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Association between the Rs3087243 polymorphism and risk for diabetes: a meta-analysis

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ABSTRACT. The aim of this study was to evaluate the association between the rs3087243 polymorphism of the cytotoxic T-lymphocyte-associated antigen-4 (*CTLA-4*) gene and the risk of type 1 diabetes mellitus (T1D). A comprehensive meta-analysis of case-control studies was conducted to determine the association between the rs3087243 polymorphism (CT60A/G) and T1D and assess the joint evidence for the abovementioned association, influence of individual studies, and evidence for publication bias. We searched PubMed, Medline, Embase, Cochrane Library, and reference lists of relevant studies up to February 2012 and contacted the authors of these studies via email. For the case-control studies, 1) the rs3087243 polymorphism was significantly associated with T1D [allele (fixed: odds ratio and 95% confidence

interval (CI) = 1.249 (1.194-1.307), P < 0.001; random: odds ratio and 95%CI = 1.601 (1.103-2.325), P = 0.013)] [genotype (GG versus GA+AA: odds ratio and 95%CI = 1.249 (1.164-1.341), P < 0.001)], 2) there was no evidence to show that this association was accounted for in any study, and 3) there was no evidence for publication bias. In conclusion, the rs3087243 polymorphism was significantly associated with T1D.

Key words: Diabetes; *CTLA-4* gene; Meta-analysis; SNPs

INTRODUCTION

The cytotoxic T-lymphocyte-associated antigen-4 (*CTLA-4)* gene is located on the long arm of the chromosome 2q33 (Brunet et al., 1987). This gene encodes a receptor expressed by activated T cells. This receptor functions as a key negative regulator of T-cell activation. Function and experimental data have recommended *CTLA-4* as a candidate gene that confers susceptibility to autoimmune disease (Greenwald et al., 2002). In some cases, T cells that encounter self-antigens may begin to express CTLA-4 molecules as a protective mechanism. CTLA-4 molecules have a high affinity for B7 molecules and deliver inhibitory signals to the T cells. CTLA-4 has a greater affinity for the B7 molecule than CD28, and it downregulates T-cell function (Leung and Linsley, 1994). Therefore, *CTLA-4* may play a crucial role in T-cell-mediated autoimmunity and thus in susceptibility to autoimmune diseases, including type 1 diabetes mellitus (T1D).

T1D is the most prevalent form of diabetes in children and young adults and results from autoimmune $CD4^+$ and $CD8^+$ T-cell-directed destruction of insulin-producing pancreatic β-islet cells in genetically susceptible individuals, leading to irreversible hyperglycemia and related complications. Several gene loci have been associated with the risk of developing T1D; among them is IDDM12, which is located on chromosome 2q3 and encodes key lymphocyte co-receptor genes, including CTLA-4, CD28, and inducible costimulator (ICOS). All these genes are in close linkage (Bartsocas et al. 2006). Many molecular epidemiologic studies have evaluated the potential role of rs3087243 (Ueda et al., 2003; Kawoura and Loannidis, 2005; Zhernakova et al., 2005; Ikegami et al., 2006; Kanazawa et al., 2006; Caputo et al., 2007; Howson et al., 2007) in susceptibility to T1D. Given the amount of accumulated data, we believe that it is important to perform a quantitative synthesis of the evidence. Therefore, we conducted a comprehensive meta-analysis on all available case-control association studies.

MATERIAL AND METHODS

Identification of eligible studies

A total of 7 references published on the association between the rs3087243 polymorphism and T1D were identified according to our inclusion criteria, involving 7085 cases and 9963 controls (Zhernakova et al., 2005; Baniasadi et al., 2006; Ikegami et al. 2006; Kanazawa et al., 2006; Howson et al. 2007; Douroudis et al., 2009; Benmansour el al., 2010). The main

characteristics of these studies are described in Table 1. Sources included MEDLINE and EMBASE (search last updated in February 2012). The search strategy was based on combinations of the terms "CTLA-4", "cytotoxic T-lymphocyte-associated antigen-4", "CD152", and "diabetes". Reference lists in retrieved articles were also screened. We excluded studies with family-based designs in which the analysis was based on linkage (Ueda et al., 2003; Howson et al., 2009).

Data extraction

The following information was independently extracted from the identified studies by two participants in the meta-analysis: first author, journal, year of publication, study design, ethnicity of the study population, clinical characteristics, genotyping method, the number of cases and controls or odds ratio (OR) and 95% confidence interval (CI), country in which the study was conducted, and confirmation of diagnosis. The results were compared and any disagreement was discussed and resolved by consensus.

Quality evaluation

All the studies included 1) discussed the association between the rs3087243 polymorphism and T1D; 2) used disease-free subjects as controls; 3) provided genotype or allele distribution in both case and control groups; 4) were independent studies, without overlap between the subject groups investigated; 5) were published in peer-reviewed journals and indexed by PubMed or cited by articles indexed by PubMed. Authors were contacted where clarification was required.

Statistical analysis

The meta-analysis examined the overall association of alleles and genotypes and the risk of T1D for the polymorphism. The effect size was represented by an OR with 95%CI. The Cochran's Q statistical test and the I^2 test were used to assess heterogeneity in combined studies. Publication bias was checked using the Begg test, and the Egger test was used for funnel plot asymmetry. Both the random-effect model and the fixed-effect model were used to calculate pooled OR with Woolf's 95%CI. P values of overall OR were generated using the *Z* test. Sensitivity analysis was conducted by removing one study at a time and analyzing the others to ensure that no single study was completely responsible for the overall results. The significance level was set at 0.05, and all P values were two-tailed. We used inverted funnel plots and the Begg-Mazumdar publication bias diagnostics (nonparametric correlation coefficient) to evaluate whether the magnitudes of the observed associations were related to the variance of each study. The meta-analysis was performed using the Comprehensive Meta Analysis software (Version 2.2.046; BIOSTAT, Englewood, NJ, USA).

RESULTS

Totally, 7 references met our criteria and 8 studied the rs3087243 polymorphism (Table 1).

Table 1. Characteristics of the studies included.

Study (references)	Year	Country	Racial descent	Polymorphisms	Case	Control
Baniasadi et al.	2006	India	North Indians	+49A/G, CT60A/G, -318C/T	130	180
Ikegami et al.	2006	Japan	Asian	+49A/G, CT60A/G	769	723
Douroudis-1 et al.	2009	Estonia	European	+49A/G, CT60A/G	170	230
Douroudis-2 et al.	2009	Finland	European	+49A/G, CT60A/G	404	725
Benmansour et al.	2010	Tunisia	African	+49A/G, CT60A/G, -318C/T	228	193
Howson et al.	2007	Great Britain	European	CT60A/G	4364	6973
Zhernakova et al.	2005	India	North Indian	CT60A/G	350	900
Kanazawa et al.	2006	Japan	Asian	CT60A/G	72	39

Allelic analysis

The eligible studies for analysis included 7085 cases and 9963 controls of the rs3087243 polymorphism (Table 1). The polymorphism was significantly associated with T1D [fixed: OR and 95%CI = 1.249 (1.194-1.307), P < 0.001; random: OR and 95%CI = 1.601 (1.103-2.325), P = 0.013] (Figure 1 and **Figure S1**).

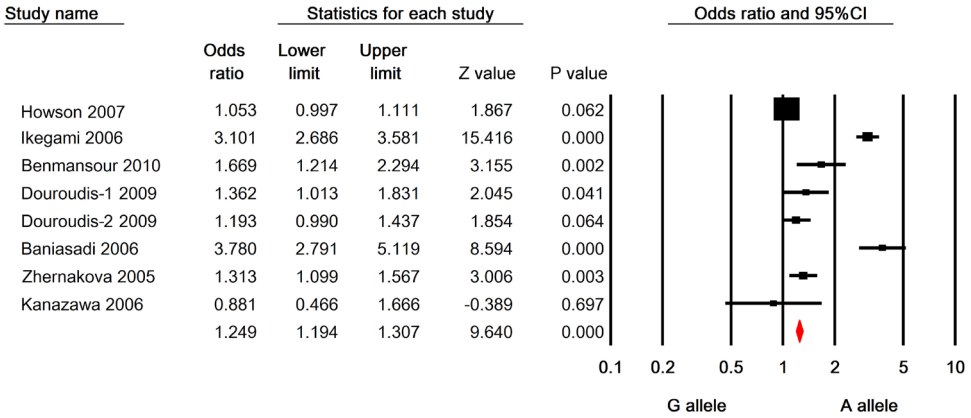

Figure 1. Meta-analysis of association studies of the CT60A/G polymorphism and diabetes (fixed model). Pooled overall OR is shown. The OR of each study is marked with a black square. Pooled OR is indicated by a red diamond.

A sensitivity analysis was conducted, and the results are shown in Figure 2. The sensitivity analysis showed that when any one of the studies was removed, the heterogeneity of the population did not change significantly, indicating that no heterogeneity existed in the population. There was no evidence to prove that the magnitude of the overall OR estimates changed in the same direction over time. Egger's funnel plots of publication bias analysis for the rs3087243 polymorphism are presented in Figure 3.

Genotypic analysis

For the genotype analysis of rs3087243, the result of GG versus GA+AA was significant [OR and 95%CI = 1.249 (1.164-1.341), P < 0.001], indicating that the GG genotype was deleterious for patients with T1D (Figure 4).

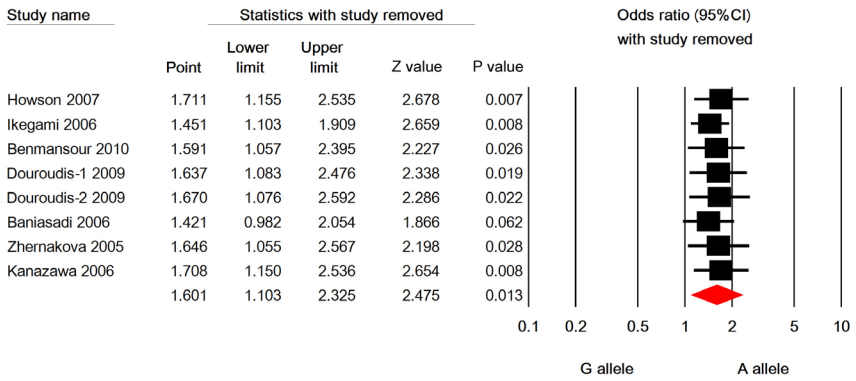

Figure 2. Sensitivity analysis of CT60A/G. When any one of the studies was removed, the heterogeneity of the population remained unchanged.

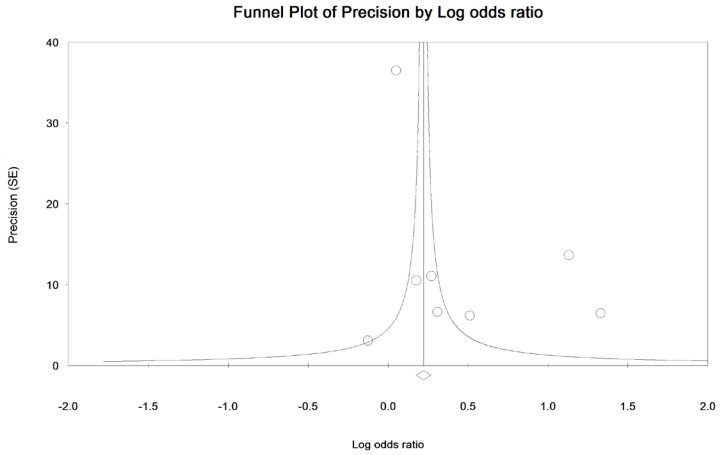

Figure 3. Egger's funnel plots of publication bias analysis for the CT60A/G polymorphism. The larger the deviation from the funnel curve of each study, the more pronounced the asymmetry. Results from small studies scatter widely at the bottom of the graph, with the spread narrowing among larger studies.

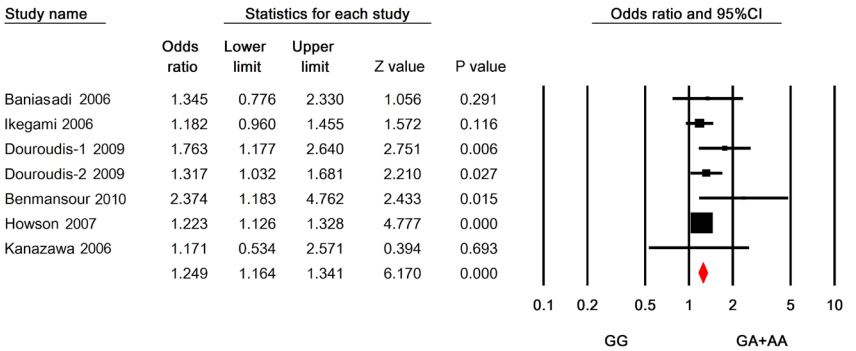

Figure 4. Meta-analysis of association studies of the CT60A/G GG/(GA+AA) and diabetes. Pooled overall OR is shown. The OR of each study is marked with a black square. Pooled OR is indicated by a red diamond.

DISCUSSION

T1D is commonly considered an organ-specific autoimmune disorder with a multifactorial background, where onset is preceded by a period of autoimmune destruction towards the insulin-producing pancreatic β-cells and with high levels of IFN-γ and TNF-β (Atkinson and Eisenbarth, 2001). However, the pathogenesis of the development and progression of T1D is very unclear at present. Because of the various and serious lifelong complications of T1D, identification of the etiologic factors is crucial in the pathogenesis of this disease. The major histocompatibility complex region explains approximately half of the genetic susceptibility to T1D, suggesting that additional determinants exist, and such determinants have been repeatedly suggested by different genome scans (Polychronakos and Li, 2011).

A number of studies indicated that variants of the *CTLA-4* gene contribute to the disease. Fine-mapping analyses have also suggested that peak linkage and association are present in the *CTLA-4* region [+49A/G, -819 C/T, and (AT)n in the 3'-UTR] (Marron et al., 1997). However, the results of genetic association studies have been confusing because of the difficulty in replicating significant associations. Different characteristics among studies, such as ethnicities, diabetes mellitus type, definition of case and control, have introduced heterogeneity and made the results of association studies difficult to interpret. This comprehensive meta-analysis included data from 8 studies, with approximately 17048 T1D cases and controls. It revealed significant evidence of the association between the rs3087243 polymorphism and T1D.

The rs3087243 polymorphism showed an overall association with T1D. The G allele and the GG genotype were positively associated with T1D in the population, implying that rs3087243 may have a significant effect on the disease and may be in linkage disequilibrium with other causative mutations.

In conclusion, the current comprehensive meta-analysis included a large sample size. The design of systematic methods and analytical approaches as well as tests of heterogeneity and sensitivity analyses have produced significant results. Since T1D is caused by the combined actions of many factors, for greater insight into its genetic component, further studies are required to confirm the role of other genes that may have a small individual effect and to identify new genetic risk factors.

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Supplementary material

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