Associations between Interleukin-1 Gene Polymorphisms and Coronary Heart Disease Risk: A Meta-Analysis

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Abstract

Objective: A great number of studies regarding the associations between IL-1B-511, IL-1B+3954 and IL-1RN VNTR polymorphisms within the IL-1 gene cluster and coronary heart disease (CHD) have been published. However, results have been inconsistent. In this study, a meta-analysis was performed to investigate the associations.

Methods: Published literature from PubMed and Embase databases were searched for eligible publications. Pooled odds ratios (ORs) with 95% confidence intervals (CIs) were calculated using random- or fixed-effect model.

Results: Thirteen studies (3,219 cases/2,445 controls) for IL-1B-511 polymorphism, nine studies (1,828 cases/1,818 controls) for IL-1B+3954 polymorphism and twelve studies (2,987 cases/2,208 controls) for IL-1RN VNTR polymorphism were included in this meta analysis. The results indicated that both IL-1B-511 and IL-1B+3954 polymorphisms were not associated with CHD risk (IL-1B-511 T vs. C: OR = 0.98, 95%CI 0.87–1.09; IL-1B+3954 T vs. C: OR = 1.06, 95%CI 0.95–1.19). Similarly, there was no association between IL-1RN VNTR polymorphism and CHD risk (*2 vs. L: OR = 1.00, 95%CI 0.85–1.17).

Conclusions: This meta-analysis suggested that there were no associations between IL-1 gene cluster polymorphisms and CHD.

Introduction

Coronary artery disease (CHD) is the most common form of cardiovascular disease which remains the leading cause of mortality and morbidity worldwide [1]. CHD accounts for 7.5 million death in 2008 [2]. CHD is an extremely complex and multifactorial disease, which is attributed to multiple genetic and environmental factors and their interactions.

It is well known that atherosclerosis is the underlying pathology of CHD through a slowly progressing lesion formation and luminal narrowing of arteries [3]. Growing evidence has suggested that inflammation plays an important role in the initiation and progression of atherosclerosis, which is recognized as a progressive inflammatory disorder [3–5]. The adventitial inflammation of advanced plaques can be attributed to the release of some enzymes, cytokines and chemokines [3,6]. Interleukin-1 (IL-1) family is a critical mediator of inflammatory response with two agonists (IL-1α and IL-1β) and one antagonist (Interleukin-1 Receptor antagonist: IL-1Ra) [7]. The IL-1 gene cluster, located within 430 kb region on chromosome 2 (2q13-21), contains IL-1A, IL-1B and IL-1RN (encoding IL-1α, IL-1β and IL-1Ra, respectively) genes [8]. Three single nucleotide polymorphisms (SNPs) of the IL-1 gene cluster have been most frequently studied in relation to CHD risk: one SNP at promoter position −511 C/T and another one in exon 5 at position +3954 C/T of the IL-1B gene and a variable number of tandem repeats (VNTR) of 86 bp polymorphism in intron 2 of IL-1RN gene [9,10], which generates a short allele with two repeats (IL-1RN*2) and long alleles with three to six repeats (IL-1RN L) [10].

To date, a great number of studies regarding the associations between IL-1 gene cluster polymorphisms and CHD risk have been published. However, results have been inconsistent [11–29]. Chen et al performed a meta-analysis in Chinese to investigate the associations between IL-1 gene polymorphisms and CHD in 2000 [30] and only seven papers were included in that meta-analysis. In addition, it did not investigate the relationship between IL-1B+3954 polymorphism and CHD risk. Therefore, in this study, we performed a meta-analysis to further clarify the associations between IL-1B-511, IL-1B+3954 and IL-1RN VNTR polymorphisms and CHD risk.

Materials and Methods

Literature and search strategy

The PubMed and Embase database searches were performed to identify all eligible articles. The search strategy involved the use of combination of the following key words: (Interleukin-1 or IL-1) and (variant or variation or polymorphism) and (coronary disease or coronary artery disease or myocardial infarct or ischemic heart disease or CHD or IHD or MI or
cardiovascular disease or heart disease OR angina). The publication languages were restricted to English and Chinese. The reference lists of retrieved articles were hand-searched. If more than one article was published using the same study data, only the study with the largest sample size was included. The literature search was updated on April 20, 2012.

Inclusion criteria and data extraction

Studies were included in the analysis if they met the following inclusion criteria: (1) a case-control or cohort study; (2) evaluating the associations of IL-1 genetic polymorphisms (include IL-1B-511, IL-1B+3954 or IL-1RN VNTR) with CHD risk; and (3) providing sufficient data for calculation of an odds ratio (OR) with 95% confidence interval (CI). The following information was extracted from each study: (1) name of first author; (2) year of publication; (3) country of origin; (4) ethnicity of the study population; (5) source of controls (population- or hospital-based); (6) sample size of cases and controls; (7) cardiovascular end point; (8) gender distribution and mean age of subjects in cases and controls; (9) genotype distributions in cases and controls; and (10) $p$ value for the test of Hardy–Weinberg equilibrium (HWE) in controls. Two authors independently assessed the articles for compliance with the inclusion criteria, and disagreement was followed by discussion until consensus was reached.

Statistical analysis

The associations between IL-1 genetic polymorphisms and CHD risk were estimated by calculating pooled ORs and 95%CI under multiplicative, co-dominant, dominant, and recessive genetic models, respectively. The significance of pooled ORs was determined by $Z$ tests ($p<0.05$ was considered statistically significant). A Q test was performed to evaluate whether the heterogeneity existed. A random- (DerSimonian-Laird method) [31] or fixed- (Mantel-Haenszel method) [32] effects model was used to calculate the pooled ORs in the presence ($p<0.10$) or absence ($p>0.10$) of heterogeneity. Meta-regression was performed to explore the potentially important sources of between-study heterogeneity. Subgroup analyses based on ethnicity, cardiovascular end point, source of controls and sample size ($n<400$ vs. $n≥400$) were also performed. Sensitivity analysis, removing one study at a time, was performed to evaluate the stability of the results. Begg’s funnel plot, a scatter plot of effect against study size, was performed using STATA version 11 (StataCorp LP, College Station, TX, USA).

Results

Characteristics of the studies

A total of 530 potentially relevant papers were identified based on the search strategy. Of these, 500 papers were excluded because of obvious irrelevance by reading their titles and abstracts. After the full texts were read, four papers were excluded because they didn’t provide sufficient data for calculation of OR with 95% CI [35–38]; two papers were excluded because of examining the associations of other genetic polymorphisms rather than the three polymorphisms studied in our analysis [39–40]; another paper was excluded because it was family-based study [41]. In addition, two reviews [30,42] and two comments [43,44] were excluded with the exception of the one by Iacoviello et al [12], which provided a new study concerning the association of IL-1RN VNTR polymorphism and CHD. Furthermore, more than one study were included in each of the two papers by Francis et al [11] and Rios et al [26], respectively, and they were considered as separate studies in the following data analysis. Thus, thirteen studies [11,14–17,19–22,26,27] on IL-1B-511 polymorphism, nine studies [16,19–24,27,29] on IL-1B+3954 polymorphism, twelve studies [11–14,18–21,25,27,28] on IL-1RN VNTR polymorphism were included in the final meta-analyses. A flow chart demonstrating the inclusion/exclusion of studies was displayed as Figure 1. The characteristics of the included studies were listed in Table 1.

Quantitative data synthesis

For IL-1B-511 polymorphism, a total of 3,219 cases and 2,445 controls were identified. Overall, the results showed no significant association between this polymorphism and CHD risk ($T$ vs. $C$: OR = 0.90, 95%CI 0.87–1.09; $TT$ vs. $CC$ : OR = 0.87, 95%CI 0.67–1.13; $TC$ vs. $CC$: OR = 0.96, 95%CI 0.84–1.09; $TT+TC$ vs. $CC$: OR = 0.93, 95%CI 0.79–1.10; $TT$ vs. $TC+CC$: OR = 0.91, 95%CI 0.74–1.12) (Table 2, Figure 2). In the subgroup analysis, there was no statistically significant association in each subgroup by ethnicity, cardiovascular end point, source of controls and sample size under all genetic models except for the association for Africans under co-dominant and dominant models (Table 2). However, the positive result of subgroup analysis by ethnicity was not reliable for Africans because only one study was performed in African patients.

For IL-1B+3954 polymorphism, six studies comprised 1,828 cases and 1,818 controls were identified. The overall result suggested no statistically significant association of this polymorphism with CHD susceptibility ($T$ vs. $C$: OR = 1.06, 95%CI 0.95–1.19; $TT$ vs. $CC$ : OR = 1.18, 95%CI 0.87–1.58; $TC$ vs. $CC$: OR = 1.12, 95%CI 0.96–1.30; $TT+TC$ vs. $CC$: OR = 1.13, 95%CI 0.98–1.30; $TT$ vs. $TC+CC$: OR = 1.12, 95%CI 0.84–1.50) (Table 3, Figure 3). In the subgroup analysis by ethnicity, cardiovascular end point, source of controls and sample size, no significant association was observed in each subgroup under all genetic models (Table 3).

For IL-1RN VNTR polymorphism, A total of 2,987 cases and 2,208 controls were identified. The overall result suggested no statistically significant association of this polymorphism with CHD risk ($*2$ vs. $L$: OR = 1.00, 95%CI 0.85–1.17; $*2/*2$ vs. $L/L$: OR = 0.86, 95%CI 0.63–1.18; $*2/L$ vs. $L/L$: OR = 0.93, 95%CI 0.81–1.07; $*2/*2 + *2/L$ vs. $L/L$: OR = 0.92, 95%CI 0.81–1.04; $*2/*2 vs. $2/L +*2/L$: OR = 0.88, 95%CI 0.81–1.04) (Table 4, Figure 4). In the further subgroup analyses based on ethnicity, there was no statistically significant association in all genetic models except for the association in Mixed population under multiplicative, co-dominant and dominant models (Table 4). However, the positive association was not reliable for Africans because only one study was performed in African patients.

Source of heterogeneity

As shown in Table 2, 3, 4, evidence for heterogeneity ($p<0.10$) between studies was found under the multiplicative, co-dominant (TT vs. CC) and dominant genetic models for IL-1B-511, under multiplicative and co-dominant ($*2/*2 + *2/L$) genetic models for IL-1RN VNTR polymorphism, respectively. No evidence for heterogeneity between studies was found for IL-1B-511 under the co-dominant (TC vs. CC) and recessive genetic models, for IL-1RN VNTR under co-dominant ($*2/L$ vs. $L/L$), dominant and
recessive genetic models and for IL-1B+3954 polymorphism under all the genetic models.

The meta-regression was conducted with the introduction of covariates including ethnicity, publication year, gender, age, sample size, source of controls and cardiovascular end point for the above mentioned polymorphisms. However, no covariate was identified as a potential source of between-study heterogeneity for any comparison.

Sensitivity analysis
Sensitivity analysis was performed by excluding one study at a time (data not shown) and the non-association results did not substantially alter.

Potential publication bias
Using Egger’s test, no publication bias could be detected for studies published on IL-1B-511 polymorphism (T vs. C: \( p = 0.461 \); TT vs. CC: \( p = 0.231 \); TC vs. CC: \( p = 0.427 \); TT+TC vs. CC: \( p = 0.776 \); TT vs. TC+CC: \( p = 0.691 \)) and IL-1RN VNTR polymorphism (*2 vs. L: \( p = 0.295 \); *2/*2 vs. L/L: \( p = 0.172 \); *2/L vs. L/L: \( p = 0.152 \); *2/*2 + *2/L vs. L/L: \( p = 0.106 \); for *2/*2 vs. *2/L +*2/L: \( p = 0.855 \)).

Discussion
Cytokines of the IL-1 family were believed to influence the inflammatory response and inflammation-related atherosclerosis, and these in turn lead to CHD and other cardiovascular diseases such as stroke [3,45,46]. Because of the effects of cytokines on inflammatory response, a series of studies have focused on the contribution of polymorphisms within the IL-1 cluster genes to the CHD risk. However, results have been contradictory. Given the relatively small sample sizes of the included individual studies for detecting the modest genetic effect, we conducted the present
Table 1. Characteristics of studies included in the meta-analysis.

| Study | Year | Country | Ethnicity | Source of controls | Sample size (case/ control) | End point | Mean age in case/ control, (years) | % of male (case/ control) | Genotype distribution | Genotype distribution | \( p_{\text{HWE}} \) * |
|-------|------|---------|-----------|-------------------|-----------------------------|-----------|-----------------------------------|--------------------------|-----------------------|-----------------------|----------------------|
|       |      |         |           |                   |                             |           |                                   |                          |                       |                       |                     |
| IL-1B-511 polymorphism |       |         |           |                   |                             |           |                                   |                          |                       |                       |                     |
| Francis11 1999 | UK | Caucasian | PB | 425/130 | CHD | 58.9/58.9 | 77.6/43 | 574b | 276c | 193b | 67c | ≥0.05 |
| Francis11 1999 | UK | Caucasian | PB | 248/102 | CHD | 61.1/57.4 | 76.5/36 | 335b | 161c | 138b | 66c | ≥0.05 |
| Vohnout14 2003 | Slovakia | Caucasian | HB | 335/205 | CHD | 59.5/56.6 | 83.6/46.8 | 151 | 152 | 32 | 90 | 89 | 26 | 0.587 |
| Licastro15 2004 | Italy | Caucasian | PB | 139/198 | MI | 65/57 | 100/100 | 65 | 60 | 14 | 46 | 65 | 11 | 0.075 |
| Iacoviello16 2005 | Italy | Caucasian | PB | 406/419 | MI | 41/40 | 85/85 | 195 | 180 | 31 | 174 | 187 | 58 | 0.495 |
| Zhang17 2006 | China | Asian | PB | 127/152 | CHD | 52/44 | 66.9/64.5 | 25 | 79 | 23 | 38 | 92 | 22 | 0.006 |
| Arman19 2008 | Turkey | Caucasian | HB | 257/170 | CHD | 58.0/51.4 | 72.4/53.5 | 75 | 130 | 52 | 51 | 74 | 45 | 0.094 |
| Geismar20 2008 | Denmark | Caucasian | HB | 96/123 | CHD | 63.8/61.8 | 69.1/62.5 | 43 | 42 | 11 | 60 | 50 | 13 | 0.095 |
| Soylu21 2008 | Turkey | Caucasian | HB | 264/117 | ACS | 58.3/52.4 | 73.1/48.7 | 81 | 128 | 55 | 28 | 58 | 31 | 0.932 |
| Zee22 2008 | USA | Caucasian | PB | 341/341 | MI | 60.2/60.1 | 100/100 | 148 | 154 | 39 | 164 | 137 | 40 | 0.172 |
| Rios26 2010 | Brazil | African | HB | 138/115 | CHD | 55.7/51.8 | 64.5/43.5 | 42 | 64 | 32 | 19 | 67 | 29 | 0.062 |
| Rios26 2010 | Brazil | Caucasian | PB | 276/138 | MI | 55.7/53.0 | 66.7/45.6 | 80 | 130 | 66 | 47 | 69 | 22 | 0.69 |
| Coker27 2011 | Turkey | Caucasian | HB | 167/235 | MI | 53.4/53.9 | 70/43 | 59 | 72 | 36 | 77 | 113 | 45 | 0.758 |
| IL-1B+3954 polymorphism |       |         |           |                   |                             |           |                                   |                          |                       |                       |                     |
| Iacoviello16 2005 | Italy | Caucasian | PB | 406/419 | MI | <50 | NA | 244 | 140 | 14 | 258 | 130 | 14 | 0.63 |
| Arman19 2008 | Turkey | Caucasian | HB | 257/170 | CHD | 58.0/51.4 | 72.4/53.5 | 151 | 91 | 15 | 93 | 68 | 9 | 0.446 |
| Geismar20 2008 | Denmark | Caucasian | HB | 96/123 | CHD | 63.8/61.8 | 69.1/62.5 | 51 | 38 | 7 | 67 | 45 | 11 | 0.393 |
| Soylu21 2008 | Turkey | Caucasian | PB | 264/117 | CHD | 58.3/52.4 | 73.1/48.7 | 157 | 93 | 14 | 69 | 41 | 7 | 0.783 |
| Zee22 2008 | USA | Caucasian | PB | 341/341 | MI | 60.2/60.1 | 100/100 | 188 | 130 | 23 | 198 | 123 | 20 | 0.877 |
| Stein23 2009 | Germany | Caucasian | HB | 54/50 | AMI | 50.8/51.7 | 92.6/94 | 48b | 24c | 48b | 21c | ≥0.05 |
| Zhu24 2009 | China | Asian | HB | 100/144 | CHD | 61.5/60.3 | 67/67.4 | 97 | 3 | 0 | 142 | 2 | 0 | 0.933 |
| Coker27 2011 | Turkey | Caucasian | HB | 167/235 | MI | 53.4/53.9 | 70/43 | 86 | 68 | 13 | 136 | 84 | 15 | 0.677 |
| Zeybek29 2011 | Turkey | Caucasian | PB | 143/213 | MI | 58.9/56.4 | 68.5/39.9 | 79 | 46 | 18 | 140 | 54 | 19 | <0.001 |
| IL-1RN VNTR polymorphism |       |         |           |                   |                             |           |                                   |                          |                       |                       |                     |
| Francis11 1999 | UK | Caucasian | PB | 425/130 | CHD | 58.9/58.9 | 77.6/43 | 628b | 222c | 201b | 59c | ≥0.05 |
| Francis11 1999 | UK | Caucasian | PB | 248/102 | CHD | 61.1/57.4 | 76.5/36 | 356b | 140c | 171b | 33c | ≥0.05 |
| Iacoviello12 2000 | Italy | Caucasian | PB | 158/153 | AMI | <50 | 81.6 | 24b | 21c | 24b | 19c | ≥0.05 |
| Zee13 2001 | USA | Caucasian | PB | 385/385 | MI | 59.6/59.5 | M | 219 | 140 | 26 | 218 | 137 | 30 | 0.199 |
| Vohnout14 2003 | Slovakia | Caucasian | HB | 335/205 | CHD | 59.5/56.6 | 83.6/46.8 | 200 | 114 | 21 | 127 | 68 | 20 | 0.02 |
| Kariz18 2007 | Slovenia | Caucasian | PB | 151/223 | MI | 59.2/66.5 | 65.6/45.7 | 87 | 49 | 15 | 134 | 75 | 14 | 0.428 |
| Arman19 2008 | Turkey | Caucasian | HB | 257/170 | CHD | 58.0/51.4 | 72.4/53.5 | 150 | 84 | 23 | 105 | 56 | 9 | 0.67 |
Table 1. Cont.

| Study          | Year | Country | Ethnicity | Source of controls | Sample size (case/control) | Mean age in case/control (year) | End point | Sample size (case/control) | Mean age in case/control (year) | Genotype distribution | P-value HWE | % of male (case/control) | Genotype distribution | % of male (case/control) |
|----------------|------|---------|-----------|-------------------|---------------------------|--------------------------------|-----------|---------------------------|--------------------------------|----------------------|-------------|--------------------------|----------------------|--------------------------|
| Geisler et al.  | 2009 | Denmark | Caucasian | HB                | 96/123                    | 63.8/61.8                      | CHD       | 11/12                     | 69.1/62.5                      | 11/12 22              | 0.001       | 11/12 22                 | 0.001       | 11/12 22                 |
| Singh et al.   | 2012 | Turkey  | Caucasian | HB                | 30/117                    | 58.3/52.4                      | ACS       | 11/12                     | 73.5/68.7                      | 14/14 22              | 0.001       | 14/14 22                 | 0.001       | 14/14 22                 |
| Fragoso et al. | 2014 | Mexico  | Mixed     | PB                | 26/268                    | 59.3/55                        | MI        | 11/12                     | 59.5/55                        | 113/90 22             | 0.001       | 113/90 22               | 0.001       | 113/90 22               |
| Coker et al.   | 2011 | Turkey  | Caucasian | HB                | 167/235                   | 51.4/53.3                      | MI        | 11/12                     | 51.4/53.3                      | 51/45 22             | 0.001       | 51/45 22                 | 0.001       | 51/45 22                 |
| Goracy et al.  | 2010 | Poland  | Caucasian | HB                | 167/117                   | 62.1/59.6                      | CHD       | 11/12                     | 68.5/66.5                      | 121/91 22           | 0.001       | 121/91 22               | 0.001       | 121/91 22               |

Notes: For IL-1B-511 and IL-1B-3954 polymorphisms, 11 = CC, 12 = CT, 22 = TT; for IL-1RN VNTR polymorphism, 11 = L/L, 12 = *2/L, 22 = *2/*2; for IL-1B-511, 11 = CC, 12 = CT, 22 = TT; for IL-1RN VNTR polymorphism, 11 = L/L, 12 = *2/L, 22 = *2/*2; for IL-1B-3954, 11 = CC, 12 = CT, 22 = TT; for IL-1RN VNTR polymorphism, 11 = L/L, 12 = *2/L, 22 = *2/*2.

meta-analysis, although there were still the limited power of meta-analysis due to size and heterogeneity of studies/patients.

Our results suggested that there was no significant association between the three polymorphisms (IL-1B-511, IL-1B-3954 and IL-1RN VNTR) within the IL-1 gene cluster and CHD risk. One previous meta-analysis also failed to suggest statistically significant associations of IL-1B-511 and IL-1RN VNTR polymorphisms with stroke risk in the overall population, with ORs and 95% CI of 1.22 (0.85–1.78) for TT vs. CC and 1.22 (0.85–1.75) for RN2/RN2 vs. RN1/RN1 [47]. In addition, the previous meta-analysis by Chen et al in Chinese showed that IL-1 gene cluster polymorphisms did not seem to affect CHD risk, with ORs and 95% CI of 1.04 (0.93–1.18) for IL-1B-511 and 1.01 (0.78–1.17) for IL-1RN VNTR under multiplicative models [30].

IL-1β, released by macrophages, platelets, and injured endothelium [48], plays a central role in the inflammatory response and its related atherosclerosis. IL-1β may act on atherosclerosis with different biological functions such as stimulating proliferation of vascular smooth muscle cells and endothelial cells [49,50], increasing expression of adhesional molecule from endothelial cells [50], modulating the endothelium to promote coagulation and thrombosis [51], stimulating the synthesis of fatty acid carrier protein by adipose tissue in vitro [52], promoting the production of some other pro-inflammatory factors such as IL-6, fibrinogen and C-reactive protein [45,46]. At the same time, IL-1Ra regulates inflammation by functioning as an endogenous inhibitor of IL-1β and competing for IL-1 receptor. Therefore, the balance between IL-1β and IL-1Ra is thought to contribute to the pathogenesis of atherosclerosis [45,46]. In addition, some evidence has indicated that elevated levels of IL-1 and IL-1Ra mRNA were observed in atherosclerotic arteries compared with normal arteries [53]. In addition, T allele of the IL-1B-511 polymorphism and 2 allele of the IL-1RN VNTR polymorphism have been associated with enhanced IL-1β production [54,55]. Nevertheless, no significant association was found between IL-1 gene cluster polymorphisms and cardiovascular diseases by meta-analysis.

Considering that CHD is a multifactorial trait and the impact of the inflammatory cytokine on CHD progress may be modulated by age, gender and some other environmental and genetic factors across different ethnicities, the subgroup analysis based on ethnicity was performed, which showed that IL-1B-511 polymorphism was only associated with CHD in Africans under co-dominant and dominant models, IL-1RN VNTR polymorphism associated in Mixed population under multiplicative, co-dominant and dominant models, respectively. However, the results were not very credible due to just one study included in the Africans and Mixed population separately.

Furthermore, the subgroup analysis indicated the positive association of IL-1RN VNTR polymorphism with ACS but not with other cardiovascular end point. However, what also needs to be pointed out is that the significant association derived from only one study and thus the result should be interpreted with caution because of the relatively small sample size or multiple testing driving false positive findings.

There are several limitations in the meta-analysis. First, our analysis was primarily based on unadjusted effect estimates and therefore the potential covariates including age, gender and environmental factors such as smoking and levels of HDL-cholesterol, which might influence the effect estimates, were not controlled for. Second, despite of evidence of between-study heterogeneity in some comparisons in our meta-analysis, none of the covariates including ethnicity, publication year, gender, age, sample size, source of controls and cardiovascular end point was identified as a potential source of heterogeneity between studies by
Table 2. Summary ORs and 95% CIs of the association between *IL-1B* -511 polymorphism and CHD risk.

| Contrasts            | No. of studies | T vs. C | No. of studies | TT vs. CC | TC vs. CC | TT vs. TC vs. CC | TT vs. TC vs. CC |
|----------------------|----------------|---------|----------------|-----------|-----------|-----------------|-----------------|
|                      |                | OR      | 95% CI         | P         | OR        | 95% CI          | P               | OR      | 95% CI          | P         | OR      | 95% CI          | P         | OR      | 95% CI          | P         |
| All                  | 13             | 0.98    | 0.87–1.09      | 0.020     | 11        | 0.87    | 0.67–1.13       | 0.039            | 0.96    | 0.84–1.09       | 0.125     | 0.93    | 0.79–1.10       | 0.069     | 0.91    | 0.74–1.12       | 0.100     |
| Studies in HWE       | 12             | 0.96    | 0.85–1.08      | 0.022     | 10        | 0.83    | 0.64–1.08       | 0.058            | 0.94    | 0.82–1.08       | 0.119     | 0.91    | 0.76–1.08       | 0.078     | 0.87    | 0.74–1.03       | 0.105     |
| Ethnicity            |                |         |                |           |           |       |                |                 |         |                |           |         |                |           |         |                |           |
| Caucasian            | 11             | 0.98    | 0.87–1.11      | 0.031     | 9         | 0.87    | 0.66–1.14       | 0.069            | 0.98    | 0.85–1.12       | 0.422     | 0.95    | 0.83–1.07       | 0.255     | 0.88    | 0.69–1.13       | 0.069     |
| Asian                | 1              | 1.20    | 0.86–1.67      | -         | 1         | 1.59    | 0.73–3.44       | -                | 1.31    | 0.73–2.35       | -         | 1.36    | 0.77–2.41       | -         | 1.31    | 0.69–2.48       | -         |
| African              | 1              | 0.73    | 0.51–1.03      | -         | 1         | 0.50    | 0.24–1.05       | -                | 0.43    | 0.23–0.82       | -         | 0.45    | 0.25–0.83       | -         | 0.90    | 0.50–1.59       | -         |
| End point            |                |         |                |           |           |       |                |                 |         |                |           |         |                |           |         |                |           |
| CHD                  | 9              | 1.01    | 0.87–1.17      | 0.050     | 7         | 0.91    | 0.64–1.29       | 0.065            | 0.99    | 0.82–1.19       | 0.150     | 0.96    | 0.75–1.22       | 0.093     | 0.93    | 0.76–1.15       | 0.183     |
| MI                   | 4              | 0.91    | 0.75–1.11      | 0.077     | 4         | 0.82    | 0.53–1.26       | 0.078            | 0.93    | 0.78–1.12       | 0.137     | 0.90    | 0.76–1.07       | 0.109     | 0.87    | 0.58–1.31       | 0.075     |
| Source of controls   |                |         |                |           |           |       |                |                 |         |                |           |         |                |           |         |                |           |
| HB                   | 7              | 0.95    | 0.84–1.06      | 0.156     | 7         | 0.88    | 0.70–1.11       | 0.123            | 0.93    | 0.78–1.12       | 0.184     | 0.93    | 0.78–1.10       | 0.152     | 0.94    | 0.76–1.15       | 0.203     |
| PB                   | 6              | 1.02    | 0.83–1.24      | 0.013     | 4         | 0.89    | 0.52–1.53       | 0.030            | 0.98    | 0.73–1.31       | 0.100     | 0.95    | 0.70–1.30       | 0.049     | 0.88    | 0.57–1.31       | 0.070     |
| Sample size          |                |         |                |           |           |       |                |                 |         |                |           |         |                |           |         |                |           |
| Small                | 6              | 0.92    | 0.80–1.06      | 0.249     | 5         | 0.82    | 0.59–1.15       | 0.192            | 0.81    | 0.56–1.17       | 0.073     | 0.82    | 0.57–1.18       | 0.057     | 0.95    | 0.72–1.27       | 0.679     |
| Large                | 7              | 1.01    | 0.86–1.19      | 0.011     | 6         | 0.89    | 0.63–1.27       | 0.024            | 1.02    | 0.88–1.19       | 0.517     | 0.98    | 0.85–1.13       | 0.264     | 0.88    | 0.63–1.22       | 0.020     |

Notes: OR, odds ratio; CI, confidence interval; P<sub>H</sub>, P value based on Q test for between-study heterogeneity; HWE = Hardy–Weinberg equilibrium; CHD = coronary artery disease; ACS = acute coronary syndrome; MI = myocardial infarction; PB, Population-based; HB, Hospital-based.

doi:10.1371/journal.pone.0045641.t002

Figure 2. Meta-analysis of the association between *IL-1B* -511 polymorphism and CHD risk (T vs. C).

doi:10.1371/journal.pone.0045641.g002
Table 3. Summary ORs and 95% CIs of the association between IL-1B +3954 polymorphism and CHD risk.

| Contrasts | No. of studies | T vs. C | OR 95% CI | P H | TT vs. CC | OR 95% CI | P H | TC vs. CC | OR 95% CI | P H | TT+C vs. CC | OR 95% CI | P H | TT vs. TC+CC | OR 95% CI | P H |
|-----------|----------------|---------|-----------|------|-----------|-----------|------|-----------|-----------|------|-------------|-----------|------|---------------|-----------|------|
| All       | 9              | 1.06    | 0.95–1.19 | 0.576 | 8         | 1.18      | 0.87–1.58 | 0.910 | 1.12      | 0.96–1.30 | 0.678 | 1.13         | 0.98–1.30 | 0.592 | 1.12         | 0.84–1.50 | 0.962 |
| Studies in HWE | 8              | 1.02    | 0.90–1.15 | 0.892 | 7         | 1.09      | 0.78–1.51 | 0.969 | 1.08      | 0.93–1.27 | 0.783 | 1.09         | 0.93–1.26 | 0.781 | 1.06         | 0.77–1.46 | 0.981 |
| Ethnicity  |                |         |           |       |           |           |       |           |           |       |             |           |       |               |           |       |
| Caucasian | 8              | 1.06    | 0.94–1.18 | 0.537 | 7         | 1.18      | 0.87–1.58 | 0.910 | 1.11      | 0.96–1.29 | 0.635 | 1.12         | 0.97–1.29 | 0.540 | 1.12         | 0.84–1.50 | 0.962 |
| Asian     | 1              | 2.18    | 0.36–13.15 | -    | 4         | 2.20      | 0.36–13.39 | -    | 1         | 0.36–13.39 | -    | -           | -           | -    | -           | -           | -    |
| End point |                |         |           |       |           |           |       |           |           |       |             |           |       |               |           |       |
| CHD       | 4              | 0.96    | 0.78–1.17 | 0.817 | 4         | 1.09      | 0.78–1.58 | 0.949 | 0.96      | 0.73–1.25 | 0.655 | 0.95         | 0.74–1.23 | 0.725 | 0.94         | 0.55–1.59 | 0.876 |
| MI        | 5              | 1.11    | 0.97–1.27 | 0.354 | 4         | 1.31      | 0.92–1.87 | 0.836 | 1.20      | 1.00–1.43 | 0.728 | 1.21         | 1.03–1.44 | 0.614 | 1.21         | 0.86–1.72 | 0.907 |
| Source of controls |                |         |           |       |           |           |       |           |           |       |             |           |       |               |           |       |
| HB        | 6              | 0.95    | 0.80–1.12 | 0.923 | 4         | 1.04      | 0.67–1.63 | 0.857 | 1.04      | 0.83–1.30 | 0.568 | 1.04         | 0.84–1.29 | 0.570 | 1.02         | 0.66–1.59 | 0.895 |
| PB        | 3              | 1.16    | 1.00–1.36 | 0.357 | 4         | 1.29      | 0.87–1.93 | 0.656 | 1.18      | 0.97–1.44 | 0.550 | 1.20         | 0.99–1.45 | 0.430 | 1.21         | 0.82–1.79 | 0.760 |
| Sample size |                |         |           |       |           |           |       |           |           |       |             |           |       |               |           |       |
| Small     | 5              | 1.15    | 0.94–1.41 | 0.456 | 4         | 1.19      | 0.73–1.95 | 0.411 | 1.21      | 0.91–1.60 | 0.574 | 1.21         | 0.92–1.57 | 0.422 | 1.10         | 0.68–1.79 | 0.520 |
| Large     | 4              | 1.02    | 0.89–1.17 | 0.563 | 4         | 1.17      | 0.80–1.70 | 0.956 | 1.09      | 0.91–1.29 | 0.479 | 1.10         | 0.93–1.30 | 0.494 | 1.13         | 0.78–1.63 | 0.986 |

Notes: OR, odds ratio; CI, confidence interval; P H, P value based on Q test for between-study heterogeneity; HWE = Hardy–Weinberg equilibrium; CHD = coronary artery disease; ACS = acute coronary syndrome; MI = myocardial infarction; PB, Population-based; HB, Hospital-based.

doi:10.1371/journal.pone.0045641.t003

Figure 3. Meta-analysis of the association between IL-1B +3954 polymorphism and CHD risk (T vs. C).
doi:10.1371/journal.pone.0045641.g003
Table 4. Summary ORs and 95%CIs of the association between IL-1RN VNTR polymorphism and CHD risk.

| Contrasts          | No. of studies | *2 vs. L | OR  (95% CI) | P<sub>H</sub> | *2*/2 vs. L/L | OR  (95% CI) | P<sub>H</sub> | *2/L vs. L/L | OR  (95% CI) | P<sub>H</sub> | *2*/2 + *2/L vs. L/L | OR  (95% CI) | P<sub>H</sub> | *2*/2 vs. *2/L +*2/L | OR  (95% CI) | P<sub>H</sub> |
|--------------------|----------------|----------|--------------|-------------|--------------|--------------|-------------|--------------|--------------|-------------|----------------------|--------------|-------------|----------------------|--------------|-------------|
| Overall (I-squared = 64.9%, p = 0.001) | 12 | 1.00 | 0.85–1.17 | 0.001 | 0.96 | 0.83–1.10 | 0.071 | 0.94 | 0.81–1.10 | 0.076 | 0.93 | 0.83–1.07 | 0.146 | 0.92 | 0.81–1.04 | 0.194 | 0.88 | 0.71–1.10 | 0.132 |
| Studies in HWE    | 8 | 1.12 | 0.94–1.32 | 0.043 | 1.06 | 0.78–1.46 | 0.215 | 1.00 | 0.84–1.20 | 0.950 | 1.02 | 0.86–1.21 | 0.727 | 1.06 | 0.78–1.44 | 0.246 |
| Ethnicity         | 11 | 1.04 | 0.90–1.20 | 0.020 | 0.94 | 0.73–1.20 | 0.127 | 1.00 | 0.86–1.16 | 0.096 | 0.99 | 0.86–1.14 | 0.207 | 0.94 | 0.74–1.19 | 0.232 |
| Mixed             | 1 | 0.66 | 0.51–0.85 | - | 0.5 | 0.33–0.94 | - | 0.59 | 0.41–0.86 | - | 0.58 | 0.41–0.82 | - | 0.68 | 0.41–1.13 | - |
| End point         |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| CHD               | 7 | 1.05 | 0.82–1.33 | 0.033 | 0.79 | 0.47–1.33 | 0.087 | 1.00 | 0.82–1.23 | 0.927 | 0.96 | 0.80–1.16 | 0.042 | 0.94 | 0.74–1.19 | 0.032 |
| MI                | 4 | 1.03 | 0.89–1.19 | 0.011 | 1.09 | 0.76–1.56 | 0.411 | 1.00 | 0.80–1.24 | 0.970 | 1.02 | 0.83–1.25 | 0.095 | 1.10 | 0.78–1.55 | 0.386 |
| ACS               | 2 | 0.79 | 0.53–1.18 | 0.07 | 0.55 | 0.33–0.94 | - | 0.59 | 0.41–0.86 | 0.242 | 0.41 | 0.24–0.72 | - | 0.68 | 0.41–1.13 | - |
| Source of controls|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| HB                | 7 | 0.97 | 0.86–1.10 | 0.176 | 0.88 | 0.66–1.19 | 0.108 | 1.00 | 0.83–1.20 | 0.968 | 0.97 | 0.82–1.15 | 0.735 | 0.89 | 0.67–1.18 | 0.116 |
| PB                | 5 | 1.06 | 0.77–1.47 | 0.000 | 0.87 | 0.49–1.55 | 0.071 | 0.85 | 0.59–1.21 | 0.063 | 0.86 | 0.58–1.55 | 0.025 | 0.88 | 0.63–1.12 | 0.165 |
| Sample size       |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Small             | 6 | 1.05 | 0.79–1.39 | 0.004 | 0.82 | 0.42–1.58 | 0.065 | 0.97 | 0.76–1.23 | 0.886 | 0.94 | 0.75–1.18 | 0.050 | 0.83 | 0.45–1.55 | 0.076 |
| Large             | 6 | 0.95 | 0.79–1.15 | 0.024 | 0.85 | 0.65–1.11 | 0.124 | 0.92 | 0.78–1.08 | 0.146 | 0.91 | 0.72–1.15 | 0.068 | 0.89 | 0.69–1.16 | 0.231 |

Notes: OR, odds ratio; CI, confidence interval; P<sub>H</sub>, P value based on Q test for between-study heterogeneity; HWE = Hardy–Weinberg equilibrium; CHD = coronary artery disease; ACS = acute coronary syndrome; MI = myocardial infarction; PB, Population-based; HB, Hospital-based.

doi:10.1371/journal.pone.0045641.t004
meta-regression. Therefore, other unknown confounding factors may help explain the between-study heterogeneity. Third, the possibility of a false negative remains due to the small size of the studies even when combined. Thus, further studies with larger sample size are required to investigate the associations. Fourth, none of the studies included in this meta-analysis considered the effect of gene-gene/environment interactions involved in the pathogenesis of CHD, this issue could not addressed in our meta-analysis. Fifth, as is known, haplotype analyses might bring out bigger net effects. However, most studies, except for the studies by Zee et al [13] and Fragoso et al [25], did not perform haplotype analyses, which impeded our further analysis. Sixth, it is conceivable, that patients with a higher inflammatory status and a polymorphism in IL1B or IL1RA have stronger association to coronary heart disease than patients without inflammation. Analysis of hsCRP in a subgroup could help to answer this question. Three studies (the study by Iacoviello et al [16], Soylu et al [21] and Coker et al [27]) provided the data about hsCRP. However, only the study by Iacoviello et al [16] provided the hsCRP-adjusted OR with 95% CI; the other two studies by Soylu et al [21] and Coker et al [27] only provided the hsCRP levels between cases and controls. Therefore, subgroup analysis of the effect of hsCRP on variant-CHD association can not yet been conducted so far.

In summary, our meta-analyses suggested that IL-1 gene cluster polymorphisms were not associated with CHD risk. More in depth researches considering gene-environment interactions and haplotype information should be conducted to further investigate these associations between IL-1 gene cluster polymorphisms and CHD risk.

Author Contributions
Conceived and designed the experiments: LZ HT. Performed the experiments: JC GL YW. Analyzed the data: JC GL YW. Contributed reagents/materials/analysis tools: JC GL YW. Wrote the paper: LZ.

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