Research Article

Polymorphisms in Acute Coronary Syndrome

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Abstract

Background: Because of their effects on inflammatory processes, some genes are candidates for association with Acute Coronary Syndrome (ACS). While a relationship between Single Nucleotide Polymorphisms (SNP) and ACS has been suggested in some populations, but not in others, genotype characterization of genes related to inflammatory reactions, becomes necessary for specific populations.

Aim: In this study, we evaluate IL6 (rs1800795), LEPR (rs6700896) and IL1b (rs16944) SNPs in a healthy population and compare the frequencies with other populations to characterize them in a region of Brazil and investigate the role of SNPs as prognostic markers in the development of ACS in futures studies.

Methods: IL6, LEPR and IL1b genes were genotyped in 295 blood donors from Fundação de Hematologia e Hemoterapia de Pernambuco. Genotyping was carried out by polymerase chain reaction, followed by DNA sequencing or enzymatic cleavage. G Williams test and odds ratio with confidence intervals of 95% were used. The significance value was $p < 0.05$.

Results: There was no difference in genotype distribution between gender or age groups. Genotypic and allelic frequencies were similar between Brazilian populations but different from most other population's analyzed ($p < 0.05$).

Conclusions: Genotypic characterization of genes that can influence the development of ACS is important, since it can provide a tool for future studies in the use of genetic markers in prevention and diagnosis of diseases.

Keywords: Acute Coronary Syndrome; Blood Donors; Prognostic Markers; Polymerase Chain Reaction; Single Nucleotide Polymorphisms.

Introduction

Genetic polymorphisms are defined as allelic variations that occur in a human population with a frequency equal to or greater than 1% and consist of insertions or deletions of DNA sequences, or substitution of a single nucleotide, named single nucleotide polymorphisms (SNPs) [1,2]. Functional SNPs in promoter regions of genes can alter transcription and expression levels of the respective protein, contributing to the development of parasitic infections [3] and chronic-degenerative diseases [4]. Amongst these diseases, Acute Coronary Syndrome (ACS) is characterized by a chronic inflammatory process (atherosclerosis) due to lipid deposits on the inner layer of the arterial wall. With a multifactorial phenotype, ACS is influenced by age, gender and others acquired risk factors: hypertension, diabetes, Dyslipidemia and family history [5,6] While recognizing and controlling the acquired risk factors is important for reducing SCA, genetic basis strategies are relevant

to contribute to the development of innovative tools for diagnosis and therapeutic actions [5,7].

Genetic polymorphisms involved in inflammatory responses have received attention in coronary heart disease. SNPs have been identified in Interleukin (IL) 6, LEPR (Leptin receptor) and IL1 genes [8,9] IL6 (-174 G / C; rs1800795) SNP, at promoter region, has been associated with ACS because it can alter the protein plasma levels and increases the inflammatory process [10] . Elliott et al. (2009) [8] described that T variant allele, in intronic region of LEPR gene (1118 C / T; rs6700896), also increases the risk of coronary heart disease. In addition, IL1 (-511 C / T; rs16944) SNP, at promoter region, has been associated with SCA because T allele variant decreases IL-1 cytokine plasma levels, reducing risk of acute myocardial infarction (AMI) [9]. Thus, the aim of this study was to evaluate SNPs in protein genes in a healthy population in northeastern Brazil, and compare the frequencies with other populations to characterize them in this region and contribute to futures studies, which aim to investigate the potential role of SNPs as prognostic markers in the development of ACS. Furthermore, the identification of such polymorphisms may contribute as an additional risk factor for this disease.

Materials and Methods

Subjects

Blood donors adults (n = 295), 51 women and 244 men, aged between 29 and 65 years (mean 47.3 ± 7.9), were randomly selected from Fundação de Hematologia e Hemoterapia de Pernambuco (HEMOPE), in Northeast Brazil. Individuals with risk factors (diabetes and hypertension arterial) related to ACS were excluded. All subjects were submitted to laboratory tests (HEMOPE) for the investigation of infectious and parasitic diseases (Human Immunodeficiency Virus, Cha gas disease, syphilis, Human T lymphotropic virus type 1 and 2 and hepatitis C). Those who presented seropositivity were excluded from the study. The Ethics Committee of HEMOPE approved the study (CAAE 03187512.2.0000.5202) and all participants signed informed consent forms. It was decided not to perform ethnic matching since previous studies in Brazilian populations have demonstrated that skin color or self-defined ethnic origins are not considered accurate as biomarkers for ancestry in Brazil [11].

Genotyping

Extracted DNA was amplified using polymerase chain reaction with appropriated primers, temperatures and amplification conditions (Table 1). As a negative control, reagents without DNA were used. The fragments were visualized on 1% agarose gel and submitted to DNA sequencing using ABI 3500xL Genetic Analyzer (Applied Bio systems, USA) to display the alleles.

Statistical analysis

The χ^2 test was used to verify if observed and expected frequencies were in accordance with Hardy-Weinberg equilibrium.

Qualitative variables were analyzed using the G Williams test and odds ratio (OR) with confidence intervals of 95%. In all tests, the level of significance was $p < 0.05$. Data analysis was performed using Bio Estat 5.0 [14].

Polymorphisms	Primers	Amplification conditions	Fragments sizes (bp)	References
IL6 (rs1800795)	F: 5' AGC CTC AAT GAC GAC CTA AGC 3' R: 5' ACT GGA GAT GTC TGA GGC TCA TT 3'	94°C – 2 mir 94°C – 1 mir 65°C – 1 mir 68°C – 1 mir 65°C – 5 min } 35 X	226	[10] Adapted
LEPR (rs6700896)	F: 5' GCC CTT CTT TCC TCA AGC CTT CC 3' R: 5' GCT CCA AAG CCA GAC AAA CTG GT 3'	95°C – 5 mir 95°C – 30 se 55°C – 30 se 68°C – 30 se 68°C – 5 min } 30 X	515	[12] Adapted
IL1 (rs1694)	F: 5' TGG CAT TGA TCT GGT TCA TC 3' R: 5' GTT TAG GAA TCT TCC CAC TT 3'	94°C – 5 min 94°C – 60 seg 55°C – 40 seg 72°C – 40 seg 74°C – 7 min } 35 X	304	[13] Adapted

bp - base pair

Table 1: Sequence of primers and amplification conditions for each polymorphism

Results

The distributions of genotype frequencies are according to Hardy-Weinberg equilibrium ($p > 0.05$). Men were the most frequent participant (82.7%). Two groups were formed according to the mean age of individuals: ≤ 47 and > 47 years old. There was no difference when compared genotype distribution among gender or age groups ($p > 0.05$). GG genotype (IL-6) was the most common in males (60.6%) and females (55.0%); CT genotype (LEPR) was more frequent in males (54.5%), while CC genotype in fe-

males (45.1%). The CT genotype (IL-1) was the most frequent in males (46.7%) and females (48.8%). GG (IL-6), CT (LEPR) and CT (IL-1) genotypes were more frequent in both age groups. There was no association risk between the genotype distributions regarding gender or age, since p > 0.05 in all analysis (Table 2). The most frequent genotypes in the northeast population were GG (IL-6), CT (LEPR) and CT (IL-1). Genotype frequencies of the present study were similar when compared to Amazon [15] and Rio de Janeiro [16] (p > 0.05). Genotype in IL6 gene, in populations from Portugal [17] Malaysia [18] Italy [13,19] China [20] and Mexico [21] were different when compared to our study (p ≤ 0.0004). For LEPR, genotype frequencies also showed statistical differences between China [22] and Egypt [12] populations (p < 0.0001). Regarding IL1 polymorfism, it was different when compared Portugal [17] Germany [23], Italy [19], Malaysia [24], Korea [25] and northeastern Brazil genotype frequencies (p ≤ 0.004) (Table 3).

Polymorphisms	Gender n = 295			Age n = 295		
Genotypes	Male n = 244 (%)	Female n = 51 (%)	p	≤ 47 years n = 179 (%)	> 47 years n = 116 (%)	p
IL6 (rs 1800795)						
GG	148 (60.6)	28 (55.0)		103 (57.5)	75 (64.6)	
GC	84 (34.4)	19 (37.2)	0.63	68 (38.0)	33 (28.5)	0.2
CC	12 (5.0)	4 (7.8)		8 (4.5)	8 (6.9)	
Alleles						
G	380 (77.9)	75 (73.5)		274 (76.5)	183 (78.9)	
C	108 (22.1)	27 (26.5)		84 (24.5)	49 (21.1)	
LEPR (rs 6700896)						
CC	73 (29.9)	23 (45.1)		60 (33.5)	36 (31.0)	
CT	133 (54.5)	20 (39.2)	0.09	94 (52.5)	59 (50.9)	0.96
TT	38 (15.6)	08 (15.7)		25 (14.0)	21 (18.1)	
Alleles						
C	279 (57.2)	66 (64.7)		214 (67.3)	131 (56.5)	
T	209 (42.8)	36 (35.3)		144 (40.2)	101 (43.5)	
IL1 (rs 16944)						
CC	75 (30.8)	13 (30.2)		57 (31.8)	33 (28.4)	
CT	114 (46.7)	21 (48.8)	0.96	87 (48.6)	53 (45.7)	0.44
TT	55 (22.5)	9 (21.0)		35 (19.6)	30 (25.9)	
Alleles						
C	264 (54.1)	47 (46.1)		201 (56.1)	119 (53.3)	
T	224 (45.9)	39 (38.2)		157 (43.8)	113 (48.7)	

p - G Williams Test

Table 2: Genotypic frequencies of polymorphisms in IL-6, IL-1, LEPR and IL-1 according to gender and age

Polymor-phisms	Country	n	Genotype frequencies n(%)			p	Allele frequencies n(%)		p
			GG	GC	CC		G	C	
IL6 (rs1800795)	Brazil - NorthEast	295	176 (59.4)	103 (35.3)	16 (5.3)	Reference	455 (77.1)	135 (22.9)	Reference
	Brazil - Amazon	77	49 (63.64)	23 (29.87)	5 (6.49)	0.6934	121 (78.6)	33 (21.4)	0.7005
	Portugal	735	319 (43.4)	324 (44.1)	92 (12.5)	< 0.0001	962 (65.4)	508 (34.6)	< 0.0001
	Malaysia	100	12 (12.0)	81 (81.0)	7 (7.0)	< 0.0001	105 (52.5)	95 (47.5)	< 0.0001
	Italy	112	45 (40.2)	51 (45.5)	16 (14.3)	0.0005	141 (62.9)	83 (37.1)	< 0.0001
	China	331	329 (99.4)	1 (0.3)	1 (0.3)	< 0.0001	659 (99.5)	3 (0.5)	< 0.0001
	Mexico	102	80 (78.4)	20 (19.6)	2 (2.0)	0.002	180 (88.2)	24 (11.8)	0.0004
			CC	CT	TT		C	T	
LEPR (rs6700896)	Brazil - NorthEast	295	96 (32.5)	153 (51.9)	45 (15.6)	Reference	345 (58.5)	243 (41.2)	Reference
	China	109	5 (4.6)	24 (22.1)	80 (73.4)	< 0.0001	34 (15.6)	184 (84.4)	< 0.0001
	Egypt	30	30 (100.0)	0 (0.0)	0 (0.0)	< 0.0001	60 (100.0)	0 (0.0)	< 0.0001
			CC	CT	TT		C	T	
IL1 (rs16944)	Brazil - NorthEast	295	91 (30.8)	138 (46.8)	66 (22.4)	Reference	320 (54.2)	270 (45.8)	Reference
	Brazil - Rio de Janeiro	44	17 (38.7)	23 (52.3)	4 (9.0)	0.0875	57 (64.8)	31 (35.2)	0.062
	Portugal	735	327 (44.5)	309 (42.0)	99 (13.5)	< 0.0001	963 (65.5)	507 (34.5)	< 0.0001
	Germany	94	43 (45.8)	41 (43.6)	10 (10.6)	0.0051	127 (67.5)	61 (32.5)	0.0012
	Italy	205	90 (43.9)	89 (43.4)	26 (12.7)	0.002	269 (65.6)	141 (34.4)	0.0003
	Malaysia	60	13 (21.6)	22 (36.7)	25 (41.7)	0.01	48 (40.0)	72 (60.0)	0.0045
	Korea	364	61 (16.8)	194 (53.3)	109 (29.9)	< 0.0001	316 (43.4)	412 (56.6)	< 0.0001

n - Number of subjects; p - G Williams Test

Table 3: Distribution of genotype frequencies of polymorphisms in different healthy populations

Discussion

In this study, it is hypothesized that the presence of polymorphisms in the IL6, LEPR and IL1 genes may contribute to the development of ACS, considering that these polymorphisms may exacerbate inflammatory responses and contribute to the formation of atherosclerosis. Blood donors have been selected for control groups in genetic studies as it is assumed that this group represents the general population. Authors have observed [26,27] that most randomly recruited blood donors are male, in agreement with our study. Swirta et al. (2015) [27] studying the genetic influence on coronary artery disease (CAD), recruited Polish blood donors as control group and verified that 85.3% were male with 34.0 years old of mean age, lower than our study (47 years old). Although our results indicated no risk association between gender and / or age in developing ACS with the studied polymorphisms, this possibility should not be excluded [28,29] Chiappelli et al. (2005) [30], in a study with Italian men over 67 years old, associated IL6 C allele with AMI and suggested that this association is age-dependent. Perhaps due to the fact that adults over 65 years were not included in the present study, we were unable to observe significant differences between the frequency of IL-6 gene polymorphism and gender (p = 0.63) or age (p = 0.20). In contrast, Jin et al. (2015) [22] found no association between LEPR polymorphism and susceptibility to cardiovascular disease in Chinese men and women of 59 years old, which support our finding, despite our mean age is lower. Besides, IL1 gene polymorphism has been associated with atherosclerosis in some populations [9,29]. Iacoviello et al. (2005) [29] stated that TT genotype conferred 64% less risk of AMI development in younger women. Also, Rios et al. (2010) [9] found that CC genotype increases the risk of CAD in individuals between 50 and 56 years old.

When comparing the genotype frequencies among Brazilian individuals from different regions, we can observe that, despite the intense miscegenation between them, the genetic characteristics

in relation to the SNPs evaluated remained similar. In the North East population of this study, the genotype distributions of IL-6 gene were different (p ≤ 0.0004) when compared to other populations, although a similarity was demonstrated in relation to the population from Amazon (p = 0.69). CC genotype was less frequent (5.3%) than in Portugal (12.5%), Malaysia (7.0%) and Italy (14.3%) populations. In a study with Pakistani patients, Satti et al. (2013) [30] demonstrated that the frequency of CC genotype, IL-6 and C - reactive protein plasma levels were higher in CAD patients than in control subjects (p = 0.0025). LEPR genotype distributions from our study were different when compared to China and Egypt populations (p < 0, 0001) [12,22]. T minor allele has been associated with a significant increase in the risk of CAD [8] which was not corroborated by Jin et al. (2015) [22], since they found no association between T allele and cardiovascular disease in Chinese patients. Interestingly, there was no evidence of this allele in the study by Swellan et al. (2012) [12] in Egyptian healthy individuals. Regarding to IL1 gene, our results were similar to genotype distributions in Rio de Janeiro (p = 0.08) [16] and CT genotype was the most frequent in both population (22.4% and 9.0%, respectively).

The CC genotype in IL1 gene was most frequent in Portugal (44.5%), Germany (45.8%) and Italy (43.9%) populations and is associated with an higher risk for CAD because it increases levels of the corresponding inflammatory cytokine, contributing to the progression of atherosclerotic plaque [29].The genotypic distributions evaluated in the blood donor of the present study, when compared to other populations, showed the diversity of the SNPs frequencies in other countries, evidencing the importance of genetic studies in specific population groups to establish a cause-effect relationship between genes and diseases.

Conclusions

Knowledge regarding the frequencies of genetic polymorphisms in a particular population may be a very useful tool for understanding and assessing the risk of development of several diseases. The investigation of risk factors, as well as genetic markers for ACS, is essential for prevention, diagnosis and treatment, and can reduce morbidity and mortality. These findings may be employed for future association studies regarding these polymorphisms with the inflammatory nature of diseases. One further explanation for the differences found with other populations may be the different cultural and ethnic backgrounds, which were not analyzed. Results showed no future risk of ACS development when using IL6, LEPR and IL1 markers. However, it was not possible to identify the presence of other risk factors for ACS, apart from hypertension and diabetes, in recruited blood donors.

A study of data of Dyslipidemia, smoking, alcohol, physical inactivity and family history would be important in order to relate with genetic polymorphisms, and thus provide a better assessment of the risk of developing ACS.

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