Original Article

Assessment of Six Polymorphic Variants as Genetic Risks for Coronary Artery Disease: A Case–Control Study

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Abstract

Background: Coronary artery disease (CAD) is the leading cause of death worldwide. Certain genetic polymorphisms play an important role in this multifactorial disease, being linked with increased risk of early onset CAD. **Objective:** To assess six genetic polymorphisms and clinical risk factors in relation to early onset nondiabetic Iraqi Arab CAD patients compared to controls. **Materials and Methods:** This case–control study recruited 40 Iraqi patients with early onset CAD and 20 healthy controls. Demographic and clinical data were reported. Six genetic variants were tested: β-fibrinogen (FGB), human platelet antigen 1 (HPA1a/b), angiotensin-converting enzyme (ACE), two variants of endothelial nitric oxide synthase (eNOS), and lymphotoxin alpha (LTA), utilizing a ready-to-use kit. **Results:** The majority of patients were older males (85%), nonsmokers (52.5%), hypertensives (57.5%), had dyslipidemia (100%), and had a family history of ischemia (77.5%). This contrasts the findings in the control group ($P < 0.001$). From the six studied polymorphisms, a statistically significant difference was found between patients and controls in relation to ACE and LTA genes ($P = 0.032$ and 0.028), respectively. None (0%) of the participants had a genetic risk score >6. There was a statistically significant association between higher clinical risk scores and CAD group; eNOS *G894T* was found to be linked with increasing age, while LTA was linked to dyslipidemia. **Conclusions:** This study aids in CAD risk stratification. There is a need for longitudinal studies assessing more genetic risks to CAD as a national CAD preventive program for high-risk Iraqi people.

Keywords: ACE, CAD, hypertension, IHD, Iraq, LTA, polymorphism

Introduction

Ischemic heart disease (IHD), also called coronary artery disease (CAD) or coronary heart disease (CHD), is the most common cause of angina, myocardial infarction, and acute coronary syndrome, and the leading cause of adult death globally, taking the lives of approximately 17.9 million each year.[1] In the vast majority of patients, CAD is caused by atherosclerosis; rarely can it occurs as the result of vasculitis, aortitis, and autoimmune connective tissue diseases.[2]

Many risk factors have been identified for atherosclerosis; unknown factors account for up to 40% of the variation in risk from one person to the other.[3] The risk factors for CADs can be grouped into major and minor, modifiable and nonmodifiable, and genetically determined and acquired.[3] These risk factors include age, sex, race, genetics, smoking, hypertension, hypercholesterolemia, diabetes mellitus, hemostatic factors, physical activity, obesity, alcohol, diet, personality, and social deprivation, among others.[2] The most common risk factors, such as hypertension, hyperlipidemia, and diabetes mellitus, are inherited in a polygenic manner.[3]

The major risk factors appear to be similar worldwide. In western countries, the incidence tends to be declining; but in Eastern Europe and much of Asia, the rates of CAD are rapidly rising in the last decades.[4]

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Factors like age, sex, and race contributed 63%–80% of prognostic performance, while modifiable risk factors contributed only modestly. However, control of the modifiable risk factors led to substantial reductions in CAD events.[5]

CAD and its risk factors can be screened, identified, and treated early. Identifying people at highest risk of CADs and ensuring they receive appropriate treatment can prevent premature deaths. Hereditary factors contribute to approximately 40% of the risk for CAD, especially so for early onset CAD.[6]

Incorporation of genetics into risk prediction offers the chance to refine risks, potentially earlier in life, toward the creation of earlier and tailored risk reduction strategies.[2]

Despite being a leading cause of death, IHD genetic studies lag behind other diseases.[5] More than 250 genes play critical roles in CAD predisposition and are involved in increasing or decreasing risks of CAD. The vast majority of them are polymorphic variants of these genes.[7]

Some genetic risk factors have extensively been studied; others are still under study. Many advances, though, have recently been made.[5]

- Beta-fibrinogen (FGB)-*455G*>A increases the risk for premature myocardial infarction (MI) and ischemic stroke and also confers elevated beta-fibrinogen plasma levels. It has an allele frequency range from about 10% to 20% in European populations and may vary in other ethnicities.[8]
- Human platelet antigen 1 (HPA1a/b; Gp IIIa; integrin beta 3 L33P): HPA1b is a risk factor for early onset MI and stroke, particularly in smokers. The HPA-1b allele frequency is around 15% in Caucasians but can be much lower in African and Asian populations.[9]
- Angiotensin-converting enzyme (ACE) 287 bp insertion/deletion (I/D) represents a risk factor for MI in elder patients and in smokers; the D allele is associated with elevated ACE activity and plasma levels. It has a frequency of approximately 50% in Caucasian populations but varies in other groups.[10]
- Endothelial nitric oxide synthase (eNOS; NOS3) is one of the most important candidate genes in CAD. eNOS-786T>C: the C allele causes a higher susceptibility to CHD and has an allele frequencies ranging from about 10% to 40%, depending on the population, while *eNOS* 894G>T (E298D): the T allele confers an increased risk for premature MI. The T allele frequency varies widely, with around 30–40% in Caucasian populations and different frequencies in other ethnic groups.[11]
- Lymphotoxin alpha (LTA) 804C>A (T26N) is in almost complete linkage with LTA 252A>G; both variants strongly act as proinflammatory and are associated with CAD. It has generally been observed around 10% to 20% in Caucasian populations but different in others.[12]

Aim of the Study

This study aims to assess the risk of six genetic variants and some clinical risk factors for early onset IHD among a group of Iraqi Arab nondiabetic patients in comparison to normal controls.

Materials and Methods

In this case–control study, two groups of individuals were recruited. About 40 patients diagnosed with early onset IHD admitted to the CCU of Ibn Al-Nafees Teaching Hospital in Baghdad represented the first group. The second group included 20 normal age- and sex-matched controls.

Early onset CAD was considered when IHD develops earlier than 50 years in males or earlier than 55 in females. Diabetics, those with chronic liver, kidney, thyroid disease, any other endocrine problem, patients on lipid-lowering drugs on admission, patients with incomplete clinical, biochemical, or molecular data were excluded from the study. The reason for this exclusion was to minimize the effect of other confounding factors potentially causing early onset CAD or its associated findings.

Basic demographic, clinical, laboratory, and radiologic findings were reported. A peripheral blood sample of 3–5 mL was aspirated from each participant, put in K_2-EDTA tubes, and stored at −20°C until the time of molecular analysis.

Molecular analysis included DNA extraction from peripheral blood samples, amplification of the extracted DNA using multiplex PCR, and then reverse hybridization of the amplification products to test strips containing allele-specific oligonucleotide probes immobilized as an array of parallel lines. The bound biotinylated sequences were detected using streptavidin-alkaline phosphatase and color substrates.

The study utilized a ready-to-use kit (CVD-StripAssay Cat. No. A 4-370), supplied from Vienna Labs Diagnostics GmbH—Austria®. Six genetic variations were tested: β-fibrinogen (FGB)-455G>A, human platelet antigen 1 (HPA1a/b), angiotensin-converting enzyme (ACE) 287 bp I/D (insertion/deletion), endothelial nitric oxide synthase (eNOS) *eNOS*-786 T>C, endothelial nitric oxide synthase (eNOS) eNOS *G894T* (E298D), and lymphotoxin alpha (LTA) C804A (T26N).

For the purpose of this study, eNOS-786 T>C will be referred to as eNOS1, while eNOS-G894T (E298D) as eNOS2.

The presence of the tested variant or its wild type can be visualized as a line on the test strip at its specified position. The appearance of the variant line only without its wild type means homozygosity for this variant, while the presence of both lines means a heterozygous state for

the variant, and the presence of only the wild-type line means a homozygous state for normal.

Statistical analysis was performed using IBM SPSS program (IBM SPSS, Inc., Chicago, IL) version 26. Data were examined by Kolmogorov–Smirnov test for the normality of data. Associations between categorical variables were assessed via cross tabulation and Chi-square test. Binary logistic regression was used to predict the value of a categorical dependent (outcome) variable. Exact tests were used to calculate the *P* value. In all statistical analyses, a *P* value < 0.05 was considered statistically significant.

This study was approved by the research ethics committee of the institution (issue No. 163 on December 29, 2020). All enrolled individuals have given their verbal consent to participate in this study and their DNA be tested provided their identities remain anonymous.

Results

About 40 Iraqi Arab nondiabetic patients with early onset IHD were recruited to this cross-sectional study along with 20 healthy controls from the general population. Their demographic and clinical data were studied and analyzed. Assessment of six genetic variants as risks for IHD was performed for both groups.

Age and sex

Patients and controls were grouped according to their ages into those <40 and those 40–55 years and were also categorized according to their sex. The majority of participants were males and belong to the 40–55 year group (85% and 77.5%, respectively). The *P* value for both was <0.05; therefore, both groups were considered age and sex-matched [Table 1].

Clinical characteristics

The majority of patients were nonsmokers (52.5%), hypertensives (57.5%), all had dyslipidemia (100%), born to nonconsanguineous marriages (57.5%), had a family history of ischemia (77.5%), did not have a family history of dyslipidemia (57.5%), and did not have a history of deep venous thrombosis (97.5%). This contrasts the findings in the control group. Hypertension, IHD, dyslipidemia, and drug history were all statistically highly significantly different between patients and controls ($P < 0.001$) [Table 2].

Molecular analysis

Molecular study of the six genetic variants, namely: β-fibrinogen (FGB)-455G>A, human platelet antigen 1 (HPA1a/b), angiotensin-converting enzyme (ACE) ACE 287 bp I/D (insertion/deletion), endothelial nitric oxide synthase (eNOS) eNOS-786 T>C (eNOS1), endothelial nitric oxide synthase (eNOS) eNOS G894T (eNOS2), and lymphotoxin alpha (LTA) LTA C804A (T26N) were

Table 1: Age and sex distribution of patients and controls

Variables		Patients		Controls		*P* value
		No.	%	No.	%	
Age (years)	<40	6	15	2	10	0.7
	40–55	34	85	18	90	
Sex	Male	31	77.5	17	85.0	0.73
	Female	9	22.5	3	15.0	

Table 2: Clinical characteristics of the enrolled patients and controls

Variables		Patients		Controls		*P* value	Odds ratio
		No.	%	No.	%		
Smoking	Yes	19	47.5	5	25.0	0.16	**2.7**
	No	21	52.5	15	75.0		
Hypertension	Yes	23	57.5	0	0	**<0.0001**	NA
	No	17	42.5	20	100		
Dyslipidemia	Yes	40	100	0	0	**<0.0001**	NA
	No	0	0	20	100		
Consanguinity	Yes	17	42.5	9	45.0	**1**	**0.9**
	No	23	57.5	11	55.0		
Family history of stroke/IHD	Yes	31	77.5	16	80.0	1	**0.86**
	No	9	22.5	4	20.0		
Family history of dyslipidemia	Yes	17	42.5	11	55.0	0.42	**0.61**
	No	23	57.5	9	45.0		
Deep venous thrombosis	Yes	1	2.5	0	0	**1**	NA
	No	39	97.5	20	100		

NA = not applicable: the risk estimate cannot be calculated

Bold values indicate any p-value less than 0.05 is considered significant

Bold values indicate any odds ratio less than 1 is considered significant

analyzed and compared between patients and controls. The zygosity status of these variants was also assessed. Both LTA C804A and ACE (D allele) were significantly linked to IHD patients group ($P < 0.05$). The remaining variants did not show a statistically significant difference [Table 3].

The combined effect of the six risky genes was also assessed; the genetic risk score was calculated for patients and control groups. These scores were calculated according to the number of risky alleles each individual had. The wild type was given 0 (no risky allele), the heterozygote was given 1 (1 risky allele), and the homozygote was given 2 (2 risky alleles) for each of the six studied genes. Therefore, the maximum number of possible risky alleles is 12 (when all genes are in homozygous state for the risky allele). Only one patient (2.5%) and none (0%) of the controls had zero risk score but none (0%) of the patients and control had a risk score of more than six. Both groups showed no statistically significant association regarding genetic risk score [Table 4].

The clinical risk scores were also calculated for patients and controls, including hypertension, dyslipidemia, smoking, and family history of ischemia. Therefore, patients and controls were divided into five groups depending on the number of risky clinical factors they had each. Only eight (20%) patients have all four clinical risks and none (0%) were free of all risky clinical factor. There was a statistically highly significant association between clinical risk scores (3 + 4) and the patients group as compared to the control group [Table 5].

To further study these genetic variants as risk factors to early onset IHD, we assessed the possible predictive value of the studied genetic risks and the clinical risk factors among the recruited individuals (patients and controls combined) by logistic regression. Result showed a statistically significant association of eNOS2

Table 3: The relative frequency of some genetic risk factors among Iraqi IHD patients and controls

Variables		Patients		Controls		*P* value
		No.	%	No.	%	
FGB-455G>A	Wild type	21	52.5	10	50.0	0.86
	Heterozygous	18	45.0	10	50.0	
	Homozygous	1	2.5	0	0	
HPA-1	aa	29	72.5	12	60.0	0.38
	ab	11	27.5	8	40.0	
ACE	II	7	17.5	9	45.0	**0.032**
	ID	17	42.5	7	35.0	
	DD	16	40.0	4	20.0	
eNOS-786T>C (eNOS1)	Wild type	22	55.0	14	70.0	0.46
	Heterozygous	16	40.0	6	30.0	
	Homozygous	2	5.0	0	0	
eNOS-298D (eNOS2)	Wild type	26	65.0	14	70.0	0.78
	Heterozygous	13	32.5	6	30.0	
	Homozygous	1	2.5	0	0	
LTA *C804A*	Wild type	18	45.0	3	15.0	**0.025**
	Heterozygous	18	45.0	10	50.0	
	Homozygous	4	10.0	7	35.0	

FGB = beta fibrinogen; HPA = human platelet antigen; ACE = angiotensin-converting enzyme; eNOS = endothelial nitric oxide; LTA= lymphotoxin alpha

The bold values are the statistically significant results

Table 4: Association between genetic risk score and the study groups

Study groups		Genetic risk score							Total
		0	1	2	3	4	5	6	
Patients	**No.**	1	5	3	7	13	9	2	**40**
	%	2.5%	12.5%	7.5%	17.5%	32.5%	22.5%	5.0%	**100.0%**
Control	**No.**	**0**	**4**	**4**	**1**	**3**	**6**	**2**	**20**
	%	0.0%	20.0%	20.0%	5.0%	15.0%	30.0%	10.0%	**100.0%**
Total	**No.**	**1**	**9**	**7**	**8**	**16**	**15**	**4**	**60**
	%	**1.7%**	**15.0%**	**11.7%**	**13.3%**	**26.7%**	**25.0%**	**6.7%**	**100.0%**

$P = 0.33$

Table 5: Association between clinical risk score and the study groups

Study groups		Clinical risk score					Total
		0	1	2	3	4	
Patients	**No.**	0	3	8	22	7	**40**
	%	0.0%	7.5%	20.0%	55.0%	17.5%	**100.0%**
Control	**No.**	4	11	5	0	0	**20**
	%	20.0%	55.0%	25.0%	0.0%	0.0%	**100.0%**
Total	**No.**	**4**	**14**	**13**	**22**	**7**	**60**
	%	**6.7%**	**23.3%**	**21.7%**	**36.7%**	**11.7%**	**100.0%**

$P \leq 0.0001$

with increasing age, while LTA was associated with the presence of IHD and dyslipidemia. The remaining genetic variants did not show a statistical significance with any clinical risk factor [Table 6]. Family history of ischemia and family history of dyslipidemia as clinical risks were also assessed, but they did not show any statistical significance, the results were omitted from Table 6.

Discussion

IHDs or CADs are categorized as complex multifactorial diseases, influenced by both genetic and nongenetic factors. These influencers affect disease occurrence, age of onset, and severity.[8] The multifactorial conditions have distinct characteristics, including sporadic occurrence with potential familial patterns, susceptibility to environmental influences, no specific sex predilection, and varying occurrence among different ethnic groups. This complexity sets them apart from clear-cut Mendelian or sex-limited genetic conditions.[13,14]

Monogenic, polygenic, and nongenetic factors may contribute to reach a threshold necessary for disease development. Some of them increase the risk and onset of CAD, including obesity, hypertension, type II diabetes mellitus, high levels of low-density lipoprotein cholesterol, and even gum disease.[15-17] Although CAD runs in families, it does not show Mendelian inheritance patterns and can occur in isolation.[18] CAD also occurs more often in men than in women,[19] and its risk is higher among African Americans than among Caucasians or Asians.[20] All of these characteristics are consistent with the classification of CAD as a multifactorial disorder.

A promising, but a difficult, area of study is the identification of genes that predispose to or directly cause CAD. The overall genetic contribution toward the development of CAD is estimated to range from 20% to 60%; genetic factors having a greater impact when CAD develops at a younger age.[21]

However, in many cases, genetic associations found in one study are not supported by subsequent studies, which make the search for definitive inferences not an easy task. These confounding results probably occur for a number of reasons, including the use of small sample sizes, sampling biases, phenotypic heterogeneity, mismatches between case and control groups, a failure to recognize gene–gene and gene–environment interactions, or racial differences.[22] The results of local and small sample-sized studies must be looked at through this perspective.

Linkage analysis, genome-wide association studies, and sequencing analysis were all used, but the most frequently used method for identifying the susceptibility genes for CAD and MI has been the case–control association studies for a candidate gene. This is the easiest methodological approach by which a candidate gene is selected based on its potential involvement in the risk for CAD.[23]

Nevertheless, studies assessing the probable statistical association of some genetic risks with early onset CAD in nonpreviously screened population remain worthy. The current study is just an example, where assessing some clinical and genetic characteristics in early onset CAD among Iraqi Arab patients and controls was tried.

Since the assessment of all the genes in relation to CAD and MI is difficult and exhaustive, a focus on the genes considered most likely to potentially contribute toward an increased risk for CAD and MI can be a practical alternative. Genes resulting in clear monogenic inherited disorders with which an increased risk of CAD is associated were not studied. The same applies for complex risky conditions, for example, type II diabetes.

In contrast to major gene mutations, which are frequently associated with high risk of disease, low-penetrance genetic polymorphisms are generally associated with minimal-to-modest increases in CAD risk. The consequences of the minimal increases in risk associated with low-penetrance genetic polymorphisms are the limited repeatability of findings reported in the literature.[24,25] In addition, acquired clinical risks can also alter the onset and severity of CAD. Some clinical risks can be affected by genetic polymorphisms themselves.[26] Furthermore, the role of protective genes and acquired factors, for example, healthy diet and habits, need not to be forgotten. The assessment of these factors altogether, considering a very large number

Table 6: Assessment of the predictive value of each genetic variant with the clinical risk factors in both study groups combined

	Age			Sex			Hypertension			Smoking			IHD			Dyslipidaemia		
Genetic variants and zygosity status	<40 No. (%)	40–55 No. (%)	*P* value	Male No. (%)	Female No. (%)	*P* value	Present No. (%)	Absent No. (%)	*P* value	Present No. (%)	Absent No. (%)	*P* value	Present No. (%)	Absent No. (%)	*P* value	Present No. (%)	Absent No. (%)	*P* value
FGB	*n* = 60			*n* = 60			*n* = 60			*n* = 60			*n* = 60			*n* = 60		
Wild type	6 (19.4%)	25 (80.6%)		26 (83.9%)	5 (16.1%)	0.558	19 (61.4%)	12 (38.7%)	0.805	18 (58.1%)	13 (41.9%)	0.490	21 (67.7%)	10 (32.3%)	0.860	21 (67.7%)	10 (32.3%)	0.860
Heterozygous	2 (7.1%)	26 (92.9%)	0.358	21 (75.0%)	7 (25%)		17 (60.7%)	11 (39.3%)		18 (64.3%)	10 (35.7%)		10 (18%)	10 (35.7%)		18 (64.3%)	10 (35.7%)	
Homozygous	0 (0%)	1 (100%)		1 (100%)	0 (0%)		1 (100%)	0 (0%)		0 (0%)	1 (100%)		1 (100%)	0 (0%)		1 (100%)	0 (0%)	
HPA-1 (a/b)	*n* = 60			*n* = 60			*n* = 60			*n* = 60			*n* = 60			*n* = 60		
Homozygous (a/a)	6 (14.6%)	35 (85.4%)	0.716	32 (78.0%)	9 (22.0%)	0.735	27 (65.9%)	14 (34.1%)	0.397	16 (39.0%)	25 (61.0%)	1	29 (70.7%)	12 (29.3%)	0.384	29 (70.7%)	12 (29.3%)	0.384
Heterozygous (a/b)	**2 (10.5%)**	17 (89.5%)		16 (84.2%)	3 (15.8%)		10 (52.6%)	9 (47.4%)		8 (42.1%)	11 (57.9%)		11 (57.9%)	8 (42.1%)		11 (57.9%)	8 (42.1%)	
ACE	*n* = 60			*n* = 60			*n* = 60			*n* = 60			*n* = 60			*n* = 60		
Homozygous (I/I)	2 (12.5%)	14 (87.5%)	1	15 (93.8%)	1 (6.3%)	0.210	4 (25%)	12 (75%)		10 (62.5%)	6 (37.5%)	0.111	7 (43.8%)	9 (56.3%)	0.073	7 (43.8%)	9 (56.3%)	0.073
Heterozygous (I/D)	3 (12.5%)	21 (87.5%)		19 (79.2%)	5 (20.8%)		8 (33.3%)	16 (66.7%)	0.164	8 (33.3%)	16 (66.7%)		17 (70.8%)	7 (29.2%)		17 (70.8%)	7 (29.2%)	
Homozygous (D/D)	3 (15%)	17 (85%)		14 (70%)	6 (30%)		11 (55%)	9 (45%)		6 (30%)	14 (70%)		16 (80%)	4 (20%)		16 (80%)	4 (20%)	
eNOS1	*n* = 60			*n* = 60			*n* = 60			*n* = 60			*n* = 60			*n* = 60		
Wild type	5 (13.9%)	31 (86.1%)	0.213	29 (80.6%)	7 (19.4%)	0.477	12 (33.3%)	24 (66.7%)	0.56	16 (44.4%)	20 (55.6%)	0.637	22 (61.1%)	14 (38.9%)	0.463	22 (61.1%)	14 (38.9%)	0.463
Heterozygous	2 (9.1%)	20 (90.9%)		18 (81.8%)	4 (18.2%)		10 (45.5%)	12 (54.5%)		7 (31.8%)	15 (68.2%)		16 (72.7%)	6 (27.3%)		16 (72.7%)	6 (27.3%)	
Homozygous	1 (50%)	1 (50%)		1 (50%)	1 (50%)		1 (50%)	1 (50%)		1 (50%)	1 (50%)		2 (100%)	0 (0%)		2 (100%)	0 (0%)	
eNOS2	*n* = 60			*n* = 60			*n* = 60			*n* = 60			*n* = 60			*n* = 60		
Wild type	3 (7.5%)	37 (92.5%)	**0.035***	32 (80%)	8 (20%)	1	16 (40%)	24 (60%)	1	17 (42.5%)	23 (57.5%)	0.384	26 (65%)	14 (35%)	1	26 (65%)	14 (35%)	1
Heterozygous	4 (21.1%)	15 (78.9%)		15 (78.9%)	4 (21.1%)		7 (36.8%)	12 (63.2%)		6 (31.6%)	13 (68.4%)		13 (68.4%)	6 (31.6%)		13 (68.4%)	6 (31.6%)	
Homozygous	1 (100%)	0 (0%)		1 (100%)	0 (0%)		0 (0%)	1 (100%)		1 (100%)	0 (0%)		1 (100%)	0 (0%)		1 (100%)	0 (0%)	
LTA	*n* = 60			*n* = 60			*n* = 60			*n* = 60			*n* = 60			*n* = 60		
Wild type	2 (9.5%)	19 (90.5%)	0.188	19 (90.5%)	2 (9.5%)	0.328	12 (57.1%)	9 (42.9%)	0.069	11 (52.4%)	10 (47.6%)	0.186	18 (85.7%)	3 (14.3%)	**0.02***	18 (85.7%)	3 (14.3%)	**0.02***
Heterozygous	6 (21.4%)	22 (78.6%)		21 (75%)	7 (25%)		9 (32.1%)	19 (67.9%)		11 (39.3%)	17 (60.7%)		18 (64.3%)	10 (35.7%)		18 (64.3%)	10 (35.7%)	
Homozygous	0 (0%)	11 (100%)		8 (72.7%)	3 (27.3%)		2 (18.2%)	9 (81.8%)		2 (18.2%)	9 (81.8%)		4 (36.4%)	7 (63.6%)		4 (36.4%)	7 (63.6%)	

Fisher's exact two-sided test was used to calculate the *P* value for all variables

FGB = beta fibrinogen; HPA = human platelet antigen; ACE = angiotensin-converting enzyme; eNOS = endothelial nitric oxide; LTA= lymphotoxin alpha; NS= not significant; * = statistically significant

of risky and protective factors, is required to calculate the actual risk of a disease. Since this is practically not possible, logically, some of these variables need to remain constant in both patients and control groups to minimize this wide variation. That is why, the results of different studies can show wide variability, even among the same population.[14]

Early onset MI constitutes nearly one-third of cases of MI among Iraqis, which is rather higher than the proportions reported in many Western countries.[23] Data about genetic risk factors of CADs in Iraq were only available from two local studies from the North[27] and Center parts of Iraq[28]; the former is inhabited mainly by Kurds, while the latter is inhabited by the Arab majority.

Both studies have assessed the role of many genetic polymorphisms as well as clinical risks in early onset CADs. This study complements the results obtained from those earlier studies.

In this study, the older males represented the majority of patients, which is quite similar to findings from all over the world, including Iraq.[27-29]

From the studied acquired clinical risks, only hypertension and dyslipidemia showed a statistically significant difference between the 60 patients and controls. Surprisingly, smoking did not show such a difference. As diabetic patients were excluded from this study, these results may contradict in parts and are similar in other parts to findings from earlier local and other international studies. Such controversy can be explained by sample size, sampling technique, and sociocultural factors.

In regard to the six studied genetic polymorphisms, only ACE (the D allele) and LTA 804C>A were found to be statistically significantly different between patients and controls; thus, they can be said to be linked to CAD.

While ACE was not found to be different comparing patients and controls in an earlier local study from Kurdistan,[27] LTA, eNOS1, and eNOS2 genes, which are considered newer markers for CAD risk, were not included in those local studies. In those two studies, FGB and HPA were not statistically significantly different between patients and controls.

In the other study of Iraqi Arabs from the center of Iraq, early onset CAD patients did not include a control group; thus, no statistical analysis could be drawn.[28]

Genetic risk scores, as calculated by the number of risky genes detected among patients and controls, were not statistically different in the study groups. This was different in relation to clinical risk scores, where a statistically significant difference was found between both study groups. In most studies, clinical risk factors appear to be more apparent than genetic risks for early onset CAD. This was evident from many studies all around the world, including the local Iraqi study from Kurdistan.[27,29,30]

None (0%) of the participants had a genetic risk score more than six. There was a statistically highly significant association between higher clinical risk scores and CAD patients group.

Many studies showed a specific association between certain risky genes and an acquired clinical factor, for example, ACE and HPA1 with smoking,[9,10] FGB, HPA1, and eNOS2 with premature MI,[8-11] or ACE with increasing age.[10] The results of the current study did not show such an association. On the contrary, the current study found that eNOS2 *G894T* was statistically associated with increasing age and LTA with IHD and dyslipidemia. These findings further support such an evidence from previous studies;[31,32] their biological and pathophysiological roles are explainable.

Endothelial cell nitric oxide synthase (eNOS) is one of the most important candidate genes in CAD.[31] All eNOS gene's isoforms are present in atherosclerosis although there is a powerful evidence pointing out to eNOS-defensive effects on vessels' wall against atherosclerosis.[33,34]

LTA is a cytokine that mediates proinflammatory responses while also participating in lipid homeostasis, and its transcriptional activity is, in part, genetically determined.[30] LTA *C804A* has been shown to be linked with increased susceptibility to diabetes mellitus, hyperinsulinemia, rheumatoid arthritis, CAD, and metabolic syndrome.[35-37] Unfortunately, these associations cannot be assessed as all diabetic CAD patients were excluded from the current study.

Knowledge about the types and frequencies of the genetic risks for early onset CAD enables health authorities the design and application of proper screening and preventive programs for the population for this fatal disease and even allows personalized targeted medicine for high-risk groups.

Conclusions

CAD is a complex multifactorial disease, where genetic polymorphisms play a significant role in its etiology. However, its genetic etiology lags behind other diseases. This study showed a statistically significant difference between ACE and LTA genes among Iraqi Arab nondiabetic patients with early onset CAD and controls. In addition, eNOS2 was found to be linked with increasing age, while LTA was linked to dyslipidemia. These findings provide further evidence to previously published studies and can aid in genetic risk assessment and risk stratification for CAD.

Limitations

This study has some limitations including a small sample size, especially the control group, the use of a nonrandom

sampling technique from one center with multiple exclusion criteria especially diabetes and later-onset CAD, as well as studying only six genetic polymorphisms out of few hundreds, all may have an impact on the types and frequencies of the studied genetic risks. Generalization cannot be made but the results remain important observational findings that require larger scale national studies in the future.

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Authors' contributions

- **BMSA:** conceptualized and designed the study, contributed to data analysis, drafted and finalized the manuscript.
- **RKUA:** collected the data and performed molecular analysis.

Availability of data and material
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflicts of interest
There are no conflicts of interest.

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