Effect of \textit{CCR5-}\textDelta{32} Heterozygosity on HIV-1 Susceptibility: A Meta-Analysis

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Abstract

Background: So far, many studies have investigated the distribution of CCR5 genotype between HIV-1 infected patients and uninfected people. However, no definite results have been put forward about whether heterozygosity for a 32-basepair deletion in CCR5 (CCR5-Δ32) can affect HIV-1 susceptibility.

Methods: We performed a meta-analysis of 18 studies including more than 12000 subjects for whom the CCR5-Δ32 polymorphism was genotyped. Odds ratio (OR) with 95% confidence interval (CI) were employed to assess the association of CCR5-Δ32 polymorphism with HIV-1 susceptibility.

Results: Compared with the wild-type CCR5 homozygotes, the pooled OR for CCR5-Δ32 heterozygotes was 1.02 (95%CI, 0.88–1.19) for healthy controls (HC) and 0.95 (95%CI, 0.71–1.26) for exposed uninfected (EU) controls. Similar results were found in stratified analysis by ethnicity, sample size and method of CCR5-Δ32 genotyping.

Conclusions: The meta-analysis indicated that HIV-1 susceptibility is not significantly affected by heterozygosity for CCR5-Δ32.

Introduction

Inter-individual variability in susceptibility to HIV-1 infection, transmission, disease progression, and response to antiviral therapy has been attributed to host variability in multiple genes [1]. CC chemokine receptor 5 (CCR5) and CXC chemokine receptor 4 (CXCR4) are co-receptors for the entry of human immunodeficiency virus type 1 (HIV-1) into target cells [2]. A natural knockout deletion of 32 bases in CCR5 gene introduces a premature stop codon resulting in truncated protein product [3]. People homozygous for CCR5-Δ32 are naturally resistant to R5 HIV infection and the heterozygous state is associated with up to 2–4 years delay in disease progression [4]. Recently, Allers et al reported that they have successfully cured a HIV infected patient through CCR5-Δ32/Δ32 stem cell transplantation [5,6]. On the other hand, the evidence for protection from HIV-1 infection among CCR5-Δ32 heterozygotes is mixed. A meta-analysis of Despina et al suggested that perinatal infection rates are not strongly determined by the number of functional CCR5 receptors in the children [7]. For adults, some studies have reported that CCR5-Δ32 heterozygotes could be protective against HIV transmission [8–15], whereas others have not confirmed that [16–28]. Therefore, we performed a meta-analysis of the accumulated data to address this question definitively.

Materials and Methods

Search Strategy and Study Selection

English database of Google Scholar (GS), PubMed and Chinese database of CNKI were searched till June 2011 using key words: CCR5-Δ32 and HIV-1. Studies satisfying the following criteria were included: case-control studies reporting the association of CCR5-Δ32 genotype with HIV-1 susceptibility, distribution of CCR5-Δ32 genotype between the cohorts was shown, not a prenatal HIV-1 infection study.

Data Extraction and Statistical Analysis

Two reviewers (SiJie Liu, Jie Wu) independently performed data extraction and then checked the results together. The following information was extracted from included studies: authors, year of publication, ethnicity, country, sample size, method of CCR5-Δ32 genotyping and CCR5-Δ32 genotype of cohorts.

Odds ratio (OR) and its 95% confidence intervals (CI) were used to evaluate the association of CCR5-Δ32 heterozygotes with HIV-1 susceptibility. Subgroups were identified by ethnicity, sample size and method of CCR5-Δ32 genotyping. A chi-square-based Q-test was carried out to assess heterogeneity across studies [29]. A P value less than 0.10 was used to denote statistical significance. Fixed effects (Mantel and Haenszel) model was employed to pool the effects of studies without heterogeneity.
otherwise the random effects (Dersimonian and Laird) model was used [30,31]. Publication bias was evaluated by Egger’s and Begg’s test with funnel plots [32,33]. Asymmetry of the funnel plot suggests publication bias. A \( P \) value less than 0.05 was used to denote statistical significance. One-way sensitivity analyses were performed to examine the influence of individual studies on meta-analysis’s results. Data were analyzed using Stata version 10.0 (StataCorp, College Station, Tex).

**Results**

Figure 1 summarized the selection process of literatures. The electronic search yielded 1232 records, after screening over titles and/or abstracts, 24 articles were selected for further review. Finally, 18 studies involving 6427 cases and 5809 controls were included in the meta-analysis. Study sample size ranged from 140 to 2605 subjects. Study characteristics of the 18 eligible studies were summarized in Table 1. Distribution of CCR5 genotype among subjects was shown in Table 2. Briefly, 9 studies involved Caucasian subjects [8,10–12,17,22–24], 4 studies involved Mongoloid subjects [16,21,25,28], 3 studies involved African subjects [8,12,24], 3 studies involved Latina subjects [12,18,27]. In addition to CCR5-\( \Delta32 \) genotype, 8 studies provided the subjects’ CCR2-64I genotype [10,16,19–21,26–28], 5 studies provided the subjects’ SDF-1 genotype [16,20,21,26,27]. All studies were done in subjects of mixed genders except that by Downer et al [24], which only included women.

Compared with the wild-type CCR5 homozygotes, the pooled OR for CCR5-\( \Delta32 \) heterozygotes was 1.02 (95%CI, 0.88–1.19, \( p = 0.073 \)) for healthy controls (HC) (figure 2a) and 0.95 (95%CI, 0.71–1.26, \( p = 0.182 \)) for exposed uninfected (EU) controls (figure 2b). There was no significant between-study heterogeneity.

We also performed stratified analysis by ethnicity, sample size and method of CCR5-\( \Delta32 \) genotyping. The results were summarized in Table 3. All the results were consistent with overall analysis and no publication bias were observed.

Sensitive analysis was conducted by deleting one study at a time to examine the influence of individual data-set to the pooled ORs. All of the corresponding pooled ORs were not materially altered (Data not shown).

**Discussion**

Meta-analysis offers a powerful method to synthesize information of independent studies with similar intention. It has been proved that CCR5-\( \Delta32 \) homozygotes are associated with near complete protection to HIV-1 infection. Moreover, published data have demonstrated that a disease-retarding effect of CCR5-\( \Delta32 \) heterozygosity in HIV-1 infected individuals [34]. Whereas it
### Table 1. Characteristics of selected studies in the meta-analysis.

| Study (year)   | Country   | Genotyping method | Ethnicity | Sample size (case/control) |
|---------------|-----------|-------------------|-----------|-----------------------------|
| Battiloro (2000) [17] | Italy      | PCR               | Caucasian | 256/806                     |
| Deng (2004) [28]   | China      | PCR               | Mongoloid | 88/119                      |
| Diaz (2000) [18]  | Colombia   | PCR               | Latina    | 29/188                      |
| Downer (2002) [24] | USA        | PCR-RFLP          | Mixed     | 929/445                     |
| Grimaldi (2002)[13] | Brazil     | PCR               | Mixed     | 113/549                     |
| Li (2003) [26]    | China      | PCR               | Mongoloid | 94/46                       |
| Liu (2004) [20]   | USA        | PCR               | Mixed     | 316/513                     |
| Lockett (1999) [19]| Britain    | PCR               | Mixed     | 86/105                      |
| Oh (2008)[8]      | Germany    | PCR               | Caucasian | 610/427                     |
| Papa (2000) [10]  | Greece     | PCR               | Caucasian | 138/239                     |
| Paz-y-Mino (2005) [27] | Ecuador   | PCR               | Latina    | 295/50                      |
| Philpott (2003) [12] | USA       | PCR-RFLP          | Mixed     | 2047/558                    |
| Takacova (2008) [22] | Slovakia | PCR               | Caucasian | 162/198                     |
| Tan (2010) [16]   | China      | PCR               | Mongoloid | 250/237                     |
| Tang (2010) [25]  | China      | PCR-LDR           | Mixed     | 245/223                     |
| Trecarichi (2006)[11] | Italy   | PCR               | Caucasian | 120/120                     |
| Veloso (2010)[23] | Spain      | PCR               | Caucasian | 184/236                     |
| Wang (2003) [21]  | China      | PCR               | Mongoloid | 330/474                     |

### Table 2. Distribution of CCR5 genotype of included studies.

| Study        | Ethnicity | HIV-infected | Healthy Controls | Exposed but uninfected |
|--------------|-----------|--------------|------------------|------------------------|
|              |           | ++/Δ¹ | ++/Δ² | +/+ | +/Δ | ++/ | +/Δ |  |
| Battiloro    | Caucasian | 232 | 24 | 744 | 62 |  |
| Deng         | Mongoloid | 88  | 0  | 117 | 2  |  |
| Diaz         | Latina    | 28  | 1  | 142 | 8  | 37 | 1  |  |
| Downer       | Mixed     | 879 | 50 | 422 | 23 |  |
| Grimaldi     | Mixed     | 103 | 10 | 520 | 29 |  |
| Li           | Mongoloid | 90  | 4  | 45  | 1  |  |
| Liu          | Mixed     | 261 | 55 | 354 | 68 | 69 | 22 |  |
| Lockett      | Mixed     | 63  | 23 | 38  | 10 | 40 | 17 |  |
| Oh           | Caucasian | 595 | 115| 352 | 75 |  |
|              | African   | 35  | 0  | 25  | 1  |  |
| Papa         | Caucasian | 132 | 6  | 216 | 23 |  |
|              | African   | 35  | 0  | 25  | 1  |  |
| Paz-y-Mino   | Latina    | 292 | 3  | 50  | 0  |  |
| Philpott     | Mixed     | 1940| 107| 513 | 45 |  |
| Takacova     | Caucasian | 137 | 25 | 164 | 34 |  |
| Tan          | Mongoloid | 226 | 24 | 222 | 15 |  |
| Tang         | Mongoloid | 221 | 24 | 209 | 14 |  |
| Trecarichi   | Caucasian | 111 | 9  | 24  | 6  |  |
| Veloso       | Caucasian | 144 | 40 | 174 | 26 | 31 | 5  |  |
| Wang         | Mongoloid | 329 | 1  | 473 | 1  |  |

¹CCR5 homozygotes.
²CCR5-Δ32 heterozygotes.

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Figure 2. Odds ratio of HIV-1 infection of CCR5Δ32 heterozygotes versus wild type CCR5 homozygotes. The area of the black square reflects the weight of each study. The diamonds represent the combined odds ratio and 95% confidence interval using the fixed effects model for (a) healthy controls (HC) and (b) exposed uninfected controls (EU).

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remained unclear if a heterozygosity for CCR5-Δ32 could affect HIV-1 susceptibility. Thus we performed this meta-analysis involving 18 eligible studies with 6427 patients and 5809 controls. The study demonstrated that CCR5-Δ32 heterozygosity has little or no protective effect against HIV-1 infection among adults. This result is similar to a previous study of perinatal HIV-1 infection [7]. Several factors might underlie the lack of observed association between CCR5-Δ32 heterozygosity and HIV-1 susceptibility. First, the expression of CCR5 is influenced by factors other than CCR5 genotype. Even an individual with CCR5-Δ32 heterozygosity could still express high level of CCR5 [35,36]. Second, susceptibility to HIV-1 infection is affected by a combination of genes besides CCR5. The CCR5-Δ32 heterozygotes couldn’t provide a full resistant to HIV-1 infection as the homozygotes. It is possible that a single Δ32 allele exerts a protective effect against HIV-1 infection only if it occurs combined with other protective factors [9].

There are a number of limitations to our study. First, although test of publication bias have generated negative results, studies solely in conference or in local journals may have been overlooked. Second, HIV-1 of X4 strain take advantage of CXCR4 as co-receptor. It has been reported that new infections in individuals are primarily established by strains that use R5 [37–39]. Currently, it is remains controversial about if HIV could use CXCR4 as co-receptor in primary HIV infection [40]. Primary infection with CXCR4-using HIV-1 strains is believed to be a rare event [41]. Thus, we might believe that in most of the cases HIV-1 R5 strain cause the initial infection, rather than the X4 strain. Although we couldn’t exclude the interference of X4 viruses, it is unlikely that virus of X4 strain would significantly affect the results. Third, controls of some studies were solely derived from healthy individuals. For studies concerning disease susceptibility, it’ll be more proper to take samples from exposed uninfected people as controls. Fourth, susceptibility to HIV-1 is influenced by multiple factors other than CCR5, they might interfere the precision of analysis.

In conclusion, our study involving more than 12000 subjects suggested that CCR5-Δ32 heterozygosity has little effect on protecting from HIV-1 infection. Therefore, other chemokine receptors and transmission mechanisms may play a more important role.

**Author Contributions**

Conceived and designed the experiments: HZZ. Performed the experiments: SJL CJK JW. Analyzed the data: SJL JW. Contributed reagents/materials/analysis tools: SJL CJK HY. Wrote the paper: SJL.

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**Table 3. Stratified analysis of CCR5-Δ32 heterozygotes and susceptibility to HIV-1.**

| Variable              | Healthy Controls | Exposed but uninfected |
|-----------------------|------------------|------------------------|
|                       | OR (95%CI)       | P                      |
| Ethnicity             |                  |                        |
| African               | 1.17 (0.71–1.93) | 0.604                  |
| Caucasian             | 1.06 (0.73–1.53) | 0.005                  |
| Latina                | 1.28 (0.62–2.61) | 0.942                  |
| Mongoloid             | 0.63 (0.39–1.01) | 0.995                  |
| Genotyping Method     |                  |                        |
| PCR                   | 0.94 (0.79–1.12) | 0.239                  |
| PCR-RFLP              | 1.32 (0.99–1.78) | 0.110                  |
| Sample Size           |                  |                        |
| >800                  | 1.09 (0.91–1.30) | 0.152                  |
| ≤800                  | 0.87 (0.65–1.16) | 0.233                  |

*P value of Q-test for heterogeneity test.

**Figure 3. Funnel plots to detect publication bias in the meta-analysis.** (a) Healthy controls considered; (b) exposed uninfected controls considered. The horizontal line indicates the pooled log odds ratio (OR) and guidelines to assist in visualizing the funnel are pooled at 95% pseudo confidence limits for this estimate.

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References

1. Kaur G, Mehra N (2009) Genetic determinants of HIV-1 infection and progression to AIDS: susceptibility to HIV infection. Toxicon; 52: 209-301.

2. O’Brien SJ, Moran JP (2000) The effect of genetic variation in chemokines and their receptors on HIV transmission and progression to AIDS. Immuno Rev; 177: 99–111.

3. Liu R, Paxton WA, Choe S, Ceradini D, Martin SK, et al. (1996) Homozygous defects in HIV-1 co-receptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell; 86: 967–77.

4. Dean M, Carrington M, Windler C, Hurtley GA, Smith MW, et al. (1996) Genetic restriction of HIV-1 infection and progression to AIDS by a deletion of the CCR5 structural gene. Science; 273: 1036–62.

5. Hutter G, Nowak D, Mounier M, Gam/AP, Schmied M, A, (2001) Long-term control of HIV by CCR5 Delta32/Delta32 stem cell transplantation. N Engl J Med; 345: 692–8.

6. Allers K, Hutter G, Hofmann J, Luddemkelper C, Rieger K, et al. (2011) Evidence for a difference in outcome of HIV-1 infection by CCR5Delta32/Delta32 stem cell transplantation. Blood; 117: 2791–9.

7. Contopoulos-Ioannidis DG, O’Brien TR, Goedert JJ, Rosenberg PS, Ioannidis JP (2003) Effect of CCR5-Delta32 heterozygosity on the risk of perinatal HIV-1 infection: a meta-analysis. J Acquir Immune Defic Synd; 32: 70–6.

8. Oh DY, Husen J, Kucherer C, Neumann K, Oh N, et al. (2000) CCR5-D32 Genotypes in a German HIV-1 Serocohetero Cohort and Report of HIV-1 Infection in a CCR5/D32 Homozygous Individual. PLoS One; 3: e2747.

9. Hladik F, Liu H, Speelman L, Livingstone-Rosendorf D, Wilson S, et al. (2005) Combined effect of CCR5 Delta32 heterozygosity and the CCR5 promoter polymorphism -2459 A/G on CCR5 expression and resistance to human immunodeficiency virus type 1 transmission. J Virol; 79: 11677–84.

10. Papa A, Papadimitriou E, Advani G, Clewley JP, Malisis N, et al. (2000) HIV-1 co-receptor CCR5 and CCR2 mutations among Greeks. FEBS Lett; 525: 87–9.

11. Trecarichi EM, Tumbarello M, de Gaetano Donati K, Tamburrini E, Cauda R, et al. (2000) Prevalence of CCR5-Delta32, CCR2b 64I and SDF1 3'A in HIV-1 infected and uninfected high-risk Uighurs in Xinjiang, China. J Acquir Immune Defic Synd; 28: 98–201.

12. Li GH, Wei FL, He Y, Sun F, Zhou ZQ, et al. (2003) Impact of the polymorphisms of CCR5, CCR2 and SDF1 on HIV71 heterosexual transmission. Chin J Microbiol Immunol; 23: 65–9.

13. Pasc-Mino C, Moellon SX, Celi AP, Wite T, et al. (2005) CCR5 Delta32, CCR2b 64I, and SDF1 3'A polymorphisms related to resistance to HIV-1 infection and disease in the Ecuadorian population. Hum Biol; 77: 21–6.

14. Deng XL, Hong KX, Chen JP, Ruan YH, Xu MY, et al. (2004) Genetic polymorphism of human immunodeficiency virus co-receptor CCR5-Delta32 and CCR2b 64I alleles in Chinese Yi Ethnic group in Sichuan. Chin J Epidemiol; 25: 050–3.

15. Cochran WG (1954) The combination of estimates from different experiments. Biometrics; 10: 9.

16. Mantel N, Haenszel W (1959) Statistical aspects of the analysis of data from retrospective studies of disease. J Nat Cancer Inst; 22: 19–48.

17. Dersimonian R, Laird N (1986) Meta-analysis in clinical trials. Control Clin Trials; 7: 37–58.

18. Egger M, Davey Smith G, Schneider M, Minder C (1997) Bias in meta-analysis detected by a simple, graphical method. BMJ; 315: 29–34.

19. Begg CB, Mazumdar M (1994) Operating characteristics of a rank correlation test for publication bias. Biometrics; 50: 889–99.

20. Ioannidis JP, Rosenberg PS, Goedert JJ, Ashon LJ, Benfield TL, et al. (2001) Effects of CCR5-Delta32, CCR2b 64I, and SDF1 3'A alleles on HIV-1 disease progression: An international meta-analysis of individual-patient data. Ann Intern Med; 135: 82–95.

21. Wu L, Paxton WA, Kasrum N, Ruffing N, Rotman JB, et al. (1997) CCR5 levels and expression pattern correlate with infectability by macrophage-tropic HIV-1 in vitro. J Exp Med; 183: 681–91.

22. de Roos HA, Blaak H, Brouwer M, Schuitemaker H (1999) CC chemokine receptor 5 genotype and susceptibility to transmission of human immunodeficiency virus type 1 in women. J Infect Dis; 180: 369–75.

23. Brdka F, Domingo P, Alonso-Villaverde C, et al. (2010) Complex cytokine polymorphisms and cytokine expression in HIV-1 infected and uninfected individuals in HIV-1 risk groups. J Acquir Immune Defic Synd; 565–74.

24. Takacova M, Negova P, Habekova M, Staneckova D (2008) Prevalence of a 32 bp deletion in the gene for human immunodeficiency virus 1 co-receptor ccr5 in slovak population. Acta Virol; 52: 61–4.

25. Veleo S, Olona M, Garcia F, Domingo P, Alonso-Villaverde C, et al. (2010) Effect of TNF-alpha genetic variants and CCR5 Delta 32 on the vulnerability to HIV-1 infection and disease progression in Caucasian Spaniards. BMC Med; 11: 3.

26. Downer MV, Hodge T, Smith DK, Qari SH, Schuman P, et al. (2002) Regional variation in CCR5-Delta32 gene distribution among women from the US HIV Epidemiology Research Study (HERS). Gene Immun; 3: 95–8.

27. Deng XL, Hoa SX, Zhang JY, Tan XH (2010) Research on the Polymorphism of HIV Infection-Related Gene CCR5-D32 in HIV Highly-Infected Uygur Population in Yili Prefecture of Xinjiang Uygur Autonomous Region. Journal of Shiley University (Natural Science); 20: 90–201.

28. Li GH, Wei FL, He Y, Sun F, Zhou ZQ, et al. (2003) Impact of the polymorphisms of CCR5, CCR2 and SDF1 on HIV71 heterosexual transmission. Chin J Microbiol Immunol; 23: 65–9.

29. Dings XL, Hong KX, Chen JP, Ruan YH, Xu MY, et al. (2004) Genetic polymorphism of human immunodeficiency virus co-receptor CCR5-Delta32 and CCR2b 64I alleles in Chinese Yi Ethnic group in Sichuan. Chin J Epidemiol; 25: 050–3.

30. Nyland TD, Goedert JJ, Rosenberg PS, Ioannidis JP (2003) Effect of CCR5-Delta32 heterozygosity on the risk of perinatal HIV-1 infection: a meta-analysis. J Acquir Immune Defic Synd; 32: 70–6.