Cytokine xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Acute coronary syndrome: Relationship between genetic variants and TIMI risk

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ARTICLE INFO

Keywords:
Acute coronary syndrome
Genetic variants
Cytokines
Risk factors

ABSTRACT

Acute Coronary Syndrome (ACS) is a multifactorial disease, including the genetic factor, caused by coronary artery obstruction by atheroma. Some genetic variants have been described as risk factors for this disease. Its early diagnosis and stratification of risk of death by Thrombolysis in Myocardial Infarction (TIMI) are important. Therefore, we evaluated variants in the *IL6R* (c950-1722C > T), *TNFa* (c.-488G > A), *LEPR* (c.2673 + 1118C > T) and *IL1b* (c.-598T > C) genes in relation to TIMI risk, cytokine serum levels, and risk factors for ACS. We selected 200 patients with ACS, 50 without ACS from the Real Hospital Português, Recife - PE, and 295 blood donors at the Fundação de Hematologia e Hemoterapia de Pernambuco (Hemope). Variants were determined by DNA sequencing or enzymatic cleavage. Cytokine levels were measured by ELISA. The most frequent risk factors found in the patients were dyslipidemia and hypertension, this latter associated with high TIMI risk ($p = 0.003$). Genotype frequencies of *IL6R* and *TNFa* differed between patients with ACS and the blood donors ($p = 0.0002$ and $p = 0.01$, respectively), and TNF-α levels differed between genotypes. The TT genotype of the *IL6R* gene is as a possible protective factor for ACS because it was significantly more present in blood donors (32.2%) than in patients with ACS (18.0%), and was more frequent in low TIMI risk (22.9%) than in the intermediate (20.2%) or high (4.9%). In patients with ACS, the TT genotype in *IL6R* was related to a lower concentration of c-reactive protein ($p = 0.03$) and troponin ($p = 0.02$), showing a less inflammatory reaction and tissue damage. The differences in the frequencies of variants in genes of medical interest among the groups show the importance of studies in specific populations groups to establish the relationship between genes and diseases.

1. Introduction

Cardiovascular diseases (DCs) are currently the leading causes of death in the world (WHO, 2016). Among them, Acute Coronary Syndrome (ACS), which includes Acute Myocardial Infarction (AMI) and Unstable Angina (IA), is a cardiovascular disease caused by obstruction of the coronary arteries by atheromatous plaque and involves clinical symptoms compatible with acute myocardial ischemia [1,2].

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https://doi.org/10.1016/j.cyto.2018.04.001
Received 11 October 2017; Received in revised form 22 January 2018; Accepted 3 April 2018

Table 1
Genotyping conditions [16–19].

Genes	*Primers*	PCR conditions	Fragment Size	Genotyping	Reference
IL6R ***(c950-1722C>T)***	F: 5' GTC GCT TTC CCT CTC CG 3' R: 5' GGA AAC CCC AAG GCA AGA GG 3'	95°C – 5 min 95°C – 30sec 59°C – 30sec 68°C – 20sec } 10X 95ºC – 30sec 57ºC – 30sec 68ºC – 20sec } 10X 95ºC – 30sec 55ºC - 30sec 68ºC - 20sec } 10X	370 bp	Enzymatic digestion – BsaHI (New England Biolabs®)	[16]
TNFa ***(c.-488G>A)***	F: 5'AGG CTT GTC CCT GCT ACC CCC 3' R: 5' TCC TCC CTG CTC CGA TTC CG 3'	95°C – 5 min 95°C – 30 seg 65°C – 30 seg 72°C – 30 seg } 30X 72°C – 2 min	363 bp	DNA Sequencing	[17]
LEPR ***(c.2673+1118C>T)***	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 min 95°C – 30 sec 55°C – 30 sec 68°C – 30 sec } 30X 68°C – 5 min	515 bp	DNA Sequencing	[18]
IL1b ***(c.-598T>C)***	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 sec 55°C – 40 sec 72°C – 40 sec } 35X 74°C – 7 min	304 bp	DNA Sequencing	[19]

ACS has a multifactorial phenotype determined by genetic factors and influenced by other risk factors, such as hypertension, diabetes, dyslipidemia, obesity and smoking. Age and gender also influence its development [3].

The identification of severity risk in ACS patients is important so that they can be benefited with a more appropriate treatment. In this sense the Thrombolysis in Myocardial Infarction (TIMI) research group proposed a rapid and practical classification that selects patients at low, moderate and high risk according to clinical data, electrocardiographic changes and biomarkers of myocardial injury, defining the best therapeutic strategy and prognostic for each case [4].

Several studies seek to describe genetic markers using molecular biology methods to identify genes that are related to coronary heart disease process and its risk factors [5,6]. Therefore, variants of genes involved in the atherosclerosis inflammatory response have received attention as a contribution to the development of innovative tools for diagnosis in ACS [7].

The presence of variants in some genes can alter their transcription and expression levels, generating different amounts of messenger RNA and the respective protein, contributing to the development of some pathologies, such as ACS [8]. Studies with variants in *Interleukin (IL)-6 receptor (IL6R), Leptin Receptor (LEPR), Tumor Necrosis Factor-alpha (TNFa)* and *IL1b* [9–11], were pointed out in association with ACS, but they presented divergent results when it comes to different study populations [12–14].

Thus, the investigation of genetic variants that are related to ACS may contribute to the identification of additional risk factors and function as markers of susceptibility and prognosis in ACS development. So, the aim of this study was to evaluate the relationship between the TIMI risk score and genes variants, inflammatory markers and myocardial injury in patients with ACS.

2. Materials and methods

2.1. Subjects

In an analytical and cross-sectional study with groups comparison, 200 patients (mean age 62.0 ± 13) with ACS admitted to Real Hospital Português (RHP), in Recife - Pernambuco, Brazil from 2012 to 2015 were recruited. All of them were submitted to electrocardiogram and dosages of injury myocardial creatine kinase MB fraction (CK-MB) and troponin and the inflammatory markers: TNF-α, IL-1 and c-reactive protein (CRP). Patients were also classified according to the TIMI risk score in accordance with Antman et al. [4].

The second group consisted of 50 patients (mean age 58.0 ± 18.9) admitted to the RHP with others cardiac disease, but not ACS. Patients with or without ACS on anti-inflammatory drugs treatment, with recent trauma, infectious process or cancer were excluded from this study. Data on the presence of risk factors for ACS, such as diabetes mellitus, systemic arterial hypertension and smoking were collected from the hospital records of the patients.

A group of 295 individuals (mean age 47.3 ± 7.9), blood donors from the Fundação de Hematologia e Hemoterapia de Pernambuco (Hemope) were formed to investigate the frequency of genetic polymorphisms in a healthy population [15]. Individuals with positive serology for HIV, Chagas disease, Hepatitis C, Syphilis and HTLV 1/2 were excluded.

Ethics Committee of RHP approved the study (CAAE 03187512.2.0000.5202) and all participants signed informed consent forms.

2.2. Genotyping

DNA extraction was performed with "illustra genomicPrep blood Mini Spin kit" and the amplification of genes fragments was done by