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Distribution of two HIV-1-resistant polymorphisms (SDF1-3′A and CCR2-64I alleles) in the Polish population

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Abstract Chemokine receptors (CCR2 and CXCR4) are used as coreceptors for entry of human immunodeficiency virus (HIV) into the target cells. Mutations in CCR2 (CCR2-64I) and stromal-derived factor SDF1 (SDF1-3′A), the primary ligand for CXCR4, exhibited a protective effect against the onset of acquired immune deficiency syndrome (AIDS). The frequency of the SDF1-3′A and CCR2-64I alleles were determined in blood donors from 16 provinces, covering the entire territory of Poland. Of 1063 individuals, 274 (25.8%) were carriers of the SDF1-3′A allele; 36 of them (3.4%) were homozygotes (SDF-3′A/A) while 238 (22.4%) were heterozygotes (SDF-3′G/A), resulting in a 14.6% frequency of the SDF1-3′A allele. Moreover, in the same group of individuals, 234 (22.0%) carried the CCR2-64I allele; 6 of them (0.6%) were homozygotes (CCR2-64I/I), and 228 (21.4%) were heterozygotes (CCR2-64V/I), resulting in an 11.3% frequency of the CCR2-64I allele. The highest frequencies of the SDF1-3′A allele were found in the northeastern provinces and in one of the western provinces of Poland. In contrast, allelic frequencies of CCR2-64I varied slightly among different provinces. The different pattern of prevalence of the SDF1-3′A and CCR2-64I alleles in Poland might suggest that the CCR2-64I allele was spread much earlier than the SDF1-3′A allele in the population of Poland.

Key words HIV-1 · SDF1-3′A allele · CCR2-64I allele · Epidemiology · Provinces of Poland

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Introduction

The discovery that the chemokine receptors CXCR4, CCR5, and CCR2B play an important role in the fusion of human immunodeficiency virus (HIV) to target CD4 cells has accelerated the search for different genetic factors that might influence HIV infection and the onset of acquired immune deficiency syndrome (AIDS) (Alkhatib et al. 1996; Doranz et al. 1996; Feng et al. 1996; Mummidi 1998; Liu et al. 1996).

It has been reported that homozygous individuals with a 32-bp deletion in the *CCR5* gene (CCR5-Δ32 mutation) are resistant to HIV infection, while heterozygotes of the CCR5-Δ32 mutation exhibit a delayed progression to AIDS (Dean et al. 1996; Eugen-Olsen et al. 1997; Sullivan et al. 2001). It has also been shown that SDF1 (stromal-derived factor), which is a primary ligand of the CXCR4 chemokine receptor, competes with HIV for binding to the target cells (Bleul et al. 1996).

The SDF1 and CCR2 mutant alleles (SDF1-3′A and CCR2-64I) might be involved in the progression to AIDS (Michael et al. 1997; Mummidi et al. 1998). The SDF1-3′A mutation was found in the untranslated region of the transcript, while the CCR2-64I allele resulted from the transition mutation G190A, which caused a substitution of valine to isoleucine (Mummidi et al. 1998). The molecular mechanism of the effect of these mutations in the SDF1 and CCR2 genes is not yet understood. The SDF1-3′A allele was recently implicated in the mobilization of the $CD34^+$ progenitor cells into the peripheral blood in humans as well as in the early onset of the type 1 diabetes mellitus, while the CCR2-64I allele conferred a lower risk for the development of sarcoidosis (Dubois-Laforgue et al. 2001a,b; Hizawa et al. 1999). The distribution of the SDF1-3′A and CCR2-64I alleles has been surveyed in East Asian and world populations (Su et al. 1999, 2000; Martinson et al. 2000). Our investigation evaluated the frequency of the SDF1-3′A and CCR2-64I alleles in a group of blood donors from 16 provinces, covering the entire territory of Poland.

Subjects and methods

Samples of peripheral blood were obtained from 1063 unrelated, healthy blood donors from the transfusion centers of the 16 administrative provinces of Poland. Genomic DNA was prepared from venous blood by a standard method (Liu et al. 1996).

The G190A nucleotide transition resulting in the formation of the CCR2-64I allele was determined by restriction fragment length polymorphism (RFLP). The DNA was amplified by polymerase chain reaction (PCR) with primers 5′-CAT CTC GTT CTC GGT TTA TCA G-3′ (forward) and 5′-GAT GAT TCC TGG GAC AGA AGC-3′ (reverse). The amplicon, 521 bp in length, was digested with restriction enzyme *Fok*I. This resulted in the formation of 134-bp, 183-bp, and 204-bp fragments (in the presence of the transition mutation), or 134-bp and 387-bp fragments (in the absence of the mutation) (Lee et al. 1998) (Fig. 1).

To determine the presence of the SDF1-3′A allele, the DNA samples were amplified by using primers 5′-AAG GAC CAC GGC TCT GAT CAG 3′ (forward) and 5′-TGG CGA CAC GTA GCA GCT TAG 3′ (reverse). The amplicon, 750 bp in length, was digested with restriction enzyme *Msp*I, resulting in the formation of 307-bp and 443-bp fragments in the absence of the transition mutation (Mummidi et al. 1998) (Fig. 1).

Statistical analysis

For each province with population n allele frequencies were calculated as

$$\frac{h + 2H}{2n},$$

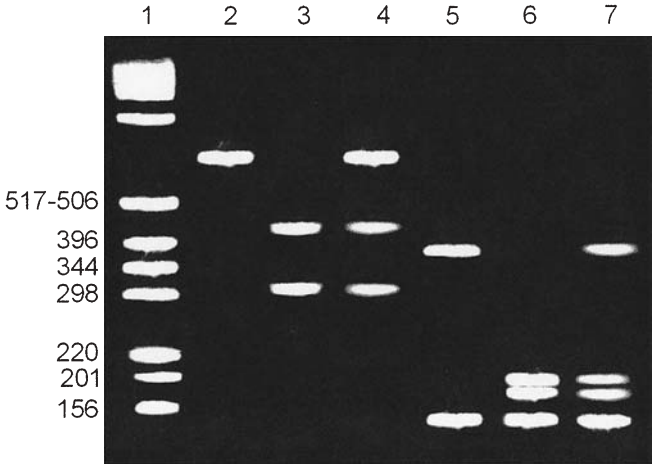

Fig. 1. Typical genotypes. The fragments of the *CCR2b receptor* and *SDF1* genes were amplified by polymerase chain reaction and identified as described in the subjects and methods section. *Lane 1*, size marker; *2*, homozygote SDF1-3′A/A (*band* 750 bp); *3*, homozygote SDF1-3′G/G (*bands* 310 and 440 bp); *4*, heterozygote SDF1-3′G/A (*bands* 310, 440, 750 bp); *5*, homozygote CCR2-64V/V (*bands* 134 and 387 bp); *6*, homozygote CCR2-64I/I (*bands* 134, 183, and 204 bp); *7*, heterozygote CCR2-64V/I (*bands* 134, 183, 204, and 387 bp)

where h and H are the numbers of heterozygotes and homozygotes, respectively. The relevance of differences in distributions of SDF1-3A′ genotypes between the provinces Warmia and Mazury, Podlasie, Lublin, Mazovia, and Lubuskie and the other provinces of Poland was tested by χ-squared test with GraphPad Prism Version 3.02 software. To assess the sample size necessary to obtain a power of 0.8 and a significance level (α) of 0.05, we used the program on line at http://www.health.ucalgary.ca/~rollin/stats/ssize/b2.html.

Results and discussion

The frequencies of the SDF1-3′A and CCR2-64I alleles were surveyed in 16 groups of blood donors from the 16 provinces of Poland. The largest group was from Mazovia and Warmia and Mazury provinces (78 donors). In most provinces, the average number of donors tested was 66. Of 1063 individuals tested, 274 (25.8%) were carriers of the SDF1-3′A allele; 36 (3.4%) were homozygous for SDF1-3′A/A, and 238 (22.4%) were heterozygous for SDF1-3′G/A (Table 1). In contrast, the same group of individuals carried 234 (22.0%) CCR2-64I alleles; 6 (0.6%) were homozygous for CCR2-64I/I and 228 (21.4%) were heterozygous for CCR2-64V/I. The highest frequencies of SDF1-3′A were found in the eastern provinces, namely, Warmia and Mazury, Podlasie, Lublin, and Mazovia (20.5%; 17.6%; 16.7%, and 15.4%, respectively) and in one western province**,** Lubuskie (19.3%) (Table 1, Fig. 2). The lowest frequencies of the SDF1-3′A allele were detected in the southeastern provinces, namely, Małopolska, Łódź, and Podkarpacie (11.5%, 11.9%, and 12.1%, respectively) (Fig. 2). The statistical relevance of the differences of SDF1-3A′ allelic frequencies between provinces Warmia and Mazury, Podlasie, Lublin, Mazovia, and Lubuskie and the rest of the provinces was evaluated by χ-squared test ($\chi^2 = 10.43$, 1 df, $P = 0.0012$). With our frequency data and using the total sample size in Warmia and Mazury, Podlasie, Lublin, Mazovia, and Lubuskie provinces (380 individuals) and the total sample size in the other provinces (683 individuals), the power and significance level (α) of the analysis were 0.8 and 0.05, respectively.

Our results indicate that the mean frequency of the SDF1-3′A allele in the population of Poland is 14.6% (N = 1063). This allelic frequency is between those of the northern European (22.0%) and Italian (15.0%) populations (Su et al. 2000).

The frequency of the CCR2-64I allele varied little among the different provinces of the country, with the mean frequency of the CCR2-64I allele in the population of Poland being 11.3% (N = 1063) (Table 1, Fig. 2).

The prevalence of the SDF1-3′A allele in world populations varies substantially, and the highest frequency has been observed in Oceanian populations (54.0%–71.0%) (Su et al. 1999, 2000). In contrast, the distribution of CCR2-64I in world populations does not vary significantly within the different continents, but the allele frequency is greatest in Africa and Asia (34.0%), but decreases in northern and

Table 1. Distribution of the SDF1-3′A and CCR-64I alleles in the 16 administrative provinces of Poland

Province	n	GR	Genotypes				Allele frequency	
			SDF1-3′ G/A	SDF1-3′ A/A	CCR2-64 V/I	CCR2-64 I/I	SDF1-3′A	CCR2-64I
Lower Silesia	61	0.90	12	2	13	0	0.131	0.107
Lublin	75	0.97	21	2	18	0	0.167	0.120
Lubuskie	75	0.92	23	3	17	0	0.193	0.113
Łódź	63	0.91	11	2	14	0	0.119	0.111
Kujawsko-Pomorskie	62	0.97	13	1	12	1	0.121	0.113
Małopolska	61	0.97	12	1	13	1	0.115	0.123
Mazovia	78	0.86	20	2	17	1	0.154	0.122
Opole	62	0.94	9	3	11	1	0.121	0.105
Podlasie	74	0.90	20	3	16	1	0.176	0.122
Podkarpacie	62	1.07	9	3	12	1	0.121	0.113
Pomerania	62	1.00	12	3	16	0	0.145	0.129
Silesia	60	0.97	11	2	13	0	0.125	0.108
Świętokrzyskie	60	0.88	12	2	13	0	0.133	0.108
Wielkopolska	67	0.97	16	1	14	0	0.134	0.104
Warmia and Mazury	78	0.95	24	4	16	0	0.205	0.103
West Pomerania	63	1.10	13	2	13	0	0.135	0.103
Total	1063	0.96	238	36	228	6	0.146	0.113

SDF1-3′G/A and CCR-64V/I represent heterozygous genotypes, SDF1-3′A/A and CCR-64I/I represent homozygous genotypes
GR, gender ratio, women/men

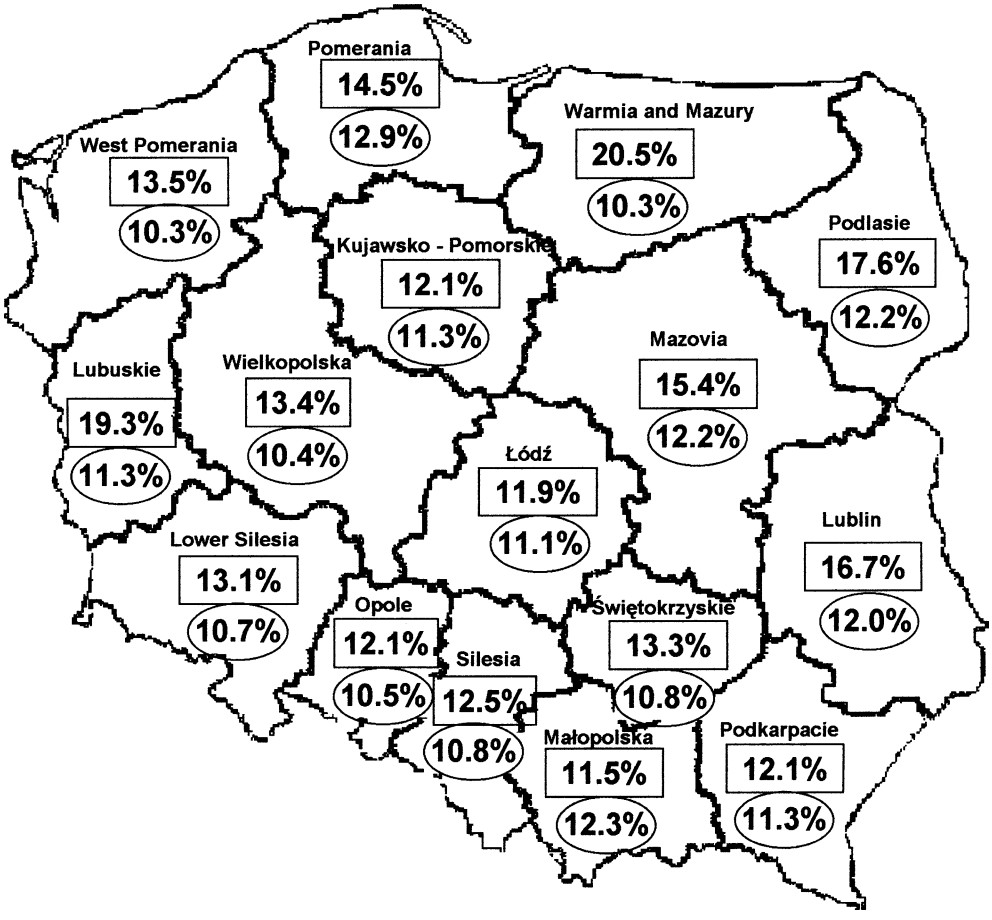

Fig. 2. Frequencies of the SDF1-3′A and CCR2-64I alleles in 16 administrative provinces of Poland. The allele frequencies of SDF1-3′A are shown in the *rectangular boxes*, and those side legend of CCR2 are within the *ovals*

southern Europe (22.0%–15.0%) (Su et al. 2000; Martinson et al. 2000).

The survey of allelic frequencies of SDF1-3′A in different provinces of Poland revealed that SDF1-3′A frequency tended to increase from the southeastern to the northeastern part of the country (Fig. 2). The high frequency of the SDF1-3′A allele in one of the western provinces could be attributed to the migration of three million Polish citizens

from what is now Lithuania, the Ukraine, and Bielorussia to the western part of Poland (mainly to Lubuskie) after World War II (Fig. 2) (Hulanicka et al. 1999; Kędzia 2001).

Despite Poland's small area compared with that of the rest of world, the different prevalence of the SDF1-3′A allele frequencies in this country reflects the trend observed in the world population. Allelic frequencies of SDF1-3′A in southeastern Asia are approximately 30.0% (Su et al. 1999). The highest frequency of the SDF1-3′A allele in Europe was observed in the northern part of that continent (22.0%) (Su et al. 2000). We also observed the highest SDF1-3′A allele frequencies in northeastern Poland, which can be explained against the historical background of the region. The common ancestral heritage of the people of Lithuania, Bielorussia, the Ukraine, Russia, and Poland may have resulted in gene flow among those populations and have led to high allelic frequencies of SDF1-3′A in this part of Poland (Hulanicka et al. 1999). Lithuania, Bielorussia, the Ukraine, and Russia link northern Europe, Asia, and Poland, and, although the allelic frequency in Lithuania, Bielorussia, and the Ukraine has not yet been recorded, we know that the average allelic frequencies of SDF1-3′A in northern Europe and Southeast Asia are approximately 22.0% and 30.0%, respectively (Su et al. 1999, 2000). Our observations can also be confirmed by recent findings in the Russian population of Moscow. It has been found that the frequency of the SDF1-3′A and CCR2-64I alleles in the Russian population of Moscow is 21.3% and 11.1%, respectively (Ryabov et al. 2002). Additionally, the distribution pattern of SDF1-3′A allele frequencies is similar to that of the CCR5-Δ32 allele in the population of Poland. We previously found the highest frequencies of the CCR5-Δ32 allele in the eastern provinces of Poland (Jagodzinski et al. 2000). Although the CCR5-Δ32 allele arose in northeastern Europe and is a younger mutation than SDF1-3′A, the high frequency of the CCR5-Δ32 allele in the eastern provinces of Poland might confirm the flow of the SDF1-3A′ allele from the northern and eastern parts of Europe.

The differences in the distribution of the SDF1-3′A allele and the moderate differences in the distribution of the CCR2-64I allele in the different provinces of Poland might suggest that the CCR2-64I allele spread earlier than the SDF1-3′A allele in the history of this country's population genetics.

We also observed that the pattern of distribution of the SDF1-3′A allele does not reflect the spread of HIV-1 infection in different provinces of the country. The spread of the disease correlates strongly with the industrialization process, and the disease is observed among drug abusers in the larger, more industrialized cities.

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