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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

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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 being 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 homozygous individuals and delayed progression to AIDS in heterozygotes (Huang *et al.*, 1996;

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Liu *et al*., 1996; O'Brien *et al*., 1997; Dean *et al*., 2002). The allele of *CCR5*-Δ32 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 populations (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). *CCR2* 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 ($t = 0.342$, $p = 0.732$; $\chi^2 = 0.794$, $p = 0.373$). 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 TGG GGG CAC GTA ATT T-3′ (forward) and 5′-CTC AAA CTC 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′-TTG CAC ATT GCA TTC 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 H_2O. 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 χ^2 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

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

			Case		*Control*			
SNPs	*MAF*	*HWE* p-*value*	n	*Frequency (%)*	n	*Frequency (%)*	*p-Value*	*OR (95% CI)*
CCR5 58755-A/G	0.157	0.772	21	11.5	36	19.8	0.031[a]	0.529 (0.295–0.948)
CCR5 58934-G/T	0.484	0.533	88	48.4	100	54.9	0.208	0.768 (0.508–1.159)
CCR5 59029-A/G	0.423	0.529	78	42.9	75	41.2	0.750	1.070 (0.706–1.622)
CCR5 59353-C/T	0.382	0.445	71	39.0	68	37.4	0.746	1.072 (0.702–1.637)
CCR5 59402-A/G	0.462	0.272	87	47.8	80	44.0	0.462	1.168 (0.773–1.764)
CCR5 59653-C/T	0.231	0.476	50	27.5	33	18.1	0.034[a]	1.710 (1.039–2.814)
CCR2-V64I(G/A)	0.209	0.476	43	23.6	33	18.1	0.197	1.397 (0.840–2.324)

[a]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.

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 the 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 2. Genotype Frequencies and Association Results of the *CCR5* Promoter and *CCR2*-V64I Polymorphisms in the Cases and Controls

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

[a]Two-sided *T*-test for the distributions of genotype and allele frequencies.
[b]Adjusted for age and gender in a logistic regression model.
[c]Significant at the level of $p < 0.05$.
HIV, human immunodeficiency virus.

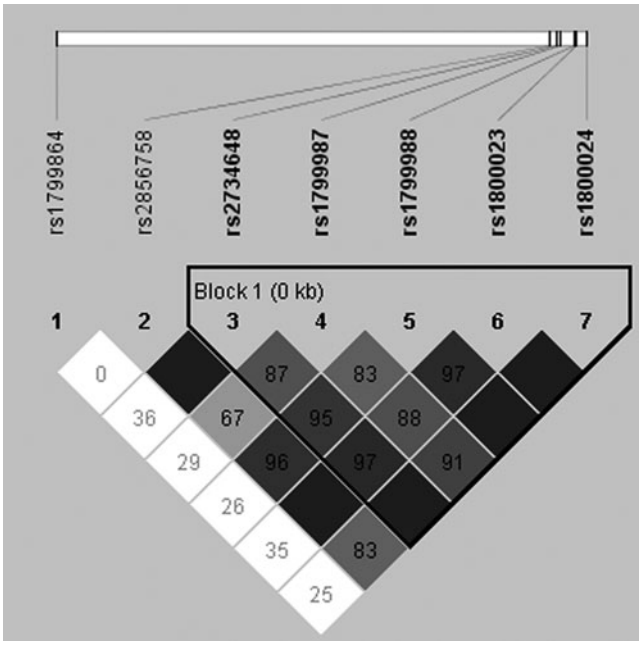

FIG. 1. LD plot of the *CCR5* promoter and *CCR2*-V64I polymorphisms. The LD plot was generated by Haploview software. The rs number (from left to right) corresponds to the SNPs (rs1799864: *CCR2*-V64I, rs2856758: *CCR5* 58755-A/G, rs2734648: *CCR5* 58934-G/T, rs1799987: *CCR5* 59029-A/G, rs1799988: *CCR5* 59353-T/C, rs41469351: *CCR5* 59356-C/T, rs1800023: *CCR5* 59402-A/G, and rs1800024: *CCR5* 59653-C/T). The level of pairwise *D′* indicating the degree of LD between two SNPs is shown in the LD structure. Strong evidence of LD is shown in black or dark gray, while weak evidence in white. CCR, chemokine receptor 5; SNP, single-nucleotide polymorphisms; LD, linkage disequilibrium.

other seven SNPs were included in the subsequent analyses. The minor allele frequencies of these SNPs (*CCR5* 58755-A/G, 58934-G/T, 59029-A/G, 59353-T/C, 59402-A/G, 59653C/T, and *CCR2*-V64I) were 11.5%, 48.4%, 42.9%, 39.0%, 47.8%, 27.5% and 23.6% in HIV-1-infected patients, and 19.8%, 54.9%, 41.2%, 37.4%, 44.0%, 18.1% and 18.1% in healthy controls, respectively (Table 1). The average allele frequency of *CCR2*-V64I was 20.9% in all subjects, which was 23.6% in the cases and 18.1% in the controls.

Association of the CCR5 *promoter and* CCR2*-V64I polymorphisms on HIV-1 infection*

The G allele of *CCR5* 58755-A/G was significantly associated with a lower risk of HIV-1 infection ($p = 0.031$, OR = 0.529, 95% CI: 0.295–0.948, Table 1). An opposite result was found between the *CCR5* 59653T allele and susceptibility to HIV-1 infection ($p = 0.034$, OR = 1.710, 95% CI: 1.039–2.814). Adjusted for age and gender, the *CCR5* 59653-CT genotype, compared with the wild-type CC genotype, was associated with higher susceptibility to HIV-1 infection (adjusted OR = 2.502, 95% CI: 1.332–4.698, Table 2). Because of the rare count (N = 3) of genotype TT in the *CCR5* 59653-C/T polymorphism, we combined genotype TT into heterozygous CT. It was found that a statistically significantly higher risk was observed in the genotypes CT/TT compared with the genotype CC (OR = 0.51, 95% CI: 0.32–0.80), suggesting the variant T allele of *CCR5* 59653-C/T increased the probability of HIV-1 infection for the carriers. No significant association was found in other SNPs.

Table 3. Haplotype Frequencies of the *CCR5* Promoter and *CCR2*-V64I Polymorphisms in the Cases and Controls

Haplotype	*Frequencies (%)*	*Frequencies in the cases (%)*	*Frequencies in the controls (%)*	χ^2 *value*	p-*Value*
TGTGC	47.9	47.6	48.1	0.007	0.932
GACAT	22.2	26.3	18.1	3.512	0.061
GACAC	12.4	10.8	14.0	0.876	0.349
GATAC	5.1	5.7	4.5	0.264	0.607
GGTAC	3.2	3.1	3.3	0.004	0.947
GGTGC	2.8	3.9	1.7	1.651	0.199
TATGC	2.6	0.1	5.1	8.899	0.003[a]
GGCAC	2.1	0.7	3.5	3.389	0.066

[a]Significant at the level of $p < 0.05$.

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%), GGTAC (3.2%), GGTGC (2.8%), 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

Table 4. Multifactor Dimensionality Reduction -Based Interaction Models of the *CCR5* Promoter and *CCR2*-V64I Polymorphisms on Human Immunodeficiency Virus-1 Infection

Model	*Training bal. acc.*	*Testing bal. acc.*	*CVC*
CCR5 59653-C/T	0.6044	0.5824[a]	10/10
CCR5 58755-A/G and 59029-A/G	0.6374	0.6209[a]	10/10
CCR5 58755-A/G, 59029-A/G and *CCR2*-V64I (G/A)	0.6825	0.6154[a]	10/10
CCR5 58755-A/G, 58934-G/T, 59029-A/G and *CCR2*-V64I (G/A)	0.6886	0.5714[a]	7/10

[a]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.

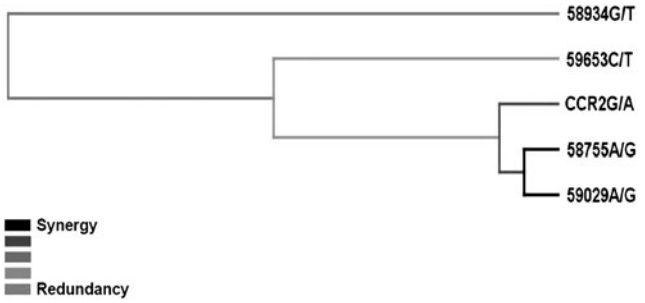

FIG. 2. Interaction dendrogram. The different color connections show the degree of interaction: synergy (black and dark gray) or redundancy (light gray).

of HIV-1infection. 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

Variable	*Genotype*	p-*Value*	*Adjusted OR (95% CI)*[a]
Age		0.823	1.004 (0.970–1.039)
Gender		0.297	1.396 (0.745–2.616)
CCR5 58755-A/G	AA	0.057	1.000
	AG		10.673 (1.373–82.972)
	GG		0.965 (0.070–13.302)
CCR5 59029-A/G	AA	0.025[b]	1.000
	AG		5.671 (1.394–23.073)
	GG		2.888 (0.689–12.110)
CCR5 58755-A/G*CCR5* 59029-A/G	AA/AA	0.014[b]	1.000
	AG/AG		0.025 (0.003–0.235)
	AG/GG		0.026 (0.001–0.544)
	GG/GG		—

[a]Adjusted for age and gender in the logistic regression model.
[b]Significant at the level of $p < 0.05$.

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* 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 Chinese Han population.

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Author Disclosure Statement

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