High Frequencies of the CCR2b-64I and SDF1-3'A Mutations with HIV Infection in Koreans

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ABSTRACT

Background: Host genetic polymorphisms in the HIV-1 co-receptor CCR5 and CCR2b and SDF-1, ligand for co-receptor CXCR4, have been known to be associated with the resistance of HIV infection and/or the delayed disease progression in HIV-infected patients. **Methods:** We examined the frequencies of SDF1-3'A and CCR2b-64I alleles of 354 Koreans including 100 HIV-uninfected persons, 13 discordant spouses of HIV-infected persons, and 241 HIV-infected persons. The genotyping assays of SDF1 and CCR2b genes were carried out by polymerase chain reaction-restriction fragment length polymorphism. **Results:** The frequencies of CCR2b-64I and SDF1-3'A alleles in Koreans were very high compared with Caucasians and blacks. Observed frequencies of CCR2b-64I and SDF1-3'A allelic variants were 25.1% and 28.7%, respectively. The frequency of the CCR2b-64I allele in Koreans was 2～4 times higher than those of other ethnic groups with the exception of Asian. The frequencies of CCR2b-64I and SDF1-3'A genotypes did not show the significant difference between HIV-infected and uninfected Koreans. However, the prevalence of CCR2b-64I genotype of the LTNP group was about two times higher than that of the remainder group ($P < 0.05$). Four (45%) out of 9 LTNPs (long-term nonprogressors) showed having the SDF1-3'A allele and 7 (78%) out of 9 LTNPs carried the CCR2b-64I allele. 3 (33%) out of 9 LTNPs had both SDF1-3'A and CCR2b-64I alleles. But none of 5 RPs (rapid progressors) appeared to have both SDF1-3'A and CCR2b-64I alleles. **Conclusion:** The different genetic backgrounds in study populations may affect the disease progression and the AIDS epidemic in each country. Further studies need to define whether high frequencies of CCR2b-64I and SDF1-3'A allelic variants may affect the HIV disease progression. **(Immune Network 2002;2(2):86-90)**

Key Words: Genetic polymorphism, CCR2b-64I allele, SDF1-3'A allele, long-term nonprogressor, HIV, Korean

Introduction

Most HIV strains use chemokine receptor molecules, CCR5 and CXCR4, as cell surface coreceptors with CD4 molecules for HIV infection and certain HIV strains use additional chemokine receptors CCR2 and CCR3 (1-3). Host genetic polymorphisms in the HIV-1 co-receptor CCR5, CCR2b and SDF-1, ligand for co-receptor CXCR4, have been known to be associated with the resistance of HIV infection and/or the delayed disease progression in HIV-infected patients (1-4). Although the most previous studies (3,4) reported that the CCR2b-64I and SDF1-3'A polymorphisms were not related with the resistance of HIV infection, Mangano et al. (5) suggested recently that CCR2b-64I allele showed the protective effect in mother-to- infant HIV-1 transmission (vertical transmission).

The interesting information of genetic polymorphism is that allelic frequencies of genetic variants are different with ethnic groups. CCR5△32 mutant is mainly restricted to Caucasian populations and is rare in African and Asian populations (6-8). In our previous study, we found that all Koreans examined including HIV-infected long-term nonprogressors (LTNPs) and rapid progressors (RPs) showed wild types for CCR5 gene (9). However, CCR2b- 64I allele

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frequency was very high (34.0%, n=53) in Mongolia population while other studies reported that it was common in all ethnic groups (10,11). In case of SDF1-3'A, the variant frequencies of African Americans was three to four fold lower than other ethnic groups (4). These studies make us to suppose that the survival time, HIV transmission rate, and disease progression may differ according to ethnic groups having different genetic polymorphism.

To investigate the correlation between host genetic polymorphism and HIV natural history, it needs the information of immunogenetic backgrounds of various ethnic groups. Therefore, we investigated the frequencies of SDF1-3'A and CCR2b-64I alleles.

Materials and Methods

Patients. The study population was composed of 100 HIV-uninfected persons, 13 discordant spouses of HIV-infected persons, and 241 HIV-infected persons including 9 long term nonprogressors (LTNPs) and 5 rapid progressors (RPs). The LTNP was defined as the patient who has lived with HIV for >7 years, CD4+ T cell count of >500 cells/mm^3, no symptoms related to HIV infection, and no history of taking anti-HIV drugs (12). The RP was defined as the patient who has lived with HIV for <5 years and CD4+ T cell counts of <200 cells/mm^3. Remainder was the HIV-infected person who did not involved in a LTNP and a RP. The discordant spouse was defined as the individual who remained HIV-uninfected despite continuous unprotected sexual contact with their HIV-infected husbands.

CCR2b-64I genotyping. Genomic DNA was isolated from cryopreserved PMBCs by using IsoQuick kit (ORCA Research, Inc. Bothell, WA). Suspended PBMCs with Reagent A was mixed with equal volume of Reagent 1 (lysis solution) followed by addition of Reagent 2 (extraction matrix) and Reagent 3 (extraction buffer). After centrifugation at 12,000x g for 5 minutes, the aqueous phase was transferred and precipitated with isopropanol and ethanol. The DNA pellets were suspended in 100µl of RNase-free water. The CCR2b-64I allele was detected by PCR-restriction fragment length polymorphism (PCR-RFLP) assay. 2µl of genomic DNA was analysed by PCR with 5'-GGATTGAACAAGGACGCATTTCCCC-3' and 5'-TTGCACATTGCATTCCCAAA GACCC-3' as forward and reverse primers (13). Samples were amplified with 100µl of PCR mixture containing 10µl of PCR buffer (Boehringer Mannheim, Germany), 8µl of dNTP (10 mM), 4µl of MgCl2 (25 mM), 4µl each of 10 pmol forward and reverse primers, and 10 unit of Taq polymerase (Boehringer Mannheim, Germany). All reactions were performed in a 100µl volume. The DNA was amplified using the following cycling scheme: 14 min of predenaturation at 94°C, 40 cycles of 30 sec at 94°C, 30 sec at 63°C, and 30 sec at 72°C, and 10 minutes elongation at 72°C. 20µl of PCR product was digested with 2 U of Fok I (New England Biolabs, Beverly, MA, USA) for 2h at 37°C and analysed on 2% agarose gel.

SDF1-3'A genotyping. 2µl of genomic DNA was analysed by PCR with primers SDF 3'UTR-F (sense, 5'-CAGTCAACCTGGGCAAAGCC-3') and SDF 3'-UTR-R (antisense, 5'-AGC TTTGGTCCTGAGAG-TCC-3') (4). Samples were amplified with 100µl of PCR mixture containing 10µl of PCR buffer (Boehringer Mannheim, Germany), 8µl of dNTP (10 mM), 4µl of MgCl2 (25 mM), 4µl each of 10 pmol SDF 3'UTR-F and SDF 3'UTR-R, and 10 unit of Taq polymerase (Boehringer Mannheim, Germany). All reactions were performed in a 100µl volume. The condition of PCR comprised 14 min of predenaturation at 94°C, 35 cycles of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C, and 10 min elongation at 72°C in thermal cycler (Perkin Elmer 2400, Norwalk, CT, USA). PCR products were verified with 2% agarose gel. PCR products were digested with Msp1 (New England Biolabs, Beverly, MA, USA) for 2h at 37°C and analysed on 2% agarose gel.

Statistical analysis. The χ^2 test or Fisher's exact test was used to compare the CCR2b-64I and SDF1-3'A genotypic and allelic frequencies between the study groups. All analyses were performed using The STATISTICA 6.0 (statsoft, Inc, Tulsa, USA). *P* values of 0.05 or less were considered significant.

Results and Discussion

The presence of the CCR2b-64I allele was determined by PCR-RFLP analysis. PCR products were digested with Fork 1 and CCR2b genotypes showed one fragment of 380 bp in the CCR2b wild type, three fragments of 165 bp, 215 bp, and 380 bp in the CCR2b-64I heterozygous type, two fragments of 165 bp and 215 bp in the CCR2b-64I homozygous type (Fig. 1). The frequencies of the CCR2b-64I allele were 30.5% in 100 normal persons, 26.9% in 13 discordant spouses of HIV-infected persons, and 22.8% in 241 HIV-infected persons (Table I). Especially, the prevalence [77.8% (7/9)] of CCR2b- 64I genotype of the LTNP group was significantly higher than 38.8% (88/227) of the remainder group ($P < 0.05$).

The presence of the SDF1-3'A allele was determined by PCR-RFLP analysis. PCR products were digested with Msp1 and SDF1 genotypes showed two fragments of 101 bp and 201 bp in the SDF-1 wild type, three fragments of 101 bp, 201 bp, and 302 bp in the SDF1-3'A heterozygous type, one fragment of 302 bp in the SDF1-3'A homozygous type (Fig. 2). The frequencies of the SDF1-3'A allele were as fol-

lows: 26.0% in 100 normal persons, 34.6% in 13 discordant spouses of HIV-infected persons, and 29.5% in 241 HIV-infected persons (Table II).

As shown in Table I and II, the frequencies of CCR2b-64I and SDF1-3'A genotypes did not show the significant difference between three groups of HIV-infected, uninfected, and discordant spouse group. But, three out of 9 LTNPs had both SDF1-

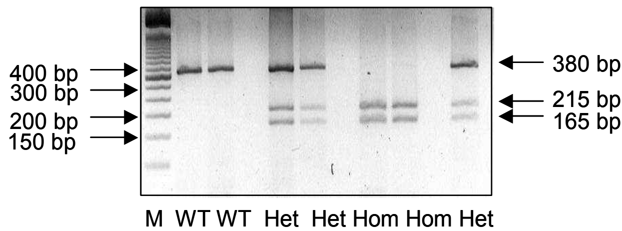

Figure 1. CCR2b-64I mutation genotyping by PCR-RFLP. Genomic DNA was amplified by PCR using the CCR2b- specific primer pair CCR2b-64IF and CCR2b-64IR which flank the point mutation sites in the first transmembrane region of CCR2b gene. The amplified products were digested with Fork I and the DNA fragments were separated by 2% agarose gel electrophoresis.M: 50bp DNA ladder marker, WT: Homozygous wild genotype (1 band of 380 bp), Het: Heterozygous genotype (3 bands of 380 bp, 215 bp, and 165 bp), Hom: Homozygous genotype (2 bands of 215 bp and 165 bp).

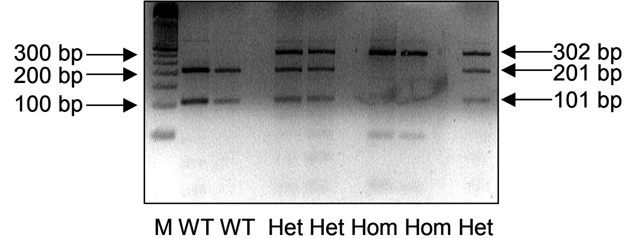

Figure 2. SDF1-3'A UTR mutation genotyping by PCR- RFLP. Genomic DNA was amplified by PCR using the SDF1- specific primer pair SDF1-3'AF and SDF1-3'AR which flank at position 801 in the 3'UTR. The amplified products were digested with Msp I and the DNA fragments were separated by 2% agarose gel electrophoresis.M: 50 bp DNA ladder marker, WT: Homozygous wild genotype (2 bands of 201 bp and 101 bp), Het: Heterozygous genotype (3 bands of 302 bp, 201 bp, and 101 bp), Hom: Homozygous genotype (1 band of 302 bp).

Table I. Frequencies of CCR2b-64I genotype in study groups

CCR2b-64I	HIV patients n=241 (%)	LTNPs n=9 (%)	RPs n=5 (%)	Discordant spauses n=13 (%)	Normal persons n=100 (%)
Genotype frequencies					
64I/64I	13 (5.4)	0 (0.0)	1 (20.0)	2 (15.4)	11 (11.0)
64V/64I	84 (34.9)	7 (77.8)	1 (20.0)	3 (23.1)	39 (39.0)
64V/64V	144 (59.8)	2 (22.2)	3 (60.0)	8 (61.5)	50 (50.0)
64I	97 (40.2)	7 (77.8)	2 (40.0)	5 (38.5)	50 (50.0)
64V	228 (94.6)	9 (100)	4 (80.0)	11 (84.6)	89 (89.0)
Allele frequencies					
64I	110[a] (22.8)	7 (38.9)	3 (30.0)	7 (26.9)	61 (30.5)
64V	372[b] (77.2)	11 (61.1)	7 (70.0)	19 (73.1)	139 (69.5)

64V: Wild type, 64I: Substitution of amino acid residue V (Valine) to I (Isoleucine) at position 64 by a G to A transition at position 190 (counting from ATG start codon) of CCR2b gene. a: RR=0.75, P<0.04, b: RR=1.1, P<0.04

Table II. Frequencies of SDF1-3'A genotype in study groups

SDF1-3'A	HIV patients n=241 (%)	LTNPs n=9 (%)	RPs n=5 (%)	Discordant spauses n=13 (%)	Normal persons N=100 (%)
Genotype frequencies					
3'A/3'A	23 (9.6)	1 (11.1)	0 (0.0)	3 (23.1)	8 (8.0)
3'G/3'A	96 (39.8)	3 (33.3)	1 (20.0)	3 (23.1)	36 (36.0)
3'G/3'G	122 (50.6)	5 (55.6)	4 (80.0)	7 (53.8)	56 (56.0)
3'A	119 (49.4)	4 (44.4)	1 (20.0)	6 (46.2)	44 (44.0)
3'G	218 (90.5)	8 (88.9)	5 (100)	10 (76.9)	92 (92.0)
Allele frequencies					
3'A	142 (29.5)	5 (27.8)	1 (10.0)	9 (34.6)	52 (26.0)
3'G	340 (70.5)	13 (72.2)	9 (90.0)	17 (65.4)	148 (74.0)

3'G: Wild type, 3'A: A G to A transition at position 801 (counting from ATG start codon) in the 3' untranslated region of SDF1 gene.

Table III. CCR2b-64I and SDF1-3'A allelic frequencies in different populations

Ethnicity	Number	Allelic frequency (%)	Reference
CCR2b-64I			
Korean	100	30.5	
Caucasian	146	7.2	Williamson *et al.* (11)
African	180	13.3	Williamson *et al.* (11)
Asian (unspecified)	40	25.0	Smith *et al.* (3)
Japanese	122	26.2	Hizawa *et al.* (14)
Chinese (Taiwan)	71	15.5	Shieh *et al.* (16)
Thai	200	15.7	Nookhai *et al.* (15)
Mongolia	53	34.0	Martinson *et al.* (10)
Hong Kong	49	17.3	Martinson *et al.* (10)
Thailand	60	17.5	Martinson *et al.* (10)
Philippines	66	5.3	Martinson *et al.* (10)
Pakistan	36	2.8	Martinson *et al.* (10)
SDF1-3'A			
Korean	100	26.0	
Caucasian	145	20.3	Williamson *et al.* (11)
African	198	1.0	Williamson *et al.* (11)
Asian (unspecified)	37	25.7	Winkler *et al.* (4)
Japanese	15	36.6	Su *et al.* (17)
Chinese (Taiwan)	63	28.6	Shieh *et al.* (16)
Thai	200	33.2	Nookhai *et al.* (15)

Allelic frequencies were calculated as (A+2B)/2N, where A is the number of heterozygous mutation genotypes, B is the number of homozygous mutation genotypes, and N is the total number of the healthy population.

3'A and CCR2b-64I alleles but none of 5 RPs appeared to have both SDF1-3'A and CCR2b-64I alleles. Mutant rate of CCR2b gene of the LTNP group was significantly higher than that of the remainder group in 241 HIV-infected persons. This result suggests that SDF1-3'A and CCR2b-64I alleles are not related with the resistance of HIV infection but may make a role for the delayed disease progression of HIV-infected Koreans.

The frequencies of CCR2b-64I and SDF1-3'A alleles in Koreans were very high compared with Caucasians and blacks. The CCR2b-64I allelic mutation in Koreans displayed a high frequency compared with Caucasians and blacks (25.1% in Koreans, 7.2% in Caucasians, and 13.3% in African-Americans, respectively). The frequency of the CCR2b-64I allele in Koreans was 2～4 times higher than those of other ethnic groups with the exception of Asian. In Asian countries, the CCR2b-64I allelic frequency of Koreans was similar to those of Mongolia and Japanese reported by Martinson et al. (10) and Hizawa et al. (14), but it was about 2 times higher than those of Thai, Hong Kong, Thailand, and Chinese reported by Martinson et al. (10), Nookhai et al. (15), and Shieh et al. (16), respectively (Table III). The allelic frequency of the SDF1-3'A gene variant in Koreans was significantly higher than 1.0% of African-Americans, but was similar to those of Thai, Chinese, and Japanese reported by Nookhai et al. (15), Shieh et al. (16), and Su et al. (17), respectively (Table III).

Our results suggest that high frequencies of CCR2b-64I and SDF1-3'A allelic variants are not closely related with the resistance of HIV infection. However, mutant rate of CCR2b gene was about two times higher in the LTNP group than in the remainder group. One of the interpretations of these phenomena is that these allelic variants may affect cell susceptibility after HIV infection. If so, the different genetic backgrounds in ethnic groups can affect the disease progression and the AIDS epidemic in each country. Therefore, we need further studies to define whether high frequencies of CCR2b-64I and SDF1-3'A allelic variants may affect the HIV disease progression.

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