Association between the CCR2-Val64Ile polymorphism and susceptibility to HIV-1 infection: a meta-analysis

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Abstract. Several studies have investigated whether the CCR2-Val64Ile polymorphism affects susceptibility to human immune deficiency virus type-1 (HIV-1), with inconclusive results. Here, we performed a meta-analysis of the literature aiming to clarify the relationship between the polymorphism of CCR2-Val64Ile and the risk of HIV-1 infection. Twelve studies with a total of 6,599 patients, including infants, were selected for inclusion in the analysis. Crude odds ratios (ORs) with 95% confidence intervals (CIs) were assessed after the collected data were pooled for analysis. The risk estimates (OR) of HIV-1 infection were calculated in a homozygote comparison (OR=1.10, 95% CI 0.79-1.53), a heterozygote comparison (OR=0.98, 95% CI 0.70-1.37), a dominant model (OR=1.06, 95% CI 0.77-1.47) and a recessive model (OR=0.98, 95% CI 0.77-1.27, by random effects model) from among the total population. Taking into account the effect of sample size, ethnicity and control population, further stratified analyses were performed. The results showed no statistically significant difference in any genetic model, with the exception of the sub-analysis of mixed ethnicity (OR=0.33, 95% CI 0.11-0.98) using heterozygote comparison. The meta-analysis clarified that the CCR2-Val64Ile polymorphism has no effect on susceptibility to HIV-1 infection in the total population.

Introduction

Nearly 40 million people have been infected by human immune deficiency virus type-1 (HIV-1) worldwide (http://data.unaids.org/pub/Report/2007/JC1299-PolicyBrief-GIPA_en.pdf). Almost 27 million people have died of acquired immuno-deficiency syndrome (AIDS) caused by HIV-1 infection; an estimated 2 million people every year. HIV prevalence (http://www.who.int/features/factfiles/hiv/facts/en/index3.html) can therefore be considered the greatest social, economic and health crisis of modern times (1).

Previous studies have reported that the polymorphisms of certain chemokines and chemokine receptors, including stromal cell derived factor-1, RANTES, chemokine receptor-2 (CCR2) and chemokine receptor-5, play important roles in HIV-1 pathogenesis and transmission (2-8). Among them, CCR2, which is located at band p21 of chromosome 3 and contains three exons distributed over 7 kb of the genomic sequence (9), has been shown to be an additional co-receptor during cellular infection of several HIV-1 strains (10).

The common polymorphism of CCR2 is a G→A nucleotide transition at position 190 (counting from the ATG start codon), which results in the translocation of valine to isoleucine at position 64 of the CCR2 protein (11). Previously, CCR2-V64I has been reported to be associated with delayed disease progression to AIDS. Patients with this V64I polymorphism developed full-blown AIDS approximately 2-4 years later than homozygous individuals carrying the wild-type allele (11-15). To date, some studies have suggested that CCR2-V64I is associated with a reduced risk of acquiring HIV-1 infection (16-21), while others suggest the opposite (22-29).

To elucidate the role of the CCR2-V64I polymorphism in HIV-1 infection, we performed a meta-analysis of all eligible related studies to obtain a decisive resolution, which may aid in understanding the level of risk of HIV-1 infection.

Materials and methods

Search strategy. Articles dated up to April 2010 were recovered from the EMBASE and MEDLINE databases via the PubMed search engine using the key words 'CCR2', 'polymorphism' and 'HIV' in various combinations. The references of the retrieved articles were perused to identify additional related studies. The search was limited to articles published in English.

Selection criteria. The following selection criteria were used to determine which studies were eligible: i) studies about the CCR2-V64I polymorphism and the risk of HIV-1 infection; ii) studies defined as case-control studies; iii) studies containing enough data; iv) studies in which genotype frequency data were available for both cases and controls.

Data extraction. The following information was extracted from the eligible studies: first author, year of publication, country of study population, ethnicity, sample size, number of HIV-1-exposed, but uninfected, individuals, and distribution of CCR2 genotypes in cases and controls.

Statistical analysis. The primary assessment of effect was estimated by the odds ratio (OR) and associated 95% confidence interval (CI). The pooled ORs were respectively calculated in four genetic models: a homozygote comparison (Ile/Ile vs. Val/Val), a heterozygote comparison (Ile/Ile vs. Val/Ile), a dominant model (Ile/Ile vs. Val/Val + Val/Ile) and a recessive model (Ile/Ile + Val/Ile vs. Val/Val). Stratified analysis was performed according to sample size, control population and ethnicity. Heterogeneity was assessed by a χ^2-based Q-statistic test (30), and was considered significant if the P-value was <0.10. In such cases, the ORs were pooled according to the random-effects model (the DerSimonian and Laird model) (31); otherwise the fixed-effects model (the Mantel and Haenszel model) was used (32). Sensitivity analyses were conducted to evaluate the stability of the results. Analysis of publication bias was conducted with the funnel plot and Egger's linear regression asymmetry test, with a P-value of <0.05 taken to indicate a statistically significant publication bias. All statistical tests were conducted with STATA version 10.0 (Stata Corporation, College Station, TX, USA).

Results

Through a search of literature, 298 relevant articles were identified, 12 of which were determined to be eligible for further analysis (Fig. 1). The 12 studies included five populations of Asian descent, four mixed populations including participants of European, African or Asian descent and/or other ethnicities, two studies involving African subjects, and one including individuals of Caucasian descent. The total number of individuals analyzed was 6,599 (3,860 HIV-infected patients and 2,739 healthy controls). Six studies involved a total of 1,204 HIV-1-exposed, but uninfected, individuals. One study was performed on infants. The 12 eligible studies are summarized in Table I.

The distribution of the CCR2-V64I genotype among the study subjects is shown in Table II. Notably, some studies had found a relatively high frequency of the CCR2-V64I allele, ranging from 0.166 to 0.279 (21,25,26).

After pooling the data from the 12 studies for meta-analysis, the results were calculated in a homozygote comparison (OR=1.10, 95% CI 0.79-1.53, P=0.908 for heterogeneity) (Fig. 2A), a heterozygote comparison (OR=0.98, 95% CI 0.70-1.37, P=0.637 for heterogeneity) (Fig. 2B), a dominant model (OR=1.06, 95% CI 0.77-1.47, P=0.915 for heterogeneity) (Fig. 2C) and a recessive model (OR=0.98, 95% CI 0.77-1.27, P=0.000 for heterogeneity, by random effects model) (Fig. 2D).

Stratified analysis was further performed to adjust for the effect of heterogeneity and to illustrate the exact relationship between the CCR2-V64I polymorphism and susceptibility to HIV-1 infection. The studies were divided into three subgroups according to control population: one subgroup of high-risk controls including 1,204 exposed, but uninfected, individuals and 3,017 HIV-1-infected patients; one subgroup of unexposed
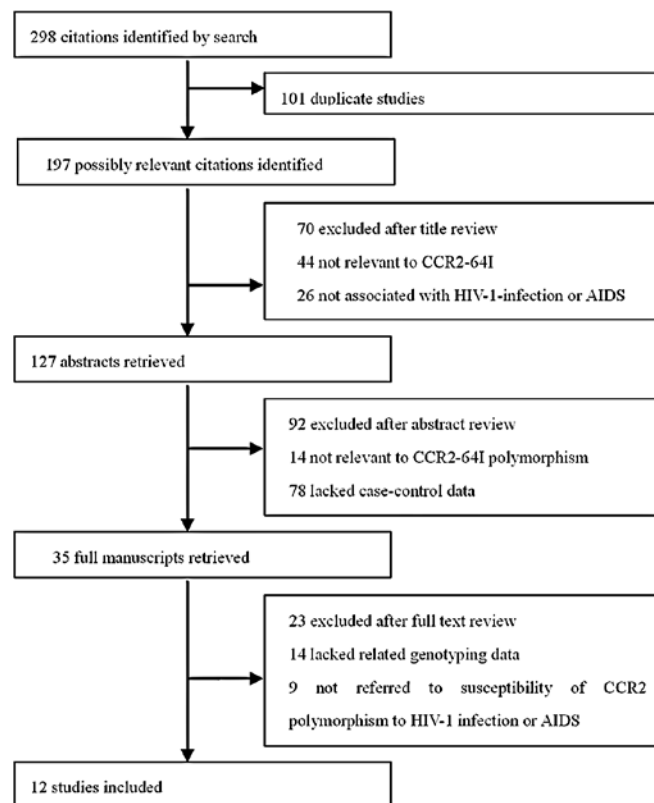
controls, including 2,754 unexposed individuals and 1,164 HIV-1-infected patients; and a final subgroup comprising patients from only one study (26), defined as a mother-to-child transmission subgroup. No evidence of a significant association between the CCR2-V64I polymorphism and susceptibility to HIV-1 infection was found in any genetic model among these subgroups (Table III). The studies were also stratified into two subgroups according to sample size: one comprising studies with more than 300 subjects; the other, studies with fewer than 300 subjects. In these subgroups, no apparent evidence of a significant association between the variant CCR2-V-64I genotype and the risk of HIV-1 infection was found (Table III). The studies were finally divided into four subgroups according to ethnicity. After pooling the data, no evidence of an association between the CCR2-V64I polymorphism and susceptibility to HIV-1 infection was found among Asian, African or Caucasian populations in any genetic model. In the mixed populations subgroup, a significantly decreased risk of HIV-1 was found in the heterozygote comparison (OR=0.33, 95% CI 0.11-0.98, P=0.352 for heterogeneity). However, no similar result was found in the homozygote comparison, the dominant model or the recessive model (Table III).

Sensitivity analysis was performed for various comparisons in the total population and all the subgroups. When one study (21) was excluded, the results became statistically significant (OR=0.61, 95% CI 0.41-0.91, P=0.196 for heterogeneity) in a recessive model among the subgroup of fewer than 300 subjects. However, the result for the recessive model in the mixed population remained insignificant (data not shown), and when another study (28) was excluded, the results in the recessive model among the Caucasian population were

Figure 1. Flow diagram of study searching and selection process.

Table I. Characteristics of included studies.

First author (reference)	Year	Country	Ethnicity	Sample size[a]	Sample size of high-risk groups[b] (case/control)
Roman	2002	Luxembourg	Mixed[c]	257/158	ND
Wang	2003	China	Asian	330/474	474
Philpott	2004	USA	Mixed[c]	1940/513	283
Paz-y-Mino	2005	Ecuador	Mixed[c]	50/222	ND
Brouwer	2005	Kenya	African	78/267[d]	ND
Carvalhaes	2005	Brazil	Mixed[c]	110/112	ND
Suresh	2006	India	Asian	49/110	35
Shrestha	2006	USA	African	100/100	100
Kaur	2007	India	Asian	180/221	ND
Wichukchinda	2008	Thailand	Asian	347/74	74
Parczewski	2009	Poland	Caucasian	168/150	ND
Tan	2010	China	Asian	251/238	238

[a]Only individuals for which genotype data was obtained. [b]HIV-1-exposed, but uninfected, individuals. [c]Individuals of European, African, Asian and/or other descent. [d]Infants. ND, no data.

Table II. Distribution of CCR2-Val64Ile genotype among HIV-1-infected cases and controls.

Author	Cases						Controls					
	Val/Val		Val/Ile		Ile/Ile		Val/Val		Val/Ile		Ile/Ile	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Roman	219	85.2	38	14.8	0	0.0	128	81.0	30	18.9	0	0.0
Wang	221	67.0	83	25.2	26	7.9	315	66.5	130	27.4	29	6.1
Philpott	1,670	86.1	251	12.9	19	1.0	445	86.7	61	11.9	7	1.4
Paz-y-Mino	20	40.0	28	56.0	2	4.0	169	76.0	40	18.0	13	6.0
Brouwer	42	53.8	32	41.0	4	5.1	191	52.0	160	43.6	16	4.4
Carvalhaes	98	89.1	12	10.9	0	0.0	84	75.0	28	25.0	0	0.0
Suresh	36	72.0	12	24.0	1	2.0	80	72.7	27	24.5	3	2.7
Shrestha	78	78.0	21	21.0	1	1.0	70	70.0	28	28.0	2	2.0
Kaur	144	80.0	32	17.7	4	2.2	173	78.2	44	19.9	4	1.8
Wichukchinda	239	68.9	95	27.4	13	3.7	49	66.2	21	28.4	4	5.4
Parczewski	136	81.0	31	18.5	1	0.6	117	78.0	31	42.8	2	1.3
Tan	130	51.8	102	40.6	19	7.6	140	58.8	85	35.7	13	5.5

also not statistically significant (data not shown). In other subgroups, sensitivity analysis revealed no significant results (data not shown).

Publication bias was assessed by Begg's funnel plot and Egger's test (data not shown) in the total population and all the subgroups. Egger's linear regression test suggested a significant publication bias in the dominant model (II vs. VV + VI) for total population (t=-2.73, P=0.026), but no evidence of publication bias in any other genetic model for the total population or subgroups (data not shown).

Discussion

In order to elucidate the association between the CCR2-V64I polymorphism and susceptibility to HIV-1 infection, we performed a meta-analysis of data from 12 eligible studies including a total of 3,860 HIV-infected patients and 2,739 controls. The results indicate that the CCR2-V64I polymorphism has no effect on the risk of HIV-1 infection among worldwide populations in any genetic model, consistent with the results of certain earlier studies (22-29). Taking into account
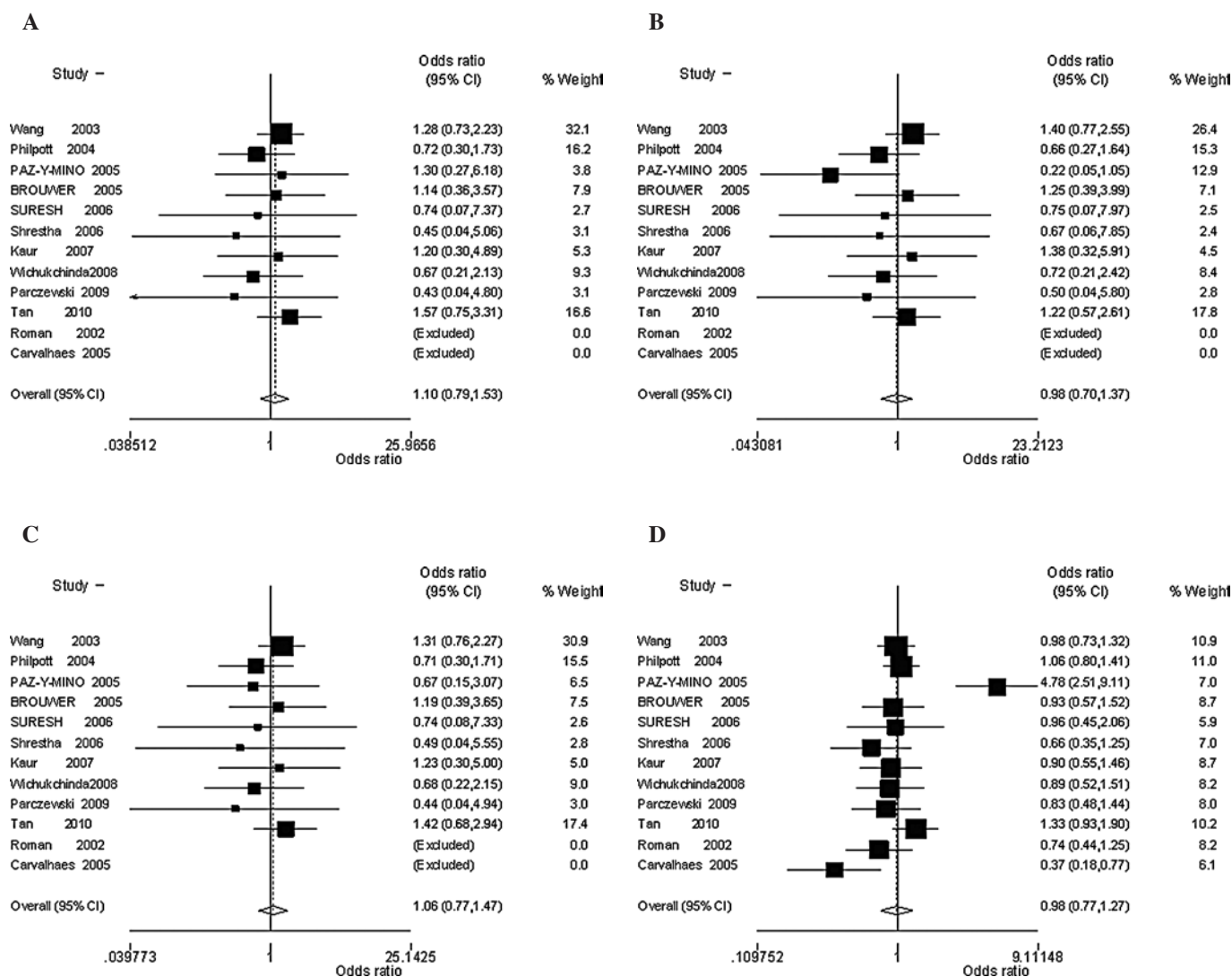
A

B

C

D

Figure 2. Meta-analysis of the relationship between the CCR2-V64I polymorphism and HIV-1 infection using (A) a homozygote comparison, (B) a heterozygote comparison, (C) a dominant model, and (D) a recessive model.

the effect of ethnicity, sample size and control population, stratified analyses were performed. The results obtained for the various subgroups did not point to an association between the CCR2-V64I polymorphism and HIV-1 infection, with one exception: populations of mixed ethnicity.

Among populations of mixed ethnicity, the CCR2-64II genotype was associated with a reduced risk of HIV-1 infection as compared to the CCR2-64VI genotype. This suggests that the association between the CCR2-V64I polymorphism and susceptibility to HIV-1 infection varies between ethnic populations. One study reported a higher frequency of CCR2-V64I genotypes in Chinese individuals relative to Caucasians (19). Other studies showed a higher frequency of the CCR2-V64I genotype among African populations relative to populations of European descent (5,11). Nevertheless, no significant association was found between the CCR2-V64I polymorphism and susceptibility to HIV-1 infection in analyses of the total population. However, among the mixed ethnic population, there were relatively few people recruited to each investigation. In conclusion, the protective effect of

CCR2-V64I on HIV-1 infection that we observed may have been due to different ethnic backgrounds and small sample size. We conclude that further large-scale analysis is required.

The entry of HIV into the CD4 lymphocyte is dependant on the binding of necessary co-receptors (4). Certain chemokine receptors have been demonstrated to play critical roles in HIV-1 infection (3). Some polymorphisms or mutations may have effects on the ability of the virus to infect cells, influencing HIV-1 infectivity (29). CCR2, which acts as a co-receptor mediating infection of some strains of HIV-1, has been found to modulate the susceptibility to HIV-1 of individuals carrying a mutation at position 64 (11). However, our present analysis found that the CCR2-V64I polymorphism has no effect on susceptibility to HIV-1 infection. According to several studies, the CCR2-64I protein preferentially dimerizes with CXCR4 polypeptides to affect HIV-1 entry and delay disease progression to AIDS (11-14). It is possible that the CCR2-V64I polymorphism is more closely associated with slower progression to AIDS than with susceptibility to HIV-1 infection.

Table III. Meta-analysis of association between the CCR2-Val64Ile polymorphism and susceptibility to HIV-1 infection.

Study groups (case/control)	No.[a]	Total sample size	II vs.VV OR (95% CI)	P-value[b]	II vs.VI OR (95% CI)	P-value[b]	II vs. VV + VI OR (95% CI)	P-value[b]	II + VI vs. VV OR (95% CI)	P-value[b]
Total	12	3860/2739	1.10 (0.79-1.53)	0.908	0.98 (0.70-1.37)	0.637	1.06 (0.77-1.47)	0.915	0.98 (0.77-1.27)[d]	0.000
Control population										
High-risk	6	3017/1204	1.16 (0.82-1.65)	0.788	1.15 (0.76-1.73)	0.900	1.17 (0.80-1.71)	0.844	1.05 (0.88-1.24)	0.407
Healthy	7	2754/1164	0.81 (0.42-1.56)	0.834	0.54 (0.27-1.08)	0.576	0.71 (0.36-1.43)	0.915	0.99 (0.61-1.62)[d]	0.000
Mother-to-child										
Transmission	1	78/267	1.14 (0.36-3.57)	-	1.25 (0.39-3.99)	-	1.19 (0.39-3.65)	-	0.93 (0.57-1.52)	-
Sample size										
≥300 subjects	8	3551/2195	1.12 (0.80-1.59)	0.762	1.11 (0.77-1.59)	0.818	1.12 (0.80-1.58)	0.802	1.00 (0.87-1.15)	0.691
<300 subjects	4	309/544	0.87 (0.27-2.75)	0.755	0.35 (0.11-1.09)	0.607	0.64 (0.21-1.99)	0.969	1.03 (0.34-3.15)[d]	0.000
Ethnicity										
Asian	5	1157/1117	1.24 (0.84-1.84)	0.791	1.22 (0.81-1.85)	0.889	1.22 (0.83-1.81)	0.844	1.04 (0.86-1.25)	0.609
African	3	1274/750	0.77 (0.36-1.65)	0.665	0.85 (0.39-1.86)	0.688	0.80 (0.38-1.70)	0.650	0.88 (0.67-1.14)	0.624
European	2	499/216	0.60 (0.10-3.62)	0.652	0.57 (0.08-3.84)	0.851	0.60 (0.10-3.65)	0.678	1.00 (0.63-1.59)	0.238
Mixed	4	929/656	1.04 (0.32-3.35)	0.692	0.33 (0.11-0.98)[c]	0.352	0.72 (0.24-2.19)	0.877	1.13 (0.43-2.95)[d]	0.000

[a]No. of comparisons. [b]P-value of Q-test for heterogeneity. [c]Statistically significant results. [d]A random effects model was used when the P-value for heterogeneity was <0.1; otherwise, a fixed-effects model was used.

Although meta-analysis is a powerful statistical method, it has certain limitations. In the present study, a publication bias existed in a dominant model among the total population. Therefore, more studies with larger sample sizes are required. Additionally, sensitivity was detected in a recessive model among some subgroups.

In conclusion, our results suggest that the CCR2-V64I polymorphism has no effect on reducing the risk of HIV-1 infection. Since the distribution of genotypes varies according to race and ethnicity, studies on more subjects recruited from specific regions worldwide are required.

Acknowledgements

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