PREDICTION OF RELATIVE RISK OF AIDS ONSET (RRA) USING COMBINED THREE-LOCUS GENOTYPE DATA IN BULGARIAN POPULATION

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ABSTRACT

Here we study the distribution of three HIV-1 resistance conferring polymorphisms - CCR5del32, CCR2-64I and SDF1-3'A in Bulgarian population uninfected with HIV. The relative risk of onset of AIDS symptoms (RRA) is calculated based on this distribution in a representative group of Bulgarians (n=95). RRA was detected to be 0.85 - which falls into the range of this characteristic for the Europeans. The relative risk values similar to that of RRA might be helpful in assessing specific epidemiological situations in population groups, especially when human genetic factors are involved.

Keywords: HIV, co-receptors, genetic polymorphism, relative risk of AIDS onset, RRA

Introduction

The main steps in contracting HIV infection involve interaction between the HIV-1 gp120/41, the CD4+ receptor and one of the chemokine co-receptors – CCR5, CXCR4 or CCR2. The presence of one of these co-receptors is absolutely needed for the virus to attach and enter the target cells. The chemokine co-receptors CCR5 and CXCR4 function as major ones, required for the entry of HIV-1 in CD4+ cells and T-tropic and macrophage-tropic strains respectively, while the CCR2 is considered a co-receptor playing a minor role in this process (9). So, the pattern of co-receptor usage defines an important characteristic of HIV-1 - namely its tropism (2). A lot of studies have indicated that mutations in genes encoding the chemokine co-receptors CCR5 and CCR2 and the only known ligand of CXCR4 - Stromal Derived Factor (SDF1) are linked to a natural genetically conferred HIV-1 resistance (6, 7, 9). The key role of one of the gene polymorphisms of CCR5 - CCR5del32 has been observed in cases being both heterozygous (CCR5/CCR5del32) and homozygous (CCR5del32/CCR5del32). Persons homozygous for CCR5del32 actually lack functional CCR5 on the cell surface and are considered insusceptible to infection with HIV-1 utilizing CCR5 as a co-receptor (R5 viruses) (10, 12**)**. R5 viruses are observed during early infection acquired by sexual transmission. On the contrary, the X4 viruses (utilizing CXCR4 co-receptors) evolve into variants that can use CXCR4 alone or both CCR5 and CXCR4 (dual-tropic or D/M viruses) and are found at late timepoints of the natural history of HIV infection (3, 13, 17). The knowledge and detection of HIV-1 tropism led to the development and therapeutical application of co-receptor antagonists (2, 5). Persons heterozygous for CCR5 (CCR5/CCR5del32) exhibit delayed progression of the infection (4). The functional insufficiencies in sequence-conserved regions of CCR2 (CCR2-64I) and SDF1 (SDF1-3 A) due to mutant alleles explained their effects on delay of AIDS symptoms (14, 16). Here we used the HIV resistance-conferring polymorphisms in mutations of three genes CCR5 (CCR5del32), CCR2 (CCR2-64I) and the ligand of CXCR4 - SDF1 (SDF1-3' A) to predict Relative Risk of AIDS (RRA) onset based on the relative hazard (RH) for HIV-1 resistance in representative group of Bulgarian population and to compare it to that of other populations and ethnic groups.

Materials and Methods

DNA samples (n=95) were taken from the collection of the Molecular Medicine Center at the Medical University, Sofia. All samples belonged to Bulgarians.

Study of polymorphism and genotyping were conducted as already described (1). Briefly, Polymerase Chain Reaction (PCR) and RFLP (Restriction Fragment Length Polymorphism) assays were carried out. PCRs and the following amplification products were subjected to restriction enzyme digestion (Msp1 for SDF1 and BsaBI for CCR2 for 4 h.). The digestion products and the PCR product for CCR5 were genotyped by agarose gel electrophoresis (for CCR5) or PAGE (for CCR2 and SDF1).

The allele frequencies (**f**) were calculated according to the formula $\mathbf{f = n/2N}$, where **n** is the allele incidence and N is the number of population studied.

In order to evaluate the risk of AIDS onset for the population screened, relative hazard (RH) was computed based on the three-locus genotypes data of each individual studied, as shown in (16). RH was estimated by the equation:

$$\mathbf{RH} = \sum \mathbf{w_i p_i},$$

where w_i is the coefficient of AIDS risk in the group and p_i is the frequency of the given allele combination (grouped in **Table 1**) and the summation covers all groups of genotypes. In the RH evaluation the AIDS-1993 definition was considered (1993 Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS among Adolescents and Adults, CDC).

Results and Discussion

As far as the protective effects of CCR5del32 and CCR2-64I were shown to be dominant, while that of SDF1-3' A is recessive (14) only persons homozygous for SDF1-3' A should be considered. In our study we found 5 persons bearing this genotype combined with heterogenous genotype for CCR5 - namely CCR5del32 (1 person) and normozygous by both CCR5 and CCR2 genotypes (4 persons).

Based on that, the allele frequencies (f) of the genes CCR5, CCR2 and SDF1 and the polymorphisms studied (SE - standard error) were estimated in the group of Bulgarian uninfected individuals as follows:

CCR5 = 0.86 CCR5del32 = 0.14 (SE=0.025)

CCR2 = 0.87 CCR2-64I = 0.13 (SE=0.024)

SDF1 = 0.80 SDF1-3'A = 0.20 (SE=0.029)

The RH value can be grouped according to the possible four types of the three-locus genotypes - **Table 1**:

TABLE 1

RH values for the possible three-locus genotypes, found according to allele frequencies. A, B and C refer to the wild type alleles of CCR5, CCR2 and SDF1 resp., a, b and c are mutant alleles at the respective loci.

Genotype	W_i
AABBC-	1.00
a-b-C-	0.65
a-BBC-	
AAb-C-	
AABBcc	0.63
a-b-cc	0.55
a-BBcc	
AAb-cc	

TABLE 2

Genotypes	Number(%)	$w_i.p_i$(%)	RRA
AABBC-	55(57.8)	57.8	0.578
a-b-C-	1(1)	0.65	0.0065
a-BBC-	11(11.6)	7.55	0.0755
AAb-C-	23(24.2)	15.75	0.1575
AABBcc	4(4.2)	2.65	0.0265
a-b-cc	-	-	-
a-BBcc	1(1)	0.55	0.0055
AAb-cc	-	-	-
total	95(100)	84.95	0.8495≈0.85

Table 2 shows the results of Relative Risk of AIDS (RRA) calculated from the frequencies of the three-locus genotypes and from the risk coefficient common for all ethnic groups. A, B and C refer to the wild type alleles of CCR5, CCR2 and SDF1 resp., a, b and c are mutant alleles at the respective loci.

The RRA value estimated (0.85) for a representative group of Bulgarian population fits well to the RRA range characteristic of other European populations (0.75 – 0.93) (11, 15). This parameter is close to RRA in some populations of Russia (8). The RH is extremely higher in South-East Asia and Africa where AIDS is known to be with higher prevalence (15) and respectively the RRA in these regions is also high.

Conclusions

RRA reflects the risk of onset of AIDS symptoms, keeping in mind the natural history of the infection, and is calculated based only on genetic data about the distribution of the three HIV-1 resistance-conferring human polymorphisms. It has nothing to do with any known history of HIV-1 infection and AIDS symptoms. Today, after introducing HAART protocols and especially the co-receptor antagonists in the clinical practice, RRA should be calculated on quite different basis. The relative risk values similar to that of RRA might be helpful in assessing specific epidemiological situations in population groups, especially when known human genetic factors are involved.

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