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Protective role of DDAH2 (rs805304) gene polymorphism in patients with myocardial infarction

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ABSTRACT

The purpose of the present study was to establish the role of *DDAH* gene polymorphisms in the risk of developing myocardial infarction (MI) in a clinical cohort of Mexican patients. One polymorphism (rs1498373) in the *DDAH1* and three in the *DDAH2* (rs805304, rs3131383, and rs805305) genes were performed by TaqMan genotyping assays in 473 patients with MI and 447 healthy unrelated controls. Similar distribution of *DDAH1* and *DDAH2* polymorphisms was observed in MI patients and healthy controls. Under a recessive model adjusted for age, gender, and obesity, the rs805304 *C* allele was associated with decreased risk of MI (OR = 0.70, 95% CI = 0.51–0.96, $P = 0.030$). The effect of the polymorphisms on various cardiovascular risk factors was analyzed. Under a recessive model adjusted for age and gender, the *DDAH2* rs805304 *C* allele was associated with decreased risk of obesity (OR = 0.35, 95% CI = 0.22–0.57, $P = 0.001$). The three *DDAH2* polymorphisms were in strong linkage disequilibrium. Our results suggest that the rs805304 *C* allele was associated with decreased risk of MI and decreased risk of obesity.

1. Introduction

Coronary artery disease (CAD), the main contributor to cardiovascular disease, is the leading cause of death. It is well known that blood flow regulates many molecular pathways in endothelial cells (EC) that can produce changes in the cellular functionality, such as apoptosis, migration, proliferation, gene and protein expression, secretion, and interaction with other cells (Ando and Yamamoto, 2009; Heo et al., 2011). In particular, nitric oxide (NO) plays an important role in the upholding of vascular homeostasis. Also, it is a very important element to form an adequate protection to the blood vessel when atherogenic processes cause a lesion. These include oxidative modification of low density lipoprotein, monocyte adhesion, platelet aggregation and proliferation of smooth muscle cells (Dimmeler et al., 1999; Wang et al., 1999). In 1992, it was reported that asymmetric dimethylarginine (ADMA) is a naturally occurring endogenous nitric oxide synthase inhibitor (Vallance et al., 1992). Increased plasma levels of ADMA have been associated with type 2 diabetes mellitus (Abbasi et al., 2001), hypercholesterolemia (Böger et al., 2000) and chronic kidney diseases (Fleck et al., 2003). Another enzyme, dimethylarginine dimethylaminohydrolase (DDAH), takes care of the depuration of ADMA. This enzyme has two isoforms (DDAH1 and DDAH2) (Leiper et al., 1999) and they are each encoded by genes positioned on chromosomes 1p22 and 6p21.3, respectively. The reduced activity of DDAH due to oxidative stress may lead to high concentration of ADMA and low level of NO in serum, thus contributing to the pathogenesis of MI (Gray et al., 2010; Stühlinger et al., 2007; Valkonen et al., 2005). High levels of circulating ADMA may have a strong influence on endothelial dysfunction. This condition is respectively linked with different cardiovascular diseases (Böger, 2003; Khalifa et al., 2012; Kim et al., 2008; Puchau et al., 2009; Sibal et al., 2010).

In the last decade, numerous studies have focused on the role of genetic predisposition in the progress of this disease. Polymorphisms in the genes that encode DDAH1 and DDAH2 have been studied in cardiovascular diseases. The results, however, have been contradictory,

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bringing up both positive and negative linkage (Achan et al., 2003; Ding et al., 2010; Gad et al., 2011; Puchau et al., 2009).

The aim of the present study was to establish the role of the DDAH gene polymorphisms in the risk of developing MI in a clinical cohort of Mexican Mestizo patients.

2. Materials and methods

2.1. Subjects

All participants provided written informed consent. The study complies with the Declaration of Helsinki and was approved by the Ethics Committee of the Instituto Nacional de Cardiología "Ignacio Chávez" (INCICH).

The study included 473 Mexican Mestizo patients with MI (283 men and 190 women, mean age 58 ± 6.23 years). MI was defined by angina symptoms with an elevation of 1 mm or more of the ST-segment as well as the development of a Q wave in two or more contiguous electrocardiographic records, and/or thrice the upper limit of serum creatine phosphokinase (CPK) MB isoenzyme (normal value = 0.6–6.3 ng/mL) in at least one sample of peripheral blood.

A group of 447 healthy individuals (259 men and 188 women, mean age 58 ± 8.71 years) that did not have any angina symptoms or have had a previous diagnosis of cardiovascular or systemic disease were used as control group. They were completely unrelated to the cases studied.

Anthropometric measures were recorded and traditional risk factors were questioned in all cases and controls who met the inclusion criteria.

We used clinical criteria in order to find those patients who had type 2 diabetes, established by either a serum glucose of 126 mg/dL or higher in two samples, a previously made diagnosis, or if they were receiving hypoglycemic treatment and/or insulin. Hypertensive patients were established by a previous diagnosis or if they were receiving antihypertensive therapy. Dyslipidemia was correspondingly defined as total cholesterol of 200 mg/dL or higher, low-density lipoprotein cholesterol ≥ 130 mg/dL or serum triglycerides of 150 mg/dL or higher. Active smokers were identified by smoking habits of 5 or more tobacco cigarettes a day or had suspended it for less than a year. Alcoholic patients were defined by a consumption of more than 6 g of alcohol every day. Body mass index was calculated by a standard formula (weight in kg / height in m^2).

Selective coronary angiography was performed by femoral access in all patients. The degree of CAD was estimated based on the number of coronary arteries with stenotic lesions. They were classified as one, two or three-vessel disease. Three-vessel meant significant stenosis in three coronary vessels; two-vessel meant significant stenosis in two coronary vessels, or a narrowing of 50% or more in the left main trunk; and involvement of one vessel was only considered hemodynamically significant with more than a 50% reduction in the diameter of an important coronary vessel such as the left anterior descending artery, circumflex artery or right coronary artery.

All subjects (patients and controls) included in the study were ethnically matched. The definition of Mexican Mestizo was established as someone who was born in Mexico, who is a descendant of the mixture of native inhabitants of the region, and of individuals of Caucasian or African origin, and who came to America during the colonization period. We therefore considered those individuals who were at least the third generation born in Mexico as Mexican Mestizos.

2.2. DNA extraction

Genomic DNA from whole blood containing EDTA was isolated by standard techniques (Lahiri and Nurnberger, 1991).

2.3. Genotyping of DDAH1 and DDAH2

The four polymorphisms of *DDAH1* and *DDAH2* genes were genotyped using 5′ exonuclease TaqMan genotyping assays on an ABI prism 7900HT Fast Real-Time PCR system (Applied Biosystems, Foster City, USA) (Table 1). The PCR reaction was performed in a 384-well plate. Each well received the following: 4 μL of genomic DNA, 5 μL of SNP reaction mixture consisting of TaqMan Universal PCR Master Mix, 0.125 μL 40× working stock of rs1498373 (assay ID: C___8864200_10), rs805305 (assay ID: C___3233673_10), rs805304 (assay ID: C___3233671_10), and rs3131383 (assay ID: C__27462642_20) and 0.7 μL DNase-free water. Universal amplification conditions: Initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 seg and annealing/extension at 60 °C for 1 min. The results were analyzed in allelic discrimination software. The Primer sequence for TaqMan assays are:

rs1498373: CACACTTTCAAACAGAAGGAAGGCA[A/G]TGTGGTAGGA TACAAGCTTGGAACT

rs805305: CCGCAGGGACTGGAAGTCCAGCCCG[C/G]GACCCGCAGG GGTTATGGGACAGAA

rs805304: CACGCCCATTCCGCCCTGCTAAGCC[G/T]CGCCCATTACAT CCAGACTGCGCCC

rs3131383: AGCTCCTCCAGCTCGGTCCTCTCCC[G/T]GGCTGGATCAG AGAGCCGCTGACTC.

The rs805304 genotypes were confirmed by sequencing PCR products using the following primers: forward: 5′-GCG CTG TTC TGA GGT CTA CGA A-3′ and reverse: 5′CCT TGT GTA GGC GAG CTC ATA TA-3′ (Gad et al., 2011). Sequences were performed by the dideoxynucleotide chain termination method and were determined using ABI PRISM 3130 analyzers (Applied Biosystems, Foster City, USA).

2.4. Statistical analysis

Allele and genotype frequencies of the four studied polymorphisms were estimated using direct counting. Hardy–Weinberg equilibrium (HWE) was calculated for each polymorphism using the chi-squared test. Statistical analysis was carried out with Stata 10.0 for Windows software and SNPStats. If the exploratory analysis showed that numerical data had a normal distribution (*Gaussian distribution*) (*test of normality* Shapiro Wilk's test, $P > 0.05$), comparison between the study groups was performed using ANOVA test. Data are presented with mean ± SD. Categorical variables were analyzed with Pearson's chi-square test and presented as absolute frequencies and proportions. Statistical significance was established as $P < 0.05$.

Logistic regression analysis was used to test for associations of polymorphisms with MI and cardiovascular risk factors. For the construction of inheritance models using logistic regression models, adjusting for age, gender, and obesity. To address multiple testing, Bonferroni correction was used considering three comparisons. According to the number of copies needed in order to modify the risk, there are five inheritance models we can define: Co-dominant, dominant, recessive, heterozygous advantage and additive model. For codominant inheritance, genotypes were coded as 1 [heterozygote; (He)], 2 [homozygote for the minor

Table 1
DDAH gene polymorphisms tested.

Gene	SNP position[a]	dbSNP[b]	Chr position (pb)	Location in gene
DDAH1	85324950 C/T	rs1498373	85563221	Intron 4
DDAH2	−449 C/G	rs805305	31805366	Promoter
	−1151 A/C	rs805304	31806067	Promoter
	−871 A/C	Rs3131383	31812273	Promoter

Order of the polymorphisms is according to the chromosomal positions.
[a] Given name according to NCBI.
[b] SNP ID in database dbSNP.