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Comparison *CCR5del32* Mutation in the *CCR5* Gene Frequencies in Russians, Tuvinians, and in Groups of HIV-Infected Individuals

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Abstract—The 32-bp deletion (*CCR5del32* mutation) in the *CCR5* (chemokine (C-C motif) receptor 5) gene, encoding CCR5 chemokine receptor, is one of the factors determining natural resistance to human immunodeficiency virus (HIV-1) infection. In the present study, the samples of Russians (n = 102), Tuvinians (n = 50), and HIV-infected individuals (n = 107) were examined for the presence of *CCR5del32* mutation in the *CCR5* gene. The *CCR5del32* allele frequency in Russians and Tuvinians constituted 7.84 and 2%, respectively. Among HIV-1 infected individuals, two groups, of macrophage-tropic HIV-1 strain- and T-cell-tropic HIV-1 strain-infected were distinguished. The *CCR5del32* allele frequency in the first group (6.45%) was lower than in the second one (8.73%). Statistical treatment of the HIV-1 infected individuals typing data showed that the difference in the *CCR5del32* allele frequencies between the groups of sexually (macrophage-tropic) and parenterally (T-cell-tropic) infected individuals observed was within the limit of random deviation.

INTRODUCTION

Natural resistance to HIV-1 infection is to a great extent associated with human major histocompatibility complex (HLA) [1, 2], as well as with chemokine receptors and their ligands, chemokines [3–10], which play crucial role in recruiting leukocytes and lymphocytes to sites of inflammation. Analysis of the effects of structural alterations in chemokine receptors and their ligands on sensitivity to human immunodeficiency virus (HIV-1) infection will provide understanding of the relationships between the virus and the cell and evaluation of the role of genetic factors in the development of natural resistance to HIV-1 infection.

It is known that the representatives of different ethnic groups and races differ in their sensitivity to HIV-1 infection. It has been also shown that some people remain uninfected, despite numerous unprotected sexual contacts with HIV-1 infected individuals [1, 3]. A crucial requirement in HIV-1 infection is expression of the CD4 receptors on the cell surface. However, the presence of only CD4 receptors is not sufficient for the infection. Penetration of viral RNA and protein complex through the cellular membrane into cytoplasm and infection requires interaction of the viral particle with additional coreceptors. It has been demonstrated in vitro that HIV-1 could use only some of chemokine receptors for the infection. It was also shown that natural resistance to HIV-1 was to a great extent associated with the functional state of CCR5 chemokine receptor (chemokine (C-C motif) receptor 5) (HIV-1 coreceptor), encoded by the *CCR5* gene [11–13]. The gene for the CCR5 receptor is mapped to human chromosome 3 region 3p21.

The CCR5 receptor is a transmembrane protein with molecular mass of 40 600 Da, and consisting of 352 amino acid residues [14]. The CCR5 is mainly found on the surfaces of activated monocytes/macrophages and memory T lymphocytes ($CD26^{High}$, $CD45RA^{-}$, $CD45R0^{+}$, $CD69^{-}$, and $CD95^{+}$). The cells expressing CCR5 receptor are infected with macrophage-tropic HIV-1 strains, which are sexually transmitted and dominate mainly at the early stages of infection. Another virus variant, T-cell-tropic HIV-1 strain, is characterized by mainly parenteral way of transmission [9].

The *CCR5del32* mutation, which is the 32-bp deletion at nucleotide position 794–825 of the *CCR5* open reading frame, represents one of the factors determining natural resistance to the infection with macrophage-tropic HIV-1 strain [14–16]. The *CCR5del32* mutation results in elimination of ten amino acid residues from

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the binding site of the CCR5 and HIV-1 surface proteins, and a frameshift. As a result, the translated receptor is functionally inactive and cannot be used by HIV-1 as a coreceptor to gain entry into the cell. This mechanism underlies protective character of the *CCR5del32* mutation. In homozygous carriers, the mutation provides protection against sexually transmitted HIV-1 infection. The *CCR5del32* heterozygous are not protected against HIV-1 infection. However, according to some data, in case of the infection, these individuals are characterized by slower disease progression compared to the individuals lacking the *CCR5del32* mutation [17].

In the present study, infected and healthy individuals from Moscow and Murmansk, as well as Tuvinians, were typed for the presence of the *CCR5del32* mutation. In addition, the frequency of this mutation in HIV-seronegative individuals from different ethnic groups, and also in HIV-seropositive patients, who were sexually (macrophage-tropic HIV-1 strain), or parenterally (T-cell-tropic HIV-1 strain) infected, was compared.

MATERIALS AND METHODS

The experiments were carried out using DIAtom™ DNA Prep 200 DNA extraction kit (IsoGene, Russia), Maxi-Taq™ and GenePak Universal DNA amplification kits (IsoGene, Russia), plasmid pBR322, the *Msp*I restriction endonuclease, and the 100 bp DNA Ladder Plus molecular size marker (MBI Fermentas, Lithuania). Peripheral blood mononuclear cells were isolated in Ficoll-Histopaque gradient (Sigma, United States), using a conventional procedure.

DNA was extracted from blood samples obtained from HIV-1 uninfected individuals (n = 238) (collection of the Institute of Immunology, Ministry of Health of the Russian Federation, Moscow) and from the samples obtained from HIV-seropositive individuals (patients of the AIDS Regional Center, Murmansk). The samples from Murmansk collection (n = 107) were divided into two groups, comprised by sexually infected (presumably, with macrophage-tropic HIV-1 strain, n = 31), and parenterally infected (presumably, with T-cell-tropic HIV-1 strain, n = 63) individuals. In 13 patients, the way of virus transmission remained uncertain. Among the HIV-1 uninfected individuals, the groups of Russians (Caucasoids, n = 102) and Tuvinians (Mongoloids, n = 50) were distinguished.

The *CCR5* gene fragment carrying the *CCR5del32* mutation was amplified with the primers selected based on the *CCR5* exon 3 sequence: CCR5F: 5'-CGCTCTACTCACTGGTGTTCA-3' and CCR5R: 5'-CATGATGGTGAAGATAAGCCTCAC-3'. Primers were synthesized at the Research-and-Production Company Litekh (Russia).

Polymerase chain reaction (PCR) with genomic DNA was performed on the Amply-4L (Biokom, Russia) thermal cycler using hot start conditions. The amplification conditions consisted of denaturation at 95°C for 0.5 min; annealing at 58°C for 0.5 min; and elongation at 74°C for 1.0 min. A total of 35 cycles were performed. PCR was started with denaturation (95°C for 4.0 min) and was finished with elongation (74°C for 7.0 min). The sizes of amplified fragments constituted 620 bp in case of the deletion absence, and 588 bp upon the deletion presence (*CCR5del32* mutation). PCR products were fractionated by electrophoresis on 6% polyacrylamide gel at 10–12 V/cm, stained with ethidium bromide, and analyzed in the UV light using the Gel Doc 1000 (Bio-Rad Laboratories, United States) gel scanner.

Statistical error of the *CCR5* allele frequencies was calculated as in [18]. Analysis of the allele frequencies and evaluation of the fit to Hardy–Weinberg proportions was performed using CHIRXC and CHIHW software programs [19]. The fit of the observed and expected genotype distributions was tested using χ^2 test.

RESULTS AND DISCUSSION

Based on the data obtained, comparative analysis of the *CCR5del32* frequency in the groups analyzed was performed (table).

The frequency of the *CCR5del32* allelic variant of the *CCR5* gene in Russians and Tuvinians constituted 7.8 and 2.0%, respectively. Homozygotes for the *CCR5del32* mutation were not found in any of the populations examined. Our results extend literature data on the *CCR5del32* frequencies among Caucasoids and Mongoloids. These results indicate that the groups examined significantly differ in the *CCR5del32* frequency. It can be, thereby, suggested that not only the groups examined, but also other ethnic groups of Russia can be differently resistant to HIV-1 infection.

The nearly equal *CCR5del32* allele frequencies in the groups of HIV-1 infected and uninfected individuals (7.94 and 6.11%, respectively), probably, indicate that the heterozygous genotype does not protect against sexually transmitted HIV-infection. In the group of HIV-1-seronegative individuals two *CCR5del32*/*CCR5del32* homozygotes were found, while there were no such homozygotes among HIV-1-positive individuals.

The *CCR5del32* allele frequency in the group of sexually infected individuals was 6.45%, while in the group of parenterally infected individuals it constituted 8.73% (table). The difference, however, was not statistically significant (χ^2 = 0.29; P = 0.78). In the group with uncertain way of infection, the allele frequency was 7.69%. The *CCR5del32*/*CCR5del32* homozygotes were found in none of the three groups.

Thus, albeit the heterozygous genotype, probably, decreases the probability of infection, it does not provide resistance to the infection, since both mutant and normal receptors are expressed on the surface of target cells. Complete protection against infection with macrophage-tropic HIV-1 strain is provided only by homozygosity for the *CCR5del32* mutation [9].

The *CCR5del32* mutation frequencies in the groups of infected and uninfected individuals from Moscow and Murmansk, and also among Tuvinians

HIV status	Group	*n*	No. of heterozygous genotypes	No. of homozygous genotypes	Frequency of the *CCR5del32* allele, %
HIV–	Russians	102	16	–	7.84
	Tuvunians	50	2	–	2.0
	Random sample	86	3	2	4.07
HIV+	Sexual channel of infection	31	4	–	6.45
	Parenteral channel of infection	63	11	–	8.73
	Uncertain channel of infection	13	2	–	7.69

Note that the ratio between the wild-type (*CCR5*) and mutant (*CCR5del32*) *CCR5* alleles is in equilibrium. Given the expected heterozygosity (H_{exp}) value of 0.146, the mean heterozygosity (H_m) was 0.158 in all of the groups examined. The test for statistical significance of the deviation of the mean observed heterozygosity from the expected one showed that this deviation was within the limits of statistical error ($\chi^2 = 0.001$). This suggests that the population examined is in equilibrium at the locus of interest. It seems likely that the *CCR5del32* mutation has no negative effect on humans, since ligands of the CCR5 receptor can use other chemokine receptors.

Analysis of the *CCR5del32* frequency in different groups of HIV-1 infected and uninfected individuals is important for the understanding of the mechanisms of HIV-1 infection and pathogenesis, and also for the prescription of the individual antivirus therapy scheme for the HIV-1 infected patients, which considers specific features of their genotypes.

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