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The *CTLA4* +49 A/G polymorphism is not associated with susceptibility to type 1 diabetes mellitus in the Portuguese population

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Summary

CTLA4 genetic polymorphisms have been associated with type 1 diabetes. We genotyped 207 patients and 249 controls for the most frequently investigated polymorphism of the *CTLA4* gene (+49A/G (rs231775)). No significant differences were observed, suggesting that this polymorphism is not strongly associated with type 1 diabetes in the Portuguese population.

Introduction

CTLA4 is a member of the immunoglobulin superfamily that is expressed on the surface of activated T cells and downregulates T-cell function (Schneider *et al*., 2006). Polymorphisms have been identified in the *CTLA4* gene and have been associated with different susceptibilities to a wide range of T-cell-mediated autoimmune disorders (Gough *et al*., 2005). Most molecular epidemiology studies have evaluated the role of the +49A/G (rs231775) single nucleotide polymorphism (SNP) that causes a threonine-to-alanine substitution in codon 17 and is associated with altered protein expression (Anjos *et al*., 2002) and T-cell activation (Maurer *et al*., 2002). This polymorphism seems to be associated with the genetic susceptibility to type 1 diabetes in several populations, although conflicting data also exist in populations of different ethnic backgrounds (Kavvoura & Ioannidis, 2005). The replication of results in populations of different ethnic backgrounds is important to confirm the association between the disease and the specific polymorphism, regardless of the genetic background of the population.

The aim of this study was to assess the contribution of this *CTLA4* polymorphism to the susceptibility to type 1 diabetes in the Portuguese population.

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Subjects and methods

The study was designed as a retrospective case–control study involving 207 Caucasian Portuguese patients with type 1 diabetes mellitus (113 males and 94 females; mean age ± standard deviation (SD) = 27.5 ± 10.2 years) that attended the outpatient clinics at the University Hospital of Coimbra (Portugal). Patients had been diagnosed on the basis of classical clinical presentation, low or undetectable levels of serum C-peptide, and presence of one or more autoantibodies (islet cell autoantibodies, autoantibodies to insulin, autoantibodies to glutamic acid decarboxylase (GAD65) or autoantibodies to the tyrosine phosphatase IA-2). Mean duration of diabetes was 11.4 ± 8.3 years (mean ± SD). The control group consisted of 249 unrelated healthy volunteers (143 males and 106 females; mean age ± SD = 36.8 ± 13.8 years) which were blood donors and hospital and faculty staff, from the same geographical region. Informed consent was obtained from patients and controls.

Leucocyte DNA was isolated from peripheral blood samples using standard methods and the detection of the +49A/G (rs231775) SNP was carried out as previously described (Donner *et al*., 1997).

The Pearson's chi-square test of independence, with one degree of freedom, was used to examine differences of genotype and allele frequencies between patients and controls. Two-tailed *P*-values were calculated, and statistical significance was set at $P < 0.05$. Odds ratios (OR) and the corresponding 95% confidence intervals (CI) were calculated for each genotype and allele. Subgroup analysis, according to the presence or absence of each of the autoantibodies, was carried out using the same method. Hardy–Weinberg equilibriums were assessed by use of the chi-square goodness-of-fit test to compare the observed and allele-based expected genotype frequencies. Power calculation was analysed using the program Power and Sample Size Calculations (version 2.1.30) (Dupont & Plummer, 1997).

Results

The distribution of *CTLA4* genotype and allele frequencies did not differ significantly between patients with type 1 diabetes mellitus and controls (Table 1). No single genotype or allele was associated with an altered risk for type 1 diabetes mellitus. Odds ratios for the putative high risk

Table 1. CTLA4 +49A/G genotype and allele frequencies in patients with type 1 diabetes mellitus and controls

Polymorphism	Controls ($n = 249$)	Patients ($n = 207$)	Odds ratio (95% CI)	*P* value
Genotype	*n* (%)	*n* (%)		
AA	111 (44.6)	82 (39.6)	0.82 (0.56–1.19)	0.285
AG	108 (43.4)	95 (45.9)	1.11 (0.77–1.60)	0.590
GG	30 (12.0)	30 (14.5)	1.24 (0.72–2.12)	0.442
Allele				
A	330 (66.3)	259 (62.6)	0.85 (0.65–1.12)	0.244
G	168 (33.7)	155 (37.4)	1.18 (0.90–1.54)	0.244

n, number; CI, confidence interval.

GG genotype and G allele were 1.24 (95% CI 0.72–2.12) ($P = 0.442$) and 1.18 (95% CI 0.90–1.54) ($P = 0.244$), respectively. No correlation was found between genotype and presence of each of the autoantibodies (data not shown). All frequencies were in Hardy–Weinberg equilibrium. Power analysis showed that the study sample size was sufficient to detect an OR of 1.47 associated with the G allele, with an estimated power of 0.8 and a type 1 error probability of 0.05.

Discussion

This is the first study of the effect of *CTLA4* polymorphisms on the susceptibility to type 1 diabetes in the Portuguese population. The results do not support the involvement of the *CTLA4* gene in the pathogenesis of type 1 diabetes in our population. This contrasts with positive associations that have been reported for the +49A/G polymorphism in case–control studies in populations from Belgium, Germany, Poland, France, Japan, China, Italy, the Philippines, Lebanon, Estonia and Iran (Zalloua *et al.*, 2004; Kavvoura & Ioannidis, 2005; Mojtahedi *et al.*, 2005). A meta-analysis of published studies indicated that the +49G allele conferred an increased risk for type 1 diabetes, with an OR of 1.45 (95% CI 1.28–1.65) (Kavvoura & Ioannidis, 2005). However, lack of association for the +49A/G polymorphism has also been reported in populations from the USA, Japan, Ghana, UK, France, Czech Republic, Morocco, Argentina, Brazil and Azerbaijan (Marron *et al.*, 1997; Caputo *et al.*, 2005; Hauache *et al.*, 2005; Kavvoura & Ioannidis, 2005; Ahmedov *et al.*, 2006).

The apparent discrepancies between the present study and other studies could be due to the genetic heterogeneity among the populations studied, to different interactions with environmental factors involved in the pathogenesis of type 1 diabetes, to limited individual study power or to other methodological issues. Furthermore, the *CTLA4* +49A/G SNP may not be the true disease-associated variant, but rather a marker in linkage disequilibrium with the causal variant, and the discrepant findings may reflect variable strengths of linkage disequilibrium in different populations.

We investigated the +49A/G polymorphism because it has been the most widely analysed *CTLA4* variant in type 1 diabetes patients from several ethnic populations (Kavvoura & Ioannidis, 2005). In addition, it is the only known SNP that causes an amino acid change (threonine to alanine), and is associated with altered protein expression (Anjos *et al.*, 2002) and T cell activation (Maurer *et al.*, 2002). However, other polymorphisms within the promoter region and 3′-untranslated region (UTR) of the gene, such as the CT60 (+6230G/A, rs3087243) SNP, might also affect protein expression and influence the risk of immune-mediated diseases (Ueda *et al.*, 2003; Perez-Garcia *et al.*, 2007; Mayans *et al.*, 2007; Muro *et al.*, 2008).

Our study was powered to detect an association, of about the same magnitude that has been reported in type 1 diabetes (Kavvoura & Ioannidis, 2005); however, a more subtle effect of the +49A/G polymorphism on susceptibility cannot be excluded in the present study.

In conclusion, our case–control study suggests that the +49A/G SNP of the *CTLA4* gene is not strongly associated with type 1 diabetes mellitus in the Portuguese population.

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