

# Polymorphism V362F (rs2304256) of tyrosine kinase 2 is not associated with childhood- or adulthood-onset type 1 diabetes in southern Brazil

## *Brief Note*

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**ABSTRACT.** Type 1 diabetes mellitus (T1D) is considered a polygenic disease that is influenced by environmental factors and autoimmune responses to autoantibodies, resulting in metabolic abnormalities. Tyrosine kinase 2 (*TYK2*) is involved in type I interferon signaling in beta cells, and *TYK2* polymorphism rs2304256 has been associated with T1D. We investigated polymorphism rs2304256 (*TYK2*) in a case-control study of Euro-Brazilians with T1D manifested during childhood and adulthood. We studied 307 individuals manifesting clinical signs of type 1 diabetes (151 children below 14 years of age and 156 adults with diagnosis after 18 years of age). The control samples consisted of 169 healthy children and 150 healthy adult subjects. T1D groups had inadequate glycemic control because fasting glucose and mean HbA1C concentrations were significantly higher than in the control groups. Real-time PCR with

TaqMan® fluorescent probes was applied for genotyping. The studied polymorphisms were in Hardy–Weinberg equilibrium. The minor allele frequency (A-allele) for children in the T1D and control groups was 24.2% (95% CI, 18 - 32) and 23.4% (95% CI, 17 - 30), respectively (P = 0.812), while for adults in the T1D and control groups values were 19.6% (95% CI, 14 - 27) and 24.7% (95% CI, 20 - 35), respectively (P = 0.127). There was no significant difference in genotypes or allelic frequencies of the polymorphism in the groups. The frequency of the minor allele of the polymorphism was similar to that found in other Caucasian populations, and different than that found in Eastern populations. In conclusion, the polymorphism rs2304256 was not associated with T1D in either group.

**Key words:** Type 1 Diabetes mellitus; Genetic susceptibility; Polymorphism; Protein tyrosine kinase 2; SNP

## INTRODUCTION

Type 1 diabetes (T1D) is a chronic autoimmune disorder characterized by destruction of pancreatic B-cells, usually leading to absolute insulin deficiency (Van Belle et al., 2011; American Diabetes Association, 2019). Although the exact pathogenesis of T1D is unknown, it is caused by the interplay of environmental factors, and several genes contributing to T1D have also been identified (Wang et al., 2017).

The tyrosine kinase 2 (*TYK2*, Chr19p13.2, OMIM 176941, 25 exons) is a member of the Janus kinase (JAK) protein family that plays a relevant role in intracellular signaling of cytokines and other molecules (Tao et al., 2011). Studies suggest that the *TYK2* gene regulates apoptotic and proinflammatory pathways in pancreatic beta cells via modulation of interferon alpha (IFN $\alpha$ ) (Marroqui et al., 2015). Furthermore, the activation of *TYK2* may induce hyper-expression of the major histocompatibility complex (MHC) class I proteins that increase the efficiency of cell antigen presentation to immune cells. In addition, *TYK2* may induce an increase in the chemokine CXCL10 (C-X-C motif chemokine 10), which is reported to play a crucial role in the recruitment of auto-aggressive T cells to pancreatic islets (Marroqui et al., 2015).

Inhibitors of protein tyrosine kinase have been shown to be associated with remission of type 1 and type 2 diabetes (Fountas A et al., 2015). Taken together, these elements point out that *TYK2* is a target for study on its association with diabetes.

The polymorphism rs2304256 C>A (V362F; Val362Phe) is located in exon 8 of the *TYK2* gene and induces a valine to phenylalanine substitution at position 362 in the JAK-homology 4 (JH4) region of *TYK2* (Kyogoku et al., 2009; Wallace et al., 2010; Lopez-Isac et al., 2016). This region is important for the interaction of *TYK2* with interferon alpha and beta-receptor subunit 1 (IFNAR1 or IFN $\alpha\beta$  receptor), as well as the maintenance of IFNAR1 expression at the cell membrane (Hellquist et al., 2009). Therefore, it has been suggested that the minor allele of rs2304256 (A/Phe) reduces *TYK2* function, resulting in decreased susceptibility to autoimmune and inflammatory diseases (Marroqui et al., 2015). The polymorphism rs2304256 was significantly associated with T1D (Wallace et al., 2010),

systemic lupus erythematosus (Sigurdsson et al., 2005; Hellquist et al., 2009), Crohn's disease (Can et al., 2015), and systemic sclerosis (Lopez-Isac et al., 2016).

We investigated a possible association of polymorphism rs2304256 in the *TYK2* gene with childhood- and adulthood-onset T1D, in a population in southern Brazil.

## MATERIAL AND METHODS

### Study subjects

The clinical cohort comprised 320 children that were  $\leq 14$  years of age, and 306 adults that were  $\geq 18$  years of Euro-Brazilian descent. The Brazilian population is an admixture (Parra et al., 2002), with Euro-Brazilians predominating in the southeast of Brazil, defined as major European influence with contributions from Italians, Spaniards and Germans (Salzano and Sans, 2014). In our study, only Euro-Brazilians were selected to improve homogeneity of the sample.

The healthy children classified as control (N = 169) were selected from public schools in Curitiba, Paraná, South Region of Brazil, and the healthy adult subjects (N = 150) were blood bank donors. The T1D subjects developed diabetes in childhood (n = 151, childhood-onset), or in adulthood (n = 156, adulthood-onset), according to the criteria of the American Diabetes Association (2019). Patients with diabetes were recruited from the Clinical Hospital of the Federal University of Paraná, Brazil. The diagnostic criteria for type 1 diabetes were those recommended by the American Diabetes Association, 2019.

The study was approved by the Ethics Committee of the Federal University of Paraná, Brazil, and written informed consent was obtained from either the subjects or their guardians (CAAE 01038112.0.0000.0102).

### Genotyping

DNA was extracted from peripheral blood leukocytes (buffy coat) using the "salting-out" method (Lahiri and Nurnberger, 1991). The concentration was adjusted to 20 ng/ $\mu$ L as determined by NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA) with A260/280 absorbance ratio between 1.6 and 1.9.

The polymorphism rs2304256 was genotyped using TaqMan® fluorescent probes (Taqman code: C\_25473911\_10) in the 7500 Fast™ Real-Time PCR (Life Technologies / Applied Biosystems, Foster City, CA, EUA). Briefly, the reaction mixture (final volume 8  $\mu$ L) contained 3.0  $\mu$ L of TaqMan Genotyping Master Mix, 1.9  $\mu$ L of ultrapure water, 0.1  $\mu$ L of fluorescent probe and 3.0  $\mu$ L of DNA (20 ng/ $\mu$ L). The reaction conditions, as recommended by the manufacturer, were 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 as measured by the software (7500 Fast SDS system software).

### Biochemical markers

Fasting glucose and creatinine were determined using the Architect Ci 8200 automated system (Abbott Diagnostics, Lake Forest, IL, USA). Glycated hemoglobin (HbA1c) was measured by immunoturbidimetry using Cobas Integra 400 plus (Roche

Diagnosics, Basel, Switzerland). All biochemical parameters were determined using reagents, calibrators, and controls according to manufacturer's instructions.

## Statistical analysis

Normality was verified with the Kolmogorov-Smirnov test. Non-normally distributed data are shown as median (interquartile range) and were compared with the Mann-Whitney U-test. The chi-square test ( $\chi^2$ ) was used to compare categorical variables. Hardy-Weinberg equilibrium and allele comparisons were calculated with the DeFinetti program (<http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>). Statistical analyses were performed using the MedCalc Statistical Software version 19 (MedCalc Software bvba, Ostend, Belgium). P-value less than 5% ( $P < 0.05$ ) was considered significant.

## RESULTS

Table 1 shows the anthropometric and laboratory data of the groups. The cohorts of childhood-onset and adulthood-onset were matched by gender. In the childhood group (comprised of children below 14 years of age), the T1D subjects were older than subjects in the control group. The age of T1D subjects were matched with that of healthy controls in adult groups.

T1D subjects with adulthood-onset showed significantly lower body mass index (BMI) than the control group. No difference in BMI was observed in the children's groups. The frequency of family history of diabetes was high (more than 65%) in both T1D groups (adult and children) (data not shown).

The median concentrations of fasting glucose (13.6 and 10.0 mmol/L) and HbA1c (9.7% and 8.6%) for T1D-childhood onset and T1D-adulthood onset demonstrated that both T1D groups showed poor glycemic control, considering a good glycemic control criterion for HbA1c ( $<7.5\%$ ). Serum creatinine concentration, which is a biomarker for kidney function, were significantly higher in both T1D groups. Additionally, in all groups (T1D and controls) the serum creatinine concentrations were within the reference interval adjusted by age, suggesting no indication of overt kidney disease in any of the subjects.

**Table 1.** Anthropometric and laboratory data of type 1 diabetes in childhood-onset, adulthood-onset, and healthy controls.

Parameters	Children ( $\leq 14$ years)			Adult ( $\geq 18$ years)		
	Control (N = 169)	T1D (N = 151)	P	Control (N=150)	T1D (N=156)	P
Age (years)	10 (10-11)	12 (9-13)	$< 0.001$	44 (40-48)	45 (34-52)	0.549
Male/female (n)	91/78	73/78	0.326*	53/97	56/100	0.918*
BMI ( $\text{kg}/\text{m}^2$ )	18 (17-20)	18 (17-21)	0.805	27 (24-30)	25 (23-28)	0.003
Fasting glucose (mmol/L)	5.2 (5.1-5.4)	13.6 (9.2-21.5)	$< 0.001$	5.3 (4.7-5.8)	10.0 (5.9-13.7)	$< 0.001$
HbA1c (%)	5.2 (5.1-5.4)	9.7 (8.7-11.1)	$< 0.001$	5.4 (5.2-5.6)	8.6 (7.6-9.6)	$< 0.001$
Creatinine ( $\mu\text{mol}/\text{L}$ )	48.6 (35.4-57.5)	61.9 (53.0-70.9)	$< 0.001$	50.4 (42.4-59.2)	75.1 (70.7-88.4)	$< 0.001$

Values are reported as median (interquartile range), or number (N) of individuals. Control, healthy subjects; T1D, childhood-onset type 1 diabetes and adulthood-onset type 1 diabetes, BMI, body mass index; HbA1c, glycated hemoglobin A1c. Probability (P), Mann-Whitney U-test or \*chi-square test. P values in bold font are significant ( $P < 0.05$ ).

Table 2 shows the genotype and allele distributions for rs2304256 of the *TYK2* gene. All groups were in Hardy-Weinberg equilibrium ( $P > 0.05$ ). There was no significant difference ( $P > 0.05$ ) in genotypes or allele frequencies of rs2304256 between T1D subjects (childhood- or adulthood-onset) and their respective controls. The dominant model (genotypes CC vs. CA+AA) or recessive model (AA vs. CC+CA) were not significantly different ( $P > 0.05$ ) among the groups (data not shown).

**Table 2.** Genotype and allele frequencies of polymorphism rs2304256 of *TYK2* gene in type 1 diabetes in childhood-onset, adulthood-onset, and healthy controls.

Genotypes	Children			Adults		
	Control (N = 169)	T1D (N = 151)	P	Control (N = 150)	T1D (N = 156)	P
rs2304256			0.965*			0.156*
C/C	98 (58.0)	86 (57.0)		83 (55.3)	103 (66.0)	
C/A	63 (37.3)	57 (37.7)		60 (40.0)	45 (28.8)	
A/A	8 (4.7)	8 (5.3)		7 (4.7)	8 (5.2)	
A-allele (95% CI)	23.4 (17-30)	24.2 (18-32)	0.812	24.7 (20-35)	19.6 (14-27)	0.127

Values are reported as N (%). 95% Confidence Interval (95% CI). Probability (P), chi-squared test for \*genotype and allele frequencies.

## DISCUSSION

T1D in adult subjects can sometimes present with better-preserved beta cell function, decreased frequency of autoantibodies, decreased prevalence of HLA class II susceptibility alleles, and increased prevalence of protective genotypes, compared to T1D diagnosed in children and adolescents (Rodacki et al., 2005). Conversely, the effect of polymorphisms in age-at-diagnosis in T1D is controversial and poorly explored. Variations in the HLA region are more associated with childhood-onset while those in non-HLA loci present with adulthood-onset (Howson et al., 2012).

Our hypothesis was that *TYK2* rs2304256 could produce different effects on T1D depending on age-at-diagnosis. Mice with deficient or mutated *TYK2* showed reduced responses in IFN type I (IFN $\alpha\beta$ ) and IL12. Lower expression of *TYK2* increased susceptibility to virus-induced diabetes mellitus in mice, suggesting a less efficient antiviral response in these animals when the production of *TYK2* is impaired (Izumi et al., 2015).

In contrast, in human pancreatic  $\beta$  cells, the inhibition of *TYK2* by specific small interfering RNAs (siRNAs) exposed to synthetic viral dsRNA (double strand RNA) showed decreased activation of the IFN1 pathway and a subsequent decrease in IFN- $\alpha$  production and MHC1 proteins, resulting in  $\beta$ -pancreatic cells that were less susceptible to apoptosis (Marroqui et al., 2015; Op de Beeck and Eizirik, 2016). A number of case control studies have been conducted to investigate the association of polymorphism rs2304256 with autoimmune and inflammatory diseases. rs2304256 decreased susceptibility to systemic sclerosis (Lopez-Isac et al., 2016), whereas there was no association with rheumatoid arthritis (Suarez-Gestal et al., 2009).

The association of rs2304256 with systemic lupus erythematosus (SLE) is controversial. A decreased susceptibility to SLE in Caucasian population was reported (Sigurdsson et al., 2005), while Asian populations (Kyogoku et al., 2009; Li et al., 2011)

showed no association between SLE and this polymorphism. Wallace et al. (2010) suggested that minor allele frequency (A allele) for polymorphism rs2304256 has a protective effect for T1D. They suggested that this polymorphism decreased the biological function of *TYK2*, and consequently decreased susceptibility to autoimmune and inflammatory diseases related to the intracellular signaling pathway of IFN $\alpha$  and interleukins, but the mechanisms behind this decrease require further study (Tao et al., 2011). The rs2304256 polymorphism showed no significant difference ( $P = 0.239$ ) in genotype and allele frequencies between the T1D groups, suggesting that this polymorphism is not associated with T1D or the onset of this disease (Table 2). Consequently, our data diverge from those published by Wallace et al. (2010) on the English population. Population background may explain this divergence, as postulated by Al-Lahham (2017) in a study on a population similar to the one used in our study.

In a study with adult Japanese, a *TYK2* promoter haplotype with 7 polymorphisms: -930G>A, -929T>A, and -104>C in promoter, 1A>G (rs17000728), 62G>A (rs17000728), and 63G>A (rs2304258) in exon 1, and the study polymorphism rs2304256 (V362F or 15597C>A), which are in linkage disequilibrium, were associated with decreased *TYK2* expression (Nagafuchi et al., 2015). This haplotype was designated “*TYK2* promoter variant.” The *TYK2* promoter variant was associated with an increased risk of T1D, with a younger age onset and an increased risk of T1D trigger by virus at diabetes onset. Decreased expression of the *TYK2* gene possibly conferred risk for the development of diabetes due to increased susceptibility to viral infection (Nagafuchi et al., 2015).

In our study, the minor allele frequency (MAF) for rs2304256 (A-allele) observed in the childhood control was 23.4 (95% CI, 17-30), and in the adult control it was 24.7% (95% CI, 20-35). This was similar to those observed in healthy Brazilian (23.6%) (Peluso et al., 2013) and European (27.9%) (Lopez-Isac et al., 2016) populations. The frequency of the A-allele in a healthy Asian population (Chinese [42.4%] (Li et al., 2011) and in Japanese [38.3%] (Kyogoku et al., 2009) was significantly higher than that obtained in our study.

In T1D groups, frequencies the of A allele were lower in the Brazilian population (24.2% children T1D and 19.6% adulthood-onset T1D) when compared with T1D in an English population (29.4%) (Wallace et al., 2010). More studies are needed to provide details about how changes in *TYK2* expression may modulate autoimmune diseases. Future studies involving genotyping in different populations with a larger sample size are required to properly define the role of *TYK2* rs2304256 on pathological processes.

In conclusion, polymorphism rs2304256 (*TYK2*) was not associated with childhood- or adulthood-onset of T1D in a population of Euro-Brazilian descent.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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