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Short communication

Functional promoter polymorphisms of the receptor for advanced glycation end products in children and adolescents with type 1 diabetes

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ABSTRACT

RAGE promoter polymorphisms are associated with increases in RAGE expression. A case-control association study was conducted involving a Euro-Brazilian population of children and adolescents with type 1 diabetes (n = 90) and healthy controls (n = 105), which were matched by sex and age. Genotyping by PCR-RFLP the −429T>C (rs1800625), −374T>A (rs1800624), and 63 bp deletion/insertion (−407 to −345 bp) showed no significant differences (P > 0.05) between the groups.

Type 1 diabetes mellitus (T1DM) is an autoimmune disease, mediated by a combination of genetic and environmental factors. Recently, the consumption of dietary advanced glycation end products (AGEs), which are pro-oxidant metabolic derivatives of non-enzymatic reactions, has been considered a potential contributor to the development of T1DM [1]. The receptor for advanced glycation end products (RAGE) is member of the immunoglobulin superfamily and binds different molecules, such as advanced glycation end products, HMGB1, amphoterin, certain S100 proteins, DNA, and RNA [2,3]. RAGE activation, which requires interaction with a ligand, promotes deleterious intracellular effects, such as immune and inflammatory responses, procoagulant effects, sustainment of tumorigenesis and metastasis development [2,4]. The RAGE gene (chromosome 6; 6p21.3) is close to a major histocompatibility complex (MHC) and is expressed in several tissues [5]. Promoter RAGE polymorphisms −429T>C (rs1800625), −374T>A (rs1800624) and 63 bp deletion (−345 to −407 bp) are associated with an increase in RAGE expression [6] and immune-related diseases, like systemic lupus erythematosus [7], Crohn's disease [8] and T1DM [9]. Fig. 1 illustrates the position of these polymorphisms in the promoter region. Interestingly, the presence of the 63 bp deletion affects the polymorphic site −374T>A in the RAGE gene promoter region. The association of RAGE promoter polymorphisms with T1DM is controversial [10]. Some studies have found an association between RAGE promoter polymorphisms with T1DM [9] or with their known complications [11–15], however, these associations have not been replicated in other populations [10,16,17]. Here we investigated the association of RAGE promoter polymorphisms with T1DM in a sample of children and adolescents from South of Brazil. The Brazilian population is admixture; therefore, the study could improve our understanding regards to RAGE functional promoter polymorphisms and T1DM.

Children (ages < 12 years old) and adolescents (ages 12–18 years old) with T1DM (patients, n = 90) were matched by sex and age with healthy subjects (controls, n = 105). These subjects were recruited from Municipal Schools in Curitiba, Parana in the South of

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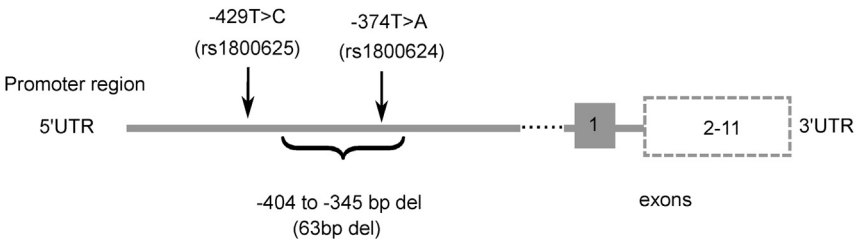

Fig. 1. Location of functional polymorphisms in the human RAGE gene promoter region. The RAGE gene (OMIM 600214) promoter region and the locations of the −429T>C, −374T>A, and the 63-bp deletion polymorphisms are indicated. Boxes represent exons 1 (gray box) and 2–11 (dashed box). Adapted from Kankova et al. [37].

Brazil. T1DM patients from the Pediatric Endocrinology Unit of the Clinical Hospital at the Federal University of Parana were classified according to ADA (2013) [18] and SBD (2012) [19] criteria. All diabetic patients were receiving insulin therapy, and were positive for at least one autoimmune antibody (GAD65, IAA, ICA or IA-2A), and showed no signs of overt kidney failure (creatinine < 88.4 μmol/L). The Ethics Committee on Human Research of our institution approved this study. Informed consent was obtained from parents/guardians of the minors, with additional assent from adolescents as required. RAGE polymorphisms were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), as described by Hudson et al. [6]. Briefly, an amplicon of 344 bp (primers: F 5′- GGG GGC AGT TCT CTC CTC -3′ and R 5′- TCA GAG CCC CCG ATC CTA TTT -3′) was cleaved by the restriction enzymes *AluI* (−429T>C) and *Tsp509I* (−374T>A). Restriction fragments were resolved by acrylamide gel (29:1) electrophoresis, while the 63-bp deletion (−345 to −407 bp) was identified using agarose gel (1.5%) electrophoresis stained with ethidium bromide. Biochemical markers were measured using an automated system (LabMax 400, Labtest SA) with reagents, calibrators and controls provided by the same manufacturer. Glycated hemoglobin A1c (HbA1c) was measured using the Tina-quant[a] HbA1c II assay (Abbott - Architect Ci8200), standardized according to National Glycohemoglobin Standardization Program (NGSP) and standardized or traceable to the Diabetes Control and Complications Trial (DCCT). The normality of continuous variables was tested with the Kolmogorov-Smirnov test. Variables with a normal distribution were reported as mean (±standard deviation) and analyzed using a Student's *t*-test. Variables with non-normal distributions were reported as the median (interquartile range; 25–75%) and were analyzed using the Mann–Whitney U test. Categorical variables were analyzed with the Chi-squared test. Hardy–Weinberg was tested using the DeFinetti program (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Linkage disequilibrium was verified with the Cubex program (http://www.oege.org/cgi-bin/cubex.py). Statistical significance was defined as $P < 0.05$. Statistical analysis was performed with the Statistica software for windows 8.1 (StatSoft Inc., Tulsa, OK, USA). The anthropometric and clinical characteristics of the two groups are shown in Table 1. Briefly, the study subjects were about 12 years old. T1DM patients were diagnosed around the age of 7 years, showed a strong family history of T1DM (63%) and had poor glycemic control (HbA1c; median 10.2%, 88.0 mmol/mol). No deviations from the Hardy–Weinberg equilibrium were found for all the studied polymorphisms in both groups ($P > 0.05$). The −429T>C and −374T>A polymorphisms were in linkage disequilibrium in the controls (D' = 1.0; $r^2 = 0.72$) and the T1DM group (D' = 1.0; $r^2 = 0.73$).

The genotype/allele distributions of the three selected polymorphisms in RAGE gene are shown in Table 2. The minor allele frequency, −374A (31%) and −429C (10%), for the control group in our study was similar to that previously reported in the Euro- and Afro-Brazilian populations in Southern Brazil by Picheth et al. [9] (−374A = 33% and −429C = 10%; Euro-Brazilians), Torres et al. [20] (−374A = 31.5% and −429C = 12.5%; Euro-Brazilians), and Cohen et al. [21] (−374A = 33% and −429C = 14%; Euro-Brazilians). The −374T>A frequency that we found in the control group was higher when compared to Asian populations (9.3–16.3%) [22–26]. Similarly, the −429C allele frequency in this study was lower (14.5% and 14.68%) in Asian populations [24,26]. The 63-bp deletion is not frequent polymorphism, varying from 0 to 6% in different population [6,20,21,24,25,27,28]. In our study, D-allele frequency in the

Table 1
Anthropometric parameters and clinical characteristics of study patients with T1DM and healthy controls.

Parameters	Control n = 105	T1DM n = 90	P Value
Age, years	11.4 ± 2.0	11.8 ± 2.1	0.234*
Male/Female, n	42/63	45/45	0.149**
Age at diagnosis, years	–	6.9 ± 3.5	–
Diabetes duration, years	–	4.7 ± 3.1	–
Family history of diabetes, n	–	57/90	–
BMI, kg/m^2	20.2 ± 3.6	18.9 ± 2.8	**0.008***
BMI, z-score	0.55 ± 0.95	0.23 ± 0.86	**0.019***
FPG, mmol/L	4.73 ± 0.56	15.60 ± 6.55	**<0.001***
HbA1C, %	5.3 (5.2–5.5)	10.2 (9.3–11.5)	**<0.001**

The values are median (Inter Quartile Range, 25%–75%) for non-normal distribution data or mean ± standard deviation for normal distributions.
BMI, body mass index; FPG, fasting plasma glycemia.
P value: Mann–Whitney U test or *Student's *t*-test or **Chi-square test.
Significant p values ($P < 0.05$) are in bold.

Table 2
Genotype and allele frequencies of RAGE promoter polymorphisms in the Euro-Brazilian population.

Genotyping	Control n = 105	T1DM n = 90	P Value
−429T>C (rs1800625)			0.566**
T/T	78 (80.4)	75 (84.3)	
T/C	19 (19.6)	33 (24.3%)	
C/C	0 (0)	0 (0)	
C-allele [95%CI]	10.0% [6–14%]	8.0% [4–12%]	0.513**
−374T>A (rs1800624)			0.415*
T/T	49 (47.1)	46 (51.1)	
T/A	35 (21.2%)	33 (24.3%)	
A/A	13 (12.6)	12 (13.3)	
T/D	3 (2.9)	1 (1.1)	
A/D	4 (3.8)	0	
A-allele [95%CI]	31.0% [25–38%]	31.0% [24–38%]	0.141**
63 bp D (−345to−407 bp)			0.071**
I/I	97 (92.4)	89 (98.9)	
I/D	7 (6.7)	1 (1.1)	
D/D	1 (0.9)	0 (0)	
D-allele [95%CI]	4.3% [2–7%]	0.6% [0–2%]	**0.020****

Values are presented as percentages as n (%).
95%CI, 95% confidence interval; I, insertion; D, deletion.
All polymorphisms are in Hardy–Weinberg equilibrium ($P > 0.05$).
P value; **Chi-square test.
P value <0.05 are significative and highlighted in bold.