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ASSOCIATION BETWEEN CORONARY ARTERY DISEASE AND POLYMORPHISMS IN THE ATTRACTIN-LIKE 1 PROTEIN AND KINESIN-LIKE PROTEIN 6 GENES

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ABSTRACT

Background: It is postulated that, in animal models, the Attractin-like 1 protein (ATRNL1) gene is correlated to energy balance and homeostasis, however, there is no information on the influence of ATRL1 polymorphism (rs180706) in humans. Our objective was to evaluate associations between polymorphisms in the ATRNL1 (rs 180706) and KIF6 (rs 20455) genes and coronary artery disease (CAD). **Materials and Methods:** A cross-sectional and analytical study was carried out from November 2018 to June 2019 that enrolled patients with CAD according to coronary angiography. **Results:** A total of 404 individuals (204 with CAD and 200 healthy controls) were evaluated.In the ATRLN1 polymorphism (rs 180706) the comparison between sick and healthy regarding the AA allele revealed 16 p (7.8%) vs 29 p (14.5%), p = 0.03 and regarding the CC allele 101 p (49.5%) vs 75 p (37.5%). In KIF6 polymorphism (RS 20455) there were no differences between healthy patients and controls. **Conclusion:** This is the first study to describe the association between ATRNL1 (rs 180706) gene polymorphism and CAD. The prevalence of the AA genotype in this polymorphism was lower in the disease group when compared to the control group.

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INTRODUCTION

Cardiovascular disease (CVD) is considered the main cause of mortality worldwide and is responsible for a significant loss of quality of life and productivity of the adult population, in addition it generates high costs for health care (Bejamin E, 2019). In Brazil, it is estimated that diseases of the circulatory system account for more than a third of deaths, with coronary artery disease (CAD) as the one with the greatest impact (Brasil, 2014). Several risk factors, such as systemic arterial hypertension (SAH), diabetes mellitus (DM), dyslipidemia (DLP), age, family history of CAD, smoking, obesity and sedentary lifestyle are classically related to atherosclerotic disease, ratified by epidemiological studies, such as the Seven Countries and the Framingham Heart Study (Menotti A, 1996; Wang Q,2005). However, numerous clinical trials have been conducted over the years and none of them were able to specifically recognize which is the most important risk factor for development and prognosis of CAD.

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This reinforces the idea that CAD is a complex and multifactorial disease with a synergism of genetic, environmental and behavioral factors, leading to its onset and progression Brasil, 2014; Menotti A, 1996). The formation of the atherosclerotic plaque begins with the aggression to the vascular endothelium by factors that enhance the shear forces in the wall of the vessels. Conformational modifications occur in the cytoskeleton of endothelial cells, which induce a cascade of intracellular signaling, leading to endothelial dysfunction (Chatzizisis, 2007). The consequence is increased permeability of the endothelium to various circulating agents, such as lipoproteins, and the appearance of leukocyte adhesion molecules, which are the triggers for the start of the inflammatory stimuli that is responsible for the progression and development of atherosclerotic lesions (Cybulsky, 1991).
Genetic contribution is an important cardiovascular risk factor and is inferred in clinical practice by reporting a family history of coronary atherosclerosis (World Health Organization, 2013). It is estimated that about 5% of AMI in patients less than 60 years old occurred due to genetic mutations, which can increase up to 20% when the acute coronary event affects even younger individuals, with an age under 45 (Hopkins, 2011).

Population studies of genetic association have identified more than 100 genes that could have a direct impact on lipid levels and are capable of forming complex DLP phenotypes (Asselbergs, 2012). Attractin protein (ATRN) and Attractin-like 1 protein (ATRNL1) are highly similar type I transmembrane proteins present in mice. The null mutant animals for the expression of the ATRN have a characteristic pleiotropic phenotype with the presence of a dark coat, neuropathies, reduction of body weight and adiposity (Walker, 2007). It is currently believed that in the human being the ATRN, in its natural serum form, mediates the spread of monocytes, modulating the interaction between T cells and macrophages, allowing a faster and more effective means for the presentation of antigens.[11] It is postulated that in animal models the Attractin-like 1 protein (ATRNL1) gene is correlated to energy balance and homeostasis, however, there is no information on the influence of ATRL1 polymorphism (rs180706) on humans (Duke-Cohan, 1998).

The Kinesin-like protein 6 (KIF6) gene encodes a protein that is a member of a family of molecular motors involved in the intracellular transport of protein complexes, cytoplasmic organelles and messenger RNA (mRNA) along the microtubule filaments. This gene encompasses a genomic region of about 390,000 base pairs on chromosome 6p21 and is expressed in several cell types and tissues, including coronary arteries and vascular cells (Shiffman, 2008). Mechanically, the structure of the KIF6 protein consists of a motor domain and a caudal domain. The motor domain can drive kinesin along the microtubules in an ATP-dependent manner. The caudal domain does not bind to its charges, such as membrane organelles, protein complexes and mRNA. The Trp719Arg is located in a coiled coil structure in the caudal domain. This variant causes a basic arginine residue to be replaced by a non-polar tryptophan residue, affecting binding of the kinesin filler (Li, 2011).

Large prospective and analytical case-control studies have reported the association of the unique nucleotide polymorphism Trp719Arg of the KIF6 gene with increased cardiovascular risk, possibly because the Arg-Trp heterodimers differ from the Arg-Arg and Trp-Trp homodimers in relation to their stability and/or transport capacity.[13] However, the data in the literature is conflicting (Li, 2011). Currently there is a need to create new alternatives for clinical screening and cardiovascular risk estimation in the population, which are capable of bringing together effectiveness, applicability, and a cost-benefit ratio to health systems (Lima, 2006). However, the establishment of genetic aspects and gene therapy are frontiers of knowledge that are being addressed and need to be incorporated into the clinical approach (Hirokawa, 1998). Thus, the objective of the present study was to evaluate associations between polymorphisms in ATRNL1 (rs 180706) and KIF6 (rs 20455) genes and the DAC.

MATERIALS AND METHODS

The study is transversal and analytical, carried out from November 2018 to June 2019.A total of 404 participants were evaluated, divided into two groups: Group I - 204 patients with coronary artery disease stenose >50% according to coronary angiography. Group II - 200 healthy controls. The study was developed in two centers.

Initially a tertiary hospital, where patients and controls were recruited and samples of 10ml of peripheral blood were collected, which were taken to laboratory (from one University), where the genotyping tests were performed. Inclusion criteria for group I were: age> 18 years, clinical diagnosis of stable CAD, ischemia-inducing test with moderate or large area of ischemic myocardium and indication for coronary angiography. For group II the inclusion criteria were: age> 18 years and be healthy. Patients with a history of previous or current cancer disease, severe liver disease, blood dyscrasia, or who refused to participate in the study, did not sign the informed consent form. This research was approved by ethical committe..

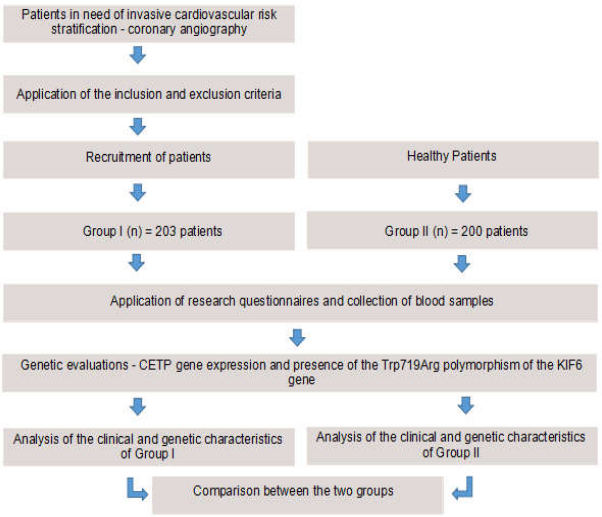
Genetic evaluation protocol

DNA extraction: DNA was extracted from the peripheral blood using the phenol-chloroform protocol. 400 microliters of the peripheral blood were added in 1 eppendorf tube, lysed in lysis solution (Alphatec Produtos químicos Ltda, DF, BR) and proteinase K (Amresco, OH, USA) at 60 ° C overnight. The first step of the extraction consisted of the addition in a ratio of 1: 1 phenol (Neon comercial Ltda, SP, BR). The aqueous phase was again recovered and added to a new tube, and the phenol-chloroform solution (1: 1) was added in two subsequent steps. The aqueous phase was then recovered and added to a new tube, and the chloroform solution (1: 1) was added. Then, the aqueous phase was recovered and added to a new tube, and the isopropyl alcohol (Dinâmica química contemporânea Ltda, SP, BR) was added (1: 1). Subsequently the DNA was eluted in 50 microliters of distilled water and immediately stored in a refrigerator at -20 °C until the genotyping assays were performed.

Detection of polymorphisms: The real-time PCR methodology was used through the TAQMAN® system for the detection of SNPs mutations, which consists of probes labeled with fluorochromes specifically designed to complement the alleles under study. The SNPs of the genes are represented by the respective probes: ATRLN1 (rs 180706) and KIF6 (rs 20455). To perform this technique, the real-time QuantStudio 5 PCR (Thermo Fisher Scientific, CA, USA) was used at the Technological Platforms Center of the Aggeu Magalhães / Fiocruz-PE Institute. Polymorphism genotyping was evaluated in QuantStudio 5 (Thermo Fisher Scientific, Foster City, CA), available at the IAM / Fiocruz Technological Platforms Nucleus, according to the manufacturer's conditions: 30 sec at 60 ° C, duration of 10 min at 95 ° C followed by 40 cycles in the PCR stage (15 sec at 95 ° C followed by 60 sec at 60 ° C) and a final 30 sec stage at 60 ° C.

Data processing and analysis: To analyze the variables, a database was built in the Excel version 2013 program, sequentially exported to the SPSS version 21 program. A descriptive statistical analysis was performed to present the results obtained. The normality tests were applied to the numerical variables. Those normal variables were presented as mean and standard deviation, while the nonnormal variables in the Gauss curve were presented as medians and maximum and minimum values. Prevalence of different genotypes was compared by Pearson's Chi-square test. The allele frequencies were estimated by the method of gene counting. Results were considered statistically significant whose descriptive levels (p values) were lower than 0.05. The presentation of the variables measured was done through tables.

The flowchart of the study is shown in Figure 1.



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, mean age in the disease and control groups were: 61.99 ± 11.2 x 38.61 ± 11.6 years (p <0.001). Table 1 presents data on gender and age characteristics in the disease versus control groups. In the disease group, the most frequent clinical variables were: hypertension (75.5%), DM (41.2%), obesity (23.5%), AMI (13.7%), Percutaneous transluminal angioplasty (TCA) (7.8%), smoking (5.4%), previous Cine (4.4%), stroke (3.4%) and last heart surgery (3.4%). As regards the genetic evaluation of the ATRLN1 polymorphism (rs 180706), the presentation of the alleles in the disease group was CC (49.5%), AC (42.6%) and AA (7.8%). In the control group, the percentages corresponded to AC (48.0%), CC (37.5%) and AA (14.5%) (p = 0.018). When analyzing the polymorphism in relation to the presence of allele A (AA + AC) x CC (absence of allele A), we observed 50.5% x 62.5% disease versus controls (p = 0.015) (Table 2). Regarding the genetic evaluation of the KIF 6 polymorphism (rs 20455), the presentation of the alleles in the disease group was AG (50%), GG (24.5%) and AA (25.5%). In the control group, the percentages corresponded to AG (51%), GG (28.5%) and AA (20.5%). When the polymorphism is analyzed for the presence of the A (AA + AG) versus GG allele (absence of the A allele) the frequency of (75.5% x 71.5%) in the disease and control groups respectively is observed. In both analyzes (p> 0.05), (table 2).

Analysis of ATRL1 polymorphism (rs 180706) in the disease group: Comparative analysis of the ATRL1 polymorphism (rs 180706) in the disease group according to gender, age and clinical profile of the patients and the genotypic patterns of the polymorphism did not find statistically significant associations - see tables 3 and 4. Considering the patients with the A (AA + AC) allele, the majority of the patients were male, accounting for 55.3% of the combined category (AA + AC) and 62.4% of CC (p = 0.307). Age averages were 62.96 ± 11.74 x 60.99 ± 10.65 years in the pooled category and CC, respectively (p = 0.145).

When grouping (AA + AC) x CC was considered, there were no statistically significant associations between the clustered genotypes and the clinical variables of the patients. In relation to the prevalence of the AA x genotype (AC + CC), according to the clinical variables in the disease group, no statistically significant associations were also recorded.

Analysis of the KIF6 polymorphism (rs 20455) in the disease group: Male was the majority in each genotype of the KIF6 polymorphism (rs 20455), with prevalences ranging from 57.8% to 60.0% (p = 0.960). The mean age of the groups AA, AG and GG were: 65.15 ± 9.9; 60.40 ± 11.04 and 61.92 ± 12.40 years respectively (table 5). The correlation between the genotypic analysis and the clinical variables of the patients was not statistically significant either - see table 6. When considering patients with the A (AA + AG) X GG allele. the majority of the patients are also male (58.4% x 60% p = 0.846) respectively. The mean age was 62.01 ± 10.86 years in the pooled category and 61.92 ± 11.23 years in the GG genotype (p = 0.086). In the comparison between the grouped variables (AA + AG) vs. GG, the prevalence of patients with a previous history of Cine (2.6% x 10% p = 0.041) is highlighted.

DISCUSSION

CVD is the leading cause of death worldwide, negatively affecting the quality of life of individuals.[1] At the national level it is no different, so this group of diseases accounts for more than a third of reported deaths, excluding external causes (Datasus, 2012). Among CVD, CAD is notorious for its broad clinical spectrum and high mortality rate. In addition, it causes great harm to society, since it directly affects the productivity of the adult population and demands high costs with health care (Brasil, 2014). 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 diet, as well as tobacco and alcohol consumption. These behavioral risk factors impact on some of the major metabolic risk factors, such as overweight, Hypertension, peripheral insulin resistance, strongly associated with atherosclerosis(Secretaria de Saúde do Estado de Pernambuco, 2016). The evolution of CAD is influenced by several genetic and environmental factors that act in synergism for the development and progression of the disease (World Health Organization, 2016). The contribution of the genetic inheritance of the individual is an important risk factor in the process of atheromatous plaque formation, being promptly considered through questioning about the patient's family history (Ministério da Saúde, 2012). Studies of genotypic patterns have been carried out with the objective of correlating genetic inheritance and phenotypic presentations of CAD, taking into account the number of vessels involved, the location and severity of atherosclerotic lesions (Hopkins, 2011) .

In the present study, 404 subjects were divided into two groups: one with 204 patients with CAD and one with 200 healthy people (control group). Men were the majority in the disease group. In addition, mean age was also higher in the disease group, compared to controls. These data are in line with those in the literature, which point out the hormonal differences between the sexes as contributory factors for the differences between these percentages. High estrogen levels, present in premenopausal women, act as a protective factor for the endothelium and reduce the formation of atheroma plaques

Table 1. Characteristics of the sample - sex and age

	Group				
Variable	Disease		Control		P value
Gender: n %					p (1) = 0,001*
Male	120	58,8	40	20,0	
Female	84	41,2	160	80,0	
Age: average ± standard deviation	61,99 ± 11,23		38,61 ± 11,60		p (2) < ,001*

(*) Significant difference at 5% (1) Through the chi-square test (2) through the Mann-Whitney test.

Table 2. Genotypic evaluation of ATLR1 (rs180706) and KIF6 (rs20455) gene polymorphism according to the groups

	Group				
Polymorphism and genotypes	Disease(n = 204)		Control (n = 200)		P value
	n	%	n	%	
ATRLN1 (rs180706)					p (1) = 0,018*
AA	16	7,8	29	14,5	p (2) = 0,033*
AC	87	42,6	96	48,0	p (2) = 0,280
CC	101	49,5	75	37,5	p (2) = 0,015*
ATRLN1 (rs180706)					p (1) = 0,015*
AA + AC	103	50,5	125	62,5	
CC	101	49,5	75	37,5	
KIF6 (rs180706)					p (1) = 0,423
AA	52	25,5	41	20,5	p (2) = 0,841
AG	102	50,0	102	51,0	p (2) = 0,412
GG	50	24,5	57	28,5	p (2) = 0,363
KIF6 (rs180706)					p (1) = 0,363
AA + AG	154	75,5	143	71,5	
GG	50	24,5	57	28,5	

(*) Significant difference at 5% (1) Through the chi-square test (2) through the chi-square test for the individual categories.

Table 3. Sex and age according to genotype of ATRL1 polymorphism (rs180706) in the disease group

	Genotype of ATRL1 polymorphism (rs180706)								
Variable	AA		AC		CC		Total Group		P value
Gender: n %									p (1) = 0,486
Male	10	62,5	47	54,0	63	62,4	120	58,8	
Female	6	37,5	40	46,0	38	37,6	84	41,2	
Age: average ± standard deviation	58,25 ± 15,63		63,83 ± 10,83		60,99 ± 10,65				p (2) = 0,081

(1) Through the chi-square test (2) through the Kruskal-Wallis test.

(Shiffman, 2008). It is emphasized that age is classically considered as an independent and unchanging risk factor for the development of CAD, being associated with more significant atherosclerotic lesions compared to younger individuals (D'Agostino, 2008). The clinical profile of the population evaluated 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.5,6 Such factors seem to lead to progressive vascular endothelial dysfunction, characterized by functional alterations and endothelial thickening (Soloperto, 2012).

Currently, during the approach of the patient with CAD, rigorous control of all risk factors is advocated. However, CAD-related morbidity and mortality remain 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 CVD hospitalizations, 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 to some extent contributes to the development of genetic research in CVD in our region. In our study, the prevalence of the ATRNL1 polymorphism (rs 180706) with the AA genotype was lower in the disease group when compared to the control group.

The prevalence of allele A (AA + AC) x CC was also lower in the group of patients with CAD. Mutant animal models (null mice for the expression of ATRN) have a characteristic pleiotropic phenotype with the presence of a dark coat, neuropathies, reduction of body weight and adiposity. It is believed that genetic mutations, which involve the loss of function or the absence of the expression of the ATRN, act indirectly stimulating the signaling pathways of melanocortin, which in turn regulates numerous biological processes, such as energy balance and homeostasis (Walker, 2007). The true role of ATRN and ATRNL1 in humans is still uncertain (Duke Cohan, 1998).It is speculated that in their natural serum form atractins mediate the spread of monocytes, allowing a faster and more effective means for the presentation of antigens ((Duke Cohan, 1998). However, the data in the literature are scarce and yet conflicting. It is known that in the pathophysiology of atherosclerosis, aggressive factors such as increased shear forces in the vascular wall and increased oxidative stress contribute to a 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 process of LDL oxidation. The LDL particles become modified in their form and function, being able to stimulate the process of appearing leukocyte adhesion molecules, maintaining the local inflammatory insult and consequently the development of the atheroma plaque (Hansson, 2005). Regarding the regulation of metabolic and energetic pathways in humans, the melanocortin signaling pathway plays a key role in the control of food intake and body weight (Yeo, 2003).

Table 4 - Evaluation of clinical variables in the disease group according to the ATRL1 gene polymorphism genotype (rs180706)

ATRL1 gene polymorphism genotype (rs180706).									
Variable	AA (n=16)		AC (n=87)		CC (n = 101)		Total group		p value
	N	%	n	%	n	%	n	%	
Arterial hypertension									p [(1)] = 0,198
Yes	12	75,0	71	81,6	71	70,3	154	75,5	
No	4	25,0	16	18,4	30	29,7	50	24,5	
Diabetes Mellitus									p [(1)] = 0,899
Yes	7	43,8	37	42,5	40	39,6	84	41,2	
No	9	56,2	50	57,5	61	60,4	120	58,8	
Dyslipidemia									p [(1)] = 0,364
Yes	3	18,8	14	16,1	10	9,9	27	13,2	
No	13	81,2	73	83,9	91	90,1	177	86,8	
Obesity									p [(1)] = 0,450
Yes	4	25,0	24	27,6	20	19,8	48	23,5	
No	12	75,0	63	72,4	81	80,2	156	76,5	
Heart attack									p [(1)] = 0,377
Yes	1	6,3	15	17,2	12	11,9	28	13,7	
No	15	93,7	72	82,8	89	88,1	176	86,3	
Stroke									p [(2)] = 0,242
Yes	-	-	1	1,1	6	5,9	7	3,4	
No	16	100,0	86	98,9	95	94,1	197	96,6	
Smoking									p [(2)] = 0,284
No	14	87,5	77	88,5	89	88,1	180	88,2	
Up to 10/day	-	-	3	3,4	8	7,9	11	5,4	
Ex-smoker	2	12,5	7	8,0	4	4,0	13	6,4	
CKD									p [(2)] = 0,193
Yes w/ hemodialysis	-	-	3	3,4	-	-	3	1,5	
No	16	100,0	84	96,6	101	100,0	201	98,5	
coronary angiography									p [(2)] = 0,404
Yes	-	-	6	6,9	3	3,0	9	4,4	
No	16	100,0	81	93,1	98	97,0	195	95,6	
PCI									p [(1)] = 0,328
Yes	-	-	9	10,3	7	6,9	16	7,8	
No	16	100,0	78	89,7	94	93,1	188	92,2	
Cardiac surgery									p [(2)] = 0,082
Yes	-	-	6	6,9	1	1,0	7	3,4	
No	16	100,0	81	93,1	100	99,0	197	96,6	

(1) Using the Chi-square test (2) through Fisher's exact test.

Table 5. Sex and age according to genotype of KIF6 polymorphism (rs 20455) in the disease group

Genotype of KIF6 polymorphism (rs 20455)									
Variable	AA		AG		GG		Total group		P value
Gender: n %									p [(1)] = 0,960
Male	31	59,6	59	57,8	30	60,0	120	58,8	
Female	21	40,4	43	42,2	20	40,0	84	41,2	
Age: average ± standard deviation	65,15 ± 9,90		60,40 ± 11,04		61,92 ± 12,40		61,99 ± 11,23		p [(2)] = 0,061

(1) Through the chi-square test (2) through the Kruskal-Wallis test.

Table 6. Evaluation of clinical variables according to genotype KIF6 polymorphism (rs 20455) in the disease group

	Gene category rs 20455								
Variable	AA		AG		GG		Total group		p value
	n	%	n	%	n	%	n	%	
Arterial hypertension									p (1) = 0,807
Yes	41	78,8	76	74,5	37	74,0	154	75,5	
No	11	21,2	26	25,5	13	26,0	50	24,5	
Diabetes Mellitus									p (1) = 0,332
Yes	17	32,7	46	45,1	21	42,0	84	41,2	
No	35	67,3	56	54,9	29	58,0	120	58,8	
Dyslipidemia									p (1) = 0,177
Yes	9	17,3	9	8,8	9	18,0	27	13,2	
No	43	82,7	93	91,2	41	82,0	177	86,8	
Obesity									p (1) = 0,243
Yes	9	17,3	29	28,4	10	20,0	48	23,5	
No	43	82,7	73	71,6	40	80,0	156	76,5	
Heart attack									p (1) = 0,354
Yes	10	19,2	11	10,8	7	14,0	28	13,7	
No	42	80,8	91	89,2	43	86,0	176	86,3	
Stroke									p (2) = 0,684
Yes	1	1,9	5	4,9	1	2,0	7	3,4	
No	51	98,1	97	95,1	49	98,0	197	96,6	
Smoking									p (2) = 0,403
No	44	84,6	94	92,2	42	84,0	180	88,2	
Up to 10/day	4	7,7	4	3,9	3	6,0	11	5,4	
Ex-smoker	4	7,7	4	3,9	5	10,0	13	6,4	
CKD									p (2) = 0,213
Yes w/ hemodialysis	-	-	1	1,0	2	4,0	3	1,5	
No	52	100,0	101	99,0	48	96,0	201	98,5	
coronary angiography									p (2) = 0,071
Yes	2	3,8	2	2,0	5	10,0	9	4,4	
No	50	96,2	100	98,0	45	90,0	195	95,6	
PCI									p (2) = 0,067
Yes	1	1,9	8	7,8	7	14,0	16	7,8	
No	51	98,1	94	92,2	43	86,0	188	92,2	
Cardiac surgery									p (2) = 0,787
Yes	1	1,9	4	3,9	2	4,0	7	3,4	
No	51	98,1	98	96,1	48	96,0	197	96,6	

(1) Using the Chi-square test (2) Using Fisher's exact test

Mutations in MC4R account for up to 6% of cases of severe early-onset obesity, with most mutations found in the heterozygous form. Some lines of research suggest that signaling through MC4R exerts an inhibitory influence on appetite and hence on the development of obesity (Yeo, 2003). Despite these findings, there is still little published information on proteins that interact with melanocortin receptors (Yeo, 2003; Ganntz, 2004, Farooqi, 2003). In mice it was shown that mutations that reduce the function of ATRN are associated with obesity, indicating that the ATRN can modulate the function of both MC1R and MC4R. [23] In humans, these findings still lack evidence (Yeo, 2003; Ganntz, 2004, Farooqi, 2003). In this context, through the interpretation of the results obtained in the present study, it is possible that the AA genotype is associated with a reduction in the activity of ATRNL1 in humans. However, since this is a hypothesis, the performance of other studies is recommended for confirmation or not, since our work evaluated the genotypic expression of ATRNL1 gene polymorphism (rs 180706), not the action or activity of the protein encoded by genotype in question. Our study did not find the presence of significant associations between the genotypic expressions of the ATRNL1 polymorphism (rs 180706) and the clinical variables of the disease group. It is worth mentioning that there were differences in prevalences higher than 10% in some situations. In the case of SAH, the AC genotype was the most associated and the CC genotype was the least.

In the subgroup of patients with a history of AMI, AC genotype was the most frequent and AA was the least frequent. There are few reports in the literature on the association of mutations related to the ATRN gene and its phenotypic presentation in humans. The first description was made by Stark et al. in 2010 regarding a deletion in the ATRNL1 gene (Stark , 2010). The case quoted spoke of a male child who was followed up for 4 years. In this period, problems related to neuropsychomotor development, presence of ventricular septal defect, dysmorphic facial features, syndactyly, and limitation of elbow movement were observed (Stark , 2010). A study by Balakrishnan et al. evaluated the synovial fluid of patients with osteoarthritis, through a proteomic analysis. Among the new proteins discovered in the synovial fluid of these patients, ATRNL1 was present. The authors suggest that there may be a correlation between the presence of these proteins and the inflammatory process present in osteoarthritis (Balakrishnan, 2014). Regarding the performance of ATRNL1 in the pathophysiology of CAD, studies have not yet been developed to prove a direct association with the disease. Thus, the true influence of the polymorphism of this gene on the phenotypic expression of CAD is not known. However, it is possible that there are common pathways that involve the pathophysiology of CAD and the action of atractins. It is well established that the monocytes migrate into the subendothelial space and differentiate into macrophages which, in turn, capture the oxidized LDL particles (Soloperto, 2012; Faludi, 2017).

Lipid-filled macrophages are called foam cells and are the main component of fatty streaks, the initial macroscopic lesions of atherosclerosis. T lymphocytes, although less numerous, through interaction with macrophages can differentiate and intensify the production of cytokines, helping to modulate the local inflammatory process (Soloperto, 2012; Faludi, 2017). In this sense, one can speculate that there is a modulating action performed by the ATRLN1, which facilitates the phagocytosis of oxidized LDL, facilitating the development of CAD, but this idea can not be confirmed with data currently available in the literature. In the present study, when the genotypic analysis of the KIF 6 polymorphism (rs 20455) was performed, we observed that the comparison between the prevalences of the homozygous and heterozygous alleles in the disease and control groups was not statistically different. The polymorphism of the KIF6 gene has been identified as a predictor of cardiovascular risk in some ethnic groups. Observational studies have confirmed the variation in allelic frequency, between men and women, and in individuals from different ethnic groups (Shiffman, 2008; Akao, 2012; Peden, 2011).Such findings were not corroborated in this study.

In the literature, the rate of the mutant allele in healthy controls was much lower when populations of European and Japanese origin were evaluated. In healthy controls in sub-Saharan Africa, the frequency of this mutation was quite high. On the other hand, in case-control studies, when populations with significant ethnic diversity were evaluated, even with a large number of participants, the association of the 719Arg mutation in an increase in cardiovascular risk could not be replicated (Shiffman, 2008; Iakoubova, 2008; Iakoubova, 2008). The present study consists of a cross-sectional, case-control analysis that evaluated a population from the northeast of our country. This is a region characterized by the occurrence of a quite pronounced miscegenation among varied ethnic groups, composed primarily of blacks, whites, and Indians. This fact gives a singular identity to this people, considered markedly miscegenated, both from the genetic point of view, as well as in social and cultural aspects (Secretaria de Saúde do Estado de Pernambuco, 2016). It is postulated that the conflicting findings in the literature could be explained by the survival and drug interaction bias, which may attenuate the comparisons between cases and controls, or even by the lack of effect of the genetic mutation on the pathophysiology of atherosclerotic disease in certain groups ethnic groups((Shiffman, 2008; Iakoubova, 2008). When the prevalence of genotypic expressions in relation to the clinical variables of the disease group was evaluated, there was also no statistically significant association. There is evidence that patients with this polymorphism may have better therapeutic effects with statins, both in terms of a greater reduction in serum LDL levels and in the modulation of the inflammatory response, thrombogenesis, and arterial vasomotor function (Peng, 2012). This could act as a confounding factor during the association analysis of the disease group. There were percentage differences above 10.0% in allele prevalence in the following situations: DM (AA and AG genotypes); obesity (AA and AG genotypes); previous ATC (AA and GG genotypes). Based on these data, it is believed that a type II statistical error may have occurred (when the analysis of the data can not reject a hypothesis, if this hypothesis is false), because the sample quantitative is reduced. When the analysis of the prevalence of the allele A (AA + AG) versus GG was performed, there was no statistical difference.

Regarding the clinical variables, only the previous history of Cine was more associated with the GG genotype, without other statistically significant associations. Data regarding the influence of the KIF6 polymorphism (rs 20455) on CAD are still very discrepant. In a meta-analysis conducted in 2012 by Peng et al., Data from more than 143,000 individuals were analyzed, showing that the presence of polymorphism was a cardiovascular risk factor in Caucasians, but with variable effects in other ethnic groups (Iakoubova, 2008). Ruiz-ramos et al. also performed a meta-analysis, and demonstrated that the KIF6 gene polymorphism would be an important risk factor for the development of AMI (Shiffman, 2008). Recently, in a meta-analysis performed by Li, Chen and Song, 50 studies were evaluated, correlating the KIF6 mutation (rs 20455) with the presence of CAD, totaling an analysis of 40,059 cases and 64,032 controls. The results of this study suggest that this polymorphism may not be associated with a greater susceptibility to CAD, but the authors reinforce the need for additional studies to confirm these results (Li, 2018). In our population data on the association of KIF6 polymorphism (rs 20455) with CVD are still scarce.

This research is a pioneer, therefore generating hypotheses, which need additional studies to confirm them. Our work was limited to evaluating genotypic expressions of ATRNL1 (rs 180706) and KIF6 (rs 20455) gene polymorphisms and their clinical correlations with CAD. The properties of the transcribed proteins from the allelic variation of said polymorphisms were not analyzed, so that we do not know for sure the mechanism of action or the activity of these proteins in the individuals. Another limiting factor of the present study is the size of the sample, which is relatively small to infer data related to a population as large as the Brazilian population. In addition, the individuals selected for the survey were from a single region of Brazil, the Northeast. It is known that because it has continental dimensions, Brazil presents a wide ethnic, social and cultural diversity, with peculiarities inherent in each one of its regions, and this fact must be considered in the interpretation of the data. This is the first study in the literature to describe the association between polymorphism in the ATRNL1 gene (rs 180706) and CAD. The prevalence of the AA genotype in the ATRNL1 gene polymorphism (rs 180706) was lower in the disease group, when compared to the control group. No associations were found between the KIF6 gene polymorphism (rs 20455) and the DAC. Thus, the hypothesis of the existence of a protective effect of the AA genotype of the ATRNL1 polymorphism (rs 180706) on the development of CAD was generated. However, additional studies are required to confirm this.

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