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Association between the -174 G/C polymorphism of the interleukin-6 gene and myocardial infarction risk: a meta-analysis

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ABSTRACT. Numerous studies have evaluated the association between the -174 G/C polymorphism in the interleukin-6 gene (*IL6*) and myocardial infarction (MI) risk. However, the results from the published studies are inconclusive. The aim of this meta-analysis was to determine whether the *IL6* -174 G/C polymorphism is associated with MI risk. A meta-analysis based on nine case-control studies was performed to address this issue. No significant associations between *IL6* -174 G/C polymorphism and MI risk were observed in any of the genetic models (CC *vs* GG: OR = 1.18, 95%CI = 0.92-1.52; CG *vs* GG: OR = 1.09, 95%CI = 0.93-1.27; dominant model: OR = 1.11, 95%CI = 0.94-1.31; recessive model: OR = 1.10, 95%CI = 0.91-1.33). Furthermore, the subgroup analysis by ethnicity did not reveal

a significant association between the *IL6* -174 G/C polymorphism and susceptibility to MI in Caucasians. In conclusion, the results indicate that the *IL6* -174 G/C polymorphism does not contribute to MI risk.

Key words: -174 G/C polymorphism; Myocardial infarction; Interleukin-6

INTRODUCTION

Coronary heart disease, especially myocardial infarction (MI), is the most common cause of death globally (Mathers and Loncar, 2006). MI usually results from the rupture of an atherosclerotic plaque with thrombus formation and occlusion of the coronary vessel, resulting in an acute reduction of blood supply to a portion of the myocardium (Dutta et al., 2012). It is estimated that more than three million people annually experience an acute ST-elevation MI, and more than four million have a non-ST-elevation MI (White and Chew, 2008). MI is known to be a complex multifactorial disorder that is associated with environmental and genetic factors. A previous INTER-HEART study identified several risk factors for MI, including family history, body mass index, smoking habits, hypertension, diabetes mellitus, and serum lipid levels (Ounpuu et al., 2001). In addition, emerging evidence indicates that genetic factors may play a critical role in the development of MI (Singh et al., 2012).

Interleukin-6 (IL-6), one of the best studied pro-inflammatory cytokines, plays a central role in immune, inflammatory, and acute-phase responses, hematopoiesis, atherogenesis, and several endocrine and metabolic processes (Hirano, 1998). The *IL6* gene, which has the chromosomal locus 7p21, spans 5 kb and contains four introns and five exons. The best characterized genetic variant of *IL6* is a G-to-C substitution at position -174 in the promoter region of *IL6* (-174 G/C or rs1800795), upstream of the transcription start site, which influences IL-6 levels *in vitro* and *in vivo* (Belluco et al., 2003; Jin et al., 2015; Xie et al., 2015; Yang et al., 2015). Because the -174 G/C polymorphism of *IL6* increases IL-6 expression, it may be associated with susceptibility to MI.

Epidemiological studies have recently focused on the association between the *IL6* -174 G/C polymorphism and MI risk. To test this important hypothesis, a number of observational studies conducted during the past decade have addressed the association between this polymorphism and the risk of MI. However, the results were inconsistent. This may in part be due to the small effect the polymorphism has on MI risk and the relatively small sample size of some published studies. Meta-analysis is a powerful tool for summarizing different studies. It can not only overcome the problem of small sample size and the inadequate statistical power of genetic studies of complex traits, but can also provide more reliable results than a single case-control study. Therefore, we performed a meta-analysis from all eligible studies to assess the association between the *IL6* gene -174 G/C polymorphism and MI.

MATERIAL AND METHODS

Literature search

PubMed and Embase database searches were performed using the following search

terms: ("interleukin-6", "IL-6" and "-174 G/C") and ("myocardial infarction" and "MI") and ("polymorphism", "SNP", "allele", and "variant"). Additional studies were identified by a manual search of the references of original studies. Eligible studies in the current meta-analysis had to meet all the following criteria: 1) the publication must have been about a case-control study referring to the association between the *IL6* -174 G/C polymorphism and MI; 2) the paper must have offered the sample size, distribution of alleles, genotypes, or other information that could help us infer the results; 3) when multiple publications reported the same or overlapping data, we used the most recent study or the one with the largest population; and 4) the publication language was confined to English.

Data extraction

Two investigators independently extracted the data and reached a consensus on all the items. For each study, the following characteristics were collected: last name of first author, year of publication, country of origin, ethnicity, numbers of genotyped cases and controls, and the counts of persons with different genotypes in cases and controls. Information on the Hardy-Weinberg equilibrium test (HWE) was also tracked or calculated if unavailable.

Statistical analysis

The strength of the association between the *IL6* -174 G/C polymorphism and MI risk was measured by odds ratios (ORs) with 95% confidence intervals (95%CIs). The pooled estimates were performed under several genetic models, including homozygote comparison (CC *vs* GG), heterozygote comparison (CG *vs* GG), dominant model (CC+CG *vs* GG), and recessive model (CC *vs* CG+GG). Subgroup analysis was performed by ethnicity. Heterogeneity was investigated and measured using the I^2 statistic; $I^2 > 50\%$ indicated evidence of heterogeneity. When heterogeneity was present, the random-effect model was used to calculate the pooled OR, whereas the fixed-effect model was used in its absence (Lau et al., 1997). Sensitivity analyses were carried out by limiting the meta-analysis to studies conforming to HWE ($P < 0.05$ of HWE was considered significant). Publication bias was investigated using a Begg's funnel plot ($P < 0.05$ was considered representative of statistically significant publication bias). All statistical tests for this meta-analysis were performed using the STATA software (version 12.0; Stata Corporation, College Station, TX, USA).

RESULTS

Study characteristics

A total of 855 potentially relevant publications up to November 2015 were systematically identified through PubMed and Embase databases. Based on our search criteria, 846 were excluded because they did not satisfy the inclusion criteria. A total of nine studies with 6778 cases and 5879 controls were included in the meta-analysis (Georges et al., 2001; Nauck et al., 2002; Bennet et al., 2003; Kelberman et al., 2004; Licastro et al., 2004; Lieb et al., 2004; Bennermo et al., 2011; Coker et al., 2011; Vakili et al., 2011). The characteristics of the selected studies are summarized in Figure 1. The year of publication of the included

studies ranged from 2001 to 2011. The HWE test was conducted on genotype distribution of the controls in all included studies, and all studies satisfied the HWE except one (Vakili et al., 2011). In addition, there were eight studies of Europeans and one study of Asians. The main characteristics of the included studies are listed in Table 1.

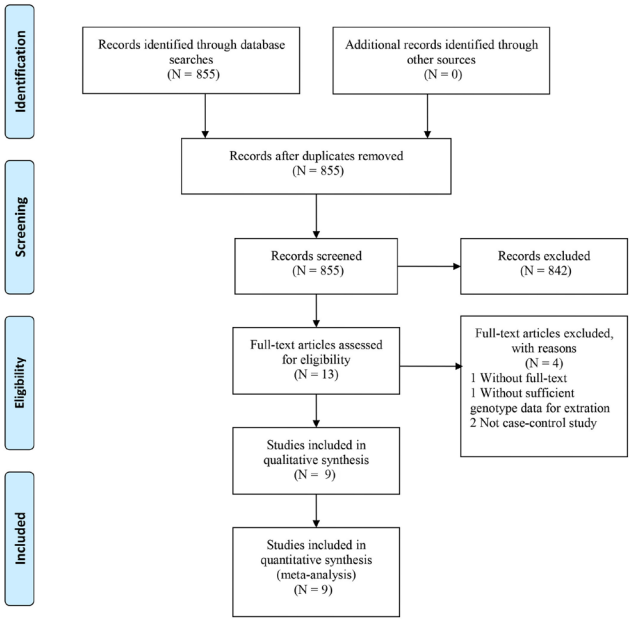

Figure 1. Flow diagram of study search and selection process.

Table 1. Characteristics of the studies included for meta-analysis.

Study included	Year	Area	Race	Cases/controls	Genotypes for cases			Genotypes for controls			HWE test
					GG	GC	CC	GG	GC	CC	
Georges	2001	France	Caucasian	1228/1344	340	680	208	462	672	210	0.18
Nauck	2002	Germany	Caucasian	1365/729	436	668	261	230	355	144	0.74
Bennet	2003	Sweden	Caucasian	1157/1500	305	577	275	398	754	348	0.80
Licastro	2004	Italy	Caucasian	138/152	35	88	15	46	44	7	0.42
Lieb	2004	Germany	Caucasian	1322/579	451	627	244	331	499	193	0.84
Kelberman	2004	Mixed	Caucasian	507/561	227	219	61	240	240	81	0.10
Bennermo	2011	Sweden	Caucasian	444/329	119	150	87	109	176	93	0.19
Coker	2011	Turkey	Caucasian	167/235	102	56	9	141	81	13	0.76
Vakili	2011	Iran	Asian	450/450	153	234	63	202	229	19	0.00

HWE = Hardy-Weinberg equilibrium.

Meta-analysis results

When all eligible studies were pooled into one dataset for the meta-analysis (Figure 2 and Table 2), we found no statistical association between the *IL6* -174 G/C polymorphism and MI risk based on any of the four genetic models (CC *vs* GG: OR = 1.18, 95%CI = 0.92-1.52; CG *vs* GG: OR = 1.09, 95%CI = 0.93-1.27; dominant model: OR = 1.11, 95%CI = 0.94-1.31;

recessive model: OR = 1.10, 95%CI = 0.91-1.33). In the stratified analysis by ethnicity, there was a similar lack of association between this polymorphism and MI risk in Caucasians (CC *vs* GG: OR = 1.03, 95%CI = 0.92-1.14; CG *vs* GG: OR = 1.06, 95%CI = 0.90-1.25; dominant model: OR = 1.06, 95%CI = 0.90-1.25; recessive model: OR = 1.00, 95%CI = 0.92-1.10). Sensitivity analysis was performed by omission of one non-HWE study (Vakili et al., 2011) and the result was not altered, indicating that our results were statistically robust (Table 2).

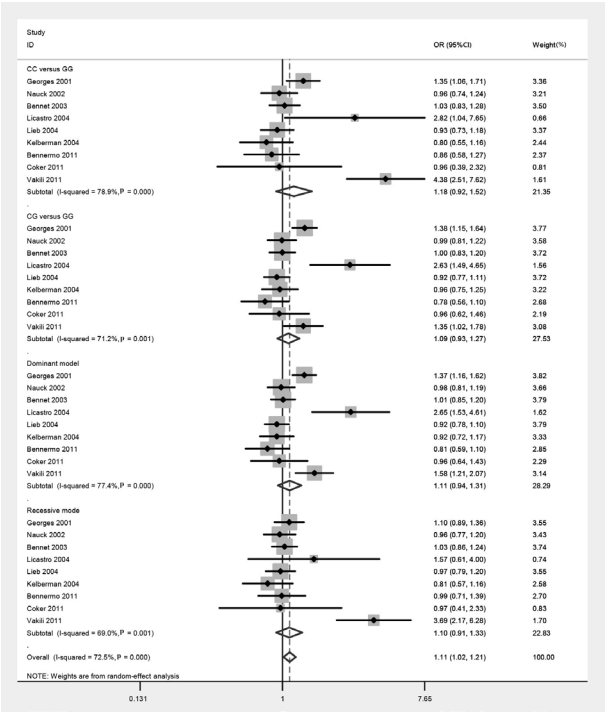

Figure 2. Forest plots of the association between the *IL6* gene -174 G/C polymorphism and myocardial infarction risk.

Table 2. Summary of different comparative results.

	N	Cases/controls	CC *vs* GG			CG *vs* GG			Dominant model			Recessive mode		
Variables			OR (95%CI)	P	I^2	OR (95%CI)	P	I^2	OR (95%CI)	P	I^2	OR (95%CI)	P	I^2
Total	9	6778/5879	1.18 (0.92-1.52)	0.00	78.9%	1.09 (0.93-1.27)	0.00	71.2%	1.11 (0.94-1.31)	0.00	77.4%	1.10 (0.91-1.33)	0.00	69.0%
Ethnicity														
Caucasian	8	6328/5429	1.03 (0.92-1.14)	0.08	43.9%	1.06 (0.90-1.25)	0.00	72.0%	1.06 (0.90-1.25)	0.00	74.2%	1.00 (0.92-1.10)	0.85	0.0%
HWE														
Yes	8	6328/5429	1.03 (0.92-1.14)	0.08	43.9%	1.06 (0.90-1.25)	0.00	72.0%	1.06 (0.90-1.25)	0.00	74.2%	1.00 (0.92-1.10)	0.85	0.0%

N = number; I^2 = inconsistency index; CI = confidence interval; OR = odds ratio; HWE = Hardy-Weinberg equilibrium.

Publication bias

We produced a Begg's funnel plot to access the publication bias of all included studies. The shape of the funnel plot seemed symmetrical (Figure 3), suggesting that there was no obvious publication bias.

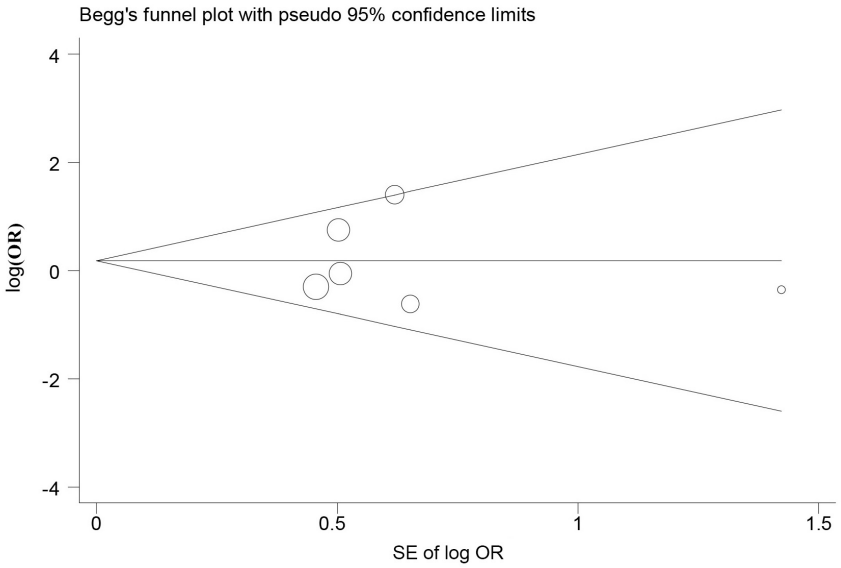

Figure 3. Begg's funnel plot for publication bias test.

DISCUSSION

MI is a multifactorial disease and its pathogenesis is not yet fully understood. Accumulated evidence indicates that MI is incontestably determined by a complex interaction of environmental and genetic factors. The -174 G/C polymorphism was studied in the promoter of the *IL6* gene, and the -174 C allele was found to be associated with lower plasma IL-6 concentration (Fishman et al., 1998). In the ECTIM study, Georges et al. (2001) first presented a significantly higher risk for MI in patients carrying the C allele. Subsequently, several studies, but not all, have confirmed a relationship between the *IL6* -174 G/C polymorphism and susceptibility to MI (Georges et al., 2001; Nauck et al., 2002; Bennet et al., 2003; Kelberman et al., 2004; Licastro et al., 2004; Lieb et al., 2004; Bennermo et al., 2011; Coker et al., 2011; Vakili et al., 2011). The current meta-analysis was performed to obtain a more adequate result by combining comparable studies, and increasing the sample size and statistical power (Wang et al., 2013).

In this study, we performed a meta-analysis to explore the association between the *IL6* -174 G/C polymorphism and MI risk among 12,657 subjects. Our meta-analysis did not show a significant association between the genotype and risk of MI. Because of the genetic and environmental differences pertaining to the subjects, we performed an ethnicity-specific subgroup analysis, and found no significant association in Caucasian populations. We could not perform stratified analysis in the Asian study by Vakili et al. (2011). Deviation of allelic distributions from HWE may have contributed to between-study heterogeneity in the sensitivity analysis (Luo et al., 2012); by limiting this meta-analysis to those studies that were consistent with HWE, we confirmed that the meta-analysis was realistic and believable. The effect of the *IL6* -174 G/C polymorphism might have a limited impact on MI. As with other diseases, the pathogenesis of MI is dependent on the synergistic reaction of multiple genes and

gene-environment interactions, and those relationships require further investigation in future studies.

Some limitations of this meta-analysis should be considered when interpreting the results. First, because of incomplete raw data or publication limitations, some relevant studies could not be included in our meta-analysis. Second, the random-effect model was used in this meta-analysis and the results must be interpreted with caution. Additionally, the genotype information stratified for the main confounding variables, such as age, gender, and exposure, was not available in the original papers, and the confounding factors might have caused unpredictable confounding bias.

In conclusion, this meta-analysis suggests that the -174 G/C polymorphism in the *IL6* gene may not be associated with MI risk. Further evaluation of the influence of gene polymorphisms on MI will require well-designed studies with large sample sizes.

Conflicts of interest

The authors declare no conflict of interest.

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