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The catalase –262C/T promoter polymorphism and diabetic complications in Caucasians with type 2 diabetes

Kátia Gonçalves dos Santos[a], Luís Henrique Canani[b], Jorge Luiz Gross[b], Balduíno Tschiedel[c], Kátia Elisabete Pires Souto[c] and Israel Roisenberg[a,*]
[a]*Genetics Department, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil*
[b]*Endocrinology Division, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil*
[c]*Endocrinology Division, Grupo Hospitalar Conceição, Porto Alegre, RS, Brazil*

Abstract. Catalase is a central antioxidant enzyme constituting the primary defense against oxidative stress. In this study, we investigated whether the functional –262C/T polymorphism in the promoter of catalase gene is associated with the presence of diabetic retinopathy (DR), diabetic nephropathy (DN) and ischemic heart disease (IHD) in 520 Caucasian-Brazilians with type 2 diabetes. The –262C/T polymorphism was also examined in 100 Caucasian blood donors. Patients underwent a clinical and laboratory evaluation consisting of a questionnaire, physical examination, assessment of diabetic complications and laboratory tests. Genotype analysis was performed using the polymerase chain reaction followed by digestion with restriction enzyme. The genotype and allele frequencies of the –262C/T polymorphism in patients with type 2 diabetes were very similar to those of blood donors (T allele frequency = 0.20 and 0.18, respectively). Likewise, there were no differences in either genotype or allele frequencies between type 2 diabetic patients with or without DR, DN or IHD. Thus, our results do not support the hypothesis that the –262C/T polymorphism is related to the development of DR, DN or IHD in patients with type 2 diabetes. Further studies are necessary to elucidate the role of catalase gene polymorphisms in the pathogenesis of diabetic complications.

Keywords: Catalase, diabetic nephropathy, diabetic retinopathy, ischemic heart disease, polymorphism, type 2 diabetes

1. Introduction

Diabetic retinopathy (DR), diabetic nephropathy (DN) and cardiovascular disease are frequent chronic complications of type 2 diabetes. DR is the leading cause of acquired blindness [7] and DN is the main cause of end-stage renal disease [11] in Western countries. Patients with type 2 diabetes are 2 to 4 times more likely than their nondiabetic counterparts to have cardiovascular disease, especially ischemic heart disease (IHD) [18]. Although both severity and duration of diabetes are strong determinants of diabetic complications, the etiology of these complications seems to be multifactorial, with an interplay between environmental and genetic risk factors [13,15,21].

Catalase (EC 1.11.1.6) is an ubiquitous enzyme present in most eukaryotic organisms. It has a very high turnover number, decomposing hydrogen peroxide into oxygen and water at a exceedingly high rate. Together with superoxide dismutases and glutathione peroxidase, catalase constitutes a primary defense against oxidative stress [10]. Interestingly, one study has reported an increased frequency of diabetes in Hungarian patients with catalase deficiency compared with both healthy relatives and the background population [9].

A common polymorphism in the promoter of human catalase gene, namely –262C/T, was described as being

*Corresponding author: Israel Roisenberg, Ph.D., Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul (UFRGS), Caixa Postal 15053, 91501-970, Porto Alegre, RS, Brasil. Tel.: +55 51 3316 6736; Fax: +55 51 3316 7311; E-mail: israberg@ufrgs.br.

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functionally associated with blood catalase levels. The erythrocyte catalase level was higher in TT homozygotes than in CC homozygotes [8]. Then, it was suggested the T allele confers a protective effect against development of oxidative stress-related diseases. In fact, catalase gene mutations have been detected in association with diabetes mellitus, hypertension and vitiligo [10]. Very recently, some studies have shown a relationship between the –262C/T polymorphism and the development of type 1 diabetes [3] and its complications [4,6,16]. However, another study have not found such an association in Danish adults with diabetes [5].

Therefore, the aim of this case-control study was to investigate whether the –262C/T polymorphism in the catalase gene promoter is associated with diabetic retinopathy (DR), diabetic nephropathy (DN) and ischemic heart disease (IHD) in Brazilians with type 2 diabetes.

2. Materials and methods

2.1. Patients

A case-control study was carried out on 520 unrelated Caucasian-Brazilian type 2 diabetic patients participating in a multicentric study in the Brazilian State of Rio Grande do Sul. All Caucasian-Brazilians were subjects of European origin (mainly from Portugal, Spain, Italy, and Germany). Type 2 diabetes was diagnosed according to the American Diabetes Association criteria [2]. The patients were defined as case or control subjects according to the presence or absence of DR, DN and IHD. The complications were analyzed separately, so the number of cases and controls varied according to the complications evaluated. Additionally, subjects in the control groups were required to have had diabetes for at least 10 years to be included in the study.

Patients [278 female, 242 male; age 61.0 ± 9.7 years (mean $\pm$ S.D.); aged between 28 and 89 years; mean duration of diabetes 14.8 ± 8.5 years] underwent a standardized clinical and laboratory evaluation that consisted of a questionnaire, physical examination, and laboratory tests. Blood pressure was measured after a 5-min rest in the sitting position using a standard mercury sphygmomanometer. Hypertension was defined as blood pressure levels $\geqslant$ 140/90 mmHg, or if the patient was on treatment with antihypertensive medication [20].

In order to obtain a reference of the allele frequency in the general population, the –262C/T polymorphism was also examined in 100 Caucasians recruited among volunteer blood donors from Hospital de Clínicas de Porto Alegre (Porto Alegre, Brazil). All subjects participating in this study gave their written informed consent, the protocol for which was approved by all hospital ethics committees.

2.2. Assessment of diabetic complications

Diabetic complications were defined according to the criteria previously described [19]. Briefly, the assessment of diabetic retinopathy (DR) was performed by ophthalmoscopic examination through dilated pupils, and fluorescein angiography was obtained when indicated. Regarding the presence of DN, the diagnosis was based on the albumin excretion rate (AER) in at least two of three consecutive 24-h timed or random spot sterile urine collections. Patients were classified as having normoalbuminuria, incipient DN (microalbuminuria) or overt DN (presence of macroalbuminuria or chronic renal failure treated by dialysis when other causes of proteinuria or renal disease were ruled out). The diagnosis of ischemic heart disease (IHD) was based on the presence of angina pectoris or possible acute myocardial infarction according to the World Health Organization questionnaire for cardiovascular disease [17], and/or on the presence of ECG abnormalities and/or on the presence of perfusion abnormalities (fixed or variable) upon myocardial scintigraphy.

2.3. Laboratory tests

Glycated hemoglobin was measured using standardized assays (reference range: 4.7–6.0%). Serum creatinine concentrations were determined by Jaffé's reaction and urinary albumin concentration by immunoturbidimetry (Sera-Pak immuno microalbuminuria; Bayer, Tarrytown, USA). Total cholesterol, HDL-cholesterol and triglycerides were measured by standard enzymatic methods.

2.4. DNA analysis

Genomic DNA was extracted from peripheral blood leukocytes by a salting-out procedure [12]. The –262C/T polymorphism was PCR amplified using primers and conditions as previously described [8]. The amplified product was digested with *Sma*I under the conditions recommended by the manufacturer (MBI

Table 1
Clinical and demographic characteristics of type 2 diabetic patients according to the presence of diabetic complications

	Diabetic retinopathy		Diabetic nephropathy		Ischemic heart disease	
	Without ($n = 164$)	With ($n = 312$)	Without ($n = 195$)	With ($n = 258$)	Without ($n = 169$)	With ($n = 177$)
Age (years)	62.3 ± 9.4	60.8 ± 9.5	62.5 ± 9.3	60.7 ± 9.5	62.4 ± 9.6	61.8 ± 8.8
Male/female	63/101	159/153(*)	66/129	147/111(**)	68/101	99/78(**)
Duration of diabetes (years)	16.3 ± 6.6	15.1 ± 9.1(*)	17.0 ± 7.0	14.4 ± 8.9(**)	16.6 ± 7.0	16.7 ± 8.2
Glycated hemoglobin (%)	6.7 ± 2.0	6.8 ± 1.7	6.7 ± 1.7	6.6 ± 1.9	6.6 ± 1.9	6.6 ± 1.9
Hypertension (%)	75.6	78.2	74.4	84.1(*)	81.6	86.4
Serum creatinine (μmol/l)	80 (35-1,025)	88 (44-919)(**)	80 (35-150)	97 (35-1,025)(**)	80 (35-849)	88 (44-1,025)(*)
Total cholesterol (mmol/l)	5.6 ± 1.2	5.7 ± 1.2	5.5 ± 1.1	5.8 ± 1.2	5.4 ± 1.0	5.7 ± 1.2
HDL-cholesterol (mmol/l)	1.2 ± 0.3	1.1 ± 0.3(*)	1.2 ± 0.3	1.1 ± 0.3(**)	1.2 ± 0.3	1.1 ± 0.3(**)
Triglycerides (mmol/l)	1.6 (0.4-10.1)	1.8 (0.4-16.3)	1.6 (0.4-5.5)	1.9 (0.5-16.6)(**)	1.7 (0.4-10.2)	1.9 (0.6-9.9)
Diabetic retinopathy (%)	–	–	42.1	76.4(**)	49.7	65.5(**)
Diabetic nephropathy (%)	32.1	67.7(**)	–	–	40.2	63.8(**)
Ischemic heart disease (%)	42.2	58.2(**)	37.3	61.1(**)	–	–

Data are expressed as mean ± S.D., median (range), and percentage.
*Comparison between cases and controls is significant at the 0.05 level (2-tailed).
**Comparison between cases and controls is significant at the 0.01 level (2-tailed).

Fermentas, St. Leon-Rot., Germany). Finally, the digested fragments were separated by electrophoresis on 6% polyacrilamide gels, followed by ethidium bromide staining, and direct visualization under ultraviolet light.

2.5. Statistical analysis

Comparisons between groups were made using the unpaired Student's t test for normally distributed variables, or the Mann-Whitney U test for variables with a skewed distribution, using the SPSS package (SPSS for Windows, version 10.0). Allele frequencies were determined by gene counting, and departures from the Hardy-Weinberg equilibrium were verified using the χ^2 test. The χ^2 test was also used to evaluate the allele and genotype distributions among groups of subjects, using PEPI program [1]. A p value < 0.05 was considered statistically significant.

3. Results

3.1. Patients

The clinical and demographic characteristics of type 2 diabetic patients according to the presence of chronic complications are summarized in Table 1. Caucasian-Brazilians with DR and/or DN were more often male, with a shorter duration of diabetes, higher levels of serum creatinine, lower levels of HDL-cholesterol and a higher prevalence of IHD than patients without microvascular complications. Moreover, subjects with DN presented a higher frequency of hypertension and higher levels of triglycerides than subjects without it. In relation to IHD, patients with this complication were more frequently male, with a higher prevalence of DR and DN, higher levels of serum creatinine, and lower levels of HDL-cholesterol as compared to patients without IHD.

3.2. Polymorphism distribution

The genotype and allele frequencies of the –262C/T polymorphism in patients with type 2 diabetes were very similar to those of blood donors (T allele frequency = 0.20 and 0.18, respectively; $p = 0.701$). The genotype frequencies were in agreement with those predicted by the Hardy-Weinberg equilibrium for both groups of subjects. Likewise, the genotype and the allele frequencies in patients with DR, DN or IHD were not significantly different from those of diabetic subjects without these complications, as shown in Table 2. Even when the comparisons between extreme phenotypes were made, e.g. normoalbuminuria versus macroalbuminuria or absence of retinopathy versus proliferative DR, no significant differences in the genotype and allele frequencies were found (data not shown).

Since there may be gender differences, we also analyzed the relationship between the –262C/T polymorphism and the diabetic complications in patients stratified by gender. The analysis revealed that there were no statistically significant differences in the frequency of the T allele between diabetic women with or without DR (19% versus 21%), DN (21% versus 19%) or IHD (19% versus 20%) ($p > 0.05$ for all comparisons). Likewise, the T allele frequency among diabetic men was very similar in those with or without DR (20%

Table 2
Genotype and allele frequencies of the –262C/T polymorphism in type 2 diabetic patients according to the presence of diabetic complications(*)

	Retinopathy		Nephropathy		Ischemic heart disease	
	Without	With	Without	With	Without	With
n	164	310	193	258	168	176
CC	103 (0.63)	205 (0.66)	123 (0.64)	169 (0.66)	115 (0.69)	108 (0.61)
CT	56 (0.34)	90 (0.29)	64 (0.33)	76 (0.29)	46 (0.27)	61 (0.35)
TT	5 (0.03)	15 (0.05)	6 (0.03)	13 (0.05)	7 (0.04)	7 (0.04)
C	0.80	0.81	0.80	0.80	0.82	0.79
T	0.20	0.19	0.20	0.20	0.18	0.21

*All comparisons between cases and controls are not significant at the 0.05 level (2-tailed).

versus 18%), DN (19% versus 22%) or IHD (23% versus 15%) ($p > 0.05$ for all comparisons). In addition, there were no differences in the genotype frequencies between women and men with or without diabetic complications ($p > 0.05$ for all comparisons).

4. Discussion

No association between the –262C/T polymorphism and DN, DR or IHD was observed in Caucasian-Brazilian type 2 diabetic patients. To our knowledge, this is the first study designed to investigate the relationship between a catalase gene polymorphism and diabetic complications in patients with type 2 diabetes. Although catalase is broadly studied, few studies have specifically investigated associations of catalase polymorphisms with diseases [10] and its interaction with environmental oxidative stimuli [14]. The –262C/T polymorphism has only recently been considered as a putative candidate gene in the susceptibility of diabetes and its complications [3–6,16].

In Russians from Moscow, the CC genotype was found to be related to increased risk of the development of type 1 diabetes [3], whereas the TT genotype was associated with a decreased risk of diabetic neuropathy [4]. Interestingly, a limited statistical evidence of an association between the –262C/T polymorphism and the presence of type 1 diabetes was found in a British family-based association study [16]. However, no evidence of such an association was observed in case-control collections in that study [16]. Apart from this, a nominal statistical significance between this catalase polymorphism and the presence of diabetic nephropathy had already been observed in a family-based study of American type 1 diabetic families of European descent [6]. However, the authors of both British and American studies concluded that their results may be false positives. Moreover, Pask et al. [16] hypothesized that the previously reported association of the –262C/T polymorphism may also have been a false positive, or a population specific association in Russians from Moscow.

It is worthwhile to note that a large study in Danish adults has found no major effects of the –262C/T polymorphism on survival, physical and cognitive functioning, as well as on several oxidative stress-mediated disorders, including IHD and diabetes [5]. Considering that the same ethiological relationships may not apply to both type 1 and type 2 diabetes and the fact that the previous studies were based on European and North-American populations, it is early to define the role of this polymorphism in the pathogenesis of chronic complications in type 2 diabetes. Apart from this, the T allele frequency observed in our study was lower than that previously found in Swedish [8] and Russian [3] healthy blood donors, suggesting that there may exist a marked population variability in the distribution of the –262C/T polymorphism.

Considering the theoretical possibility that the –262C/T polymorphism is associated with diabetes and its complications only in specific ethnic groups [3,4], one limitation of the present study should be taken into account. In spite of including only subjects who had been born in the State of Rio Grande do Sul and had at least two European grandparents, it is possible that there is some degree of genetic admixture in the Caucasian-Brazilian population. Thus, our results should be viewed with caution.

In conclusion, our results do not support the hypothesis that the –262C/T polymorphism in the catalase gene promoter is related to the development of diabetic retinopathy, diabetic nephropathy or ischemic heart disease in Caucasians with type 2 diabetes. Further studies are necessary to elucidate the role of this polymorphism in the pathogenesis of diabetic complications.

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