Association Between *IL6*-174 G/C Polymorphism and Graves' Disease: A Systematic Review and Meta-Analysis

Danyal Imani[1], Ramazan Rezaei[1], Bahman Razi[2], Shahab Alizadeh[3], and Mahdi Mahmoudi[4,5]

[1] Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

[2] Department of Hematology, School of Align Medicine, Tehran University of Medical Sciences, Tehran, Iran

[3] Department of Cellular and Molecular Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran

[4] Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran

[5] Rheumatology Expert Group (REG), Universal Scientific Education and Research Network (USERN), Tehran, Iran

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Abstract- Several studies have evaluated the association between interleukin-6 (IL-6) -174 G/C polymorphism and Graves' disease (GD); however, the results have been inconsistent. In the current study, a meta-analysis was performed to assess the association of *IL6* -174 G/C polymorphism with Graves' disease. Medline, EMBASE, and Web of Science databases were searched to identify all eligible studies published before August 2016. Odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were calculated to assess the strength of association in dominant, recessive, allelic, homozygotes contrast, and heterozygotes contrast models. A total of four case-control studies with 554 GD cases and 1201 healthy controls were included in this meta-analysis. In the combined analysis, the results showed significant association between the *IL6* -174 G/C polymorphism and the risk for GD in dominant model (OR=1.39, 95% CI: 1.07-1.80), recessive model (OR=2.75, 95% CI: 1.01-7.55) and homozygote contrast model (OR=3.25, 95% CI: 1.1-9.58). No publication bias was found in the current study (all $P>0.05$). The meta-analysis results suggested that the *IL6* -174 G/C polymorphism was indicated to be associated with the risk of GD. Further studies are warranted to confirm these results.

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Keywords: Interleukin-6; Polymorphism; Graves; Meta-analysis

Introduction

Graves' disease (GD), a common autoimmune disorder, is one of the main causes of hyperthyroidism and is characterized by the existence of thyrotoxicosis, diffuse goiter, and infiltrative ophthalmopathy (1,2). The exact etiology and pathogenesis of GD is not clear, however, the involvement of intrathyroidal thyrotropin receptor antibodies has been described in various studies (3,4). Thyroid stimulating hormone (TSH) receptor antibodies (TRAb) are produced through the incorporation of immunological processes (5). Upon stimulation and activation of interferon-γ-producing inflammatory cells, thyroid follicular cells express major histocompatibility complex class II molecules, allowing them to further present antigens, such as TSH receptors, to the auto-reactive T cells (6). Eventually, the activated T cells can facilitate the activation and differentiation of B cells which lead to the formation of antibodies (7). Furthermore, intrathyroidal inflammatory cells can secrete different pro-inflammatory cytokines to initiate local inflammation and help withstand the autoimmune process (8-10).

Obviously, in addition to the TRAb, pro-inflammatory cytokines play an important role in the development of GD (11,12). IL-6 is a major pleiotropic pro-inflammatory cytokine (13). Accumulating results of various studies suggest that IL-6 participates in the pathogenesis of GD (10). The human *IL6* gene, which is located on chromosome 7p21, encodes a protein of the pro-inflammatory cytokines (14). This gene spans 5 kb and contains five exons and four introns (15). Almost 50 single nucleotide polymorphisms (SNPs) in the promoter region of the human *IL6* gene have been described (16, 17). These polymorphisms are suggested to be associated with the basal levels of cytokine (18). Therefore, the

Corresponding Author: M. Mahmoudi
Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran
Tel: +98 21 88220067, Fax: +98 21 88220067, E-mail address: mahmoudim@tums.ac.ir

frequently studied SNP at position -174 (*IL6* -174 G/C) can alter transcriptional regulation and cytokine levels and affect inflammatory conditions (15). Previous studies have demonstrated that *IL6* -174 G/C polymorphism is associated with the onset and progression of several human diseases including neuroblastoma (19), psoriasis (20), systemic lupus erythematosus (21), coronary heart disease (22), and nephritis (23).

Internationally, several genetic studies have evaluated the association between *IL6* -174 G/C polymorphism and the susceptibility of individuals to GD, but the results have been inconsistent and inconclusive, probably because of different study populations and limited sample sizes. Although Anvari *et al*., (10) and Amirzargar *et al*., (2) reported significant associations between the *IL6* polymorphism and GD, Bednarczuk *et al*., (24) and Duraes *et al*., (25) failed to find such associations. Thus, this meta-analysis of all eligible case-control studies were performed to obtain a more precise estimate of the effect of *IL6* -174 G/C polymorphism and the risk of GD.

Materials and Methods

The present meta-analysis was performed in accordance with Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines (26).

Searches and data sources

A systematic literature search of the electronic databases Medline, EMBASE, and Web of Science was conducted to identify eligible studies published prior to August 2016 that explored the relationship between *IL6* -174 G/C polymorphism and GD risk. The following search terms were used: (interleukin 6 OR IL-6) AND (Graves' disease) AND (polymorphism OR polymorphisms OR SNP OR variation OR mutation). The search was restricted to articles written in the English language and studies of human populations. The abstracts of the retrieved articles were fastidiously evaluated to determine whether they contained information on the topic of interest.

Inclusion criteria

Studies were enrolled in the current meta-analysis based on conformance to the following inclusion criteria: (1) case-control studies that assessed the association of the *IL6* gene -174 G/C polymorphism with the risk of Graves' disease, and (2) studies that supplied genotype or allele frequencies for the *IL6* -174 G/C polymorphism in both cases and controls to calculate the odds ratio and 95% confidence interval. Accordingly, the following exclusion criteria were used: (1) abstracts, reviews, comments, and letters; (2) repeated studies and studies with overlapping subjects; and (3) studies in which genotype frequencies were not reported. The identified studies were independently reviewed by two investigators for eligibility based on the predefined criteria; any discrepancies between the reviewers were resolved by discussion.

Data extraction and quality assessment

For each study, the first author's last name, year of publication, ethnicity of participants, numbers of cases and controls, genotyping method, and frequencies of genotypes in cases and controls were recorded. The Newcastle-Ottawa Scale (NOS) was used to evaluate the methodological quality (27). This quality assessment tool judges studies on the basis of a star system. A study awarded 0-3, 4-6, or 7-9 was considered to be a low, moderate, or high-quality study, respectively. The data extraction and quality assessment were evaluated by two reviewers, and any discrepancies were resolved by consensus and discussion between the two authors.

Statistical analysis

For each study, deviations from Hardy-Weinberg equilibrium (HWE) were assessed using the $\chi 2$ test (28). ORs and their corresponding 95% CI were used to assess the strength of the association between *IL6* -174 G/C polymorphism and GD risk in five genetic models: homozygote contrast (CC vs. GG), heterozygote contrast (GC vs. GG), dominant model (CC+GC vs. GG), recessive model (CC vs. GC+GG), and allelic model (C vs. G). The presence of heterogeneity between studies was calculated using the chi-square-based Q-test; significance was set at a level of $P<0.1$. The inconsistency index I^2 was calculated to assess the variation caused by heterogeneity (29). If heterogeneity existed among the individual studies in the present meta-analysis, the pooled OR was assessed using the random-effects model. A sensitivity analysis was conducted to assess the influence of studies deviating from HWE on the pooled results. The potential publication bias was estimated by the funnel plot. The funnel plot asymmetry was assessed by Egger's linear regression test and Begg's test (significance was set at a level of $P<0.05$) (30). All statistical analyses were performed using STATA (version 13.0; Stata Corporation, College Station, TX) and SPSS (version 23.0; SPSS, Inc., Chicago, IL).

Results

Characteristics of eligible studies

The procedures for including/excluding potential studies are presented in Figure 1. The initial search retrieved 131 potentially relevant studies. Based on the inclusion criteria, four case-control studies with 554 cases and 1201 healthy controls were included in the final meta-analysis (2,10,24,25). The selected studies had been conducted in different countries: two studies were conducted in Iran (2,10), one in Portugal (25), and one in Poland (24). The studies' publication years ranged from 2004 to 2010. According to the criteria of the NOS, all included studies had an overall good methodological quality with total scores ranging from 7 to 9. The general characteristics and allele and genotype distributions of studies included in this meta-analysis are shown in Tables 1 and 2.

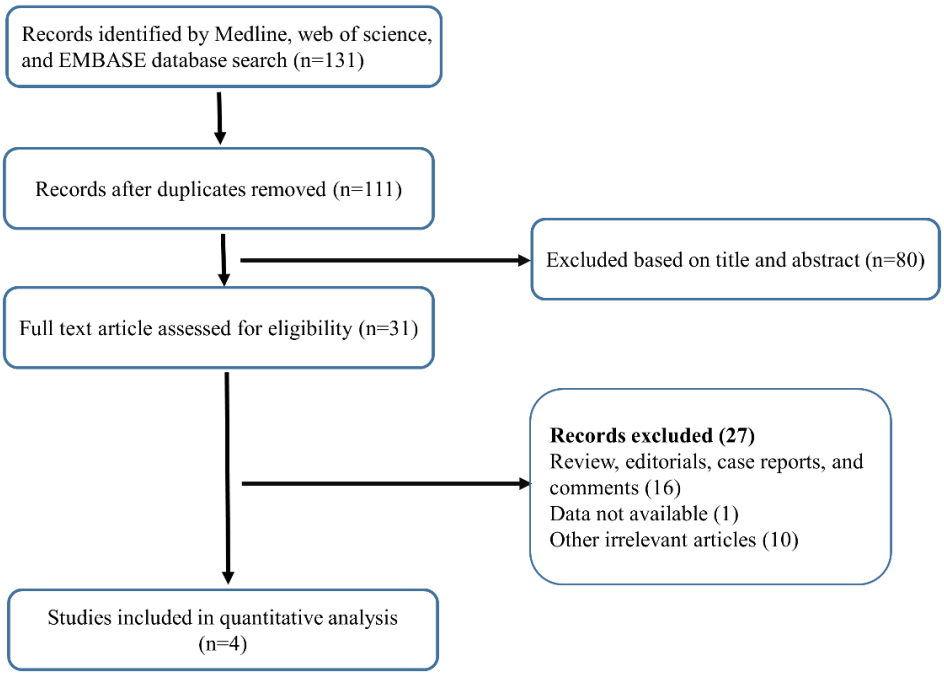

Figure 1. Flow diagram of study selection

Table 1. The characteristics of the studies included in the meta-analysis of the Graves' disease

Study author	Year	Country	ethnicity	Sex	Cases/controls	Case age/control age (Mean±SD)	Genotype method	Quality score
Anvari *et al.*,	2010	Iran	Caucasian	Both sexes	107/140	NR/NR	PCR–RFLP	7
Amirzargar *et al.*,	2010	Iran	Caucasian	Both sexes	57/140	NR/NR	PCR–RFLP	7
Bednarczuk *et al.*,	2004	Poland	Caucasian	Both sexes	279/186	43/47	PCR–RFLP	8
Duraes *et al.*,	2014	Portugal	Caucasian	Both sexes	111/735	45.1±16/49.0±16	PCR–RFLP	7

NR, not reported

Table 2. Distribution of genotypes and alleles among GD patients and controls

Study author	Graves' disease cases					Healthy control					*P*-HWE	MAF
	GG	GC	CC	G	C	GG	GC	CC	G	C		
Anvari *et al.*,	17	63	27	97	117	42	93	4	177	101	<0.001	0.36
Amirzargar *et al.*,	13	34	10	60	54	42	93	4	177	101	<0.001	0.36
Bednarczuk *et al.*,	85	138	56	308	250	58	101	27	217	155	0.11	0.41
Duraes *et al.*,	37	61	13	135	87	319	324	92	962	508	0.49	0.34

P-HWE, P for Hardy–Weinberg equilibrium; MAF, minor allele frequency of control group

Meta-analysis of *IL6* −174 G/C polymorphism and Graves' disease

The pooled results and heterogeneity tests of the association between the 174 G/C polymorphism at the *IL6* gene and risk of Graves' disease in different models are shown in Table 3. A significant association was found between the *IL6* -174 G/C polymorphism and risk of Graves' disease in dominant model (OR=1.39, 95%CI: 1.07-1.80) (Figure 2), recessive model (OR=2.75, 95%CI: 1.01-7.55) (Figure 3) and homozygote contrast model (OR=3.25, 95%CI: 1.1-9.58) (Figure 4). More ever, our result suggested there was not association between the *IL6* -174 G/C polymorphism and risk of Graves' disease in the allelic model (OR=0.85, 95%CI: 0.54-1.32) and heterozygote contrast (OR=1.27, 95%CI: 0.96-1.67). Since significant evidence of heterogeneity was observed in the recessive model, allelic model and homozygote contrast a random-effects model was applied. In contrast, there was no heterogeneity among studies in other models; thus, a fixed-effects model was applied.

Table 3. Main results of the pooled ORs in meta-analysis of the *IL6* -174 G/C polymorphism

Genetic model	Sample size	Test of association		Test of heterogeneity		Test of publication bias Begg's		Egger's	
	Case/Control	OR	95%CI	I^2 (%)	*P*	Z	*P*	t	*P*
Dominant model	554/1201	1.39	1.07-1.80	28.7	0.24	0.68	0.49	1.11	0.38
Recessive model	554/1201	2.75	1.01-7.55	81.7	0.001	0.68	0.49	2.34	0.14
Allelic model	554/1201	0.85	0.54-1.32	81.3	0.001	0.68	0.49	0.81	0.50
Homozygote contrast	554/1201	3.25	1.11-9.58	80.5	0.002	0.68	0.49	3.37	0.07
Heterozygote contrast	554/1201	1.27	0.96-1.67	17.1	0.30	0.0	1	0.48	0.71

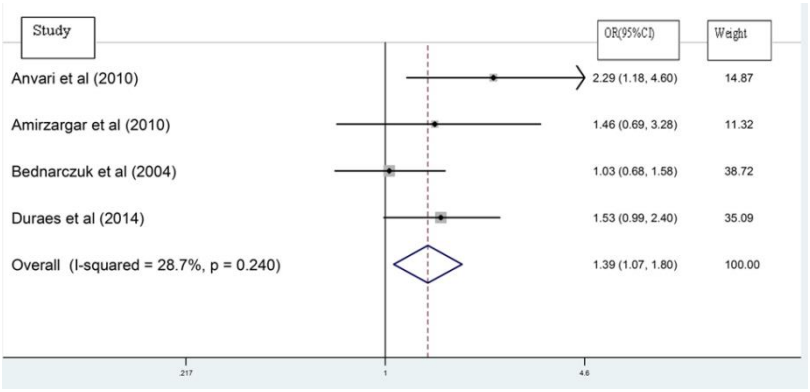

Figure 2. Forest plot of association between the -174 G/C SNP in dominant model and Graves' disease risk

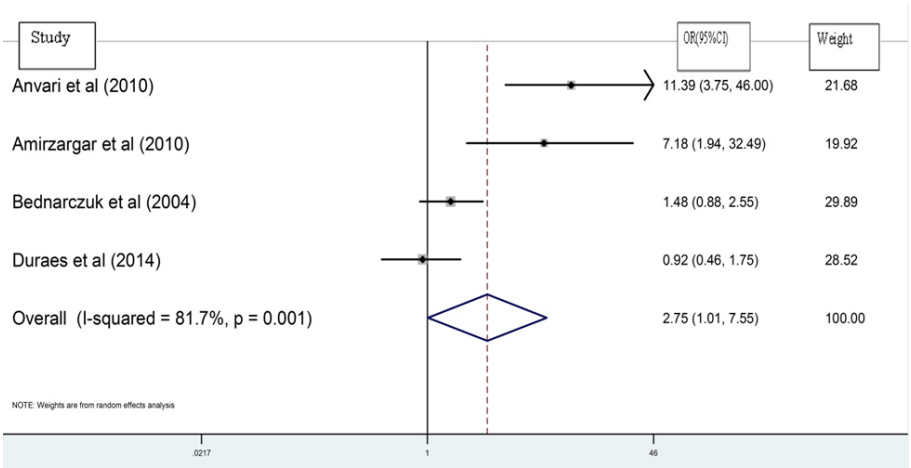

Figure 3. Forest plot of association between the -174 G/C SNP in recessive model and Graves' disease risk

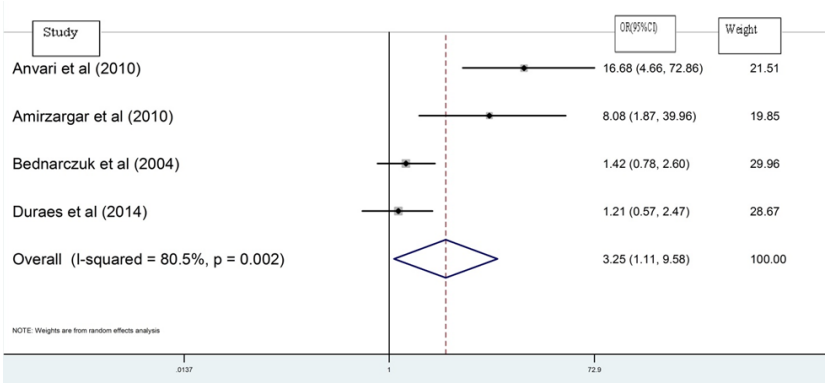

Figure 4. Forest plot of association between the -174 G/C SNP in homozygote contrast model and Graves' disease risk

Evolution of publication bias

The Begg's test did not reveal any significant evidence of publication bias for any of the genetic models, which implied that the publication bias was low in the present meta-analysis (all $P>0.05$).

Discussion

Graves' disease (GD) is a common autoimmune disorder in which the thyroid gland produces too much thyroid hormone, leading to an increased metabolic rate (31). The cause of GD is unclear; however, genetic susceptibility interacting with environmental factors are suggested to be involved in GD progression (32). Similarly, the inflammatory response is also related to Graves' disease. IL-6 is one of the confirmed major pleiotropic pro-inflammatory cytokines associated with Graves' disease, and enhanced serum levels of IL-6 have been found in patients with GD (33). Recently, several studies have focused on the association between the *IL6* gene -174 G/C polymorphism and Graves' disease; however, the results of these studies are controversial. Meta-analysis is a powerful method that enhances the statistical power of the analysis by increasing the sample size from that of individual studies. The current meta-analysis was performed to explore the association between the -174 G/C polymorphism in the *IL6* gene and the risk of Graves' disease. The results suggested that there was a significant association between the *IL6* -174 G/C polymorphism and Graves' disease risk. Accumulating evidence describes an intimate association between IL-6 and autoimmune diseases (34). Polymorphisms in the *IL6* -174 G/C promoter region have been associated with the development of various chronic and inflammatory diseases. IL-6 was initially known as a B cell growth factor and potent inducer of plasma cell differentiation (35). It promotes antibody production by promoting the capabilities of CD4 T cells through enhancing the production of IL-21 (36,37). Therefore, considering its important function in the growth and differentiation of lymphocytes, IL-6 might augment the development and production of thyroid receptor antibodies during the course of GD. In this regard, Grubeck-Loebenstein *et al*., demonstrated that lymphocytes infiltrate and follicular cells synthesize IL-6 in the thyroid glands of GD patients (38). In addition, Zheng *et al*., identified IL-6 mRNA in human epithelial cells from thyroid tissue and confirmed the in vivo secretion of IL-6 in these cells, suggesting the involvement of this event in the pathogenesis of human thyroid disease (38).

The present study has some limitations. First, the number of studies included in this meta-analysis was limited; because of that, a subgroup analysis to assess possible differences caused by participant age, sex, or race could not be performed. Second, this meta-analysis was restricted to English-language publications, which may have allowed the exclusion of some relevant publications in other languages. Third, because of a lack of original data, the potential interactions between gene-gene and gene-environmental factors could not be further evaluated; this deficiency might have affected the results. Finally, this meta-analysis was based on crude estimates without adjustment for confounders, which also limited the current study.

The present meta-analysis provides pooled results of the available evidence for the relationship between the *IL6* -174 G/C polymorphism and the risk for Graves' disease. To the best of the authors' knowledge, this is the first systematic study of the association between the *IL6* -174 G/C polymorphism and Graves' disease risk using meta-analysis. This meta-analysis suggested that the *IL6* -174 G/C polymorphism may be associated with the risk of Graves' disease. Large-scale case-control and

population-based association studies should be performed to validate the risk identified in the current meta-analysis, investigate the effects of other *IL6* gene polymorphisms, and examine potential gene-gene and gene-environment interactions on Graves' disease risk.

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