# PREVALENCE OF A 32 BP DELETION IN THE GENE FOR HUMAN IMMUNODEFICIENCY VIRUS 1 CO-RECEPTOR CCR5 IN SLOVAK POPULATION

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**Summary. –** The chemokine (C-C motif) receptor 5 (CCR5) represents a major co-receptor for macrophage-tropic Human immunodeficiency virus 1 (HIV-1) strains. A 32 bp deletion mutant allele in the CCR5 gene (CCR5-Δ32) provides to homozygotes a strong resistance to HIV-1 infection. In this study, the prevalence of CCR5-Δ32 in 200 HIV-1-negative and 162 HIV-1-positive individuals was determined. The presence of CCR5-Δ32 in blood samples was detected by PCR. CCR5-Δ32 was found to be homozygous in 1% and heterozygous in 17% of the HIV-1-negative individuals. In HIV-1-positive patients, no homozygous CCR5-Δ32 was found, while heterozygous CCR5-Δ32 occurred in 15.4% patients. Thus, no significant difference between HIV-1-negative and HIV-1-positive individuals was found, what is in accord with the findings obtained in other EU countries. The results of this study do not indicate that a relatively low incidence of HIV-1 infection in Slovakia could be caused by the CCR5-Δ32 mutation.

**Key words:** Human immunodeficiency virus 1; CCR5 co-receptor; Δ32 mutation; Slovakia

## Introduction

HIV-1 is the etiologic agent of acquired immunodeficiency syndrome (AIDS) resulting from the destruction of CD4+ lymphocytes in an infected individual. The entry of HIV-1 into its target cells is mediated by the viral envelope glycoproteins such as gp120, which binds to the cellular receptor CD4 resulting in a conformational change that exposes the variable loop 3 in gp120 and permits subsequent interaction with a chemokine receptor. The primary cellular receptor for all strains of HIV-1 is CD4 molecule, but strain-specific chemokine receptors are required as co-receptors for fusion and entry (Sattentau and Weiss, 1988). CXCR4 is a co-receptor for HIV-1 strains that infect T-cell lines (T-tropic strains) and CCR5 is a co-receptor for HIV-1 strains that infect macrophages and activated T cells (M-tropic strains) (Alkhatib *et al*., 1996; Doranz *et al*., 1996). Viruses that infect T-cell lines are frequently found in the late-stage of HIV-1 infection and utilize the chemokine receptor CXCR4, while viruses that infect macrophages utilize receptor CCR5 and are present throughout the infection.

The CCR5 gene has been mapped in the short arm of chromosome 3 amongst a group of genes that encode multiple chemokine receptors (Samson *et al*., 1996b). The importance of the chemokine receptors in the pathophysiology of HIV-1 infection became apparent at the time of discovery that an individual homozygous for CCR5-Δ32 cannot synthetize a functional CCR5 protein. In general, these individuals were not found among HIV-1-positive patients (Dean *et al*., 1996; Liu *et al*., 1996; Samson *et al*., 1996a). Furthermore, in persons who are heterozygous for the mutation, the rate of progression of HIV-1 infection is slower than in those without the mutation (Marmor *et al*., 2001; Dean *et al*., 1996).

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**Abbreviations:** AIDS = acquired immunodeficiency syndrome; CCR5 = chemokine (C-C motif) receptor 5; Δ32 = 32 bp deletion in the CCR5 gene; CCR5-Δ32 = 32 bp deletion mutant allele in the CCR5 gene; CXCR4 = chemokine (C-X-C motif) receptor 4; HIV-1 = Human immunodeficiency virus 1; UNAIDS = United Nations Programme on HIV/AIDS

Findings of Hummel *et al*. (2005) indicated that this mutation was prevalent already among prehistoric Europeans. These results also argue against the possibility that plague was a major selective force that caused a rapid increase in CCR5-Δ32 frequencies. Kremeyer *et al*. (2005) also assumed that the medieval plague pandemics did not contribute to an increase of the frequency of the CCR5-Δ32. If there had been a positive selection of this allele, it was likely to have occurred before the 14th century and before the arrival of the plague in Europe. CCR5-Δ32 mutation appears to have originated in northeastern Europe, because it occurs with an average allelic frequency of 10% in North American Caucasians coming from Europe. It was not found in native Asians and Africans (Gibejová, 2000).

According to UNAIDS (United Nations Programme on HIV/AIDS) about 40 million people are living with HIV/AIDS all over the world (www.unaids.org). Slovakia with the population about 5 millions belongs to the countries with a relatively low prevalence of HIV-1 infection that is the lowest in central Europe. At the end of June 2008 there were 345 registered HIV-1-infected people in Slovakia and only 245 of them were Slovaks.

The aim of our study was to examine the prevalence of CCR5-Δ32 co-receptor in the Slovak population.

## Materials and Methods

*Blood samples* of 200 HIV-1-negative (100 men and 100 women) and 162 HIV-1-positive (138 men and 24 women) patients were collected.

*PCR.* Cell DNA were prepared from blood samples by commercial kit NucleoSpin® Blood QuickPure (Macherey-Nagel). PCR amplification was performed either by the use of the kit EliGene CCR5 polymorphism Δ (Elisabeth Pharmacon) or by using the following primers: CCR5-forward 5'-GTCTTCATTACACCTG CAGCTC-3' and CCR5-reverse 5'-GTGAAGATAAGCCTCA CAGCC-3' (Beretta *et al*., 2000). After 5 mins of denaturation at 94°C, the reaction was carried out in a 20 µl volume of the Easy-Caps PCR PreMix (Ecoli) in 35 cycles (denaturation at 94°C for 40 secs, annealing at 60°C for 40 secs, and strand extension at 72°C for 40 secs) followed by the final extension step at 72°C for 10 mins. Amplified products were separated by electrophoresis in 2% agarose gel (Amresco®) and visualized with ethidium bromide (0.5 µl/ml). Two fragments of 199 bp for the wild type allele of CCR5 and 168 bp for the CCR5-Δ32 allele were obtained (Fig. 1b).

## Results and Discussion

CCR5-Δ32 mutation was found to be homozygous in 1% of HIV-1-negative individuals and heterozygous in 17% of

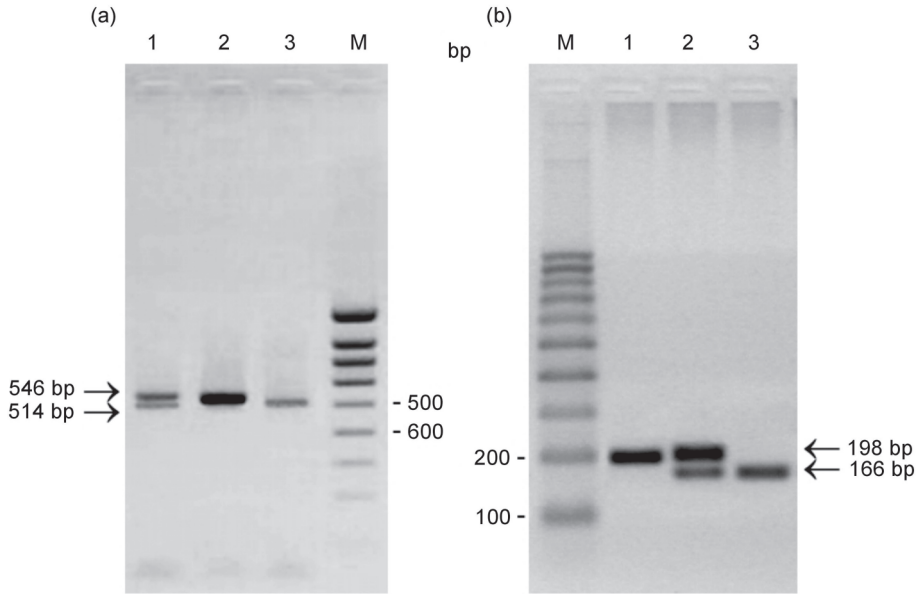


**Fig. 1**

**Detection of CCR5 polymorphism by PCR**

(a) PCR performed using the EliGene CCR5 Kit. Heterozygous mutant genotype CCR5/CCR5-Δ32 (lane 1), homozygous wild-type genotype CCR5/CCR5 (lane 2), homozygous mutant genotype CCR5-Δ32/CCR5-Δ32 (lane 3). (b) PCR performed using the primers. Homozygous wild-type genotype CCR5/CCR5 (lane 1), heterozygous mutant genotype CCR5/CCR5-Δ32 (lane 2), homozygous mutant genotype CCR5-Δ32/CCR5-Δ32 (lane 3). DNA size markers (lanes M). Arrows indicate fragments specific for wild-type (198 respectively 546 bp) and mutant (166 respectively 514 bp) alleles.

**Table 1. Prevalence of CCR5-Δ32 in HIV-1-negative and HIV-1-positive participants in Slovakia**

| CCR5 polymorphism | No. of participants (%) | | | | | |
|---|---|---|---|---|---|---|
| | HIV–1-negative | | | HIV-1-positive | | |
| | Women | Men | Total | Women | Men | Total |
| Homozygous wild-type genotype CCR5/CCR5 | 81 (81) | 83 (83) | 164 (82) | 19 (79.2) | 118 (85.5) | 137 (84.6) |
| Homozygous mutant genotype CCR5-Δ32/CCR5-Δ32 | 1 (1) | 1 (1) | 2 (1) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Heterozygous mutant genotype CCR5/CCR5-Δ32 | 18 (18) | 16 (16) | 34 (17) | 5 (20.8) | 20 (14.5) | 25 (15.4) |
| Total | 100 | 100 | 200 | 24 | 138 | 162 |

HIV-1-negative individuals. The majority of HIV-1-negative persons (82%) were homozygous for the wild-type allele. In the group of HIV-1-positive patients no homozygosity of CCR5-Δ32 was found. Heterozygosity of CCR5-Δ32 in HIV-1-positive patients was 15.4%, what is similar number as in HIV-1-negative individuals (Table 1). According to the obtained results the frequency of CCR5-Δ32 deletion allele in Slovak population was calculated to 9.5%. The Δ32 allele frequency in men was similar to that in women (9% vs. 10%).

The prevalence of CCR5-Δ32 mutation differs according to the geographical region. The prevalence of CCR5-Δ32 mutation in the Czech population is similar to that observed in our study (0.3% homozygotes vs. 21% heterozygotes) (Drábek and Petrek, 1998). German study revealed the presence CCR5-Δ32 mutation in 16.2% from 737 HIV-1-negative and in 17.5% from 463 HIV-1-positive individuals (Oh *et al*., 2008).

Correlation between CCR5-Δ32 allele frequency and latitude for the populations in 35 different locations in Europe, Middle East, and North Africa was studied. The correlation coefficient r = 0.795 was highly significant ($p < 10^{-9}$). The highest CCR5-Δ32 allele frequency was found in Sweden, Norway, Denmark, Finland, and Iceland, where the mean value 13.4% was significantly higher than the mean value 8.18% for all populations studied. The lowest CCR5-Δ32 allele frequencies were found in Greece, Cyprus, Turkey, Daghestan, and Morocco, where the mean value was 4.5% (Lucotte 2001).

Similarly, the study of Libert *et al*. (1998) found similar results in 18 European populations with the highest CCR5-Δ32 allele frequency in Finland and Moldavia (16%) and the lowest in Sardinia (4%). Moreover, in the countries of the former Soviet Union, the frequencies of the alleles CCR5-Δ32 was 15, 12, and 12% for Russia, Ukraine, and Belarus, respectively (Kozhekbaeva *et al*., 2004). In contrast, the inability to detect CCR5-Δ32 among Asians and other Pacific Islander groups suggested that other mechanisms are responsible for resistance to HIV-1 infection in these populations. The frequency of CCR5-Δ32 heterozygosity was 16.8% in Caucasians, 5.6% in Puerto Rican Hispanics, and 1.8% in Pacific Islanders. However, the absence of CCR5-Δ32 was found in Asians and Africans (Lu *et al*., 1999). Until now, only 12 cases of HIV-1-infected CCR5-Δ32 homozygous individuals have been described worldwide. Large-scale studies conducted among Caucasians indicate that individuals who are homozygous for this deletion mutation are protected against HIV-1 infection despite multiple high-risk exposures (Oh *et al*., 2008; Lu *et al*., 1999).

No significant differences in genotype distributions and CCR5-Δ32 allele frequencies between HIV-1 infected and uninfected participants were found in the study in Luxembourg (Roman *et al*., 2002). On the other hand, in Poland was found higher prevalence of CCR5-Δ32 among seronegative participants (13.6%) compared to HIV-1-infected patients (9.7%), but this difference did not attain statistical significance (Wasik *et al*., 2005). Surprisingly, parallel study in Poland revealed three times higher prevalence of CCR5-Δ32 in HIV-1-negative population compared to HIV-1-positive individuals (Piasecki *et al*., 2005).

In our study, a significant difference in the incidence of CCR5-Δ32 mutation between HIV-1-negative population and HIV-1-positive patients was not found. Results of the study do not indicate that relatively low prevalence of HIV-1-infection in Slovakia could be due to the presence of CCR5-Δ32 mutation in our population.

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