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CDKN2B-AS (rs2891168), SOD2 (rs4880), and PON1 (rs662) polymorphisms and susceptibility to coronary artery disease and type 2 diabetes mellitus in Iranian patients: A case-control study

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Abstract

Background and Aims: Coronary artery disease (CAD) is a devastating illness and primary cause of death worldwide that arises from a combination of genetic and environmental factors. Several large-scale studies found that 9p21.3, superoxide dismutase 2 (SOD2), and paraoxonase 1 (PON1) polymorphisms increase type 2 diabetes mellitus (T2DM) and/or coronary artery disease (CAD) risk. Our research aimed to investigate whether the SNPs of the 9p21.3 locus (rs28911698), SOD2 (rs4880), and PON1 (rs662) genes were associated with the risk of T2DM and/or CAD in the Iranian population.

Methods: In this case-control study four group subjects including patients with CAD non-T2DM, with CAD and T2DM, non-CAD with T2DM, and non-CAD non-T2DM were recruited to the study from 2019 to 2020. Molecular analysis was carried out by allele specific-polymerase chain reaction (AS-PCR) technique for rs4880, Taqman genotyping assay for rs2891168, and PCR followed by restriction fragment length polymorphism (PCR-RFLP) technique for rs662.

Results: The rs2891168 polymorphism presented an elevated risk of CAD in non-T2DM with CAD and with T2DM CAD groups compared to the non-T2DM non-CAD group with GG genotype and dominant model after adjustment ($p < 0.05$). G-allele in *PON1* rs662 polymorphism associated with increased risk of T2DM in T2DM non-CAD, and T2DM CAD groups compared to non-T2DM non-CAD group with dominant model, GG and AG genotypes ($p < 0.05$). However, *SOD2* rs4880 polymorphism presented no significant association with the development of diabetes or CAD.

Conclusion: These results provide a prime witness that rs2891168 and rs662 gene variants might have a possible increased risk of CAD and T2DM occurrence,

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respectively. To obtain more definitive and accurate results in this area, further research is required.

KEYWORDS
CDKN2B-AS, coronary artery disease (CAD), polymorphism, PON1, SOD2, T2DM

1 | INTRODUCTION

Type 2 diabetes mellitus (T2DM), a major worldwide health concern, is a multifactorial metabolic condition defined by a high level of blood glucose. It arises as a result of decreased insulin production, insulin resistance, or both, leading to poor carbohydrate, fat, and protein metabolism. In 2017, over 450 million individuals worldwide were diagnosed with diabetes, a figure that is anticipated to rise to nearly 700 million by 2045.[1] Recent research has shown that Iranians have a genetic vulnerability to T2DM, which might be attributable to an increase in obesity incidence, urbanization, and lifestyle changes.[2] T2DM is also a substantial risk factor for coronary artery disease (CAD) in the general population.[3] Microvascular complications of T2DM are a major cause of mortality in T2DM, which is mostly caused by CAD and stroke. According to studies, diabetes has been associated with a higher risk of developing CAD and myocardial infarction (MI).[4] T2DM development and progression, as well as its associated complications such as nephropathy, neuropathy, and retinopathy, are hypothesized to be impacted by genetic susceptibility as well as environmental variables.[5] Single nucleotide polymorphisms (SNPs) have emerged as prospective genetic indicators of choice in this field due to their large effect and approximately equivalent distribution in human genomes, and have been used by scientists to map loci and locate disease-causing genes.[3] The genetic foundation of T2DM is undeniable. According to the GWAS Catalog (https://www.ebi.ac.uk), over 130 genome-wide association studies (GWAS) have been done, with over 1600 SNPs correlations with various diabetes symptoms described. As a result, several studies are being conducted throughout the world to uncover candidate and susceptibility genes for T2DM. However, only a few studies for T2DM susceptibility genes in Middle Eastern populations, including Iranians, have been described.

Recently, several GWAS revealed that *CDKN2B-AS* (rs2891168), *SOD2* (rs4880), and *PON1* (rs662) genetic variants were linked to the risk of T2DM and/or CAD. The locus 9p21.3 is one of the earliest identified and robust loci in the human genome and has an important role in the pathophysiology of T2DM and CAD. The genes located in this region have a role in the proliferation of inflammatory and vessel smooth muscle cells (VSMCs) that are important in atherosclerosis.[6] Several large-scale studies showed that some variants in this locus predispose to both T2DM and CAD by transcription level alteration. Previously, the rs2891168 genetic polymorphism has been identified as one of the strongest associations with CAD.[7] In an Italian population-based study, it was found that rs2891168 was associated with CAD, but it was not shown to be related to T2DM.[8] In another study on Saudi Arabian patients with CAD, it was found that individuals with the G-risk allele of rs2891168 were significantly more likely to develop MI than those with A-allele.[9]

According to our literature review, there is evidence that oxidative stress (OS) plays a part in the development of T2DM and CAD in Iranians.[10–12] Therefore, functional polymorphisms in OS-related genes may have an association with a predisposition to T2DM and/or CAD. The superoxide dismutase (SOD) is an antioxidant enzyme that contributes to the elimination of reactive oxygen species such as superoxides and hydroxyl radicals.[13] These antioxidants' genetic polymorphisms may contribute to the development of T2DM and CAD diseases.[14] The SOD-2 enzyme is encoded by the MnSOD (or SOD2) gene. This enzyme in the mitochondrial matrix captures oxygen radicals generated by redox reactions and electron transport in the mitochondria. The rs4880 is a synonymous (C/T) variation located on chromosome 6q25 and position 47 (exon2) of the *MnSOD* gene.

PON1, a high-density lipoprotein-bound antioxidant enzyme, protects LDL from oxidative damage.[15] PON1's antioxidant qualities prompted a substantial investigation into its function in the onset and development of T2DM and atherosclerosis. The oxidative alteration of LDL is thought to be a crucial initiating and progressing event in the onset and advancement of T2DM and CAD events.[16] The rs662 mutation causes glutamine (Q) to arginine (R) (A/G) substitution at amino acid position 192, which was reported to reduce PON1 enzymatic activity toward paraoxon.

Low activity may raise the risk of T2DM and/or CAD by reducing HDL's capacity to block LDL oxidation, and genetic variants have been discovered to reduce HDL's ability to force cholesterol efflux from macrophages and reverse cholesterol transport from peripheral tissues. Several recent studies showed rs662 association with diabetes and CAD.[17,18] The rs662 SNP indicates that HDL has a protective impact against LDL oxidation, and 192QQ homozygous is particularly useful in reducing the buildup of lipid peroxides on LDL.[17]

The *CDKN2B-AS*, *SOD2*, and *PON1* polymorphisms are thought to be potential variants for CAD and T2DM due to their functions in lipid metabolism, endothelial function, and angiogenesis. Moreover, there are few data on the association of these SNPs with the risks of CAD and/or T2DM in the Iranian population sample. This study is the first in the Southeast of Iran to look at the selected polymorphisms associated with T2DDM and CAD. Therefore, this study aims to evaluate three SNPs within the 9p21.3 genetic locus (rs2891168), *SOD2* (rs4880), and *PON1* (rs662) in patients with diabetes and CAD in an Iranian population.

2 | MATERIALS AND METHODS

2.1 | Subjects

Between January 2019 and August 2020, individuals from two separate centers in Kerman province, Iran (Shafa and Mehregan, Kerman) were admitted to the research. The protocol of the study was carried out in accordance with the Helsinki Declaration and was approved by Kerman Medical University's Ethical Committee (Approval No. IR.KMU.REC.1396.1977). Every person provided written consent for participation and data publication. All participants' demographic information, lifestyle habits, and medical history were collected using a standardized questionnaire. Clinical and laboratory tests such as systolic and diastolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), fasting blood glucose (FBS), cell blood count (CBC), and blood lipids profile provided further clinical and biochemical data. Obesity was defined as having a BMI ≥ 30 kg/m^2. Hyperlipidemia was identified as the presence of one of the following conditions: (i) low-density lipoprotein-cholesterol (LDL-c) >130 mg/dL; (ii) triglyceride (TG) > 150 mg/dL; (iii) total cholesterol (TC) >200 mg/dL; (iv) high-density lipoprotein-cholesterol (HDL-c) <40 mg/dL; and (v) a history of hyperlipidemia medical therapy. SBP/DBP over 140/90 mmHg was considered hypertension, as was the current usage of antihypertensive medication. Furthermore, FBS levels ≥126 mg/dL following an 8-h fast and/or the use of antidiabetic medication were accepted as diagnostic criteria for diabetes. Based on angiography findings, CAD was classified as a considerable luminal narrowing (50% or more) in at least one primary coronary artery, whereas individuals with no plaque were thought to be free of CAD.

The control group comprises of 90 patients with FBS levels <126 mg/dL and no history of diabetes therapy. Obesity, smoking, hyperlipidemia, hypertension, cardiovascular illnesses, nervous system diseases, liver and renal diseases, malignancy or neoplastic disorders, metabolic disorders, autoimmune diseases, and chronic infection were all exclusion criteria.

T2DM patients without CAD include 90 people whose FBS is ≥126 mg/dL or who are taking diabetic medication but have no history or indications of CAD. Any sort of cardiovascular illness, obesity, smoking, hyperlipidemia, hypertension, nervous system disorders, cancer, renal and liver diseases, autoimmune diseases, and chronic infection were all exclusion criteria.

CAD patients without T2DM included 90 patients whose FBS is <126 mg/dL and ≥50% narrowing in a major coronary artery. Obesity, smoking, hyperlipidemia, hypertension, lung illnesses, renal disease, liver disease, malignancy, endocrine disease, metabolic disorders, and autoimmune diseases were all exclusion criteria.

T2DM patients with CAD comprised 90 individuals with FBS ≥126 mg/dL or diabetic medication and 50% narrowing of a primary coronary artery. Obesity, smoking, hyperlipidemia, hypertension, lung illnesses, liver and renal diseases, cancer, metabolic abnormalities, and autoimmune diseases were all exclusion criteria. Our sample size (90 in each group) is sufficient and surpasses the projected number of samples (70 per group) necessary to achieve 90% statistical power.

2.2 | DNA extraction and genotyping

Blood samples were collected in vacuum tubes containing ethylene diamine tetraacetic acid (EDTA). Genomic DNA extraction was performed from leucocytes using the Lahiri and Nurnberger protocol[19] and quantified by NanoDrop ND-2000c spectrophotometer (Thermo Fisher Scientific). The genotyping for rs4880, rs2891168, and rs662 were determined using Allele Specific-Polymerase Chain Reaction (AS-PCR), TaqMan® assay (Applied Biosystems), and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), respectively. For rs4880 and rs662, the primer sequences were adopted from previous studies[20,21] and presented in Table 1. The Biometra T advanced PCR thermal cycler (Analytik Jena) was used for the amplification. PCR program was as follows: initial denaturation at

TABLE 1 The primer sequences, melting temperature (T_m), and product sizes for PCR-RFLP and tetra ARMS-PCR analysis.

SNP	Primer sequence (5′ → 3′)	Primer length (bp)	T_m (°C)	RE	Product size (bp)
rs662	F: 5′-GGGACCTGAGCACTTTTATGGC-3′	22	67.7	AlwI	AA 99
					AG 99, 66, 33
	R: 5′-CATCGGGTGAAATGTTGATTCC-3′	22	67.2		GG 66,33
rs4880	Inner F: 5′-CACCAGCACTAGCAGCATGT-3′	19	64	-	TT 514, 189
	Outer F: 5′-CACCAGCACTAGCAGCATGT-3′	20	60		TC 514
	Inner R: 5′-CCTGGAGCCCAGATACCCTAAAG-3′	23	66		CC 514, 366
	Outer R: 5′-ACGCCTCCTGGTACTTCTCC-3′	20	63		

Abbreviations: Bp, base pair; F, forward; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; R, reverse; RE, restriction enzyme; SNP, single-nucleotide polymorphism.

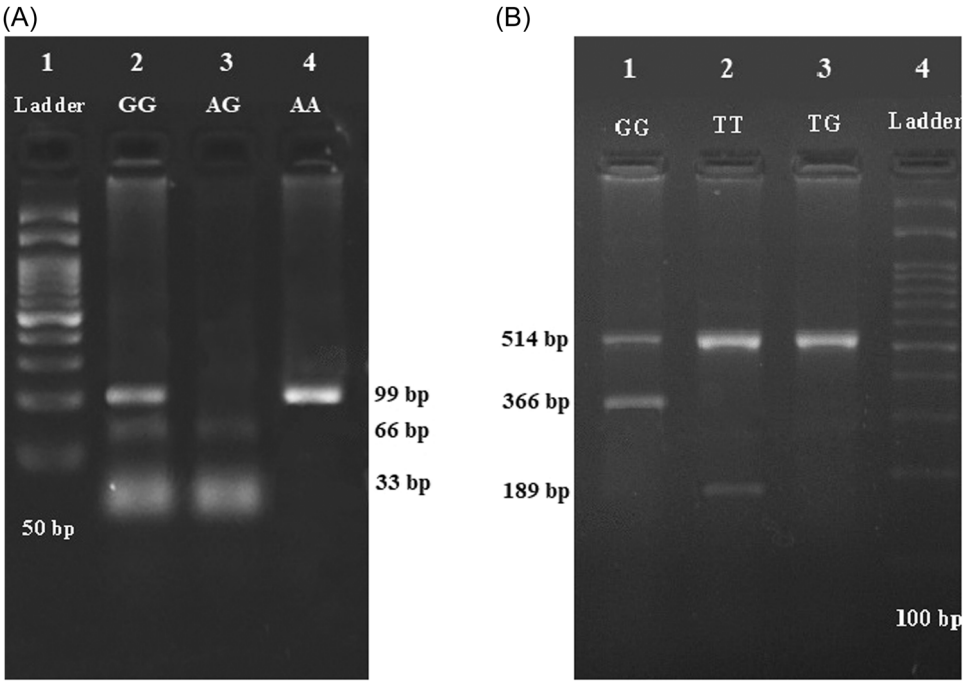

FIGURE 1 The 2% agarose gel showing (A) paraoxonase 1 (PON1) rs662 A/G genotypes (Lane 1: 50 bp ladder, Lane 2: GG genotype, Lane 3: AG genotype, and Lane 4: AA genotype), and (**B**) SOD2 rs4880 G/T genotypes (Lane 1: GG genotype, Lane 2: TT genotype, Lane 3: TG genotype, and Lane 4: 100 bp ladder).

95°C for 6 min (hold cycle), 35 cycles of denaturation at 95°C for 30 s, annealing of 60°C (rs4880), and 65°C (rs662) for 45 s, and extension at 72°C for 30 s. The last extension phase lasted 10 min at 72°C. For rs662, the products were digested by AlwI restriction enzyme (Thermo Fisher Scientific) at 37°C for 24 h. The PCR product (for rs4880) and digested fragments (for rs662) were electrophoretically separated on 2.5% (w/v) agarose gel and then visualized by Safe Stain (Yekta Tajhiz Azma) under UV light using a gel documentation system (Bio-Rad). A representative image of PCR products is shown in Figure 1A,B.

The ABI Step One Plus (Applied Biosystems) real-time PCR equipment was used to genotype rs2891168 SNP. Amplification was performed according to the manufacturer's instructions, with the steps outlined: 95°C for 10 min (hold cycle); 40 cycles of denaturation at 95°C for 15 s followed by annealing/extension at 60°C for 60 s. The TaqMan Genotyper Software v1.6 (Thermo Fisher Scientific) was used for genotype analysis. showing an allelic discrimination plot for rs2891168 polymorphism.

2.3 | Statistical analysis

The data were analyzed with IBM SPSS 20.0 software (SPSS Inc.). Data were assessed for normality using Kolmogorov–Smirnov test. Quantitative data were expressed as mean ± standard deviation (mean ± SD) and categorical variables were expressed as frequency. The Student's *t*-test or the Mann–Whitney test was used to compare quantitative variables. The χ^2 test or Fisher's exact test was used to compare categorical variables. Hardy–Weinberg equilibrium was tested for rs4880, rs662, and rs2891168 genetic variants using the χ^2 test. Based on the number of copies required to modify the risk, four genetic models such as codominant, dominant, recessive, and log-additive were used to calculate the allele/genotype frequencies and odds ratio (OR) at a 95% confidence interval (95% CI) by using SNPstats online tool (http://bioinfo.iconcologia.net/SNPstats). Univariate logistic regression analysis was used to get the adjusted odds ratio (aOR) for the connection between illness and risk factors. For statistical significance, the threshold of significance two-sided *p*-value < 0.05 was used. *p*-Values for multiple testing were adjusted using the Bonferroni adjustment for the number of tests.

3 | RESULTS

3.1 | Clinical profiles of study participants

Demographic and clinical features of the participants of each group are presented in Table 2. There was no notable difference between the four groups in terms of age, sex, and smoking status (*p*-value < 0.05). A significant difference between groups was observed in terms of BMI, FBS, and serum creatinine level (*p*-value < 0.05), but no significant difference was observed in terms of urea, sodium, potassium, TC, TG, HDL-cholesterol, LDL-cholesterol, and VLDL-cholesterol (*p*-value > 0.05).

TABLE 2 Baseline demographic and clinical characteristics of four groups (no-T2DM/no-CAD, T2DM/no-CAD, no-T2DM/CAD, and T2DM/CAD).

Characteristics		no-T2DM/no-CAD (n = 90)	T2DM/no-CAD (n = 90)	no-T2DM/CAD (n = 90)	T2DM/CAD (n = 90)	p-Value
Age (years) (Mean ± SD)		56.64 ± 10.92	56.87 ± 9.54	58.35 ± 10.08	57.53 ± 9.66	0.573
Sex n (%)	Male	46 (51.1)	48 (53.3)	50 (55.5)	52 (57.8)	0.826
	Female	44 (48.9)	42 (46.7)	40 (44.5)	38 (42.2)	
BMI (kg/m^2) (Mean ± SD)		24.15 ± 4.22	24.35 ± 4.49	25.13 ± 4.62	26.89 ± 5.37	**0.008**
Smoking status	Yes	19	21	28	25	0.419
	No	71	69	62	65	
HbA1c		13.23 ± 2.53	13.29 ± 3.04	13.63 ± 2.94	13.97 ± 3.22	0.214
S. creatinine (mg/dL)		0.99 ± 0.23	0.98 ± 0.19	1.11 ± 0.21	1.08 ± 0.18	**0.018**
Urea (mg/dL)		34.12 ± 6.92	35.02 ± 10.04	35.98 ± 10.29	36.77 ± 10.74	0.512
Sodium (mg/dL)		139.0 ± 3.22	139.5 ± 3.14	140.12 ± 2.91	139.40 ± 2.93	0.121
Potassium (mg/dL)		4.31 ± 0.42	4.34 ± 0.41	4.51 ± 0.45	4.27 ± 0.37	0.134
FBS (mg/dL)		89.65 ± 12.58	102.22 ± 10.02	94.65 ± 12.66	99.93 ± 10.30	**<0.001**
TC (mg/dL)		141.18 ± 41.63	141.95 ± 31.9	144.98 ± 43.13	137.90 ± 38.84	0.712
TG (mg/dL)		104.51 ± 35.7	119.11 ± 58.8	109.23 ± 46.74	114.84 ± 67.46	0.456
HDL-C (mg/dL)		40.62 ± 9.5	38.26 ± 9.29	39.05 ± 12.24	37.50 ± 12.42	0.304
LDL-C (mg/dL)		79.01 ± 31.0	86.44 ± 37.5	83.75 ± 35.37	77.48 ± 32.84	0.634
VLDL-C (mg/dL)		20.92 ± 6.43	23.82 ± 7.22	21.84 ± 6.12	23.02 ± 8.25	0.407

Note: Categorical variables are expressed as number (percentage) of subjects and continuous variables are presented as mean ± SD. Bold p-values are statistically significant (p-value < 0.05).

Abbreviations: BMI, body mass index; CAD, coronary artery disease; FBS, fasting blood sugar; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; N, number; SD, standard deviation; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TG, triglycerides; VLDL-C, very low-density lipoprotein cholesterol.

3.2 | Allele and genotype frequencies distribution between four groups

Table 3 presented the details of allele and genotype frequencies of the three polymorphisms (*CDKN2B-AS* rs2891168 A > G, *SOD2* rs4880 T > C, and *PON1* rs662 A > G) in the four studied groups. Regarding genotypic distribution, all of the study groups (no-T2DM/no-CAD, T2DM/no-CAD, no-T2DM/CAD, and T2DM/CAD), were consistent with expectations under HWE for the three polymorphisms (Table 4).

Compared with no-T2DM/no-CAD group, *CDKN2B-AS* rs2891168 A > G exhibited significant differences in the homozygous (GG), heterozygous (AG) and dominant models in the no-T2DM/CAD patients, with the highest odds ratio in the dominant model (AOR = 2.38, 95% CI: 1.22–4.68, p-value = 0.014; Table 5). We also found significant differences in the homozygous (GG) and dominant models in the T2DM/CAD group compared with no-T2DM/no-CAD group (AOR = 2.43, 95% CI: 1.03–5.79, p-value = 0.047 and AOR = 2.07 95% CI: 1.09–3.89 p-value = 0.032, respectively); however, these values were not significant after Bonferroni correction (p-value < 0.016). The frequency of the G-allele was markedly higher than the A-allele in the no-T2DM/CAD and T2DM/CAD groups compared to the no-T2DM/no-CAD group (p-value < 0.05). Although, no statistical association was observed between the no-T2DM/no-CAD and T2DM/no-CAD groups. Even after Bonferroni correction (p-value < 0.016), the associations in the G-allele and dominant model were significant. For the *SOD2* rs4880 T > C, the frequency of the C-allele was not noticeably different in comparison with the T-allele between groups (p-value > 0.05). Similarly, there was no notable difference in the genotypes or genetic models between the four groups (p-value > 0.05). Therefore, the rs4880 polymorphism was not associated with the risk of CAD and/or T2DM. In the case of *PON1* rs662 A > G, the G-allele was considered a risk allele in T2DM/no-CAD and T2DM/CAD groups compared with no-T2DM/no-CAD group (p-value = 0.008 and < 0.001, respectively). Moreover, an increased risk of rs662 polymorphism was found in both T2DM/no-CAD and T2DM/CAD groups before and after

adjustment in homozygous, heterozygous, recessive, and dominant models (p-value < 0.05). After Bonferroni adjustment, the correlations for G-allele, homozygous, and dominant models remained significant in the T2DM/no-CAD group (p-value < 0.016); and in the T2DM/CAD group, the associations remained significant for G-allele, homozygous, recessive and dominant models (p-value < 0.016).

TABLE 3 The association of CDKN2B-AS1 (rs2891168), SOD2 (rs4880), and PON1 (rs662) selected genetic variants with risk of CAD.

Genotypes/ alleles	no-T2DM/ no-CAD (n = 90)	T2DM/ no-CAD (n = 90)	no-T2DM/ CAD (n = 90)	T2DM/ CAD (n = 90)
CDKN2B-AS1 (rs2891168)				
AA	34	31	18	20
AG	41	40	48	48
GG	15	19	24	22
A	109	102	84	88
G	71	78	96	92
SOD2 (rs4880)				
TT	28	25	22	24
TC	44	45	50	49
CC	18	20	18	17
T	100	95	94	97
C	80	85	86	83
PON1 (rs662)				
AA (QQ)	46	28	36	25
AG (QR)	36	42	40	43
GG (RR)	8	20	14	22
A (Q)	128	98	112	93
G (R)	52	82	68	87

Abbreviations: CAD, coronary artery disease; N, number of subjects; Rs, reference SNP; T2DM, type 2 diabetes mellitus.

4 | DISCUSSION

Today, heart disease is currently the main cause of mortality in the world and approximately takes 17.9 million lives each year.[22] There are several types of heart disease such as Arrhythmia, Atherosclerosis, Cardiomyopathy, Congenital heart defects, heart infections, and CAD, and different terms are used for them.[23] CAD is the situation in which plaque buildup in the coronary arteries and it's sometimes known as ischemic heart disease.[4] In the past few years, many studies have been done to understand the physiological mechanism of CAD development. Environment and genetics play an important role in the contraction of cardiovascular disease. The understanding of disease-causing factors allows us to better prognosis and determine the treatment strategies of patients in the future. The conventional risk factors for CAD are high LDL cholesterol, low HDL cholesterol, high blood pressure, first-degree family history, smoking, diabetes mellitus (DM), and so on. CAD is one of the long-term complications of DM and it seems for many reasons, including metabolic factors such as hyperglycemia, dyslipidemia, and insulin resistance, which leads to abnormal function of endothelial cells, vascular smooth muscle, impaired platelet function, and abnormal coagulation.[24] It is well-known stress oxidative and enzymes involved in its pathways such as SOD2 and PON1 contribute to endothelial dysfunction and the development of cardiovascular disease. PON1 acts as an antioxidant factor, it's associated with HDL that metabolizes organophosphates and prevents the onset of coronary artery disease. Also, SOD2 protects cells by catalytically scavenging harmful superoxide radicals.[25,26] Moreover, clinical and experimental studies indicate that the oxidative stress pathway is associated with susceptibility to T2DM, insulin resistance, impaired glucose tolerance, and related complications through decreased activity of antioxidant enzymes.

Until now, several biological pathways have been identified and well-understood that are related to the development of DM and CAD.[27,28] GWAS and case-control studies have found more than 200 genetic loci that are associated with DM and/or CAD susceptibility in humans.[7,29] However, causal genes, variants, and molecular pathways have not been wholly identified for most loci. Polymorphisms are the most common genetic variants that repeat in more than 1% of the

TABLE 4 Characteristics of selected polymorphisms and HWE of each group.

NCBI rs#	Chr. position[a]	Gene	Location	Major/ minor allele	1000 Genome MAF frequency	p-Value (HWE)[b]			
						No-T2DM/ no-CAD	T2DM/ no-CAD	no-T2DM/CAD	T2DM/ CAD
rs2891168	chr9:22098620	CDKN2B-AS1	Intronic	A/G	0.40	0.659	0.367	0.498	0.672
rs4880	chr6:159692840	SOD2	Exon 2	T/C	0.41	0.924	0.976	0.282	0.364
rs662	chr7:95308134	PON1	Exon 6	G/A	0.46	0.802	0.574	0.604	0.680

Abbreviations: Chr, chromosome; HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; Rs, reference SNP.

[a]Chromosomal position data was based on NCBI genome build 38.p12.

[b]The p-value for HWE was calculated from the genotype data in our cases and controls. p < 0.05 is not consistent with HWE.

TABLE 5 The association of CDKN2B-AS1, SOD2, and PON1 selected genetic variants and the risk of T2DM and CAD.

Genotypes/ genetic models/alleles	T2DM/no-CAD				no-T2DM/CAD				T2DM/CAD			
	p-Value	COR (95% CI)	*p*-Value[a]	AOR (95% CI)[a]	*p*-Value	COR (95% CI)	*p*-Value[a]	AOR (95% CI)[a]	*p*-Value	COR (95% CI)	*p*-Value[a]	AOR (95% CI)[a]
CDKN2B-AS1 (rs2891168)												
AA	-	Reference	-	Reference	-	Reference	-	Reference	-	Reference	-	Reference
AG	0.839	1.07 (0.55–2.05)	0.847	1.06 (0.53–2.03)	**0.027**	2.21 (1.09–4.48)	**0.033**	2.19 (1.08–4.45)	0.051	1.99 (0.99–3.97)	0.062	1.92 (0.98–3.88)
GG	0.439	1.39 (0.60–3.19)	0.444	1.37 (0.58–3.22)	**0.011**	3.02 (1.27–5.15)	**0.015**	2.99 (1.25–5.11)	**0.036**	2.49 (1.05–5.87)	**0.047**	2.43 (1.03–5.79)
Recessive	0.447	1.33 (0.63–2.83)	0.454	1.30 (0.59–2.78)	0.106	1.81 (0.88–3.75)	0.111	1.78 (0.84–3.71)	0.199	1.61 (0.77–3.36)	0.210	1.56 (0.72–3.31)
Dominant	0.641	1.15 (0.62–2.12)	0.651	1.17 (0.65–2.09)	**0.009**	2.42 (1.24–4.74)	**0.014**	2.38 (1.22–4.68)	**0.024**	2.12 (1.10–4.08)	**0.032**	2.07 (1.09–3.89)
Overdominant	0.880	0.95 (0.53–1.72)	0.773	0.93 (0.51–1.69)	0.297	1.36 (0.76–2.45)	0.305	1.33 (0.73–2.41)	0.297	1.36 (0.76–2.45)	0.286	1.41 (0.79–2.51)
A	-	Reference	-	Reference	-	Reference	-	Reference	-	Reference	-	Reference
G	0.454	1.17 (0.77–1.78)	0.460	1.15 (0.74–1.74)	**0.008**	1.75 (1.15–2.66)	**0.014**	1.72 (1.14–2.61)	**0.026**	1.60 (1.05–2.43)	**0.035**	1.53 (1.04–2.38)
SOD2 (rs4880)												
TT	-	Reference	-	Reference	-	Reference	-	Reference	-	Reference	-	Reference
TC	0.695	1.14 (0.58–2.26)	0.709	1.12 (0.55–2.21)	0.294	1.44 (0.72–2.88)	0.308	1.39 (0.70–2.82)	0.450	1.29 (0.65–2.56)	0.463	1.22 (0.59–2.49)
CC	0.607	1.24 (0.54–2.86)	0.597	1.27 (0.58–2.91)	0.582	1.27 (0.53–3.00)	0.572	1.32 (0.56–3.11)	0.824	1.10 (0.46–2.59)	0.833	1.08 (0.47–2.43)
Recessive	0.715	1.14 (0.55–2.34)	0.694	1.16 (0.57–2.29)	1.00	1.00 (0.48–2.07)	>0.99	0.99 (0.47–2.01)	0.850	0.93 (0.44–1.94)	0.862	0.95 (0.46–1.88)
Dominant	0.623	1.17 (0.62–2.23)	0.633	1.14 (0.65–2.25)	0.319	1.39 (0.72–2.68)	0.327	1.34 (0.69–2.59)	0.511	1.24 (0.69–2.24)	0.519	1.27 (0.63–2.32)
Overdominant	0.881	1.04 (0.58–1.87)	0.872	1.05 (0.56–1.93)	0.371	1.30 (0.72–2.34)	0.359	1.37 (0.76–2.28)	0.456	1.25 (0.69–2.24)	0.468	1.30 (0.73–2.31)
T	-	Reference	-	Reference	-	Reference	-	Reference	-	Reference	-	Reference
C	0.596	1.12 (0.73–1.69)	0.605	1.10 (0.70–1.64)	0.525	1.14 (0.75–1.73)	0.534	1.12 (0.72–1.67)	0.750	1.06 (0.70–1.62)	0.761	1.05 (0.68–1.53)
PON1 (rs662)												
AA	-	Reference	-	Reference	-	Reference	-	Reference	-	Reference	-	Reference
AG	**0.038**	1.91 (1.00–3.66)	**0.045**	1.80 (1.03–3.52)	0.273	1.42 (0.75–2.65)	0.281	1.38 (0.72–2.53)	**0.019**	2.19 (1.13–4.24)	**0.027**	2.11 (1.10–3.98)
GG	**0.003**	4.10 (1.59–9.53)	**0.009**	3.95 (1.52–9.23)	0.107	2.23 (0.84–5.91)	0.111	2.20 (0.79–5.78)	**<0.001**	5.06 (1.96–13.01)	**0.001**	4.87 (1.82–12.56)
Recessive	**0.016**	2.92 (1.21–7.07)	**0.023**	2.83 (1.18–6.78)	0.177	1.88 (0.75–4.75)	0.183	1.81 (0.69–4.54)	**0.006**	3.36 (1.41–8.04)	**0.011**	3.23 (1.28–7.82)
Dominant	**0.006**	2.31 (1.26–4.25)	**0.012**	2.23 (1.19–3.89)	0.135	1.56 (0.86–2.83)	0.128	1.61 (0.82–2.71)	**0.001**	2.71 (1.46–5.04)	**0.008**	2.60 (1.38–4.92)
Overdominant	0.367	1.31 (0.72–2.37)	0.354	1.42 (0.65–2.44)	0.546	1.20 (0.66–2.16)	0.531	1.29 (0.75–2.30)	0.293	1.37 (0.76–2.47)	0.302	1.26 (0.67–2.35)

(Continues)

TABLE 5 (Continued)

Genotypes/genetic models/alleles	T2DM/no-CAD				no-T2DM/CAD				T2DM/CAD			
	p-Value	COR (95% CI)	*p*-Value[a]	AOR (95% CI)[a]	*p*-Value	COR (95% CI)	*p*-Value[a]	AOR (95% CI)[a]	*p*-Value	COR (95% CI)	*p*-Value[a]	AOR (95% CI)[a]
A	-	Reference	-	Reference	-	Reference	-	Reference	-	Reference	-	Reference
G	**0.001**	2.05 (1.33–3.18)	**0.008**	1.96 (1.28–3.08)	0.075	1.49 (0.96–2.32)	0.083	1.42 (0.97–2.42)	**<0.001**	2.30 (1.49–3.55)	**<0.001**	2.22 (1.41–3.47)

Note: Bold *p*-values are statistically significant ($p < 0.05$).

Abbreviations: AOR (95% CI), adjusted odds ratio (95% confidence interval); CAD, coronary artery disease; COR (95% CI), crude odds ratio (95% confidence interval); Rs, reference SNP; T2DM, type 2 diabetes mellitus.

[a]AOR (95% CI) and *p*-values were obtained from multivariate analyses after adjusting for age, sex, BMI, smoking, hyperlipidemia, TC, LDL-C, and HDL-C.

population and are related to multifactorial diseases like T2DM and CAD. Here, we investigated the prevalence of three independent polymorphisms in four separate groups. To our knowledge, there is no previous study on the relationship between polymorphisms *CDKN2B-AS*, *SOD2*, and *PON1* and the risk of T2DM/CAD in the Iranian population.

In this study, we found that rs2891168 is significantly associated with CAD. We used TaqMan allelic discrimination which is one of the most developed genotyping techniques to detect genotypes. Several previous GWASs confirmed that rs2891168 is associated with CAD.[7,30] The rs2891168 SNP on chromosome 9p21.3 is found in the intronic sequence of the CDKN2B-AS gene, which is thought to be a susceptibility locus for the pathogenesis of CAD. Though the function of CDKN2BAS is not completely known, its level of expression has been associated with CAD and it seems allelic modification, changes CDKN2BAS production in the blood and finally increases the risk for CAD development.[31–33] As mentioned in the result, rs2891168 was significantly associated with CAD but we couldn't find any association between rs2891168 and T2DM. These findings were consistent with previous reports.[8] After comparing no-T2DM/no-CAD and T2DM/no-CAD groups, the distribution of G-allele was relatively similar in both groups and our result might happen because of the distinct process of causing T2DM.

In the case of *PON1* rs662 A > G, the GG, and AG genotypes were more frequent among the T2DM/no-CAD and T2DM/CAD groups compared to no-T2DM/no-CAD group, showing the G-allele may be a risk factor for T2DM susceptibility. As a result, we assume that rs662 polymorphism is not a risk factor for CAD even though it may result in a T2DM predisposing factor. The role of paraoxonase1 (PON1) in the inhibition of atherosclerosis is remarkable. Oxidative stress and inflammation convert LDL to oxidized low-density lipoprotein (ox-LDL) and it can be internalized by macrophages. PON1 hydrolyses ox-LDL to LDL and promotes cholesterol efflux from macrophage, and through this pathway inhibits atherosclerosis. According to previous studies, it seems the G allele is less effective than the A allele in oxidation and it can be considered as a risk factor for CAD.[34,35] Randa et al.[36] reported that individuals with the GG genotype show 9-fold risks of developing CAD as compared to an individual with an AG genotype that demonstrates four-fold risks. Studies show a contradictory result about the association of rs662 and CAD, but it might be associated due to structural change in protein and subsequently altered activity of PON1.[37] Based on our study, unlike rs2891168, the allelic distribution of rs662 was notably different in the T2DM/no-CAD group compared to no-T2DM/no-CAD group. It supports previous studies that rs662 reduces PON1 activity and concentration which leads to glucose tolerance and insulin resistance in T2DM patients.[38–40] It is supposed that OS is induced by decreased PON1 concentration and/or activity, which in turn causes muscle cells to take in less blood glucose and become insulin-resistant.

For the SOD2 rs4880 T > C, the frequency of the C-allele was not statistically different in comparison with the T-allele between groups. Similarly, we did not find a remarkable difference between

the genotypes or genetic models in the four groups. According to our study, it may be that rs4880 doesn't have any association with CAD risk, although several previous studies showed that *SOD2* polymorphism can increase CAD risk by stress-related lipid abnormalities. *SOD2* rs4880 T > C, substitute valine with alanine at position 16 of signal peptide and subsequently lead to more efficient import of SOD2 into the mitochondrial matrix.[41] In 2008, Fujimoto et al. showed that the alanine variant could increase the SOD2 ability to neutralize superoxide radicals by its increased activity.[42] It seems *SOD2* polymorphism is not directly related to coronary atherosclerosis, although more study is needed to confirm this hypothesis.

We faced some limitations in this study. First, different ethnic groups live in Iran, and each of them has a variety of allele frequencies, but we just could only reach individuals from southeast of Iran and it probably affected the results. Second, our study only shows an association between three effective SNPs of CDKN2B-AS, SOD2, and PON1 genes in T2DM and CAD development, not a causal relationship. Additionally, we couldn't investigate the influence of these SNPs on gene expression or function in this study. Third and last, our study design does not consider the presence of other mutations, polymorphisms, and the action of conventional risk factors that could influence the TD2M and/or CAD development. CAD is a multifactorial disease that is influenced by both genetic and environmental factors. Genetic association studies like this create potential genetic knowledge about the processes and pathways involved in CAD. Gene expression is affected by one's genetic background, interaction with other genes, and environmental factors. It sounds like further studies are recommended in this field to discover the secret of multifactorial disease pathophysiology like CAD.

5 | CONCLUSIONS

This study investigates the association between rs2891168, rs4880, and rs662 gene polymorphisms and the prevalence of T2DM and CAD in an Iranian population for the first time and suggests that genetic polymorphisms in the 9p21.3 locus and *PON1* gene may have a relationship with the risk of CAD and T2DM, respectively. Although the results of the present study suggest that polymorphisms in the 9p21.3 locus and *PON1* gene could be prognostic factors useful for CAD and T2DM prevention, further epidemiologic studies involving a larger cohort of subjects and functional studies should be performed to validate and expand these findings.

AUTHOR CONTRIBUTIONS

Abolfazl Yari: Investigation; methodology; software; writing—original draft. **Zahra M. Karam**: Data curation; investigation; methodology; writing—original draft. **Seyed M. E. Meybodi**: Formal analysis; investigation; writing—original draft. **Marzieh L. Sargazi**: Formal analysis; validation; writing—original draft. **Kolsoum Saeidi**: Funding acquisition; project administration.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available because of privacy or ethical restrictions.

TRANSPARENCY STATEMENT

The lead author Kolsoum Saeidi affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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REFERENCES

1. Cho NH, Shaw JE, Karuranga S, et al. IDF diabetes Atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract*. 2018;138:271-281.
2. Bandarian F, Omidvar M, Razi F, et al. Iran diabetes research roadmap (IDRR) study; knowledge gap in genetic research on diabetes mellitus in Iran: a review article. *Iran J Public Health*. 2017;46:53-59.
3. Khera AV, Kathiresan S. Genetics of coronary artery disease: discovery, biology and clinical translation. *Nat Rev Genet*. 2017;18:331-344.
4. Goodarzi MO, Rotter JI. Genetics insights in the relationship between type 2 diabetes and coronary heart disease. *Circ Res*. 2020;126(11):1526-1548.
5. Bae J, Kim IJ, Hong SH, et al. Association of endothelial nitric oxide synthase polymorphisms with coronary artery disease in Korean individuals with or without diabetes mellitus. *Exp Ther Med*. 2010;1(4):719-724.
6. Kojima Y, Downing K, Kundu R, et al. Cyclin-dependent kinase inhibitor 2B regulates efferocytosis and atherosclerosis. *J Clin Invest*. 2014;124(3):1083-1097.
7. Nikpay M, Goel A, Won HH, et al A comprehensive 1000 genomes-based genome-wide association meta-analysis of coronary artery disease. *Nat Genet*. 2015;47(10):1121-1130.
8. Gori F, Specchia C, Pietri S, et al. Common genetic variants on chromosome 9p21 are associated with myocardial infarction and type 2 diabetes in an Italian population. *BMC Med Genet*. 2010;11(1):60.
9. Al-Ama M, Ahmed A, Awan ZA, et al. The CDKN2BAS polymorphism rs2891168 is associated with increased risk of myocardial infarction in a Saudi Arabian population. *Middle East J Med Genet*. 2018;7(1):39-45.

10. Moasser E, Kazemi-Nezhad SR, Saadat M, Azarpira N. Study of the association between glutathione S-transferase (GSTM1, GSTT1, GSTP1) polymorphisms with type II diabetes mellitus in Southern of Iran. *Mol Biol Rep*. 2012;39(12):10187-10192.
11. Andalib S, Vaseghi G, Motavallian A, et al. Association of polymorphism of ser311cys paraoxonase-2 gene with type 2 diabetes mellitus in Iran. *Int J Prev Med*. 2013;4(5):517-522.
12. Pourkeramati A, Mehrjardi EZ, Tezerjani MD, Seifati SM. Association of GSTP1, GSTT1 and GSTM1 gene variants with coronary artery disease in Iranian population: a case-control study. *Int J Gen Med*. 2020;13:249-259.
13. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid. *Alexand J Med*. 2018;54(4):287-293.
14. Decharatchakul N, Settasatian C, Settasatian N, et al. Association of genetic polymorphisms in SOD2, SOD3, GPX3, and GSTT1 with hypertriglyceridemia and low HDL-C level in subjects with high risk of coronary artery disease. *PeerJ*. 2019;2019(8):e7407. doi:10.7717/peerj.7407
15. Gupta N, Binu KBK, Singh S, et al. Low serum PON1 activity: an independent risk factor for coronary artery disease in North-West Indian type 2 diabetics. *Gene*. 2012;498(1):13-19.
16. Decharatchakul N, Settasatian C, Settasatian N, et al. Association of combined genetic variations in SOD3, GPX3, PON1, and GSTT1 with hypertension and severity of coronary artery disease. *Heart Vessels*. 2020;35(7):918-929.
17. Wamique M, Ali W, Himanshu D. Association of SRB1 and PON1 gene polymorphisms with type 2 diabetes mellitus: a case control study. *Int J Diabetes Dev Ctries*. 2020;40(2):209-215.
18. Godbole C, Thaker S, Kerkar P, Nadkar M, Gogtay N, Thatte U. Association of PON1 gene polymorphisms and enzymatic activity with risk of coronary artery disease. *Fut Cardiol*. 2021;17(1):119-126.
19. Lahiri JNJ DK. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res*. 1991;11(19):5444.
20. Ruiz-Sanz JI, Aurrekoetxea I, Matorras R, Ruiz-Larrea MB. Ala16Val SOD2 polymorphism is associated with higher pregnancy rates in in vitro fertilization cycles. *Fertil Steril*. 2011;95(5):1601-1605.
21. Hassan MA, Al-Attas OS, Hussain T, et al. The Q192R polymorphism of the paraoxonase 1 gene is a risk factor for coronary artery disease in Saudi subjects. *Mol Cell Biochem*. 2013;380(1-2):121-128.
22. Mensah GA, Roth GA, Fuster V. The global burden of cardiovascular diseases and risk factors. *J Am Coll Cardiol*. 2019;74:2529-2532.
23. Mackay J, Mensah G. *The Atlas of Heart Disease and Stroke*. 2004. https://books.google.com/books?hl=en&lr=&id=JagK-qIWaZoC&oi=fnd&pg=PA13&ots=2nUWbXKRXv&sig=HyvBaHt7fBZ1S2vCQB9AujIMoYs
24. Al-Nozha MM, Ismail HM, Al Nozha OM. Coronary artery disease and diabetes mellitus. *J Taibah Univ Med Sci*. 2016;11:330-338.
25. Pytel E, Olszewska-Banaszczyk M, Koter-Michalak M, Broncel M. Increased oxidative stress and decreased membrane fluidity in erythrocytes of CAD patients. *Biochem Cell Biol*. 2013;91(5):315-318.
26. Peng JR, Lu TT, Chang HT, Ge X, Huang B, Li WM. Elevated levels of plasma superoxide dismutases 1 and 2 in patients with coronary artery disease. *BioMed Res Int*. 2016;2016:1-9.
27. Cole JB, Florez JC. Genetics of diabetes mellitus and diabetes complications. *Nat Rev Nephrol*. 2020;16:377-390.
28. Zheng Q, Ma Y, Chen S, Che Q, Chen D. The integrated landscape of biological candidate causal genes in coronary artery disease. *Front Genet*. 2020;11:320.
29. Xue A, Wu Y, Zhu Z, et al. Genome-wide association analyses identify 143 risk variants and putative regulatory mechanisms for type 2 diabetes. *Nat Commun*. 2018;9(1):2941.
30. Broadbent HM, Peden JF, Lorkowski S, et al. Susceptibility to coronary artery disease and diabetes is encoded by distinct, tightly linked SNPs in the ANRIL locus on chromosome 9p. *Hum Mol Gen*. 2008;17(6):806-814.
31. Jarinova O, Stewart AFR, Roberts R, et al. Functional analysis of the chromosome 9p21.3 coronary artery disease risk locus. *Arterioscler Thromb Vasc Biol*. 2009;29(10):1671-1677.
32. Holdt LM, Beutner F, Scholz M, et al. ANRIL expression is associated with atherosclerosis risk at chromosome 9p21. *Arterioscler Thromb Vasc Biol*. 2010;30(3):620-627.
33. Harismendy O, Notani D, Song X, et al. 9p21 DNA variants associated with coronary artery disease impair interferon-γ signalling response. *Nature*. 2011;470(7333):264-268.
34. Regieli JJ, Jukema JW, Doevendans PA, et al. Paraoxonase variants relate to 10-year risk in coronary artery disease. *J Am Coll Cardiol*. 2009;54(14):1238-1245.
35. MacKness B, MacKness MI, Arrol S, Turkie W, Durrington PN. Effect of the human serum paraoxonase 55 and 192 genetic polymorphisms on the protection by high density lipoprotein against low density lipoprotein oxidative modification. *FEBS Lett*. 1998;423(1):57-60.
36. Mohamed RH, Mohamed RH, Karam RA, Abd El-Aziz TA. The relationship between paraoxonase1-192 polymorphism and activity with coronary artery disease. *Clin Biochem*. 2010;43(6):553-558.
37. Zhang Z, Ou J, Cai P, et al. Association between the PON1 Q192R polymorphism and coronary heart disease in Chinese. *Med*. 2018;97(26):e11151. doi:10.1097/MD.0000000000011151
38. El-Lebedy D, Kafoury M, Abd-El Haleem D, Ibrahim A, Awadallah E, Ashmawy I. Paraoxonase-1 gene Q192R and L55M polymorphisms and risk of cardiovascular disease in Egyptian patients with type 2 diabetes mellitus. *J Diab Metab Disord*. 2014;13(1):124.
39. Garin M, James R, Garin MC, et al. Paraoxonase polymorphism Met-Leu54 is associated with modified serum concentrations of the enzyme. A possible link between the paraoxonase gene and increased risk of cardiovascular dise. *Am Soc Clin Investig*. 1997;92(1):62-66.
40. Rejeb J, Omezzine A, Rebhi L, et al. Association of PON1 and PON2 polymorphisms with pon1 activity and significant coronary stenosis in a Tunisian population. *Biochem Genet*. 2013;51(1-2):76-91.
41. Sutton A, Khoury H, Prip-Buus C, Cepanec C, Pessayre D, Degoul F. The Ala16Val genetic dimorphism modulates the import of human manganese superoxide dismutase into rat liver mitochondria. *Pharmacogenetics*. 2003;13(3):145-157.
42. Fujimoto H, Taguchi J, Imai Y, et al. Manganese superoxide dismutase polymorphism affects the oxidized low-density lipoprotein-induced apoptosis of macrophages and coronary artery disease. *Eur Heart J*. 2008;29(10):1267-1274.

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