



CETP TaqIB Polymorphism, Serum Lipid Levels And Risk Of Atrial Fibrillation: A Case-Control Study

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Abstract

The cholesteryl ester transfer protein (CETP) mediates the transfer of cholesteryl esters from high-density lipoproteins (HDL) to triglyceride (TG)-rich lipoproteins. A consistent number of investigations has suggested an association between the TaqIB polymorphism of the CETP gene, plasma HDL-C levels and the risk of cardiovascular disease, but the results are controversial.

The aim of this study was to determine if the TaqIB polymorphism might be related to the presence of atrial fibrillation (AF).

We conducted a case-control study, enrolling 109 Caucasian unrelated patients coming from Salento (Southern Italy) with documented AF and 109 controls selected from the same ward. The CETP TaqIB genotypes were determined by RFLP-PCR.

The subjects with the B2B2 genotype seem to be more susceptible to AF development (OR=2.28, 95% CI 1.06-4.89, p=0.032). The AF incidence is higher if we consider only the female subgroup (OR=5.14, 95% CI 1.57-16.82, p=0.0061). In the AF female subgroup the B2B2 patients had a statistically significant decrease of HDL-C levels (1.50 ± 0.35 vs 2.07 ± 0.42 ; p=0.012) and statistically higher TG levels (1.34 ± 0.46 vs 0.77 ± 0.14 ; p=0.027) and TG/HDL-C ratio (2.14 ± 0.80 vs 0.88 ± 0.23 ; p=0.007) when compared to B2B2 female control subjects. When we analyzed the linkage between the TaqIB polymorphism and the promoter variant (-629C/A), we found that 100% of the B2 alleles of the TaqIB polymorphism were associated with the A alleles of the -629 promoter polymorphism in our subjects.

This study suggests that in post-menopausal women atrial fibrillation could be promoted by the association of CETP B2B2/AA genotype with higher triglycerides values.

Introduction

The cholesteryl ester transfer protein (CETP) is a major determinant of the high-density lipoprotein (HDL) variability in the general population. This protein facilitates the exchange of cholesteryl esters from HDLs to triglyceride (TG)-rich lipoproteins and low-density lipoproteins (LDLs) in exchange to TGs, decreasing the HDL plasma level.¹ In human, CETP is expressed predominantly in the liver, spleen and adipose tissue.² Detectable levels of CETP can be observed in the small intestine, adrenal glands, heart, kidneys and skeletal muscle.³ The human CETP gene is located on chromosome 16q21 and consists of 16 exons. Several single nucleotide polymorphisms (SNPs) in the CETP gene have been identified. The most widely studied CETP variant, denoted TaqIB (rs708272), is the silent base change affecting the 279th nucleotide in the first intron of the gene.⁴ This was the first genetic variation related to

HDL-C plasma levels. The less common B2 allele (absence of the TaqI restriction site) has been associated with a lower CETP mass and with a higher HDL-C level compared to the more common B1 allele.⁵ Though this polymorphism has been shown to be consistently associated with HDL-C concentration, it does not directly influence CETP concentration or function. In Caucasian population the TaqIB polymorphism has been found to be in complete linkage with -629C/A (rs1800775), a polymorphism located in the promoter region of the CETP gene.⁶ This promoter polymorphism has been shown to influence CETP gene expression and this could account for the associations found between TaqIB polymorphism and plasma CETP mass and HDL-C concentration.⁶ The -629A allele has been associated with lower CETP mass and higher HDL-C than the -629C allele as demonstrated by 50% reduction in transcriptional activity in a reporter construct comprising the CETP promoter and a luciferase reporter.⁷ Since its first description, a consistent number of investigations has suggested an association between the TaqIB polymorphism, plasma HDL-C level and the risk of cardiovascular disease^{8,9} although controversy exists on this.¹⁰ SNPs in the CETP gene were also investigated for their association with atrial fibrillation (AF). Using the multifactor-dimensionality reduction method Asselbergs and coworkers showed, for the first time, that the B1B1

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genotype was associated with a decreased HDL-C level and the development of AF in presence of albuminuria, elevated C-reactive protein, renal dysfunction and ischemic heart disease.¹¹ On the contrary, using a total of 2145 cases with AF and 4073 controls from Germany, Sinner and coworkers could not reliably replicate this association.¹² In both studies the promoter variant has not been investigated and the lipid profile was not considered.

The aim of the present study was to determine the prevalence of TaqIB polymorphism in a cohort coming from Salento, that is a Mediterranean country, with a racially homogeneous population of Caucasian origin and to investigate the association of the CETP /TaqIB polymorphism with plasma HDL-C level and the development of AF. We found that in our population the B2B2 genotype was associated with the risk of AF in females.

Materials and Methods

Subjects

109 Caucasian unrelated patients, coming from Salento (Southern Italy), with documented AF and 109 controls, selected from the same ward, were enrolled in this study between January 2011 and June 2012. Patients with hyperthyroidism, moderate to severe valve disease, heart failure (greater than grade NYHA II) and with lone AF were excluded. The presence of AF was determined by patient's history, serial electrocardiograms or 48 hours ambulatory ECG monitoring. Patients with palpitations without ECG documentation of the arrhythmia were excluded from both patients and control groups.

We classified AF in paroxysmal when arrhythmia is self-terminating in <7 days, persistent when an episode lasts longer than 7 days or requires termination by pharmacological or electrical cardioversion, and permanent when the presence of arrhythmia is accepted by the patient.

Transthoracic echocardiogram was performed to assess left atrial and left ventricular dimensions, left ventricular ejection fraction and to detect significant valvular heart disease (at least moderate to severe). Information regarding the use of lipid-lowering drugs and smoking was obtained using a checklist. Body mass index (BMI) was calculated as the ratio between the weight and height squared (Kg/m²). Serum levels of total cholesterol, HDL cholesterol, triglycerides, glucose, C-reactive protein, interleukin-6 and urinary albumin excretion were determined in each patient. The study was approved by the local ethics committee and conducted in accordance with the guidelines of the declaration of Helsinki. An informed consent prior to participation was obtained from all subjects.

Hypertension was defined as a systolic blood pressure \geq 140 mmHg or a diastolic blood pressure \geq 90 mmHg or the use of antihypertensive medications. Diabetes was defined as a fasting plasma glucose level $>$ 7.0 mmol/l or a non-fasting plasma glucose level $>$ 11.1 mmol/l or the use of antidiabetic medications. Presence of ischemic heart disease was defined as prior myocardial infarction and/or angina with hospitalization and/or an infarct and/or major ischemia patterns on the electrocardiogram. Smoking was categorized as no smoking or current smoking (current or stopped $<$ 1 year ago). Chronic obstructive pulmonary disease (COPD) diagnosis was based on the presence of a post-bronchodilator FEV1/FVC $<$ 0.70 and we classified COPD as mild (FEV1 $>$ 80% predicted), moderate (50% $<$ FEV1 $<$ 80% predicted), severe (30% $<$ FEV1 $<$ 50% predicted) and very severe (FEV1 $<$ 30% predicted). Kidney failure (KF) diagnosis

was based on a level of GFR $<$ 15 mL/min/1.73 m² or a need for initiation of kidney replacement therapy (dialysis or transplantation) for treatment of complications caused by a decreased GFR.

Laboratory Measurements

The urinary albumin excretion rate was measured as the mean of two 24-h urine collections, and urinary albumin concentrations were determined by nephelometry. Blood samples were collected from subjects, after a 12- to 14-hour fast, into tubes containing 0.1% EDTA. High-sensitive C-reactive protein (CRP) was determined by nephelometry, interleukin-6 (IL-6) by immunochemiluminescence and all lipid parameters (total and HDL-C and TG) by colorimetric/spectrophotometric procedure. LDL-cholesterol was estimated from quantitative measurements of total and HDL-C and TG using the empirical relationship of Friedewald.

DNA Analysis

DNA extraction was carried out on total blood using Archive Pure DNA Blood Kit (5-PRIME, Hamburg, Germany) according to the

Table 1: Demographic data and clinical features of the study population

		Controls n = 109	AF patients n = 109	p value
Demographic	Male sex, n (%)	53 (48.6%)	47 (43.1%)	0.3414
	Age (years)	74 \pm 10	75 \pm 10	0.4612
	BMI (kg/m ²)	28 \pm 5	27 \pm 4	0.1045
	Current smoking	36 (33.0%)	41 (37.6%)	0.4831
Clinical history	NYHA class, n (%)			0.3717
	I	66 (60.5%)	61 (56.0%)	
	II	43 (39.5%)	48 (44.0%)	
	IHD, n (%)	34 (31.2%)	13 (11.9%)	0.0005
	Hypertension, n (%)	78 (71.6%)	83 (76.1%)	0.4465
	Diabetes, n (%)	24 (22.0%)	20 (18.3%)	0.5060
	KF, n (%)	20 (18.3%)	23 (21.1%)	0.6151
	COPD, n (%)			0.8155
	Mild	26 (23.9%)	21 (19.3%)	
	Moderate	65 (59.6%)	68 (62.4%)	
	Severe	13 (11.9%)	16 (14.7%)	
	Very severe	5 (4.6%)	4 (3.6%)	
Echo	AP left atrial diameter, mm	43.8 \pm 6.7	45.1 \pm 7.2	0.1690
	SI left atrial diameter, mm	52.8 \pm 6.4	53.3 \pm 4.1	0.4929
	ML left atrial diameter, mm	37.4 \pm 6.6	38.3 \pm 4.2	0.2310
	LV EF, (%)	55 \pm 7	54 \pm 6	0.2587
Laboratory	C-reactive protein, mg/dl	1.17 \pm 2.04	0.99 \pm 1.82	0.4926
	Urinary albumin excretion, mg/L	84 \pm 204	75 \pm 166	0.7212
	IL-6, pg/ml	15.47 \pm 45.77	13.39 \pm 25.41	0.6787
	Total cholesterol, mmol/L	4.50 \pm 1.14	4.28 \pm 0.93	0.1199
	Triglycerides, mmol/L	1.37 \pm 0.68	1.19 \pm 0.54	0.0315
	HDL-cholesterol, mmol/L	1.41 \pm 0.44	1.46 \pm 0.35	0.3542
	LDL-cholesterol, mmol/L	2.46 \pm 1.01	2.25 \pm 0.79	0.0887
Total cholesterol/HDL-cholesterol	3.34 \pm 0.99	3.13 \pm 1.16	0.1520	
	Triglycerides /HDL-cholesterol	2.52 \pm 1.67	2.02 \pm 1.17	0.0111
Drug therapy	Statin use, n (%)	76 (35%)	44 (20%)	0.0001

AF, atrial fibrillation; BMI, body mass index; IHD, ischemic heart disease; KF, kidney failure; COPD, chronic obstructive pulmonary disease; AP, antero-posterior; SI, supero-inferior; ML, medio-lateral; LV EF, left ventricular ejection fraction; IL-6, interleukin-6.

manufacturer's recommended protocol. Subsequently, CETP TaqIB (rs708272, G/A) genotyping was performed in all participants. Briefly, a fragment of 1018 bp of the intron 1 of the gene was amplified by PCR (polymerase chain reaction) with the use of primers designed on the NCBI Reference Sequences NT_010498 (pos 10608262-10610237) (forward 5'-GTGTGCCGCATCACCAAG-3', reverse 5'-TCTCGCCGTGATATCTG-3') followed by TaqI digestion. The resulting DNA fragments were the 707 and 311 bp in length corresponding to the wild type B1 allele, and the intact 1018-bp in length corresponding to the uncut B2 allele. For the -629C>A (rs1800775) genotyping, amplification of a 909 bp fragment of the 5'-flanking region of the CETP gene was carried out using PCR with the primers designed on the same Reference Sequences (forward 5'-CTGAGGAGGCAGCACTTGG-3', reverse -CAGCAGGGCCAGGGTCAG-3'). After the amplification, the generated PCR products were purified by PureLink PCR Purification Kit (INVITROGEN, Carlsbad, USA) and used directly for both strand DNA sequencing by a commercial service. DNA sequence data were processed and analyzed using the blast program (<http://www.ncbi.nlm.nih.gov/BLAST>).

Statistical Analysis

Continuous data are expressed as mean \pm standard deviation; categorical data are expressed as percentage. A goodness of fit test for normality and a Brown-Forsythe or Levene test for homogeneity of variances were used to assess the applicability of parametric tests. Differences between mean were evaluated by Student's t-test for the normally distributed continuous variables, or by non-parametric test for non-normal distribution (Mann-Whitney for two subgroups and Kruskal-Wallis for more than two subgroups). Differences in genotype frequencies and other categorical data between cases and controls were compared with Fisher's exact test (mid-p exact p-value). Consistency of genotype frequencies with the Hardy-Weinberg equilibrium (HWE) was tested using a chi-squared goodness-of-fit test on a contingency table of observed versus expected genotype frequencies in cases and controls. Post-hoc evaluations, where necessary, were made by means of the Bonferroni correction. A two-sided p value <0.05 was considered significant for all tests.

Results

Characteristics of the Study Population

Table 1 provides a summary of the characteristics of our study population. We excluded patients with hyperthyroidism, valvular disease, heart failure (greater than grade NYHA II) and with lone AF. No significant differences were observed between the case patients and the control subjects with regard to demographic data and common cardiovascular risk factors, such as hypertension, diabetes and smoking. No differences also exist between the two groups with regard to NYHA classification, kidney failure presence and stages of COPD. Therefore, the two populations appear to be homogeneous, with the exception of a lower percentage of subjects with ischemic heart disease in the AF group (p=0.0005).

The levels of IL-6, CRP as well as urinary albumin excretion were no significantly different between the two groups. HDL-C levels were similar in both case and control groups. The plasma TG levels and TG/HDL-C ratio were significantly lower in the AF group compared to the control group (p=0.0315 and p=0.0111, respectively).

76 subjects of the control group (35%) and 44 patients with AF

(20%) were treated with the lipid lowering drug statins (p=0.0001).

Among the 109 patients with arrhythmia, 51 (46.8%) were affected by paroxysmal AF, 39 (35.8%) by persistent AF and 19 (17.4%) by permanent AF.

Frequency of the CETP TaqIB Polymorphism

The genotype distribution of CETP TaqIB polymorphism is shown in Table 2. B1 and B2 were respectively used to denote the presence and the absence of the restriction site for the enzyme TaqI in intron 1. In the general population, the AF group had a higher percentage of both B2B2 and B1B1 genotypes and a lower percentage of B1B2 genotype compared to the control group and these differences were significant (p=0.0342). On the contrary no significant differences in B2 allele frequency were found between the two groups. When the genotypes were stratified by gender, we found no significant differences in the male subgroup. Conversely in the female subgroup, the difference of B2B2 genotype between AF and control subjects significantly increased (p=0.0059). A post-hoc analysis with Bonferroni's correction for pair-wise comparisons confirmed the significant differences between control subjects and AF patients for the B2B2 (p=0.015) and B1B2 genotypes (p=0.019). We also observed the increase of B2 allele frequency in AF patients with respect to control subjects but the difference was not significant. The genotype distribution of case and control group was in the Hardy-Weinberg equilibrium.

According to these data the subjects with the B2B2 genotype seem to be more susceptible to AF development (OR=2.28, 95% CI 1.06-4.89, p=0.032). The AF incidence is higher if we consider only the female subgroup (OR=5.14, 95% CI 1.57-16.82, p=0.0061).

We then examined the distribution of CETP genotypes in relation to the different forms of atrial fibrillation (paroxysmal, persistent, permanent) without observing any statistically significant difference (p=0.0917).

Effect of the CETP TaqIB Polymorphism on Serum Lipid Levels in Female Population

The effect of CETP TaqIB polymorphism on lipid parameters in the female population is shown in Table 3. In total female population the B2B2 individuals showed a trend towards increase in HDL-C compared to B1B1 and B1B2 subjects, although the difference was not statistically significant. The increase in HDL levels in B2B2 subjects was more evident when we considered the control subgroup. In this case we have found a difference, close to statistical significance (p=0.086), in the HDL-C levels in B2B2 subjects with respect to B1 carriers. The increased HDL-C levels were concomitant with a reduction in TG levels and TG/HDL-C ratio. For both parameters, the difference was statistically significant (p=0.005 and p=0.019 respectively). Differently in the AF subgroup the B2B2 patients had a statistically significant decrease of HDL-C levels (1.50 ± 0.35 vs 2.07 ± 0.42 ; p=0.044) and statistically higher TG levels (1.34 ± 0.46 vs 0.77 ± 0.14 ; p=0.006) and TG/HDL-C ratio (2.14 ± 0.80 vs 0.88 ± 0.23 ; p=0.004) when compared to B2B2 control subjects. Therefore in B2B2 females, the higher TG levels contrast the raising of HDL-C levels.

Sequence Analysis of the CETP Promoter Region

To investigate the -629C>A polymorphism that has been found to be in complete linkage with the TaqIB variant, the DNA sequence between -827 to +36 bp of the CETP gene was amplified in all

B2B2 females (18 AF patients and 5 controls) and in 23 B1B1 AF females randomly selected. The 909-bp PCR produced from all the typed patients was directly sequenced. The sequence analysis showed that all the B2B2 subjects exhibited the -629AA genotype, while the B1B1 subjects showed the CC genotype (16/23), the CA genotype (5/23) or the AA genotype (2/23). Therefore in our subjects the 100% of the B2 alleles of the TaqIB polymorphism was associated with the A alleles of the -629 upstream promoter polymorphism, while the 80% of the B1 alleles of the TaqIB polymorphism was associated with the C alleles. These results indicate that in our female population the -629A allele was completely concordant with the TaqIB2 variant.

Discussion

AF is the most common arrhythmia found in everyday clinical practice. The majority of patients with AF has underlying heart disease, such as valvular heart disease, hypertension, or left ventricular dysfunction. However, some patients develop AF in absence of any known risk factor. Family studies have revealed that gene mutations with a mendelian hereditary pattern underlie rare forms of AF. In addition, numerous reports have suggested associations between genetic polymorphisms and common forms of AF¹³⁻¹⁶ but the identified variants were not often replicated in independent populations.

The CETP TaqIB polymorphism is very common in the population and seems to play an important role in cardiovascular disease.¹⁷ Although HDL cholesterol levels are higher about 10% in B2B2 genotype compared to B1B1, the association of this polymorphism with cardiovascular disease has not been established unequivocally. Some studies have shown a protective effect,^{18,19} others have highlighted the association with adverse cardiovascular events²⁰ or have found no association.

Our study reported that the TaqIB2 allele and the concordant -629A allele of the CETP gene are associated with a higher incidence of AF in general population, particularly in females with increased TG levels (OR=5.14, 95% CI 1.57-16.82, p=0.0061). The role of the CETP TaqIB polymorphism on AF has not yet been unequivocally reported. Asselbergs and coworkers showed for the first time an

Table 2: TaqIB polymorphism: genotype and allele frequencies in the study population

	Controls	AF patients	p value
Total	n=109	n=109	
B2B2	14 (12.8%)	25 (23.0%)	0.0342
B1B2	62 (56.9%)	44 (40.4%)	
B1B1	33 (30.3%)	40 (36.6%)	
B2 allele	90 (41.3%)	94 (43.1%)	0.4634
Male	n=53	n=47	
B2B2	9 (17.0%)	7 (14.9%)	0.7120
B1B2	29 (54.7%)	23 (48.9%)	
B1B1	15 (28.3%)	17 (36.2%)	
B2 allele	47 (44.3%)	37 (39.4%)	0.1256
Female	n=56	n=62	
B2B2	5 (9.0%)	18 (29.0%)	0.0059
B1B2	33 (59.0%)	21 (33.9%)	
B1B1	18 (32.0%)	23 (37.1%)	
B2 allele	43 (38.4%)	57 (46.0%)	0.0591

AF, atrial fibrillation

Table 3: Impact of TaqIB genotype on serum lipid levels in the female population

	B2B2	B1B2	B1B1
Total (n = 118)			
TC, mmol/L	4.44 ± 1.13	4.56 ± 1.02	4.42 ± 0.74
HDL-C, mmol/L	1.61 ± 0.42	1.45 ± 0.30	1.52 ± 0.45
LDL-C, mmol/L	2.27 ± 0.93	2.43 ± 1.03	2.33 ± 0.59
TG, mmol/L	1.23 ± 0.47	1.17 ± 0.64	1.09 ± 0.57
TC/HDL-C	2.82 ± 0.66	3.24 ± 0.91	3.04 ± 0.75
TG/HDL-C	1.89 ± 0.88	2.21 ± 1.32	1.87 ± 1.19
Controls (n = 56)			
TC, mmol/L	5.08 ± 1.29	4.66 ± 1.11	4.28 ± 0.88
HDL-C, mmol/L	2.07 ± 0.42¶	1.46 ± 0.30	1.59 ± 0.63
LDL-C, mmol/L	2.13 ± 0.89	2.38 ± 1.23	2.21 ± 0.65
TG, mmol/L	0.77 ± 0.14**	1.19 ± 0.30	1.02 ± 0.29
TC/HDL-C	2.5 ± 0.7	3.3 ± 1.1	2.9 ± 1.0
TG/HDL-C	0.88 ± 0.23††	2.46 ± 1.35	1.70 ± 0.97
AF patients (n = 62)			
TC, mmol/L	4.28 ± 1.07	4.44 ± 0.92	4.52 ± 0.60
HDL-C, mmol/L	1.50 ± 0.35¶	1.44 ± 0.31	1.47 ± 0.26
LDL-C, mmol/L	2.15 ± 0.89	2.49 ± 0.76	2.42 ± 0.53
TG, mmol/L	1.34 ± 0.46**	1.14 ± 0.52	1.14 ± 0.59
TC/HDL-C	2.9 ± 0.7	3.1 ± 0.7	3.1 ± 0.6
TG/HDL-C	2.14 ± 0.80††	1.89 ± 1.24	2.00 ± 1.34

AF, atrial fibrillation; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides.

¶p=0.044; **p=0.006; ††p=0.004 between B2B2 AF patients and control subjects

association between CETP B1B1 genotype, decreased HDL-C level and AF.¹¹ On the contrary, a study done in 8141 subjects showed that the TaqIB polymorphism was not associated with an increased risk of AF disease¹²

The strength of the present study lies in the selection of the participants based on their ethnic background, recruited carefully after evaluation of AF condition by patient's history, serial electrocardiograms or ambulatory ECG monitoring, with AF onset occurring after 65 years of age in almost all the patients. It is important to note the difference in age between our population and those recruited by Asselbergs¹¹ and Sinner.¹² More than 90% of our patients was over 65 years, while all the subjects enrolled in the other two studies were less than 65 years. We know that the prevalence and incidence of atrial fibrillation increase with age.²¹ so a potential misclassification of referent subjects that will later develop AF may bias the results of the two studies. Moreover, they didn't stratify their population according to gender, whereas we observed the most significant data in the female subgroup. In addition, Sinner sample consisted predominantly of males (over 70%).

Although it has been recognized that inflammation may facilitate the development of AF,²² our cohort could represent a selected group of patients in whom AF is associated with an abnormal lipid metabolism rather than inflammation. In this case the TG/HDL-C ratio is a better indicator than other measurements as CRP and IL-6, and polymorphisms in genes involved in lipid metabolism, as CEPT, may have a role in the onset of the disease.

In our general population the frequency of B2 allele and B2B2 genotype were the same of other Caucasian populations²³ but

the frequency of the B2B2 genotype (9%) in our female control subgroup was lower compared to those reported in different studies: in the Framingham Offspring study the B2B2 genotype frequency in females was 18.2%.²⁴ Park and coworkers in their Korean cohorts discovered that the B2B2 genotype frequency was 22.7%.²⁵

Moreover in our B2B2 females it was observed a modification of the HDL-C levels by TG. The B2B2 individuals tended to have higher HDL-C levels and significant lower TG levels and TG/HDL-C ratio compared to B1B1 and B1B2 subjects (Table 3). Conversely in the B2B2 AF females we found significant decreased HDL-C levels and a significant higher plasma TG level and TG/HDL-C ratio compared to B2B2 controls.

When we performed sequence analysis of the CETP promoter to identify the functional polymorphisms located 629 bp upstream to the ATG codon, we observed the presence of the -629AA genotype in all the 23 B2B2 females. Thus, the -629A allele of the promoter region and the B2 allele of the TaqIB polymorphism were concordant in our population and might account for the observed increased HDL-C level in control females and the decreased HDL-C level in AF female patients with a higher TG level.

Our idea is that in the presence of hypertriglyceridemia there is a modification of the relationship between CETP and HDL-C levels. Our findings are consistent with those of Tato²⁶ and Föger²⁷ who showed that among hypertriglyceridemic individuals HDL-C concentration was dependent by CETP levels, while the contribution of CETP was less in normotriglyceridemic subjects. So, while in normotriglyceridemia reverse cholesterol transport by HDL-cholesteryl-ester-selective-uptake pathway, a non-CETP pathway, is important, in the presence of hypertriglyceridemia the CETP pathway becomes more important. But lower circulating CETP levels in B2B2 subjects could reduce the generation of small pre- β -HDL particles that stimulate the cellular cholesterol efflux. Low CETP levels could therefore reduce the removal of cellular cholesterol. This protein also regulates the cholesterol traffic directly at cellular level, as macrophages present in atherosclerotic lesions produce CETP. Zheng²⁸ has shown that voltage-dependent ion channels and lipids in close proximity form functional units and no-phospholipidic molecules, as cholesterol, can change opening/closing state of such channels.

The increase in triglyceride levels after the menopausal transition could account for the data we obtained in the female subgroup. In fact a longitudinal study reported an increase of 16% in triglyceride values.²⁹ In NHANES the gap in triglyceride levels between men and women narrowed in the 50- to 59-year age group, and from 60 years onward women had higher levels than men.³⁰ This was confirmed in our population, in which emerged a statistically significant difference between men and women, with higher triglyceride levels in the female subgroup (1.37 ± 0.59 vs 1.19 ± 0.48 , $p=0.0212$).

The low number of our sample may represent a limitation of the study we conducted. Despite this, our work was performed on patients from a well-defined geographical area and in these association studies the genetic background is particularly important. In addition, the selection mode plays a key role. In atrial fibrillation, whose prevalence and incidence increase with age, it may be important to select patients taking into account this parameter, as an age too low can lead to a potential misclassification of enrolled subjects, which may later develop arrhythmia, distorting the results of the study. However, to better define the associations observed in our work,

further studies are required in larger populations, also belonging to other geographical areas.

Conclusions:

The results of our study indicate significant association of TaqIB2 and the concordant -629A alleles of CETP gene with lower HDL-C levels, higher TG levels and TG/HDL-C ratio in a subset of postmenopausal females coming from Salento (Southern Italy).

This lipid profile, added to the negative effects caused by CETP lower plasmatic levels induced by polymorphism, such as pre- β -HDL particles reduction, reduced cellular cholesterol removal, cellular membrane fluidity modification, could have promoted the onset of the arrhythmia in our population.

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