RESEARCH

Association of methylation status of *ABCA1/G1* genes with the risk of coronary artery disease

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Abstract

Background: ATP-binding cassette transporters A1/G1 (ABCA1/G1) is a main regulator of HDL (high-density lipoprotein) formation and reverse cholesterol transport. Impaired *ABCA1/G1* genes function may seriously affect cholesterol homeostasis, leading to increased risk of cardiovascular disease. In the present study, the association of *ABCA1/G1* genes methylation status with the risk of coronary artery disease (CAD), risk factors of CAD, and serum level of lipid parameters was investigated.

This study was conducted on 70 CAD patients and 40 control subjects. All CAD subjects with diabetes mellitus were excluded. The promoter methylation status of *ABCA1/G1* genes was determined by the methylation-specific polymerase chain reaction (MS-PCR) method and serum lipid parameters were assessed using commercial kits.

Results: *ABCA1* promoter methylation was higher in CAD group compared to the control participants (80% vs. 60%). Hypermethylation of the *ABCA1* gene significantly increases the risk of CAD in the total population (OR 3.886, 95% CI (1.181–12.791), $p = 0.026$). *ABCG1* methylation status showed no difference between CAD and control subjects. In addition, no significant association was noted between methylation status of *ABCA1/G1* and serum level of lipid profile.

Conclusions: Altogether, our study shows that *ABCA1* gene promoter hypermethylation may increase the risk of CAD, which may help identify people at risk of developing CAD.

Keywords: Apolipoproteins ATP-binding cassette transporter, Coronary artery disease, DNA methylation

Introduction

Coronary artery disease (CAD), the most common form of cardiovascular disease (CVD), is one of the main causes of mortality and morbidity worldwide [1]. Atherosclerosis, the leading cause of CAD, is a chronic inflammatory disorder with lipid deposition in coronary arteries that in turn develop angina pectoris, myocardial infarction (MI), and death [2].

Genetic and environmental factors such as poor diet, a family history of CAD, aging, smoking, hypertension, diabetes, and dyslipidemia are considered as risk factors for CAD. Dyslipidemia, as a key predictor of atherosclerotic cardiovascular disease, is defined as an increase in plasma total cholesterol (TC), triglyceride (TG), low-density lipoprotein-cholesterol (LDL-C), and decrease in high-density lipoprotein-cholesterol (HDL-C) [3]. CAD is treated mainly through lifestyle changes, such as exercising, eating a healthy diet, and controlling body weight. In some cases, the use of drugs that lower cholesterol and

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blood pressure, as well as beta blockers and aspirin are also prescribed [4].

The inverse association between HDL-C and CVD risk is well established [5]. However, previous studies have failed to show the beneficial effect of increasing HDL-C on CVD outcome improvement [6]. One reason that should be considered is the complexity of HDL metabolism.

ATP-binding cassette transporters A1/G1 (*ABCA1/G1*) is one of the most important genes that associated with HDL metabolism [7]. ABCA1/G1 as a member of ATP-binding cassette (ABC) superfamily is responsible for HDL-C formation through cholesterol efflux from non-hepatic peripheral tissues (e.g., macrophages and vascular smooth muscle cells) to extracellular lipid acceptors [8]. *ABCA1/G1* genes are crucial for cholesterol homeostasis and plasma HDL-C level regulation. Dysfunction of these genes will dramatically impair cholesterol and lipid transport, leading to a higher risk for premature CAD [9]. *ABCA1* gene mutations were shown to be involved in the pathogenesis of Tangier disease (TGD, OMIM 205400) [10] and familial hypoalphalipoproteinemia (FHA, OMIM 604091) characterized by the absence of plasma HDL-C and increased susceptibility to CAD [11]. Furthermore, pieces of evidence indicate that upregulation of *ABCA1/G1* genes can significantly alleviate macrophage lipid deposition and atherogenesis [10, 12].

Apolipoprotein A1 (ApoA1) is a key protein component of HDL particle and acts as the main acceptor for cholesterol efflux by ABCA1 [13]. Previous studies demonstrate increase serum level of apolipoprotein B (ApoB) and decrease level of ApoA1 are significantly associated with enhanced cardiovascular event [14, 15]. In addition, it has been shown that ApoA1 and ApoB level and ApoB/ApoA1 ratio are more accurate markers of cardiovascular risk compared to LDL-C and TC [16].

The exact regulatory mechanisms of ABCA1/G1 are still unknown. It has been well established that DNA methylation plays a critical role in gene expression regulation [17]. In general, gene promoter hypomethylation can upregulate gene expression, whereas gene promoter hypermethylation can suppress gene expression [18]. Previous studies have stated that differential promoter methylation in candidate genes contributes to CAD risk variability [19, 20].

Although *ABCA1* and *ABCG1* play key roles in regulating HDL-C level, few works were available regarding epigenetic modification of these genes in CAD [21, 22]. The present study aimed to investigate the association between *ABCA1* and *ABCG1* genes methylation status and the risk of CAD. It is well demonstrated that diabetes can significantly alter the methylation status of genes [23], so in the present work, CAD patients without diabetes were selected. The methylation alteration of ABCG1 in diabetes mellitus patients was shown previously [24, 25]. Another aim of our study was to evaluate the association of the methylation status of *ABCA1/G1* genes with serum levels of lipids, apolipoproteins, and patients' clinical characteristics.

Method and materials
Study subjects
This cross-sectional study was performed on 110 patients suffering from chest pain and dyspnea. Sample size was determined according to the following formula [26]:

$$n_1 = kn_2$$
$$n_2 = \left(\frac{Z_{\frac{\alpha}{2}} + Z_{\beta}}{p_1 - p_2}\right)^2 \times \left(\frac{p_1 q_1}{k} + p_2 q_2\right)$$
$$k = 3, \ p_1 = 0.9, \ p_2 = 0.296$$

The participants were selected among people who underwent coronary angiography for the first time. Subjects were excluded if they had any malignancy, chronic liver and kidney diseases, inflammatory disease, thyroid disease, any history of cardiovascular disease, and operation in the last 2 months. Subjects with diabetes mellitus were also excluded. Patients were defined as a diabetic if they had taken antidiabetic medications, previously diagnosed as diabetic, had fasting blood sugar equal or greater than 126 mg/dl or positive results of an oral standard glucose tolerance test [27].

According to angiography results, participants were divided into two groups: CAD group with at least 50% stenosis in at least one major coronary artery ($n = 70$) and control group with no stenosis in coronary arteries ($n = 40$). The control group had a completely normal angiogram [28]. The study protocol was in compliance with the Declaration of Helsinki and was approved by the local ethics committee (Ref: IR.HUMS.REC.1394. 82, Bandar Abbas, Iran, 2018). Informed consent was obtained from all subjects before being recruited for the study.

Biochemical analyses
Overnight fasting venous blood samples were obtained at admission, before administration of angiography contrast agent. The blood was collected in tubes and allowed 30 min to clot at room temperature. The serum was separated by centrifugation at 3000 rpm for 10 min and stored at -80 °C [29]. Blood samples containing anticoagulant (K_2EDTA 1 mg/ml) were used for genomic DNA extraction. All lipid indexes were measured using standard commercial kits (Pars Azmoon Company, Tehran, Iran) and BS-800 automatic biochemical analyzer (Mindray Company, UK). Total cholesterol (kit lot No: 97003)

and triglyceride (kit lot No:97002) were determined using enzymatic CHOD-PAP and GPO-PAP colorimetric methods, respectively. LDL-C was assessed according to the Friedewald formula, in subjects with TG > 400 mg/dl [30]. In those subjects with TG more than 400 mg/dl, a direct method using a commercial kit (lot No:97001) was used to determine LDL-C. Serum HDL-C (kit lot No:97011) was assessed by homogeneous assay. The serum levels of ApoA1 (kit lot No: 96012) and ApoB (kit lot No:96013) were assessed using auto analyzer BS-800 (Mindray Company, UK) by the immunoturbidimetric method and standard commercial kits (Pars Azmoon Company, Tehran, Iran). The inter-assay coefficient of variation (%CV) for triglyceride, total cholesterol, HDL-C, and LDL-C was 2.8, 2.9, 1.0, and 2.3, respectively.

Genomic DNA extraction, bisulfite treatment, and methylation-specific PCR

Genomic DNA was extracted from whole blood using the salting-out method [31]. Quantity and quality of DNA were assessed by the Nanodrop spectrophotometer (NanoDrop 2000c, Thermo Fisher Scientific, USA) and gel electrophoresis, respectively. For each sample, 2 μg of extracted DNA was used for bisulfite treatment [32]. In bisulfite modification, unmethylated cytosine converts to uracil while methylated cytosine will remain unchanged. After purification of modified DNA with commercial columns (Roche Company, Germany), methylation of *ABCA1* and *ABCG1* was assessed using methylation-specific polymerase chain reaction method (MS-PCR) and specific primers for methylated and unmethylated regions (Table 1). All PCR reagents (cat No:YT1591) were from Yekta Taghiz company (Tehran, Iran).

PCR reaction in 50 μl volume for methylated primers of both *ABCA1/G1* was contained: 5 μl PCR buffer (10X), 3 μl MgCl$_2$ 50 mM, 1 μl dNTP (10 mM), 0.4 μl Taq polymerase (5 U/μl), 1.25 μl of each forward and reverse primers (10 μM), and 2 μl of modified DNA. PCR reaction in 50 μl volume for *ABCA1/G1* unmethylated primers consisted of 5 μl PCR buffer (10X), 1.5 μl MgCl$_2$ 50 mM, 1 μl dNTP (10 mM), 0.25 μl Taq polymerase (5 U/μl), 2.5 μl of each forward and reverse primers

(10 μM), and 2 μl of modified DNA. The conditions for PCR amplification were as follows: an initial denaturation step at 95 °C for 2 min followed by 35 cycles consisting of denaturation at 95 °C for 15 s, annealing at 58.5 °C (ABCA1 methylated primer), 51 °C (*ABCA1* unmethylated primer), 54 °C (methylated *ABCG1* primer), and 48 °C (unmethylated *ABCG1* primer) for 30 s, and extension at 72 °C for 30 s. Following these cycles, the final extension was performed at 72 °C for 5 min. Commercially human methylated and non-methylated DNA set (Zymo Research Company, USA, Cat No:D5011) were used as control. Ultimately, for determining the methylation pattern, PCR products were run on 2% agarose gel (Pars Tous Biotechnology company, Mahshad, Iran, cat No: B111192) stained with GelRed (Biotinum company, Fremont USA, cat No:41001) and visualized under UV illumination (Fig. 1).

Statistical analyses

Data normality was checked for all continuous variables using the Shapiro–Wilk test. Anthropometric and laboratory characteristics of participants were reported as mean±SD for continuous variables with normal distribution, as median (IQR) for continuous variables with non-normal distribution and as number/percentage for categorical variables. The comparison of anthropometric and laboratory data with normal distribution was carried out using independent t test and for not normally distributed data, the Mann–Whitney U test was used. For evaluation of categorical variables, the Chi-square test was performed for normally distributed data and Mann–Whitney U test was performed for data with non-normal distribution. Log transformation was performed for non-normal distributed variables. To determine the relation of *ABCA1/G1* methylation and clinical variables, each variable was separately entered in a univariate logistic regression model; then, the variables which had a $p < 0.2$ were selected and entered into a multiple regression model using forward stepwise method and odds ratios (OR), 95% confidence intervals (CI), and p value was estimated. The correlation coefficient was employed

Table 1 Primers characteristics for methylation-specific PCR

Gene	Forward primer(5′–3′)	Tm	Reverse primer (5′–3′)	Tm	Product size (bp)
ABCA1 M	5′TTTGATCGATAGTAATTTTTGC 3′	51.29	5′AAAAATCGACTCGACTCTACG 3′	55.88	126
ABCA1 U	5′GGTTTTGATTGATAGTAATTTTTGT 3′	53.09	5′AAAAAAAATCAACTCAACTCTACA 3′	53.59	126
ABCG1 M	5′ATTTGTATTGTGATATCGACGAGAC 3′	57.38	5′CTTACCTCCTCGATTCTAAACGTAC 3′	58.80	251
ABCG1 U	5′CTTACCTCCTCGATTCTAAACGTAC 3′	58.80	5′AACTTACCTCCTCAATTCTAAACATAC 3′	57.33	251

M methylated-specific primers, *U* unmethylated-specific primers
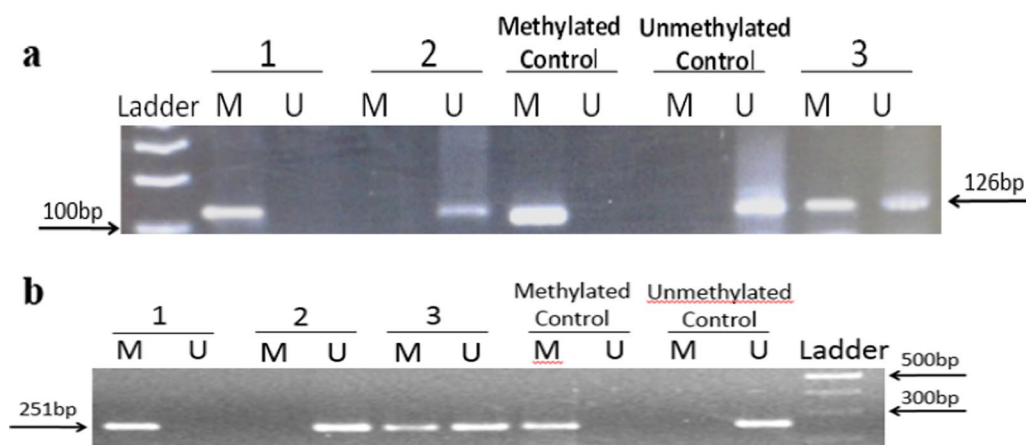
Fig. 1 The electrophoresis gel photograph of methylation status analysis of the *ABCA1/G1* gene promoter. **a** Lanes 1,2, and 3 represent the methylated, unmethylated, and hemimethylated status of the *ABCA1* gene in three participants, respectively. **b** Lanes 1,2, and 3 represent the methylated, unmethylated, and hemimethylated status of the *ABCG1* gene in three participants, respectively. As a standard methylated and unmethylated control, commercially methylated and unmethylated DNA control were used, respectively. M: methylated-specific primer, U: unmethylated-specific primer

by two-tailed Pearson's correlation analysis to examine the correlation of ApoA1, ApoB, and ApoB/ApoA1 with lipid profiles. All statistical analyses were performed using SPSS software version 16.0 (SPSS Inc. Chicago, IL, USA) and were reported two-tailed. P values < 0.05 were considered statistically significant.

Results

Anthropometric and laboratory parameters of the study population based on CAD and gender subgroup analysis

The anthropometric, clinical, and laboratory parameters of the study participants are shown in Table 2. The mean age of control and CAD groups were 51.63 ± 10.53 and 54.84 ± 8.18 years, respectively ($p = 0.10$). The mean of systolic blood pressure was markedly higher in CAD group than in control group. Subgroup analysis according to gender shows that BMI and systolic blood pressure among women were significantly higher than men ($P ˂ 0.05$). The methylation status of the *ABCA1* gene promoter (at cg05286874) was detected in 80% of CAD and 60% of control group and the differences were statistically significant ($P = 0.023$). Furthermore, the *ABCA1* gene promoter was hypermethylated in 85.7% of women and 66.7% of men ($P = 0.037$). 60% of participants in the control group and 41.4% of subjects in the CAD group were hypermethylated for *ABCG1* ($P > 0.05$). Also, *ABCG1* methylation (chr21:42296214-42296464) was detected in 44% of men and 57.1% of women but the differences were not statistically significant.

Association between *ABCA1* and *ABCG1* methylation and CAD risk factors

According to Table 3, 70% ($56/80 \times 100 = 70\%$) of participants with *ABCA1* promoter hypermethylation suffer from CAD, whereas just 46.66% ($14/30 \times 100 = 46.6\%$) of the study subjects with unmethylated *ABCA1* had CAD. This difference remains significant even after adjustment for potential confounders that can affect *ABCA1* promoter methylation including age, gender, smoking, and aspirin medication. The ratio of male to female in the unmethylated group is markedly higher than in the methylated group. 62.5% ($50/80 \times 100 = 62.5\%$) of subjects with methylated status of *ABCA1* were men while in the unmethylated group, 83.3% ($25/30 \times 100 = 83.3\%$) were men. There was no association between *ABCA1* promoter methylation and serum level of lipids and apolipoproteins. According to Table 4, the percentage of men with *ABCA1* promoter hypermethylation in the CAD group was markedly higher than in the control group ($p = 0.021$). The same pattern of *ABCA1* methylation was observed for women but the differences among women were not significant (Table 4).

The association between *ABCG1* methylation pattern and CAD-related risk factors is summarized in Table 5. 54.72% ($29/53 \times 100 = 54.72\%$) of subjects in the methylated group had CAD while 71.93% ($41/57 \times 100 = 71.93\%$) of the unmethylated group suffered from CAD and the difference was partially significant (p value $= 0.061$). Lipid profile and apolipoproteins show no significant association with *ABCG1* methylation status. The result of gender subgroup analysis is presented in Table 6.

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Table 2 Anthropometric and laboratory parameters of all participants based on subgroup analysis by CAD and gender

Variables	Control (40)	CAD (70)	P	Men (75)	Women (35)	*p*
Age (year)	51.63 ± 10.53	54.84 ± 8.18	0.100	52.67 ± 9.22	55.83 ± 8.87	0.093
Gender (M/F)	23/17	52/18	0.069	–	–	–
Male, n (%)	23 (57.5)	52 (74.3)	–	–	–	–
Female, n (%)	17 (42.5)	18 (25.7)	–	–	–	–
BMI (kg/m^2)	25.44 ± 5.09	25.06 ± 4.20	0.674	24.14 ± 4.32	27.45 ± 4.17	< 0.001
Systolic blood pressure (mmHg)	127.22 ± 22.32	136.21 ± 20.80	0.036	128.71 ± 18.13	142.03 ± 25.88	0.008
Diastolic blood pressure (mmHg)	78.63 ± 11.36	82.34 ± 10.12	0.079	80.53 ± 10.19	81.97 ± 11.77	0.513
Blood pressure history, n (%)	15 (37.5)	30 (42.9)	0.583	26 (34.7)	19 (40.9)	0.51
Smoking, n (%)	20 (50)	45 (64.3)	0.143	50 (66.7)	15 (42.9)	0.018
Aspirin medication, n (%)	34 (85)	58 (82.9)	0.896	61 (81.3)	31 (88.6)	0.339
Statin medication, n (%)	28 (70)	51 (72.9)	0.749	51 (68)	28 (80)	0.193
ABCA1 methylation, n (%)	24 (60)	56 (80)	0.023	50 (66.7)	30 (85.7)	0.037
ABCG1 methylation, n (%)	24 (60)	29 (41.4)	0.061	33 (44)	20 (57.1)	0.199
TG (mg/dl)	102.50 (80.75–164.50)	139.50 (98.00–178.50)	0.278	132 (91–180)	134 (95–160)	0.760
Total cholesterol (mg/dl)	156.10 ± 42.55	157.50 ± 37.96	0.895	153.93 ± 39.86	163.54 ± 38.45	0.236
HDL-C (mg/dl)	41.82 ± 10.32	41.66 ± 12.64	0.946	40.35 ± 11.91	44.63 ± 11.16	0.077
LDL-C (mg/dl)	85.96 ± 38.72	84.94 ± 39.79	0.897	85.42 ± 38.73	85.07 ± 40.84	0.966
ApoA1 (mg/dl)	133.10 ± 25.25	129.38 ± 25.16	0.457	127.80 ± 25.55	137.01 ± 23.36	0.073
ApoB (mg/dl)	79.88 ± 28.35	85.31 ± 26.81	0.320	82.43 ± 25.10	85.28 ± 32.01	0.644
ApoB/ApoA1	0.62 ± 0.26	0.69 ± 0.26	0.207	0.67 ± 0.23	0.65 ± 0.31	0.698

Continuous variables with normal and non-normal distribution are presented as mean ± SD and median (IQR), respectively. Independent Student's t test was performed for normally distributed data and Mann–Whitney *U* test for non-normally distributed data. Categorical variables are described as number (percentage) using Chi-square test

CAD coronary artery disease, *BMI* body mass index, *ABCA1* ATP-binding cassette transporters A1, *ABCG1* ATP-binding cassette transporters G1, *TG* triglyceride, *HDL-C* high-density lipoprotein-cholesterol, *LDL-C* low-density lipoprotein-cholesterol, *ApoA1* apolipoprotein A1, *ApoB* apolipoprotein B

Correlation of serum ApoA1, ApoB, and ApoB/ApoA with the study variables

Bivariate correlation analysis of ApoA1, ApoB, and ApoB/ApoA1 with anthropometric and laboratory variables in the study population is shown in Table 7. A positive significant correlation between serum ApoA1 level and age ($r = 0.337$, $P < 0.001$), systolic blood pressure ($r = 0.261$, $P = 0.006$), and HDL-C ($r = 0.578$, $P < 0.001$) was indicated. Additionally, serum ApoB had a positive significant correlation with TG ($r = 0.321$, $P = 0.001$), TC ($r = 0.534$, $P < 0.001$), and LDL-C ($r = 0.372$, $P < 0.001$). Moreover, a significant negative correlation between ApoB/ApoA1 ratio and age ($r = -0.200$, $P = 0.036$) and HDL-C ($r = -0.278$, $P = 0.003$) was noted. Finally, a significant positive correlation between ApoB/ApoA1 ratio and TG ($r = 0.232$, $P = 0.015$), TC ($r = 0.386$, $P < 0.001$), and LDL-C ($r = 0.342$, $P < 0.001$) were observed.

Discussion

The aim of the present investigation was to assess the methylation status of *ABCA1/G1* in non-diabetic CAD patients and control participants. Also, the association of methylation pattern of these genes with CAD and CAD-related risk factors, especially serum levels of HDL-C,

ApoA1, ApoB, and ApoB/ApoA1, are shown. To the best of our knowledge, this is the first report in which the methylation status of *ABCA1/G1* in non-diabetic CAD patients was studied.

In our study, the methylated form of the *ABCA1* gene was higher in CAD group than control subjects. We have shown the association of *ABCA1* gene promoter hypermethylation with non-diabetic coronary artery disease patients. In agreement with our results, Ghaznavi et al. showed an association between ABCA1 gene hypermethylation and the risk of CAD [21]. However, in their study, the control group was selected among healthy participants without angiography confirmation. Therefore, the presence of coronary artery stenosis could not be ruled out in their control subjects [21]. Moreover, Guay et al. observed that *ABCA1* gene promoter methylation has a direct correlation with CAD occurrence and aging among men [22]. In line with Guay et al. study, we showed that *ABCA1* methylation was associated with older age and a higher risk of CAD. As mentioned before, we found a higher percentage of ABCA1 methylation among women than men. However, in Guay et al. investigation the methylation status of ABCA1 was not compared between women and

Table 3 Association of *ABCA1* gene promoter methylation status with anthropometric and laboratory parameters

Variables	Methylation status		Univariate logistic regression		Multiple logistic regression	
	M (80)	*U* (30)	OR(95%CI)	*p*	OR(95%CI)*	*P**
Age (year)	54.96 ± 8.12	50.23 ± 10.99	1.059 (1.010–1.111)	0.038	0.948 (0.901–0.997)	0.046
Gender (M/F)	50/30	25/5	0.333 (0.115–0.964)	0.037	2.795 (0.914–8.544)	0.071
CAD (yes/no)	56/24	14/16	2.667 (1.126–6.315)	0.023	3.294 (1.233–8.799)	0.017
BMI (kg/m^2)	25.61 ± 4.55	24.08 ± 4.32	1.084 (0.980–1.199)	0.115	0.929 (0.832–1.038)	0.193
Systolic blood pressure (mmHg)	134.81 ± 22.10	127.97 ± 20.11	1.016 (0.995–1.037)	0.141	0.990 (0.965–1.015)	0.427
Diastolic blood pressure (mmHg)	81.33 ± 10.05	80.10 ± 12.38	1.011 (0.971–1.052)	0.595		
Blood pressure history (yes/no)	36/44	9/21	1.909 (0.779–4.679)	0.154	0.806 (0.295–2.200)	0.674
Smoking (yes/no)	47/33	18/12	0.949 (0.404–2.233)	0.905	1.388 (0.533–3.615)	0.502
Aspirin medication (yes/no)	69/11	23/7	1.909 (0.662–5.503)	0.226		
Statin medication (yes/no)	59/21	20/10	1.405 (0.567–3.483)	0.462		
TG (mg/dl)	134 (91–180.25)	164 (131.75–192)	2.253 (0.326–15.563)	0.413		
Total cholesterol (mg/dl)	154.39 ± 40.34	163.93 ± 36.90	0.994 (0.983–1.005)	0.261		
HDL-C(mg/dl)	42.65 ± 10.94	39.22 ± 13.71	1.027 (0.988–1.067)	0.175	0.989 (0.950–1.030)	0.586
LDL-C (mg/dl)	80.85 ± 39.40	97.22 ± 36.80	0.989 (0.979–1.000)	0.051	1.011 (1.000–1.023)	0.058
ApoA1 (mg/dl)	130.65 ± 22.67	130.94 ± 31.22	1.000 (0.983–1.016)	0.958		
ApoB (mg/dl)	83.16 ± 27.27	83.80 ± 28.12	0.999 (0.984–1.015)	0.913		
ApoB/ApoA1	0.65 ± 0.25	0.68 ± 0.29	0.703 (0.143–3.451)	0.664		

M methylated, *U* unmethylated, *CAD* coronary artery disease, *BMI* body mass index, *ABCA1* ATP-binding cassette transporters A1, *ABCG1* ATP-binding cassette transporters G1, *TG* triglyceride, *HDL-C* high-density lipoprotein-cholesterol, *LDL-C* low-density lipoprotein-cholesterol, *ApoA1* apolipoprotein A1, *ApoB* apolipoprotein B

*OR and *P* value after adjustment for age, gender, smoking, and aspirin medication

Continuous variables with normal and non-normal distribution are presented as mean ± SD and median (IQR), respectively. Independent Student's t test was performed for normally distributed data and Mann–Whitney *U* test for non-normally distributed data. Categorical variables are described as number (percentage) using Chi-square test. To determine the relation of *ABCA1/G1* methylation and clinical variables, each variable was separately entered in a univariate logistic regression model; then, the variables which had a *p* < 0.2 were selected and entered into a multiple regression model

Table 4 Association between *ABCA1* promoter methylation status and CAD risk based on subgroup analysis by gender

Methylation status	CAD	Non-CAD	OR(95%CI)	*p*	OR(95%CI)*	*P**
Study participants						
Methylated	56	24	2.667 (1.126–6.315)	0.023	3.886 (1.181–12.791)	0.026
Unmethylated	14	16				
Sex (male)						
Methylated	39	11	3.273 (1.167–9.178)	0.021	3.203 (1.097–9.348)	0.033
Unmethylated	13	12				
Sex (female)						
Methylated	17	13	5.231 (0.521–52.551)	0.129	2.546 (0.183–35.373)	0.486
Unmethylated	1	4				

*OR and *P* value after adjustment for age, gender, smoking, and aspirin medication

Odds ratio (OR) and 95% confidence interval (CI) were estimated using logistic regression model

men, because their study was only conducted on men subjects [22]. In another study, the researchers have reported that *ABCA1* gene promoter methylation was higher in familial hypercholesterolemia (FH) patients with CAD history rather than FH subjects without CAD history. FH is related to more susceptibility to CAD while our participants had no inherited abnormality [19].

Our results revealed that *ABCG1* methylation in the control group was higher than in CAD group. However, the data were not statistically significant. In contrast to our finding, Peng et al. observed that *ABCG1* gene hypermethylation increases the risk of CAD [26]. There are no consistent data about the role of *ABCG1* in CAD pathogenesis. Pieces of evidence reveal the protective role of *ABCG1* against CAD development

Table 5 Association of *ABCG1* gene promoter methylation status with anthropometric and laboratory parameters

Variables	Methylation status		Univariate logistic regression		Multiple logistic regression	
	M (53)	U (57)	OR(95%CI)	p	OR(95%CI)*	P*
Age	55.17 ± 8.51	52.28 ± 9.65	1.036 (0.933–1.081)	0.100	1.025 (0.98–1.071)	0.286
Gender (M/F)	33/20	42/15	0.589 (0.262–1.325)	0.199	0.756 (0.322–1.772)	0.519
CAD (yes/no)	29/24	41/16	2.121 (0.961–4.679)	0.061	0.450 (0.189–1.068)	0.070
BMI (kg/m^2)	25.07 ± 4.81	25.31 ± 4.28	0.988 (0.909–1.074)	0.782		
Systolic blood pressure (mmHg)	133.72 ± 20.88	132.23 ± 22.60	1.003 (0.986–1.021)	0.721		
Diastolic blood pressure (mmHg)	81.53 ± 9.30	80.49 ± 11.89	1.009 (0.974–1.046)	0.613		
Blood pressure history (yes/no)	23/30	22/35	1.220 (0.570–2.611)	0.609		
Smoking (yes/no)	27/26	38/19	0.519 (0.240–1.122)	0.094	0.547 (0.238–1.259)	0.156
Aspirin medication (yes/no)	47/6	45/12	2.089 (0.722–6.040)	0.168	2.203 (0.721–6.737)	0.166
Statin medication (yes/no)	40/13	39/18	1.420 (0.614–3.285)	0.411		
TG (mg/dl)	132(91–161.5)	134(94–180.5)	0.722 (0.134–3.907)	0.709		
Total cholesterol (mg/dl)	156.98 ± 34.76	157.00 ± 43.76	1.000 (0.991–1.010)	0.998		
HDL-C (mg/dl)	41.73 ± 12.16	41.70 ± 11.56	1.000 (0.969–1.033)	0.990		
LDL-C (mg/dl)	87.04 ± 34.96	83.70 ± 43.07	1.002 (0.993–1.012)	0.658		
ApoA1 (mg/dl)	131.30 ± 23.86	130.20 ± 26.48	1.002 (0.987–1.017)	0.818		
ApoB (mg/dl)	86.45 ± 28.03	80.44 ± 26.67	1.008 (0.994–1.022)	0.250		
ApoB/ApoA1	0.69 ± 0.28	0.64 ± 0.25	2.028 (0.47–8.748)	0.343		

M methylated, *U* unmethylated, *CAD* coronary artery disease, *BMI* body mass index, *ABCA1* ATP-binding cassette transporters A1, *ABCG1* ATP-binding cassette transporters G1, *TG* triglyceride, *HDL-C* high-density lipoprotein-cholesterol, *LDL-C* low-density lipoprotein-cholesterol, *ApoA1* apolipoprotein A1, *ApoB* apolipoprotein B

*OR and P value after adjustment for age, gender, smoking, and aspirin medication

Continuous variables with normal and non-normal distribution are presented as mean ± SD and median (IQR), respectively. Independent Student's t test was performed for normally distributed data and Mann–Whitney *U* test for non-normally distributed data. Categorical variables are described as number (percentage) using Chi-square test. To determine the relation of *ABCA1/G1* methylation and clinical variables, each variable was separately entered in a univariate logistic regression model; then, the variables which had a $p < 0.2$ were selected and entered into a multiple regression model

Table 6 Association between *ABCG1* promoter methylation status and CAD risk based on subgroup analysis by gender

Methylation status	CAD	Non-CAD	OR (95%CI)	p	OR (95%CI)*	P*
Study participants						
Methylated	29	24	2.121 (0.961–4.679)	0.061	0.0.450 (0.189–1.068)	0.070
Unmethylated	41	16				
Sex (male)						
Methylated	19	14	2.702 (0.984–7.417)	0.050	2.594 (0.905–7.437)	0.076
Unmethylated	33	9				
Sex (female)						
Methylated	10	10	1.143 (0.299–4.367)	0.845		
Unmethylated	8	7				

*OR and P value after adjustment for age, gender, smoking, and aspirin medication

Odds ratio (OR) and 95% confidence interval (CI) were estimated using logistic regression model

[10, 12], while some researchers had documented the deleterious effect of *ABCG1* in atherosclerosis and CAD development [33, 34]. On the other hand, in the aforementioned studies, a considerable percentage of the CAD subjects had also diabetes and based on available data diabetes could alter the genes methylation pattern [23, 35, 36]. Therefore, to find a real association between *ABCA1/G1* and the risk of CAD it is essential to exclude CAD patients with diabetes. In the present work, we excluded all diabetic subjects in both CAD and control groups. This could markedly strengthen the validity of our results and can more accurately reveal the association of *ABCA1/G1* methylation with the risk of CAD.

Table 7 Correlation of ApoA1, ApoB, and ApoB/ApoA1 with anthropometric and laboratory variables

Variables	ApoA1		ApoB		ApoB/ApoA1	
	Pearson coefficient	**P**	**Pearson coefficient**	**P**	**Pearson coefficient**	**P**
Age	0.337	0.000	− 0.013	0.889	− 0.200	0.036
BMI	− 0.064	0.506	0.063	0.514	0.089	0.353
Systolic blood pressure	0.261	0.006	0.002	0.986	− 0.112	0.244
Diastolic blood pressure	0.066	0.493	0.016	0.866	− 0.007	0.941
TG	0.109	0.258	0.321	0.001	0.232	0.015
Total cholesterol	0.176	0.065	0.534	0.000	0.386	0.000
HDL-C	0.578	0.000	0.029	0.762	− 0.278	0.003
LDL-C	− 0.004	0.966	0.372	0.000	0.342	0.000

The correlation coefficient was employed by two-tailed Pearson's correlation analysis to examine the correlation of ApoA1, ApoB, and ApoB/ApoA1 with lipid profiles and clinicodemographic parameters

ApoA1 apolipoprotein A1, *ApoB* apolipoprotein B, *BMI* body mass index, *TG* triglyceride, *HDL-C* high-density lipoprotein-cholesterol, *LDL-C* low-density lipoprotein-cholesterol

We did not find any association of *ABCA1/G1* methylation status with lipid profile, ApoA1, ApoB, and ApoB/ApoA1 ratio. In Guay et al. study, higher *ABCA1* methylation is related positively to TC, LDL-C, and TG levels in men older than 61 years but not in younger participants [22]. In the present work, the mean age of participants in all groups was lower than 54 years old. Albeit, there are inconsistent shreds of evidence about the relationship between *ABCA1/G1* methylation status and serum lipid profile level. Some researchers suggested the positive correlation between *ABCA1/G1* gene methylation and TG and LDL-C and others show a negative correlation between *ABCA1/G1* gene methylation and HDL-C [19, 22, 37].

A growing body of evidence had indicated the ratio of ApoB/ApoA1 as a better predictor for CAD than TC/HDL-C [38]. In the present work, we fail to indicate the significant association between ApoB/ApoA1 and CAD risk or between *ABCA1/G1* methylation and serum lipid profile level. This lack of association may be partly related to antihyperlipidemic medications that were used by most of our study participants. In fact, 70% of the control group and 72.9% of CAD patients received statins for modulating their serum cholesterol.

Our study had some limitation. First, we had a limited sample size. Other studies with a larger sample size are required to confirm our results. Second, our participants took some medications such as statins and aspirin which might affect the methylation status of ABCA1/G1 genes.

Future directions

Accumulating evidence demonstrate that hypermethylation and hypomethylation of DNA can lead to changes in gene expression. In the future work, it is better to study the methylation status of ABCA1/G1 genes along with mRNA and protein expression level in order to show the more precise impact of the methylation change of these genes on the risk of CAD.

Conclusion

Altogether, our study showed that *ABCA1* gene promoter hypermethylation may increase the risk of CAD, which may help identify people at risk of developing CAD. However, we could not show any association between methylation status of *ABCA1/G1* and serum level of APOA1, APOB, and lipid profile. Therefore, other basic and clinical studies are needed to show the effect of methylation status of these genes on the serum level of lipid parameters.

Abbreviations
ABCA1/G1: ATP-binding cassette transporters A1/G1; CAD: Coronary artery disease; MS-PCR: Methylation-specific polymerase chain reaction; CVD: Cardiovascular disease; MI: Myocardial infarction; TC: Total cholesterol; TG: Triglyceride; LDL-C: Low-density lipoprotein-cholesterol; HDL-C: High-density lipoprotein-cholesterol; ApoA1: Apolipoprotein A1; ABC: ATP-binding cassette; FH: Familial hypercholesterolemia.

Author contributions
EM was involved in data collection, and performed the experiment. FE helped in data collection, performed the experiment, analyzed the data, and wrote the manuscript. MM analyzed the data; MA E studied conception or design. SK performed the experiment, and wrote the manuscript. MK studied conception or design. HZ studied conception or design. HT studied conception or design. EE contributed to supervision, studied conception or design, helped in critical revision or editing of the article. All authors read and approved the final manuscript.

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Declarations

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