

GENETIC POLYMORPHISM OF THE CHEMOKINE CO-RECEPTORS CCR5, CXCR4 AND CCR2 IN BULGARIANS LIVING WITH HIV

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ABSTRACT

The aim of this study was to investigate the most spread genetic polymorphisms of the chemokine co-receptors CCR5, CXCR4 and CCR2 – namely the allele frequency of CCR5del32, SDF-1 3'A and CCR2 V64I in 177 Bulgarians living with HIV, as well as to correlate the results to the clinical course of HIV-infection and the same allele frequency for general population. The persons studied were of different duration of HIV-infection – registered during the period 1987-2004. Fourteen persons (7.9%) showed slow progression to AIDS, received therapy after no treatment for 7 – 10 years and were considered Long Term Survivors (LTSs). All HIV(+)s have been screened for CCR5 (CCR5del32) and 48 - for CXCR4 (SDF-1) (SDF-1 3'A) and CCR2 (CCR2 V64I) polymorphisms. No one from HIV positives has been found with a homozygous - CCR5del32/CCR5del32 - status, 6 out of all studied (3.2%) had heterozygous (CCR5/CCR5del32) genotype (only two LTSs). Seven out of 48 persons studied (14.6 %) had homozygous (SDF-1 3'A/SDF-1 3'A) genotype, (3 – LTSs), another 12 (25%) showed heterozygosity for CCR2 (CCR2/CCR2V64I - 2 – LTSs). The LTSs heterozygous by CCR5 (CCR5/CCR5del32), those displaying (SDF-1 3'A/SDF-1 3'A) homozygosity and both (13.33%) demonstrating (CCR2/CCR2V64I) heterozygous genotype were different persons. Although low numbers of persons studied, the results obtained coincide well to the literature ones. The genetic studies will be used to predict the results of HAART treatment as well as the onset to AIDS.

Key words: HIV, co-receptors, genetic polymorphism

Introduction

HIV is a multifactorial infection, representing a lifetime long fight between the causative agent – Human Immunodeficiency Virus (HIV) and the host (6). Similarly to other retroviral infections, the intensive research on HIV-replication dynamics *in vivo* during the last years has clearly defined both viral and human/host factors influencing transmission, course, progression and outcome of infection (6, 11).

HIV-transmission depends on biological properties of viral strains, as well as on the individual susceptibility. On the other hand, the latter is closely associated to a range of immunological and genetic factors. Among those, a number of chemokine receptors have been identified to function as co-receptors for HIV-1 entry in human CD4+ T-lymphocytes and monocytes (16). CCR5 is a chemokine receptor, whose α -chemokine ligands participate in chemotaxis of lymphocytes during inflammation. CCR5-protein is expressed on the surface of monocytes, macrophages, memory T-cells and dendritic cells, as well as on microglial cells correlating to the ability of HIV to infect the central nervous system (7). This is the major co-receptor, necessary for macrophage-tropic (M-tropic) non-syncytium inducing (NSI) HIV-1 strains entry (5). A complicated interaction between HIV-1 glycoprotein gp120, CD4 and CCR5 on the surface of CD4+ cells is crucial to HIV entry. As known, M-tropic strains are associated predominantly

with sexual transmission of HIV. Detailed studies of the coding region of CCR5 showed a variety of genetic polymorphisms. 32-base pair deletion (CCR5del32 mutation) in one or both alleles seems the most common polymorphism. When occurring in both alleles (homozygous pattern), this polymorphism leads to a formation of truncated, non-functional receptor protein, incapable to bind gp120 during the adhesion of HIV to the cell (20). The same mutation could also exist in only one allele (heterozygous pattern), leading to a limited production of the functional protein.

The homozygous (CCR5del32/CCR5del32) individuals were reported insusceptible to infection with M-tropic HIV-1 strains (8). Data showed that heterozygous (CCR5del32/CCR5) individuals had better prognosis during natural course of infection compared to those bearing “wild type” (CCR5/CCR5) alleles. The individuals with heterozygous pattern show a decreased expression of CCR5 on the cell surface and are more frequently considered as Long Term Non Progressors (LTNPs) compared to patients with a rapid/moderate progression of infection (4). In addition, HIV-infected heterozygous individuals also demonstrate better response to Highly Active Anti Retroviral Therapy (HAART) and decreased lymphoma incidence (19). These data clearly indicate that CCR5 density on cell surface could be a limiting factor for HIV-replication both *in vitro* and *in vivo*.

SDF-1 (Stromal Derived Factor) is another genetic factor influencing the course of HIV-infection. The coding gene is located in 10th chromosome and its product is the ligand of CXCR4 co-receptor. Studies on the gene polymorphism and its relation to the course of HIV-infection have led to discovery of polymorphic allele - SDF-1 3'A, containing inversion in promoting region. This inversion results in lowered expression of the gene product and incapability of binding to CXCR4, so preventing formation of functionally active protein complex. This leads to a delay to AIDS (especially if combined with heterozygous pattern of CCR5), but does not protect the individual from infection and re-infection with M-tropic variants. SDF-1 3'A allele is recessive and therefore such phenotype appears only if homozygous pattern is presented. The heterozygous status does not confer protective ability, i.e. displays practically the same phenotype as the wild type (23).

CCR2 is the next chemokine co-receptor participating in chemotaxis between lymphocytes and extrinsic agents, analogous to CCR5. The coding gene is located in proximity to that of CCR5, therefore the two genes are co-inherited. An amino acid replacement (CCR2 V64I) has been studied in relation to progression to AIDS. Comparison of individuals displaying wild types of CCR5 and CCR2 to those with homo- and/or hetero-zygous patterns of both genes display considerable delay of progression of HIV-infection, but mostly in presence only of non-syncytium inducing (NSI; M-tropic) HIV-1 variants. In heterozygous CCR2 V64I patients with genetic defects in CCR5 a predominance of syncytium inducing (SI; T-tropic) HIV-1 variants is observed. This fact shows that the influence of CCR2 polymorphism is extended over CCR5-exploring HIV-variants (18).

Genetic restrictions of HIV-1 infection and progression to AIDS requires meeting of criteria for Long Term Non Progressors (LTNPs) and Long Term Survivors (LTSs). A number of authors define LTNPs as asymptomatic for 10 or more years, with normal absolute CD4 T cell counts, below detection viral loads and remaining therapy naive. LTSs are individuals who have been infected with HIV also for several years (typically 7-10 or more years), with a damaged immune system and/or opportunistic infections and receiving therapy (2). Some authors include LTNPs in the group of LTSs (2). According to literature, 5-10% of all people living with HIV fall into the LTNP category, displaying no evidence of disease progression despite 10-15 years of documented HIV-infection (3, 10, 15).

Since it is clear that genetic factors influence both natural course of infection and presumably the response to HAART, it has been hypothesized that the effect of therapy could be to some extent genetically determined (14). This fact raises the need for a further study of chemokine co-receptors in persons, living with HIV and receiving HAART.

Here, for the first time, we describe the most spread genetic polymorphisms of the chemokine co-receptors CCR5, CXCR4 and CCR2 – namely the allele frequency of CCR5del32, SDF-1 3'A and CCR2 V64I in 177 Bulgarians living with HIV, as well

as the correlation of the results to the clinical course of HIV-infection and the same allele frequency for general population. Earlier, when we started studies of the same genetic factors (1) we considered 9 persons as LTNPs. During the following years their HIV disease progressed toward immune deficiency and they were given HAART. We believe this could be a reason for revision and addition of new genetic data linked to the course of infection. Today we consider these persons LTSs, not LTNPs. The second reason to present the current results and to compare them to those for general population is the fact that no or limited genetic co-receptors polymorphism data are published for the South-East European region (Balkan countries) (14). Such data would be of interest, because population migration in this district has occurred during last centuries quite often. Also, minority populations studied by us earlier (21) live in relative isolation although often migrating.

Additionally, it has been recently demonstrated that the protection effect of CCR5del32 and CCR2 I64V alleles on HIV-1 disease progression varies with duration of infection (15). All this encouraged us to study further the impact of host genetic factors in HIV disease.

Here we describe for the first time the most spread genetic polymorphisms of CCR5, CXCR4 (SDF-1) and CCR2 and their combinations in Bulgarians living with HIV with differing clinical course of infection.

Materials and Methods

Patient population

In this study 177 Bulgarians, living with HIV with different duration of infection were included, 14 out of them (7.9%) were clinically detected as LTSs. The blood samples (Gutrie cards) were taken in the Hospital of Infectious Diseases, Sofia. All participants were studied for CCR5del32 polymorphism and 48 out of them (only 11 LTSs for genetic polymorphisms of CXCR4 SDF-1 (SDF-1 3'A) and CCR2 (CCR2 V64I).

The following control groups were studied:

1. a representative group for CCR5del32 genetic polymorphism. The group consists of 900 Bulgarian residents.
2. a non-representative group for CXCR4 (SDF-1 3'A) including 100 Bulgarians chosen randomly.

No control group for CCR2 polymorphism in non-infected persons is represented in this paper.

Genetic analysis

For genetic analysis of all samples Gutrie cards with peripheral blood samples were used. First, direct DNA amplification from dried blood spots punched from the Gutrie cards without prior DNA extraction was tried. The results obtained however were inconclusive, so DNA-extraction had to be undertaken.

DNA was isolated from Gutrie cards using phenol: chloroform: isoamyl alcohol (25:24:1) method according to protocol described elsewhere.

To study CCR5 genetic polymorphism extracted DNA (50-100ng/μl) was amplified by PCR according to previous adaptation (not published) and the amplification products further were analyzed and visualized in horizontal 3% agarose gel-electrophoresis. This agarose gel could separate between 198bp (wild type) and 166bp (homo- or heterozygous pattern). The primers used were according to (16).

For study of CXCR4 (SDF-1) genetic polymorphisms, restriction analysis with the restrictase MspI (18) following the PCR amplification was performed followed by horizontal 3% agarose gel-electrophoresis. The SDF-1 3'A mutation alters the specific site for MspI restrictase, thus preventing it from cleaving the 294bp PCR product. The agarose gel could separate between 101bp, 193 (wild type) and 294bp (SDF – 1 3'A). The primers used were according to (18).

The CCR2 genetic polymorphisms were also studied by restriction analysis with the restrictase Bsa BI (22) following the PCR amplification. Analysis was then performed by 8% polyacrylamide gel-electrophoresis. The CCR2 V64I mutation creates site, recognized by Bsa BI restrictase. The latter cleaves the 128bp PCR product, creating 110bp fragment. The polyacrylamide gel could separate between 128bp (wild type) and 110bp (CCR2 V64I). The primers used were according to (22).

Statistical methods

Zero Hypothesis (H_0) was used to compare the frequencies studied. Initially this method determines first the Significance level (α) for this data. This parameter represents the risk to discard H_0 , even if true. In our study we used $\alpha = 0.05$ (5%).

The probability P was then estimated. When $P > \alpha$, H_0 is accepted, i.e. there is no statistically significant difference between compared groups. When $P < \alpha$, H_0 is discarded, i.e. there is statistically significant difference between compared groups.

The P value depends on the parameter χ^2 . Standard variation analysis software was used for calculating of χ^2 and P for the results obtained for allelic and genotype frequencies.

Results and Discussion

CCR5 genetic polymorphism

Fig. 1 shows different patterns of CCR5 genetic polymorphism - amplification products of 198bp and 166bp in 3% agarose-gel electrophoresis.

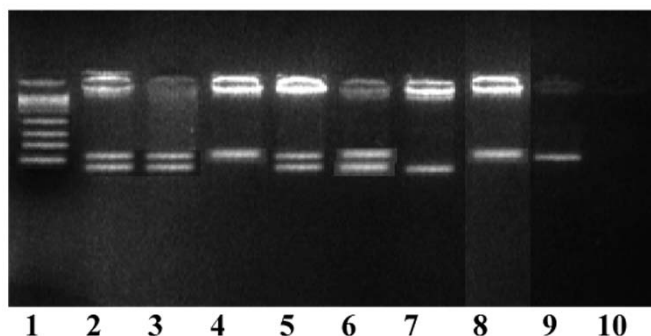


Fig. 1. An example of PCR analysis of CCR5 amplification products in 3% agarose gel:

Lane 1 – Ladder; **Lanes 4, 8, 9** – wild type (CCR5/CCR5); **Lanes 2, 3, 5, 6** – heterozygous type (CCR5/CCR5del32); **Lane 7** – homozygous type (CCR5del32/CCR5del32); **Lane 10** – blank gel control;

Fig. 2 shows a 3% agarose gel representing the results found for 16 persons. As seen, in this gel wild type and heterozygous patterns of CCR5 can be seen. No homozygous pattern has been found either in this or in any other person in this study.

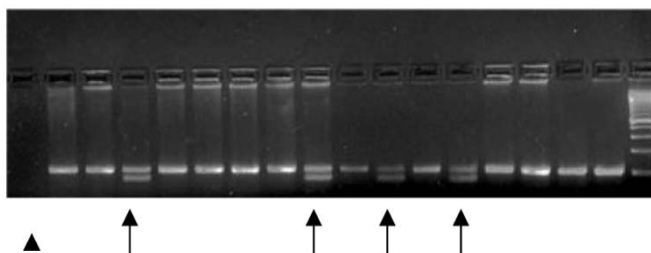


Fig. 2. Agarose gel electrophoresis representing wild type and heterozygous patterns of CCR5 in Bulgarians living with HIV. The lanes marked by arrows represent heterozygous pattern of CCR5, lanes marked by triangle – blank gel control, all the rest lanes – wild type of CCR5

TABLE 1

Association of CCR5 genetic polymorphism to the course of infection in Bulgarians living with HIV, n=177

A No. (%) of HIV-infected studied; course of infection	B No. (%) of heterozygous CCR5 pattern	C No. (%) of wild CCR5 pattern
Persons with slow progression –LTS; n=14 14/177 (8.5 %)	2/177(1.1 %) 2/14 (14.3 %)	12/177(6.8 %) 12/14(85.7 %)
Persons with rapid/moderate progression 163/177 (91.5 %) n=163	4/177 (2.2 %) 4/163 (2.5 %)	159/177 (89.8 %) 159/163 (97.5 %)
Total number studied 177	6/177 (3.4 %)	171/177 (96.7 %)

TABLE 2

Comparison of CCR5del32 allelic frequency between HIV-infected and non-infected Bulgarians

Population studied	Wild type CCR5/CCR5	Heterozygous CCR5del32/CCR5	Homozygous CCR5del32/CCR5del32	CCR5del32 allelic frequency and standard error (SE) according to the number of chromosomes studied
Persons, living with HIV (n=177)	N=171	n=6	n = 0	1,7% ± 0.7% (n=354)
Non-infected person (n=901)	N= 784	n=110	n =7	7% ± 0.9% (n=1802)

 $\chi^2 = 14.05$; $P=0.0002$

Totally, among 177 persons, living with HIV, none had CCR5del32 homozygous status, six out of them showed heterozygosity (only three – LTSs) and 171 – “wild type”. The CCR5del32 allelic frequency was 1.7%. The fact that nobody among those living with HIV was found with homozygous pattern of CCR5 (CCR5del32/CCR5del32) confirms the data reported about genetic insusceptibility of these individuals to HIV-infection (8).

As seen, only 2 (14.29%) out of 14 LTSs showed heterozygous genotype. The other 12 LTSs (85.71%) demonstrated “wild type” of the gene coding for CCR5. Additionally, the remaining 4 persons with heterozygous pattern (CCR5/CCR5del32) (66.66%) clinically were not identified as LTSs. The association between CCR5 genetic polymorphism found and the course of infection is presented on **Table 1**.

Table 1 clearly shows that only 14.3 % of LTSs studied demonstrated heterozygous CCR5 pattern (Column B). The majority of 12 LTSs (85.7%) showed wild type CCR5 (Column C). A significant difference ($p<0.05$) between the results in columns B and C was found.

On the other hand, it is evident that the heterozygous CCR5 profile is not the only one responsible for the slow progression to AIDS. Six persons out of all studied (3.4%) were found with heterozygous CCR5del32 genotype, but only 3 out of them belonged to LTSs (Column B). This fact shows that although linked to slow progression of HIV-infection, this polymorphism is not the only one, associated with this clinical condition. Therefore, this research should be extended and further study of polymorphism of other human genetic factors should be included in order to clarify the clinical significance of different genetic polymorphism combinations. Additionally, it becomes also clear that the heterozygous CCR5 profile is not typical for the individuals with rapid/moderate progression (Column B vs. C – $p < 0.05$).

It was of interest to compare the CCR5del32 allelic frequency found in HIV-infected persons to the same frequency in Bulgarian general population. The data about the Bulgarian population were published earlier by (21) and the comparison is shown on **Table 2**.

The data show statistically significant difference in CCR5del32 allelic frequency between HIV-infected (1.7%) and non-infected (7%) Bulgarians ($p<0.05$).

The exploring of zero-hypothesis (H_0) for comparison of CCR5del32 allelic frequencies in persons, living with HIV and non-infected persons showed level of significance lower than 0,05, so rejecting the lack of difference between the two compared groups originally postulated by the zero-hypothesis (H_0). In other words, this means that the allelic CCR5del32 frequency in non-infected persons is remarkably higher than in persons living with HIV. This conclusion, as well as the lack of homozygous (CCR5del32/CCR5del32) persons in the group of those living with HIV, confirms and extends the significance of genetic factors, responsible for the HIV insusceptibility.

CXCR4 (SDF-1) genetic polymorphisms

Fig. 3 represents an example of electrophoretic profile of the amplification product of the gene coding for SDF-1 after MspI restrictase treatment.

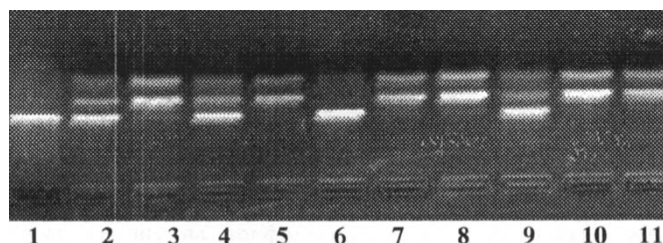


Fig. 3. Electrophoresis in 3% agarose gel of SDF-1 amplification products after restriction with Msp I

Lane 1 – 294 bp control representing SDF-1 3'A allele; **Lanes 3, 5, 7, 8, 10, 11** – wild type (SDF-1/SDF-1) – 101 bp and 193 bp fragments only; **Lanes 2, 4, 9** – heterozygous type (SDF-1/SDF-1 3'A) – 101bp, 193bp and 294bp fragments; **Lane 6** – homozygous type (SDF-1 3'A/SDF-1 3'A) 294bp fragment only (no restriction);

The results for CXCR4 (SDF-1) genetic polymorphisms are shown on **Table 3**.

The data show a frequency of homozygous pattern (SDF-1 3'A/SDF-1 3'A) in persons living with HIV (7 out of 48, 14.58%) comparable to that in general population (9%) – $p=0,3$ According to them, so far there is no dependence of this

TABLE 3

Polymorphism of the gene coding for SDF-1 in Bulgarians living with HIV (n=47)

$\chi^2 = 1.39$; $P = 0.2378$

SDF-1 Genotype	A n=37 n ;(%)	B n=11 n ;(%)	C n=100 (n ; %)
No. of persons studied with wild type SDF-1 and (%)	11/48(22.91 %) 11/37 (29.73 %)	4/48(8.33 %) 4/11(36.36 %)	56 (56 %)
No. of persons studied with heterozygous type and (%)	21/48 (43.75 %) 21/37 (56.76 %)	5/48 (10.41 %) 5/11 (45.45 %)	35 (35 %)
No. of persons studied with homozygous type and (%)	4/48 (8.33 %) 4/37 (10.81 %)	3/48 (6.25 %) 3/11 (27.27%)	9 (9 %)
Total No. of persons studied for SDF-1 genotype and (%)	37/48 (77.08 %)	11/48 (22.91%)	100 (100%)

A – HIV(+) persons with rapid/moderate progression to AIDS

B – HIV(+) persons – LTSs

C – Control group Bulgarians – HIV(–) persons

homozygous status and the susceptibility to HIV. These data should be reviewed after computing on the basis of the three-locus genotype (CCR5del32, SDF-1 3'A and CCR2 V64I) for each individual studied (both infected and uninfected) and evaluation of relative hazard (RH) values.

Additionally, higher frequency of homozygous pattern (SDF-1 3'A/SDF-1 3'A) in LTSs (27.3%) compared to the group of persons with moderate or rapid progression (10.81%) was observed, but without statistical significance ($p = 0.2378$), probably due to the low number of LTSs.

According to literature (22), the homozygosity in SDF-1 (SDF – 1 3'A/ SDF – 1 3'A) only is most likely associated with better prognosis due to later AIDS onset and lower viral load. It is important to compare the distribution of SDF-1 3'A polymorphism in HIV(+) persons with slow progression to AIDS (45.8%) to that of the general Bulgarian population (26.5%) – there is a statistically significant difference ($p = 0.04$). The distribution of SDF – 1 3'A in general population varies from population to population and Bulgaria has a relatively high, but within the parameters found (range 2.9% - 36.6%), allele frequency (23). Therefore, although low number of LTSs studied, their SDF-1 3'A polymorphism data confirm those published.

To evaluate risk of AIDS onset and even for HIV infection for the Bulgarian population, the relative hazard (RH) value should be computed on the basis of three-locus genotype for each individual. The RH values for each of the possible three-locus genotypes should evaluate the expected insusceptibility to HIV and risk for AIDS onset from genetical point of view.

CCR2 genetic polymorphisms

Fig. 4. represents an example of electrophoretic profile of the amplification product of the gene coding for CCR2 after BsaBI restrictase treatment.

The results for CCR2 genetic polymorphisms are shown on **Table 4.**

In the literature the significance of CCR2-V64I genetic polymorphism alone is not completely clear. As seen, in our study there is no significant difference by this defect between the group of rapid/moderate progressors to AIDS and LTSs ($P = 0.4414$). However, it is well known that cases, where homozygosity in CCR2 V64I coexists with heterozygosity in CCR5 are associated with lower levels of viremia during early chronic HIV-1 infection (9). Moreover, patients with both defects show better response to HAART. So, further study of response to HAART in relation to genetic polymorphism of co-receptor genes seems worth doing.

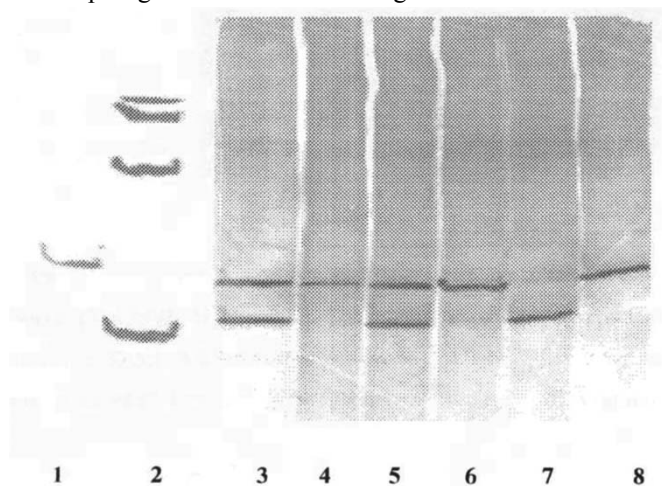


Fig. 4. Electrophoresis in 8% polyacrylamide of CCR2 amplification products after restriction with BsaBI

Lane 1 – 128 bp control; **Lane 2** – molecular marker; **Lanes 4, 6, 8** – wild type (CCR2/CCR2) – 128bp fragment only; **Lanes 3, 5** – heterozygous type (CCR2/CCR2 V64I) – 110 bp and 128 bp fragments; **Lane 7** – homozygous type (CCR2 V64I/CCR2 V64I) 110 bp fragment only (no restriction).

To elucidate the role and significance of the combined genetic polymorphisms for the slow course of HIV infection of

TABLE 4

Polymorphism of the gene coding for CCR2 in Bulgarians living with HIV (n=48)

CCR2 Genotype	A (n=37)	B n=(11)
No. of persons studied with wild type CCR2 and (%)	26/48 (54.17 %) 26/37 (70.27 %)	9/48 (18.75 %) 9/11 (81.82 %)
No. of persons studied with heterozygous type and (%)	10/48 (20.83 %) 10/37 (27.03 %)	2/48 (4.17 %) 2/11 (18.18 %)
No. of persons studied with homozygous type and (%)	1/48 (2.08 %) 1/37 (2.7 %)	0/48 (0 %) 0/11 (0 %)
Total No. of persons studied for CCR2 genotype and (%)	37/48 (77.08 %)	11/48 (22.92 %)

 $\chi^2 = 0.59$; P=0.4414

A – HIV(+) persons with moderate or rapid progression to AIDS

B – HIV(+) persons with slow progression to AIDS (LTSs)

TABLE 5

Polymorphism of the genes coding for CCR5, SDF-1 and CCR2 in LTSs-Bulgarians living with HIV

LNSs	CCR5 pattern	SDF-1 pattern	CCR2 pattern
1	CCR5/CCR5del32	SDF-1/SDF-1 3'A	CCR2/CCR2
2	CCR5/CCR5del32	SDF-1/SDF-1	CCR2/CCR2
3	CCR5/CCR5	SDF-1 3'A/SDF-1 3'A	CCR2/CCR2
4	CCR5/CCR5	SDF-1 3'A/SDF-1 3'A	CCR2/CCR2
5	CCR5/CCR5	SDF-1 3'A/SDF-13'A	CCR2/CCR2
6	CCR5/CCR5	SDF-1/SDF-1	CCR2/CCR2-V64I
7	CCR5/CCR5	SDF-1/SDF-1	CCR2/CCR2-V64I
8	CCR5/CCR5	SDF-1/SDF-13'A	CCR2/CCR2
9	CCR5/CCR5	SDF-1/SDF-13'A	CCR2/CCR2
10	CCR5/CCR5	SDF-1/SDF-13'A	CCR2/CCR2
11	CCR5/CCR5	SDF-1/SDF-13'A	CCR2/CCR2
12	CCR5/CCR5	–	–
13	CCR5/CCR5	–	–
14	CCR5/CCR5	–	–

all three co-receptors, comparison of genetic patterns of LTS so far studied, has been done (**Table 5**).

As seen, 2 out of all 14 LTSs show heterozygous CCR5/CCR5del32 pattern (Nos.1, 2, 3), including one displaying heterozygous SDF-1/SDF-1 3'A pattern (No. 1). Another two (Nos. 7 and 8) display heterozygous CCR2/CCR2-V64I pattern. On the other hand, three LTSs (Nos. 4, 5, 6) show homozygous SDF-1 3'A/SDF-1 3'A pattern, none of them heterozygous for CCR5del32. Surprisingly, four LTSs (Nos. 9, 10, 11, 12) demonstrate heterozygous SDF-1/SDF-1 3'A pattern combined with wild type of the other two co-receptor genes. The only explanation for this could be the relatively high incidence of SDF-1 3'A allele in Bulgarian population.

After completing and publishing of the results obtained here together with the first results of genetic polymorphism of CCR5 in population groups in Bulgaria (21) and in a little group of HIV-infected untreated LTSs (1) a recommendation could be drawn to evaluate risk of AIDS onset and even for HIV infection for the Bulgarian population, using the genetic

data and respective RH values. Latter should be computed on the basis of three-locus genotype for each individual, separately for the general population and the HIV(+) persons. The RH values for each of the possible three-locus genotypes should evaluate the expected insusceptibility to HIV for the general population and risk for AIDS onset from the HIV-infected persons from genetical point of view.

Although referring to little number of HIV-infected people, the data obtained are in agreement with those published already. We feel encouraged in performing further studies to link the genetic polymorphisms of the three most often used co-receptors in HIV-infection to the response to HAART in the Bulgarians living with this infection.

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