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The Occurrence of HIV-1 Resistance Biomarker Among Two Cohorts from Poland

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Abstract

Approximately 1% of the Caucasians shows resistance to HIV-1 infection conditioned by 32bp deletion in CCR5 gene. Homozygotes are almost totally resistant, but heterozygotes have delay the progression to AIDS. Due to the constantly increase of HIV infection in Poland we examined the incidence of del32 allele in two cohorts (south-western and south-eastern region of country) that remarkably differs in the incidence of HIV. Among the individuals from the south-western region, we detected 7 homozygotes (2.6%), in compare with individuals from the south-eastern area of Poland, where we found 1 homozygote (0.4%). The prevalence of CCR5del32 allele in the group from the south-western region was estimated at 11.6%, while in the group from the south-eastern region was assessed at 9.7%. Differences in the prevalence of genotypes and alleles between regions were not statistically significant. Our results were discussed in relation to the incidence of HIV infection in Poland. We conclude that occurence of CCR5del32 biomarker does not reflect the incidence of HIV in the examined regions.

Introduction

In the aetiology of HIV-1 infection, some genetic factors may be important, for example the gene variants, which encode chemokine receptors: CCR5, CCR2 and CXCR4 - SDF-1 ligand receptor [1]. The human β-chemokine receptor is encoded by CCR5 gene located in the 3p21.3 locus. *CCR5* consists of four exons and two introns; exon 4 contains ORF (open reading frame). The deletion of 32 bp in the ORF causes the formation of non-functional protein and protects homozygotes against HIV-1 R5 infection [2-5]. This alteration decreases, in heterozygotes, risk of infection [6] and delays progression into AIDS for about 2-3 years [3,5,7]. There are some evidence that the resistance of this type is related to the Caucasian due to spreading epidemies of diseases with similar pathogenesis to HIV infection. One hypothesis states that people with the deletion of 32 bp in CCR5 gene could have survived the episemies, and allele with deletion could been inherited by progeny. Bubonic plaque (1347 – 1351 year) [8] as well as smallpox could have also had a selective pressure on chemokine receptors [9,10]. An alternative hypothesis states that allele with deletion could have been conveyed by Vikings between 8th and 10th century [11]. Nowadays, the CCR5del32 biomarker occurs approximately in 1% of the Caucasian population [2].

The aim of our study was to examine the occurrence of CCR5del32 biomarker in two population groups from the south-western and south-eastern regions of Poland. Our results were compared with epidemiology data of HIV infection in this region.

Materials and Methods

The study involved 268 individuals from the south-western (dolnośląskie) region of Poland and 252 individuals from the south-eastern (podkarpackie) region, altogether 520 subjects chosen randomly. The group was matched according to sex and age. The age of respondents was in the range between 20-29 years. DNA was obtained from peripheral blood lymphocytes (268 sample), buccal cells (125 samples) and hair follicle cells (13 samples). DNA from peripheral blood lymphocytes was isolated by fenol–chloroform extraction; from buccal cells and hair follicle cells by alkaline lysis according to Bolla [12] and Klintshaar methods with minor modifications [13].

Molecular analysis of CCR5del32 (c.794-925del) variant was carried out with PCR technique, using primers described previously by Liu et al. [4]. The PCR reaction mixture in the total volume of 10 µl per tube contained: 0.2 mM dATP, dCTP, dGTP and dTTP, 0.5 U/µl OptiTaq DNA Polymerase (EURx, Poland), 1x *Taq* polymerase buffer; 1.5 mM $MgCl_2$, 0.5µM Fwd primer and 0.5µM Rev primer. PCR conditions were: 25 seconds at 95°C, 25 seconds at 62°C, 25 seconds at 72°C, 35 cycles. Results of PCR amplification were visualized on 2% agarose gel stained with SYBR® Safe DNA gel stain (Invitrogen, Carlsbad, CA, USA). Band of about 182 bp was characteristic for wild type homozygotes (wt/wt), about 150 bp band was characteristic for homozygotes with 32 bp deletion (del32/del32), two bands (182 bp and 150 bp) were characteristic for heterozygote (wt/del32).

Analysis of the frequency of del32 variant in the *CCR5* gene was assessed using the Pearson $\chi 2$ test. Statistical analysis of data was performed using StatSoft, Inc. (2005) STATISTICA, version 7.0, http://www.statsoft.com. This study protocol was approved by the Ethics Committee of the Faculty of Medicine, University of Rzeszow, Poland.

Results

Among the individuals from the south – western region of Poland deletion was detected in 55 (20.5%) cases, including 7 homozygotes (2.6%). In individuals from the south - eastern region deletion was found in 48 (19%) subjects, including one homozygous case (0.4%). The prevalence of CCR5del32 was assessed at 11.6% and 9.7% (south - western and south - eastern province respectively, see Table 1). The differences in the prevalence of genotypes and alleles between regions

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	Region			
	south-western	south - eastern	Total	p value
Genotypes				
wt/wt	213 (79.5%)	204 (81%)	417(80.2%)	
wt/del32	48 (17.9%)	47 (18.6%)	95 (18.3%)	0.12
del32/del32	7 (2.6%)	1 (0.4%)	8 (1.5%)	
Total	268	252	520	
Alleles				
del32	62 (11.6%)	49 (9.7%)	111 (10.7%)	0.33
wt	474 (88.4%)	455 (90.3%)	929 (89.3%)	
Total	536	504	1040	

Table 1: The *CCR5/CCR5*del*32* frequency and genotype distribution in the south-western and south-eastern Polish population.

were not statistically significant (p = 0.12 and p = 0.33 respectively). Our results were discussed in relation to the incidence of HIV infection in Poland.

Discussion

Recently in Poland, has been noticed increasing amount of infections caused by HIV, especially in western region of Poland [14]. In the face, the growing number of reported HIV infections it is important to predict the factors likely to influence the incidence of infections. Faced with an increasing number of reported HIV infections is a selection of important factors that may affect the incidence of infections. One of such factors is the genetic resistance caused by 32 bp deletion in CCR5 gene can have the effect on limitation of infection. In Europe the prevalence of 32 bp deletion in the human β-chemokine receptor gene is ranked between 16% in the North Europe to 4% in the South Europe [15-25]. In Poland the frequency of deletion was assessed at 10.9% [26].

In our study we investigated the incidence of *CC5*del32 variant in two distant cohorts from the south-western and south-eastern region of Poland that differ in the incidence of HIV. In south – western region HIV infections were observed in 4.1 per 100'000 inhabitants (in 2004 – 2008) and were the greatest in Poland. In turn, in south - eastern region HIV was found in 0.6 per 100'000 inhabitants and was one of the smallest amounts of infected cases in Poland [14]. We observed slightly higher frequency of del32 allele in the south-western territory than in the south-eastern. However the prevalence of CCR5del32 variants between regions were not differs significant. Moreover the frequency of deletion in the south-western and the south-eastern regions are not compatible with the incidence of infections in these regions. We conclude that occurrence of CCR5del32 biomarker does not reflect the incidence of HIV in the examined regions. Studies on larger groups could explain this phenomenon.

Very similar distribution of CCR5del32 variant among inhabitants of this distinct region can be the result of the recently described genetic homogeneity of Polish population [27]. On the other hand, it is possible that the comparable values of del32 allele frequency can be the cause of increasing migration of people from the south-eastern to south-western Poland after the Second World War [26].

Due to the relatively high incidence of deletion in the study groups (about 20% individuals) should be noted, that the deletion can have the impact on incidence of other diseases. The latest studies revealed that CCR5del32 variant is a modifying pathogenetic factor in type I diabetes [28]. By analyzing morbidity type I diabetes on population level, should be taken into account CCR5del32 biomarker as risk factor.

This finding might be useful for epidemiological researches of HIV infections and other diseases like diabetes. Moreover our data may be useful for planning prevention efforts.

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