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The Genetic Associations and Epistatic Effects of the CCR5 Promoter and CCR2-V64I Polymorphisms on Susceptibility to HIV-1 Infection in a Northern Han Chinese Population

Jiapeng Lu,1,2,* Aijuan Sheng,3 Youxin Wang,1,2,* Ling Zhang,1,2 Jingjing Wu,1,2 Manshu Song,1,2 Yan He,1,2 Xinwei Yu,1,2 Feifei Zhao,1,2 Yezhou Liu,1,2 Shuang Shao,1,2 Jie Lan,4 Hao Wu,3 and Wei Wang2,4,5

The outcome of human immunodeficiency virus (HIV)-1 infection and course to AIDS are variable among individuals. Both chemokine receptor 5 (CCR5) and CCR2 gene polymorphisms play essential roles in the susceptibility of HIV-1 infection. To investigate the main and epistatic effects of the CCR5 promoter and CCR2-V64I polymorphisms on HIV-1 infection in the Northern Han Chinese, subjects of 91 HIV-1-infected patients and 91 health controls were recruited. Single-nucleotide polymorphisms (SNPs) in the CCR5 promoter region and CCR2-V64I variants were genotyped. In the single-locus analysis, CCR5 58755-G and CCR5 59653-T alleles were significantly associated with HIV-1 infection (odds ratio [OR] = 0.529, 95% confidence interval [CI]: 0.295–0.948; OR = 1.710, 95% CI: 1.039–2.814). After adjustment with age and gender, subjects with the CCR5 59653-CT genotype showed the increased risk of HIV-1 infection compared with those with the wild-type CC genotype (adjusted OR = 2.502; 95% CI: 1.332–4.698). No positive association was observed in other SNPs. Haplotype-based association analysis revealed that the haplotype TATGC was associated with the susceptibility to HIV-1 infection (p = 0.003). Besides, we found the significant epistatic effects between the CCR5 58755-A/G and CCR5 59029-A/G polymorphisms associated with the lower risk of HIV-1 infection. In addition, we also identified the best three-factor interaction model, including the CCR5 58755-A/G, 59029-A/G, and CCR2-V64I polymorphisms, indicating that there were also strong gene–gene interactions between the CCR5 promoter and CCR2 polymorphisms on the susceptibility of HIV-1 infection. These findings contribute to understanding the genetic mechanism for the susceptibility of HIV-1 infection in Northern Han Chinese.

Introduction

The history of human immunodeficiency virus (HIV)-1 infection and the course of AIDS have demonstrated considerable variability in individuals. This is partially due to the extreme heterogeneity in immune responsiveness. Some individuals develop AIDS within 2–3 years and are named as rapid-progress individuals. Nevertheless, some individuals are uninfected despite having been in contact with virus and others maintain normal CD4 counts for 10 years or more (Pantaleo and Fauci, 1996). The interindividual variability observed in terms of HIV-1 infection is regulated by multiple host genetic factors, for example, chemokine receptors (CCR), and their ligands, some of which are directly involved in the procedure of HIV-1 cell entry (Arenzana-Seisdedos and Parmentier, 2006). Several studies have reported that these gene variants might influence susceptibility to HIV-1 infection and progression to AIDS in different populations (Hogan and Hammer, 2001; Arenzana-Seisdedos and Parmentier, 2006). Among the host factors, the CCR5 promoter and CCR2-V64I polymorphisms have remarkable contribution to HIV-1 infection or the progression of HIV to AIDS, in different ethnic populations (Hogan and Hammer, 2001).

CCR5, the HIV-1 coreceptor, mediates the viral envelope fusion with the membrane of CD4+ T cells (Berger et al., 1998). The CCR5 gene is located in the 3p21.3 region of human genome (Samson et al., 1996b). The 32-bp deletion of the CCR5 gene in the coding sequence leads to effectively protection against HIV-1 infection in homoyzgous individuals and delayed progression to AIDS in heterozygotes (Huang et al., 1996;
The allele of CCR5-A32 is common in the European populations, but almost absent from the African and Asian populations (Martinson et al., 1997). In addition, the CCR5 promoter polymorphisms, CCR5 59029-G homozygotes, were associated with delayed disease progression compared with the CCR5 59029-A homozygotes (McDermott et al., 1998; Clegg et al., 2000; Knudsen et al., 2001; Kaur et al., 2007); while CCR5 59353-C was reported to be associated with HIV pathogenesis in the Korean population (Jang et al., 2008), as well as with increased risk of vertical transmission of HIV-1 infection in Brazilian children (de Souza et al., 2006). In the Northern Chinese population, no significant association was found on the single-nucleotide polymorphism (SNPs) in the CCR5 promoter region. However, a novel haplotype (GGTAC: CCR5 58934-G/T, 59029-A/G, 59353-C/T, 59402-A/G, and 59653-C/T) was significantly associated with HIV-1 infection (Xu et al., 2010).

CCR2 is the receptor for the β-chemokine macrophage chemoattractant protein 1–4 (MCP-1–4) acting as a coreceptor of rare R5 and dual-tropic R5X4 viruses to invade host cells (Simmons et al., 1996). CCCR2 is closely linked with the CCR5 gene, with 17.5-kb downstream distance (Samson et al., 1996a). A mutant of the CCR2 gene at position 64 in the first transmembrane segment, a valine (V) of the receptor replaced by an isoleucine (I), has been reported (Smith et al., 1997). The frequency of the CCR2-64I allele was 10%–20% among different populations (Su et al., 1998). In the overall Chinese Han population, the frequency of the CCR2-64I allele was ~20% (Wang et al., 2001). According to the results of different studies, CCR2-64I had no influence on the incidence of HIV-1 infection, but heterozygotes were found to display slower progression to AIDS compared to homozygotes for the wild type (Smith et al., 1997; Rizzardi et al., 1998; Easterbrook et al., 1999). A recent study identified that the allele frequencies of CCR2-64I were 14.7% in the HIV-1-infected patients and 18.6% in the healthy controls in the Northern Chinese population, and confirmed the association of the CCR2-64I allele with slower disease progression (Xu et al., 2009).

In addition to the main effects of individual SNPs, complex diseases may be influenced by SNP-SNP interaction, which is named as epistasis (Cordell, 2009). The finding of SNP-SNP interaction could provide valuable information on the underlying biochemical and biological pathways of HIV infection (Moore, 2003). Based on the reports that the protective effect of the CCR2-64I allele may attribute to the link with the variants in the CCR5 promoter region (Carrington et al., 1999; Gonzalez et al., 1999), it may be assumed that the interaction of the CCR5 promoter and the CCR2 gene might take an indispensable effect on the process of HIV-1 infection in the Chinese Han population.

This study aims to detect the frequencies of seven CCR5 promoter region polymorphisms (CCR5 58755-A/G, 58934-G/T, 59029-A/G, 59353-T/C, 59356-C/T, 59402-A/G, and 59653-C/T), and CCR2-V64I in a Northern Han Chinese population, and to investigate the main effects and epistatic effects on susceptibility to HIV-1 infection.

**Materials and Methods**

**Study subjects**

This study recruited a total of 91 HIV-1-infected patients (45 men and 46 women) from Beijing YouAn Hospital, Capital Medical University. The mean age was 40.08±7.30 years old (range 25–55 years). The average CD4+ T lymphocyte count was 395.4 cells/μL (range 186–759 cells/μL). Among them, 81 (89.0%) cases were infected through blood contact; 6 (6.6%) individuals were infected through sexual transmission; and 4 (4.4%) cases were not clear with respect to the transmission route. The HIV-1-infected patients were identified by western blotting. Matching age and gender with HIV-1-infected patients, 91 healthy controls (51 men and 40 women) were collected from the Physical Health Examination Centre, Beijing TongRen Hospital, Capital Medical University. The mean age was 40.55±10.95 years old (range 22–58 years). Both HIV-1-infected patients and healthy controls are Northern Han Chinese. The distributions of age and gender were not significantly different between the cases and controls (t=0.342, p=0.732; χ²=0.794, p=0.373). Informed consent was obtained from each participant, and the study was approved by the ethics committee of the Capital Medical University (Beijing, China).

**DNA preparation and SNP genotyping**

Whole-genome DNA was extracted by the DNeasy Blood & Tissue Kit (Qiagen) according to the standard protocol. We designed amplification primers for the seven CCR5 promoter and CCR2-V64I polymorphisms using NCBI/Primer-BLAST. The primer sequences of the CCR5 promoter polymorphisms were 5′-TAG GAT GAT GGG GGG CAC CTA ATT T-3′ (forward) and 5′-CTC AAA TTC CCT GCA CCT TAG ACT A-3′ (reverse), while for the CCR2-V64I polymorphism, the primer sequences were 5′-GGA TTG AAC AAG GAC GCA TTT CCC C-3′ (forward) and 5′-TTT CAC ATT GTC CCA AAG ACC C-3′ (reverse). Polymerase chain reactions (PCRs) were executed in a 50-μL volume containing 5 μL 10× PCR buffer, 4 μL dNTPs (2.5 mM), 1 μL forward primer (20 μM), 1 μL reverse primer (20 μM), 2 μL genomic DNA, 0.25 μL Taq polymerase (5 U/μL, TaKaRa), and 36.75 μL deionized H₂O. PCRs were initiated by denaturation at 95°C for 5 min, followed by 40 cycles: 30 s at 95°C, 30 s at the optimal annealing temperature, and 60 s at 72°C, and then PCR products were prolonged for 6 min at 72°C and were finally held at 4°C. The PCR products were then separated by 2.0% agarose gel electrophoresis. All amplified DNA fragments were resequenced by Sangon Corp.. The genotypes of our investigated polymorphisms were derived from comparing the CCR5 and CCR2 gene sequences (GenBank accession Nos: AF031237 and AB119271, respectively).

**Statistical analysis**

The distributions of the allele and genotype frequencies were analyzed using the χ² test. The single-locus association and SNP-SNP interactions were estimated by computing the odds ratios (ORs) and 95% confidence intervals (CIs) with adjustment for age and gender using logistic regression analysis. All of those analyses were completed by SPSS13.0 software. Deviations from the Hardy–Weinberg equilibrium in the controls were tested online (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). The frequencies of the haplotypes and the association analyses were evaluated using Haploview software 4.1. p < 0.05 was considered statistically significant.

To identify the SNP-SNP interactions, the linkage disequilibrium (LD) of each two loci was calculated in both cases and
controls (Zhao et al., 2006). Gene–gene interactions were determined by nonparametric multifactor dimensionality reduction (MDR) software (version 2.0; Computational Genetics Laboratory, Dartmouth Medical School, Hanover, NH; www.epistasis.org). The MDR approach is a model-free and nonparametric analysis method for gene–gene and gene–environment interaction studies in case–control and discordant sib-pair study designs (Hahn et al., 2003). We applied the MDR method for case–control study designs using the 10-fold cross-validation procedure 10 times, with a different number of factors, to reduce the chance of observing spurious results due to chance divisions of the data. Empirical \( p \)-values were based on the number of prediction errors estimated among the 1000 simulations that were as small as or smaller than the observed prediction errors. The best MDR model was selected as the one with maximum average testing balanced accuracy and also high cross-validation consistency (CVC) (Ritchie et al., 2001; Hahn et al., 2003).

### Results

**Allele and genotype frequencies**

The genotype distribution of all investigated SNPs followed the Hardy–Weinberg equilibrium in controls (\( p > 0.05 \), Table 1). The T allele of CCR5 59356-C/T was unable to be found, so this SNP was excluded from analysis. All of the

<table>
<thead>
<tr>
<th>SNP</th>
<th>MAF</th>
<th>HWE p-value</th>
<th>Case n</th>
<th>Frequency (%)</th>
<th>Control n</th>
<th>Frequency (%)</th>
<th>p-Value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR5 58755-A/G</td>
<td>0.157</td>
<td>0.772</td>
<td>21</td>
<td>11.5</td>
<td>36</td>
<td>19.8</td>
<td>0.031&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.529 (0.295–0.948)</td>
</tr>
<tr>
<td>CCR5 58934-G/T</td>
<td>0.484</td>
<td>0.533</td>
<td>106</td>
<td>54.9</td>
<td>106</td>
<td>54.9</td>
<td>0.208</td>
<td>0.768 (0.508–1.159)</td>
</tr>
<tr>
<td>CCR5 59029-A/G</td>
<td>0.423</td>
<td>0.529</td>
<td>78</td>
<td>42.9</td>
<td>75</td>
<td>41.2</td>
<td>0.750</td>
<td>1.070 (0.706–1.622)</td>
</tr>
<tr>
<td>CCR5 59353-C/T</td>
<td>0.382</td>
<td>0.445</td>
<td>71</td>
<td>39.0</td>
<td>68</td>
<td>37.4</td>
<td>0.746</td>
<td>1.072 (0.702–1.637)</td>
</tr>
<tr>
<td>CCR5 59402-A/G</td>
<td>0.462</td>
<td>0.272</td>
<td>87</td>
<td>47.8</td>
<td>80</td>
<td>44.0</td>
<td>0.462</td>
<td>1.168 (0.773–1.764)</td>
</tr>
<tr>
<td>CCR5 59653-C/T</td>
<td>0.231</td>
<td>0.476</td>
<td>50</td>
<td>27.5</td>
<td>33</td>
<td>18.1</td>
<td>0.034&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.710 (1.039–2.814)</td>
</tr>
<tr>
<td>CCR2-V64I(G/A)</td>
<td>0.209</td>
<td>0.476</td>
<td>43</td>
<td>23.6</td>
<td>33</td>
<td>18.1</td>
<td>0.197</td>
<td>1.397 (0.840–2.324)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Significant at the level of \( p < 0.05 \).

HWE, Hardy–Weinberg equilibrium in controls; OR, odds ratio; 95% CI, 95% confidence interval; MAF, minor allele frequencies; SNP, single-nucleotide polymorphisms.

### Table 1. Allele Frequencies of the CCR5 Promoter and CCR2-V64I Polymorphisms in the Cases and Controls

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype/allele</th>
<th>HIV-1 infected</th>
<th>Healthy control</th>
<th>p-Value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Adjusted OR (95% CI)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR5 58755-A/G</td>
<td>AA</td>
<td>71 (78.0)</td>
<td>59 (64.8)</td>
<td>0.099</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>19 (20.9)</td>
<td>28 (30.8)</td>
<td>0.558 (0.281–1.109)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>1 (1.1)</td>
<td>4 (4.4)</td>
<td>0.198 (0.021–1.830)</td>
<td></td>
</tr>
<tr>
<td>CCR5 58934-G/T</td>
<td>GG</td>
<td>24 (26.4)</td>
<td>17 (18.7)</td>
<td>0.413</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>46 (50.5)</td>
<td>48 (52.7)</td>
<td>0.701 (0.332–1.481)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>21 (23.1)</td>
<td>26 (28.6)</td>
<td>0.586 (0.250–1.373)</td>
<td></td>
</tr>
<tr>
<td>CCR5 59029-A/G</td>
<td>AA</td>
<td>11 (12.1)</td>
<td>14 (15.4)</td>
<td>0.750</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>56 (61.5)</td>
<td>47 (51.6)</td>
<td>1.591 (0.654–3.868)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>24 (26.4)</td>
<td>30 (33.0)</td>
<td>1.079 (0.411–2.834)</td>
<td></td>
</tr>
<tr>
<td>CCR5 59353-C/T</td>
<td>CC</td>
<td>12 (13.2)</td>
<td>11 (12.1)</td>
<td>0.944</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>47 (51.6)</td>
<td>46 (50.5)</td>
<td>0.969 (0.387–2.430)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>32 (35.2)</td>
<td>34 (37.4)</td>
<td>0.885 (0.341–2.298)</td>
<td></td>
</tr>
<tr>
<td>CCR5 59402-A/G</td>
<td>AA</td>
<td>17 (18.7)</td>
<td>15 (16.5)</td>
<td>0.689</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>53 (58.2)</td>
<td>50 (54.9)</td>
<td>0.953 (0.429–2.116)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>21 (23.1)</td>
<td>26 (28.6)</td>
<td>0.725 (0.293–1.791)</td>
<td></td>
</tr>
<tr>
<td>CCR5 59653-C/T</td>
<td>CC</td>
<td>44 (48.4)</td>
<td>62 (68.1)</td>
<td>0.015&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>44 (48.4)</td>
<td>25 (27.5)</td>
<td>2.502 (1.332–4.698)&lt;sup&gt;†&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>3 (3.2)</td>
<td>4 (4.4)</td>
<td>1.029 (0.218–4.864)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CT/TT</td>
<td>47</td>
<td>29</td>
<td>2.296 (1.250–4.218)&lt;sup&gt;†&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>CCR2-V64I(G/A)</td>
<td>GG</td>
<td>51 (56.0)</td>
<td>62 (68.1)</td>
<td>0.171</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>37 (40.7)</td>
<td>25 (27.5)</td>
<td>1.835 (0.967–3.483)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Two-sided \( T \)-test for the distributions of genotype and allele frequencies.

<sup>b</sup>Adjusted for age and gender in a logistic regression model.

<sup>c</sup>Significant at the level of \( p < 0.05 \).

HIV, human immunodeficiency virus.

### Table 2. Genotype Frequencies and Association Results of the CCR5 Promoter and CCR2-V64I Polymorphisms in the Cases and Controls
It was found that a statistically significantly higher risk was associated with a lower risk of HIV-1 infection for the carriers. No significant association was found in other SNPs.

In CCR5 promoter region, five SNPs (CCR5 58755-A/G, 58934-G/T, 59029-A/G, 59353-T/C, and 59402-A/G) had strong evidence of LD (Fig. 1). We selected the haplotypes with frequencies above 1.0%, and eight haplotypes were identified (Table 3). The most common CCR5 haplotype was TGTGC (47.9%), followed by GACAT (22.2%), GACAC (12.4%), GATAC (5.1%), GTTGC (3.2%), TATGC (2.6%), and GGCAC (2.1%). The frequency of TATGC haplotype in HIV-1-infected patients was significantly lower than that of controls (0.1% vs. 5.1%, \( \chi^2 = 8.899, p = 0.003 \)).

### Interaction of the CCR5 promoter and CCR2-V64I variants on HIV-1 infection

All \( D' \) values among the CCR5 promoter and CCR2-V64I polymorphisms were larger than 0.90 in the patients, but weak LD was observed in the controls (five \( D' \) values were <0.80, and data were not shown), indicating that epistatic effects are involved in the genetic effects on the susceptibility.

### Multifactor Dimensionality Reduction -Based Interaction Models of the CCR5 Promoter and CCR2-V64I Polymorphisms on Human Immunodeficiency Virus-1 Infection

<table>
<thead>
<tr>
<th>Model</th>
<th>Training bal. acc.</th>
<th>Testing bal. acc.</th>
<th>CVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR5 58755-C/T</td>
<td>0.6044</td>
<td>0.5824*</td>
<td>10/10</td>
</tr>
<tr>
<td>CCR5 58755-A/G and 59029-A/G</td>
<td>0.6374</td>
<td>0.6208*</td>
<td>10/10</td>
</tr>
<tr>
<td>CCR5 58755-A/G and CCR2-V64I (G/A)</td>
<td>0.6825</td>
<td>0.6154*</td>
<td>10/10</td>
</tr>
<tr>
<td>CCR5 58755-A/G, 58934-G/T, 59029-A/G and CCR2-V64I (G/A)</td>
<td>0.6886</td>
<td>0.5714*</td>
<td>7/10</td>
</tr>
</tbody>
</table>

*Multifactor dimensionality reduction model is considered statistically significant (\( p<0.05 \)) if testing accuracy is greater than testing accuracy cutoff based on a 1,000-fold permutation test for up to three-factor interactions.

Training bal. acc., training balance accuracy; testing bal. acc., testing balance accuracy; CVC, cross-validation consistency.
of HIV-infection. Then, the potential interaction model of the CCR5 promoter and CCR2-V64I polymorphisms was estimated using the MDR method (Table 4 and Fig. 2). The CCR5 59653-C/T polymorphism was the strongest single factor for the risk of HIV-1 infection with testing accuracy = 0.5824 and CVC = 10/10. Yet, according to the selected principle of best model, the best interaction model was the two-factor model (CCR5 58755-A/G and 59029-A/G) with an improved testing accuracy to 62.09%, a perfect CVC of 10, and statistically significant ($p = 0.001$). The strongest interaction between CCR5 58755-A/G and 59029-A/G was directly shown in black in the interaction dendrogram (Fig. 2). In addition, it was observed that the three-factor model (CCR5 58755-A/G, 59029-A/G, and CCR2-V64I) was with less testing accuracy (0.6154) and complete CVC. The CCR2-V64I polymorphism was situated on the same branch with the best two-factor model (dark gray in Fig. 2), suggesting that there were also strong gene–gene interactions between the CCR5 promoter and CCR2 polymorphisms on the susceptibility of HIV-1 infection.

Finally, logistic regression models were evaluated in the best interaction model identified by MDR (Table 5). The CCR5 59029-A/G polymorphism as a main effect was a statistically significant predictor for the risk of HIV-1 infection ($p = 0.025$; AG vs. AA: OR = 5.671; 95% CI: 1.394–23.073), indicating that CCR5 59029-AG genotype carriers were more vulnerable to HIV-1 infection in a Northern Han Chinese population. The interaction between CCR5 58755-A/G and CCR5 59029-A/G was significantly associated with the susceptibility of HIV-1 infection ($p = 0.014$). The interaction showed the protective effect on the risk of HIV-1 infection in a Northern Han Chinese population (AG*AG vs. AA*AA: OR = 0.025; 95% CI: 0.003–0.235; AG*GG vs. AA*AA: OR = 0.026; 95% CI: 0.001–0.544).

**Discussion**

Although most genetic susceptibility studies focus on the gene’s main effects on HIV-1 infection, the SNP-SNP interaction may account for the variability of the risk of HIV-1 infection, as well. In this study, it was observed that the CCR5 58755-A/G and CCR5 59653-C/T variants have their main effects on susceptibility to HIV-1 infection in a Northern Han Chinese population. In addition, significant epistatic effects were obtained between the CCR5 58755-A/G and 59029-A/G polymorphisms on the risk of HIV-1 infection in Northern Han Chinese.

The G allele frequency of CCR5 58755-A/G was 15.7% in all investigated individuals, which was substantially higher than results reported before (Xu et al., 2010). Statistically significant allelic difference was found between cases and controls ($p = 0.031, OR = 0.529, 95\% CI: 0.295–0.948$), suggesting that the G allele of CCR5 58755-A/G is a protective factor for HIV-1 infection in a Northern Han Chinese population. Furthermore, the CCR5 59653-T allele is significantly associated with a higher risk of HIV-1 infection ($p = 0.034, OR = 1.710, 95\% CI: 1.039–2.814$). After logistic regression analyses adjusted for both age and sex, we observed the CCR5 59653-CT genotype to be significantly associated with increased susceptibility to HIV-1 infection among a Northern Han Chinese population. The combined genotypes CT/TT also contribute to the higher risk of HIV-1 infection, indicating that the T allele of CCR5 59653 has a negative effect on HIV-1 infection for its carriers. No other associations between the allelic and genotypic frequencies and susceptibility to HIV-1 infection reached statistical significance ($p > 0.05$), which is not consistent with previous reports in other ethnic populations (Kostrikis et al., 1999; Clegg et al., 2000; de Souza et al., 2006; Kaur et al., 2007). Some scholars accepted the concordant conclusion that HIV-1-infected individuals carrying the CCR5 59029-GG genotype progress to AIDS more slowly than those carrying

**Table 5. Logistic Regression Model of Main Effect and Gene–Gene Interaction on Human Immunodeficiency Virus-1 Infection Risk Based on Multifactor Dimensionality Reduction**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Genotype</th>
<th>p-Value</th>
<th>Adjusted OR (95% CI)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td>0.823</td>
<td>1.000 (0.797–1.109)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td>0.297</td>
<td>1.396 (0.745–2.616)</td>
</tr>
<tr>
<td>CCR5 58755-A/G</td>
<td>AA</td>
<td>0.057</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td></td>
<td>10.673 (1.373–82.972)</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td></td>
<td>0.965 (0.070–13.302)</td>
</tr>
<tr>
<td>CCR5 59029-A/G</td>
<td>AA</td>
<td>0.025(^b)</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td></td>
<td>5.671 (1.394–23.073)</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td></td>
<td>2.888 (0.689–12.110)</td>
</tr>
<tr>
<td>CCR5 58755-A/G*CCR5 59029-A/G</td>
<td>AA/AA</td>
<td>0.014(^b)</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>AG/AG</td>
<td></td>
<td>0.025 (0.003–0.235)</td>
</tr>
<tr>
<td></td>
<td>AG/GG</td>
<td></td>
<td>0.026 (0.001–0.544)</td>
</tr>
</tbody>
</table>

\(^a\) Adjusted for age and gender in the logistic regression model.
\(^b\) Significant at the level of $p < 0.05$.  

**FIG. 2.** Interaction dendrogram. The different color connections show the degree of interaction: synergy (black and dark gray) or redundancy (light gray).
CCR5 59029-AA (McDermott et al., 1998; Clegg et al., 2000; Knudsen et al., 2001; Kaur et al., 2007). In Brazilian children, the presence of the CCR5 59353-TT genotype indicated a trend for increased risk of vertical transmission of HIV-1 infection (de Souza et al., 2006). The CCR5 59356-T allele is associated with an increased incidence of perinatal HIV-1 transmission in the African-American and sub-Saharan African populations (Kostrikis et al., 1999; Singh et al., 2008). In the Korean population, no significant differences of CCR5 59029-G/A, 59353-T/C, and 59402-A/G between HIV-1-infected patients and controls were found (Jang et al., 2008).

Although the CCR2-V64I polymorphism does not affect susceptibility to HIV-1 infection, HIV-1-infected persons carrying the CCR2-V64I allele show a slower progression to AIDS (Smith et al., 1997; Anzala et al., 1998; Kostrikis et al., 1998; Wasik et al., 2005). However, some studies do not support this conclusion (Lee et al., 1998; Mummidi et al., 1998). In this study, the frequency of the CCR2-64I allele is 23.6% for cases and 18.1% for controls, respectively, and no significant association is identified between CCR2-V64I and HIV-1 infection (p = 0.197). An in vivo study reported that protein encoded by the CCR2-V64I mutant can cross-regulate the receptor-signaling process of the major HIV-1 coreceptors CCR5 and CXCR4, and thus reduce the interaction between HIV-1 and CCR5, as well as CXCR4, explaining the effect of the CCR2-V64I polymorphism on progression to AIDS (Lee et al., 1998). It has been suggested that there is an LD between CCR2-V64I and other mutations in the CCR5 promoter region (Smith et al., 1997; Kostrikis et al., 1998; Lee et al., 1998). In this study, CCR2-V64I has complete LD with the CCR5 58755A/G, 59029-A/G, 59353-T/C, and 59402-A/G polymorphisms in a Northern Han Chinese population, suggesting that CCR2-V64I and CCR5 promoter polymorphisms may jointly influence the process of HIV-1 infection.

The distribution of haplotypes in CCR5 promoter is highly variable in different populations, and a number of CCR5 haplotypes have been shown to influence HIV-1 infection (Arenzana-Seisdedos and Parmentier, 2006). In the Caucasian populations, the haplotype ACCAC is associated with both increased risk and an accelerated course of infection (Li et al., 2005), but not among African-Americans (Mummidi et al., 2000). We observed that a haplotype TATGC of the CCR5 promoter region is significantly different between HIV-1-infected patients and healthy controls, showing that haplotype TATGC is associated with higher susceptibility of HIV-1 infection in a Northern Han Chinese population.

According to the LD results of the targeted loci through pairwise analyses, different LD degree between cases and controls was found, suggesting that there is an SNP-SNP interaction influencing the risk of HIV-1 infection in a Northern Han Chinese population. In the two-factor model of MDR methods, the strongest interaction is between the CCR5 58755-A/G and CCR5 59029-A/G polymorphisms, showing that the CCR5 58755A/G and CCR5 59029-A/G polymorphisms jointly contribute to the susceptibility of HIV-1 infection in a Northern Han Chinese population. The logistic regression analysis further validated that the interaction of the CCR5 58755-A/G and CCR5 59029-A/G polymorphisms decreases the susceptibility of HIV-1 infection in the Han Chinese population. In addition, the potential interaction effects of the CCR5 promoter and CCR2-V64I polymorphism on the risk of HIV-1 infection were also examined in the three-factor model, including CCR5 58755-A/G, CCR5 59029-A/G, and CCR2-V64I polymorphisms. To some extent, there are significant interactions between the CCR5 promoter region and the CCR2 gene affecting HIV-1 infection in a Northern Han Chinese population.

There are two limitations in this study. Our findings are only from a Northern Han Chinese population, so it is uncertain whether these results are relevant to other ethnic groups. Secondly, MDR and logistic analyses cannot explain the biological mechanism of this observed gene–gene interaction. Therefore, further research at a biochemical level is required to confirm the results of the present study.

In conclusion, this study detects an associated relationship of the CCR5 promoter and CCR2-V64I polymorphisms with the susceptibility of HIV-1 infection at allelic, genotypic, and haplotypic levels. Individuals with the A allele of CCR5 58755-A/G, T allele of CCR5 59653-C/T, or TATGC haplotype are more susceptible to HIV-1 infection in Northern Han Chinese. Furthermore, this study suggests that the epistatic effects of the CCR5 58755-A/G and CCR5 59029-A/G polymorphisms significantly decrease susceptibility of HIV-1 infection in Northern Han Chinese. These findings expand the understanding of the genetic mechanism of the CCR5 promoter and CCR2-V64I polymorphisms contributing to the risk of HIV-1 infection in a Northern Han Chinese population.

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Author Disclosure Statement

No competing financial interests exist.

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