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The Branched-chain Amino Acid Transaminase 1 -23C/G Polymorphism Confers Protection Against Acute Coronary Syndrome

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ABSTRACT

Background: Previous studies have shown an association between polymorphisms of the *BAT1-NF-κB* inhibitor-like-1 *(NFKBIL1)-LTA* genomic region and susceptibility to myocardial infarction and acute coronary syndrome (ACS). **Objective:** The objective of the study was to study the role of three polymorphisms in the *BAT1, NFKBIL1,* and *LTA* genes on the susceptibility or protection against ACS; we included a group of cases-controls from Central Mexico. **Methods:** The *BAT1* rs2239527C/G, *NFKBIL1* rs2071592T/A, and *LTA* rs1800683G/A polymorphisms were genotyped using a 5' TaqMan assay in a group of 625 patients with ACS and 617 healthy controls. **Results:** Under a recessive model, the *BAT1* -23C/G (rs2239527) polymorphism showed an association with protection against ACS (odds ratio = 0.56, and p-corrected = 0.019). In contrast, the genotype and allele frequencies of the *NFKBIL1* rs2071592T/A and *LTA* rs1800683G/A polymorphisms were similar between ACS patients and controls and no association was identified. **Conclusion:** Our data suggest an association between the *BAT1* -23C/G polymorphism and protection against ACS in Mexican patients. (REV INVEST CLIN. 2020;72(1):19-24)

Key words: Acute coronary syndrome. BAT. Single-nucleotide polymorphism.

INTRODUCTION

Atherosclerosis is a complex chronic disease of the arterial wall with multifactorial etiology and is the etiological basis of most cardiovascular events, including coronary artery disease (CAD)[1,2]. Acute coronary syndrome (ACS) comprises a spectrum of obstructive CAD which most commonly arises from plaque rupture and/or erosion, leaving the vulnerable lipid-rich core exposed to the circulation, resulting in activation of platelets, and the coagulation cascade leading to acute thrombotic occlusion. There are three major subtypes of ACS: unstable angina, non-ST-elevation myocardial infarction (NSTEMI), and STEMI. Each

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subtype represents a different stage in the spectrum of disease. Plaque erosion with subendocardial ischemia represents the majority of cases of unstable angina and NSTEMI, while plaque rupture and complete thrombotic occlusion, of an epicardial coronary, with associated transmural infarction, are characteristic of STEMI[3,4]. A growing body of evidence suggests that the genetic component is an important risk factor for ACS. In this way, some studies have identified an association between single-nucleotide polymorphisms (SNPs) located in *branched-chain amino acid transaminase 1 (BAT1),* NF-κB inhibitor-like-1 *(NFKBIL1)* and/or *lymphotoxin alpha (LTA)* and MI or CAD[5-8]. A first study conducted in Japanese population identified an association between various BAT1, NFKBIL1 and LTA *single nucleotide polymorphisms* (SNPs) polymorphisms, and MI susceptibility. For example, the *BAT1* -23G/C (rs2239527), *NFKBIL1* -63T/A (rs2071592), and *LTA* -162G/A (rs1800683 or 10G/A) SNPs showed similar odds ratio (OR) (1.6), 95% confidence interval (CI) (1.25-2.03), and p-value (≈ 0.0004) because they were in high linkage disequilibrium (LD)[5]. In addition, the *LTA* -162G/A also showed an association with CAD susceptibility (OR 1.2, 95% CI: 1.00-1.50, and p = 0.047) in Chinese population[8]. However, other studies have not replicated these findings[9-12]. The *BAT1, NFKBIL1,* and *LTA* genes are located in the 6p21.3 region. *BAT1* encodes a nuclear protein is called HLA-B-associated transcript 1, which negatively regulates the expression of interleukin (IL)-1, tumor necrosis factor (TNF), and IL-6[13,14]; *NFKBIL1* encodes a protein homologous to IκB (κB inhibitors), which regulates the expression of nuclear factor-kappa B transcription factor, TNF, IL-1, and IL-6[15]; and *LTA* encodes a protein involved in the inflammatory and immunological response[16]. *LTA* binds to their cognates, TNF receptors (TNFR1) and TNFR2, and regulates the expression of anti-apoptotic proteins to prevent cell death, inflammatory response, and cell differentiation[17]. Thus, these three proteins are involved in various inflammatory processes.

Given the inconsistencies of genetic association between *BAT1, NFKBIL1* or *LTA,* and ACS or MI, our study aimed to determine whether the *BAT1* -23C/G, *NFKBIL1* -63T/A, and *LTA* -162G/A polymorphisms (the choice of these variants in these three genes was due to the almost complete LD [≈r^2] among them) confer risk or protection against ACS in Mexican patients.

MATERIAL AND METHODS

Subjects

All subjects included in this study were ethnically matched and were considered Mexican Mestizo only those individuals whose families had been born in Mexico for three generations, including their own. A Mexican Mestizo is defined as someone born in Mexico, who is a descendant of the original autochthonous inhabitants of the region, and of individuals of Caucasian (predominantly Spaniards) and/or African origin, who came to the American continent during the 16th century. The ethnical characteristics of the studied groups were demonstrated analyzing 265 ancestry informative markers (AIMs). The results showed a similar background between patients and controls. Patients showed 55.8%, 34.4%, and 9.8% of Amerindian, Caucasian, and African ancestry, respectively, whereas controls showed 54.1%, 35.8%, and 10.1% of Amerindian, Caucasian, and African ancestry, respectively. Our study included 1242 Mexican Mestizos, 625 (501 men and 124 women, with a mean age of 58.2 ± 10.4 years) patients with ACS and 617 (468 men and 149 women, with a mean age of 54.01 ± 7.69 years) controls (individuals who had no history of ACS or CAD, symptoms or previous diagnosis of cardiovascular disease). Five hundred and two patients presented MI, and 123 had unstable angina. The ACS patients were diagnosed according to the World Health Organization and the American Heart Association/American College of Cardiology criteria[18,19]. Both cases and controls were referred from the Instituto Nacional de Cardiología (INC) Ignacio Chávez, Mexico City. All the patients and controls provided written informed consent. Our study was approved by the Ethics and Research Committee of INC.

DNA extraction

We used the DNA extraction method proposed by Lahiri and Nurnberger[20].

Genetic analysis

The *BAT1* -23C/G (rs2239527), *NFKBIL1* -63T/A (rs2071592), and *LTA* -162G/A (rs1800683) SNPs were genotyped using a TaqMan SNP genotyping

Table 1. Baseline clinical characteristics of the studied individuals

Parameter	β-coefficient	OR	p-value
Clinical characteristics	ACS patients (n[%]) (n=625)	Healthy controls (n[%]) (n=617)	p-value
Men*	501 (80)	468 (76)	NS
High blood pressure (mmHg)	265 (42)	82 (13)	<0.001
Type 2 diabetes mellitus*	189 (42)	63 (14)	<0.001
Dyslipidemia*	288 (46)	216 (35)	<0.001
Smoking*	224 (36)	141 (23)	<0.001
Alcohol*	139 (22)	441 (71)	<0.001
	Median (percentile 25-75)	Median (percentile 25-75)	
Age (years)	58 (51-65)	54 (49-59)	0.01
BMI (kg/m^2)	27.2 (24.7-29.3)	28.1 (25.4-30.5)	0.01

*(n[%]) Number and proportion of subjects with the clinical characteristic in both groups.

assay on a 7900HT fast real-time polymerase chain reaction system according to manufacturer's instructions (Applied Biosystems, Foster City, USA). Thermo cycling conditions were as follows: initial denaturation at 95°C for 10 min (1 cycle) followed by 40 cycles at 95°C for 15 sec (denaturation) and at 60°C for 1 min (annealing/extension). Sequenced samples of different genotypes were included as positive controls.

Statistical analysis

The Mann–Whitney U-test was used to compare continuous variables between cases and controls. For categorical variables, we used Chi-square or Fisher's exact tests. We analyzed the association of the three polymorphisms with ACS by logistic regression and under the codominant, dominant, recessive, overdominant, and additive genetic models. Multiple logistic models were constructed to identify the variables associated with ACS. $p < 0.05$ was considered statistically significant. The haplotypes and LD of the *BAT1* -23C/G, *NFKBIL1* -63T/A, and *LTA* -162G/A polymorphisms were obtained using the Haploview program (V 4.1, Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge, MA, USA). ADMIXTURE software was used to evaluate 265 AIMs in our study population. Hardy-Weinberg equilibrium (HWE) was obtained using Finnet software (https://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Statistical data analysis was performed with SPSS version 18.0 (SPSS, Chicago, Illinois) statistical package.

RESULTS

Characteristics of the study population

Baseline characteristics of the ACS patients and controls included in our study are shown in Table 1. As expected, ACS patients presented a higher frequency of type 2 diabetes (T2D), high blood pressure, dyslipidemia, and smoking habit, and a lower frequency of alcohol habit than controls.

Genotype frequencies and association analysis

Observed frequencies of the *BAT1* -23C/G, *NFKBIL1* -63T/A, and *LTA* -162G/A polymorphisms were in HWE for both cases and controls ($p > 0.05$). The distribution of the allele and genotype frequencies of *NFKBIL1* -63T/A, and *LTA* -162G/A was similar in cases and controls, and no association was detected (Table 2). Nonetheless, under the genetic recessive model adjusted by gender, age, body mass index (BMI), high blood pressure, T2D, dyslipidemia, alcohol

Table 2. Association of the *BAT1*, *NFKBIL1*, and *LTA* SNPs with ACS

Gene and single nucleotide polymorphism	Genotype frequency n (%)			MAF	Model	OR (95%CI)	pC
				***BAT1* -23 C/G (rs2239527)**			
Control	***CC***	***CG***	***GG***				
(n=617)	272 (44.1)	266 (43.1)	79 (12.8)	0.34	Codominant	0.54 (0.33-0.91)	0.06
					Dominant	0.84 (0.62-1.15)	0.27
					Recessive	0.56 (0.35-0.91)	**0.019**
ACS	283 (45.2)	281 (45.0)	61 (9.8)	0.32	Heterozygous	1.06 (0.79-1.44)	0.69
(n=625)					Log-additive	0.80 (0.64-1.00)	0.05
				***NFKBIL1* -63 T/A (rs2071592)**			
Control	***TT***	***TA***	***AA***				
(n=617)	268 (43.4)	275 (44.6)	74 (12.0)	0.34	Codominant	0.68 (0.41-1.13)	0.33
					Dominant	0.87 (0.64-1.19)	0.38
					Recessive	0.71 (0.44-1.14)	0.16
ACS	282 (45.1)	277 (44.3)	66 (10.6)	0.33	Heterozygous	1.01 (0.74-1.36)	0.97
(n=625)					Log-additive	0.86 (0.68-1.08)	0.18
				***LTA* -162 G/A (rs1800683)**			
Control	***GG***	***GA***	***AA***				
(n=617)	260 (42.1)	274 (44.4)	83 (13.5)	0.36	Codominant	0.62 (0.38-1.01)	0.15
					Dominant	0.83 (0.61-1.13)	0.25
					Recessive	0.65 (0.41-1.03)	0.06
ACS	279 (44.6)	276 (44.2)	70 (11.2)	0.33	Heterozygous	1.01 (0.75-1.37)	0.94
(n=625)					Log-additive	0.82 (0.65-1.03)	0.81

ACS: Acute Coronary Syndrome, MAF: minor allele frequency, OR: odds ratio, CI: confidence interval, *p*C: p-corrected. The p-values were calculated from logistic regression analysis, and the ORs were adjusted for gender, age, body index mass (BMI), high blood pressure, type 2 diabetes, dyslipidemia, alcohol consumption, and smoking. Bold numbers indicate significant associations.

consumption, and smoking habit, the *BAT1* -23C/G polymorphism showed an association with protection against ACS (OR = 0.56, 95% CI = 0.35-0.91, and p-corrected = 0.019) (Table 2).

LD analysis

The alleles of the *BAT1* -23C/G, *NFKBIL1* -63T/A, and *LTA* -162G/A polymorphisms showed a high LD ($r^2 \approx 0.9$, data not shown). Meanwhile, the distribution of haplotypes was similar between cases and controls, and no association was identified with this inflammatory disease (Table 3).

DISCUSSION

We analyzed three SNPs located in the *BAT1, NFKBIL1,* and *LTA* genes in a group of ACS patients and controls. In the literature, association studies between *BAT1* -23C/G, *NFKBIL1* -63T/A, and *LTA* -162G/A and susceptibility or protection to different cardiovascular diseases are scarce and controversial[5-12,21]. For example, two previous studies showed an association with susceptibility between the *LTA* -162G/A and A252G polymorphisms and MI in Japanese population[5,6]. However, other studies (where only *LTA* A252G was evaluated) conducted in the same

Table 3. Haplotype (*BAT1* -23C/G, *NFKBIL1* -63 T/A, and *LTA* -162 G/A) frequencies (%) in ACS patients and healthy controls

Combination of alleles of the three single nucleotide polymorphisms	ACS (n=625)	Controls (n=617)	Odds ratio	95% Confidence interval	p-value
Haplotype	Hf	Hf			
H1 (CTC)	65.3	62.9	1.10	0.94-1.30	0.21
H2 (GAA)	30.6	32.5	0.91	0.77-1.08	0.29
H3 (CAA)	1.4	1.1	1.29	0.62-2.67	0.48

The order of the polymorphisms in the haplotypes is according to the positions in the chromosome (rs2239527, rs2071592, and rs1800683). Hf: haplotype frequency, ACS: Acute Coronary Syndrome.

population did not replicate this finding[10,22]. In addition, other studies in European-derived populations (patients from Germany and UK), the *LTA* A252G SNP was not associated with MI susceptibility[9,11]. Contrary to this finding, in another group of ACS patients from Germany, the TCGATCAGA haplotype carrying the *BAT* -23G, *NFKBIL1* -63A, and *LTA* -162A minor alleles (underlined alleles, respectively) showed an association with protection against MI[7]. Regarding ACS, as far as we know, only one study has been published, and in that report, the authors did not identify any association between *LTA* A252G and ACS (although a trend toward an association with protection was identified; p = 0.06)[12]. Similar to those results, we did not identify any association between this variant and ACS. On the other hand, as far as we know, the *NFKBIL1* -63T/A polymorphism has not been evaluated in ACS patients. Our data suggest that this variant is not a risk or protection factor for ACS in the Mexican population. In contrast, we identified an association between the *BAT1* -23GG genotype and protection against ACS under the recessive genetic model. As far as we know, this is one of the few studies that describe the association of this polymorphism with protection against ACS. In line with our results, Koch et al. reported that the *BAT1* -23GG genotype conferred protection (OR = 0.78) against MI in a German population[7]. In addition, Gnjec et al. reported that the -23 *GG* genotype of *BAT1* -23 C/G was associated with reduced risk of Alzheimer's disease in Caucasian population (OR = 0.43)[23]. On the other hand, Mendonça et al. reported in patients infected with *Plasmodium vivax* that the -23 *G* allele was associated with reduced clinical manifestations of malaria in Brazilian populations[14].

A functional study showed that the *BAT1* -23G minor allele (we identified an association between the *BAT1* -23GG genotype and protection against ACS) affects the binding of OCT1 (a transcription factor) suggesting a biological role of this allele on the *BAT1* expression[24]. That same study showed that the Ying Yang 1 (YY1) transcription factor might bind indirectly with the *BAT1* – 23G allele[24]. OCT1 is ubiquitously expressed in various tissues and cells and can positively or negatively regulate the expression of different genes involved in inflammatory process[25], while YY1 suppresses or activates the expression of several genes depending on the features of the promoter or cells[24]. A previous study reported by Mordvinov et al. showed that the OCT1/YY1 complex is involved in the negative regulation of IL-5 expression in human T cells[26]; it is possible that this complex leads to a decrease in the expression of other pro-inflammatory cytokine genes.

We recognize that our work has limitations, such as the fact that we studied only one polymorphism in each gene *(BAT1, NFKBIL1,* and *LAT).* In addition, the different ancestry of the populations may have biased the association (or no associations) observed between *BAT1* -23C/G and ACS. Thus, additional studies in other populations are necessary to understand the role of this variant in ACS. In summary, our data suggest that the *NFKBIL1* -63T/A and *LTA* -162G/A polymorphisms are not risk or protection factors for ACS, while *BAT1* -23C/G is associated with protection against ACS in a sample from Mexico.

Finally, our data suggests that BAT1 -23C/G is a protection factor for ACS in Mexican patients.

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