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Impact of Thrombomodulin Polymorphism −33G>A on Acute Myocardial Infarction Risk and Circulating Inflammatory Markers

ABSTRACT

Background: There is increasing evidence that thrombomodulin (THBD) polymorphisms, along with inflammatory markers [i.e., C-reactive protein (CRP), fibrinogen, albumin], may increase the risk of acute myocardial infarction (AMI). The aim of the study was to investigate the role of the THBD −33G>A polymorphism (rs1042579) as a marker of AMI risk and to correlate it with serum levels of inflammatory markers.

Methods: Case−control study of 277 AMI patients and 329 healthy controls. A binary logistic regression analysis was performed to evaluate the association between the parameters studied and AMI risk.

Results: The frequencies of genotypes AA, GA, and GG of the THBD −33G>A polymorphism were 31.4%, 45.5%, and 23.1% in patients and 21.6%, 44.1%, and 34.3% in controls. A significant association was found between the AA genotype of the THBD −33G>A polymorphism (AA: OR = 2.011, 95% CI 1.561-3.074, $P < .001$) or A allele (A: OR = 1.725, 95% CI 1.493-2.510, $P < .001$) and AMI risk. A backward stepwise logistic regression method combining AMI status as the dependent variable and conventional risk factors (age, smoking, arterial hypertension (HTA), diabetes, dyslipidemia, CRP, albumin, fibrinogen, serum angiotensin converting enzyme (ACE) activity, serum malondialdehyde, conjugated dienes, glutathione peroxidase, cardiac troponin-I (cTnI) and THBD AA genotype) as independent variables showed that the most predictive risk factors for AMI were smoking, HTA, albumin, fibrinogen, CRP, ACE activity, cTnI, and the THBD AA-genotype with odds ratios of 2.942, 2.203, 2.352, 1.323, 1.652, 1.014, 2.105, and 3.781 respectively. The AA genotype was associated with increased diastolic blood pressure, CRP, ACE activity, and albumin levels.

Conclusions: The study shows that the THBD -33G>A polymorphism should be included in the stratification of AMI risk.

Keywords: Acute myocardial infarction, THBD −33G>A polymorphism, albumin, fibrinogen, serum ACE activity, C-reactive protein, cardiac troponin-I

ORIGINAL INVESTIGATION

INTRODUCTION

Acute myocardial infarction (AMI) is a multicausal disease resulting from interactions between genetic background and environmental risk factors. Acute myocardial infarction is a focal ischemic damage of the myocardium due to stenosis or occlusion of a coronary artery.[1] The development of systems biology and the associated systemic literature review have ushered in a new era of AMI research, which is critical for the success of preventive measures.[2] In recent years, a large number of single nucleotide polymorphisms (SNPs) have been identified and assessed for their AMI risk. The genetic components involved have not yet been definitively identified, but several studies have investigated the effects of candidate SNPs on AMI risk.[3-6]

Thrombomodulin (THBD) is a multifunctional transmembrane protein that plays a key role in regulating several biological processes. It is mainly expressed on the surface of endothelial cells.[7] Thrombomodulin plays several key roles in endothelial cells by mediating endothelial thromboresistance. When THBD is expressed on the luminal surface of vascular endothelial cells, it's primary function is to promote

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an anticoagulant state and prevent unwanted blood clotting. Thrombomodulin binds to thrombin, thereby enhancing the activation of protein C and the thrombin-activatable fibrinolysis inhibitor, which has anticoagulant, antifibrinolytic, and anti-inflammatory effects on the vascular wall.[8] Thrombomodulin has been shown to have anti-inflammatory and immunomodulatory properties. It can inhibit allergic airway inflammation and regulate dendritic cell function. Thrombomodulin also appears to play a role in protecting endothelial cells from cytotoxicity.[7] Recent research suggests that THBD can mediate endothelial cell sprouting and angiogenesis through interactions with the FGFR1 receptor, suggesting a potential role for THBD in vascular remodeling and repair.[8] Since THBD is expressed throughout the vascular endothelium, it is considered a key molecule for integrating various biological processes such as coagulation, immunity, and cell proliferation to protect the vasculature from injury and promote healing.[8]

Thrombomodulin serves as a central regulator to maintain hemostatic balance by inhibiting thrombotic activity and contributing to anti-inflammatory processes. Its interactions with thrombin and other molecules underscore its diverse role in vascular biology and immune response modulation. Its diverse properties highlight the endothelium as a dynamic organ with tightly coordinated mechanisms to respond to various stresses. The impact of the THBD polymorphism on THBD levels and activity is not completely clear. Over the last 2 decades, studies have investigated the association between these polymorphisms and AMI risk and other cardiovascular diseases.[4,9] Despite these studies, the impact of the –33G>A polymorphism on the pathophysiology of AMI is still unclear.

Measuring multiple biomarkers and using point-of-care markers may accelerate current diagnostic protocols to assess patients at risk for AMI. Circulating levels of inflammatory markers, including C-reactive protein (CRP), fibrinogen, and albumin, are associated with the risk of vascular disease and overall mortality resulting from atherothrombosis and its clinical complications.[10,11] Therefore, we conducted a case-control study on the influence of the SNP –33G>A in THBD (rs1042579) on AMI pathophysiology and the association of –33G>A with the levels of various inflammatory markers.

METHODS

Subjects

A case–control study was conducted between July 2018 and December 2019 in 277 unrelated Tunisian patients with non-ST-segment elevation myocardial infarction (NSTEMI). This study was approved by the Ethical Committee (CER-SVS/ISBM 007/2023), and informed consent was obtained from all patients before their enrollment. Acute myocardial infarction was defined according to the World Health Organization criteria based on symptoms, electrocardiogram (ECG) findings, and cardiac enzyme abnormalities. All included patients had acute coronary syndrome. Cardiac catheterization and coronary angiography were performed according to standard procedures. Non-ST-segment elevation myocardial infarction was diagnosed by the absence of ST-segment elevation, the presence of ischemic ST-segment or T-wave changes over 24 hours with positive cardiac enzymes, and a normal ECG. The control group consisted of 329 unrelated individuals matched to the AMI group for age, sex, and geographical origin. During the hospital stay, all patients completed a standard questionnaire to assess lifestyle habits, cardiovascular risk factors, and current treatment.

Arterial hypertension (HTA) was defined as elevated systolic (>140 mm Hg) or diastolic (>90 mm Hg) blood pressure or current use of antihypertensive medication. The definition of diabetes was based on fasting blood glucose ≥7.0 mmol/L or current treatment. Dyslipidemia was defined as elevated total cholesterol (TC) and/or elevated low-density lipoprotein (LDL) cholesterol and/or decreased high-density lipoprotein (HDL) cholesterol, and/or increased triglycerides (TG), and treatment for dyslipidemia in the past 2 weeks. Body mass index (BMI) was classified as normal (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2), or obese (BMI >30 kg/m^2).

Patients with stable or unstable anginal chest pain without AMI, congenital heart disease, valvular heart disease, cardiomyopathy, viral myocarditis, sarcoidosis, or severe arrhythmias were excluded from the study. Serum angiotensin-converting enzyme (ACE) activity was determined in patients who were not taking ACE inhibitors or angiotensin II receptor antagonists for the treatment of HTA.

Blood was collected from all subjects after an overnight fast. After blood separation, serum was immediately stored at 20°C. For biochemical analyses of plasma glucose concentration, HbA1c, TC, TG, HDL, LDL, albumin, fibrinogen, and CRP, validated methods were applied using Randox kits (Randox Laboratories-Antrim UK). Angiotensin-converting enzyme activity and cardiac troponin-I (cTn-I) were measured as described by Mehri et al.[3,5]

Genotyping

Genomic DNA was extracted from white blood cells using the standard salt precipitation method.[12] For analysis of the THBD polymorphism –33G>A, PCR and restriction fragment length polymorphism were performed using a PCR cycler (GeneAmp 2700; Applied Biosystem, Singapore).

The total volume of each sample mixture (20 µL) contained 50 ng DNA, 0.5 µmol/L of forward primer (5′-GGC CAG GGC TCG AGT TTA TAA AGG C-3′) or reverse primer (5′-CGG GGA CAG TCG TCT GTT ACA G-3′), 0.2 mM dNTP, 2 mM $MgCl_2$, and 0.05 U/µL of Taq enzyme in an appropriate buffer.[13] The PCR procedure consisted of 5 minutes at 94°C for

HIGHLIGHTS

- The thrombomodulin polymorphism –33G>A (AA-genotype) is associated with an increased risk of myocardial infarction.
- The thrombomodulin polymorphism –33G>A (AA-genotype) is also associated with increased DBP, CRP, ACE activity, and increased albumin levels.
- Risk stratification for myocardial infarction should include a search for thrombomodulin polymorphisms.

initial denaturation, followed by 32 cycles of 94°C for 40 seconds, annealing at 64°C for 30 seconds, extension at 72°C for 40 seconds, and a final extension for 8 minutes at 72°C. The PCR product was a 107 bp DNA fragment. Digests (10 µL) containing 5 units of restriction enzyme (StuI, from MBI Fermentas GMBH, Germany) were incubated for 12 hours, i.e., overnight, at 37°C for the PCR products. Digests were separated on a 3% agarose gel and visualized by ethidium bromide staining. Homozygous GG has 1 band (259 bp), homozygous AA has 2 bands (24 and 235 bp), and heterozygous GA has 3 bands (259, 235, and 24 bp).

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 15.0 for Windows was used for statistical analysis. All variables were tested for normality with the Kolmogorov–Smirnov test. Categorical variables were expressed as numbers (percentages) and were compared with the χ^2 test. Continuous variables with a non-Gaussian distribution were compared with the non-parametric Mann–Whitney analysis, and these data were expressed as median with interquartile range (25^{th} and 75^{th} percentiles). The frequencies of genotypes and alleles for the THBD polymorphism were compared between patient and control groups using the χ^2 test. Odds ratios (OR) and 95% CI were calculated using Woolf's method.[14] A binary logistic regression analysis (backward stepwise logistic regression method) was performed to determine the independent correlations of the evaluated parameters and to determine their relationship with AMI. The strength of associations between parameters was assessed using Pearson's rank correlation coefficient test. A *P*-value <.05 was considered statistically significant.

We declare that no artificial intelligence and assistive technologies were used for the creation of this work.

RESULTS

Clinical Characteristics of the Study Population

Biochemical and clinical data from AMI patients and controls are summarized in Table 1. Age and gender were similar in both groups. The incidence of diabetes, HTA, dyslipidemia, and smoking was higher in AMI patients than in controls. Triglycerides, TC, LDL, and fasting glucose concentrations were higher in patients than in controls. Systolic and diastolic blood pressure were higher in patients than in controls ($P < .001$). Acute myocardial infarction patients were more obese than controls ($P = .001$). Serum CRP and cTnI levels and ACE activity were higher in AMI patients ($P < .001$). A statistically significant increase in serum malondialdehyde (MDA) and conjugated dienes (CD) was found in AMI patients ($P < .001$). Glutathione peroxidase (GPx) activity was significantly lower in AMI patients compared to controls ($P < .001$).

Table 1. Clinical and Biochemical Features of AMI Patients and Control Subjects

Variables	AMI Patients (n = 277)	Control Subjects (n = 329)	*P*
Age, years	63 [43-84]	63 [35-87]	.700
Sex (M/F)	146/131	174/155	.515
Smokers	100 (36.4)	64 (19.6)	<.001
Hypertension	122 (44)	32 (9.7)	<.001
Dyslipidemia	117 (42.2)	13 (4.0)	<.001
Diabetes	127 (45.8)	55 (16.7)	<.001
DBP, mm Hg	94 [55-156]	83 [65-99]	<.001
SBP, mm Hg	149 [100-215]	129 [85-155]	<.001
Fasting glucose, mmol/L	6.6 [3.8-13]	4.5 [2.1-7.45]	<.001
HbA1c, %	7.3 [3.2-13.9]	5.3 [2-11.1]	<.001
Triglycerides, mmol/L	2.8 [1.0-9.0]	1.2 [0.5-5.8]	<.001
Total cholesterol, mmol/L	8 [5-12]	4.2 [1.7-7.3]	<.001
HDL-C, mmol/L	2 [0.8-2.7]	1 [0.9-2]	<.001
LDL-C, mmol/L	5.7 [2.6-9.4]	2.4 [2-4.5]	<.001
CRP, mg/L	6.9 [0.45-118]	1.15 [0.1-5.4]	<.001
BMI, kg/m^2	27.3 [20.4-39.4]	26.7 [16-40]	.001
Serum ACE activity, U/L	97.8 [57-174]	88.5 [20-145]	<.001
cTnI, mg/L	45 [30-49.8]	0.0 [0.0-0.0]	<.001
MDA, µM	0.47 [0.10-0.94]	0.20 [0.1-0.87]	<.001
CD, µmol of hydroperoxide mg of protein	168 [45-389]	88.5 [20-199]	<.001
GPx, U/mg of protein	32.6 [12.2-74]	49.7 [16-81.6]	<.001

Data are ratio, number (percentage), or median [interquartile range]. ACE, angiotensin-converting enzyme; BMI, body mass index; CD, conjugated dienes; CRP, C-reactive protein; cTnI, cardiac troponin-I; DBP, diastolic blood pressure; GPx, the antioxidant enzyme, glutathione peroxidase; HDL, high density lipoprotein; LDL, low density lipoprotein; MDA, malondialdehyde; SBP, systolic blood pressure.

Implication of the THBD –33G>A Polymorphism on AMI Risk

The genotype distributions of the 3 variants of the THBD gene were in Hardy–Weinberg equilibrium in both controls and AMI patients. The frequency of the THBD –33G>A polymorphism was significantly increased in AMI patients compared to controls ($\chi^2 = 18.16$; $P = .001$). The frequencies of the AA, GA, and GG genotypes were 31.4%, 45.5%, and 23.1% in AMI patients and 21.6%, 44.1%, and 34.3% in controls. The frequencies of the A allele were 54.1% and 44.9% in the AMI and control groups, respectively. A significant association was also found between the AA genotype (AA: OR = 2.011, 95% CI 1.561-3.074, $P < .001$) or the A allele (A: OR = 1.725, 95% CI 1.493-2.510, $P < .001$) of the THBD –33G>A polymorphism (Table 2).

Binary logistic regression analysis with AMI status as the dependent variable and conventional risk factors (age, smoking, HTA, diabetes, dyslipidemia, TC, TG, CRP, albumin, fibrinogen, serum ACE activity, MDA, DC, GPx, cTnI and THBD AA genotype) as independent variables revealed that the most predictive AMI risk factors were smoking, HTA, albumin, fibrinogen, CRP, serum ACE activity, cTnI, and AA genotype with odds ratios of 2.942, 2.203, 2.352, 1.323, 1.652, 1.014, 2.105, and 3.781, respectively (Table 3). Table 4 shows the characteristics of patients with the THBD –33G>A

Table 2. Thrombomodulin –33G>A Gene Polymorphism in AMI Patients Compared to Control Subjects

	OR	95% CI	*P*
Genotypes			
GG	1	–	–
AA	2.011	1.561-3.074	<.001
GA	1.124	0.458-1.875	.901
Allele			
A	1.725	1.493-2.510	<.001

OR, odds ratio.

polymorphism. THBD genotypes were associated with age, smoking, HTA, dyslipidemia, cTnI, ACE activity, MDA, DC, and GPx levels. The AA genotype was associated with increased DBP, CRP, serum ACE activity, and albumin levels. No correlation was found between THBD genotypes and any parameters in the controls.

Correlation Between Albumin, Fibrinogen and Other Parameters

Spearman's rank correlation analysis to evaluate the correlations between albumin, fibrinogen, and other parameters in AMI patients showed that albumin was negatively correlated with fibrinogen ($r = -0.391$; $P = .001$) (Figure 1). In addition, CRP was positively correlated with fibrinogen ($r = 0.217$; $P = .001$) and negatively correlated with albumin ($r = -0.511$; $P = .001$).

Table 3. Results of the Binary Logistic Regression Analysis

Variables	OR	95% CI	*P*
Age	0.023	0.290-0.055	.485
Smoking	2.942	0.065-3.391	.009
Hypertension	2.203	1.791-2.842	.004
Diabetes	0.511	1.732-2.174	.699
Dyslipidemia	0.501	1.291-2.386	.515
Triglycerides	0.346	0.118-1.171	.170
Total cholesterol	0.254	0.492-0.830	.360
Albumin	2.352	1.601-3.736	.002
Fibrinogen	1.323	1.152-2.137	.046
CRP	1.652	1.252-2.363	.041
Serum ACE activity	1.014	0.0261-1.044	.028
cTnI	2.105	0.953-3.121	.001
MDA	1.311	0.834-4.110	.214
CD	0.113	0.019-0.681	.234
GPx	0.035	0.015-0.068	.121
THBD AA genotype	3.781	2.770-4.313	.001

Adjustment was performed for confounding factors including age, smoking, HTA, diabetes, dyslipidemia, triglycerides, total cholesterol, albumin, fibrinogen, serum ACE activity, MDA, CD, GPx, cTnI, and THBD AA genotype.
ACE, angiotensin-converting enzyme; CD, conjugated dienes; CRP, C-reactive protein; cTnI, cardiac troponin-I; GPx, glutathione peroxidase; MDA, malondialdehyde; OR, odd ratio; THBD, thrombomodulin.

Table 4. Demographic and Biochemical Characteristics of AMI Patients in the Study Across Thrombomodulin Gene Polymorphism –33G>A Genotypes

Variables	AA (n = 87)	GA (n = 126)	GG (n = 64)	*P*
Sex (M/F)	49/38	59/67	38/26	.188
Smoking	22 (25.3)	39 (31.5)	39 (60.9.4)	< .001
Hypertension	59 (67.8)	42 (33.3)	21 (32.8)	< .001
Dyslipidemia	31 (35.6)	67 (53.2)	19 (29.7)	.003
Diabetes	43 (49.4)	54 (42.9)	30 (46.9)	.760
Fasting glucose (mmol/L)	7 [3.8-13]	6.6 [3.7-10]	6.5 [4-12]	.575
HbA1c (%)	7.1 [3.3-13.9]	7.6 [3.12-13.8]	7.2 [3.2-13.7]	.894
SBP (mm Hg)	151 [104-215]	148 [100-211]	148 [100-212]	.068
DBP (mm Hg)	94 [66-139]	92 [55-156]	92.5 [62-125]	.592
Triglycerides (mmol/L	2.7 [1.0-9]	3 [1.5-8.4]	2.5 [1.4-6.6]	.091
Total cholesterol (mmol/L)	8 [5.4-12]	8.0 [5-12]	8 [5.3-11.9]	.497
HDL-C (mmol/L)	2 [1.2-2.5]	2 [0.8-2.7]	2 [1.2-2.4]	.274
LDL-C (mmol/L)	5.6 [3-9.4]	5.7 [2.6-9.4]	5.8 [3-9.4]	.398
CRP (mg/L)	7.6 [1.12-81.9]	7.47 [0.4-107]	4.9 [0.6-118]	.841
BMI (kg/m^2)	26.3 [20.4-37.3]	27.3 [20.4-37.4]	30.3 [22.1-39.4]	.207
cTnI (mg/L)	45 [30-49.7]	41 [30-49.8]	47.7 [33.5-49.7]	<.001
Crp (mg/L)	7 [5-8]	7 [5.75-8]	7 [5-8]	.523
Fibrinogen (g/L)	4.1 [0.8-10.1]	4.1 [0.1-10]	4 [0.1-9.8]	.054
Albumin (g/L)	58.9 [40.2-65.1]	58.6 [40.2-65.1]	42.7 [58-65]	.965
Serum ACE activity (U/L)	97.8 [67-168.7]	92 [59-174]	100 [57.3-164.3]	.023
MDA (µM)	0.31 [0.1-0.9]	0.48 [0.1-0.94]	0.59 [0.18-0.93]	<.001
DC (µmol of hydroperoxide/mg of protein)	134 [45-300]	178 [54-389]	245 [119-363]	<.001
GPx (U/mg of protein)	16.9 [12.2-20.5]	35.8 [20.5-52.2]	60 [52.7-74.5]	<.001

Data are ratio, number (percentage), or median [interquartile range].
ACE, angiotensin-converting enzyme; BMI, body mass index; CD, conjugated dienes; CRP, C-reactive protein; cTnI, cardiac troponin-I; DBP, diastolic blood pressure; GPx, the antioxidant enzyme, glutathione peroxidase; HDL, high density lipoprotein; LDL, low density lipoprotein; MDA, malondialdehyde; SBP, systolic blood pressure.

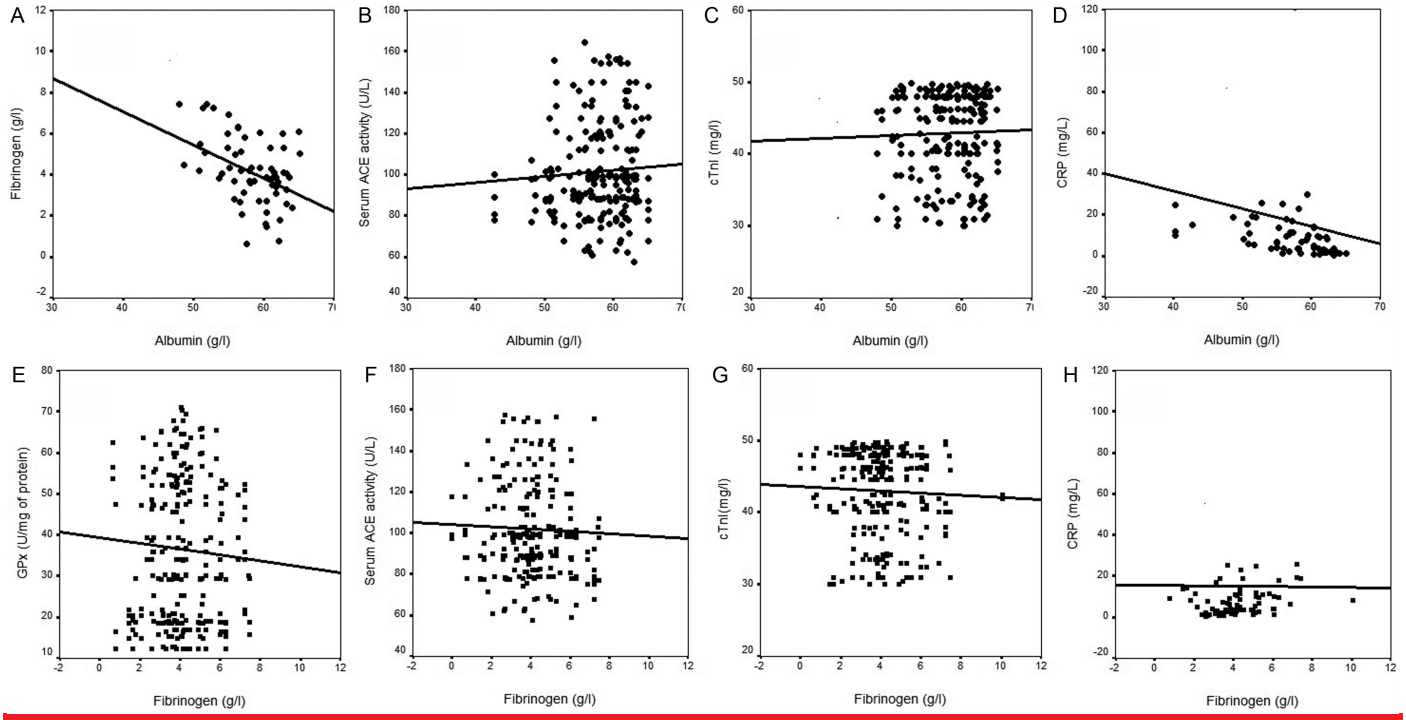

Figure 1. Scatterplot illustrating the Pearson's rank correlations between (1) Albumin and (a) Fibrinogen ($r = -0.391$; $P = .001$), (b) serum ACE activity ($r = 0.057$; $P = .401$), (c) cTnI ($r = 0.047$; $P = .486$), (d) CRP ($r = -0.511$; $P = .001$) and (2) Fibrinogen and (e) GPx ($r = -0.031$; $P = .624$), (f) serum ACE activity ($r = -0.020$; $P = .754$), (g) cTnI ($r = -0.043$; $P = .497$), (h) CRP ($r = 0.217$; $P = .001$) in AMI patients.

DISCUSSION

This study investigated the role of the THBD −33G>A polymorphism (rs1042579) as a marker of AMI risk in relation to inflammatory marker levels and AMI prevention strategies. The results of this study should be considered as a starting point for further studies on larger populations to make more conclusive statements in this regard. The strengths of the present study are that: (1) it is the first study on the association between TM −33G/A polymorphism and AMI risk in the Tunisian population, and (2) the frequencies for the polymorphism were in Hardy–Weinberg equilibrium, indicating the absence of population bias. In addition, the present study fulfills most of the criteria of a good genetic association study as suggested by Hattersley and McCarthy.[15]

In this study, the THBD −33G>A polymorphism was associated with an increased risk of AMI. Compared with GG homozygotes, individuals with the AA genotype or the A allele had an increased risk of experiencing AMI. Furthermore, logistic regression analysis showed that the AA genotype could be a predictor of AMI in the Tunisian population, independent of age, gender, cardiovascular risk factors, and other clinical characteristics. Studies in Chinese, Taiwanese, Korean, and North Indian populations confirmed our findings.[6,16-21] A meta-analysis, including 5493 cases of coronary heart disease (CHD) and 8297 controls from 14 case–control studies investigated the association of the TM −33G/A polymorphism with CHD risk.[21] In the AMI subgroup analyses, patients with the TM −33G/A polymorphism had an increased risk of AMI, suggesting that the TM −33G/A polymorphism is critical for AMI development.[21] In contrast, studies in Javanese,[22] Han Chinese,[13] Asians,[21] and Bahrainis[23] reported no such association with AMI. It was suggested that different lifestyles, diets, and environments were responsible for this discrepancy. Further investigation of the association of this polymorphism with other exonic and intronic variants of the THBD gene and with the range of risk factors in different ethnic populations may provide insights into the importance of the THBD gene in regulating endothelial dysfunction and help prevent such modulations of AMI risk.

The present study also showed that the AA genotype of the THBD −33G>A polymorphism is associated with increased DBP, CRP, serum ACE activity, and albumin concentrations. Albumin was negatively correlated with fibrinogen in AMI patients. C-reactive protein was positively correlated with fibrinogen and negatively with albumin. On the other hand, HTA, diabetes, dyslipidemia, and smoking were independent risk factors for AMI. An interaction between smoking and THBD −33G/A polymorphism also increased the risk of AMI in North Indian and Taiwan cohorts.[16, 20] In these studies, smokers carrying the THBD −33G>A polymorphism had an almost 10-fold increased risk of AMI compared with non-smokers who were not carriers.[16, 20]

Arterial hypertension, smoking, diabetes, physical inactivity, obesity, hyperlipidemia, poor diet, and excessive alcohol consumption combine to significantly increase the production of reactive oxygen species. Angiotensin-II (Ang-II) is a central component of the renin-angiotensin-aldosterone pathway and is generated primarily from the precursor

angiotensinogen through the sequential action of renin and ACE. Angiotensin-II controls blood pressure by regulating body fluid volume. Excessive oxidative stress negatively affects myocardial cells and leads to complications in target tissue and organs. The effects of Ang-II include the activation of nuclear transcription factors that specifically stimulate the expression of cytokines, chemokines, nitric oxide, cyclooxygenase-2, and adhesion molecules. These factors, which increase under the influence of Ang-II, stimulate low-density lipoprotein oxidation through the activation of macrophages and increase the production of CRP, fibrinogen, and other acute-phase reactants in the liver.[24-27] In general, elevated CRP and fibrinogen levels are associated with increased cardiovascular risk and mortality. [24,28-31] C-reactive protein is considered a marker of generalized atherosclerosis[32] and is often used to stratify cardiovascular risk.[33] Some studies also found that fractional forms, i.e., the trimeric and dimeric subunits of CRP, may be specific early biomarkers for the assessment of acute myocardial tissue damage.[34,35]

The relationship between fibrinogen concentration and cardiovascular risk and mortality is unclear.[29] In response to thrombin production, THBD acts as a thrombin receptor on the endothelium and reduces the ability of thrombin to convert fibrinogen to fibrin and to activate platelets. The thrombin–THBD complexes then activate protein-C, and activated protein-C inactivates coagulation factors Va and VIIIa, leading to suppression of thrombin formation.[36] Thrombomodulin forms a high-affinity complex with thrombin and plays an essential role in both anticoagulation, anti-inflammation,[25] and in the protein-C anticoagulant pathway. Thrombomodulin is an integral glycoprotein of the endothelial cell membrane that binds to thrombin in the presence of calcium ions, reducing its specificity to fibrinogen. This polymorphism has previously been associated with plasma-soluble THBD and is functionally important in patients at cardiovascular risk.[37] Thrombomodulin acts as an important physiological anticoagulant and deficiency of this protein can lead to excessive thrombus formation.[38,39] The cause of elevated CRP and fibrinogen concentrations in individuals with CVD has not yet been identified.

Study Limitations

A limitation of the study is that the number of subjects was relatively small and not sufficient to generate the necessary power to draw definitive conclusions. Larger studies are needed to assess the association between TM –33G/A polymorphisms and AMI risk in different ethnic groups. According to our results, ethnicity appears to be one of the most important factors determining the association between THBD polymorphism and AMI risk. Therefore, a meta-analysis of the Arab population is needed to better understand the associations between TM variant –33G/A and AMI risk. Meta-analyses are useful methods for studying associations between genetic factors and diseases because they use a quantitative approach to combine the results of different studies on the same topic, allowing for more reliable conclusions. A second limitation is the limited number of markers of antioxidant activity and inflammation examined. A third limitation is that THBD activity was not determined.

CONCLUSION

This study shows that the THBD –33G>A polymorphism, together with elevated albumin and fibrinogen concentrations, may become an important component of the clinical assessment of AMI risk. Further studies with larger sample sizes are needed to confirm these results.

Ethics Committee Approval: The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the Ethical Committee (CER-SVS/ISBM 007/2023) on 20th January 2023.

Informed Consent: All participants gave written informed consent. All methods were carried out in accordance with relevant guidelines and regulations.

Peer-review: Externally peer-reviewed.

Author Contributions: R.C. and W.K.: Conception and design of the study. S.H.: acquisition and analysis of data. S.M. and J.F.: writing, review, and revision of the manuscript. M.H.: study supervision. All authors read and approved the final manuscript.

Declaration of Interests: The authors have no conflicts of interest to declare.

Availability of data and materials: The data sets generated and analyzed during the present study are available from the corresponding author upon reasonable request. Patients were recruited from the Fattouma Bourguiba Hospital, Monastir, Tunisia.

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