The Review Of
DIABETIC
STUDIES OPEN ACCESS

# Genetic Association Of Arginase 1 Gene Polymorphism (Rs60389358, And Rs2781666) With Valvular Heart Diseases Of Type 2 Diabetic

**Shaema Saadon Fadhil [1], and Jwan A. Zainulabdeen[2]**

*[1,2] Department of Chemistry, College of Sciences, University of Baghdad, Baghdad, Iraq. [1] E- mail: shimaa.s@sc.uobaghdad.edu.iq*
*[2] E- mail: jwanbiochem@yahoo.com*
**Corresponding Author E- mail: shimaa.s@sc.uobaghdad.edu.iq*

## Abstract

**Background:** Most prevalent metabolic disease (Type 2 diabetes; T2DM) can accelerate the development of valvular heart diseases (VHD) and that Arginase 1(Arg1) is an important enzyme involved in many metabolic pathways. **Objective**. The association of Arg1 genetic polymorphisms as well as its functional effects on VHD with and without T2DM were estimated. **Methods:** samples of blood were collected from T2DM, heart valves with T2DM (VHDM) and without (VHD) patients, as well healthy controls. In this work, two Arg1 SNPs (rs60389358 and rs2781666) and their sequencing were examined to assess the allele frequencies in addition to the genotype for the chosen collections. **Results:** Data showed that the genotype as well as the allele of both SNPs were non- significant (P >0.05) for T2DM and VHD group compared to controls. However, the C and T allele frequencies for the rs60389358 SNP in VHDM patients were significantly different; OR and 95% Cl (0.34, (0.12 -0.92) and 2.96(1.09 -8.01), P < 0.05 respectively) compared to controls. Moreover, the rs2781666 SNP in VHDM patients was significant with controls comparison (in genotype frequencies in GG and TT genotype; OR,,and 95% Cl (0.14 ,(0.03-0.65) ,and 7.36,(1.34- 40.55),P < 0.05 ,respectively ). As well, the G and T allele; OR,and 95%CI (0.21, (0.08-0.53) , and (4.90 ,(1.90-12.67),P < 0.001 ,respectively in comparison with controls**. Conclusions:** Genetic variations of the Arg1 gene may play a role as a potential predisposing factor for T2DM disease complications and its progression to VHD susceptibility risk in the Iraqi population.

**Keywords:** Arginase1**,** Polymorphism, Type 2 diabetes, and valvular heart diseases.

## Introduction

Type 2 diabetes mellitus (its abbreviation is T2DM) considered as one of the most common and widespread chronic metabolic diseases that characterized by high blood glucose levels due to insulin resistance in peripheral tissues (muscles and liver) with progressive impaired the secretion of insulin via pancreatic beta cells [1-2]. Environmental and genetic factors participate in the onset and progress of this disease, which is often related to obesity, physical inactivity, unhealthy diet, and the genetic influences inherited from the family members [3-4]. This disease damages to blood vessels due to inflammation, endothelial dysfunction, and oxidative stress, leading to serious complications, including atherosclerosis, myocardial infarction, stroke, and valvular heart diseases [5-6]. Meanwhile, heart disease is more likely to occur in people with diabetes at a younger age than in those without diabetes. Therefore, diabetes adults are about twice probably to develop stroke or heart diseases than non-diabetic adults [7].

The valvular heart disease (VHD) considered as a prevalent form of cardiovascular diseases (CVD), that leads to death and disability worldwide. An increase in the prevalence VHD among adults over 18 years of age has been associated with age, which currently stands at 2.5% [8]. The four heart valves work to move the blood in the right direction. The most common type of VHD worldwide is aortic (AV) and mitral valve (MV) disease because pressure is higher on the left side of the heart. This may have two effects: the valves lose their elasticity and may not close or open adequately, and regurgitation

occurs, impairing the heart's ability to pump blood [9]. Valves are influenced by numerous environmental factors like smoking status and alcohol consumption; account for 34% of all cases of native aortic disease [10-11]. Genomics' functional involvem -ent in research is believed to contribute to an estimated incidence of CVD between 20% and 60% [12]. Therefore, it is possible that VHD are transmitted from parents to children, as the inheritance rate is estimated at 89% [13].

Liver extensively expressed Arginase 1(Arg1) which is located on 6q 23, it is accountable in urea cycle, furthermore it present in multiple extra hepatic tissues, such as cardiovascular system, in endothelial cells, vascular smooth muscle cells, and macrophages [5]. It has an important role in the initiation, growth, and complications of much diseases including diabetic and coronary heart diseases [14]. Moreover, Arg 1 over expression could be harmful by cases platelet aggregation, vascular smooth muscle cell proliferation, and leukocyte adhesion leading to dysfunction of the endothelial; noted in vascular disorders like VHD [15]. Where, synthase nitric oxide endothelial and Arg 1 compete on L-arginine, which may be a factor in this effect [5]. The human Arg1 gene was examined for single nucleotide polymorphisms (SNPs) different populations are linked CVD. There have been reports of common SNPs in Arg1 rs278166 G/T conflicting observations [16]. The aim of the present work is to explore the association between Arg 1 gene (rs60389358 and rs2781666 SNPs) in VHD Iraqi diabetic and non-diabetic patients

## Material and method

**Blood samples:** The samples (blood) were collected from the participants including T2DM, and VHD patients who visited the Ibn Al Bitar Cardiac Surgery Center in Baghdad, Iraq. The study included 80 people who were divided into: The patients were 20 people with T2DM, 20 people with VHD of two types (AV and MV) without T2DM(VHD), 20 people VHD with T2DM(VHDM), as well as 20 healthy controls (C), during the period from October 2023 until April 2024. Age and sex were matched between samples. Smoking status and alcohol consumption were eliminated. The protocol of this work was approved by Research Ethics Committee from college of Science/University of Baghdad (Ref. CSEC /1123/0114 on November 19,2023).

**Genomic DNA extraction:** Total genomic DNA of blood samples was extracted via the Norgen (Canada); the kit of blood DNA extraction.

**Primer Sequence:** A newly designed SNPs primer by National Center for Biotechnology Information (NCBI) from (Macrogen Corporation - Korea)., gene number NG-007086were selected. The primer set sequence , the gene and SNPs names, the products sizes of the polymerase chain reaction (PCR) were listed in Table 1 .

**Table 1: Oligonucleotide primer used for the amplification of Arginase 1 gene**

| SNP name | The primer | The sequence ( 5`- 3`) | The Tm ($^0$C) | The product Size (bp.) | Reference |
|---|---|---|---|---|---|
| rs60389358 rs2781666 | Forward primer | TTCACACATGAGGGTAA ATGG | 56.1 | 661 | Newly Design |
| | Reverse primer | GGGGATACAGCAGACAA AATT | 56.5 | | |

**The PCR**: A total volume of (25 µL) was performed for this reaction; Table 2 show the cycle parameters. To separate PCR products and ladder marker; an one % agarose gel was used for electrophoresis (60 minutes at 115 volts). The gel was examined (After staining via Red Safe dye) under a UV transilluminator of the gel documentation system (using a 100 bp DNA; the molecular size of the bands was estimated).

**Table 2: PCR conditions for amplification of Arg1 promoter**

| Cycle No. | Stage | Temperature(ºC) | Time |
|---|---|---|---|
| 1 | The initial Denaturation | 94 | 3 minutes |
| 35x | The denaturation | 94 | 30 seconds |
| | The annealing | 50 | 30 seconds |
| | The extension | 70 | 45 seconds |
| 1 | The final Extension | 70 | 7 minutes. |
| | The hold | 4 | 7 minutes. |

**Single-nucleotide polymorphism selection and genotyping:** The amplification products for the Arg 1 gene were sent to Korea Genetics for Sanger sequencing to determine SNPs. Sequence analysis of the FASTA files was performed (http://www.geneious.com) ; the Geneious software.

**Statistical Analysis:** The statistical comparisons between the groups were done by applying the SPSS (version 22). The Hardy-Weinberg equilibrium (HWE)was applied to investigate the allelic frequencies. Moreover, a number of methodologies like: Fisher's exact test, Pearson Chi-Square, Student's t-test, and genotype-phenotype association were applied for identifying genotypes in group's samples.

**Results and Discussion**

Agarose gel electrophoresis for Arg1 gene PCR amplified products (SNPs rs60389358, and rs2781666) showed a single band of 661bp in size, as shown in Figure 1.

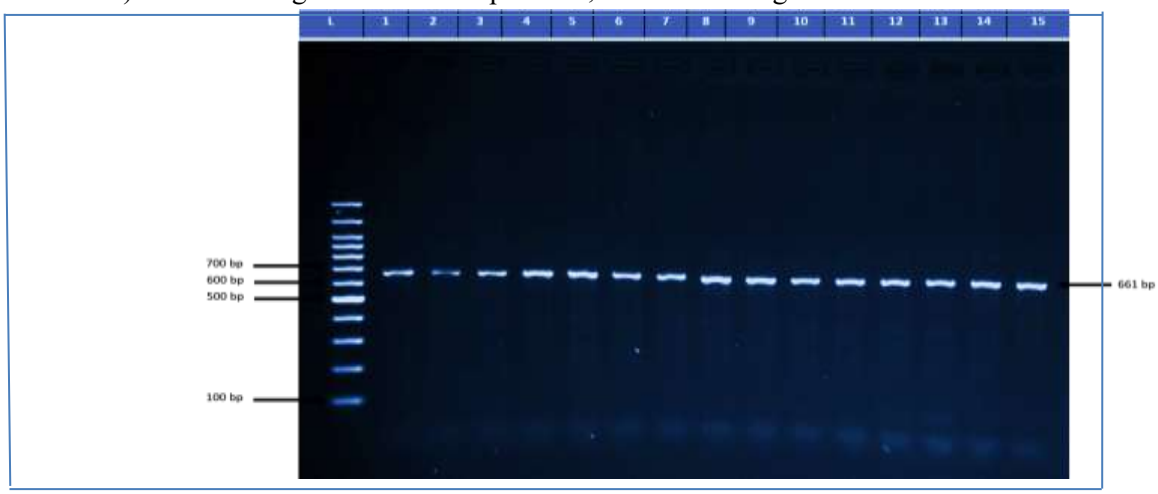


**Figure 1:** The gel electrophoresis for Arg1 gene PCR products (SNP rs60389358 and rs2781666) on 1% agarose at 115V for 60 minutes showing bands of 661 bp (Lane L: 100bp DNA ladder; Lanes 1-4: samples of controls; Lanes 5-7: samples of T2DM ;Lanes 8-11:samples of VHD without T2DM patients ;Lanes 12-15:samples of VHD with T2DM patients).

These Two SNPs with polymorphic frequencies (rs60389358, [C/T]; Chromosome 6:13189 3557; Nucleotide Location 4193) and (rs27816 66 [G/C/T]; Chromosome 6:131893559; Nucleotide Location 4195) were assigned in the DNA sequence of the PCR-amplified region (661 bp). Two SNP sequencing results are noted in Figure 2, which had the genotype C/T and G/T, respectively.

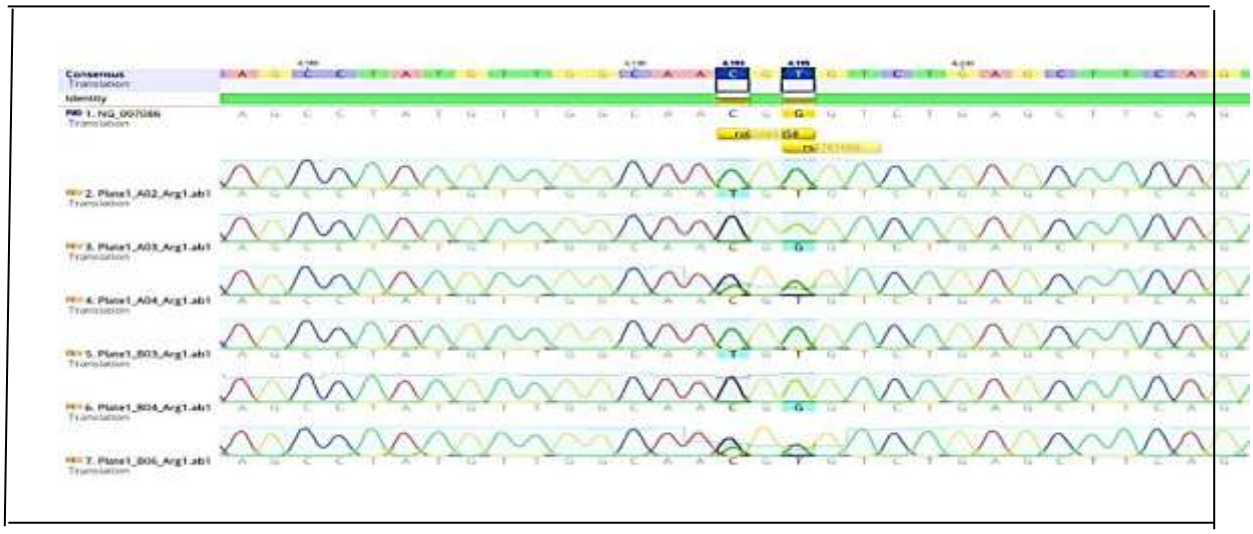

**Figure 2:** Allelic variation analysis of (rs60389358 and rs2781666) SNPs located in Arg1 gene using Sanger sequencing method.

In the present study, the distribution of both alleles and genotypes frequencies of Arg1 SNPs (rs60389358, and rs2781666) genotypes among Iraqi patients with T2DM, VHD, VHDM and C groups. The possible association of these two SNPs of Arg1 gene with susceptibility to these diseases has been investigated.

The allocations of genotype as well as allele frequencies of (rs60389358, and rs2781666 SNPs) between T2DM and C groups are reported in following Table (Table 3).

**Table 3: The genotype with allele frequencies for the two SNPs of ARG1 gene and their HWE inT2DM patients and controls.**

| Arg1 gene SNPs Genotype and allele frequency | T2DM Patients N=20 (%) | Controls N=20(%) | OR | 95%CI | P-value |
|---|---|---|---|---|---|
| **rs60389358 genotype frequency** | | | | | |
| **CC** | 10(50%) | 13(65%) | 0.54 | 0.15 -1.92 | 0.3394NS |
| **CT** | 8(40%) | 6(30%) | 1.56 | 0.42 - 5.76 | 0.5084NS |
| **TT** | 2(10%) | 1(5%) | 2.11 | 0.18 - 25.35 | 0.5557NS |
| **HWE P–value** | 0.8314NS | 0.7799NS | -- | -- | -- |
| **rs60389358 allele frequency** | | | | | |
| **C** | 28(70%) | 32(80%) | 0.58 | 0.21 - 1.63 | 0.3043NS |
| **T** | 12(30%) | 8(20%) | 1.71 | 0.61-4.79 | 0.3043NS |
| **rs2781666 genotype frequency** | | | | | |
| **GG** | 12(60%) | 11(55%) | 1.23 | 0.35 - 4.31 | 0.7492NS |
| **GT** | 4(20%) | 7(35%) | 0.46 | 0.11- 1.94 | 0.2930NS |
| **TT** | 4(20%) | 2(10%) | 2.25 | 0.36- 13.97 | 0.3841NS |
| **HWE P–value** | 0.0192* | 0.5846NS | -- | -- | -- |
| **rs2781666 allele frequency** | | | | | |
| **G** | 28(70%) | 29(72.5%) | 0.89 | 0.34 - 2.33 | 0.8049NS |
| **T** | 12(30%) | 11(27.5%) | 1.13 | 0.43 - 2.98 | 0.8049NS |

Single nucleotide polymorphism (SNPs), Reference SNP cluster ID (rs), Confidence interval (CI), Odds ratio (OR), Hardy-Weinberg equilibrium (HWE), Significant* ($P \leq 0.05$), Significant** ($P \leq 0.01$), Non-significant(NS)**.**

In the case of (rs60389358) SNPs for T2DM patients in comparison with C group:

The study found non-significant analysis (P> 0.05) in genotype frequency as well as allele frequency in heterozygote CT and homozygotes (mutant) CC, TT. The genotype frequency of CC for T2DM decreased in comparison with the C group (50% and 65%), while it increased in CT (40% and 30%) and TT (10% and 5%) genotypes. On the other hand, the odd ratio, and 95% CI were 0.54 (0.15 -1.92), respectively for CC, whilst 1.56 (0.42 - 5.76), respectively for CT, and 2.11 (0.18 - 25.35), for TT genotype respectively. Furthermore, the allele frequency distribution of this SNP polymer -phism in T2DM, and C groups were (70%, and 80%) for C allele and for T allele were (30, and 20%); the increasing frequency of T allele in T2DM was in parallel with decreasing in C allele frequency, in contrast differences in the two allele frequencies were noted for C group. Concerning the odd ratio, and 95% CI were 0.58(0.21 - 1.63), respectively for C allele, as well they were 1.71(0.61 - 4.79), respectively for T allele. Fisher's exact possibility assessment of a relationship's significance was selected because it permits possibility correction and is not impacted by small numbers (less than five). The (rs60389358) SNP distributions was non significantly (P>0.05) deviate from HWE in T2DM patient and also for C groups.

Arg1 SNP (rs2781666) is associated with different genotypes and alleles as shown in Table 3. homozygotes GG, Heterozygote mutant GT and homozygotes mutant TT, as well as allele G and T indicated non-significantly relation with T2DM patients (P>0.05). The genotype frequency of GT for T2DM decreased in comparison with the C group (20% and 35%), while it increased in GG (60% and 55%) and TT (20% and 10%) genotypes. Either, the genotype results were (GG genotype: OR 1.23, 95% CI 0.35 - 4.31, GT genotype: OR 0.46, 95%CI 0.11 - 1.94) and (TT genotype: OR 2.25, 95%CI 0.36- 13.97). On other hand, the allele frequency distribution of this SNP polymorphism in T2DM, and C groups were (70%, and 72.5%) for G allele and for T allele were (30%, and 27.5%); the increasing frequency of T allele in T2DM was in parallel with decreasing in C allele frequency, in contrast differences in the two allele frequencies were noted for C group. Concerning the odd ratio, and 95%CI were 0.89(0.34 - 2.33), respectively for G allele, as well they were 1.13(0.43 - 2.98), respectively for T allele. Table 3 indicates that only T2DM patients with Arg1 SNPs (rs2781666) were in HWE with (P≤0.05). The distributions of genotype as well as allele frequencies of (rs60389358, and rs2781666 SNPs) are compared between VHD patients and C groups. As shown in the following Table (Table 4).

**Table 4: The genotype with allele frequencies for the two SNPs of ARG1 gene and their HWE in VHD patients and controls.**

| Arg1 gene SNPs Genotype and allele frequency | Patient (VHD) N=20 (%) | Controls N=20(%) | OR | 95%CI | P-value |
|---|---|---|---|---|---|
| **rs60389358 genotype frequency** | | | | | |
| **CC** | 12(60%) | 13(65%) | 0.81 | 0.22 - 2.91 | 0.326NS |
| **CT** | 5(25%) | 6(30%) | 0.78 | 0.19 - 3.13 | 0.7235NS |
| **TT** | 3 (15%) | 1(5%) | 3.35 | 0.32 - 35.37 | 0.3142NS |
| **HWE P–value** | 0.0953NS | 0.7799NS | -- | -- | **--** |
| **rs60389358 allele frequency** | | | | | |
| **C** | 29(72.5%) | 32(80%) | 0.66 | 0.23-1.87 | 0.4321NS |
| T | 11(27.5%) | 8(20%) | 1.52 | 0.54 - 4.29 | 0.4321NS |
| **rs2781666 genotype frequency** | | | | | |
| **GG** | 6(30%) | 11(55%) | 0.35 | 0.10 - 1.29 | 0.1142NS |
| **GT** | 9(45%) | 7(35%) | 1.52 | 0.43 - 5.43 | 0.5195NS |
| **TT** | 5(25%) | 2(10%) | 3.00 | 0.51 - 17.74 | 0.2257NS |
| **HWE P–value** | 0.662NS | 0.5846NS | -- | -- | **--** |
| **rs2781666 allele frequency** | | | | | |
| **G** | 21(52.5%) | 29(72.5%) | 0.42 | 0.17 - 1.06 | 0.0672NS |
| **T** | 19(47.5%) | 11(27.5%) | 2.39 | 0.94 - 6.05 | 0.0672NS |

Inspecting rs60389358 SNP (C/T) and rs2781666 (G/T) of the Arg1 gene in VHD patients and controls revealed non- significant (P>0.05) differences in the distribution of both frequencies (genotype and allele).

In rs60389358 SNP: CC and CT genotypes frequencies decreased in VHD compared to the C group (60%,65%) and (25%,30%), respectively. while the frequency increased in the TT genotype (15%,5%). Concerning the odd ratio, and 95% CI were 0.81(0.22 - 2.91), respectively for CC, as well they were 0.78 (0.19 - 3.13), respectively for CT genotype, while TT genotype were 3.35 (0.32 - 35.37). However, a decreased frequency of C allele and an increased frequency of T allele were observed in VHD patients, respectively. On the other hand, odd ratio of C allele was 0.66 (0.23 - 1.87), respectively and 1.52(0.54 - 4.29) for T allele, As shown in the Table 4. The rs60389358 SNP distributions did not significantly (P > 0.05) deviate from HWE in the VHD patient and C groups. Also the study examined the genotype and alleles frequencies of the Arg1 SNP rs27816666 and found that there were less homozygous GG than expected (30%), while there were more in heterozygotes GT and homozygotes mutant TT (45% and 25%), respectively in VHD patients than C group. The GG genotype an odd ratio, and 95% CI were 0.35(0.10 -1.29), respectively, while 1.52 (0.43 - 5.43), respectively for GT, whilst 3.00(0.51 - 17.74), respectively for TT genotype. The G allele and T mutant were found in (52.5%) and (47.5%) of VHD patients and (72.5%), (27.5%) for controls, respectively; between the two groups, frequency differences were statistically (P>0.05) non-significant. On the other hand, odd ratio of G was 0.42 (0.17 - 1.06), respectively and 2.39(0.94 - 6.05) for T allele. The rs2781666 SNPs distributions did not significantly P>0.05 deviate from HWE in VHD patient and C group. So that the OR of TT genotype higher than genotype GT for SNPs. Although there are no significant statistical differences between patients and control.

Moreover, genotype and allele frequencies Arg1 SNP (rs60389358) and (rs2781666) were studied for VHDM patients and compared with C group, as shown in the following Table (Table 5).

**Table 5: The genotype with allele frequencies for the two SNPs of ARG1 gene and their HWE in VHDM patients and controls.**

| Arg1 gene SNPs Genotype and allele frequency | Patient (VHDM) N=20 (%) | Controls N=20(%) | OR | 95%CI | P-value |
|---|---|---|---|---|---|
| **rs60389358 genotype frequency** | | | | | |
| **CC** | 7(35%) | 13(65%) | 0.29 | 0.08 - 1.06 | 0.0618NS |
| **CT** | 9(45%) | 6(30%) | 1.91 | 0.52 - 7.00 | 0.3297NS |
| **TT** | 4(20%) | 1(5%) | 4.75 | 0.48 - 46.91 | 0.1823NS |
| **HWE P–value** | 0.7229NS | 0.7799NS | -- | --- | --- |
| **rs60389358 allele frequency** | | | | | |
| **C** | 23(57.5%) | 32(80%) | 0.34 | 0.12 - 0.92 | 0.0330* |
| **T** | 17(42.5%) | 8(20%) | 2.96 | 1.09 - 8.01 | 0.0330* |
| **rs2781666 genotype frequency** | | | | | |
| **GG** | 3(15%) | 11(55%) | 0.14 | 0.03-0.65 | 0.0121* |
| **GT** | 8(40%) | 7(35%) | 1.24 | 0.34-4.46 | 0.7441NS |
| **TT** | 9(45%) | 2(10%) | 7.36 | 1.34-40.55 | 0.0218* |
| **HWE P–value** | 0.5888NS | 0.5846NS | --- | --- | --- |
| **rs2781666 allele frequency** | | | | | |
| **G** | 14(35%) | 29(72.5%) | 0.21 | 0.08-0.53 | 0.0011** |
| **T** | 26(65%) | 11(27.5%) | 4.90 | 1.90-12.67 | 0.0011** |

The results indicate that there were non- significant (P>0.05) differences frequencies in homozygotes CC, heterozygotes CT and homozygotes mutant TT for rs60389358 SNP between VHDM patients and C group, as indicated in above Table. Whilst CC genotype frequency is less in VHDM patients compared to control. While CT and TT genotype had higher levels in patients. Patients VHDM have frequency 35% and OR of 0.29 (0.08 - 1.06) for CC genotype, while 45% have OR of 1.91 (0.52 - 7.00)

for the CT genotype, whereas 20% and OR of 4.75(0.48 - 46.91) for TT genotype. The C allele was found in (57.5%) and OR of 0.34 (0.12 - 0.92), respectively, while 42.5% and OR of 2.96 (1.09 - 8.01), respectively for T allele mutant; difference in frequency were significant ( P<0.05 )between the two mentioned groups, but the distributions of rs60389358 SNPs differ (HWE ) but not significantly .

The case of rs2781666 SNP for VHDM patients in comparison with C group: The study found significant analysis (P< 0.05) in genotype frequencies in homozygotes GG and heterozygote mutant TT, while non-significant in heterozygote GT. The genotype frequency of GG for VHDM decreased in comparison with the C group (15% and 55%), while it increased in GT (40% and 35%) and TT (45% and 10%) genotypes. On the other hand, the OR, and 95% CI were 0.14(0.03 -0.65), respectively for GG genotypes, whilst 1.24 (0.34 - 4.46), respectively for GT genotypes, and 7.36 (1.34 - 40.55), respectively for TT genotypes. The G allele frequency was found (35%) and (72.5%), respectively. While, T allele was (65%) and (27.5%) in VHDM patients and C group, respectively. The results indicate a highly significant (P<0.001) difference between the two groups, Whereas frequency were decrease for G allele and increase for T allele. On the other hand, the OR, and 95% CI were 0.21(0.08-0.53), 4.90(1.90-12.67), respectively.The rs2781666 SNP distributions deviate but did significantly (P>0.05) from HWE in the VHDM patient as well as C groups
, as indicated by the OR of TT genotype (higher than GT). Present study demonstrated an observed relation of the rs2781666 SNP with the occurrence of VHDM disease.

**Discussion**

As far as we know, the current study was the first that investigate the relation of (rs60389358 and rs2781666) SNPs for Arg1 gene in the T2DM, VHD, and VHDM diseases, therefore, it was important to study their relation with the diseases in Iraqi patients. According to previous results shown in Table 3, it was discovered that T2DM patients had higher levels of heterozygote CT, homozygote mutant TT genotype and mutant T allele than control indicating that the (rs60389358) SNP may be a possible risk factors for T2DM patients. The reports regarding Arg1 genetic variants associated with T2DM are still scarce. Our finding is correspond with the only previous study conducted in patients with atherosclerosis[5], As well as, results appears that may lead to T2DM when patients are homozygotes of mutant TT(OR was 2.25) more from when patients are heterozygote of mutant GT(OR was 0.46) for SNP rs2781 666.These results are consistent with a previous work by Shah et al. on Pakistani T2DM patients that show a significant association of the Arg1 gene SNP (rs2781666) with T2DM, they found an elevation in Arg1 levels in T2DM patients with variant genotypes for the SNP [17] . Where Polymorphism frequencies and their effects on disease are known to be influenced by ethnic background, giving a suggestion that the existence of T allele performs as an effective risk factor for T2DM. These two SNPs may not be directly associated with developing T2DM, but rather with certain risk factors and related traits in the population. The SNP use disease susceptibility tests tend to be clinically justified because the diseases represent greater socioeconomic burdens on the healthcare system [5].

As shown in Table 4, The VHD disease can manifest when people possess a high a homozygous mutant TT ratio and mutant T allele in rs60389358 SNP. While, results revealed a notable relation between the rs2781666 SNP and the occurrence of VHD disease with high percentage in individuals possessing heterozygous GT and homozygous mutant TT genotype and mutant T allele, may be possible risk factor for VHD patients. This is consistent with a study in the Han Chinese population which showed that the SNP rs2781666 in Arg1 may confer a predisposition to the risk and features of cirrhotic cardiomyopathy [18]. A study of the Tunisian population, the rs2781666 Arg 1 gene polymorphism was significantly correlated with acute myocardial infarction [19], Recently, the SNP rs2781666 showed a significant relation with the risk of coronary heart disease [20]. Although there is some non-statistical significance between the patients group and the C group. This may be because of the small sample size of cases under study.

According to previous results shown in Table 5., The VHDM can manifest when people possess had higher levels of CT, TT genotype and mutant T allele than control for rs6038 9358 SNP.This is agreement with the study of Suhad, et al [5], as cytosine nucleotide was substituted by thymine. As well as, it was discovered that VHDM patients had higher levels of GT, TT genotype and mutant T allele than C group in rs2781666SNP, indicating that it is closely related to the disease, it may serve as a risk factor for VHDM patients. These results have been observed in other ethnic populations, such as coronary artery disease patients with T2DM of Polish origin [21]. In contrast, others indicated a non-significant relation with this disease [22]. A study in Iraqi patients subjected to percutaneous coronary intervention showed a significant and consistent association of Arg1 limited to rs60389358 and rs2781666 SNPs [5]. Although there is some non-statistical significance between patients and the C group, further investigation is warranted to ascertain the true nature of these findings.

In this study, it was found that an increased that both the heterozygous (GT, CT) genotype and the homozygous mutant TT genotype, as well as the mutant T allele in patients with T2DM, VHD, and VHDM except decreased GT genotype in T2DM and CT genotype in VHD when compared with C group in two SNPs. Although, that there were some non- significant variations between these freque -ncies. However, the risk may be increased by the increased T allele and the TT genotype (for the patients of the three groups), and may potentially serve as a risk factor for patients. Genetic changes in DNA composition lead to polymorphisms in multiple genes that behaves by changing amino acids and/or change how a gene-encoded protein performs [23]. The results may be related to the detrimental impact of Arg1overexpression, at least in endothelial cells, because the polymorphism location of rs60389358 and rs2781666 are on the promoter region of the gene and it may cause modifying in Arg1 expression [24-25]. An increase in Arg1 with VHD is associated with several pathological mechanisms, most of which are linked to endothelial dysfunction, chronic inflamm -ation, and CVD [26]. In addition, studies have shown that Arg1 gene expression increases with diabetes, which may have some significance due to its development, as the risk of MI is increased especially with the rs2781666 variant genotype including some French populations [27-28], While rs60389358 was observed in other ethnic groups that were not polymorphic in this SNP [5]. Previous studies have implicated Arg1 in endothelial cell dysfunction in aging,hypertension, diabetes as well as ischemic diseases [27,17,29]. Arginase-1 expression is active (functionally) in human endothelial cells, and thus any endothelial dysfunction may cause T2DM. Because elevated Arg1 may lead to decreased nitrite and nitrate concentrations, resulting in nitric oxide deficiency, which leads to decreased nitric oxide mediated dilation and deterioration of vascular function. On the other hand, the insulin signaling pathway may lead to upregulation of Arg1, reducing the level of Arg1and thus limiting nitric oxide synthesis [30-31]. It may be related with elevated risk of inflammation, due to the insidious effects of hyperglycemia on endothelial injury and activation of macrophages, monocytes, inflammatory cytokines and growth factors in T2DM 17,32].

## Conclusions

Our findings suggest significant associations between the Arg 1 gene rs60389358 and rs2781666 SNPs and VHD in patients with T2DM. The current research demonstrates that Arg1 gene polymorphisms at rs60389358 and rs2781666 may have a role in the occurence of T2DM as well as its progression to VHD susceptibility risk in the Iraqi population.

## Acknowledgments

The authors thank the staff of the Ibn Al Bitar Cardiac Surgery Center in Baghdad, Iraq. Additionally, they appreciated the participant's cooperation who gave blood samples and information.

## Conflict of interests

The authors did not declare any conflicts of interest.

## References

[1] Gieroba B, Kryska A, Bartnicka A S. Type 2 diabetes mellitus – conventional therapies and future perspectives in innovative treatment.Biochemistry and Biophysics Reports, 2025; 42 (102037):1-16 .

[2] Nouri M A, and Altaee M F. Gene Expression and Genetic Polymorphism of TCF7L2 and CDKAL1 Genes in a Sample of Iraqi Patients with Diabetes Mellitus Type 2. (Phd), University of Baghdad, 2025.

[3] Mahajan A, Taliun D, Thurner M, Robertson N R, et al. Fine-mapping type 2 diabetes loci to single-variant resolution using high-density imputation and isletspecific epigenome maps. Nature Genetics, 2018;50 (11):1505–1513.

[4] Ramadhani F A, and Cahyati W H. Artikel Type 2 Diabetes Mellitus (Case Study at Insan Rizkillah Clinic). Asian Journal of Healthcare Analytics, 2023;2(1):247-254.

[5] Ibrahim S A. Jasim H M, Zainulabdeen J A. Association of Arginase I Gene Polymorphism with the Risk of Atheroscle -rosis in a Sample of Iraqi Patients, Indian Journal of Public Health Research & Development,2019;10(6):85-90.

[6] Ibrahim R K, Ghudhaib K K , and Dyab Allawi A A . Studying the Effect of Sex on CTGF and TGF-B1 Levels and Some Relevant Parameters in Iraqi Diabetic Patients with Glomerular and Renal Tubular Fibrosis. Ibn Al-Haitham Journal for Pure and Applied Sciences,2024; 37(2): 260–269.

[7] Chen Y, Xiao F, Wang R. Calcified aortic valve disease complicated with and without diabetes mellitus: the underlying pathogenesis., Rev. Cardiovasc. Med. ,2022; 23(1):7.

[8] Wang Y T, Tao J. Maimaiti A, Adi D, Yang Y N, et al. Prevalence of valvular heart diseases and associated risk factors in Han, Uygur and Kazak population in Xinjiang, China. PLoS ONE, 2017; 12(4): e0174490.

[9] Qianhong Lu, Junxing Lv,Yunqing Ye , et al . Prevalence and impact of diabetes in patients with valvular heart disease., iScience.,2024;27(3):109084.

[10] Huang N, Zhuang Z, Liu Z, Huang T. Observational and genetic associations of modifiable risk factors with aortic valve stenosis: a prospective cohort study of 0.5 million participants. Nutrients,2022;14(11): 2273.

[11] Rahma M M and Salman A. D. Heart Disease Classification–Based on the Best Machine Learning Model. Iraqi Journal of Science, 2022; 63(9):3966-3976.

[12] Tiziana C, Andrea B, Alvaro G, Maria G A. DNA Damage and Repair in Atherosclerosis: Current Insights and Future Perspectives. Int. J. Mol. Sci.,2012;13(12): 16929-16944.

[13] Coffey S, Roberts-Thomson R, Brown A, Carapetis J, Chen M, et.al. Global epidemiology of valvular heart disease., Nat Rev Cardiol.,2021;18(12):853–864.

[14] Yao L, Bhatta A, Xu Z M, Chen J J, et al. Obesity-induced vascular inflammation involves elevated arginase activity. Am J. Physiol Regul Integr Comp Physiol. ,2017; 313(5): R560–R571.

[15] Jasieckaby A J, Siekierzycka A, Płoska A, Dobrucki I T and Kalinowski L. Endothelial Dysfunction Driven by Hypoxia-The Influence of Oxygen Deficiency on NO Bioavailability, Biomolecules,2021;11(7): 982.

[16] Ali Shah S F, Khan M J, Iqbal T, Akram S, Waheed F et al. Arginase-1 Variants and the Risk of Familial Coronary Artery Disease in Subjects Originating from PakistanIn, Genetic Testing and Molecular Biomarkers,2019;23(1):1-5.

[17] Ali Shah S F, Iqbal T, Naveed N, Akram S, et al. ARG1 single nucleotide polymorphisms rs2781666 and rs2781665 confer risk of type 2 diabetes mellitus. EXCLI J.,2018;17:847–855.

[18] Yu S, Sun L, Jiang J, He X, Zhou Q. Common variants in AGR1 genes contributed to the risk and traits of cirrhotic cardiomyopathy in the Han Chinese population, Biomark Med ,2022; 16 (5) 331-340.

[19] Younes S, Shi Z, Zayed H. Genetic variations associated with coronary artery disease and myocardial infarction in the Arab world: a systematic review and meta-analysis., Highlights in BioScience ,2020;3:1-21.

[20] Sitinjak B D P, Murdaya N, Rachman T A, Zakiyah N, Barliana M I. The Potential of Single Nucleotide Polymorphisms (SNPs) as Biomarkers and Their Association with the Increased Risk of Coronary Heart Disease: A Systematic Review, Vasc Health Risk Manag.,2023;5 (19):289-301.

[21] Buraczynska M, Zakrocka I. Arginase Gene Polymorphism Increases Risk of Diabetic Retinopathy in Type 2 Diabetes Mellitus Patients. J. Clin. Med.,2021;10(22) :5407.

[22] Meroufel D, Dumont J, Benchekor S M, Benhammamouch S, et al. Characterization of arginase 1 gene polymorphisms in the Algerian population and association with blood pressure. Clin Biochem.,2009;42(10-11): 1178–1182.

[23] Abed R M, Abdulmalek H W, Yaaqoob L A, Altaee M F, Kamona Z K. Genetic Polymorphism of TLR5 and TLR6 in Iraqi Patients with Heart Failure Disease, Iraqi Journal of Science,2023;64(4):1662-1674.

[24] Buga G M, Singh R, Pervin S, Rogers N E, Schmitz D A. Arginase activity in endothelial cells: inhibition by NG-hydroxy-L-arginine during high-output NO production. Am. J. Physio.l,1996;271(5):H1988-H1998.

[25] Yoo H W, M.D. Genetic testing in clinical pediatric practice. Korean J. of Pediatrics, 2010; 53(3):273-285.

[26] A. S, Momani M S. Plasma arginase activity is elevated in type 2 diabetic patients. Biomed Res. ,2017;28(9):4102–4106.

[27] Beleznai T, Feher A, Spielvogel D, Lansman S L, Bagi Z, et al. Arginase1 contributes to diminished coronary arteriolar dilation in patients with diabetes. Am J Physiol Heart Circ Physiol. ,2001;300(3): H777–H783.

[28] Sediri Y, Kallel A, Ali S B, Omar S, Mourali M S, et al. Association of rs27816 66 G/T polymorphism of arginase I gene with myocardial infarction in Tunisian male population. Clin Biochem ,2010;43(1/2):106-109.

[29] Dumont J, Zureik M, Cottel D, Montaye M, Ducimetière P, et al. Association of arginase 1 gene polymorphisms with the risk of myocardial infarction and common carotid intimamedia thickness. J Med Genet. ,2007;44(8):526–531.

[30] Ali Shah S F, Iqbal T, Qamar R, Rafiq M A, Hussain S. ARG1 gene polymorphisms and their association in individuals with essential hypertension: a case-control study. DNA Cell Biol.,2018;37(7):609–616.

[31] Bagi Z, Feher A, Dou H, Broska Z. Selective upregulation of arginase1 in coronary arteries of diabetic patients. Front Imm -unol. ,2013;4: 293.

[32] Dahham Z M, Haddad N I A. Correlation Between Gene Expression of Interferon Regulatory Factor-5 and Disease Activity Index in Systemic Lupus Erythematosus Iraqi Patients, Iraqi Journal of Science, 2023; 64(2):605-619.