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UNIDAD ACADÉMICA DE CIENCIAS QUÍMICO BIOLÓGICAS

MAESTRÍA EN CIENCIAS BIOMÉDICAS

POLIMORFISMOS EN LOS GENES *APOE* Y *LDLR* Y SU RELACIÓN CON EL SÍNDROME METABÓLICO EN MUJERES GUERRERENSES.

T E S I S

QUE PARA OBTENER EL GRADO DE

MAESTRÍA EN CIENCIAS BIOMÉDICAS

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ESTE TRABAJO SE REALIZÓ EN EL LABORATORIO DE ENFERMEDADES CRÓNICO DEGENERATIVAS DE LA UNIDAD ACADÉMICA DE CIENCIAS QUÍMICO BIOLÓGICAS DE LA UNIVERSIDAD AUTÓNOMA DE GUERRERO EN CHILPANCINGO, GUERRERO, MÉXICO.

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Correlation of ApoE4 with rs688 SNP in the *LDLR* gene with LDL-c and triglycerides levels of atherogenic risk

ÍNDICE

Pages

Dear Dr. Gabriel Cahua-Pablo,

You have been listed as a Co-Author of the following submission:

Journal: Gene
Title: Correlation of ApoE4 with rs688 SNP in the LDLR gene on levels of LDL-c and triglycerides of atherogenic risk
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Correlation of ApoE4 with rs688 SNP in the *LDLR* gene with LDL-c and triglycerides levels of atherogenic risk

Abstract

Background: The Dyslipidemia are the major risk factors in the development of coronary heart disease (CHD gene-environment and gene-gene interactions have been involved. Apolipoprotein E (ApoE) and the receptor for low density lipoproteins (LDLR) contribute to the lipoproteins excretion by the liver, thus reducing the cholesterol, triglycerides (TG) and lipoproteins levels. Three ApoE isoforms have been identified; the ApoE4 with cardiovascular disease, diabetes and elevated cholesterol, low density lipoprotein cholesterol (LDL-c) and TG. The aim of this study was to evaluate the association of ApoE isoforms and the SNP rs688 in the *LDLR* gene with CHD risk factors in women from the state of Guerrero, Mexico.

Methods: A cross-sectional study in 400 women with genetic ancestry and residents of the state of Guerrero were evaluated. Anthropometric and blood pressure were recorded. Fasting glucose, cholesterol, triglycerides, HDL-c and LDL-c were assessed. The polymorphism in rs429358 and rs7412 *APOE* and the SNP rs688 the *LDLR* gene were performed by real time PCR using TaqMan probes.

Results: ApoE3 isoform was the most frequent (76.2%). A significant association of LDL-c (OR=3.2, 95%CI:1.9-5.6), and TG (OR=1.7, 95%CI:1.1-3.0) of atherogenic risk in women carriers of the ApoE4 compared with ApoE3 carriers. This risk was consistent with those having both markers elevated (OR=3.2,

95%CI:1.6-6.4). We found significant interaction between ApoE4 with CT genotype (OR=5.4, 95%CI: 2.2-13.4) or TT (OR=6.2, 95%CI: 1.6-24.0) compared with women carrying the ApoE3.

Conclusions: Our results suggest that the ApoE4 isoform could significantly influence the metabolism of lipids, delaying the removal of blood lipoproteins, suggesting that this process involving a joint effect between the ApoE4 isoform and the LDL receptor on the increase in LDL-c.

Keywords: ApoE isoforms, polymorphisms, LDL- receptor, LDL-c, Triglycerides

1. Introduction

Coronary heart disease (CHD) is the most common disease among cardiovascular diseases in developing countries, and the major cause of death in men and women around the world. CHD is multifactorial, involving both genetic and environmental factors as well as its possible interactions. The main cause of CHD is atherosclerosis, defined as a disease of blood vessels that involves degenerative and regenerative processes that affect the intimate initially, and the middle layer of the arteries in a later stage, mainly due to infiltration and deposition of lipid on the walls of the arteries (1).

Experimental investigations, epidemiological and genetic forms of hypercholesterolemia indicate that elevated low-density lipoprotein cholesterol (LDL-c) is the major cause of CHD. Moreover, prospective studies indicate that high serum levels of triglycerides (TG) are also an independent risk factor for CHD. The contributing factors to high LDL-c or TG include overweight, physical inactivity,

smoking, excessive alcohol consumption, high carbohydrate diets, severe diseases such as type-2 diabetes (T2D), chronic renal failure, nephrotic syndrome, drugs (corticosteroids, estrogen, retinoid, beta adrenergic blocking agents), and genetic disorders (2).

Apolipoprotein E (ApoE) plays an important role in lipid metabolism which is involved in lipoprotein uptake through interaction with LDL receptors (LDLR). The association of several metabolic disorders with various single nucleotide polymorphisms (SNPs) in the *APOE* gene has been reported. The SNP rs429358 causes the change of cysteine for arginine at position 112 (Cys112Arg), whereas the SNP rs7412 changes the arginine for cysteine at position 158 (Arg158Cys), giving rise to the three most common isoforms of ApoE: ApoE2 (Cys112 and Cys158), ApoE3 (Cys112 andArg158) and ApoE4 (Arg112and Arg158) (3-5). In addition, it has been observed that *LDLR* gene mutations lead to elevated blood levels of LDL-c, and as a result increased risk for the development of atherosclerosis and ischemic heart disease. Genetic association studies have reported several *LDLR* gene polymorphism associated with the variation in the concentration of serum lipids. The rs688 SNP, located in exon 12, has been associated with increased blood cholesterol levels in different populations (6, 7).

Different ApoE isoforms differ in their affinity LDLR binding, the ApoE4 binds with slightly higher affinity to ApoE3, whereas ApoE2 presents much lower binding affinity in comparison to the other isoforms (8). Despite the low LDLR binding of the ApoE2, most ApoE2-carriying individuals have low levels of LDL-c and a low risk of atherosclerosis (9). Moreover, in Chinese population, carrying ApoE4 isoform was found to be positively associated with metabolic syndrome prevalence, and ApoE4

carriers were more likely to have high blood pressure and hyperglycemia in men (10). Notably, it has been reported that individuals who only carry the ApoE3 isoform have 20% lower risk of CHD compared with carriers of isoform ApoE2, whereas the ApoE4 carriers have the highest risk (11).

Other reports have associated the high levels of ApoE4 with decreased TG and HDL-c (12). This study was aimed to evaluate the association between ApoE isoforms and the SNP rs688 in the *LDLR* gene with CHD risk factors in women from Guerrero, Mexico. Our results together indicate that the association between ApoE4 isoform and the SNP rs688 in *LDLR* gene could have an effect on the elevation of LDL-c and triglyceride levels.

2. Material and methods

2.1. Study population

We studied a total of 400 women between 30 and 65 years old, genetically unrelated, native and residents of the state of Guerrero, Mexico, whose parents and grandparents were also born in the Guerrero State. Sociodemographic data were obtained through a questionnaire. The project was approved by The Ethic Committee of the Autonomous University of Guerrero. All women agreed to participate in the study by means of written informed consent.

2.2. Anthropometric measurements and laboratory analyses

For each woman weight, height, waist circumference (WC) and blood pressure were measured. Venous blood sample was obtained for the determination of biochemical measurements and DNA extraction. The concentrations of glucose, total cholesterol, HDL-c, LDL-c and triglycerides were measured using conventional enzymatic methods with commercially available kits.

2.3. Genotyping

Genomic DNA was extracted from peripheral blood leucocytes using the non-enzymatic rapid technique (13). Genotyping of the SNPs rs429358 and rs7412 *APOE* gene, and rs688 *LDLR* gene was based on the 5'nuclease assay, using PCR with specific TaqMan assay for each SNP assay (7500HT Real-Time, Life Technology, Applied Biosystems). Verification of the genotypes was performed in duplicate at 10% of samples.

2.4. Statistical analysis

The X^2 test or Fisher exact test was used for comparison between strata of LDL-c and TG with the genotype frequencies of the SNPs rs429358 (388T>C) and rs7412 (526C>T) in the *APOE* gene and the rs688 (1773C>T) in the *LDLR* gene. Hardy-Weinberg equilibrium was verified using the chi-square test with one degree of freedom. The Kruskal-Wallis test was required for comparison of medians between CHD risk factors and the ApoE isoforms. Logistic regression models, unadjusted and adjusted, were constructed to assess the association between CHD risk factors with the ApoE isoforms. Statistical analysis was performed using

STATA software (v.11.1). All statistical tests were two-sided and a value of $p < 0.05$ was considered statistically significant.

3. Results

The median age of women was 46 years (38-53), 54.8% had abdominal adiposity, serum glucose levels were 80 mg/dl, and over 77% of women had decreased levels of HDL -c (Table 1).

3.1. *Classification of ApoE isoforms*

The group studied was classified at high risk atherogenic using blood levels of LDL-c (≥160 mg/dl) and TG (≥150 mg/dl). The SNPs evaluated were in equilibrium genic proposed by Hardy-Weinberg. Based on the analysis of genotypes of rs429358 (388T>C) and rs7412 (526C>T) in the *APOE* gene; genotypes were determined as follows: E2/E2 (TT/TT), E2/E3 (TT/CT), E2/E4 (CT/CT), E3/E3 (TT/CC), E3/E4 (CT/CC), and E4/E4 (CC/CC), starting from those that were classified into three groups: ApoE2 (E2/E2, E2/E3, E2/E4), ApoE3 (E3/E3), and ApoE4 (E3/E4, E4/E4), based on the classification made by other authors (3, 14).

3.2. Genotype frequencies

The ApoE3 isoform was the most frequent (76.2%), followed by ApoE4 (17.8%) and ApoE2 (6%). It was observed that in women with LDL-c ≥160 mg/dl and/or TG

≥150 mg/dl the frequency of TC or CC genotype (dominant inheritance model) of SNP rs429358 in the *APOE* gene, and the frequency of the ApoE4 was significantly higher compared to women who had decreased levels of LDL-c and/or TG. Moreover, the CT genotype of SNP rs688 in the *LDLR* gene was the most prevalent (45.5%), followed by CC (37%) and TT (17.5%). No significant differences were found between these genotypes with high levels of LDL-c and/or TG (Table 2).

3.3. Association between ApoE isoforms with LDL-c and TG atherogenic risk

We found significant differences in women who had high levels of LDL-c (≥160 mg/dl) and TG (≥150 mg/dl) (Table 3), when comparing ApoE isoforms with different CHD risk factors. Notably, women who are carriers of the ApoE4 isoform had 1.7 times the risk of having high TG (OR=1.7, 95%CI: 1.1-3.0) and 3.3 times more likely to have LDL-c of atherogenic risk (OR=3.3, 95%CI: 1.9 -5.7) compared with carriers of the ApoE3 isoform. Such risk was consistent with those who presented both markers of risk (OR=3.2, 95%CI: 1.6-6.5) (Table 4).

In assessing the joint effect between ApoE isoforms with rs688 SNP genotypes in the *LDLR* gene on the levels of LDL-c ≥160 mg/dl, we found significant association between the ApoE4 isoform with the CT genotype (OR=5.4, 95%CI: 2.2-13.4) or TT (OR=6.2, 95%CI: 1.6-24.0) compared with women carrying the ApoE3 isoform (Table 5).

4. Discussion

It is widely known that dyslipidemia contribute significantly to the development of CHD, mainly elevated blood levels LDL-c and triglyceride and decreased HDL-c. Various genes involved in the development of CHD have been proposed in genetic association studies, such as the *APOE* gene and the risk of cardiovascular disease. We evaluated the association of the ApoE isoforms with dyslipidemias and its relationship to a common variant in the *LDLR* gene.

In this study, it was found that all three isoforms of ApoE are present in women of Southwestern Mexico, and as in other studies, the most frequently found isoform of ApoE was ApoE3 (76.2%), considered as the wild form of the protein, followed by ApoE4 and ApoE2 (15, 16). Moreover women who are carriers of the ApoE4 isoform showed higher frequencies in the elevated levels of LDL-c ($p<0.001$) and TG mg/dl ($P=0.041$) compared with E2 and E3 isoforms, these results are consistent with other studies in different populations (10, 14). Several reports indicate that the increase in carotid artery intima-media thickness (CA-IMT) is a predictor of atherosclerosis; some studies have found association between the ApoE4 isoform with CA-IMT (17, 18). We found significant association between ApoE4 isoform with elevated levels of LDL-c and TG, even in women who had high levels of both atherogenic risk markers compared with the ApoE3 carriers.

In Indian population, the E4 allele has a high occurrence of dyslipidemia (19). Similar results were found in patients with cardiovascular disease without diabetes and lipid-lowering therapy, who had a positive association between ApoE4 carriers with the prevalence of metabolic syndrome and high blood pressure (20). In

another interesting study in Canadian ethnic groups, the ApoE4 isoform was associated with elevated LDL-c, Apo B, Apo B/ApoA, and decreased ApoA as well as with decreased levels of HDL-c (21). Iranian population showed that ApoE4 increased the risk of coronary artery disease and T2D. Similar results were observed in Japanese (22, 23), Canadian (24) and Chinese populations (25). In our study we found that women, who carry the ApoE4 isoform, were associated with atherogenic risk levels of LDL-c and TG, compared with women carrying the ApoE3. It is noteworthy the fact that we found a joint effect between E4 isoform with CT or TT genotype of SNP rs688 the *LDLR* gene on the increase in LDL-c, novel finding that has not been reported and could signify a possible interaction gene -gene involved in the development of metabolic disorders.

Experiments in primary culture of hepatocytes show that the low affinity of ApoE2 toward LDLR increases ApoE levels in circulation, transferring to VLDL and increasing the amount of ApoE2 in these, which facilitates their uptake mediated by the LDLR and heparan sulfate proteoglycan. Meanwhile, the high affinity LDL receptor-ApoE4, causes is confined to the surface of hepatocytes and ApoE4 plasma levels are low, and consequently the very low density lipoproteins (VLDL) have low concentrations of ApoE. VLDL that are low in ApoE stick to the hepatocyte surface without being internalized, and the kidnapping of these VLDL causes exposed to the action of lipases and subsequently converted to remnants and LDL, also release free fatty acids in circulation which will re-synthesized TG raising their serum levels. ApoE4 is characterized by the rapid removal and down-regulation by the LDL receptor, leading to increased plasma levels of LDL-c and TG (9).

The mechanism by which the ApoE isoforms contributing to the risk of cardiovascular disease is still poorly understood, and is attributed to the structural differences of isoforms arising starting from the different alleles (E2, E3 and E4), producing differences the affinity for LDLR in the order of ApoE4>ApoE3>ApoE2, which impact on plasma cholesterol levels (26, 27). However, despite the low affinity binding of the ApoE2, most individuals carrying this isoform have low levels of LDL-c and a low risk for atherosclerosis, and it has been reported that 5% to 10% ApoE2 homozygotes develop type-III hyperlipoproteinemia (5, 28). Also, it is relevant the association of the ApoE4 isoform with high levels of LDL-c and increased risk of atherosclerosis (29).

In conclusion, our results suggest that the ApoE4 isoform could significantly be involved in the development of atherosclerosis and CHD risk. These findings also suggest that ApoE4 can significantly influence the metabolism of lipids, delaying the removal of blood lipoproteins, suggesting that this process involving a joint effect between the ApoE4 isoform and the LDL receptor on the increase in LDL-c. More detailed studies are needed to evaluate these effects.

Abbreviations

CHD: coronary heart disease; ApoE: apolipoprotein E; LDL-c: low density lipoprotein cholesterol; LDLR: LDL receptor; TG: triglycerides; HDL-c: high density lipoprotein cholesterol; PCR: polymerase chain reaction; SNP: Single nucleotide polymorphism.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

GCP, MC, OMH, MALV, DLAO, JACP, LCAR, COP, DHS, MEMG, and EFA: contributed equally to this work. All authors read and approved the final manuscript.

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Table 1. Sociodemographic and clinical characteristics of the women studied

Characteristic	n =400
Age (y)	46 (38-53)
Body mass index (kg/m^2)	27.3 (24.5-30.4)
Abdominal obesity, n (%)	219 (54.8)
% Body fat	36.7 (31.3-41)
% Water	44.4 (41.6-47.9)
Systolic blood pressure (mm Hg)	117 (108-127)
Glucose (mg/dl)	80 (72-89.5)
≥ 110, n (%)	49 (12.3)
Total cholesterol (mg/dl)	168 (145-192)
≥ 200, n (%)	74 (18.5)
Triglycerides (mg/dl)	123.8 (91.9-166)
≥ 150, n (%)	141 (35.2)
LDL-c (mg/dl)	112.9 (88.1-155.8)
≥ 160, n (%)	94 (23.5)
HDL-c (mg/dl)	39.3 (32-48.9)
< 50, n (%)	310 (77.5)
Exercise, n (%)	203 (50.8)

Data are reported as medians (25-75th percentile) or n (%).

Table 2. Genotypic and allelic frequencies of the SNPs rs429358 and rs7412 in *APOE* gene and SNP rs688 in the *LDLR* gene and ApoE isoforms related to LDL-c and triglycerides

SNPs	Total n=400	**LDL-c (mg/dl)** < 160 n=306	≥ 160 n=94	**P**[1]	**Triglycerides (mg/dl)** <150 n=259	≥150 n=141	**P**[1]
APOE gene, n (%)							
rs429358 (388T>C)							
TT	325 (81.3)	262 (85.6)	63 (67.0)	**<0.001**	218 (84.2)	107 (75.9)	0.125
TC	71 (17.8)	43 (14.1)	28 (29.8)		39 (15.1)	32 (22.7)	
CC	4 (1)	1 (0.3)	3 (3.2)		2 (0.8)	2 (1.4)	
TC+CC	75 (18.8)	44 (14.4)	31 (33.0)	**<0.001**	41 (15.9)	34 (24.1)	**0.043**
Alleles							
T	721 (90.1)	567 (92.6)	154 (81.9)		475 (91.7)	246 (87.2)	
C	79 (9.9)	45 (7.4)	34 (18.1)		43 (8.3)	36 (12.8)	
EHW	P=0.96	P=0.58	P=0.50		P=0.86	P=0.82	
rs7412 (526C>T)							
CC	374 (93.5)	284 (92.8)	90 (95.7)	0.561	239 (92.3)	135 (95.7)	0.105
CT	25 (6.3)	21 (6.9)	4 (4.3)		20 (7.7)	5 (3.6)	
TT	1 (0.2)	1 (0.3)	0		0	1 (0.7)	
CT+TT	26 (6.5)	22 (7.2)	4 (4.3)	0.313	20 (7.7)	6 (4.3)	0.179
Alleles							
C	773 (96.6)	589 (96.2)	184 (97.9)		498 (96.1)	275 (97.5)	
T	27 (3.4)	23 (3.8)	4 (2.1)		20 (3.9)	7 (2.5)	
EHW	P=0.40	P=0.37	P=0.83		P=0.52	P=0.06	
LDLR gene, n (%)							
rs688 (1773C>T)							
CC	148 (37)	119 (38.9)	29 (30.9)	0.157	92 (35.5)	56 (39.7)	0.431
CT	182 (45.5)	139 (45.4)	43 (45.7)		124 (47.9)	58 (41.1)	
TT	70 (17.5)	48 (15.7)	22 (23.4)		43 (16.6)	27 (19.2)	
CT+TT	252 (63)	187 (61.1)	65 (69.1)	0.158	167 (64.5)	85 (60.3)	0.406
Alleles							
C	478 (59.8)	377 (61.6)	101 (53.7)		308 (59.5)	170 (60.3)	0.09
T	322 (40.2)	235 (38.4)	87 (46.3)		210 (40.5)	112 (39.7)	
EHW	P=28	P=0.49	P=0.44				
ApoE isoforms, n (%)							
ApoE2	24 (6)	246 (80.4)	59 (62.8)	**<0.001**	202 (78.0)	103 (73.0)	**0.041**
ApoE3	305 (76.2)	20 (6.5)	4 (4.2)		19 (7.3)	5 (3.6)	
ApoE4	71 (17.8)	40 (13.1)	31 (33.0)		38 (14.7)	33 (23.4)	

[1] X^2 test or Fisher exact test; HWE: Hardy-Weinberg equilibrium

Table 3. Relationship of the ApoE isoforms with risk factors for coronary heart disease

Factor	ApoE2 n=24	ApoE3 n=305	ApoE4 n=71	p
Body mass index (kg/m^2)	27.6 (26.0-30.4)	27.1 (24.4-30.3)	28.2 (25.9-32.4)	0.219[1]
% Body fat	37.5 (33.3-40.5)	35.9 (30.8-40.7)	37.6 (34.6-42)	0.110[1]
Abdominal obesity, n (%)	17 (70.8)	160 (52.5)	42 (59.2)	0.157[2]
BP systolic/diastolic (mmHg)				
≥ 130/85, n (%)	5 (20.8)	79 (25.9)	20 (28.2)	0.776[2]
Glucose (mg/dl)				
≥ 110 y/o diabetes, n (%)	4 (16.7)	37 (12.1)	8 (11.3)	0.778[2]
Cholesterol (mg/dl)				
≥ 200, n (%)	4 (16.7)	54 (17.7)	16 (22.5)	0.622[2]
HDL-c (mg/dl)				
< 50, n (%)	19 (79.2)	234 (76.7)	57 (80.3)	0.795[2]
LDL-c (mg/dl)				
≥ 160, n (%)	4 (16.7)	59 (19.3)	30 (42.9)	**<0.001**[2]
Triglycerides (mg/dl)				
≥ 150, n (%)	5 (20.8)	103 (33.8)	33 (46.5)	**0.041**[2]
LDL-c ≥160 mg/dl and TG ≥150 mg/dl, n (%)	0	24 (7.9)	13 (18.6)	**0.002**[2]

The data indicate median (p25-p75) or n (%). [1] Kruskal Wallis test; [2]X^2 test or Fisher exact test.

Table 4. Association of ApoE isoforms with the atherogenic risk factors

Factor	ApoE3	ApoE2	P	ApoE4	P
		OR (95%CI)[1]		OR (95%CI)[1]	
Abdominal obesity	1.0	2.3 (0.9-5.9)	0.071	1.3 (0.8-2.3)	0.291
BP ≥130/85 mm Hg	1.0	0.8 (0.3-2.2)	0.658	1.1 (0.6-2.1)	0.660
Glucose ≥110 mg/dl	1.0	0.4 (0.1-3.3)	0.419	0.9 (0.3-2.2)	0.750
TG ≥150 mg/dl	1.0	0.5 (0.2-1.5)	0.220	1.7 (1.1-3.0)	**0.041**
HDL-c <50 mg/dl	1.0	1.1 (0.4-3.1)	0.839	1.1 (0.6-2.2)	0.676
LDL-c ≥160 mg/dl	1.0	0.8 (0.3-2.5)	0.747	**3.3 (1.9-5.7)**	**<0.001**
TG ≥150 mg/dl and LDL-c ≥160 mg/dl	1.0	0.5 (0.1-4.0)	0.533	**3.2 (1.6-6.5)**	**0.001**

[1]Ajusted for age and body mass index, except from abdominal obesity

Table 5. Joint effect of ApoE isoforms and SNP rs688 *LDLR* gene on elevated levels in LDL-c

ApoE Isoforms	SNP rs688	LDL-c ≥160 mg/dl OR (95%CI)[1]	P
E3	CC	1.0	
E3	CT	1.6 (0.8-3.2)	0.166
E3	TT	2.2 (1.0-4.9)	0.052
E2	CC	1.8 (0.3-9.3)	0.501
E2	CT	ND	
E2	TT	12.4 (1.1-145.4)	0.045
E4	CC	3.9 (1.3-9.7)	**0.01**
E4	CT	5.4 (2.2-13.4)	**<0.001**
E4	TT	6.2 (1.6-24.0)	**0.008**

[1]Ajusted for age and body mass index