Association between the CTLA-4 +49A/G polymorphism and Graves' disease: A meta-analysis

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Received March 31, 2012; Accepted June 14, 2012

DOI: 10.3892/etm.2012.618

Abstract. The +49A/G polymorphism of the cytotoxic T-lymphocyte-associated antigen-4 gene (CTLA-4) has been associated with Graves' disease (GD). However, results have been inconsistent. The aim of this study was to quantitatively summarize the evidence for CTLA-4 +49A/G polymorphism and GD. Electronic search of PubMed was conducted to select studies. Case-control studies containing available genotype frequencies of CTLA-4 +49 were chosen, and odds ratio (OR) with 95% confidence interval (CI) was used to assess the strength of this association. Forty-two case-control studies including 8,288 cases and 9,372 controls were identified. Three studies were eliminated from the total 42 studies due to a p-value <0.05 (p-value for Hardy-Weinberg equilibrium in control group) in these studies which induced significant publication bias. The overall results suggested that the variant genotypes were highly associated (p<0.01) with GD risk in all genetic models (additive model: OR, 1.443; 95% CI, 1.319-1.578; p<0.001; recessive model: OR, 1.589; 95% CI, 1.396-1.808; p<0.001; dominant model: OR, 1.621; 95% CI, 1.430-1.837; p<0.001). Similarly, in the subgroup analyses for ethnicity (Caucasian, Asian), the results were positive. This meta-analysis suggests that the CTLA-4 +49A/G polymorphism is highly associated (p<0.01) with increased risk of GD, especially in Caucasians and Asians. To validate this association, further studies with larger participants worldwide are needed to examine associations between this polymorphism and GD.

Introduction

Graves' disease (GD) is one of the autoimmune thyroid diseases (AITDs) which affect 5% of the general population (1). GD is an autoimmune antibody-mediated, thyroid-specific autoimmune disease which causes thyroid gland tumefaction. GD patients make antibodies to the thyroid-stimulating hormone receptor leading to hyperthyroidism. People of Western countries (~1.2%) and 0.25-1.09% of people of China are afflicted with GD (2,3). Although environmental factors, such as infection (4) and stress, are very important in the process of Graves' disease in susceptible individuals, one study in twins revealed that ~80% of the predisposition to GD is due to genetic factors (5). Several genetic loci have been implicated in the susceptibility to this disease. One of the associated genes is the cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) gene which consists of 4 exons and 3 introns. In 1997, Yanagawa et al (6), Marron et al (7) and Donner et al (8) initially reported that there was an association between CTLA4 and Graves' disease. The CTLA-4 gene is located on the long arm of chromosome 2q33 and belongs to the immunoglobulin superfamilly. Since the CTLA-4 protein transmits an inhibitory signal to T-cells, it has a strong susceptibility in autoimmunity. One of the CTLA-4 gene polymorphisms is located on exon 1 +49, which causes a threonine to alanine substitution in codon 17 (codon 17 T/A). To date, the CTLA-4 +49A/G polymorphism has been studied in different and numerous groups in humans, and a potential association with GD has been found in many results (6-36). However, some results suggest that there is no association between CTLA-4 +49A/G polymorphism and GD (37-46). Thus, the results are still inconsistent. Another problem is that these published studies only refer to a rather modest sample size that limits their significance. Utilizing the advantage of meta-analysis, a powerful method for quantitatively summarizing different study results, we combined the data for analysis and increased the sample size to a reasonable level. In this study, we conducted a meta-analysis to quantitatively assess the effect of the CTLA-4 +49A/G polymorphism on the risk of GD.

Materials and methods

Publication search. PubMed was searched using the terms ‘CTLA 4’, ‘Graves’ and ‘polymorphism’ or ‘CTLA4’, ‘Graves’ and ‘polymorphism’ or ‘cytotoxic T lymphocyte’, ‘Graves’ and ‘polymorphism’ (the last search update was on March 11, 2012). Case-control studies containing available genotype frequencies of 49A/G were chosen. Additional studies were
identified by a manual search of the references of the original studies.

**Statistic analysis.** For the control group of each study, the observed genotype frequencies of the CTLA-4 +49A/G polymorphism were assessed for Hardy-Weinberg equilibrium using the $\chi^2$ test. The strength of association between the +49A/G polymorphism of the CTLA-4 gene and GD was assessed by calculating crude odds ratios (ORs) with 95% confidence intervals (CIs). The pooled ORs were performed for the additive genetic model (G vs. A), dominant model (G/G+G/A vs. A/A) and recessive model (G/G vs. G/A+A/A), respectively. Heterogeneity assumption was checked by a $\chi^2$-based Q-test. A p-value of <0.05 for the Q-test indicated a lack of heterogeneity among the studies; the summary OR estimate of each study was calculated by the random effects model (47,48). The potential for publication bias was examined by Begg's test (funnel plot method) and Egger's linear regression test (p<0.05 was considered representative of statistical significance) (49). All statistical analyses were performed with Stata software (version 11.0; Stata Corporation, College Station, TX).

**Results**

**Eligible studies.** We identified 42 case-control studies concerning the association between the CTLA-4 +49A/G polymorphism and GD, which included 8,288 GD cases and 9,372 controls. These data were used in our meta-analysis (Table I). The distribution of genotypes in the controls of all the studies was in agreement with Hardy-Weinberg equilibrium.

**Meta-analysis.** The results of the association between the CTLA-4 +49A/G polymorphism and GD and the heterogeneity test are shown in Table II. The overall results suggest that the variant genotypes were highly associated (p<0.01) with GD risk in all genetic models [additive model: OR, 1.443; 95% CI, 1.319-1.578; p<0.001 (Fig. 1); recessive model: OR, 1.589; 95% CI, 1.396-1.808; p<0.001 (Fig. 2); dominant model: OR, 1.621; 95% CI, 1.430-1.837; p<0.001 (Fig. 3)]. Similarly,
### Table I. Distribution of the CTLA-4 +49A/G genotype for patients with Graves’ disease and the controls.

| Population          | Ethnicity or Study | Year | GD A/A | GD A/G | GD G/G | Control A/A | Control A/G | Control G/G | P-value  |
|---------------------|--------------------|------|--------|--------|--------|-------------|-------------|-------------|----------|
| Caucasian South Indian | Veeramuthumari et al (9) | 2011 | 11     | 37     | 32     | 29          | 25          | 26          | 0.000819 |
| Asian               | Thai               | 2011 | 22     | 49     | 61     | 26          | 73          | 54          | 0.875319 |
| Asian               | Chinese Han        | 2010 | 104    | 730    | 1030   | 156         | 823         | 945         | 0.211832 |
| Asian               | Japanese           | 2009 | 62     | 143    | 210    | 142         | 358         | 295         | 0.067982 |
| Caucasian           | Iranian            | 2009 | 48     | 43     | 14     | 75          | 25          | 3           | 0.606930 |
| Caucasian           | Iranian            | 2009 | 114    | 71     | 20     | 75          | 25          | 3           | 0.606930 |
| Others              | Brazilian          | 2008 | 43     | 58     | 15     | 39          | 32          | 7           | 0.905523 |
| Asian               | Chinese            | 2008 | 7      | 73     | 97     | 18          | 77          | 97          | 0.633099 |
| Asian               | Thai               | 2007 | 15     | 69     | 124    | 18          | 77          | 97          | 0.846451 |
| Asian               | Chinese            | 2006 | 2      | 29     | 58     | 7           | 26          | 27          | 0.211832 |
| Caucasian           | Turkish            | 2006 | 48     | 38     | 11     | 42          | 34          | 14          | 0.120930 |
| Asian               | Chinese            | 2006 | 33     | 95     | 135    | 32          | 89          | 75          | 0.520341 |
| Asian               | Korean             | 2006 | 16     | 112    | 160    | 30          | 197         | 244         | 0.240107 |
| Asian               | Taiwanese          | 2005 | 8      | 53     | 46     | 15          | 58          | 28          | 0.091603 |
| Caucasian           | Turkish            | 2005 | 29     | 33     | 15     | 43          | 48          | 7           | 0.189953 |
| Caucasian           | Italian            | 2005 | 59     | 68     | 23     | 139         | 138         | 24          | 0.201228 |
| Asian               | Japanese           | 2005 | 17     | 25     | 1     | 78          | 88          | 34          | 0.287293 |
| Asian               | Taiwanese          | 2004 | 18     | 72     | 81     | 11          | 50          | 87          | 0.316477 |
| Caucasian           | Lebanese           | 2004 | 8      | 23     | 3     | 24          | 14          | 0           | 0.163933 |
| Caucasian           | Polish             | 2004 | 32     | 50     | 17     | 50          | 84          | 20          | 0.964800 |
| Caucasian           | White              | 2003 | 88     | 139    | 74     | 146         | 158         | 45          | 0.825642 |
| Asian               | Japanese           | 2003 | 1      | 6      | 13     | 12          | 27          | 21          | 0.539129 |
| Caucasian           | Iranian            | 2003 | 21     | 49     | 20     | 30          | 53          | 30          | 0.510214 |
| Caucasian           | Polish             | 2003 | 73     | 123    | 66     | 77          | 85          | 32          | 0.303455 |
| Asian               | Japanese           | 2003 | 28     | 140    | 151    | 15          | 63          | 34          | 0.067423 |
| Asian               | Chinese            | 2002 | 3      | 54     | 66     | 23          | 59          | 76          | 0.069793 |
| Caucasian           | USA                | 2002 | 22     | 67     | 31     | 30          | 36          | 14          | 0.576150 |
| Asian               | Japanese           | 2002 | 32     | 62     | 50     | 38          | 46          | 26          | 0.107271 |
| Caucasian           | Tunisian           | 2001 | 31     | 63     | 50     | 26          | 94          | 85          | 0.998814 |
| Caucasian           | UK                 | 2001 | 136    | 262    | 86     | 192         | 198         | 34          | 0.081624 |
| Others              | African, American, Hispanic, Asian | | | | | | | | |
| Asian               | Korean             | 2000 | 5      | 35     | 57     | 26          | 75          | 98          | 0.061219 |
| Others              | Not specified      | 2000 | 8      | 29     | 8      | 15          | 23          | 5           | 0.390573 |
| Caucasian           | Moscow             | 2000 | 6      | 22     | 50     | 25          | 38          | 30          | 0.081864 |
| Others              | African-American   | 2000 | 20     | 25     | 4      | 23          | 19          | 5           | 0.718804 |
| Caucasian           | UK                 | 1999 | 122    | 192    | 65     | 164         | 171         | 28          | 0.067423 |
| Caucasian           | White              | 1998 | 23     | 37     | 13     | 47          | 37          | 16          | 0.069793 |
| Caucasian           | German, Canadian   | 1998 | 22     | 56     | 25     | 52          | 48          | 21          | 0.096985 |
| Asian               | Japanese           | 1998 | 11     | 44     | 57     | 58          | 197         | 170         | 0.938310 |
| Asian               | Japanese           | 1997 | 11     | 64     | 78     | 34          | 88          | 78          | 0.287293 |
| Asian               | Chinese            | 1997 | 1      | 11     | 16     | 6           | 39          | 49          | 0.632129 |
| Caucasian           | German, Canadian   | 1997 | 81     | 161    | 63     | 135         | 149         | 41          | 0.990935 |

*a-value for Hardy-Weinberg equilibrium in the control group. GD, Graves’ disease.*
in subgroup analyses for ethnicity (Caucasians, Asians), the results were positive.

**Table II. ORs and 95% CI for the CTLA-4 +49A/G polymorphism for different genetic models in patients with Graves' disease.**

| Genetic model          | Population   | Pooled OR (95% CI) | P-value | Heterogeneity P-value | Begg's test P-value | Egger's test P-value |
|------------------------|--------------|--------------------|---------|-----------------------|---------------------|----------------------|
|                        |              |                    |         |                       |                     |                      |
| Additive               |              |                    |         |                       |                     |                      |
| (G vs. A)              | Asian        | 1.347 (1.203-1.507) | <0.001  | 0.003                 | 0.323               | 0.373                |
|                        | Caucasian    | 1.543 (1.324-1.798) | <0.001  | <0.001                | 0.426               | 0.788                |
|                        | Others       | 1.458 (1.157-1.837) | 0.001   | 0.845                 | 0.174               | 0.505                |
|                        | Overall      | 1.443 (1.319-1.578) | <0.001  | <0.001                | 0.255               | 0.642                |
| Recessive              |              |                    |         |                       |                     |                      |
| (G/G vs. A carriers)   | Asian        | 1.476 (1.267-1.721) | <0.001  | 0.003                 | 0.621               | 0.506                |
|                        | Caucasian    | 1.770 (1.386-2.260) | <0.001  | <0.001                | 0.791               | 0.586                |
|                        | Others       | 1.487 (0.931-2.376) | 0.097   | 0.773                 | 0.174               | 0.275                |
|                        | Overall      | 1.589 (1.396-1.808) | <0.001  | <0.001                | 0.978               | 0.965                |
| Dominant               |              |                    |         |                       |                     |                      |
| (G carriers vs. A/A)   | Asian        | 1.431 (1.227-1.670) | <0.001  | 0.349                 | 0.187               | 0.196                |
|                        | Caucasian    | 1.727 (1.419-2.102) | <0.001  | <0.001                | 0.344               | 0.860                |
|                        | Others       | 1.739 (1.254-2.412) | 0.001   | 0.850                 | 1.000               | 0.705                |
|                        | Overall      | 1.621 (1.430-1.837) | <0.001  | 0.001                 | 0.113               | 0.166                |

**Figure 2. Forest plot of ORs of the G/G genotype when compared to the A allele carriers (G/A+A/A) (recessive model) in the Graves' patients. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the study-specific weight. The diamond represents the pooled OR and 95% CI. OR, odds ratio; CI, confidence interval.**

**Publication bias.** Funnel plot and Egger's test were performed to estimate the publication bias of studies. The results of Egger's
test provided statistical evidence for funnel plot symmetry (for G/G+G/A vs. A/A, p=0.166) (Table II).

Discussion

This meta-analysis examined the association of the CTLA-4 +49A/G polymorphism with GD and included 8,288 GD cases and 9,372 controls. Three studies were eliminated from the total 42 studies due to a p-value of <0.05 (p-value for Hardy-Weinberg equilibrium in control group) in these studies which induced significant publication bias. The results of Egger's test provided statistical evidence for funnel plot symmetry (for G/G+G/A vs. A/A, p=0.166). The overall results suggest that the variant genotypes were highly associated (p<0.01) with GD risk in all genetic models (additive model: OR, 1.443; 95% CI, 1.319-1.578; p<0.001; recessive model: OR, 1.589; 95% CI, 1.396-1.808; p<0.001; dominant model: OR, 1.621; 95% CI, 1.430-1.837; p<0.001). Similarly, in subgroup analyses for ethnicity (Caucasians, Asians), the results were positive.

GD is a disease with significant clinical consequences. The mechanism of GD is still relatively unknown. Although environmental factors, such as infection (4) and stress, are important in the process of Graves' disease in susceptible individuals, one study in twins suggests that ~80% of the predisposition to GD is due to genetic factors (5). Single nucleotide polymorphisms (SNPs) can be used as a tool for investigating genetic variations and disease susceptibility. GD is an autoimmune antibody-mediated, thyroid-specific autoimmune disease. The CTLA-4 protein can transmit an inhibitory signal to T-cells and has a strong susceptibility in autoimmunity. CTLA-4 protein has recently been described as a gatekeeper of conjugation timing and reduced conjugation may protect against prolonged contact periods of cytotoxic T lymphocytes with autoantigen-defined targets (50). It has been in the centre of attention for its key role in autoimmunity. The +49A/G polymorphism is one of the CTLA-4 three forms of polymorphisms. To date, a multitude of different studies were carried out concerning the association between the CTLA-4 +49A/G polymorphism and GD, but the results are inconsistent. In many studies (6-36) the results are positive, however in others (37-46) the results are negative.

This meta-analysis revealed a highly significant (p<0.01) association between the CTLA-4 +49A/G polymorphism and
GD risk, in both Asian and Caucasian subgroups. In conclusion, this meta-analysis suggests that the CTLA-4 +49A/G polymorphism is potentially associated with the risk of GD among Caucasians and Asians. Future, well-designed, large scale studies are necessary to validate this association in different populations.

Acknowledgements
This work was financially supported by the National Science Foundation of China (nos. 30960152, 30871232, 31170735), the Nature Science Foundation of Yunnan Province (no. 2008C043M) and the Fund of State Key Laboratory of Genetics Resources and Evolution (no. GREKF10-07).

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