

# Protective Effect of *CCR5-Δ32* Against HIV Infection by the Heterosexual Mode of Transmission in a Polish Population

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## Abstract

Effects of chemokine receptor alleles (*CCR5-Δ32* and *CCR2-64I*) on susceptibility to human immunodeficiency virus (HIV) infection were studied in a Polish population. The *CCR5* and *CCR2* genotypes were determined for 311 healthy, HIV-negative individuals (control group), 121 exposed to HIV infection but uninfected (EU group), and 470 HIV-positive patients. The frequency of the alleles in the control group was calculated as 0.12 for both *CCR5-Δ32* and *CCR2-64I*. The logistic regression method was used to analyze the effects of the described factors. A protective effect was observed for the *CCR5-Δ32* allele but only in the case of heterosexual exposure. Prevalence of the *CCR5-Δ32/+* genotype in HIV<sup>+</sup> patients infected via the heterosexual route ( $n=61$ ; 8.2%) was much lower than in the control group ( $n=311$ ; 21.5%); in the heterosexually exposed uninfected group it was slightly higher ( $n=28$ ; 25%). This suggested that in this mode of infection, the native *CCR5* expression level was crucial for establishment of infection. Individuals with the *CCR5-Δ32* allele have more than three times less chance of infection in the case of HIV heterosexual exposure (odds ratio, 3.37; 95% confidence interval, 1.055–10.76). However, a protective effect of the *CCR5-Δ32/+* genotype was not observed in the case of intravenous drug users (IDUs). The rates of the genotype were similar in HIV-infected IDU individuals ( $n=356$ ; 17.7%) and in exposed uninfected patients ( $n=84$ ; 15.5%), not significantly different from control group. No effect of the *CCR2* genotype was observed. The analysis revealed that the important factor increasing infection risk was, in particular, hepatitis C virus (HCV) infection (odds ratio, 12.9). Moreover, the effect of HCV infection was found to be age dependent. Susceptibility to HIV infection resulting from HCV positivity became weaker (6% per year) with increasing age.

## Introduction

THE IMPORTANCE OF CHEMOKINE receptors in human immunodeficiency virus (HIV) infection has become recognized.<sup>1–3</sup> It is known that these receptors in their functional form act as HIV coreceptors and are essential for the establishment of new infection. Infection of a cell with non-functional or inaccessible coreceptors is unlikely.<sup>4</sup> CC chemokine receptor type 5 (*CCR5*) is the main coreceptor used by the virus in the case of primary infection of an individual. The *CCR5-Δ32* allele is particularly important because it is relatively common in white populations.<sup>5,6</sup> The geographic distribution of *CCR5-Δ32* was found to be irregular, with the highest frequency observed in northern Europe.<sup>5,6</sup>

*CCR5-Δ32* homozygosity was found to give almost complete resistance to HIV infection.<sup>3,4,7–9</sup> There have been only 12 reported cases of infection of homozygous persons.<sup>10</sup> As only 1–2% of white individuals have a protective genotype, the impact of the allele on the population is limited.<sup>11</sup> On the other hand, such a genotype was connected with severe course of West Nile virus<sup>12,13</sup> and tick-borne encephalitis virus infection.<sup>12,14</sup> Furthermore, *CCR5-Δ32* heterozygosity was frequently found among patients with critical illness after influenza A/H1N1 2009 infection.<sup>15</sup>

Individuals with a *CCR5+Δ32* heterozygous genotype are susceptible to HIV. However, the significance of the genotype for infection progression is not clear. Most of the reports showed beneficial effects on prolongation of the asymptomatic period,

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TABLE 1. BASELINE CHARACTERISTICS OF STUDIED GROUPS

	Control group	EU group	HIV <sup>+</sup> group
Number of individuals [ <i>n</i> ]	311	121	470
Age range (average) [years]	5–71 (33.5)	3–50 (30.5)	0–59 (30.7)
Sex (female/male) [ <i>n</i> (%)]	161/150 (51.7/48.3%)	41/80 (33.8/66.2%)	158/312 (33.6/66.4%)
Homosexual exposure [ <i>n</i> (%)]	nc	2 (1.7%)	35 (7.5%)
Heterosexual exposure [ <i>n</i> (%)]	nc	33 (27.2%)	61 (13.0%)
Intravenous drug users [ <i>n</i> (%)]	nc	84 (69.4%)	359 (76.4%)
Other type of exposure [ <i>n</i> (%)]	nc	2 (1.7%)	15 (3.1%)
HCV <sup>+</sup> [ <i>n</i> (%)]	nt	77 (63.6%)	356 (75.7%)
HCV <sup>-</sup> [ <i>n</i> (%)]	nt	39 (32.2%)	109 (23.2%)
No data for HCV coinfection [ <i>n</i> (%)]	nt	5 (4.2%)	5 (1.1%)

EU, exposed uninfected; HCV, hepatitis C virus; nc, not concerned; nt, not tested.

probably due to a reduced expression level of the protein.<sup>7,8,16</sup> The differences may be connected with other factors influencing HIV progression, including coinfections or drug use. Moreover, other genetic factors might interfere with the effect of *CCR5-Δ32*, as was suggested for *SDF-1*.<sup>17</sup>

Among other chemokine receptor polymorphisms, excluding rare ones, only the *CCR2-64I* allele was found to play a role in slowing infection progression, although it had no effect on susceptibility to infection.<sup>7,8,11,18</sup> Moreover, this allele has been identified as a risk factor for cancer.<sup>19–21</sup>

In our previous work we have found a positive correlation of acid-labile interferon- $\alpha$  level<sup>22</sup> and inhibition of vesicular stomatitis virus replication<sup>23</sup> with progression of HIV infection. However, these factors have no effect on susceptibility to infection as genetic factors may have. Many polymorphisms have been studied in this respect, often resulting in ambiguous conclusions.<sup>24</sup>

The aim of this study was to determine the frequency of chemokine polymorphisms *CCR5-Δ32* and *CCR2-64I* in a Polish population and to evaluate whether these polymorphisms had an impact on susceptibility to HIV-1 infection depending on the route of infection.

## Materials and Methods

### Patients

Three groups of patients from the Lower Silesia region of Poland were analyzed. The first group consisted of 311 people who were healthy, HIV-negative, and unexposed to HIV (the control group). These individuals were selected on the basis of anamnesis, showing no risk behavior. They were recruited from among blood donors and healthy volunteers with no reported infection or other severe diseases (on the basis of anamnesis), age matched to the HIV-positive group. Moreover, taking into consideration the fact that only a small percentage of the Polish population is HIV infected (up to 0.1%<sup>25</sup>), we could reasonably assume that these people were not exposed to HIV. Genotyping of this group provided information about frequencies of tested alleles in the studied population.

The second group (EU, exposed uninfected) was composed of 121 individuals exposed to HIV but seronegative. In all cases in that group, exposure was long-lasting and repeated in character. Thirty-five individuals in the EU group were long-term partners of HIV-infected people [2 homosexual (EU-HO)

and 33 heterosexual (EU-HT)]. Eighty-four people were intravenous drug users (EU-IDU) with a history of addiction and thus a risk of HIV infection lasting on average 13.7 years (1.5–36 years). About 90% of EU-IDU were HCV positive. In the EU group there were also two seronegative children of HIV-infected mothers despite the lack of vertical transmission prophylaxis.

The third analyzed group consisted of 470 HIV-positive people in various stages of infection (HIV<sup>+</sup>). All of them were patients of the Department and Clinic of Infectious Diseases, Hepatology, and Acquired Immune Deficiencies (Wrocław Medical University, Wrocław, Poland). HIV-positive patients were selected on the basis of confirmed diagnosis. All such patients were included in the study. According to the route of infection HIV<sup>+</sup> patients were divided into three main groups: 35 men who had sex with men (HIV-HO), 61 people infected by the heterosexual route (HIV-HT), and 359 infected intravenous drug users (HIV-IDU). There were also three children infected from mothers; one person infected during blood transfusion; one patient with hemophilia, infected after taking blood products; and 10 people with an undefined route of HIV transmission. Characteristics of the studied groups are shown in Table 1. To estimate the impact of studied genetic factors on susceptibility to HIV infection depending on the mode of transmission, a comparison of these features in EU and HIV<sup>+</sup> groups was performed.

### Genotyping

Five milliliters of peripheral EDTA-anticoagulated blood was taken from all tested individuals and frozen at  $-20^{\circ}\text{C}$  until DNA isolation. Genomic DNA was extracted from blood samples by digestion with proteinase K and purification on Qiagen columns according to the manufacturer's protocol (QIAamp DNA blood mini kit; Qiagen, Hilden, Germany). The DNA concentration and purity were determined with a BioPhotometer (Eppendorf, Hamburg, Germany).

**CCR5.** The genotypes of *CCR5* were determined by polymerase chain reaction (PCR). A primer pair (SP4.760, 5'-CTT CAT TAC ACC TGC AGC TCT-3', and PM6.492, 5'-CAC AGC CCT GTG CCT CTT CTT C-3') was used to obtain a 182-bp amplicon from the human *CCR5* gene and a 150-bp fragment from the mutational form of this gene.<sup>26</sup> The assay was done with a 10- $\mu\text{l}$  reaction volume containing 0.25 U of Hot-StarTaq DNA polymerase (Qiagen), 2.25 mM  $\text{MgCl}_2$  solution

(Qiagen), 200 μM dNTPs (Invitrogen, Carlsbad, CA), 0.5 μM CCR5 primer mix (Symbios, Straszyn, Poland), and about ~30 ng of DNA sample. The PCR was run on a thermocycler T3000 (Biometra, Goettingen, Germany) under the following conditions: 95°C for 15 min; 32 cycles at 94°C for 60 s, 58°C for 30 s, and 72°C for 30 s; and a final extension at 72°C for 4 min. The PCR products were separated by 2% agarose (AppliChem, Darmstadt, Germany) gel electrophoresis at 140 V for 15 min. The agarose gels were stained with ethidium bromide (0.5 mg/ml) and visualized under ultraviolet (UV) light.

**CCR2.** The detection of CCR2 and CCR2-64I was performed by PCR and, in some cases when PCR alone did not give clear results, PCR restriction fragment length polymorphism (RFLP) methods.

In the first case a 10-μl tetraprimer PCR was performed: 0.25 U of HotStarTaq DNA polymerase (Qiagen), 200 μM MgCl<sub>2</sub> solution (Qiagen), a 0.4 μM concentration of primers CCR2-V and CCR2-I, a 0.06 μM concentration of primers CCR2-U and CCR2-L (Symbios), 200 mM dNTP mix (Invitrogen), and ~30 ng of specimen DNA. The PCR protocol included the following parameters: 95°C for 15 min; 22 cycles (first set) of 94°C for 30 s, 64°C for 30 s, and 72°C for 1 min; 28 cycles (second set) of 94°C for 30 s, 43°C for 30 s, and 72°C for 1 min; and a final extension at 72°C for 7 min. PCR amplification was performed with a Biometra thermocycler T3000. The PCR products were separated by 2% agarose gel electrophoresis at 140 V for 20 min and visualized under UV light. During the first set of cycles a 445-bp region of CCR2 was amplified with CCR2-U (5'-GAT CAT GGA TTG CGG TGT TTG TGT TGT-3') and CCR2-L (5'-CAC ATT GCA TTC CCA AAG ACC CAC TCA-3'); this served as an internal control for the quality of the PCR amplification and as a template for the subsequent amplification.

During the second set of cycles, the allele-specific amplification (CCR2-V, 5'-TTT ATT AAG ATG AGG AC-3' and CCR2-I, 5'-GGC AAC ATG CTG GTC A-3') resulted in a 313-bp product specific for the CCR2-V allele and in a 164-bp product specific for the CCR2-I allele.<sup>27</sup>

**Detection of CCR2 and CCR2-64I allele by RFLP.** The PCR products were obtained during amplification with a primer pair (CCR2-F, 5'-TTG TGG GCA ACA TGA TGG-3' and CCR2-R, 5'-GAG CCC ACA ATG GGA GAG TA-3').<sup>28</sup> The reaction was done in 10 μl and PCR conditions were as follow: 0.25 U of FastStar Taq DNA polymerase (Roche Diagnostics, Mannheim, Germany), 2.25 mM MgCl<sub>2</sub> solution (Roche Diagnostics), a

0.7 μM concentration of each primer (Symbios), 200 μM dNTPs (Invitrogen), and ~30 ng of specimen DNA. The conditions were as follows: 95°C for 6 min; 3 cycles at 94°C for 25 s, 48°C for 20 s, and 72°C for 30 s; 40 cycles at 94°C for 25 s, 55°C for 30 s, and 72°C for 15 s; and a final extension at 72°C for 5 min. PCR was performed in a Biometra thermocycler T3000. The digestion of PCR products was performed with a 15-μl reaction volume containing 3.75 U of BseGI (FokI) (Fermentas, Vilna, Lithuania) and 10 μl of post-PCR mix. The reaction was performed at 55°C for 2 h in a Biometra Thermoblock TB1. The restriction products were separated by 2% agarose gel electrophoresis at 140 V for 40 min and visualized under UV light. The restriction products were 128 bp for the wild-type and 110 and 18 bp for the CCR2-64I allele.

#### Data analysis

Genotypes and allele frequencies were counted according to a formula based on the Hardy-Weinberg principle. Genotypes and allele frequencies were calculated in control ( $n=311$ ), EU ( $n=121$ ), and HIV<sup>+</sup> ( $n=470$ ) groups. To analyze the impact of chemokine polymorphisms CCR5-Δ32 and CCR2-64I on the susceptibility to HIV infection, logistic regression at general linear model's scheme was used. A comparison of genetic and nongenetic factors in EU ( $n=114$ ) and HIV<sup>+</sup> ( $n=452$ ) groups was performed. The numbers in this analysis were smaller than in the previous one because of the lack of complete data for several individuals. Besides CCR5-Δ32 and CCR2-64I effects, the impact of route of exposure to HIV (homosexual, heterosexual, and intravenous drug use), sex, age, and HCV coinfection were also tested. Variables were included in the model if  $p < 0.05$ . All statistical analyses were performed using the platform R-CRAN version 2.8.1 (www.r-project.org).

## Results

### Frequency of CCR5-Δ32 allele

CCR5 and CCR5-Δ32 allele frequencies were studied in HIV-infected patients (HIV<sup>+</sup>,  $n=470$ ) who were under the care of the Department and Clinic of Infectious Diseases, Hepatology, and Acquired Immune Deficiencies (Wroclaw Medical University). The patients were mainly from the Lower Silesia region of Poland. The second investigated group comprised exposed but uninfected individuals (EU,  $n=121$ ). The results were compared with those of unexposed, uninfected healthy people forming a control group ( $n=311$ ) (Table 2).

TABLE 2. FREQUENCIES OF CCR5 AND CCR2 GENOTYPES IN HIV-INFECTED AND EXPOSED UNINFECTED PATIENTS IN COMPARISON TO CONTROL GROUP CONSISTING OF UNEXPOSED UNINFECTED INDIVIDUALS

Studied group	CCR5					CCR2				
	Number of individuals (rate)		Allele frequency			Number of individuals (rate)		Allele frequency		
	CCR5 +/+	CCR5 +/Δ32	CCR5- Δ32/Δ32	CCR5- Δ32	CCR5+	CCR2 +/+	CCR2 +/64I	CCR2- 64I/64I	CCR2- 64I	CCR2+
HIV <sup>+</sup> ( $n=470$ )	394 (83.83)	76 (16.17)	0	0.08	0.92	367 (78.09)	97 (20.64)	6 (1.28)	0.12	0.88
EU ( $n=121$ )	94 (77.69)	25 (20.66)	2 (1.65)	0.12	0.88	88 (72.73)	30 (24.79)	3 (2.48)	0.15	0.85
Control ( $n=311$ )	240 (77.17)	67 (21.54)	4 (1.29)	0.12	0.88	238 (76.53)	70 (22.51)	3 (0.96)	0.12	0.88

EU, exposed uninfected.

CCR5-Δ32 allele frequency in control and EU groups was calculated as 0.12. There was no difference between the groups ( $p=0.9$ ) according to Fisher's exact test. The groups conform to the Hardy-Weinberg principle ( $p=0.993$ ). The principle was rejected in the case of the HIV<sup>+</sup> group. The difference compared with the control and EU groups ( $p=0.0104$ ) resulted from the lack of CCR5-Δ32/Δ32 homozygotes and smaller number of heterozygotes than expected ( $p=0.00058$ ). This suggested that CCR5-Δ32 had a protective effect that occurred at the moment of exposure to HIV.

#### Frequency of CCR2-64I allele

Studies similar to the previous analysis were done for CCR2 alleles: wild 64V and 64I. Frequencies of the CCR2-64I allele in control and HIV<sup>+</sup> groups were determined as 0.12, and in the EU group as 0.15 (Table 2). All three groups conform to the Hardy-Weinberg principle ( $p=0.8928$ ) and show no significant differences in the allele frequencies ( $p=0.5497$ ), so no protective effect of CCR2-64I was observed.

#### Analysis of susceptibility to HIV infection

To analyze susceptibility to HIV infection, a comparison of HIV<sup>+</sup> and EU groups was made. The characteristics of the patients are shown in Table 3. The total number of patients was smaller than in previous analyses because individuals without complete data (mainly mode of infection and HCV status) were omitted.

Logistic regression was used for analysis of impact of studied factors (CCR5-Δ32 and CCR2-64I alleles, routes of

TABLE 3. CHARACTERISTICS OF HIV-INFECTED AND EXPOSED UNINFECTED PATIENTS INCLUDED IN THE STUDY ON SUSCEPTIBILITY TO HIV INFECTION

Characteristics of studied group	EU	HIV <sup>+</sup>
Numbers of individuals	114	452
Average age [years]	31.02	30.90
Sex [n (%)]		
Female	36 (31.6)	151 (33.4)
Male	78 (68.4)	301 (66.6)
Mode of infection [n (%)]		
Homosexual	2 (1.7)	35 (7.7)
Heterosexual	28 (24.6)	61 (13.5)
IDU	84 (73.7)	356 (78.8)
HCV infection [n (%)]		
-	37 (32.5)	98 (21.7)
+	77 (67.5)	354 (78.3)
CCR5 [n (%)]		
CCR5 +/+	92 (80.7)	377 (83.4)
CCR5 +/Δ32	20 (17.5)	75 (16.6)
CCR5-Δ32/Δ32	2 (1.8)	0 (0.0)
CCR2 [n (%)]		
CCR2 +/+	82 (71.9)	353 (78.1)
CCR2 +/64I	29 (25.4)	93 (20.6)
CCR2-64I/64I	3 (2.6)	6 (1.3)

Analysis of effect of transmission was made for individuals with all needed data available. Because of that the number of patients included in the study was lower than that in the genotype frequency determination (Table 2).

EU, exposed uninfected; IDU, intravenous drug user; HCV, hepatitis C virus.

TABLE 4. FACTORS SELECTED BY LOGISTIC REGRESSION METHOD AS HAVING AN EFFECT ON SUSCEPTIBILITY TO HIV INFECTION

Tested factor	Impact on susceptibility to HIV infection	OR	CI 95%	p
HCV infection	↑	12.90	4.69–35.48	0.00002
Homosexual exposure	↑	7.69	1.88–31.48	0.007017
CCR5-Δ32 allele in case of heterosexual exposure	↓	3.37	1.055–10.76	0.044913

↑, infection risk increased; ↓, infection risk reduced; OR, odds ratio; CI, confidence intervals.

exposure to HIV, sex, age, and HCV coinfection) (Table 4) on the susceptibility to HIV infection. A protective effect was found for the CCR5-Δ32 allele but only in the case of heterosexual exposure. No effect of CCR2 genotype was observed. The analysis revealed that important factors increasing infection risk were homosexual exposure and particularly HCV infection [odds ratio (OR), 12.9]. Moreover, the effect of HCV infection was found to be age dependent. Susceptibility to HIV infection resulting from HCV positivity became weaker (6% per year) with increasing age.

#### Effect of CCR5 genotype on susceptibility to infection in context of exposure mode

A detailed analysis of CCR5 genotypes was performed in HIV<sup>+</sup> and EU groups, which additionally were divided into subgroups defined by common transmission modes (homosexual, heterosexual, and connected with intravenous drug use) (Table 5). CCR5-Δ32/Δ32 homozygotes were found only among EU patients (two cases), which was predictable. However, heterozygote frequency analysis should show differences. In IDU patients no differences between infected and uninfected groups were observed. The majority of patients belonged to this group, so equal frequency of the allele left no doubt that CCR5 genotype exerted no effect in this type of exposure.

In the smallest group of homosexual exposure differences observed were statistically insignificant. There were only two individuals in the EU subgroup. An increase of their number would be favorable for analysis, but the specificity of this group made it practically difficult.

A statistically significant difference in CCR5-Δ32 frequency was found between infected and uninfected individuals exposed by the heterosexual route. Prevalence of the CCR5-Δ32/+ genotype in HIV<sup>+</sup> patients (HIV-HT) was much lower than in the control group (8.2 vs. 21.5%); in the EU-HT group it was slightly higher (25%). This suggested that in this route of infection native CCR5 expression level was crucial for establishment of infection. Individuals with the CCR5-Δ32 allele have more than three times less chance of infection in the case of HIV exposure (OR, 3.37; Table 4).

#### Discussion

The first genetic factor discovered as affecting HIV infection was CCR5-Δ32, which was an allele of the gene encoding

TABLE 5. ANALYSIS OF CCR5 GENOTYPE IN HIV-INFECTED AND EXPOSED UNINFECTED PATIENTS: EFFECT OF MODE OF EXPOSURE

Mode of exposure	Mode of Exposure					
	Homosexual		Heterosexual		Intravenous drug use	
	HIV-HO (n=35)	EU-HO (n=2)	HIV-HT (n=61)*	EU-HT (n=28) <sup>a</sup>	HIV-IDU (n=356)	EU-IDU (n=84)
Number of CCR5+/+	28	2	56	20	293	70
Number of CCR5+/ $\Delta$ 32	7	0	5	7	63	13
Number of CCR5- $\Delta$ 32/ $\Delta$ 32	0	0	0	1	0	1
CCR5+/+ rate [%]	80.00	100	91.80	71.43	82.30	83.30
CCR5+/ $\Delta$ 32 rate [%]	20.00	0	8.20	25.00	17.70	15.50
CCR5- $\Delta$ 32/ $\Delta$ 32 rate [%]	0	0	0	3.57	0	1.20
CCR5- $\Delta$ 32 frequency	0.10	0	0.04	0.16	0.09	0.09
CCR5 frequency	0.90	1.00	0.96	0.84	0.91	0.91

<sup>a</sup>Significant difference at  $p < 0.05$ .

HO, homosexual; EU, exposed uninfected; HT, heterosexual; IDU, intravenous drug user.

the CCR5 coreceptor essential for R5 strains.<sup>3,7</sup> Shortened CCR5- $\Delta$ 32 protein is inactive both as a chemokine and an HIV receptor.<sup>7,29</sup> Soon after the discovery of the allele, significant differences in its geographic distribution were found. The allele was not found in native populations of sub-Saharan Africa, East Asia, or America. However, its frequency in whites was determined as 2–20%; in Europe it is highest in northern countries and becomes lower southward.<sup>30</sup>

The frequency of the CCR5- $\Delta$ 32 allele in Poland was estimated as 10.9% by Jagodziński and colleagues.<sup>31</sup> The highest values were found in the northeast (13.2–14%) and western regions (13–13.3%). The latter is probably because of postwar migrations from the east. Our result for the Lower Silesia region (southwest Poland) was 12%, calculated by analysis of 311 individuals. It is a slightly lower value than that obtained by Jagodziński and colleagues. They found the allele present at a frequency of 13%, but only 46 people were analyzed.<sup>31</sup> Zawicki and Witas<sup>32</sup> determined the frequency of CCR5- $\Delta$ 32 in ancient DNA isolated from Polish medieval samples (eleventh to fourteenth centuries). The result (5.06%) suggested that during less than 1000 years the allele frequency increased 2-fold.<sup>32</sup>

CCR5- $\Delta$ 32 homozygotes are almost completely resistant to HIV infection. They can be infected only by T-tropic X4 or X4R5 strains, rarely engaged in primary infection. Also, M-tropic R5 strains can use a coreceptor other than CCR5. However, cases of such infection seem to be exceptional.<sup>9,33</sup> In practice, the role of mutation CCR5- $\Delta$ 32 was shown by Hütter and colleagues,<sup>34,35</sup> presenting a case of an HIV-infected patient who received hematopoietic stem cell transplantation from a homozygous CCR5- $\Delta$ 32 donor. The viral load, which was under the detection level for several months after the interruption of antiretroviral therapy, suggested at least complete suppression of HIV.<sup>34,35</sup>

The protective role of CCR5- $\Delta$ 32 homozygosity can be seen in some cases of considerable exposure without infection. Such a case of long-term heterosexual exposure with no other protection factors known was described by us (unpublished results). However, the protective effect of CCR5- $\Delta$ 32/ $\Delta$ 32 homozygotes seems not to be significant at the population level because of their low occurrence (1–3%<sup>9,32</sup>; 1.29% in our study) (Table 2). There was no consensus as to whether CCR5+/ $\Delta$ 32

heterozygosity had an effect on HIV infection. Some reports showed no protective effect of this genotype<sup>7,8,36</sup>; others reported at least a partial protective effect in different modes of exposure.<sup>16,30,37,38</sup> In our study, CCR5- $\Delta$ 32 allele frequencies in the control and EU group (12%; Table 2) were higher than in HIV-infected patients (8%). In the latter group no homozygous CCR5- $\Delta$ 32/ $\Delta$ 32 case was found. However, two such cases were detected in the EU group. In this group, the percentage of CCR5+/ $\Delta$ 32 heterozygotes (20.7%) was found to be higher than in HIV-infected patients (16.2%). The results suggested a protective effect of the CCR5- $\Delta$ 32 allele, including heterozygotes.

The detailed analysis using the logistic regression model showed that the protective effect was to be shown in the case of heterosexual exposure only. A clear illustration of this protection was the comparison of two heterosexually exposed groups: HIV-infected and noninfected. The frequency of the CCR5- $\Delta$ 32 allele in these groups was 4% and 16%, respectively. The infection risk of homozygous CCR5+/+ individuals was found to be more than three times higher (OR, 3.37) than that of heterozygous patients. Such an effect was not observed in the case of other analyzed exposure routes: homosexual and connected with intravenous drug use.

In the case of homosexual individuals, the EU group was too small, mainly because of difficulties with reaching these people (healthy individuals in informal relationships are usually not likely to attend any studies). In the case of the IDU group the number of individuals was high enough to evaluate statistical analysis. The patients in this group (infected and uninfected) were generally in poor condition, with many viral and nonviral infections. HCV infection was common in the IDU group and the infection usually preceded HIV infection, so it might be one of factors overcoming the protective effect of CCR5- $\Delta$ 32. The other reason for these differences was probably the fact that more viral particles could reach sensitive cells with active CCR5 receptors during homosexual or IDU exposure. There is generally lower risk of infection in the case of heterosexual exposure. Probably the decreased amount of functional receptors (in CCR5+/ $\Delta$ 32 individuals) was enough to make effective infection difficult during this type of exposition. Such a protective effect was not observed during massive,

frequent and long-term exposure as it is in the case of the homosexual route of infection and IDU.

Both *CCR2-64* alleles (*64V* and *64I*) are used as HIV coreceptors by certain viral strains. However, *CCR2-64I* was found to have an anti-HIV effect, which might be due to the formation of complexes with CCR5 protein.<sup>8</sup> *CCR2-64I* allele frequency in the Polish population of Lower Silesia was estimated as 12% (Table 2). This is comparable to the 10–25% determined for different geographic locations.<sup>8</sup> Most of the available data showed no effect of *CCR2-64I* mutation on susceptibility to HIV infection.<sup>39</sup> Our results are in line with those data (Tables 2 and 3). The protective effect was noted only in perinatal infection,<sup>36</sup> which was not the case in our study.

Our results confirmed that among chemokine receptors the role of *CCR5* alleles was predominant in protection against HIV infection. *CCR5-Δ32* was found to be one of the factors important for establishment of long-term nonprogression.<sup>40</sup> The protective effects of the *CCR5-Δ32* allele in heterosexual exposure were shown according to Italian<sup>16</sup> and Zambian populations.<sup>41</sup> Our results, referring to Poland, suggest that the effect is common worldwide. Moreover, our study suggested that the protective role of the *CCR5-Δ32/+* genotype in cases of strong exposure, for example, connected with intravenous drug use could be excluded.

#### Author Disclosure Statement

No competing financial interests exist.

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