Scavenger Receptor Class B Type 1 Gene rs5888 Single Nucleotide Polymorphism: Association with Risk and Severity of Premature Coronary Artery Disease

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ABSTRACT

Background: Many different genetic associations with premature coronary artery disease (PCAD) have been identified. One exonic single nucleotide polymorphism (SNP) rs5888 of Scavenger receptor class B type1 (SCARB 1) gene has been linked to lipid traits as well as subclinical atherosclerosis.

Objective: To investigate the association between SNP rs5888 of SCARB 1 gene and PCAD risk and severity.

Patients and Methods: This study was conducted on 50 Egyptian patients diagnosed as PCAD by coronary angiograph (PCAD group) and (50) age- and sex-matched non-CAD subjects who showed no luminal stenosis in coronary angiographic served as a control group (non-CAD group). Gensini score was calculated as an indication for atherosclerosis severity for patients only. Detection of rs5888 polymorphism of SCARB 1 gene was done by real-time polymerase chain reaction (PCR) and high resolution melting analysis (HRM).

Results: The mutant TT and CT genotypes were more frequently distributed in PCAD group compared to non-PCAD group (34 % and 48 % vs. 26 % and 18 %, respectively). The (CT) genotype conferred a positive risk for PCAD in males (OR: 6.117, 95% CI (1.744-21.452); $p<0.01$). T allele conferred a positive risk for PCAD (OR: 2.564, 95% CI (1.440-4.543); $p<0.05$). Highest Gensini score was found in PCAD patients with (TT) genotype ($p<0.01$).

Conclusion: The mutant (CT) genotype and the T allele of SCARB 1 SNP rs5888 were associated with increased risk of PCAD and the mutant (TT) genotype was associated with disease severity calculated by Gensini score.

Keywords: PCAD, SCARB 1, genotypes, Gensini.

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)]. 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 reaching 27.7% of all causes of premature mortality[(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)].

In addition, apart from these modifiable factors, accumulating evidences have shown close associations of genetic polymorphisms in candidate genes with the risk of PCAD[(5)]. Moreover, studying the genetic predisposition associated with plaque production showed that disease progression may be avoided by following the established preventive measures with altering patients' lifestyles [(6)].

Scavenger receptor class B type1 (SCARB 1) 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 [(8)].

SCARB 1 receptor is encoded on SCARB 1 gene which is located on 12q24.31. Various SCARB 1 polymorphisms in humans have been shown to be associated with altered serum lipid profile [(9)]. One exonic single nucleotide polymorphism (SNP) (rs5888) within SCARB 1 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)]. Therefore, SCARB 1 gene is considered as an attractive marker for CAD [(11)].

PATIENTS AND METHODS

This study was conducted on 50 patients with premature coronary artery disease (PCAD) newly diagnosed by coronary angiography showing luminal stenosis as an evidence for atherosclerosis in at least one coronary artery or major branch segment in their epicardial coronaries [(12)] "Group 1", and a control

Received: 16 /5/2020
Accepted: 28/6/2020

group of 50 age- and sex-matched apparently healthy non-CAD subjects who showed no luminal stenosis in coronary angiographic results; "Group 2".

Both groups were referred to Cardiology Department Catheterization (Cath) lab of Ain Shams University Hospitals.

Group 1 mean age was 46.7 ± 5.7 years with subclassification into: male subgroup (n= 33, mean age of 47± 5.8 years) and female subgroup (n=17, mean age of 46± 5.5 years).

Gensini score was calculated as an indication for atherosclerosis severity [13].

Group 2 mean age was 47.4±7.1 years. This group was also subclassified into male subgroup (n= 30, mean age of 45.5±6.9 years) and female subgroup (n=20, mean age of 45.2±7.7 years).

Ethical approval:

Verbal informed consents were obtained from all participants before enrollment in the study.

The study protocol was approved by the Researcher Ethics Committee at Faculty of Medicine, Ain shams University (FWA 000017585), number FMASU MD 157/2016.

Exclusion Criteria: patients previously on lipid lowering drugs and diabetic patients.

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, in addition to general and local examination, coronary angiography and laboratory investigations that included full lipid profile (total cholesterol, triglycerides and HDL-C that were assayed on AU680 Beckman coulter, USA) and LDL-C that was calculated using Friedewald equation as well as detection of rs5888 polymorphism of SCARB 1 gene by real-time polymerase chain reaction (PCR) and high resolution melting analysis (HRM) through the following three steps:

Step 1: DNA extraction

Three milliliters of peripheral blood were taken in a sterile EDTA K3 coated vacutainer tube for extraction of DNA. DNA was extracted using QIAamp DNA blood mini kit (QIAGEN, Strasse 1, 40724 Hilden, Germany), according to manufacturer instructions. The extracted DNA was kept at -20ºC till used for SCARB 1 rs5888 polymorphism detection.

Step2: DNA amplification by high resolution melting analysis- PCR (HRM-PCR)

The High-resolution melting PCR (HRM- PCR) was done using the *Type-it HRM* PCR Kit (Qiagen, USA) on the Rotor-Gene 5plex HRM instrument (Qiagen, USA) by using the following primers [11]:

-Forward primer: CTTGTTTCTCTCCCATCCTCA
-Reverse primer: GAGTGTGCCTCCTGGTTAG

Positive and negative controls were treated as cases and worked in the same run in order to be used as a reference in result interpretation. Controls were selected according to their history and clinical data; the selected samples underwent sequencing in order to obtain their exact nucleotide sequence. Sequencing was done using the same primers as used for PCR. Nucleotide sequencing was detected using a BigDye Terminator v1.1 Cycle Sequencing Kit and 3500 genetic analyzer (Applied Biosystems, Thermo-scientific; Singapore). Data analysis was done using *https://blast.ncbi.nlm.nih.gov/Blast.cgi* website. Results of sequencing yielded a case with homozygous mutant genotype (TT) serving as positive control and another case with wild genotype (CC) serving as negative control.

Step3: Single nucleotide polymorphism detection by HRM analysis

The HRM data analysis was done using Rotor-Gene Q Series Software 2.3.1 (Build 49, Qiagen, USA). Data were presented in two formats: the normalized plot and the difference plot. The melting curves were normalized and temperature shifted in order to create the normalized plot which allows samples to be directly compared **(Figure 1A)**. The normalized plot is the graph in which the amount of fluorescence (due to the intercalating dye remaining at any temperature point) is expressed as a fraction of the amount prior to data acquisition. [14]

Difference plots were generated by selecting a negative control as the baseline and the fluorescence of all other samples was plotted relative to this sample. Significant differences in fluorescence were indicative of mutations **(Figure 1B)**. Each mutant allele had its own distinctive melting curve when compared to the wild-type allele. The distinct melting curves of the mutant became more apparent when data were represented in a difference plot format than in a normalized plot. [15]

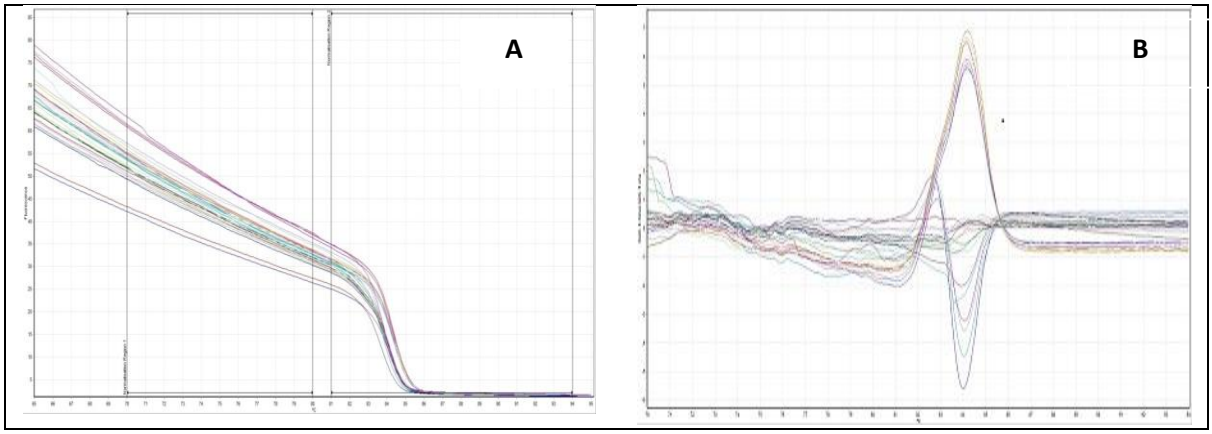


Figure (1): HRM curve showing SCARB 1 gene mutated samples, negative samples and wild type controls (**A:** Normalized HRM curve**, B**: Difference plots)

Statistical data analysis

Data analysis was done using IBM SPSS statistics (V. 25.0, IBM Corp., USA, 2017-2018). For comparing qualitative data in different groups, Chi Squared test/ Fisher exact test was used. Wilcoxon rank sum test and Kruskal Wallis test were used for comparing of non-parametric data between two groups and three genotypes; respectively. Measurement of association of data between different groups was assessed by means of Odds ratio test. A probability value (p value) < 0.05 was considered significant and p < 0.01 was considered highly significant.

RESULTS

Statistical comparison between the two studied groups regarding the demographic data revealed that PCAD patients had significantly more hypertension, smoking, family history of PCAD compared to control group. In addition, there was a highly significant increase in patients' group regarding lipid profile in both male and female populations **(Table 1)**.

Table (1): Descriptive and comparative statistics of demographic data and lipid profile in PCAD patients compared to non-CAD controls

Parameter		(PCAD Patients) (n=50)	(non- CAD control) (n=50)	X^2 / Z	p value
		n (%) / Median (Q1-Q3)	n (%) / Median (Q1-Q3)		
Hypertension	**All**	26 (52 %)	6 (12 %)	18.382	< 0.01
	Male	20 (60.6%)	4 (13.3%)	14.891	< 0.01
	Female	6 (35.3%)	2 (10%)	3.469	0.063
Smoking	**All**	24 (48 %)	12 (24 %)	6.250	<0.05
	Male	24 (72.7%)	12 (40%)	6.873	< 0.01
	Female	0 (0%)	0 (0%)	-	-
Family history of PCAD	**All**	24 (48 %)	0 (0 %)	31.579	< 0.01
	Male	16 (48.5%)	0 (0%)	19.497	< 0.01
	Female	8 (47.1%)	0 (0%)	12.008	0.01
Total Cholesterol (mg/dL)	**All**	211.5 (188.5-250.25)	147.5 (125-178.5)	7.012	< 0.01
	Male	213 (192.5-251.5)	155 (140-185)	5.465	< 0.01
	Female	205 (178-248.5)	142 (123-173.5)	4.359	< 0.01
HDL-C (mg/dL)	**All**	38 (35-41)	47 (44.5-53)	-7.705	< 0.01
	Male	37 (34.5-40)	46 (43-53)	-6.286	< 0.01
	Female	41 (38-43.5)	48 (46-53)	-4.618	< 0.01
LDL-C (mg/dL)	**All**	141 (112-176)	74.8 (52-106)	7.235	< 0.01
	Male	145 (122-179)	85.5 (65-107)	5.629	< 0.01
	Female	135 (108-169)	59.6 (47.5-90.5)	4.435	< 0.01
Triglycerides (mg/dL)	**All**	160 (152-183)	140 (119-149)	5.617	< 0.01
	Male	160(150-178)	142.5 (120-149)	3.862	< 0.01
	Female	178 (153-190)	136.5 (117-147.5)	4.027	< 0.01

X^2: Chi square test or Fisher exact test, Z: Wilcoxon rank sum test, Q1: 25 percentile, Q3: 75 percentile, Number of male patients: 33, Number of female patients: 17, Number of male controls: 30, Number of female controls: 20

The frequency of the wild type (CC) was higher in the non-PCAD group than patients' group (Table 2) and it can be considered as a negative risk factor for PCAD for all patients (Table 3).

Table (2): Descriptive and comparative statistics of the SCARB 1 rs5888 genotype frequency among patients versus non-CAD controls

All Subjects				
Genotype	**PCAD Group (n=50) n (%)**	**Non-CAD Group (n=50) n (%)**	χ^2	**p value**
CC	9 (18%)	28 (56%)	17.108	**< 0.01**
CT	24 (48%)	9 (18%)		
TT	17 (34%)	13 (26%)		
All males				
Genotype	**PCAD Group (n=33) n (%)**	**Non-CAD Group (n=30) n (%)**	χ^2	**p value**
CC	7 (21.2%)	17 (56.7%)	11.302	**< 0.01**
CT	16 (48.5%)	4 (13.3%)		
TT	10 (30.3%)	9 (30%)		
All females				
Genotype	**PCAD Group n=17 (n %)**	**Non-CAD Group n=20 (n %)**	χ^2	**P value**
CC	2 (11.8%)	11 (55%)	7.548	**< 0.05**
CT	8 (47.1%)	5 (25%)		
TT	7 (41.2%)	4 (20%)		

X²: Chi square test

Regarding the heterozygous mutant genotype (CT), it was statistically more frequently distributed in PCAD patients compared to control subjects (48 % vs. 18 %) and it can be considered as positive risk factor for PCAD in all patients and in male subgroup but not in females. Moreover, the homozygous mutant genotype (TT) was more distributed in PCAD patients when compared to non-CAD subjects (34 % vs. 26 %), however it is not associated with PCAD compared with non-TT genotypes (**Table 3**).

Table (3): Association analysis of rs5888 genotypes frequencies in patients' group and non-CAD control group

All Subjects			
	SCARB 1 rs5888 Genotypes		
	CC vs. non-CC	**CT vs. non -CT**	**TT vs. non-TT**
χ^2	15.487	10.176	0.762
p value	**< 0.01**	**<0.01**	0.383
OR (95 % CI)	**0.172 (0.069-0.429)**	**4.205 (1.692-10.448)**	1.466 (0.619-3.469)
All males			
	SCARB 1 rs5888 Genotypes		
	CC vs. non-CC	**CT vs. non-CT**	**TT vs. non-TT**
χ^2	8.376	8.961	0.00
p value	**< 0.01**	**<0.01**	> 0.05
OR (95 % CI)	**0.205 (0.068-0.620)**	**6.1176 (1.744-21.452)**	1.014 (0.345-2.979)
All females			
	SCARB 1 rs5888 Genotypes		
	CC vs. non-CC	**CT vs. non-CT**	**TT vs. non-TT**
χ^2	7.537	1.962	1.973
p value	**< 0.01**	> 0.05	> 0.05
OR (95 % CI)	**0.109 (0.019-0.608)**	2.666 (0.664-10.703)	2.800 (0.649-12.06)

OR: odds ratio, 95% CI: 95% confidence interval, X²: Chi square test

In our study, no statistically significant difference was found between males and females PCAD patients regarding SCARB 1 genotype frequencies; the distribution, n (%), of genotypes CC, CT and CT in PCAD males versus females were 7 (77.8%), 16 (66.7%) and 10 (58.8%) versus 2 (22.2%), 8 (33.3%) and 7 (41.2%); respectively; X^2=0.951; p>0.05.

Regarding allele frequencies of studied SCARB 1 SNP, the frequency distribution of T alleles was higher in patients' group when compared to control group and it confers a positive risk to PCAD. On the other hand, the higher frequency of distribution of C allele was found in controls (non-PCAD) group when compared to patients' group conferring a negative risk for PCAD **(Table 4)**.

Table (4): Descriptive and comparative statistics between PCAD group and non-CAD group regarding allele frequencies of studied SCARB 1 SNP

SCARB 1 SNP	PCAD Group (allele n=100) n (%)	Non-CAD Group (allele n=100) n (%)	χ^2	p value	OR (95 % CI)
C allele	42 (42%)	65 (65%)	10.632	**<0.01**	**0.389** (0.220-0.690)
T allele	58 (58%)	35 (35%)			**2.564** (1.448-4.541)

X^2: Chi square test
OR: odds ratio
95% CI: 95% confidence interval

Assessment of SCARB 1 gene SNP (rs 5888) genotype distribution in PCAD patients in relation to patients' lipid profile showed no difference between the three genotypes regarding all tests ($p>0.05$), whereas, a highly significant difference was detected among SCARB 1 genotypes regarding Gensini score ($p < 0.01$) **(Table 5).**

Table (5): Descriptive and comparative statistics of genotype frequencies of SCARB 1 gene SNP (rs 5888) in PCAD patients in relation to patients' lipid profile and Gensini score

Parameter		CC	CT	TT	H*	p value
		Median (Q1-Q3)				
Cholesterol (mg/dL)	**All**	207 (165-251)	207 (195-237)	240 (179-284)	3.326	> 0.05
	Male	212 (185-254)	208 (195-237)	242 (176-297)	1.94	> 0.05
	Female	181 (172)	207 (182-234)	227 (181-286)	1.714	> 0.05
HDL-C (mg/dL)	**All**	39 (34.5-42.5)	38.5 (35-41)	37 (33-40.5)	0.483	> 0.05
	Male	37 (34-40)	37.5 (33-40)	37 (34.5-41)	0	> 0.05
	Female	42.5 (40)	41 (39-44)	39 (28-41)	2.205	> 0.05
LDL-C (mg/dL)	**All**	140 (92.5-188)	137 (127-166)	158 (108-215)	1.484	> 0.05
	Male	142 (91-196)	142 (129-171)	163 (102-2240	0.681	> 0.05
	Female	103.5 (94)	135 (112-160)	158 (109-220)	3.05	> 0.05
Triglycerides (mg/dL)	**All**	155 (145-169)	160 (150-178)	173 (157-190)	4.572	> 0.05
	Male	153 (140-156)	160 (138-177)	172 (159-202)	5.511	> 0.05
	Female	175 (160)	166 (151-195)	187 (152-190)	0.57	> 0.05
Gensini Score	**All**	10 (2.5-20)	41 (28-49.5)	92 (79-107.5)	38.873	**< 0.01**
	Male	10 (0-20)	35 (27-47)	99.5 (81-128)	25.837	**< 0.01**
	Female	17.5 (5)	41.5 (33-55.5)	82 (60-96)	12.765	**< 0.01**

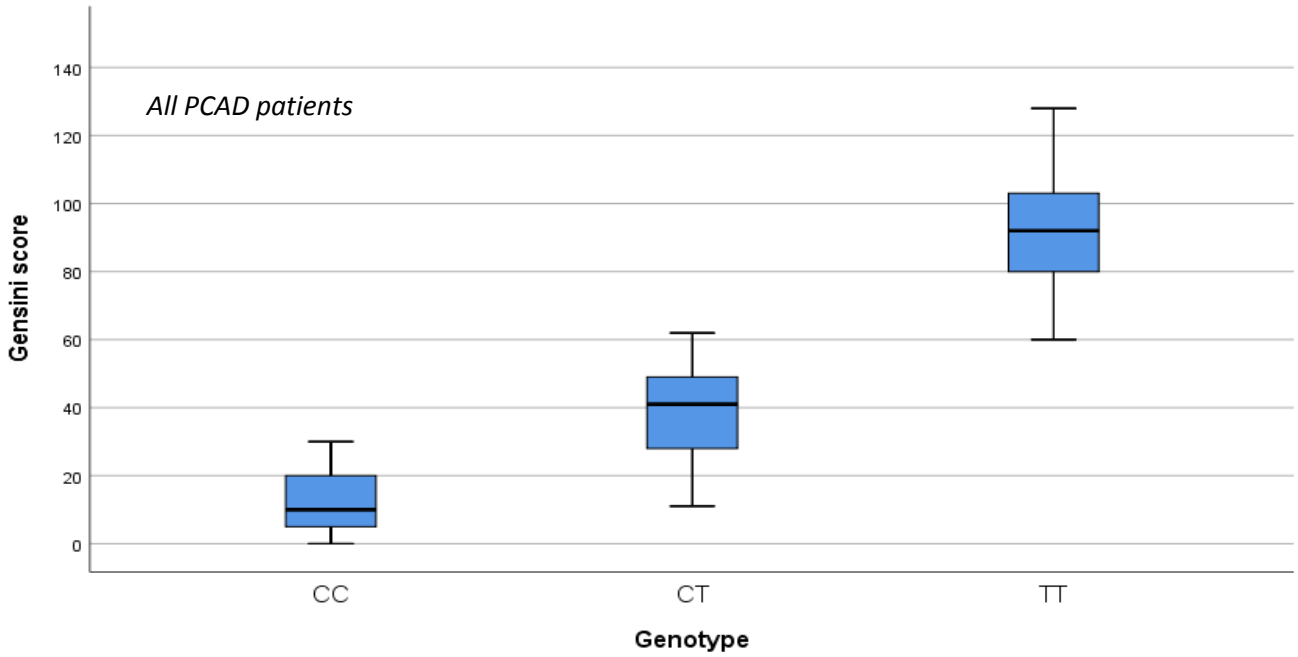
Q1: 25 percentile, Q3: 75 percentile
*H: Kruskal Wallis test

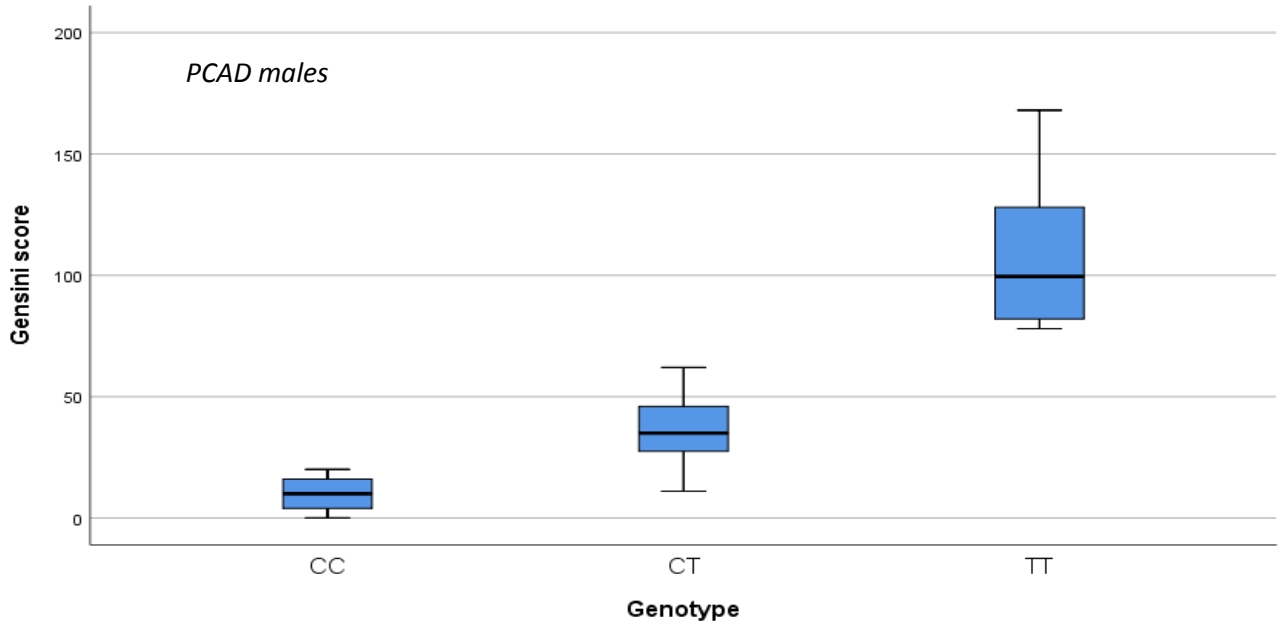
Gensini score in PCAD group was described in median (25-75 percentile). Its score was 44 (25-80.5) in all patients, 42 (20-80) in male patients and 56 (35.5-81) in female patients. Gensini score revealed a significant stepwise increase in PCAD patients from (CC) genotype to (CT) and (TT) genotypes (**Figure 2**). The highest score was detected in the homozygous mutant form (TT) when compared to (CC) and (CT) genotypes in all PCAD group, in males subgroup of patients and in females subgroup of patients as well (**Table 6**).

Table (6): Comparison statistics of SCARB 1 rs5888 SNP genotypes in association with PCAD severity by Gensini score

All PCAD patients:			
Parameter	**CC vs. CT**	**CC vs. TT**	**CT vs. TT**
Z	-3.766	-4.129	-5.35
P value	**< 0.01**	**< 0.01**	**< 0.01**
Among males:			
Parameter	**CC vs. CT**	**CC vs. TT**	**CT vs. TT**
Z	-3.349	-3.424	-4.221
P value	**< 0.01**	**< 0.01**	**< 0.01**
Among females:			
Parameter	**CC vs. CT**	**CC vs. TT**	**CT vs. TT**
Z	-1.713	-2.058	-3.249
P value	> 0.05	**< 0.05**	**< 0.01**

Z: Wilcoxon rank sum test





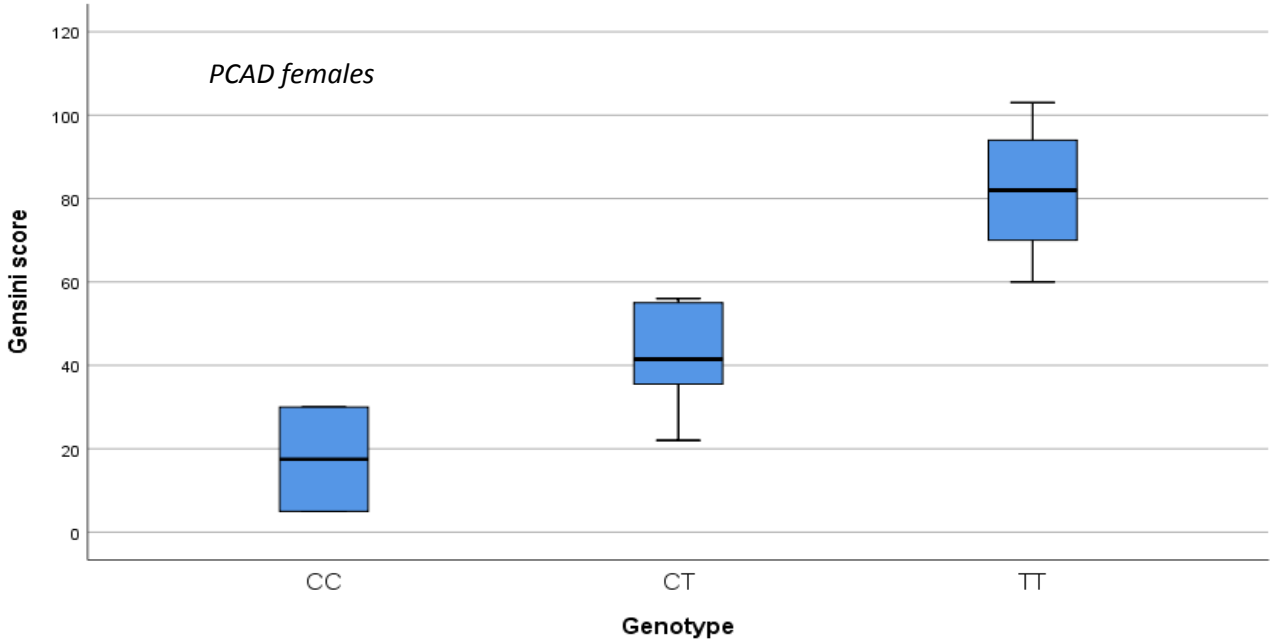


Figure (21): SCARB 1 rs5888 SNP genotypes in association with Gensini score.

In addition, Gensini score was found to be higher in heterozygous mutant (CT) form when compared to the wild form (CC) in all PCAD group and in males' subgroup of patients, but with no difference in score values between both CC and CT genotypes in female subgroup of patients.

DISCUSSION

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 are important for better defining individuals at risk for cardiovascular events [(16)].

SCARB 1 is a cell surface receptor that typically binds multiple ligands. It is encoded by the SCARB1 gene located on chromosome 12q24.31 [(17)]. It was the first HDL receptor to be identified and it mediates the selective transport of lipids, such as cholesterol esters from HDL into cells [(18)].

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 genetic studies in various populations have discovered multiple SCARB 1 variants and reported their relationship with lipid traits, and subclinical atherosclerosis and incidence of coronary artery disease [(17)].

In our study, the frequency of the wild genotype (CC) of SCARB 1 was higher in the non-PCAD group than patients' group and it can be considered as a protective genotype for CAD in patients' population including both male and female subgroups.

The homozygous and heterozygous mutations (TT and CT genotypes) were statistically more frequently distributed in PCAD patients compared to non- PCAD subjects. However, only the CT genotype was considered as significant positive risk factor for PCAD in males but not in females.

These results came in accordance with **Nagarajan *et al.*** [(19)] who studied 148 Indian CAD patients and 162 controls, they found that CT genotype had higher odds of developing myocardial infarction.Moreover, **Wu and his colleagues** [(20)] 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% vs. 5.2 %). They added that the TT genotype had higher risk for CAD.

The relation between SCARB 1 studied SNP and PCAD can be linked to their role in lipid metabolism. **Constantineau *et al.*** [(10)] found that the rs5888 SNP affected SCARB 1 RNA secondary structure, which changed its ability to undergo productive protein translation leading to significantly lower SCARB 1 protein expression; thus leading to dysfunctional HDL particles and/or increased LDL levels in circulation fastening the process of atherosclerosis.

Moreover, SCARB 1 function was significantly lower in macrophages expressing the rs5888 variant[(10)]. However, more researches are required to investigate the exact mechanism and biological basis of possible role of rs5888 variant in pathogenesis of CAD needs to be investigated.

On contrary to our findings, **Zeng *et al.*** [(21)] conducted a study on 295 Chinese CAD patients and 312 controls and showed no significant differences in

allele frequency and genotype distribution of SCARB 1 rs5888 SNP between control subjects and patients with CAD.

In addition, **Rejeb *et al.*** [22] studied 316 patients with more than 50 % luminal stenosis in coronary angiography in Tunisian population and found that the 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.

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.

For example, the precise definition of phenotype in studies on CAD association is of essential importance because individuals with coronary artery stenosis who are clinically silent may be classified as controls leading to a higher likelihood of null results.

To avoid this potential bias in the current study, we defined phenotype based on objective angiographic documentation of coronary artery status, where PCAD patients were defined as those with evidence of atherosclerotic lesions and non- CAD were those with negative angiographic findings.

Our study results have shown that the C allele was more frequently distributed among control group and it can be considered as a significant negative risk factor for PCAD. On the other hand, the T allele was more distributed among PCAD group and it can be considered as a significant positive risk factor for PCAD.

These findings came in accordance with **Goodarzynejad *et al.*** [11] study on Iranian population that included 505 PCAD patients and 546 controls. They found that T allele in comparison with C allele had 1.3-fold increased risk for PCAD.

Moreover, the results of our study showed no statistical difference between the distribution of the studied genotypes of SCARB 1 in male and female subgroups of the PCAD patients, this came in accordance with the findings of **Goodarzynejad *et al.*** [11].

Regarding the association of lipid profile and SCARB 1 genotype in patients in our study, there wasn't any statistically significant difference in the distribution of rs5888 genotypes even after analyzing data separately for males and females.

Our results agreed with those of **Goodarzynejad *et al.*** [11], **McCarthy *et al.*** [23] on American group of patients (n= 371) and **Cerda *et al.*** [24] in their study on Brazilian patients (n=332) who found no associations between SCARB 1 rs5888 (C > T) genotypes and lipid profile in their studied patients.

However, the association between SCARB 1 gene and lipid profile is complex and still debated. As significant sex-dependent associations between SCARB 1 genetic variations with lipid profile has been previously shown by **Stanislovaitiene *et al.*** [25] in their study on 463 Lithuanian patients who found that females with the TT genotype (homozygous mutant) had significantly lower LDL- cholesterol in comparison to the CC carriers (wild type). They explained their findings by a likely interaction between SCARB 1 genotype and estrogen dependent regulation of SCARB 1 expression or by a possible effect of sex hormones on factors affecting the function of SCARB 1 [25].

Further investigations are needed to test the relationship between SCARB 1 polymorphisms associated with lipid metabolism (e.g. exon 1 and intron 5) and the risk of CAD in both male and female populations.

Regarding the severity of PCAD that was assessed in our patients using Gensini score, our study revealed a significant stepwise increase among 3 genotypes, being highest in PCAD patients with the homozygous mutant genotype (TT) compared to PCAD patients with heterozygous mutant genotype (CT) and PCAD patients with wild type (CC) among all patients and in both male and female subgroup of patients.

However, our results didn't agree with **Goodarzynejad *et al.*** [11] who found no association for the studied genotypes with the severity of PCAD. They explained this null result by the fact that rs5888 SNP might be in linkage disequilibrium (LD) with another functional mutation in the SCARB 1 gene, and the LD pattern at this region is different among populations.

Accordingly, further studies on the associations of the SCARB 1 rs5888 SNP and severity of atherosclerosis related diseases in different ethnic populations are needed.

The results of our study showed that the overall prevalence of cardiovascular risk factors including hypertension, smoking and family history was higher in PCAD group when compared to control group. These findings go with the findings of **Goodarzynejad *et al.*** [11].

The importance of environmental risk factors in CAD is explained by the fact that over 90% of CAD events occur in individuals with at least 1 risk factor. Moreover, the absence of one of the major risk factors predicts a much lower risk of CAD [26].

Limitation of the study

This study is a pilot study done on Egyptian patients with PCAD and further studies on the associations of the SCARB 1 rs5888 SNP and severity of atherosclerosis related diseases in different ethnic populations are needed in a large scale. In addition, follow up of PCAD patients is recommended to

provide information on the role of SCARB 1 on PCAD prognosis and survival.

Conclusion

The study has shown that the wild (CC) genotype and the C allele confer a negative risk for PCAD. On the other hand, the mutant (CT) genotype together with the T allele of SCARB 1 SNP rs5888 were associated with increased risk of PCAD. Moreover, a significant association was observed between SCARB 1 SNP rs5888 genotypes (TT) and (CT) and disease severity calculated by Gensini score.

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