Contribution of CYP27B1 and CYP24A1 Genetic Variations to the Incidence of Acute Coronary Syndrome and to Vitamin D Serum Level

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Contribution of CYP27B1 and CYP24A1 Genetic Variations to the Incidence of Acute Coronary Syndrome and to Vitamin D Serum Level

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Running Title: CYP271B1, CYP24A1, vitamin D and acute coronary syndrome

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Abstract

**Background:** Cardiovascular diseases remain a major public health burden worldwide. It was reported that vitamin D protects the cardiovascular system through several mechanisms mainly by hindering atherosclerosis development. Genetic variations in vitamin D metabolic pathway were found to affect vitamin D levels. This study aims at investigating the association between single nucleotide polymorphisms (SNPs) in genes involved in vitamin D metabolism; *CYP27B* and *CYP24A1*; 25(OH)D levels, and susceptibility to acute coronary syndrome (ACS).

**Subjects/Methods:** 185 patients and 138 healthy controls were recruited. *CYP24A1* rs2762939 was genotyped using Fast Real time PCR, while *CYP24A1* rs4809960 and *CYP27B1* rs703842 were genotyped using polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP). 25(OH)D$_3$ and 25(OH)D$_2$ levels were measured using ultra-performance liquid chromatography tandem mass spectrum (UPLC-MS/MS).

**Results:** Vitamin D level was significantly lower in patients than controls ($p<0.05$). The GG genotype of rs2762939 was significantly associated with the risk of ACS development, but not correlated to the vitamin D level. Rs4809960 and rs703842 genetic variations were not associated with ACS nor with 25(OH)D level.

**Conclusion:** The genetic variant rs2762939 of *CYP24A1* is remarkably associated with ACS. Meanwhile, the variants rs4809960 and rs703842 are not associated with ACS incidence.

**Keywords:** Myocardial infarction -25(OH)D -Single Nucleotide Polymorphism -*CYP24A1* -*CYP27B1*. 
Introduction

Cardiovascular disease (CVD) represents an immense burden worldwide. It is responsible for the death of 17.3 million per year which accounts for 30% of the reported worldwide mortality where 80% of these cases occur in low and middle income developing countries (Mozaffarian et al. 2016).

Almost half of the CVD deaths are attributed to acute coronary syndrome (ACS) (Zhao and Winget 2011). On the other hand, vitamin D deficiency is common worldwide, approximately 50% of human race globally have low vitamin D levels (Nair and Maseeh 2012). Vitamin D deficiency is classically known to cause rickets in children and osteomalacia in adults (Ellfolk 2008). However, its deficiency does not only affect the skeletal system but also perturbs different body organs through altering the expression of about 10% of our genome (Morris and Anderson 2010).

According to the US endocrine Society Guidelines, vitamin D deficiency is defined as circulating 25(OH)D concentrations below 20ng/ml, whereas insufficiency includes those with 25(OH)D levels between 20-30 ng/ml, and sufficiency comprises concentrations above 30 ng/ml (Abu el Maaty and Gad 2013; Abu el Maaty et al. 2016).

Accumulating epidemiological studies suggest that there is an association between vitamin D and CVD. Several studies have shown that individuals with low level of 25(OH)D had higher risk for developing myocardial infarction (MI) (Abu el Maaty et al. 2015; Judd and Tangpricha 2009; Scragg et al. 1990).

Vitamin D is cardioprotective, it is involved in different pathways that lower the risk of atherosclerosis (Abu el Maaty and Gad 2013; Kienreich et al. 2013; Menezes et al. 2014).

Vitamin D is mainly obtained through exposure of skin cells to UV-B-rays or through diet or supplementation. There are two forms of vitamin D (D$_2$ and D$_3$) both undergo the same metabolism to become active (Abu el Maaty and Gad 2013). The first step occurs in the liver by 25-hydroxylase enzyme encoded by CYP2R1 gene to produce 25(OH)D (Bikle 2014) followed by the addition of another hydroxyl group at carbon 1 by 1-α hydroxylase encoded by CYP27B1 producing 1α,25(OH)$_2$D (calcitriol) (Abu el Maaty and Gad 2013), the active and most potent form of all metabolites (Ellfolk 2008; Pilz et al. 2013). Calcitriol is degraded by 24-hydroxylase encoded by CYP24A1 producing calcitroic acid (Ellfolk 2008).

1,25(OH)$_2$D binds to a steroid nuclear receptor known as vitamin D receptor (VDR), expressed in different organs and cells including osteoblast, chondrocytes, musculoskeletal system, immune, endothelial cells, cardiomyocytes and parathyroid gland (DeLuca 2014). This complex dimerizes with the retinoid X-receptor (RXR) forming a
heterodimer which acts as a transcription factor (Abu el Maaty and Gad 2013) and elicits a biological response (Ellfolk 2008; Morris and Anderson 2010).

On the genetic level, rs703842 is located in the promoter of CYP27B1 carried on chromosome 12q13-14 (Sundqvist et al. 2010). While rs4809960 and rs2762939 are located in non-coding regions of CYP24A1 gene (Morrison et al. 2011) on chromosome 20q13.2 (Wang et al. 2015). SNPs in CYP24A1 have been associated with coronary artery disease and asthma (Ramnath et al. 2013). The present study explored the triangular relationship between the aforementioned SNPs, circulating 25(OH)D level and the risk of ACS development in the Egyptian population.

Material and Methods

Subjects:

185 ACS patients were randomly recruited in the study. Samples were obtained from inpatients' and outpatients' settings of the National Heart Institute in Cairo. They were selected based on history of MI, percutaneous coronary intervention, and coronary catheterization-verified CAD, with age range between 22-70 years old. Few patients had diabetes or hypertension (table 1). Patients with acute or chronic disease such as renal failure, liver failure or other cardiovascular diseases were excluded from the study. ACS was diagnosed by the presence or absence of significant ST-segment elevations on electrocardiogram (ECG) (STEMI / No ST-elevation) and through the measurement of cardiac biomarkers (Troponins and / or CK-MB). Age and sex-matched controls (n=138) were recruited from healthy volunteers free from any diagnostic signs of ACS. These samples were obtained from the blood bank at the Children’s Cancer Hospital 57357 in Cairo, Egypt. Medical records were collected for each participant in the study. All participants were Egyptians and had controlled blood pressure below 140/90 mmHg; some patients were under anti-hypertensive medication not known to affect the level of 25(OH)D. Subjects characteristics are shown in table 1. The study was approved by the ethical committee of the Hospitals and the German University in Cairo.

Blood samples were collected in plain (no additives) and EDTA-coated vacutainers. Serum vacutainers were centrifuged for 20 min at 4000 rpm at 4°C and serum was used for 25(OH)D determination using UPLC-MS/MS and EDTA vacutainers were used for DNA extraction using ABIOPure Total DNA Blood/Cell/Tissue Extraction kit.
(Alliance Bio Inc. Washington, USA). Each sample was tested for DNA concentration and purity using Thermoscientific Nanodrop 2000 spectrophotometer.

Selection of SNPs in CYP24A1 and CYP27B1:

The SNPs for the present study were selected based on prior association with ACS disease or with 25(OH)D levels in serum in few ethnic groups. However, they have not been studied before on the Egyptian population. CYP24A1 rs2762939 was genotyped using Fast Real-Time PCR. DNA sample was diluted to 11.25μL with DNase-free water to deliver a DNA mass of 20ng per well. 40X SNP Genotyping assay C___1915653_20 (Applied Biosystems, Massachusetts, USA) was diluted to produce 20X working stock of SNP Genotyping assay. To each PCR tube, 12.5μL of Taqman Master mix(Applied Biosystem, Foster City, CA, USA), 1.25μL of 20XSNP genotyping assay were added to 11.25μL DNA solution and genotyped using Stratagene Mx3000P/Mx3005P qPCR Agilent Genomics real-time qPCR system.

The thermal profile was as follows: step 1; in which activation of AmpliTaq Gold DNA polymerase enzyme takes place; 95°C for 10 min, followed by 40 cycles, composed of denaturation and annealing/extension at 92°C for 15 sec and 60°C for 1 min respectively. Each allele was expressed by a specific fluorescence. On the other hand, CYP24A1 rs4809960 and CYP27B1 rs703842 were genotyped using polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) and viewed under UV light on a 2% agarose gel. Primers were designed using the National Center for Biotechnology Information primer designing tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and purchased from Thermo Fisher Scientific, Waltham, MA, USA.

For CYP24A1 rs4809960, the primers (5’→3’) used were forward CCCTTTCTCTTTTCTTTTACTCCT and reverse: AGGAGCTGTGTCCAGAATTGG. The PCR cycle consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycle consisting of denaturation, annealing and extension of 95°C for 30 sec, 50°C for 45 sec and 72°C for 45 sec respectively. Finally, extension step at 72°C for 10 min. The PCR product was 966bp long. CutSmart NlaIII restriction enzyme (New England Biolabs, Ipswich, Massachusetts, USA) was used for genotyping according to the manufacturer’s instructions. NlaIII cuts twice at the T-allele and once at the C-allele in the PCR product. In other words, for the T-allele 3 bands were produced 607, 314, and 45bp while as for the C-allele 2 bands of 921 and 45bp were produced (Fig.1).
For the last SNP, CYP27B1 rs703842, the sequence of forward and reverse primers was: 
5' TGGGCAAGGACTTATTGGACC3' and 5' AAGGAAGAGCAAGACCCAC3' respectively. The PCR set up was similar to that used for rs4809960 except that the annealing temperature was set to 61°C. The PCR product was 472bp long, which was then digested using Fastdigest FspBI (Thermoscientific, USA) according to the manufacturer’s protocol. FspBI cuts at the C-allele only, it was also found to cut at another site in the PCR product apart from the SNP. To clarify, for the T-allele, 2 bands were observed at 412 and 60bp while as for the C-allele 3 bands were obtained of 241, 171, and 60bp (Fig.2). Random samples were reanalyzed to confirm the accuracy of the method for the 3 SNPs. Reanalysis was carried out through sequencing of few samples for the genetic variant rs4809960. For rs2762939, some samples were analyzed twice on 2 different occasions by RT-PCR. Regarding rs703842, reanalysis included selection of random samples, then digestion by restriction enzyme, and view under UV light on agarose gel (PCR-RFLP). All new results were similar to the ones obtained earlier (100%).

**Determination of 25(OH)D:**

25(OH)D was extracted from serum samples of both studied groups using C18 Solid Phase Extraction Cartridges (Thermoscientific, USA). Concentration was determined using Acquity UPLC-PDA-MS/MS (Waters Corporation Milford, MA, USA). Multiple reaction monitoring (MRM) mode was using Mass Lynx™ mass spectrometry software at transitions 401.35 > 365 for 25(OH)D$_3$ and 413.35 > 107 for 25(OH)D$_2$. Quality control samples (pooled serum samples) were analyzed with batches of study samples to monitor analytical precision. The assay was validated for sensitivity, intra-and inter-day accuracy and precision (Sedky et al. 2018).

**Statistical analysis:**

Analyses were performed using GraphPad Prism statistics software Version 5.03 (GraphPad Software, Inc.). Association between the SNPs and ACS was based on both a genotypic and allelic model through calculation of odds ratio (OR) using Fischer’s T-test. Comparison of 25(OH)D levels between the studied groups was performed using the Mann–Whitney U test, while comparison of 25(OH)D levels among the different genotypes of the studied SNPs was performed using Kruskal-Wallis test. The Hardy–Weinberg equilibrium (HWE) was calculated for the 3 SNPs (table 2). Shapiro-wilk test for normality was used for testing normality of distribution of quantitative variables. Statistical significance was defined at a p-value of less than 0.05. Multivariate logistic regression analysis was performed on results that showed significant p-value to eradicate the influence of other confounding factors such as age, sex, BMI and smoking.
Results

Relationship between SNPs and ACS risk.

*CYP24A1* rs2762939 and ACS: A suggestive relationship between the *CYP24A1* SNP rs2762939 and ACS was observed. Genotype distribution and allele frequency of *CYP24A1* rs2762939 were highly associated with the risk of development of the disease \( p=0.0435, 0.0130 \) respectively (table 3, fig. 3). Based on the dominant model, it was shown that the *GG* individuals have 2.2 folds risk to ACS. Multivariate logistic regression analysis showed that rs2762939 is an independent factor associated with risk of ACS development \( p=0.039 \).

*CYP24A1* rs4809960 and ACS:
As for rs4809960, by studying the genotype and allele distribution not statistically significance was found among both studied groups \( p>0.05 \) (table 3).

*CYP27B1* rs703842 and ACS:
Genotype distribution pattern and allele frequency did not show a significant difference between ACS patients and healthy controls \( p>0.05 \) (table 3).

SNPs and levels of 25(OH)D:

25(OH)D level among both study groups:
The results suggest that increasing concentrations of 25(OH)D were significantly associated with reduced incidence of ACS. 25(OH)D2 and 25(OH)D3 levels were significantly lower in patients than in controls (table 4) \( p<0.0001 \). The total mean of 25(OH)D was 2.5 times lower in patients.

Rs2762939 and 25(OH)D:
A trend was observed in which the GG genotype showed the lowest levels of 25(OH)D3, and total 25(OH)D in patients. However, these differences in vitamin D levels were not significant on calculating the mean 25(OH)D3, and total 25(OH)D for each of the studied group \( p>0.05 \) (table 5).
Rs4809960 and 25(OH)D:
There was no correlation between 25(OH)D$_3$, 25(OH)D$_2$ and total 25(OH)D levels and the genetic variation in CYP24A1 rs4809960 among patients and controls (p >0.05) (table 5).

Rs703842 and 25(OH)D:
There was no significant difference in the levels of 25(OH)D$_3$, 25(OH)D$_2$ and total 25(OH)D and the genetic variation of CYP27B1 rs703842 in patients (p >0.05) (table 5).

Discussion:
Owing to the fact that VDRs are present in virtually all cells including myocytes and endothelial cells; vitamin D is considered a modulator to several systems including the cardiovascular system (Abu el Maaty and Gad 2013). Some SNPs in CYP2R1 and CYP27B1 genes have been studied and were found to have an impact on the development of multiple sclerosis or have been associated with low levels of 25(OH)D$_3$ (Scazzone et al. 2018; Agnello et al. 2017). Vitamin D affects inflammation and immune cells involved in atherosclerosis. T-helper 1(Th1) cells promote inflammation while T-helper 2(Th2) are anti-atherogenic through the secretion of IL-5, IL-10, IL-13. Vitamin D acts through shifting the immune response towards the effect of Th2 away from Th1 (Kassi et al. 2013). Vitamin D decreases the expression of renin, thus lowers blood pressure (Kienreich et al. 2013). It has anti-inflammatory action through decreasing the activity of NF-κB (Menezes et al. 2014) and increasing the expression of endothelial nitric oxide synthase (eNOS) (Abu el Maaty and Gad 2013). The increase in NO is very beneficial as it is a potent vasodilator and inhibitor of platelet aggregation (Menezes et al. 2014). A case-control study was conducted on New Zealand population showed that myocardial infarction is inversely associated with the level of 25-hydroxy vitamin D$_3$ (Scragg et al. 1990). Moreover, a retrospective analysis done by the Intermountain Heart Collaborative (IHC) Study Group showed that low levels of vitamin D were highly associated with coronary artery disease and myocardial infarction (Kassi et al. 2013). In support to other studies (Abu el Maaty et al. 2016), our results showed that 25(OH)D was significantly lower in ACS patients than controls (p<0.05).

CVDs are considered as complex diseases, where both environmental and genetic factors contribute to their incidence. Hence, it became important to undergo association studies that correlate CVDs with genes; in which SNPs are the most used marker (Lewis and Knight 2012).
The current work aimed to study the impact of *CYP24A1* rs2762939, rs4809960 and *CYP27B1* rs703842 genetic variations on 25(OH)D levels and the risk of ACS development. Our research group further studied SNPs in *CYP2R1* gene involved in vitamin D pathway on the same study group assigned to the current work (Sedky et al. 2018). Other studies have focused on SNPs in *CYP2R1* and have found an association between rs10766197 and multiple sclerosis (Scanzzone et al. 2018).

Results showed that GG genotype of rs2762939 was highly associated to the risk of ACS development based on the genotype distribution and allele frequency in both studied groups. However, it was not statistically associated with 25(OH)D level. In harmony with those results, a meta-analysis study on Amish population and two other studies on European populations showed that the G-allele was associated with a higher coronary artery calcification quantity which predicts CVD and this variant was not significantly associated with 25(OH)D levels (Shen et al. 2010).

Moreover, another study showed that rs2762939 was not associated to 25(OH)D level but associated to 1,25(OH)\textsubscript{2}D (Hibler et al. 2015).

Although an association between GG genotype rs2762939 and lower levels of vitamin D would have given a clear explanation linking this SNP with the incidence of ACS.

Nevertheless, it can be that this variant affects the expression of *CYP24A1* locally in the endothelium and these may not be correlated to the circulating level of 25(OH)D (Shen et al. 2010). Hence, further investigations should be done to study the correlation between genetic variations in the vitamin D metabolism and the vascular system. Another cohort study done on Norwegian MI patients and healthy controls showed no association between rs2762939 with incidence of MI nor with 25(OH)D level (Jorde et al. 2012). On the contrary, a study done on healthy young adults from the United Arab Emirates found that the GG genotype was significantly higher in subjects with a sufficient concentration of 25(OH)D, showing that the G-allele is protective (Al Anouti et al. 2017).

However, this difference in results can be attributed to the fact that the present study was done on Egyptians, who belong to a different ethnic group than Arabs, hence can differ genetically.

For *CYP24A1* rs4809960, the current study showed no relation between the genotype distribution and the risk of development of ACS. There was also no association between this variant in any of the studied groups with 25(OH)D levels. This SNP has not been studied before in correlation to the CVD. In consistency with our results, a study done on Danish healthy children and adults found no association between the SNP and 25(OH)D level (Nissen et al. 2005).
Reimers et al. also reported that rs4809960 is not a genetic determinant for the 25(OH)D levels (Reimers et al. 2015).

Our findings showed that rs703842 was not a risk factor for ACS development and not a determinant for the vitamin D level. To our knowledge, no studies have linked rs703842 to ACS; however few linked it to the risk of multiple sclerosis development (Jiang et al. 2016; Sundqvist et al. 2010). A large study performed on multiple sclerosis patients and healthy controls from the United States of America and the United Kingdom revealed that rs703842 was not associated with 25(OH)D level (Simon et al. 2011). On the other hand, rs703842 has been associated with 25(OH)D₃ levels in Canadian population (Sundqvist et al. 2010). Other variants in CYP27B1 have been investigated by other research groups and have found no significant difference between these variants and the risk of multiple sclerosis development, however, the disease was highly associated with low levels of 25(OH)D₃. Nevertheless, in vitro studies have shown that these variants affect CYP27B1 function and decrease vitamin D level (Agnello et al. 2017).

There were both strengths and limitations to this study. Strengths include that this was the first investigation of CYP27B1 and CYP24A1 SNPs in relation to ACS development and was conducted on the Egyptian population. However, limitations include a small sample size for both studied groups.

**Conclusion**

In summary, our findings show that low level of 25-hydroxy vitamin D in serum is linked to the risk of development of ACS in the Egyptian population. Moreover, GG genotype in rs2762939 in CYP24A1 is significantly associated to ACS development representing a 2.2 higher risk, but it is not associated to vitamin D level. As for rs4809960 and rs703842, both were not associated to vitamin D level or to the risk of ACS development.

Further studies should be implemented on studying the genetic variations in vitamin D metabolism pathway and the vascular system.

**Acknowledgement**

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Statement of competing interests:

The authors declare no potential conflict of interest.

Declaration:

The authors declare that the experiment has been reviewed and approved by German University in Cairo’s ethics review committee, and that the subjects have given informed consent prior to participating in the study.
References:


Table 1: Patients and healthy controls characteristics

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<th>Acute Coronary Syndrome patients</th>
<th>Healthy controls</th>
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<tr>
<td></td>
<td>Overall N=185</td>
<td>Overall N=138</td>
</tr>
<tr>
<td>Gender</td>
<td>Male N=133</td>
<td>Male N=123</td>
</tr>
<tr>
<td></td>
<td>Female N=52</td>
<td>Female N=15</td>
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<tr>
<td>Age</td>
<td>54.8±0.7</td>
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</tr>
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<td></td>
<td>53.6±0.8</td>
<td>49.2± 0.9</td>
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<tr>
<td></td>
<td>57.7±1.5</td>
<td>51.6± 3.1</td>
</tr>
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<td>BMI (Kg/m²)</td>
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<td>22.1± 0.2</td>
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<tr>
<td></td>
<td>24.7 ± 0.4</td>
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<tr>
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<td>27.3 ± 0.6</td>
<td>22.7± 0.4</td>
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<tr>
<td></td>
<td>2 (1%)</td>
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</tr>
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<td>23 (44%)</td>
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<td>97 (73%)</td>
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Age and BMI are expressed as mean ± SEM.
Table 2: Hardy Weinberg equilibrium for the three SNPs. MAF is the major allele frequency, M/m: Major/minor and X2 is the goodness of fit “Chi-square”.

<table>
<thead>
<tr>
<th>SNP</th>
<th>HWE, P</th>
<th>MAF %</th>
<th>M / m</th>
<th>X²</th>
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<tbody>
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<td>rs2762939</td>
<td>0.962</td>
<td>41%</td>
<td>G / C</td>
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</tr>
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<td>rs4809960</td>
<td>0.178</td>
<td>24%</td>
<td>T / C</td>
<td>1.807</td>
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<td>rs703842</td>
<td>0.184</td>
<td>17%</td>
<td>T / C</td>
<td>1.763</td>
</tr>
</tbody>
</table>
Table 3: Genotype distribution and allele frequency for rs2762939, rs4809960 and rs703842 in the studied group

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype distribution</th>
<th>Control</th>
<th>ACS patients</th>
<th>OR(95%CI)</th>
<th>P-value (Fisher’s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rs2762939</td>
<td>GG</td>
<td>36(35%)</td>
<td>91(50%)</td>
<td>GG vs CC</td>
<td>2.262(1.058-4.835)</td>
</tr>
<tr>
<td></td>
<td>GC</td>
<td>49(48%)</td>
<td>73(40%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>17(17%)</td>
<td>19(10%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rs4809960</td>
<td>TT</td>
<td>60(61%)</td>
<td>99(56%)</td>
<td>TT vs CC</td>
<td>1.576</td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>31(31%)</td>
<td>63(35%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>8(8%)</td>
<td>14(8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rs703842</td>
<td>TT</td>
<td>98(71%)</td>
<td>134(72%)</td>
<td>TT vs CC</td>
<td>0.8927</td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>34(25%)</td>
<td>46(25%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>6(4%)</td>
<td>5(3%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| OR = odds ratio; 95% CI = 95% confidence interval.
Odds ratios were used to test for the association of the genotype with the disease. The similarity of both distributions suggests that none of the observed genotypes or alleles is found to affect coronary artery disease incidence.
Table 4: 25(OH)D$_3$ and 25(OH)D$_2$ and total 25(OH)D in the study groups:

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ACS patients</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH) D$_3$(ng/ml) Mean ± SEM</td>
<td>31.63 ± 0.94</td>
<td>13.62 ± 0.38</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>25(OH)D$_2$(ng/ml) Mean ± SEM</td>
<td>11.86 ± 7.13</td>
<td>3.75 ± 0.14</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total 25(OH) D (ng/ml) Mean ± SEM</td>
<td>43.48 ±1.06</td>
<td>17.37 ± 0.42</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Table 5: Association of the genotypes rs2762939, rs4809960, and rs703842 with the circulating levels of 25(OH)D$_3$, 25(OH)D$_2$ and total 25(OH)D

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>25(OH)D$_3$ Mean± SEM (ng/mL)</th>
<th>P-value</th>
<th>25(OH)D$_2$ Mean± SEM (ng/mL)</th>
<th>P-value</th>
<th>Total 25(OH)D Mean ± SEM (ng/mL)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rs2762939</td>
<td>Patients</td>
<td>GG 12.9±0.6, 13.9 ±0.6, 14.4 ±1.0</td>
<td>0.4487</td>
<td>GC 4.0±0.3, 3.8 ±0.2, 3.2 ±0.3</td>
<td>0.1621</td>
<td>GC 16.9 ±0.7, 17.7 ±0.6, 17.7 ±1.1</td>
<td>0.8242</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GC 13.9±0.6, 13.9 ±0.6, 14.4 ±1.0</td>
<td></td>
<td>GC 3.8±0.3, 3.8 ±0.2, 3.2 ±0.3</td>
<td></td>
<td>GC 17.7 ±0.7, 17.7 ±0.6, 17.7 ±1.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC 14.4±1.0, 14.4 ±1.0, 14.4 ±1.0</td>
<td></td>
<td>CC 3.2±0.3, 3.2 ±0.3, 3.2 ±0.3</td>
<td></td>
<td>CC 17.7 ±1.1, 17.7 ±1.1, 17.7 ±1.1</td>
<td></td>
</tr>
<tr>
<td>Rs2762939</td>
<td>Controls</td>
<td>GG 32.4 ±1.8, 32.9 ±1.7, 30.9 ±2.8</td>
<td>0.6951</td>
<td>GC 11.9±0.9, 10.5 ±0.7, 14.3±1.6</td>
<td>0.0378</td>
<td>GC 44.0 ±1.7, 43.4 ±1.6, 45.2 ±3.4</td>
<td>0.4759</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GC 32.9±1.7, 32.9 ±1.7, 30.9 ±2.8</td>
<td></td>
<td>GC 10.5±0.7, 10.5 ±0.7, 14.3±1.6</td>
<td></td>
<td>GC 43.4 ±1.6, 43.4 ±1.6, 45.2 ±3.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC 30.9±2.8, 30.9 ±2.8, 30.9 ±2.8</td>
<td></td>
<td>CC 14.3±1.6, 14.3 ±1.6, 14.3±1.6</td>
<td></td>
<td>CC 45.2 ±3.4, 45.2 ±3.4, 45.2 ±3.4</td>
<td></td>
</tr>
<tr>
<td>Rs4809960</td>
<td>Patients</td>
<td>TT 13.5±0.5, 13.7±0.7, 13.1±1.4</td>
<td>0.9449</td>
<td>TC 3.7±0.2, 3.7±0.2, 3.7±0.6</td>
<td>0.8451</td>
<td>TC 17.2±0.5, 17.4±0.8, 16.8±1.5</td>
<td>0.9742</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC 13.7±0.7, 13.7±0.7, 13.1±1.4</td>
<td></td>
<td>TC 3.7±0.2, 3.7±0.2, 3.7±0.6</td>
<td></td>
<td>TC 17.4±0.8, 17.4±0.8, 16.8±1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC 13.1±1.4, 13.1±1.4, 13.1±1.4</td>
<td></td>
<td>CC 3.7±0.6, 3.7±0.6, 3.7±0.6</td>
<td></td>
<td>CC 16.8±1.5, 16.8±1.5, 16.8±1.5</td>
<td></td>
</tr>
<tr>
<td>Rs4809960</td>
<td>Controls</td>
<td>TT 32.6±1.4, 32.6±2.3, 29.0±4.0</td>
<td>0.8740</td>
<td>TC 12.0±0.7, 10.3±0.9, 16.4±3.6</td>
<td>0.2528</td>
<td>TC 44.7±1.5, 42.9±2.0, 45.4±2.8</td>
<td>0.4708</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC 32.6±2.3, 32.6±2.3, 29.0±4.0</td>
<td></td>
<td>TC 10.3±0.9, 10.3±0.9, 16.4±3.6</td>
<td></td>
<td>TC 42.9±2.0, 42.9±2.0, 45.4±2.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC 29.0±4.0, 29.0±4.0, 29.0±4.0</td>
<td></td>
<td>CC 16.4±3.6, 16.4±3.6, 16.4±3.6</td>
<td></td>
<td>CC 45.4±2.8, 45.4±2.8, 45.4±2.8</td>
<td></td>
</tr>
<tr>
<td>Rs703842</td>
<td>Patients</td>
<td>TT 13.8±0.5, 13.0±0.7, 15.6±0.7</td>
<td>0.1535</td>
<td>TC 3.7±0.2, 3.9±0.2, 4.6±0.7</td>
<td>0.2580</td>
<td>TC 17.5±0.5, 16.8±0.8, 20.2±1.1</td>
<td>0.1093</td>
</tr>
<tr>
<td></td>
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<td>TC 13.0±0.7, 13.0±0.7, 15.6±0.7</td>
<td></td>
<td>TC 3.9±0.2, 3.9±0.2, 4.6±0.7</td>
<td></td>
<td>TC 16.8±0.8, 16.8±0.8, 20.2±1.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC 15.6±0.7, 15.6±0.7, 15.6±0.7</td>
<td></td>
<td>CC 4.6±0.7, 4.6±0.7, 4.6±0.7</td>
<td></td>
<td>CC 20.2±1.1, 20.2±1.1, 20.2±1.1</td>
<td></td>
</tr>
<tr>
<td>Rs703842</td>
<td>Controls</td>
<td>TT 29.5±1.0, 36.7±2.1, 34.3±4.5</td>
<td>0.0107</td>
<td>TC 12.3±0.8, 11.3±1.0, 7.5±3.3</td>
<td>0.1513</td>
<td>TC 41.8±1.3, 48.0±1.9, 41.8±1.8</td>
<td>0.0706</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC 36.7±2.1, 36.7±2.1, 36.7±2.1</td>
<td></td>
<td>TC 11.3±1.0, 11.3±1.0, 11.3±1.0</td>
<td></td>
<td>TC 48.0±1.9, 48.0±1.9, 48.0±1.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC 34.3±4.5, 34.3±4.5, 34.3±4.5</td>
<td></td>
<td>CC 7.5±3.3, 7.5±3.3, 7.5±3.3</td>
<td></td>
<td>CC 41.8±1.8, 41.8±1.8, 41.8±1.8</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM and p ≤ 0.05 is considered significant.
Figure 1: 2% agarose gel electrophoresis representing the digestion of PCR product of CYP24A1 rs4809960 using NlaIII. Lane 1: 100bp DNA ladder (Bioline, London, UK)

51x22mm (300 x 300 DPI)
Figure 2: 2% agarose gel electrophoresis representing the digestion of PCR product of CYP27B1 rs703842 with FspBI. Lane 1: 100bp DNA ladder (Bioline, London, UK)

49x17mm (300 x 300 DPI)
Figure 3: Genotype distribution of rs2762939 SNP of CYP24A1 gene

P<0.05

Figure 3: Genotype distribution of rs2762939 among both study groups
Acute coronary syndrome

CYP24A1 rs2762939
CYP24A1 rs4809960
CYP27B1 rs703842

“GG Genotype”

81x60mm (300 x 300 DPI)