ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 10, Issue, 01, pp. 33496-33500, January, 2020

RESEARCH ARTICLE OPEN ACCESS

POLYMORPHIMS IN THE CHOLESTERYL ESTER TRANSFER PROTEIN GENE IN PATIENTS WITH CORONARY ARTERY DISEASE

***[1]Augusto Ferreira Correia, [1,2]Dinaldo Cavalcanti de Oliveira, [1]Carolina Gomes Cavalcanti de Oliveira, [3]Walter Lins Barbosa Junior, [3]Maria Eduarda Azevedo Acioli and [3]Luydson Richardson Silva Vasconcelos**

[1]Hospital Ilha do Leite. HAPVIDA
[2]Hospital das Clinicas. Universidade Federal de Pernambuco
[3]Instituto Ageu Magalhães. Fundação Oswaldo Cruz

ARTICLE INFO

Article History:
Received 20th October, 2019
Received in revised form
08th November, 2019
Accepted 21st December, 2019
Published online 31st January, 2020

Key Words:

Cholesterol ester transfer protein, Genotype, Polymorphisms, Coronary artery disease, Atherosclerosis.

***Corresponding author:**
Augusto Ferreira Correia

ABSTRACT

Introduction: Cholesterol ester transfer protein (CETP) is a plasma glycoprotein responsible for mediating the transfer of cholesterol esters and phospholipids, from high density LP (HDL) to low density LP, in exchange for TG. **Materials and Methods:** This is a cross-sectional, prospective and analytical study which enrolled 204 patients with coronary artery disease and 200 healthy controls. Evaluate the prevalence of polymorphisms in the CETP gene and its relationship with clinical characteristics. **Results:** Most patients analyzed in each group regarding the polymorphism of the CETP gene had the GG genotype (73.0% in the Disease group and 72.5% in the Control group), followed by those with the AG genotype (25.0% Control and 23.0% in the Disease group). The minority in each group had the AA genotype. Among patients with atherosclerosis, the prevalence of GG was higher in women and in obese patients. **Conclusions:** The AA genotype was less frequent in the patients and in the controls. This study did not identify any clinical characteristics more associated with AA genotype or A allele. However, in obese patients and in women GG genotype was more prevalent.

Citation: ***Augusto Ferreira Correia, Dinaldo Cavalcanti de Oliveira, Carolina Gomes Cavalcanti de Oliveira et al.*** **2020.** "Polymorphims in the cholesteryl ester transfer protein gene in patients with coronary artery disease*", International Journal of Development Research*, 10, (01), 33496-33500.

INTRODUCTION

The identification of genetic characteristics related to DLP was researched. Epidemiological data showed that a greater expression of the gene responsible for the cholesterol ester transfer protein (CETP), correlates with a higher incidence of cardiovascular disease (CVD) (Niu, 20015). CETP is a plasma glycoprotein responsible for mediating the transfer of cholesterol esters and phospholipids, from high density LP (HDL) to low density LP, in exchange for TG. (Kaestner, 2009). Many restriction polymorphisms in DNA fragment length (RFLP, restriction fragment length polymorphisms) have been described for the CETP gene, the mutation called Thermobius aquaticus IB (TaqIB), RFLP, which has been most widely studied. The terms B1 and B2 are used to indicate the presence and absence, respectively, of the TaqI restriction site.(Li, 2012; Kaestner, 2009). Although the data in the literature are still conflicting, some studies have shown that the B2 allele was associated with an increase in HDL levels and a reduction in CETP levels and activity, representing a slight deficiency in its activity. The B1 allele, however, has been associated with a decrease in HDL and an increase in CETP levels and activity (Kaestner, 2009; Kuivenhoven, 1997).

Objectives: Evaluate the prevalence of polymorphisms in the cholesteryl ester transfer protein gene in the study population, identify and compare the genotypic pattern of polymorphisms in the CETP gene betwen patients with coronary artery disease and controls, describe and compare clinical characteristics of the patients according to with the genotypic pattern of the polymorphism in the CETP gene.

MATERIALS AND METHODS

This is a cross-sectional, prospective and analytical study which enrolled a total (n) of 404 participants, subdivided into two groups:

Group I: 204 patients with clinical indication of coronary angiography
Group II: 200 healthy controls, matched by sex and age.

Inclusion criteria

Group I: Age > 18 years, with a clinical diagnosis of stable CAD, ischemia-inducing test with moderate or large area of ischemic myocardium despite of optimal medical therapy, so clinical indication for coronary angiography.
Group II: Healthy people wiht age > 18 years

Exclusion criteria: Patients with a history of previous or current cancer, severe liver disease, blood dyscrasia, immunological disease and dementia.

Study phases: The clinical characteristics were collected through the application of questionnaires DNA was extracted from peripheral blood, using the phenol-chloroform protocol. 400 microliters of peripheral blood were added in 1 eppendorf tube, lysed in a lysis solution (Alphatec Chemicals Ltd., DF, BR) and proteinase K (Amresco, OH, USA) at 60 ºC overnight. The first stage of extraction consisted of adding phenol in a 1: 1 ratio (Neon comercial Ltda, SP, BR). The aqueous phase was again recovered and added to a new tube, with the addition of the phenol-chloroform solution (1: 1) in two subsequent steps. Then the aqueous phase was recovered and added to a new tube, with the addition of the chloroform solution (1: 1). Then, the aqueous phase was recovered and added to a new tube, with the addition of isopropyl alcohol (Dinâmica Química Contemporânea Ltda, SP, BR) (1: 1). Subsequently, the DNA was eluted in 50 microliters of distilled water and immediately stored in a refrigerator at - 20 ºC, until the genotyping tests were carried out. The real-time PCR methodology, using the TAQMAN® system, was used to detect SNPs mutations, which consists of probes labeled with fluorochromes designed specifically to complement the alleles under study.

To perform this technique, QuantStudio 5 real-time PCR (Thermo Fisher Scientific, CA, USA) was used. The polymorphism genotyping was evaluated in QuantStudio 5 (Thermo Fisher Scientific, Foster City, CA, USA), according to the manufacturer's conditions: 30 sec at 60ºC, duration 10 min at 95ºC, followed by 40 cycles in the PCR stage (15 sec at 95ºC followed by 60 sec at 60ºC) and a final stage of 30 sec at 60ºC.

Data processing and analysis: For the analysis of the variables, a database was done in the Excel version 2013 program, sequentially exported to the SPSS version 21 program. The Shapiro Wilk test was applied and as the numerical variables were normal they were presented as mean and standard deviation. Categorical variables were presented as absolute value and percentage. Categorical variables were compared using Pearson's Chi-square test. Allele frequencies were estimated using the gene count method. To compare numerical variable T student test was applied. Value of $p \leq 0,05$ was considered significant.

Ethical aspects: The project was approved by Ethical Committee, according to the Declaration of Helsinki. All individuals who participated in the research previously signed the informed consent form.

RESULTS

In this study, 404 individuals were evaluated, of which 204 were patients with CAD (disease group) and 200 healthy controls (control group). The percentage of male patients between the disease and control groups was 58.8% x 20.0% (p = 0.001) respectively. In addition, the mean ages in the disease and control groups were: 61.99 ± 11.2 x 38.61 ± 11.6 years (p <0.001). Most patients analyzed in each group regarding the polymorphism of the CETP gene had the GG genotype (73.0% in the Disease group and 72.5% in the Control group), followed by those with the AG genotype (25.0% Control and 23.0% in the Disease group).

Table 1. Clinical characteristics and padrões genotípicos do polimorfismo do gene CETP in patients with coronary artery disease

Variable	AA (n = 8)		AG (n = 47)		GG (n =149)		Population		p
	n	%	n	%	n	%	n	%	
Hypertension									
Yes	5	62.5	40	85.1	109	73.2	154	75.5	p = 0.1
No	3	37.5	7	14.9	40	26.8	50	24.5	
Diabetes Mellitus									
Yes	3	37.5	17	36.2	64	43.0	84	41.2	p = 0.7
No	5	62.5	30	63.8	85	57.0	120	58.8	
Dyslipidemia									
Yes	2	25.0	6	12.8	19	12.8	27	13.2	p = 0.6
No	6	75.0	41	87.2	130	87.2	177	86.8	
Obesity									
Yes	-	-	7	14.9	41	27.5	48	23.5	p=0.05
No	8	0	40	85.1	108	72.5	156	76.5	
Previous MI									
Yes	2	25.0	5	10.6	21	14.1	28	13.7	p = 0.5
No	6	75.0	42	89.4	128	85.9	176	86.3	
Stroke									
Yes	1	12.5	-	-	6	4.0	7	3.4	p = 0.1
No	7	87.5	47	0	143	96.0	197	96.6	
Smoking									
Yes	2	25	5	10.6	17	11.4	23	11.8	p= 0.3
No	6	75.0	42	89.4	132	88.6	180	88.2	
CKD									
Yes	-	-	-	-	3	2.0	3	1.5	p = 1
No	8	0.0	47	0	146	98.0	201	98.5	

CETP: Cholesterol ester transfer protein, **MI:** Myocardial infarction, **CKD:** Chronic kidney disease

The minority in each group had the AA genotype. There were no significant differences between groups in relation to the CETP gene. Table 1 shows the comparative statistical analysis of the clinical characteristics of the patients in the disease group according to the genotypic patterns of the CETP gene polymorphism. There was no statistically significant association.

Considering the patients with the A allele (AA + AC), Table 2 shows that the distribution by sex was approximately equal while in the group of those who had the GG allele, the majority (63.9%) were female , a difference that shows a significant association between sex and genotype (p = 0.017). Age and gender comparison as grouped category (AA + AG) and in the GG category are shown in Table 2.

Table 2. Comparison of age and gender according to presence or absence of the allele G in patients with coronary artery disease

Variable	AA + AG (n = 55)		GG (n = 149)		Population		p
Gender: n %							
Male	54	49.1	106	36.1	160	39.6	p = 0,01
Female	56	50.9	188	63.9	244	60.4	
Mean age ± SD	52.88 ± 17.41		50.12 ± 15.87		50,87 ± 16.32		p = 0,3

SD: Standard deviation

Table 3. Comparison ofClinical characteristics according to presence or absence of the allele A in patients with coronary artery disease

Variable	AA + AG (n = 55)		GG (n = 149)		All patients		p
	n	%	n	%	n	%	
Hypertension							
Yes	45	81,8	109	73,2	154	75,5	p = 0,2
No	10	18,2	40	26,8	50	24,5	
Diabetes Mellitus							
Yes	20	36,4	64	43,0	84	41,2	p = 0,3
No	35	63,6	85	57,0	120	58,8	
Dyslipidemia							
Yes	8	14,5	19	12,8	27	13,2	p = 0,7
No	47	85,5	130	87,2	177	86,8	
Obesity							
Yes	7	12,7	41	27,5	48	23,5	p = 0,02
No	48	87,3	108	72,5	156	76,5	
Previous MI							
Yes	7	12,7	21	14,1	28	13,7	p = 0,8
No	48	87,3	128	85,9	176	86,3	
Stroke							
Yes	1	1,8	6	4,0	7	3,4	p = 0,6
No	54	98,2	143	96,0	197	96,6	
Smoking							
Yes	7	12,7	17	11,4	24	11,8	p = 0,5
No	48	87,3	132	88,6	180	88,2	
CKD							
Yes	-	-	3	2,0	3	1,5	p = 0,5
No	55	0	146	98,0	201	98,5	

MI: Myocardial infarction, CKD: Chronic kidney disease

Table 4. Comparison of Clinical characteristics according to presence or absence of the allel Gin patients with coronary artery disease

Variable	AA (n = 8)		AG+GG (n =196)		All patients		p
	N	%	n	%	n	%	
Hypertension							
Yes	5	62.5	149	76	154	75,5	p = 0.4
No	3	37.5	47	24	50	24,5	
Diabetes Mellitus							
Yes	3	37.5	81	41.3	84	41,2	p = 1
No	5	62.5	115	58.7	120	58,8	
Dyslipidemia							
Yes	2	25	25	12.8	27	13.2	p = 0.2
No	6	75	171	87.2	177	86.8	
Obesity							
Yes	-	-	48	24.5	48	23.5	p = 0.2
No	8	100	148	75.5	156	76.5	
Previous MI							
Yes	2	25.0	26	13.3	28	13.7	p = 0.3
No	6	75.0	170	86.7	176	86.3	
Stroke							
Yes	1	12.5	6	3.1	7	3.4	p = 0.2
No	7	87.5	190	96.9	197	96.6	
Smoking							
Yes	2	25	24	11.2	24	11.8	p = 0.2
Não	6	75	174	88.8	180	88.2	
CKD							
Yes	-	-	3	1.5	3	1.5	p = 1
No	8	100	193	98.5	201	98.5	

MI: Myocardial infarction, CKD: Chronic kidney disease

When the group (AA + AG) x GG was considered, obesity was the only variable with a significant association with the subgroups of the genotypes. For this variable, it is highlighted that the prevalence of patients with obesity was higher among patients with the GG genotype than the group (27.5% x 12.7%), as shown in Table 3 Evaluating the results of the prevalence of the genotype AA x (AG + GG), according to the clinical variables in the disease group, no significant associations were found, as shown in Table 4.

DISCUSSION

The evolution of CAD is influenced by several genetic and environmental factors, which act synergistically for the development and progression of the disease.34 The contribution of the individual's genetic heritage is an important risk factor in the process of forming atheromatous plaque, being promptly considered through questioning about the patient's family history (MinisterioSaude, 2012). Studies of genotypic patterns have been carried out with the objective of correlating the genetic inheritance and phenotypic presentations of CAD, taking into account the number of vessels involved, the location and severity of atherosclerotic lesions (Hopkins, 2011). Regarding CVDs, a group of genes characterized by the development of so-called monogenic diseases stands out. In these cases, the variant in a single gene clearly explains the phenotype, being frequently associated with metabolic changes that, in turn, constitute an important aspect of the pathophysiology of the CAD (García-Giustiniani, 2016; Freitas, 2015). However, even in the case of monogenic pathologies, the mutations or polymorphisms identified can cause important variations in phenotypic expressions, which usually hinder the diagnosis (Peters, 2011).

In the present study, 404 individuals were evaluated, divided into two groups: one with 204 patients with CAD (disease group) and the other with 200 healthy people (control group). Men were the majority in the disease group. In addition, the mean age was also higher in the disease group, compared to controls. The majority of the patients analyzed in each group regarding the polymorphism of the CETP gene had the GG genotype (73.0% in the Disease group and 72.5% in the Control group), followed by those with the AG genotype (25.0% Control and 23.0% in the Disease group). The minority in each group had the AA genotype. It is noteworthy that age is classically considered as an independent and immutable risk factor for the development of CAD, being associated with more significant atherosclerotic lesions, compared to younger individuals (D'Agostinho, 2008). There was a significant association between the female sex and the GG genotype. This fact may be associated with a possible increase in cardiovascular risk in females triggered by this genotype. Literature data point to hormonal differences between the sexes as contributing factors to a lower cardiovascular risk in women. The elevated estrogen levels, present in women before menopause, act as a protective factor for the endothelium and reduce the formation of atheroma plaques (Freitas, 2015).

The association of the TaqIB mutation and low plasma HDL levels, could play a relevant role in CAD. In addition, other studies have shown that the presence of TaqIB and / or other polymorphisms in the CETP gene could influence the response to statins, regardless of serum HDL levels. However, such effects have not yet been associated with a gender predilection (Kaman, 2015). When the cluster (AA + AG) x GG was considered, the prevalence of patients with obesity and women were higher among patients with the GG genotype than the grouped one. Regarding obesity this fact is in agreement with the data in the current literature, which consider obesity as the result of a genetic predisposition and lifestyle characteristics of individuals (Faludi, 2017). However, our finding about woman needs more studies to better understanding. The progressive increase in the prevalence of CAD occurs, in part, due to the negative effects of the globalization process, rapid urbanization, sedentary life and high calorie food, in addition to the consumption of tobacco and beverages (Bejamin, 2019). These behavioral risk factors impact on some of the main metabolic risk factors, such as being overweight, SAH, peripheral insulin resistance, strongly associated with atherosclerosis (Almahnoud, 2016). Numerous studies have been carried out over the years, however none of them has been able to specifically recognize which is the most important risk factor for CAD development, which suggests a complex and multifactorial pathophysiological pathway (AHA; Menotti, 1996).

In the present study, the clinical profile of the assessed population is considered to be at high risk for CAD and cardiovascular events, which is in agreement with epidemiological studies, such as the Seven Countries and the Framingham Heart Study (Menotti, 1996; Wang, 2005). Such factors seem to lead to progressive vascular endothelium dysfunction, characterized by functional changes and endothelial thickening (Soloperto, 2012). Currently, when approaching the patient with CAD, strict control of all risk factors is recommended. However, the morbidity and mortality related to CAD remains at worrying levels, since the first clinical manifestations usually appear at an advanced stage of the disease, contributing to the occurrence of unfavorable outcomes such as AMI and the development of HF (Menotti, 1996). The high risk clinical profile of some patients for cardiovascular events and the increasing number of hospitalizations for CVD in the state of Pernambuco, despite efforts to reduce the prevalence of these diseases, may suggest a genetic aspect that has not yet been elucidated as a factor involved. Therefore, our study, in a way, contributes to the development of genetic investigations in CVD in our region.
t is known that in the pathophysiology of atherosclerosis, aggressive factors such as increased shear forces on the vascular wall and increased oxidative stress contribute to greater permeability of the endothelium. This allows the passage of cells from the immune system to the subendothelial space, where they will act in the LDL oxidation process. LDL particles become modified in their form and function, being able to stimulate the process of the emergence of leukocyte adhesion molecules, maintaining the local inflammatory insult and, consequently, the development of atheroma plaque (Hansson, 2005).

CETP is responsible for intermediating the transfer of cholesterol esters and phospholipids, from HDL to low density LP, in exchange for TG (Kaestner, 2009). In this sense, it can be inferred that the GG genotype is associated with an increase in CETP transcription or even with an increase in the activity of this enzyme, since the increase in CETP levels and activity has been associated with decreased HDL and increased cardiovascular risk (Kaestner, 2009; Kuivenhoven, 1997). In conclusion, the AA genotype was less frequent in the patients and in the controls. This study did not identify any clinical characteristics more associated with AA genotype or A allele.

However, in obese patients and in women GG genotype was more prevalent. This study was limited to evaluating the genotypic expressions of the polymorphisms in the cholesteryl ester transfer protein gene and their clinical correlations with CAD. The properties of the transcribed proteins were not analyzed from the allelic variation of the referred polymorphisms, so that we are not sure about the mechanism of action or the activity of these proteins in individuals. Another limiting factor in the present study is the sample size.

REFERENCES

Almahnoud K, Rei Y. Secretaria Estadual de Saúde. Secretaria executiva de vigilância em Saúde. Diretoria Geral de Promoção, Monitoramento e Avaliação da vigilância em Saúde. Perfil Socioeconômico, Demográfico e Epidemiológico: Pernambuco 2016. 1ª Ed. portal.saude.pe.gov.br/

Amit VK, Sekar K. Genetics of Coronary Artery Disease: Discovery, Biology and Clinical Translation. Nat Ver Genet. 2018:18:331-334

Bejamin EJ, Munter P, Alonson A, *et al*.Heart Disease and Stroke Statistics. 2019 Update: A Report From the American Heart Association. Circulation. 2019;139:e56-e528

D'Agostino RB, Vasan RS, Pencina MJ, *et al*. Generalcardiovascularriskprofile for use in primary care: the Framingham Heart Study. Circulation. 2008:117:743-53

Faludi AA, Izar MCO, Saraiva JFK, *et al*. Atualização da diretriz brasileira de dislipidemias e prevenção da aterosclerose. 2017. http://publicacoes.cardiol.br/2014/diretrizes/2017/02_DIRETRIZ_DE_DISLIPIDEMIAS.pdf

Freitas RG, Campana EM, Pozzan R, *et al*. APOE and LDLR Gene Polymorphisms and Dyslipidemia Tracking. Rio de Janeiro Study. Arq Bras Cardiol. 2015;104:468–474.

García-Giustiniani D, Stein R. Genetics of Dyslipidemia. Arq Bras Cardiol. 2016; 106:434–438.

Hansson GK. Inflammation, Atherosclerosis, and Coronary Artery Disease. N Engl J Med 2005;352:1685-1695.

Hopkins PN, Toth PP, Ballantyne CM, Rader DJ .National Lipid Association Expert Panel on Familial Hypercholesterolemia. Familialhypercholesterolemias: prevalence, genetics, diagnosis and screening recommendations from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. *J Clin Lipidol.* 201;5(3 Suppl):S9-17.

Kaestner S, Patsouras N, Spathas DH, Flordellis CS, Manolis AS. Lack of association between the cholesteryl ester transfer protein gene--TaqIB polymorphism and coronary restenosis following percutaneous transluminal coronary angioplasty and stenting: a pilot study. Angiology. 2010;61:338-343.

Kaman D, İlhanN, İlhanN, AkbulutM.TaqIB and Severity of Coronary Artery Disease in the Turkish Population: A Pilot Study. Bosn J Basic Med Sci. 2015:15; 9-13.

Kuivenhoven JA, de Knijff P, Boer JM, *et al*.. Heterogeneity at the CETPgene locus. Influence on plasma CETP concentrations and HDL cholesterollevels. Arterioscler Thromb Vasc Biol. 1997; 17:560-568.

Li YY, Wu XY, Xu J, Qian Y, Zhou CW, Wang B. ApoA5 -1131T/C, FgB -455G/A, -148C/T, and CETP Taq IBgenepolymorphisms and coronaryarterydisease in the Chinesepopulation: a meta-analysis of 15,055subjects. *Mol Biol Rep.* 2013; 40:1997-2014.

Menotti A, Keys A, Blackburn H, *et al*. Comparison of Multivariate Predictive Power of Major Risk Factors for Coronary Heart Diseases in Different Countries: Results From Eight Nations of the Seven Countries Study, 25-year Follow-Up. J Cardiovas Risk. 1996:3;69-75.

Ministério da Saúde. Rede Interagencial de Informações para a Saúde (RIPSA). Indicadores e dados básicos (IDB). Indicadores de mortalidade. http://tabnet.datasus.gov.br/cgi/idb2012/matriz.htm

Niu W, Qi Y. Circulatingcholesteryl ester transfer protein and coronary heart disease: mendelian randomization meta-analysis. Circ Cardiovasc Genet. 2015 Feb;8(1):114-121.

Peters BJ, Pett H, Klungel OH, *et al*. Geneticvariability within the cholesterolloweringpathway and the effectiveness of statins in reducing the risk ofMI. Atherosclerosis. 2011; 217:458-464.

Soloperto G, Casciaro S. Progress in atherosclerotic plaque imaging. World J Radiol. 2012;4:353–371.

Wang Q. Molecular Genetics of Coronary Artery Disease. Curr Opin Cardiol. 2005:20;182-188.

****