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Research Article

The 1057 C>A Acetylcholinesterase Variability is Associated with Risk for Coronary Atheromatosis and Acute Coronary Syndrome

Marina F. de Mattias[1]; Rodrigo S. Kojima[1]; Agnaldo B. Chies[1]; Igor R.C. Bienert[2]; Eduardo A. Donadi[3]; Pedro B. Andrade[2]; Priscilla B. Oliveira[1]; Antonio Fabron JR.[1,5]; Marcelo O. Ruiz[5]; Ana C. P. Latini[6] and Wilson Baleotti JR.[4]*

[1]Department of Medicine, FAMEMA, Brazil

[2]Department of Hemodynamics and Interventional Cardiac, FAMEMA, Brazil

[3]Ribeirao Preto of the University of São Paulo, FMRP-USP, Brazil

[4]Department of immunohaematology, FAMEMA, Brazil

[5]Diagnosticos do Brasil, Brazil

[6]Lauro de Souza Lima Institute, Brazil

Abstract

Introduction: Acute Coronary Syndrome (ACS) is a consequence of long-term endothelial dysfunction, which has been associated with decreased vasodilator mediators, such as nitric oxide and Acetylcholine (ACh).

Acetylcholinesterase (*ACHE*) degrades ACh and plays an important role on the control of the anti-inflammatory cholinergic pathway.

Objective: To assess the relationship between the *ACHE* YT polymorphism (rs1799805) on the severity of ACS in a Brazilian population.

Methodology: We studied 83 patients presenting ACS undergoing coronary angiography and 408 unrelated healthy individuals from the same region. We also associated the *ACHE* YT polymorphism with the presence of comorbidities, electrocardiographic features, the angiographic Syntax score, and the plasma concentrations of Nitrite/Nitrate (NOx).

Results: The frequency of the YT*1/YT*2 genotypes was not different between the ACS and controls. The YT*1/YT*2 genotype was overrepresented in patients exhibiting the ST elevation when compared to those without ST elevation (p=0.0295, OR=4.61, 1.1646-18.2910). The YT*1/YT*2 genotypes were not associated with the Syntax index and the plasma concentrations of nitrite/nitrate.

Conclusion: Patients admitted to the emergency room presenting the YT*1/YT*2 *ACHE* genotype exhibited increased frequency of the ST-segment elevation, which is associated with a worse prognosis.

Keywords: Acute coronary syndrome; Acetylcholinesterase YT polymorphism; Cartwright system; Cholinergic anti-inflammatory pathway; Nitric oxide levels

Abbreviations

ACS: Acute Coronary Syndrome; Ach: Acetylcholine; *ACHE*: Acetylcholinesterase; NOx: Nitrite/Nitrate; STE-ACS: Acute coronary syndrome with ST elevation; NSTE-ACS: Acute coronary syndrome with Non ST Elevation; AMI: Acute Myocardial Infarction; ECG: Electrocardiogram; MR: Myocardial Revascularization; NO: Nitric Oxide; SNP: Single-Nucleotide Polymorphisms; CAD: Coronary Artery Disease; STE-AMI: ST-Segment Elevation Acute Myocardial Infarction; NSTE-AMI: Non-ST Segment Elevation Acute Myocardial Infarction

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***Corresponding author:** Marina Francischine de Mattias, Department of Medicine, Faculty of Medicine of Marilia -FAMEMA, Brazil

Introduction

According to the American Heart Association and American College of Cardiology, Acute Coronary Syndrome (ACS) is considered an ischemic myocardial event, due to the abrupt reduction of blood flow in coronary artery [1]. Patients with ACS are electrocardiographically classified into two groups: presenting (STE-ACS) or not ST segment elevation (NSTE-ACS). NSTE-ACS patients may present an Acute Myocardial Infarction (AMI) without ST elevation (NSTE-AMI) or Unstable Angina (UA), when there is no increase in myocardial necrosis biomarkers (troponin) [2].

AMI is characterized by the damage of myocardium (increased troponin) associated with at least one of the following signs or symptoms: acute myocardial ischemia symptoms, ischemic Electrocardiogram (ECG) changes or coronary thrombus identification [3].

The investigation of ACS should be performed through diagnostic coronary angiography, evaluating the number of lesions

and percentage of luminal obstruction. The complexity of coronary obstructions can be assessed using the Syntax score, which combines anatomical variables, creating an accurate prediction of mortality to guide the use of transluminal coronary angioplasty or Myocardial Revascularization (MR) [4].

Multiple factors may influence the pathogenesis of ACS, including the cholinergic autonomic nervous system, which in addition to stimulating the release of Nitric Oxide (NO) in the vascular bed, also presents an anti-inflammatory activity, [5,6] mediated by the stimulation of the vagus nerve, releasing the neurotransmitter Acetylcholine (ACh) [7]. This anti-inflammatory action occurs through the binding of ACh to the α7AChR macrophage receptor accompanied by activation of the JAK-STAT signaling, which induces macrophages to produce less pro-inflammatory cytokines, such as TNF-α, IL-1β and IL-6 [8,9]. Vagus nerve stimulation can occur in several ways, such as a reflex to acute inflammation or electrical direct stimulation of the nerve [10,11]. The induction of cardiac ischemia in dogs increases the release of Ach mediated by the vagus and reduces the post-infarction necrotic area extension, accompanied by lower concentrations of inflammatory cytokines (TNF-α, IL-6) and neutrophilic infiltration in the myocardium. The increased survival of the stimulated group suggests that an efficient cholinergic tone decreases inflammation and positively affects AMI outcome [12,13]. The α7AChR nicotinic receptor is also present on endothelial cells. Studies *in vivo* and *in vitro* have shown that the cholinergic system suppresses the activation of pro-inflammatory cytokines, leading to increased anti-inflammatory response. The blockage of adhesion molecule (ICAM-1, VCAM-1 and E-selectin) expression impairs the leukocyte recruitment and the release of pro-inflammatory cytokines, such as the decrease of TNF-α [14].

Inhibition of the inflammatory response through cholinergic system can help preventing cardiovascular diseases. Inflammatory condition generates an oxidative stress in the vascular endothelium, leading to a reduction in NO bioavailability and, consequently, to endothelial dysfunction. Finally, endothelial dysfunction leads to an imbalance between endothelial-derived relaxation and constriction factors, narrowing blood vessels, favoring the formation of atheromatous plaques [15]. Pharmacological stimulation of the cholinergic system through a α7AChR agonist decreases plaque formation in the aorta artery of rats, decreasing the progression of atherosclerosis [16].

Since Ach plays an important anti-inflammatory role, increased action of Acetylcholinesterase (*ACHE*) may lead to a low grade systemic inflammatory condition, especially in nervous system, kidneys and blood vessels [17-19], predisposing to a pro-atherogenic state [20]. Additionally, the activity of *ACHE* is directly related to NO synthesis and its increased activity may be a risk factor for endothelial dysfunction. An experimental study reported that *ACHE* inhibitors increased vagal function, as well as NO concentration [21]. In this context, *ACHE* mutations that increase its Ach activity could predispose to cardiovascular diseases.

A large part of the human genetic variation is due to the occurrence of Single-Nucleotide Polymorphisms (SNP), which may modify the translated protein, being associated with complex diseases such as phaeochromocytoma [22] or systemic lupus erythematosus [23]. One of the SNPs at the *ACHE* gene (on chromosome 7q22) is associated with the YT blood group (also called Cartwright system). Due to blood transfusion incompatibility, the red blood cell YT antigens were identified as two variants of *ACHE*, encoded by two different alleles: YT*1 (natural) and YT*2 (rs1799805, exon 2 polymorphism, codon 353). The YT*1 allele is characterized by a Histidine at codon 353 and the YT*2 translocation encodes an Asparagine (SNP1057C>A) [24]. Besides erythrocytes, the *ACHE* gene is primarily expressed at the neuromuscular synapse clefts [25].

ACHE may present a differential function according to gender, ethnicity, and polymorphisms [26]. Increased activity of *ACHE* leads to higher degradation of ACh, causing higher systemic inflammation and lower NO production, interfering in vasodilation and predisposing to cardiovascular disease. Indeed, the presence of the additional intronic variability at the *ACHE* rs2571598 allele has been associated with increased rate of coronary artery disease [27]. Therefore, the exonic 1057C>A variation that recognizably modifies the *ACHE* function may also be associated with atheromatous coronary and the ACS. The present study aims to analyze the association of the 1057C>A *ACHE* polymorphism (YT*2 allele) in ACS patients, stratified according to clinical, electrocardiographic, and angiographic AMI outcomes, as well as the NO bioavailability.

Material and Methods

Study population and design

This unicentric exploratory study was approved by the Ethics Committee of the University Hospital FAMEMA (CAAE 60170916.8.0000.5413). All patients who underwent coronary angiography from 2016 to 2018 (n=83), who were admitted with ACS at the Hemodynamic laboratory, exhibiting or not ST-segment elevation, were selected. Whole blood samples (4 mL, collected using EDTA) were collected between 30 days to one year after the procedure and patients in continuous or recent use of nitrate were excluded. A questionnaire was applied to the patients to document other comorbidities that could interfere with the results. Major comorbidities found in patients included Systemic Arterial Hypertension (SAH) (84.3%), dyslipidemia (59%) and Diabetes Mellitus (DM) (41%). Almost half of the group of patients had a positive family history for Coronary Artery Disease (CAD) (44.6%). 30.1% of the patients analyzed had previous ACS. A group 408 blood donors, exhibiting no previous ACS history was also studied.

DNA extraction

DNA extraction was performed using from the Illustra blood genomicPrep Mini Spin Kit (GE Healthcare, Buckinghamshire, UK).

Syntax score

Patients were evaluated according to the Syntax Score, an angiographic tool used to classify the complexity of coronary artery disease. This tool combines anatomical and prognostic variables, creating an accurate prediction of mortality to guide the treatment.

YT*1/YT*2 Genotyping

YT*1/YT*2 genotyping was performed by real-time PCR technique (Taqman SNP genotype assay: assay ID C_8786419_20; catalog 4351379, Applied Biosystems, Carlsbad, CA) using a pair of primers aligned with the genomic sequence of exon 2 of the *ACHE/YT gene* (located on chromosome 7q22) and a probe that recognizes codon 353 (CAC), in which the 1057C>A (rs1799805) transversion occurs.

Quantification of plasma NOx concentration-nitrate reductase assay

Due to the short half-life, NO quantification must be conducted

by measuring the concentration of its oxidation products, NO2-andNO3-, which are more stable, and present in plasma, erythrocytes and in various tissues. A combined measurement of NO2- and NO3- (NOx) was performed as an alternative to assess NO, using the Griess reagents, as previously reported (Giustarini et al. [28]).

Statistical analysis

Statistical analyses were performed using the R 3.6.0 (R Core Team, 2019) [29] and the MedCalc software [30], and the graphs were built using the ggplot2 package (WICKHAM, 2009). For the comparisons between two categorical variables with two categories (tables 2 × 2), the two tailed Fisher's exact test was used, and for the other cases, the Chi-Square test. A significance level of 5% was adopted for all cases.

The descriptive statistics for quantitative variables are given as mean and standard deviations or averages and confidence intervals of 95%. For qualitative variables, absolute and relative frequencies were considered.

For the logistic regression analysis between blood donors and patients, the Hosmer and Lemeshow model, 2000 was used, whose answer is the presence or absence of YT*1/YT*2 variable sites.

Results

The healthy controls were younger (34.2 ± 11.1 years) than ACS patients (60.7 ± 9.3 years (p<0.001), predominating males (69.6% *vs.* 30.45) (p<0.01). The frequency of the YT alleles taken as double (YT*1/YT*1and YT*2/YT*2homozygosis) and single doses (YT*1/YT*2 heterozygosis) did not differ between patients and controls (p=0.202). The stratification of patients and controls regarding the frequency of the YT*1/YT*2 variations considering age and gender (younger or older than 40 years) did not show differences, as well as the stratification between patients with and without the polymorphism (web appendix 1). In contrast, the stratification of patients according to ECG features (STE-ACS *vs.* NSTE-ACS) and the YT*1/YT*2 genotype showed that the mutant allele was increased in patients exhibiting STE-ACS when compared to NSTE-ACS (p=0.029), and an OR=4.61 (1.1646-18.2910). The mean NOx levels and the Syntax score were not significantly different between patients exhibiting or not YT*1/YT*2 genotype. No association was observed between NOx levels and the Syntax score (Table 1).

Discussion

This is a pilot exploratory study about the functional assessment of *ACHE* YT polymorphism (rs1799805) influencing the cholinergic anti-inflammatory pathway and its correlation with ACS. One of the purposes of the study was to characterize for the first time this polymorphism in the population with ACS, to serve as a basis for future research. No similar study has been found in the literature until now.

The frequency of the *ACHE* polymorphism in the control group in this study (7.8% YT*1/YT*2) is closely similar to that observed in other Brazilian studies, which reported frequencies ranging from 8.4% [31] to 9.18% [32] of the YT*1/YT*2 genotype. In the present study, the YT*2/YT*2 genotype was not found, possibly due to the low frequency if this genotype that is more frequent in the Jewish population [33]. Regarding the *ACHE* polymorphisms in ACS, only the rs2571598 variable site was overrepresented in Italian patients with coronary artery disease [OR1.76 (95% CI 1.17-2.65)] [27].

Although the present study did not demonstrate an increase in the incidence of ACS in the YT*1/YT*2 group, it was shown that the presence of the polymorphism was correlated with a more severe electrocardiographic presentation (STE-ACS YT*1/YT*2 50% *vs.* YT*1/YT*1 17, 8%-p-value= 0.029).This may partially be explained by the change in the function of polymorphic acetylcholinesterase which reduces the bioavailability of ACh, which has anti-inflammatory action, leading to increased production of macrophage pro-inflammatory cytokines, such as TNF-α, IL-1β and IL-6 [8,9]. This could cause an increase in platelet aggregation factors and total obstruction of the lumen of the coronary arteries.

Changes in the cholinergic anti-inflammatory pathway associated with *ACHE* polymorphisms could cause a reduction in the NO bioavailability (endothelial function marker), since the released NO would react with other radical species generated by inflammation [34]. This reaction generates inactive forms of NO, which do not have a vasodilating action, causing vascular obstruction. In this study, we observed a non-significant reduction in NOx in patients with the polymorphism [YT*1/YT*2 89.4 (68.0; 110.8) *vs.* YT*1/YT*1 105.5 (88.3; 122.8)], generating further evidence that the *ACHE* polymorphism may be related to higher rates of ACh degradation. Further studies should be performed with more invasive and accurate methods to measure the relation between *ACHE* polymorphism and endothelial dysfunction, such as intracoronary infusion of acetylcholine [35].

Despite the presence of the *ACHE* polymorphisms associated with a higher incidence of STE-ACS, this study showed no relationship between polymorphism and the Syntax score (p=0.743). The literature reports that the presence of high Syntax score is associated with great complexity of coronary atherosclerotic disease, worsening endothelial dysfunction. The present study did not demonstrate a relationship

Table 1: Frequency of the YT*1/YT*2 genotype in patients stratified according to: i) The presence (STE-ACS) or not (NSTE-ACS) of the ST-segment elevation in acute coronary syndrome, ii) The clinical diagnosis (ST-segment elevation acute myocardial infarction: STE-AMI, non-ST segment elevation acute myocardial infarction: NSTE-AMI, or unstable angina), iii) NOx levels, and iv) the Syntax score.

		Genotype		Total	p-value
Diagnosis		**YT*1/YT*1**	**YT*1/YT*2**		
ECG	STE-ACS	13/73 (17.8%)	5/10 (50.0%)	18/83 (21.7%)	**0.029***
	NSTE-ACS	60/73 (82.2%)	5/10 (50.0%)	65/83 (78.3%)	
Clinical Diagnosis	STE-AMI	13/73 (17.8%)	5/10 (50.0%)	18/83 (21.7%)	0.068
	NSTE-AMI	47/73 (64.4%)	4/10 (40.0%)	51/83 (61.4%)	
	Unstable angina	13/73 (17.8%)	1/10 (10.0%)	14/83 (16.9%)	
NOx (µmol/L)		105.5 (88.3; 122.8)	89.4 (68.0; 110.8)	103.6 (88.3; 118.)	0.24
Score Syntax		12.3 (9.7; 14.9).	11.9 (6.3; 17.6)	12.2 (9.9; 14.6)	0.46
	Mild	63/73 (86.3%)	9/10 (90.0%)	72/83 (86.7%)	0.743
	Moderate	6/73 (8.2%)	1/10 (10.0%)	7/83 (8.4%)	
	Severe	4/73 (5.5%)	0/10 (0.0%)	4/83 (4.8%)	

*Odds Ratio (OR) = 4.61 (1.1646-18.2910)

between the polymorphism and the occurrence of a more complex atherosclerotic disease. Notably, most patients in the study had an angiographic profile of mild complexity.

The major limitations of the present study are related to i) the limited number of ACS patients, since samples could only be collected 30 days after the acute event in the absence of a recent use of vasodilator drugs derived from nitrate, and ii) the unavailability of the *ACHE* activity measurement.

Conclusion

The *ACHE*/*YT* (rs1799805) polymorphism was reported for the first time in association with ACS. Patients admitted to the emergency room presenting the YT*1/YT*2 *ACHE* genotype exhibited increased frequency of the ST-segment elevation, which is associated with a worse prognosis.

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