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Cytotoxic T lymphocyte antigen-4 (*CTLA-4*) rs231775 and rs5792909 polymorphisms are not associated with adult- and childhood-onset type 1 diabetes in a Southern Brazilian population

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ABSTRACT. Several studies have described an association between cytotoxic T-lymphocyte antigen-4 (*CTLA-4*) gene polymorphism and type 1 diabetes mellitus (T1D) in some ethnic populations, and a lack of association in other populations. Differences in the contribution of the genetic background of T1D onset are age dependent. We conducted a case-control study of a T1D Brazilian population, in which a possible association of rs231775 (+49A/G) and rs5792909 (-318C/T) polymorphisms in *CTLA-4* with T1D was evaluated. These polymorphisms were genotyped in 150 childhood-onset (age ≤ 14 years old) and 150 adult-onset (age >18 years) patients with diabetes and non-diabetic healthy individuals (150 children and 150 adults). PCR-restriction fragment length polymorphism (rs5792909) and TaqMan® fluorescent probe (rs231775) methodologies were used for genotyping. The polymorphisms were in Hardy-Weinberg equilibrium. There was no difference in genotype and allele frequency between the patients with T1D and non-diabetic controls. The frequencies for childhood-T1D and adulthood-T1D, for the rs231775 G-allele (95% CI) were 39.0% (31–47) and 37.3% (30–46), and for the T-allele of the rs5792909 polymorphism they were 5.0% (3–7%) and 2.7% (1–4%), respectively. We did not

identify an association of *CTLA-4* rs231775 and rs5742909 polymorphisms with adult- or childhood-onset T1D in a Euro-Brazilian population in Southern Brazil.

Key words: *CTLA-4*; Type 1 diabetes; Childhood-onset; Adult-onset; Polymorphism; Brazilian population

INTRODUCTION

Type 1 diabetes **(**T1D) is a heterogeneous disorder with a distinct pathogenesis in age onset, due to a combination of genetic and/or environmental factors (Gillespie and Long, 2019). Epidemiological studies have shown that T1D is not only a childhood disease and suggest that 42–65% of patients with T1D may develop clinical signs of diabetes after age 20. Moreover, there is increasing evidence to indicate that T1D, especially when developed in adulthood, is clinically and immunologically heterogeneous (Gillespie and Long, 2019).

Major genetic risk factors of T1D are located within the class II human leukocyte antigen (HLA) region; however, approximately 60 recognized non-HLA loci also affect disease susceptibility, including cytotoxic T lymphocyte-associated antigen-4 (*CTLA-4*) (Robertson and Rich, 2018).

CTLA-4, also named CD152, a glycoprotein receptor expressed by both $CD4^+$ and $CD8^+$ T cells, binds to a pair of ligands (CD80 and CD86) expressed on the surface of antigen presenting cells (APCs), and interactions of these ligands with CTLA-4 inhibit T cell responses, although the precise mechanisms are not fully understood. Dysregulated expression of CTLA-4 leads to immune homoeostasis imbalance and autoimmunity (Rowshanravan et al., 2018). Monoclonal inhibitors of CTLA-4 were associated with rare immune checkpoint inhibitor induction of diabetes mellitus (Quandt et al., 2020). Gerold et al. (2011) demonstrated in an animal model that the reduction in soluble CTLA-4 (sCTLA-4) expression, a splicing variant, impairs the function of Treg cells, promoting an increased risk of autoimmune diabetes.

CTLA-4, located on chromosome 2 (2q33), consists of four exons and three introns and has been implicated in several autoimmune diseases, including T1D (Nistico et al., 1996; Vaidya and Pearce, 2004; Douroudis et al., 2009). Although several polymorphisms have been repeatedly associated with the disease, various studies did not confirm this association (Bouqbis et al., 2003; Kisand and Uibo, 2012; Tavares et al., 2015). In addition, Abe et al. (1999) have reported that *CTLA-4* polymorphism is associated with the mode of onset of T1D in a Japanese population.

A common polymorphism in the *CTLA-4* coding region is +49AG (rs231775), which is a conversion of threonine to an alanine amino acid in exon one, in the signal peptide (Kantarova and Buc, 2007). The Thr17Ala change results in incomplete glycosylation in the endoplasmic reticulum, leading to retrograde transport of a portion of the molecules into the cytoplasm for degradation. This results in reduced amounts of CTLA-4 (Ala17) at the cell surface, which may explain the reduced inhibitory function of CTLA-4 reported in +49G allele carriers (Anjos et al., 2002). This polymorphism is associated with numerous autoimmune diseases (Wang et al., 2017), such as Graves' disease (Si et al., 2012) and T1D (Bouqbis et al., 2003; Turpeinen et al., 2003; Haller et al., 2004; Douroudis et al., 2009; Benmansour et al., 2010; Jin et al., 2015).

Another well-studied polymorphic marker that has drawn considerable attention is a C-to-T substitution at position -318 (rs5992909) of the promoter region (Deichmann et al., 1996). Previous studies have shown that a T allele at position −318 of the *CTLA-4* promoter is associated with increased transcriptional activity (Anjos et al., 2002), explaining the association of the T (-318) allele with a lower risk of a variety of autoimmune diseases. Chistiakov et al. (2006) have suggested that the (-318) single nucleotide polymorphism (SNP) modifies a putative binding site for lymphoid enhancer-binding factor 1 (LEF1), such that it alters the stimulating effect of LEF1 on the expression of the *CTLA-4* promoter. However, the association of the −318C/T polymorphism in the promoter region with autoimmunity is weak and inconsistent (Heward et al., 1998; Wang et al., 2002; Huber et al., 2008).

We investigate the prevalence of the *CTLA-4* +49A/G (rs231775) and -318C/T (rs5742909) gene polymorphisms in adult- and childhood-onset T1D in a case–control study in a Southern State Euro-Brazilian population.

MATERIAL AND METHODS

Subjects

A total of 600 unrelated subjects in southern Brazil, classified as children (n = 300; ≤ 14 years old) or adults (n = 300; ≥18 years old) were selected for the study. Patients with childhood-onset T1D (n = 150) were randomly selected from the Clinical Hospital of the Federal University of Parana (HC-UFPR), Parana, Brazil. The control ("healthy" children, n = 150) were randomly selected from a public elementary school network from the Curitiba, Parana, Brazil, with the same ethnic characteristics as the T1D group. Patients with adult-onset T1D (n = 150), who developed T1D after they were 18 years old, were selected from the Clinical Hospital – UFPR; the controls ("healthy" adults, n = 150) were selected from blood bank donors at the Clinical Hospital of the Federal University of Parana (HC-UFPR), Parana, Brazil.

Subjects were considered healthy (control groups, inclusion criteria) when they showed no relevant medical conditions, with an emphasis on the absence of acute or chronic diseases, such as cardiovascular and kidney diseases or diabetes, as well as laboratory parameters (hemogram, urinalysis, and routine serum chemistry) within the reference interval adjusted for age and sex. A diabetes diagnosis was established according to the criteria of the International Society for Pediatric and Adolescent Diabetes (Mayer-Davis et al., 2018) and the American Diabetes Association (ADA, 2020).

The Brazilian population is a genetic admixture (trihybrid) from European, African and Native American populations (Salzano and Sans, 2014). The southern region of Brazil, such as in Parana State, had a greater European (or Caucasian) contribution (Moura et al., 2015). The study population mostly self-reported as having white skin and European ancestry and was classified as Euro-Brazilian (81%). Other contributions were African-Brazilian (18%) and Orientals and Native Americans (1%).

All the recruited subjects gave written informed consent. The Federal University of Parana's Ethics Committee approved this study (CAAE 01038112.0.0000.0102 and 24676613.6.0000.0102).

Genotyping

The *CTLA-4* +49A/G (rs231775) polymorphism was analyzed using the Taqman (C_2415786_20) SNP genotyping assay (Applied Biosystems, Foster City, CA, USA). The -318 C/T (rs5742909) SNP was amplified using PCR from genomic DNA, using the following primers: forward 5′-AAATGAATTGGACTGGATGGT-3′ and reverse 5′-TTACGAGAAAGGAAGCCGTG-3′. Genomic DNA (100 ng) was amplified in each 20 µL PCR reaction containing 0.2 mmol/L of each dNTP (Invitrogen, Carlsbad, California, EUA), 0.1 U of Taq DNA polymerase (Invitrogen), 1× PCR buffer (Invitrogen), and 0.5 pmol/L of each primer. The reaction mixture was first heated at 94°C for 2 min and then amplified for 30 cycles in a PCR thermocycler (Eppendorf, Mastercycler gradient, Connecticut, EUA) by denaturation at 94°C for 30 s, annealing at 60°C for 30 s and extension at 72 C for 30 s in each cycle, followed by final cycle at 72°C for 7 min. A restriction digest (final volume, 10 µL) was incubated at 37°C for 3 h, containing 1.0 µL (~4–10 ng) PCR products mixed with 0.2 µL (2 U) *Mse*I (10 U/µL; New England Biolabs, Hitchin, UK) and 1× CutSmart™ Buffer (50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 100 µg/mL bovine serum albumin, pH 7.9). DNA fragments were separated using 15% polyacrylamide (29:1) gel electrophoresis and stained with ethidium bromide (Figure 1). All gels presented unequivocal genotyping.

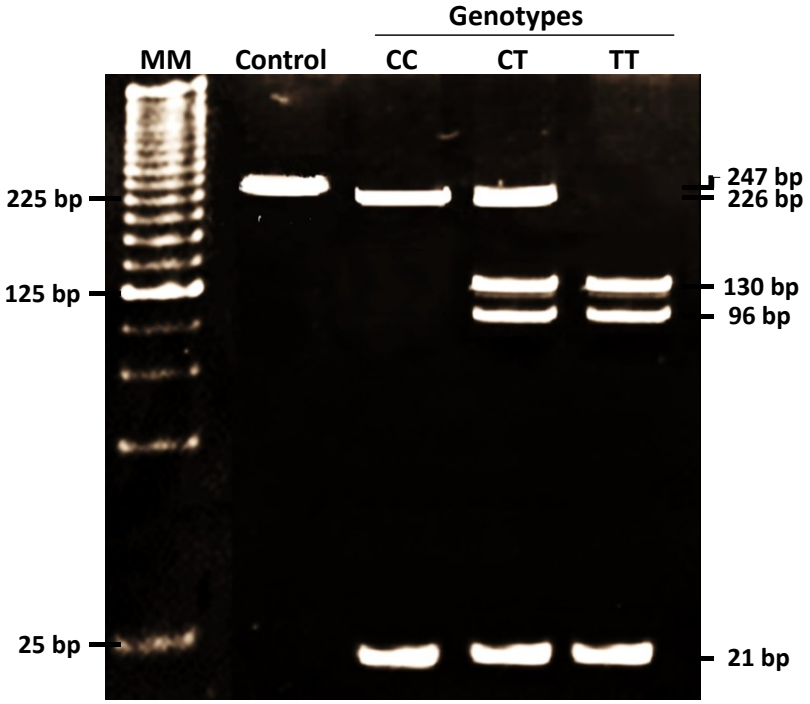

Figure 1. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) electrophoretic pattern of the genotype of *CTLA-4* promoter -318C/T (rs5742909). Restriction digest with *Mse*I. Lanes, MM (25 bp DNA ladder); Control, PCR product without digestion (247 bp); CC, CC genotype (226 bp and 21 bp); CT, CT genotype (21 bp, 96 bp, 130 bp, and 226 bp) and TT, TT genotype (21 bp, 96 bp, and 130 bp); (2). Right, molecular mass of the restriction fragments; Left, relevant ladder molecular mass. Polyacrylamide (29:1) gel (15%) electrophoresis in 1× Tris/Borate/EDTA buffer, stained with ethidium bromide.

Statistical analysis

Normality was tested using the Kolmogorov–Smirnov test. Parameters with normal distributions were compared using a Student's *t*-test (two-tailed) for independent samples. The Mann–Whitney *U* test was used to compare parameters with non-normal distributions. Categorical variables were compared using a chi-square test. Verification of the Hardy-Weinberg equilibrium (HWE), genotypic and allelic frequencies, as well as the 95% confidence interval (95% CI) for the minor allele frequencies (MAFs) were performed using the program DeFinetti (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl).

Comparisons of allele frequencies with other populations were considered similar when the MAFs were within the 95% CI limits. Frequencies above or below the 95% CI were considered different. All calculations were performed using MedCalc version 17.6 (MedCalc Statistical Software Bvba, Ostend, Belgium). A probability less than 5% ($P < 0.05$) was considered significant for all tests.

RESULTS

The demographic, clinical and laboratory characteristics of the studied groups are presented in Table 1. The children and adult groups were matched by gender and body mass index. Patients with childhood-T1D were slightly and significantly older than controls (median difference 1 year old; P=0.013). Adult patients with T1D were matched by age. The BMI z-scores were significantly reduced (P=0.015) in patients with childhood-onset T1D, as expected.

Table 1. Anthropometric, clinical, and laboratory characteristics for children and adults with type 1 diabetes and respective non-diabetic controls.

Parameters	Children Controls n = 150	T1D n = 150	P	Adults Controls n = 150	T1D n = 150	P
Age (years)	10 (10–11)	11 (9–13)	0.013	44 (40–49)	45 (34–52)	0.692
Gender (M/F)	81/69	73/77	0.355**	56/94	52/98	0.630**
BMI (kg/m^2)	19.2 ± 4.0	18.6 ±3.0	0.157*	27.0 ± 5.9	26.1 ± 6.0	0.192*
BMI z-score	0.53 ± 1.06	0.24 ± 0.99	0.015*	-	-	-
Age at diagnosis (years)	-	6.5 ± 3.4		-	27.9 ± 9.3	
Time of DM onset (years)	-	4.1 ± 3.1	-	-	14.8 ± 10.7	-
DM family history, %	-	71.2		-	69.3	
Fasting glycemia (mmol/L)	5.1 (4.7–5.4)	13.0 (9.5–19.1)	< 0.001	5.3 (4.7–5.8)	10.4 (5.9–13.8)	< 0.001
HbA1C (%)	5.2 (5.1–5.4)	9.7 (8.7–11.1)	< 0.001	5.4 (5.2–5.6)	8.8 (7.6–9.7)	< 0.001
1,5 AG (µmol/L)	183 (157–233)	18 (12–29)	< 0.001	150 (125–178)	25 (14–36)	< 0.001
Albumin (g/L)	42 (40–46)	43 (40–44)	0.780	39 (38–40)	41 (38–43)	< 0.001
Creatinine (µmol/L)	48.6 (35.4–57.5)	61.9 (53.0–70.7)	< 0.001	49.5 (42.4–59.2)	75.1 (70.7–88.4)	< 0.001

Results are presented as means ± 1-standard deviation; medians (interquartile range, 25%–75%); n (number of individuals); or %.T1D, type 1 diabetes (childhood- or adult-onset); Controls, non-diabetic "healthy" subjects; Gender, M, male; F, female; BMI, body mass index; Time in years since diabetes (DM) onset; DM family history, families with a history (parents, brothers, grandparents) of diabetes; HbA1C, hemoglobin A1c; 1,5 AG, 1,5-anhydroglucitol. Probability (*P*), Mann-Whitney *U* test; *Student *t*-test (two-sided); or **Chi-square test. $P < 0.05$ is significant and highlighted in bold.

Poor glycemic control was evident in both diabetic groups, demonstrated by glycated hemoglobin and 1,5 anhydroglucitol (good glycemic control criteria, hemoglobin A1c [HbA1c] < 7.5% and 1,5-AG > 61 mmol/L). All subjects showed no critical liver dysfunction or malnutrition, as tested by serum albumin. Additionally, serum creatinine concentrations did not characterize acute kidney failure in all study groups.

The frequencies of alleles and genotypes of the *CTLA-4* +49A/G (rs231775) and -318 C/T (rs5742909) polymorphisms in the studied groups are presented in Table 2. All genotypic frequencies were in Hardy-Weinberg equilibrium. The genotypic and allelic frequencies of both polymorphisms were not significantly different between patients with T1D (child and adult) and their respective controls. Comparisons of MAFs for both polymorphisms between controls and between T1D groups showed no significant difference.

Table 2. Allele and genotype distribution of *CTLA-4* polymorphisms in patients with childhood-type 1 and adult-type 1 diabetes and controls.

Gene *CTLA-4* Polymorphisms	Genotypes Alleles	Children Controls n = 150	T1D n = 150	P	Adults Controls n = 150	T1D n = 150	P
rs231775 A>G	A/A	53 (35.5)	61 (40.4)	0.510	60 (40.0)	56 (37.1)	0.155
(+49AG)	A/G	73 (50.3)	61 (40.4)		62 (41.4)	76 (51.0)	
	G/G	21 (14.2)	28 (19.2)		28 (18.6)	18 (11.9)	
MAF	G-allele, %	39.3	39.0	0.933*	39.3	37.3	0.614*
	[95% CI]	[31–48]	[31–47]		[31–48]	[30–46]	
Dominant	AA vs AG+GG	53/94	61/89	0.414	60/90	56/94	0.635
Recessive	GG vs AG+AA	21/129	28/122	0.274	28/122	18/132	0.109
rs5742909 C>T	C/C	137 (91.3)	135 (90.0)	0.510	134 (89.4)	142 (94.4)	0.186
(-318 CT)	C/T	12 (8.0)	15 (10.0)		15 (10.0)	8 (5.6)	
	T/T	1 (0.7)	0 (0)		1 (0.6)	0 (0 %)	
MAF	T-allele, %	4.7	5.0	0.849*	5.7	2.7	0.067*
	[95% CI]	[2–7]	[3–7]		[3–8]	[1–4]	
Dominant	CC vs CT+TT	137/13	135/15	0.414	134/16	142/8	0.089
Recessive	TT vs CC+TT	1/149	0/150	0.317	1/149	0/150	0.317

Values are reported as n (%). Probability (*P*), chi-squared test (χ^2) for genotype and *allele frequencies; 95% CI = 95% confidence interval. Hardy-Weinberg equilibrium (*P*-value) for rs231775 in child controls (0.450), childhood-onset T1D (0.075), in adult controls (0.101) and adult-onset T1D (0.310) and for rs5742909 in child controls (0.217), for childhood-onset T1D (0.424), in adult controls (0.428) and adult-onset T1D (0.730). MAF, minor allele frequency; T1D, type 1 diabetes.

Additionally, the study did not reveal any significant association between rs231775 genotypes with serum biomarkers, such as glycemia, HbA1c, 1,5 anhydroglycitol, creatinine, and lipid profile (data not shown). The low MAF in rs5742909 did not allow analysis of biomarker associations.

DISCUSSION

T1D is a very heterogeneous syndrome, with considerable variability in the age of onset, in abrupt onset, and in the profile of autoantibodies (Gillespie and Long, 2019). Ethnic heterogeneity of the effect of *CTLA-4* on T1D has been observed (Wang et al., 2014). The *CTLA-4* gene (IDDM12) is located near two other T1D susceptibility regions, IDDM7 (2q31), IDDM13 (2q34), and the genes encoding CD28 and islet tyrosine phosphate, which may be considered candidate T1D susceptibility genes. This closeness to other susceptibility genes and regions may explain the weakness or absence of *CTLA-4* association with T1D in some populations (Marron et al., 1997; Badenhoop et al., 1999; Larsen et al., 1999).

First observed in Italian subjects (Nistico et al., 1996), and confirmed subsequently by case control and family studies, *CTLA-4* polymorphic variants have been linked with

T1D pathogenesis (Vaidya and Pearce, 2004, Douroudis et al., 2009). While this association is detected in various ethnic groups (Douroudis et al., 2009), it appears more likely to be Caucasian selective (Wells et al., 2001) and absent from non-Caucasians (Baniasadi et al., 2006; Balic et al., 2009). A report from the Type 1 Diabetes Genetics Consortium on 2,300 affected sib pair families demonstrated that among the 24 SNPs genotyped in the *CTLA-4* region, only the +49AG and CT60 SNPs are replicated in the nine combined collections (Qu et al., 2009).

Similarly, Abe et al. (1999) have reported a significant association between *CTLA-4* polymorphism allele G (+49AG) rs231775 and the mode of onset of T1D in Japanese patients. The results of both studies indicated that the presence of the G allele in *CTLA-4* might be involved in determining the mode of onset of T1D in Japanese patients. Addicionaly, the G-allele of rs231775 was associated with a reduced risk for for insulin autoantibody (IAA) as the first appearing cell autoantibody in children born to a mother with gestational respiratory infection (Lynch et al., 2018), affecting the risk and progression to T1D.

Therefore, the Brazilian population was evaluated to determine if there were associations between *CTLA-4* +49A/G (rs231775) and -318 C/T (rs5742909) polymorphisms with T1D, as well as diagnosis age of disease.

The genotype, allele frequencies and the models dominant and recessive, for rs231775, did not differ between children and adult groups, suggesting that this polymorphism was not associated with T1D in the study population (Table 2). The lack of association with T1D in this study is in agreement with other studies carried out in the Portuguese (Lemos et al., 2009), Argentinean (Caputo et al., 2005), Colombian (Rodriguez et al., 2015), Chilean (Balic et al., 2009), North Indian (Baniasadi et al., 2006), Korean (Jung et al., 2009), Moroccan (Bouqbis et al., 2003) populations.

Studies that evaluated Brazilian populations with different genetic background from the Northeast (Recife city) (Tavares et al., 2015) and Southeast (São Paulo city and Minas Gerais State) (Hauache et al., 2005; Ferreira et al., 2009) showed no association of this polymorphism with childhood-onset T1D, in agreement with our results. According to Souza et al (2019), the frequencies of European/African descendants vary in each of the cited regions, and characterized as Minas Gerais (Euro 70.5%; Afro 24.5%), São Paulo city (Euro 67%; Afro 21.8%) and Recife city (Euro 60%; Afro 23%), sample from Parana State (Euro 81%; Afro 18%).

There was similarity in the MAFs in this study (~39%) for the control and T1D groups with the Indian (35.8% and 37.7%) (Baniasadi et al., 2006), Chilean (34.4% and 35.6%) (Balic et al., 2009), Brazilian Northeast (35.0% and 38.0%) (Tavares et al., 2015) and American populations (46.4%; dbSNP). However, the frequencies are lower than those described for Asian (69.9 and 68.3%) (Jung et al., 2009) and Iranian populations (49.7 and 58.9%) (Khoshroo et al., 2017) and higher than those described for the Moroccan population (28 and 28%) (Bouqbis et al., 2003).

The genotype and allele frequencies for the *CTLA-4* polymorphism -318C/T (rs5742909) in the studied groups are shown in Table 2. There was no significant difference between the genotypic and allelic frequencies, suggesting that the 5742909 polymorphism of the *CTLA-4* gene was not associated with T1D in the South-Brazilian population.

The rs5742909, -318T allele and CT/TT genotype are associated with risk for T1D in a Tunisian (Benmansour et al., 2010) and an American (Steck et al., 2005) population. In

our study, and in accordance with the frequencies described in the Estonian (Kisand and Uibo, 2012), Korean (Jung et al., 2009), Argentinean (Caputo et al., 2005), and Chilean populations (Balic et al., 2009), the distribution of CT and TT genotypes is similar in the control and T1D groups (Table 2).

In the Caucasian population, a consistent association between the rs5742909 polymorphism of the *CTLA-4* gene and T1D is not found (Heward et al., 1998). A higher T-allele frequency is expected, as it is associated with higher promoter activity (Wang et al., 2002). Nevertheless, the absence of the rare genotype TT is not surprising, since the genotype frequencies in many populations are not significantly different from zero (Gonzalez-Escribano et al., 1999).

Anjos et al. (2004) found a joint polymorphic association between polymorphisms -318C/T (rs5742909) and +6230A/G (rs3084243). In a study carried out in patients with T1D in the Canadian population, neither polymorphism was responsible for a functional effect, although the -318C/T polymorphism showed a possible underlying mechanism in the promoter region of *CTLA-4* (Anjos et al., 2004). Baniasadi et al (2006) observed a possible association between T1D and the polymorphisms -318C/T and -1661A/G (rs4553802) of the promoter region of *CTLA-4* in various populations. The strong linkage imbalance between the polymorphisms in the 2q33 region of *CTLA-4* makes it difficult to determine which alleles or combinations of alleles are directly responsible for the observed associations (Douroudis et al., 2009).

The rs5742909 -318T allele frequency in the control groups in this study (~5%) is lower than that described for other European (9.1 %; dbSNP) and Asian (10.7%) (Kisand and Uibo 2012) populations, but similar to that described for the Brazilian population in the Southeast region (7–8%) (Guzman et al., 2005; Oliveira et al., 2011), and higher than that of Africans (1.8%; dbSNP) (Table 2).

The allelic variation that influences the development of T1D and chronic complications is a historical one of ancestry, taxes of migration, and interbreeding between people (Spanakis and Golden, 2013). Most genetic studies in T1D are performed in homogeneous populations. Therefore, it is important to carry out studies in mixed populations, such as in Brazil, with the objective to determine the relationship between ancestry and allelic frequencies.

The population genetic differences and the high heterogeneity of T1D may be responsible for the variation among diverse studies of association with the studied polymorphisms (Chen et al., 2013). The positive association results were found mostly in Caucasians; therefore we expected that the Southern Brazilian population studied, with the highest Caucasian (Euro-Brazilian) background, compared with other published Brazilian studies previously mentioned, could be revealing. Additionally, we studied type 1 diabetes with childhood- and (adult-onset), pathological processes that present consistent genetic differences, offering a new level of information. Previously, with a similar population, we showed that the Protein Tyrosine Phosphatase non-Receptor 22 (*PTPN22*) rs2476601 was associated with T1D only in childhood- and not in adulthood-onset diabetes (Campos et al., 2021). Our results reinforce the importance of replicating association studies between a complex disease as T1D and the specific polymorphisms, in different populations.

We did not find an association of *CTLA-4* +49A/G (rs231775) and -318 C/T (rs5742909) polymorphisms with adult- or childhood-onset T1D in an Euro-Brazilian population in southern Brazil.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abe T, Takino H, Yamasaki H, Ozaki M, et al. (1999). CTLA4 gene polymorphism correlates with the mode of onset and presence of ICA512 Ab in Japanese type 1 diabetes. *Diabetes Res. Clin. Pract.* 46(2): 169-175.

ADA (2020). Standards of Medical Care in Diabetes-2020. *Diabetes Care.* 43(Suppl 1): S1-S2.

Anjos S, Nguyen A, Ounissi-Benkalha H, Tessier MC, et al. (2002). A common autoimmunity predisposing signal peptide variant of the cytotoxic T-lymphocyte antigen 4 results in inefficient glycosylation of the susceptibility allele. *J. Biol. Chem.* 277(48): 46478-46486.

Anjos SM, Tessier MC and Polychronakos C (2004). Association of the cytotoxic T lymphocyte-associated antigen 4 gene with type 1 diabetes: evidence for independent effects of two polymorphisms on the same haplotype block. *J. Clin. Endocrinol. Metab.* 89(12): 6257-6265.

Badenhoop K, Donner H, Pani M, Rau H, et al. (1999). Genetic susceptibility to type 1 diabetes: clinical and molecular heterogeneity of IDDM1 and IDDM12 in a german population. *Exp. Clin. Endocrinol. Diabetes.* 107 Suppl 3: S89-92.

Balic I, Angel B, Codner E, Carrasco E, et al. (2009). Association of CTLA-4 polymorphisms and clinical-immunologic characteristics at onset of type 1 diabetes mellitus in children. *Hum. Immunol.* 70(2): 116-120.

Baniasadi V, Narain N, Goswami R and Das SN (2006). Promoter region -318 C/ T and -1661 A/G CTLA-4 single nucleotide polymorphisms and type 1 diabetes in North Indians. *Tissue Antigens.* 67(5): 383-389.

Benmansour J, Stayoussef M, Al-Jenaidi FA, Rajab MH, et al. (2010). Association of single nucleotide polymorphisms in cytotoxic T-lymphocyte antigen 4 and susceptibility to autoimmune type 1 diabetes in Tunisians. *Clin. Vaccine Immunol.* 17(9): 1473-1477.

Bouqbis L, Izaabel H, Akhayat O, Perez-Lezaun A, et al. (2003). Association of the CTLA4 promoter region (-1661G allele) with type 1 diabetes in the South Moroccan population. *Genes Immun.* 4(2): 132-137.

Campos LP, Graciolo V, Welter M, Lopes MS, et al. (2021). Research Article The IL18 rs1946518 and PTPN22 rs2476601 polymorphisms are not associated with adult- and childhood-onset type 1 diabetes mellitus. *Genet. Mol. Res.* 19(4): 11.

Caputo M, Cerrone GE, Lopez AP, Villalba A, et al. (2005). Cytotoxic T lymphocyte antigen 4 heterozygous codon 49 A/G dimorphism is associated to latent autoimmune diabetes in adults (LADA). *Autoimmunity.* 38(4): 277-281.

Chen Z, Fei M, Fu D, Zhang L, et al. (2013). Association between cytotoxic T lymphocyte antigen-4 polymorphism and type 1 diabetes: a meta-analysis. *Gene.* 516(2): 263-270.

Chistiakov DA, Savost'anov KV, Turakulov RI, Efremov IA, et al. (2006). Genetic analysis and functional evaluation of the C/T(-318) and A/G(-1661) polymorphisms of the CTLA-4 gene in patients affected with Graves' disease. *Clin. Immunol.* 118(2-3): 233-242.

Deichmann K, Heinzmann A, Bruggenolte E, Forster J, et al. (1996). An Mse I RFLP in the human CTLA4 promotor. *Biochem. Biophys. Res. Commun.* 225(3): 817-818.

Douroudis K, Laine AP, Heinonen M, Hermann R, et al. (2009). Association of CTLA4 but not ICOS polymorphisms with type 1 diabetes in two populations with different disease rates. *Hum. Immunol.* 70(7): 536-539.

Ferreira AC, Gomes KB, Sampaio IB, Oliveira VC, et al. (2009). Type 1 diabetes susceptibility determined by HLA alleles and CTLA-4 and insulin genes polymorphisms in Brazilians. *Arq. Bras. Endocrinol. Metabol.* 53(3): 368-373.

Gerold KD, Zheng P, Rainbow DB, Zernecke A, et al. (2011). The soluble CTLA-4 splice variant protects from type 1 diabetes and potentiates regulatory T-cell function. *Diabetes.* 60(7): 1955-1963.

Gillespie KM and Long AE (2019). What Have Slow Progressors Taught Us About T1D-Mind the Gap! *Curr. Diab. Rep.* 19(10): 99.

Gonzalez-Escribano MF, Rodriguez R, Valenzuela A, Garcia A, et al. (1999). CTLA4 polymorphisms in Spanish patients with rheumatoid arthritis. *Tissue Antigens.* 53(3): 296-300.

Guzman VB, Morgun A, Shulzhenko N, Mine KL, et al. (2005). Characterization of CD28, CTLA4, and ICOS polymorphisms in three Brazilian ethnic groups. *Hum. Immunol.* 66(7): 773-776.

Haller K, Kisand K, Nemvalts V, Laine AP, et al. (2004). Type 1 diabetes is insulin -2221 MspI and CTLA-4 +49 A/G polymorphism dependent. *Eur. J. Clin Invest.* 34(8): 543-548.

Hauache OM, Reis AF, Oliveira CS, Vieira JG, et al. (2005). Estimation of diabetes risk in Brazilian population by typing for polymorphisms in HLA-DR-DQ, INS and CTLA-4 genes. *Dis. Markers.* 21(3): 139-145.

Heward JM, Allahabadia A, Carr-Smith J, Daykin J, et al. (1998). No evidence for allelic association of a human CTLA-4 promoter polymorphism with autoimmune thyroid disease in either population-based case-control or family-based studies. *Clin. Endocrinol. (Oxf).* 49(3): 331-334.

Huber A, Menconi F, Corathers S, Jacobson EM, et al. (2008). Joint genetic susceptibility to type 1 diabetes and autoimmune thyroiditis: from epidemiology to mechanisms. *Endocr. Rev.* 29(6): 697-725.

Jin P, Xiang B, Huang G and Zhou Z (2015). The association of cytotoxic T-lymphocyte antigen-4 + 49A/G and CT60 polymorphisms with type 1 diabetes and latent autoimmune diabetes in Chinese adults. *J. Endocrinol. Invest.* 38(2): 149-154.

Jung MH, Yu J, Shin CH, Suh BK, et al. (2009). Association of cytotoxic T lymphocyte antigen-4 gene polymorphisms and HLA class II alleles with the development of type 1 diabetes in Korean children and adolescents. *J. Korean Med. Sci.* 24(6): 1004-1009.

Kantarova D and Buc M (2007). Genetic susceptibility to type 1 diabetes mellitus in humans. *Physiol. Res.* 56(3): 255-266.

Kisand K and Uibo R (2012). LADA and T1D in Estonian population - two different genetic risk profiles. *Gene.* 497(2): 285-291.

Larsen ZM, Kristiansen OP, Mato E, Johannesen J, et al. (1999). IDDM12 (CTLA4) on 2q33 and IDDM13 on 2q34 in genetic susceptibility to type 1 diabetes (insulin-dependent). *Autoimmunity.* 31(1): 35-42.

Lemos MC, Coutinho E, Gomes L, Bastos M, et al. (2009). The CTLA4 +49 A/G polymorphism is not associated with susceptibility to type 1 diabetes mellitus in the Portuguese population. *Int. J. Immunogenet.* 36(3): 193-195.

Lynch KF, Lee HS, Torn C, Vehik K, et al. (2018). Gestational respiratory infections interacting with offspring HLA and CTLA-4 modifies incident beta-cell autoantibodies. *J. Autoimmun.* 86: 93-103.

Marron MP, Raffel LJ, Garchon HJ, Jacob CO, et al. (1997). Insulin-dependent diabetes mellitus (IDDM) is associated with CTLA4 polymorphisms in multiple ethnic groups. *Hum. Mol. Genet.* 6(8): 1275-1282.

Mayer-Davis EJ, Kahkoska AR, Jefferies C, Dabelea D, et al. (2018). ISPAD Clinical Practice Consensus Guidelines 2018: Definition, epidemiology, and classification of diabetes in children and adolescents. *Pediatr. Diabetes.* 19 Suppl 27: 7-19.

Moura RR, Coelho AV, Balbino Vde Q, Crovella S, et al. (2015). Meta-analysis of Brazilian genetic admixture and comparison with other Latin America countries. *Am. J. Hum. Biol.* 27(5): 674-680.

Nistico L, Buzzetti R, Pritchard LE, Van der Auwera B, et al. (1996). The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. Belgian Diabetes Registry. *Hum. Mol. Genet.* 5(7): 1075-1080.

Oliveira LC, Ramasawmy R, Borges JD, Marin ML, et al. (2011). Frequency of single nucleotide polymorphisms of some immune response genes in a population sample from Sao Paulo, Brazil. *Einstein (Sao Paulo).* 9(3): 359-366.

Qu HQ, Bradfield JP, Grant SF, Hakonarson H, et al. (2009). Remapping the type I diabetes association of the CTLA4 locus. *Genes Immun.* 10 Suppl 1: S27-32.

Quandt Z, Young A and Anderson M (2020). Immune checkpoint inhibitor diabetes mellitus: a novel form of autoimmune diabetes. *Clin. Exp Immunol.* 200(2): 131-140.

Robertson CC and Rich SS (2018). Genetics of type 1 diabetes. *Curr. Opin. Genet. Dev.* 50: 7-16.

Rodriguez A, Alfaro JM, Balthazar V and Pineda Trujillo N (2015). Association analysis of PTPN22, CTLA4 and IFIH1 genes with type 1 diabetes in Colombian families. *J. Diabetes.* 7(3): 402-410.

Rowshanravan B, Halliday N and Sansom DM (2018). CTLA-4: a moving target in immunotherapy. *Blood.* 131(1): 58-67.

Salzano FM and Sans M (2014). Interethnic admixture and the evolution of Latin American populations. *Genet. Mol. Biol.* 37(1 Suppl): 151-170.

Si X, Zhang X, Tang W and Luo Y (2012). Association between the CTLA-4 +49A/G polymorphism and Graves' disease: A meta-analysis. *Exp. Ther. Med.* 4(3): 538-544.

Souza AM, Resende SS, Sousa TN and Brito CFA (2019). A systematic scoping review of the genetic ancestry of the Brazilian population. *Genet. Mol. Biol.* 42(3): 495-508.

Spanakis EK and Golden SH (2013). Race/ethnic difference in diabetes and diabetic complications. *Curr. Diab. Rep.* 13(6): 814-823.

Steck AK, Bugawan TL, Valdes AM, Emery LM, et al. (2005). Association of non-HLA genes with type 1 diabetes autoimmunity. *Diabetes.* 54(8): 2482-2486.

Tavares NA, Santos MM, Moura R, Araujo J, et al. (2015). Association of TNF-alpha, CTLA4, and PTPN22 polymorphisms with type 1 diabetes and other autoimmune diseases in Brazil. *Genet. Mol. Res.* 14(4): 18936-18944.

Turpeinen H, Laine AP, Hermann R, Simell O, et al. (2003). A linkage analysis of the CTLA4 gene region in Finnish patients with type 1 diabetes. *Eur. J. Immunogenet.* 30(4): 289-293.

Vaidya B and Pearce S (2004). The emerging role of the CTLA-4 gene in autoimmune endocrinopathies. *Eur. J. Endocrinol.* 150(5): 619-626.

Wang J, Liu L, Ma J, Sun F, et al. (2014). Common variants on cytotoxic T lymphocyte antigen-4 polymorphisms contributes to type 1 diabetes susceptibility: evidence based on 58 studies. *PLoS One.* 9(1): e85982.

Wang K, Zhu Q, Lu Y, Lu H, et al. (2017). CTLA-4 +49 G/A Polymorphism Confers Autoimmune Disease Risk: An Updated Meta-Analysis. *Genet. Test Mol. Biomarkers*. 21(4): 222-227.

Wang XB, Kakoulidou M, Giscombe R, Qiu Q, et al. (2002). Abnormal expression of CTLA-4 by T cells from patients with myasthenia gravis: effect of an AT-rich gene sequence. *J. Neuroimmunol.* 130(1-2): 224-232.

Wells AD, Walsh MC, Bluestone JA and Turka LA (2001). Signaling through CD28 and CTLA-4 controls two distinct forms of T cell anergy. *J. Clin. Invest.* 108(6): 895-903.