

Apolipoprotein CIII polymorphism and triglyceride levels of a Japanese population living in Southern Brazil

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Apolipoprotein CIII (apo-CIII) participates in the regulation of triglyceride-rich lipoprotein metabolism. Several polymorphic sites have been detected within and around the apo-CIII gene. Here, we examined the relationship between apo-CIII *Sst*I polymorphism (CC, CG, GG genotypes) and plasma triglyceride (TG) levels in a group of 159 Japanese individuals living in Southern Brazil. The sample was divided into a group of Japanese descendants (N = 51) with high TG (HTG; >200 mg/dL) and a group of Japanese descendants (N = 108) with normal TG (NTG; <200 mg/dL). TG and total cholesterol levels were analyzed by an enzymatic method using the Labtest-Diagnostic kit and high- and low-density lipoproteins by a direct method using the Labtest-Diagnostic kit and DiaSys Diagnostic System International kit, respectively. A 428-bp sequence of apo-CIII gene was amplified using oligonucleotide primers 5' GGT GAC CGA TGG CTT CAG TTC CCT GA 3' and 5' CAG AAG GTG GAT AGA GCG CTG GCC T 3'. The PCR products were digested with a restriction endonuclease *Sst*I. Rare G allele was highly prevalent in our study population (0.416) compared to Caucasians (0.00-0.11). G allele was almost two times more prevalent in the HTG group compared to the NTG group ($P < 0.001$). The genotype distribution was consistent with the Hardy-Weinberg equilibrium. There was a significant association between rare G allele and HTG in Japanese individuals living in Southern Brazil as indicated by one-way ANOVA, $P < 0.05$.

Key words: Triglycerides; *Sst*I polymorphism; Apolipoprotein CIII; Japanese population

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Introduction

Cardiovascular disease (CVD) is the most common cause of death in industrialized countries with evidence that high total cholesterol (TC) or triglyceride (TG) and low high-density lipoprotein (HDL) are independent risk factors. In the last 10 years, evidence has increased for the importance of genetic and environmental factors in determining plasma lipid and lipoprotein concentrations (1).

We have shown that Japanese descendants living in Brazil have a lipid profile that is different from non-Japa-

nese controls (2). They have a higher tendency to develop ischemic heart disease and have increased TG, TC and low-density lipoprotein (LDL) levels and low HDL levels. An alteration in apolipoprotein CIII (apo-CIII) could explain these data. The inheritance of CVD is polygenic and specific mutations at several candidate genes have been shown to be associated with altered lipid levels in subjects with severe dyslipidemia and with increased CVD risk. However, these mutations account for only a small fraction of the genetic variability in the general population. (3)

Apo-CIII, a 79-amino acid glycoprotein, is synthesized

predominantly in the liver and to a lesser degree in the intestine. It is present on very-low-density lipoproteins (VLDL) and chylomicron remnants, and to some extent on HDL (4). Several lines of evidence suggest that apo-CIII plays a major role in the regulation of TG-rich lipoprotein catabolism (5). *In vitro*, apo-CIII inhibits lipoprotein lipase, a rate-limiting enzyme for TG hydrolysis, resulting in the delayed catabolism of TG-rich particles that leads to higher plasma TG levels. Additional copies of the human apo-CIII gene in transgenic mice were associated with hypertriglyceridemia, whereas the absence of the gene in knock-out mice leads to reduced TG (6,7).

Several polymorphic sites have been detected within and around the apo-CIII gene. The most extensively studied is the *SstI* polymorphism, due to a C → G substitution at nucleotide 3238, in the 3' untranslated region of the gene (8). This transversion generates two alleles: S1 and S2. The frequency of the less common allele (S2) varies among different ethnic groups. In several case-control studies, this *SstI* polymorphism has been associated with high triglyceride (HTG), similar to the Japanese population (N = 40; P < 0.025), Arabs of Kuwait (N = 38; P < 0.001) and Caucasians (N = 52; P < 0.001) (9-12).

The objective of the present study was to determine the association of apo-CIII *SstI* polymorphism and plasma lipids in a group of volunteer Japanese descendants from Southern Brazil because HTG is one of the underlying risk factors for CVD progression, and there is no information available on Japanese descendants living in Brazil.

Material and Methods

Population

A total of 159 healthy Japanese descendants living in Southern Brazil, with no pre-existing medical conditions (i.e., diabetes, atherosclerosis or heart disease), participated. They shared common socio-cultural background and comparable Japanese dietary habits. Subjects who had blood glucose above 110 mg/dL were excluded. The mean age was 64 ± 12 years. All subjects had Japanese origin only. The study was approved by the Ethics Committee of the Hospital de Clínicas de Porto Alegre and all subjects gave written informed consent to participate.

A 20-mL blood sample was collected by venipuncture from each participant after a 12-h fast. Samples were centrifuged at 5000 rpm for 10 min and plasma was frozen at -40°C for further analysis.

Groups

The individuals were divided into two groups: 51 Japanese descendants with TG above 200 mg/dL (HTG; 53.7%

females) and 108 Japanese descendants with TG below 200 mg/dL (normal TG, NTG; 51.6% females). The mean blood pressure of the HTG group was 143/85 mmHg and in the NTG group it was 139/82 mmHg (P > 0.05).

Lipid assays

TC and TG were determined by an enzymatic method using the Labtest-Diagnostic kit (Lagoa Santa, MG, Brazil). HDL cholesterol (HDL-C) and LDL cholesterol (LDL-C) were determined by a direct method using the enzymes cholesterol oxidase and esterase with the Labtest-Diagnostic kit and DiaSys Diagnostic System International kit (Holzheim, bei Lemburg, Germany), respectively. VLDL cholesterol (VLDL-C) values were calculated by the TG/5 formula. Body mass index was calculated as weight (kg)/height (m²). These determinations were performed with Cobas Mira (Roche, Switzerland) equipment.

DNA analysis

Genomic DNA was isolated from peripheral blood leukocytes by standard methods (11). A 428-bp sequence of apo-CIII gene was amplified by polymerase chain reaction in a thermal cycler (PTC-100, MJ Research Inc., USA), using oligonucleotide primers 5' GGT GAC CGA TGG CTT CAG TTC CCT GA 3' and 5' CAG AAG GTG GAT AGA GCG CTG GCC T 3'. DNA templates were denatured at 95°C for 5 min and then subjected to 35 cycles with temperature cycle consisting of 1 min denaturing at 95°C, 40 s annealing at 58°C, and 40 s extension at 72°C. The polymerase chain reaction products were digested with 5 units of *SstI* and the fragments separated by electrophoresis on a 5.0% (w/v) agarose gel (Fisher Scientific, USA). After electrophoresis, the gel was stained with ethidium bromide for 20 min and DNA fragments were visualized by UV illumination. The resulting fragments used to identify the G allele were 269 and 159 bp and 428 bp for the unhydrolyzed C allele.

Statistical analysis

The biochemical characteristics of the individuals in various genotype groups were reported as means \pm SD. To determine if there was a significant difference between groups, the following statistical tests were used: one-way ANOVA followed by the Tukey test when necessary, and the Student *t*-test to compare two groups. Allelic frequencies were estimated by gene-counting method. The sample size-dependent standard error of allele was calculated in terms of 95% confidence intervals of the estimates. Chi-square goodness-of-fit was used to confirm the agreement of the observed genotype frequencies with those expected (Hardy-Weinberg equilibrium) for various groups. Chi-

square test was applied to compare genotype frequencies between the two groups. All tests were performed using the SPSS-PC software, version 8.0.

Results

The comparison of plasma lipid and lipoprotein data of the HTG and NTG groups is given in Table 1. There were statistically significant differences between the groups for TC, HDL-C, VLDL-C, TG, TG/HDL-C, and TC/HDL-C. No significant differences between groups were detected for age, systolic or diastolic pressures, body mass index, LDL-C, glucose, or LDL-C/HDL-C. Note that the population was separated into 2 groups on the basis of TG levels: < or >200 mg/mL.

The genotype and allele distribution of apo-CIII polymorphism in the study population is shown in Table 2. The frequency of G allele in the study population was 0.416.

The distribution of genotypes and alleles of apo-CIII polymorphism between the NTG and HTG groups is reported in Table 3. There was a significant difference in the genotype distribution between the NTG and HTG groups (chi-square = 14.11, d.f. = 2, $P < 0.001$). CC genotype was significantly more frequent in the NTG group compared to the HTG group (39.8 vs 15.6%). Conversely, GG genotype was almost three times more prevalent in the HTG group compared to the NTG group (27.4 vs 9.3%, $P < 0.05$). However, no significant difference was observed for the CG frequency between the two groups (HTG: 56.8 vs NTG: 50.9%). A total of 84.4% of HTG (43/51) were G carriers of which 32.5% (14/43) were homozygotes. Comparatively, the NTG group had 60.2% (65/108) G carriers, of which only 15.3% (10/65) were G homozygotes. Thus, the G allele was almost two times more frequent in the HTG group than the NTG group ($P < 0.05$) as shown in Table 3. Logistic analysis revealed a significant association of apo-CIII G allele with hypertriglyceridemia ($P < 0.05$).

The intergenotypic variations in lipid profile in the HTG, NTG and total subjects are reported in Table 4. TC, VLDL-C, TG levels, and TG/HDL-C and TC/HDL-C ratios were significantly different among genotypes in the HTG group ($P < 0.05$). TC, VLDL-C and TG levels in the GG genotype, although tending to be higher for the total number of subjects, did not differ significantly between HTG and NTG. In particular, the GG individuals were associated with the highest concentration of TG followed by CG and then by CC in the HTG group. We observed the same association with TC, VLDL-C levels and TG/HDL-C ratio. No significant differences were observed in LDL-C, HDL-C, TC/HDL-C, and LDL-C/HDL-C ratio within any group.

Table 1. Comparison of Japanese descendants with high (HTG; >200 mg/dL) and normal (NTG; ≤200 mg/dL) triglyceride levels.

Parameter	HTG (N = 51)	NTG (N = 108)
Age (years)	63.1 ± 12.4	65.8 ± 12.4
Systolic pressure (mmHg)	143.7 ± 17.6	139.1 ± 18.8
Diastolic pressure (mmHg)	85.2 ± 10.1	82.5 ± 10
BMI (kg/m ²)	24.1 ± 2.9	23.3 ± 3.1
TC (mg/dL)	207 ± 41	193 ± 31*
HDL-C (mg/dL)	45.1 ± 9.3	49.9 ± 10.7*
LDL-C (mg/dL)	109 ± 36	116 ± 30.8
VLDL-C (mg/dL)	56 ± 17	29 ± 6*
TG (mg/dL)	284 ± 85	146 ± 34*
Glucose (mg/dL)	101 ± 29	105 ± 29
TG/HDL-C	6.7 ± 3.1	3.0 ± 1.1*
TC/HDL-C	4.7 ± 1.1	4.0 ± 0.9*
LDL-C/HDL-C	2.4 ± 0.8	2.4 ± 0.8

Data are reported as mean ± SD. BMI = body mass index; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; VLDL-C = very-low-density lipoprotein cholesterol; TG = plasma triglyceride. * $P < 0.05$ compared to high triglyceride level (Student *t*-test).

Table 2. Frequency distribution of genotypes and alleles of apo-CIII *SstI* polymorphism in 159 Japanese descendants.

Genotype	Observed genotype frequency	Expected genotype frequency	Allele frequency (95%CI)
CC	51	54.23	C = 0.584 (0.46-0.70)
CG	84	77.26	G = 0.416 (0.29-0.53)
GG	24	27.51	

Test for Hardy-Weinberg equilibrium: $\chi^2 = 1.2282$, d.f. = 2, $P = 0.5411$. 95%CI = confidence interval at 95%.

Table 3. Apo-CIII *SstI* polymorphism and triglyceride levels in Japanese descendants with high (HTG; >200 mg/dL) and normal (NTG; ≤200 mg/dL) triglyceride levels.

Frequency	Genotype/allele	HTG (N = 51)	NTG (N = 108)
Genotype	CC	8 (15.6%)	43 (39.8%)
	CG	29 (56.8%)	55 (50.9%)
	GG	14 (27.6%)	10 (9.3%)
Allele	C	0.441	0.653
	G	0.559	0.347

Genotype: $\chi^2 = 14.11$, d.f. = 2, $P = 0.00086$; allele: $\chi^2 = 6.39$, d.f. = 1, $P = 0.0115$.

Discussion

Human apo-CIII gene expression is controlled by positive and negative elements that are spread throughout the apo-AI-CIII-AIV gene cluster on the long arm of chromosome 11 (13). Various restriction fragment length polymorphisms in and around the human apo-CIII gene have been associated with hypertriglyceridemia in several distinct populations (14). The present study on *Sst*I polymorphism was carried out on a random sample of 159 Japanese individuals living in Southern Brazil and separated into 7

Table 4. Intergenotype variation in the lipid profile in Japanese descendants with high (HTG; >200 mg/dL) and normal (NTG; ≤200 mg/dL) triglyceride levels and the total study population.

Groups	Genotypes		
	CC	CG	GG
TC			
NTG	193 ± 31	192 ± 36	197 ± 33
HTG	183 ± 26	212 ± 50	227 ± 43*
Total	189 ± 29	199 ± 34	203 ± 36
LDL-C			
NTG	118 ± 28	114 ± 34	117 ± 33
HTG	93 ± 24	113 ± 47	121 ± 43
Total	108 ± 28	114 ± 31	117 ± 35
HDL-C			
NTG	46 ± 11	50 ± 11	52 ± 10
HTG	43 ± 10	47 ± 8	41 ± 6
Total	45 ± 10	49 ± 10	49 ± 9
VLDL-C			
NTG	29 ± 7	29 ± 7	31 ± 6
HTG	54 ± 18	54 ± 14	72 ± 15*
Total	39 ± 18	37 ± 17	39 ± 19
TG			
NTG	143 ± 37	141 ± 33	157 ± 31
HTG	269 ± 92	270 ± 73	365 ± 76*
Total	195 ± 89	186 ± 85	198 ± 96
TC/HDL-C			
NTG	4.3 ± 1	3.9 ± 1	3.9 ± 0.7
HTG	4.4 ± 0.9	4.6 ± 0.9	5.6 ± 1.5*
Total	4.3 ± 0.9	4.1 ± 1	4.2 ± 1
TG/HDL-C			
NTG	3.2 ± 1	3.0 ± 1	3.1 ± 0.8
HTG	6.9 ± 3.9	6.0 ± 2.7	8.9 ± 2.4*
Total	4.7 ± 3	4.0 ± 2.5	4.2 ± 2.7
LDL-C/HDL-C			
NTG	2.6 ± 0.8	2.3 ± 0.8	2.3 ± 0.7
HTG	2.2 ± 0.7	2.4 ± 0.8	3.0 ± 1.3
Total	2.5 ± 0.8	2.3 ± 0.8	2.4 ± 0.8

Data are reported as mean ± SD in mg/dL. TC = total cholesterol; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; VLDL-C = very-low-density lipoprotein cholesterol; TG = plasma triglyceride.

*P < 0.05, GG compared to CC and CG (one-way ANOVA).

groups on the basis of TG levels. The genotype distribution was consistent with the Hardy-Weinberg equilibrium. We attempted to determine if there is an association of apo-CIII *Sst*I polymorphism with TG levels. Japanese individuals having TG levels up to 200 mg/dL made up the NTG group and more than 200 mg/dL made up the HTG group. GG Japanese individuals had the highest levels of TG followed by CG and CC in the HTG study population. A significantly higher frequency of G allele in the HTG group compared to the NTG group suggests a strong association of the G allele with higher levels of TG. This association has been reported in studies carried out with Caucasians (15), Chinese (16), Mayans (17), Japanese (living in Japan) (18), Koreans (13), Arabs (19), and Asian Indians (20). In the present study, the G allele frequency in NTG and HTG subjects were 0.347 and 0.559, respectively. The G allele frequency is consistent with ranges reported for Chinese (0.30-0.43) (16), Japanese (0.25-0.48) (18), and Indians (0.36) (20), but is higher than those reported for Caucasians in whom the G allele frequency was 0.00-0.11 (3,19-22). The clinical significance of possessing this allele has been demonstrated in some case-control studies that showed a 2- to 5-fold increase in frequency in patient groups with premature coronary heart disease and peripheral vascular disease compared with control groups (23,24).

The biochemical basis for the association of G allele with hypertriglyceridemia has yet to be established. Dallinga-Thie et al. (25,26) and Shoulders et al. (27,28) reported an association between levels of apo-CIII and G allele. The *Sst*I polymorphism is located in the 3'untranslated region of apo-CIII gene. Therefore, it is more likely that G allele is not etiological but in linkage disequilibrium with other causative mutation hitherto unknown in apo-CIII nearby involved in determining the TG levels. It has been suggested that certain haplotypes generated from *Sst*I polymorphism and promoter polymorphism of apo-CIII gene may protect from or predispose to hypertriglyceridemia. In addition, *Sst*I polymorphism may also influence mRNA stability (13). It has been speculated that the linkage disequilibrium between this polymorphic site and the causative mutations is weakened or absent in some populations (27).

Apo-CIII provides a strong negative charge on the surface of lipoproteins preventing nonspecific interactions with the cell surface (29) and perhaps with other lipoproteins. This may act to reduce futile cycles in TG transport by preserving the particles for high affinity interactions such as with lipoprotein lipase or specific cell surface receptors, such as those binding to apo E or apo B. Plasma concentrations of apo-CIII in human populations correlate well with TG levels (4). *In vivo* apo-CIII modulates the

postprandial managements of TG (7) and inhibits the hepatic uptake of VLDL remnants (30). The genetically determined deficiency of apo-CIII has been shown to increase the rate of TG clearance from plasma by 6- to 7-fold (31). Over-expression of apo-CIII produces hypertriglyceridemia in transgenic mouse models via inhibition of clearance of TG-rich particles (32). It is now clear that normal physiological systems responsible for TG transport are partially determined by the plasma concentration of apo-CIII (33).

In conclusion, the results of the present study indicate a high prevalence of rare G allele of apo-CIII gene in HTG Japanese individuals. This allele was more frequent in our study population compared to Caucasians, as previously reported by others. Since HTG is considered to be a risk factor for CVD, long-term follow-up of these subjects may allow us to evaluate the association of apo-CIII SstI polymorphism with the risk of developing coronary artery disease.

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