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Letter to the Editor

Polymorphism rs2476601 in the *PTPN22* gene is associated with type 1 diabetes in children from the South Region of Brazil

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Dear Editor,

Type 1 diabetes (T1D) is a multigenic autoimmune disease characterized by T-cell-dependent destruction of pancreatic B-cells (Roep, 2003). The protein tyrosine phosphatase, non-receptor type 22 (lymphoid) (*PTPN22*) gene encodes the lymphoid tyrosine phosphatase (LYP) protein, which is the main negative regulator of proximal T cell receptor/B cell receptor (TCR/BCR) signaling, and a powerful inhibitor of T-cell activation (Vang et al., 2005; Bottini et al., 2006). *PTPN22* is well-known as the third major genetic locus that contributes to the risk of development of T1D (Hasegawa et al., 2004).

PTPN22 is located on chromosome 1p13.2 and comprises 24 exons (Tavares et al., 2015). The *PTPN22* gene polymorphism rs2476601 is located in exon 14 and is associated with T1D (Dultz et al., 2009; Lee and Song, 2013; Rodriguez et al., 2015; Tavares et al., 2015) and other autoimmune diseases (Dieude et al., 2008).

The polymorphism rs2476601, also known as R620W (Trp → Arg) or (c.1858T > C), is a missense mutation in the P1 domain, which reduces the binding capacity of LYP to tyrosine kinase Csk (Cloutier and Veillette, 1999; Fiorillo et al., 2010). However, the precise mechanism by which this variant affects the function of *PTPN22* gene and confers susceptibility to autoimmune disease is unknown. A few studies have suggested that the R620W variant is a mutant with loss-of-function mutation, while other evidences suggest a gain-of-function mutation (Vang et al., 2005; Rieck et al., 2007; Zikherman et al., 2009; Fiorillo et al., 2010; Zhang et al., 2011; Burn et al., 2016).

Our study aimed to evaluate the association between the *PTPN22* polymorphism rs2476601 and T1D in children from a Brazilian population from the South Region of Brazil.

The clinical cohort comprised 320 unrelated Euro-Brazilian children, matched on the basis of gender. The clinical subjects were classified as healthy controls (n = 169) and T1D patients (n = 151), according to the criteria of American Diabetes Association (2017), which are as follows: (i) random plasma glycemia ≥11.1 mmol/L, (ii) glycated hemoglobin (HbA_{1C}) ≥6.5%, and (iii) fasting plasma glucose ≥7.0 mmol/L. All patients showed a few clinical symptoms of acute hyperglycemia at diagnosis (polyuria, polydipsia, and ketoacidosis), and at least one positive test for one autoantibody such as islet cell cytoplasmic autoantibodies (ICA), insulin autoantibodies (IAA), glutamic acid decarboxylase autoantibodies (GAD65), and insulinoma-associated-2 autoantibodies (IA-2A).

We selected subjects aged ≤14 years old for the cohort, in accordance with International Diabetes Federation (IDF) criterion for children (IDF, 2015). The healthy control group was selected from public schools in Curitiba, Paraná, South Region of Brazil. The absence of familial history or any clinical symptom for diabetes, as well as the concentration of HbA_{1C} ≤ 5.7% (39 mmol/mol) were considered as the criteria to select healthy control group. The children affected by T1D had the same background and were undergoing treatment at the Clinical Hospital of the Federal University of Paraná, Brazil. The familial history for diabetes with predominance of type 2 diabetes (occurrence in parents, grandparents, brothers, and sisters) was observed at a high frequency (65.6%) in T1D group.

Our research was approved by the Ethics Committee of the Federal University of Paraná. The polymorphism rs2476601 was genotyped using real-time PCR fluorescent probes (TaqMan code: C_16021387_20) and genotyping was performed using a 7500 Fast™ Real-Time PCR System (Life Technologies/Applied Biosystems, Foster City, CA, USA). Reagents such as Master Mix®, Genotyping Assay®SNPs, and other real-time PCR materials were provided by Applied Biosystems. The quality of genotyping was assessed to be ≥99%. Data analysis was performed using Statistica software for Windows (Version 8.0, StatSoft Inc., Tulsa, OK, USA), and a probability of ≤5% ($P < 0.05$) was considered significant for all analyses.

Table 1 shows the anthropometric and clinical data of the groups included in the clinical cohort. The polymorphism was in Hardy-Weinberg equilibrium in both the groups and was associated with T1D (Table 2). Children with the A-allele show a 3.0-fold (95% confidence interval: 1.5–6.0) higher probability of developing T1D than children without an A-allele.

The frequencies of the A-allele were extremely low in Chinese and African individuals than in Euro-Brazilian individuals. We observed variability in the frequency of the A-allele among Caucasians. Italians showed similar frequencies, whereas Estonians and Polish people showed higher

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Table 1
Anthropometrics and clinical characteristics in healthy children, or children with type 1 diabetes mellitus.

Parameters	Control (n = 169)	T1D (n = 151)	P
Age, years	10 (10 − 11)	12 (9–13)	< **0.001***
Male/female, n	91/78	73/78	0.326**
BMI, kg/m^2	18 (17–20)	18 (17–21)	0.805*
Z-score	0.53 ± 1.06	0.24 ± 0.99	**0.015**
Percentile	72 (45–90)	62 (31–84)	**0.014***
DKA at diagnostic, %	–	70.2	**–**
Family history of diabetes, %	–	65.6	–
Fasting glycemia, mmol/L	5.1 ± 0.6	14.2 ± 6.6	< **0.001**
HbA_{1C}, %	5.2 (5.1–5.4)	9.7 (8.7–11.1)	< **0.001***
Creatinine, μmol/L	48.6 (35.4–57.5)	61.9 (53.0–70.7)	< **0.001***

Values represented are mean ± standard deviation, median (interquartile range, 25th–75th) or n.
BMI - body mass index; Z-score (calculated using http://reference.medscape.com/calculator/body-mass-index-percentile-boy), DKA - Diabetic ketoacidosis; P - probability according to Students *t*-test (two-tailed), *Mann-Whitney *U* test, or **Chi-square test.
Significant P-values (< 0.05) are in bold.

Table 2
Genotype and allele frequencies for *PTPN22* rs2476601 in children.

Gene/ polymorphism	Genotype allele	Control children n = 169	T1D children n = 151	P
PTPN22, rs2476601				**0.002**
	G/G	158 (93.5)	122 (80.8)	
	G/A	10 (5.9)	28 (18.5)	
	A/A	1 (0.6)	1 (0.7)	
MAF	A-allele [95% CI]	3.6 [2–6]	9.9 [7–13]	**0.001**
Dominant model	GG vs. GA + AA	158/11	122/29	**0.001**

Values represented are n (%); MAF - minor allele frequencies, 95% CI - 95% confidence interval.
P - probability, chi-square test.
Hardy-Weinberg equilibrium (P-value) for children in healthy controls (0.077) and for T1D (0.656).
Dominant model (prevalent homozygous genotype vs. others).
Odds ratio for allele, 3.0 (95% CI, 1.5–6.0).

Table 3
Allele frequency of the *PTPN22* rs2476601 polymorphism and a literature review.

Population	n	A-allele frequencies (%)	References
Euro-Brazilian T1D	151	9.9 [7–13]	This study
Euro-Brazilian Control	169	3.6 [2–6]	
Northeastern Brazilian T1D	205	8	(Tavares et al., 2015)
Northeastern Brazilian Control	308	3	
Italian T1D	558	10.4	(Petrone et al., 2008)
Italian Control	545	4.6	
Egyptian T1D	150	10	(Abdelrahman et al., 2016)
Egyptian Control	165	4.8	
Poland T1D	175	**18.9**	(Okruszko et al., 2012)
Poland Control	151	**12.6**	
Estonian T1D	154	**26**	(Kisand and Uibo, 2012)
Estonian T2D	260	**20**	
Estonian LADA	65	**13**	
Estonian Control	229	**14**	
Chinese Han LADA	229	**0.2**	(Liu et al., 2012)
Chinese Han Control	210	**0**	
Iranian T1D	99	**6.1**	(Abbasi et al., 2017)
Iranian Control	100	**0**	
Japanese	168	**1.8**	(NCBI, 2017)
Africans	1322	**0.3**	

n, sample size; LADA, Latent Autoimmune Diabetes of Adulthood; control, healthy patients.
95% CI, 95% of confidence interval.
In bold, frequencies outside the 95% CI for the control group.