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ORIGINAL ARTICLE

# The Frequency of CCR5del32 Mutation in Populations of Russians, Tatars and Bashkirs of Chelyabinsk Region, Russia

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**Abstract** The distribution of genetic variants associated with natural resistance to viral infections can vary among human ethnic groups due to evolutionary factors, defining the different epidemiologic background of world populations. The polymorphisms, defining the natural resistance to HIV-infection and the rate of progression up to AIDS, are very important since epidemic is still on rise. We have studied the distribution of allele and genotype frequencies of *CCR5delta32* mutation in major populations inhabiting Chelyabinsk region of the Russian Federation. Genetic survey included the population of 509 potential blood marrow donors: Russians ($N = 300$), Bashkirs ($N = 118$) and Tatars ($N = 91$). The genotyping assay was performed using real-time polymerase chain reaction (real-time PCR). The genotypes were defined by melting curve analysis. The *CCR5delta32* allele and *CCR5delta32/delta32* genotype are presented in population of Russians in Chelyabinsk region with the frequencies of $F_x = 10.83\%$ and $P_x = 1.67$, for the *CCR5delta32* allele and its homozygosity, respectively. In populations of Bashkirs and Tatars *CCR5delta32* allele and *CCR5delta32/delta32* genotype are presented at lower frequencies of $F_x = 6.36\%$/$P_x = 0.85$ and $F_x = 7.14\%$/$P_x = 1.10$, respectively. These data are consistent with the theory of northern origin of the *CCR5delta32* mutation.

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## Introduction

The worldwide spread of human immunodeficiency virus (HIV) remains the most urgent problem in the field of infectious diseases treatment and prevention. Recent survey of Russian health defense authorities' estimates the total number of HIV-infected people in the Russian Federation as one million (Central Research Institute of Epidemiology of Rospotrebnadzor 2016). With reference to "Rospotrebnadzor's" monitoring "Data on prevention of HIV-infection, hepatitis B and C, identification and treatment of HIV-infected people" the number of people who had died because of HIV-infection by the 31st of December 2015 had reached 212 579 (12.9% more than for the same period in 2014) (Central Research Institute of Epidemiology of Rospotrebnadzor 2016).

Several independent studies report that there are certain allelic variants of human genes which can influence the individual susceptibility to HIV-infection and progression to acquired immune deficiency syndrome (AIDS). Among them there are C–C chemokine receptor genes, playing the role of viral entry cofactors—*CCR5* and *CCR2*, as well as *SDF1/CXCL12* gene coding stromal cell derived factor 1 (chemokine (C–X–C motif) ligand 12) (Dean et al. 1996; Libert et al. 1998). The most significant associations with natural resistance to HIV/AIDS are reported for *CCR5delta32* (rs333), *CCR2-64I* (rs1799864) and *SDF1-3′A* (rs1801157) (Kofiadi et al. 2007).

Glycoproteins gp120 and gp41 of HIV membrane connect with the cell's surface due to their high similarity to

*CD4*—the main viral receptor. The cell-virus interaction is also mediated by *CCR5* co-receptor, which is expressed on macrophages, or *CXCR4*, expressed on T lymphocytes depending on viral strain tropism. This interaction initiates conformational changes initiating the fusion process.

The main HIV subspecies in primary infection is *R5-HIV-1*, which uses *CCR5* receptor, multiplying predominantly in T cells, macrophages, dendritic and microglia cells. The human *CCR5* gene maps to the short arm of chromosome 3p21.31. For the first time the *CCR5delta32* polymorphism was described in context of natural resistance to HIV in 1988 (Hütter et al. 2009). It is represented by a 32 bp deletion causing a frameshift, preterm stop of translation and loss of three transmembrane domains of the protein. This truncation causes a loss of protein's function and blockage of interaction with viral glycoproteins in homozygotes. Thus, *CCR5delta32* homozygotes become resistant to *R5-HIV-1* infection. Heterozygotes have lower *CCR5* expression than wild-type homozygotes, and hence, are characterized by decreased replication of virus, and slower rates of disease progression (Hütter et al. 2009).

The activation of T helpers by chemokines in HIV-resistant individuals is decreased. As a result, they are characterized by more severe course of some viral infections including West Nile Fever (Lim and Murphy 2011), tick-borne encephalitis (Kindberg et al. 2008), flu (Keynan et al. 2010) etc., but at the same time their hematopoietic cells give less complications upon transplantation (Borinskaya et al. 2012).

The highest frequencies of *CCR5delta32* deletion (15–18%) was reported in Russians and Ugro-Finnic-speaking populations (Estonians, Finns, Mordvins) (Aseev et al. 1997; Kalev et al. 2000; Limborska et al. 2002; Yudin et al. 1998). In South European and Indian populations the frequency of *CCR5delta32* is much lower (2–5%), and in Africa, Southeast Asia, Iran and indigenous populations of the New World it is almost absent (Borinskaya et al. 2012; Jagodzinski et al. 2000; Libert et al. 1998; Martinson et al. 1997; Stephens et al. 1998; Su et al. 2000; Zare-Bidaki et al. 2015).

Presumably, *CCR5delta32* polymorphism appeared in the north-west of Europe and was spread with human migrations. Another explanation is natural selection because of epidemics, which were protected against by this polymorphism (Libert et al. 1998). Under assumption of equal efficiency of selection in different regions, the most probable place of *CCR5delta32* variant origin is either southern Finland (Borinskaya et al. 2012) or Spain or northern Germany (Novembre et al. 2005).

Considering the biological role of *CCR5delta32* polymorphism, as well as potential therapeutic effect of transplantations from homozygous donors, the aim of our study was to identify *CCR5delta32* allele and genotype frequencies in major populations of Chelyabinsk region of the Russian Federation.

## Materials and Methods

We have studied 509 healthy adults (18–45 years old, 334 men and 175 women) without HIV/AIDS history. The samples were collected in the framework of "Chelyabinsk Stem Cell Donor Registry". HLA typing results were obtained from the Chelyabinsk Stem Cell Donor Registry. Informed consent was obtained from the participants. The subjects of the study were divided into three groups based on ethnic origin: Russians ($N = 300$), Bashkirs ($N = 118$) and Tatars ($N = 91$). Ethnic affiliation was defined by analysis of official papers and collection of genealogical anamnesis data up to the third generation.

DNA was extracted with Protrans DNA Box 500 reagent (Protrans, Germany) according to manufacturer instructions. Genotyping was performed by adjacent probe real-time PCR assay made in co-operation with DNA-Technology Company (DNA-Technology, Ltd, Russia). The assay employs two differentially labeled allele-specific fluorescent probes each specific to one of the two alleles of a gene and one quenching probe downstream of the target sequence and adjacent to allele-specific probes. Once hybridized to a target sequence, fluorescent probes become inactivated (quenched). The temperature of probes is raised sequentially up to the so called melting temperature, when half of the probes become denatured, thus no more quenched and, therefore, provide fluorescent signal. The generated fluorescent signal, which was measured and registered at every step of the melting process, increased proportionally to the number of denatured probes. Software based data analysis allows reliable detection of genotypes. The genotyping was performed on CFX-96 "Real Time" thermal cycler (Bio-Rad, USA) by following program: 94 °C—30 s, 67 °C—15 s for five cycles; 94 °C—5 s, 67 °C—15 s for 45 cycles, with fluorescence measurement at melting stage in range of 25–75 °C.

The following parameters were analyzed:

1. Frequency of allelic variant ($F_x$)—the sum of (number of homozygotes × 2) and number of heterozygotes, divided by (number of all samples × 2);
2. Genotype frequency ($P_x$)—% of samples positive for a given genotype (e.g., del/del).

Statistical significance of differences was evaluated with $\chi^2$ and exact Fisher tests. The results were considered significant at $p \leq 0.05$.

## Results

We have identified 7 homozygotes and 79 heterozygotes for *CCR5delta32* polymorphism (Table 1).

The obtained data suggests that *CCR5delta32* allele is higher in Russian population than in Tatars and significantly higher than in Bashkirs ($p < 0.05$) inhabiting Chelyabinsk region of the Russian Federation. It was reported previously that distribution of the *CCR5delta32* polymorphism was characterized by negative gradient from the North to South-East of Europe which could be explained by northern origin of this variant (Borinskaya et al. 2012). Our results correlate with other reports. In the genetic survey of cord blood samples ($N = 2260$) from Pokrovsky stem cell bank, 25 samples homozygous for *CCR5delta32* polymorphism (1.11%) and 439 heterozygous ones (19.42%) were identified. All *CCR5delta32/delta32* were reported to coincide with HLA alleles most frequent for Russian north-west populations (unpublished data). In population genetic study by Kofiadi et al. (2007), covering 11 populations in different regions of Russia and neighboring countries the highest frequency of homozygous genotype was reported for Russian Pomors from Arkhangelsk region (3.1%) and at lower level in Tatars (1.0%).

The lower incidence of *CCR5delta32* allele in Bashkirs ($F_x = 6.36\%$) and Tatars ($F_x = 7.14\%$) correlates with previously reported data showing decreased levels of *CCR5delta32* in populations of Asian ancestry (Su et al. 2000).

The data represented in Table 2 confirm independent inheritance of HLA and CCR5 genotypes under study. The distribution of HLA allele families in the *CCR5delta32* homozygous individuals could not be determined due to the small number of cases. All *CCR5delta32* homozygous donors have widely distributed HLA alleles, and all *CCR5delta32* homozygotes were HLA heterozygotic.

## Discussion

We have shown the frequency of the *CCR5* mutation in the registry of potential stem cell donors of the Chelyabinsk region (Russians, Tatars, Bashkirs) in our investigation. The unique properties of the defective protein product of the *CCR5delta32* allele (i.e. lack of the receptor for HIV-1) suggest that bone marrow transplantation from donors with this mutation may be a cure for HIV-1 infected patients (Hammer et al. 2006; McDermott et al. 2010). One successful (a patient from Berlin) and several unsuccessful bone marrow transplants with a homozygous mutation of *CCR5* were described (Duarte et al. 2015; Kuritzkes 2016). Since the frequency of this mutation is very low (Libert et al. 1998), it is very difficult to find such donors, it is necessary to make an inventory of all umbilical units. According to Petz et al. (2015), gene tests on material from cord blood banks showed further 300 units with this mutation among 25,000 units (i.e., about 0.8%).

A small number of donors from around the world with the *CCR5* mutation dictates the search for other therapeutic approaches, in particular, genetic modification of autologous (or unrelated) stem cells homozygous mutation or variant to use maraviroc to block *CCR5* (Kuritzkes 2016). Additionally, as CCR5/CCR5delta32 individuals are much more frequent than CCR5delta32 homozygotes and, therefore, much more likely to find HLA-matched recipients, their cells could be genetically modified to achieve CCR5delta32 homozygosity and then used for hematopoietic stem cell transplantation (Petz et al. 2015).

On the other hand, it has been shown previously, that the characteristics of the immune system of persons with *CCR5* mutation (donors and recipients of allogeneic stem cells) may have its effect on the course of post-transplant period (Bogunia-Kubik et al. 2007, 2015).

It was shown that the incidence of Epstein–Barr virus reactivation 2–3 months after transplantation was significantly lower in patients carrying the *CCR5delta32* allele (Bogunia-Kubik et al. 2007), while recipient and/or donor *CCR5delta32* homozygosity associated with a higher risk for graft-versus-host disease development (Bogunia-Kubik et al. 2015).

Summarizing, the *CCR5delta32* allele and the *CCR5delta32/delta32* genotype are present in population of Russians from the Chelyabinsk region at frequencies of $F_x = 10.83\%$ and $P_x = 1.67$, respectively. In populations of Bashkirs and Tatars *CCR5delta32* allele and *CCR5delta32/delta32* genotype are present at lower levels

**Table 1** The distribution of allele and genotype frequencies for the *CCR5delta32* polymorphism in populations of Russians, Bashkirs and Tatars

| Group | *N* | Genotype frequency (%) | | | Allele frequency (%) | |
|---|---|---|---|---|---|---|
| | | *ins/ins* | *ins/del* | *del/del* | *ins* | *del* |
| Russians | 300 | 80.0 | 18.33 | 1.67 | 89.17 | 10.83 |
| Bashkirs | 118 | 88.14 | 11.01 | 0.85 | 93.64 | 6.36 |
| Tatars | 91 | 86.81 | 12.09 | 1.10 | 92.86 | 7.14 |

*N* number of individuals tested

**Table 2** HLA-genotypes in donors of the *CCR5delta32/delta32* genotype

| | HLA-genotype |
|---|---|
| Russians | |
| Donor no. 1 | A*03,*24; B*07,*15; C*03,*07; DRB1*04,*15; DQB1*06,*03:02 |
| Donor no. 2 | A*01,*26; B*08,*27; C*03,*07; DRB1*01,*03; DQB1*02,*05 |
| Donor no. 3 | A*02,*02; B*13,*15; C*03,*06; DRB1*07,*13; DQB1*02,*06 |
| Donor no. 4 | A*01,*26; B*08,*27; C*07,*07; DRB1*01,*15; DQB1*05:01,*06:02 |
| Donor no. 5 | A*02,*11; B*44,*51; C*05,*14; DRB1*07,*08; DQB1*02,*04 |
| Bashkirs | |
| Donor no. 6 | A*24; B*35,*40; C*03,*12; DRB1*07,*08; DQB1*02,*03:02 |
| Tatars | |
| Donor no. 7 | A*03,*24; B*15,*35; DRB1*01,*13; DQB1*05:01,*06:03–08 |

of $F_x = 6.36\%/P_x = 0.85$ and $F_x = 7.14\%/P_x = 1.10$, respectively.

In conclusion, identification of the *CCR5* genotypes in blood marrow donors allows to create a subgroup of donors homozygous for deletion who can be the candidates for involvement in HIV stem cell based treatment trials. However, due to low fraction of *CCR5delta32*-positive potential donors, very high polymorphism of HLA, and lack of linkage disequilibrium between *CCR5* and *HLA*, this approach would be limited to a very small minority of patients.

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