Original Article

Association between the Xba I polymorphism of APOB gene and plasma lipid level in Mexican patients with coronary artery disease

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INTRODUCTION

The constituent lipids of the human body are mainly phospholipids, cholesterol, triglycerides (TG) and cholesteryl esters; they are transported through blood forming lipoprotein complexes of lipids and one or more proteins, called apolipoproteins, and are under a continuous synthesis/degradation turnover. Low density lipoprotein (LDL) is the product of metabolism of very low density lipoprotein (VLDL), and LDL is built by 75% lipid (cholesterol esters and cholesterol) and remaining 25% protein. Increased LDL levels are associated with cardiovascular disease.¹ It is known that dyslipidemia results from interactions between genetics and environmental (particularity diet, exercise and tobacco smoking) factors, in different populations. The biological, genetics and diet risk factors, may explain the differences in the coronary artery disease (CAD) risk in several populations. It has been observed that there exist factors in the diet that can be altering the lipids concentrations such as: high concentration of lipids, low ingest of vegetables and fruits.²

APOB is essential in the maintenance of cholesterol homeostasis, and is the major protein element of LDL. It serves as the ligand for the recognition and catabolism of plasma LDL by LDL-receptor.³ Elevated levels of serum APOB are associated with an increased risk of premature atherosclerosis.⁴ APOB circulates in two distinct forms (apoB100 and apoB48), encoded by a single gene.

Some studies, that consider polymorphisms of the apolipoprotein B (APOB) gene as risk factors for coronary artery disease (CAD), have reported discordant results. The aim of the present study was to search for associations between plasma lipid profiles with the DNA Xba I polymorphism of the APOB gene in CAD patients diagnosed by angiography (CAD+). In the present study we compared 114 Mexican patients (80 men and 34 women) with CAD+ and 132 control patients (59 men and 73 women) without evidence of ischemia or arterial damage (CAD-).

The frequency of X+/X+ genotype of Xba I polymorphism, in CAD+ group, was 23% (26/114) compared with 8% (11/132) in the CAD- (OR 3.25, p = 0.002). The patients with X+/X+ for the Xba I genotype APOB gene had higher concentration of triglycerides (TG) and VLDL in plasma than CAD- (p < 0.05). The genotype X+/X+ in the CAD had an effect increasing the TG and VLDL plasma levels when compared with individuals with X-/X- and X-/X+ genotypes. The present study indicated that the X+X+ genotype of Xba I polymorphism is associated with CAD+ patients and high plasma levels of TG and VLDL, in the Mexican population.

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There are polemical studies that have reported the allele, and conversely its absence determines the glutamine to a UAA (stop) codon in bowel by a novel posttranscriptional RNA editing a CAA allele lacking the C- terminal domain of apoB100. ApoB48 lacks the C-terminal domain of apoB100. As a result, it does not bind to the LDL-receptor. Different polymorphisms in the APOB gene have been identified, and some of them have been associated with total cholesterol, LDL cholesterol, high density cholesterol (HDL) and VLDL cholesterol levels.

The smaller form, apoB48, is produced from the small antithymine, which does not change the amino sequence, located within exon 26 of the gene, at the 2,488th nucleotide (ACC_ACT). The substitution of cytosine by thymine, which does not change the amino sequence, creates an Xba I restriction site that characterizes the X+ allele, and conversely its absence determines the X- allele. There are polemical studies that have reported the allele lacking the Xba I site (X-) and/or its homozygous genotype (X-/X-) as more frequent in survivors of myocardial infarction and in patients with CAD than in controls (12). However other reports correlated the allele X+ with CAD patients. In the present study, the APOB alleles detected with the Xba I restriction enzymes were examined for association with CAD by evaluating their frequency distributions in patients with CAD (CAD+) and compared to patients with the confirmed absence of this disease (CAD-).

MATERIAL AND METHODS

Study population

A total of 114 (80 men and 34 women) CAD+ patients and 132 control patients (59 men and 73 women) without ischemia or arterial damage (CAD-) were diagnosed by coronary angiography at the Servicio de Cardiología, Hospital de Especialidades, Centro Médico Nacional de Occidente (CMNO), Instituto Mexicano del Seguro Social (IMSS), Guadalajara, Jalisco; México.

The CAD+ group comprised patients with recurrent acute ischemic syndrome and angiographically proven CAD (with at least one coronary stenosis with ≥50% of narrowing of the luminal diameter). The CAD- group comprised consecutive patients being catheterized for clinical reasons and presenting non-cyanogenic congenital cardiopathy or valvulopathies. All patients in the CAD- group had angiographically normal coronaries. All coronary angiograms were analyzed visually by two experienced interventional cardiologists according to conventional American Heart Association methods.

All participants in the study signed the consent form. This study was approved by the Ethics Committee of the Centro de Investigación Biomédica de Occidente, CMNO, IMSS, Guadalajara, Jalisco; México.

Whole blood (5 ml) was collected without anticoagulant and the serum was used for the determination of lipids and lipoproteins. The blood sample was taken after 12 hour of fasting. The levels of total cholesterol (TC) and TG were obtained by an enzymatic procedure (Roche Diagnostic GmbH, Mannheim, Germany) using a Cobas Mira S autoanalyzer (Roche). HDL-cholesterol (HDL) was measured by an enzymatic method (Roche) in the supernatant after precipitation with phosphotungstate-MgCl2 (Roche). LDL-C (LDL) levels were estimated by the method of Friedewald et al.15

Determination of DNA polymorphism

Genomic DNA was extracted from peripheral blood samples (4ml with EDTA anticoagulant) according to standard protocols.16 The presence of X- allele in exon 26 of the APOB gene was determined by PCR amplification in a total volume of 15 μL containing 200 μM dNTPs, 1 pmol of primers, 1.5 mM MgCl2, and 2.5 U Taq polymerase. The primers were 5'-GGGACTATTCAGAAGCTAA-3' and 5'-TCAGTCAGAAGTCCGAGAAG-3'. These primers amplify the exon 26 of the human apoB gene, yielding a 710-bp fragment. Cycling conditions consisted of an initial melting temperature of 94°C (4 min), followed by 35 cycles of melting (94°C, 1 min), annealing (58°C, 3 min), and extension (72°C, 3 min).

The amplified product was subjected to restriction enzyme analysis with Xba I (New England Biolabs, Beverly, MA), according to the manufacturer’s instructions. The PCR products were separated by electrophoresis using 6% polyacrylamide gels (29:1), followed by silver staining. The wild-type allele (X-) produced a single 710-bp fragment, while the mutant allele (X+) produced 433-bp and 277-bp fragments.

Statistical analysis

Differences in means values between CAD patients and control group were analyzed by Student t-test and present as means ± standard deviation (SD), chi-square test was used to compare discrete variables. Allelic frequencies were calculated by gene counting. Comparison of observed and expected genotypes was analyzed by Hardy-Weinberg equilibrium. The means and standard deviations of plasma level lipids were analyzed by parametric analysis, to determine differences using an independent sample t-test and a two-way ANOVA. The odds ratio (OR) was calculated as a measure of relative risk. A multivariate logistic regression was performed considering CAD as dependent variable and the following independent variables: Xba I genotypes, gender, hypertension, tobacco consumption, TC, and TG. For this analysis numbers were assigned to the variables (CAD- = 0, CAD+ = 1; female = 0, male = 1; absence of hypertension = 0, presence = 1; absence of tobacco consumption = 0, presence = 1). The Xba I genotypes were classified as X+/X+ (=1) and other genotypes as 0. TC and TG were classified as 0 when plasma levels were below to 200 mg/dL and 150 mg/dL, respectively, and as 1 when plasma levels were equal or over to 200 mg/dL for TC and 150 mg/dL for TG. HDL was classified as 1 when plasma levels were below 35 mg/dL, and as 0 when they were equal or over 35 mg/dL. LDL was classified as 0 when plasma levels were below 100 mg/dL, and as 1 when plasma levels were equal or over 100 mg/dL. VLDL was classified as 0 when plasma levels were below 26 mg/dL, and as 1 when levels were equal or over. A p-value of less than 0.05 is consid-
considered statistically significant. All tests were performed using the SPSS version 11 (Chicago IL) for windows.

RESULTS

The characteristics of the CAD+ and CAD- groups, stratified by gender are listed in Table 1. The two groups contained middle-aged participants [mean in male 64.0±9.5 and female 62.6±10.8 in CAD+ group (p=0.509), and 55.3±5.8 and female 54.8±5.8 in CAD- group (p=0.580)]. The tobacco smoking status was significantly different (p=0.035) in the CAD- group stratified by gender.

The APOB Xba I genotypes of the study groups are presented in Table 2. Xba I genotype frequency and their interaction with lipid levels in CAD+ and CAD- patients are shown.
shown in the Table 2(up). The frequency of genotype $X^+/X^+$ was 8% (11/132) among CAD-, and 23% (26/114) among the CAD+ group. The CAD+ patients showed a significantly higher frequency of $X^+/X^+$ genotype, with an OR of 3.25 (95% CI=1.44-7.4; $p=0.002$).

The CAD+ patients with the $X^+/X^+$ genotype had statistically significant higher TG (121±112), and VLDL (47.2±25.1), than CAD- (110±28.6 and 21.9±5.59 respectively) ($p<0.05$). When the $X-$/X- participants was compared between the study groups we observed statistical significance in TG, LDL and VLDL levels ($p<0.05$) among CAD+ and CAD- participants. We also observed significant differences in TG, LDL, HDL and VLDL levels ($p<0.05$) between CAD+ and CAD- participants with the $X^+/X^+$ genotype (Table 2(low)).

In the analysis of CAD+ and CAD- together with Xba I genotype association, we observed that, those individuals with $X^+/X^+$ genotype had higher levels of TG (182±106) and VLDL (39.6±24.1) than those individuals with genotypes $X-$X- and X-/X+ (140±77.3 and 28.0±15.3) respectively ($p<0.05$) (Table 3).

The multivariable logistic regression analysis which considered gender as the dependent variable in the CAD+ and CAD- groups, showed as risk factor de both LDL level in CAD+ group with OR of 3.39 (95%CI=1.23-8.8; $p=0.017$) and tobacco in CAD- group with OR of 2.43 (95%CI=1.1-5.4; $p=0.030$).

**DISCUSSION**

CAD is a multifactorial disease that may differ in each race or ethnic population. This way the prevalence of CAD vary widely among different population, and the frequencies of the APOB gene polymorphisms have been reported to vary among ethnic groups. Thus, we investigated the association of plasma lipid levels with polymorphism Xba I of the APOB gene in Mexican CAD patients. Tobacco consumption, the conventional risk factor, was significantly different in our patients with CAD+ compared with the CAD- group by gender ($p<0.05$).

APOB mutations might affect the plasma lipid responses or the plasma APOB concentrations during dietary modifications by altering APOB secretion, structural stability, affinity for the LDL receptor, or interactions of APOB-containing lipoproteins with other lipoproteins, cells or enzymes. The physiologic role of the APOB Xba I polymorphism in the codon 2488 in exon 26 is still unclear, but it has been observed that this polymorphism may be associated with the Ag system, as well as with serum APOB, cholesterol, and TG levels in different studies. The Ag systems are based on antigens a1/d, c/g, h/i, t/z, and x/y, which appeared to be products of five closely linked allele pairs. Different combinations of Ag system have been associated with serum lipid levels. It has also been observed that the changes in the polarity of the amino acids, in the protein structure and its relation to function, can influence the molecular function of the protein in lipid metabolism.

The polymorphism alters plasma lipid concentrations and LDL catabolism even though it does not alter the amino acid sequence. In our study, the genotype distribution of the CAD group showed association between the CAD+ group in comparison with the CAD- group ($p<0.05$). These data are in accordance with some previous studies where the $X^+$ allele was associated with CAD.

The lipid profile of the CAD+ group was more atherogenic than that of the CAD- group. It has been postulated that a high plasma TG concentration together with small dense LDL particles are associates with premature CAD, the atherogenic LDL particles originating from VLDL. An increase in total TG, a decrease in HDL cholesterol levels and an association of elevated total, LDL cholesterol and TG and low HDL cholesterol with the increasing extension of CAD have been reported. Lowering of VLDL cholesterol and TG has been reported to be associated with a slowing down of the progression of coronary atherosclerosis in young AMI survivors.

Our study confirms the earlier associations between the presences of the Xba I cutting site and the elevated TG levels, and part of the effect might be due to the slightly lower LDL catabolic rate in the individuals with the Xba I cutting site. It has been demonstrated that the Xba I polymorphism modifies dietary fat and cholesterol responses in individuals with the Xba I cutting site. This individuals are more responsive to a low-fat, low cholesterol diet than those lacking the cutting site.
may therefore serve an important function in regulating metabolic processes relating to lipid homeostasis, atherosclerosis, and hemostasis. 31

We would like to point out that in our study, we did not consider the nutritional status of the patients; however it is well known that the Mexican diet is rich in lipids and deficient in bran and vegetables.

In conclusion, the results of this study suggest that, at least in the Mexican population, the APOB Xba I polymorphism of the APOB gene is to be a useful marker for CAD patients.

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AUTHOR DECLARATIONS

The manuscript has been seen and approved by all of the authors. There is no conflict of interest with regards to this manuscript.

REFERENCES


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罹患冠心病的墨西哥患者之脂蛋白酶元 B 基因 Xba I 多型性與血脂濃度的相關性

有些研究認為脂蛋白酶元 B(APOB)的基因多型性為冠狀動脈疾病的危險因子，但其研究結果並不一致。而此研究的目的在於尋找出由血管攝影診斷有冠心病的患者，其脂蛋白酶元 B 基因上的 Xba I 多型性與血脂的相關性。在此研究中比較 114 位(80 位男性；34 位女性)罹患冠心病(CAD+)的墨西哥患者與 132 位(59 位男性；73 位女性)沒有心肌缺血及動脈損傷的控制組(CAD-)。在患有冠心病的組別，其 Xba I 多型性的基因型為 X+/X+ 的比例為 23%，而控制組為 8%(OR=3.25；p=0.002)。在 Xba I 基因型為 X+X+ 的 CAD+患者相較於控制組，有較高的血漿三酸甘油酯及極低密度脂蛋白(VLDL)(p<0.05)。當與基因型為 X- X- 或 X-X+ 的冠心病患者比較時，X+X+基因型的 CAD+患者，顯現較高的血漿三酸甘油酯及極低密度脂蛋白濃度。本研究指出在墨西哥族群中，Xba I 的基因型為 X+/X+ 者，與冠心病患者的高濃度血漿三酸甘油酯及極低密度脂蛋白具有相關。

關鍵字：脂蛋白酶元 B、膽固醇、基因多型性、血脂、墨西哥人