The ABCA1 R230C Variant Affects HDL-cholesterol Levels and Body Mass Index in the Mexican Population: Association with Obesity and Obesity-Related Comorbidities

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Short running title: ABCA1 gene variant is associated with obesity

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ABSTRACT

Although the ATP binding cassette transporter 1 (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-C levels to seek functional variants. A highly frequent non-synonymous variant (R230C) was identified in low HDL-C but not in high HDL-C 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 was found in 20.1% of these individuals. The variant was significantly associated not only with decreased HDL-C and Apo A-I levels, but also with obesity (OR=2.527; *P*=0.005), the metabolic syndrome (OR=1.893; *P*=0.0007) and type 2 diabetes (OR=4.527; *P*=0.003). All these associations remained significant after adjusting for admixture (*P*=0.011, *P*=0.001, *P*=0.006, respectively). This is the first study reporting the association of an ABCA1 variant with obesity and obesity-related comorbidities, being epidemiologically relevant in the Mexican population.

Coronary heart disease (CHD) is one of the leading causes of death in developed countries (1) and in Mexico (2). HDL cholesterol (HDL-C) plasma levels show a strong inverse relationship with coronary heart disease (CHD) (3), and the cardioprotective effect of HDL-C has been attributed mainly to the key role in reverse cholesterol transport (RCT) (4). The ATP-binding cassette transporter A1 (ABCA1) is a major determinant of plasma HDL-C levels in humans (5). Homozygous or compound heterozygous ABCA1 mutations cause Tangier disease, an autosomal recessive disorder characterized by very low plasma HDL-C levels, whereas heterozygous mutations cause the milder familial hypoalfalipoproteinemia (FHA) (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 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 upon differentiation of 3T3-L1 pre-adipocytes to mature adipocytes (9). ABCA1 has also been implicated in phenotypes such as Alzheimer Disease (10), type 2 diabetes (T2D) (11) and Scott Syndrome (12). Under this premise, ABCA1 could play a role in traits other than HDL-C levels.

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

A nationwide survey revealed that the most frequent dyslipidemia in Mexican individuals is low HDL-C plasma concentrations (20). In addition to hypoalphalipoproteinemia, T2D and the metabolic syndrome (MS) 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-C levels). A non-synonymous sequence change (R230C) previously reported as a rare variant or mutation causing FHA in an Oji-Cree individual (22) was found to be strikingly common in Mexican individuals with low HDL-C levels. This led us to screen for the presence of R230C and analyze its effect on HDL-C 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-C levels)

The initial study population included two groups of unrelated Mexican Mestizo individuals attending the Endocrinology Laboratory of the Instituto Nacional de Ciencias

Médicas y Nutrición Salvador Zubirán (INCMNSZ). The low HDL-C group included 40 individuals (22 female; 18 male) with HDL-C levels below the 10^{th} percentile according to age and gender in the Mexican population (20). Exclusion criteria for this group included: T2D, hypertriglyceridemia (TG levels > 150 mg/dl), obesity (BMI > 30 mg/dl), smoking habit, use of lipid lowering drugs, or other pathological conditions known for altering HDL-C levels such as nephropathy, liver disease and thyroid disorders. The high HDL-C group included 34 individuals (19 female; 15 male) with HDL-C levels above the 90^{th} percentile according to age and gender 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 non-pregnant women and 154 men) working at 4 different governmental institutions in Mexico City (INCMNSZ, Instituto Nacional de Neurología y Neurociencias Manuel Velasco Suarez, Universidad Nacional Autónoma de México, and Universidad Autónoma Metropolitana-Iztapalapa) aged 20 to 69 years, including 34 previously undiagnosed T2D 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 was classified as middle socio-economical status. Smoking was defined as current, former, or never. The educational level was classified into two categories: primary/secondary and preparatory/university. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters squared). Weight, height and waist circumference of participants were measured by trained personnel. Obesity was defined as BMI $\geq$30 kg/m^2. 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 located in Northern Mexico; 88 Mazahuas from the state of Mexico and 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 South-eastern Mexico.

This project was approved by the Institutional Committee of Biomedical Research in Humans of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ). All individuals gave written informed consent prior to 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 the 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-hour fast. Glucose was measured using the glucose oxidase method; serum total cholesterol and triglycerides were measured using an enzymatic method; HDL-C levels were assessed using phosphotungstic acid and $Mg2^{+}$; plasma insulin was determined by radioimmunoassay; and plasma Apo A-I and Apo B were measured using a commercially available kit (Beckman). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from fasting glucose and insulin measures (24).

T2D was diagnosed according to World Health Organization criteria (25). We examined two definitions of the metabolic syndrome (MS): 1) 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), 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 ≥150 mg/dl or lipid-lowering treatment, HDL cholesterol <40mg/dl in men or <50 mg/dl in women, blood pressure ≥130/85 mmHg or treatment with blood pressure-lowering medications, and fasting glucose ≥ 100 mg/dl or treatment for diabetes. 2) International Diabetes Federation (IDF) (28), 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-C) was amplified with specific oligonucleotides sets as previously described (22), and 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 the TaqMan assay and allelic discrimination performed on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems).

Ancestry Informative Markers (AIMs) Genotyping and Ancestry Estimation

Because the Mexican mestizo population is admixed, it was necessary to analyze AIMs to assess whether any association could be confounded by population stratification. A panel of 10 AIMs (rs4884, rs2695, rs17203, rs2862, rs3340, rs722098, rs203096, rs223830, rs1800498 and rs281478) distinguishing mainly Amerindian and European ancestry (δ>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 AIM exceeded 95%, and no discordant genotypes were observed in 47 duplicate samples.

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

Statistical Analysis

All calculations were performed using the SPSS v10.0 (SPSS Chicago, Il) statistical package. Means $\pm$ SD and frequencies of baseline characteristics were calculated. Since fasting serum insulin and triglyceride levels as well as HOMA-IR indices were not normally distributed, they were log-transformed for analysis, but geometric means were presented in results tables. Analysis of covariance was used to construct a model for quantitative traits. Age, gender, smoking, educational level, plasma triglyceride levels, BMI, HDL-C, Apo A-I and type 2 diabetes (T2D) were included as covariates, when appropriate; and genotype was included as a fixed factor in the model (GLM Univariate).

The ADMIXMAP program was used to test the possible effect of population stratification on associations with obesity, the metabolic syndrome and T2D (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 upon individual admixture, and allows the inclusion of covariates such age, sex, BMI, HDL, Apo A-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 the X^2 test.

RESULTS

Association with hypoalphalipoproteinemia

R230C genotype frequencies according to HDL levels (low HDL-C group [≤10 percentile] and high HDL group [≥90 percentile]) are given in Table 1. R230C/C230C genotypes were significantly more frequent in the low HDL-C than the high HDL-C group (45% vs. 2.9%, *P*=0.00006,

P=0.0005 after adjusting for admixture).

Association with HDL/Apo A-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-C and Apo A-I levels were observed in individuals with R230C/C230C genotypes (44.4 ± 11.1 mg/dl and 131.9 ± 24.4 mg/dl, respectively) as compared to those with the R230R genotype (48.7 ± 13.8 mg/dl and 141.1 ± 23.8 mg/dl; *P*=0.024 and 0.001, respectively). Interestingly, R230C/C230C individuals displayed higher average BMI and waist measurements (29.3 ± 6.4 kg/m^2 and 93.1 ± 14.5 cm, respectively) than R230R individuals (27.1 ± 5.3 and 90.1 ± 13.1 cm; *P*=0.005 and 0.048, respectively). The inverse correlation between BMI and HDL-C/Apo A-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-C and Apo A-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 Apo A-I levels > 130 mg/dl (mean Apo A-I levels of R230C carriers), the association with higher BMI remained significant (*P*=0.018).

Association with Obesity and Obesity-related Comorbidities

Table 3 shows the genotype distribution in obese (BMI ≥30 kg/m^2) and non-obese individuals (BMI <25 kg/m^2). 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 (OR=2.428; 95% CI 1.548-3.706; *P*=0.011). In addition, HDL and Apo A-I serum levels were not significantly different in R230C/C230C obese and non-R230C obese individuals (42.9 ± 10.3 mg/dl and 134.9 ± 20.3 mg/dl vs. 43.7 ± 8.5 mg/dl and 135.7 ± 27.2 mg/dl; *P*=0.380 and 0.242, respectively).

Disturbed lipoprotein metabolism as low HDL-C concentrations is a key feature of the metabolic syndrome. The R230C genotype distribution in subjects with and without MS is presented in Table 3, which includes 86 more MS cases and controls not previously included in the general Mestizo population. R230C/C230C genotypes were significantly more frequent in MS than in non-MS subjects according to both ATP-III criteria (OR=1.893; 95% CI 1.483-2.460; *P*=0.0007) and IDF criteria (OR=1.775; 95% CI 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 (OR=1.833; 95% IC 1.405-2.425; *P*=0.001; and 1.745; 95% IC 1.328-2.298; *P*=0.005, respectively).

Because the R230C variant was more frequent in diabetic individuals of the overall population, we compared the R230C genotype distribution in T2D and non-T2D subjects. Since the mean age at diagnosis in diabetic patients was 44.7 ± 9.75 years, we included only controls aged above 50 years. R230C/C230C genotypes were significantly more frequent in T2D than in non-T2D individuals (41.2%

vs. 11.1%), showing a 4.527 fold increased risk for T2D (95% CI 2.474-8.499; *P*=0.003 adjusted for sex, BMI, HDL-C, Apo A-I and educational level). The association with T2D remained significant after adjusting for admixture (*P*=0.006). Moreover, while BMI was significantly higher in R230C than in non-R230C diabetic individuals (32.3 ± 4.8 vs. 27.8 ± 5.1 Kg/m^2; *P*=0.048), HDL and Apo A-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 5 Mexican Amerindian populations are shown in Table 4. R230C genotype frequencies did not significantly deviate from the 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-C formation is well known, the understanding of its role in other cell types is still limited. The high frequency of a functional ABCA1 variant (R230C) in the Mexican population provides a unique opportunity to assess associations with HDL-C and Apo A-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 database predict that the probability of functional impairment according to the substitution position-specific evolutionary conservation score is 78% (34), and the Polyphen program 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 FHA mutations are clustered (35); 2) the arginine at position 230 is conserved between species; 3) the nature of the aminoacids involved is very different: while arginine is basic, positively charged and hydrophilic, cysteine is hydrophobic and contains a sulfhydril 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-C levels), but also in metabolic functions other than HDL-C formation.

HDL-C and Apo A-I levels

The effect of ABCA1 mutations on HDL-C serum concentrations is well known, and R230C exerted a modest but clear HDL-C/Apo A-I-decreasing effect. We infer that R230C affects cholesterol efflux in HDL-forming cells because it was significantly associated with decreased HDL-C and Apo A-I levels regardless of age, gender, smoking, BMI, triglyceride levels and educational level. In agreement with our finding, a linkage study seeking quantitative trait loci (QTL) for HDL-C levels in Mexican Americans reported a modest peak (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-C 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 could be 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-C levels (<40 mg/dl). Because more than 50 different genes are involved in regulating HDL-C (37), the presence of functional variants in others genes (such as cholesterol ester transfer protein or hepatic lipase) could increase HDL-C levels, and may explain why one R230C heterozygote was found in the high HDL-C levels among individuals bearing ABCA1 mutations in a heterozygous form (15). Moreover, HDL-C and Apo A-I levels were very similar in homo and heterozygotes, showing that the effect of this variant on HDL-C levels is not dose-dependent. This is consistent with previous observations that ABCA1 gene mutations affecting HDL-C levels in humans may cause only dominant negative phenotypes with no gene dosage effect, due to 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 the observed for lowering HDL-C levels. To our knowledge, this is the first time the ABCA1 gene is 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-C levels, very close to the ABCA1 gene locus (36, 39). In addition, when individuals with Apo A-I levels < 130 mg/dl (mean Apo A-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 independently of its role in regulating HDL-C and Apo A-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 pre-adipocyte differentiation and evidence suggesting the presence of adipocyte-specific mechanisms regulating ABCA1 at the posttranscriptional level. In addition, while 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-C levels and cardiovascular risk (41). In addition to these previously described associations, we found a significant association of R230C with obesity, and thus sought an association with the MS. This association was significant even after adjusting for age, gender and educational level. Interestingly, while 21.7% of R230C heterozygotes had MS, all 8 C230C homozygotes met both IDF and ATP-III criteria for MS. Although the number of homozygotes is reduced, this suggests an allele dose defect for this trait.

We also found a significant association of R230C with T2D in the Mexican Mestizo population. Although 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 T2D. ABCA1 is regulated by a transcriptional regulatory network including several proteins and drugs involved in the lipid and glucose metabolism (42, 43), and insulin is known to downregulate ABCA1 expression *in vitro*, while glucose upregulates ABCA1 expression in leukocytes *in vivo* (44, 45). There is one study reporting association of ABCA1 with T2D, where an at-risk diplotype was found in the Japanese population (11). Because serum HDL-C levels were similar in the at-risk and non-at risk groups, the authors suggested that ABCA1 may have influence on the pathophysiology of T2D independently of serum HDL-C 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 T2D 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 AIMs 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 T2D 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 level, obesity and obesity-related comorbidities.

High Prevalence of R230C in Amerindian Populations

It has been suggested that genetic susceptibility to obesity and T2D 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 2-fold

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 FHA, 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 firstly, a much more remote origin, possibly among the first humans crossing the Bëring Strait; and secondly, 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. Further 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-C 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: Kbiosciences (http://www.kbioscience.co.uk), ADMIXMAP (http://www.ucd.ie/genepi/software.html), Polyphen (http://genetics.bwh.harvard.edu/pph).

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Table 1. R230C genotype and allele frequencies in Mexican individuals with hypo and hyper-alphalipoproteinemia.

	Low HDL-C *n* (%)	High HDL *n* (%)	*P*
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)	

**P*-value comparing R230C/C230C vs. R230R.

Table 2. Clinical and biochemical parameters in R230C carriers and non-carriers in the general population of Mexico City.

Characteristic	R230R	R230C/C230C	*P**
Number (429)	343	86	
Males (%)	37.0	31.5	
Age (years)	40.1 ± 12.8	40.2 ± 10.5	0.767
Smoker (%)	26.3	28.4	0.586†
Menopause (%)	8.9	7.4	0.717†
BMI (kg/m^2)	27.1 ± 5.3	29.3 ± 6.4	0.005
Waist (cm)	90.1 ± 13.1	93.1 ± 14.5	0.048
Obesity (%)	38.5	64.8	0.003†
Diabetes mellitus (%)	5.8	16.3	0.003†
SBP (mmHg)	119.4 ± 18.9	119.6 ± 15.6	0.924
DBP (mmHg)	79.3 ± 10.4	80.0 ± 11.7	0.738
Fasting glucose (mg/dl)	97.3 ± 33.7	105.0 ± 43.5	0.346
Fasting insulin (μU/ml)	10.5 ± 7.6	11.6 ± 8.0	0.674
HOMA-IR	2.65 ± 3.3	3.0 ± 2.5	0.712
Cholesterol (mg/dl)	211.0 ± 42.9	207.2 ± 41.1	0.803
Triglycerides (mg/dl)	184.7 ± 140.6	191.2 ± 141.9	0.913
HDL-C (mg/dl)	48.7 ± 13.8	44. 4 ± 11.1	0.024
ApoA-I (mg/dl)	141.1 ± 23.8	131.9 ± 24.4	0.001
Apo B (mg/dl)	112.7 ± 28.9	112.9 ± 28.3	0.929

Data are means ± SE or n (%). **P*-value comparing R230C/C230C vs. R230R, adjusted for age, sex, BMI, triglyceride levels, smoking and educational level. †Fisher's exact two-tailed test.

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

Metabolic Trait	Genotype *n* (%)			
	R230R	R230C/ C230C	OR	*P*
Obese subjects	84 (71.2)	34 (28.8)	2.527	0.005*
Non-obese	139 (86.8)	21 (13.2)		
MS‡ subjects	143 (73.7)	51 (26.3)	1.893	0.0007†
Non-MS	268 (84.8)	48 (15.2)		

**P*-value adjusted for age, sex, smoking, HDL-C, Apo A-I, educational level and T2D. †*P*-value adjusted for age, sex, smoking and educational level. ‡According to ATP-III criteria. MS = metabolic syndrome. The associations remained significant after adjusting for admixture (*P*=0.011 and 0.001 for obesity and MS, respectively).

Table 4. R230C genotype and allele frequencies in Amerindian populations of Mexico.

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

**P*-value when compared allelic frequencies of the Mestizos. NS=Non-significant.