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ORIGINAL ARTICLE

Association of lipid metabolism-related gene promoter methylation with risk of coronary artery disease

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Abstract

Background: Coronary artery disease (CAD) is a complex disease that is influenced by environmental and genetic factors. Lipid levels are regarded as a major risk factor for CAD, and epigenetic mechanisms might be involved in the regulation of CAD development. This study was designed to investigate the association between the DNA methylation status of 8 lipid metabolism-related genes and the risk of CAD in the Chinese Han population.

Methods: A total of 260 individuals were sampled in this study, including 120 CAD cases and 140 normal healthy controls. DNA methylation status was tested via targeted bisulfite sequencing.

Results: The results indicated a significant association between hypomethylation of the APOC3, CETP and APOC1 gene promoters and the risk of CAD. Individuals with higher methylation levels of the APOA5 and LIPC gene promoters had increased risks for CAD. In addition, ANGPTL4 methylation level was significantly associated with CAD in males but not females. There were no significant differences in the methylation levels of the APOB and PCSK9 gene promoters between CAD patients and controls.

Conclusions The methylation status of the APOC3, APOA5, LIPC, CETP and APOC1 gene promoters may be associated with the development of CAD.

Keywords Coronary artery disease · Lipid metabolism · Methylation

Introduction

Coronary artery disease (CAD) is a common chronic inflammatory disease that has been recognized as the leading cause of death worldwide [1]. In China, it is estimated that 700,000 people died from CAD every year [2]. Blood lipid levels, including triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and serum total cholesterol (TC) levels, have been identified as important independent risk factors for CAD [3]. Recent advances have started to reveal the genetic architecture of CAD and have shown that genetic variants and epigenetic regulation of lipid metabolism-related genes also contribute to CAD etiology [4–6].

DNA methylation is a biological process in which gene expression is regulated by the recruitment of proteins involved in gene repression or by inhibition of the binding of transcription factors to DNA without changing the DNA sequence. Aberrant promoter hypermethylation and hypomethylation may be associated with risks of various diseases, including cardiovascular, cancer and metabolic diseases [7–9]. Several previous studies have revealed that the methylation signatures of critical genes may play a role in CAD development [4–6].

In this study, we aimed to investigate the association of the methylation status of 8 lipid metabolism-related genes (ANGPTL4, APOC3, APOA5, APOB, LIPC, CETP, PCSK9 and APOC1) with the risk of CAD development in the Chinese Han population.

Materials and methods

Study population.

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The participants in this study were recruited from Shanghai Renji Hospital between 2018 and 2020. A total of 120 CAD patients (88 male and 32 female) and 140 non-CAD controls (93 male and 47 female) were included in this study. The criterion for CAD diagnosis was at least one of the major segments of the coronary arteries (right coronary, left circumflex, or left anterior descending artery) with at least 50% organic stenosis based on coronary angiography. All unaffected controls were determined to be free of CAD. All participants were genetically unrelated Chinese Han individuals from Shanghai. This study was approved by the Medical Ethics Committee of Renji Hospital affiliated with the Shanghai Jiaotong University School of Medicine and compliant with the principles set forth by the Declaration of Helsinki. Written informed consent was obtained from all subjects. Blood samples (5 ml) were collected from the participants into EDTA tubes and then stored at − 80 °C for further use.

DNA extraction, bisulfite conversion and targeted bisulfite sequencing.

Genomic DNA was extracted from whole blood with a Tiangen DNA extraction kit (Tiangen Ltd., Beijing, China) according to the manufacturer's instructions. DNA quality and concentration were analyzed using electrophoresis and a NanoDrop spectrophotometer (NanoDrop Technologies, Houston, TX, USA). Bisulfite conversion of 200 ng genomic DNA was performed by the EZ DNA Methylation-Gold Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's standard protocol. For each gene, PCR primers were designed specifically for bisulfite-converted DNA using MethPrimer [10]. PCR primers were synthesized by Shanghai Free Biotechnology Co., Ltd. (Shanghai, China). Multiplex PCR of target CpG regions was performed, and the products were sequenced with Illumina NovaSeq sequencing instruments (Novogene, Beijing, China). A mean sequencing depth of > 500X was achieved for all samples. CpG sites were named according to their relative distance (in bp) to the transcriptional start site (TSS) (with negative distances upstream from the TSS). The methylation level of each CpG site was calculated as the percentage of the methylated cytosines over the total tested cytosines. The average methylation level was calculated using the methylation levels of all measured CpG sites within the gene.

Statistical analysis

Statistical analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5 software (GraphPad Software Inc., San Diego, CA, USA). The correlation between DNA methylation and CAD was assessed using an independent sample t test and expressed as the mean ± standard deviation (SD). Both the average gene methylation data and the methylation data for the individual CpG loci were analyzed. Stratified analyses based on sex were carried out. A P value < 0.05 was considered statistically significant.

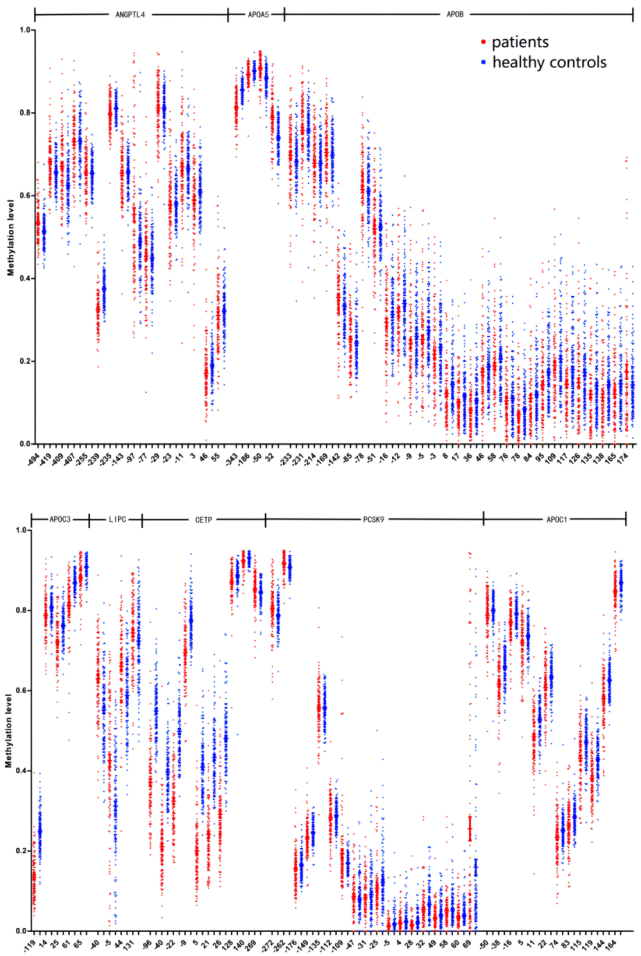

Fig. 1 Methylation levels of patients and healthy controls

Results

A total of 120 CAD patients and 140 healthy controls were recruited for this study. Targeted bisulfite sequencing was used to assess a total of 98 CpG sites in 8 lipid metabolism-related gene (ANGPTL4, APOC3, APOA5, APOB, LIPC, CETP, PCSK9 and APOC1) promoters. The methylation levels of each CpG site were compared between patients and healthy controls (Fig. 1). The methylation levels of 14 sites were significantly higher in the case group than in the control group, while the methylation levels of 37 sites were significantly lower in the case group than in the control group (supplementary Table 1).

The mean methylation level of each gene was calculated (Table 1). As shown in Table 1, significantly decreased

Table 1 Mean methylation levels (%) of candidate genes in cases and controls

Gene	CAD	Non-CAD	P value
ANGPTL4	57.42 ± 3.72	56.93 ± 2.68	0.233
APOC3	66.75 ± 3.14	71.84 ± 1.99	**1.17E-26**
APOA5	85.07 ± 2.03	84.5 ± 1.76	**0.022**
APOB	27.78 ± 7.06	28.77 ± 6.24	0.258
LIPC	61.37 ± 7.07	54.35 ± 6.11	**1.59E-15**
CETP	48.7 ± 4.58	62.13 ± 4.69	**1.33E-49**
PCSK9	21.46 ± 2.48	21.33 ± 1.86	0.672
APOC1	55.95 ± 3.94	59.05 ± 2.41	**8.40E-12**

Methylation levels (%) are reported as the means ± SDs, and P values less than 0.05 are shown in bold

Furthermore, subgroup analyses based on sex were carried out, which demonstrated that the results for APOC3, LIPC, CETP and APOC1 remained significant in both males and females, those for while APOA5 remained significant only in males (Table 2). ANGPTL4 was hypermethylated in males but not in females. No significant association was identified for APOB or PCSK9 methylation in the stratified analysis

methylation levels of the APOC3, CETP and APOC1 genes were observed in the case group compared with the control group. Most CpG sites were hypomethylated in APOC3 (5 of 5), CETP (9 of 10) and APOC1 (11 of 12). Significantly increased methylation levels of the APOA5 and LIPC genes were observed in the case group compared with the control group. For LIPC, 3 of 4 CpG sites were hypermethylated. For APOA5, 2 CpG sites were hypomethylated, and 2 CpG sites were hypermethylated. However, no significant correlations between methylation level and CAD were observed in ANGPTL4, APOB or PCSK9.

Discussion

CAD is a complex disease that is influenced by environmental, biochemical, and genetic risk factors. Lipoprotein metabolism disorder is a causal risk factor for cardiovascular diseases in the general population. DNA methylation, the most widely studied epigenetic mechanism, plays an important role in the etiology of human disease. Recent studies revealed that DNA methylation changes in gene promoters might be implicated in the development of CAD. In this study, we investigated the methylation status of a subset of lipid metabolism-related genes in CAD patients and control subjects in the Chinese Han population via targeted bisulfite sequencing.

The results support the hypothesis that epigenetic changes within lipid metabolism-related genes might account for blood lipid profile variability and could be a molecular mechanism explaining the pathogenesis of CAD. Different methylation statuses of the APOC3, APOA5, LIPC, CETP and APOC1 gene promoters were observed between CAD patients and healthy controls. DNA methylation could serve as a biomarker for predicting the risk of CAD [11–13].

APOC3 encodes a protein component of TG-rich lipoproteins (TRLs) and plays a role in promoting the hepatic secretion of TRLs. APOC3 is an inhibitor of lipoprotein lipase (LPL) enzyme activity and prevents TRL clearance [14, 15]. Loss-of-function APOC3 mutations were associated with low plasma TG levels and reduced risk of cardiovascular disease [16, 17]. Genetic variation in the promoter region of the APOC3 gene was associated with increased risks of hypertriglyceridemia, metabolic syndrome and CAD [18–20]. Overexpression of the APOC3 gene in transgenic animals induces severe hypertriglyceridemia, while APOC3 gene deletion results in hypotriglyceridemia [21–24]. In this study, the APOC3 gene was hypomethylated in CAD patients. The methylation level of CpG-119 in the APOC3 gene promoter was significantly lower in the CAD group than in the control group (13.5% vs. 24.5%) and might lead to higher gene expression.

The APOC1 gene encodes a member of the apolipoprotein C1 family and resides within the APOE/APOC1/APOC2 gene cluster. This gene is predominantly expressed in the liver, lung, skin, spleen, adipose tissue, and brain [25]. APOC1 plays an important role in high-density lipoprotein (HDL) and very-low-density lipoprotein (VLDL) metabolism. APOC1 is a very potent and highly selective inhibitor of cholesteryl ester transfer protein (CETP) in plasma [26, 27]. Transgenic analysis revealed that increased expression

Table 2 Mean methylation levels (%) of candidate genes in males and females

	Males			Females		
Gene	CAD	Non-CAD	P value	CAD	Non-CAD	P value
ANGPTL4	57.76 ± 3.98	56.61 ± 2.59	**0.024**	56.47 ± 2.71	57.55 ± 2.76	0.089
APOC3	66.68 ± 3.28	71.67 ± 1.98	**1.40E-18**	66.96 ± 2.79	72.19 ± 1.99	**1.62E-12**
APOA5	85.16 ± 1.98	84.47 ± 1.59	**0.015**	84.84 ± 2.21	84.55 ± 2.07	0.587
APOB	27.68 ± 7.51	28.1 ± 6.14	0.697	28.04 ± 5.89	30.11 ± 6.3	0.168
LIPC	62.05 ± 6.69	54.01 ± 5.78	**5.23E-15**	59.55 ± 7.82	55.02 ± 6.74	**0.008**
CETP	48.3 ± 4.38	61.5 ± 4.36	**1.44E-37**	49.9 ± 4.99	63.54 ± 5.13	**4.25E-14**
PCSK9	21.51 ± 2.32	21.09 ± 1.94	0.252	21.35 ± 2.89	21.8 ± 1.58	0.488
APOC1	55.74 ± 4.09	58.46 ± 2.28	**3.51E-07**	56.54 ± 3.48	60.23 ± 2.25	**7.93E-06**

Methylation levels (%) are reported as the means ± SDs, and P values less than 0.05 are shown in bold

of APOC1 inhibits the hepatic uptake of lipoproteins and results in combined hyperlipidemia [28–30]. In this study, 12 CpG sites in APOC1 were analyzed, and 11 CpG sites from − 38 to + 164 were hypomethylated in CAD patients. The methylation level of CpG-50 was not significantly different between the case and control groups.

CETP mediates the transfer of cholesteryl ester from HDL to other lipoproteins and promotes the formation of TG-rich and CE-poor HDL particles. Genetic variation in CETP has been reported to be associated with HDL-C levels [28–33. CETP deficiency was associated with slow progression of CAD, high HDL-C level, low HDL-TG levels and a larger HDL particle size [34, 35]. CETP inhibitors effectively reduce LDL-C levels and increase HDL-C levels and may be effective in reducing atherosclerosis and cardiovascular events [36]. In this study, 9 of 10 CpG sites from − 94 to + 140 in the CETP gene were hypomethylated in CAD patients. The methylation level of CpG + 269 did not differ significantly between the case and control groups.

LIPC encodes hepatic triglyceride lipase, which participates in the hydrolysis of TGs and phospholipids and is mainly expressed in and secreted from the liver. Variants in the promoter region of LIPC were reported to be correlated with high HDL-C levels [37, 38]. It has been reported that in familial hypercholesterolemia, subjects with a previous history of CAD had higher LIPC DNA methylation levels than those without a CAD history [39]. Our study revealed that 3 of 4 CpG sites from − 40 to + 44 in the LIPC gene were hypermethylated in CAD patients. The methylation level of CpG + 131 did not differ significantly between the case and control groups.

The APOA5 gene plays an important role in the regulation of blood triglyceride levels and is regarded as a major risk factor for coronary heart disease. Genome-wide methylation analysis revealed that APOA5 was hypomethylated in children with obesity [40]. APOA5 hypomethylation is also involved in aortic valve stenosis (AVS) [41]. Genetic variation in the APOA5 gene was associated with the levels of plasma lipids and an increased risk of cardiovascular disease [42, 43]. The mean methylation level of the APOA5 gene was relatively high in CAD patients compared with the control group. However, CpG − 50 and CpG + 32 were hypermethylated, while CpG − 343 and CpG − 186 were hypomethylated.

DNA methylation affects gene expression and regulates lipid metabolism. However, the cause of methylation variation is still poorly understood. DNA methylation signature was previously shown to be partially inherited. The methylation pattern can be affected by nearby single nucleotide polymorphisms (SNPs) [44, 45]. It has been revealed that the interaction of genetic and epigenetic variation contributes to the development of complex diseases [46]. In addition to genetic variants, environmental and lifestyle modifications are also considered to be potential causes of DNA methylation diversity [47–49]. Previous reports showed that a high-fat diet introduced DNA methylation changes in skeletal muscle and subcutaneous adipose tissue. Consumption of a high-fat diet is also associated with an increased risk of metabolic diseases [50, 51]. Hahn found that dietary restriction remodels DNA methylation patterns and gene expression, particularly of genes involved in lipid metabolism [52]. In addition, physical activity, smoking and drinking also induce DNA methylation variations [53–56].

In conclusion, targeted bisulfite sequencing was used to assess the methylation status of 8 lipid metabolism-related candidate genes in patients diagnosed with CAD and control subjects without CAD. We revealed that the methylation levels of the APOC3, CETP and APOC1 gene promoters were lower in the CAD group than in the control group. The methylation levels of the APOA5 and LIPC gene promoters were higher in the CAD group than in the control group. Our findings support the hypothesis that DNA methylation of lipid-related genes plays a role in the development of CAD and provide some new insight for the prevention and treatment of CAD. However, there are some limitations to this study. The expression levels of the target genes were not investigated, so we could not determine whether promoter methylation affects gene expression. Moreover, variations in the gene regions and DNA methylation might have dual effects on disease development. Further study of a larger sample with expression and genotyping data is needed to confirm our results.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11033-022-07789-0.

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