of the corresponding genes [51–53].

DNA methylation is more typical for down-regulation of the expression of miRNA genes, than protein-coding genes. According to various studies, 11–30% of miRNA genes and only 1–2% of protein-coding genes are methylated [15, 50, 54]. In addition, approximately 50% of miRNA genes harbor CpG islands and, therefore, can be regulated via DNA methylation [55, 56].

However, the regulation of miRNA expression through DNA methylation also occurs outside the CpG islands [55]. In particular, the expression of intronic miRNAs can be controlled by modification of their own promoter regions in tissue- and cell-specific manner. The own promoters of intronic miRNAs are genome regions with low CpG dinucleotide content, enriched with TATA-boxes and various transcription factors binding sites [16]. In addition, the changes of DNA methylation in the enhancers can also modulate miRNA expression [57].

When examining atherosclerotic plaques of various arteries, researchers mainly focus their attention on the analysis of DNA methylation in the promoter region of protein-coding genes. Only a few studies have revealed changes in DNA methylation level of miRNA genes in atherosclerotic plaques [58–63].

In particular, using the Illumina Infinium Human Methylation27 BeadChip, a decrease in the methylation level of the CpG island (located in the region of the *HOXD4* gene promoter), which harbor the coding region of the *MIR10B* gene, was found in the cells of coronary and carotid atherosclerotic plaques, as well as in peripheral blood leukocytes of patients with advanced atherosclerosis compared to intact internal thoracic arteries and great saphenous veins [58, 62].

It should be noted that miR-10b expression is altered in whole human arteries affected by atherosclerosis [37, 40]. *Mir10b* knockout mice were resistant to experimental atherosclerosis after high-fat-high-cholesterol diet and partial carotid artery ligation [64]. As shown in cell cultures, the up-regulation of miR-10b in endothelial cells (ECs) acts through the target genes *LTBP1* and *HOXD10* and leads to an

increased ECs migration and angiogenesis, as well as to decreased anti-proliferative ability of type II ECs against SMCs. Increased expression of miR-10b in macrophages through the target genes *ABCA1* and *ABCG1* leads to decreased cholesterol efflux and consequently reverse cholesterol transport, and this miRNA effect to the target gene *TIP30* increases the proliferation of SMCs [64–67].

Researchers from China evaluated the DNA methylation level, miRNA and mRNA expression in pooled *post mortem* coronary artery samples of advanced atherosclerotic plaques and intact arteries using the arrays GoldenGate Methylation Cancer Panel I (Illumina, USA), 22K human genome array chip (CapitalBio Corp, USA) and miRCURY LNA Array (Exiqon, USA). They identified not only genes, cellular processes and signaling pathways previously considered as involved in atherosclerosis, but also miR-519d [59].

Another study compared the methylation level of individual CpG sites in aortic and carotid atherosclerotic plaques with intact aortic tissues using Illumina HumanMethylation450 BeadChip microarray. Aortic samples were taken *post mortem* from the same individuals, and the carotid arteries were obtained during endarterectomy. All plaques were stable, classified as types III-VI according to the American Heart Association classification. The *MIR23B* gene was identified among the differentially methylated gene between atherosclerotic plaques and intact aortic tissues. Alterations in both the DNA methylation level in the *MIR23B* region and its expression in arteries have been confirmed by alternative methods [60].

The researchers from Finland evaluated the level and pattern of DNA methylation in atherosclerotic plaques of femoral arteries and intact internal thoracic arteries using the methyl-cytosine dependent DNA precipitation followed by next-generation sequencing. They also compared the results with the data from the expression microarrays [61]. More than 140 regions of miRNA genes were hypomethylated in atherosclerotic plaques, including atherosclerosis-related miRNAs (miR-10b, -27b and -758). Significant hypomethylation has been detected at imprinted 14q32 locus which contains a large cluster of miRNA genes. Increased expression of this miRNA cluster (miR-127, -136, -410, -431, -432 and -433) has also been shown in atherosclerotic plaques. Thus, this is one of the first studies established an association between DNA methylation and miRNA expression in atherosclerotic plaques.

However, a recent study revealed significant differences in the expression of the 14q32 miRNA cluster between healthy tissues of blood vessels of various localizations ($n = 109$). The DNA methylation level of the three CpG dinucleotide regions (differentially methylated regions—DMRs) at this locus is also highly variable between different vessels and associated with their pathological changes, independently of the primary or mature miRNA expression [68].

Ehrlich et al. used whole-genome bisulfite sequencing of three aortic samples and revealed hypermethylation of the *CARMN* (*MIR143HG*) enhancer region, 26 kb in length, in the aorta affected by atherosclerosis compared to the intact aorta and other tissues. They also found a potential association of changes in DNA methylation in the analyzed region with miR-143 and miR-145 expression [63].

At the same time, the analysis of the association of 283 miRNAs expression with methylation of more than 400000 CpG sites in whole blood cells obtained from 3565 patients with CAD in the Framingham Heart Study revealed an association of differential methylation of 227 CpG sites (cis-eQTM) with expression of 40 nearby miRNAs at the level of significance $p < 0.01$ [69]. Most frequently, cis-eQTMs were located in the promoter and polycomb-repressed regions; and 60% of them were inversely associated with the expression of a miRNA nearby. The Mendelian randomization analysis additionally identified 58 of cis-miR-eQTMs-miRNA pairs, in which case changes in DNA methylation appear to affect miRNA expression. These cis-eQTMs include cg18089426, which affects the miR-127 expression. However, increased cis-eQTM methylation levels in the "body" regions of miRNA genes resulted in enhanced transcription. Notably, a positive association of DNA methylation and miRNA expression was observed for cg06000878 and miR-100, as well as for cg11682508 and miR-133a [69].

Thus, DNA methylation changes in the region of miRNA genes (miR-10b, miR-23b, 14q32 locus (miR-127, -136, -410, -431, -432 and -433), miR-143 and miR-145) in atherosclerotic plaque cells have been analyzed in the few studies performed on whole and differently localized vessels. The specificity of the DNA methylation profile in atherosclerotic plaques of various vascular beds remains unclear, including profile of miRNA genes, as well as association with the cellular composition of arteries and clinical traits of atherosclerosis. Moreover, the assessment of the role of DNA methylation changes of miRNA genes in human atherosclerotic plaques is complicated by the dynamic nature of the disease, as well as high cellular heterogeneity of vascular tissues. Therefore, the use of animal models and cell cultures is an obligatory addition to such studies.

DNA POLYMORPHYSM IN THE miRNA GENE REGIONS IN THE ATEROSCLEROSIS-ASSOCIATED DISEASES

Genome-wide association studies have shown that most of the single nucleotide polymorphisms (SNPs) that associated with atherosclerosis are localized in non-coding DNA regions and/or in regulatory ele-

ments [70]. Considering that genetic predisposition plays an important role in the risk of atherosclerosis-associated diseases, polymorphism in the miRNA genes (in their regulatory regions, in pri- and pre-miRNAs, in the regions of mature miRNA nucleotide sequence), genes of miRNA biogenesis and miRNA target genes may have a functional significance for pathology formation [71].

It is expected that the number of polymorphic variants in the miRNA genes and, especially, in the miRNA biogenesis gene is smaller than in miRNA target genes, since miRNAs are conservative and short in length. In the nucleotide sequence of 1075 pre-miRNAs, 2257 SNPs have been identified and 246 SNPs have been found in the target mRNA recognition regions (seed-region) of 207 miRNAs [72]. At the same time, about 180000 SNPs are located in the 3'UTR region of protein-coding genes, so, theoretically, a large number of these genetic variants can be associated with miRNAs through mRNAs [73].

Associations of DNA polymorphisms in the miRNA gene regions with clinical complications of coronary and carotid atherosclerosis, such as CAD and myocardial infarction, ischemic stroke, and coronary artery restenosis have been studied to date (Table 2). The majority of studies are devoted to rs2910164:G>C (*MIR146A*) and rs3746444:A>G (*MIR499A; MIR499B*).

The association of the rs2910164:G>C (*MIR146A*) polymorphism with coronary artery atherosclerosis was studied mainly in Chinese population (Table 2). The separate studies have found an increased risk of CAD and ischemic stroke in carriers of the CC genotype and the C allele of this polymorphism. However, a meta-analysis of the results obtained in Asian populations has revealed interpopulation differences in the association of this polymorphism with different pathological phenotypes of coronary and carotid atherosclerosis [77, 79, 80, 86]. It is also possible that there is a difference in the molecular genetic mechanisms of the development of atherosclerotic plaque in the coronary and carotid arteries.

The only study that examined European population (in Germany) showed the association of CC genotype of rs2910164 with the risk of coronary artery restenosis rather than the coronary atherosclerotic lesions [81]. Since restenosis is a well-defined phenotype that differs from atherosclerosis by a rapid rate of development, it is assumed that epigenetic mechanisms may be more closely involved in the pathogenesis of restenosis compared to atherosclerosis. On the other hand, given the lower frequency of the C allele of rs2910164 in Europeans than in Asians (0.22 vs. 0.39 in ExAC database [98]), the result obtained may be associated with insufficient statistical power of the study.

The G allele and the GG genotype of rs3746444:A>G (*MIR499A*; *MIR499B*) was associated with the risk of MI and IS in the Chinese Han, South Korean and Iranian populations (Table 2). However, in Turkey the risk of coronary, carotid, and peripheral artery atherosclerosis was higher in carriers of A allele of rs3746444. The contradictions in the findings of the studies can be related to many factors, including, first of all, a small sample size and insufficient statistical power (in the latter study), as well as differences in the ethnic origin of individuals and their burden of comorbid diseases.

The functional consequences of polymorphic variant will depend on its localization in the miRNA gene. A proportional change in the total number of miRNAs and, consequently, in the expression of their target genes occurs when SNPs are localized in the genes of miRNA biogenesis. However, to date, there are no large-scale studies analyzed the associations of DNA polymorphism in the genes of miRNA biogenesis with atherosclerosis-associated cardiovascular diseases.

SNPs localized in the seed region of the mature miRNA have the strongest effect on miRNA binding to their mRNA targets. These genetic variants lead to strengthening or weakening of miRNA–mRNA binding, and can disrupt the binding of miRNAs to their canonical targets and/or make them bind to new mRNA target. For example, rs2168518:G>A, that is located in the seed region of miR-4513, is associated with different cardiometabolic phenotypes (serum glucose and lipid levels, blood pressure) and risk of CAD [97, 99]. It has been shown that the A allele of rs2168518 polymorphism decreases the level and activity of miR-4513, thereby up-regulating *GOSR2* gene, which product provides protein transport in the Golgi compartments [97].

The polymorphism in the 3'-UTR of protein-coding genes results in loss of miRNA binding site on mRNA and an increase in transcript/protein level or, conversely, in creation of a new mRNA:miRNA binding site and a decrease in transcript/protein level. These SNPs are of interest for disease association analysis since some variants have high population frequencies or there are strong differences in the frequency of SNPs in different populations.

In particular, the *APOC3* gene encodes the apolipoprotein C3, which is involved in triglyceride metabolism. Decreased APOC3 levels in the blood plasma of individuals result in lower triglyceride levels and reduced risk of coronary artery disease. The polymorphism rs4225:G>T is located in the 3'-UTR of the *APOC3* gene. According to the 1000 Genomes database, the frequency of T allele is 0.21 in East Asians, 0.61 in Europeans, and 0.07 in Africans [100]. In the Chinese population, individuals with TT genotype of rs4225 had lower APOC3 and triglyceride levels in plasma; this genotype also reduced risk of CAD, since the T allele is indirectly associated with APOC3 translation suppression through the interaction of its mRNA and miR-4271 [101].

The DNA polymorphism in the regulatory region (promoter, enhancer) of miRNA gene can lead to

Table 2. Associations of DNA polymorphisms in the miRNA gene regions with clinical complications of coronary and carotid atherosclerosis

miR	SNP	SNP localization*	Risk allele/ genotype	Atherosclerosis phenotype	Population	Sample size (case/control)	Reference
let-7	rs13293512	8.5 kb upstream from *MIRLET7A1* gene	TT	IS	Chinese Han	329/357	[74]
-27a	rs895819	In *MIR27A* gene body	GG	MI	Chinese Han	287/646	[75]
-146a	rs2910164	In *MIR146A* gene body	G/GG	Atherosclerosis of coronary, carotid and peripheral arteries	Turkish	150/145	[76]
			CC	CVD risk for South Korean and Indian, but CVD protection for the Chinese	Meta-analysis (Asian populations)	5433/6278 (12 studies)	[77]
			C/ GC + CC	CAD	Chinese Han	295/283	[78]
			CC	CAD	Meta-analysis (different populations)	13186/14497 (30 studies)	[79]
			CC	CAD	Meta-analysis (different populations)	3138/3097 (8 studies)	[80]
			CC	Coronary restenosis	Caucasian (Germany)	101/105	[81]
			CC	Carotid atherosclerosis	Chinese	596/379	[82]
			C/CC	IS, large arteries atherosclerosis subtype	Chinese Han	368/381	[83]
			C	IS	Chinese Han	297/300	[84]
			G	IS	South Korean	678/553	[85]
			G/GG	IS risk for South Korean only	Meta-analysis (different populations)	3138/3097 (8 studies)	[80]
			G/GG	IS risk for South Korean only	Meta-analysis (Asian populations)	2254/2506 (5 studies)	[86]
			GG	Recurrent IS	Chinese	1139/1585	[87]

Table 2. (Contd.)

miR	SNP	SNP localization*	Risk allele/ genotype	Atherosclerosis phenotype	Population	Sample size (case/control)	Reference
-149	rs2292832	In *MIR149* gene body	T/TC + CC	CAD	South Korean	522/535	[88]
-196a2	rs11614913	In *MIR196A2* gene body	CC + CT	Complications of CAD	Chinese Han	956/620	[89]
			C	MI	Caucasian (Russia)	325/185 (initial sample), 203/280 (validation sample)	[90]
			TT	IS risk for Chinese only	Meta-analysis (Asian populations)	1873/1856 (4 studies)	[86]
-200b	rs7549819	1.5 kb upstream from *MIR200B* gene	TT + TC	IS	South Korean	523/400	[91]
-423	rs6505162	In *MIR423* gene body	A/CA	CAD	Indian	100/117	[92]
-499	rs3746444	In *MIR499A* and *MIR499B* genes body	A	Atherosclerosis of coronary, carotid and peripheral arteries	Turkish	150/145	[76]
			GG	MI	Chinese Han	919/889	[93]
			G	IS	Iranian	470/489	[94]
			G	IS	Chinese Han	296/391	[95]
			GG	IS risk for Chinese only	Meta-analysis (Asian populations)	1505/1475 (3 studies)	[86]
-618	rs2682818	In *MIR618* gene body	GT + TT	Recurrent IS	Chinese	74/779	[96]
-4513	rs2168518	In *MIR4513* gene body	A	CAD	Meta-analysis (CARDIoGRAMplusC4D, GWAS)	63746/130681	[97]

* SNP localization is given in relation to the miRNA gene.
IS – ischemic stroke, MI – myocardial infarction, CVD – cardiovascular diseases, CAD – coronary artery disease.

impaired expression levels of individual miRNA. Analysis of genome-wide association studies (GWAS) data revealed 69 miRNAs located within 100 kb of SNPs associated with alteration in blood lipid levels. Functional analysis on model animals confirmed the association of two miRNAs (miR-128-1 and miR-148a) with the regulation of lipid metabolism [102].

Genetic variants in pri- and pre-miRNAs affect the processing of individual miRNAs and, consequently, alter the level of mature miRNAs and their binding to target mRNAs. In particular, the polymorphism that was most actively studied in researches focused on atherosclerosis and its complication (CAD, MI, IS) is rs2910164:C>G (Table 2). The rs2910164 polymorphism leads to the G>C nucleotide substitution in the pre-miRNA-coding sequence of *MIR146A* gene and then in "passenger chain" of mature miR-146a-3p (or miR-146a*). As a result, the processing and the conformation of pre-miR-146a secondary structure and its stability are impaired, and the production of mature miR-146a in the U2OS cell line decreases [103]. It was shown that CC genotype and the C allele of rs2910164 are associated with a decreased expression of miR-146a in cells, that increases mRNA levels of its targets (*IRAK1, TRAF6*), contributing to the proinflammatory profile and, therefore, the risk of disease. However, the miR-146a expression is higher in coronary and carotid atherosclerotic plaques than in intact vessels [28, 34, 37]. The target genes and molecular mechanisms (proliferation, apoptosis and migration of SMCs, modulation of immune response) by which miR-146a acts in atherosclerosis-associated cells are being actively studied [104, 105].

The rs11614913:C>T (*MIR196A2*) variant also belongs to the SNPs in pre-miRNA. The functional effect of this variant is associated with impaired processing of pre-miR-196a2, alteration of the mature miR-196a2 level and subsequent binding of this miRNA to target mRNAs. The C allele and CC+CT genotypes of rs11614913 are associated with the risk of MI in Russians and with the complicated course of CAD in the Chinese population [89, 90]. However, a meta-analysis of South Asian populations showed that, on the contrary, TT+TC genotypes of rs11614913 are associated with an increased risk of IS in Chinese, but not in South Koreans [86].

In contrast to SNPs, the association of copy number variations (CNVs) with the miRNAs and their regulatory network almost have not been studied both in normal condition and in atherosclerotic lesions. A comparison of miRNA loci and CNVs revealed 289 miRNA genes localized in the CNV regions (miRNA-CNVs) [106]. The functional effect of CNVs in the miRNA genes consists of a change in their expression – an increase or decrease, or in the absence of changes in the gene dosage [107]. However, we found only one association study of miRNA-CNVs with cardiovascular diseases [108]. This study showed the association of CNVs in the miR-93, -122 and -192 genes with CAD in combination with type 2 diabetes mellitus.

Previously, Nazarenko M.S. et al. revealed an approximately 39 kb deletion in 3q29 chromosomal region (chr3:195425875-195464424; GRCh37/hg19 genome assembly) in 5 patients with coronary artery atherosclerosis in combination with metabolic syndrome. This deletion includes the *MIR570* and *MUC20* genes. According to the 1000 Genomes Consortium (Phase 3) database, the population frequency of CNVs in this region varies from 24 to 78% [109]. Moreover, the enlargement of CNVs in this region is associated with increased relative expression of miR-570 in blood mononuclear cells of relatively healthy individuals [110]. In addition, CNV in the miR-570 genomic region is associated with the risk of congenital heart disease [111]. It is possible that this CNV can be considered as a candidate for atherosclerotic lesions.

Thus, most association studies of polymorphism in the miRNA gene regions (miR-let-7, miR-27a, -146a, -149, -196a2, -200b, -423, -499, -618, -4513) with pathological phenotypes of coronary and carotid atherosclerosis has been performed in Asian populations. The rs2910164:G>C (*MIR146A*) and rs3746444:A>G (*MIR499A; MIR499B*) polymorphisms receive the most attention. However, the results of these works are controversial (Table 2). It is possible that, as in other common diseases, a significant role in the predisposition to atherosclerotic arterial disease is played by ethnic differences, cumulative effect of many genetic variants, ethnicity and the differences in the comorbidity of individuals and in molecular genetic mechanisms of atherosclerotic lesions formation in different vascular beds.

CONCLUSION

Impairment of miRNA biogenesis, expression and its regulatory network with target genes is actively studied, since it will allow to identify miRNAs that may be selective therapeutic targets or biomarkers for prediction/prognosis of clinical complications of atherosclerosis, such as coronary artery disease, myocardial infarction, chronic cerebral ischemia, and ischemic stroke [8, 112–115]. However, the involvement of miRNA regulome in the molecular genetic mechanisms of atherosclerosis development in the arteries of different vascular beds is poorly understood. The study of miRNA biogenesis, expression and its regulatory network with target genes in humans in vivo is based on the analysis of a single molecular level (miRNA expression, DNA methylation, genetic polymorphism).

The expression of a wide range of miRNAs (miR-let-7f, miR-1, -16, -21, -24, -29b, -106b, -133a, -145, -146a, -155, -223, -486) changes in the tissues of the arteries and blood plasma/serum of individuals with coronary and carotid atherosclerosis. These miRNAs

have a large number of target genes, including *EGFR*, *ABI2*, *IGF1R*, *NFAT5*, *BRI3BP*, *VEGFA*, *CCND1*, *SP1*, *MYC*, *NOTCH2*, and protein products of these genes are involved in angiogenesis, cell migration and adhesion, apoptosis, and immune response activation.

DNA methylation can control the expression of many genes, including miRNA genes. The studies related to the analysis of changes in DNA methylation levels of miRNA genes (miR-10b, miR-23b, 14q32 locus (miR-127, -136, -410, -431, -432 and -433), miR-143 and miR-145) in atherosclerotic plaques are few in number and were performed on whole arteries of different blood vessels. On the other hand, DNA polymorphism can affect the function and expression of miRNA (similar to other regulatory molecules). The majority of association studies of polymorphism in miRNA genes (miR-let-7, miR-27a, -146a, -149, -196a2, -200b, -423, -499, -618, -4513) with pathological phenotypes of coronary and carotid atherosclerosis were performed mainly for individual SNPs in Asian populations and have contradictory results.

A comprehensive understanding of the molecular mechanisms of common diseases, including CVD, can be provided by multi-omics studies that analyze the interaction of genetic, epigenetic, transcriptomic, proteomic, metabolomic, and exposome factors [116, 117]. In particular, the epigenome is closely related to the genome, since genetic variants in the region of CpG sites alter the DNA methylation profile and transcription factor binding, and those in the miRNA genes modify their structure or expression. Moreover, miRNAs themselves can be targets for epigenetic modifications in the case when the DNA methylation level changes in the promoter region of miRNA gene, which leads to alterations in their expression. In turn, miRNAs are able to regulate the DNA methylation level by regulating DNA methyltransferases or methyl-CpG binding proteins. The application of such an integrative approach to the analysis of common diseases can complement the existing knowledge about their pathogenesis, as well as generate new ideas, including identification of biomarkers that are informative for assessment of disease severity and predicting its complications, as well as for identification of key therapeutic molecular targets, that will provide the basis for precision medicine.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflicts of interest.

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REFERENCES

1. Libby P., Buring J.E., Badimon L., Hansson G.K., Deanfield J., Bittencourt M.S., Tokgözoğlu L., Lewis E.F. 2019. Atherosclerosis. *Nat. Rev. Dis. Primers.* **5** (1), 56. https://doi.org/10.1038/s41572-019-0106-z
2. Basatemur G.L., Jørgensen H.F., Clarke M., Bennett M.R., Mallat Z. 2019. Vascular smooth muscle cells in atherosclerosis. *Nat. Rev. Cardiol.* **16** (12), 727–744. https://doi.org/10.1038/s41569-019-0227-9
3. Chinetti-Gbaguidi G., Colin S., Staels B. 2015. Macrophage subsets in atherosclerosis. *Nat. Rev. Cardiol.* **12** (1), 10–17. https://doi.org/10.1038/nrcardio.2014.173
4. Raitoharju E., Oksala N., Lehtimäki T. 2013. MicroRNAs in the atherosclerotic plaque. *Clin. Chem.* **59** (12), 1708–1721. https://doi.org/10.1373/clinchem.2013.204917
5. Andreou I., Sun X., Stone P.H., Edelman E.R., Feinberg M.W. 2015. miRNAs in atherosclerotic plaque initiation, progression, and rupture. *Trends. Mol. Med.* **21** (5), 307–318. https://doi.org/10.1016/j.molmed.2015.02.003
6. Feinberg M.W., Moore K.J. 2016. MicroRNA regulation of atherosclerosis. *Circ. Res.* **118**(4), 703–720. https://doi.org/10.1161/CIRCRESAHA.115.306300
7. Kucher A.N., Nazarenko M.S. 2017. Role of microRNA in atherogenesis. *Kardiologiya.* **57** (9), 65–76.
8. Fasolo F., Di Gregoli K., Maegdefessel L., Johnson J.L. 2019. Non-coding RNAs in cardiovascular cell biology and atherosclerosis. *Cardiovasc. Res.* **115** (12), 1732–1756. https://doi.org/10.1093/cvr/cvz203
9. Friedman R.C., Farh K.K.-H., Burge C.B., Bartel D.P. 2009. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res.* **19** (1), 92–105. https://doi.org/10.1101/gr.082701.108
10. Catalanotto C., Cogoni C., Zardo G. 2016. MicroRNA in control of gene expression: An overview of nuclear functions. *Int. J. Mol. Sci.* **17** (10), 1712. https://doi.org/10.3390/ijms17101712
11. Lu Y., Thavarajah T., Gu W., Cai J., Xu Q. 2018. Impact of miRNA in atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **38** (9), e159–e170. https://doi.org/10.1161/ATVBAHA.118.310227
12. Kozomara A., Birgaoanu M., Griffiths-Jones S. 2019. miRBase: From microRNA sequences to function. *Nucleic Acids Res.* **47** (D1), D155–D162. https://doi.org/10.1093/nar/gky1141
13. Laffont B., Rayner K.J. 2017. MicroRNAs in the pathobiology and therapy of atherosclerosis. *Can. J. Cardiol.* **33** (3), 313–324. https://doi.org/10.1016/j.cjca.2017.01.001
14. Olena A.F., Patton J.G. 2010. Genomic organization of microRNAs. *J. Cell. Physiol.* **222** (3), 540–545. https://doi.org/10.1002/jcp.21993
15. Morales S., Monzo M., Navarro A. 2017. Epigenetic regulation mechanisms of microRNA expression. *Biomol. Concepts.* **8** (5–6), 203–212. https://doi.org/10.1515/bmc-2017-0024
16. Marsico A., Huska M.R., Lasserre J., Hu H., Vucicevic D., Musahl A., Orom U., Vingron M. 2013. PROmiRNA:

A new miRNA promoter recognition method uncovers the complex regulation of intronic miRNAs. *Genome Biol.* **14** (8), R84. https://doi.org/10.1186/gb-2013-14-8-r84

17. Chakraborty C., Das S. 2016. Profiling cell-free and circulating miRNA: A clinical diagnostic tool for different cancers. *Tumour Biol.* **37** (5), 5705–5714. https://doi.org/10.1007/s13277-016-4907-3
18. Wong L.L., Wang J., Liew O.W., Richards A.M., Chen Y.T. 2016. MicroRNA and heart failure. *Int. J. Mol. Sci.* **17** (4), 502. https://doi.org/10.3390/ijms17040502
19. Hinske L.C., França G.S., Torres H.A., Ohara D.T., Lopes-Ramos C.M., Heyn J., Reis L.F., Ohno-Machado L., Kreth S., Galante P.A. 2014. miRIAD-integrating microRNA inter- and intragenic data. *Database (Oxford).* **2014**, bau099. https://doi.org/10.1093/database/bau099
20. Huang Z., Shi J., Gao Y., Cui C., Zhang S., Li J., Zhou Y., Cui Q. 2019. HMDD v3.0: A database for experimentally supported human microRNA-disease associations. *Nucleic Acids Res.* **47** (D1), D1013–D1017.
21. Sharma H., Estep M., Birerdinc A., Afendy A., Moazzez A., Elariny H., Goodman Z., Chandhoke V., Baranova A., Younossi Z.M. 2013. Expression of genes for microRNA-processing enzymes is altered in advanced non-alcoholic fatty liver disease. *J. Gastroenterol. Hepatol.* **28** (8), 1410–1415. https://doi.org/10.1111/jgh.12268
22. Cipollone F., Felicioni L., Sarzani R., Ucchino S., Spigonardo F., Mandolini C., Malatesta S., Bucci M., Mammarella C., Santovito D., de Lutiis F., Marchetti A., Mezzetti A., Buttitta F. 2011. A unique microRNA signature associated with plaque instability in humans. *Stroke.* **42** (9), 2556–2563. https://doi.org/10.1161/STROKEAHA.110.597575
23. Lovren F., Pan Y., Quan A., Singh K.K., Shukla P.C., Gupta N., Steer B.M., Ingram A.J., Gupta M., Al-Omran M., Teoh H., Marsden P.A., Verma S. 2012. MicroRNA-145 targeted therapy reduces atherosclerosis. *Circulation.* **126** (11, Suppl. 1), S81–S90. https://doi.org/10.1161/CIRCULATIONAHA.111.084186
24. Nazari-Jahantigh M., Wei Y., Noels H., Akhtar S., Zhou Z., Koenen R.R., Heyll K., Gremse F., Kiessling F., Grommes J., Weber C., Schober A. 2012. MicroRNA-155 promotes atherosclerosis by repressing Bcl6 in macrophages. *J. Clin. Invest.* **122** (11), 4190–4202. https://doi.org/10.1172/JCI61716
25. Santovito D., Mandolini C., Marcantonio P., De Nardis V., Bucci M., Paganelli C., Magnacca F., Ucchino S., Mastroiacovo D., Desideri G., Mezzetti A., Cipollone F. 2013. Overexpression of microRNA-145 in atherosclerotic plaques from hypertensive patients. *Expert. Opin. Ther. Targets.* **17** (3), 217–223. https://doi.org/10.1517/14728222.2013.745512
26. Di Gregoli K., Jenkins N., Salter R., White S., Newby A.C., Johnson J.L. 2014. MicroRNA-24 regulates macrophage behavior and retards atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **34** (9), 1990–2000. https://doi.org/10.1161/ATVBAHA.114.304088
27. Bazan H.A., Hatfield S.A., O'Malley C.B., Brooks A.J., Lightell D., Jr., Woods T.C. 2015. Acute loss of miR-221 and miR-222 in the atherosclerotic plaque shoulder accompanies plaque rupture. *Stroke.* **46** (11), 3285–3287. https://doi.org/10.1161/STROKEAHA.115.010567
28. Cao J., Zhang K., Zheng J., Dong R. 2015. MicroRNA-146a and -21 cooperate to regulate vascular smooth muscle cell proliferation via modulation of the Notch signaling pathway. *Mol. Med. Rep.* **11** (4), 2889–2895. https://doi.org/10.3892/mmr.2014.3107
29. Maitrias P., Metzinger-Le Meuth V., Massy Z.A., M'Baya-Moutoula E., Reix T., Caus T., Metzinger L. 2015. MicroRNA deregulation in symptomatic carotid plaque. *J. Vasc. Surg.* **62** (5), 1245–50.e1. https://doi.org/10.1016/j.jvs.2015.06.136
30. Di Gregoli K., Mohamad Anuar N.N., Bianco R., White S.J., Newby A.C., George S.J., Johnson J.L. 2017. MicroRNA-181b controls atherosclerosis and aneurysms through regulation of TIMP-3 and elastin. *Circ. Res.* **120** (1), 49–65. https://doi.org/10.1161/CIRCRESAHA.116.309321
31. Bildirici A.E., Arslan S., Özbilüm Şahin N., Berkan Ö., Beton O., Yilmaz M.B. 2018. MicroRNA-221/222 expression in atherosclerotic coronary artery plaque versus internal mammarian artery and in peripheral blood samples. *Biomarkers.* **23** (7), 670–675. https://doi.org/10.1080/1354750X.2018.1474260
32. Gong F.H., Cheng W.L., Wang H., Gao M., Qin J.J., Zhang Y., Li X., Zhu X., Xia H., She Z.G. 2018. Reduced atherosclerosis lesion size, inflammatory response in miR-150 knockout mice via macrophage effects. *J. Lipid Res.* **59** (4), 658–669. https://doi.org/10.1194/jlr.M082651
33. Jin H., Li D.Y., Chernogubova E., Sun C., Busch A., Eken S.M., Saliba-Gustafsson P., Winter H., Winski G., Raaz U., Schellinger I.N., Simon N., Hegenloh R., Matic L.P., Jagodic M., et al. 2018. Local delivery of miR-21 stabilizes fibrous caps in vulnerable atherosclerotic lesions. *Mol. Ther.* **26** (4), 1040–1055. https://doi.org/10.1016/j.ymthe.2018.01.011
34. Raitoharju E., Lyytikäinen L.P., Levula M., Oksala N., Mennander A., Tarkka M., Klopp N., Illig T., Kähönen M., Karhunen P.J., Laaksonen R., Lehtimäki T. 2011. miR-21, miR-210, miR-34a, and miR-146a/b are up-regulated in human atherosclerotic plaques in the tampere vascular study. *Atherosclerosis.* **219** (1), 211–217. https://doi.org/10.1016/j.atherosclerosis.2011.07.020
35. Miller C.L., Haas U., Diaz R., Leeper N.J., Kundu R.K., Patlolla B., Assimes T.L., Kaiser F.J., Perisic L., Hedin U., Maegdefessel L., Schunkert H., Erdmann J., Quertermous T., Sczakiel G. 2014. Coronary heart disease-associated variation in TCF21 disrupts a miR-224 binding site and miRNA-mediated regulation. *PLoS Genet.* **10** (3), e1004263. https://doi.org/10.1371/journal.pgen.1004263
36. Wang R., Dong L.D., Meng X.B., Shi Q., Sun W.Y. 2015. Unique microRNA signatures associated with early coronary atherosclerotic plaques. *Biochem. Biophys. Res. Commun.* **464** (2), 574–579. https://doi.org/10.1016/j.bbrc.2015.07.010
37. Xue Y., Wei Z., Ding H., Wang Q., Zhou Z., Zheng S., Zhang Y., Hou D., Liu Y., Zen K., Zhang C.Y., Li J., Wang D., Jiang X. 2015. MicroRNA-19b/221/222 induces endothelial cell dysfunction via suppression of

PGC-1α in the progression of atherosclerosis. *Atherosclerosis*. **241** (2), 671–681. https://doi.org/10.1016/j.atherosclerosis.2015.06.031

38. Markus B., Grote K., Worsch M., Parviz B., Boening A., Schieffer B., Parahuleva M.S. 2016. Differential expression of microRNAs in endarterectomy specimens taken from patients with asymptomatic and symptomatic carotid plaques. *PLoS One*. **11** (9), e0161632. https://doi.org/10.1371/journal.pone.0161632
39. Parahuleva M.S., Lipps C., Parviz B., Hölschermann H., Schieffer B., Schulz R., Euler G. 2018. MicroRNA expression profile of human advanced coronary atherosclerotic plaques. *Sci. Rep*. **8** (1), 7823. https://doi.org/10.1038/s41598-018-25690-4
40. Berkan Ö., Arslan S., Lalem T., Zhang L., Şahin N.Ö., Aydemir E.I., Korkmaz Ö., Eğilmez H.R., Çekin N., Devaux Y. 2019. Regulation of microRNAs in coronary atherosclerotic plaque. *Epigenomics*. **11** (12), 1387–1397. https://doi.org/10.2217/epi-2019-0036
41. Hao L., Wang X.G., Cheng J.D., You S.Z., Ma S.H., Zhong X., Quan L., Luo B. 2014. The up-regulation of endothelin-1 and down-regulation of miRNA-125a-5p, -155, and -199a/b-3p in human atherosclerotic coronary artery. *Cardiovasc. Pathol*. **23** (4), 217–223. https://doi.org/10.1016/j.carpath.2014.03.009
42. Katano H., Nishikawa Y., Yamada H., Yamada K., Mase M. 2018. Differential expression of microRNAs in severely calcified carotid plaques. *J. Stroke Cerebrovasc. Dis*. **27** (1), 108–117. https://doi.org/10.1016/j.jstrokecerebrovasdis.2017.08.009
43. Brennan E., Wang B., McClelland A., Mohan M., Marai M., Beuscart O., Derouiche S., Gray S., Pickering R., Tikellis C., de Gaetano M., Barry M., Belton O., Ali-Shah S.T., Guiry P., et al. 2017. Protective effect of let-7 miRNA family in regulating inflammation in diabetes-associated atherosclerosis. *Diabetes*. **66** (8), 2266–2277. https://doi.org/10.2337/db16-1405
44. Kern F., Aparicio-Puerta E., Li Y., Fehlmann T., Kehl T., Wagner V., Ray K., Ludwig N., Lenhof H.P., Meese E., Keller A. 2021. miRTargetLink 2.0–interactive miRNA target gene and target pathway networks. *Nucleic Acids Res*. **49** (W1), W409–W416. https://doi.org/10.1093/nar/gkab297
45. Chang L., Zhou G., Soufan O., Xia J. 2020. miRNet 2.0: Network-based visual analytics for miRNA functional analysis and systems biology. *Nucleic Acids Res*. **48** (W1), W244–W251. https://doi.org/10.1093/nar/gkaa467
46. Kern F., Fehlmann T., Solomon J., Schwed L., Grammes N., Backes C., Van Keuren-Jensen K., Craig D.W., Meese E., Keller A. 2020. miEAA 2.0: Integrating multi-species microRNA enrichment analysis and workflow management systems. *Nucleic Acids Res*. **48** (W1), W521–W528. https://doi.org/10.1093/nar/gkaa309
47. Huang H.Y., Lin Y.C., Li J., Huang K.Y., Shrestha S., Hong H.C., Tang Y., Chen Y.G., Jin C.N., Yu Y., Xu J.T., Li Y.M., Cai X.X., Zhou Z.Y., Chen X.H., et al. 2020. miRTarBase 2020: Updates to the experimentally validated microRNA-target interaction database. *Nucleic Acids Res*. **48** (D1), D148–D154. https://doi.org/10.1093/nar/gkz896
48. STRING: Protein–Protein Interaction Networks Functional Enrichment Analysis. 2021. https://string-db.org/.
49. Kanuri S.H., Ipe J., Kassab K., Gao H., Liu Y., Skaar T.C., Kreutz R.P. 2018. Next generation microRNA sequencing to identify coronary artery disease patients at risk of recurrent myocardial infarction. *Atherosclerosis*. **278**, 232–239. https://doi.org/10.1016/j.atherosclerosis.2018.09.021
50. Loginov V.I., Rykov S.V., Fridman M.V., Braga EA. 2015. Methylation of miRNA genes and oncogenesis. *Biochemistry* (Moscow). **80** (2), 145–162.
51. Chhabra R. 2015. miRNA and methylation: A multifaceted liaison. *Chembiochem*. **16** (2), 195–203. https://doi.org/10.1002/cbic.201402449
52. Ma J., Hong L., Chen Z., Nie Y., Fan D. 2014. Epigenetic regulation of microRNAs in gastric cancer. *Dig. Dis. Sci*. **59** (4), 716–723. https://doi.org/10.1007/s10620-013-2939-8
53. Piletič K., Kunej T. 2016. MicroRNA epigenetic signatures in human disease. *Arch. Toxicol*. **90** (10), 2405–2419. https://doi.org/10.1007/s00204-016-1815-7
54. Kunej T., Godnic I., Ferdin J., Horvat S., Dovc P., Calin G.A. 2011. Epigenetic regulation of microRNAs in cancer: An integrated review of literature. *Mutat. Res*. **717** (1–2), 77–84. https://doi.org/10.1016/j.mrfmmm.2011.03.008
55. Baer C., Claus R., Plass C. 2013. Genome-wide epigenetic regulation of miRNAs in cancer. *Cancer Res*. **73** (2), 473–477. https://doi.org/10.1158/0008-5472.CAN-12-3731
56. Wang Z., Yao H., Lin S., Zhu X., Shen Z., Lu G., Poon W.S., Xie D., Lin M.C., Kung H.F. 2013. Transcriptional and epigenetic regulation of human microRNAs. *Cancer Lett*. **331** (1), 1–10. https://doi.org/10.1016/j.canlet.2012.12.006
57. Bell R.E., Golan T., Sheinboim D., Malcov H., Amar D., Salamon A., Liron T., Gelfman S., Gabet Y., Shamir R., Levy C. 2016. Enhancer methylation dynamics contribute to cancer plasticity and patient mortality. *Genome Res*. **26** (5), 601–611. https://doi.org/10.1101/gr.197194.115
58. Markov A.V., Nazarenko M.S., Koroleva Yu.A., Lebedev I.N., Sleptsov A.A., Frolov A.V., Popov V.A., Barbarash O.L., Barbarash L.S., Puzyrev V.P. 2014. Methylation level in the promoter region of gene *HOXD4* in patients with atherosclerosis. *Med. Genet*. **13** (1), 39–42.
59. Wang Z., Guo D., Yang B., Wang J., Wang R., Wang X., Zhang Q. 2014. Integrated analysis of microarray data of atherosclerotic plaques: Modulation of the ubiquitin-proteasome system. *PLoS One*. **9** (10), e110288. https://doi.org/10.1371/journal.pone.0110288
60. Zaina S., Heyn H., Carmona F.J., Varol N., Sayols S., Condom E., Ramírez-Ruz J., Gomez A., Gonçalves I., Moran S., Esteller M. 2014. DNA methylation map of human atherosclerosis. *Circ. Cardiovasc. Genet*. **7** (5), 692–700. https://doi.org/10.1161/CIRCGENETICS.113.000441
61. Aavik E., Lumivuori H., Leppänen O., Wirth T., Häkkinen S.K., Bräsen J.H., Beschorner U., Zeller T.,

Braspenning M., van Criekinge W., Mäkinen K., Ylä-Herttuala S. 2015. Global DNA methylation analysis of human atherosclerotic plaques reveals extensive genomic hypomethylation and reactivation at imprinted locus 14q32 involving induction of a miRNA cluster. *Eur. Heart. J.* **36** (16), 993–1000. https://doi.org/10.1093/eurheartj/ehu437

62. Sleptcov A.A., Koroleva I.A., Frolov A.V., Popov V.A., Barbarash O.L., Puzyrev V.P. 2015. A comparison of genome-wide DNA methylation patterns between different vascular tissues from patients with coronary heart disease. *PLoS One.* **10** (4), e0122601. https://doi.org/10.1371/journal.pone.0122601
63. Ehrlich K.C., Lacey M., Ehrlich M. 2019. Tissue-specific epigenetics of atherosclerosis-related *ANGPT* and *ANGPTL* genes. *Epigenomics.* **11** (2), 169–186. https://doi.org/10.2217/epi-2018-0150
64. Nakahara M., Kobayashi N., Oka M., Nakano K., Okamura T., Yuo A., Saeki K. 2018. miR-10b deficiency affords atherosclerosis resistance. *bioRxiv*. 248641.
65. Shen X., Fang J., Lv X., Pei Z., Wang Y., Jiang S., Ding K. 2011. Heparin impairs angiogenesis through inhibition of microRNA-10b. *J. Biol. Chem*. **286** (30), 26616–26627. https://doi.org/10.1074/jbc.M111.224212
66. Wang D., Xia M., Yan X., Li D., Wang L., Xu Y., Jin T., Ling W. 2012. Gut microbiota metabolism of anthocyanin promotes reverse cholesterol transport in mice via repressing miRNA-10b. *Circ. Res*. **111** (8), 967–981. https://doi.org/10.1161/CIRCRESAHA.112.266502
67. Yu X., Li Z., Chen G., Wu W.K. 2015. MicroRNA-10b induces vascular muscle cell proliferation through Akt pathway by targeting TIP30. *Curr. Vasc. Pharmacol.* **13** (5), 679–686. https://doi.org/10.2174/1570161113666150123112751
68. Goossens E.A.C., de Vries M.R., Simons K.H., Putter H., Quax P.H.A, Nossent A.Y. 2019. miRMap: Profiling 14q32 microRNA expression and DNA methylation throughout the human vasculature. *Front Cardiovasc. Med*. **6**, 113. https://doi.org/10.3389/fcvm.2019.00113
69. Huan T., Mendelson M., Joehanes R., Yao C., Liu C., Song C., Bhattacharya A., Rong J., Tanriverdi K., Keefe J., Murabito J.M., Courchesne P., Larson M.G., Freedman J.E., Levy D. 2020. Epigenome-wide association study of DNA methylation and microRNA expression highlights novel pathways for human complex traits. *Epigenetics*. **15** (1–2), 183–198. https://doi.org/10.1080/15592294.2019.1640547
70. Edwards S.L., Beesley J., French J.D., Dunning A.M. 2013. Beyond GWASs: Illuminating the dark road from association to function. *Am. J. Hum. Genet.* **93** (5), 779–797. https://doi.org/10.1016/j.ajhg.2013.10.012
71. Borghini A., Andreassi M.G. 2018. Genetic polymorphisms offer insight into the causal role of microRNA in coronary artery disease. *Atherosclerosis.* **269**, 63–70. https://doi.org/10.1016/j.atherosclerosis.2017.12.022
72. Miao Y.R., Liu W., Zhang Q., Guo A.Y. 2018. lncRNASNP2: An updated database of functional SNPs and mutations in human and mouse lncRNAs. *Nucleic Acids Res*. **46** (D1), D276–D280. https://doi.org/10.1093/nar/gkx1004
73. Moszyńska A., Gebert M., Collawn J.F., Bartoszewski R. 2017. SNPs in microRNA target miRNA sites and their potential role in human disease. *Open Biol.* **7** (4), 170019. https://doi.org/10.1098/rsob.170019
74. Zhang L., Yang J., Xue Q., Yang D., Lu Y., Guang X., Zhang W., Ba R., Zhu H., Ma X. 2016. An rs13293512 polymorphism in the promoter of let-7 is associated with a reduced risk of ischemic stroke. *J. Thromb. Thrombolysis.* **42** (4), 610–615. https://doi.org/10.1007/s11239-016-1400-1
75. Cai M.Y., Cheng J., Zhou M.Y., Liang L.L., Lian S.M., Xie X.S., Xu S., Liu X., Xiong X.D. 2018. The association between pre-miR-27a rs895819 polymorphism and myocardial infarction risk in a Chinese han population. *Lipids Health Dis*. **17** (1), 7. https://doi.org/10.1186/s12944-017-0652-x
76. Oner T., Arslan C., Yenmis G., Arapi B., Tel C., Aydemir B., Sultuybek G.K. 2017. Association of NFKB1A and microRNAs variations and the susceptibility to atherosclerosis. *J. Genet.* **96** (2), 251–259. https://doi.org/10.1007/s12041-017-0768-9
77. He Y., Yang J., Kong D., Lin J., Xu C., Ren H., Ouyang P., Ding Y., Wang K. 2015. Association of miR-146a rs2910164 polymorphism with cardio-cerebrovascular diseases: A systematic review and meta-analysis. *Gene.* **565** (2), 171–179. https://doi.org/10.1016/j.gene.2015.04.020
78. Xiong X.D., Cho M., Cai X.P., Cheng J., Jing X., Cen J.M., Liu X., Yang X.L., Suh Y. 2014. A common variant in pre-miR-146 is associated with coronary artery disease risk and its mature miRNA expression. *Mutat. Res*. **761**, 15–20. https://doi.org/10.1016/j.mrfmmm.2014.01.001
79. Bastami M., Choupani J., Saadatian Z., Zununi Vahed S., Mansoori Y., Daraei A., Samadi Kafil H., Masotti A., Nariman-Saleh-Fam Z. 2019. Polymorphisms and risk of cardio-cerebrovascular diseases: A systematic review and meta-analysis. *Int. J. Mol. Sci.* **20** (2), 293. https://doi.org/10.3390/ijms20020293
80. Bao M.H., Xiao Y., Zhang Q.S., Luo H.Q., Luo J., Zhao J., Li G.Y., Zeng J., Li J.M. 2015. Meta-analysis of miR-146a polymorphisms association with coronary artery diseases and ischemic stroke. *Int. J. Mol. Sci.* **16** (7), 14305–14317. https://doi.org/10.3390/ijms160714305
81. Hamann L., Glaeser C., Schulz S., Gross M., Franke A., Nöthlings U., Schumann R.R. 2014. A micro RNA-146a polymorphism is associated with coronary restenosis. *Int. Immunogenet*. **41** (5), 393–396. https://doi.org/10.1111/iji.12136
82. Shen J., Zhang M., Sun M., Tang K., Zhou B. 2015. The relationship of miR-146a gene polymorphism with carotid atherosclerosis in Chinese patients with type 2 diabetes mellitus. *Thromb. Res*. **136** (6), 1149–1155. https://doi.org/10.1016/j.thromres.2015.10.013
83. Zhu R., Liu X., He Z., Li Q. 2014. miR-146a and miR-196a2 polymorphisms in patients with ischemic stroke in the northern Chinese han population. *Neurochem. Res.* **39** (9), 1709–1716. https://doi.org/10.1007/s11064-014-1364-5

84. Zhong H., Cai Y., Cheng J., Cai D., Chen L., Su C., Li K., Chen P., Xu J., Cui L. 2016. Apolipoprotein E epsilon 4 enhances the association between the rs2910164 polymorphism of miR-146a and risk of atherosclerotic cerebral infarction. *J. Atheroscler. Thromb.* **23** (7), 819–829. https://doi.org/10.5551/jat.32904

85. Jeon Y.J., Kim O.J., Kim S.Y., Oh S.H., Oh D., Kim O.J., Shin B.S., Kim N.K. 2013). Association of the miR-146a, miR-149, miR-196a2, and miR-499 polymorphisms with ischemic stroke and silent brain infarction risk. *Arterioscler. Thromb. Vasc. Biol.* **33** (2), 420–430. https://doi.org/10.1161/ATVBAHA.112.300251

86. Zhu J., Yue H., Qiao C., Li Y. 2015. Association between single-nucleotide polymorphism (SNP. in miR-146a, miR-196a2, and miR-499 and risk of ischemic stroke: A meta-analysis. *Med. Sci. Monit.* **21**, 3658–3663. https://doi.org/10.12659/msm.895233

87. Qu J.Y., Xi J., Zhang Y.H., Zhang C.N., Song L., Song Y., Hui R.T., Chen J.Z. 2016. Association of the microRNA-146a SNP rs2910164 with ischemic stroke incidence and prognosis in a Chinese population. *Int. J. Mol. Sci.* **17** (5), 660. https://doi.org/10.3390/ijms17050660

88. Sung J.H., Kim S.H., Yang W.I., Kim W.J., Moon J.Y., Kim I.J., Cha D.H., Cho S.Y., Kim J.O., Kim K.A., Kim O.J., Lim S.W., Kim N.K. 2016. miRNA polymorphisms (miR-146a, miR-149, miR-196a2 and miR-499. are associated with the risk of coronary artery disease. *Mol. Med. Rep.* **14** (3), 2328–2342. https://doi.org/10.3892/mmr.2016.5495

89. Zhi H., Wang L., Ma G., Ye X., Yu X., Zhu Y., Zhang Y., Zhang J., Wang B. 2012. Polymorphisms of miRNAs genes are associated with the risk and prognosis of coronary artery disease. *Clin. Res. Cardiol.* **101** (4), 289–296. https://doi.org/10.1007/s00392-011-0391-3

90. Osmak G.Zh., Matveeva N.A., Titov B.V., Favorova O.O. 2018. The myocardial infarction associated variant in the *MIR196A2* gene and presumable signaling pathways to involve miR-196a2 in the pathological phenotype. *Mol. Biol.* (Moscow). **52** (6), 872–877.

91. Kim J., Choi G.H., Ko K.H., Kim J.O., Oh S.H., Park Y.S., Kim O.J., Kim N.K. 2016. Association of the single nucleotide polymorphisms in microRNAs 130b, 200b, and 495 with ischemic stroke susceptibility and post-stroke mortality. *PLoS One.* **11** (9), e0162519. https://doi.org/10.1371/journal.pone.0162519

92. Jha C.K., Mir R., Elfaki I., Khullar N., Rehman S., Javid J., Banu S., Chahal S. 2019. Potential impact of microRNA-423 gene variability in coronary artery disease. *Endocr. Metab. Immune Disord. Drug Targets.* **19** (1), 67–74. https://doi.org/10.2174/1871530318666181005095724

93. Chen C., Hong H., Chen L., Shi X., Chen Y., Weng Q. 2014. Association of microRNA polymorphisms with the risk of myocardial infarction in a Chinese population. *Tohoku J. Exp. Med.* **233** (2), 89–94. https://doi.org/10.1620/tjem.233.89

94. Darabi H., Salmaninejad A., Jaripour M.E., Azarpazhooh M.R., Mojarrad M., Sadr-Nabavi A. 2019. Association of the genetic polymorphisms in immunoinflammatory microRNAs with risk of ischemic stroke and subtypes in an Iranian population. *J. Cell. Physiol.* **234** (4), 3874–3886. https://doi.org/10.1002/jcp.27159

95. Liu Y., Ma Y., Zhang B., Wang S.X., Wang X.M., Yu J.M. 2014. Genetic polymorphisms in pre-microRNAs and risk of ischemic stroke in a Chinese population. *J. Mol. Neurosci.* **52** (4), 473–480. https://doi.org/10.1007/s12031-013-0152-z

96. Zhang Z., Xu G., Cai B., Zhang H., Zhu W., Liu X. 2017. Genetic variants in microRNAs predict recurrence of ischemic stroke. *Mol. Neurobiol.* **54** (4), 2776–2780. https://doi.org/10.1007/s12035-016-9865-7

97. Ghanbari M., de Vries P.S., de Looper H., Peters M.J., Schurmann C., Yaghootkar H., Dörr M., Frayling T.M., Uitterlinden A.G., Hofman A., van Meurs J.B., Erkeland S.J., Franco O.H., Dehghan A. 2014. A genetic variant in the seed region of miR-4513 shows pleiotropic effects on lipid and glucose homeostasis, blood pressure, and coronary artery disease. *Hum. Mutat.* **35** (12), 1524–1531. https://doi.org/10.1002/humu.22706

98. Reference SNP (rs. Report: rs2910164). 2021. https://www.ncbi.nlm.nih.gov/snp/rs2910164.

99. Li Q., Chen L., Chen D., Wu X., Chen M. 2015. Influence of microRNA-related polymorphisms on clinical outcomes in coronary artery disease. *Am. J. Transl. Res.* **7** (2), 393–400.

100. Ensembl rs4225 SNP Allele: Frequency count. 2021. https://www.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=11:116832455-116833455;v=rs4225;vdb=variation;vf=164407333.

101. Hu S.L., Cui G.L., Huang J., Jiang J.G., Wang D.W. 2016. An APOC3 3'UTR variant associated with plasma triglycerides levels and coronary heart disease by creating a functional miR-4271 binding site. *Sci. Rep.* **6**, 32700. https://doi.org/10.1038/srep32700

102. Wagschal A., Najafi-Shoushtari S.H., Wang L., Goedeke L., Sinha S., deLemos A.S., Black J.C., Ramírez C.M., Li Y., Tewhey R., Hatoum I., Shah N., Lu Y., Kristo F., Psychogios N., et al. 2015. Genome-wide identification of microRNAs regulating cholesterol and triglyceride homeostasis. *Nat. Med.* **21** (11), 1290–1297. https://doi.org/10.1038/nm.3980

103. Jazdzewski K., Murray E.L., Franssila K., Jarzab B., Schoenberg D.R., de la Chapelle A. 2008. Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. *Proc. Natl. Acad. Sci. U. S. A.* **105** (20), 7269–7274. https://doi.org/10.1073/pnas.0802682105

104. Wang D., Atanasov A.G. 2019. The microRNAs regulating vascular smooth muscle cell proliferation: A minireview. *Int. J. Mol. Sci.* **20** (2), 324. https://doi.org/10.3390/ijms20020324

105. Sun X., Icli B., Wara A.K., Belkin N., He S., Kobzik L., Hunninghake G.M., Vera M.P., MICU Registry, Blackwell T.S., Baron R.M., Feinberg M.W. 2012. MicroRNA-181b regulates NF-κB-mediated vascular

inflammation. *J. Clin. Invest.* **122** (6), 1973–1990. https://doi.org/10.1172/JCI61495

106. Dweep H., Georgiou G.D., Gretz N., Deltas C., Voskarides K., Felekkis K. 2013. CNVs-microRNAs interactions demonstrate unique characteristics in the human genome. An interspecies *in silico* analysis. *PLoS One.* **8** (12), e81204. https://doi.org/10.1371/journal.pone.0081204
107. Marcinkowska M., Szymanski M., Krzyzosiak W.J., Kozlowski P. 2011. Copy number variation of microRNA genes in the human genome. *BMC Genomics.* **12**, 183. https://doi.org/10.1186/1471-2164-12-183
108. Sohrabifar N., Ghaderian S., Vakili H., Ghaedi H., Rouhani B., Jafari H., Heidari L. 2021. MicroRNA-copy number variations in coronary artery disease patients with or without type 2 diabetes mellitus. *Arch. Physiol. Biochem.* **127** (6), 497–503. https://doi.org/10.1080/13813455.2019.1651340
109. Nazarenko M.S., Sleptcov A.A., Lebedev I.N., Skryabin N.A., Markov A.V., Golubenko M.V., Koroleva I.A., Kazancev A.N., Barbarash O.L., Puzyrev V.P. 2017. Genomic structural variations for cardiovascular and metabolic comorbidity. *Sci. Rep.* **7**, 41268. https://doi.org/10.1038/srep41268
110. Lins T.C. de L. 2014. Variação estrutural no número de cópias e sua implicação na expressão de microRNA em humanos. https://repositorio.unb.br/handle/10482/16506.
111. Xing H.J., Li Y.J., Ma Q.M., Wang A.M., Wang J.L., Sun M., Jian Q., Hu J.H., Li D., Wang L. 2013. Identification of microRNAs present in congenital heart disease associated copy number variants. *Eur. Rev. Med. Pharmacol Sci.* **17** (15), 2114–2120.
112. Chen L.J., Lim S.H., Yeh Y.T., Lien S.C., Chiu J.J. 2012. Roles of microRNAs in atherosclerosis and restenosis. *J. Biomed. Sci.* **19** (1), 79. https://doi.org/10.1186/1423-0127-19-79
113. Schober A., Weber C. 2016. Mechanisms of microRNAs in atherosclerosis. *Annu. Rev. Pathol.* **11**, 583–616. https://doi.org/10.1146/annurev-pathol-012615-044135
114. Johnson J.L. 2019. Elucidating the contributory role of microRNA to cardiovascular diseases (a review). *Vascul. Pharmacol.* **114**, 31–48. https://doi.org/10.1016/j.vph.2018.10.010
115. Tao J., Xia L., Cai Z., Liang L., Chen Y., Meng J., Wang Z. 2021. Interaction between microRNA and DNA methylation in atherosclerosis. *DNA Cell Biol.* **40** (1), 101–115. https://doi.org/10.1089/dna.2020.6138
116. Mens M., Maas S., Klap J., Weverling G.J., Klatser P., Brakenhoff J., van Meurs J., Uitterlinden A.G., Ikram M.A., Kavousi M., Ghanbari M. 2020. Multi-omics analysis reveals microRNAs associated with cardiometabolic traits. *Front Genet.* **11**, 110. https://doi.org/10.3389/fgene.2020.00110
117. Vohra M., Sharma A.R., Prabhu B.N., Rai P.S. 2020. SNPs in sites for DNA methylation, transcription factor binding, and miRNA targets leading to allele-specific gene expression and contributing to complex disease risk: A systematic review. *Public Health Genomics.* **23** (5–6), 155–170. https://doi.org/10.1159/000510253