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## Do Human Polyoma Viruses and Human Immunodeficiency Virus Share Common Co-receptors?

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Keywords:	human polyoma viruses (HPoV - JCV, BKV), HIV co-receptors (CCR5, CCR2, CXCR4), Bulgarians living with HIV



TABLE I. Genetic Polymorphisms of CCR5, CXCR4(SDF1) and CCR 2 in HIV infected Bulgarians co-infected or not infected with HPoV

HIV infected patients		Genetic polymorphism patterns of chemokine co-receptors in HIV infected Bulgarians (n = 49)								
		CCR5			CXCR4 (SDF1)			CCR2		
		Wild type CCR5/CCR5 n, (%)	Heterozygous CCR5/CCR del 32 n, (%)	Homozygous CCR del 32/CCR del 32 n, (%)	Wild type SDF1-G/G n, (%)	Heterozygous SDF1-G/A n, (%)	Homozygous SDF1-A/A n, (%)	Wild type V/V n, (%)	Heterozygous V/I n, (%)	Homozygous I/I n, (%)
Co-infected with HPoV (n = 27)	BKV	11 (91.7)	1 (8.3)	0 (0)	7 (58.3)	3 (25.0)	2 (16.7)	9 (75.0)	3 (25.0)	0 (0)
	JCV	11 (91.7)	1 (8.3)	0 (0)	9 (75.0)	2 (16.7)	1 (8.3)	9 (75.0)	3 (25.0)	0 (0)
	BKV +JCV	3 (100)	0 (0)	0 (0)	2 (66.7)	0 (0)	1 (33.3)	2 (66.7)	1 (33.3)	0 (0)
Not infected with HPoV (n = 22)		22 (100)	0 (0)	0 (0)	10 (45.4)	11 (50.0)	1 (4.6)	13 (59.0)	9 (40.9)	0 (0)

TABLE II. Distribution of genetic polymorphim/s in patients with HIV co-infected or not infected with HPoV

Patients groups	Genetic polymorphisms found	Number (%)	Average CD4+T cells count around the moment of HPoV detection
Persons with HIV co-infected with HPoV (n=27)	Wild genotype (wt) by all three co-receptors	10 (37.0)	738 (180 - 1014)**
	Polymorphism/s in one or two genes	17 (63.0)	602 (218- 1473)**
Persons with HIV not infected with HPoV (n=22)	Wild genotype (wt) by all three co-receptors	5 (22.7)*	558 (272 - 1054)***
	Polymorphism/s in one or two genes	17(77.3)*	573 (253 - 955)***

\* P = 0.03; \*\* P = 0.47; \*\*\* P = 0.89

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3 1 **Do Human Polyoma Viruses and Human Immunodeficiency Virus Share Common Co-**  
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6 2 **receptors?**

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8 3 Kalin Borissov<sup>1</sup>, Iliya Tsekov<sup>2</sup>, Rayna Gavazova<sup>3</sup>, Zlatko Kalvatchev<sup>2</sup>, Radka Argirova<sup>1</sup>  
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39 16 **Running head:** Human Polyoma Viruses and HIV Share Common Co-receptors?  
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3 1 ABSTRACT  
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5 2 Host and/or viral factors involved in human polyomavirus (HPoV) infection in persons living  
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7  
8 3 with HIV remain unknown. A hypothesis is outlined suggesting the importance of the co-  
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10 4 receptors CCR5, CCR2 and CXCR4 not only for HIV, but also for HPoV. Functionally capable  
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12 5 receptors coded by wild type (wt) genotypes could facilitate internalization of HPoV in the cell  
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14 6 resulting in brain and/or kidney infection/s in HIV infected individuals.

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17 7 Forty nine Bulgarians with HIV, all treated by HAART, without neurological and/or kidney  
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19 8 disorders, were tested for JCV and BKV and genotyped for CCR5(CCR5del32), CCR2(CCR2-  
20  
21 9 64I) and CXCR4(SDF1-3'A). In 27/49 (55.1%) individuals a co-infection with HPoV was  
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23 10 identified - BKV in 12/49 (24.5%), JCV - in another 12/49 (24.5%) and both viruses – in 3/49  
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25 11 (6.1%). A high frequency of wt CCR5 was found in patients with HPoV (91.7% for BKV and  
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27 12 JCV and in 100% with both viruses). V/V of CCR2 was presented in 75% for BKV and JCV and  
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29 13 in 66.7% for BKV plus JCV. SDF1-3'G/G predominated in JCV infected patients (75%), while  
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31 14 G/A and A/A genotypes were more frequent in patients with BKV (41.7%).

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34 15 Also, 21/22 (95.4%) persons without HPoV infection were heterozygous for SDF1 and CCR2.  
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36 16 The number of individuals bearing wt of all co-receptors in the group of persons not infected with  
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38 17 HPoV was lower ( $p=0.03$ ) than that with polymorphism/s in one or two genes (SDF1 and CCR2)  
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40 18 in the same group.

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42 19 The results suggest a probable role of co-receptors used by HIV to facilitate infection with HPoV.  
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50 21 **Key Words:** human polyoma viruses (HPoV - JCV, BKV), HIV co-receptors (CCR5, CCR2,  
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52 22 CXCR4), Bulgarians living with HIV  
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## INTRODUCTION

When Highly Active Antiretroviral Therapy (HAART) for infection with HIV was introduced in 1996, a number of viral and bacterial opportunistic infections associated earlier with severe immunodeficiency, such as those caused by cytomegalovirus or the mycobacterium avium complex, were reduced drastically [Hoffmann, 2005]. Nevertheless, some problems associated with the diagnosis and treatment of other opportunistic infections remained, among them the progressive multifocal leukoencephalopathy [Hoffmann, 2005].

In contrast to infection with cytomegalovirus or with the mycobacterium avium complex progressive multifocal leukoencephalopathy does not always indicate the final stages of HIV infection. Although CD4+T cells number is usually below 100/ $\mu$ l when clinical disease develops, the progressive multifocal leukoencephalopathy may also occur when the CD4+ T-cell count is above 200/ $\mu$ l. Since HAART was introduced, the decrease in the incidence of the progressive multifocal leukoencephalopathy was not as frequent as other opportunistic infections. However, progressive multifocal leukoencephalopathy is now probably the second most common neurological opportunistic infection, after cerebral toxoplasmosis, in persons infected with HIV [Antinori et al., 2001].

The human polyomaviruses (HPoV), known as JCV, BKV, WUV, KIV and MCC, are small, naked, DNA containing viruses. Infection with HPoV is almost ubiquitous, asymptomatic and lifelong. However, reactivation during immunosuppression, associated with mutations in the transcriptional control region, that upregulates viral replication, can cause life-threatening disease [Cole and Conzen, 2001]. Thus, it has been considered that JCV, the causative agent of progressive multifocal leukoencephalopathy, is activated and the disease becomes symptomatic only when cellular immunity is impaired. JCV reaches the central nervous system via leukocytes,

1 where it destroys cells containing myelin sheath [Hoffmann, 2005]. On the other side, HIV-1 can  
2 also infect oligodendrocytes, the cells producing myelin, resulting in production of infectious  
3 virus [Albright et al., 1996].

4 BKV causes productive infection in the urinary tract. Approximately 80% of adults  
5 worldwide are seropositive for BKV, though there is no evidence that BKV causes any disease in  
6 the immune competent host. There is strong evidence for the association of BKV with  
7 hemorrhagic cystitis, nephritis and especially with graft failure in renal transplant patients. BKV  
8 remains latent in lymphocytes, the urogenital tract and brain but may be reactivated if the host  
9 becomes immunocompromised [Vallbracht et al., 1993]. Little is known about the role of the  
10 other HPoV (WU, KI and MC) identified recently in immunocompromised individuals [Sharp et  
11 al., 2009].

12 A number of host genetic factors, including genotypes of the chemokine co-receptors  
13 (CCR5, CCR2) and the SDF1, the only natural ligand of the CXCR4 co-receptor, have been  
14 identified. They have an impact on susceptibility to HIV-1 infection, as well as, on progression to  
15 AIDS and death [Singh et al., 2003; Su et al., 2000].

16 The brain and kidney are among the organs favoring the persistence of HPoV. This means  
17 that viruses harbored there are able to evade immunological surveillance [Nathanson and  
18 Gonzales-Scarano, 2007]. It appears this happens through restricted access of lymphocytes to the  
19 brain across the blood-brain barrier or to infected glomerular epithelial cells across the  
20 subendothelial basement membrane.

21 The onset and course of a HPoV related disease in persons infected with HIV is in most  
22 cases considered to be associated with a severe impairment of the immune system and especially  
23 of cell-mediated immunity. Although HAART was introduced more than a decade ago, some  
24 infections, especially the progressive multifocal leucoencephalopathy remain a problem. So,



1 factors of human and/or of viral origin should be presented and analyzed to explain the observed  
2 discordance. Today, a limited number of studies are dedicated to interactions between HPoV and  
3 HIV and still less – to the impact of host genetic factors for acquiring and development of these  
4 opportunistic infections in persons infected with HIV [Doerries, 2004; Guerini et al. 2008].

5       The aim of this study was to demonstrate the presence of HPoV (JCV and/or BKV) in  
6 Bulgarians infected with HIV but without neurological and/or kidney disorders and to analyze the  
7 possible role of genetic polymorphisms of three chemokine co-receptors: CCR5 (CCR5del32),  
8 CXCR4 (SDF1-3'A) and CCR2 (CCR2-64I), in relation to the presence/absence of two HPoV:  
9 JCV and BKV. Several reports discuss the receptors that can be used by HPoV. Infections with  
10 JCV, BKV as well as SV40 could be blocked by pretreatment of cells with neuraminidase,  
11 suggesting the receptors are sialoglycoproteins [Greenlee and O'Neill, 2002]. A recent research  
12 determined that the serotonergic receptor 5HT2AR could be utilized by JCV in glial cells  
13 [Elphick et al., 2004]. Nevertheless, investigation of the effect of risperidone (a potent 5HT(2A)R  
14 blocker) on JCV infected cells, failed to determine a significant difference between treated and  
15 naive cells, questioning the effectiveness of this substance for patients with progressive  
16 multifocal leucoencephalopathy [Chapagain et al., 2008]. Therefore, JCV might employ  
17 additional entry mechanisms. Similarly to other naked viruses, internalization of HPoV in the cell  
18 differs substantially from the entry of enveloped viruses like HIV. There is no need for  
19 membrane fusion for JCV and BKV to penetrate the cell. It has been shown that HPoV use  
20 caveolar endocytosis, a receptor-dependent process [Cole and Conzen, 2001]. On the other hand,  
21 before infecting macrophages and microglia in the brain, polyomaviruses could be transferred  
22 by leukocytes. Viruses might exploit the co-receptors (CCR5, CXCR4 and/or CCR2), as well as  
23 CD4 + receptors and MHC class I molecules of CD8 cells, respectively.

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3 1 Recently, HIV-1 binding sites were discovered in brain, i.e. chemokine co-receptors could  
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5 2 facilitate the infection of macrophages and microglia not only with HIV [Cartier et al, 2005; Kaul  
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7 3 et al., 2001], but according to the proposed hypothesis, also with HPoV. If this really occurs, it  
8  
9 4 should be demonstrated that the CCR5, CXCR4 and/or CCR2 co-receptors presented on CD4+ T  
10  
11 5 cells and on other cells involved in HIV-1 brain/kidney persistent infection, are functionally  
12  
13 6 capable, i.e. are coded by wild type (wt) genotype resulting in active receptor proteins, not in  
14  
15 7 truncated ones. On the contrary, internalization of HPoV in the cell should not be supported by  
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17 8 polymorphism/s in co-receptor gene/s, probably conferring absence of HPoV infection even in  
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19 9 patients with HIV induced immunodeficiency.  
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## MATERIALS AND METHODS

### Patients and Study Design

Forty nine blood samples collected on Gutrie cards from Bulgarians living with HIV (36 males and 13 females), with an average duration of HIV-1 infection 12.54 years (22 – 4), all of them on treatment by HAART, with no clinical evidence for neurological and/or kidney disorders, were enrolled in this study. Approval for the study was obtained from the Independent Ethics Committee of National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria. DNA was isolated from the Gutrie cards by phenol:chlorophorm:isoamylalcohol (25:24:1) method. For every person the CD4+T cell count around the moment of detection of BKV and/or JCV was registered (TABLE II).

### Identification of HPoV Sequences in Peripheral Blood Leucocytes

For detection of JCV and BKV, qualitative real-time PCR assays were employed. BKV viral genomic sequences were documented using a novel Light Upon Extension (LUX) primer system as demonstrated before [Slavov et al., 2008]. For detection of JCV the primer pair JCT1 and JCT2 [MacKenzie et al., 2003] was used, modified for a protocol with SYBR-Green I. As this is a non-specific detection system (intercalating dye) and in order to verify the specificity of the amplified fragments, a dissociation analysis was performed. Only samples showing both an increase in the fluorescent signal and a characteristic dissociation pattern with a specific melting temperature of 75.7<sup>0</sup>C, were considered positive. Measures were taken according to the molecular biology guidelines in order to prevent contamination, including the use of DNA free negative controls in each run.

## Chemokine Co-receptor Genotyping

Study of polymorphism and genotyping of CCR5 (CCR5del32), CXCR4 – SDF1 (SDF1-3'G/A) and CCR2 (V64I) were conducted as already described [Borissov et al., 2007]. Briefly, PCR and Restriction Fragment Length Polymorphism assays were carried out. Following the amplification reaction, the products were subjected to restriction enzyme digestion with Msp1 for SDF1 and BsaBI for CCR2 for 4 h. The digestion products and the PCR product for CCR5 were analyzed by agarose gel electrophoresis (for CCR5) or PAGE (for CCR2 and SDF1). Statistical Chi-square ( $\chi^2$ ) analysis was used to compare the genotype frequency distribution within the groups of persons infected with HIV and/or HPoV. Fisher's exact test was performed to compare the average number of absolute CD4+T cell counts, as appropriate. A cut-off of  $p \leq 0.05$  was considered significant.

## RESULTS

### Genetic Polymorphisms in HIV Infected Patients with HPoV Infection(s)

Genotyping of DNA of 49 individuals was performed for CCR5 (CCR5del32), SDF1 – 3’G (SDF1-3’G/A) and CCR2 (CCR2-V64I) in order to evaluate their involvement in HIV and/or HPoV infection. Specific BKV and/or JCV sequences were identified in peripheral blood leucocytes of 27/49 (55.1%) Bulgarians infected with HIV. No neurological symptoms and/or kidney dysfunction were observed in the patients at the time point of genetic studies. BKV as well as JCV were found in 12/49 (24.5%) of the studied DNA samples. BKV plus JCV were detected in 3/49 (6.12%). A high frequency of wt form of CCR5 (TABLE I) was found in patients with HIV co-infected with HPoV (11 out of 12 BKV and JCV positive samples – 91.7% and 3 out of 3 – 100% for the double infected, respectively). The results obtained for wild V/V genotype of CCR2 were 9 out 12 (75%) for each BKV and JCV and 2 out of 3 (66.7%) for the BKV plus JCV positive samples (TABLE I). In the samples tested none was found with homozygous (CCR2-64I/CCR2-64I) genotype. It is of special interest that 3/12 (25%) from the BKV and JCV infected persons showed heterozygous, CCR2/CCR2-64I, genotype. The wild genotype of SDF1-3’G/G (TABLE I) predominated in patients with JCV (75%), while G/A and A/A genotypes were found more frequently in patients with BKV (41.7%).

### Genetic Polymorphisms in HIV Infected Patients without HPoV Infection(s)

Genetic polymorphisms profile of all three studied co-receptors in 22 out of 49 (44.9%) patients living with HIV, but not infected with HPoV is represented in TABLE I, too. The results clearly demonstrate that 21 out of all 22 (95.4%) HIV infected individuals without HPoV show heterozygous patterns of two co-receptors – CXCR4 (SDF1-3’A/SDF1-3’ G/A) and CCR2 (CCR2/CCR2-64I). Only one person (1 out 22 – 4.6%) has a homozygous pattern for the ligand

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3 1 of CXCR4: SDF1-3' A/A. It is worth noting that double genetic polymorphisms - those of SDF1  
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5 2 (CXCR4) and CCR2 - predominate. The explanation and importance of this finding is still  
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8 3 unclear and needs further investigation.  
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10 4 The distribution of genetic polymorphim/s in persons infected with HIV, co-infected or  
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12 5 not with HPoV is demonstrated in TABLE II. A significant difference ( $p=0.03$ ) was found  
13  
14 6 between the wt carrying persons (for all three co-receptors) and those with polymorphism/s in  
15  
16 7 one or two genes coding for chemokine co-receptors within the group of individuals infected with  
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18 8 HIV but not infected with HPoV. No statistical difference was found when comparing the  
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20 9 CD4+T positive counts in all patients groups (all patients were treated by HAART of different  
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22 10 duration and protocols).  
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## DISCUSSION

Data are presented on the possible role of the most important co-receptors for HIV – CCR5, CXCR4 (SDF1) and CCR2, for polyomaviruses' cell entry in persons infected with HIV, without clinical disease or symptoms characteristic for JCV and/or BKV. Recently, synergism between HIV and JCV has been demonstrated in vitro [Hoffmann, 2005]. For example, the HIV-1-encoded Tat protein, essential for the establishment of a productive HIV-1 infection, has been found to be a potent activator of the JCV promoter. BKV T antigen is able to transactivate the HIV-1 Long Terminal Repeat. Although HIV-1 expression in oligodendrocytes is regularly low, the Tat protein appears to be secreted by HIV-1-infected cells. When localized in JCV-infected oligodendrocytes, it could induce transcription on Tat-responsive genes [Doerries, 2004].

Popik et al.(2002) demonstrated the involvement of T-cell lipid rafts in the process of HIV attachment [Popik et al., 2002]. According to these studies, independently of the presence of CD4 [Millan et al., 1999] and CCR5 [Manes et al., 1999] in detergent-resistant membrane microdomains , the distribution of CD4, CCR5, and CXCR4 receptors in T-cell lipid rafts remains largely unknown. A study using immunogold electron microscopy, has shown clearly the existence of homogenous microclusters of CD4 and chemokine co-receptors separated by distances of less than 100 nm, thus suggesting that these HIV-1 receptors may colocalize in rafts [Singer et al. 2001]. Also, it has been shown that the assembly and budding of different viruses, including HIV-1 [Nguyen and Hildreth, 2000; Ono and Freed, 2001], take place in lipid rafts. Moreover, the same authors found that HIV-1 particles produced by infected T cells incorporate raft-resident proteins and glycolipids into the viral envelope Further, it has been demonstrated that raft-colocalized receptors may be crucial for entry of different pathogens, including SV40 [Parton and Lindsay, 1999; Pelkmans et al., 2001] and the murine leukemia virus [Lu, Silver,

1 2000]. Bearing all these facts in mind, a hypothesis was outlined suggesting the participation of  
2 co-receptors used by HIV infected cells for internalization of HPoV in the cells of the brain and  
3 kidney of persons with HIV infection. In order to verify this hypothesis two groups of individuals  
4 infected with HIV were compared – co-infected and not infected with HPoV. No  
5 immunodeficiency at the time of HPoV detection was found (TABLE II).

6 Data presented on TABLE I demonstrate that more than 50% of HPoV and HIV infected  
7 persons bear normozygous (wt) co-receptors. Heterozygosity in all co-receptors genes should be  
8 considered as restricted expression but not as a lack of receptor proteins. These data support the  
9 hypothesis by demonstrating the presence of functionally capable co-receptor proteins in  
10 individuals infected with HIV and positive for HPoV. But this hypothesis should sound more  
11 strongly if the restricted expression or even lack of more than one co-receptor protein was found  
12 in persons not infected with HPoV. This conferred the special interest to see the distribution of  
13 genetic polymorphism/s of the studied co-receptors in the group of persons infected with HIV but  
14 not infected with HPoV (TABLE I and TABLE II).

15 The number of persons bearing wild type of all three studied co-receptors in the group of  
16 persons not infected with HPoV but with HIV infection, is statistically lower ( $p=0.03$ ) than that  
17 of persons with polymorphism/s in one or two genes (G/A and/or A/A in SDF1 and V/I in CCR2)  
18 in the same group (TABLE II). No similar difference was observed in the group of HPoV-  
19 infected persons (TABLE II). Additionally, only 4 out of 27 (14.9%) of all HPoV (JCV and  
20 BKV) positive individuals were found to have a homozygous profile (SDF1-A/A) meaning no  
21 expression of the SDF1 gene (TABLE I). This result suggests the probable role of both co-  
22 receptors with a restricted or even lacking expression could facilitate the internalization of HPoV  
23 in the cell. Guerini FR et al. (2008) described an association of G/A and A/A polymorphisms of  
24 SDF1 in patients with HIV-related progressive multifocal leucoencephalopathy (JCV positive),



1 but not in HIV infected persons without JCV. The results concerning SDF1 polymorphisms  
2 documented in the present study differ from those observed by Guerini FR et al. (2008): all  
3 patients with JCV had both no lesions characteristic for progressive multifocal  
4 leucoencephalopathy, and no immunosuppression (all of them received HAART) and possibly no  
5 JCV and/or BKV activation. At the same time, HpoVs are ubiquitous and wide spread in the  
6 general population [zur Hausen, 2008] and the failure to identify any viral sequences in a number  
7 of samples (TABLE II) arises several questions. It might be considered that the  
8 immunocompromised state of individuals living with HIV should lead to a rather higher rate of  
9 JCV/BKV presence than the observed one. The possible explanation of these findings probably  
10 lies in the studied material. Detection of specific HPoV sequences in peripheral leucocytes was  
11 demonstrated in the current study. According to current knowledge, before the development of  
12 viremia and further dissemination, HPoV viruses are found mainly in urothelial cells (Doerries,  
13 2004). Actually, the results for the presence of HPoV in the group of HIV-infected persons  
14 resemble positivity rates documented in healthy blood donors [Kalvatchev et al., 2009].

15 As for the CCR5 co-receptor, it is well established that two types of genetic  
16 polymorphisms are observed: in the open reading frame - CCR5del32; and in the gene regulatory  
17 region. Here only the genetic polymorphism in the open reading frame but not in the regulatory  
18 region – especially the 59029 – G/A, for which its role together with CCR5del32 has been  
19 recently confirmed to be associated with disease progression and neurological impairment  
20 [Singh et al., 2003], was studied. On the other hand, CCR5 is a co-receptor that facilitates the  
21 entry of HIV in CD4+ T cells and the macrophage lineage. Similar to Guerini et al. (2008), no  
22 differences in CCR5del32 polymorphism (heterozygosity) between HPoV infected and  
23 uninfected persons with HIV infection were found in the present study. The same is valid for the  
24 wild type of the CCR5 suggesting the presence of a functioning co-receptor. As individuals with

1 absent or incomplete expression of one or more chemokine co-receptors have a lower chance to  
2 become infected with certain types of HIV and to progress to AIDS [Su et al., 2000], it is possible  
3 that the restricted expression of co-receptors might also have a protective role in relation to the  
4 HPoV infection and its associated disease. It could be also theorized that if the HPoV exploit the  
5 same co-receptors as those used by HIV, new opportunities for protection and treatment of these  
6 opportunistic infections could be provided. Additional research and prospective monitoring of  
7 HIV-infected persons with or without progressive multifocal leucoencephalopathy are required.

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