J Mol Neurosci (2014) 53:150–157
DOI 10.1007/s12031-014-0238-2

Effect of E670G Polymorphism in *PCSK9* Gene on the Risk and Severity of Coronary Heart Disease and Ischemic Stroke in a Tunisian Cohort

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Received: 28 November 2013 / Accepted: 13 January 2014 / Published online: 6 March 2014

Abstract The association of E670G (rs505151) polymorphism in *PCSK9* gene with an increased risk of coronary artery disease (CAD) and ischemic stroke (IS) was reported in previous studies. We investigated the effect of the E670G (rs505151) on the risk of CAD and IS in a Tunisian cohort. Genotyping of the PCSK9 E670G was performed using polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) and then confirmed by direct sequencing. The frequency of the 670G allele was significantly higher in the CAD than in the no-CAD subgroup (0.132 vs. 0.068, p=0.030). As expected, the incidence of E670G was significantly important in IS subgroup than control group (0.122 vs. 0.073, p=0.032). Furthermore in CAD patients, the 670G carriers showed significantly increased plasma total cholesterol and LDL-cholesterol levels compared to E670 carriers (6.78 [6.47–7.00] vs. 4.92 [4.02–5.46] mmol/l, p<0.0001 and 4.60 [4.00–5.04] vs. 3.00 [2.22–3.70] mmol/l p=0.001, respectively). The risk and severity of CAD were significantly increased in 670G carriers between no-CAD subgroup and CAD patients presenting a stenosis ≥50 % in two or three major coronary arteries (0.068 vs. 0.198, p= 0.001, OR=3.39 [1.55–7.37]). The E670G polymorphism of the PCSK9 gene is mainly associated with a increased risk and severity of CAD and IS in Tunisian cohort.

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Keywords Coronary artery disease · *PCSK9* gene · E670G polymorphism

Introduction

Coronary artery disease (CAD) and stroke are the two major manifestations of atherosclerotic processes, which are expected to remain one of the leading causes of mortality until at least 2030 (Mathers and Loncar 2006). Elevated plasma levels of low density lipoprotein-cholesterol (LDL-C) have consistently been shown to be a risk factor for the development of atherosclerosis (Hobbs et al. 1990). Plasma concentrations of LDL-C are determined primarily by the activity of the LDL receptor (LDLR) in the liver. LDLR activity is controlled at the post-translational level by targeted degradation through the action of proprotein convertase subtilisin/kexin type 9 (PCSK9) (Mousavi et al. 2009; Seidah et al. 2003).

PCSK9, also known as NARC1 (neural apoptosis-regulated convertase 1), is the ninth member of the proprotein convertase (PC) family (Seidah et al. 2003). The human *PCSK9* gene is located on chromosome 1p32.3; it encompasses 12 exons and encodes a 692 amino acid glycoprotein. PCSK9 is synthesized as an inactive zymogen, pro-PCSK9 (73 kDa) and contains a signal peptide, a prodomain (residues 31–152) and a catalytic domain (residues 153–451) followed by a C-terminal domain (residues 452–692) (Lambert et al. 2009). PCSK9 undergoes intra-molecular autocatalytic processing at FAQ152↓SIP site in the endoplasmatic reticulum (ER) to form a 14-kDa prodomain and a 63-kDa mature PCSK9 (Seidah and Prat 2007). After cleavage, the prodomain remains closely attached to the catalytic domain of PCSK9 blocking the substrate-binding site (Seidah et al. 2003; Hampton et al. 2007; Kwon et al. 2008). Autocatalytic cleavage is required for trafficking PCSK9 from the ER to the

secretory pathway (Lagace et al. 2006; Cunningham et al. 2007). PCSK9 acts as a molecular chaperone that binds to the epidermal growth factor–like domain A of LDL receptor (LDLR) and promotes LDLR degradation through an endosomal/lysosomal pathway (Benjannet et al. 2004; Zhang et al. 2007). PCSK9 may also regulate ApoB-containing lipoprotein production and ApoB secretion (Ouguerram et al. 2004; Sun et al. 2005), and promote production of nascent very low-density lipoprotein (VLDL) in the fasting state (Lambert et al. 2006).

It was shown that human mutations affecting the level of PCSK9 and/or its activity toward the LDLR resulted in either hypercholesterolemia or hypocholesterolemia (Abifadel et al. 2009). Gain-of-function (GOF) mutations in PCSK9 reduces LDLR expression on the cell surface, which inhibits cellular uptake of serum LDL-cholesterol and ultimately elevates serum cholesterol that leads to autosomal dominant hypercholesterolemia (ADH) and premature atherosclerosis (Chan and Watts 2012). Conversely, a loss of function (LOF) mutations promotes LDL-cholesterol uptake and lowers serum cholesterol and LDL-cholesterol (LDLC) and confer protection against cardiovascular disease (Abifadel et al. 2003; Cohen et al. 2005).

PCSK9 is a highly polymorphic gene with over 40 non-synonymous, exonic single nucleotide polymorphisms (SNPs) reported in humans (Abifadel et al. 2007). Recent advances have revealed a large number of genetic variants of PCSK9 that may modulate plasma cholesterol levels either positively or negatively, therefore influencing the risk of atherosclerosis. A common SNP, E670G (rs505151) in exon 12 of PCSK9, result in the substitution of glutamate for a glycine residue at position 670 in the protein (Aung et al. 2011). Previous studies suggested a key role for the E670G polymorphism in determining plasma LDL-C levels and the severity of coronary atherosclerosis in an American population (Chen et al. 2005). However, subsequent studies conducted in Caucasian and African populations failed to find this association (Kotowski et al. 2006; Polisecki et al. 2008; Scartezini et al. 2007). In addition, the E670G polymorphism has been reported to be associated with higher serum lipid parameters (TC, LDL-C, HDL-C and ApoB) in the Han population (Aung et al. 2011). Furthermore, in a European population the 670G carriers showed significantly increased LDL in men but not in women (Evans and Beil 2006). More recently, the presence of the 670G allele was significantly associated with an increased risk of large-vessel atherosclerosis (LVA) stroke (Abboud et al. 2007) and the intima media thickness (IMT) (Norata et al. 2010).

In this study, we aimed to determine the relative frequency of the E670G (rs505151) of the *PCSK9* gene, their association with serum lipid levels and the risk of CAD and IS in a Tunisian population.

Patients and Methods

Patients and Controls

A total of 258 patients, recruited through the Cardiovascular Department of Fattouma Bourguiba hospital (Monastir, Tunisia), were diagnosed by angiography. Subjects were defined with CAD when presenting a stenosis ≥50 % in at least one major coronary artery. Patients who had inflammatory diseases, valvular heart disease, cancers or rheumatoid arthritis were excluded. The Control group included 232 subjects from a health professional Department, undergoing a routine checkup, all free of any history of obesity, hypertension, dyslipidemia, diabetes mellitus (DM), or CAD.

The ischemic stroke (IS) subgroup included 114 patients, recruited through the emergency Department at Fattouma Bourguiba University Hospital in Monastir, Tunisia. IS was defined as the rapid development of focal or global cerebral function's disturbance, with symptoms lasting 24 h or longer, or leading to death, with no apparent cause other than vascular origin. Stroke subtype determined according to TOAST criteria.

Subjects with hypertension were defined by a systolic blood pressure (SBP) ≥140 mmHg, or diastolic blood pressure (DBP) ≥90 mmHg, or both and/or the current use of antihypertensive drugs (Wang et al. 2005). Diabetic subjects were defined by a fasting plasma glucose ≥7.0 mmol/l, or by the use of anti-diabetic drugs (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 2003). The dyslipidemia defined by either high cholesterol levels (TC >5.17 mmol/l), or high triglycerides levels (TG >1.70 mmol/l), or both, or by the use of hypocholesterolemic drugs. According to the diagnosis, each patient (diabetic, hypertension and dyslipidemia) was treated independently with specific drugs. Obese subjects were defined by a body mass index (BMI) ≥30.0 kg/m^2. This study was approved by the hospital ethics committee. All participants were of Tunisian origin and gave their informed consent for this study.

Biochemical Analysis

Blood samples were taken for biochemical analysis following overnight fasting. Serum total cholesterol (TC), triglyceride (TG) and HDL cholesterol (HDL-C) concentrations were determined at accredited clinical laboratories using routine clinical methods. LDL-cholesterol (LDL-C) concentrations were calculated using the Friedewald equation (Friedewald et al. 1972).

DNA Analysis

Genomic DNA was prepared from white blood cells using the salting-out method (Miller et al. 1988). Genotypes for the

variant E670G were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) reaction. Exon 12 of *PCSK9* gene was amplified by PCR using the following primers: 5′-GATGTCGGAGGGAG AAATGA -3′ (forward) and 5′-GGCACCCAGAGTGAGT GAGT-3′ (reverse). The amplification conditions were: 94 °C for 3 min followed by 94 °C/55 °C/72 °C each one for 30 s for 30 cycles and a final extension at 72 °C for 5 min. The PCR fragment was digested with 2 U of *Sau*96I restriction enzyme (New England BioLabs). The A allele of the E670G produced band sizes of 287, 69, 51, 33, 30, 18 and 6 bp, and the G allele produced band sizes of 215, 72, 69, 51, 33, 30, 18 and 6 bp. The digestion products were separated on 8 % polyacrylamide gel. The bands were visualised on UV Transilluminator after staining with ethidium bromide. Six samples (AA, AG and GG genotypes in two, respectively) detected by the PCR-RFLP were also confirmed by direct sequencing. Sequences were performed through a partnership with La Plate-Forme Séquençage et Génomique (Institut Cochin, Paris, http://cochin.inserm.fr/) Electrophoregrams were analyzed using PolyPhred 5.04 software (Stephens et al. 2006).

Statistical Analyses

All statistical analysis was performed using version 11.0 of the Statistical Package for the Social Sciences (SPSS; SPSS Inc., Chicago, IL, USA). Data are presented as means ± standard deviation for continuous variables, and medians followed by interquartile ranges (25–75 %) for variables with a skewed distribution. Qualitative variables were expressed as percentages. Differences between two groups were evaluated by the parametric unpaired Student's *t*-test for continuous variables, or the non-parametric Mann–Whitney test for discontinuous variables. Qualitative variables, allele frequencies and Hardy–Weinberg equilibrium were assessed by the Pearson's χ^2 test. Linear multiple regression analysis was used to define factors affecting the risk and severity of CAD and IS in this population. A p value of <0.05 was considered significant.

Results

Participant Characteristics

In this study, we divided the group of 258 patients, according to their angiographic analysis, into a subgroup of 66 patients without CAD and a subgroup of 192 patients with CAD (presenting a stenosis ≥50 % in at least one major coronary artery). We observed several differences between the groups of patients (Table 1). As expected, several common risk factors for CAD were significantly different between the two subgroups: age, gender, low HDL-C, high LDL-C and diabetes. Other CAD risk factors, such as high triglyceride levels, hypertension and cigarette smoking (current smokers), were not significantly different. Surprisingly, obesity was significantly less frequent in the CAD subgroup. We observed a significantly difference between the IS and controls group among age, gender, cigarette smoking (former smokers). As expected, obesity and diabetes were significantly more frequent in the IS group (Table 1).

Genotyping of the rs505151 Polymorphism

Through a PCR-RFLP method used to analyze E670G polymorphism, the genotype were determined according to the presence or absence of the enzyme restriction sites, when an A to G transversion at 670 locus of the PCSK9 gene. One sample, for each one of the three different genotypes identified (A/A, A/G, G/G), was confirmed by Sanger sequencing.

Genotypic and Allelic Frequencies

The genotypic and allelic frequencies of E670G polymorphism in exon 12 of the PCSK9 gene are shown in Table 2. Among the 258 patients, were 203 AA, 48 AG, and seven GG. Among the 232 controls, were 199 AA, 32 AG, and one GG. Among the 114 IS, 90 were AA, 20 AG, and four GG.

The distributions of the E670G among the three subgroup (patients, control and IS) of this study did not deviate from the Hardy–Weinberg equilibrium (p=0.879, p=0.926 and p=0.877, respectively). The G allele frequency was 0.120, 0.085 and 0.122 among the patients, the controls and the IS, respectively.

Association of E670G Variant with Increased LDL-C and Diabetes

We found a possible association between E670G polymorphism and the risk of CAD and DM. The frequency of the 670G allele was significantly higher in the DM than in the no-DM subgroup (0.151 vs. 0.091, p=0.036). Similarly, the incidence of G allele of E670G polymorphism was more frequent in CAD than in the no-CAD subgroup (0.132 vs. 0.068, p=0.030). In addition, the frequency of 670G allele was significantly higher in IS subgroup than the control group (0.122 vs. 0.073, p=0.032).

The association of the 670G allele with a increased in LDL-C levels was observed in the present study. For the group of patients, the variation of LDL-C for E670G polymorphism was 3.00 mmol/l for A/A genotype and 3.20 mmol/l for A/G+G/G genotype. This increase in the LDL-C level was statistically significant (p=0.034). Interestingly, CAD patients with G allele showed significantly increased total cholesterol and LDLC levels when compared to A allele carriers (6.78 [6.47–7.00] vs. 4.92[4.02–5.46] mmol/l, $p<0.0001$ and 4.60 [4.00–

Table 1 Demographic, clinical and biological characteristics of the different subgroups of patients and controls

	No CAD (*N*=66)	CAD (*N*=192)	*p* value	IS (*N*=114)	Controls (*N*=232)	*p* value
Age (years)	55 [52–65]	61 [55–67]	0.027*	66 [54.5–76.50]	49.0 [45–55]	<0.0001*
% Male	74.2	84.7	0.051§	57.4	74.0	<0.009§
% Current smokers	47.0	46.6	NS§	42.1	41.3	NS§
% Former smokers	9.1	21.2	0.001§	17.5	11.9	<0.0001§
BMI (kg/m^2)	28.21±4.67	27.08±4.15	0.037#	26.00±5.68	25.00±3.77	NS#
% Obese	27.3	15.9	NS§	31.5	13.3	0.006§
Fasting glucose[a]	8.56±4.57	8.81±4.52	NS#	9.36±4.97	4. 97±0.62	<0.0001#
% Diabetics	46.6	62.3	0.029§	48.1	0	–
% Hypertension	36.2	39.1	NS§	55.3	0	–
Cholesterol[a]	4.62 [3.96–5.43]	5.00 [4.10–5.50]	NS*	4.40 [3.87–5.00]	4.28 [3.50–5.62]	NS*
Triglycerides[a]	1.72±0.87	1.90±1.11	NS#	1.56±0.72	1.37±0.61	0.052#
HDL-C[a]	1.23±0.36	1.01±0.35	<0.0001§	1.29 ±0.39	1.29±0.61	NS#
LDL-C[a]	2.58 [2.00–3.35]	3.10 [2.39–3.74]	0.014*	2.61 [2.17–3.20]	2.32 [1.63–3.29]	NS*

[a] Levels are in mmol/l

*One-tailed *p* values were obtained with Mann–Whitney test for quantitative variables with a skewed distribution

One-tailed *p* values were obtained with unpaired Student's *t* test for quantitative continuous variables

§ One-tailed *p* values were obtained with Pearson's χ^2 test for qualitative variables

5.04] vs. 3.00 [2.22–3.70] mmol/l, p=0.001, respectively). The association of the E670G polymorphism with DM was showed in the CAD subgroup when the mean fasting glucose level was greater in the G allele carries (12.88±8.09) than the A allele carriers (8.50±4.37), with a significant difference (p=0.012).

Among the IS subgroup, the variation of CT and LDL-C was associated with the E670G polymorphism. The G allele carriers showed significantly increased total cholesterol and LDLC levels when compared to A allele carriers (6.23 [6.07–6.47] vs. 4.10 [3.41–5.11] mmol/l, p=0.002 and 4.14 [3.88–4.32] vs. 2.13 [1.60–2.95] mmol/l, p=0.001, respectively).

The Association of E670G Polymorphism with Risk and Severity of CAD

To investigate the association of the G allele with a risk of CAD, we divided the group of 258 patients into three subgroups: no-CAD (N=66), presenting a stenosis ≥50 % in one major coronary artery (N=109) [CAD-1], and presenting a stenosis ≥50 % in two or three major coronary arteries (N=83) [CAD-2,3]. Among these three new subgroups, the frequency of the G allele progressively increased (0.068, 0.082, and 0.198, respectively), and this increase was significant between the no-CAD and the CAD-2,3 and CAD-1 vs. CAD-2,3 subgroups (p=0.001, OR=3.39 [1.55–7.37] and p=0.008, OR=2.57 [1.49–5.09], respectively).

Multiple regression analysis was performed to extract factors determining the risk and the severity of CAD and IS. Conventional CAD risk factors and the G670 allele were treated as independent variables, and we compared all of them between CAD and no-CAD, IS and controls, no-CAD and CAD-2,3. As expected, conventional factors such as age, gender, LDL-C and HDL-C significantly modulate the risk of CAD. Age, gender and LDL-C increase the CAD risk (p=0.018, OR=1.04 [1.00–1.07]; p=0.041, OR=1.91 [0.97–3.77]; p=0.040, OR=1.29

Table 2 Genotypic and allelic distributions of E670G (rs505151) polymorphism in CAD and no-CAD patients, IS and controls

	No CAD (*N*=66)	CAD (*N*=192)	*p* value	DM (*N*=132)	No DM (*N*=126)	*p* value	IS (*N*=114)	Controls (*N*=232)	*p* value
E670G (rs72555377)									
A/A	57 (0.863)	148 (0.770)	0.935	98 (0.724)	104 (0.825)	0.060	90 (0.789)	199 (0.857)	0.020
A/G	9 (0.136)	37 (0.192)	0.251	28 (0.212)	21 (0.166)	0.278	20 (0.175)	32 (0.137)	0.298
A/G+G/G	9 (0.136)	44 (0.229)	0.107	34 (0.257)	22 (0.174)	0.106	24 (0.210)	33 (0.142)	0.109
G/G	0 (0.00 %)	7 (0.036)	0.251	6 (0.455)	1 (0.079)	0.052	4 (0.035)	1 (0.0043)	0.020
Allele G	0.068	0.132	0.030	0.151	0.091	0.036	0.122	0.073	0.032

Data presented are number (percentage)

[0.98–1.69]). HDL-C decrease the CAD risk ($p<0.001$, OR= 0.20 [0.09–0.44]). The G670 allele also appears to be an independent factor increasing the CAD risk ($p=0.030$, OR=3.51 [1.26–5.40]). For the severity of CAD, age, gender, LDL-C, HDL-C and DM significantly modulate the severity of CAD and the G670 allele also appears to be an independent factor which increased the severity of CAD ($p=0.025$, OR=2.33 [1.20–4.98]). In addition, age, gender and obesity significantly increasing the risk of IS, ($p<0.001$, OR=1.13 [1.09–1.17]; $p=0.010$, OR=1.43 [1.00–2.27]; $p=0.007$, OR=2.59 [1.29–5.21], respectively). The G670 allele also appears to be an independent factor increasing the IS risk ($p=0.005$, OR=21.83 [2.49–30.06]).

Discussion

Our investigation aimed to determinate the effect of a E670G (rs505151) polymorphism in exon 12 of *PCSK9* gene in a group of 258 patients diagnosed by angiography and in group of 114 patients with IS, and to analyze the association between E670G and DM, the risk of CAD.

The division of this group of patients into the CAD and the no-CAD subgroups revealed classical risk factors for CAD such as age, gender (Abdel-Maksoud et al. 2012), low HDL-C (Zhang et al. 2008), high LDL-C (Gotto and Moon 2012) and DM (Saely and Drexel 2013), but not high triglyceride levels, hypertension, cigarette smoking and obesity. One explanation for this surprising observation is the small size of the sample which limits the power of this analysis to only strong risk factors.

Our findings showed a higher frequency of G allele of E670G polymorphism in CAD subgroup (0.132). Among the other subgroups (no-CAD, IS and controls), the frequency of G allele (0.068, 0.122 and 0.073) was less than that observed in the CAD subgroup. Previous findings showed that the frequency spectrum of E670G polymorphism varied significantly among different races/ethnicities (Aung et al. 2011; Evans and Beil 2006; Norata et al. 2010). Interestingly, the incidence of G allele observed in our study was different from values reported in the TexGen population (0.044) and that reported for the LCAS (0.074) by Chen et al. (2005). In addition, the frequency of the G allele in patients with polygenic hypercholesterolemia (0.11) selected from Universitätsklinikum Hamburg–Eppendorf Martinistrasse, Hamburg, Germany (Evans et al. 006), was slightly less than that observed in the CAD patients subgroup. In contrast to our finding, the 670G carrier in Chinese Taiwanese was identified less frequently in patients with CAD than in controls (9.9 % vs. 11.9 %), but the difference was not significant in a multivariable logistic regression analysis (Hsu et al. 2009). The discrepancy between the current study and other reports may be due to the fact that this study included CAD patients with different clinical and biological characteristics (Table 3).

Recently, it has been shown that PCSK9 is also expressed in human atherosclerotic plaques. In vitro, PCSK9 secreted by vascular smooth muscle cells reduced LDLR expression and LDL-C uptake of human and murine macrophages, which might result in vascular lipid accumulation and oxidation and indicating a possible direct action of PCSK9 on atherosclerotic plaque development and composition (Ferri et al. 2012).

On the other hand, we extend the previous finding reported by Abboud et al., who have mainly focused on the impact of E670G polymorphism on the risk of IS. The incidence of G allele tended to be higher in IS subgroup of our study and in LVA stroke patients (0.108) selected from the Belgium Stroke Study. This finding is clearly a direct effect of G670

Table 3 Biological characteristics of the CAD and IS patients according to E670G (rs505151) polymorphism

	Total patients (*N*=258)			CAD patients (*N*=192)			IS patients (*N*=114)		
	AA	AG+GG	*p*- value	AA	AG+GG	*p*- value	AA	AG+GG	*p*- value
Variables									
Fasting glucose[a]	8.44±4.23	9.29±5.55	0.224[#]	8.39±4.33	12.88±8.09	0.010[#]	7.19±5.18	9.68±4.93	0.257[#]
Cholesterol[a]	4.91 [4.00–5.50]	5.08 [4.18–5.65]	0.110*	4.88 [4.04–5.44]	6.78 [6.47–7.00]*	<0.001	4.10 [3.41–5.11]	6.23 [6.07–6.47]	0.002*
Triglycerides[a]	1.78±0.99	1.67±0.76	0.429[#]	1.69±0.86	1.97±0. 96	0.405[#]	1.58±0.75	1.47±0.58	0.681[#]
HDL-C[a]	1.07±0.38	1.03±0.52	0.432[#]	1.00±0.36	1.03±0.16	0.863[#]	1.35±0.40	1.04±0.19	0.031[#]
LDL-C[a]	3.00 [2.20–3.67]	3.20 [3.00–4.00]	0.034*	3.10 [2.38–3.70]	4.60 [4.00–5.04]	0.001*	2.13 [1.60–2.95]	4.14 [3.88–4.32]	0.001*

[a] Levels are in mmol/l

*One-tailed *p* values were obtained with Mann–Whitney test for quantitative variables with a skewed distribution

[#] One-tailed *p* values were obtained with unpaired Student's *t* test for quantitative continuous variables

polymorphism on cerebrovascular disease, especially in IS, and suggests that the risk is mediated by the severity of intracranial atherosclerosis (Abboud et al. 2007). Our study proved this association via the logistic multiple regression analysis. In accordance with this observation, a Canadian study found a 2-fold increased expression of the PCSK9 in the human frontal cortex of autopsy-confirmed Alzheimer's disease cases compared with age-matched controls (Belanger Jasmin 2011). Furthermore, Rousselet et al. (2011) showed that PCSK9 and LDLR are co-expressed in mouse brain during development and at adulthood. In addition, they provide the first demonstration that endogenous PCSK9 regulates the levels of LDLR during mouse brain development and following IS (Rousselet et al. 2011).

Besides lipid metabolism, PCSK9 may also interfere with glucose metabolism. Recently, several data have emphasized a possible link between PCSK9 and glucose homeostasis. Indeed, fasting–refeeding experiments in mice have demonstrated that hepatic PCSK9 expression is induced by insulin in a SREBP-1c-dependent-manner (Costet et al. 2006). Noteworthy, in pancreatic tissue of PCSK9(−/−) mice, expression of LDLR is increased while insulin levels are reduced, resulting in hyperglycemia and glucose intolerance (Mbikay et al. 2010). Thus, we also analyzed the association between E670G and DM. It is noteworthy that patients with DM present a significantly higher frequency of G allele than patients with no-DM. To our knowledge, the increase in body fat, especially the intra-abdominal adipose tissue is a major contributor to the insulin resistance and is associated with chronic diseases such as type 2 diabetes, CAD, metabolic syndrome and stroke (Yin et al. 2012). In contrast to our finding, the incidence of 670G polymorphism was significantly less in subject with overweight than normal group. An intriguing result is that 670G polymorphism may associate with increased fasting glucose level in CAD subgroup patients (Yin et al. 2012). Subsequently, analysis of a larger cohort is required to definitively establish a significant association between G670 variant and DM.

Previous findings in patients with coronary stenosis (Chen et al. 2005) or hypercholesterolemia (Scartezini et al. 2007) showed that the E670G variant was associated with increased plasma total cholesterol, LDL cholesterol, and apolipoprotein B levels, a finding not confirmed in an elderly population with pre-existing vascular disease or prevalence of three major risk factors (Polisecki et al. 2008). In this present report, carriers of the G allele showed LDL-C levels 6.6 % higher than non-carriers. In particular, the greater increased levels of LDL-C and CT was shown in CAD subgroup than the other subgroup (no-CAD and controls group). Contradictory to our finding, Hsu et al. (2009) failed to find a cholesterol-raising effect of the E670G in a Taiwanese population sample, and the study of Kotowski et al. (2006) also failed to find such an association between plasma levels of LDL-C and the E670G substitution in the Dallas Heart Study. In line with our results, Norata et al. (2010) showed that the 670G carriers were associated with increased plasma TC, LDL-C, and ApoB levels in the general population. Another association was determined by Evans and Beil (2006), wherein the PCSK9 670G allele was associated with increased LDL-C levels in men, but not in women, of European origin. More fundamentally, Chen et al. (2005) showed that PCSK9 E670G was an important determinant of plasma LDL-C levels exerting a dose effect (GG _ EG _ EE), accounting for 3.5 % of its variability. Plasma total cholesterol, apolipoprotein B, and lipoprotein (a) levels were also associated with the E670G variant (Chen et al. 2005).

Our observations raise an important report to replicate the association between E670G polymorphism and risk and severity of atherosclerosis. It is well admitted that 670G allele is associated with an increased risk of CAD. The same finding was shown, where the presence of the 670G allele was stratified according to the apoE gene alleles, and apoE2–PCSK9-670EE carriers showed a more favorable plasma lipid profile and decreased IMT compared with the apoE4–PCSK9-670G carriers. This is the first demonstration of an apoE by PCSK9 GOF mutation interaction in a general population and supports the relative protective effect of the apoE2 allele (Norata et al. 2010). It is consistent with the findings of Chen et al. (2005) both for the effect of the E670Gvariation as part of a haplotype on LDL-C and on severity of CAD in the Lipoprotein Coronary Atherosclerosis Study (LCAS) in Houston. However, the association between the PCSK9 E670G SNP and CAD risk was not confirmed in other studies (Kotowski et al. 2006; Scartezini et al. 2007; Huang et al. 2009).

In conclusion, our study clearly demonstrates the association between the E670G variant and an increased LDL-C levels and risk of CAD and IS. Our findings suggest a direct role of PCSK9 on atherogenesis. This study presents several limitations, including the small size of the cohort and subgroup of patients especially the DM patients and IS patients, which is why these findings need to be investigated in larger populations to better clarify the trend of the association observed.

Acknowledgments We are indebted to the patients for their cooperation. This work was supported by grants from the Tunisian Ministries of Higher Education and Scientific Research.

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