

# The ATP-Binding Cassette Transporter A1 R230C Variant Affects HDL Cholesterol Levels and BMI in the Mexican Population

## Association With Obesity and Obesity-Related Comorbidities

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Although ATP-binding cassette transporter A1 (ABCA1) is well known for its role in cholesterol efflux and HDL formation, it is expressed in various tissues, where it may have different functions. Because hypoalphalipoproteinemia is highly prevalent in Mexico, we screened the ABCA1 coding sequence in Mexican individuals with low and high HDL cholesterol levels to seek functional variants. A highly frequent nonsynonymous variant (R230C) was identified in low-HDL cholesterol but not in high-HDL cholesterol individuals ( $P = 0.00006$ ). We thus assessed its frequency in the Mexican-Mestizo general population, seeking possible

associations with several metabolic traits. R230C was screened in 429 Mexican Mestizos using Taqman assays, and it was found in 20.1% of these individuals. The variant was significantly associated not only with decreased HDL cholesterol and apolipoprotein A-I levels but also with obesity (odds ratio 2.527,  $P = 0.005$ ), the metabolic syndrome (1.893,  $P = 0.0007$ ), and type 2 diabetes (4.527,  $P = 0.003$ ). All of these associations remained significant after adjusting for admixture ( $P = 0.011$ ,  $P = 0.001$ , and  $P = 0.006$ , respectively). This is the first study reporting the association of an ABCA1 variant with obesity and obesity-related comorbidities as being epidemiologically relevant in the Mexican population. *Diabetes* 56:1881–1887, 2007

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ABCA1, ATP-binding cassette transporter A1; apo, apolipoprotein; ATP-III, National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, Adult Treatment Panel III; CHD, coronary heart disease; IDF, International Diabetes Federation; INCMNSZ, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán.

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Coronary heart disease (CHD) is one of the leading causes of death in developed countries (1) and in Mexico (2). HDL cholesterol plasma levels show a strong inverse relationship with CHD (3), and the cardioprotective effect of HDL cholesterol has been attributed mainly to the key role in reverse cholesterol transport (4). ATP-binding cassette transporter A1 (ABCA1) is a major determinant of plasma HDL cholesterol levels in humans (5). Homozygous or compound heterozygous ABCA1 mutations cause Tangier disease, an autosomal recessive disorder characterized by very low plasma HDL cholesterol levels, whereas heterozygous mutations cause the milder familial hypoalphalipoproteinemia (6–8). The identification of the defective Tangier disease gene as ABCA1 has contributed substantially to the understanding of its role as a key transporter of cellular cholesterol and phospholipids across cell membranes to acceptor molecules such as apolipoprotein (apo) A-I. Interestingly, ABCA1 is widely expressed throughout many animal tissues, where it may have multiple and diverse functions (5). In this regard, ABCA1 expression is strongly induced on differentiation of 3T3-L1 preadipocytes to mature adipocytes (9). ABCA1 has also been implicated in phenotypes such as Alzheimer's disease (10), type 2 diabetes (11), and Scott's syndrome (12). Under this premise, ABCA1 could play a role in traits other than HDL cholesterol levels.

Several groups have investigated whether common ABCA1 variants are associated with HDL cholesterol levels and CHD, with inconsistent results (13–16). More recently, subjects with the lowest and highest HDL levels were screened for single nucleotide polymorphisms and mutations by sequencing the entire ABCA1 coding region (17–19). Using this approach, ~10% of HDL-deficient Caucasian individuals were found to be heterozygous for ABCA1 mutations. Altogether, their findings suggest that both mutations and single nucleotide polymorphisms in ABCA1 contribute to variation in HDL cholesterol and apoA-I in the general population.

A nationwide survey revealed that the most frequent dyslipidemia in Mexican individuals is low HDL cholesterol plasma concentrations (20). In addition to hypoalphalipoproteinemia, type 2 diabetes and the metabolic syndrome are highly prevalent in this population (21). In an attempt to assess genetic factors that may contribute to the high prevalence of hypoalphalipoproteinemia in the Mexican population, we screened the coding sequence of ABCA1 in extreme phenotype groups (lowest and highest HDL cholesterol levels). A nonsynonymous sequence change (R230C) previously reported as a rare variant or mutation causing familial hypoalphalipoproteinemia in an Oji-Cree individual (22) was found to be strikingly common in Mexican individuals with low HDL cholesterol levels. This led us to screen for the presence of R230C and analyze its effect on HDL cholesterol levels and several other clinical/metabolic traits in the general population of Mexico City.

## RESEARCH DESIGN AND METHODS

### Extreme phenotype populations (low and high HDL cholesterol levels).

The initial study population included two groups of unrelated Mexican-Mestizo individuals attending the Endocrinology Laboratory of the Salvador Zubiran National Institute of Medical Sciences and Nutrition (INCMNSZ). The low-HDL cholesterol group included 40 individuals (22 female, 18 male) with HDL cholesterol levels <10th percentile according to age and sex in the Mexican population (20). Exclusion criteria for this group included type 2 diabetes, hypertriglyceridemia (triglyceride levels >150 mg/dl), obesity (BMI >30 mg/dl), smoking habit, use of lipid-lowering drugs, or other pathological conditions known for altering HDL cholesterol levels, such as nephropathy, liver disease, and thyroid disorders. The high-HDL cholesterol group included 34 individuals (19 female, 15 male) with HDL cholesterol levels >90th percentile according to age and sex in the Mexican population (20). Exclusion criteria for this group included alcoholism, treatment with steroids, cholestasis, nephropathy, or thyroid disorders.

**Mexican-Mestizo general population.** The Mexican-Mestizo group is the result of admixture between Native American and European (Spanish) populations, which occurred during the Spanish colony, with a much smaller contribution of African groups. Because over the last century Mexico City has been a site of massive immigration, receiving inhabitants from all around the country, this group may be considered as representative of the overall Mexican population. Only individuals born in Mexico whose parents and grandparents identified themselves as Mexican Mestizos were included in this group. The study population comprised 429 unrelated Mexican Mestizos (275 nonpregnant women and 154 men) working at four different governmental institutions in Mexico City (INCMNSZ, Manuel Velasco Suarez National Institute of Neurology and Neurosurgery, National Autonomous University of Mexico and Autonomous Metropolitan University-Iztapalapa) aged 20–69 years, including 34 previously undiagnosed type 2 diabetic patients (7.9%). Individuals on lipid-lowering drugs or with pathological conditions such as nephropathy, liver disease, and thyroid disorders were excluded from the study.

Questionnaires were used to obtain information on socioeconomic status, educational level, medical history, medication, and tobacco use. The vast majority of individuals were classified as middle socioeconomic status. Smoking was defined as current, former, or never. The educational level was classified into two categories: primary/secondary and preparatory/university. BMI was calculated as weight (in kg) divided by height (in m<sup>2</sup>). Weight, height, and waist circumference of participants were measured by trained personnel.

Obesity was defined as BMI  $\geq 30$  kg/m<sup>2</sup>. Hypertension was defined as systolic blood pressure >140 mmHg and/or a diastolic blood pressure >90 mmHg or the use of oral antihypertensive therapy (23).

**Amerindian populations.** The Amerindian populations currently residing in Mexico descend from pre-Hispanic Amerindians and have remained isolated since the Spanish colony. All Amerindian individuals and their ancestors (two generations) had been born in the same community and spoke their own native language. The study included 37 Yaquis from the state of Sonora in northern Mexico, 88 Mazahuas from the state of Mexico, 67 Teneek from San Luis Potosí in central Mexico, 35 Purépechas from the state of Michoacán in western Mexico, and 40 Mayans from the state of Yucatan, in southeastern Mexico.

This project was approved by the institutional committee of biomedical research in humans of INCMNSZ. All individuals gave written informed consent before their inclusion in the study. The local authorities of Amerindian populations gave their approval to participate in the study, and a translator was used as needed.

**Biochemical parameters.** The Endocrinology and Metabolism Department of INCMNSZ performed all biochemical laboratory measurements using standardized procedures. This laboratory is certified for standardization of tests by the External Comparative Evaluation of Laboratories Program of the College of American Pathologists. The measurements were performed with commercially available standardized methods in blood samples obtained after a 12-h fast. Glucose was measured using the glucose oxidase method, serum total cholesterol and triglycerides were measured using an enzymatic method, HDL cholesterol levels were assessed using phosphotungstic acid and Mg<sup>2+</sup>, plasma insulin was determined by radioimmunoassay, and plasma apoA-I and apoB were measured using a commercially available kit (Beckman). Homeostasis model assessment of insulin resistance was calculated from fasting glucose and insulin measures (24).

Type 2 diabetes was diagnosed according to World Health Organization criteria (25). We used two definitions of the metabolic syndrome. First, we used the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III [ATP-III]) (26,27) definition, by the presence of at least three or more of the following abnormalities: waist girth >102 cm in men or >88 cm in women, triglycerides  $\geq 150$  mg/dl or lipid-lowering treatment, HDL cholesterol <40 mg/dl in men or <50 mg/dl in women, blood pressure  $\geq 130/85$  mmHg or treatment with blood pressure-lowering medications, and fasting glucose  $\geq 100$  mg/dl or treatment for diabetes. Second, we used the International Diabetes Federation (IDF) (28) definition, using ethnicity-specific cutoff points for elevated waist circumference (>90 cm in men and >80 cm in women).

**DNA sequencing.** Genomic DNA was extracted from peripheral blood leukocytes using the salt-chloroform extraction method (29). The coding sequence and proximal promoter region of the ABCA1 gene of all individuals with extreme phenotypes (low and high HDL cholesterol) was amplified with specific oligonucleotides sets as previously described (22), and they were sequenced using ABI Prism BigDye Terminators version 3.1 on an ABI3100 automated sequencer according to the manufacturer's protocol (Applied Biosystems).

**R230C variant genotyping.** The general Mexican-Mestizo and Amerindian populations were screened for the R230C variant by TaqMan assay, and allelic discrimination was performed on an ABI Prism 7900HT sequence detection system (Applied Biosystems).

**Ancestry informative markers genotyping and ancestry estimation.** Because the Mexican-Mestizo population is admixed, it was necessary to analyze ancestry informative markers to assess whether any association could be confounded by population stratification. A panel of 10 ancestry informative markers (rs4884, rs2695, rs17203, rs2862, rs3340, rs722098, rs203096, rs223830, rs1800498, and rs281478) distinguishing mainly Amerindian and European ancestry ( $\delta > 0.44$ ) was screened in extreme phenotype and general Mexican-Mestizo populations (30,31). Genotyping was performed by KBiosciences (Hertfordshire, U.K.) using a KASPar assay system. Genotyping call rates of each ancestry informative marker exceeded 95%, and no discordant genotypes were observed in 47 duplicate samples.

No ancestry informative marker showed significant departure from Hardy-Weinberg equilibrium. The mean admixture proportions of the population were estimated as 69.9 (95% CI 67.2–72.5) Amerindian, 25.1 (22.3–27.8) European, and 4.97 (3.89–6.12) African. Although Amerindian ancestry was associated with obesity and the metabolic syndrome, these associations were not statistically significant (odds ratio [OR] 2.886, 95% CI 0.542–14.440; and 1.340, 0.79–2.492; respectively).

**Statistical analysis.** All calculations were performed using SPSS version 10.0 (SPSS, Chicago, IL) statistical package. Means  $\pm$  SD and frequencies of baseline characteristics were calculated. Because fasting serum insulin and triglyceride levels as well as homeostasis model assessment of insulin

TABLE 1  
R230C genotype and allele frequencies in Mexican individuals with hypo- and hyperalphalipoproteinemia

	Low HDL cholesterol	High HDL	<i>P</i>
Genotype			
R230R	22 (55.0)	33 (97.1)	0.00006*
R230C	15 (37.5)	1 (2.90)	—
C230C	3 (7.5)	0	—
Allele			
R230	59 (73.8)	67 (98.5)	0.00002
C230	21 (26.2)	1 (1.5)	—

Data are *n* (%). \**P* value comparing R230C/C230C vs. R230R.

resistance indexes were not normally distributed, they were log-transformed for analysis, but geometric means were presented in Table 2. ANCOVA was used to construct a model for quantitative traits. Age, sex, smoking, educational level, plasma triglyceride levels, BMI, HDL cholesterol, apoA-I, and type 2 diabetes were included as covariates, when appropriate, and genotype was included as a fixed factor in the model (univariate general linear model).

The AdmixMap program was used to test the possible effect of population stratification on associations with obesity, the metabolic syndrome, and type 2 diabetes (32,33). Because the Mexican-Mestizo population derived mainly from Amerindian and European (Spanish) populations, the model used included two primary parental populations. AdmixMap fits a logistic regression model of the trait on individual admixture, and it allows the inclusion of covariates such as age, sex, BMI, HDL, apoA-I, and other potential confounders. Previously reported Amerindian and European ancestral allele frequencies were used for the analysis (30,31).

Differences in allele and genotype frequencies among Mexican populations and Hardy-Weinberg equilibrium were tested by  $\chi^2$  test.

## RESULTS

**Association with hypoalphalipoproteinemia.** R230C genotype frequencies according to HDL levels (low-HDL cholesterol group [ $\leq 10$ th percentile] and high-HDL cholesterol group [ $\geq 90$ th percentile]) are given in Table 1. R230C/C230C genotypes were significantly more frequent in the low-HDL cholesterol than the high-HDL cholesterol group (45 vs. 2.9%,  $P = 0.00006$ ,  $P = 0.0005$  after adjusting for admixture).

**Association with HDL/apoA-I levels and BMI in the general population.** The relationship of the R230C variant with anthropometric and biochemical measurements is shown in Table 2. Significantly lower HDL cholesterol and apoA-I levels were observed in individuals with R230C/C230C genotypes ( $44.4 \pm 11.1$  and  $131.9 \pm 24.4$  mg/dl, respectively) as compared with those with the R230R genotype ( $48.7 \pm 13.8$  and  $141.1 \pm 23.8$  mg/dl,  $P = 0.024$  and  $0.001$ , respectively). Interestingly, R230C/C230C individuals displayed higher average BMI and waist measurements ( $29.3 \pm 6.4$  kg/m<sup>2</sup> and  $93.1 \pm 14.5$  cm, respectively) than R230R individuals ( $27.1 \pm 5.3$  and  $90.1 \pm 13.1$  cm,  $P = 0.005$  and  $0.048$ , respectively). The inverse correlation between BMI and HDL cholesterol/apoA-I levels (also observed in this population) is well known. Because both parameters showed association with the R230C variant, we additionally adjusted all other parameters for HDL cholesterol and apoA-I levels. Although the significance of the association with waist circumference dropped to  $P = 0.182$ , the association with BMI remained significant after this adjustment ( $P = 0.019$ ). Moreover, including only individuals with apoA-I levels  $>130$  mg/dl (mean apoA-I levels of R230C carriers), the association with higher BMI remained significant ( $P = 0.018$ ).

TABLE 2  
Clinical and biochemical parameters in R230C carriers and noncarriers in the general population of Mexico City

Characteristic	R230R	R230C/C230C	<i>P</i> *
<i>n</i>	343	86	—
Males	37.0	31.5	—
Age (years)	40.1 $\pm$ 12.8	40.2 $\pm$ 10.5	0.767
Smoker	26.3	28.4	0.586 <sup>†</sup>
Menopause	8.9	7.4	0.717 <sup>†</sup>
BMI (kg/m <sup>2</sup> )	27.1 $\pm$ 5.3	29.3 $\pm$ 6.4	0.005
Waist (cm)	90.1 $\pm$ 13.1	93.1 $\pm$ 14.5	0.048
Obesity	38.5	64.8	0.003 <sup>†</sup>
Diabetes	5.8	16.3	0.003 <sup>†</sup>
SBP (mmHg)	119.4 $\pm$ 18.9	119.6 $\pm$ 15.6	0.924
DBP (mmHg)	79.3 $\pm$ 10.4	80.0 $\pm$ 11.7	0.738
Fasting glucose (mg/dl)	97.3 $\pm$ 33.7	105.0 $\pm$ 43.5	0.346
Fasting insulin ( $\mu$ U/ml)	10.5 $\pm$ 7.6	11.6 $\pm$ 8.0	0.674
HOMA-IR	2.65 $\pm$ 3.3	3.0 $\pm$ 2.5	0.712
Cholesterol (mg/dl)	211.0 $\pm$ 42.9	207.2 $\pm$ 41.1	0.803
Triglycerides (mg/dl)	184.7 $\pm$ 140.6	191.2 $\pm$ 141.9	0.913
HDL cholesterol (mg/dl)	48.7 $\pm$ 13.8	44.4 $\pm$ 11.1	0.024
ApoA-I (mg/dl)	141.1 $\pm$ 23.8	131.9 $\pm$ 24.4	0.001
ApoB (mg/dl)	112.7 $\pm$ 28.9	112.9 $\pm$ 28.3	0.929

Data are the means  $\pm$  SE or %. *N* = 429. \**P* value comparing R230C/C230C vs. R230R, adjusted for age, sex, BMI, triglyceride levels, smoking, and educational level; <sup>†</sup>Fisher's exact two-tailed test. DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment of insulin resistance; SBP, systolic blood pressure.

**Association with obesity and obesity-related comorbidities.** Table 3 shows the genotype distribution in obese (BMI  $\geq 30$  kg/m<sup>2</sup>) and nonobese individuals (BMI  $< 25$  kg/m<sup>2</sup>). R230C/C230C genotypes were associated with an increased risk of obesity (OR 2.527, 95% CI 1.667–3.819,  $P = 0.005$ ). This association remained significant after adjusting for admixture (2.428, 1.548–3.706,  $P = 0.011$ ). In addition, HDL and apoA-I serum levels were not significantly different in R230C/C230C obese and non-R230C obese individuals ( $42.9 \pm 10.3$  and  $134.9 \pm 20.3$  mg/dl vs.  $43.7 \pm 8.5$  and  $135.7 \pm 27.2$  mg/dl,  $P = 0.380$  and  $0.242$ , respectively).

Disturbed lipoprotein metabolism as low HDL cholesterol concentrations is a key feature of the metabolic syndrome. The R230C genotype distribution in subjects with and without metabolic syndrome is presented in Table 3, which includes 86 more metabolic syndrome case and control subjects not previously included in the general Mestizo population. R230C/C230C genotypes were significantly more frequent in metabolic syndrome than in non-metabolic syndrome subjects according to both ATP-III criteria (OR 1.893, 95% CI 1.483–2.460,  $P = 0.0007$ ) and IDF criteria (1.775, 1.370–2.336,  $P = 0.003$ ). The association of the R230C variant with the metabolic syndrome remained significant after adjusting for admixture under both criteria (1.833, 1.405–2.425,  $P = 0.001$ ; and 1.745, 1.328–2.298,  $P = 0.005$ , respectively).

Because the R230C variant was more frequent in diabetic individuals in the overall population, we compared the R230C genotype distribution in type 2 diabetic and non-type 2 diabetic subjects. Because the mean age at diagnosis in diabetic patients was  $44.7 \pm 9.75$  years, we included only control subjects aged  $>50$  years. R230C/C230C genotypes were significantly more frequent in type 2 diabetic than in non-type 2 diabetic individuals (41.2 vs.

TABLE 3  
Association of the R230C variant in the ABCA1 gene with obesity and the metabolic syndrome

Metabolic trait	Genotype		OR	P
	R230R	R230C/C230C		
Obese subjects	84 (71.2)	34 (28.8)	2.527	0.005*
Nonobese	139 (86.8)	21 (13.2)	—	—
Metabolic syndrome subjects‡	143 (73.7)	51 (26.3)	1.893	0.0007†
Non-metabolic syndrome	268 (84.8)	48 (15.2)	—	—

Data are n (%). \*P value adjusted for age, sex, smoking, HDL cholesterol, ApoA-I, educational level, and type 2 diabetes; †P value adjusted for age, sex, smoking, and educational level; ‡according to ATP-III criteria. The associations remained significant after adjusting for admixture (P = 0.011 and 0.001 for obesity and metabolic syndrome, respectively).

11.1%), showing a 4.527-fold increased risk for type 2 diabetes (95% CI 2.474–8.499, P = 0.003, adjusted for sex, BMI, HDL cholesterol, apoA-I, and educational level). The association with type 2 diabetes remained significant after adjusting for admixture (P = 0.006). Moreover, although BMI was significantly higher in R230C than in non-R230C diabetic individuals (32.3 ± 4.8 vs. 27.8 ± 5.1 kg/m<sup>2</sup>, P = 0.048), HDL and apoA-I serum levels showed no significant differences between these groups (P = 0.262 and 0.082, respectively). Genotype frequencies did not significantly deviate from the Hardy-Weinberg equilibrium in any group.

**Genotypic and allelic frequencies in Mexican-Mestizo and Amerindian populations.** Allele and genotype frequencies of the R230C variant in Mexican-Mestizos and five Mexican Amerindian populations are shown in Table 4. R230C genotype frequencies did not significantly deviate from Hardy-Weinberg equilibrium in any group, except in Yaquis, where an excess of C230C homozygotes was found. ABCA1 C230 allele frequencies were significantly higher in all Mexican Amerindians (except Mazahuas) than in Mexican Mestizos (P < 0.02).

**DISCUSSION**

Although the role of ABCA1 in cholesterol efflux and HDL cholesterol formation is well known, the understanding of its role in other cell types is still limited. The high frequency of a probably functional ABCA1 variant (R230C) in the Mexican population provides a unique opportunity to assess associations with HDL cholesterol and apoA-I levels, as well as with other clinical/metabolic traits. The R230C variant has not been functionally tested, so the marker under study may be in linkage disequilibrium with a causative genomic variant within or proximal to the study locus. However, data from the PANTHER (Protein Analysis Through Evolutionary Relationships) database predict that the probability of functional impairment is 78%, according to the substitution position-specific evolutionary conservation score (34), and the Polyphen pro-

gram predicts the variant as possibly damaging. Other facts strongly suggest that the variant is functional: 1) R230C occurs at the first extracellular loop, where Tangier and familial hypoalphalipoproteinemia mutations are clustered (35); 2) the arginine at position 230 is conserved between species; and 3) the nature of the amino acids involved is very different: whereas arginine is basic, positively charged, and hydrophilic, cysteine is hydrophobic and contains a sulfhydryl group. Data from our analysis in a sample of the general population of Mexico City strongly suggest that this variant has a role not only in cholesterol efflux (lowering HDL cholesterol levels) but also in metabolic functions other than HDL cholesterol formation.

**HDL cholesterol and apoA-I levels.** The effect of ABCA1 mutations on HDL cholesterol serum concentrations is well known, and R230C exerted a modest but clear HDL cholesterol/apoA-I–decreasing effect. We infer that R230C affects cholesterol efflux in HDL-forming cells because it was significantly associated with decreased HDL cholesterol and apoA-I levels, regardless of age, sex, smoking, BMI, triglyceride levels, and educational level. In agreement with our finding, a linkage study seeking quantitative trait loci for HDL cholesterol levels in Mexican Americans reported a modest peak (logarithm of odds [LOD] score 1.4) on chromosome 9q, near marker D9S299 located very close to the ABCA1 locus (36). Although we found a few other possibly damaging ABCA1 coding sequence variants in the initial group of patients with the lowest 10% HDL cholesterol levels (data not shown), each variant has been found in only one patient. Thus, the high frequency of R230C in Mexicans may be responsible for this modest peak.

R230C does not seem to abolish cholesterol efflux in HDL-forming cells, but it could be a functional variant. The bases for this statement are 1) not one of the eight C230C homozygotes had a Tangier phenotype and 2) not all heterozygotes or homozygotes had low HDL cholesterol levels (<40 mg/dl). Because >50 different genes are involved in regulating HDL cholesterol (37), the presence of

TABLE 4  
R230C genotype and allele frequencies in Amerindian populations of Mexico

Group	n	Genotype			Allele		P*
		R230R	R230C	C230C	R230	C230	
Yaqui	37	0.703	0.189	0.108	0.797	0.203	0.013
Teenek	67	0.671	0.299	0.030	0.821	0.179	0.015
Mazahua	88	0.818	0.170	0.011	0.903	0.097	NS
Purépecha	35	0.629	0.314	0.057	0.786	0.214	0.007
Mayan	40	0.450	0.525	0.025	0.718	0.288	0.00001
Mestizos	429	0.799	0.183	0.018	0.891	0.109	—

\*P value when compared with allelic frequencies of the Mestizos.

functional variants in others genes (such as cholesterol ester transfer protein or hepatic lipase) could increase HDL cholesterol levels, and this may explain why one R230C heterozygote was found in the high-HDL cholesterol group (15). Moreover, HDL cholesterol and apoA-I levels were very similar in homo- and heterozygotes, showing that the effect of this variant on HDL cholesterol levels is not dose dependent. This is consistent with previous observations that ABCA1 gene mutations affecting HDL cholesterol levels in humans may cause only dominant-negative phenotypes with no gene dosage effect because of the quaternary structure of ABCA1, in which alterations by gene defects affect the minimum functional unit (38).

**Obesity and obesity-related comorbidities.** The association of R230C with BMI in Mexican Mestizos was more significant than that observed for lowering HDL cholesterol levels. To our knowledge, this is the first time the ABCA1 gene has been associated with obesity. Once again in agreement with our results, a linkage study of obesity in Mexican Americans revealed a peak (LOD score 2.1) on chromosome 9p with the same marker (D9S299) previously linked to HDL cholesterol levels, very close to the ABCA1 gene locus (36,39). In addition, when individuals with apoA-I levels <130 mg/dl (mean apoA-I levels of R230C carriers) were excluded from the analysis, the association remained significant, strongly suggesting that the role of ABCA1 in the pathophysiology of obesity is independent of its role in regulating HDL cholesterol and apoA-I levels.

Information on the function of ABCA1 in the adipocyte is still limited. Le Lay et al. (9) analyzed ABCA1 gene expression and regulation in 3T3-L1 adipocytes, finding a strong induction of ABCA1 during preadipocyte differentiation and evidence suggesting the presence of adipocyte-specific mechanisms regulating ABCA1 at the posttranscriptional level. In addition, although no changes in total cholesterol content were observed, there was a shift in cholesterol intracellular distribution during differentiation. The authors suggest that the low ability of adipocytes for cholesterol efflux fits with cholesterol being a signaling molecule in fat cells. Further studies are required to understand the role of ABCA1 and the R230C variant in adipocytes.

Obesity is a risk factor for several other metabolic traits (40). ABCA1 has been proposed as a candidate gene for the metabolic syndrome because of evidence suggesting associations with insulin resistance in addition to HDL cholesterol levels and cardiovascular risk (41). In addition to these previously described associations, we found a significant association of R230C with obesity, and thus we sought an association with the metabolic syndrome. This association was significant even after adjusting for age, sex, and educational level. Interestingly, although 21.7% of R230C heterozygotes had metabolic syndrome, all eight C230C homozygotes met both IDF and ATP-III criteria for metabolic syndrome. Although the number of homozygotes was reduced, this suggests an allele dose defect for this trait.

We also found a significant association of R230C with type 2 diabetes in the Mexican-Mestizo population. Although this result should be interpreted with caution because of the low number of diabetic individuals included ( $n = 34$ ), it is not unreasonable to speculate on a possible role of ABCA1 in the pathogenesis of type 2 diabetes. ABCA1 is regulated by a transcriptional regula-

tory network including several proteins and drugs involved in lipid and glucose metabolism (42,43), and insulin is known to downregulate ABCA1 expression in vitro, whereas glucose upregulates ABCA1 expression in leukocytes in vivo (44,45). There is one study reporting association of ABCA1 with type 2 diabetes, where an at-risk diplotype was found in the Japanese population (11). Because serum HDL cholesterol levels were similar in the at-risk and non-at-risk groups, the authors suggested that ABCA1 may have influence on the pathophysiology of type 2 diabetes independent of serum HDL cholesterol levels. Interestingly, in a recent study, mice in which ABCA1 was specifically inactivated in pancreatic  $\beta$ -cells displayed markedly impaired glucose tolerance and defective glucose-stimulated insulin secretion in vivo and in vitro (46). However, this association deserves further analysis under a carefully controlled design before drawing conclusions. The high frequencies of type 2 diabetes and R230C in the Mexican-Mestizo population provide a unique opportunity to more thoroughly analyze this association in the diabetic population.

Because the Mexican-Mestizo population is admixed, it was necessary to determine whether population stratification could have affected our findings. Although we only applied 10 ancestry informative markers to obtain admixture estimates, the results of the group admixture estimates from our Mexican-Mestizo general population cohort agree with the results in a previous admixture study in Mexico City (47). Even though Amerindian ancestry may be considered a risk factor for these metabolic traits, the associations of R230C with obesity, the metabolic syndrome, and type 2 diabetes remained significant after adjusting for admixture. Thus, it appears that ABCA1 R230C or some other variant in linkage disequilibrium with it represents a significant risk factor for low HDL levels, obesity, and obesity-related comorbidities.

**High prevalence of R230C in Amerindian populations.** It has been suggested that genetic susceptibility to obesity and type 2 diabetes in Mexicans is probably related to their Amerindian heritage (40,48). The high frequency of R230C in the Mexican population suggests that this could be one of several gene variants contributing to this genetic susceptibility. In most Mexican Amerindian populations analyzed, the allele and genotype frequencies of R230C were approximately twofold higher than in Mexican Mestizos, as would be expected because of the admixture. Interestingly, R230C seems to be found exclusively in Amerindian and Amerindian-derived populations such as Mexican Mestizos. It has not been found in African, European, Chinese, South Asian, or Inuit populations (17–19,22). The variant was first reported in an Oji-Cree individual with familial hypoalphalipoproteinemia, and because it was found in 2 of 80 more Oji-Crees, the authors suggested it arose recently in that population (22). Nevertheless, the notoriously higher frequency of R230C in Amerindians of Mexico suggests, first, a much more remote origin, possibly among the first humans crossing the Bering Strait, and, second, that R230C may have somehow been selected in Amerindians. Among the possible selective advantages are 1) R230C could be an energy-saving allele favorable in famine or insufficient food availability and 2) based on the finding that a homozygous ABCA1 gene deletion confers complete resistance against cerebral malaria in mice (49), it is possible to speculate that R230C could also confer protection against certain infectious and/or thrombotic disorders involving vesiculation. Fur-

ther population genetics and functional studies are needed to confirm this.

In conclusion, the R230C variant is apparently a marker informative for Amerindian ancestry, which is also significantly associated with low HDL cholesterol levels, obesity, and obesity-related comorbidities, although further studies are required to confirm these associations. Functional studies both in vitro and in vivo are required to further understand the role of ABCA1 and the R230C variant in these metabolic traits.

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#### APPENDIX

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Electronic database information for KBiosciences can be found online at <http://www.kbioscience.co.uk>, for ADMIXMAP at <http://www.ucd.ie/genepi/software.html>, and for Polyphen at <http://genetics.bwh.harvard.edu/pph>.

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