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Original article

Assessment of genetic polymorphism associated with ATP-binding cassette transporter A1 (ABCA1) gene and fluctuations in serum lipid profile levels in patients with coronary artery disease

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ABSTRACT

Background: Coronary artery disease (CAD) is one of the common genetic and clinical risk factors associated with cardiovascular and multifactorial disorder. ATP-binding cassette transporter A1 (ABCA1) gene plays an important role in lipid metabolism and in multiple studies associated with CAD. However, more studies are needed to identify the exact role of single nucleotide polymorphisms which may cause CAD.
Objectives: The aim of this study is to investigate the genetic association of polymorphism g.1051G > A in the *ABCA1* gene with CAD patients in the Saudi population.
Methods: We included 315 confirmed CAD cases, and 205 non-CAD or control subjects in this case-control study. DNA isolation was carried out for all registered participants and the polymorphism g.1051G > A was genotyped with Polymerase Chain Reaction followed by Restriction Fragment Length Polymorphism analysis with EcoNI restriction enzyme.
Results: Modifiable risk factors such as Body Mass Index, smoking and diabetes were strongly associated and non-modifiable risk factors such as hypertension (Systolic Blood Pressure and Diastolic Blood Pressure) and serum analysis such as Fasting Blood Glucose, Total cholesterol (TC), Triglyceride (TG) and LDL-c were significantly associated in CAD cases ($p < 0.05$). Allele (OR-1.73;95% CI:1.33–2.26; $p = 0.0004$), GA vs GG (OR-2.26; 95% CI: 1.53–3.35; $p = 0.0003$ and dominant inheritance pattern (OR-2.23; 95% CI:1.56–3.20; $p = 0.00009$ was strongly associated with CAD cases and control subjects. The

Abbreviations: CAD, Coronary artery disease; ABCA1, ATP-binding cassette transporter A1; PCR, Polymerase Chain Reaction.

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frequency level of use of atorvastatin was significantly different among GG, GA and AA subjects. Additionally, TC and TG levels were influenced by the presence of g.1051G > A polymorphism.
Conclusion: The polymorphism g.1051G > A in the gene *ABCA1* is closely associated with the existence of the CAD subjects. This polymorphism could also affect the serum levels of the lipid profile, suggesting a possible occurrence of CAD in the Saudi population.

1. Introduction

Coronary artery disease (CAD) is commonly known to cause premature mortality worldwide (Alshammary et al., 2021). The CAD prevalence rate is extremely high despite all of the precautionary methods that have been provided to the public. In 2015, CAD was responsible for 17.9 million of all death occurrences globally (Ruan et al., 2018). It is well known that the outcomes of congestive heart failure caused by CAD are worse than those caused by Acquired Immune Deficiency Syndrome (AIDS) or cancer (McMurray and Stewart, 2000). There are several factors that could cause CAD, including family history, lifestyle and the environment. Other risk factors include but are not limited to hypertension, diabetes, obesity and high cholesterol (McMurray and Stewart, 2000).

Previous genetic studies showed that most genetic variants that are associated with CAD were located close to specific genes. These genes have various roles in regulating the metabolism of triglyceride-rich lipoproteins (TRLs) and low-density lipoproteins (LDLs), thus underlining their roles in causing CAD (Toth, 2016). In addition, a whole exome sequencing study investigated mutations in 9 genes that were previously linked to CAD occurrence. The results from this study revealed destructive mutations in the LDLR gene, which were associated with an increased incidence of CAD (Do et al., 2015). Recently, several genome-wide association studies (GWAS) have linked numerous SNPs in non-coding regions of various genes with CAD occurrence. Although it was proposed that these SNPs could cause CAD through the stimulation of atherosclerosis, the exact mechanisms require further investigation (Christiansen et al., 2017). To date, GWAS have identified 161 genetic loci that are related to the causation of CAD. The genetic locus chr9p21.3 has the strongest effect as a risk factor of CAD development. This locus has multifarious SNPs that can be found in the CDKN2B-AS1 locus of the non-coding RNA. SNPs in this locus were also linked to other disorders, such as atherosclerosis and ischaemic stroke (Giral et al., 2018). A study that was performed on European and Non-European subjects documented a number of genetic loci that could lead to CAD. These loci have SNPs in an abundant number of CAD genes, including but not limited to BRAP, ATP2B1, PPAP2B, APOB and ABCG8 (McPherson, 2014). Nevertheless, a network analytical study has prioritized CAD candidate genes through bioinformatics tools. This study revealed the likelihood of 481 genes causing CAD based on their top scores in GWAS significance and included HLA-DQA1, SAYSD1, EXOC6B, LRP1, NDUFA4L2, SHMT2 and CRABP1.

Recent studies have focused on the effects of various mutations in the *ABCA1* gene, which is also known as ATP-binding-cassette-transporter-A1, facilitates the transfer of lipid-derived molecules across the cell membrane. This mechanism is essential in maintaining preventive measures against atherosclerosis and ensuring cellular cholesterol homeostasis (Kyriakou et al., 2007). Previous studies have suggested a link between elevated levels of triglycerides and low levels of HDL cholesterol, which could lead to CAD. Although single nucleotide polymorphisms (SNPs) that target the coding sequence in ABCA1 could affect the severity of CAD, SNPs in the non-coding sequence could disrupt ABCA1 expression. Four out of 12 SNPs, including C69T, G-191C, InsG319 and C-17G, were linked to coronary events. A study conducted in a Chinese population revealed that SNPs in the ABCA1 gene have an effect on the modulation of lipid metabolism. Therefore, it could increase the risk of developing CAD (Guo et al., 2011). Based on the results of previous discoveries, it is clear that SNPs in the ABCA1 gene may have an impact on causing CAD. However, the effects of these SNPs are still not fully understood. The current study aims to provide insight into the effects of the g.1051G > A polymorphism of the *ABCA1* gene and the profile of plasma lipids and their interaction with the frequency of CAD in the Saudi population.

2. Materials and methods

2.1. Ethics & confidentiality

The present research followed the guidelines set by the institutional review boards of King Abdullah Medical City (KAMC) and Umm Al-Qura University (UQU). All methods were ethical according to law stipulations of KAMC and UQU. Informed written consent was obtained from each individual. This research has been performed in line with good clinical practice.

2.2. Study population

Three hundred fifteen CAD patients were recruited from King Abdullah Medical City- KAMC and Al-Noor Specialist Hospital that is located in the western region in Saudi Arabia between September 2013 and May 2015. The CAD cases were enrolled within the hospital premises according to the full investigation achieved. Patients with CAD who suffered from diabetes and/or hypertension were excluded from this study as well as patients who had CAD as a secondary effect from other diseases. All patients in this study were adults with ages ranging from 30 to 70 years old. They have also been diagnosed with>50% stenosis. Without any history of peripheral vascular disease, CAD and stroke, age and gender controls were picked about 205 subjects. Our research also excluded patients with malignancies and other autoimmune diseases. The inclusion and exclusion criteria for CAD cases and controls were described in our recent publication (Bogari et al., 2020).

2.3. Clinical evaluation and blood testing

During the recruitment, all patients were examined for their age, gender, health and history. A 10-mL sample of 3.2% sodium citrate solution was obtained and then centrifuged for the blood test. The split plasma and blood samples were transferred to the laboratory-KAMC and the Al Noor Hospital for clinical variables in Makkah, Saudi Arabia to evaluate their levels and a full blood count.

2.4. DNA extraction and molecular genotyping

Genomic DNA was extracted from peripheral blood samples using a Whole Blood Genomic DNA Purification kit from Thermo

Scientific (Dreieich, Germany) and stored at − 20 °C (Khan et al., 2019). The extracted DNA was subjected to molecular genotyping using the restriction fragment length polymorphism technique (RFLP) for the *ABCA1 gene* c.g.1051G > A variant. The primer sequence was as follows: forward: 5′-GTA TTT TTG CAA GGC TAC CAG TTA CAT TTG ACA A-3′, and reverse: 5′-GAT TGG CTT CAG GAT GTC CAT GTT GGA A-3′. The primers were adopted from previously published article (Clee et al., 2001) and from local manufacturer, primers were synthesized (Ig Biosystems, Saudi Arabia). The amplification was carried out using a Veriti thermal cycler from Life Technologies (Darmstadt, Germany) with Promega GoTaq Master Mix (Walldorf, Germany) with the following programming conditions: 95 °C for 5 min, followed by 37 cycles of 95 °C for 30 s, 47 °C for 30 s, and 72 °C for 1 min, and a final extension step at 72 °C for 10 min. The selected primers produced a DNA fragment of 177 base pairs. Subsequently, 3 U of EcoNI (CCTACCAA**G/A**GGAG) restriction endonuclease from New England BioLabs (Frankfurt am Main, Germany) was added to 20 μl of amplified products to a total volume of 25 μl and incubated at 37 °C for overnight. Electrophoretic separation was carried out for the digestion products on a 3% agarose gel with ethidium bromide, and the bands were then visualized using an ultraviolet light cabinet (Rao et al., 2015). DNA fragments of 177 bp, 107 bp and 70 bp were released. There was a single 170-bp product for GG homozygotes; 3 bands for GA heterozygotes of 170 bp, 107 bp and 70 bp; and 2 bands for AA homozygotes of 107 bp and 70 bp (Fig. 1). Validation was performed with Sanger sequencing analysis (Fig. 2) based on our recent publication (Alharbi et al., 2021).

2.5. Statistical considerations and analysis

Data were analyzed using statistical software SPSS 21.0 (IBM Inc., Chicago, United States). The interpretation of the categorical variables was using descriptive statistics (frequencies and percentages). The Chi-square (χ^2) test of Pearson was used to compare CAD cases and controls as well as to distribute categorical study variables across the three genotypes of the g.1051G > A (GG, GA & AA) *ABCA1* gene. The χ^2 test is commonly applied for testing the relationships between the categorical variables and the null hypothesis of χ^2 test is that no relationship exists on the categorical variables in the independent population. All the frequencies were counted with the method of gene counting and the χ^2 test was applied to validate the departures from the equilibrium Hardy-Weinberg (HWE). The genotype distribution of g.1051G > A polymorphism has been contrasted by the χ^2 test using openepi software between CAD cases and subject controls (Khan et al., 2014). Two-sided experiments aimed at statistical significance. The bonferroni correction has been applied to the polymorphism g.1051G > A for different inheritance modes and different genotypes (Alharbi et al., 2014). A non-parametric Kruskal-Wallis test was used to compare the mean ranges of skewed quantitative variables with the three *ABCA1* gene products g.1051G > A (GG, GA, and AA). A p-value of < 0.05 has been used to describe the results of significant correlations.

3. Results

3.1. Clinical data

Table 1 shows the basic demographic characteristics of CAD cases and subjects of control. This study was conceived as a balanced sample of age and gender which indicates that both of them are not significantly associated ($p > 0.05$). CAD appears to be associated more often with BMI, smoking, diabetes, HTN, SBP, and DBP compared to non-CAD cases/healthy controls. Serum samples include fasting blood glucose, positive association of lipid profile parameters of TC, TG and LDL-c ($p < 0.05$). In CAD cases, HDL-c is not related and showed non-significant association when compared with controls ($p > 0.05$).

3.2. Genetic analysis of g.1051G > A genotyping

In the investigated CAD cases the genotypical distribution was consistent with HWE ($p = 0.5461$). The genotypical distribution of the g.1051G > A polymorphism between CAD cases and control subjects was shown in Table 2. The frequencies of the genotypes

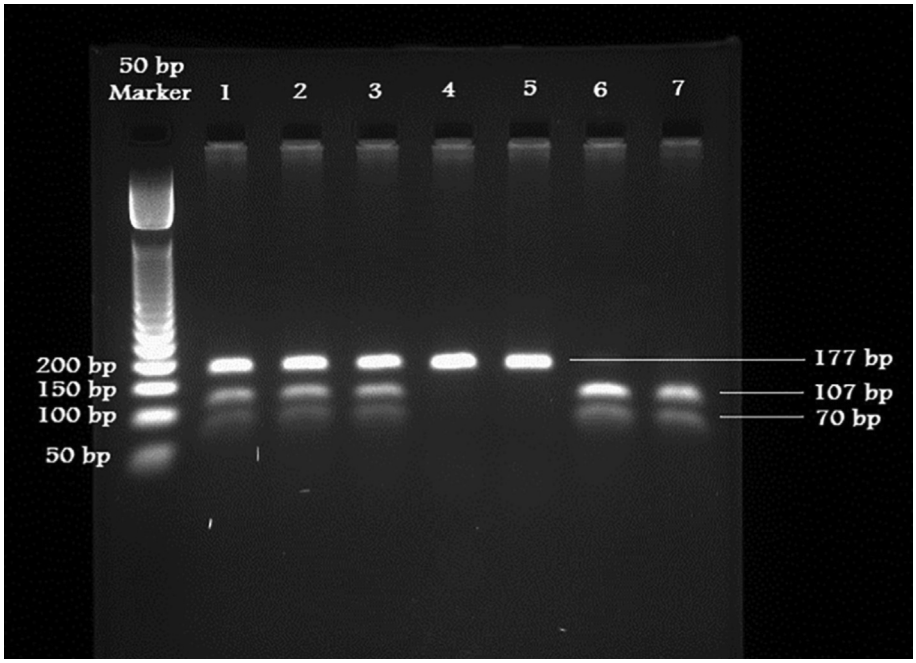

Fig. 1. A 3% agarose gel picture represents the g.1051G > A polymorphism in *ABCA1* gene.

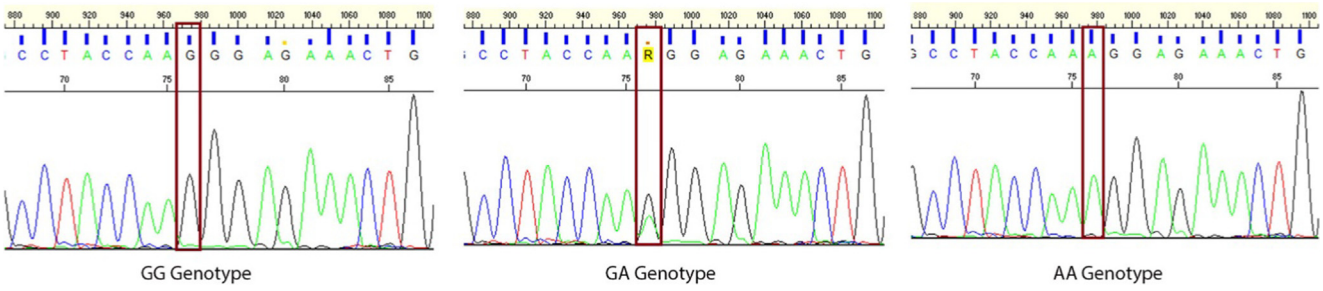

Fig. 2. Sanger sequencing analysis for g.1051G > A polymorphism in *ABCA1* gene.

Table 1
Clinical characteristics between control subjects and CAD cases.

Variables	CAD cases (n = 315)	Controls (n = 205)	P-values
Age (Mean ± SD)	59.7 (10.87)	58.4 (9.28)	p = 0.157
BMI (Mean ± SD)	29.26 (4.11)	26.26 (6.49)	p = 0.002
Gender (Male%)	206 (65.4%)	119 (58%)	p = 0.055
Smoking (%)	150 (47.6%)	58 (28.3%)	p = 0.001
Exercise (%)	72 (22.9%)	67 (32.7%)	p = 0.009
Diabetes (%)	187 (59.4%)	35 (17.1%)	p = 0.001
Fasting glucose (mg/dl)	140.64	83.56	p = 0.001
Hypertension (%)	221 (70.2%)	41 (20%)	p = 0.001
SBP (mmHg)	146.47	122.03	p = 0.002
DBP (mmHg)	91.84	74.25	p = 0.003
Event Stroke (%)	72 (22.9%)	7 (3.4%)	p < 0.001
Total Cholesterol (mg/dl)	154.17 (23.6)	140.88 (18.09)	p = 0.003
HDL-c (mg/dl)	39.95 (8.05)	41.14 (8.65)	p = 0.111
LDL-c (mg/dl)	115.89 (38.28)	88.6 (30.18)	p = 0.001
Triglycerides (mg/dl)	145.97 (60.27)	120.12 (22.71)	p = 0.001

GG, GA and AA were 35.9%, 46.3% and 17.8% in CAD cases and 55.6%, 31.7% and 12.7% in control subjects. Allele (OR-1.73; 95 % CI:1.33–2.26; p = 0.0004) and various genotype frequencies (AA vs GG: OR-2.17; 95 %CI: 1.27–3.70; p = 0.003, GA + AA vs GG: OR-2.23; 95 %CI: 1.56–3.20; p = 0.00009) were significantly associated within the cases and controls. After adjusting the age, BMI and sex between the CAD cases and controls, bonferroni correction was calculated and found a significant association between genotypes (GA vs GG: OR-2.5; 95 %CI: 1.4–3.3; p = 0.001) and genetic inheritance models (GA + AA vs GG: OR-2.2; 95 %CI:1.8–3.5; p = 0.00006 & GA vs AA + GG: OR-2.0; 95 %CI: 1.5–2.9; p = 0.0003). The measurement of sample and power size in g.1051G > A polymorphism was found to be 56%. The interaction was picked into the R219 K polymorphism after modification of the potential confounding factors.

3.3. Comparison of the use of medicines

The use of different medicines among these CAD subjects was observed, and its frequency of use was compared across the three products of the *ABCA1 gene* c.g.1051G > A. Out of 9 different medicines, the frequency of use of two medicines (atorvastatin and nitroglycerin) was significantly different among the subjects who had GG, GA, and AA. That is, 65 out of 91 (71.4%) with GG and 94 out of 117 (80.3%) with GA were using atorvastatin when compared with subjects with AA in which 28 out of 47 (59.5%) were using it, which was statistically significant (p = 0.022). Additionally, 9 out of 117 (7.7%) subjects with GA were using nitroglycerin when compared with subjects with GG (1 out of 91) and subjects with AA (1 out of 47), which was statistically significant (p = 0.048) (Table 3).

3.4. Comparison of the distribution and mean rank values

The plasma levels of LDL, cholesterol, HDL and triglycerides were compared across the three genotypes of the *ABCA1 gene* c.g.1051G > A in which a statistically significant difference was observed for the categories of cholesterol. That is, the proportions of borderline high cholesterol (52.4%) and high cholesterol (81.8%) were higher in persons with GA and GG than those with AA (p = 0.023). No statistical significance was observed for the other 3 variables across the three genotypes of the *ABCA1 gene* g.1051G > A (Table 4). The mean ranks of all clinical variables

Table 2
g.1051G > A polymorphic genotype and allele frequencies in the *ABCA1* gene in CAD cases and control subjects and Bonferroni correction.

Genotypes	CAD Cases (n = 315)	Controls (n = 205)	OR (95 %CI)[a]	P-Value	OR (95 %CI)[b]	P-Value
GG	113 (35.9%)	114 (55.6%)	Reference	Reference	1.0	1.0
GA	146 (46.3%)	65 (31.7%)	OR-2.26 (95 %CI: 1.53–3.35)	0.0003	OR-2.2 (95 %CI: 1.4–3.3)	0.0001
AA	56 (17.8%)	26 (12.7%)	OR-2.17 (95 %CI: 1.27–3.70)	0.003	OR-2.4 (95 %CI: 1.5–4.0)	0.001
GA + AA vs GG	202 (64.1%)	91 (44.4%)	OR-2.23 (95 %CI: 1.56–3.20)	0.00009	OR-2.5 (95 %CI: 1.8–3.5)	0.00006
GA vs GG + AA	146 (46.3%)	65 (31.7%)	OR-1.86 (95 %CI: 1.28–2.68)	0.0008	OR-2.0 (95 %CI: 1.5–2.9)	0.0003
AA vs GG + GA	56 (17.8%)	26 (12.7%)	OR-1.48 (95 %CI: 0.90–2.46)	0.11	OR-1.6 (95 %CI: 1.1–2.7)	0.08
G allele	372 (0.59)	293 (0.71)	Reference	Reference	–	–
A allele	258 (0.41)	117 (0.29)	OR-1.73 (95 %CI: 1.33–2.26)	0.0004	–	–

[a]Indicates 95% CI with crude odds ratio and [b]indicates Bonferroni correction adjusted with age, BMI and gender.

Table 3
Comparison of the use of medicines across three products of the *ABCA1 gene* c.g.1051G > A in CAD study subjects.

Name of medicines	*ABCA1 gene* c.g.1051G > A			X^2-value	p-value
	GG	GA	AA		
Amodipine (yes/no)(n = 110;143;54)	23/87	26/1117	9/45	0.514	0.774
Enoxaparinclexame(yes/no)(n = 111;143;54)	14/96	16/127	7/47	0.190	0.909
Aspirin (yes/no)(N = 91;117;47)	69/22	92/25	31/16	2.917	0.233
Atorvastatin (yes/no)(n = 91;117;47)	65/26	94/23	28/19	7.657	0.022*
Rosuvastatin (yes/no)(n = 91;117;47)	7/84	2/115	3/44	4.447	0.108
Clopidoreal (yes/no)(n = 91;117;47)	43/48	60/57	23/24	0.338	0.845
Pantoprazole (yes/no)(n = 91;117;47)	48/43	67/50	24/23	0.697	0.706
Nitroglycerin (yes/no)(n = 91;117;47)	1/90	9/108	1/46	6.058	0.048*
Perindopril arginine (yes/no)(n = 91;117;47)	33/58	47/70	21/26	0.947	0.623

*Statistically significant.

Table 4
Comparison of the distribution of clinical variables across three genotypes of **the** ABCA1 gene c.g.1051G > A in CAD study persons

Classification based on biochemical variables	*ABCA1 gene* c.g.1051G > A			X^2-value	p-value
	GG N = 113	GA N = 146	AA N = 55		
LDL-C (mg/dl)					
Optimum (>100)	70(36.3)	91(47.2)	32(16.6)		
Near optimum (100–129)	19(29.7)	34(53.1)	11(17.2)		
Borderline high (130–159)	13(32.5)	17(42.5)	10(25.0)	9.130	0.166
High (≤160)	11(64.7)	4(23.5)	2(11.8)		
T. Chol (mg/dl)					
Desirable (>200)	90(34.5)	122(46.7)	49(18.8)		
Borderline high (200–239)	14(33.3)	22(52.4)	6(14.3)	11.292	0.023*
High (<=240)	9(81.8)	2(18.2)	0(0)		
HDL-C (mg/dl)					
Major risk factor low (>40)	70(34.3)	93(45.6)	41(20.1)		
Moderate (40–60)	38(40.0)	46(48.4)	11(11.6)	3.466	0.483
Protective (<60)	5(33.3)	7(46.7)	3(20.0)		
Triglycerides (mg/dl)					
Normal (>150)	76(34.2)	100(45.0)	46(20.7)		
Borderline high (150–200)	16(41.0)	18(46.2)	5(12.8)	6.018	0.198
High (<200)	21(39.6)	28(52.8)	4(7.5)		

*Statistically significant (AA genotype has been opted only 55 instead of 56).

(LDL, CH, HDL, TG, PPBS, WBC, HB, RBC, BUN and albumin) were compared among the three genotypes of the *ABCA1 gene* g.1051G > A, where a statistically significant difference was observed for TG, which indicates that the persons with GG and GA had higher TG values when compared with those with AA (p = 0.047) (Table 5).

Table 5
Comparison of the mean rank values of clinical variables across three genotypes of the **ABCA1 gene** c.g.1051G > A in CAD study persons.

Clinical variables	*ABCA1 gene* c.g.1051G > A			p-value
	GG	GA	AA	
LDL	154.51	156.92	159.49	0.943
CH	158.10	154.64	143.69	0.619
HDL	166.69	152.76	133.69	0.081
TG	153.78	160.77	126.54	0.047*
PPBS	152.74	161.68	156.16	0.580
WBC	158.69	158.73	151.79	0.875
HB	160.45	150.82	169.17	0.398
RBC	163.72	150.14	164.25	0.403
Bun	167.42	154.10	143.24	0.209
Albumin	157.30	156.66	160.15	0.588

*Statistically significant.

3.5. Relation of risk factors associated with disease and genotyping of g.1051G > A in CAD-patients

In Table 6, when performing the χ^2-analysis, we tested the relationship between smoking vs. DM and HTN in the CAD cases and found to be insignificant association in DM (p = 0.35) and HTN (p = 0.58). At the same time, in Table 7, we cross-checked the g.1051G > A polymorphism with good and bad compliances of HTN in the CAD cases and found a non-significant association (p = 0.68) when the χ^2 analysis was applied.

4. Discussion

Studies have been carried out in this current case - control study on the genetic association between g.1051G > A polymorphism in the gene *ABCA1* and CAD in the Saudi population. The genetic interactions demonstrated the strong significant association between Allele, genotypes, inheritance pattern dominant and recessive. The parameters of the lipid profile (TC, TG and LDL-c levels were correlated with genotype levels in CAD patients. There were no significant differences in sex and age between CAD cases and controls as this study was chosen for similar age and genders involved in the study with respect to the presence or absence of the

Table 6
Correlation between smoking vs DM and HTN in CAD cases.

Relation between Smoking vs DM, vs HTN			Smoking		Total	Chi-Square Tests P value
			No	Yes		
DM	No	n	63	65	128	0.352*
		%	49.2%	50.8%	100%	
	Yes	n	102	85	187	
		%	54.5%	45.5%	100%	
HTN	No	n	47	47	94	0.581*
		%	50.0%	50.0%	100%	
	Yes	n	118	103	221	
		%	53.4%	46.6%	100%	
Total		n	165	150	315	
		%	52.4%	47.6%	100%	

*Insignificant statistically at α 0.05%.

Table 7
Relation between g.1051G > A polymorphism and HTN levels in CAD cases.

Relation between Genotyping and HTN			HTN		Total	Chi-Square Tests P value
			No	Yes		
Genotyping	GG	n	31	82	113	0.681
		%	27.4%	72.6%	100%	
	GA	n	44	102	146	
		%	30.1%	69.9%	100%	
	AA	n	19	37	56	
		%	33.9%	66.1%	100%	
Total		n	94	221	315	
		%	29.8%	70.2%	100%	

*Insignificant statistically at α 0.05%.

mutated allele in the *ABCA1* gene. This result was similar to results from another study where no association between the mutated gene *ABCA1* and hypertension (Yao et al., 2016) was found. However, there was a significant correlation between Diabetes Mellitus and the g.1051G > A variant in the *ABCA1* gene. A large number of patients whose genome contains polymorphisms have shown T2DM. This result suggested that patients carrying the variant g.1051G > A were more likely to develop DM. By comparison, the association between the possibility of developing T2DM and the *ABCA1* variant rs2230806 was confirmed by a study conducted on an Asian population. This research conducted a *meta*-analysis and concluded that having the minor A allele could affect the prevalence of T2DM in senior patients in Asia (Jung et al., 2018a). Although the minor allele rs2230806 in *ABCA1* has an impact on type II DM prevalence, according to the findings of a recent study (Cai et al., 2017), it has shown no influence on mild cognitive impairment (MCI) associated with DM.

The gene *ABCA1* (OMIM: 600046) is known to be a membrane protein involved in membrane transport. The gene *ABCA1* plays one of the key roles in an efflux of cholesterol from peripheral tissues transferred to the liver. The gene ABCA1 occurs on human chromosome region9q31.1. Due to the lack of HDL-c in plasma, the molecular function of genetic mutations involved in the *ABCA1* gene was shown to be involved in the pathogenesis of Tangier disease characteristics and the propensity to develop premature CVD. There have been numerous variants in the *ABCA1* gene and one of the well-known variants is rs2230806 (g.1051G > A or p. R219 K) SNP which appears on exon-7 and includes amino acid alterations from lysine to arginine and these alterations occur as a result of the substitution of G-A in the *ABCA1* gene at position 1051 (Ghaznavi et al., 2018;Karimian et al., 2020).

The results from this study also presented a significant difference in cholesterol levels among patients carrying the g.1051G > A variants and the controls. The carriers of the wild-type allele and the carriers of the heterozygous allele had higher cholesterol levels than the homozygous carriers of the g.1051G > A variant. Furthermore, the levels of TG were significantly higher in CAD patients who were heterozygous for the g.1051G > A variant and patients who carried the wild-type allele when compared to the levels in the AA allele carriers. There was no significant difference in the levels of HDL and LDL between g.1051G > A carriers and wild-type carriers. However, HDL levels were higher in patients carrying the AA allele. This finding is consistent with another study that showed an association between the g.1051G > A variant in the *ABCA1* gene and reduced HDL levels. The study concluded that the presence of the A allele could reduce the risk of developing CAD (Mokuno et al., 2015;Srinivasan et al., 2003)

Several case-control studies conducted around the globe in polymorphism g.1051G > A and found both positive and negative associations in the CAD. A recent *meta*-analysis study was conducted with g.1051G > A polymorphism in the CAD disease involving 43 different CAD case-control studies, and confirmed that K allele was significantly associated with reduced CAD risk in Asian and Caucasian populations (Fan et al., 2020). Our finding, however, was not in line with this review of *meta*-analysis and one of the explanations might be difference in an ethnicity. In addition to CAD, the g.1051G > A polymorphism was also used as *meta*-analysis studies in T2DM (Jung et al., 2018b) and Atherosclerosis (Yin et al., 2014), and confirmed negative association in T2DM and positive association with atherosclerosis. Our study was conducted in a relatively small subpopulation in the Saudi population's western region, and the generalizability of current findings to other Saudi populations may require a further complementary study with a larger sample size from other Saudi Arabian regions. Nonetheless, with the associated polymorphism (g.1051G > A), a few studies with small and large sample sizes have been published. Nonetheless, research by Zargar et al (2013) showed an insignificant correlation with large sample size between the CAD cases

and Cyrus et al (2016). Whereas in our study we have chosen a modest sample size of 315 CAD cases and found to be associated with them, and one of the main reasons for significant association is to recruit Saudi subjects for CAD cases and healthy controls from western Kingdom area. In the Saudi population with C69T polymorphism, Alharbi et al (2013) conducted the case-control study in T2DM cases and found to be associated in the *ABCA1* gene.

The study's strength was to include 315 CAD cases and 205 healthy Saudi-born controls. Another big strength of this study was drug definition in the patients with CAD. There are several limitations to this study, such as (i) lacking family history (ii) lack of evidence and (iii) performing only one SNP. The other limitations could be identified in the current study: (iv) specific coding SNPs similar to p.(V771 M), p.(I883 M) and p.(R1587 K) were not investigated in the *ABCA1* gene and their relationship to the production of CADs, and (v) the functional effects of the g.1051G > A variant in the cholesterol efflux assay need to be examined to establish the cell impact.

5. Conclusion

CAD is one of the most prevalent diseases that endangers human life. The focus of updated studies was on revising the genetic causes of CAD and establishing a correlation between common genetic variants and CADs. In conclusion, the current study was established as the initial studies to show genetic association in the CAD patients with g.1051G > A polymorphism in the *ABCA1* gene conducted in the Saudi population. By its lipid profile results, this study defines a correlation between the genetic variant g.1051G > A in the *ABCA1* gene and the CAD. The A allele carriers have higher HDL than the wild type carriers, thereby reducing the risk for these patients to develop CAD later in life. Future studies in the entire ethnicities should be conducted to rule out the role of genetic polymorphism in the CAD and may be useful as a genetic marker.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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