

## The functional polymorphisms -429T>C and -374T>A of the *RAGE* gene promoter are not associated with gestational diabetes in Euro-Brazilians

I.C.R. Santos<sup>1</sup>, D.R. Daga<sup>1</sup>, H.R. Frigeri<sup>1</sup>, R.R. Réa<sup>2</sup>, A.C.R. Almeida<sup>2</sup>, E.M. Souza<sup>3</sup>, F.O. Pedrosa<sup>3</sup>, C.M.T. Fadel-Picheth<sup>1</sup> and G. Picheth<sup>1</sup>

<sup>1</sup>Departamento de Patologia Médica, Universidade Federal do Paraná, Curitiba, PR, Brasil

<sup>2</sup>Serviço de Endocrinologia e Metabologia do Paraná, Hospital de Clínicas, Curitiba, PR, Brasil

<sup>3</sup>Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná, Curitiba, PR, Brasil

Corresponding author: G. Picheth  
E-mail: gpicheth@ufpr.br

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**ABSTRACT.** The receptor for advanced glycation end products (*RAGE* or *AGER*) is a multiligand member of the immunoglobulin superfamily. *RAGE* is expressed in several tissues, including human myometrium, chorionic villi and placenta. Advanced glycation end products are the best studied ligands of *RAGE*; they have pro-inflammatory actions in human gestational tissues, increasing oxidative stress and the release of cytokines and prostaglandins. We

investigated the association of *RAGE* gene promoter polymorphisms -429T>C (rs1800625) and -374T>A (rs1800624) with gestational diabetes. A sample of 750 unrelated European origin pregnant Brazilian women were classified as nondiabetic (control group, N = 600) or having gestational diabetes (N = 150) according to American Diabetes Association 2009 criteria. Genotyping was performed by PCR-RFLP. The frequencies of the rare alleles -429C (6.3 versus 9.1%) and -374A (26 versus 30%) were not significantly different between the gestational diabetes patients and healthy pregnant women. Also, the -429T>C and -374T>A polymorphisms were not associated with body mass index, lipid profile, fasting glycemia, HbA1C, or insulin requirement. We found that functional promoter polymorphisms of the *RAGE* gene were not associated with gestational diabetes or its complications in these Euro-Brazilian patients.

**Key words:** Gestational diabetes; Single nucleotide polymorphisms; Genetic polymorphisms; *RAGE*; *AGER*

## INTRODUCTION

The receptor for advanced glycation end products (AGEs), *RAGE* or *AGER*, is a multi-ligand member of the immunoglobulin superfamily (Schmidt et al., 1996). *RAGE* is expressed in several tissues including human myometrium, chorionic villi and placenta (Lappas et al., 2007) and is involved in diabetes complications such as tissue injury, sustained inflammation and vascular complications (Bierhaus et al., 2005; Bierhaus and Nawroth, 2009). AGEs are the best studied ligands of *RAGE* that also bind  $\beta$ -amyloid peptide, S100/calgranulins and amphoterin (Bierhaus et al., 2005).

It has been shown that AGEs have pro-inflammatory actions in human gestational tissues, increasing oxidative stress, release of cytokines and prostaglandins (Lappas et al., 2007; Pertynska-Marczewska et al., 2009), suggesting an involvement of the AGE-*RAGE* interaction in gestational diabetes pathogenesis.

The *RAGE* gene is on the chromosome 6p21.3 and presents about 50 polymorphisms (Hudson et al., 2001a; NCBI, 2009). Association studies of *RAGE* polymorphisms with diabetes, cardiovascular disease and inflammatory diseases such as rheumatoid arthritis showed controversial results (Bucciarelli et al., 2002; Kankova et al., 2005; Lindholm et al., 2006; Bierhaus and Nawroth, 2009).

The single nucleotide polymorphisms, -429T>C (rs1800625) and -374T>A (rs1800624), of the promoter region of *RAGE* were found to increase its expression 2- and 3-fold, respectively, *in vitro* (Hudson et al., 2001b), electing these genetics variations as targets for association studies involving *RAGE* and its effects.

Here we have investigated the association of -429T>C and -374T>A polymorphisms with gestational diabetes mellitus (GDM), a pathology present in about 4% of pregnant women in Brazil. The study was approved by the University's Human Research Ethics Committee of the Universidade Federal do Paraná.

## MATERIAL AND METHODS

A sample of 750 unrelated pregnant Euro-Brazilian women were classified as non-diabetic (control group, N = 600) or having GDM (N = 150) according to American Diabetes Association 2009 criteria. The diagnostic was performed between the 24-28th week of gestation. The follow-up for glycemic control of the diabetic patients included fasting glucose and glycated hemoglobin (HbA1C) measurement monthly. The body mass index (BMI), presence of hypertension, insulin requirement, and serum levels of the lipid profile and creatinine were also determined. Patients with kidney failure or cardiac diseases were excluded.

Biochemical parameters were measured by standard automated methods (Architect, Abbott). HbA1C was measured by ion-exchange high performance liquid chromatography (Variant, Bio-Rad).

DNA was extracted from blood by a salting-out procedure (Lahiri and Nurnberger Jr., 1991). Primers used to amplify from the position -590 to -246 region of the *RAGE* promoter gene and polymerase chain reaction (PCR) conditions have been described elsewhere (Hudson et al., 2001a).

Genotyping was performed by PCR-RFLP (restriction fragment length polymorphism) using *AluI* (Invitrogen) and *Tsp509I* (New England Biolabs), for -429T>C and -374T>A variations, respectively, as described by Hudson et al. (2001a,b). RFLPs were resolved using 10% polyacrylamide gel electrophoresis, stained with ethidium bromide and registered with a UVP BioChemi system. Subjects with the 63-bp deletion (-407 to -345 bp) in the *RAGE* promoter gene (about 2% of both groups) were not included in the analyses.

Comparisons of biochemical parameters with normal distribution were performed by the *t*-test for independent variables and triglyceride levels, and log-transformed for statistical analysis. Other non-continuous variables were compared by the chi-square test.

Genotypes were compared by the two-tailed Fisher exact test (RxC software). Allele frequencies and Hardy-Weinberg equilibrium were tested using the chi-square test (<http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>).

Regression analyses (standard, forward and backward) were applied to associate the polymorphisms with all studied parameters. The dominant model (homozygous wild-type genotype versus other genotypes) was analyzed for both polymorphisms. The recessive model (homozygous rare genotype versus other genotypes) was studied only for the -374T>A polymorphism.

All statistical analyses were performed with the Statistica for windows software, version 8.0 (StatSoft Inc., Tulsa).  $P < 0.05$  was considered to be significant.

## RESULTS

The subjects' characteristics are shown in Table 1. GDM patients were significantly older, heavier and more hypertensive than the control group. Of the biochemical parameters studied, total cholesterol, LDL cholesterol and triglyceride levels were significantly higher in GDM patients. Fasting glucose and HbA1C levels suggested that GDM patients were under good glycemic control.

**Table 1.** Clinical and biochemical characteristics of the groups studied.

Characteristics	Control (N = 600)	GDM (N = 150)	P
Age (years)	24.9 ± 6.2	31.7 ± 6.1	<0.001 <sup>+</sup>
Body mass index (kg/m <sup>2</sup> )	24.8 ± 4.4	33.5 ± 6.2	<0.001 <sup>+</sup>
Hypertension (%)	1.3%	32%	<0.001*
Total cholesterol (mM)	5.1 ± 1.3	5.9 ± 1.2	<0.001 <sup>+</sup>
HDL cholesterol (mM)	1.4 ± 0.4	1.5 ± 0.4	0.24 <sup>+</sup>
LDL cholesterol (mM)	3.0 ± 1.1	3.2 ± 1.1	0.006 <sup>+</sup>
Triglycerides (mM)	1.4 ± 0.6	2.5 ± 0.8	<0.001**
Creatinine (μM)	71.6 ± 6.4	65.3 ± 9.3	<0.001 <sup>+</sup>
Fasting glucose (mM)	4.5 ± 0.4	5.5 ± 0.9	<0.001 <sup>+</sup>
HbA1C (%)	-	5.7 ± 0.9	-
Insulin requirement (%)	-	33.8%	-

Data are reported as means ± SD or %. <sup>+</sup>t-test for independent variables; \*chi-square test; \*\*log-transformation. GDM = gestational diabetes mellitus.

The genotype and allele frequencies are shown in Table 2. All variants are in the Hardy-Weinberg equilibrium and no significant difference was observed among the studied groups related to the polymorphisms.

**Table 2.** Genotype and allele frequencies of RAGE gene promoter polymorphisms in the absence (control) or presence of gestational diabetes mellitus (GDM).

Polymorphisms		Control (N = 600)	GDM (N = 150)	P*
-429T>C (rs1800625)	T/T	486 (81.0%)	131 (87.3%)	0.100
	T/C	109 (18.2%)	19 (12.7%)	
	C/C	5 (0.8%)	0 (0%)	
	H-W	0.681	0.407	
C allele frequency	[95%CI]	0.09 [0.08-0.12]	0.06 [0.04-0.09]	(χ <sup>2</sup> ) 0.054
-374T>A (rs1800624)	T/T	289 (48.2%)	85 (56.7%)	0.161
	T/A	258 (43.0%)	52 (34.7%)	
	A/A	53 (8.8%)	13 (8.6%)	
	H-W	0.669	0.225	
A allele frequency	[95%CI]	0.30 [0.28-0.33]	0.26 [0.21-0.31]	(χ <sup>2</sup> ) 0.141

Genotypes are reported as number with percent in parentheses. \*Two-tailed Fisher exact test or chi-square test (χ<sup>2</sup>). H-W = Hardy-Weinberg equilibrium; 95%CI = 95% confidence interval.

Also, the -429T>C and -374T>A polymorphisms were not associated with BMI, lipid profile, fasting glycemia, HbA1C, or insulin requirement as revealed by regression analysis in dominant or recessive models (data not shown).

## DISCUSSION

GDM patients were reported to be associated with older age, higher BMI, hypertension, and increased levels of the lipid profile, especially triglycerides, when compared to healthier pregnant women (Langer et al., 2005; Wiznitzer et al., 2009). So, the characteristics of the studied population presented in Table 1 were similar to other studies.

The frequencies of the rare -429C allele (6.3%, 95%CI = 4-9%) observed for the GDM group were lower than those reported for Euro- and Afro-Brazilians with type 2 diabetes (12%; Dos Santos et al., 2005) or Brazilians with type 1 diabetes (19.1%; Picheth et al., 2007). On the other hand, these frequencies are similar to those reported for healthy Brazilian subjects (10%; Picheth et al., 2007).

For the -374A allele (26.0%, 95%CI = 21-31%) the frequencies observed in the GDM group were similar to those reported for Euro- and Afro-Brazilians with type 2 diabetes (31-24%; Dos Santos et al., 2005) and for other Caucasian populations (NCBI, 2009).

The association of *RAGE* polymorphisms with diabetes (types 1 and 2) and its complications is controversial.

The -429C allele was associated with type 1 diabetes (Picheth et al., 2007) and to diabetic retinopathy (Hudson et al., 2001a), while other studies showed no association of this allele with diabetes (Dos Santos et al., 2005; Kankova et al., 2005).

The -374A allele was associated with a protective effect against cardiovascular disease (Falcone et al., 2004; Dos Santos et al., 2005) or with the severity of this disease (Picheth et al., 2007). On the other hand, different reports did not find an association of the -374A allele with cardiovascular disease or diabetes (Hudson et al., 2001a,b; JiXiong et al., 2003; Kirbis et al., 2004; Kankova et al., 2005).

Multiple regression analyses did not show any association with the studied *RAGE* polymorphisms in both groups studied with the variables present in Table 1 (data not shown). Also the frequency of insulin requirement (33.8%), a marker for pregnancy complication, was not different from those reported for other Caucasian populations (Nizard and Ville, 2009) and was not associated with the studied polymorphisms.

To our knowledge, this study is the first report of these polymorphisms in patients with gestational diabetes. Our data show that the functional promoter polymorphisms of the *RAGE* gene are not associated with gestational diabetes or its complications in Euro-Brazilian patients.

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