Relationship Between Serum C-reactive protein Levels and Apolipoprotein E Gene Polymorphism: A Meta-Analysis

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Abstract

**Background and Aim:** Apolipoprotein E refers to a polymorphic protein involved in lipoprotein transport and metabolism. However, the relationship between Apo E gene polymorphism and serum CRP levels remains unclear. The present meta-analysis aimed to comprehensively assess the relationships between Apo E gene polymorphism and serum CRP levels.

**Method:** Comprehensive search of all relevant documents published in PubMed, EMBASE, Web of science, Cochrane Library before April 2020. Calculate the standard deviation of mean (SMD) and 95% confidence interval (CI) with a random effects model. Compare serum CRP levels between different Apo E genotypes and isoforms. Use funnel plots to assess publication bias of studies involved.

**Results:** On the whole, 9 studies including 173 different Apo E genotypes comparisons were involved in the present meta-analysis. Overall, the serum CRP level of the apo E ε3ε4 and ε4ε4 genotypes exhibit lower CRP levels as compared with ε3ε3 (OR= -0.21, 95% CI: -0.25, -0.16, P<0.00001; OR= -0.32, 95% CI: -0.39, -0.25, P<0.00001); Apo E ε2ε2 and ε2ε4 display higher CRP levels as compared with ε4ε4 (OR= 0.38, 95% CI: 0.16, 0.60, P=0.0006; OR= 0.38, 95% CI: 0.27, 0.49, P<0.00001). Moreover, Apo E E4 isoforms achieve lower CRP levels than E2 and E3 (OR= 0.22, 95% CI: 0.17, 0.26, P<0.00001; OR= 0.22, 95% CI: 0.15, 0.29, P<0.00001).

**Conclusions:** As revealed from the present meta-analysis, serum CRP levels are different between different Apo E gene polymorphism, and Apo E genotypes with a higher risk of atherosclerosis exhibit lower serum CRP levels.

Introduction

Atherosclerosis (AS) refers to a common and serious disease, and the main pathological basis of ischemic cardio-cerebrovascular disease (e.g., coronary heart disease, cerebrovascular disease and thromboembolic disease). As early as 1986, Professor Ross of the University of Washington School of Medicine first proposed AS to be an inflammatory disease as well as an excessive defense response to injury. Though the inflammatory mechanism of atherosclerotic thrombosis is unclear, whereas nowadays increasing studies [1–3] have reported that the inflammation response may critically impacts the formation of atherosclerosis, and several biomarkers of inflammation have become tools to predict future cardiovascular adverse events.

C-reactive protein (CRP) refers to a phylogenetically highly conserved plasma protein, which has been long considered an exquisitely sensitive systemic marker of inflammation and tissue damage. As indicated from extensive research, CRP could participate in the systemic response to inflammation [4] and has a wide range of promoting arteriosclerosis [5], capable of assessing the risk of atherosclerotic diseases (e.g., myocardial infarction, stroke and peripheral artery disease) [6, 7].

Apolipoprotein E (Apo E) refers to one of the major plasma polymorphic lipoproteins participate in lipoprotein synthesis, secretion, processing and metabolism. The synthesis of apo E is regulated by three alleles located at one locus, i.e., ε2, ε3 and ε4, with each allele corresponding to a major isomer produces three homozygotes (ε2ε2, ε3ε3 and ε4ε4) and three heterozygotes (ε2ε3, ε2ε4 and ε3ε4), a total of six common phenotypes, and lead to three isoforms, E2, E3 and E4. Apo E genotype displays a linear relationship to the risk of coronary heart disease and other diseases [8]. Moreover, several studies [9, 10] reported that the presence of apo E can affect the expression of inflammatory molecules.

A question is raised that whether CRP id causally related to apo E gene polymorphism, or it is CRP merely a marker of potential atherosclerosis. The biological mechanism between apo E genotype and atherosclerosis remains unclear. Given this, clarifying the relationship between apo E and CRP may help gain insights into the relationship between apo E and atherosclerosis.

Methods
Search strategy

The authors of this study searched all relevant studies assessing the association of apo E polymorphism and CRP in PubMed, EMBASE, Web of science, Cochrane Library by two independent investigators. All included studies were published before the end of April 2020. A combination of subject words and free words was adopted to determine the search strategy, the key terms used for searching included (“Apolipoproteins E” OR “Apo E” OR “Apolipoprotein E Isoproteins”) AND (“Polymorphism, Genetic” OR “Genetic Polymorphisms”) AND (“C-Reactive Protein” OR “Protein, C-Reactive”).

Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) Study related to apo E and CRP; (2) Case–control or cohort study; (3) With specific information presented on apo E genotype; (4) Provide the data of CRP; and (5) Studies with full-text. Specific to the exclusion criteria: (1) Review, case reports, animal studies, abstracts and repeated literature; (2) No specific apo E genotype information provided; (3) No CRP data provided.

Data Extraction and Quality Assessment

Two independent researchers (Wang and He) extracted the following data from each selected study, including first author, year of publication, country of region, apo E genotype distribution and genotype number, level of CRP. Disagreements are resolved by discussion and consensus.

The study involved in the evaluation was recommended with the evaluation items recommended by Agency for Healthcare Research and Quality (AHRQ). The evaluation criteria included 11 items, which were answered with "Yes", "No" and "Unclear". If the answer is "No" or "Unclear", then the item score is 0; if the answer is "Yes", the item score is 1. Quality evaluation: low quality = 0–3; medium quality = 4–7; high quality = 8–11.

Statistical Analysis

The relationships between apo E gene polymorphism and CRP was assessed by standard mean difference (SMD) and 95% confidence interval (CI). A P value of less than 0.05 was considered to exhibit significance. I² was adopted to test the heterogeneity among studies. The pooled statistic was counted with the random effect model. Moreover, Funnel plots were exploited to assess the publication bias by performing Begg test and Egger test. Furthermore, all statistical analyses were conducted by using Cochrane Review Manager (Rev Man, Version 5).

Results

Study characteristics

Figure 1 indicates that a total of 340 studies were initially retrieved, and 331 studies were excluded. Nine studies were involved into the present meta-analysis for the relationship between the level of CRP and apo E gene polymorphism. Five studies (including 12 comparisons) were recruited in $\varepsilon_2\varepsilon_2$ versus $\varepsilon_3\varepsilon_3$ comparison. Seven studies (covering 18 comparisons) recruited in $\varepsilon_3\varepsilon_3$ versus $\varepsilon_4\varepsilon_4$ comparison. Seven studies (including 18 comparisons) were employed in $\varepsilon_3\varepsilon_4$ versus $\varepsilon_3\varepsilon_3$ comparison. Nine [11–19] studies (with 20 comparisons involved) were recruited in E2 versus E3, E4 versus E3 and E2 versus E4 comparisons.

Quantitative synthesis
The Genotype analysis showed that apo E ε3ε4 and ε4ε4 genotypes exhibit lower CRP levels as compared with ε3ε3 genotypes (SMD = -0.21, 95% CI: -0.25, -0.16, P < 0.00001; SMD = -0.32, 95% CI: -0.39, -0.25, P < 0.00001), (Fig. 2C and D; Table 2). Apo E ε2ε4 genotype also exhibit lower CRP levels as compared with ε3ε3 genotypes, though there was no statistical difference (SMD = -0.03, 95% CI: -0.13, 0.06, P = -0.50), (Fig. 2B; Table 2). Meanwhile apo E ε2ε2 and ε2ε4 genotypes display higher CRP levels as compared with ε4ε4 genotypes (SMD = 0.38, 95% CI: 0.16, 0.60, P = 0.0006; SMD = 0.38, 95% CI: 0.27, 0.49, P < 0.00001), (Fig. 2E and F; Table 2). It seemed that apo E ε4 allele has lower CRP levels as compared with other genotypes.

Table 1
Characteristics of included studies

| Study          | Year | Country      | Apo E Genotype | Apo E Isoforms | Quality Score |
|----------------|------|--------------|----------------|----------------|---------------|
| Martiskainen et al | 2018| Finland      | -              | 3008 98 1474 219 416 3608 1966 | 7             |
| Martiskainen et al | 2017| Czech        | - 10 63        | -              | 10 63 21 4    | 4             |
| Martiskainen et al | 2016| Taiwan       | -              | -              | 81 339 96 7   | 7             |
| Wu et al       | 2015| South Korea  | 80 1980 13592 221 2879 173 | 2060 13813 3052 | 8             |
| Yun et al      | 2011| Germany      | 52 789 3835 135 1268 93 976 3835 136 | 7             |
| Grammer et al  | 2010| Australia    | 7 141 805 33 267 25 148 838 292 | 7             |
| Golledge et al | 2010| Czech        | 42 708 4126 122 1155 77 750 4248 1232 | 6             |
| Judson et al   | 2004| USA          | 6 82 900 34 488 59 88 934 547 | 7             |
Table 2
Meta-analysis of relationship between the level of CRP and apolipoprotein E gene polymorphism

| Genotype comparison | Comparisons | p-value | SMD    | 95% CI      | Model |
|---------------------|-------------|---------|--------|-------------|-------|
| ε2ε2 versus ε3ε3    | 12          | 0.91    | -0.01 | -0.15, 0.13 | Random|
| ε2ε3 versus ε3ε3    | 17          | 0.91    | -0.00 | -0.04, 0.04 | Random|
| ε2ε4 versus ε3ε3    | 18          | 0.50    | -0.03 | -0.13, 0.06 | Random|
| ε2ε2 versus ε4ε4    | 12          | 0.0006  | 0.38   | 0.16, 0.60  | Random|
| ε2ε4 versus ε4ε4    | 18          | <0.00001| 0.38   | 0.27, 0.49  | Random|
| ε3ε4 versus ε3ε3    | 18          | <0.00001| -0.21  | -0.25, -0.16| Random|
| ε4ε4 versus ε3ε3    | 18          | <0.00001| -0.32  | -0.39, -0.16| Random|
| E2 versus E3        | 20          | 0.78    | -0.01 | -0.04, 0.03 | Random|
| E3 versus E4        | 20          | <0.00001| 0.22   | 0.17, 0.26  | Random|
| E2 versus E4        | 20          | <0.00001| 0.22   | 0.15, 0.29  | Random|

Abbreviations: SMD, standard deviation of mean; CI, confidence interval.

As also revealed from the analysis results, apo E E4 isoforms achieve lower CRP levels than E2 and E3 isoforms (SMD = 0.22, 95% CI: 0.17, 0.26, P < 0.00001; SMD = 0.22, 95% CI: 0.15, 0.29, P < 0.00001), (Fig. 3B and C; Table 2). No significant difference is identified in CRP level between Apo E E2 subtype and E3 subtype (SMD= -0.01, 95% CI: -0.04, 0.03), (Fig. 3A; Table 2). This further verifies that the CRP level of apo E ε4 allele is lower than that of other genotypes.

Discussion

Atherosclerosis refers to a spontaneous vascular embolism disease, highly correlated with the levels of plasma cholesterol and low-density lipoprotein cholesterol (LDL-C). It acts as the main cause of coronary heart disease, cerebral infarction, peripheral vascular disease and other diseases. Over the past few years, numerous studies [20] reported that apo E gene polymorphism is associated with atherosclerotic disease, Bennet et al.[8] suggested a linear relationship between apolipoprotein genotype and LDL-C level and coronary heart disease risk. Apolipoprotein E is recognized as a polymorphic protein, critically regulating the stable state of human cholesterol by regulating the intake of chylomicrons, significantly low density lipoproteins, medium density lipoproteins and several high density lipoproteins [21, 22]. The apo E gene has 3 alleles, i.e., ε2, ε3 and ε4, constituting a total of 6 different genotypes, including 3 homozygous types (ε2ε2, ε3ε3 and ε4ε4) and 3 heterozygous types (ε2ε4, ε3ε4 and ε2ε3). A compared with the ε3ε3 genotype, the most common genotype, the risk of coronary heart disease carrying the ε4 allele is higher, while the ε2 allele exhibits the neutral risk [23]. Over the past few years, the inflammatory response is considered a vital factor in the development of atherosclerosis, stimulating the formation of atherosclerosis, reducing the stability of damaged atherosclerotic plaques, and forming occlusive thrombi[24]. Alan R. Tall et al.[25] considered that hypercholesterolemia leads to the accumulation of cholesterol in macrophages, thereby promoting inflammation.

CRP, a marker of inflammation, has been shown in multiple prospective studies to have a risk prediction effect on atherosclerotic diseases (e.g., myocardial infarction, stroke, peripheral arterial disease)[7]. Treatment with statins therapy can effectively down-regulate LDL-C and CRP levels, thereby reducing cardiovascular events and mortality [26]. It is an
independent predictor of future cardiovascular events, though the causal relationship between serum CRP levels and atherosclerotic disease remains unclear. Apo E as a lipid transport protein has been suggested to be related to immune regulation and inflammation of the disease as well [27–29]. A question is raised that whether, there are any connections between them.

In comparison with other genotypes, apo E ε4 allele carriers have relatively high levels of TC, triglycerides, and LDL [30], and the risk of CHD in apo E E4 isoform is also higher than others [31]. Interestingly, in the present meta-analysis, we found that the apo E ε4 allele, as a high-risk genotype of atherosclerotic disease, has lower CRP levels than other carriers. Among the three isoforms of apo E, the CRP level of E4 is lower than that of the other two as well. As a high-risk factor for atherosclerotic disease, CRP is not positively correlated with apo E E4 genotype as expected. It is generally known that cholesterol is synthesized via the mevalonate pathway [32], and statins have been suggested to down-regulate CRP expression in liver cells by inhibiting the mevalonate pathway [33]. Chasman et al. [34] reported that the trend for plasma levels of apo E protein with apo E allele was more similar to that for CRP. Carriers of the apo E ε4 allele are capable of absorbing cholesterol more efficiently than those of other genotypes [35], so their cholesterol biosynthesis is hindered. Accordingly, this study speculates that in carriers of the apo E ε4 allele, CRP levels are lowered by inhibiting mevalonate pathway, suggesting that the mechanism of apo E genotype and CRP on atherosclerotic disease are different and independent. In other words, apo E gene polymorphism shows that association with plasma CRP levels, whereas no causal relationship is identified. Since apo E and CRP are both produced by the liver, the expression of the mentioned proteins may be derived from the regulation of similar factors, whereas there is currently no evidence to prove this.

Though CRP is an independent risk factor for atherosclerosis, numerous studies [36, 37] have doubted the predictive effect of CRP on atherosclerosis. There is no causal relationship between them, and it is not conclusively evidenced that lowering CRP levels can prevent atherosclerosis. CRP is of low negative predictive value and cannot be employed to exclude diseases for its sensitivity difference. Though CRP is still recommended as a routine test for patients with atherosclerosis [38], its effect in guiding treatment remains controversial. Moreover, this study considers that the predicting effect of the apo E E4 genotype on the risk of atherosclerosis may be masked by low CRP levels, and adopting CRP as a biomarker to assess the risk of atherosclerosis may underestimate the risk of carriers of ε4 alleles.

**Conclusions**

In brief, the results here demonstrate that serum CRP levels are different between different apo E gene polymorphism, apo E genotypes with a higher risk of atherosclerosis exhibit lower serum CRP levels, whereas the prediction of atherosclerosis risk by CRP for people with different apo E genotypes require in-depth studies.

**Declarations**

**Consent for publication**

Not applicable.

**Availability of data and materials**

All data generated or analyzed during this study are included in this published article.

**Competing interests**

All authors declare that they have no conflicts of interest.
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Not applicable.

Authors' contribution
Lei Wang and Xiaoyin He conceived and designed the review. Lei Wang and Yuanyuan He performed Literature search and data collection. Rnkun Wang completed statistical analysis. Lihua Zhang was a major contributor in writing the manuscript. Shaokui Shi was responsible for critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Competing interests
All authors declare that they have no conflicts of interest.

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Figures
Figure 1

Study Flowchart
Figure 2

(A) Forest plot of $\varepsilon_2\varepsilon_4$ vs $\varepsilon_3\varepsilon_3$. (B) Forest plot of $\varepsilon_2\varepsilon_4$ vs $\varepsilon_3\varepsilon_3$. (C) Forest plot of $\varepsilon_2\varepsilon_4$ vs $\varepsilon_3\varepsilon_3$. (D) Forest plot of $\varepsilon_2\varepsilon_4$ vs $\varepsilon_3\varepsilon_3$. (E) Forest plot of $\varepsilon_2\varepsilon_4$ vs $\varepsilon_3\varepsilon_3$. (F) Forest plot of $\varepsilon_2\varepsilon_4$ vs $\varepsilon_3\varepsilon_3$. 

A

B

C
Figure 3

(A) Forest plot of E2 vs E3. (B) Forest plot of E2 vs E4. (C) Forest plot of E3 vs E4.

Supplementary Files

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