

Association of Scavenger Receptor Gene with Premature Coronary Artery Disease

Manal Abd Al Aziz, Wessam El Sayed Saad, Hoda Ahmad Abdelsattar, Adham Ahmad Abd El Tawab, Marium El Sayed Ahmad Fathi

Clinical Pathology Department, Faculty of Medicine, Ain Shams University

Corresponding author: Marium El Sayed Ahmad Fathi, E-mail: cutemar2@hotmail.com

ABSTRACT

Background: Coronary artery disease (CAD) is one of the greatest causes of morbidity and mortality worldwide. It is the principal threat to health in countries in Africa and the Middle East and one of the leading causes of disease burden in developing countries. Scavenger receptor class B type1 (SCARB1) is a multi-ligand cell surface receptor. This membrane protein facilitates the uptake of cholesterol esters from high-density lipoprotein cholesterol (HDL-C) and drives cholesterol from tissues to the liver in the various stages of reverse cholesterol transport pathway.

Aim: The aim of this work is to study the association of rs5888 polymorphism of SCARB1 gene and premature coronary artery disease.

Patients and Methods: PCAD group included 20 patients newly diagnosed angiographically with premature coronary artery disease, and non-PCAD group that included 20 age and sex matched non-CAD individuals who showed no luminal stenosis in coronary angiographic results.

Results: The frequency of the wild type (CC) was higher in the control group (60%) than patients' group (25%) and it can be considered as a negative risk factor for PCAD (OR: 0.71, 95% CI [0.60-0.82], $p < 0.01$). The homozygous and heterozygous mutations (TT & CT) were statistically more frequently distributed in PCAD patients compared to control subjects (30 % and 45 % respectively), however only the CT genotype was considered as positive risk factor for PCAD (OR: 4.02, 95% CI[1.96-10.54], $p = 0.01$).

Conclusion: Allele frequencies of studied SCARB 1 SNP revealed a higher frequency of distribution of T alleles in patients' group when compared with control group; on the other hand it shows the higher frequency of distribution of C alleles in control group when compared to patients' group.

Keywords: PCAD, SCARB 1

INTRODUCTION

Coronary artery disease (CAD) is a leading cause of morbidity and mortality all over the world, affecting millions of people in both developed and developing countries⁽¹⁾. In Egypt, cardiovascular diseases accounted for 46% of all-causes of mortality with 107.2 thousand deaths in 2012, where nearly half of these deaths occur at an age less than seventy years⁽²⁾.

Premature coronary artery disease (PCAD) is defined as CAD which manifests for the first time under the age of 55 years for males and under 65 years for females⁽³⁾. Risk factors for CAD are both environmental and genetic; environmental factors contributing to the development of CAD includes obesity, hypercholesterolemia, alcohol intake, smoking, diabetes and hypertension. Hypercholesterolemia arising from abnormal lipid metabolism has been considered to be one of the most key risk factors for CAD pathogenesis⁽⁴⁾.

Moreover, apart from these modifiable factors, accumulating evidences have shown close associations of genetic polymorphisms in candidate genes with the risk of CAD, especially the early-onset CAD. In patients with PCAD, the role of genetic risk factors is expected to be more important than that of environmental factors⁽⁵⁾.

In PCAD patients, where the disease progresses at an earlier age than usual; studying the genetic predisposition to this vulnerable plaque production showed that progression may be avoided by following established preventive measures and altering patients' lifestyles⁽⁶⁾.

Scavenger receptor class B type1 (SCARB1) is a multi-ligand cell surface receptor expressed both on macrophages and on liver cells, indicating a major role for clearance of excess cholesterol from the body⁽⁷⁾. This membrane protein facilitates the uptake of cholesterol esters from high-density lipoprotein cholesterol (HDL-C) and drives cholesterol from tissues to the liver in the various stages of reverse cholesterol transport pathway and this makes SCARB1 gene an attractive marker for CAD⁽⁸⁾.

SCARB1 receptor is encoded on SCARB1 gene which is located on 12q24.31. Various SCARB1 polymorphisms in humans have been shown to be associated with altered serum lipid profile⁽⁹⁾. One exonic single nucleotide polymorphism (SNP) (rs5888) within SCARB1 gene has been linked to lower the receptor expression and function. This SNP is a "C" to "T" substitution at cDNA position 1050 base position on exon 8⁽¹⁰⁾.

AIM OF THE WORK

The aim of this work is to study the association of rs5888 polymorphism of SCARB1 gene and premature coronary artery disease.

PATIENTS AND METHODS

The study was carried out at Clinical Pathology and Cardiology Departments, Ain Shams University Hospital.

This study was conducted on the following groups:

Group A: Premature Coronary Artery Disease (PCAD) Patients (n = 20):

This group included 20 patients newly diagnosed angiographically with premature coronary artery disease. They were recruited from the cardiology department- angiography unit, whom age is less than 55 years for male patients and 65 years for female patients. Selection was based on their angiographic results showing luminal stenosis as an evidence for atherosclerosis in at least one coronary artery or major branch segment in their epicardial coronaries⁽¹¹⁾.

Group B: Non coronary artery disease (Non-CAD) Control Group (n = 20):

This group included 20 age and sex matched non-CAD individuals who showed no luminal stenosis in coronary angiographic results.

Exclusion criteria: Patients on lipid lowering drugs were excluded from the study.

Methods:

All individuals included in this study were subjected to the following after an informed consent: 1- Full history taking focusing on risk factors of coronary artery disease including: Family history of premature coronary disease, dyslipidaemia, and hypertension and smoking. 2- Thorough clinical examination: general and local examination. 3- Radiological investigations including: a. Coronary angiography. 4- Laboratory investigations including: Full lipid profile (total cholesterol, triglycerides, HDL and LDL) and fasting blood glucose. Detection of rs5888 polymorphism of SCARB1 gene by real-time polymerase chain reaction (PCR) and high-resolution melting analysis (HRM).

Approval statement: The study protocol was approved by the Researcher Ethics Committee at faculty of Medicine, Ain shams university.

Statistical analysis: Data analysis was done using IBM SPSS statistics (V. 25.0, IBM Corp., USA, 2017-2018).

RESULTS

Table (1) shows the descriptive and comparative statistics of the demographic data, laboratory findings and angiographic results of the studied PCAD patients and the non- CAD control group including positive history of hypertension, smoking, family history of PCAD and lipid profile.

Statistical comparison between the two groups regarding the demographic data revealed that PCAD patients have a higher strong history of hypertension, smoking, family history of PCAD compared to control group (p value < 0.001). In addition, there was a highly significant difference between patients' group and control group regarding lipid profile (P < 0.001).

Tables (2 &3) show the descriptive and comparative statistics of genotype frequencies of SCARB1 gene SNP (rs 5888) in PCAD patients and control groups. The frequency of the wild type (CC) was higher in the control group (60%) than patients' group (25%) and it can be considered as a negative risk factor for PCAD (OR: 0.71, 95% CI [0.60-0.82], p < 0.01). The homozygous and heterozygous mutations (TT & CT) were statistically more frequently distributed in PCAD patients compared to control subjects (30% and 45 % respectively), however only the CT genotype was considered as positive risk factor for PCAD (OR: 4.02, 95% CI[1.96-10.54], p= 0.01).

Table (4) shows the descriptive and comparative statistics between PCAD patients and control groups regarding allele frequencies of studied SCARB 1 SNP revealed a higher frequency of distribution of T alleles in patients' group when compared with control group, on the other hand it shows the higher frequency of distribution of C alleles in control group when compared to patients' group.

Table (1): Descriptive and comparative statistics of the demographic data, lipid profile and Gensini score among PCAD patients versus healthy controls.

Parameter	Group I (Patients)(n=20) Median (Q1-Q3)	Group II (control) (n=20) Median (Q1-Q3)	Z	p value
Total Cholesterol (mg/dL)	191.5 (191.5-230.25)	127.5 (105-158.5)	7.01	< 0.001
HDL-C (mg/dL)	28 (25-31)	37 (34.5-43)	-7.70	< 0.001
LDL-C (mg/dL)	126.2 (97-160.95)	59.8 (37.35-91.15)	7.23	< 0.001
Triglycerides (mg/dL)	140 (132-163)	120 (120.25-128.75)	5.61	< 0.001
Hypertension (n;%)	13 (65 %)	6 (30 %)	18.38	< 0.001
Smoking (n;%)	10 (50 %)	7 (35 %)	6.25	< 0.05
Family history of PCAD (n;%)	12 (60 %)	2 (10 %)	31.57	< 0.001

Table (2): Descriptive and comparative statistics of the genotype frequency among patients versus healthy controls.

Genotype	Group I (Patients') (n=20)		Group II (Control) (n=20)		χ^2	P value
	N	(%)	N	(%)		
CC	5	(25%)	12	(60%)	17.10	<0.001
CT	9	(45%)	3	(15%)		
TT	6	(30%)	5	(25%)		

Table (3): Comparative Statistics of Genotypes Frequencies of the Studied Gene SNP in patients' group and control group.

Genotype Parameter	SCARB 1 rs5888		
	CC vs. non CC	CT vs.non CT	TT vs non TT
χ^2	15.48	10.17	0.762
P value	< 0.001	<0.01	< 0.383
OR (CI)	0.71 (0.60-0.82)	4.02 (1.96-10.54)	-

Table (4): Descriptive and comparative statistics between PCAD patients and control groups regarding allele frequencies of studied SCARB 1 SNP.

SCARB 1 SNP	Group I (n=40) N (%)	Group II (n=40) N (%)	χ^2	P value
C allele	19 (47.5%)	27 (67.5%)	3.26	0.01
T allele	21 (52.5%)	13 (32.5%)		

DISCUSSION

According to the World Health Organization Regional office report, there is an increased prevalence of CAD in Egypt which is

responsible for about 47% of all deaths among Egyptians in the year 2012⁽²⁾.

The institute of health metrics and evaluation in the year 2016 has declared that ischemic heart disease was the leading cause of premature mortality in Egypt with increase 27.7% from the year 2005⁽¹²⁾.

Atherosclerotic CAD comprises a broad spectrum of clinical entities that include asymptomatic subclinical atherosclerosis and its clinical complications, such as angina pectoris, myocardial infarction (MI) and sudden cardiac death.

Clinical and population-based studies have demonstrated that genetic factors play important roles in CAD and MI. The phenomenon of family clustering of CAD was repeatedly reported in the 1950s and 1960s. **Slack and Evans** demonstrated that a history of early onset ischemic heart disease (IHD) of first degree relatives was significantly associated with and predicted early onset IHD (< 55 in men and < 65 in women)⁽¹³⁾. The subsequent Framingham Heart Study (FHS) confirmed that a family history of premature CAD, defined as the presence of a first degree relative with a diagnosis of CAD at < 55 years of age in men and < 65 years of age in women, is an independent risk factor for CAD⁽¹⁴⁾.

While there are many traditional and novel risk markers associated with CAD, yet a large gap for CAD risk prediction remains present. Epidemiological evidence points to an approximate 50% genetic susceptibility to the disease. Many different genetic associations with CAD have been identified through family and population based analyses, and genetic risk markers may be important for better defining individuals at risk for cardiovascular events⁽¹⁵⁾.

Regarding SCARB 1, it was shown to be the first HDL receptor to be identified and to mediate the selective transport of lipids, such as cholesterol esters from HDL, from circulating lipoproteins into cells. It is encoded by the SCARB1 gene located on chromosome 12q24.31. Several genetic studies in various populations have discovered multiple SCARB1 variants and reported their relationship with lipid traits, and subclinical atherosclerosis and incidence of coronary artery disease⁽¹⁶⁾.

Various SCARB1 polymorphisms in humans have been shown to be associated with altered serum lipid profile but their influence on CAD development or severity is still unclear⁽⁹⁾. One exonic SNP (rs5888) within SCARB 1, has been linked to lower its protein expression and

function. This SNP is a “C” to “T” substitution at position 1050 on exon 8. Several studies on rs5888 polymorphism and the risk of CAD have been previously published with contradictory results. There is even controversy about which allele confers increased risk. **Wu et al.** found that the individuals with TT genotype were associated with increased CAD risk⁽¹⁷⁾; whereas others reported that the T allele or the T allele carrier was associated with decreased risk of CAD^{(18) (19)}.

To our knowledge, no study on SCARB 1 gene polymorphisms in premature coronary artery disease has been conducted to date in Egypt.

In the light of the previous postulation, the objective of our study was to investigate the association of rs5888 polymorphism of SCARB1 gene and premature coronary artery disease and to assess its relationship with the severity of the disease.

This study was conducted on twenty (20) patients with premature coronary artery disease who were recruited from cardiology Department of Ain Shams University Hospitals in addition to twenty (20) age and sex matched non coronary artery disease controls. All individuals included in this study were subjected to full history taking focusing on risk factors of coronary artery disease including: Family history of premature coronary disease, hypertension and smoking. Thorough clinical examination including general and local examination. Coronary angiography and Laboratory investigations including: Full lipid profile (total cholesterol, triglycerides, HDL-C and LDL-C) and Detection of rs5888 polymorphism of SCARB1 gene by real-time polymerase chain reaction (PCR) and high resolution melting analysis (HRM).

The results of our study showed that the overall prevalence of cardiovascular risk factors including hypertension, smoking and family history was higher in patients' group than in control group. These findings go with the findings of **Goodarzynejad et al., 2016** in their study on Iranian population that included 505 PCAD patients and 546 controls⁽⁸⁾.

Moreover, the results of our study showed that The frequency of the wild type (CC) was higher in the control group (60%) than patients' group (25%) and it can be considered as a negative risk factor for PCAD (OR: 0.71, 95% CI [0.60-0.82], $p < 0.01$). The homozygous and heterozygous mutations (TT & CT) were statistically more frequently distributed in PCAD

patients compared to control subjects (30 % and 45 % respectively), however only the CT genotype was considered as positive risk factor for PCAD.

These results came in accordance with **Arul et al.** in his study on 148 Indian CAD patients and 162 control subjects. They found that CT genotype had higher odds of developing myocardial infarction (OR: 2.04, 95% CI [1.17-3.56], $p < 0.01$)⁽²⁰⁾.

In addition, **Wu and his colleagues 2013** conducted a study on 601 CAD patients and 582 healthy controls and concluded that frequency of TT genotype was higher in CAD patients than in controls (8.8% and 5.2 % respectively) however in their study, only TT genotype had high risk for CAD (OR: 1.76, 95% CI [1.03-3.01], $p < 0.05$ for TT vs. CC) and not the heterozygous mutant type (CT)⁽¹⁷⁾.

As regards the wild form (CC), our study has shown that it can be considered as a protective genotype for CAD in patients population with (OR: 0.172, 95% CI[0.0693-0.04295], $p < 0.001$)

However, this result was neither consistent with the results of **Zenga et al.** who conducted a study on 295 Chinese CAD patients and 312 controls and showed no significant differences in allele frequency and genotype distribution between control subjects and patients with CAD⁽²¹⁾, nor with the results of **Rejeb et al.** in his study on 316 patients in Tunisian population and found that CC genotype was significantly higher in diseased group and that carriers of the mutant forms (CT and TT) carry approximately 41 % lower risk of coronary events⁽¹⁸⁾.

Such disparity in study results may be attributed to methodological heterogeneity (differences in study designs, sample sizes, definition of the phenotype, age and gender), different genetic background, and differences in the nature of various populations.

Our study results have shown that the C allele was more frequently distributed among control group (67.5%). As for the T allele, it was more distributed among PCAD group (52.5%) and it can be considered as a risk factor for PCAD.

This result came in accordance with **Goodarzynejad et al.** in their study on Iranian population that included 505 PCAD patients and 546 controls and they found that T allele was associated with increased odds of PCAD (OR: 1.3, 95% CI [1.00-1.50], $p < 0.05$)⁽⁸⁾.

CONCLUSION

Allele frequencies of studied SCARB 1 SNP revealed a higher frequency of distribution of T alleles in patients' group when compared with control group, on the other hand it shows the higher frequency of distribution of C alleles in control group when compared to patients' group.

REFERENCES

- Xu S, Cheng J, Li N, Chen Y, Cai M and Tang S (2015):** The association of APOC4 polymorphisms with premature coronary artery disease in a Chinese Han population. *Lipids in Health and Disease*, 14:63-72.
- World Health Organization (2012)** [Internet]: Country statistics and global health estimates by WHO and UN partners. Non communicable diseases country profiles. Available from: <http://www.who.int/gho/countries/egy.pdf?ua=1>
- De Sutter J, De Bacquer D, Kotseva K, Sans S, Pyörälä K and Wood D (2003):** Screening of family members of patients with premature coronary heart disease. Results from the EUROASPIRE II family survey. *Eur. Heart J.*, 24(3):249–257.
- Abd El-Aziz T, Mohamed R and Hagrass H (2014):** Increased risk of premature coronary artery disease in Egyptians with ABCA1 (R219K), CETP (TaqIB), and LCAT (4886C/T) genes polymorphism. *J. Clin. Lipidol.*, 8(4):381–389.
- Cengel A and Tanindi A (2009):** Myocardial infarction in the young. *J. Postgrad. Med.*, 55 (4):305–313.
- Klein L and Nathan S (2003):** Coronary artery disease in young adults. *Journal of the American College of Cardiology*, 41(4):529-531.
- Van Eck M, Pennings M Hoekstra M, Out R and Van Berkel T (2005):** Scavenger receptor B1 and ATP-binding cassette transporter A1 in reverse cholesterol transport and atherosclerosis. *Curr. Opin. Lipidol.*, 16(3):307–315.
- Goodarzynejad H, Boroumand M, Behmanesh M, Ziaee S and Jalali A (2016):** The rs5888 single nucleotide polymorphism in scavenger receptor class B type 1 (SCARB1) gene and the risk of premature coronary artery disease: a case-control study. *Lipids in Health and Disease*, 15:7-16.
- Mineo C and Shaul P (2012):** Functions of scavenger receptor class B, type I in atherosclerosis. *Curr. Opin. Lipidol.*, 23(5):487–493.
- Constantineau J, Greason, E, West M, Filbin M, Kieft J and Carletti M (2010):** A synonymous variant in scavenger receptor, class B, type I gene is associated with lower SR-BI protein expression and function. *Atherosclerosis*, 210(1):177–182.
- Neeland J, Patel R, Eshtehardi P, Dhawan S, and Quyyumi A (2012):** Coronary angiographic scoring systems: an evaluation of their equivalence and validity. *Am. Heart J.* 164(4): 547–552.
- Institute of health metrics and evaluation (IHME) (2016):** Available from: <http://www.healthdata.org/egypt>
- Slack J and Evans KA (1966):** The increased risk of death from ischaemic heart disease in first degree relatives of 121 men and 96 women with ischaemic heart disease. *J Med Genet.*, 3: 239-257
- Schildkraut JM, Myers RH, Cupples LA, Kiely DK and Kannel WB (1989):** Coronary risk associated with age and sex of parental heart disease in the Framingham Study. *Am J Cardiol.*, 64: 555-559
- Dai X, Wiernek S, Evans JP, Runge MS (2016):** Genetics of coronary artery disease and myocardial infarction *World J Cardiol.*, 8(1): 1-23
- Niemsiri V, Wang X, Pirim D& Radwan ZH (2015):** Impact of Genetic Variants in Human Scavenger Receptor Class B, *Curr. Opin. Lipidol.* 7(6), 838–847.
- Wu DF, Yin RX, Cao XL, Chen WX, Aung LH and Wang W (2013):** Scavenger receptor class B type 1 gene rs5888 single nucleotide polymorphism and the risk of coronary artery disease and ischemic stroke: a case-control study. *Int J Med Sci.*, 10(12):1771–7.
- Rejeb J, Omezzine A, Boumaiza I, Rebhi L, Kacem S AND Rejeb NB (2012):** Association of three polymorphisms of scavenger receptor class BI gene (exon8, exon1, intron5) with coronary stenosis in a coronary Tunisian population. *Gene*, 511(2):383–8.

19. **Stanislovaitiene D, Lesauskaite V, Zaliuniene D, Smalinskiene A, Gustiene O and Zaliaduonyte-Peksiene D (2013):** SCARB1 single nucleotide polymorphism (rs5888) is associated with serum lipid profile and myocardial infarction in an age- and gender-dependent manner. *Lipids Health Dis.*,12:24.
20. **Arul Jothi KN, Abinaya M, Suruthi Abirami B, George M, Elangovan S and Devi A (2017):** SCARB1 rs5888 c.1050C>T Polymorphism and the Risk of Hypercholesterolemia and Myocardial Infarction in Indian Tamil Population. *Pakistan journal of zoology*,5: 319–325
21. **Zenga TT, Tangb DJ, Yea YX, Sua,J, Jianga H (2017):** Influence of SCARB1 gene SNPs on serum lipid levels and susceptibility to coronary heart disease and cerebral infarction in a Chinese population. *Gene*, 626.319–325.