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*Taq*1*B CETP* Polymorphism and Cardiovascular Risk in an Endogamous Population of Diabetic Men: A Study in Santa Rosa Del Conlara, San Luis, Argentina

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Abstract

Objectives: Type 2 diabetes mellitus (T2DM) patients are at increased risk of cardiovascular diseases (CVDs). Several polymorphisms in the *cholesterol ester transfer protein* (*CETP*) gene have been reported. The aim of this study was to determine the distribution and effect of the *Taq*1*B* polymorphism in the *CETP* gene on clinical and biochemical indicators of CVD risk in a population of endogamous-T2DM men. Methods: 102 men (57.5 ± 9.3 years old) inhabitants of Santa Rosa del Conlara, San Luis, Argentina, were recruited and assigned into two groups (22 control and 80 T2DM). Further, these two groups were subdivided according to their *Taq*1*B CETP* gene genotypes (*i.e.*, B1B1, B1B2 and B2B2). Clinical and fasting-plasma biochemical indicators of CVD risk were measured and their association with the B1 allele was determined. Results: Compared to control, T2DM men had more central obesity, hypertension, atherogenic index, insulin resistance and poorly controlled diabetes. Compared to T2DM men having the B2 allele, those T2DM men having the B1 allele have increased risk of CVD as assessed by systolic blood pressure (156 ± 16.0 vs 135.8 ± 19.2, $p = 0.015$), atherogenic index (6.15 ± 1.3 vs 4.4 ± 0.7, $p = 0.0008$), HDL-c levels (38.9 ± 5.3 vs 64.4 ± 8.2, $p < 0.0001$) and insulin resistance (HOMA-IR, 5.78 ± 3.0 vs 2.4 ± 0.78, $p = 0.004$). Interestingly, only body mass index ($r = -0.559$, $p = 0.01$) and HDL-c concentration ($r = -0.492$, $p = 0.02$) negatively correlated with CVD risk in the endogamous population of B1B1 and B1B2 T2DM men. Conclusion: The B1 allele of the *CETP* gene predicts cardiovascular complications in an

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endogamous population of T2DM men.

Keywords

Cholesteryl Ester Transfer Protein, *Taq*1B Polymorphism, Endogamous Type 2 Diabetic Men, Cardiovascular Disease Risk, Santa Rosa del Conlara-San Luis-Argentina

1. Introduction

Type 2 diabetes mellitus (T2DM) patients have an increased risk of developing cardiovascular diseases (CVDs) [1]. This increased risk of CVDs is associated with alterations in the lipid profile in plasma. Indeed, lipoprotein profile in T2DM patients is characterized by increased plasma concentrations of triglyceride (TG)-rich lipoprotein particle remnants, small dense low-density lipoprotein-cholesterol (LDL-c) particles, but decreased concentrations and size of high-density lipoprotein-cholesterol (HDL-c) [2]. Therefore, changes in the traffic and tissue deposition of lipids in T2DM patients may predict the risk of CVDs.

Cholesteryl ester transfer protein (CETP) is one of the major proteins that regulate the exchange of lipids between circulation and tissues [3] [4]. CETP regulates the bidirectional exchange of cholesteryl esters (CE) and TG between all plasma lipoprotein particles [2] [5]. CETP activity results in an overall transfer of CEs from HDL-c, an Apo-A1-containing lipoprotein, to apolipoprotein B (apoB) contained in very-low-density lipoprotein (VLDL) and LDL-c, which causes an atherogenic lipid profile [3]. Therefore, increased CETP protein and/or activity in humans may cause a lipid profile that is potentially antiatherogenic [6] [7]. Because CETP plays a central role in the composition and size of HDL-c, subtle changes in its synthesis, and consequently activity, may alter CVD risk [6] [8] [9].

Single nucleotide polymorphisms (SNPs) in the *CETP* gene that determine decreased CETP activity and/or mass are associated with elevated concentrations of HDL-c and decreased CVD risk [4]. A number of SNPs have been described in the *CETP* gene (Accession id: ENSG00000087237) [10]-[12]. Most of these SNPs are associated with low plasma CETP protein and high HDL-c concentrations in plasma [10] [13]. Interestingly, environmental factors have been shown to contribute to the association strength between these SNPs of the *CETP* gene and HDL-c concentrations [10] [14]-[16]. One of the most frequent SNPs in the *CETP* gene is that located at the 227th nucleotide in its first intron (rs708272 or Taq1B, NG_008952.1:g.5454G>A) [17]. This SNP results in the disruption of a restriction site for the restriction enzyme *Taq*1 (B2 allele) [10]. The heterozygous B1B2 genotype is the most frequent in most populations [18]. The Taq1B polymorphism is characterized by a silent base change from a G (designed as B1) to an A (designed as B2) nucleotide, at the 277th nucleotide in the intron 1 of the *CETP* gene, and possesses a restriction site for the endonuclease *Taq*1. The B2 allele of this SNP (absence of restriction site) has been associated with decreased CETP mass, increased HDL-c concentrations and decreased cardiovascular risk and metabolic syndrome [6] [14] [17] [19]. Therefore, the Taq1B polymorphism of the *CETP* gene is likely to have an impact on lipid profile and thus determine CVD risk in T2DM patients, but reports on the subject in endogamous populations are limited [20] [21]. Importantly, endogamy is an important factor that may explain the increased incidence of genetic-associated metabolic complications in T2DM populations [18] [21] [22].

The results from epideliological studies have, like-wise, been equivocal, with some studies suggesting that the association of the CETP Taq1B SNP with plasma HDL-c concentrations may be population specific [23] [24] and highly influenced by gender [13], environmental factors such as diet, alcohol consumption [25] and smoking [26]. In the general population, the Taq1B polymorphism of the *CETP* gene is known to affect both CETP activity and HDL-c concentrations, perhaps independently [27]. In a population of 406 T2DM patients, Durlach *et al.* [20] showed that the Taq1B polymorphism of the CETP gene has an impact on HDL-c concentrations in male patients only, female displayed equally high concentrations independent of genotype. That study showed that B2-homozygotes men had less incidence of CVD. In addition, the association of CETP Taq1B genotype with plasma HDL-c and CVD risk may be population specific, and highly influenced by dietary, environmental and genetic backgrounds [6]. Indeed, a study in a Tunisian population of 172 T2DM patients shown that the B1 allele was more associated with low HDL-c concentrations than other populations [6]. The association between

the B2 allele and microangiopathy in a French and a Japanese population of T2DM patients showed contrasting results regarding the effect of gender [28]. These and other studies suggested the importance of determining in specific ethnic populations the association between gender, CETP Taq1B genotypes, biochemical profile and CVD risk in order to improve therapeutic and interventions.

The population of Santa Rosa del Conlara, a small city (~4000 inhabitants) located in the northwest region of the province of San Luis-Argentina, has shown an incidence of T2DM in adults of 13.5%, which is higher than any other region in the country (*i.e.*, 7.5%) (MS Ojeda, *et al*. unpublished results). These patients have increased risk of CVDs, however whether a particular Taq1B genotype, and associated atherogenic lipid profile, may affect the risk of CVDs in this population is unknown.

Herein we investigated the impact of *Taq*1*B* genotypes on clinical and biochemical parameters that may determine changes in the risk of CVDs in an endogamous population of T2DM men. Studies like this are rare in the literature and will provide interesting data regarding the association of the CETP Taq1B genotype and CVD risk in a unique population of T2DM men that will help improving health care policies. We found that, compared to control patients, endogamous T2DM men have more central obesity, atherogenic index (AI), insulin resistance, lower HDL-c, and higher systolic blood pressure (SBP). Although endogamous T2DM men have the same frequency of *Taq*1B allele and genotypes of the *CETP* gene, those with the B1 allele are at increased risk of CVDs, more likely due to an atherogenic lipid profile, insulin resistance and hypertension.

2. Methods

2.1. Population Studied

The present study was carried out in accordance with the guidelines of the Helsinki Declaration. Hundred and two (102) un-related men (57.5 ± 9.3 years old) inhabitants of Santa Rosa del Conlara were enrolled during their regular annual health check-up. The protocol followed in this study was approved by the local Institutional Review Board, and a written informed consent was obtained from each man to be enrolled. During an initial interview with each patient, they were asked for diseases, medication and smoking histories. Exclusion criteria included liver, kidney and thyroid diseases, as well as the use of anti-lipemic drugs. The American Diabetes Association's criteria [29] were used to grouping this population in control (n = 22, 55.4 ± 9.7 years old) and T2DM (n = 80, 58.2 ± 9.1 years old) patients.

2.2. Clinical and Anthropometric Measurements

For each subject enrolled, height (meters), weight (Kg) and waist and hip circumferences (cm) were recorded following routine procedures. Height and weight were measured to the nearest 0.5 cm and 0.1 kg, respectively. The body mass index (BMI) was calculated as weight divided by height squared (Kg/m^2). Those men with a BMI equal or greater than 30 Kg/m^2 were considered as obese. Waist/hip ratio (WHR) was calculated as a measurement of central obesity. Blood pressure was measured in the right arm after a 10-min rest in the supine position. Hypertension was diagnosed as a systolic blood pressure (SBP) equal or greater than 130 mmHg and a diastolic blood pressure equal or greater than 85 mmHg.

2.3. Blood Sampling

Fating blood samples were obtained from patients that had fasted for at least 12 h. Blood was collected in plastic tubes containing 0.1% EDTA. Plasma and blood cells (pellets) were separated by centrifugation at 2400 rpm for 20 min at room temperature. After separation, plasma and packed blood cells were aliquoted and stored at −20˚C until use.

2.4. Biochemical Measurements

Fasting plasma glucose (FPG) was measured by using a glucose oxidase method with a commercial enzymatic kit (Wiener Laboratories, Rosario, Argentina). Fasting plasma insulin (INS) concentration was measured by an electrochemiluminiscence immunoassay (Roche Diagnostics, Mannheim, Germany). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as follow: fasting insulin (μIU/mL) × fasting glucose (mmol/L)/22.5. Glycated hemoglobin (HbA1c) concentration was measured with a coupled ionic-exchange

chromatography/spectrophotometric assay (BioSystems, Barcelona, Spain). Total cholesterol (TC), TG and HDL-c concentrations were measured using commercial kits by following manufacturer's instructions (Wiener Laboratories). LDL-c was calculated with the Friedewald formula [30]. Apo A-I concentration in the plasma was measured with an immunoturbidimetric assay (Diffu-Plate, Biocientífica, Buenos Aires). The atherogenic index (AI) was calculated by using the following formula: TC (mg/dL)/HDL-c (mg/dL).

2.5. Analysis of Taq1B Polymorphism of the CETP Gene

DNA was extracted from packed blood cells using the Qiagen QiAmp Mini Kit (Valencia, CA). CETP *TaqB*1 alleles were determined as described by Fumeron *et al*. [31]. Briefly, a 535 bp fragment in the first intron of the CETP gene (GenBank accession number NM_000078) that includes the rs708272 SNP was amplified by the polimerasa chain reaction (PCR). Oligonucleotide primers used here were as follow: forward 5'-CACTAGCCC-AGAGAGAGGAGTGCC-3' and reverse 5'-CTGAGCCCAGCCGCACACTAAC-3'. Each amplification reaction included 100 ng of genomic DNA, 20 pmol of each PCR primer and 1 unit of AmpliTaq Gold DNA polymerase (Perkin-Elmer, Waltham, MA). These reactions were performed in a buffer containing 1.5 mmol/L $MgCl_2$, 50 mmol/L KCl, 20 mmol/L Tris-HCl (pH 8.4), and 200 pmol/L of each deoxynucleotide triphosphate. The template DNA was denatured for 3 minutes at 95˚C before undergoing 30 cycles of amplification. Each amplification cycle included: denaturation for 30 seconds at 95˚C, primer annealing for 30 seconds at 60˚C, and extension for 45 seconds at 72˚C; followed by a final extension at 72˚C for 5 minutes.

The Taq1B allele of the *CETP* gene in each sample was determined by digesting 10 µL of the PCR product with one unit of *Taq*1 restriction endonuclesase (Promega, Madison, WI) for 2 hours at 65˚C. The resulting digest was separated by electrophoresis in a 2% agarose gel. The digestion products were revealed by ethydium bromide staining of the gel and visualized under ultraviolet light. Visualization of two DNA fragments of the *Taq*1 treated amplicon at 174 and 361 bp indicates a B1 allele (presence of the restriction site), whereas an intact 535 bp indicates a B2 allele (absence of the restriction site). See **Figure 1**.

2.6. Statistical Analysis

Chi-square test was used to check adjustment of the data to the Hardy-Weinberg equilibrium and to compare the allelic frequencies between control and T2DM men. These allelic frequencies in our patients were in a Hardy-Weinberg equilibrium. Statistical analyses were first performed for all patients and then for control and T2DM men by separate. Data are shown as either mean values ± standard deviation (SD), absolute values or percentages (%). To analyze the association between the B1 allele of the *CETP* gene and indicators of CVD risk a Student *t*-test was used when variables were continuous, whereas a Fisher's exact test was used for the categorical variables. STATA version 6 (SataCorp LP, College Station, TX) was used for statistics. A $p < 0.05$ was considered to be statically significant.

3. Results

3.1. Clinical, Anthropometric and Biochemical Characteristics of the Study Population

Hundred and two (102) men living in Santa Rosa del Conlara, were enrolled in this study. This study included 22 control and 80 T2DM unrelated men. Clinical, anthropometric and biochemical data are shown **Table 1**. Enrolled patients were 57.5 ± 9.3 years old and had a BMI of 30.0 ± 4.2 Kg/m^2. Compared with control patients,

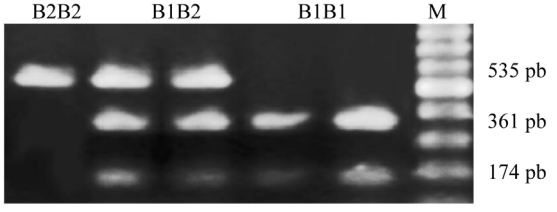

Figure 1. Agarose-gel electrophoresis showing typical patterns of the Taq1B polymorphism of the *CETP* gene. M, molecular weight marker.

Table 1. Selected clinical, anthropometric and biochemical indicators of CVD risk.

	All (n = 102)	Control (n = 22)	T2DM (n = 80)	*p*
Age (years)	57.5 ± 9.3[†]	55.4 ± 9.7	58.2 ± 9.1	0.2090
BMI (kg/m^2)[‡]	30.0 ± 4.2	27.1 ± 4.7	30.7 ± 3.6	0.0002
WHR	0.90 ± 0.1	0.77 ± 0.1	0.93 ± 0.1	<0.0001
SBP (mmHg)	137.7 ± 19.8	125.7 ± 10.5	141.0 ± 20.5	0.0010
TG (mg/dL)	254.0 ± 106.0	125.6 ± 11.4	281.3 ± 96.5	<0.0001
TC (mg/dL)	221.0 ± 50.3	180.8 ± 32.6	232.0 ± 48.8	<0.0001
LDL-c (mg/dL)	124.8 ± 28.0	99.7 ± 25.0	131.5 ± 25.0	<0.0001
HDL-c (mg/dL)	43.3 ± 9.1	49.7 ± 3.1	41.5 ± 9.5	<0.0001
Apo A-I (mg/dL)	125.8 ± 7.4	120.3 ± 6.4	131.4 ± 8.5	0.3240
AI	5.3 ± 1.6	3.57 ± 0.8	5.76 ± 1.5	<0.0001
FPG (mg/dL)	180.0 ± 70.8	88.4 ± 9.4	194.4 ± 68.3	<0.0001
HbA1c (%)	8.5 ± 2.4	5.6 ± 0.6	8.5 ± 2.3	<0.0001
HOMA-IR	4.66 ± 4.3	2.33 ± 1.1	5.30 ± 4.7	0.0040

[†]Data are shown as mean ± SD; [‡]Abbreviations used here are: AI, atherogenic index = TC(mg/dL)/HDL-c(mg/dL); Apo, apolipoprotein; BMI, body mass index; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDL-c, high density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-c, low density lipoprotein-cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; WHR, waist-to-hip ratio.

T2DM men had higher BMI, WHR, and SBP. These data suggest T2DM endogamous men included in this study are obese and hypertensive. Compared to control, T2DM men had higher FPG, HbA1c and HOMA-IR, which together indicate that these patients were insulin resistant and also that their glycemia was poorly controlled. Compared to control, T2DM patients had a larger AI, higher fasting concentrations of atherogenic lipids (*i.e.*, TC, TG and LDL-c) and lower concentrations of HDL-c. These data suggest that these patients had increased risk of CVD, such as atherosclerosis.

3.2. Genotype Frequencies

Table 2 shows the genotype and allelic frequencies (%) and absolute number of patients having each genotype in all, control and T2DM men. The observed allelic frequencies were in Hardy-Weinberg equilibrium. DNA analysis showed that 22.5% of all our patients were homozygous carriers of the B1 allele (B1B1 genotype), whereas 67.4% were heterozygous carriers of the B1 and B2 alleles (B1B2 genotype). Only 9.8% of all our patients were homozygous carriers of the B2 allele (B2B2 genotype). Genotype frequencies, as percentages, of the B1B1:B1B2:B2B2 in control and T2DM men were 13.6:77.3:9.1 and 25:65:10, respectively. Genotype and allele frequencies between control *versus* T2DM patients were not different.

3.3. Association between CVD Risk Indicators and Taq1B Polymorphism of the CETP Gene in the Population of Endogamous Men

Table 3 shows the association of Taq1B polymorphism of the *CETP* gene and CVD risk indicators in control and T2DM men by separate. Compared to control men having the B2B2 genotype, those control patients having the B1 allele had more central obesity (WHR, $p = 0.025$), lower HDL-c ($p = 0.004$) and insulin resistance (HOMA-IR, $p = 0.025$). With the exception of WHR, the values of HDL-c ($p < 0.0001$) and HOMA-IR ($p = 0.004$) were more significant in T2DM patients with the B1B1/B1B2 genotypes than in those T2DM with the B2B2 genotype. The presence of at least one B1 allele caused T2DM men having higher SBP ($p = 0.015$), AI ($p = 0.0008$), TC ($p = 0.01$) and TG ($p = 0.003$), however, these indicators were not affected by the TaqB1 CETP genotype in control men. **Supplementary Table 1** shows the analysis of the association of CVD risk in control

Table 2. Genotype and allelic frequencies.

	All (n = 102)	Control (n = 22)	T2DM (n = 80)	OR (95% CI)	*p*
Genotypes [†]					
B2B2	10 (9.8%)	2 (9.1%)	8 (10.0%)	1	
B1B1	23 (22.5%)	3 (13.6%)	20 (25.0%)	1.667 (0.232 - 11.934)	0.608[§]
B1B2	69 (67.4 %)	17 (77.3%)	52 (65.0%)	0.765 (0.147 - 3.957)	0.748
B1B1/B1B2	92 (89.9%)	20 (90.9%)	72 (90.9%)	0.900 (0.176 - 4.580)	0.898
Alleles [‡]					
B2	0.44	0.48	0.42	1	
B1	0.56	0.52	0.57	0.789 (0.456 - 1.397)	0.517[¶]

[†]Percentages, odds ratio (OR) and p with respect to B2B2 genotype (shown as 1.0); [‡]Percentages, OR and p with respect to B2 allele (shown as 1.0); [§]Significance when comparing the frequency of the B1B1 and B1B2 with respect to the B2B2 genotype; [¶]Significance when comparing the frequency of the B1 allele with respect to the B2 allele.

Table 3. Selected indicators of CVD risk according *Taq*1*B* genotypes of CETP in the study population[†].

	Control (n = 22)[‡]			T2DM (n = 80)		
Variables	**B1B1/B1B2 n = 20** (90%)	**B2B2 n = 2** (9%)	***p***	**B1B1/B1B2 n = 72** (90%)	**B2B2 n = 8** (10%)	***p***
Age (years)	56.1 ± 9.8	47.0 ± 1.4	0.084	58.7 ± 7.2	53.2 ± 7.5	0.108
Smoking Status	12 (60 %)	2 (100%)	-	22 (30.5 %)	3 (37.5%)	-
BMI[c] (kg/m^2)	27.5 ± 4.7	24.2 ± 1.5	0.190	32.3 ± 3.3	30.6 ± 3.7	0.445
WHR	0.78 ± 0.09	0.67 ± 0.02	0.025	0.94 ± 0.06	0.92 ± 0.06	0.428
SBP	126.1 ± 10.2	122.6 ± 11.3	0.599	156.1 ± 16.0	135.8 ± 19.2	0.015
AI	4.1 ± 0.2	4.3 ± 0.6	0.280	6.15 ± 1.3	4.4 ± 0.7	0.0008
TC (mg/dL)	208.1 ± 19.7	188.1 ± 25.6	0.156	248.6 ± 37.7	212.2 ± 45.6	0.01
TG (mg/dL)	126.6 ± 11.2	115.2 ± 3.3	0.055	300.6 ± 78.2	272.6 ± 44.2	0.003
LDL-c (mg/dL)	109.8 ± 15.5	102.5 ± 1.9	0.384	141.1 ± 21.4	135.3 ± 26.1	0.499
HDL-c (mg/dL)	43.7 ± 3.4	50.5 ± 5.0	0.004	38.9 ± 5.3	64.4 ± 8.2	<0.0001
FPG (mg/dL)	88.4 ± 9.9	85.5 ± 5.2	0.579	198.8 ± 69.4	182.4 ± 39.5	0.513
INS mU/ml	11.5 ± 2.9	6.7 ± 1.6	0.006	21.8 ± 10.7	19.6 ± 7.0	0.563
HOMA-IR	2.46 ± 1.1	1.05 ± 0.36	0.025	5.78 ± 3.0	2.40 ± 0.78	0.004
HbA1c	5.6 ± 0.6	5.1 ± 0.5	0.212	8.6 ± 2.4	7.4 ± 1.0	0.142

[†]Data shown as percentage of the total, absolute values and mean values ± SD; [‡]Abbreviations used here are: AI, atherogenic index; Apo, apolipoprotein; BMI, body mass index; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; INS, insulin; HDL-c, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance index; LDL-c, low density lipoprotein-cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; WHR, waist/hip ratio.

and T2DM men having either a B1B1/B1B2 or B2B2 genotype. Data shown indicate that SBP ($p = 0.002$) and AI ($p < 0.0001$) are different between control and T2DM patients having at least one B1 allele (*i.e.*, B1B1 or B1B2 genotype). TC ($p < 0.0001$ vs $p = 0.03$), TG ($p < 0.0001$ vs $p = 0.002$), LDL-c ($p < 0.0001$ vs $p = 0.006$), HDL-c ($p < 0.0001$ vs $p = 0.002$), FPG ($p < 0.0001$ vs $p = 0.0007$), insulin (INS, $p = 0.003$ vs $p = 0.005$) and HbA1c ($p < 0.0001$ vs $p = 0.009$) were more significant when B1B1/B1B2 control and T2DM were compared, regarding the same comparison between B2B2 control and T2DM men. The HOMA-IR difference was more significant in control/T2DM men harboring the B2B2 genotype ($p = 0.0002$) than in control/T2DM men having

the B1B1/B1B2 genotypes ($p = 0.002$).

3.4. Correlation Analysis of the B1 Allele and CVD Risk Indicators in T2DM Men

Table 4 shows a correlation analysis between the B1 allele and the most common CVD risk indicators in T2DM men. Data had a normal distribution according the Kolmogorov-Smimov test. As expected, the B1 allele negatively correlated with HDL-c ($r = -0.492$, $r^2 = 0.242$, $p = 0.02$) and BMI ($r = -0.559$, $r^2 = 0.313$, $p = 0.01$) in T2DM men, but not in control men. Our analysis did not show any correlation of the B1 allele of the *CETP* gene and other common CVD risk indicators, such as WHR, SBP, AI and HOMA-IR.

4. Discussion

We studied a possible association between *Taq*1B CETP genotypes and indicators of CVD risk in an endogamous population of control and T2DM men inhabitant of Santa Rosa del Conlara City, San Luis, Argentina. This population is unique because the prevalence of T2DM in this region is almost twice as any other region in the country (13.5% vs 7.5%) (Ojeda, MS, unpublished data). Importantly, our data suggest that those T2DM men having a B1 allele of the *CETP* gene have a lipid profile consistent with an increased risk of CVDs.

A number of previous studies have shown that the Taq1B polymorphism of the CETP gene is associated with vascular complications of T2DM ([1] [28] and references therein). Changes in the synthesis of CETP lead to changes in the concentration, size and composition of HDL-c particles [5]. Another research team reported that the B1B1 and B2B2 genotypes of the *CETP* gene were associated with high and low plasma mass/activity of CETP, respectively [6] [13]. Besides the uniqueness of our study carried out in an endogamous population of control and T2DM men, our data show a relationship between the different Taq1B alleles of the *CETP* gene and anthropometric, clinical and biochemical variables in T2DM unrelated men. Kawasaki *et al*. [32] reported an association between *Taq*1*B* polymorphism of CETP and HDL-c plasma concentrations in T2DM patients. Moreover, ethnicity plays an important role in the association between CETP polymorphisms, T2DM incidence and CVD risk [18] [28] [33]. Our present study goes beyond those studies by showing the frequency and distribution of *Taq*1*B*-CETP alleles and genotypes in a well defined and homogeneous population of endogamous T2DM unrelated men.

Table 4. Correlation between the B1 allele of the CETP gene and selected indicators of CVD risk[†].

		r	r^2	*p*
Indicators of CVD risk[‡]				
BMI[§]	Control	0.868	0.753	0.330
	T2DM	−0.559	0.313	0.010
WHR	Control	0.693	0.480	0.512
	T2DM	−0.088	0.008	0.710
SBP	Control	0.832	0.692	0.374
	T2DM	−0.159	0.025	0.501
HDL-c	Control	0.945	0.893	0.212
	T2DM	−0.492	0.242	0.020
AI	Control	−0.988	0.977	0.097
	T2DM	0.046	0.002	0.846
HOMA-IR	Control	−0.776	0.602	0.434
	T2DM	−0.128	0.0002	0.957

[†]The distribution of the population of data was determined using the Kolmogorov-Smirnov test. Data had a normal distribution (homogeneous). [‡]The correlation between the Taq1B B1 allele of the CETP gene and selected indicators of CVD risk was determined using the Spearman's test (non-parametric). r, is the correlation coefficient and r^2 is the coefficient of determination. [§]Abbreviations used here are: AI, atherogenic index; BMI, body mass index; HDL-c, high density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance index; SBP, systolic blood pressure; WHR, waist-to-hip ratio.

Factors such as gender, life-styles, smoking status and BMI are known to affect the association between the Taq1B polymorphism of the *CETP* gene and risk of CVDs [16] [34] [35]. Our study showed an association between the B1 allele, central adiposity, hypertension and HDL-c concentrations in T2DM endogamous men. The frequencies (%) of B1B1:B1B2:B2B2 genotypes in our T2DM men were 25:65:10, respectively, which was similar to that one reported in The Framingham Offspring Study [36] that showed a similar distribution of B1B1: B1B2:B2B2 (*i.e.*, 30:51:19). In addition, Park *et al*. [37], in their Korean cohort reported similar B1B1:B1B2: B2B2 frequencies in men 35.5:50:14.5. Obviously, ethnic, cultural, demographic, geographic and nutritional factors may impact the association between *Taq*1*B* CETP genetic variants, lipid parameters and the incidence of CVD in T2DM men.

Durlach *et al*. [20] showed that T2DM patients with the B2 allele had significantly less CVD risk than those having the B1 allele of the *CETP* gene. Our study showed that those T2DM men having a B2B2 genotype had higher plasma HDL-c concentrations. These findings agree with the association between HDL-c concentrations and Taq1B polymorphism of the *CETP* gene in the Veterans Affairs HDL-c Intervention Trial [13]. However, men who have the B1 allele may be at increased risk for metabolic complications due to the larger central adiposity and altered distribution of cholesteryl esthers (CE) and TG [38]-[40]. The cholesterol exchange process from HDL to non-HDL lipoproteins is mediated by CETP that facilitates the incorporation of CE into TG-rich lipoproteins as part of the reverse cholesterol transport pathway [5]. Indeed, the beneficial effects of HDL-c on the cardiovascular system have been attributed to its ability to remove cellular cholesterol, as well as its anti-inflammatory, anti-oxidant and anti-thrombotic properties [41] [42]. All these properties of HDL-c can improve endothelial function and inhibit atherosclerosis, thereby reducing the risk of CVDs [43].

The association of the *Taq*1*B* CETP genotype and HDL-c concentrations is widely recognized in T2DM patients [44]-[46]; however, our population is unique in a number of aspects. For instance, its endogamous nature may explain the high incidence of T2DM, and CVD related death, among the adult population [22]. The present study in an endogamous population shows that the B1B1 and B1B2 genotypes are associated with lower HDL-c concentrations and increased CVD risk in T2DM men.

Our study suggests that our population of endogamous T2DM men having at least one Taq1 B1 allele of *CETP* gene are at increased risk of CVDs. In this regard, an association between CETP Taq1B polymorphism and HDL-c concentrations was more evident in T2DM patients with higher intake of total fat, saturated fat, and monosaturated fat. Lowering fat and increasing carbohydrate intake to keep the caloric balance may help reducing the effect of the B1 allele on HDL-c concentration and consequently reducing CVD risk [47]. Identification of T2DM B1 homozygous patients in this endogamous population will help deciding whether the treatment with inhibitors of CETP activity, such as anacetrapib [48] [49], may benefit by increasing HDL-c concentrations, and thus reducing CVD risk. On the other hand, cigarette smoking has been identified as an independent and preventable risk factor for CVDs. HDL-c is susceptible to oxidative modifications by cigarette smoking, which makes HDL-c become dysfunctional and lose its cardioprotective properties in smokers [50]. Thus, reducing cigarette smoking, one of the most preventable causes of CVD risk associated to CETP activity, especially in B1-homozygous T2DM patients in a genetically homogeneous population such as our endogamous population may be beneficial.

Consequently, aggressive management to modulate CETP mass and/or activity by nutritional (reducing fat intake and increasing carbohydrate intake), behavioural (reducing cigarette smoking) or pharmacological (inhibitors of CETP activity) means are required to reduce the risk of CVDs in T2DM men harbouring the B1 allele of the *CETP* gene [41]. Importantly, our study also emphasizes the importance of genotyping T2DM patients according the Taq1B polymorphism of the CETP gene in order to establish efficient prevention policies to reduce the risk of CVDs in T2DM patients. Finally, genotyping of the Taq1B polymorphism of the *CETP* gene can be of high predictive and interventional value of cardiovascular complications in our, and probably other, endogamous population of T2DM men.

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Disclosure

Authors have not conflict of interest to disclose

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Abbreviations

Apo, apolipoprotein; BMI, body mass index; CE, cholesteryl esther; CVD, cardiovascular disease; CETP, cholesteryl ester transfer protein; FPG, fasting plasma glucose; HbA1c, glycated haemoglobin; HDL, high density lipoprotein; LDL, low density lipoprotein; OR, odds ratio; SNP, single nucleotide polymorphism; T2DM, type 2 diabetes; TG, triglycerides; TC, total cholesterol; WHR, waist-hip ratio

Supplementary Table 1. Effect of Taq1B CETP genotypes on CVD risk of the study population[a].

	B1B1/B1B2			B2B2		
Variables	**Control n = 20**	**T2DM n = 72**	***p***	**Control n = 2**	**T2DM n = 8**	***p***
Age (years)	56.1 ± 9.8	58.7 ± 7.2	0.269	47.0 ± 1.4	53.2 ± 7.5	0.224
Smoking Status	12 (60%)	22 (30.5%)	-	2 (100%)	3 (37.5%)	-
BMI[b] (kg/m^2)	27.5 ± 4.7	30.6 ± 3.7	0.002	24.2 ± 1.5	31.7 ± 2.7	0.003
WHR	0.78 ± 0.09	0.94 ± 0.06	<0.0001	0.67 ± 0.02	0.92 ± 0.06	<0.0001
SBP	126.1 ± 10.2	141.1 ± 20.8	0.002	122.8 ± 11.7	135.8 ± 19.2	0.181
AI	4.1 ± 0.2	6.15 ± 1.3	<0.0001	4.3 ± 0.6	4.4 ± 0.7	0.721
TC (mg/dL)	176.4 ± 30.8	226.5 ± 47.2	<0.0001	212.0 ± 15	278.6 ± 50	0.03
TG (mg/dL)	126.6 ± 11.2	278.5 ± 98.6	<0.0001	122.7 ± 12.8	290.7 ± 85.2	0.002
LDL-C (mg/dL)	99.5 ± 15.5	135.8 ± 22.1	<0.0001	101.5 ± 3	141.6 ± 22.1	0.006
HDL-C (mg/dL)	44.7 ± 1.7	38.9 ± 5.3	<0.0001	49.9 ± 3.5	61.1 ± 12.3	0.002
FPG (mg/dL)	88.4 ± 9.9	192.6 ± 50.2	<0.0001	84.7 ± 6.7	182.4 ± 39.5	0.0007
HOMA-IR	2.46 ± 1.1	6.2 ± 5.4	0.002	1.0 ± 0.3	4.4 ± 0.78	0.0002
INS (mU/ml)	11.5 ± 2.9	18.0 ± 10.7	0.003	6.8 ± 3.0	19.6 ± 7.0	0.005
HbA1c	5.6 ± 0.6	8.6 ± 2.4	<0.0001	5.2 ± 0.4	8.2 ± 1.8	0.009

[a]Data shown as percentage of the total, absolute values and mean values ± SD. [b]Abbreviations used here are: AI, atherogenic index; Apo, apolipoprotein; BMI, body mass index; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance index; INS, fasting plasma insulin; LDL-C, low density lipoprotein-cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; WHR, waist/hip ratio.