Genetic Determinants of Cardiovascular Disease: The Endothelial Nitric Oxide Synthase 3 (eNOS3), Krüppel-Like Factor-14 (KLF-14), Methylenetetrahydrofolate Reductase (MTHFR), MiRNAs27a and Their Association with the Predisposition and Susceptibility to Coronary Artery Disease

Rashid Mir 1,* , Imadeldin Elfaki 2,* , Jamsheed Javid 1 , Jameel Barnawi 1 , Malik A. Altayar 1,*, Salem Owaid Albalawi 3, Mohammed M. Jalal 1,*, Faris J. Tayeb 1,*, Aadil Yousif 1, Mohammad Fahad Ullah 1,*, and Faisel M. AbuDuhier 1,*. 

1 Prince Fahd Bin Sultan Research Chair, Department of Medical Lab Technology, Faculty of Applied Medical Sciences, University of Tabuk, Tabuk 71491, Saudi Arabia
2 Department of Biochemistry, Faculty of Science, University of Tabuk, Tabuk 71491, Saudi Arabia
3 Department of Cardiology, King Fahd Specialist Hospital, Tabuk 71491, Saudi Arabia
* Correspondence: rashidmirtabuk@gmail.com (R.M.); elfakiimadeldin@gmail.com (I.E.)

Abstract: Coronary artery disease (CAD) is an important cause of death worldwide. CAD is caused by genetic and other factors including hypertension, hyperlipidemia, obesity, stress, unhealthy diet, physical inactivity, smoking and Type 2 diabetes (T2D). The genome wide association studies (GWASs) have revealed the association of many loci with risk to diseases such as cancers, T2D and CAD. Nitric oxide (NO) is a potent vasodilator and is required for normal vascular health. It is produced in the endothelial cells in a reaction catalyzed by the endothelial NO synthase (eNOS). Methylenetetrahydrofolate reductase (MTHFR) is a very important enzyme involved in metabolism of folate and homocysteine, and its reduced function leads to cardiovascular disease. The Krüppel-like factor-14 (KLF-14) is an important transcriptional regulator that has been implicated in metabolic syndrome. MicroRNA (MiRNAs) are short non-coding RNAs that regulate the gene expression of proteins involved in important physiological processes including cell cycle and metabolism. In the present study, we have investigated the potential impact of germline pathogenic variants of endothelial eNOS, KLF-14, MTHFR, MiRNA-27a and their association with risk to CAD in the Saudi population. Methods: Amplification Refractory Mutation System (ARMS) PCR was used to detect MTHFR, KLF-14, miRNA-27a and eNOS3 genotyping in CAD patients and healthy controls. About 125 CAD cases and 125 controls were enrolled in this study and statistical associations were calculated including p-value, risk ratio (RR), and odds ratio (OD). Results: There were statistically significant differences (p < 0.05) in genotype distributions of MTHFR 677 C>T, KLF-14 rs972283 G>A, miRNA27a rs895819 A>G and eNOS3 rs1799983 G>T between CAD patients and controls. In addition, our results indicated that the MTHFR-TT genotype was associated with increased CAD susceptibility with an OR 2.75 (95%) and p < 0.024. Moreover, the miRNAs27a-GG genotype protects from CAD risk with an OR = 0.31 (0.016), p = 0.016. Our results also indicated that eNOS3 -GT genotype is associated with CAD susceptibility with an OR = 2.65, and p < 0.003. Conclusion: The MTHFR 677C>T, KLF14 rs972283 G>A, miRNAs27a A>G, and eNOS3 rs1799983 G>T genotypes were associated with CAD susceptibility (p < 0.05). These findings require verification in future large-scale population based studies before these loci are used for the prediction and identification of individuals at risk to CAD. Weight control, physical activity, and smoking cessation are very influential recommendations given by clinicians to the at risk individuals to reduce or delay the development of CAD.
Keywords: coronary artery disease (CAD); endothelial nitric oxide synthase 3 (eNOS); Krüppel-like factor-14 (KLF-14); methylenetetrahydrofolate reductase (MTHFR); MicroRNA; tetra-primer amplification refractory mutation system (T-ARMS); Hardy–Weinberg disequilibrium (HWD)

1. Introduction

Cardiovascular disease (CVD) is a very important cause of morbidity and death worldwide including the kingdom of Saudi Arabia (KSA) [1]. In 2019, the WHO estimated that CVD was the cause of 17.9 million deaths which represented more than 30% of all deaths in that year. It has been reported that in the Gulf Cooperation Council which including the KSA, about 45% of deaths are caused by CVD [2]. Coronary artery disease (CAD) is one of the important types of CVD [3]. CVD is caused by lifestyle and genetic risk factors [4]. Lifestyle risk factors include unhealthy diet, physical inactivity, obesity, smoking and type 2 diabetes (T2D) [4]. The genome wide association studies have indicated the association of certain loci with CAD, diabetes, and other diseases in different populations [5–15].

Nitric oxide (NO) is an important signaling molecule with a short half-life in body fluids [16]. NO is a crucial mediator of endothelial function [17,18]. It is synthesized in the vascular endothelial cells in a reaction catalyzed by endothelial NO synthase (eNOS) [17,18]. Physiological production of NO by eNOS has been linked to a healthy cardiovascular system, and reduced bioavailability of NO has been associated with CVD [17,18]. NO maintains the homeostasis of cardiovascular cells and has anti-hypertensive properties. Moreover, NO protects vasculature from white blood cell immigration and adhesion as well as platelet aggregation [17]. Single nucleotide variations (SNVs) of eNOS gene were associated with diseases such as Multiple sclerosis and uterine cervical cancer in Iranian and Chinese populations, respectively [19,20]. Recently, the eNOS rs1799983 SNP were associated elevated susceptibility to hypertension [21].

The role of transcription factors is crucial. Krüppel-like factor 14 (KLF-14) is one of the members of the Krüppel-like family (KLF) and is a transcription factor from the third group of KLFs that inhibits the transcription of genes by binding to the deoxynucleic acid with Sin3A [22]. The latter is a transcriptional co-suppressor [22]. KLF-14 is an important metabolic transcriptional regulator that has been implicated in metabolic syndrome [22,23]. KLF-14 protects against the inflammatory process in endothelial cells via the inhibition of signaling pathway of NF-κB [24]. KLF-14rs972283 SNV was reported to be associated with T2D [25]. Moreover, SNVs in KLF-14 gene were associated with lipid profiles, status of blood pressure, resistance to insulin and metabolic syndrome [26].

MTHFR is a very important enzyme involved in the metabolism of folate, homocysteine, nucleotide synthesis, and methylation of membranes, DNA, protein and lipids [27,28]. Increased blood levels of homocysteine have been demonstrated to be associated with increased risk to CVD [29]. The C677T SNV is found at exon 4 which replaces valine to alanine at codon 222 [30], and decreases the catalytic activity of MTHFR [30]. Furthermore, the MTHFR 677 SNV was reported to be associated with psoriasis, CAD, diabetes, and neurological disease [30,31].

MicroRNAs are short noncoding RNA molecules that modulate the expression of genes and are involved in many pathophysiological processes [32]. MiR-27a regulates Peroxisome proliferator-activated receptor Gamma (PPARγ) [33]. PPARγ has been implicated in CVD [34]. The miRNAs27a rs895819 SNV was reported to be associated with susceptibility to myocardial infarction (MI) and breast cancer in the Chinese population [35,36].

The genome wide association studies (GWAS) have helped in uncovering the linkage between certain loci and diseases including diabetes, CVD, cancers, and others [5,6,10,13,14,37]. In the current study, we investigated the association of pathogenic gene variants of eNOS rs1799983, KLF14rs972283, MTHFR 677, and miR-27a rs895819 with the risk to CAD in the Saudi Arabian population.
2. Materials and Methods

This study addresses clinically confirmed cases of CAD in the population from the Tabuk region, Saudi Arabia. This project is approved by the ethics committee, University of Tabuk (No: UT-91-23-2020). Informed consent was obtained before collection of samples from all participants.

2.1. Patient Selection Criteria

2.1.1. Criteria for Inclusion of Subjects in this Study

We selected cases conducting elective angiography for the diagnosis of stable angina at the King Fahad Specialist Hospital, KSA. Other tests were also conducted such as electrocardiogram (ECG or EKG), ambulatory electrocardiography, Holter monitoring, X-ray, echocardiogram (echo) for chest, cardiac computed tomography (CCT), test for exercise stress and myocardial perfusion imaging (MPI) or Multigated acquisition scan (MUGA).

The subjects were divided according to the result of the coronary angiographic to either significant coronary artery disease or ischemic heart disease (stenosis ≥ 50%) or no ischemic heart disease (no stenosis or stenosis < 50%). Cases with cancers, diabetes, or other chronic disorders have been excluded from this project.

2.1.2. Healthy Controls

Healthy controls were subjects attending King Fahd Specialist Hospital for regular checkups. The healthy controls were apparently healthy with no history of cardiovascular disease, or any other chronic diseases. Blood biochemistry tests were also done for the healthy controls. All subjects filled the informed consent form.

2.2. Genomic DNA Extraction

The Genomic DNA from each subject was purified with the DNeasy Blood K (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. The DNA amount was quantified with the NanoDrop, and DNA integrity was assessed with agarose gel electrophoresis. Then DNA was kept at 25 °C till genotyping was performed.

2.2.1. Genotyping of MTHFR 677, KLF-14, miRNAs27a and eNOS3 SNPs

The genotyping of MTHFR 677, KLF-14, miRNAs27a and eNOS3 was determined by ARMS-PCR using tetra-primers (Table 1).

| Direction | Sequence | Product Size | AT |
|-----------|----------|--------------|----|
| KLF-14 FO | 5’-GTCA$	ext{TAGGCATCAACACGCTAGATATTG}$GT-3’ | 437 bp | 60 °C |
| KLF-14 RO | 5’-CTACAGGACCAACTCAAATTATGAGGT-3’ | | |
| KLF-14 FI-(G allele) | 5’-CATTGTATACCTGGAAAAATCTCTACATG-3’ | 274 bp | |
| KLF-14 RI-(A allele) | 5’-TAGCTAAAATAAGTATGCGCCATGCCT-3’ | 221 bp | |

| Direction | Sequence | Product Size | AT |
|-----------|----------|--------------|----|
| miRNAs27a FO | 5’-GGCTTGACCCCTGTTCCTCCTGCTGAACCT-3’ | 353 bp | 63 °C |
| miRNAs27a RO | 5’-CTGCTTCCGTCAAAATCACATTGCCA-3’ | | |
| miRNAs27a FI-(G allele) | 5’-GAACTTACCTGACAAGCACTGGACTTGG-3’ | 184 bp | |
| miRNAs27a-(A allele) | 5’-CTTAGCTGCTTGAGCAGGGTGCCCA-3’ | 226 bp | |
Table 1. Cont.

| Direction          | Sequence                                      | Product Size | AT  |
|--------------------|-----------------------------------------------|--------------|-----|
| ARMS primers for MTHFR 677 C>T rs1801133 genotyping | MTHFR677FO 5'-AAGCATATCAGTCATGAGCCCAGCC-3' | 224 bp       | 58°C|
|                    | MTHFR677RO 5'-GGGAAGAAACTCAGGAATCTGACGAC-3' |              |     |
|                    | MTHFR677 FI-C 5'-AGAGAGAAGTAGTCGCTGGGGGT-3' | 101 bp       |     |
|                    | MTHFR677 RI-T 5'-AAGAAAAGCTGCTGATGATGAAATAGG-3' | 177 bp       |     |

ARMS primers for eNOS3-rs1799983 (Glu298Asp) G>T genotyping

| Direction          | Sequence                                      | Product Size | AT  |
|--------------------|-----------------------------------------------|--------------|-----|
|                    | eNOS3-FO 5'-AGCCTCGGTGAGATAAAGGATG-3'         | 701 bp       | 60°C|
|                    | eNOS3-RO 5'-GCTGCTGAGGCCCCCCAGATAAAG-3'       |              |     |
|                    | eNOS3-FL-G 5'-GCTGCTGAGGCCCCCCAGATAAAG-3'     | 475 bp       |     |
|                    | eNOS3-RI-T 5'-GCAGAGGAAGAGTTCTGAGGAGA-3'     | 271 bp       |     |

2.2.2. Preparation of PCR Mix

The PCR reaction was conducted in 12 µL that contained sample DNA (50 ng), F0—0.10 µL, R0—0.10 µL, F1—0.10 µL, R1—0.10 µL (25 pmol of desired primer), and 6 µL Green Master Mix for PCR (2X) (Cat# M712C, Promega, Madison, WI, USA). Then water free from nuclease was added to bring the total volume to 12 µL.

2.2.3. PCR Conditions

The conditions were: initial denaturation at 95 °C (08 min), then 32 cycles of denaturation at 94 °C (34 s); annealing temperatures for 44 s MTHFR 677 C>T (58 °C), KLF 14 C>T (60 °C), miRNAs27a A>G (63 °C), eNOS3-G>T (60 °C) for 45 s, extension at 72 °C for 35 s, and a final extension 72 °C for 10 min.

2.2.4. Gel Electrophoresis and PCR Product Visualization

Products of the PCR were run in agarose gel electrophoresis (1.5%) stained with SYBR safe dye. The gel was visualized on a UV transilluminator from Bio-Rad, Hercules, CA, USA.

2.2.5. MTHFR 677 C>T rs1801133 Genotyping

The external primers F0 and R0 amplify the external region of the MTHFR gene, producing a band of 224 bp that serves as a control for DNA soundness. Primers F0 and R1 amplify the T allele, producing a band of 177 bp, and primers F1 and R0 generate a band of 101 bp from the C allele.

2.2.6. KLF-14 rs972283 G>A Genotyping

The external primers F1 and R1 amplify the external region of the KLF-14, producing a band of 437 bp that serves as a control for DNA soundness. Primers F0 and R1 amplify the A allele, producing a band of 221 bp, and primers F1 and R0 producing a band of 274 bp from the G allele.

2.2.7. MiRNAs27ars895819 A>G Genotyping

The primers F0 and R0 amplify the external region of the miR-27, producing a band of 353 bp that is used as a control for DNA soundness. Primers F0 and R1 amplify the A allele, giving a band of 226 bp, and primers F1 and R0 produce a band of 184 bp from the G allele.

2.2.8. eNOS3-rs1799983 (Glu298Asp) G>T Genotyping

Primers F1 and R1 flank the exon of the eNOS3, producing a band of 701 bp that serves as a control for DNA integrity. Primers F0 and R1 amplify the T allele, producing a band of 271 bp, and primers F1 and R0 produce a band of 475 bp from the T allele.
2.3. Statistical Analyses

The differences of genotype distributions of the SNPs MTHFR 677 C>T, KLF-14 rs972283 C>T, miR-27a rs895819 A>G and eNOS3-rs1799983 G>T in CAD subjects and controls were calculated with Chi-square test, and checked with Hardy–Weinberg equilibrium (HWE). The associations between the genotypes and alleles of the SNVs and the risk to CAD were estimated by the odds ratios (ORs), risk ratios (RRs) and risk differences (RDs) with 95% confidence intervals (CIs). A p-value < 0.05 was regarded as statistically significant. All statistical analyses were conducted with SPSS 16.0.

3. Results
3.1. Demographic Features of CAD Patients

Table 2 summarizes the demographic trends observed in 125 consecutive CAD patients. From a group having 125 CAD profiles, the clinical data was collected for all 125 cases available; however, patients were stratified based on age into above 50 years (n = 30, 24%) or ≤50 years (n = 95, 76%) as summarized in Table 2. Out of 125 CAD cases, 84 were males and 41 females.

Table 2. Demographic features of CAD patients and controls.

| Parameters                  | CAD | Controls | Controls |
|-----------------------------|-----|----------|----------|
|                             | N = 125 | %       | N = 125 | %       |
| Male                        | 84   | 67.2     | 76       | 74.75   |
| Female                      | 41   | 32.8     | 49       | 47.75   |
| Age < 50                    | 95   | 76       | 70       | 68.75   |
| Age > 50                    | 30   | 24       | 55       | 53.75   |
| Cholesterol ≤ 200 (mg/dL)   | 60   | 48       |          |         |
| Cholesterol > 200 (mg/dL)   | 65   | 52       |          |         |
| LDL ≤ 100 (mg/dL)           | 74   | 59.2     |          |         |
| LDL > 100 (mg/dL)           | 51   | 40.8     |          |         |
| HDL ≤ 40 (mg/dL)            | 55   | 44       |          |         |
| HDL > 40 (mg/dL)            | 70   | 56       |          |         |
| TGL ≤ 150 (mg/dL)           | 80   | 64       |          |         |
| TGL > 150 (mg/dL)           | 45   | 36       |          |         |
| Creatinine < 1.35 mg/dL     | 74   | 59.2     |          |         |
| Creatinine > 1.35 mg/dL     | 51   | 40.8     |          |         |
| C-reactive protein < 10 mg/L| 53   | 42.4     |          |         |
| C-reactive protein > 10 mg/L| 72   | 57.6     |          |         |
| Hypertension                | 52   | 41.6     |          |         |
| No hypertension             | 73   | 58.4     |          |         |
| T2D                         | 55   | 44       |          |         |
| No T2D                      | 70   | 56       |          |         |
| Smoking (Yes)               | 70   | 56       |          |         |
| Smoking (No)                | 55   | 44       |          |         |
| Obesity                     | 40   | 32       |          |         |
| No Obesity                  | 83   | 66.4     |          |         |
| Myocardial infarction       | 72   | 57.6     |          |         |
| No Myocardial infarction    | 53   | 42.4     |          |         |
Lipid biomarkers: Results indicated that 52% had cholesterol higher than 200 mg/dL, 40.8% had LDL levels higher than 100 mg/dL, 56% had HDL levels higher than 40 mg/dL, whereas 36% had TGL higher than 150 mg/dL as depicted in Table 2. About 40.8% CAD patients had higher level of serum creatinine >1.35 mg/dL and 57.6% CAD patients had higher level of serum C-reactive protein > 10 mg/L. Out of 125 CAD cases, 41.6% were having hypertension, 44% had T2D (+), 32% had obesity and 57.6% had MI. Out of 125 cases, 56% of subjects were smokers.

3.2. Hardy–Weinberg Equilibrium for Genotype Distributions and Allele Frequencies

There was no deviation observed for the genotype distributions and allele frequencies of the SNPs for the four genes: MTHFR 677 C>T, KLF 14 rs972283 C>T, miR-27a rs895819 A>G and eNOS3-rs1799983 G>T in the control group. Therefore, the genotyping results were reviewed from the 10% of the samples randomly chosen from the normal control group, which reflected the accuracy rate of more than 99%.

3.3. Statistical Analysis of MTHFR 677 C>T, KLF 14G>A, miRNAs27a-A>G and eNOS3G>T Genotypes with CAD Patient Susceptibility

At the time of analysis, KLF-14-rs972283 G>A, eNOS3-rs1799983 G>T and miRNAs27ars895819 A>G SNP were studied in 125 CAD cases and 125 controls, and MTHFR 677 C>T gene polymorphism was analyzed in 116 CAD cases and 125 healthy controls.

3.4. Comparative Statistical Analysis of MTHFR 677 C>T Genotypes Assessed for CAD Patients and Controls

The MTHFR 677 C>T genotype frequency in CAD patients and controls was CC (57.75%), CT (37.93%) and TT (12%) and controls CC (63.2%), CT (24.8%) and TT (4.48%) respectively (Table 3). The results for MTHFR 677 C>T gene variation was statistically significant (\( p < 0.045 \)) between CAD patients and controls. It was also noted that the T allele had a higher frequency among CAD patients than in healthy individuals (0.31 vs. 0.17) (Table 3).

Table 3. Association between MTHFR 677 C>T genotypes between CAD patients and healthy controls.

| Subjects | GG (%) | GA (%) | AA (%) | Df | \( X^2 \) | G | A | \( p \) Value |
|----------|--------|--------|--------|----|--------|---|---|-------------|
| Cases    | 67 (57.75) | 44 (37.93) | 14 (12.06) | 2 | 6.11 | 0.69 | 0.31 | 0.045      |
| Controls | 79 (63.2)  | 31 (24.8)  | 06 (4.48)  | 0.83 | 0.17 |

3.5. The Association between MTHFR 677 C>T Genotypes and Risk to Patients with CAD Cases Estimated through Multivariate Analysis Based on OR and RR (CI = 95%)

As summarized in Table 4, our results demonstrated that in the codominant model, the MTHFR-TT genotype was associated strongly with increased CAD patient susceptibility with an OR of 2.75, (95%) CI = (1.0018 to 7.5558), RR = 1.80 (0.9084 to 3.5814), and \( p < 0.049 \); whereas no association with CAD susceptibility was reported for the MTHFR-CT genotype with an OR of 1.67, (95%) CI = (0.9529 to 2.9393), and \( p < 0.73 \). We also observed a significant association between the MTHFR-CC and MTHFR-(CT+TT) genotypes in the dominant inheritance model that may be linked to a greater CAD susceptibility with an OR = 1.84, (95% CI) (1.0930 to 3.1257), and \( p < 0.021 \). Moreover, no association was found between the MTHFR-(CT+TT) and MTHFR-TT genotypes in the recessive inheritance model. The allelic comparison found the MTHFR -T allele to be significantly associated with CAD susceptibility with an OR of 1.82, (95% CI) (1.1816 to 2.8041), RR 1.39 (1.0777 to 1.813), and \( p < 0.006 \) (Table 4).
Table 4. Association of MTHFR 677 C>T genotypes with CAD Susceptibility.

| Genotypes | Healthy Controls (N = 116) | CAD Cases (N = 125) | OR (95% CI) | Risk Ratio (RR) | p Value |
|-----------|---------------------------|---------------------|-------------|----------------|---------|
|           |                           |                     |             |                |         |
| Codominant inheritance model |               |                     |             |                |         |
| MTHFR-CC  | 79                        | 67                  | 1 (ref.)    | 1 (ref.)       |         |
| MTHFR-CT  | 31                        | 44                  | 1.67 (0.9529 to 2.9393) | 1.30 (0.9618 to 1.7817) | 0.073   |
| MTHFR-TT  | 06                        | 14                  | 2.75 (1.0018 to 7.5558) | 1.80 (0.9084 to 3.5814) | 0.049   |
| Dominant inheritance model |               |                     |             |                |         |
| MTHFR-CC  | 79                        | 67                  | 1 (ref.)    | 1 (ref.)       |         |
| MTHFR-(CT+TT) | 37                | 58                  | 1.84 (1.0930 to 3.1257) | 1.38 (1.0367 to 1.8618) | 0.021   |
| Recessive inheritance model |               |                     |             |                |         |
| MTHFR-(CT+TT) | 110          | 111                 | 1 (ref.)    | 1 (ref.)       |         |
| MTHFR-TT  | 06                        | 14                  | 2.31 (0.8574 to 6.2359) | 1.65 (0.8385 to 3.2829) | 0.09    |
| Allele    |                           |                     |             |                |         |
| MTHFR-C   | 189                       | 178                 | 1 (ref.)    | 1 (ref.)       |         |
| MTHFR-T   | 42                        | 72                  | 1.82 (1.1816 to 2.8041) | 1.39 (1.0777 to 1.8130) | 0.006   |

Statistically significant p-values with in bold.

3.6. The Association of MTHFR 677 C>T Genotypes with CAD Patient Characteristics

There was no significant association between MTHFR 677 C>T genotypes assessed for the age and gender of the CAD patients (p>0.05), as reported in Table 5. However, the association between MTHFR 677 C>T genotypes and serum cholesterol (mg/dL) of CAD patients was significant (p = 0.003). In addition, a significant association was found between MTHFR 677 C>T genotypes and LDL-C (mg/dL) of CAD patients (p < 0.0018). Moreover, HDL-C (mg/dL) of CAD patients did not show significant association with MTHFR 677 C>T genotypes (p = 0.560). Similarly, the statistical analysis of the correlation between MTHFR 677 C>T genotypes and creatinine (mg/dL) in CAD patients did not show any significant association (p =0.313). MTHFR 677 C>T genotypes and CRP (mg/L) in CAD patients revealed a significant association (p < 0.033). A significant correlation of MTHFR 677 C>T genotypes was however observed with hypertension (p = 0.002), T2D (p = 0.0067), obesity (p < 0.022) and MI (p = 0.0001), which was also the case with smoking (p = 0.005) in CAD patients (Table 5).

Table 5. The association of MTHFR 677 C>T genotypes with CAD patients characteristics.

| Parameters     | N= | GG | GA | AA | X² | Df | p Value |
|----------------|----|----|----|----|----|----|---------|
| Male           | 84 | 45 | 30 | 09 | 0.07 | 2 | 0.96    |
| Female         | 41 | 22 | 14 | 05 |      |   |         |
| Age < 50       | 95 | 48 | 38 | 09 | 4.34 | 2 | 0.11    |
| Age > 50       | 30 | 19 | 06 | 05 |      |   |         |
| Cholesterol ≤ 200 (mg/dL) | 60 | 23 | 27 | 10 | 11.24 | 2 | 0.003   |
| Cholesterol > 200 (mg/dL) | 65 | 44 | 17 | 04 |      |   |         |
| LDL-C ≤ 100 (mg/dL) | 74 | 30 | 34 | 10 | 12.60 | 2 | 0.0018  |
| LDL > 100 (mg/dL) | 51 | 37 | 10 | 04 |      |   |         |
| HDL-C ≤ 40 (mg/dL) | 55 | 28 | 19 | 08 | 1.13 | 2 | 0.560   |
| HDL-C > 40 (mg/dL) | 70 | 39 | 25 | 06 |      |   |         |
Table 5. Cont.

| Parameters                  | N= | GG | GA | AA | $X^2$ | Df | p Value |
|-----------------------------|----|----|----|----|------|----|---------|
| TGL ≤ 150 (mg/dL)           | 80 | 40 | 33 | 07 | 4.04 | 2  | 0.136   |
| TGL > 150 (mg/dL)           | 45 | 27 | 11 | 07 |      |    |         |
| Creatinine < 1.35 mg/dL     | 74 | 36 | 30 | 08 | 2.32 | 2  | 0.313   |
| Creatinine > 1.35 mg/dL     | 51 | 31 | 14 | 06 |      |    |         |
| C-reactive protein < 10 mg/L| 53 | 23 | 20 | 10 | 6.69 | 2  | 0.033   |
| C-reactive protein > 10 mg/L| 72 | 44 | 24 | 04 |      |    |         |
| Hypertension                | 52 | 19 | 27 | 06 | 11.92| 2  | 0.002   |
| No hypertension             | 73 | 48 | 17 | 08 |      |    |         |
| T2D                         | 55 | 36 | 11 | 08 | 10   | 2  | 0.0067  |
| No T2D                      | 70 | 31 | 36 | 06 |      |    |         |
| Smoking (Yes)               | 70 | 31 | 34 | 05 | 13   | 2  | 0.005   |
| Smoking (No)                | 55 | 36 | 10 | 09 |      |    |         |
| Obesity                     | 40 | 15 | 17 | 08 | 7.8  | 2  | 0.020   |
| No Obesity                  | 83 | 52 | 27 | 06 |      |    |         |
| Myocardial infarction (MI)  | 72 | 29 | 37 | 06 | 19.51| 2  | 0.0001  |
| No Myocardial infarction (MI)|53 | 38 | 7  | 8  |      |    |         |

Statistically significant p-values in bold.

3.7. Association of KLF-14 rs972283 G>A Genotypes between Cases and Controls

The frequency of KLF-14 rs972283 G>A genotypes in CAD patients and controls was GG (46.4%), GA (22.4%) and AA (31.2%) and controls GG (40%), GA (48%) and AA (12%), respectively, as reported in Table 6. The KLF-14 rs972283 G>A polymorphic gene variation found between cases and healthy individuals was statistically significant ($p < 0.045$). It was reported that the allele A had a higher frequency among CAD patients than in healthy individuals (0.43 vs. 0.36) as shown in Table 6.

Table 6. Association of CAD patients and controls for KLF-14 rs972283 G>A genotypes.

| Subjects       | N= | GG (46.4%) | GA (22.4%) | AA (31.2%) | $X^2$ | G | A | p Value |
|----------------|----|------------|------------|------------|------|---|---|---------|
| Cases          | 125| 58 (46.4%) | 28 (22.4%) | 39 (31.2%) | 6.43 | 0.57 | 0.43 | 0.045   |
| Controls       | 125| 50 (40%)   | 60 (48%)   | 15 (12%)   | 2    | 0.64 | 0.36 |         |

3.8. Multivariate Analysis Predicting the Association of KLF-14 rs972283 G>A Genotypes with the Risk to Patients with CAD Cases

This analysis is based on a logistic regression-like odd ratio (OD), and a risk ratio (RR) with 95% CI was used to estimate the association between the KLF14 rs972283 G>A genotypes and risk of CAD patients—the data are summarized in (Table 7). As reported in the codominant model, the KLF14-AA genotype was demonstrated to be associated with increased CAD susceptibility with an OR of 2.24, (95%) CI = (1.1070 to 4.5383), RR = 1.66 (1.0358 to 2.6817), and $p < 0.024$; whereas the KLF14-GA genotype was shown to be a potential protective marker for CAD risk with an OR 0.40, (95%) CI = (0.2237 to 0.7234), and $p < 0.002$. Our study found no association between the KLF14-GG and KLF14 (GA+AA) genotypes in the dominant inheritance model with an OR = 0.77 and $p < 0.30$. Interestingly, a strong association also existed between the KLF14+(GG+GA) and KLF14-AA genotypes in the recessive inheritance model with an OR of 3.32, (95%) CI = (1.7207 to 6.4275), RR = 2.02 (1.2914 to 3.1608), and $p = 0.0004$. The KLF14-A allele was not associated with CAD susceptibility with an OR of 1.30 and $p < 0.14$ (Table 7).
Table 7. Association of KLF-14 rs972283 G>A genotypes with risk to CAD.

| Genotypes   | Healthy Controls (N = 125) | CAD Cases (N = 125) | OR (95% CI) | Risk Ratio (RR) | p Value |
|-------------|----------------------------|---------------------|-------------|-----------------|---------|
| Codominant inheritance model |                          |                     |             |                 |         |
| KLF14-GG    | 50                        | 58                  | 1 (ref.)    | 1 (ref.)        |         |
| KLF14-GA    | 60                        | 28                  | 0.40 (0.2237 to 0.7234) | 0.67 (0.5297 to 0.8704) | 0.002   |
| KLF14-AA    | 15                        | 39                  | 2.24 (1.1070 to 4.5383) | 1.66 (1.0358 to 2.6817) | 0.024   |
| Dominant inheritance model |                          |                     |             |                 |         |
| KLF14-GG    | 50                        | 58                  | 1 (ref.)    | 1 (ref.)        |         |
| KLF14-(GA+AA) | 75                      | 67                  | 0.77 (0.4663 to 1.2718) | 0.87 (0.6787 to 1.1320) | 0.30    |
| Recessive inheritance model |                          |                     |             |                 |         |
| KLF14-(GG+GA) | 110                     | 86                  | 1 (ref.)    | 1 (ref.)        |         |
| KLF14-AA    | 15                        | 39                  | 3.32 (1.7207 to 6.4275) | 2.02 (1.2914 to 3.1608) | 0.0004  |
| Allele      |                           |                     |             |                 |         |
| KLF14-G     | 160                       | 144                 | 1 (ref.)    | 1 (ref.)        |         |
| KLF14-A     | 90                        | 106                 | 1.30 (0.9130 to 1.8758) | 1.14 (0.9520 to 1.3800) | 0.1431  |

Statistically significant p-values in bold.

3.9. The Association of KLF-14 rs972283 G>A Genotypes with CAD Patients Demographic and Clinical Variables

As displayed in Table 8, there was no association between KLF-14 rs972283 G>A genotypes and the age and gender of the CAD patients (p > 0.05). However, the analysis of the association between KLF-14 G>A genotypes and serum cholesterol (mg/dL) of CAD patients showed a significant association (p = 0.0032). Similar results were found for KLF-14 G>A genotypes and its association with serum LDL-C (mg/dL) of CAD cases (p < 0.002). Moreover, our results showed that there was no significant association between KLF-14 G>A genotypes and serum HDL-C (mg/dL) of CAD patients with a (p = 0.26). On the other hand, a significant association was found between KLF-14 G>A genotypes and serum triglyceride TG (mg/dL) of CAD patients, with p = 0.008. The statistical analysis of the correlation between KLF-14 G>A genotypes and CRP < 10 mg/L in CAD patients demonstrated a significant association (p = 0.0001). A significant correlation was also found between KLF-14 G>A genotypes and hypertension (p = 0.002), T2D (p = 0.0002) and MI (p = 0.009). As mentioned, no significant correlation existed between KLF-14 G>A genotypes and obesity (p = 0.10) in CAD patients (Table 8).

3.10. Association of miRNAs27a rs895819 A>G Genotypes in a Correlation Study between CAD Cases and Healthy Individual Controls

The frequency of miRNAs27a rs895819 A>G genotypes in CAD cases and healthy individuals was GG (52%), GA (42.4%) and AA (5.6%), and controls GG (44.8%), GA (40%) and AA (15.2%), respectively (Table 9). The miR-27a rs895819 A>G gene polymorphic variations analyzed for CAD cases and controls was reported to be statistically significant (p < 0.032). As shown in Table 9, the frequency of the allele A was higher among CAD cases than in than in healthy individuals (0.73 vs. 0.65).
Table 8. The association of KLF-14 rs972283 G>A genotypes with CAD patient characteristics.

| Parameters                        | N= | GG | GA | AA   | X²   | Df | p Value |
|-----------------------------------|----|----|----|------|------|----|---------|
| CAD Patients                      | 125| 58 | 28 | 39   |      |    |         |
| Male                              | 84 | 34 | 20 | 30   | 3.84 | 2  | 0.146   |
| Female                            | 41 | 24 | 08 | 09   |      |    |         |
| Age < 50                          | 95 | 46 | 20 | 29   | 0.73 | 2  | 0.69    |
| Age > 50                          | 30 | 12 | 8  | 10   |      |    |         |
| Cholesterol ≤ 200 (mg/dL)         | 60 | 21 | 21 | 18   | 11.46| 2  | 0.0032  |
| Cholesterol > 200 (mg/dL)         | 65 | 37 | 07 | 21   |      |    |         |
| LDL ≤ 100 (mg/dL)                 | 74 | 25 | 22 | 27   | 12.2 | 2  | 0.0022  |
| LDL > 100 (mg/dL)                 | 51 | 33 | 06 | 12   |      |    |         |
| HDL ≤ 40 (mg/dL)                  | 55 | 43 | 18 | 21   | 2.67 | 2  | 0.263   |
| HDL > 40 (mg/dL)                  | 70 | 15 | 10 | 18   |      |    |         |
| TG ≤ 150 (mg/dL)                  | 80 | 35 | 13 | 32   | 9.6  | 2  | 0.008   |
| TG > 150 (mg/dL)                  | 45 | 23 | 15 | 07   |      |    |         |
| Creatinine < 1.35 mg/dL           | 74 | 30 | 16 | 28   | 3.95 | 2  | 0.138   |
| Creatinine > 1.35 mg/dL           | 51 | 28 | 12 | 11   |      |    |         |
| C-reactive protein < 10 mg/L      | 53 | 13 | 15 | 25   | 18.44| 2  | 0.0001  |
| C-reactive protein > 10 mg/L      | 72 | 45 | 13 | 14   |      |    |         |
| Hypertension                      | 52 | 21 | 13 | 18   | 1.3  | 2  | 0.522   |
| No hypertension                   | 73 | 37 | 15 | 21   |      |    |         |
| T2D                               | 55 | 16 | 17 | 22   | 11.95| 2  | 0.0002  |
| No T2D                            | 70 | 42 | 11 | 17   |      |    |         |
| Smoking (Yes)                     | 70 | 26 | 16 | 28   | 6.9  | 2  | 0.031   |
| Smoking (No)                      | 55 | 32 | 12 | 11   |      |    |         |
| Obesity                           | 40 | 13 | 11 | 16   | 4.59 | 2  | 0.100   |
| No Obesity                        | 83 | 45 | 17 | 23   |      |    |         |
| Myocardial infarction (MI)        | 72 | 31 | 23 | 18   | 9.41 | 2  | 0.009   |
| No Myocardial infarction (MI)     | 53 | 27 | 5  | 21   |      |    |         |

Table 9. Association of miRNAs27a rs895819 A>G genotypes in CAD cases and controls.

| Subjects         | N=  | AA | AG %  | GG %  | Df  | X²  | A   | G   | p Value |
|------------------|-----|----|-------|-------|-----|-----|-----|-----|---------|
| Cases            | 125 | 65 | 52%   | 53    | (42.4%) | 7 | 5.6% |    | 0.73 | 0.27 | 0.032 |
| Controls         | 125 | 56 | 44.8% | 50    | (40%)  | 19| 15.2%|    | 0.65 | 0.35 |        |

3.11. Multivariate Analysis for Predicting the Association between miRNAs27a rs895819 A>G Genotypes and Risk to Patients with CAD Cases

This analysis is based on a logistic regression-like OR or risk ratio RR with 95% CI and was used to estimate the association between the miR-27a rs895819 A>G genotypes and risk of CAD patients. As reported in Table 10, we found that in the codominant model, the miRNAs27a-GA genotype was not associated with CAD susceptibility with an OR of 0.91, (95%) CI = (0.5669 to 1.5038), RR = 0.95 (0.7258 to 1.2501), and p < 0.738; whereas miR-27a-GG genotype has a significance of protective biomarker for CAD risk with an OR of 0.31, (95%) CI = (0.1243 to 0.8104), and p < 0.016. There is no association observed between miRNAs27a-AA and miRNA27a-(GA+GG) genotypes in the dominant inheritance model with an OR = 0.74 and p < 0.25. The miRNA27a-GG vs (AA+GA) genotypes in the recessive
The inheritance model also acts as a protective biomarker for CAD susceptibility with an OR of 0.33, (95% CI = (0.1338 to 0.8184), RR = 0.62(0.4801 to 0.8076) p = 0.016. In addition, in allelic comparison, miRNA27a-G allele acts as a protective biomarker against the CAD susceptibility with an OR of 0.67 and p < 0.04 (Table 10).

Table 10. Association of miRNAs27a rs895819 A>G gene variations with CAD susceptibility.

| Genotypes         | Healthy Controls (N = 125) | CAD Cases (N = 125) | OR (95% CI)     | Risk Ratio (RR) | p-Value |
|-------------------|-----------------------------|---------------------|-----------------|-----------------|---------|
| Codominant Dominant inheritance model |                 |                     |                 |                |         |
| miRNA27a-AA       | 56                          | 65                  | 1 (ref.)        | 1 (ref.)        |         |
| miRNA27a-GA       | 50                          | 53                  | 0.91 (0.5669 to 1.5038) | 0.95 (0.7258 to 1.2501) | 0.738   |
| miRNA27a-GG       | 19                          | 07                  | 0.31 (0.1243 to 0.8104) | 0.63 (0.4682 to 0.8567) | 0.016   |
| Dominant inheritance model |                 |                     |                 |                |         |
| miRNA27a-AA       | 56                          | 65                  | 1 (ref.)        | 1 (ref.)        |         |
| miRNA27a-(GA+GG)  | 69                          | 60                  | 0.74 (0.4556 to 1.2320) | 0.86 (0.6735 to 1.1116) | 0.25    |
| Recessive inheritance model |               |                     |                 |                |         |
| miRNA27a-(AA+GA)  | 106                         | 118                 | 1 (ref.)        | 1 (ref.)        |         |
| miRNA27a-GG       | 19                          | 07                  | 0.33 (0.1338 to 0.8184) | 0.62 (0.4801 to 0.8076) | 0.016   |
| Allele            |                             |                     |                 |                |         |
| miRNA27a-A        | 162                         | 183                 | 1 (ref.)        | 1 (ref.)        |         |
| miRNA27a-G        | 88                          | 67                  | 0.67 (0.4601 to 0.9873) | 0.82 (0.6927 to 0.9876) | 0.04    |

3.12. The Association of miRNAs27a rs895819 A>G Genotypes with CAD Patients Demographic and Clinical Variables

The study found no significant association between miRNA27a rs895819 A>G genotypes with either the age or the gender of the CAD patients (p > 0.05), as shown in Table 11. By contrast, we did find the association between miRNA27a A>G genotypes and serum cholesterol (mg/dL) of CAD patients (p = 0.046). Similarly, a significant association between miRNA27a A>G genotypes and serum LDL-C (mg/dL) of CAD patients was also reported (p < 0.006). However, no association between miRNA27a A>G genotypes and serum HDL-C (mg/dL) was reported for CAD patients (p = 0.66). A significant association between miR-27a A>G genotypes and serum TAGS (mg/dL) of CAD patients have been reported (p = 0.0019). The statistical analysis of the correlation between miRNA27a A>G genotypes and C-reactive protein < 10 mg/L in CAD patients revealed a significant association (p = 0.121). A significant correlation was found between miRNA27a A>G genotypes and hypertension (p = 0.0001), T2D (p = 0.0001) and MI (p = 0.005). Further the study did not find any correlation between miRNA27a A>G genotypes and obesity (p = 0.49) in CAD patients (Table 11).

3.13. Association of eNOS3-rs1799983 G>T Genotypes in the Comparative Study between CAD Cases and Controls

The eNOS3-rs1799983 G>T genotypes frequency was GG (30.4%), GT (65.6%) and TT (4%) in CAD patients, and for controls were GG (50.4%), GT (41.6%) and TT (8%) as shown in Table 12. The miR-27a rs895819 A>G polymorphic gene variation exhibited between CAD cases and healthy individuals in the control group was statistically significant
(p < 0.0007). In addition, as shown in Table 12, the frequency of the allele A was higher among CAD patients than in healthy individuals (0.37 vs. 0.29).

**Table 11.** Association of miRNA-27a rs895819 A>G genotypes with respect to the clinical feature of coronary artery disease patients.

| Parameters               | N=  | AA  | GA  | GG  | X²  | Df  | p-Value |
|--------------------------|-----|-----|-----|-----|-----|-----|---------|
| 125                      |     | 65  | 53  | 7   |     |     |         |
| Male                     | 84  | 47  | 33  | 4   | 1.68| 2   | 0.43    |
| Female                   | 41  | 18  | 20  | 3   | 1.68| 2   | 0.431   |
| Age < 50                 | 95  | 49  | 43  | 3   | 1.68| 2   | 0.431   |
| Age > 50                 | 30  | 16  | 10  | 4   | 1.68| 2   | 0.431   |
| Cholesterol ≤ 200 (mg/dL)| 60  | 28  | 31  | 1   | 6.6 | 2   | 0.046   |
| Cholesterol > 200 (mg/dL)| 65  | 37  | 22  | 6   |     |     |         |
| LDL ≤ 100 (mg/dL)        | 74  | 47  | 23  | 4   | 10.12| 2   | 0.006   |
| LDL > 100 (mg/dL)        | 51  | 18  | 30  | 3   |     |     |         |
| HDL ≤ 40 (mg/dL)         | 55  | 30  | 23  | 2   | 0.81 | 2   | 0.66    |
| HDL > 40 (mg/dL)         | 70  | 35  | 30  | 5   |     |     |         |
| TG ≤ 150 (mg/dL)         | 80  | 38  | 41  | 1   | 12.48| 2   | 0.0019  |
| TG > 150 (mg/dL)         | 45  | 27  | 12  | 6   |     |     |         |
| Creatinine < 1.35 mg/dL  | 74  | 44  | 26  | 4   | 4.21 | 2   | 0.121   |
| Creatinine > 1.35 mg/dL  | 51  | 21  | 27  | 3   |     |     |         |
| C-reactive protein < 10 mg/L | 53 | 29  | 22  | 2   | 0.7  | 2   | 0.70    |
| C-reactive protein > 10 mg/L | 72 | 36  | 31  | 5   |     |     |         |
| Hypertension             | 52  | 15  | 33  | 4   | 19.01| 2   | 0.0001  |
| No hypertension          | 73  | 50  | 20  | 3   |     |     |         |
| T2D                      | 55  | 17  | 34  | 4   | 17.63| 2   | 0.0001  |
| No T2D                   | 70  | 48  | 19  | 3   |     |     |         |
| Smoking (Yes)            | 70  | 30  | 35  | 5   | 5.4  | 2   | 0.06    |
| Smoking (No)             | 55  | 35  | 18  | 2   |     |     |         |
| Obesity                  | 40  | 18  | 20  | 2   | 1.39 | 2   | 0.499   |
| No Obesity               | 85  | 47  | 33  | 5   |     |     |         |
| Myocardial infarction (MI)| 72 | 46  | 24  | 2   | 10.32| 2   | 0.005   |
| No Myocardial infarction (MI) | 53 | 19  | 29  | 5   |     |     |         |

**Table 12.** Association of eNOS3-rs1799983 G>TSNV between cases and controls.

| Subjects       | N=   | GG  | GA  | AA  | Df  | X²  | G  | A  | p Value |
|----------------|------|-----|-----|-----|-----|-----|----|----|---------|
| Cases          | 125  | 38  | 82  | 5   | 2   | 14.57| 0.63| 0.37| 0.0007  |
| Controls       | 125  | 64  | 52  | 9   | 2   | 0.71 | 0.37| 0.29|         |

3.14. *Multivariate Analysis to Estimate the Association between eNOS3-rs1799983 G>T Genotypes and Risk to Patients with CAD Cases*

This analysis was based on a logistic regressionlike OR, and RR with 95% CI was calculated to estimate the association between the eNOS3-rs1799983 G>T genotypes and risk of CAD patients as displayed in Table 13. The study found that in the codominant model, the eNOS3-GT genotype was associated strongly with CAD susceptibility with
an OR of 2.65, (95%) CI = (1.5619 to 4.5162), RR = 1.61(1.2468 to 2.0969), and \( p < 0.0003 \); whereas the eNOS3-TT genotype did not show any association with CAD risk with an OR 0.93, (95%) CI = (0.292 to 2.99), and \( p < 0.74 \). Additionally, a strong association was still observed between the eNOS3-GG and eNOS3-(GT+TT) genotypes in the dominant inheritance model with an OR = 2.40 and \( p < 0.0009 \). There was no association observed between the eNOS3-(GG+GT) and eNOS3-TT genotypes in the recessive inheritance model with an OR of 0.53, (95%) CI = (0.174 to 1.650), RR = 0.76 (0.506 to 1.153). The allelic comparison found allele eNOS3–T was associated strongly with the susceptibility to CAD with an OR of 1.49 and \( p < 0.036 \) (Table 13).

Table 13. Association of eNOS3-rs1799983 (Glu298Asp) gene variations with CAD susceptibility.

| Genotypes       | Healthy Controls (N = 125) | CAD Cases (N = 125) | OR (95% CI) Risk Ratio(RR) | p-Value |
|-----------------|-----------------------------|---------------------|---------------------------|---------|
| Codominant inheritance model |                             |                     |                           |         |
| eNOS3-GG        | 64                          | 38                  | 1 (ref.)                  | 1 (ref.)|
| eNOS3-GT        | 52                          | 82                  | 2.65 (1.5619 to 4.5162)   | 1.61 (1.2468 to 2.0969) | 0.0003 |
| eNOS3-TT        | 09                          | 05                  | 0.93 (0.2920 to 2.9985)   | 0.97 (0.644 to 1.3800)  | 0.74   |
| Dominant inheritance model |                             |                     |                           |         |
| eNOS3-GG        | 64                          | 38                  | 1 (ref.)                  | 1 (ref.)|
| eNOS3-(GT+TT)   | 61                          | 87                  | 2.40 (1.4319 to 4.5162)   | 1.52 (1.1931 to 1.9424) | 0.0009 |
| Recessive inheritance model |                             |                     |                           |         |
| eNOS3-(GG+GT)   | 116                         | 120                 | 1 (ref.)                  | 1 (ref.)|
| eNOS3-TT        | 09                          | 05                  | 0.53 (0.1748 to 1.6503)   | 0.76 (0.5067 to 1.1538) | 0.277  |
| Allele          |                             |                     |                           |         |
| G               | 180                         | 158                 | 1 (ref.)                  | 1 (ref.)|
| T               | 70                          | 92                  | 1.49 (1.0268 to 2.1834)   | 1.23 (1.0062 to 1.5096) | 0.036  |

3.15. Association of eNOS3-rs1799983 (Glu298Asp) G>T Genotypes with Demographic and Clinical Variables for CAD Patients

The study showed no significant association between eNOS3-rs1799983 G>T genotypes with either the age or the gender of the CAD patients (\( p > 0.05 \)), as reported in Table 14. The statistical analysis exhibited a significant association between eNOS3 G>T genotypes and serum cholesterol (mg/dL) in CAD patients (\( p = 0.031 \)). Similarly, a significant association between eNOS3 G>T genotypes and serum TAGs (mg/dL) in CAD patients was also reported (\( p = 0.014 \)). There was no significant association reported between eNOS3 G>T genotypes and serum HDL-C (mg/dL)/serum LDL-C (mg/dL) in CAD patients (\( p = 0.07 \) and 0.07, respectively). The statistical analysis of the correlation between eNOS3 G>T genotypes and CRP < 10 mg/L in CAD patients displayed a significant association (\( p = 0.0008 \)).

A significant correlation was found between eNOS3 G>T genotypes and hypertension (\( p = 0.0001 \)), T2D (\( p = 0.029 \)) and MI (\( p = 0.002 \)). There was also a significant correlation reported between eNOS3 G>T genotypes and obesity (\( p = 0.004 \)) in CAD patients (Table 14).
Table 14. Association of eNOS3-rs1799983 (Glu298Asp) G>T genotypes with respect to CAD case characteristics.

| Parameters                               | N= | GG  | GA  | AA  | X²  | Df | p Value |
|------------------------------------------|----|-----|-----|-----|-----|----|--------|
| Male                                     | 125| 38  | 82  | 5   |     |    |        |
| Female                                   | 84 | 30  | 51  | 3   | 3.43| 2  | 0.18   |
| Age < 50                                  | 95 | 25  | 66  | 4   | 3.12| 2  | 0.210  |
| Age > 50                                  | 30 | 13  | 16  | 1   |     |    |        |
| Cholesterol ≤ 200 (mg/dL)                | 60 | 25  | 33  | 2   | 6.92| 2  | 0.031  |
| Cholesterol > 200 (mg/dL)                | 65 | 13  | 49  | 3   |     |    |        |
| LDL ≤ 100 (mg/dL)                        | 74 | 28  | 44  | 2   | 5.11| 2  | 0.077  |
| LDL > 100 (mg/dL)                        | 51 | 10  | 38  | 3   |     |    |        |
| HDL ≤ 40 (mg/dL)                         | 55 | 20  | 31  | 4   | 5.06| 2  | 0.079  |
| HDL > 40 (mg/dL)                         | 70 | 18  | 51  | 1   |     |    |        |
| TG ≤ 150 (mg/dL)                         | 80 | 30  | 49  | 01  | 8.43| 2  | 0.014  |
| TG > 150 (mg/dL)                         | 45 | 8   | 33  | 4   |     |    |        |
| Creatinine < 1.35 mg/dL                 | 74 | 25  | 47  | 2   | 1.57| 2  | 0.45   |
| Creatinine > 1.35 mg/dL                 | 51 | 13  | 35  | 3   |     |    |        |
| C-reactive protein < 10 mg/L             | 53 | 24  | 25  | 04  | 14.36| 2 | 0.0008 |
| C-reactive protein > 10 mg/L             | 72 | 14  | 57  | 01  |     |    |        |
| Hypertension                             | 52 | 26  | 23  | 3   | 18.13| 2 | 0.0001 |
| No hypertension                          | 73 | 12  | 59  | 2   |     |    |        |
| T2D                                      | 55 | 10  | 42  | 03  | 7.08| 2  | 0.029  |
| No T2D                                   | 70 | 28  | 40  | 02  |     |    |        |
| Smoking (Yes)                            | 70 | 22  | 45  | 03  | 0.13| 02 | 0.930  |
| Smoking (No)                             | 55 | 16  | 37  | 02  |     |    |        |
| Obesity                                  | 40 | 20  | 19  | 01  | 10.07| 2 | 0.0047 |
| No Obesity                               | 85 | 18  | 63  | 04  |     |    |        |
| Myocardial infarction (MI)               | 72 | 30  | 38  | 4   | 12.37| 2 | 0.0021 |
| No Myocardial infarction (MI)            | 53 | 08  | 44  | 01  |     |    |        |

4. Discussion

CVD including CAD is an important cause of death worldwide. Our results showed that the MTHFR 677 C>T SNV was associated with increased CAD risk (Tables 2 and 3). This result is consistent with previous investigations [31,38,39]. The MTHFR is crucial for metabolism of folate, replication of DNA, and methylation of DNA and proteins [40]. It is required for synthesis of proteins and nucleic acids and for cellular homeostasis [39]. The 5,10-methylenetetrahydrofolate is converted to 5-methyltetrahydrofolate by the MTHFR [41]. In the synthesis of protein, the 5-methyltetrahydrofolate donates the CH³ to convert the homocysteine to methionine [42]. It has been reported that the C677T C>T SNV reduces the activity of the MTHFR enzyme [39,43]. In addition, it has been reported that increased serum homocysteine is a risk factor for CVD in young cases [38,44]. The increased serum homocysteine is accompanied by oxidative stress, stimulates the aggregation of platelet, and induces the dysfunction of endothelial cells, increasing hypercoagulability, stress of endoplasmic reticulum and the proliferation of blood vessel smooth muscular cells [38,44]. These lead to atherosclerosis and CAD [44].
Our results showed that there was a significant difference in MTHFR 677 C>T SNV in cases with hyperlipidemia and cases with normal lipid profile (Table 4). Hyperlipidemia is a classical risk factor for CAD [45,46]. Our results indicated that the MTHFR 677 C>T genotype distribution is significantly different between cases with increased and cases with normal CRP (Table 5). The CRP is an inflammatory marker, and elevated levels of CRP were associated with CAD [47]. The role of inflammation in induction of atherosclerosis and CAD is well-established [48]. Previous studies conducted in mice have reported that the reduced MTHFR can alter inflammatory responses and lipid metabolism in hepatocytes [49,50].

We also found a significant difference in the MTHFR 677 C>T SNV genotype distribution in cases with and without T2D (Table 5). Indeed, such an association of MTHFR 677 C>T SNV with T2D and diabetic nephropathy have also been observed in Asian populations [51,52]. MTHFR is very important in metabolism of folate and involved in diabetes and its complications [51,53]. As shown in the results, there was a significant difference in the MTHFR 677 C>T SNV genotype distribution in cases with and cases without hypertension (Table 5). Similarly, in the Irish population, the association of MTHFR 677 TT genotype with increased blood pressure independently of homocysteine has been reported [54], and additionally, the treatment with the riboflavin that is a co-factor of MTHFR can reduce blood pressure in hypertensive cases [54]. The hypertension leads to CAD [55].

Our result indicated that the KLF-14 rs972283 G>A SNV was associated with CAD (Tables 3 and 4). The potential role the KLF-14 rs972283 G>A SNV in the induction of CVD (through dyslipidemia) has also been shown in the Chinese population [56]. A recent study has reported the association of KLF-14 SNVs with metabolic syndrome such as CVD and Type 2 diabetes (T2D) [22]. It has been reported that the KLF14 mediates signaling of lipid and that SNVs in KLF genes influence the KLF roles in maturation of adipocytes, in the hepatocytes (lipid metabolism), in regulation of blood vessels and in angiogenesis [22]. Moreover, it has been demonstrated that the KLF-14 expression might be involved in the formation of atherosclerotic lesion [57]. A significant difference in KLF-14 rs972283 G>A genotype distribution has been observed in patients with normal lipid profile compared to patients with hyperlipidemia (Table 8). Furthermore, significant differences were also seen in patients without T2D and MI compared with patients with T2D and MI (Table 7). Studies have supported the role of KLF-14 genetic variants in induction of metabolic syndrome including obesity, insulin resistance, T2D and CVD [22]. This is since the KLF-14 (and other KLF members) regulates the metabolism of glucose and lipid and is involved in adipogenesis, in differentiation and in the function of adipocytes [22]. Results also showed that there was a significant difference in KLF-14 rs972283 G>A SNV between cases with normal and those with elevated CRP (Table 8). This result is expected hence increased CRP is a biomarker of CVD. The CRP enhances the inflammation and the damage of the blood vessels [58]. It has been reported that normal expression of KLF-14 suppresses the transcription of the signaling pathway of NF-kappa-B and thereby inhibits the inflammation in vascular endothelium mediated by the macrophages [22]. Moreover, KLF-14 prevents endothelial cells from the inflammatory stresses [22]. The role of blood vessel inflammation in the development of CVD is well-established [48].

Our results showed that the GG genotype and the G allele of the miRNAs27a rs895819 A>G were associated with reduced risk to CAD (Tables 5 and 6). A study indicating the association of A allele of the miRNAs27a rs895819A>G with increased risk to MI has been reported in the Chinese Han population wherein the role of high-density lipoprotein-cholesterol (HDL-C levels) has been noticed [35]. The miRNAs27a rs895819 A>G SNV is a loop-stem structure SNV influencing the function of the mature miRNAs27a [59]. It has been reported that the G allele of the miR-27a rs895819 was associated with decreased expression of the mature miRNAs27a [59]. Our results indicated that the miRNAs27a rs895819 A>G genotype distribution was significantly different between cases with normal lipid levels and those with hyperlipidemia (Table 11). It is known that the metabolism of lipids is also regulated by miRNAs27a through the targeting of the nuclear receptor,
retinoid X receptor alpha, which has a role in adipogenesis [60,61]. MiRNAs27a also reported targeting the ATP-binding cassette sub-family A member1 (ABCA1) which is involved in the metabolism of cholesterol [61,62]. Results also showed that there was significant difference in miRNAs27a rs895819 A>G genotype distribution in cases with and those without T2D (Table 11). A previous study in an in vivo model has reported that miRNAs27a regulates the PPAR-γ-Pi3K/AKT-GLUT4 signaling axis and has an active role in insulin signaling and T2D development [63]. Our results are also consistent with a study conducted in the Iranian population which reported that the C allele of the miRNAs27a rs895819 confers protection against T2D [64].

As shown, the eNOS3-rs1799983 G>T SNV was associated with CAD (Tables 12 and 13), which has also been indicated by some previous investigations [21]. The eNOS is an important effector of the cGMP pathway and produces nitric oxide (NO) by converting L-arginine to L-citrulline [65]. The Genes (eNOS) encoding the endothelial NOS was reported as an important gene for cardiovascular physiology since it is crucial for the enzyme activity of the NOS and NO production [21,66]. The NO is a blood vessels relaxing factor produced by the endothelial cells [16]. It is a vasodilator regulating the blood pressure, blood vessel tone and hemodynamics [16]. Dysregulation of NO is implicated in CVD including atherogenesis and hypertension [16,67]. The T allele of the eNOS3-rs1799983 G>T SNV (Asp298Glu) was reported to reduce the enzyme activity of the eNOS [65,67,68]. Future protein–protein interaction (PPI) studies [69,70] are recommended to examine the effect of eNOS3-rs1799983 on the enzyme catalytic activity and eNOS3 PPI interactions network. Our results showed that the eNOS3-rs1799983 G>T SNV genotype distribution was significantly different in cases with normal and those with elevated lipid profile (Table 14). In addition, there was a significant difference in eNOS3-rs1799983 G>T SNV genotype distribution between obese cases and non obese cases (Table 14). Results also showed that the T allele eNOS3-rs1799983 G>T SNV is associated with CAD (Table 12). The study by Luo et al., 2019, has also reported the association of NOS3 rs1799983 with CAD [71], and that the T allele of the rs1799983 is associated with reduced NO levels and abnormal levels of lipids in blood leading to the development of CAD [71]. Population based studies in different countries have also demonstrated the association of eNOS3-rs1799983 G>T with coronary slow flow phenomenon in Iranians and CAD in Egyptians [68,72]. The eNOS has important roles in regulation of obesity and the general metabolism by stimulating mitochondrial biogenesis and activity in adipose tissues [73]. As mentioned in our results, there was a significant difference in eNOS3-rs1799983 G>T SNV between patients with and without T2D (Table 14). The normal NO signaling associates with glucose homeostasis mediated by insulin [16]. A few years ago, Chen et al., 2016, indicated that polymorphism at the eNOS3 is associated with diabetes and diabetic nephropathy in Chinese and Iranian populations [74,75]. Another study reported that overexpression of the eNOS3 does not ameliorate systemic insulin resistance [73]. The role of the eNOS3 polymorphism in the development of insulin resistance and T2D require further investigations. Results indicated that the eNOS3-rs1799983 G>T SNV genotype distribution was significantly different in cases with and those without hypertension and MI (Table 14). NO is a potent vasodilator, and it suppresses the activation and adhesion of platelets to the vascular endothelium [16]. Moreover, NO targets the mitochondria and reduces the generation of the reactive oxygen species (ROS) and decrease the mitochondrial calcium [16]. The accumulation of ROS and calcium are implicated in the progression of CAD [16,76]. Furthermore, NO protects against MI by contributing in ischemic preconditioning [16], and the physiologic levels of NO enhances lipolysis of adipocyte and oxidation of fatty acid in skeletal muscles as well as in myocardium [16,77].

5. Conclusions

Taken together, our results showed that the MTHFR 677C>T, KLF-14 rs972283 G>A, miRNAs27a rs895819 A>G and eNOS3-rs1799983 G>T SNVs are associated with CAD susceptibility in the Saudi population. To the best of our knowledge, this is the first study
that has comprehensively evaluated the association of the eNOS, KLF-14, MTHFR, and miRNAs27a SNVs with the risk of coronary artery diseases in the given population. These findings require verification in future large-scale population-based studies before these loci are used for prediction and identification of individuals at risk to CAD. In addition, further studies merging these SNVs to evaluate their combination effects on CVD susceptibility are recommended: such studies will greatly improve the diagnosis of CAD and the prediction of individuals who are susceptible to this disease.

Author Contributions: Conceptualization, R.M., I.E., J.J., J.B., M.A.A., S.O.A., M.M.J., F.J.T., A.Y., M.F.U., F.M.A.; methodology, R.M., I.E., J.J.; formal analysis, R.M., I.E., J.J.; investigation, R.M., I.E., J.J.; F.M.A.; data curation, J.J., F.M.A. and S.O.A.; original draft preparation, R.M. and I.E.; writing—review and editing, R.M., I.E. and M.F.U.; supervision, I.E. and R.M.; project administration, R.M. and I.E.; funding acquisition, R.M., I.E., J.J., J.B., M.A.A., S.O.A., M.M.J., F.J.T., A.Y., M.F.U., F.M.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: This research study was ethically approved by the research ethics committee of the University of Tabuk. The ethical approval was obtained from the local ethics committee of the University of Tabuk (Decision No: UT-91-23-2020) and in accordance with human subjects, and complied with the principles of the Helsinki Declaration. Informed consent was obtained before collecting samples from all patients and control subjects.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All the data associated with the current study has been presented in this manuscript.

Conflicts of Interest: We thank CAD patients and healthy controls for their participation in this study.

Acknowledgments: The authors declare no conflict of interest.

References
1. Libby, P.; Buring, J.E.; Badimon, L.; Hansson, G.K.; Deanfield, J.; Bittencourt, M.S.; Tokgozoglu, L.; Lewis, E.F. Atherosclerosis. Nat. Rev. Dis. Prim. 2019, 5, 56. [CrossRef]
2. Alhabib, K.F.; Batais, M.A.; Almigbal, T.H.; Alshamiri, M.Q.; Altaradi, H.; Rangarajan, S.; Yusuf, S. Demographic, behavioral, and cardiovascular disease risk factors in the Saudi population: Results from the Prospective Urban Rural Epidemiology study (PURE-Saud). BMC Public Health 2020, 20, 1213. [CrossRef] [PubMed]
3. Stewart, J.; Mannathan, G.; Wilkinson, P. Primary prevention of cardiovascular disease: A review of contemporary guidance and literature. JRSM Cardiovasc. Dis. 2017, 6, 2048004016687211. [CrossRef] [PubMed]
4. Said, M.A.; Verweij, N.; van der Harst, P. Associations of Combined Genetic and Lifestyle Risks With Incident Cardiovascular Disease and Diabetes in the UK Biobank Study. JAMA Cardiol. 2018, 3, 693–702. [CrossRef] [PubMed]
5. Elfaki, I.; Mir, R.; Abu-Duhier, F.M.; Jha, C.K.; Ahmad Al-Alawy, A.I.; Babakr, A.T.; Habib, S.A.E. Analysis of the Potential Association of Drug-Metabolizing Enzymes CYP2C9*3 and CYP2C19*3 Gene Variations With Type 2 Diabetes: A Case-Control Study. Curr. Drug Metab. 2020, 21, 1152–1160. [CrossRef] [PubMed]
6. Elfaki, I.; Mir, R.; Abu-Duhier, F.; Alotaib, M.; Alalawy, A.; Barnawi, J.; Babakr, A.; Mir, M.; Mirghani, H. Clinical Implications of MiR128, Angiotensin I Converting Enzyme and Vascular Endothelial Growth Factor Gene Abnormalities and Their Association with Susceptibility to T2D. J Pers. Med. 2021, 11, 861. [CrossRef]
7. Elfaki, I.; Almutairi, F.; Mir, R.; Khan, R.; Abu-Duhier, F. Cytochrome P450 CYP1B1*2 gene and its association with T2D in Tabuk population, Northwestern region of saudi arabia. Asian J. Pharm. Clin. Res. 2018, 1, 55–59. [CrossRef]
8. Elfaki, I.; Mir, R.; Almutairi, F.M.; Duhier, F.M.A. Cytochrome P450: Polymorphisms and Roles in Cancer, Diabetes and Atherosclerosis. Asian Pac. J. Cancer Prev. 2018, 19, 2057–2070. [CrossRef]
9. Jha, C.K.; Mir, R.; Elfaki, I.; Javid, J.; Babakr, A.T.; Banu, S.; Chahal, S.M.S. Evaluation of the Association of Omentin 1 rs2274907 A>T and rs2274908 G>A Gene Polymorphisms with Coronary Artery Disease in Indian Population: A Case Control Study. J. Pers. Med. 2019, 9, 30. [CrossRef] [PubMed]
10. Jha, C.K.; Mir, R.; Banu, S.; Elfaki, I.; Chahal, S.M.S. Heterozygosity in LDLR rs2286761 and rs72658855 Gene is Associated with Increased Risk of Developing Coronary Artery Disease in India—A Case-Control Study. Endocr. Metab. Immune Disord.-Drug Targets 2020, 20, 388–399. [CrossRef] [PubMed]
11. Mir, R.; Elfaki, I.; Duhier, F.M.A.; Alotaib, M.A.; AlAlawy, A.I.; Barnawi, J.; Babakr, A.T.; Mir, M.M.; Mirghani, H.; Hamadi, A.; et al. Molecular Determination of miRNA-126 rs4636297, Phosphoinositide-3-Kinase Regulatory Subunit 1-Gene Variability rs7713645, rs706713 (Tyr73Tyr), rs3730089 (Met326Ile) and Their Association with Susceptibility to T2D. J. Pers. Med. 2021, 11, 861. [CrossRef]
12. Mir, M.M.; Mir, R.; Alghamdi, M.A.A.; Wani, J.I.; Elfaki, I.; Sabah, Z.U.; Alhujaily, M.; Jeelani, M.; Marakala, V.; Alharthi, M.H.; et al. Potential impact of GCK, MIR-196A-2 and MIR-423 gene abnormalities on the development and progression of type 2 diabetes mellitus in Asir and Tabuk regions of Saudi Arabia. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 2012, 159B, 981–986. [CrossRef] [PubMed]

13. Husemoen, L.L.; Skaaby, T.; Jorgensen, T.; Thuesen, B.H.; Fenger, M.; Grarup, N.; Sandholt, C.H.; Hansen, T.; Pedersen, O.; Linneberg, A. MTHFR C677T genotype and MTHFR polymorphisms and risk of type 2 diabetes mellitus: A global meta-analysis. *Hum. Immunol.* 2014, 75, 342–347. [CrossRef] [PubMed]

14. Wang, J.; Zhang, J.; Lomberk, G.A.; Zhou, Z.; Sun, L.; Mathison, A.J.; Garcia-Barrio, M.T.; Zhang, J.; Zeng, L.; et al. Perhexiline activates KLF14 and reduces atherosclerosis by modulating ApoA-I production. *J. Clin. Investig.* 2015, 125, 3819–3830. [CrossRef] [PubMed]

15. Saetre, P.; Grove, J.; Borglum, A.D.; Mors, O.; Verge, T.; Andreassen, O.A.; Vares, M.; Agartz, I.; Terenius, L.; Jonsson, E.G. Differential Genetic and Epigenetic Effects of the KLF14 Gene on Body Shape Indices and Metabolic Traits. *Int. J. Mol. Sci.* 2020, 21, 385. [CrossRef] [PubMed]

16. Liew, S.C.; Gupta, E.D. Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism: Epidemiology, metabolism and its clinical applications. *Cardiology* 2012, 122, 55–68. [CrossRef]

17. Siragusa, M.; Thole, J.; Bibi, S.I.; Luck, B.; Loot, A.E.; de Silva, K.; Wittig, I.; Heidler, J.; Stingl, H.; Randriamboavongy, V.; et al. Nitric oxide maintains endothelial redox homeostasis through PKM2 inhibition. *EMBO J.* 2019, 38, e100938. [CrossRef] [PubMed]

18. Qian, J.; Fulton, D. Post-translational regulation of endothelial nitric oxide synthase in vascular endothelium. *Front. Physiol.* 2013, 4, 347. [CrossRef] [PubMed]

19. Heidari, M.M.; Khatami, M.; Tahamtan, Y. Molecular Analysis of rs2070744 and rs1799983 Polymorphisms of NOS3 Gene in Iranian Patients With Multiple Sclerosis. *Basic Clin. Neurosci.* 2017, 8, 279–284. [CrossRef] [PubMed]

20. Hung, W.C.; Wu, T.F.; Ng, S.C.; Lee, Y.C.; Shen, H.P.; Yang, S.F.; Wang, P.H. Involvement of endothelial nitric oxide synthase gene variants in the aggressiveness of uterine cervical cancer. *J. Cancer* 2019, 10, 2594–2600. [CrossRef] [PubMed]

21. Shi, J.; Liu, S.; Guo, Y.; Liu, S.; Xu, J.; Pan, L.; Hu, Y.; Liu, Y.; Cheng, Y. Association between eNOS rs1799983 polymorphism and hypertension: A meta-analysis involving 14,185 cases and 13,407 controls. *BMJ Cardiovasc. Disord.* 2021, 21, 385. [CrossRef] [PubMed]

22. Yang, Q.; Civelek, M. Transcription Factor KLF14 and Metabolic Syndrome. *Front. Cardiovasc. Med.* 2020, 7, 91. [CrossRef]

23. Guo, Y.; Fan, Y.; Zhang, J.; Lomberk, G.A.; Zhou, Z.; Sun, L.; Mathison, A.J.; Garcia-Barrio, M.T.; Zhang, J.; Zeng, L.; et al. Perhexiline activates KLF14 and reduces atherosclerosis by modulating ApoA-I production. *J. Clin. Investig.* 2015, 125, 3819–3830. [CrossRef] [PubMed]

24. Heidari, M.M.; Khatami, M.; Tahamtan, Y. Molecular Analysis of rs2070744 and rs1799983 Polymorphisms of NOS3 Gene in Iranian Patients With Multiple Sclerosis. *Basic Clin. Neurosci.* 2017, 8, 279–284. [CrossRef] [PubMed]

25. Wang, J.; Zhang, J.; Lomberk, G.A.; Zhou, Z.; Sun, L.; Mathison, A.J.; Garcia-Barrio, M.T.; Zhang, J.; Zeng, L.; et al. Perhexiline activates KLF14 and reduces atherosclerosis by modulating ApoA-I production. *J. Clin. Investig.* 2015, 125, 3819–3830. [CrossRef] [PubMed]

26. Wu, S.; Hsu, L.A.; Teng, M.S.; Chou, H.H.; Ko, Y.L. Differential Genetic and Epigenetic Effects of the KLF14 Gene on Body Shape Indices and Metabolic Traits. *Int. J. Mol. Sci.* 2022, 23, 4165. [CrossRef] [PubMed]

27. Igari, S.; Ohtaki, A.; Yanamaka, Y.; Sato, Y.; Yohda, M.; Noguchi, K.; Yamada, K. Properties and crystal structure of methylenetetrahydrofolate reductase from Thermus thermophilus HB8. *PloS ONE* 2011, 6, e23716. [CrossRef]

28. Saetre, P.; Grove, J.; Borglum, A.D.; Mors, O.; Verge, T.; Andreassen, O.A.; Vares, M.; Agartz, I.; Terenius, L.; Jonsson, E.G. Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and age at onset of schizophrenia: No consistent evidence for an association in the Nordic population. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 2012, 159B, 981–986. [CrossRef] [PubMed]

29. Yang, Q.; Bailey, L.; Clarke, R.; Flanders, W.D.; Liu, T.; Yesupriya, A.; Khoury, M.J.; Friedman, J.M. Prospective study of methylenetetrahydrofolate reductase (MTHFR) variant C677T and risk of all-cause and cardiovascular disease mortality among 6000 US adults. *Am. J. Clin. Nutr.* 2012, 95, 1245–1253. [CrossRef] [PubMed]

30. Liew, S.C.; Gupta, E.D. Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism: Epidemiology, metabolism and the associated diseases. *Eur. J. Med. Genet.* 2015, 58, 1–10. [CrossRef]

31. Husemoen, L.L.; Skaaby, T.; Jorgensen, T.; Thuesen, B.H.; Fenger, M.; Grarup, N.; Sandholt, C.H.; Hansen, T.; Pedersen, O.; Linneberg, A. MTHFR C677T genotype and MTHFR polymorphisms and risk of general population without mandatory folic acid fortification. *Eur. J. Nutr.* 2014, 53, 1549–1559. [CrossRef] [PubMed]

32. Improta Caria, A.C.; Nonaka, C.K.V.; Pereira, C.S.; Soares, M.B.P.; Macambira, S.G.; Souza, B.S.F. Exercise Training-Induced Changes in MicroRNAs: Beneficial Regulatory Effects in Hypertension, Type 2 Diabetes, and Obesity. *Int. J. Mol. Sci.* 2018, 19, 3608. [CrossRef] [PubMed]

33. Kim, S.Y.; Kim, A.Y.; Lee, H.W.; Son, Y.H.; Lee, G.Y.; Lee, J.W.; Lee, Y.S.; Kim, J.B. miR-27a is a negative regulator of adipocyte differentiation via suppressing PGAR expression. *Biochem. Biophys. Res. Commun.* 2010, 392, 323–328. [CrossRef] [PubMed]

34. Chandra, M.; Miriyala, S.; Panchatcharam, M. PGAR expression and Its Role in Cardiovascular Diseases. *PPAR Res.* 2017, 2017, 6404638. [CrossRef]

35. Cai, M.Y.; Cheng, J.; Zhou, M.Y.; Liang, L.L.; Lian, S.M.; Xie, X.S.; Xu, S.; Liu, X.; Xiong, X.D. The association between pre-miR-27a rs985819 polymorphism and myocardial infarction risk in a Chinese Han population. *Lipids Health Dis.* 2018, 17, 7. [CrossRef] [PubMed]

36. Liu, Y.; Gyi, Y.F.; Liao, W.Y.; Zhang, Y.Q.; Zhang, X.B.; Huang, Y.P.; Wu, F.M.; Huang, Z.; Lu, Y.F. Association between miR-27a rs985819 polymorphism and breast cancer susceptibility: Evidence based on 6118 cases and 7042 controls. *Medicine* 2021, 100, e23834. [CrossRef]
37. Charmet, R.; Duffy, S.; Keshavarzi, S.; Gyorgy, B.; Marre, M.; Rossing, P.; McKnight, A.J.; Maxwell, A.P.; Ahluwalia, T.V.S.; Paterson, A.D.; et al. Novel risk genes identified in a genome-wide association study for coronary artery disease in patients with type 1 diabetes. Cardiovasc. Diabetol. 2018, 17, 61. [CrossRef]

38. Bouzidi, N.; Hassine, M.; Fodha, H.; Ben Messaoud, M.; Maatouk, F.; Gami, H.; Ferchichi, S. Association of the methylene-tetrahydrofolate reductase gene rs1801133 C677T variant with serum homocysteine levels, and the severity of coronary artery disease. Sci. Rep. 2020, 10, 10064. [CrossRef]

39. Rahguebeer, S.; Matsha, T.E. Methyleneetetrahydrofolate (MTHFR), the One-Carbon Cycle, and Cardiovascular Risks. Nutrients 2021, 13, 4562. [CrossRef]

40. Crider, K.S.; Yang, T.P.; Berry, R.J.; Bailey, L.B. Folate and DNA methylation: A review of molecular mechanisms and the evidence for folate's role. Adv. Nutr. 2012, 3, 21–38. [CrossRef]

41. Vidmar Golja, M.; Smid, A.; Karas Kuzelicki, N.; Trontelj, J.; Gersak, K.; Mlinaric-Rascan, I. Folate Insufficiency Due to MTHFR Deficiency Is Bypassed by 5-Methyltetrahydrofolate. J. Clin. Med. 2020, 9, 2836. [CrossRef] [PubMed]

42. Vaccaro, J.A.; Naser, S.A. The Role of Methyl Donors of the Methionine Cycle in Gastrointestinal Infection and Inflammation. Healthcare 2021, 10, 61. [CrossRef] [PubMed]

43. Baba, S.M.; Shah, Z.A.; Javaid, K.; Pandith, A.A.; Rasool, J.; Geelani, S.A.; Baba, R.A.; Amin, S.; Mohammad, G. Methylenetetrahydrofolate dehydrogenase gene C677T polymorphism and type 2 diabetes mellitus (T2DM) susceptibility. Mol. Genet. Genom. Med. 2019, 7, e1020. [CrossRef]

44. Chen, C.J.; Yang, T.C.; Chang, C.; Lu, S.C.; Chang, P.Y. Homocysteine is a bystander for ST-segment elevation myocardial infarction: A case-control study. BMC Cardiovasc. Disord. 2018, 18, 33. [CrossRef] [PubMed]

45. Mir, R.; Elfaki, I.; Frah, E.A.M.; Alzahrani, K.J.; Mir, M.M.; Banu, S. Clinical correlations of Lipid Profiles with the Age and Gender in the Coronary Artery Disease Patients: A study of 3878 CAD patients from India. Endocr. Metab. Immune Disord.-Drug Targets 2022, 22, 440–452. [CrossRef] [PubMed]

46. Nelson, R.H. Hyperlipidemia as a risk factor for cardiovascular disease. Prim. Care 2013, 40, 195–211. [CrossRef]

47. Strang, F.; Schunkert, H. C-reactive protein and coronary heart disease: All said–is not it? Mediat. Inflamm. 2014, 2014, 757123. [CrossRef]

48. Fioranelli, M.; Bottaccioli, A.G.; Bottaccioli, F.; Bianchi, M.; Rovesti, M.; Roccia, M.G. Stress and Inflammation in Coronary Artery Disease: A Review Psychoneuroendocrineimmunology-Based. Front. Immunol. 2018, 9, 2031. [CrossRef]

49. Leclerc, D.; Christensen, K.E.; Cauvi, O.; Yang, E.; Fournelle, F.; Bahous, R.H.; Malysheva, O.V.; Deng, L.; Wu, Q.; Zhou, Z.; et al. Mild Methyleneetetrahydrofolate Reductase Deficiency Alters Inflammatory and Lipid Pathways in Liver. Mol. Nutr. Food Res. 2019, 63, e1801001. [CrossRef]

50. Christensen, K.E.; Mikael, L.G.; Leung, K.Y.; Levesque, N.; Deng, L.; Wu, Q.; Malyshewa, O.V.; Best, A.; Caudill, M.A.; Greene, N.D.; et al. High folic acid consumption leads to pseudo-MTHFR deficiency, altered lipid metabolism, and liver injury in mice. Am. J. Clin. Nutr. 2015, 101, 646–658. [CrossRef]

51. Moll, S.; Varga, E.A. MTHFR Mutations. Circulation 2015, 132, e6–e9. [CrossRef] [PubMed]

52. Chen, H.; Wei, F.; Wang, L.; Zhang, Z.; Meng, J.; Jia, L.; Sun, G.; Zhang, R.; Li, B.; Yu, H.; et al. MTHFR gene C677T polymorphism and type 2 diabetic nephropathy in Asian populations: A meta-analysis. Int. J. Clin. Exp. Med. 2015, 8, 3662–3670. [PubMed]

53. Ward, M.; Hughes, C.F.; Strain, J.J.; Reilly, R.; Cunningham, C.; Moll, A.M.; Horigan, K.; Casey, M.; McCarrick, K.; O’Kane, M.; et al. Impact of the common MTHFR 677C→T polymorphism on blood pressure in adulthood and role of riboflavin in modifying the genetic risk of hypertension: Evidence from the JINGO project. BMC Med. 2020, 18, 318. [CrossRef] [PubMed]

54. Weber, T.; Lang, I.; Zweiker, R.; Horn, S.; Wenzel, R.R.; Watschinger, B.; Slany, J.; Eber, B.; Roithinger, F.X.; Metzler, B. Hypertension and coronary artery disease: Epidemiology, physiology, effects of treatment, and recommendations: A joint scientific statement from the Austrian Society of Cardiology and the Austrian Society of Hypertension. Wien. Klin. Wochenschr. 2018, 130, 467–479. [CrossRef] [PubMed]

55. Huang, P.; Yin, R.X.; Huang, K.K.; Zeng, X.N.; Guo, T.; Lin, Q.Z.; Wu, J.; Wu, D.F.; Li, H.; Pan, S.L. Association of the KLF14 rs4731702 SNP and serum lipid levels in the Guangxi Mulao and Han populations. BioMed Res. Int. 2015, 2015, 646–658. [CrossRef] [PubMed]

56. Xu, Q.; Chen, T.J.; He, C.Y.; Sun, L.P.; Liu, J.W.; Yuan, Y. MiR-27a rs895819 is involved in increased atherosclerotic gastritis risk, improved gastric cancer progression and negative interaction with Helicobacter pylori. Sci. Rep. 2017, 7, 14307. [CrossRef] [PubMed]

57. Yang, Z.; Cappello, T.; Wang, L. Emerging role of microRNAs in lipid metabolism. Acta Pharm. Sin. B 2015, 5, 145–150. [CrossRef]

58. Shirasaki, T.; Honda, M.; Shimakami, T.; Horii, R.; Yamashita, T.; Sakai, Y.; Sakai, A.; Okada, H.; Watanabe, R.; Murakami, S.; et al. MicroRNA-27a regulates lipid metabolism and inhibits hepatitis C virus replication in human hepatoma cells. J. Virol. 2013, 87, 5270–5286. [CrossRef]
62. Yan, H.; Cheng, L.; Jia, R.; Yao, H.; Wu, H.; Shen, Y.; Zhang, Y.; Hao, P.; Zhang, Z. ATP-binding cassette sub-family a member1 gene mutation improves lipid metabolic abnormalities in diabetes mellitus. *Lipids Health Dis.* 2019, 18, 103. [CrossRef] [PubMed]

63. Chen, T.; Zhang, Y.; Liu, Y.; Zhu, D.; Yu, J.; Li, G.; Sun, Z.; Wang, W.; Jiang, H.; Hong, Z. MiR-27a promotes insulin resistance and mediates glucose metabolism by targeting PPAR-gamma-mediated PI3K/AKT signaling. *Aging* 2019, 11, 7510–7524. [CrossRef] [PubMed]

64. Ghaedi, H.; Tabasinezhad, M.; Alipoor, B.; Shokri, F.; Movafagh, A.; Mirfakhraie, R.; Omrani, M.D.; Masotti, A. The pre-mir-27a variant rs895819 may contribute to type 2 diabetes mellitus susceptibility in an Iranian cohort. *J. Endocrinol. Invest.* 2016, 39, 1187–1193. [CrossRef] [PubMed]

65. Kondkar, A.A.; Azad, T.A.; Sultan, T.; Osman, E.A.; Almobarak, F.A.; Al-Obeidan, S.A. Association of endothelial nitric oxide synthase (NOS3) gene polymorphisms with primary open-angle glaucoma in a Saudi cohort. *PLoS ONE* 2020, 15, e0227417. [CrossRef] [PubMed]

66. Zhuo, M.L.; Huang, Y.; Chen, J.Z.; Sun, L.H.; Yang, R.F.; Chen, H.Z.; Lv, X.; Li, H.L.; Wei, Y.S.; Liu, G.; et al. Endothelium-specific overexpression of human IC53 downregulates endothelial nitric oxide synthase activity and elevates systolic blood pressure in mice. *Cardiovasc. Res.* 2009, 84, 292–299. [CrossRef] [PubMed]

67. Wang, X.L.; Sim, A.S.; Wang, M.X.; Murrell, G.A.; Trudinger, B.; Wang, J. Genotype dependent and cigarette specific effects on endothelial nitric oxide synthase gene expression and enzyme activity. *FEBS Lett.* 2000, 471, 45–50. [CrossRef] [PubMed]

68. Karimi, Y.; Sehati, F.; Sarreshtedari, A.; Mirzad, M.; Khalili, Y.; Kiani, R.; Taheri Baigan, E.; Hosseini Moghadam, M.; Mehrvarz, F.; Bakhshandeh, H.; et al. Endothelial nitric oxide synthase Asp298Glu (894G/T) gene polymorphism as a possible risk factor for the coronary slow flow phenomenon among Iranians. *BMC Cardiovasc. Disord.* 2022, 22, 300. [CrossRef] [PubMed]

69. Fragoza, R.; Das, J.; Wierbowski, S.D.; Liang, J.; Tran, T.N.; Liang, S.; Beltran, J.F.; Rivera-Erick, C.A.; Ye, K.; Wang, T.Y.; et al. Extensive disruption of protein interactions by genetic variants across the allele frequency spectrum in human populations. *Nat. Commun.* 2019, 10, 4141. [CrossRef] [PubMed]

70. Elfaki, I.; Knitsch, A.; Matena, A.; Bayer, P. Identification and characterization of peptides that bind the PPIase domain of Parvulin17. *J. Pept. Sci.* 2013, 19, 362–369. [CrossRef] [PubMed]

71. Luo, Z.; Jia, A.; Lu, Z.; Muhammad, I.; Adenrele, A.; Song, Y. Associations of the NOS3 rs1799983 polymorphism with circulating nitric oxide and lipid levels: A systematic review and meta-analysis. *Postgrad. Med. J.* 2019, 95, 361–371. [CrossRef] [PubMed]

72. Arafa, S.; Abdelsalam, S.; El-Gilany, A.H.; Mosaad, Y.M.; Abdel-Ghaffar, A. Endothelial nitric oxide synthase Glu 298 Asp (G894T) and Apolipoprotein E gene polymorphism as possible risk factors for coronary heart disease among Egyptians. *Egypt. Heart J.* 2018, 70, 393–401. [CrossRef] [PubMed]

73. Sansbury, B.E.; Cummins, T.D.; Tang, Y.; Hellmann, J.; Holden, C.R.; Harbeson, M.A.; Chen, Y.; Patel, R.P.; Spite, M.; Bhatnagar, A.; et al. Overexpression of endothelial nitric oxide synthase prevents diet-induced obesity and regulates adipocyte phenotype. *Circ. Res.* 2012, 111, 1176–1189. [CrossRef] [PubMed]

74. Chen, F.; Li, Y.M.; Yang, L.Q.; Zhong, C.G.; Zhuang, Z.X. Association of NOS2 and NOS3 gene polymorphisms with susceptibility to type 2 diabetes mellitus and diabetic nephropathy in the Chinese Han population. *IUBMB Life* 2016, 68, 516–525. [CrossRef] [PubMed]

75. Garme, Y.; Saravani, R.; Galavi, H.R. Association of nitric oxide synthase 3 gene polymorphism with the risk of type 2 diabetes. *Biomed. Rep.* 2017, 7, 85–89. [CrossRef] [PubMed]

76. Moris, D.; Spartalis, M.; Spartalis, E.; Karachaliou, G.S.; Karaolannis, G.I.; Tsourouflis, G.; Tsilimigras, D.I.; Tzatzaki, E.; Theocharis, S. The role of reactive oxygen species in the pathophysiology of cardiovascular diseases and the clinical significance of myocardial redox. *Ann. Transl. Med.* 2017, 5, 326. [CrossRef] [PubMed]

77. Jobgen, W.S.; Fried, S.K.; Fu, W.J.; Meininger, C.J.; Wu, G. Regulatory role for the arginine-nitric oxide pathway in metabolism of energy substrates. *J. Nutr. Biochem.* 2006, 17, 571–588. [CrossRef] [PubMed]