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ORIGINAL ARTICLE

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Distribution of Δ32 alelle of the *CCR5* gene in the population of Poland

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Abstract The chemokine receptor CCR5 constitutes a major co-receptor for the R5 strains of HIV-1, and a mutant allele of the *CCR5* gene, especially in the homozygous form Δ32/Δ32, confers resistance against infection by the virus. The frequency of the Δ32 allele was determined in blood donors from 16 provinces, covering the entire territory of Poland. Among 861 individuals 182 (21.1%) were carriers of the mutated allele; 7 of them (0.8 %) were homozygotes Δ32/Δ32, and 175 (20.3%) were heterozygotes +/Δ32, resulting in a 10.9% frequency of the Δ32 allele. The highest frequencies of the mutated allele were found in the eastern and western provinces, and the lowest frequencies of the Δ32 allele were detected in the provinces in the center of the country. This pattern of distribution may reflect the migration of the population from the eastern territories of Poland to the western part of the country after World War II.

Key words HIV-1 · CCR5-Δ32 allele · Epidemiology · Provinces of Poland

Introduction

The entry of HIV to target cells is initiated by the high-affinity binding of envelope glycoprotein 120 (gp120) to the CD4 receptor, followed by fusion with the cell membrane and deposition of the viral core in the cytoplasm (Sattentau et al. 1988). It has been demonstrated that the fusion process requires seven transmembrane loop, G-protein-coupled chemokine receptors (Feng et al. 1996; Alkhatib et al. 1996) binding chemokines: RANTES, MIP-α, MIP-β, and SDF-1. The X4 strain of HIV-1 uses the CXCR4 receptor to facilitate the entry of the virus (Feng et al. 1996), whereas the R5 strain uses the Cysteine-Cysteine (C-C)-linked CCR5 receptor in the fusion process (Alkhatib et al. 1996; Dragic et al. 1996). Closely related chemokine receptors, CCR2 and CCR3, can also act as co-receptors for some other HIV-1 strains (Horuk 1999). It is known that the majority of primary HIV-1 isolates, in an early stage of the disease, use CCR5 as a co-receptor, whereas, during progression to acquired immunodeficiency syndrome (AIDS), the CXCR4 co-receptor is used (Connor et al. 1997). Resistance to infection by HIV-1 has been described in individuals who remain seronegative despite repeated exposure to the virus (Dean et al. 1996; Paxton et al. 1996). This observation suggested that genetic factors may be involved in susceptibility to infection by HIV-1 and that mutations in the chemokine receptor genes may confer resistance to AIDS. It has been noted that $CD4^+$ lymphocytes from some HIV-1-exposed individuals, who have remained uninfected, are resistant to infection by the virus, suggesting that a defect in the co-receptors, or their expression, may protect from infection (Dean et al.1996; Paxton et al. 1996; Liu et al. 1996). The relevance of HIV-1 co-receptors to genetically determined resistance became apparent when mutations in the *CCR5* gene were discovered (Liu et al. 1996). The gene, encoding CCR5, is localized in the p21.3 region of chromosome 3, within a cluster including most of the other C-C-linked chemokine receptor genes (Dean et al. 1996). Among alleles of the *CCR5* gene, the most common mutated variant contains a 32-bp deletion, in the region encoding the second extracellular loop of the chemokine receptor (Liu et al. 1996).

This mutation causes a frame shift at amino acid 185, resulting in non-functional protein, both as chemokine receptor and HIV-1 co-receptor (Liu et al. 1996). Other genetic variants are very rare and constitute less than 1% of the total number of the mutations (Horuk 1999). Dean et al. (1996) found 17 homozygotes Δ32/Δ32 in 612 HIV-1-exposed, antibody-negative individuals (2.8%). These results were confirmed by other groups (Paxton et al. 1996;

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Samson et al. 1996), leading to the suggestion that homozygotes Δ32/Δ32 resist infection, while infected heterozygotes +/Δ32 were more resistant to the development of AIDS than +/+ individuals (Eugen-Olsen et al. 1997). Despite numerous reports on the frequency of the Δ32 allele in the population of most European countries (Libert et al. 1998; Lucotte and Mercier 1998), the distribution of this mutation in the population of Poland has not been studied.

The present investigation was designed to determine the frequency of the Δ32 allele in a large group of blood donors from 16 provinces covering the entire territory of Poland.

Materials and methods

Samples of peripheral blood were obtained from 861 unrelated, apparently healthy, blood donors, from major transfusion centers located in all 16 administrative provinces of Poland. Genomic DNA was prepared from venous blood by a standard method (Liu et al. 1996). The *CCR5* gene fragments, 182 bp in length, flanking the site of the deletion were amplified, under optimal conditions, by polymerase chain reaction (PCR), using previously applied (Liu et al. 1996) *CCR5*-specific forward and reverse primers: SP4.760, 5-CCT CAT TAC ACC TGC AGC TCT-3′ and PM6.942, 5′-CAC AGC CCT GTG CTT CTT CTT-3′. The amplification products were separated by electrophoresis in 2.5% agarose-1000 gels (GibcoBRL, Grand Island NY, USA) and visualized by staining with ethidium bromide.

Results and discussion

The *CCR5* gene fragments, 182 bp in length, covering the site of the deletion were amplified, under optimal conditions, by PCR. Under these conditions, the amplification products obtained from the DNA of individuals homozygous for the Δ32 allele (Δ32/Δ32), showed a single band 150 bp in length, whereas, in heterozygotes (+/Δ32), two bands (182 bp and 150 bp) were evidenced. In homozygotes (+/+), upon electrophoresis of the amplified fragment, a single band, 182 bp in length, was observed (Fig. 1).

The frequency of the Δ32 allele was determined in 16 groups of blood donors, averaging about 50 individuals in each, from 16 provinces of Poland, covering the entire territory of the country. The largest group was from Wielkopolska province (174 donors). In most provinces, the number of donors tested was between 40 and 50. Among 861 individuals, 182 (21.1%) were carriers of the mutated allele; 7 (0.8 %) were homozygotes Δ32/Δ32, and 175 (20.3%) were heterozygotes +/Δ32 (Table 1). The highest frequencies of the mutated allele were found in the eastern provinces: Podlasie, Lublin, and Mazovia (14.0%, 13.3%, and 13.2%, respectively) and in the western provinces: Lower Silesia and Lubuskie (13.0% and 13.3%, respectively) (Fig. 2). The lowest frequencies of the Δ32 allele (8.9% to 10.2%) were detected in the provinces in the central and southern parts of the country (Fig. 2). The differences in the allelic frequency distribution pattern can be explained against the background of the history of the country. For one hundred and fifty years Poland was annexed by Russia, Germany, and the Austro-Hungarian Empire, and

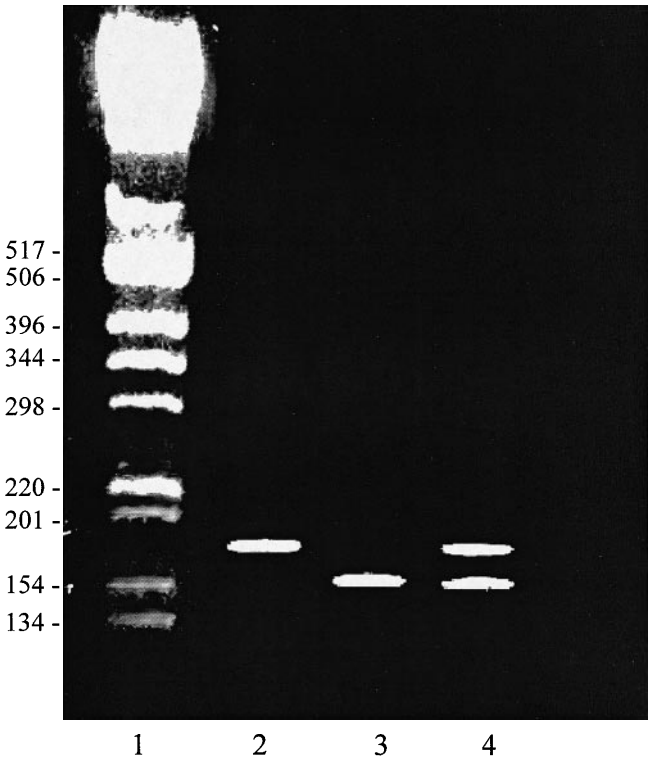

Fig. 1. Typical genotypes. The fragments of the *CCR5* receptor gene were amplified by polymerase chain reaction (PCR) and identified as described in the Materials and methods section. *1*, Size marker; *2*, homozygote +/+ (single band 182 bp); *3*, homozygote Δ32/Δ32 (single-band 150 bp); *4*, heterozygote +/Δ32 (two bands, 182 bp and 150 bp)

Table 1. Distribution of the Δ32 allele in 16 administrative provinces of Poland

Province	*n*	Genotypes		Δ32 allele frequency
		+/Δ32	Δ32/Δ32	
Lower Silesia	46	12	0	0.130
Lublin	45	6	3	0.133
Lubuskie	49	13	0	0.133
Łódź	40	9	0	0.112
Kujawsko-Pomorskie	39	9	0	0.115
Małopolska	49	8	1	0.102
Mazovia	68	14	2	0.132
Opole	39	7	0	0.090
Podlasie	50	14	0	0.140
Podkarpacie	40	10	0	0.125
Pomerania	46	9	0	0.098
Silesia	43	8	0	0.093
Świętokrzyskie	44	9	0	0.102
Wielkopolska	174	29	1	0.089
Warmia and Mazury	50	11	0	0.110
West Pomerania	39	7	0	0.090
Total	861	175	7	0.109

+/Δ32 and Δ32/Δ32 represent heterozygous and homozygous genotypes, respectively; *n*, number of individuals

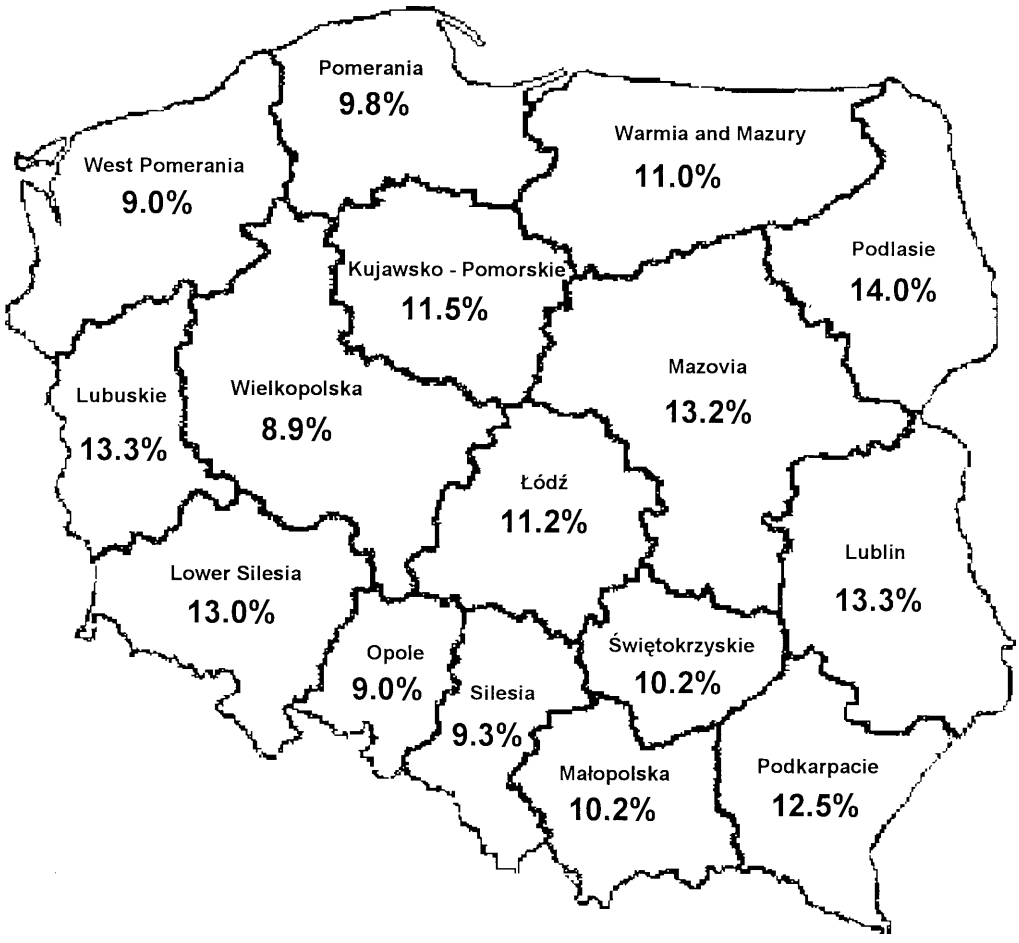

Fig. 2. Frequencies of the Δ32 allele in the 16 provinces of Poland

the eastern part of Poland belonged to Russia. The increased allelic frequencies in the eastern part of Poland may reflect the gene flow from the North-Eastern European part of Russia. After World War I, Poland gained independence. Following World War II, Poland was formed in new territory, and people were moved from the eastern territories to the western part of the country (the contemporary provinces of Lubuskie and Lower Silesia). Based on our history, we presume that the pattern of allelic frequency distribution may reflect the migration of the population from the eastern territories of Poland to the western part of the country after World War II, whereas the autochthonous population occupies the central part of the country. Our results indicate that the mean frequency of the Δ32 allele in the population of Poland is 10.9% (n = 861). This allelic frequency can be placed between that of the Lithuanian population (11.5%) and that of the population of Western European countries (9.2%–10.1%) (Liu et al. 1996; Libert et al. 1998; Lucotte and Mercier 1998). The distribution pattern of the allelic frequencies does not reflect resistance to HIV-1 infection. We observed the highest incidence of HIV-1 infection in the biggest cities, in drug-abuser cohorts. The incidence of HIV-1 infection has a strong relationship with the industrialisation process, rather than reflecting the distribution pattern of allelic frequencies. The 150 years of common history of Poland's eastern territories and Russia may have resulted in gene flow and higher allelic frequencies in this part of Poland, providing evidence that the Δ32 allele may have migrated mainly from the North-East.

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