OncoTargets and Therapy

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ORIGINAL RESEARCH

Association between chemotactic chemokine ligand 5 -403G/A polymorphism and risk of human immunodeficiency virus-1 infection: a meta-analysis

This article was published in the following Dove Press journal:
OncoTargets and Therapy
7 April 2015
Number of times this article has been viewed

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Background: The association between chemotactic chemokine ligand 5 (*CCL5*) -403G/A gene polymorphism and human immunodeficiency virus-1 (HIV-1) infection has been illustrated among several case-control studies, but the conclusions are still inconsistent. Here we performed a meta-analysis to estimate the association.

Methods: The published studies based upon the association between *CCL5* -403G/A polymorphism and HIV-1 infection were retrieved from PubMed, Embase, and China National Knowledge Infrastructure database. Quantitative synthesis, including pooled odds ratios (ORs) and 95% confidence intervals (CIs), was performed for all genetic models.

Results: A total of ten studies consisting of 5,127 subjects were included for this meta-analysis. There was no association found between -403G/A polymorphism and HIV-1 infection in the overall analysis under any genetic models. Further stratified by ethnicity, our analysis showed that -403A/A polymorphism significantly decreased the susceptibility to HIV-1 infection in three models: the dominant model (AA+AG vs GG: OR =0.44, 95% CI =0.21–0.94) among Africans, the homozygous model (AA vs GG: OR =0.62, 95% CI =0.242–0.90), and the recessive model (AA vs GG+AG: OR =0.62, 95% CI =0.45–0.93) among Asians.

Conclusion: We found that only Asians and Africans with *CCL5* -403A/A polymorphism could be resistant to HIV-1 infection. However, further studies should be performed to evaluate this association on ethnic basis against control groups consisting of individuals who have once been exposed to HIV-1 but are seronegative.

Keywords: *CCL5*, polymorphism, susceptibility, HIV-1, meta-analysis

Introduction

The human immunodeficiency virus (HIV) is a retrovirus that infects the cells of the immune system, destroying and impairing their function. As reported by World Health Organization, there were approximately 35.3 million people living with HIV in 2012 (http://www.who.int/mediacentre/factsheets/fs360/en/). HIV infection has been one of the most serious public health threats all over the world. However, the risk and natural course of HIV-1 disease are different among individuals.[1] It is well known that the genetic variants could affect the susceptibility to HIV-1 infection, transmission, disease progression, and antiviral therapy.[2] Genomic studies have shown that the allelic variants of chemokine receptors and their natural ligands that are related to HIV-1 entry can affect the susceptibility and progression of HIV-1 infection.[3–6]

Chemotactic chemokine ligand 5 (CCL5), which is also called regulated on activation, normal T cell expressed and secreted (RANTES), belongs to the family of

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http://dx.doi.org/10.2147/OTT.S78581

chemotactic chemokines. As an important chemokine, CCL5 is mostly involved in immune regulatory and inflammatory activities owing to its ability to recruit, activate, and co-stimulate T-cells and monocytes.[7,8] By conjugating to macrophage inhibitory protein-1α (MIP-1α) and -1β (MIP-1β), CCL5 plays a critical protective role in HIV-1 infection by blocking the access of the HIV-1 envelope glycoprotein gp120 to chemotactic chemokine receptor 5 (CCR5).[9] -403G>A (rs2107538) polymorphism, which has been identified in the promoter region of the gene *CCL5*, is associated with transcriptional activity, and subsequently affects its protein expression in human cell lines,[10] so that -403G/A polymorphism is considered a potential susceptibility factor to HIV-1 infection based upon its function.

Up to now, many reports have been published evaluating the association between *CCL5* -403G/A polymorphism and HIV-1 infection among different ethnic populations, but the results are in conflict. Several possible factors contribute to this inconsistency, including sample size, ethnic diversity, and others. Meta-analysis conducted by pooling the data from different studies would provide better statistical power to address the above-mentioned shortcomings posed by different studies, especially those due to an inadequate sample size.[11]

Hence, in order to provide a precise and comprehensive evaluation of the association between *CCL5* -403G/A polymorphism and the risk of HIV-1 infection, we performed a meta-analysis by pooling data from published studies.

Materials and methods

Literature search strategy

We retrieved documents from major electronic databases, including PubMed, Embase web database, and China National Knowledge Infrastructure, using various combinations of the following keywords: "HIV or HIV-1 or AIDS or human immunodeficiency viruses," "RANTES or CCL5 or chemotactic chemokine ligand", and "polymorphism or polymorphisms or mutation or variant" (last search update October 1, 2014). We then evaluated the potential studies by reviewing their titles and abstracts, and the documents were retrieved in duplication by two independent investigators (He and Li) according to the search strategy.

Inclusion and exclusion criteria

We examined the titles and abstracts of the articles to ensure that all articles included in the current meta-analysis met all the following three inclusion criteria: (a) case-control studies were conducted to evaluate the association between *CCL5* -403G/A polymorphism and susceptibility to HIV-1 infection, and the controls were healthy individuals without a history of exposure to HIV-1, (b) the studies provided distribution data of -403G/A polymorphism in the case-control population, and (c) the original articles were published either in English or in Chinese. In addition, we selected the studies with the largest sample size when there was a subject overlap among different studies. Some studies were excluded because they: (a) were either merely reviews or irrelevant to human subjects; (b) overlapped with other studies in terms of data; or (c) only involved cases or control group.

Data extraction

According to the inclusion and exclusion criteria listed above, two investigators (He and Li) reviewed and extracted the information from all eligible articles independently. Disagreements were resolved by discussion between the two investigators. Another investigator (Tang) was invited to the discussion if they did not reach an agreement. The following details of eligible articles were collected: the first author, year of publication, original country, ethnicity, number of HIV-1 patients and healthy individuals, and the distributions of *CCL5* -403G/A polymorphism in all subjects.

Statistical analysis

We used crude odds ratios (ORs) with their 95% confidence intervals (CIs) to evaluate the strength of association between the gene *CCL5* -403G/A polymorphism and the risk of HIV-1 infection. The allelic model (A vs G), homozygous model (AA vs GG), dominant model (AA+AG vs GG), and recessive model (AA vs AG+GG) were used for quantitative analysis. Further, stratification analysis was performed based on ethnicity. Heterogeneity between studies was assessed by χ^2-based *Q*-test. We used a fixed-effect model (Mantel–Haenszel) to evaluate the summary ORs if the *P*-value for heterogeneity was more than 0.05, indicating an absence of heterogeneity. Otherwise, we used the random-effect model (DerSimonian and Laird) to evaluate the summary ORs. We performed the Hardy–Weinberg equilibrium (HWE) test to assess the departure of frequencies of *CCL5* polymorphism from the expected values by χ^2-test in controls. Sensitivity analysis was performed by deleting one study at once to test the stability of the pooled results. We assessed the possible publication bias by Begg's funnel plot and Egger's test.

The Stata software package (version 9.2; College Station, TX, USA) was used for all statistical tests in this meta-analysis. A result was defined as statistically significant when the *P*-value was less than 0.05.

Results

Study characteristics

As the flow chart in Figure 1 shows, a total of 279 articles were retrieved through an initial search of the selected electronic databases. After screening the titles and abstracts, 14 of the 279 articles were obtained, and further data were extracted from these 14 articles. During the extraction of data, one study was excluded due to insufficient data on the distribution of *CCL5* -403G/A polymorphism,[12] and another study was excluded on account of duplicate data.[13] Further, two studies and the data from the two studies concerning Caucasians were excluded because of inconsistency with HWE.[14–17] Finally, a total of ten studies were considered suitable for meta-analysis.[15,16,18–25] Among them, there were four studies of Asians, four studies of Caucasians, and three studies of Africans. One of the studies involved both Asians and Africans.[15] The total number of samples was 5,127, which consisted of 2,306 HIV-1 patients and 2,821 healthy controls. The details of eligible studies are listed in Table 1.

Quantitative synthesis

The main results of this meta-analysis and the heterogeneity test are shown in Table 2 and Figure 2. In the overall analysis, no association between the -403G/A polymorphism and the risk of HIV-1 infection were observed in any genetic models. When further stratified by ethnicity, the analysis showed that the -403A/A polymorphism significantly decreased the risk of HIV-1 infection in the dominant model (AA+AG vs GG: OR =0.44, 95% CI =0.21–0.94, *P*=0.002 for heterogeneity test) among Africans, also in the homozygous model (AA vs GG: OR =0.62, 95% CI =0.242–0.90, *P*=0.175 for heterogeneity test), and the recessive model (AA vs GG+AG: OR =0.62, 95% CI =0.45–0.93, *P*=0.261 for heterogeneity test) among Asians.

Sensitivity analysis

Sensitivity analysis was performed to assess the stability of the pooled ORs in the total population and all the subgroups under all genetic models. The results became statistically significant (OR =0.58, 95% CI =0.36–0.94, *P*=0.002 for the heterogeneity test) in the homozygous model in the overall analysis, when one study of German subjects (Caucasian) was deleted.[21] However, the result remained stable for other genetic models.

Publication bias

The publication bias was evaluated by Begg's rank correlation method and Egger's weighted regression method.

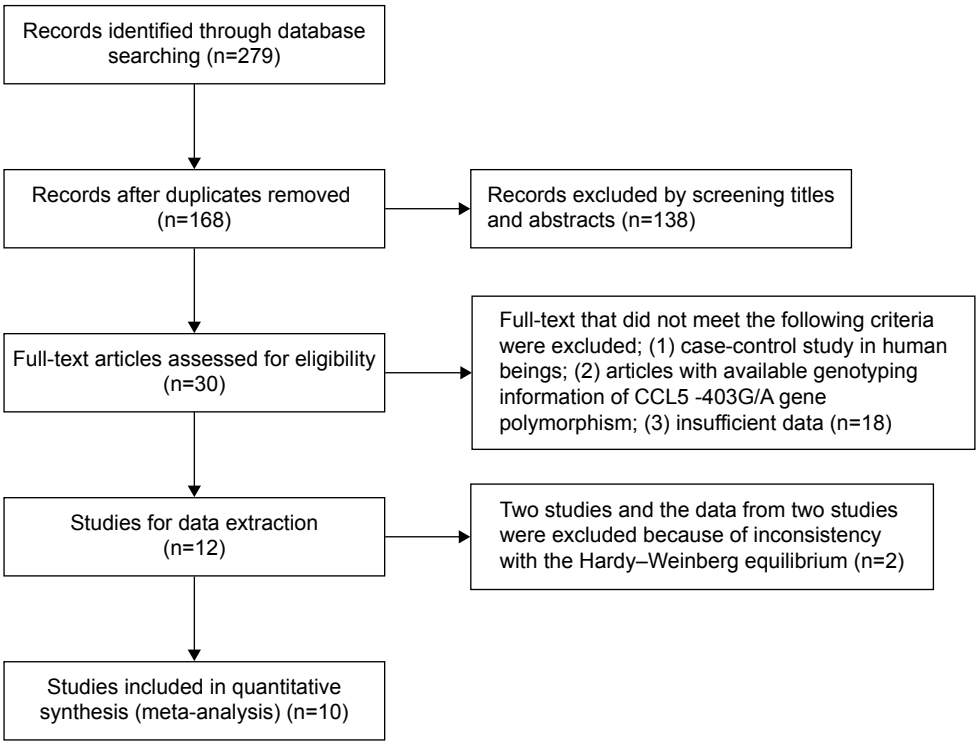

Figure 1 Flow chart of study inclusion.
Abbreviation: CCL5, chemotactic chemokine ligand 5.

Table 1 Major characteristics of the studies included in this meta-analysis

First author [Ref][a]	Year	Country	Ethnicity	HIV-1 infected patients (AA/AG/GG)	Healthy controls (AA/AG/GG)	P_{HWE}[b]
Liu et al[15]	1999	Japan, People's Republic of China, Thailand	Asian	8/23/35	45/111/113	0.05
		Ivory Coast	African	6/26/55	24/19/11	0.06
McDermott et al[18]	2000	NA[c]	Caucasian	10/121/218	6/44/101	0.66
Gonzalez et al[16]	2001	NA[c]	African	68/184/151	98/245/117	0.15
Fernandez et al[19]	2003	Spain	Caucasian	13/132/295	3/32/65	0.69
Zhao et al[20]	2004	People's Republic of China	Asian	20/123/106	159/527/405	0.56
Ahlenstiel et al[21]	2005	Germany	Caucasian	8/69/128	1/31/77	0.26
Shrestha et al[22]	2006	America	African	19/46/35	18/51/30	0.65
Vidal et al[23]	2006	Spain	Caucasian	3/51/105	3/30/65	0.84
Suresh et al[24]	2006	India	Asian	2/11/36	2/14/59	0.31
Rathore et al[25]	2008	Korea	Asian	9/55/135	11/74/230	0.11

Notes: [a]The ref was referred to the reference numbers in this study; [b]The *P*-value of the Hardy–Weinberg equilibrium; [c]Not available.
Abbreviations: HIV, human immunodeficiency virus; Ref, reference, AA/AG/GG, the genotype frequencies of -403G/A polymorphism.

The visual inspection of funnel plot asymmetry is shown in Figure 3, which presents no significant publication bias in any genetic models.

Discussion

Meta-analysis offers a powerful method to synthesize similar types of data obtained from independent studies with similar targets.[26] Up to now, the association between chemokines and their receptor gene polymorphisms and the risk of HIV-1 infection has been investigated by many studies. A recent meta-analysis suggests that the *RANTES* -28G allele might play a role in the resistance to HIV-1 infection among Asians,[27] and a lower copy number of *CCL3L1* might increase the susceptibility to HIV-1 infection,[28] while other two genes, polymorphisms *CCR2*-Val64Ile and *CCR5*-Δ32, have not been shown to have any association with HIV-1 infections.[29,30]

The association between *CCL5* -403G/A polymorphism and the risk of HIV-1 infection had been presented in many studies, but the results were inconsistent. Here we performed a meta-analysis to find a more definitive conclusion. Finally, a total of ten studies composed of 5,127 subjects were pooled for the analysis. We found that -403A/A decreased the risk of HIV-1 infection in the dominant model among Africans, also in the homozygous model, and the recessive model among Asians.

The interactions between the viral envelope glycoproteins, the CD4 receptor, and HIV-1 coreceptors are critical for the entry of HIV-1 into $CD4^+$ cells. As the primary HIV-1 coreceptors, the chemokine receptors, CCR5 and CXCR4, can emerge during the later stages of HIV-1 infection by using their functional regions, R5 in HIV-1 strains and Cy in X4-HIV-1 strains, respectively. By competitive binding and downregulation of CCR5, CCL5 can inhibit HIV-1 cell entry and replication.[31] It has also been reported that variants in the HIV-1 coreceptors and their natural ligands have been shown to change HIV-1 susceptibility, transmission, and progression.[3,4] At the genetic level, the mutant allele -403A can decrease the rate of $CD4^+$ T cell, and demonstrated increased promoter activity when tested by luciferase reporter gene assay in both U937 and Jurkat cell lines.[10,13] Here is the probable mechanism to explain that -403A/A could be resistant to HIV-1 infection.

Table 2 Results of *CCL5* -403G/A polymorphism on the risk of HIV-1 infection

Variables	N[a]	A vs G		AA vs GG		AA+AG vs GG		AA vs AG+GG	
		OR (95% CI)	Phet[b]	OR (95% CI)	Phet	OR (95% CI)	Phet	OR (95% CI)	Phet
Total	11	0.84 (0.66–1.08)	<0.001	0.63 (0.39–1.02)	<0.001	0.84 (0.63–1.11)	<0.001	0.70 (0.46–1.06)	0.006
Ethnicity									
African	3	0.50 (0.23–1.09)	<0.001	0.31 (0.09–1.16)	<0.001	0.44 (0.21–0.94)	0.002	0.45 (0.15–1.38)	<0.001
Asian	4	0.92 (0.68–1.25)	0.043	0.62 (0.42–0.90)	0.175	0.91 (0.74–1.11)	0.100	0.62 (0.45–0.93)	0.261
Caucasian	4	1.10 (0.90–1.14)	0.458	1.07 (0.55–2.07)	0.421	1.13 (0.89–1.43)	0.559	1.02 (0.53–1.97)	0.435

Notes: [a]Number of studies included in the meta-analysis; [b]The *P*-value of *Q*-test for heterogeneity test.
Abbreviations: CCL5, chemotactic chemokine ligand 5; HIV, human immunodeficiency virus; OR, odds ratio; CI, confidence interval.

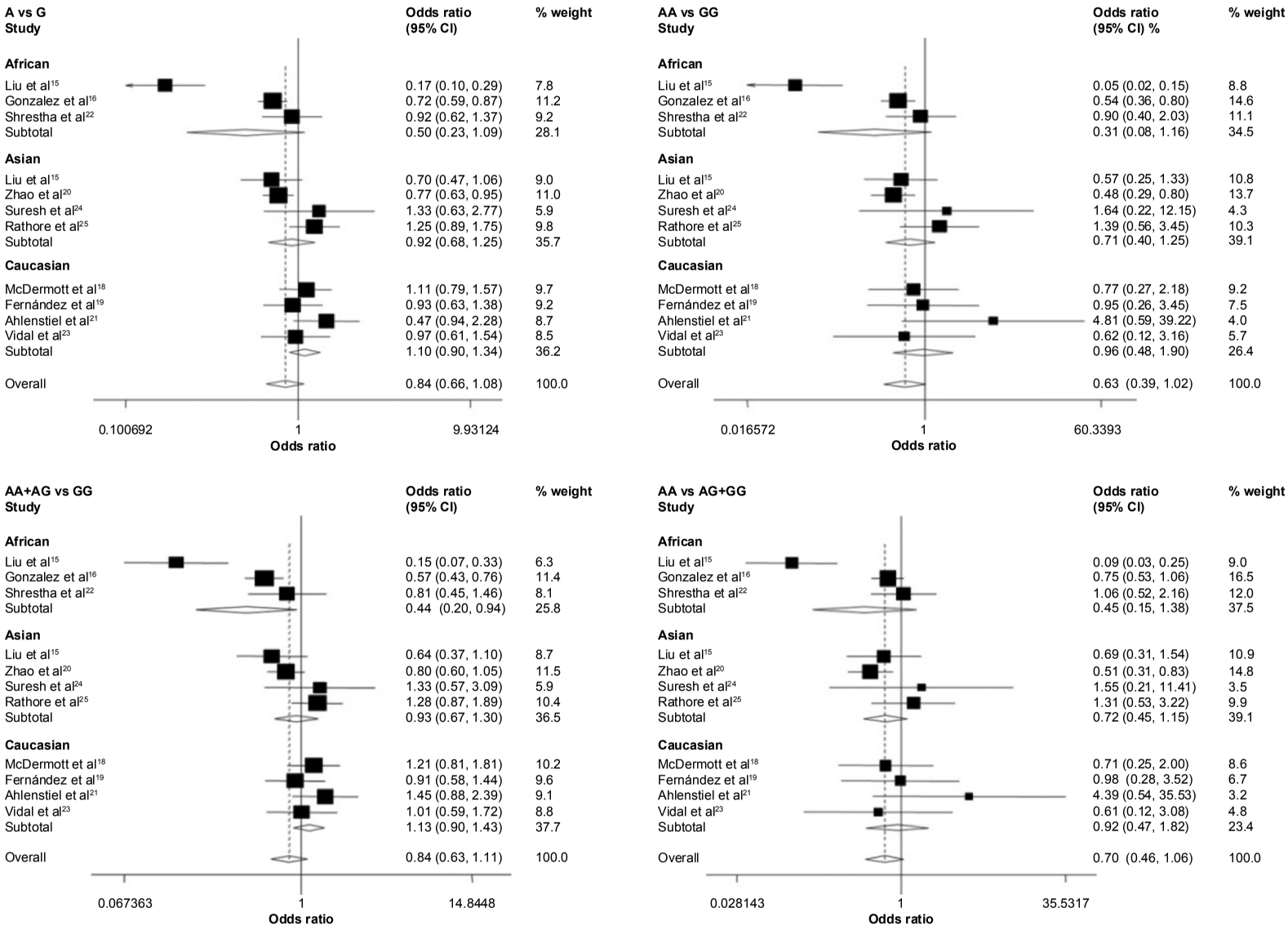

Figure 2 Forest plot analysis for assessing HIV-1 infection risk associated with *CCL5* -403G/A polymorphism under all genetic models by using the random-effect model.
Abbreviations: HIV, human immunodeficiency virus; CCL5, chemotactic chemokine ligand 5; CI, confidence interval.

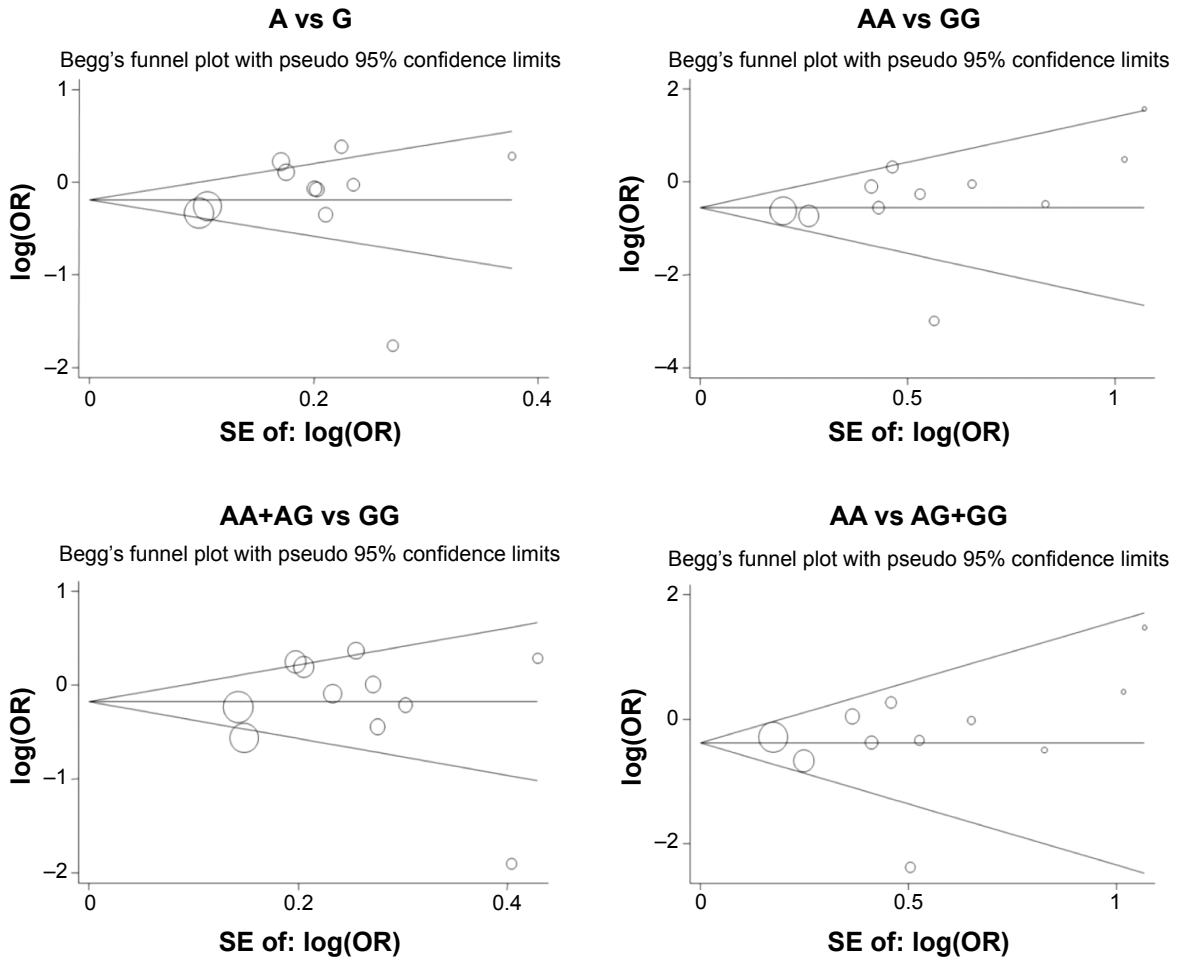

Figure 3 Funnel plots to detect publication bias in this meta-analysis.
Abbreviations: SE: standard error; OR, odds ratios; A vs G, allelic model; AA vs GG, homozygous model; AA+AG vs GG, dominant model; AA vs AG+GG, recessive model.

Heterogeneity is very common when the meta-analysis is conducted between the genetic association studies. In this study, we also found inter-study heterogeneity in the overall analysis. Several factors contribute to heterogeneity, such as the different genetic backgrounds of subjects, and the function and frequency of gene polymorphisms vary among different ethnic individuals.[32] Although the meta-analysis is a powerful statistical method, limitations are still inevitable. First, we included studies published in English and Chinese only, and the studies were retrieved from selected electronic databases. Relevant reports published in other languages and indexed in other electronic databases might have been ignored in this study. Second, sensitivity analysis demonstrated unstable results; thus, we should draw conclusions cautiously sometimes. Third, publication bias could not be excluded. The most common reason for publication bias is that the negative results in genetic association studies are hard to be published. In this study, we could get sufficient data from one study because of the negative result.[32] Fourth, gene–gene and gene–environment interactions are well known to affect host susceptibility to HIV-1 infection. In fact, many genes and their variants have been reported to be associated with HIV-1 infection, but enough evidence is not available to eliminate these confounding factors. Socioeconomic factors also play a critical role in the prevalence of HIV-1 infection and its progression. Finally, the route of transmission could be essential for the impact of polymorphisms in HIV-transmission. The HIV-1 exposed but seronegative individuals are at high risk of HIV infection, including commercial sex workers, people with hemophilia, discordant couples, intravenous drug users, and infants born to HIV-infected mothers. We had retrieved no more than 211 HIV-exposed but seronegative individuals consisting of 50 controls with hemophilia,[13] 82 heterosexual contact individuals,[24,25] and 79 male homosexuals.[18] It is not suitable to pool the data for meta-analysis. Finally, the controls in this study were almost healthy individuals.

Conclusion

In conclusion, this meta-analysis involving ten case-control studies suggests that the *CCL5* -403A/A genotype might be resistant to HIV-1 infection among Asians and Africans. While there are few limitations as shown above, we should make this conclusion cautiously. Further studies should be performed to evaluate this association among different ethnic populations against controls who have HIV-1-exposed history but remain seronegative.

Acknowledgment

We sincerely thank Dr Yao at the Nanchang University for extending help in revising the article draft.

Disclosure

The authors report no conflicts of interest in this work.

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