Lack of an association between interleukin-6 gene promoter polymorphisms (−174G/C, −572G/C) and ischemic heart disease and/or ischemic stroke: A meta-analysis

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The association between single-nucleotide polymorphisms −174G/C (rs1800795) and −572G/C (rs1800796) in the interleukin-6 (IL-6) gene promoter region and ischemic heart disease (IHD)/ischemic stroke (IS) remains controversial and ambiguous. In this study, we performed a more precise estimation of the relationship by a meta-analysis based on currently available evidence from literature. To assess the effect of IL-6 polymorphisms (−174G/C, −572G/C) on IHD/IS susceptibility, a meta-analysis of 30 available studies was performed through May 2010. Summary odds ratios and their 95% confidence intervals for IL-6 polymorphisms and IHD/IS were estimated using fixed- and random-effects models when appropriate. Heterogeneity and publication bias were evaluated. When available studies were pooled into the meta-analysis, there was no significant association between IL-6 polymorphisms (−174G/C, −572G/C) and IHD/IS in any comparison model (CC vs GG, GC vs GG, dominant, and recessive models). Subgroup analyses results were consistent with the main analyses by ethnicity, ischemic types, quality score, and genotyping methods. Ethnicity (European studies) and quality score (low-quality studies) might be important sources of heterogeneity for −174G/C. However, metaregression analysis did not reveal that the foregoing characteristics could explain the r² in any comparison model. We could not identify the sources of heterogeneity for −572G/C. The present meta-analysis suggests that IL-6 promoter polymorphisms (−174G/C, −572G/C) were unlikely to be associated with risk of IHD and/or IS.

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1. Introduction

Interleukin-6 (IL-6), a proinflammatory and immunoregulatory cytokine, is a multifunctional protein principally involved in the genesis and maintenance of the inflammatory response. It is derived from diverse tissues, including fibroblasts, monocytes, adipocytes, and endothelial cells [1,2]. Recently, accumulating evidence indicated that high serum levels of IL-6 were associated with a worse prognosis in patients with unstable angina, acute myocardial infarction (MI), and ischemic stroke (IS) [3,4]. It was indicated that IL-6 was involved in atherosclerosis, cardiovascular disease, and inflammatory response to IS [5–8].

The IL-6 gene, located on chromosome 7p21 in humans, is composed of 5 exons, 4 introns, and a proximal promoter region [9]. The IL-6 promoter polymorphisms at positions −174 (rs1800795) and −572 (rs1800796) have been extensively studied, and evidence demonstrates that the 2 polymorphisms are associated with several diseases [10–12]. Ischemic heart disease (IHD) and IS are complex diseases influenced by genetic and environmental factors. A number of molecular epidemiologic studies have been performed to evaluate the association between the 2 polymorphisms and IHD/IS in diverse populations. Some authors have reported that the polymorphisms are associated with IHD/IS [9,10,13–22]; however, other studies demonstrated converse results, even in the same population [23,24]. It might be that a single study had lower statistical power to detect the overall effects; hence, a quantitative synthesis of the combined data from different studies was used to evaluate the association between IL-6 polymorphisms and IHD/IS. In our study, the present systematic review and meta-analysis were performed on all published case–control studies to estimate the association between IL-6 polymorphisms (−174G/C, −572G/C) and IHD/IS and to quantify the heterogeneity between the individual studies, as well as to investigate the existence of potential publication bias.
### Characteristics of eligible studies

<table>
<thead>
<tr>
<th>First author (reference)</th>
<th>Origin</th>
<th>Ethnicity</th>
<th>Ischemic phenotype</th>
<th>Sample size (case/control)</th>
<th>Genotyping methods</th>
<th>Matching criteria</th>
<th>Polymorphism(s) investigated</th>
<th>HWE 174G/C</th>
<th>HWE 572G/C</th>
<th>Quality score</th>
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<tr>
<td>IS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
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<td>Tong et al. [10]</td>
<td>Han Chinese</td>
<td>Asian</td>
<td>IS</td>
<td>648/648</td>
<td>TaqMan</td>
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<td>Yes</td>
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<tr>
<td></td>
<td>Uyghur Chinese</td>
<td>Asian</td>
<td>IS</td>
<td>100/100</td>
<td>TaqMan</td>
<td>Age, gender</td>
<td>−174G/C, −572G/C</td>
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<tr>
<td>Yamada et al. [15]</td>
<td>Japanese</td>
<td>Asian</td>
<td>ACI</td>
<td>1,140/2,010</td>
<td>PCR-SSCP</td>
<td>BMI and smoking</td>
<td>−174G/C, −572G/C</td>
<td>NA</td>
<td>Yes</td>
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<tr>
<td>Banerjee et al. [42]</td>
<td>Indian</td>
<td>Asian</td>
<td>IS</td>
<td>112/212</td>
<td>PCR-RFLP</td>
<td>Age, gender, same area</td>
<td>−174G/C,</td>
<td>Yes</td>
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<td>Karahan et al. [65]</td>
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<td>Asian</td>
<td>PAS</td>
<td>86/83</td>
<td>PCR-RFLP</td>
<td>Same area</td>
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<td>Austrian</td>
<td>Caucasian</td>
<td>IS/TIA</td>
<td>404/415</td>
<td>MS-PCR</td>
<td>Smoking</td>
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<td>Chamarro et al. [49]</td>
<td>Spanish</td>
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<td>IS</td>
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<td>Irishman</td>
<td>IS</td>
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<td>Revilla et al. [55]</td>
<td>Spanish</td>
<td>Caucasian</td>
<td>IS</td>
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<td>Rosner et al. [53]</td>
<td>Caucasian-Brazilians</td>
<td>American</td>
<td>CAD</td>
<td>276/138</td>
<td>PCR-RFLP</td>
<td>—</td>
<td>−174G/C, −572G/C</td>
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<td>Asian</td>
<td>CAD</td>
<td>210/232</td>
<td>PCR-RFLP</td>
<td>Age, gender</td>
<td>−174G/C, −572G/C</td>
<td>Yes</td>
<td>NA</td>
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<td>Sekuri et al. [52]</td>
<td>Trukese</td>
<td>Asian</td>
<td>CAD</td>
<td>115/105</td>
<td>PCR-RFLP</td>
<td>Age, gender</td>
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<td>NA</td>
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<td>Jia et al. [48]</td>
<td>Chinese</td>
<td>Asian</td>
<td>CHD</td>
<td>231/210</td>
<td>Real-time PCR</td>
<td>Age</td>
<td>−572G/C</td>
<td>NA</td>
<td>No</td>
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<td>Park et al. [14]</td>
<td>Korean</td>
<td>Englishman</td>
<td>CAD</td>
<td>168/166</td>
<td>SNAPShot</td>
<td>Age, gender</td>
<td>−572G/C</td>
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<td>Smith et al. [13]</td>
<td>Pole</td>
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<td>CABG</td>
<td>121/221</td>
<td>HIFMEECH</td>
<td>Age, gender</td>
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<td>Caucasian</td>
<td>CABG</td>
<td>142/121</td>
<td>PCR-RFLP</td>
<td>Age, gender</td>
<td>−174G/C, −572G/C</td>
<td>Yes</td>
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<td>Myśliwińska et al. [66]</td>
<td>Pole</td>
<td>Caucasian</td>
<td>CV</td>
<td>116/383</td>
<td>SSP-PCR</td>
<td>2 groups control</td>
<td>−174G/C,</td>
<td>Yes</td>
<td>5</td>
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<tr>
<td>Densen et al. [56]</td>
<td>Englishman</td>
<td>Caucasian</td>
<td>CV</td>
<td>320/100</td>
<td>PCR-RFLP</td>
<td>Age</td>
<td>−174G/C, −572G/C</td>
<td>Yes</td>
<td>NA</td>
<td>5.5</td>
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<td>Sie et al. [58]</td>
<td>Dutch(man)</td>
<td>Caucasian</td>
<td>CHD</td>
<td>243/1,929</td>
<td>TaqMan</td>
<td>−</td>
<td>−174G/C, −572G/C</td>
<td>Yes</td>
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<td>Chiappelli et al. [33]</td>
<td>Dutch(woman)</td>
<td>Caucasian</td>
<td>CHD</td>
<td>220/3,292</td>
<td>TaqMan</td>
<td>−</td>
<td>−174G/C, −572G/C</td>
<td>Yes</td>
<td>NA</td>
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<td></td>
<td>Northern Italian</td>
<td>Caucasian</td>
<td>MI</td>
<td>138/204</td>
<td>PCR-RFLP</td>
<td>Age, gender, same area</td>
<td>−174G/C,</td>
<td>Yes</td>
<td>5.5</td>
<td></td>
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<tr>
<td></td>
<td>Southern Italian</td>
<td>Caucasian</td>
<td>MI</td>
<td>66/53</td>
<td>PCR-RFLP</td>
<td>Age, gender, same area</td>
<td>−174G/C,</td>
<td>Yes</td>
<td>5.5</td>
<td></td>
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<tr>
<td>Lieb et al. [51]</td>
<td>Germans</td>
<td>Northern European</td>
<td>MI</td>
<td>1,322/1,023</td>
<td>PCR-RFLP</td>
<td>MONICA control</td>
<td>−174G/C, −572G/C</td>
<td>Yes</td>
<td>NA</td>
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<tr>
<td>Kelberman et al. [17]</td>
<td>Germans</td>
<td>Northern European</td>
<td>MI</td>
<td>229/244</td>
<td>PCR-RFLP</td>
<td>Age, gender</td>
<td>−174G/C, −572G/C</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td></td>
<td>Southern European</td>
<td>Caucasian</td>
<td>MI</td>
<td>278/278</td>
<td>PCR-RFLP</td>
<td>Age, gender</td>
<td>−174G/C, −572G/C</td>
<td>Yes</td>
<td>Yes</td>
<td>8</td>
</tr>
<tr>
<td>Bennet et al. [41]</td>
<td>Swedish(man)</td>
<td>Caucasian</td>
<td>MI</td>
<td>812/1,013</td>
<td>DASH</td>
<td>Age, man</td>
<td>−174G/C, −572G/C</td>
<td>Yes</td>
<td>Yes</td>
<td>6.5</td>
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<td></td>
<td>Swedish(woman)</td>
<td>Caucasian</td>
<td>MI</td>
<td>345/487</td>
<td>DASH</td>
<td>Age, woman</td>
<td>−174G/C, −572G/C</td>
<td>Yes</td>
<td>Yes</td>
<td>6.5</td>
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<tr>
<td>Nauck et al. [23]</td>
<td>Germans</td>
<td>Caucasian</td>
<td>CAD</td>
<td>2575/729</td>
<td>PCR-RFLP</td>
<td>−</td>
<td>−174G/C, −572G/C</td>
<td>Yes</td>
<td>NA</td>
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<tr>
<td>Basso et al. [24]</td>
<td>Germans</td>
<td>Caucasian</td>
<td>CAD</td>
<td>498/1,109</td>
<td>PCR-SSCP</td>
<td>Age, gender, smoking</td>
<td>−174G/C,</td>
<td>Yes</td>
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<td>Humphries et al. [18]</td>
<td>Englishman</td>
<td>Caucasian</td>
<td>CAD</td>
<td>160/2,560</td>
<td>PCR-SSCP</td>
<td>Age, man</td>
<td>−174G/C, −572G/C</td>
<td>Yes</td>
<td>Yes</td>
<td>10</td>
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<tr>
<td>Georges et al. [9]</td>
<td>Northern Irishman</td>
<td>Caucasian</td>
<td>MI</td>
<td>186/172</td>
<td>PCR-SSCP</td>
<td>Age, MONICA</td>
<td>−174G/C, −572G/C</td>
<td>Yes</td>
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<td>8</td>
</tr>
</tbody>
</table>

The C allele frequency was estimated based on the numbers of genotypes. NA = not applicable.

*The number of subjects in each genotype was estimated based on the reported proportions.*
2. Subjects and methods

2.1. Publication search

We searched the PubMed, Medline, and Web of Science databases for all articles on the association between IL-6 polymorphisms and IHD/IS (updated to 26 May 2010). The following terms were used in this search: “interleukin 6” or “interleukin-6” or “IL-6” or “IL6”) and ("genetic variant" or "genetic variation" or “polymorphism”) and (“coronary heart disease” or “coronary artery disease” or “myocardial infarction” or “unstable angina” or “stable angina” or “ischemic heart disease” or “cerebral infarction” or “cerebral ischemia” or “stroke”). All searched studies were retrieved, and their references were checked for other relevant publications. Review articles were also searched to identify additional eligible studies. Only published studies with full-text articles were included: studies investigating the association between the IL-6 polymorphisms (−174G/C, −572G/C) and any IHD/IS event, studies published in English, studies reporting odds ratios (ORs) or data for their calculation, and case–control or cross-sectional studies.

Studies were excluded if they used study designs other than case–control or cross-sectional methods (e.g., prospective cohort of patients without healthy control subjects) [25–30], did not present relevant data to calculate the OR and its variance, or examined patients without healthy control subjects [25–30]. For overlapping studies, only the study with the largest sample numbers of cases and controls, genotyping methods, matching variables, and evidence of Hardy–Weinberg equilibrium (HWE) using online software [http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl]. A p value less than 0.05 of HWE was considered significant. All data from eligible studies are presented in Table 1. Different ethnicity descents were categorized as Asian, Caucasian, and African.

2.2. Data extraction

The following information was recorded for each study: first author, year of publication, study origin, ischemic types, ethnicity, numbers of cases and controls, genotyping methods, matching variables, and evidence of Hardy–Weinberg equilibrium (HWE) using online software [http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl]. A p value less than 0.05 of HWE was considered significant. All data from eligible studies are presented in Table 1.
2.3. Quality score assessment

The quality of the included studies was evaluated independently by 3 investigators (Ma, Liu, and Peng) according to a set of predetermined criteria (Table 2), which was modified from previous studies [39,40], and disagreements were resolved by consensus among the 3 authors. Scores ranged from 0 (lowest) to 10 (highest), and studies with scores ≥ 6 were classified as high-quality studies, whereas studies with scores < 6 were classified as low-quality studies.

2.4. Statistical analysis

Summary ORs and corresponding 95% confidence intervals (95% CIs) were estimated for each polymorphism in different comparison models, including CC versus GG, GC versus GG, GC/CC versus GG (dominant), and CC versus GG/GC (recessive) models. For studies that had cells with no counts [9,10,17,18,41,42], we added 1 for each cell in those studies to determine the genetic model [40].

The Q test and I² statistics were used to assess statistical heterogeneity among studies [43,44]. If the result of the Q test was P < 0.1 and I² < 50%, indicating the absence of heterogeneity, then a fixed-effects model (the Mantel–Haenszel method) was used to estimate the summary ORs [45]; otherwise, the random-effects model (the DerSimonian and Laird method) was used [46]. To explore sources of heterogeneity among studies, we performed stratified and logistic metaregression analyses. The following study characteristics were examined: sample size (≥500 and >500 subjects), genotyping methods (polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) and no PCR-RFLP), ethnicity, quality score, type of controls (blood donors, healthy subjects), non-IHD or IS patients, and neighbor-based sample, and ischemic types.

Sensitivity analysis was mainly performed by sequential omission of individual studies. For each polymorphism, publication bias was evaluated using a funnel plot and Egger’s regression asymmetry test [47].

All analyses were performed using Stata software, version 11.0 (Stata Corp., College Station, TX). All p values were two-sided. To ensure the reliability and the accuracy of the results, 2 authors entered the data into the statistical software programs independently with the same results.

3. Results

3.1. Study characteristics

Overall, 30 studies met the criteria. The main characteristics of the studies are presented in Table 1. Twenty studies (23 subgroups) evaluated the −174G/C variant, 7 studies (11 subgroups) evaluated the −174G/C and −572G/C variants, and only 3 studies evaluated the −572G/C variant. Twenty-seven studies (34 subgroups), including 12,305 cases and 20,106 controls, were available for the meta-analysis of IL-6 −174G/C and 5,475 cases and 9,537 controls for IL-6 −572G/C (14 subgroups from 10 studies). The sample size in these studies varied considerably, ranging from 119 to 3,512 individuals. Twenty-six subgroups were conducted in Caucasian populations, 6 in Asians, and 2 in Africans for −174G/C; 5 studies were conducted in Asians and 9 in Caucasians for −572G/C. Twenty-four subgroups with IHD and 10 subgroups with IS were conducted for −174G/C, 11 subgroups with IHD, and 3 subgroups with IS for −572G/C. Several genotyping methods were used, including PCR-RFLP, TaqMan, PCR/single-strand conformation polymorphism (SSCP), and SNaPShot. The genotype distributions among the controls of all studies were consistent with HWE except for the study of Jia et al. (HWE = 0.02) [48].

Most reports presented demographic information regarding cases and controls. The controls were younger than the cases in 8
studies [15,18,19,23,37,49–51]. Age and sex matching was described in 13 studies [9,10,17,20–22,24,35,42,52–55].

3.2. Meta-analysis results

The meta-analyses suggested that the −174G/C polymorphism was not associated with IHD/IS in all comparison models (CC vs GG, GC vs GG, dominant, and recessive models; Table 3). We failed to identify any significant association between the −174G/C polymorphism and IHD/IS in all comparison models through subgroup analyses according to ethnicity, ischemic types, quality score, and genotyping methods (Table 3). For the −572G/C polymorphism, there was no significant difference in all comparison models (Table 4). Subgroup analyses also indicated no significant association between the −572G/C polymorphism and IS/IHD based on ethnicity and ischemic types. However, we determined that the CC genotype was associated with an increased IHD/IS risk compared with the GG genotype in high-quality studies (OR = 1.32; 95% CI 1.02–1.70; p = 0.034) but not in low-quality studies (Table 4). Nevertheless, stratification analyses in high-quality score studies did not indicate any association between the −572G/C polymorphism and IHD and IS (data not shown).

3.3. Heterogeneity analysis

For the −174G/C polymorphism, most I² values of heterogeneity were greater than 50% and no p_Q values were greater than 0.10, which indicated statistically significant heterogeneity among studies. To explore the sources of heterogeneity, we performed stratified analyses of sample size, genotyping methods, ethnicity, quality score, and ischemic types. We determined that ethnicity and quality score but not sample size, genotyping methods, and ischemic types might substantially influence the initial heterogeneity (Table 3). However, metaregression analyses did not indicate that the foregoing characteristics could explain the I² in any comparison models (data not shown).

All I² values decreased obviously and p_Q values were greater than 0.10 after excluding the studies of Chiappelli et al. (Northern Italy) [33], Flex et al. [35], Georges et al (France) [9], and Densem et al. [56] (I²_CC vs GG = 21.2%, p_Q = 0.153; I²_GC vs GG = 0%, p_Q = 0.506; I²_Dominant = 1.9%, p_Q = 0.436; I²_Recessive = 16.2%, p_Q = 0.217), and Galbraith plots spotted the outlier of the 4 studies (Fig. 1C). The significance of summary ORs for the −174G/C polymorphism in different comparison models in the overall population and subgroup analyses was not influenced by omitting the 4 studies. In the overall population analyses, p_OR values of CC versus GG, GC versus GG, dominant, and recessive models were 0.801, 0.646, 0.611, and 0.989, respectively. The heterogeneity of some comparison models remained significant in stratified analyses after excluding the 4 studies; for example, p_Q values of CC versus GG and recessive models in Caucasians were 0.041 and 0.08, respectively, and p_Q values of IS were 0.081 and 0.04, respectively. However, the heterogeneity was disappeared after excluding the foregoing studies and study of Revilla et al. [55], and Galbraith plots spotted the outlier of the 5 studies (Fig. 1); the significance of summary ORs in all comparison models was also not influenced by omitting those 5 studies (Fig. 2). At the same time, the 5 studies were all Caucasian studies.

Fig. 1. Galbraith plot of −174G/C polymorphism and IHD/IS risk in different contrast models. (A) The studies of Revilla et al. [55], Densem et al. [56], Flex et al. [35], Chiappelli et al. (Northern Italy) [33], and Smith et al. [13] were outliers in the CC vs GG model. (B) The studies of Chiappelli et al. (Northern Italy) [33], Georges et al (French) [9], and Humphries et al. [18] were outliers in the GC vs GG model. (C) The studies of Chiappelli et al. (Northern Italy) [33], Flex et al. [35], Georges et al. (France) [9], and Densem et al. [56] were outliers in the dominant model. (D) The studies of Revilla et al. [55], Densem et al. [56], and Flex et al. [35] were outliers in the recessive model.
Fig. 2. Forest plot of IHD/IS risk associated with the IL-6 −1474C/G polymorphism in different contrast models after excluding the 5 studies of Chiappelli et al. (Northern Italy) [33], Flex et al. [35], Georges et al. (France) [9], Densem et al. [56], and Revilla et al. [55]. (A) CC vs GG model; (B) GC vs GG model; (C) dominant model; and (D) recessive model.
Fig. 2. (continued)
and the studies of Revilla et al. [55], Densem et al. [56], Flex et al. [35], and Chiappelli et al. (Northern Italy) [33] were low-quality studies (Table 1).

For the $-572G/C$ polymorphism, there was no statistically significant heterogeneity in all comparison models except the recessive model (Table 4). $I^2$ and $p_Q$ values were 0% and 0.813 after excluding the studies of Yamada et al. [15] and Tong et al. (Han Chinese) [10]. Galbraith plots spotted the outlier of the 2 studies (Fig. 3A). The pooled ORs were not influenced by omitting the 2 studies in all comparison models ($p_{OR}$ values of CC vs GG, GC vs GG, dominant, and recessive models were 0.674, 0.631, 0.718, and 0.704, respectively). However, stratified analyses indicated heterogeneity (CC versus GG, dominant, and recessive models in Asian populations: $p_Q = 0.033, 0.097$, and 0.087, respectively; $I^2 = 70.6, 57.1$, and 59.1%, respectively; Fig. 3B). Results indicated that the 2 studies were not completely the sources of the heterogeneity. The CC genotypes were associated with a significantly increased IHD/IS risk when compared with the GG genotype ($OR = 1.32; 95\% CI 1.03–1.68, p_{OR} = 0.03, p_Q = 0.48, I^2 = 0\%$) after excluding the study of Jia et al. because of deviation from HWE [48]. However, heterogeneity was significant under the recessive model in the overall population ($p_Q = 0.002, I^2 = 62.1\%$) and IS patients ($p_Q < 0.001, I^2 = 93.2\%$).

3.4. Sensitivity analysis

Sensitivity analysis was performed by sequential omission of individual studies. For each polymorphism, the significance of pooled ORs in all comparison models and subgroup analyses was not influenced excessively by omitting any single study (data not shown).

3.5. Publication bias

We reported no obvious publication bias for any of the polymorphisms in all comparison models ($-572G/C$, Egger’s test $p > 0.55$; $-174G/C$, Egger’s test $p > 0.10$).

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**Fig. 3.** Galbraith plot of $-572G/C$ polymorphism and IHD/IS risk and Forest plot of $-572G/C$ polymorphism after excluding the studies of Yamada et al. [15] and Tong et al. (Han) [10]. (A) The studies of Yamada et al. [15] and Tong et al. (Han) [10] were outliers in the recessive model. (B) Forest plot after excluding the studies of Yamada et al. [15] and Tong et al. (Han) [10] in the recessive model.
4. Discussion

The association between the IL-6 promoter polymorphisms (–174G/C and –572G/C) and IHD/IS has been widely studied. However, some studies supported the conclusion that risk for various types of IHD or IS was associated with the polymorphisms, whereas other studies drew converse conclusions. We performed a systematic review and meta-analysis to clarify the relationship between the polymorphisms and susceptibility to IHD/IS.

In this meta-analysis, 30 studies (34 subgroups for –174G/C, 14 subgroups for –572G/C) on IL-6 polymorphisms were performed to provide the most comprehensive assessment of the association between the polymorphisms and IHD/IS. Our data did not support a genetic association between the polymorphisms and IHD/IS risk in overall populations. It is possible that the individual polymorphisms could be associated with IHD or IS; the genetic effect would be very small and ORs would need to be less than 1.10 to detect an association in all comparison models [57]. In addition, we did not find evidence of an association between the –174G/C polymorphism and IHD/IS based on ethnicity, ischemic types, and quality score. The results indicated that the polymorphism might not contribute to IHD/IS risk, which helped to explain the observed interindividual differences with regard to IHD or IS susceptibility. Our results were consistent with the studies of Sie et al., which indicated that the –174 G/C genotype was not associated with coronary heart disease (CHD) risk in subjects older than 54 years of age [58].

Interestingly, we determined that the CC genotype of –572 G/C was associated with a higher IHD/IS risk than the GG genotype in high-quality studies but not in low-quality ones. Stratification analyses did not indicate any significant associations in all comparison models according to ischemic types in high-quality studies. Although the reason for these discrepancies remains unknown, some possibilities should be considered. For example, high-quality studies were more reasonable than low-quality studies were, with regard to representative populations, source of controls, etc., than low-quality studies. We considered that the –572G/C polymorphism might be associated with IHD/IS; however, more convincing evidence, such as larger sample size, number of studies, and ethnicity, is required to draw solid conclusions.

IL-6 –174 G/C and –572G/C polymorphisms were associated with IL-6 production or protein expression both in vivo and in vitro [11,12]. Some studies demonstrated that the –174G allele and GG genotype were related to a higher IL-6 expression level than the –174C allele and CC genotype and –174G allele and GC genotype were more responsive to induce such as lipopolysaccharide and IL-1 [11,59,60], however, other studies reported conflicting results [61,62]. The activity of the –174 position might be affected by the nearby polymorphic site –572 in the promoter [12,15]; the C allele of –572G/C was also associated with higher IL-6 concentrations [63]. We were unable to fully understand this discrepancy; it might be associated with genetic heterogeneity or ethnic and geographic variations. A complex interactive effect on IL-6 expression might exist for the 2 polymorphisms; the current meta-analysis did not indicate a significant association for both polymorphisms, investigated separately.

Heterogeneity analysis of –174 G/C suggested significant heterogeneity in all comparison models. By stratification analyses, there was obvious heterogeneity among studies with regard to sample size, genotyping methods, and ischemic types, but a lack of heterogeneity in high-quality studies and Asian populations (Table 3). Results indicated that ethnicity (European studies) and quality score (low-quality studies) but not sample size, genotyping methods, and ischemic types might substantially influence the initial heterogeneity. It might be that IHD and IS were mainly caused by atherosclerosis; sample size and genotyping methods did not influence heterogeneity in this meta-analysis. However, meta-regres-

sion analysis did not reveal that ethnicity and quality score could explain the $I^2$ in all comparison models. We determined that all $I^2$ decreased obviously and $p_q$ values were greater than 0.1 after excluding the studies of Georges et al. (France) [9], Densen et al. [56], Flex et al. [35], and Chiappelli et al. (Northern Italy) [33] in the overall population; however, the heterogeneity was still significant in some comparison models after excluding the 4 studies. Heterogeneity was eliminated after excluding the 4 studies and that of Revilla et al. [55]. In addition, the significance of summary ORs was not influenced by omitting the 5 studies in all comparison models in both overall population analyses and subgroup analyses. At the same time, the 5 studies were all European studies, and the studies of Revilla et al. [55], Densen et al. [56], Flex et al. [35], and Chiappelli et al. (Northern Italy) [33] were low-quality studies. The results indicated that the 5 studies might be the major source of the heterogeneity for the –174G/C polymorphism.

Significant heterogeneity was found in the recessive model ($I^2 = 64.4, p_q < 0.001$) for the –572G/C polymorphism. We determined that the $I^2$ and $p_q$ values were 0% and 0.813 in the overall population after excluding the studies of Yamada et al. [15] and Tong et al. (Han Chinese) [10]; Galbraith plots spotted the outlier for these 2 studies in the recessive model (Fig. 3A). However, stratified analysis demonstrated heterogeneity, for example, CC versus GG, dominant, and recessive models for Asian populations. When the study of Jia et al. [48], which was inconsistent with HWE, was excluded, heterogeneity was not significantly changed. The results indicated that the study of Tong et al. (Han Chinese) [10], Yamada et al. [15] or Jia et al. [48] was not the source of heterogeneity. The heterogeneity might be the result of the small number of studies included in the meta-analysis [64].

This meta-analysis must be interpreted with caution at the present time because of some limitations. First, the overall outcomes were based on individual unadjusted ORs, whereas a more precise evaluation should be adjusted by potentially suspected factors, including age, gender, smoking status, and environmental factors. In some studies, individuals who were unmatched by age and gender later developed IHD/IS within the age range in the control group. The results would hence underestimate the OR association with the genotype. Second, these estimations were obtained by pooling the studies with regard to heterogeneity. However, heterogeneity provided the opportunity to identify factors that modified the genotype. We still identified the source of heterogeneity by stratified analysis. Pooling studies with different results would lead to a high degree of heterogeneity, but might result in hazardous or invalid estimates. Third, genotyping accuracy and quality control measures were not well documented in some reports. The small number of studies and sample size limited the ability to conduct more meaningful subgroup analyses. The unavailability of raw data from the original studies limited the evaluation of gene–environment interactions. Fourth, the non-English literature and some original studies without related data were excluded which might limit analysis. Finally, we cannot exclude the possibility that the results were biased because of undetected stratification in the original case–control samples.

In summary, the present meta-analyses did not support a prominent association of the IL-6 promoter polymorphisms (–174G/C, –572G/C) with IHD/IS. However, the –572G/C polymorphism might be associated with IHD/IS in high-quality studies based on current published studies. More convincing evidence is required to draw solid conclusions on the relation between the –572G/C polymorphism and IHD/IS.

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