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Original Article

Apolipoprotein M gene polymorphisms in childhood-onset type 1 diabetes in southern Brazil

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Abstract: Type 1 diabetes mellitus (T1DM), associated with autoimmune destruction of pancreatic β cells, is observed in children and adolescents. Objective: We investigated the potential association of the apolipoprotein M (*APOM*) polymorphisms rs707921, rs805264, rs805296, rs805297, and rs9404941 in childhood-onset T1DM (n = 144) and compared them to those in healthy (mostly Euro-Brazilian) children (n = 168). Methods: This project was approved by the Ethics Committee of the Federal University of Parana (CAAE 24676613.6.0000.0102). Genotyping was performed using PCR-restriction fragment length polymorphisms (rs805296 and rs9404941) and TaqMan probes (rs707921, rs805264, and rs805297). Results: All polymorphisms were in Hardy-Weinberg equilibrium. In the codominant model, no significant differences ($P > 0.05$) were observed in genotype and allele frequencies between healthy controls and children with T1DM. The minor allele frequencies (95% CI) for healthy subjects were rs707921 (A, 10.7%; 7-14%), rs805264 (A, 6.5%; 4-9%), rs805296 (C, 3.6%; 2-6%), rs805297 (A, 22.6%; 22-31%), and rs9404941 (C, 2.7%; 1-4%). The frequencies of the rs805297 A allele and rs805296 C allele were similar to those of other Caucasian populations; both the rs707921 and rs805264 A alleles were similar to American and Latin American populations, whereas that of the rs9404941 C allele was lower than that observed in the Caucasian and Asian populations. Conclusions: Haplotype analysis suggests that rs805297-C, rs9404941-T, rs805296-T, rs805264-G, and rs707921-C conferred risk (OR: 4.25; 95% CI: 1.81-10.1) to childhood-onset T1DM in the Euro-Brazilian population.

Keywords: Diabetes *mellitus*, polymorphism, *APOM* gene, Euro-Brazilian, childhood-onset diabetes

Introduction

Type 1 diabetes mellitus (T1DM), a heterogeneous and polygenic immune-mediated disorder, is one of the most common endocrine and metabolic conditions occurring during childhood [1]. The human leukocyte antigen (HLA) region is the major locus responsible for susceptibility to T1DM, and more than 50 non-HLA loci contribute to this susceptibility [2].

APOM (OMIM 606907) encodes a 26 kDa protein (apoM), a member of the lipocalin superfamily, and is located on chromosome 6p21.33, which is adjacent to the major histocompatibility complex (MHC) class III region, and it is possible that apoM may have immune property [3-5]. Therefore, apoM may be involved in the pathogenesis of T1DM.

Xu et al. [6] suggested that cytokines such as platelet-activating factor and transforming growth factor alpha affect the mRNA expression levels of *APOM*. *APOM* was found to be associated with diabetes by analyzing ~2500 single nucleotide polymorphisms (SNPs) located in the MHC region [7].

Human apoM is predominantly present in high-density lipoprotein (HDL) and, to a lesser extent, in triglyceride-rich and low-density lipoprotein (LDL) [3, 8], with plasma levels of approximately

1 μM [9]. Sphingosine 1-phosphate (S1P), a secondary messenger of five G protein-coupled receptors (S1P receptor [S1PR1-5]), binds to the hydrophobic binding pocket of apoM [10]. ApoM is important for the delivery of S1P to S1P receptors [11, 12] and the apoM/S1P complex participates in the regulation of triglyceride metabolism and endothelial cell barrier maintenance [13, 14]. ApoM enables the transformation of preβ-HDL into mature HDL particles and lipoprotein turnover, especially LDL; therefore, it has been suggested to exert anti-inflammatory and atheroprotective effects, in part via improved cholesterol efflux and antioxidative effects [15-19]. Moreover, because of the ubiquitous and overlapping expression of S1P receptors in multiple organs and systems, S1P has been implicated in several immune-mediated disorders [20-22]. ApoM is associated with endothelial functions and inflammatory processes [5, 23, 24].

It has been proposed that the apoM/S1P complex is critical for HDL antidiabetic activity, encompassing protection against insulin resistance, promotion of insulin secretion, enhanced β-cell survival, and inhibition of hepatic glucose production [25]. *APOM* is expressed in the liver and proximal renal tubules and its gene expression is regulated by transcription factors involved in hepatic lipid and glucose metabolism, suggesting a link between these two metabolic pathways [26]. The genetic influence of *APOM* on the progression of diabetes was assessed by examining the potential association between *APOM* polymorphisms and diabetes. *APOM* polymorphisms are associated with numerous diseases, including T2DM [27-29], chronic obstructive pulmonary disease [30], and systemic lupus erythematosus [31].

The C allele of the rs805296 (T-778C) polymorphism may increase promoter activity and is associated with the risk of T1DM in both Han Chinese and Swedish populations [32], and with T2DM in Han Chinese populations [33]. In the current study, we selected five apoM polymorphisms: three from the promoter region (rs805296, rs805297, and rs9404941) and two intronic polymorphisms, rs707921 in intron 5 and rs805264 in intron 1, having the main transcript of *APOM1* as a reference. We aimed to evaluate the association of *APOM* gene polymorphisms in children with T1DM and healthy controls in southern Brazil.

Materials and methods

Subjects

A total of 312 children were included in this study and divided into two groups: healthy children (control, n = 168) and those with childhood-onset T1DM (n = 144), with both age groups between 7 and 14 years. The groups were matched by gender. Individuals comprised mostly of Euro-Brazilian (85%) and Afro-Brazilian (13%) populations, with a small percentage of Oriental populations. Diabetes was classified according to the criteria established by the International Society for Pediatric and Adolescent Diabetes (ISPAD) [34], the American Diabetes Association [35], and the Brazilian Society of Diabetes [36]. Participants were recruited from a public hospital and school in southern Brazil after obtaining written consent from their parents. This study was approved by the Ethics Committee of the Federal University of Parana (CAAE 24676613.6.0000.0102: https://plataformabrasil.saude.gov.br/login.jsf;jsessionid=40CFEB49A8B249A561D2027C6E771F71.server-plataformabrasil-srvjpdf130).

Patients who developed T1DM before the age of 18 years old and showed no overt kidney disease verified by serum creatinine levels were included in the study. Among those selected, those who had infectious processes and anemia, verified by blood count, urinalysis, and laboratory parameters, were excluded.

For the control group, individuals without clinical and laboratory signs of any ongoing pathological processes were included.

Healthy children (controls) were selected from public schools in Curitiba city, State of Parana, South Region of Brazil. In the same region, patients with T1DM were recruited from the Clinical Hospital of the Federal University of Parana, Brazil between 2014 and 2017.

Clinical and laboratory data

Blood samples were collected in EDTA (BD Vacutainer® K_3EDTA; BD, Franklin Lakes, NJ, USA) and tubes with separating gel and clot activator (BD Vacutainer® SST® II Advance; BD). The separated serum was stored at -80°C.

Routine laboratory tests, such as determination of blood glucose, HbA1c, and lipid profile,

were performed for all samples using an automated LabMax 400 (Labtest Diagnostic SA, Lagoa Santa, MG, BR) with reagents, calibrators, and controls appropriate for the automated system.

Serum apoM levels were measured using a sandwich ELISA provided by ElabScience (E-EL-H0473; Elabscience Biotechnology, Inc., Houston, TX, USA). Briefly, a micro-ELISA plate was pre-coated with an antibody specific to human apoM. A biotinylated detection antibody specific for human apoM and avidin-horseradish peroxidase conjugate were used to generate the chromogen. Serum samples were diluted 1:20 in saline solution (154 mmol/L NaCl). Absorbance was measured at 450 nm ± 2 nm. The ApoM concentration was derived from the sigmoidal standard curves fitted via nonlinear regression analysis, which was developed using the recombinant apoM protein ranging from 0.31 to 20 ng/mL. To obtain the results in μmol/L, the concentration in ng/mL was divided by 21,05263 (MW 21 KDa).

Genotyping

DNA was extracted from whole blood (K_3 EDTA) after centrifugation (white cell pellets) using the salting-out method, as previously described by Lahiri and Nurnberger (1991) [37], and the levels were normalized to 20 and 100 ng/μL for qPCR and PCR-restriction fragment length polymorphism (PCR-RFLP) techniques, respectively. In this study, only samples with 280/260 nm absorbance ratio between 1.8 and 2.0 were used (NanoDrop; Thermo Scientific, Waltham, MA, USA). Genotyping of rs707921, rs805264, and rs805297 was performed using qPCR with specific fluorescent probes (C_7514748_10, C_7514753_10, and C_7514748_10) using the TaqMan system (Applied Biosystems, Foster City, CA, USA). Genotyping experiments were performed using a Fast™ 7500 instrument (Applied Biosystems) using reagents (Master Mix® and GenotypingAssay® SNP Applied Biosystems) and other supplements provided by the manufacturer.

The rs805296 and rs9404941 polymorphisms were genotyped using the PCR-RFLP protocol described by Niu et al. [33]. The primers used were: forward 5'-AGACAGAGTCTCTGTCGCCCAAG-3' and reverse 5'-GCCAAGGTGGGCGGATGGCTTGA-3'. The PCR reaction system (20 μL) included a final concentration of 0.8 U Platinum®Taq polymerase (Invitrogen, Waltham, MA, USA), 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.25 μM of each primer, 1× PCR buffer, and 100 ng of genomic DNA template. PCR was performed using a Veriti 96 Well Thermal Cycler (Applied Biosystems) under the following parameters: one cycle of denaturation at 94°C for 2 min, 32 cycles of denaturation at 94°C for 30 s, annealing at 70°C for 30 s, and extension at 72°C for 60 s.

PCR products were subjected to restriction endonuclease *Rsa*I (Invitrogen) that cleaves the rs805296 polymorphism, and *Hae*III (Promega, Madison, WI, USA) that cleaves rs9404941, after which they were incubated overnight in a water bath at 37°C. DNA fragments obtained from the restriction reactions were separated using 12% polyacrylamide gel (29:1) electrophoresis (Miniprotean 3 vat; Bio-Rad, Hercules, CA, USA) (100 × 75 × 0.75 mm) and stained with ethidium bromide.

Statistical analyses

Normality for continuous variables was checked with Kolmogorov-Smirnov. Variables with normal distribution were expressed as mean and standard deviation and compared with Student's t-test (two-tailed). Non-normal distribution variables were presented as median and interquartile range (25-75%) and compared using the Mann-Whitney U test. Categorical variables were presented as count (n) and/or percentage (%) and compared using the chi-square test. To assess the association between the polymorphisms and biomarkers, Pearson or Spearman correlation analysis, as appropriated, and ANOVA (one-way) were applied.

The DeFinetti program (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) was used for Hardy-Weinberg (H-W) equilibrium calculations, allele and genotype frequencies, and 95% confidence intervals (95% CI). MedCalc Statistical Software version 19.1 (MedCalc Software bv, Ostend, Belgium) was used for the statistical analysis. HaploView 4.1 software was used to analyze the linkage disequilibrium (LD). *APOM* haplotypes were assessed using ARLEQUIN 3.11 (http://cmpg.unibe.ch/software/arlequin3/).

A probability of < 5% ($P < 0.05$) was considered statistically significant.

Table 1. Anthropometric and laboratory characteristics of the study groups

Parameters	Control (n = 168)	T1DM (n = 144)	P-value
Age (years)	10.0 (10.0-11.0)	11.0 (9.5-13.0)	**< 0.001**
Gender, M/F	90/78	68/76	0.264**
Weight (kg)	38.3 (33.3-45.2)	38.4 (30.1-50.4)	0.961
Height (m)	1.4 (1.3-1.5)	1.4 (1.3-1.5)	0.516
BMI (kg/m^2)	19.5 ± 4.4	18.7 ± 2.9	0.454*
BMI Z-score	0.5 ± 1.0	0.2 ± 0.9	**0.020***
T1DM age at diagnosis, years	-	6.9 (3.8-9.0)	-
Nonfasting glycemia (mmol/L)	5.1 (4.6-5.4)	13.6 (9.4-18.7)	**< 0.001**
HbA1c (%)	5.2 (5.1-5.4)	9.7 (8.7-11.1)	**< 0.001**
1,5-Anhydroglucitol (μmol/L)	183 (156-233)	18 (12-29)	**< 0.001**
Total cholesterol (mmol/L)	3.8 (3.3-14.1)	4.5 (3.8-5.1)	**< 0.001**
HDL-cholesterol (mmol/L)	1.3 (1.1-1.5)	1.4 (1.2-1.7)	**< 0.001**
LDL-cholesterol (mmol/L)	2.1 (1.8-2.3)	2.5 (2.1-3.1)	**< 0.001**
Triglycerides (mmol/L)	1.1 (0.7-1.4)	0.8 (0.6-1.0)	**< 0.001**
ApoM (μmol/L)	2.46 ± 0.97	1.88 ± 0.74	**< 0.001***
Albumin (g/L)	41 (40-45)	42 (40-44)	0.654
Total protein (g/L)	72 (68-77)	71 (68-74)	**0.023**
Creatinine (μmol/L)	44 (27-53)	62 (53-71)	**< 0.001**

Values are presented as mean ± SD, median (interquartile range, 25-75%), or %, -, with no information available. Control: healthy children; T1DM: children with T1DM; BMI: body mass index. P-values were calculated using the Mann-Whitney U test, *Student's t-test (two-tailed), or **chi-square test. Significant P-values (P < 0.05) are marked in bold.

Results

Anthropometric and laboratory data are reported in **Table 1**. Groups were matched according to sex. No statistical differences (P > 0.05) were observed in height, weight, or BMI between the patients with T1DM and healthy controls. Patients with T1DM were leaner considering the Z-score (0.2 ± 0.9 vs. 0.5 ± 1.0; P = 0.020) and were slightly older (median 11 years) than those in the control group (median 10 years).

Patients with T1DM presented with poor glycemic control. All glycemic marker levels were higher than the established guidelines for pediatric age groups, such as HbA1c < 7.0%, glycemia < 7.2 mmol/L, postprandial glycemia < 10 mmol/L [35, 36, 38], and 1,5-anhydroglucitol > 61 μmol/L [39].

T1DM patients had higher levels of total cholesterol, HDL cholesterol (HDL-C), and LDL-C, but this level does not characterize dyslipidemia, as the LDL-C values are within the accepted risk level (< 2.6 mmol/L) [35]. A similar profile was observed for renal markers (creatinine) with average levels within the reference intervals in children (28-63 μmol/L), suggesting that no subjects with overt kidney disease were included [40].

The albumin and total protein levels indicated that none of the groups presented with relevant liver disease, kidney damage, or nutritional disorders.

The apoM levels were significantly (P < 0.001) lower (approximately 23% in median) in children with T1DM compared to those in the controls (1.88 vs. 2.46 μmol/L, respectively).

The genotyping results and allele frequencies of the study groups related to the rs707921, rs805264, rs805296, rs805297, and rs9404941 polymorphisms are listed in **Table 2**. The genotype frequencies of all polymorphisms studied in both groups were in H-W equilibrium (P > 0.05).

Patients with T1DM and nondiabetic control subjects had similar genotype distributions and allelic frequencies for rs805264 (G+203A), rs805296 (T-778C), rs805297 (C-1065A), and rs9404941 (T-855) in the co-dominant model (**Table 2**).

Table 2. Genotyping and allele frequencies for *APOM* polymorphisms rs805296, rs805297, rs9404941, rs707921, and rs805264 in the study groups

Polymorphisms	Genotype alleles	Control (*n* = 168)	T1DM (*n* = 144)	*P*-value
rs805296	T/T	156 (92.9)	139 (96.6)	0.154
T > C	T/C	12 (7.1)	5 (3.4)	
	C/C	0 (0)	0 (0)	
	H-W (P)	0.964	0.983	
	C allele, % [95% CI]	3.6 [2-6]	1.7 [0-3]	0.160
rs805297	C/C	93 (55.6)	89 (61.8)	0.068
C > A	C/A	60 (35.5)	51 (35.4)	
	A/A	1 (8.9)	4 (2.8)	
	H-W (P)	0.732	0.795	
	A allele, % [95% CI]	26.8 [22-32]	20.5 [16-25]	0.065
Dominant*	CC vs. CA + AA	93 vs. 75	92 vs. 56	0.238
Recessive**	AA vs. CC + CA	15 vs. 153	04 vs. 144	**0.021**
rs9404941	T/T	159 (94.7)	140 (97.3)	0.240
T > C	T/C	9 (5.3)	4 (2.7)	
	C/C	0 (0)	0 (0)	
	H-W (P)	0.971	0.986	
	C allele, % (95% CI)	2.7 [1-4]	1.4 [0-3]	0.260
rs707921	C/C	134 (79.7)	127 (88.2)	0.084
C > A	C/A	32 (19.1)	17 (11.8)	
	A/A	2 (1.2)	0 (0.0)	
	H-W (P)	0.954	0.451	
	A allele, % (95% CI)	10.7 [7-14]	5.9 [3-9]	**0.031**
rs805264	G/G	148 (88.1)	127 (88.2)	0.740
G > A	G/A	18 (10.7)	17 (11.8)	
	A/A	2 (1.2)	0 (0.0)	
	H-W (P)	0.106	0.451	
	A allele, % (95% CI)	6.5 [4-9]	5.9 [3-9]	0.407

Values are presented as n (%). Controls: healthy children; T1DM: children with type 1 diabetes. All polymorphisms were in Hardy-Weinberg equilibrium ($P > 0.05$). 95% CI: 95% confidence interval. *Dominant model and **Recessive model. Probability (P), chi-square test; significance $P < 0.05$ (in bold).

In polymorphism rs707921 (C+1871A), the frequency of the A allele was higher ($P = 0.031$) in controls than that in patients with T1DM (10.7% vs. 5.9%, respectively); nevertheless, the genotype frequencies were not significantly different (P = 0.084) (**Table 2**).

The rs805297 genotype distribution differed significantly between the control group and subjects with T1DM only in the recessive model ($P = 0.021$), and the homozygous minor AA allele of rs805297 was associated with a significantly lower risk of T1DM (**Table 2**).

Figure 1 shows that rs805297 is in a complete LD (D' = 100) with rs805264, rs805296, and rs805264 (D' = 93). In addition, rs805296 and rs707921 (D' = 93) as well as rs805264 and rs707921 (D' = 93) are in a strong LD.

Table 3 shows three common haplotypes constructed by these five polymorphisms in Brazilian populations. The frequency of C-T-T-G-C haplotype (h1) in nondiabetic controls was significantly lower than that patients with T1DM (82.1% vs. 95.1%, respectively; $P < 0.001$).

Discussion

Mughal et al. [41] did not find any differences in apoM levels between patients with T1DM and healthy controls, but HNF1A-MODY individuals had lower levels of ApoM compared to both T1DM (1.37 [0.26], $P = 3.1 \times 10^{-18}$) and control

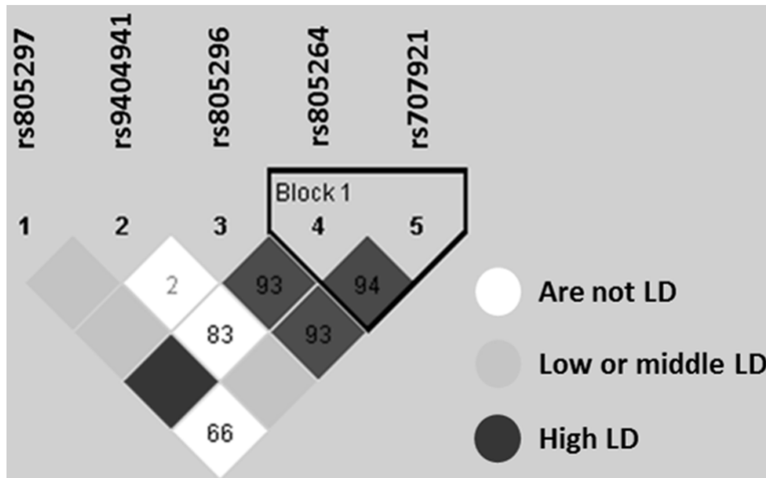

Figure 1. Linkage disequilibrium (LD) of five polymorphisms studied. Five polymorphisms, rs805297 (1), rs9404941 (2), rs805296 (3), rs805264 (4), and rs707921 (5), were selected for this study. Intensity of LD is reflected in color and numeric value (D') of each box. Numbers in squares indicate D' index (%). Dark gray squares without numbers indicate perfect LD (D' = 100% or 1) between corresponding SNPs.

(1.34 [0.22], $P = 7.2 \times 10^{-19}$) groups. Frej et al. [42] did not find any differences in apoM levels between T1DM and control groups. This could be attributed to a difference between the age of diagnosis evaluated in the current study (11.0; 9.5-13.0 years) and those of Mughal et al. (25.5 ± 11.5 years) [41] and Frej et al. (45.2 ± 1.4 years) [42]. Plasma apoM levels were 9% lower in patients with T2DM than those in healthy controls [43].

In women, apoM levels were weakly correlated with age (r = 0.24, $P < 0.001$). Therefore, a common reference interval for the whole population was not possible and could explain the variation in apoM levels found in the literature (0.91 ± 0.22 - 1.37 ± 0.33 μmol/L) to healthy adult individuals [41, 44-46]. In addition, it has been reported that apoM levels are approximately 30% higher in serum than those in plasma [45].

Finally, as reported by Yao Mattisson and Christoffersen [23], the quantification of plasma levels of human apoM is challenging owing to the lack of robust and reliable methods for analysis. The nature of the apoM protein, with a hydrophobic signal peptide anchoring it to the phospholipid layer of multiple lipoproteins, makes it difficult to develop specific antibodies, reliable assay conditions, and control materials.

In the healthy group (age ≥18 years), plasma apoM levels were positively associated with HDL-C and LDL-C levels [46]. In addition, it has been reported that there is a positive association (r = 0.28-0.42) between plasma apoM and LDL-C levels [43, 45, 46]. Individuals with cardiovascular diseases and control subjects did not show significant differences in plasma apoM levels [45]. However, it has been proposed that ApoM may be a useful biomarker for predicting the progression of diabetic nephropathy in T2DM [47] and T1DM [48].

The expression stimuli in the liver is mediated by inflammatory processes, transcription factors, and hormones [49]. Drexler et al. [49] showed that plasma ApoM is a potential biomarker of glomerular mRNA expression of deficiency and is strongly associated with clinical outcomes in glomerular diseases. In addition, polymorphisms in the APOM promoter region may increase promoter activity [32]. Therefore, a complex system of interactions exists among transcription factors, hormones, and functional polymorphisms. The effects of different modulators on each other need to be further assessed.

The current 3% global increase in T1DM occurrence per year is well documented [50]. This rapid increase strongly suggests that the environmental effects on gene susceptibility contribute to the evolving epidemiology of T1DM [51]. Therefore, genetic studies of these polymorphisms are crucial to replicate these findings and assess their effects on the pathogenesis of T1DM in populations with diverse genetic backgrounds and environments.

We analyzed the general distribution of alleles and genotypes of the five polymorphisms in *APOM* in both the case and control groups; however, the results did not support an association between these five polymorphisms and T1DM susceptibility in the Euro-Brazilian population (**Table 2**).

Table 3. Haplotypes resulting from combination of polymorphisms rs805297, rs9404941, rs805296, rs805264, and rs707921 in the study groups

Haplotype	*APOM* polymorphisms					Prevalence (%)		*P*-value
	rs805297 C/A	rs9404941 T/C	rs805296 T/C	rs805264 G/A	rs707921 C/A	CTRL (*n* = 168)	T1DM (*n* = 144)	
h1	C	T	T	G	C	82.1	95.1	**< 0.001**
h2	A	T	T	G	C	44.6	38.2	0.249
h3	C	T	T	A	A	5.4	7.6	0.412

Haplotypes with a frequency > 5% in the study groups. CTRL: control, healthy children; T1DM: children with type 1 diabetes; Probability (*P*), chi-square test; significance $P < 0.05$ (in bold). Mutated alleles of SNPs in each haplotype are underlined. Odds ratio for h1 haplotype; 4.25 (95% CI, 1.81-10.1).

The AA genotype of rs805297 was associated with a significantly lower risk of T1DM (**Table 2**), suggesting that the A allele confers protection from the disease. In concordance with this result, the AA allele was also found at a higher frequency in the control group than in T1DM patients in both Chinese and Swiss populations [32]. Importantly, our study reported a limited number of individuals with the rs707921 and rs805297 AA genotypes, which significantly reduced the statistical power of this comparison.

Wu et al. [32] found an association between T1DM and rs805296 in both Han Chinese and Swedish populations, but not between T1DM and rs805297 or rs9404941, despite the similar genotype distribution of these two polymorphisms and the different prevalence of T1DM between Chinese and Swedish populations. Wu et al. [32] found a higher C allele frequency in the T1DM group in Chinese (10.5%) and Swedish (8.1%) populations than in the Euro-Brazilian (1.7%) population in the present study (**Table 2**). The rs805296 C allele frequency in the control group (Han Chinese, 5.1% and Swedish, 3.0%) was in accordance with our results within the CI (2-6%); however, it was lower than that reported in other Chinese populations (between 6.9 and 13.2%), particularly in the Han Chinese [52-56].

The low frequency of the C allele observed in the present study in rs9404941 was similar to that in the African population (0.7%); however, it was lower in European (7.1%), American (4.7%), and Asian (24.4%) populations [57].

The frequencies of the A allele rs707921 (10.7%; 95% CI: 7-14) and rs805264 (6.5%; 95% CI: 4-9) in the control group were similar to those described in American (9.1% and 8.1%, respectively) and Latin American (7.1% and 5.3%, respectively) populations, higher than European (3.4% and 3.1%, respectively) populations, and lower than Asian (20.9% and 20.6%, respectively) populations [57-59].

Regarding the LD of *APOM* variants (**Figure 1**), this study revealed that rs805297 was in complete LD (D' = 100) with rs805264. The rs805296 and rs805264 (D' = 93); rs805296 and rs707921 (D' = 93) and rs805264 and rs707921 (D' = 93) variants was in a strong LD. Studies on Chinese populations have found no LD between rs805297, rs9404941, and rs805296 (D' ≤ 0.734) [32], or between different variants, rs805297 and rs9404941 (D' = 82) [27].

The subjects with h1 haplotype have an increased risk for T1DM (OR: 4.25; 95% CI: 1.81-10.1) (**Table 3**). In Chinese populations, the rs805297-A, rs9404941-T, and rs805296-T allelic haplotype and rs805297-C and rs9404941-C allelic haplotype indicate a high risk of T1DM (odds ratio [OR] [95% CI] = 0.71 [0.53-0.95], $P = 0.016$) [32] and T2DM (OR [95% CI] = 1.62 [1.29-2.16], $P < 0.001$) [27], respectively.

ApoM has been suggested to play a role in lipid metabolism. Studies have revealed that the *APOM* rs805296 C allele polymorphism is associated with higher cholesterol levels in patients with coronary artery disease and healthy subjects in the Han Chinese population [32, 55]. The mechanisms by which the CT and CC genotypes of the rs805296 polymorphism contribute to higher total cholesterol levels remain unknown. Presumably, different alleles in the proximal promoter region of *APOM* may enhance its expression and further affect lipoprotein metabolism.

We analyzed the association of rs805297 genotypes with the levels of glycemia, HbA1c, lipid profile, and apoM using ANOVA. However, no significant association was observed (data not shown). Total cholesterol, HDL-C, LDL-C, and triglyceride levels remained unaffected by the rs805297 polymorphism in *APOM* genotypes, which was in accordance with a study by Zhang et al. [28].

However, we found a weak correlation between rs805296 and HDL-C levels ($r = 0.1967$, $P = 0.021$; data not shown), which is in accordance with the evidence revealing that *APOM* expression and plasma HDL-C levels are linked [15, 60].

We were unable to identify any relevant associations between the polymorphisms and biomarkers. We hypothesized that two major factors may have affected these analyses. First, the sample size is relatively small. Second, the frequency of the rare allele in our population was low for rs707921, rs805264, rs805296, and rs9404941, which affected the power calculation.

Nevertheless, this is the first study to provide information on the genotypic and allelic frequencies of polymorphisms rs707921, rs805264, rs805296, rs805297, and rs9404941 of *APOM* in the Euro-Brazilians population. However, further investigation is required in other ethnic groups to confirm and enhance our findings.

These differences may be related to the varying incidences of T1DM in various countries. Most of these differences cannot be easily explained by the allele frequencies of the polymorphisms studied, including class II HLA and non-HLA risk genes; however, environmental factors and gene-environment interactions may explain these differences and may also be relevant to the genetic effects detectable in each geographical area [61]. Indeed, the *APOM* rs805396 (T-778C) polymorphism was strongly associated with T1DM in both Han Chinese and Swedish populations [32] and with T2DM in Han Chinese populations [27, 33]; however, these results were not replicated in northern China [28]. An important confounding factor in T1DM is the random distribution of subjects with rapid or slow natural disease [61].

The present study is a prospective one involving a relatively small sample size that needs to be increased to confirm our findings. Brazil has a mixed population, and similar future studies will be required in other regions of the country to identify the contribution of these polymorphisms to T1DM and expand knowledge of this population diversity.

In summary, the present study provides evidence that the *APOM* C-T-T-G-C haplotype of rs805297, rs9404941, rs805296, rs805264, and rs707921 confers an increased risk of childhood-onset T1DM in the Euro-Brazilian population.

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Disclosure of conflict of interest

None.

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