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ORIGINAL ARTICLE

Relation of Vaspin rs2236242 Gene Polymorphism to Type-2 Diabetic Patients with Coronary Artery Disease

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) as well as coronary artery disease (CAD) are highly prevalent and often coexist, especially among high-risk populations like Egypt. Recent research suggested that genetic variants, involving the vaspin (SERPINA12) gene, could play a role in the development of T2DM and the related vascular complications. This work aimed to assess the association of the vaspin rs2236242 gene polymorphism with T2DM and the presence of CAD in Egyptian patients, and to assess its potential as a genetic risk factor.

Methods: We carried out this case-control study on 90 participants divided into three groups: healthy controls (n=30), T2DM patients without CAD (n=30), and T2DM patients with CAD (n=30). Clinical, biochemical, and genetic data were collected. Genotyping of Vaspin rs2236242 was performed using TARMS PCR. Gel electrophoresis was utilized for analyzing the PCR products following the amplification of the Vaspin rs2236242 polymorphism.

Results: The TA genotype frequency of vaspin rs2236242 was significantly higher in T2DM (56.7%) and T2DM with CAD (83.3%) versus controls (23.3%) ($P < 0.001$), and A allele frequency also increased across groups ($P < 0.001$). No AA genotype was detected. TA carriers had significantly higher fasting glucose, HbA1c, and blood pressure, especially among the CAD group ($P < 0.01$). The TA genotype increased the risk of T2DM (OR = 4.5, $P < 0.001$) and T2DM with CAD (OR = 5.5, $P < 0.001$); the A allele was also a significant risk factor for both outcomes.

Conclusion: This study suggests that the vaspin rs2236242 gene polymorphism, particularly the TA genotype, may play a significant role in the development of type 2 diabetes mellitus, especially in patients who also have coronary artery disease.

Keywords: Type 2 Diabetes; Coronary Artery Disease; Vaspin rs2236242 ;Gene Polymorphism.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a progressive metabolic disorder, resulting from both insulin resistance and impaired insulin secretion. Its global prevalence continues to rise due to increasing obesity, sedentary lifestyles, and aging populations[1]. According to the International Diabetes Federation (2025), an estimated 529 million adults worldwide are affected, with projections reaching 783 million by 2045. Egypt has one of the world's highest diabetes prevalence rates at

22.4% among adults [2]. T2DM is the predominant form of diabetes and a major cause of premature death, primarily due to its vascular complications such as coronary artery disease (CAD)[3]. Individuals with T2DM have a two- to four-fold increased risk of developing CAD, which remains the leading cause of mortality in this group [5,6].

Recent studies highlight the role of adipokines, such as vaspin—a serine protease inhibitor encoded by the SERPINA12 gene—in modulating metabolic and vascular risk. Vaspin levels are elevated in obesity and insulin resistance, and animal and human studies suggest it may protect against metabolic and endothelial dysfunction [8–10]. The rs2236242 single nucleotide polymorphism (SNP) in SERPINA12 has been implicated in T2DM and CAD, but findings are inconsistent and may vary by ethnicity and population [9,11,12].

Despite increasing interest, data are scarce regarding the relationship between the vaspin rs2236242 polymorphism and CAD among Egyptian T2DM patients. This study aimed to evaluate the association of vaspin rs2236242 with T2DM and CAD risk in an Egyptian population, addressing a key gap in the regional literature.

METHODS

This case-control study was conducted at the Medical Biochemistry and Molecular Biology, Internal Medicine, and Cardiology Departments, Faculty of Medicine, Zagazig University, between May 2024 and May 2025.

The study included a total of 90 participants, divided equally into three groups. The three study groups were matched for age and sex to minimize confounding effects. BMI was not used as a matching criterion, as differences in BMI were of interest for analysis regarding their relationship with T2DM, CAD, and the vaspin rs2236242 genotype. Group 1 consisted of 30 healthy controls, Group 2 included 30 patients diagnosed with type 2 diabetes mellitus (T2DM) but without coronary artery disease (CAD), and Group 3 comprised 30 patients with both T2DM and confirmed CAD. The sample size calculation was based on an assumed frequency of the vaspin rs2236242 gene polymorphism in diabetic versus control groups, which was estimated at 65% and 32%, respectively. OpenEpi software was used for sample size estimation, considering 80% power and a 95% confidence interval [9].

The research ethics board of the Faculty of Medicine at Zagazig University approved the study, and all participants gave written informed consent. As a component of the Code of Ethics for Research Involving Humans, the Declaration of Helsinki ensures that the work was performed in compliance with its provisions. Before this study could begin, we obtained approval from the Institutional Review Board (IRB 168/5 - March 2024).

Patients diagnosed with T2DM, with or without CAD, and seen at the Internal Medicine or Cardiology Departments were included. According to the World Health Organization, type 2 diabetes can be diagnosed with fasting plasma glucose levels of 126 mg/dL or higher, 2-hour postprandial plasma glucose levels of 200 mg/dL or higher, a HbA1c level of 6.5% or higher, or random blood glucose levels of 200 mg/dL or higher. The European Society of Cardiology established criteria for the diagnosis of acute coronary syndrome. These criteria include a 30-minute history of ischemic chest pain, distinctive ECG abnormalities, and dynamic variations in myocardial troponin levels. Inclusion criteria included that the patient had sufficient renal, hepatic, and respiratory function [2]. Patients with controlled hypertension or dyslipidemia were included in the study, as these conditions are frequently associated with T2DM and CAD, and their inclusion reflects the typical clinical spectrum of these patient populations.

Exclusion criteria comprised patients with chronic liver, kidney, or respiratory diseases; those with inflammatory disorders or using anti-inflammatory drugs such as NSAIDs and corticosteroids within one month prior to enrollment; patients with valvular heart disease, aortic aneurysms, heart failure, malignancy, type 1 diabetes, or other comorbidities that

could affect the study outcomes; and those who declined to give consent or were uncooperative. Each subject underwent a comprehensive history and clinical examination, with a special focus on risk factors such as age, gender, family history of diabetes and ischemic heart disease, hypertension, smoking, chest pain, and previous myocardial infarction. Physical examination included assessment of pulse rate, rhythm, and blood pressure.

Electrocardiography was performed to assess ischemic changes, and coronary angiography was used for definitive identification of atherosclerotic vessels. The diagnosis of CAD was confirmed by coronary angiography, with inclusion limited to patients who demonstrated at least one significant coronary artery stenosis of greater than 50%. Blood samples were collected after an overnight fast of 8–12 hours. A total of 8 ml of venous blood was drawn under aseptic conditions. Three milliliters were collected in EDTA tubes for genomic DNA extraction and HbA1c estimation, one milliliter in sodium fluoride tubes for plasma glucose estimation, three milliliters in plain tubes for serum separation and lipid profile analysis, and one milliliter in sodium fluoride tubes for postprandial glucose testing. All blood samples were processed promptly. Plasma and serum samples were separated within 2 hours of collection and stored at –80°C until biochemical analysis. Whole blood samples for DNA extraction were stored at 4°C and processed within 24 hours.

Laboratory investigations included fasting and postprandial plasma glucose, HbA1c, and a lipid profile involving triglycerides, total cholesterol, LDL-cholesterol, as well as HDL-cholesterol. Plasma glucose was measured using an enzymatic colorimetric method following the procedure described by Trinder [13]. HbA1c was estimated using a sandwich immunoassay (Boditech ichroma™ HbA1c kit), and serum total cholesterol and HDL-cholesterol were assessed using standard colorimetric methods [14,15]. Serum triglycerides were determined enzymatically as previously described [16]. LDL-cholesterol was calculated using the Friedewald formula [17]. For genetic analysis, the vaspin rs2236242 single-nucleotide polymorphism was detected by Tetraprimer amplification refractory mutation system PCR (T-ARMS PCR) [18]. Genomic DNA was extracted from whole blood using a commercial spin-column kit, and its concentration and purity were checked by measuring absorbance at 260 and 280 nm. Only samples with an A260/A280 ratio between 1.7 and 1.9 were considered pure [19]. Using suitable controls, PCR amplification was performed in a volume totaling 20 µl. First, denaturation; then, annealing; then, extension; and last, extension; these were the conditions of the cycling process.

Amplification Refractory Mutation System Polymerase Chain Reaction (ARMS-PCR) was used to identify the Vaspin gene polymorphism (rs2236242). This method relied on primers that were made to amplify the T or A allele specifically. To be more precise, primer F1 was used to target the T allele (AAGACGCCGCTTCTGTGCACT),and primer R1 was used to target the A allele (CCAGGGACCCAGGATAACTTGCT). Outer forward primer F0 (sequence: GGAGGCAGACCAGGCACTAGAAA) and outer reverse primer R0 (sequence: ACCATCTCTGGCTTCAGGCTTC) were added to the allele-specific primers to aid in the amplification process. Genotyping and accurate detection of the vaspin (rs2236242) single-nucleotide polymorphism were made possible by combining these primers.

For genotyping, the T-ARMS PCR protocol was used. The cycling conditions were as follows: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 62°C for 45 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 7 minutes. Each PCR run included a negative control (no DNA template) to detect contamination and a positive control sample with a previously confirmed genotype to validate amplification and allele detection.

Gel electrophoresis was employed to analyze the PCR products following the amplification of the vaspin rs2236242 polymorphism. To begin, a 1X Tris-Acetate-EDTA (TAE) buffer was prepared by diluting a 50X stock solution, which had been made by dissolving Tris base, glacial acetic acid, and EDTA in distilled water to a final volume of one liter. For the agarose gel, a 2% concentration was achieved by dissolving 2 grams of agarose in 100 ml of the 1X TAE buffer, heating the mixture until fully dissolved, and then allowing it to cool to around 65°C before incorporating ethidium bromide for DNA visualization. The gel was poured into a tray, left to set at room temperature, and then 10 microliters of each PCR product were carefully loaded into individual wells.

A 50 base-pair DNA ladder was used as a molecular size marker throughout the electrophoresis technique, which was carried out using a Maxicell EC 360 underwater gel system. After the electrophoresis was finished, the DNA bands were observed by utilizing a transilluminator to expose the ethidium bromide-stained gel to ultraviolet light. The resulting images were then recorded for further study. The AA genotype was not detected in any samples, but the TT and TA genotypes could be identified by their distinct banding patterns. Alleles T, A, and control were expected to have PCR products with sizes of 174, 248, and 378 base pairs, respectively, as shown on the 2% agarose gel.

Statistical analysis

Data analysis was performed using SPSS version 20 (IBM, USA). Descriptive statistics included mean and standard deviation for numerical variables, while frequencies and percentages were used for categorical data. For inferential analysis, ANOVA was applied to compare means across more than two groups, and the independent t-test assessed differences between two groups. Relationships between qualitative variables were examined using the chi-square test or odds ratio, with Fisher's exact test used when expected cell counts were low. Statistical significance was set at $p \leq 0.05$, with values above 0.05 considered non-significant and those below 0.001 deemed highly significant. Genotype–phenotype associations were assessed using univariate comparisons. Multivariate analysis, such as logistic regression adjusting for confounders (e.g., age, BMI, hypertension), was not performed.

RESULTS

Baseline Characteristics

Significant differences were revealed between the three groups as regards BMI, hypertension history, smoking status, and both systolic and diastolic blood pressures ($p < 0.05$). Group 3 (T2DM with CAD) had the highest BMI compared to Groups 1 and 2 ($p < 0.05$). Hypertension was significantly more common in both diabetic groups than in controls ($p < 0.001$), and smoking was also more prevalent among people with diabetes, especially those with CAD ($p = 0.02$). Both systolic and diastolic blood pressures were highest in Group 3 ($p < 0.001$) with non-significant differences in age or sex distribution (Table 1).

Biochemical Markers

Diabetic groups exhibited substantially higher fasting blood glucose, 2-hour postprandial glucose, and HbA1c than controls ($p < 0.001$). Lipid profiles revealed significantly increased total cholesterol, triglycerides, and LDL-C in diabetic patients, particularly those with CAD ($p < 0.001$), while HDL-C was lowest in Group 3 ($p < 0.001$). Non-HDL cholesterol increased significantly across the groups ($p < 0.001$) (Table 2).

Genotype Distribution

Analysis of rs2236242 genotypes revealed highly significant differences in distribution among the groups ($\chi^2 = 21.86$, $p < 0.001$). The TT genotype predominated in controls (76.7%) but decreased in frequency to 43.3% in T2DM without CAD and 16.7% in T2DM with CAD. Conversely, the TA genotype increased from 23.3% in controls to 56.7% in T2DM without CAD and 83.3% in T2DM with CAD ($p < 0.001$ for all pairwise comparisons). Allelic analysis showed that T allele frequency declined from 88.3% (controls) to 71.6%

(T2DM without CAD) and 58.4% (T2DM with CAD), while the A allele increased from 11.6% to 28.4% and 41.6%, respectively ($p < 0.001$ for most pairwise comparisons) (Table 4). Notably, the AA genotype was not detected in any of the study participants across all groups. This may be attributed to the low frequency of the A allele in this population or the sample size.

Genotype–Phenotype Correlation

A significant correlation was observed between rs2236242 polymorphism and the risk of T2DM and CAD. The TA genotype conferred a 7.7 - fold higher risk of developing T2DM or T2DM with CAD compared to the TT genotype (OR = 7.7; 95% CI: 2.79–21.06; $p < 0.0001$). Carriers of the A allele had a 4.08-fold increased risk (OR = 4.08; 95% CI: 1.70–9.76; $p = 0.005$). (Table 5).

Among T2DM patients without CAD, TA genotype carriers exhibited higher systolic and diastolic blood pressure, fasting and postprandial glucose, HbA1c, and triglycerides compared to TT genotype (all $p < 0.05$). In T2DM patients with CAD, the TA genotype was associated with higher BMI, blood pressure, fasting and postprandial glucose, HbA1c, triglycerides, LDL-C, non-HDL cholesterol, CPK–MB, and troponin, and lower HDL-C (all $p < 0.05$). No significant genotype-related differences were observed for ECG changes or the number of affected vessels in either diabetic group (Table 6).

Figure 1 shows gel electrophoresis of PCR products for the Vaspin rs2236242 gene polymorphism, stained with ethidium bromide. A 50 bp DNA ladder was used as the molecular size marker. Lanes 1, 3, 4, and 6 show the TA genotype, indicated by three bands at 378, 248, and 174 bp. Lanes 2, 5, and 7 show the TT genotype, with two bands at 378 and 174 bp.

Table 1: Demographic, Clinical, and Blood Pressure Characteristics of the Studied Groups

Variable	Group 1 Control (n=30)	Group 2 T2DM without CAD (n=30)	Group 3 T2DM with CAD (n=30)	Test	P Value	LSD (Pairwise Comparisons)
Age (years)	47.08±6.48 (29–55)	54.9±4.1 (40–62)	57.4±3.3 (50–73)	F=34.49	0.07NS	0.3[1], 0.2[2], 0.2[3]
BMI (kg/m²)	20.39±4.31 (20–27)	26.22±8.5 (21–37)	34.62±6.32 (28.3–40.9)	F=32.87	<0.05*S	0.04[1], 0.05[2], 0.03[3]
Sex				χ^2=1.69	0.42NS	
Male	13 (43.4%)	16 (53.4%)	18 (60%)			
Female	17 (56.6%)	14 (46.6%)	12 (40%)			
Smoking				χ^2=13.16	0.02*	0.01[1], 0.05[2], 0.03[3]
No	25 (83.3%)	22 (73.3%)	21 (70%)			
Yes	5 (16.7%)	8 (26.7%)	9 (30%)			
History of HTN				χ^2=12.65	<0.001**	<0.001**[1], <0.001**[2], <0.001**[3]
Negative	30 (100%)	20 (66.7%)	17 (56.6%)			
Positive	0 (0%)	10 (33.3%)	13 (43.4%)			
SBP (mmHg)	119.34±6.99 (100–130)	132.85±8.17 (120–150)	174.67±6.63 (160–180)	F=438.06	<0.001**	<0.001**[1], <0.001**[2], <0.001**[3]
DBP (mmHg)	74.61±9.47 (56–93)	87.30±6.64 (75–100)	102.67±9.74 (93–115)	F=72.54	<0.001**	<0.001**[1], <0.001**[2], 0.006**[3]

SD: standard deviation; BMI: body mass index; LSD: least significant difference (post-hoc pairwise comparison); NS: non-significant (P > 0.05); S: significant (P < 0.05); *: significant; **: highly significant
(P < 0.01); SBP: systolic blood pressure; DBP: diastolic blood pressure; HTN: hypertension; T2DM: type
2 diabetes mellitus; CAD: coronary artery disease; F: ANOVA F statistic; χ^2: chi-square statistic. LSD pairwise comparisons: [1]: Group 1 vs. Group 2; [2]: Group 1 vs. Group 3; [3]: Group 2 vs. Group 3.

Table 2: Glycemic Indices and Lipid Profile of the three Studied Groups

Variable	Group 1 Control (n=30)	Group 2 T2DM without CAD (n=30)	Group 3 T2DM with CAD (n=30)	Test	P Value	LSD (Pairwise Comparisons)
FBG (mg/dl)	83.66±11.41 (65–110)	199.38±54.5 (115–295)	271.5±50.9 (220–340)	F=132.53	<0.001**	<0.001**[1], <0.001**[2], 0.02**[3]
2hPPBG (mg/dl)	108.68±15.71 (82–140)	290.15±80.5 (160–435)	380.29±51.28 (320–460)	F=171.81	<0.001**	<0.001**[1], <0.001**[2], 0.001**[3]
HbA1c (%)	4.48±0.62 (3.5–5.3)	7.23±1.6 (5.8–9.6)	9.31±0.94 (8.37–10.25)	F=128.80	<0.001**	<0.001**[1], <0.001**[2], 0.003**[3]
TC (mg/dl)	140.05±36.51 (120–180)	187.39±38.59 (145–231)	246.51±46.0 (200–301)	F=48.39	<0.001**	0.005**[1], <0.001**[2], 0.001**[3]
TG (mg/dl)	78.18±4.57 (69–125)	127.89±39.5 (88–167)	154.01±30.64 (130–192)	F=49.47	<0.001**	<0.001**[1], <0.001**[1], 0.001**[3]
HDL-C (mg/dl)	69.89±9.73 (50–89)	58.86±18.40 (40–77)	33.18±12.48 (21–46)	F=50.60	<0.001**	0.01*[1], <0.001**[2], 0.001**[3]
LDL-C (mg/dl)	83.3±27.78 (78–129)	111.59±37.16 (74–149)	219.2±29.4 (149–260)	F=143.15	<0.001**	0.003**[1], <0.001**[2], <0.001**[3]
Non-HDL Cholesterol (mg/dl)	120±12.78 (112–135)	132.27±10.24 (122–143)	137.67±19.84 (125–160)	F=10.41	<0.001**	0.005**[1], <0.001**[2], 0.001**[3]

SD: standard deviation; FBG: fasting blood glucose; 2hPPBG: 2 hours post-prandial blood glucose; HbA1c: glycated hemoglobin; TC: total cholesterol; TG: triglycerides; HDL: high density lipoprotein; LDL: low density lipoprotein; LSD: least significant difference (post-hoc pairwise comparison); NS: non-significant (P > 0.05); S: significant (P < 0.05); *: significant; **: highly significant (P < 0.01); T2DM: type 2 diabetes mellitus; CAD: coronary artery disease; F: ANOVA F statistic. LSD pairwise comparisons: [1]: Group 1 vs.Group 2; [2]: Group 1 vs. Group 3; [3]: Group 2 vs. Group 3.

Table 3: Comparison between three studied groups regarding ECG.

Variable	Group 1 Control (*n*=30)	Group 2 T2DM without CAD (*n*=30)	Group 3 T2DM with CAD (*n*=30)	χ^2 test	LSD	P
Normal (N%)	**30(100%)**	**30(100%)**	**6(20%)**	**65.45**	**N/A *1 <0.001*2 <0.001 3**	**<0.001****
ST elevation (N%)	**0(0%)**	**0(0%)**	**9(30%)**			
ST depression(N%)	**0(0%)**	**0(0%)**	**10(33.4%)**			
T wave inversion(N%)	**0(0%)**	**0(0%)**	**5(16.6%)**			

T2DM – Type 2 Diabetes Mellitus, CAD – Coronary Artery Disease, LSD – Least Significant Difference

Table 4. Distribution of genotypes of rs2236242 among the three studied groups.

	Group 1 Control (n=30)		Group 2 T2DM without CAD (n=30)		Group 3 T2DM with CAD (n=30)		χ^2 test	P	LSD
	N	%	N	%	N	%			
TT	23	76.7	13	43.3	5	16.7	**21.86**	**<0.001****	**<0.001** 1 <0.001** 2 0.003**3**
TA	7	23.3	17	56.7	25	83.3		**<0.001****	**<0.001** 1 <0.001** 2 <0.001** 3**
T allele	53	88.3	43	71.6	35	58.4	**13.68**	**<0.001****	**<0.001** 1 <0.001** 2 <0.001** 3**
A allele	7	11.6	17	28.4	25	41.6		**<0.001****	**<0.001** 1 <0.001**2 0.003** 3**

LSD: P1: Group 1 versus Group 2, P2: Group 1 versus Group 3, P3: Group 2 versus Group 3. Data are presented as mean ± standard deviation. **: highly significant (P value < 0.01).

Table 5: Association analysis for vaspin rs2236242 gene polymorphism, T2DM and coronary artery disease risk

	Group 1 Control (n=30)		Group 2 T2DM without CAD (n=30)		Group 3 T2DM with CAD (n=30)		OR (95% CI)	p Value
	N	%	N	%	N	%		
TT	23	76.7	13	43.3	5	16.7	Reference (1.00)	0.0001
TA	7	23.3	17	56.7	25	83.3	7.7 (2.79–21.06)	
Allele Distribution								
T allele	53	88.3	43	71.6	35	58.4	Reference (1.00)	0.005
A allele	7	11.6	17	28.4	25	41.6	4.08 (1.70–9.76)	

CI: confidence interval; OR: odds ratio; T2DM: type 2 diabetes mellitus; CAD: coronary artery disease.

Table 6: Relation of Vaspin rs2236242 Genotypes with Clinical, Laboratory, ECG, and Angiographic Parameters

Clinical and Laboratory Parameters among T2DM without CAD (Group 2) by Vaspin rs2236242 Genotype				
Parameter	**TT (N=13)**	**TA (N=17)**	**t-test**	**P value**
Age (years)	**47.8 ± 4.9**	**49.1 ± 5.1**	**0.71**	**0.48 NS**
BMI (kg/m2)	**25.6 ± 10.7**	**26.7 ± 10.8**	**0.27**	**0.79 NS**
Systolic BP (mmHg)	**122.24 ± 13.19**	**139.3 ± 8.17**	**4.32**	**0.001**S**
Diastolic BP (mmHg)	**84.3 ± 7.1**	**89.6 ± 6.9**	**2.21**	**0.03* S**
FBG (mg/dl)	**160.08 ± 24.6**	**230.2 ± 53.7**	**4.64**	**0.001**S**
2h PPBG (mg/dl)	**271.4 ± 73.8**	**328.5 ± 61.4**	**2.25**	**0.03* S**
HbA1c %	**6.48 ± 2.13**	**9.04 ± 2.16**	**3.51**	**0.001**S**
TC (mg/dl)	**185.0 ± 49.09**	**190.0 ± 39.19**	**0.36**	**0.72 NS**
TG (mg/dl)	**115.2 ± 37.5**	**137.6 ± 41.2**	**2.2**	**0.03* S**
HDL-C (mg/dl)	**57.16 ± 17.49**	**60.16 ± 19.49**	**0.44**	**0.66 NS**
LDL-C (mg/dl)	**105.92 ± 34.53**	**115.92 ± 39.53**	**0.78**	**0.44 NS**
Non-HDL Cholesterol	**130 ± 10.78**	**134 ± 9.78**	**1.13**	**0.27 NS**
Clinical, ECG, and Angiographic Characteristics among T2DM with CAD (Group 3) by Vaspin rs2236242 Genotype				
Parameter	**TT (N=5)**	**TA (N=25)**	**t-test/χ^2**	**P value**
Age (years)	**58.13 ± 7.85**	**60.0 ± 7.15**	**0.62**	**0.54 NS**
BMI (kg/m2)	**32.2 ± 3.6**	**35.1 ± 3.5**	**2.2**	**0.03* S**
Systolic BP (mmHg)	**168.0 ± 6.0**	**176.0 ± 6.0**	**3.27**	**0.001**S**
Diastolic BP (mmHg)	**91.0 ± 10.0**	**105.0 ± 8.0**	**3.71**	**0.001**S**
Acute CAD (N,%)	**2 (40%)**	**10 (40%)**	**0.00**	**1.0 NS**
Chronic CAD (N,%)	**3 (60%)**	**15 (60%)**		
Normal ECG (N,%)	**3 (33.3%)**	**6 (28.5%)**	**2.31**	**0.5 NS**
ST elevation (N,%)	**2 (22.2%)**	**8 (38%)**		
ST depression (N,%)	**1 (20%)**	**2 (9.5%)**		
T wave inversion (N,%)	**0 (0%)**	**5 (24%)**		
One vessel (N,%)	**3 (60%)**	**7 (28%)**	**3.68**	**0.15 NS**
Two vessels (N,%)	**2 (40%)**	**7 (28%)**		
Multi vessels (N,%)	**0 (0%)**	**11 (44%)**		
Laboratory Parameters among T2DM with CAD (Group 3) by Vaspin rs2236242 Genotype				
Parameter	**TT (N=5)**	**TA (N=25)**	**t-test**	**P value**
FBG (mg/dl)	**205.76 ± 57**	**284.65 ± 38.9**	**3.74**	**<0.001**S**
2h PPBG (mg/dl)	**306.74 ± 60.0**	**395.0 ± 35.0**	**4.25**	**<0.001**S**
HbA1c %	**7.89 ± 0.65**	**9.6 ± 0.7**	**6.86**	**<0.001**S**
TC (mg/dl)	**229.55 ± 42.75**	**249.9 ± 46.7**	**1.11**	**0.27 NS**
TG (mg/dl)	**124.05 ± 41.35**	**160.0 ± 25.0**	**2.43**	**0.02* S**
HDL-C (mg/dl)	**48.58 ± 15.5**	**30.1 ± 10.0**	**3.65**	**0.001**S**
LDL-C (mg/dl)	**190.0 ± 52.0**	**225.0 ± 38.0**	**2.2**	**0.03 S**
Non-HDL Cholesterol	**126 ± 15.78**	**146 ± 17.0**	**2.25**	**0.03 S**
CPK–MB (ng/ml)	**7.8 ± 1.6**	**10.8 ± 1.6**	**4.74**	**<0.001**S**
Troponin (pg/ml)	**210.8 ± 12.7**	**284.8 ± 19.6**	**9.43**	**<0.001**S**

SD: standard deviation; BMI: body mass index; BP: blood pressure; FBG: fasting blood glucose; 2h PPBG: 2 hours post-prandial blood glucose; HbA1c: glycated hemoglobin; TC: total cholesterol; TG: triglycerides; HDL-

C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; CPK–MB: creatine phosphokinase-MB; NS: non-significant (P > 0.05); S: significant (P < 0.05); *: significant; **: highly significant (P < 0.01); CAD: coronary artery disease.

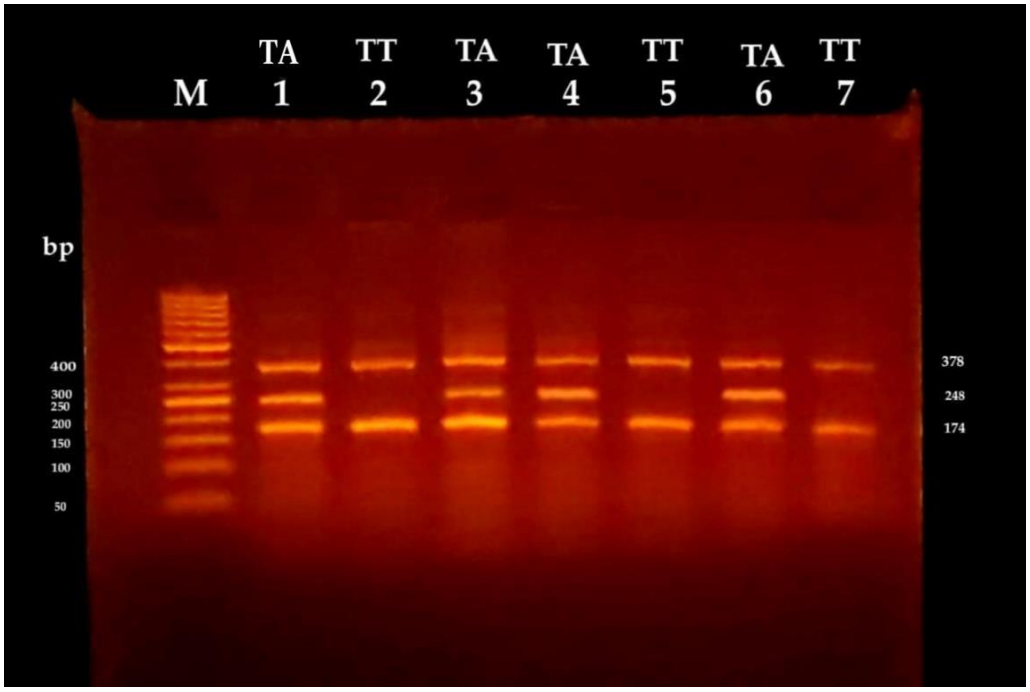

Figure (1): Agarose gel electrophoresis picture stained with ethidium bromide showing the PCR product in which there was the analysis of the Vaspin rs2236242 gene polymorphism. Lanes (1,3,4,6): TA genotype showing the presence of three bands 378+248+174bp. Lanes (2,5,7): TT genotype showing the presence of two bands 378 +174 bp.

DISCUSSION

In this study, we examined the clinical and genetic associations of the vaspin rs2236242 gene polymorphism in Egyptian patients with T2DM and CAD, aiming to clarify its contribution to cardiometabolic risk in a high-prevalence setting. The careful matching of age and sex across the three studied groups minimized confounding, thereby allowing more reliable interpretation of genetic and metabolic correlations. Notably, we observed that BMI was significantly higher in patients with both T2DM and CAD, compared to other groups. This is in line with prior reports indicating that obesity remains a central risk factor for the progression of both T2DM and atherosclerotic cardiovascular disease, reinforcing the importance of adiposity in the pathogenesis of these conditions [19–22]. Several studies from different ethnic backgrounds have similarly highlighted the synergistic role of increased BMI in the development and worsening of both diabetes and coronary events, even after adjustment for other risk factors.

The prevalence of traditional cardiovascular risk factors, such as hypertension and smoking, was also found to be higher in diabetic patients, particularly those with CAD, in our study. This agrees with findings from recent regional studies, which consistently report increased rates of hypertension, smoking, and metabolic syndrome among T2DM patients with cardiovascular complications [23,24]. Blood pressure measurements, both systolic and diastolic, increased progressively across study groups and were highest in the T2DM with CAD group, highlighting the cumulative vascular burden faced by these patients.

Beyond clinical risk factors, the present study's most notable finding was the distinct pattern of vaspin rs2236242 genotype distribution among the study groups. The TT genotype was

predominant among controls but became progressively less common with increasing metabolic and cardiovascular disease burden. The absence of the AA genotype in our cohort is consistent with several studies from Middle Eastern and North African populations, where the frequency of the A allele is generally low [20,24,25]. However, it is also possible that the lack of AA homozygotes reflects the modest sample size in the present study, which may not capture rare genotypes. Larger studies are needed to more accurately assess the distribution of all vaspin rs2236242 genotypes in this population. The allele frequency analysis further reinforced these patterns, as the A allele was much more prevalent in T2DM and CAD groups than in healthy controls, with both genotypic and allelic associations reaching statistical significance.

Our findings demonstrate that the TA genotype of the vaspin rs2236242 polymorphism is associated with a higher prevalence of T2DM and CAD, as well as more adverse metabolic and cardiovascular profiles in this Egyptian cohort. These results are in agreement with previous studies from Turkish, Chinese, and Egyptian populations, which also report increased cardiometabolic risk in carriers of the A allele or TA genotype [19,23,25:29]. The higher frequency of hypertension, dyslipidemia, and poor glycemic control among TA carriers in our study further supports the potential pathogenic role of this variant.

The mechanistic basis for these associations appears increasingly clear in light of recent research. Vaspin, encoded by the SERPINA12 gene, is an adipokine predominantly produced in visceral and subcutaneous fat, but also expressed in other metabolic tissues. It is thought to act as a compensatory molecule in states of metabolic stress, enhancing insulin sensitivity, reducing systemic inflammation, and protecting vascular endothelium [6,8,34]. The rs2236242 SNP, especially the presence of the A allele, has been linked to reduced vaspin expression or function, with downstream effects that may exacerbate insulin resistance, promote dyslipidemia, and accelerate atherogenesis [22,30,31]. Studies have demonstrated that lower circulating vaspin levels are associated with higher fasting glucose, poorer glycemic control, adverse lipid profiles, and more frequent cardiovascular events [19,29,35,36].

Vaspin has been shown to inhibit the serine protease kallikrein 7, which is involved in the degradation of insulin-sensitizing peptides, as well as to suppress pro-inflammatory pathways such as NF-κB. This may result in decreased vascular cell adhesion molecule expression and a reduction in endothelial dysfunction, both crucial mechanisms in the development of atherosclerosis and CAD [8,37]. Animal and human studies alike have documented that vaspin-deficient or low-vaspin states are characterized by enhanced vascular inflammation, oxidative stress, and endothelial apoptosis, further supporting the proposed protective role of vaspin [33].

The present study also demonstrated that the TA genotype was linked with more unfavorable lipid profiles, higher triglycerides and non-HDL cholesterol, along with greater blood pressure and cardiac biomarker elevations, especially in CAD patients. These clinical trends match those reported in Egyptian, Turkish, and Chinese studies, where A allele carriers showed worse metabolic outcomes and a greater burden of cardiovascular risk factors [19,22,24,25]. However, some reports found conflicting patterns regarding which genotype conferred higher risk for adverse lipid profiles, highlighting again the possible impact of ethnicity, environment, and comorbidities on gene expression and metabolic regulation [30].

A key limitation of our work is that serum vaspin concentrations were not measured, precluding direct correlation between genotype and protein expression or function. Although literature data suggest lower vaspin levels in A allele carriers, this gap restricts the strength of causal conclusions in our population. We strongly recommend that future studies incorporate both genetic and serum vaspin measurements to more definitively elucidate the functional impact of rs2236242 variants on metabolic and cardiovascular risk

[19,27,29,30]. For example, Sathyaseelan et al. [29] found that T2DM patients with acute coronary syndrome had lower vaspin levels compared to those without ACS, and these lower levels correlated negatively with CK-MB and blood pressure. Other reports have shown similar patterns, linking low vaspin to poor glycemic control, worse lipid profiles, and increased inflammatory markers in high-risk populations [34,36].

Despite accumulating evidence, not all studies agree on the direction of these associations. Some research in Iranian and Iraqi populations has found that the A allele might be associated with lower fasting glucose or HbA1c, or that the TA genotype is more frequent among healthy controls [23,28]. These contrasting results may reflect complex interactions between genetic background, dietary patterns, lifestyle factors, and environmental exposures. It is also possible that sample size and methodology influence the observed associations, underscoring the need for larger, multiethnic studies to clarify the role of vaspin polymorphisms across different settings [38].

The strength points of this study include careful matching, robust genotyping, and a multi-group comparison that captures the spectrum from healthy controls to those with T2DM and CAD. However, limitations exist: the sample size, while adequate for detecting moderate effects, may not capture rare genotypes; serum vaspin concentrations were not measured, preventing direct correlation with genotype; and the cross-sectional design precludes causal inference. An important limitation of our study is that genotype–phenotype associations were examined using only univariate analysis. We did not perform multivariate adjustment for potential confounding variables such as age, BMI, or hypertension. Thus, the observed associations between the TA genotype and adverse metabolic parameters may be partly influenced by these confounders, and future studies employing multivariate regression are needed to validate these findings.

CONCLUSION

This study suggests that the vaspin rs2236242 gene polymorphism, particularly the TA genotype, may play a significant role in the development of type 2 diabetes mellitus, especially in patients who also have coronary artery disease. The increased frequency of the TA genotype among diabetic patients with CAD points to its potential as a genetic risk factor for both metabolic and cardiovascular complications. However, our findings should be interpreted in light of certain limitations, including the relatively small sample size and the lack of serum vaspin measurements, which precluded direct genotype–phenotype correlations. Future research should include larger, prospective cohort studies and functional investigations of vaspin expression to clarify the biological mechanisms underlying these associations and to validate the clinical utility of this polymorphism as a genetic risk marker in diverse populations.

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

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Citation

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