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The IL18 rs1946518 and PTPN22 rs2476601 polymorphisms are not associated with adult- and childhood-onset type 1 diabetes mellitus

L.P. Campos[1]; V. Graciolo[1]; M. Welter[1]; M.S. Lopes[1]; S. Nesi-França[2]; G. Picheth[1]; and F.G.M. Rego[1]

[1] Programa de Pós-graduação em Ciências Farmacêuticas, Universidade Federal do Paraná, Curitiba, PR, Brasil
[2] Unidade de Endocrinologia Pediátrica, Departamento de Pediatria, Universidade Federal do Paraná, Curitiba, PR, Brasil

Corresponding author: F.G.M. Rego
E-mail: rego@ufpr.br; fgmrego@gmail.com

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ABSTRACT. Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by the destruction of pancreatic β-cells. Interleukins such as interleukin 18 (IL-18) and protein tyrosine phosphatase, nonreceptor type 22 (PTPN22) have been found to be associated with immune related diseases. We investigated a possible association of polymorphisms in IL-18 gene rs1946518 and PTPN22 rs2476601 with T1DM diagnosed in children (aged ≤14 years) and adults (aged ≥18 years) via a case-control study. In Euro-Brazilian children (n = 320) and adults (n = 291), patients with T1DM and healthy individuals (control) were genotyped for rs1946518 and rs2476601 using fluorescent probes (Taqman system). All groups were in the Hardy-Weinberg equilibrium. No significant differences were observed in the genotypes and allele frequencies for both polymorphisms. For the IL-18 rs1946518 A-allele, the minor allele frequencies for children, adults, healthy individuals, and T1DM were 49% (95% CI, 43.0–55.0), 47.5% (40–52), 47.9% (43–53), and 51% (45–57), respectively. For the PTPN22 rs2476601 T-allele in adults, controls and T1DM had frequencies of 7.3% (4–10) and 6.7% (4–10). In conclusion, these polymorphisms were not associated with T1DM onset in children or adults in this population.

Key words: Adult-onset T1DM; Childhood-onset T1DM; Polymorphisms; IL-18; PTPN22; Case-control study

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is an autoimmune disease caused by the immune-mediated destruction of pancreatic β-cells (Yoon and Jun, 2005). Approximately 550,000 children aged 0–14 years have T1DM, with approximately 90,000 new cases diagnosed each year, worldwide; in Brazil, about 51,500 children and adolescents in this age group present T1DM (IDF, 2019). The precise incidence of new-onset T1DM in those aged above 20 years remains unknown; however, a large proportion of new-onset T1DM has been seen in adults (Weng et al., 2018).

T1DM is a polygenic condition, with major genetic risk factors located within the class II HLA region; however, around 60 recognized non-HLA loci have been associated with this disease (Robertson and Rich, 2018). Nevertheless, the genetic basis correlations among children, adolescents, and adults with T1DM individuals remain unclear (Zhu et al., 2019).

Autoimmune T1DM is believed to be a T helper 1 (Th1) lymphocyte-mediated disease occurring due to interferon gamma (IFN-γ) production. Different pathways induce IFN-γ expression in different cell types, including the interleukin 18 (IL18)-mediated signaling pathway (Esmailbeig and Ghaderi, 2017). Moreover, several factors may contribute to the destruction of insulin-producing β-cells, including the cytokines secreted by T helper 1 and 2 (Th1 and Th2), subsets of T helper (CD41) cells (Yoon and Jun, 2005). As *IL18* plays a pivotal role in autoimmunity, it is considered as a strong candidate for influencing T1DM (Esmailbeig and Ghaderi, 2017).

With increasing onset age, the clinical course of the disease tends to become milder and β-cell destruction is slower than that in childhood-onset T1DM (Ilonen et al., 2019). Analysis of T cells from the peripheral blood of patients with newly diagnosed T1DM revealed different cytokine expression patterns when stimulated by islet autoantigens depending on their age at diagnosis (Arif et al., 2014).

The human *IL18* gene is located on 11q23.1, and it contains six exons over 20.8 kb. The *IL18* sequenced analyses revealed 91 polymorphic loci within a region of 23 kb, including the proximal promoter and 500 bp downstream of the gene (Thompson and Humphries, 2007). Moreover, five polymorphisms (G-656T, C-607A, G-137C, Tþ113G, and Cþ127T) located in the *IL18* promoter altered the transcriptional activity (Giedraitis et al., 2001, Kretowski et al., 2002).

IL18 overproduction in T1DM may be attributable to polymorphisms in regions with regulatory functions, and it is known that the polymorphism -607 C/A (rs1946518) located in the *IL18* promoter region alters *IL18* promoter activity by changing its transcription activity (Giedraitis et al., 2001, Kretowski et al., 2002). This polymorphism disrupts the potential cAMP-responsive element-binding protein-binding site, and higher promoter activity is associated with the -607C allele compared to the607A allele (Giedraitis et al., 2001).

PTPN22 (protein tyrosine phosphatase, nonreceptor type 22) is another gene that encodes a key regulator of the immune response and is associated with various autoimmune diseases, including T1DM (Carr et al., 2009). The *PTPN22* gene is a protein-coding gene

that belongs to the nonreceptor class 4 group of the protein tyrosine phosphatase family. This gene is located on chromosome 1p13 and encodes a protein known as lymphoid-specific intracellular phosphatase (LYP). This protein binds to the adapter protein called calcineurin B-like, which is primarily involved in the negative selection of thymocytes and regulation of peripheral T cell activation. Variants in the *PTPN22* gene are associated with the dysregulation of T-cell maturation, thereby leading to the development of autoimmune diseases, including T1DM (Ramu et al., 2019).

It has been reported that a polymorphism (SNP) in the *PTPN22* gene, C1858T (rs2476601), causes an R620W amino acid substitution that restricts the ability of Lyp to negatively regulate T cell receptor signaling and causes alterations in the B cell receptor signal transduction (Rodriguez et al., 2015).

The genetic information gathered to date is dominated by pediatric-onset T1DM in European-ancestral populations. Therefore, genetic evaluation of non-European populations at all stages of the etiologic process in T1DM should identify the novel genetic factors that can be used to predict the genetic risk of T1DM (Robertson and Rich, 2018). For instance, we found an association between the polymorphism rs2476601 in the PTPN22 gene with childhood-onset T1DM in the Brazilian population (Welter et al., 2018).

The present study aimed to assess the role of polymorphisms rs1946518 *IL18* and rs2476601 *PTPN22* in susceptibility to childhood and adult-onset T1DM in a Euro-Brazilian population.

MATERIAL AND METHODS

Study subjects

The clinical cohort and control groups, matched by gender, comprised 320 children aged ≤14 years and 291 adults aged ≥18 years with Euro-Brazilian ancestry. Healthy children classified as controls (n = 169) were selected from public schools in Curitiba, Paraná, South Region of Brazil, and healthy adult subjects (n = 150) were randomly selected from the blood bank donors at the Clinical Hospital of the Federal University of Paraná (HC-UFPR), Parana, Brazil. The T1DM subjects developed diabetes in childhood (n = 151, childhood-onset) or adulthood (n = 141, adult-onset), based on the criteria of the International Society for Pediatric and Adolescent Diabetes (ISPAD) (Mayer-Davis et al., 2018) and the American Diabetes Association (ADA 2020). Patients with T1DM were recruited from the Clinical Hospital of the Federal University of Paraná, Brazil. All the recruited subjects provided written informed consent. The study population showed predominantly European ancestry and self-reported having white skin, being classified as Euro-Brazilians (Krieger et al., 1965, Pena et al., 2011). The study was approved by the Ethics Committee of the Federal University of Paraná, Brazil (CAAE 01038112.0.0000.0102 and 24676613.6.0000.0102).

Genotyping

Blood samples were collected from all subjects in ethylenediaminetetraacetic acid anticoagulant treated tubes. Total genomic DNA was extracted from the whole peripheral blood of all subjects using the salting-out method, as previously described (Lahiri and Nurnberger, 1991). To extract DNA, the $A_{260/280}$ ratio (accepted >1.8) and concentration

(adjusted to 20 ng/μL) were measured with a Nano Drop 2000 spectrophotometer (Eppendorf, Germany).

The genotyping of *IL18* rs1946518 and *PTPN22* rs2476601 was performed using fluorescent probes TaqMan® (Taqman code: C_2898460_10 and C_16021387_20) in the 7500 Fast™ Real-Time PCR (Life Technologies/Applied Biosystems, Foster City, CA, EUA). Briefly, the reaction mixture (final volume 8 μL) comprised 3.0 μL TaqMan Genotyping Master Mix, 1.9 μL ultrapure water, 0.1 μL of fluorescent probes, and 3.0 μL DNA (20 ng/μL) in each well of a 96-well plate. The reaction conditions were recommended by the manufacturer: 10 min at 95°C (1 cycle), followed by 50 cycles of 15 s at 95°C and 90 s at 60°C. The genotyping quality was >98% for all samples measured by the software (7500 Fast SDS system software).

Clinical and laboratory data

Laboratory routine assays were determined by using an automated system (Architect Ci8200; Abbott Diagnostics, Santa Clara, CA) or Labmax 400 (Labtest, Lagoa Santa, MG) with reagents, calibrators, and controls provided by the manufacturer. Concentrations of 1,5-anhydroglucitol were measured enzymatically (GlycoMark, Inc., New York, NY). Glycated hemoglobin (HbA1c) was measured via immunoturbidimetry using Cobas Integra 400 plus (Roche Diagnostics, Basel, Switzerland).

Statistical analyses

Normality was tested using the Kolmogorov–Smirnov test. Parameters with normal distributions were compared using the Student's t-test (2-tailed) for independent samples. The Mann–Whitney U test was used to compare parameters with non-normal distributions. Categorical variables were compared using the chi-square test. Verification of the Hardy–Weinberg equilibrium), genotypic and allelic frequencies, as well as 95% confidence interval (95% CI) for the minor allele frequency (MAF) were performed using the program DeFinetti (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Odds ratios were calculated using http://vassarstats.net/index.html.

The allele frequencies in other populations were considered similar when the frequency of the MAF was within the 95% CI limit. All calculations were performed using the MedCalc version 17.6 (MedCalc Statistical Software bvba, Ostend, Belgium). A P value < 0.05 was considered as statistically significant for all tests.

RESULTS

The children and adult groups were matched by gender. Adults were additionally matched by age (Table 1). The childhood-onset T1DM group (12 years) was older ($P < 0.001$) than the control group (10 years). The childhood-onset T1DM group presented 2.8 times more diabetic ketoacidosis compared with adult-onset (70.2 vs. 24.7%, respectively) during diagnosis. The T1DM groups revealed poor glycemic control, considering a good glycemic control criterion for HbA1c (<7.0 %) and fasting glucose (< 7.2 mmol/L) (Table 1) (ADA 2020).

Albumin was found at significantly higher concentrations in the adult-onset T1DM group than in the control group; however, both results were within the reference range. The concentrations of creatinine were significantly higher in the T1DM group (children and adults) than those in their respective control groups (Table 1). In contrast, the creatinine concentration in both T1DM groups suggested no manifestation of renal damage, as the levels were close to the reference range (Ceriotti et al., 2008).

Table 1. Anthropometric and laboratory data in the child and adult groups.

Parameters	**Control (*n* = 169)**	**Children Childhood-onset T1DM (*n* = 151)**	***P value***	**Controls (*n* = 150)**	**Adult Adult-onset T1DM (*n* = 141)**	**P *value***
Gender male/female	91/78	73/78	0.303**	53/97	48/93	0.817**
Age (years)	10 (10–11)	12 (9–13)	<0.001	44.0 (40–49)	45.0 (34–52)	0.602
BMI (kg/m²)	18 (17–20)	18 (17–21)	0.805	27.0 ± 4.2	25.8 ± 4.3	0.018*
Z-score	0.53 ± 1.06	0.24 ± 0.99	0.015*	-	-	-
Diabetes duration (years)	-	4.1 ± 3.1	-	-	15.2 ± 10.7	<0.001*
DKA at diagnostic, %	-	70.2	-	-	24.7	<0.001**
DM Family history (%)	-	65.6	-	-	69.2	0.473**
Glycemia (mmol/L)	5.1 ± 0.6	14.2 ± 6.6	<0.001*	5.3 (4.7–5.9)	9.5 (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
Albumin (g/L)	42 (40–46)	43 (40–44)	0.780	39 (38–40)	41 (38–43)	<0.001
Creatinine (µmol/L)	48.6 (35–57)	61.9 (53–71)	<0.001	49.5 (42–59)	72.5 (71–88)	<0.001

Values are reported as means ± standard deviation, median (interquartile range), or number (*n*) of individuals. Control, healthy subjects; T1DM, type 1 diabetes subjects; M, male; F, female; BMI, body mass index; Z-score (calculated using http://reference.medscape.com/calculator/body-mass-index-percentile-boy); DKA, diabetic ketoacidosis; HbA1c, glycated hemoglobin A1c. Probability (*P*), Mann–Whitney U-test, * Student's t-test (two-tailed), or **chi-square test. *P*-values in bold are significant ($P < 0.05$).

The allele and genotype frequencies of *IL18* –607C (rs1946518) were similar in all groups studied; therefore, they were not associated with T1DM regardless of the age of disease onset (Table 2).

The frequencies of C1858T (rs1946518) genotypes and alleles were not significantly different between T1DM adult patients and the control group. Moreover, rs1946518 was not associated with adult-onset T1DM. The genotype frequencies of the *IL18* –607C (rs1946518) and *PTPN22* C1858T (rs2476601) were in accordance with Hardy–Weinberg equilibrium in all groups (Tables 2 and 3).

Table 2. Genotype and allele frequencies for the polymorphism rs1946518 *IL18* in the study groups.

Genotypes	Control (*n* = 169)	Childhood-onset T1DM (*n* = 151)	P value	Control (*n* = 150)	Adult-onset T1DM (*n* = 141)	P value
rs1946518						0.251
C/C	50 (29.6)	39 (25.8)		31 (20.6)	41 (29.0)	
C/A	76 (45.0)	76 (50.3)	0.616	85 (56.6)	71 (50.4)	
A/A	43 (25.4)	36 (23.9)		34 (22.8)	29 (20.6)	
A allele	47.9	49.0	0.785	51.0	45.7	0.205
[95%CI]	[43.0–53.0]	[43.0–55.0]		[45.0–57.0]	[40.0–52.0]	
Dominant model CC vs. CA + AA	50/119	39/112	0.454	119/31	41/100	0.097
Recessive model AA vs. CC + CT	43/126	36/115	0.740	24/116	102/29	0.916

Values are reported as *n* (%). Probability (P), chi-squared test for genotype and allele frequencies 95% CI = 95% confidence interval. Dominant model (CC vs. CA + AA). Recessive model (AA vs. CA + CC). Hardy–Weinberg equilibrium (*P*-value) for rs1946518 in healthy children controls (0.198), for childhood-onset T1DM (0.931), in adult healthy controls (0.101), and adult-onset T1DM (0.863)

Table 3. Genotype and allele frequencies for the polymorphism rs2476601 in *PTPN22* in the study groups.

Genotypes	Control (*n* = 150)	Adult-onset T1DM (*n* = 141)	*P value*
rs2476601			0.211
C/C	128 (85.0)	124 (87.9)	
C/T	22 (15.0)	15 (10.7)	
C/C	0 (0)	2 (1.4)	
T allele	7.3	6.7	0.779
[95% CI]	[4.0–10.0]	[4.0–10.0]	
Dominant model CC vs. CT + TT	130/22	124/15	0.324
Recessive model TT vs. CT + CC	0/150	2/139	0.451*

Values of genotypes are *n* (%); 95% CI: 95% confidence interval; *P*-value, Probability, χ^2 test and *χ^2 Yates corrected. Genotypic frequencies according to the Hardy–Weinberg equilibrium (χ^2 test). SNP, rs2476601 control Group ($P = 0.332$), and T1DM ($P = 0.069$)

DISCUSSION

In this study, we investigated the role of polymorphisms rs1946518 *IL18* and rs2476601 *PTPN22* in susceptibility to childhood and adult-onset T1DM in the Euro-Brazilian population. Brazilians are an admixture population mostly composed of Europeans, Africans and Native Americans (Gomes et al., 2018). This genetic background could explain the differences of alleles frequencies when compared to other populations, even with populations with more similar ancestors such as Caucasians, as in the case of the population studied in this work. Notably, allele frequencies for single nucleotide polymorphisms (SNPs) can vary by ethnic group, sometimes drastically (Ioannidis et al., 2004).

Interleukins (ILs) play a pivotal role in immune-related disorders. Proinflammatory cytokines, such as TNF-α, IL-1β, IFN-γ, IL-1α, IL-6, and IL-18, have been implicated in the pathogenesis of T1DM (Grunnet et al., 2009). Individual differences during inheritance of polymorphic cytokine genes (*IL6*, *IL18*, *INF-γ*) cause individual variations in the immune response. Therefore, cytokine gene polymorphisms may reflect or control the severity and

duration of inflammation, and thus, the progression of various autoimmune diseases, including T1DM (Thompson and Humphries, 2007).

In a previous study, we showed that IL-6R rs2228145 was associated with T1DM development in adulthood (Campos et al., 2019).

Cytokine polymorphisms that modulate the degree of destruction and age of T1DM onset have already been described (Ide et al., 2002). Moreover, a significant difference was observed in the genotype distribution at position −137 *IL18* (rs187238) between patients depending on their age at T1DM onset (Mojtahedi et al., 2006, Altinova et al., 2010); however, the associations were contradictory. Altinova et al. (2010) found that polymorphism was associated with younger onset age, whereas Mojtahedi et al. (2006) found that it was associated only in older patients. Therefore, in the present study, we evaluated -607 *IL-18* (rs1946518) in patients with adult- and childhood-onset T1DM.

Several studies have evaluated the influence of *IL18* genetic variation on T1DM risk and have found dissonant results. This may be due to the influence of other genetic and environmental factors, which are likely to differ among the ethnic groups, or another gene locus nearby could be a region conferring major susceptibility to T1DM in humans, as the *IL18* gene locus does not do the same (Nolan et al., 1998). Therefore, is not surprising that *IL18* polymorphisms do not contribute to T1DM in certain populations. Boëchat-Fernandes et al. (2019) showed the rs1946518 A-allele from IL18 gene predisposes to early-onset T1DM in a Brazilian population with predominantly African ancestry subjects; nevertheless this effect was not observed in Brazilians with European or Native Amerindian ancestry. These findings reinforce the differences according to ethnic origin, especially for the admixed Brazilian population. Additionally, the IL18 rs1946518 was associated with other types of diabetes, such as increased prevalence of type 2 diabetes (Huang et al., 2010) and an increased risk to gestational diabetes (Tarnowski et al., 2017).

The genotypic and allelic frequencies did not differ between the study groups (Table 2). Similar results were found in other studies with Caucasian (Novota et al., 2005, Szeszko et al., 2006, Hadzija et al., 2013), Turkish (Altinova et al., 2010), Iranian (Mojtahedi et al., 2006), and Brazilian populations (Tavares et al., 2013). Some studies did not report any association between different 607 IL18 (rs1946518) genotypes and the onset age in adult T1DM patients (Altinova et al., 2010), or T1DM in adults or Latent Autoimmune Diabetes of the Adult (LADA) (Novota et al., 2005), which was in accordance with our results; however, significant associations were found with T1DM in Asian populations (Dong et al., 2007, Lee et al., 2015).

The observed MAF (A) frequencies in the Brazilian population (Table 2) reveal that the healthy (child 43–53% and adult 45–57%; 95% CI) and T1DM (child 43–55% and adult 40–52%; 95% CI) do not differ from the Northeast Brazilian population (control 46% and T1DM 43%) (Tavares et al., 2013); nevertheless, they were higher than those observed in the African (control 35%) (dbSNP), European (control 38.1% and T1DM 39.2%) (Szeszko et al., 2006), and Turkish (control 35.6% and T1DM 35.7%) populations (Altinova et al., 2010).

PTPN22 encodes the lymphoid specific phosphatase (Lyp) (Cohen et al., 1999), which prevents spontaneous T-cell activation (Hill et al., 2002), and hence *PTPN22* deficiencies might induce proliferation of autoreactive lymphocytes in autoimmune-mediated diabetes (Rodriguez et al., 2015); however, the β-cell destruction is slower in adult-onset T1DM than in childhood-onset T1DM (Ilonen et al., 2019).

In a previous study, we already assessed rs2476601 of the *PTPN22* gene in a population of children with T1DM (Welter et al., 2018). The context of the present study offers the possibility of expanding the evidence and analyzing this polymorphism in the adult-onset T1DM.

No significant difference was observed in comparing genotypes (P = 0.217) and allelic frequencies (P = 0.704); therefore, it is suggested that rs2476601 of the *PTPN22* gene is not associated with adult-onset T1DM in this population (Table 3); however, this polymorphism was associated with childhood-onset T1DM in a similar ethnic population by our research group. Analysis of the child cohort revealed that the frequency of the PTPN22 risk allele (1858T) was significantly different in patients (9.9%) than that in the controls (3.6%; OR = 3.0, 95% CI = 1.5–6, P = 0.01) (Welter et al., 2018).

The DR3/DR4-DQ302 risk genotype for T1DM is present more frequently in individuals with early disease onset (Caillat-Zucman et al., 1992). In contrast, reports of the effects of the age-on-diagnosis and non-HLA loci interaction are contradictory, with positive reports largely confined to studies involving small sample sizes (Todd et al., 2007; Espino-Paisan et al., 2011a; Espino-Paisan et al., 2011b).

Considering rs2476601 polymorphism, certain studies report that it is not related to the diagnosis age (Todd et al., 2007; Chagastelles et al., 2010; Howson et al., 2012; Tavares et al., 2015). Tavares et al. (2015) assessed the Brazilian population and found that the average age for the C/C and C/T + T/T genotypes in the dominant model for rs2476601 polymorphism did not differ significantly. Although Howson et al. (2012) did not find a relationship between this polymorphism and the age at diagnosis of the disease, they observed gene–gene interaction, an association between the DR3/DR4-DQB1*0302 genotype and rs2476601 polymorphism (P < 0.001). Differently, with a population with the same ancestry background as the one studied in this study, we have showed that TYK2 (tyrosine kinase 2) polymorphism rs2304256 was not associated with T1DM or the age of diabetes onset (Graciolo et al., 2019).

Type 1 diabetes is determined by a combination of environmental and genetic factors, whose may change with age (Robertson and Rich 2018). Relatively few studies have focused on late-onset T1DM, particularly in adults. Some studies found association between the rs1893217 in PTPN2 and rs2542151 near PTPN2 with T1D risk, especially an earlier T1D onset (Espino-Paisan et al., 2011a, Peng et al., 2015, Chen et al., 2019). The GWAS-derived GRS (genetic risk score) significantly predicted T1D risk and was associated with age in a Chinese population (Zhu et al., 2019). The correlations of the genetic basis among children, adolescents, and adults with T1DM in Brazilian individuals are still unclear. Knowledge of the genetic characteristics for each age group and ethnicity will be critical in order to facilitate early intervention in T1DM.

In this study, the frequency of the rs2476601 T allele in the T1DM group was 6.7% (95% CI, 4–10) (Table 3). This frequency was similar to the childhood-onset T1DM in South Brazil (9.9%) (Welter et al., 2018), Northeast Brazil (8.0%) (Tavares et al., 2015), and Italian (4.9%) (Bottini et al., 2004) populations. The frequency of the T allele in the control group (7.3%, 95% CI, 4–10) (Table 3) was similar to that in the populations in the region of Rio Grande do Sul, Brazil (5.2%) (Chagastelles et al., 2010), Italy (5.9%) (Saccucci et al., 2008), Spain (6.7%) (Santiago et al., 2007), and Germany (8.0%) (Dultz et al., 2009). The frequency of both groups was higher than that reported for African (0.3%) and Asian (1.7%) populations.

Our results suggest that the polymorphism IL18 –607C (rs1946518) is not associated with both childhood and adult T1DM, and PTPN22 C1858T (rs2476601) is not associated with adult T1DM in the Euro-Brazilian population, South Brazil.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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