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Association of interleukin-6 circulating levels with coronary artery disease: A meta-analysis implementing mendelian randomization approach ☆

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ABSTRACT

Background: We aim to investigate whether the association between circulating interleukin 6 (IL-6) levels and the risk for coronary artery disease (CAD) is robust and perhaps even causal by a meta-analysis implementing mendelian randomization approach with *IL-6* gene G–174C polymorphism as an instrument.
Methods: Data were available from 19 articles encompassing 9417 CAD patients and 15982 controls. A random effects model was applied irrespectively of between-study heterogeneity, and publication bias was examined using a funnel plot and the corresponding statistics.
Results: Overall, comparison of *IL-6* gene alleles –174C with –174G had 4% increased risk for CAD (95% confidence interval [95% CI]: 0.97–1.10; P = 0.285), accompanying marginal heterogeneity (I^2 = 38.3%; P = 0.033). This association was potentiated in dominant model as odds ratio (OR) reached 1.08 (95% CI: 0.96–1.22; P = 0.204) and heterogeneity was significant (I^2 = 58.4%; P < 0.0005). Subgroup analysis by ethnicity indicated that carriers of –174C allele were associated with a 12% increased risk for CAD in prospective studies involving White populations (OR = 1.12; 95% CI: 0.95–1.33; P = 0.184), whereas the association in East Asians was remarkably reversed with 37–46% reduced risk. Relative to –174GG homozygotes, carriers of –174C allele had an overall 0.24 pg/ml high circulating IL-6 levels (P = 0.047). The predicted OR for 1 pg/ml elevation in IL-6 levels was 1.60 (95% CI: 1.44–1.72; P < 0.01) in prospective studies involving White populations. Publication biases were absent for all comparisons (P > 0.1).
Conclusion: Our findings provided strong evidence on the causal association of circulating IL-6 levels with the development of CAD in White populations.

1. Introduction

Coronary artery disease (CAD) is one of the common causes of death and disability in developed countries, accounting for up to 40% of lethal events [1]. A strong genetic underpinning presents in CAD: men with 2 or more affected parents or siblings relative to without family history have a 3.4-fold increased risk of developing myocardial infarction [2]. Genetic determinants contributing to huge amount of sporadic CAD, however, remain unclear. It is universally accepted that CAD is atherosclerosis of the coronary arteries, producing blockages in the vessels which nourish the heart itself [3]. Inflammation plays a major role in the development and progression of atherosclerosis [4,5], implicating the logical candidacy of inflammatory cytokines for CAD prediction.

Interleukin 6 (IL-6) acts as a multifunctional cytokine with an essential role in bridging the inflammatory and atherosclerotic processes [6,7], and its mRNA levels in atherosclerotic arteries are 10 to 40 times higher than that in nonatherosclerotic vessels [8]. Increased production of IL-6 involves in the development or progression of CAD because elevated IL-6 levels are associated with increased risk and severity of CAD [9–11], leaving the open question that whether this association is causal or reflects residual confounding. The increasing availability of polymorphic DNA markers has expanded great efforts to identify which specific loci in *IL-6* gene might have functional potentials in affecting its final bioavailability. Several variants in *IL-6* gene have been identified, with the G–174C (rs1800795) polymorphism in the promoter region having been the most extensively examined in association with CAD, although this claim is controversial and inconclusive, possibly due to methodological limitations, including inadequate sample size, patient selection, ethnicity of the populations studied, and lack of adjustments for confounders [12,13].

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As meta-analysis is a reliable way to resolve discrepancies in genetic association studies and in an effort to clarify earlier inconclusive results, we evaluated the association of *IL-6* gene G–174C polymorphism with both circulating IL-6 levels and the risk for CAD, and investigated the potential sources of between-study heterogeneity and the existence of publication bias. If this polymorphism is found to be predictive of both CAD and circulating IL-6 levels, we next plan to evaluate the perhaps causal association of circulating IL-6 levels with CAD by implementing mendelian randomization approach with G–174C polymorphism as an instrument to circumvent residual confounding and reverse causation. The mendelian randomization approach is based on Mendel's second law, stating that given the random assortment of alleles at the time of gamete formation, association between a genetic polymorphism and a disease outcome provides robust evidence of the causal nature of the phenotype influenced by the polymorphism [14,15].

2. Methods

2.1. Search strategy for identification of studies

We searched relevant articles from PubMed and EMBASE, as well as China Biological Medicine (http://sinomed.imicams.ac.cn/index.jsp) and Wanfang (http://www.wanfangdata.com.cn) databases before April 2011. The subject terms were expressed in Boolean combinations: (interleukin 6 OR IL-6) AND (coronary heart disease OR isch[a]emic heart disease OR myocardial infarction OR atherosclerosis OR arteriosclerosis OR coronary stenosis OR coronary artery disease OR coronary disease). We restricted our search spectrum to articles with full text, written in English or Chinese language, and performed in human subjects. After browsing the title, keywords, and abstract (where available) of all relevant papers, we decided whether data on the topic of interest were included. If the paper could not be rejected with certainty, we scanned the full text of the paper for evaluation. The bibliographies in articles and reviews including meta-analysis were also manually searched to identify the citations of articles on the same topic. Where studies included more than one subgroup with homogenous characteristics such as ethnicity and disease type, each subgroup was considered separately.

2.2. Inclusion/exclusion criteria

Articles were qualified if they examined the hypothesis that *IL-6* gene G–174C polymorphism was associated with CAD risk or circulating IL-6 levels or both, if they followed a prospective or retrospective study design, and if they provided information on G–174C genotype counts between patients and controls for determining odds ratio (OR) and the corresponding 95% confidence interval (95% CI) or on circulating IL-6 levels across G–174C genotypes for standard mean difference (SMD) and its 95% CI. In this meta-analysis, CAD end points were defined as myocardial infarction, atherosclerosis, angina pectoris, percutaneous transluminal coronary angioplasty, coronary artery bypass graft surgery, and severe stenosis on coronary angiography. Where there were duplicate or overlapping publications from the same study group, the most complete results were abstracted.

2.3. Extracted information

Data were extracted independently and entered into separate databases by two authors (W.N. and Y.Q.) from each qualified study: first author's last name, publication year, ethnicity, diagnostic criteria, baseline characteristics of the study population where available (including age, gender, body mass index or BMI, circulating IL-6 levels), and *IL-6* gene G–174C genotype counts in patients and controls. The units of circulating IL-6 levels were standardized to pg/ml. For consistency, quantitative variables were expressed as mean ± standard deviation (SD) or median (interquartile range); standard error was converted to SD.

2.4. Statistical analysis

Considering the low frequency of –174CC genotype and to maximize the statistical power, we assessed the association of G–174C polymorphism with CAD under only allelic and dominant models. Crude OR and SMD with 95% CI were used for genetic contrasts between patients and controls, and circulating IL-6 levels across G–174C genotypes under dominant model. The random effects model using the DerSimonian and Laird method was employed to combine the individual effect size estimates to calculate pooled weighted ORs, and the estimate of heterogeneity was determined using the Mantel–Haenszel model [16].

The between-study heterogeneity was explored by the χ^2 test, and the quantity of heterogeneity was assessed by the inconsistency index I^2 statistic (ranging from 0 to 100%), which is defined as the percentage of the observed between-study variability that is due to heterogeneity rather than chance. In this method, higher values suggest the existence of heterogeneity [17,18]. The visual funnel plots, as well as Egger's test and Begg–Mazemdar test, were used to assess publication bias. Egger's test can detect funnel plot asymmetry by determining whether the intercept deviates significantly from zero in a regression of the standardized effect estimates against their precision [19]. Begg–Mazumdar test can quantify any possible correlation between the natural logarithm of the OR and the weight of the study [20].

Cumulative meta-analysis was conducted to identify the influence of the first published study on the subsequent publications, and the evolution of the combined estimates over time according to the ascending date of publication. To identify potentially influential studies, sensitivity analysis was undertaken by removing an individual study each time to check whether any of these estimates can bias the overall estimate.

It is widely accepted that genetic association studies are more closely relevant to randomized trials than other types of epidemiological study due to the Mendel's second law of independent assortment of alleles which theoretically should not be confounded by environmental or behavioral factors [21,22]. In mendelian randomization analysis, risk estimate was computed from the ratio of the coefficient of the association between a polymorphism and a disease to that of the association between the polymorphism and biomarker as a reflection of the potential causal effect of circulating IL-6 levels on CAD risk.

Satisfaction of G–174C genotypes with Hardy–Weinberg proportions was performed using the χ^2 test or Fisher's exact test in control groups. Probability less than 0.05 was judged significantly except for the I^2 statistic, Egger's and Begg–Mazumdar tests, where a significance level of less than 0.1 was chosen. Data management and statistical analyses were performed using STATA version 11.0 for Windows.

3. Results

3.1. Search results and study characteristics

The initial search yielded 103 potentially relevant articles. After applying our inclusion/exclusion criteria, 19 articles were selected for inclusion in the final analysis [5,23–40]. A diagram schematizing the selection process of identified and excluded articles with specification of reasons is presented in Fig. 1. Of these 19 articles involving 23 study populations, 16 populations were performed in Whites [5,23–33,35,36], 5 in East Asians [34,37–40], and 2 in Middle Eastern populations [33,35]. Twelve out of 23 populations were described as in prospective design [5,23–25,28–32,35–37], and 11 populations were in retrospective design [26,27,33,34,38–40]. Finally, data from 9417 CAD patients and 15982 controls were analyzed.

Data on circulating IL-6 levels across G–174C genotypes were available from 7 populations involving 2911 patients and 3459 controls. The frequency of –174C allele ranged from 27.27% to 52.90% in White patients and from 27.36% to 52.12% in White controls, and from 0 to 10.87% in East Asian patients and from 0.38% to 16.25% in East Asian controls. Taking control groups into account, genotype distributions of G–174C polymorphism were in Hardy–Weinberg equilibrium for all qualified populations. The baseline characteristics of study populations are summarized in Table 1.

3.2. Overall association of IL-6 gene G–174C polymorphism with CAD

Under allelic model, an overall comparison of *IL-6* gene –174C allele with –174G allele had only 4% increased risk for CAD (95% CI: 0.97–1.10; P = 0.285), accompanying marginal heterogeneity between studies ($I^2 = 38.3\%$; P = 0.033) (Fig. 2) and low probability of publication bias as reflected by the suggestive symmetry of funnel plot (Fig. 3), as well as the Egger's test (P = 0.441) and Begg–Mazemdar test (P = 0.77). Under dominant model, the magnitude of association was potentiated, albeit nonsignificant, with OR reaching 1.08 (95% CI: 0.96–1.22; P = 0.204) (Fig. 2); however, between-study heterogeneity tingled this association ($I^2 = 58.4\%$; P < 0.0005). The visual funnel plot inspection (Fig. 3), along with the Egger's (P = 0.291) and Begg–Mazemdar (P = 0.581) tests, indicated no signs of publication bias. Cumulative analysis suggested no evidence for the first published study that reported a potentially significant result and then trigged the subsequent replication for both allelic and dominant models; likewise sensitivity analysis showed that no single studies influenced the pooled results significantly (data not shown).