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Effect of *APOB* gene polymorphisms on body mass index, blood pressure, and total cholesterol levels

A cross-sectional study in Mexican population

Ana Gabriela Colima Fausto, PhD[a], Jaqueline Topete, BS[b], Juan Ramón González García, PhD[b], Teresita de Jesús Hernández Flores, PhD[b,c], Sergio Yair Rodríguez Preciado, PhD[d], María Teresa Magaña Torres, PhD[b,*]

Abstract

APOB gene polymorphisms are considered risk factors for the development of dyslipidemia, hypertension, and cardiovascular disease (CVD) in several populations. In Mexico, these pathologies are frequent and studies regarding this gene are scarce. The aim of this cross-sectional study was to determined genotype, allele, and haplotype frequencies of *APOB* polymorphisms and performed analyses of association among the biochemical, hemodynamic, anthropometrical, and genetic variables. Blood samples were taken from 361 subjects from unselected Mexican population for biochemical analysis and for deoxyribonucleic acid extraction; besides blood pressure and body mass index (BMI) were measured. *APOB* polymorphisms rs934197, rs533617, rs693, and rs1042031 were genotyped by polymerase chain reaction (PCR)-restriction fragment length polymorphism; whereas, rs17240441 and c.66_67insCTGCTG were genotyped by PCR followed by electrophoresis. Genotype and allele frequencies were obtained by simple counting and deviations from Hardy-Weinberg equilibrium (HWE) were calculated by chi-square test. The effect of the polymorphisms on the quantitative variables was determined using analysis of variance, Student's *t* test, Pearson's and Spearman's correlations and multiple linear regression models. All the polymorphisms were within HWE. Frequencies of mutated alleles were highly heterogeneous: rs934197-T 33.6%, rs17240441-D 39.3%, c.66_67insCTGCTG-I 3.9%, rs533617-G 0.9%, rs693-T 40.5%, and rs1042031-G 17.3%. Chronic degenerative diseases were frequent in the studied population: overweight-obesity 55.1%, dyslipidemia 45.8%, and hypertension 23.5%. The association analyses showed that despite adjustments for age and sex the mutated alleles rs934197-T, rs1042031G, c.66_67-insCTGCTG-I, and rs533617-G, were related to lower values of BMI, total cholesterol (TC), systolic blood pressure, and diastolic blood pressure, respectively. All polymorphisms analyzed except rs533517 and c.66_67insCTGCTG showed high frequencies of the mutated allele, making them useful for association studies. Our results revealed that, *APOB* gene polymorphisms could be contributing to the development of several chronic diseases, such as essential hypertension, dyslipidemias, obesity, among others. However, specific studies with each pathology are needed to know the possible implications of the polymorphisms.

Abbreviations: BMI = body mass index, CVD = cardiovascular disease, DBP = diastolic blood pressure, ENSANUT = national health and nutrition survey, HDL-C = high-density lipoprotein cholesterol, HWE = Hardy-Weinberg equilibrium, LDL-C = low-density lipoprotein cholesterol, PCR = polymerase chain reaction, TC = total cholesterol, TG = triglycerides.

Keywords: *APOB* polymorphisms, blood pressure, body mass index, chronic diseases, lipid levels, Mexican population

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[a] School of Medicine, Universidad Autónoma de Guadalajara, Guadalajara, Jalisco, México, [b] División de Genética, Centro de Investigación Biomédica de Occidente, Instituto Mexicano del seguro social, Guadalajara, Jalisco, México, [c] Doctorado en Genética Humana, Universidad de Guadalajara, Guadalajara, Jalisco, México, [d] Centro Universitario de los Valles, Universidad de Guadalajara, Guadalajara, Jalisco, México.

**Correspondence: María Teresa Magaña Torres, División de Genética, Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco 44340, México (e-mail: aganamt@gmail.com).*

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1. Introduction

The *APOB* gene is located on chromosomal band 2p24.1, contains 29 exons and 28 introns, and encodes a glycoprotein involved in maintaining the normal homeostasis of serum cholesterol levels. In the plasma there are 2 main isoforms of the APOB protein: the first is APOB-100 (4563 aa), highly relevant for the constitutive formation of very low-density lipoproteins in the liver; it plays an important role in maintaining the structural integrity of lipoprotein particles and functions as a recognition signal for the internalization of low-density lipoprotein cholesterol (LDL-C) by the LDL receptor. The second is APOB48 (2153 aa), a truncated form of APOB corresponding to nearly 48% of the N-terminal sequence, which acts upon the assembly of chylomicrons and their secretion from the enterocyte.[1,2]

Several polymorphisms in the *APOB* gene have been associated with dyslipidemia, hypertension, and cardiovascular disease (CVD); however, results are heterogeneous and even inconsistent among populations (Table 1).[1–6] Despite chronic degenerative diseases are frequent in Mexican population, studies of the *APOB* gene are scarce. Of all of the *APOB* single-nucleotide polymorphisms reported, we paid particular attention on 6 variants, of which 5 have been worldwide studied (Table 1).[1–6] We here report genotype, allele, and haplotype frequencies of 6 *APOB* gene polymorphisms in general population. In addition, we performed an association analysis of these variants with lipid levels and other variables.

2. Methods

2.1. Subjects

During the years 2007 to 2010, different studies were carried out in order to identify patients with some chronic degenerative diseases such as dyslipidemia, hypertension, diabetes and obesity. An invitation to participate in the study (without considering any selection factor) was made to families from the general population of Northwestern Mexico, who were unaware of their state of health. Through continued inclusion, 361 individuals from 99 families were recruited. This analytical cross-sectional study protocol was designed based on the guidelines outlined in the Declaration of Helsinki and approved by the research and ethics committees of our Institute (National Scientific Research Commission). Blood samples for biochemical and molecular analyzes were taken after obtaining informed consent from each participant.

2.2. Biomarkers analyzed

A clinical history was performed for each individual, which included information on demographic aspects, lifestyle factors, and familial and personal health histories. Weight (Tanita model BF-682T digital scale) and height (SECA CEO/123 height rod) were determined to calculate body mass index (BMI); and blood pressure was measured using a mercury sphygmomanometer according to the guidelines established by Mexican Official Standard 2009.[7] Blood samples were collected from each participant after at least 12 hours of fasting and 72 hours without consuming alcohol. Total cholesterol (TC), triglycerides (TG), and high-density lipoproteins cholesterol (HDL-C) values were determined by enzymatic methods with Serapack kit; whereas, LDL-C was calculated using Friedewald's equation. In those patients with TG values above 400 mg/dL, LDL-C was directly quantified. Glucose, creatinine and uric acid were measured with ADVIA kit.

2.3. Molecular analyses

Genomic deoxyribonucleic acid was obtained using the method proposed by Gustincich et al[8] with slight modification. Genotyping of the polymorphisms rs934197, rs533617, rs693, and rs1042031 was performed by polymerase chain reaction (PCR) and restriction enzymes (Table 2). Polymorphisms rs17240441 (W1 > D) and c.66_67insCTGCTG (W2 > I) were amplified in 1 PCR reaction with the same primer pair (both polymorphisms are separated by only 17 bp) and the PCR products were visualized in 8% polyacrylamide gels, showing 1 of the 4 expected combinations (Table 2). Electrophoretic results were confirmed selecting several random samples and sequencing them with the BigDye® Terminator v3.1 kit.

2.4. Statistical analyses

Genotype and allele frequencies were determined by simple counting and deviations from Hardy-Weinberg equilibrium (HWE) were calculated by chi-square test. The distribution of genotypic frequencies of 5 out of the 6 studied polymorphisms were compared with the data available from 26 populations (7 African, 10 Asian, 5 European, and 4 American) in the 1000 Genomes Project. Haplotypes were established by familial segregation or, in case of elevated heterozygosity, by the Bayesian algorithm. Linkage disequilibrium between loci pairs of all polymorphisms was calculated with haplotype frequencies and a strong disequilibrium was deduced when 2 loci showed values of $r^2 > 0.33$. All these analyses were performed with Arlequin v.3.1 software.

Prevalence of dyslipidemias was estimated considering the values proposed by Aguilar-Salinas et al[9] and described in the Mexican Official Standard 2012[10] (hypercholesterolemia: TC > 200 mg/dL, LDL-C > 130 mg/dL and TG < 200 mg/dL;

Table 1

Association studies of *APOB* polymorphisms.

Polymorphism	Location	Allele	Effect
rs934197 (–516C>T)	Promoter	T	Associated with increases the transcription rate of the *APOB* gene, high levels of LDL-C and APOB; as well as with the presence of carotid atherosclerotic disease.[3,4]
rs17240441 (c.35_43delTGGCGCTGC)	Exon1	D	Associated with high levels LDL-C.[5]
c.66_67insCTGCTG	Exon1	I	No association studies
rs533617 (c.5768A>G)	Exon 26	G	Related with high levels of LDL-C during a low fat and cholesterol diet in men.[3]
rs693 (c.7545C>T)	Exon 26	T	Associated with higher concentrations of total cholesterol, LDL-C and triglycerides.[2] In Mexican population was related with risk to develop coronary artery disease.[1]
rs1042031 (c.12541A>G)	Exon 29	G	Associated with elevated LDL-C and low HDL-C. It is a risk factor for primary hypertension in Mexican population.[2,6]

D = deletion, HDL-C = high-density lipoprotein cholesterol, I = insertion, LDL-C = low-density lipoprotein cholesterol.

hypertriglyceridemia: TC < 200 mg/dL, LDL-C < 130 mg/dL and TG > 200 mg/dL; mixed hyperlipidemia: TC ≥ 200 mg/dL, LDL-C ≥ 130 mg/dL and TG ≥ 200 mg/dL; hypoalphalipoproteinemia: HDL-C < 40 mg/dL).

Moreover, frequencies of diabetes mellitus type 2 (Glucose > 126 mg/dL), essential hypertension (systolic (>140 mm Hg) or diastolic (>90 mm Hg) blood pressure), and hyperuricemia (men > 7 mg/dL and women > 6 mg/dL) were calculated.

The effect of the polymorphisms on the quantitative variables was determined by an analysis of variance for genotypes, followed by post hoc tests Bonferroni or Dunnett T3. Allelic mean values of each polymorphism were compared by Student's *t* test. Pearson's and Spearman's correlations were performed to compare quantitative and qualitative variables. In multiple linear regression models variables were adjusted for age and sex. A *P* value < .05 was considered statistically significant and SPSS v.20 software was used for the analysis. In silico analyses for predicting the impact of rs533617 (p.H1923R) and rs1042031 (p.E4181K) on APOB protein were performed with the PolyPhen-2 program.[11]

3. Results

3.1. Genotype and allele frequencies

A total of 361 deoxyribonucleic acid samples were successfully amplified. Allelic and genotypic frequencies of the 6 *APOB* gene polymorphisms were calculated based on 168 unrelated individuals (Table 3) and the remaining 193 subjects were used to establish the phase of haplotype segregation. The frequencies of the mutant homozygote genotypes of rs934197, rs17240441 and rs693 were higher than 10% and the minor alleles of these SNP´s exceeded 30%, whereas for rs533617 and c.66_67insCTGCTG polymorphisms, mutant homozygote genotypes were not detected and frequencies of minor alleles did not exceed 5%. All the polymorphisms were within HWE expectations (*P* > .05) (Table 3).

3.2. Comparison of genotypic frequencies with other populations

Genotypic frequencies of 5 out of the 6 polymorphisms studied were compared with data available in the 1000 Genomes

Table 2

Primers and enzymes used for the analysis of the *APOB* gene polymorphisms.

SNP	Primer sequence 5' to 3'	Restriction enzyme	Electrophoretic fragments (bp)	
rs934197 (–516C>T)	CTCTGCTTTTCCTCGTCCG GCACCCACACCCTAATCCT	*Ear*I	C/C T/T	319 259, 60
rs533617 (c.5768A>G)	TGTCTTCCGTTCTGTAATGGC GGTCTTGAGTTTCCAGGTGC	*Rsa*I	A/A G/G	274 49, 225
rs693 (c.7545C>T)	GGTTGGATTTATTGATGATGCTGT GCAAGAGTCCACCAATCAGAAATG	*Xba*I	C/C T/T	249 367, 125
rs1042031(c.12541A>G)	GGCCATTAGGCAAATTGATGA GGAAACTGGAATCTGGGGAAG	*Eco*RI	G/G A/A	188, 73 261
rs17240441 *(c.35_43del9bp)/ †c.66_72insCTGCTG	AGCTGGCGATGGACCCGC CACTCACCGGCCCTGGCG	–	§W1/W2 W1/I D/W2 D/I	97 103 88 94
rs17240441 (c.35_43del9bp)/ c.66_72insCTGCTG	‡AGTGCCCTTCTCGGTTGCT CCCTCCTCAGCCCCTCCAT	–	376	

bp = base pairs, SNP = single-nucleotide polymorphism.

*rs17240441 (c.35_43del9bp): D = allele with deletion, W1 = allele without deletion or reference sequence allele.

†c.66_67insCTGCTG: I = allele with insertion, W2 = allele without insertion or reference sequence allele.

‡Primers used for sequencing both polymorphisms. §Haplotype combination obtained with the 2 polymorphisms. bp = base pairs.

Table 3

Genotypic and allelic frequencies of 6 polymorphisms in the *APOB* gene in Mexican population.

n = 168 individuals

Polymorphism	rs934197C>T			†rs17240441			‡c.66_72insCTGCTG			rs533617A>G			rs693C>T			rs1042031G>A		
		n	%		n	%		n	%		n	%		n	%		n	%
Genotype	CC	75	44.6	W1W1	64	38.1	W2W2	155	92.3	AA	165	98.2	CC	64	38.1	GG	116	69
	CT	73	43.5	W1D	76	45.2	W2I	13	7.7	AG	3	1.8	CT	72	42.9	GA	46	27.4
	TT	20	11.9	DD	28	16.7	II	0	0	GG	0	0	TT	32	19	AA	6	3.6
*P value	.73			.5			.6			.91			.15			.59		

n = 336 chromosomes

		n	%		n	%		n	%		n	%		n	%		n	%
Allele	C	223	66.4	W1	204	60.7	W2	323	96.1	A	333	99.1	C	200	59.5	G	278	82.7
	T	113	33.6	D	132	39.3	I	13	3.9	G	3	.9	T	136	40.5	A	58	17.3

*P value of Hardy Weinberg Equilibrium.

†rs17240441 (c.35_43del9bp): D = allele with deletion, W1 = allele without deletion or reference sequence allele.

‡c.66_67insCTGCTG: I = allele with insertion, W2 = allele without insertion or reference sequence allele.

Project. Polymorphisms rs934197, rs17240441, and rs693 showed frequencies significantly different from those observed in Asian and African populations ($P < .01$). Genotype frequency of rs1042031 polymorphism was significantly different from frequencies detected in Peruvian and all Asian populations ($P < .01$), except Indian Telugu. In addition, our rs533617 genotypic frequency showed differences only with those reported in Finnish and Toscani populations ($P < .01$).

To our knowledge, the c.66_72insCTGCTG variant is not yet registered in any database. However, we compare our results with previously reported data of 3 different populations: individuals with Mexican ancestry (random samples: W2W2 = 169, W2I = 12),[12] Brazilian indigenous individuals (Gaviões: W2W2 = 27, W2I = 3; Zoró: W2W2 = 26, W2I = 3),[13] and Africans from Benin (W2W2 = 823, W2I = 1).[14] In none of these populations the mutated homozygote genotype (II) was observed. When comparing the genotypic and allelic frequencies obtained in this study with those previously reported, significant differences are evidenced regarding the African population ($P < .001$).

3.3. Haplotype analysis

We established haplotypes with the 6 polymorphisms ordering them from 5' to 3' as follows: rs934197, rs17240441, c.66_67insCTGCTG, rs533617, rs693, and rs1042031 (Table 4). In total, 375 independent chromosomes were obtained (336 from 168 unrelated individuals, and the remaining 39 selected from 193 first-degree relatives sharing at least 1 of the 2 *APOB* genes). Twenty-one different haplotypes were observed; the most common being CW1W2ACG and TDW2ATG, with frequencies of 32.5% and 23.5%, respectively. Seventeen combinations had frequencies lower than 5% (Table 4).

Linkage disequilibrium evaluation revealed significant results in 3 pairs of loci: rs934197-rs17240441 ($r^2 = .48$, $P < .001$), rs934197-rs693 ($r^2 = .34$, $P < .001$), and rs17240441-rs693 ($r^2 = .33$, $P < .001$). Whereas, polymorphisms c.66_67insCTGCTG, rs533617 and rs1042031 showed linkage equilibrium with all the sites. However, the first 2 had low frequencies of the mutated allele, 3.9% and .9%, making difficult the detection of disequilibrium.

In silico analysis with the PolyPhen-2 program on the p.H1923R and p.E4181K variants revealed a score of.997 (probably damaging) and.012 (benign), respectively.

3.4. Sociodemographic characteristics and frequency of chronic degenerative diseases

Table 5 shows the values of the lipid profile and blood chemistry of the 361 individuals (51 under 18 years) included in the study; of which, 153 were men and 208 women, with a mean age of 38.4 ± 17.9 (range 5–81 years). Overweight-obesity was observed in 55.1% (n = 199) of the individuals (BMI > 25); whereas, 6.7% (n = 24) had low weight (BMI < 18.5).

We calculated the prevalence of dyslipidemias considering the reference values previously reported[9,10] and found 164 affected individuals (45.8%). The most frequent abnormality was hypoalphalipoproteinemia (n = 99), which was present as an isolated condition in 58 individuals and combined with another dyslipidemia in 41 subjects: 6 with hypercholesterolemia, 13 with mixed hyperlipidemia and 22 with hypertriglyceridemia. Hypercholesterolemia (45/358, 12.6%), mixed hyperlipidemia (24/358, 6.7%) and hypertriglyceridemia (37/358, 10.3%) showed similar frequencies. After analyzing the medical records, we estimated the prevalence of essential hypertension to be of 23.2% (66/310) and for type 2 diabetes mellitus of 11.3% (35/310).

3.5. Effect of studied polymorphisms on biomarkers analyzed

The effect of 6 *APOB* polymorphisms on the studied variables was analyzed with 127 unrelated individuals, all adults (range 18–81 years) and without having received pharmacological treatment. Statistically significant results of correlation analysis between the biomarkers are described in Table 6. TG and TG/HDL-C ratio showed significant correlation with all variables except for the age.

Table 4

Haplotypes at the *APOB* gene locus and their frequencies observed in Mexican population.

			†c.66-67insCTGCTG	rs533617	rs693	rs1042031	Chromosomes	
Haplotype	rs934197 C>T	*rs17240441 W1>D	W2>I	A>G	C>T	G>A	n	%
1	C	W1	W2	A	C	G	122	32.5
2	T	D	W2	A	T	G	88	23.5
3	C	W1	W2	A	C	A	45	12
4	C	W1	W2	A	T	G	33	8.8
5	C	D	W2	A	T	G	18	4.8
6	C	D	W2	A	C	G	17	4.5
7	T	D	W2	A	C	G	11	2.9
8	C	W1	I	A	C	A	8	2.1
9	T	W1	W2	A	T	G	7	1.9
10	C	W1	I	A	C	G	4	1.1
11	T	W1	W2	A	C	G	4	1.1
12	T	W1	W2	A	C	A	4	1.1
13	T	D	W2	A	C	A	3	.8
14	T	D	W2	A	T	A	3	.8
15	C	W1	W2	A	T	A	2	.5
16	C	W1	I	A	T	G	1	.27
17	C	W1	W2	G	C	G	1	.27
18	C	W1	I	G	C	G	1	.27
19	T	W1	W2	A	T	A	1	.27
20	C	D	W2	A	C	A	1	.27
21	T	W1	W2	G	C	G	1	.27
Total							375	100

*rs17240441 (c.35_43del9bp): D = allele with deletion, W1 = allele without deletion or reference sequence allele.

†c.66-67insCTGCTG: I = allele with insertion, W2 = allele without insertion or reference sequence allele.

The results statistically significant of analysis of variance, Student's *t* tests, correlations and linear regression models between biomarkers and the polymorphisms are shown in Tables 7 and 8. Subjects heterozygous for rs533617 variant displayed lower diastolic blood pressure (DBP) than wild homozygous individuals. Furthermore, BMI and TC values were lower in individuals with rs934197-T and rs1042031-A alleles, respectively. These associations remained significative after adjusting by age and sex in the linear regression models.

4. Discussion

4.1. Genotype, allele, and haplotype frequencies

Mutated homozygote genotypes of polymorphisms rs934197, rs17240441, and rs693 had frequencies higher than 10%, which makes them useful for further association studies. The distribution of the genotype frequencies of our population was similar with some European and American populations. These results are consistent with the origin of the Mexican mestizo population.[15]

In addition, the c.66_72insCTGCTG mutated allele showed a frequency of 3.9% in our population, similar to Brazilian indigenous individuals, but significantly different from Africans ($P < .001$).[9–11] Twenty-one haplotypes were observed, standing out by their frequency the combinations CW1W2ACG and TDW2ATG with 32.5% and 23.5%, respectively (Table 4). Although there is no information in the literature to compare these data, it is important to mention that the 2 most frequent haplotypes are composed of at least 3 variants that have been associated with increased values of TC, LDL-C, TG, and blood pressure.

4.2. Frequency of chronic degenerative diseases in the study population

We found that 55.1% of the individuals had overweight or obesity, prevalence significantly lower than that observed in the ENSANUT (National Health and Nutrition Survey) report (68.6%, $P < .001$).[16] Hypertension and dyslipidemias, which are important risk factors for CVD, were detected with frequencies of 23.2% and 45.8%, respectively. The frequency of hypertension observed in our study was similar to that reported in Mexican adults registered during 2000 to 2006 (23.2% vs 31.6%), ($P = .18$).[17] With the exception of hypertriglyceridemia (31.5% vs 34.1%, $P = .32$), all the dyslipidemias were significantly less frequent in this study than those reported in ENSANUT.[16] These differences in the results may due to the methods and/or equipment used to quantify lipoproteins, although we cannot rule out the type of population analyzed. In our study only Northwestern Mexican population was included and the ENSANUT report incorporated individuals from all states of the country.[16] It is important to note that, in spite of our including underage individuals in the analysis, the age variable did not influence such analysis since minors were excluded from calculations to obtain dyslipidemia percentages, which rendered the same results at the end. Independently from these differences, it is evident that dyslipidemias are a health problem in Mexico; and in order to explain its high prevalence, different studies have analyzed the genetic component in patients clinically and biochemically well characterized. However, strong genetic evidence has not been found in multifactorial or polygenic dyslipidemias.[18,19]

In this study, uric acid had an average value of 5.1 ± 1.5 mg/dL, and hyperuricemia was present in 15.9% (17/107) of men (values >7 mg/dL) and in 10.5% (17/162) of women

Table 5

Biochemical and anthropometric characteristics of the studied population.

Variable	n	Mean ± SD	†% affected individuals	Reference values
BMI (kg/m^2)	361	26.2 ± 5.6	*61.8	18.5–24.9
Total cholesterol (mg/dL)	358	182.3 ± 30.7	26.8	<200
Triglycerides (mg/dL)	358	140.88 ± 91.02	17.3	<200
HDL-C (mg/dL)	357	46.3 ± 11.4	27.7	>40
LDL-C (mg/dL)	358	108.0 ± 34.4	21.2	<130
Glucose (mg/dL)	310	91.8 ± 30.7	11.3	<126
Uric acid (mg/dL)	269	5.1 ± 1.5	12.6	<7.0
Creatinine (mg/dL)	269	.84 ± .21	‡4.8	0.52–1.2

Abbreviations: BMI = body mass index, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol.

*55.1% overweight-obesity and 6.7% low weight.

†Percentage of individuals that showed values different from those of reference.

‡0.74% high creatinine levels and 4.1% low creatinine levels.

Table 6

Significant correlations between the quantitative variables.

Variable*	Age	BMI	TC	TG	HDLC	Glucose	Uric acid	Creatinine	SBP	DBP	TG/HDL
Age	*					.024			<.001	.001	
BMI		*		<.001	.005	.001	<.001		<.001	<.001	<.001
TC			*	<.001					.015		.006
TG		.401	.333	*	<.001	.001	<.001	.011	<.001	<.001	<.001
HDLC		.256		.361	*	.021	<.001	<.001		.002	<.001
Glucose	.220	.323		.317	.229	*	.029				<.001
Uric acid		.492		.421	.509	.228	*	<.001	.029	.005	<.001
Creatinine				.263	.363		.473	*		.008	<.001
SBP	.472	.500	.241	.452			.233		*	<.001	<.001
DBP	.342	.505		.536	.305		.296	.281	.731	*	<.001
TG/HDLC		.396	.243	.913	.630	.446	.482	.353	.410	.536	*

BMI = body mass index, DBP = diastolic blood pressure, HDLC = high-density lipoprotein cholesterol, SBP = systolic blood pressure, TC = total cholesterol, TG = triglycerides.

*Above and below the diagonal the significant *P* values and correlation values are shown, respectively.

Table 7

Effect of *APOB* gene polymorphisms on quantitative variables studied in Mexican population.

Polymorphism	Variable	Genotype/allele		Genotype/allele		*P* value
rs533617	DBP (mm Hg)	AA	77.9	AG	65.0	.04
rs934197	BMI (kg/m^2)	C	27.8	T	26.3	.02
rs1042031G>A	TC (mg/dL)	G	186.1	A	171.9	.02
rs533617A>G	DBP (mmHg)	A	77.8	G	65.0	.04

Polymorphism	Variable	Correlation coefficient (rho)	*P* value
rs934197C>T	BMI (kg/m^2)	–.212	.021
rs1042031G>A	TC (mg/dL)	–.186	.037
rs533617A>G	DBP (mm Hg)	–.191	.05

BMI = body mass index, DBP = diastolic blood pressure, TC = total cholesterol.

Table 8

Linear regression models.

Variable	Allele	Constant	Beta	*P*
TC (mg/dL)	rs1042031-A	188.1	–14.2	.02
BMI (kg/m^2)	rs934197-T	27.7	–4.0	<.001
	rs17240441-D		3.0	.003
DBP (mm Hg)	rs533617-G	66.2	–13.1	.03

BMI = body mass index, DBP = diastolic blood pressure, TC = total cholesterol.

(values >6 mg/dL). However, Hayden and Tyagi suggested that values higher than 4.0 mg/dL of uric acid should be considered as 1 of the multiple factors capable of damaging the endothelium of arterial vessels, which is associated to several chronic degenerative diseases.[20] The latter is relevant for our population since 76.6% of our study group showed uric acid values higher than 4.0 mg/dL.

4.3. Correlation between biomarkers analyzed

There is little information about the biochemical, hemodynamic, and anthropometric biomarkers that alone or in additive form contribute to the development of chronic disease in Mexico. In general, most biomarker studied increase their values with age; however, in our study, only systolic and diastolic blood pressure, and glucose values correlated with age (Table 6), which probably indicates that the other biomarkers are modified by lifestyle and/or genetic factors in the Mexican population.

The BMI showed a positive correlation with systolic blood pressure, DBP, uric acid, TG and glucose; but also, it had a negative correlation with HDL-C. These results are similar to those reported by Camacho-Camargo, who observed that overweight or obese adolescents had 8.8-fold higher risk for developing essential hypertension than individuals with normal or low BMI.[21]

Particularly in Mexican population, prevalence of essential hypertension has been 1.3-fold higher in adults with obesity (42.3%) than in adults with normal BMI (18.5%)[16]; it has been reported that for every 10 kg of weight, there is a 3 mm Hg-increase in blood pressure,[22] which agrees with our findings. Moreover, the phenotype composed by high TG and low HDL-C, common in our population, is associated with insulin resistance[23] which could explain the increase in glucose levels.

An abnormal TG/HDL-C ratio indicates an atherogenic lipid profile and confers risk to develop insulin resistance, metabolic syndrome, hypertension and coronary disease. High TG/HDL-C ratios were found in 52.9% of men (>3.5) and in 49.3% of women (>2.5), showing mean values of 6.2 ± 2.3 and 3.9 ± 1.4, respectively. These values did not show significant differences with those reported in Argentine population with cardiometabolic risk (men 5.4 ± 2, $P = .82$ and women 4.1 ± 1.8, $P = .95$).[24,25] On the other hand, the TG/HDL-C ratio was significantly correlated with all the biomarkers included in this study. The same relation was observed with BMI, TC, HDL-C, TG and uric acid levels in other populations.[25,26] A direct correlation of TG/HDL-C ratio with glucose levels has not been previously observed; however, this ratio has been strongly associated with insulin resistance,[25] and low levels of HDL-C were associated with an increase in blood glucose. In patients with type 2 diabetes mellitus, this relation (low HDL-C and high glucose) can modify 2 important mechanisms for glucose metabolism: insulin secretion by pancreatic B cells and glucose uptake independent of insulin.[27] These results show the possible utility of the TG/HDL ratio in detecting a population with high risk for developing different diseases. However, in each population it would be necessary to establish the values that could allow identifying the largest number of individuals at risk.

Uric acid was associated to all the studied variables except for TC (Table 6). Peng et al found it related to TG and HDL-C; they hypothesize that uric acid could play a very important role in lipid metabolism, insulin resistance, inflammation and, early-onset hypertension, highlighting a possible role of this variable in the metabolic syndrome[26]

Diastolic blood pressure was correlated with all biomarkers except TC and glucose. It is well known that blood pressure values are directly related to age, BMI and triglycerides; and, inversely with HDL-C levels.[28] High HDL-C levels could influence the reduction of DBP, since this lipoprotein promotes lipid catabolism and stimulates endothelial cells for the production of different molecules (anti-inflammatory, anti-apoptotic, anti-thrombotic, and nitric oxide agents),[29] which in turn, directly or indirectly, regulate the blood pressure.

Elevated TG levels were associated with an increase in glucose, TC, BMI, HDL-C, as well as systolic blood pressure and DBP. Dyslipidemia, diabetes, hypertension, as well as abdominal obesity are characteristics of the metabolic syndrome. This syndrome is a health problem in Mexico with a prevalence of 26.6% in adults over 20 years of age.[30]

High frequencies of these pathologies in our population demand for a timely diagnosis and an adequate treatment to avoid complications, such as ischemic heart disease, the main cause of death in Mexico. Unfortunately, the number of affected individuals continues growing.

4.4. Effect of the polymorphisms on biomarkers analyzed

We found that polymorphism rs934197 was associated with BMI. Individuals carrying T allele had lower BMI values than those with C allele. This variant is located in the promoter region and it has been reported that T allele induces a significant increase in the transcription of *APOB* gene[31]; and consequently, in both an increased very low-density lipoproteins secretion and a greater availability of fatty acids, which could be stored in adipocytes, thus favoring a rise in the BMI. Nevertheless, this is contrary to what was observed in the present study, therefore, in order to know the relationship between T allele and the reduction of BMI, further studies are required.

In the rs533617 polymorphism, subjects heterozygous displayed lower DBP than the wild homozygous. The association was evident since the 4 applied statistical tests were significant (Table 7 and 8). This polymorphism presents very low frequencies in the populations, which is why it has not been included in studies of association. However, the analysis with the PolyPhen-2 program predicted that the amino acid change generated by this polymorphism (H1923R) is harmful (score.997).

Polymorphism rs1042031 has been studied in several populations with diverse pathologies and it has also been associated with blood lipid levels, but the results have been controversial. These discrepancies could be caused by differences in ethnic background. Benn et al 2008 demonstrated that the A allele increases LDL-C catabolism,[32] which is congruent with our results. We found this allele to be associated with low cholesterol levels and several studies have revealed similar results with low levels of TC, LDL-C and TG.[32,33] In contrast, there are also diverse reports where this allele is related to high levels of TC, LDL-C, Lipoprotein (a) and HDL-C.[2,34] In addition, it has been observed as a risk factor for the development of CVD[35] and hypertension,[5] although it has been suggested that its association with these diseases is given through a pathway different from lipid metabolism.

In conclusion, all polymorphisms analyzed, except rs533517 and c.66_67insCTGCTG, showed high frequencies of the mutated allele, which may render them useful in association studies. Our results revealed that in the Mexican population rs934197-T, rs533517-G, and rs1042031-A alleles were associated with lower BMI, DBP, and TC, respectively. Therefore, common variants in the *APOB* gene could be contributing to the development of several chronic diseases, such as essential hypertension, dyslipidemias, obesity, among others, where the quantitative variables studied are relevant. However, specific studies with each pathology are needed to know the possible implications of such polymorphisms.

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Author contributions

Conceptualization: María Teresa Magaña Torres.
Data curation: Ana Gabriela Colima Fausto, Jaqueline Topete.
Formal analysis: Ana Gabriela Colima Fausto, Jaqueline Topete, María Teresa Magaña Torres.
Funding acquisition: María Teresa Magaña Torres.
Investigation: Juan Ramón González García, María Teresa Magaña Torres.
Methodology: Teresita de Jesús Hernández Flores, Sergio Yair Rodríguez Preciado, María Teresa Magaña Torres.
Project administration: María Teresa Magaña Torres.
Resources: María Teresa Magaña Torres.
Software: Sergio Yair Rodríguez Preciado.
Supervision: Juan Ramón González García, María Teresa Magaña Torres.
Validation: María Teresa Magaña Torres.
Visualization: Juan Ramón González García, María Teresa Magaña Torres.
Writing – original draft: Ana Gabriela Colima Fausto, Teresita de Jesús Hernández Flores, María Teresa Magaña Torres.
Writing – review & editing: Ana Gabriela Colima Fausto, Juan Ramón González García, Teresita de Jesús Hernández Flores, Sergio Yair Rodríguez Preciado, María Teresa Magaña Torres.

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