Association between the interleukin-6 genetic polymorphism 174 G/C and thrombosis disorder risk

Meta-analysis of 10,549 cases and 19,316 controls

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Abstract

Studies investigating the association between interleukin-6 (IL-6) gene-174 G/C polymorphism (rs1800795) and thrombosis disorder risk reported conflicting results. The aim of our study was to assess the association between the IL-6 gene 174 G/C polymorphisms and the risk of thrombosis disorders.

By pooling all studies, there was marginal association between and the risk of thrombotic disorders (1.09[0.97–1.22]), arterial thrombotic disorders (1.08[0.96–1.23]), and myocardial infarction (MI, 1.14[0.99–1.32]) under dominant genetic effect (C carriers vs GG). In subgroup analyses stratified by ethnicity, study scale, thrombotic category, and country, the results indicated that IL-6 gene-174 G/C polymorphism was significantly associated with increased risk of thrombotic disorders given the conditional such as Asians, large sample-sized, MI, population-based, and Indian studies (C carriers vs GG: 1.39 [1.13–1.72] and C allele vs G allele: 1.36 [1.19–1.56] for Asian; C carriers vs GG: 1.15 [1.01–1.31] and C allele vs G allele: 1.12 [1.01–1.23] for large sample-sized studies; C allele vs G allele: 1.10 [1.03–1.18] for population-based studies; and C carriers vs GG: 1.40 [1.19–1.66] for Indian studies). We did not observe significant association between IL-6-174 G/C and the risk of Caucasians, small sample-sized studies, stroke and venous studies, and other country studies.

This meta-analysis suggests that IL-6 gene-174 G/C polymorphism may be marginally associated with risk of thrombotic disorders, arterial disorders, MI especially for Asian, Indian, population-based, and large sample-sized studies. More studies with larger sample size and well-designed studies might be warranted.

Abbreviations: CI = confidence interval, CRP = C-reactive protein, HWE = Hardy–Weinberg equilibrium, IL-6 = interleukin-6, IS = ischemic stroke, MI = myocardial infarction, NOS = Newcastle–Ottawa Scale, OR = odds ratio.

Keywords: IL-6, meta-analysis, polymorphism, thrombosis disorders

1. Introduction

Arterial and venous thrombotic disorders, including myocardial infarction (MI) and ischemic stroke (IS) which are common and frequently fatal events, are hypothesized to share some etiologic pathway. Despite recent advances in effective prevention and acute thrombotic disorders intervene is a vital strategy to reduce the overall burden of thrombotic disorders worldwide. Epidemiologic studies show the favored model and the polygenic foundation for the pathophysiologic mechanism of thrombotic disorders is an interaction between genetic/cultural background and identified risk factors.[1,2] Numerous genetic/congenital, acquired, and environmental risk factors can keep the balance in favor of coagulation, which exists between fibrinolytic and anticoagulant forces and procoagulant (clotting) forces, predisposing to the pathologic thrombi formation in arteries (e.g., MI, IS) and veins (e.g., deep venous thrombosis).[3] Subsequent thrombi may lead to detach and embolize to obstruct a distant blood vessel or block blood flow at the site of thrombi formation and define the risk of thrombotic disorders (e.g., pulmonary embolism, embolic stroke).[3]

Arterial, venous thrombotic disorders and system inflammation are 2 closely correlated entities, which measured by predisposing factors (such as genetic, acquired, and environmental factors) and
activity or quantity of natural anticoagulant molecules in human plasma and tested for specific human gene defects.[15] Interleukin-6 (IL-6) is a pleiotropic cytokine related with atherosclerosis and cardiovascular diseases which may also be a pivotal mediator in the inflammatory response to cerebral ischemia.[14] The human IL-6 gene is mapped to chromosome 7p21-24 region,[5] containing of 4 introns and 5 exons. Among the mutations described, the 174 G/C (namely rs1800795), polymorphism in the IL-6 promoter region was detected the association with tuberculosis,[6] Alzheimer disease,[7] and multiple sclerosis,[8] although other reports failed to confirm these relationships.[9,10]

A common single nucleotide polymorphism at position -174 (IL-6-174 G/C, namely rs1800795) of the IL-6 gene promoter is demonstrated to impact the adherence of the glucocorticoid receptor and then results in repressive transcriptional activation.[13,14] Recently, increasing studies reported the role of this polymorphism in the predisposition to thrombotic disorders including MI, IS, and venous thromboembolism.[12,14,15] However, the conclusions are rather inconsistent, partially caused by the relative statistics power which stems from small sample size and diverse origins of incorporated studies. To our best knowledge, there is no meta-analysis involving in the IL-6 gene-174 G/C polymorphism and the risk of whole thrombotic disorders available up to now. Therefore, we carried out the meta-analysis to explore the relationship between IL-6 gene-174 G/C polymorphism and the susceptibility to thrombotic disorders based on the eligible published papers.

2. Methods

2.1. Publication search

This study was with approval by the Ethics Committee of Huazhong University of Science and Technology and Shidong Hospital, thus we assessed the association between the IL-6 genetic polymorphism 174 G/C and thrombosis disorder risk using meta-analysis. All published literatures investigating the association between polymorphism of IL-6 gene and the risk of thrombotic disorders were systematically searched using several electronic databases (Pubmed, EBASE, and ISI Web of Science database) as of June 1, 2015 using the following search terms: “myocardial infarction” or “stroke or venous thrombosis,” “pulmonary embolism” and “interleukin-6” or “IL-6” in combination with “polymorphism,” “mutation” or “variant.” Selection criteria in our meta-analysis incorporated: the association of 174 G/C polymorphism of IL-6 gene and risk of thrombotic disorders in English articles must be evaluated, detailed genotype frequency in participants (cases and controls) to assess odds ratios (ORs) and corresponding 95% confidence intervals (CIs), and studies with no deviation from Hardy–Weinberg equilibrium (HWE) in genotype distribution of the control subjects were included. For the exclusion criteria, we used as follows: without original data for the calculation of ORs and the corresponding 95% CIs in case and control studies; we incorporated only the largest or most recent studies when overlapping or repeat publications; and papers classified as reviews, abstracts, or case reports.

2.2. Data extraction

Two authors (HR and YY) independently extracted all potentially eligible reports and reached an agreement on all information. In case of disagreement, a 3rd author (YZ) would check these studies. The following information were collected and applied from the studies: 1st author, publication year, ethnicity/race, thrombotic disorder category, source of control, genotyping approaches, total number of case and control participants, and genotype distributions in all subjects of cases and controls.

2.3. Statistical analysis

For each study, by using an Internet-based program (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl), we first examined whether the genotype distribution in controls was according to HWE. The strength of the association between the G/C polymorphism and thrombotic risk was measured by ORs and 95% CI. The statistical significance of summary OR was determined with Z-test. We first estimated the risk of with 3 models including recessive model (CC vs GG + GC) and dominant model (CC + GC vs GG) and then evaluated variant genotype CC and compared with the wild-type GG homozygote. We also estimated the risks of C allele versus G allele and GC versus GG. A Chi-squared-based Q-statistic test was used to identify the statistical heterogeneity across studies (presence of heterogeneity was considered if $P < 0.10$). In the condition of absent of statistical heterogeneity (when the $P$-value is >0.10), a random-effect model was applied to the pooled OR of each study was calculated by the fixed-effects model (Mantel–Haenszel method); otherwise the fixed-effect model was selected (DerSimonian and Laird method). Z test was carried out to determine the statistical significance of pooled ORs.[13] In addition to overall analysis, subgroup evaluations were conducted according to ethnic group and thrombotic disorder category. Sensitivity analysis was performed through removing each study in turn to evaluate the stability of the results.[14]

Potential publication bias was analyzed by the funnel plot of the ORs versus their standard errors.[15] The Begg test and Egger test were also carried out to statistically observe the publication bias.[16,17] All statistical tests were analyzed by using the software STATA 12.0 (STATA Corporation, College Station, TX).

3. Results

As presented in Fig. 1, a total of 34 eligible and no overlap case–control reports in 29 research articles, assessing 10,549 cases and 19,316 controls were incorporated in our current meta-analysis, and there were 18 studies about MI[2,12,18–29] in 14
polymorphism and risks of thrombotic disorders (OR [95% CI]: 1.14 [0.99–1.32] for C carriers vs GG, $P_{\text{heterogeneity}} < 0.01$; 1.08 [0.99–1.18] for C allele vs G allele, $P_{\text{heterogeneity}} < 0.01$, Fig. 2), marginally associated with the risk of arterial thrombotic disorders (1.08 [0.95–1.23] for C carriers vs GG, $P_{\text{heterogeneity}} < 0.01$), but not associated with IS (0.95 [0.73–1.24] for C carriers vs GG, $P_{\text{heterogeneity}} < 0.01$) and venous thrombotic disorders (1.17 [0.97–1.42] for C carriers vs GG, $P_{\text{heterogeneity}} = 0.99$).

When analyzing for ethnicity, IL-6 gene-174 G/C was suggested to be associated with risks for the thrombotic disorders for Indians (1.99 [0.82–5.09] for CC vs GG, $P_{\text{heterogeneity}} = 0.15$; 1.39 [1.13–1.72] for C carriers vs GG, $P_{\text{heterogeneity}} = 0.26$; 1.36 [1.18–1.56] for C allele vs G allele, $P_{\text{heterogeneity}} = 0.11$), but not for Caucasians (0.97 [0.85–1.09] for CC vs GG, $P_{\text{heterogeneity}} = 0.003$; 1.05 [0.93–1.18] for C carriers vs GG, $P_{\text{heterogeneity}} < 0.01$) (Supplemental Fig. 1, http://links.lww.com/MD/B89).

When analyzing by countries, we found that IL-6 gene-174 G/C was associated with risks of the thrombotic disorders for India (1.40 [1.19–1.65] for C carriers vs GG, $P_{\text{heterogeneity}} = 0.17$), but not associated with Sweden (1.03 [0.91–1.15] for C allele vs G allele, $P_{\text{heterogeneity}} = 0.12$), not for German (0.98 [0.87–1.10] for CC vs G carriers, $P_{\text{heterogeneity}} = 0.94$), Italians (0.67 [0.36–1.25] for CC vs GG, $P_{\text{heterogeneity}} < 0.01$), and reduce the risk of English

### Table 1

Characteristics of studies included in our meta-analysis.

| First author | Disease | Country | Ethnic origin | Year | No. of cases | No. of controls | MAF (controls) |
|--------------|---------|---------|---------------|------|--------------|----------------|---------------|
| Humphries    | MI      | UK      | Caucasians    | 2001 | 40           | 827            | 0.95          |
| Georges (1)  | MI      | Sweden  | Caucasians    | 2001 | 170          | 231            | 0.95          |
| Georges (2)  | MI      | Germany | Caucasians    | 2001 | 45           | 47             | 0.95          |
| Nauck        | MI      | Germany | Caucasians    | 2002 | 436          | 230            | 0.95          |
| Bennis (1)   | MI      | Sweden  | Caucasians    | 2003 | 210          | 278            | 0.95          |
| Bennis (2)   | MI      | Sweden  | Caucasians    | 2003 | 95           | 120            | 0.95          |
| Licastro     | MI      | Sweden  | Caucasians    | 2004 | 35           | 46             | 0.95          |
| Lieb         | MI      | Germany | Caucasians    | 2004 | 451          | 331            | 0.95          |
| Kebelerman (1)| MI     | UK      | Caucasians    | 2004 | 89           | 71             | 0.95          |
| Kebelerman (2)| MI     | UK      | Caucasians    | 2004 | 138          | 169            | 0.95          |
| Rixner       | MI      | USA     | Caucasians    | 2005 | 204          | 822            | 0.95          |
| Chiappelli (1)| MI     | UK      | Caucasians    | 2005 | 35           | 101            | 0.95          |
| Chiappelli (2)| MI     | France  | Caucasians    | 2005 | 36           | 26             | 0.95          |
| Sie (1)      | MI      | Italy   | Caucasians    | 2006 | 44           | 673            | 0.95          |
| Sie (2)      | MI      | Italy   | Caucasians    | 2006 | 29           | 1142           | 0.95          |
| Bonnermo     | MI      | Sweden  | Caucasians    | 2010 | 119          | 109            | 0.95          |
| Coker        | MI      | Turkey  | Caucasians    | 2011 | 102          | 141            | 0.95          |
| Binwas       | MI      | India   | Asian         | 2014 | 348          | 407            | 0.95          |
| Revilla      | IS      | Spain   | Caucasians    | 2002 | 27           | 37             | 0.95          |
| Pola         | IS      | Italy   | Caucasians    | 2003 | 56           | 28             | 0.95          |
| Greisenegger | IS      | Australia | Caucasians  | 2003 | 81           | 76             | 0.95          |
| Ren          | IS      | Italy   | Caucasians    | 2004 | 100          | 56             | 0.95          |
| Karahan      | IS      | Turkey  | Asians        | 2004 | 54           | 55             | 0.95          |
| Chamarro     | IS      | Spain   | Caucasians    | 2004 | 104          | 46             | 0.95          |
| Bolding      | IS      | Ireland | Caucasians    | 2004 | 33           | 123            | 0.95          |
| Laloucque    | IS      | Austria | Caucasians    | 2006 | 143          | 156            | 0.95          |
| Banerjee     | IS      | India   | Asians        | 2007 | 123          | 156            | 0.95          |
| Tong         | IS      | China   | Asians        | 2010 | 99           | 98             | 0.95          |
| Chakraborty  | IS      | India   | Asian         | 2013 | 57           | 73             | 0.95          |
| Xuan         | IS      | China   | Asian         | 2014 | 205          | 246            | 0.95          |
| Pieroni      | VTE     | Greece  | Caucasians    | 2006 | 217          | 232            | 0.95          |
| Vormittag    | VTE     | Australia | Caucasians  | 2006 | 72           | 50             | 0.95          |
| Beckers      | VTE     | Netherlands | Caucasians  | 2010 | 38           | 116            | 0.95          |
| Matos        | VTE     | Brazil  | Caucasians    | 2011 | 70           | 75             | 0.95          |

HWE = Hardy–Weinberg equilibrium, IL-6 = interleukin-6, IS = ischemic stroke, MAF = minimum allele frequency, MI = myocardial infarction, VTE = venous thromboembolism.

*P* heterogeneity $< 0.01$.
sample size more than 400 (1.15 [1.01–1.19]), G/C was associated with the risks of thrombotic disorders with NOS score and study design were performed. We found that ORs for population-based studies and hospital-based studies and C carriers vs GG C vs G

| Indexes | No. of studies | CC vs GG OR (95%CI) | CC vs GC OR (95%CI) | CC vs G carriers OR (95%CI) | C carriers vs GG OR (95%CI) | C vs G OR (95%CI) |
|---------|----------------|---------------------|---------------------|----------------------------|-----------------------------|------------------|
| Total   | 34             | 1.03 (0.87–1.22)    | 0.97 (0.85–1.09)    | 0.99 (0.86–1.13)           | 1.09 (0.97–1.21)            | 1.05 (0.96–1.14) |
| Ethnicity |                |                     |                     |                            |                             |                  |
| Caucasians | 28             | 0.99 (0.84–1.18)    | 0.95 (0.83–1.07)    | 0.96 (0.84–1.10)           | 1.05 (0.93–1.18)            | 1.01 (0.93–1.10) |
| Asians   | 6              | 1.99 (0.82–3.09)    | 1.03 (0.74–2.29)    | 1.48 (0.79–2.07)           | 1.39 (1.13–1.72)            | 1.3m6 (1.18–1.56) |
| Country  |                |                     |                     |                            |                             |                  |
| Sweden   | 4              | 1.05 (0.85–1.30)    | 1.03 (0.89–1.19)    | 1.04 (0.90–1.20)           | 1.03 (0.82–1.29)            | 1.03 (0.91–1.15) |
| English  | 4              | 1.00 (0.64–1.55)    | 0.81 (0.66–0.992)   | 0.85 (0.69–1.06)           | 1.31 (0.76–2.25)            | 1.09 (0.81–1.46) |
| India    | 3              | 1.88 (0.24–14.0)    | 1.40 (0.23–8.61)    | 1.73 (0.24–12.7)           | 1.40 (1.19–1.65)            | 1.40 (0.96–2.05) |
| Germany  | 3              | 0.97 (0.67–1.50)    | 0.90 (0.89–1.11)    | 0.98 (0.87–1.10)           | 0.99 (0.95–1.03)            | 0.90 (0.94–1.03) |
| Italy    | 5              | 0.63 (0.23–1.7)     | 0.67 (0.36–1.25)    | 0.64 (0.29–1.41)           | 0.84 (0.43–1.62)            | 0.80 (0.47–1.36) |
| Design   |                |                     |                     |                            |                             |                  |
| HB       | 6              | 0.58 (0.28–1.20)    | 0.66 (0.41–1.07)    | 0.62 (0.35–1.11)           | 0.79 (0.52–1.19)            | 0.77 (0.53–1.10) |
| PB       | 28             | 1.12 (0.99–1.27)    | 1.04 (0.96–1.13)    | 1.06 (0.98–1.15)           | 1.15 (1.03–1.29)            | 1.10 (1.03–1.18) |
| Diseases |                |                     |                     |                            |                             |                  |
| MI       | 18             | 1.06 (0.93–1.21)    | 1.01 (0.92–1.11)    | 1.02 (0.94–1.11)           | 1.14 (0.99–1.32)            | 1.08 (0.99–1.19) |
| Stroke   | 12             | 0.85 (0.48–1.50)    | 0.88 (0.58–1.33)    | 0.88 (0.54–1.42)           | 0.95 (0.73–1.24)            | 0.96 (0.73–1.22) |
| Venous   | 4              | 1.14 (0.83–1.58)    | 0.96 (0.58–1.67)    | 1.03 (0.76–1.40)           | 1.17 (0.97–1.42)            | 1.10 (0.96–1.28) |
| Thrombosis| 30             | 1.02 (0.65–1.22)    | 0.97 (0.85–1.10)    | 0.98 (0.85–1.13)           | 1.08 (0.95–1.23)            | 1.04 (0.94–1.14) |
| Sample size |          |                     |                     |                            |                             |                  |
| ≤400     | 25             | 0.98 (0.75–1.28)    | 0.90 (0.74–1.10)    | 0.93 (0.75–1.15)           | 1.05 (0.88–1.25)            | 1.01 (0.88–1.15) |
| >400     | 9              | 1.13 (0.97–1.31)    | 1.05 (0.95–1.16)    | 1.08 (0.96–1.21)           | 1.15 (1.01–1.31)            | 1.12 (1.01–1.23) |
| NOS score |                |                     |                     |                            |                             |                  |
| ≥6       | 31             | 1.00 (0.84–1.19)    | 0.96 (0.84–1.08)    | 0.97 (0.84–1.11)           | 1.07 (0.95–1.19)            | 1.03 (0.94–1.12) |
| <6       | 3              | 1.68 (1.05–2.69)    | 1.24 (0.54–2.80)    | 1.36 (0.69–2.08)           | 1.37 (0.62–2.01)            | 1.30 (0.95–1.77) |

95% CI = 95% confidence interval, HB = hospital-based case–control studies, NOS = Newcastle–Ottawa Scale, OR = odds ratio, PB = population-based case–control studies.

Table 2: Summary ORs and 95% CIs of the association rs1800795 polymorphism and thrombosis risk.

The above results suggested that the effect of 174 G/C on the risk of thrombotic disorders could be modulated by country/area, ethnicity, sample scale, and control subject resource. Details are shown in Table 3.

3.1. Sensitivity analyses and publication bias

Sensitivity analysis was conducted to assess the stability of the result by removing individually 1 study at a time, and then reanalysis was conducted. The results were not altered, which indicated that our results were reliable. Through applying asymmetry of funnel plots, the Begg test, and Egger test, we did not find publication bias for analyzing CC versus GG (P_{pbg} = 0.65, Pegger = 0.47), CC versus G carriers (P_{pbg} = 0.14, Pegger = 0.11), and C carrier versus GG (P_{pbg} = 0.91, Pegger = 0.22) (shown in Table 3 and Fig. 3).

4. Discussion

Various genetic risk factors are known to increase the susceptibility to thrombotic disorders. Increasing evidence reveals a “cross-talk” between the coagulation and inflammatory...
### Table 3

Heterogeneity and publication bias test for meta-analysis of rs1800795 polymorphism and thrombosis risk.

| Indexes                     | No. of studies | \( P_{\text{her}} \) | \( I^2 \) % | \( P_{\text{begg}} \) | \( P_{\text{pegger}} \) | \( P_{\text{her}} \) | \( I^2 \) % | \( P_{\text{begg}} \) | \( P_{\text{pegger}} \) | \( P_{\text{her}} \) | \( I^2 \) % | \( P_{\text{begg}} \) | \( P_{\text{pegger}} \) |
|-----------------------------|----------------|---------------------|-------------|-----------------|---------------------|---------------------|-------------|-----------------|---------------------|---------------------|-------------|-----------------|---------------------|
| **Total**                   | 34             | <0.01               | 67.8        | 0.64            | 0.50                | <0.01               | 56.9        | 0.26            | 0.15                | <0.01               | 70.6        | 0.65            | 0.17                |
| **Ethnicity**               |                |                     |             |                 |                     |                     |             |                 |                     |                     |             |                 |                     |
| Caucasians                  | 28             | <0.01               | 69.3        | 0.68            | 0.52                | <0.01               | 60.0        | 0.44            | 0.15                | <0.01               | 71.2        | 0.28            | 0.15                |
| Asians                      | 6              | 0.15                | 41.4        | 1.00            | 0.31                | 0.26                | 24.4        | 0.73            | 0.02                | 0.16                | 38.8        | 1.00            | 0.77                |
| **Country**                 |                |                     |             |                 |                     |                     |             |                 |                     |                     |             |                 |                     |
| Sweden                      | 4              | 0.2                 | 35.2        | 1.00            | 0.77                | 0.94                | 0.31        | 0.76            | 0.63                | 0.63                | 0.73        | 0.96            | 0.03                |
| UK                          | 4              | 0.077               | 56.2        | 0.73            | 0.26                | 0.835               | 0.73        | 0.557           | 0.03                | 0.03                | 0.308       | 0.154           | 0.01                |
| India                       | 3              | 0.033               | 70.8        | 1.00            | 0.95                | 0.071               | 62.2        | 0.97            | 0.03                | 0.07                | 69.6        | 1.00            | 0.958               |
| Germany                     | 0.776          | 0                   | 2.96        | 0.88            | 0.08                | 0.962               | 0.10        | 0.827           | 0.94                | 0.94                | 0.10        | 0.218           | 0.62                |
| Italy                       | 5              | <0.01               | 91.7        | 0.806           | 0.8                | 0.001               | 79.8        | 0.975           | 0.01                | 0.01                | 88.4        | 0.806           | 0.889               |
| **Design**                  |                |                     |             |                 |                     |                     |             |                 |                     |                     |             |                 |                     |
| HB                          | 6              | <0.01               | 88.5        | 1.00            | 0.61                | 0.01                | 75.4        | 0.80            | 0.62                | <0.01               | 84.5        | 0.45            | 0.65                |
| PB                          | 28             | 0.05                | 328         | 0.83            | 0.60                | 0.45                | 1.00        | 0.02            | 0.11                | 0.35                | 8.0         | 0.12            | 0.18                |
| **Diseases**                |                |                     |             |                 |                     |                     |             |                 |                     |                     |             |                 |                     |
| MI                          | 18             | 0.08                | 335         | 0.20            | 0.70                | 0.71                | 0.41        | 0.04            | 0.67                | 0.07                | 0.41        | 0.15            | 0.09                |
| stroke                      | 12             | <0.01               | 58.6        | 0.59            | 0.03                | <0.01               | 73.1        | 0.37            | 0.19                | <0.01               | 82.6        | 0.86            | 0.3                |
| VTE                         | 4              | 0.68                | 0           | 0.73            | 0.50                | 0.07                | 58.2        | 1.00            | 0.47                | 0.08                | 32.6        | 0.74            | 0.36                |
| Arterial                    | 30             | <0.01               | 71.2        | 0.62            | 0.61                | <0.01               | 45.8        | 0.09            | 0.09                | <0.01               | 61.8        | 0.24            | 0.11                |
| **Sample size**             |                |                     |             |                 |                     |                     |             |                 |                     |                     |             |                 |                     |
| <400                        | 25             | <0.01               | 71.6        | 0.92            | 0.44                | 0.002               | 52.0        | 0.75            | 0.29                | <0.01               | 63.2        | 0.35            | 0.81                |
| >400                        | 9              | 0.10                | 40.5        | 0.92            | 0.44                | 0.008               | 48.0        | 0.75            | 0.29                | 0.24                | 22.7        | 0.35            | 0.81                |
| **NOS score**               |                |                     |             |                 |                     |                     |             |                 |                     |                     |             |                 |                     |
| ≥6                          | 31             | <0.01               | 69.2        | 0.02            | 0.20                | 0.004               | 45.5        | 0.01            | 0.09                | <0.01               | 60.9        | 0.11            | 0.13                |
| <6                          | 3              | 0.8                 | 0           | 1.00            | 0.49                | 0.075               | 61.4        | 1.00            | 0.19                | 0.483               | 84.6        | 0.09            | 0.39                |

HB = hospital-based case-control studies, MI = myocardial infarction, NOS = Newcastle-Ottawa Scale, PB = population-based case-control studies, VTE = venous thromboembolism.

1. \( P_{\text{her}} \) for heterogeneity. If \( P < 0.10 \), random effects model was used, otherwise, fixed effects model was used. \( I^2 \) calculated by %.

2. \( I^2 \) was the abbreviations of \( I^2 \) (%).
In addition, variants in genes related to inflammation may predispose to thrombotic disorders. Recent reports reveal that the vessel wall is often unmarred, although venous thrombosis is accompanied by a low blood flow and shear rate.\[46-49\] Be independent of venous thromboembolism, arterial thrombotic diseases, including MI and stroke, have a correlation with platelet aggregation stem from the split of an atherosclerotic plateau at sites in the artery vessels where the shear stress is high, stimulating the formation of a blood clot and the subsequent obstruction of the blood vessel.\[48,49\] Inflammatory processes take a pivotal part in the pathogenesis of atherosclerosis.\[47,50\] The inflammatory cytokines perform a multitude of functions within the inflammatory pathway.\[50\] IL-6 is a pleiotropic inflammatory cytokine.\[50\] It plays an important part in the acute-phase response and inflammatory cascade, such as upregulation of acute-phase proteins like C-reactive protein,\[50\] which has been observed to be related to the risk of coronary heart disease. IL-6 is able to initiate coagulation through tissue factor and indirectly by the endothelium.\[11\] IL-6 was capable of engendering tissue factor expression on monocytes in vitro,\[11\] and was demonstrated to play a key role in the activation of coagulation\[11\] and the subsequent and potential thrombosis diseases in animal models.

According to Fishman the G/C polymorphism at position -174 of the gene IL-6 has the potential to influence the binding of the glucocorticoid receptor, and therefore it has ability to repress transcriptional activation.\[5,11\] It is significant that the alteration from a G to a C at position -174 causes a potential binding site for the transcription factor NF-1, a repressor of IL-6 gene expression.\[11\] The C allele and further the CC genotype create in the cell and indirectly by the endothelium.\[31\] The results of this meta-analysis indicated that of the 19 loci showed the correlation at P \(<\ 1 \times 10^{-6}\) in the combination of stage 1 and 2 analysis, with 13 of them having the genome-wide significance, namely APOB, ABCG5-ABC8, FURIN-FES, FLT1, GUCY1A3, IL6R, KCNK5, LPL, PLG, TRIB1, SLC22A4-SLC22A5, TRIB1, VAMP5-VAMP8-GGCX, and ZEB2-AC074093.1.\[53,54\] NINDS Stroke Genetics Network (SiGN) and International Stroke Genetics Consortium (ISGC) confirmed a novel site (G allele at rs12122341) at 1p13.2 near ISFAN2 which was correlated with large artery atherosclerosis-related stroke, and their data also supported robust relationships with IS for 4 other loci which have been demonstrated in published studies, including HDAC9 for large artery atherosclerosis stroke, and
ZFHX3 and PITX2 for cardioembolic stroke.[55] Germain et al.[56] reported that the common mutations left to be confirmed are not uniformly distributed across the genome and chromosome 20, itself, could attribute to 7% of the total genetic mutation for venous thrombosis. The findings emerged among different studies may be due to different genetic or culture backgrounds, and environmental factors.

Heterogeneity existed in overall analysis under the recessive model due to the differences in thrombotic disorder category, genetic backgrounds, and environmental exposures which existed among different ethnicities/cultures and in difference study groups. Another potential factor contributing to heterogeneity was minor allele frequencies difference between studies. Subsequently, sensitivity and subgroup analyses were explored to investigate the underlying causes. Our sensitivity analysis revealed that even after excluding studies with a small number of cases (n < 100), the results of our main meta-analysis remain unchanged.

As far as we know, the present meta-analysis involving 10,549 cases and 19,316 controls was the most comprehensive to date to investigate the relation between the IL-6-174G/C polymorphism and thrombosis susceptibility. Our finding suggested that the IL-6-174G/C polymorphism was not associated with the thrombosis risk both in Caucasian and Asian populations, which were in line with the conclusion of the previous meta-analysis by Kumar et al.[57] and inconsistent with Jin et al.’s results.[58] Compared to the previous study, our meta-analysis has some potential strength. First, we had the largest sample size. Second, this meta-analysis incorporated all types of thrombotic disorders into pooled analysis, while the meta-analyses from Kumar and Jin et al only investigated MI/stroke susceptibility.

Our subgroup meta-analysis suggested that IL-6-174G/C was not associated with the stroke risk, which is consistent with the study conducted by Kumar et al.[57] The pooled ORs were performed in our meta-analysis for allelic comparison (C vs G), dominant model (CC + GC vs GG), recessive model (CC vs GC + GG), homozygote comparison (CC vs GG), and heterozygote comparison (GC vs GG), respectively. The study out of HWE conducted by Tuttolomondo et al.[59] was excluded in present meta-analysis. Deviation from HWE can be due to laboratory/ genetic error, population stratification, selection bias in the choice of controls, and confounding factors unaccounted for.[60] It is suggested that the analysis without studies not conforming to HWE would be more valid.[61]

Prior published articles have revealed conflicting results regarding the association of the IL-6-174G/C polymorphism with MI risks. Significant association between the IL-6-174G/C polymorphism and MI development has been identified in the study by Jin et al.[58] Such discrepancy may be caused from different population, sample sizes, and stratification. As stroke analysis, we excluded studies in which HWE was absent in the controls as well.

In addition, our updated analysis incorporated more studies with a larger sample and subgroup analyses. Beside to exclusion to the studies departure from HWE and 5 analysis model for MI, noticeably, the previous meta-analysis[58] only contained published data from prior to 2011, involving 5429 cases and 4823 controls. Fewer studies were included in these meta-analyses compared with ours, probably due to insufficient attention in the search process or screening methods, which might lead selection bias. Moreover, small studies showed a risk factor for 174C allele. Small sample with limited subjects is usually accompanied with selection biases, and is powerless to deny or support an association.

When explaining the results, several limitations of the current meta-analysis should be acknowledged. First, we only studied IL-6G/C polymorphism in IL-6 gene in present meta-analysis, thus, we cannot exclude the potential effect that other haplotypes or polymorphisms in IL-6 gene might also be implicated in the risk of thrombosis disorders. Second, all original studies were from Asians and Caucasians and data involving other ethnicities were limited; thus, our conclusion may not be applied to all ethnic groups and participants incorporating African American should be examined the relationship to consume result in present the meta-analysis. Third, raw data were not listed by other life factors such as smoking, diet preference, and physical activities, because lack of sufficient information could be provided from the original papers, which prevents us from interpreting any interaction between IL-6-174G/C and other factors on thrombosis disorders. Fourth, due to the study sample size for some subgroup and unpublished data (so-called “gray literature”), the current meta-analysis was limited to exclude the possibility of type I and type II errors and contribution to the power analysis.

To our best knowledge, this study is the first comprehensive meta-analysis till now to explore the correlation between the IL-6 gene-174G/C polymorphism and over risk of thrombosis disorders. It provided evidence of the association between IL-6 gene -174G/C polymorphism and risk of thrombotic disorders, supporting the hypothesis that the IL-6-174G/C polymorphism may be a predisposed marker for arterial thrombotic disorders including MI, Asians including Indian. However, additional larger and more case-control studies are required to confirm our conclusions. Since no study was conducted in an Africans, it is essential that larger and well-designed and multicentric studies incorporating African population should be carried out to further examine the association. Moreover, further studies investigating the effect of haplotypes and gene-environment interactions can ultimately provide a better and comprehensive finding of the associations between the IL-6 gene-174G/C polymorphism and the risk of thrombotic disorders. Future analyses should be carried out in large-scale population studies and should examine the potential effect stratified by age and smoke status in different ethnicity and populations.

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