The role of mannose-binding lectin gene polymorphisms in susceptibility to HIV-1 infection in Southern Brazilian patients

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Objective: This study investigates the role of mannose-binding lectin (MBL) in the susceptibility to HIV-1 infection analyzing polymorphisms located at the *MBL2* promoter and exon 1 regions.

Materials and methods: The prevalence of *MBL2* variant alleles was investigated in 410 HIV-1-infected patients from the South Brazilian HIV cohort and in 345 unexposed uninfected healthy individuals. The promoter variants were genotyped using polymerase chain reaction with sequence-specific primers (PCR-SSP) and exon 1 variants were analyzed by real-time PCR using a melting temperature assay and were confirmed by PCR-restriction fragment length polymorphism (RFLP). *MBL2* genotypic and allelic frequencies were compared between HIV-1-infected patients and controls using the chi-squared tests.

Results: The analyses were performed subdividing the individuals according to their ethnic origin. Among Euro-derived individuals a higher frequency of the LX/LX genotype was observed in patients when compared to controls ($P < 0.001$). The haplotypic analysis also showed a higher frequency of the haplotypes associated with lower MBL levels among HIV-1-infected patients ($P = 0.0001$). Among Afro-derived individuals the frequencies of LY/LY and HY/HY genotypes were higher in patients when compared to controls ($P = 0.009$ and $P = 0.02$).

Conclusions: An increased frequency of *MBL2* genotypes associated with low MBL levels was observed in Euro-derived patients, suggesting a potential role for MBL in the susceptibility to HIV-1 infection in Euro-derived individuals.

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Introduction

The acquired immune deficiency syndrome (AIDS) became known worldwide in the 1980s, when the first epidemic signals appeared. Without treatment, life expectancy of patients diagnosed with AIDS is around 2 years [1,2]. Interestingly, humans show a remarkable variation in vulnerability to HIV infection, probably due to genetic and immunologic factors. HIV transmission depends on infectivity factors (for example, the higher the

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viral load, the greater the chance of transmission) as well as on host susceptibility [3].

There are two distinct HIV types, HIV-1 and HIV-2, though in Brazil HIV-1 is much more prevalent than HIV-2 [4,5]. Infectivity depends on HIV-1 concentration and presence and number of cells infected with the virus in body fluids, although the viral and cell requirements for HIV-1 transmission remain poorly understood [5]. By 2008, there were 33.4 million people living with HIV worldwide. In that year, 2.7 million new cases were reported and 2 million died from the disease worldwide [6]. In Brazil, by 2009 approximately 544 thousand cases of the disease had been reported, since the first case in 1980 [2]. The resistance to infection reflects the combination of genetic factors, innate and acquired immuno-resistance. Among the genetic factors associated with HIV resistance, homozygosis of the delta 32 allele of the *CCR5* gene is the best known [5].

In recent years, there has been an emerging interest in mannose-binding lectin (MBL) due to its central role as a recognition molecule in the complement system. MBL is a member of a family of proteins called collectins and is characterized by the presence of both a collagenous region and a lectin domain [7,8]. This protein is produced by the liver and is capable of binding mannan, gram-negative bacteria and envelope glycoproteins of viruses [9]. After binding to a pathogen, MBL goes through a conformational change and activates associated mole-cules, such as MBL-associated serine proteases (MASP-1, MASP-2 and MASP-3), resulting in complement activation by the lectin pathway [10,11]. Since HIV gp120 protein is highly glicosylated, it has been suggested that MBL could be involved in HIV-1 binding and opsonization [12].

Mannose-binding lectin is also known as a paradox of innate immunity. High MBL serum levels are useful in the immune response against extracellular pathogens. MBL recognizes pathogens and triggers phagocytosis, opsoni-zation and elimination of the microorganism. Conversely, MBL-facilitated opsonization and phagocytosis may increase the infectivity of some intracellular pathogens. Thus, it could be said that, in the case of intracellular pathogens, low MBL levels offer an advantage to the host by hindering the infection [9,12–14]. The human mannose-binding lectin gene (*MBL2*) is located on chromosome 10 (q11.2-q21) and contains four exons. Polymorphisms located at exon 1 are reported to interfere with protein conformation, resulting in altered collage-nous regions and, as a consequence, inhibiting oligomer-ization. This leads to low serum MBL levels and impaired protein function [12,15,16]. *MBL2* gene exon 1 region contains three functional single-nucleotide polymor-phisms (SNPs), at codon 52 (CGT to TGT, Arg→Cys, referred as 'D' allele), at codon 54 (GGC to GAC, Gly→Asp, 'B' allele) and at codon 57 (GGA to GAA,

Gly→Glu, 'C' allele). A wild-type sequence is called allele 'A', and the presence of any of these variants is defined as allele '0' [17,18]. Additional polymorphisms in the promoter and 5'UTR regions of the *MBL2* are also associated to reduced serum MBL levels [19]. The H/L polymorphism is located at -550 bp, X/Y is found at -221 bp (both are G to C nucleotide substitutions), and P/Q is located at position +4 of the 5'UTR (C/T). These three polymorphisms are in strong linkage disequilibrium with exon 1 SNPs, and seven more common haplotypes have been described: HYPA, LYQA, LYPA, LXPA, LYPB, LYQC and HYPD [7,20–22].

Several mechanisms associate MBL to HIV infection. MBL can promote activation of inflammatory cells and, by doing so, induce the depletion of T CD4$^+$ cells [23]. It has been shown that HIV-infected patients carrying *MBL2* mutations had a significantly shorter lifespan after AIDS diagnosis [24]. Heggelund *et al.* [25] suggested that MBL can increase HIV replication, inducing persistent inflammatory response. Also, the 'B' allele was associated with disease progression, and two studies found association of exon 1 genotypes with HIV vertical transmission [26–28]. Although the mechanism of MBL−HIV interaction has not as yet been fully understood, most studies suggest that MBL deficiency is a risk factor for HIV infection. However, these findings were not replicated in all populations analyzed, and some studies do not evidence a role for MBL in the susceptibility to HIV infection [9,23]. In our study we genotyped 410 HIV-positive patients and 345 healthy blood donors, both from Southern Brazil, looking for a possible association between *MBL2* polymorphisms and HIV infection.

Materials and methods

Patients and controls
HIV-infected patients were consecutively enrolled at the South Brazilian HIV cohort (SOBRHIV) from January 2004 through November 2005, in Porto Alegre, capital of Brazil's southernmost state [29]. The inclusion criteria were as follows: asymptomatic HIV-infected individuals on highly active antiretroviral therapy (HAART) for at least 1 year (HAART prescribed according to Brazilian guidelines at that time) [2]; HIV-1 RNA load less than 50 copies/ml determined by the technical Versant HIV-1 RNA 3.0 assay/bDNA automation system in 340 bDNA Analyzer (Bayer, Germany); and over 18 years of age. Exclusion criteria were: pregnancy, current use of drugs that could be associated to body changes such as corticosteroids or anabolizing steroids, and mental illness. Written informed consent was signed by all individuals and the protocol was approved by the Hospital de Clínicas de Porto Alegre Ethics Research Committee.

Table 1. Demographic characteristics of the study group.

Characteristics	HIV-infected individuals ($n = 410$)	Control group ($n = 345$)
Sex (male/females)	224/186	243/102
Age (years $\pm$ SD and min-max)	42.98 ± 9.42 (19–73)	43.43 ± 7.78 (20–62)
Ethnicity (European/African-derived)	239/171	244/101
HCV co-infection (*n*, %)	103 (25.1%)	NA
HBV co-infection (*n*, %)	17 (4.1%)	NA

NA, not apply.

A total of 410 samples from HIV-infected individuals, 224 men and 186 women, with ages ranging from 19 to 73 years were obtained. Patients were classified as European or African-derived according to phenotypic characteristics of individuals and ethnicity data of parents/grandparents reported by the participants in an appropriate questionnaire. The issue concerning skin color-based classification criteria adopted in Brazil is well documented and has been already assessed by our group in previous studies [30,31]. In total, 239 individuals were classified as European-derived and 171 were classified as African-derived.

The control group was formed by 345 unexposed, uninfected healthy blood donors, 244 being European-derived and 101 African-derived, from the urban population of Porto Alegre, the capital of the southernmost state of Brazil. The demographic characteristics of both groups are described in Table 1. All patients and controls participating in this study gave their written informed consent. The genomic DNA for molecular characterization was obtained from 5-ml peripheral blood samples collected with ethylenediaminetetraacetic acid (EDTA) and purified through a salting-out procedure as described by Lahiri and Nurnberger [32].

Exon 1 genotyping
MBL2 exon 1 polymorphisms genotyping was performed by melting temperature assay following the protocol of Arraes *et al.* [27]. After melting temperature genotyping, A/0 and 0/0 genotypes were confirmed by polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) assay [33], and the specific presence of 'B', 'C' and 'D' alleles was defined.

Promoter region genotyping
The promoter region of the *MBL2* gene spanning the -550 (L or H) and -221 (X or Y) polymorphisms was amplified by PCR with sequence-specific primers (PCR–SSP) as described by Neonato *et al.* [34], using specific primers named: L forward, X reverse, H forward and Y reverse. Four simultaneous reactions were performed for each sample, using different combinations of primers (LX, LY, HX and HY), to identify the haplotypes. The amplified fragments were visualized in 2% agarose gels. In addition, a control gene (cytochrome P450 debrisoquine, *CYP2D6*) was amplified in all the reactions.

Statistical analysis
MBL2 genotypic distribution was determined by direct counting. The genotypic frequencies were compared to Hardy−Weinberg expectations using chi-squared tests. *MBL2* allelic frequencies were compared between patients and controls using the chi-squared test and adjusted residuals were also calculated. The significance level was set as $\alpha = 0.05$ (two-tailed). All statistical analyses were performed with SPSS 15.0 (SPSS Inc., Chicago, Illinois, USA) and WinPepi 10.0 softwares.

Results

Data from the literature concerning *MBL2* polymorphic variant frequencies revealed a high level of interethnic diversity. For this reason, our analyses were performed subdividing the individuals according to ethnic origin [33,35]. The European-derived group included 244 healthy individuals and 239 HIV-positive patients. In this group, we first evaluated exon 1 *MBL2* polymorphisms (Table 2). The frequency of *MBL2* 0/0 homozygote genotype was 0.053 in controls and 0.042 in HIV-positive patients; no statistical difference was found. All A/0 and 0/0 individuals were then genotyped in order to identify 'B', 'C' and 'D' alleles. The analyses of the allelic frequencies showed a higher frequency of the 'D' allele in HIV-positive patients, when compared to controls ($P = 0.002$).

We also compared the frequency of *MBL2* promoter polymorphisms (Table 3). Among healthy individuals, 0.041 were genotyped as X/X against 0.122 of the HIV-positive patients ($P = 0.001$). The analysis of the haplotypes showed a higher frequency of the LY haplotype in the control group, when compared to HIV-infected patients ($P = 0.048$). The haplotypic combination frequencies were also compared between cases and controls, showing a higher frequency of the LX/LX genotype in the HIV-infected patients when compared to healthy individuals (0.122 and 0.041, respectively; $P = 0.001$).

Finally, we grouped *MBL2* exon 1 and promoter genotypes and calculated combined genotypes classifying them in three classes, associated with MBL serum levels (as described in the literature): high serum MBL levels (HYA/A and LYA/A), intermediate serum MBL levels (LXA/LXA, HYA/0 and LYA/0) and deficient serum MBL levels (LXA/0 and 0/0) [12]. *MBL2* combined

Table 2. Distribution of *MBL2* exon 1 polymorphisms allelic and genotypic frequencies in HIV-1-infected individuals and controls, according to ethnicity.

Genotypes	European-derived		African-derived	
	Control freq (n) n = 244	HIV+ freq (n) n = 239	Control freq (n) n = 101	HIV+ freq (n) n = 171
A/A	0.607 (148)	0.544 (130)	0.584 (59)	0.526 (90)
A/0	0.340 (83)	0.414 (99)	0.366 (37)	0.409 (70)
0/0	0.053 (13)	0.042 (10)	0.050 (5)	0.064 (11)
	χ^2, P = 0.233		χ^2, P = 0.630	
	n = 244	n = 229	n = 101	n = 167
A/A	0.607 (148)	0.568 (130)	0.584 (59)	0.539 (90)
A/0				
A/B	0.246 (60)	0.223 (51)	0.099 (10)	0.192 (32)
A/C	0.037 (9)	0.039 (9)	0.218 (22)	0.132 (22)
A/D	0.057 (14)	0.127 (29)	0.050 (5)	0.072 (12)
0/0				
B/C	0.020 (5)	0.000 (0)	0.010 (1)	0.006 (1)
B/D	0.008 (2)	0.018 (4)	0.000 (0)	0.018 (3)
C/D	0.000 (0)	0.004 (1)	0.000 (0)	0.012 (2)
B/B	0.025 (6)	0.013 (3)	0.010 (1)	0.030 (5)
C/C	0.000 (0)	0.004 (1)	0.030 (3)	0.000 (0)
D/D	0.000 (0)	0.004 (1)	0.000 (0)	0.000 (0)

No statistical comparison was performed on these data due to the small number of individuals in each subgroup

Alleles	n = 488	n = 468	n = 202	n = 334
A	0.777 (379)	0.767 (359)	0.767 (155)	0.736 (246)
B	0.162 (79)	0.130 (61)	0.065 (13)[b]	0.138 (46)[b]
C	0.029 (14)	0.026 (12)	0.144 (29)[c]	0.075 (25)[c]
D	0.033 (16)[a]	0.077 (36)[a]	0.025 (5)	0.051 (17)
	χ^2, P = 0.016		χ^2, P = 0.003	

0 allele = B + C + D alleles; A allele = wild type; A/A genotype, high serum MBL levels; A/0 genotype, Intermediate serum MBL levels; 0/0 genotype, lower serum MBL levels.
[a]Adjusted residual, P = 0.003.
[b]Adjusted residual, P = 0.009.
[c]Adjusted residual, P = 0.01.

genotypes are shown in Table 4. The frequency of genotypes associated with high serum MBL levels was 0.574 in healthy individuals and 0.460 in HIV-positive patients (P = 0.012). Conversely, frequency of genotypes associated with lower serum MBL levels was 0.053 in healthy individuals and 0.174 in HIV-positive patients (P < 0.0001).

The same approach was used to analyze the African-derived individuals (101 healthy individuals and 171 HIV-positive patients). Both patients and controls showed a similar distribution of *MBL2* exon 1 polymorphisms (grouped as allele 'A' and allele '0') (Table 2). When the analysis was conducted using 'B', 'C' and 'D' alleles separately, we observed an increased frequency of 'B' allele in patients (0.138) with respect to the controls (0.065; P = 0.009). Conversely, an increased frequency of 'C' allele was detected in controls in comparison to patients (0.144 and 0.075, respectively; P = 0.01) (Table 2).

Concerning the *MBL2* promoter region, a higher frequency of the LY/LY haplotypic combination was observed among healthy individuals (0.390), compared with HIV-positive patients (0.158; P < 0.001); also, an increase in the LY/HY and HY/HY genotypes in patients

(0.316 and 0.164, respectively) was observed in comparison with controls (0.18 and 0.07, respectively) (P = 0.015 and P = 0.026, in that order). These findings reflected *MBL2* haplotypic frequencies [see, for example, a frequency of 0.60 of the LY haplotype in the controls against 0.39 in HIV-positive patients (P < 0.001), and the frequency of 0.37 of the HY haplotype in patients, compared with 0.19 in controls (P < 0.001)]. When genotypes were examined, H/L and H/H were more frequent in HIV-positive individuals (0.42 and 0.16), compared to 0.24 and 0.07 in healthy individuals (P = 0.003 and P = 0.026), whereas the L/L genotype had a frequency of 0.69 among controls and 0.41 in patients (P < 0.0001) (Table 3). When *MBL2* exon 1 and promoter polymorphisms were grouped and combined genotypes analyzed, no statistically significant differences were observed.

We also performed analysis subdividing HIV-infected individuals according to co-infection with hepatitis B and C, considering that these infections have similar routes of transmission, making co-infection a common event. In the group of HIV-infected individuals, 17 patients were co-infected with hepatitis B virus (0.04) and 103 were co-infected with hepatitis C virus (0.25). Yet, we did not find statistical differences (data not shown).

Table 3. *MBL2* promoter polymorphisms frequencies in patients and controls, according to ethnicity.

	European-derived		African-derived	
	Control freq (n)	HIV+ freq (n)	Control freq (n)	HIV+ freq (n)
Haplotypic combination	n = 244	n = 237	n = 100	n = 171
LX/LX	0.041 (10)[a]	0.122 (29)[a]	0.060 (6)	0.105 (18)
LX/LY	0.193 (47)	0.135 (32)	0.240 (24)	0.152 (26)
LX/HY	0.102 (25)	0.110 (26)	0.060 (6)	0.105 (18)
LY/LY	0.180 (44)	0.169 (40)	0.390 (39)[b]	0.158 (27)[b]
LY/HY	0.336 (82)	0.291 (69)	0.180 (18)[c]	0.316 (54)[c]
HY/HY	0.148 (36)	0.173 (41)	0.070 (7)[d]	0.164 (28)[d]
	χ^2, P = 0.018		χ^2, P < 0.001	
Haplotypes	n = 488	n = 474	n = 200	n = 342
LX	0.188 (92)[e]	0.245 (116)[e]	0.210 (42)	0.234 (80)
LY	0.445 (217)[f]	0.382 (181)[f]	0.600 (120)[g]	0.392 (134)[g]
HY	0.367 (179)	0.373 (177)	0.190 (38)[h]	0.374 (128)[h]
	χ^2, P = 0.054		χ^2, P < 0.001	
Genotypes	n = 244	n = 237	n = 100	n = 171
L/L	0.414 (101)	0.426 (101)	0.690 (69)[j]	0.415 (71)[j]
H/L	0.438 (107)	0.401 (95)	0.240 (24)[k]	0.421 (72)[k]
H/H	0.148 (36)	0.173 (41)	0.070 (7)[l]	0.164 (28)[l]
	χ^2, P = 0.626		χ^2, P < 0.0001	
X/X	0.041 (10)[i]	0.122 (29)[i]	0.060 (6)	0.105 (18)
X/Y	0.295 (72)	0.245 (58)	0.300 (30)	0.258 (44)
Y/Y	0.664 (162)	0.633 (150)	0.640 (64)	0.637 (109)
	χ^2, P = 0.004		χ^2, P = 0.390	

HY haplotype, high serum MBL levels; LX haplotype, lower serum MBL levels; LY haplotype, intermediate serum MBL levels.
[a]Adjusted residual, χ^2, P = 0.001.
[b]Adjusted residual, χ^2, P < 0.001.
[c]Adjusted residual, χ^2, P = 0.015.
[d]Adjusted residual, χ^2, P = 0.026.
[e]Adjusted residual, χ^2, P = 0.034.
[f]Adjusted residual, χ^2, P = 0.048.
[g]Adjusted residual, χ^2, P < 0.001.
[h]Adjusted residual, χ^2, P < 0.001.
[i]Adjusted residual, χ^2, P = 0.001.
[j]Adjusted residual, χ^2, P < 0.0001.
[k]Adjusted residual, χ^2, P = 0.003.
[l]Adjusted residual, χ^2, P = 0.026.

Discussion

Mannose-binding lectin is an important molecule in the first-line defense of the organism, and considering its ability to bind to HIV-1 gp120 glycoprotein, several studies have evaluated functional *MBL2* polymorphisms in different populations in the context of HIV infection, although the conclusions were somewhat conflicting [37−39].

In the present study, we investigated the association between *MBL2* gene polymorphisms and the susceptibility to HIV infection in patients and ethnicity-matched controls from Southern Brazil subdividing the individuals according to their ethnic origin [40].

Among European-derived individuals we observed a higher frequency of the 'D' allele in HIV-positive patients when compared to the controls. Nevertheless,

Table 4. *MBL2* combined genotypes related to MBL serum levels in HIV-1 patients and healthy controls, according to ethnicity.

	Euro-descendant		Afro-descendant	
	Control Freq (n)	HIV+ Freq (n)	Control Freq (n)	HIV+ Freq (n)
Combined genotypes	n = 244	n = 235	n = 100	n = 167
High	0.574 (140)[a]	0.460 (108)[a]	0.540 (54)	0.443 (74)
Intermediate	0.373 (91)	0.366 (86)	0.290 (29)	0.395 (66)
Deficient	0.053 (13)[b]	0.174 (41)[b]	0.170 (17)	0.162 (27)
	χ^2, P < 0.0001		χ^2, P = 0.202	

Combined genotypes: High serum MBL levels (LYA/A, HYA/A); Intermediate serum MBL levels (LXA/LXA, LYA/0, HYA/0); Deficient serum MBL levels (LXA/0, 0/0).
[a]Adjusted residual, P = 0.012.
[b]Adjusted residual, P < 0.0001.

considering 'B', 'C' and 'D' alleles grouped under the designation of '0' allele, similar frequencies were observed in patients and controls. Two independent studies, performed in Spanish and Colombian HIV-positive individuals, also reported the lack of association between *MBL2* exon 1 polymorphisms and HIV infection [41,42]. Conversely different findings reporting an increased frequency of *MBL2* 0/0 genotype among HIV-positive individuals or the association with disease progression in children have been reported in different ethnic groups [27,43–47].

In spite of showing similar results to our study for the exon 1 polymorphisms, however, neither the study with Spanish patients nor the investigation in Colombian individuals analyzed the *MBL2* promoter region polymorphisms. The analyses of the *MBL2* promoter region polymorphisms in patients and ethnicity-matched controls from Southern Brazil showed an increased frequency of the LX/LX haplotypic combination in HIV-positive individuals (0.122) when compared to the healthy individuals (0.041), indicating a higher frequency of the genotype related with low MBL levels in HIV-positive patients. Promoter haplotypes and genotypes frequencies also suggested a tendency of increased frequency of LX haplotype and X/X genotype in HIV-positive individuals with respect to the controls, indicating the increased presence of X allele, related to low serum MBL levels in patients. Taken together, these results suggest a possible role for these SNPs associated with low MBL levels, in HIV infection.

It is interesting to note that other studies also hypothesized a role of the X/X genotype in the context of HIV infection. For instance, the X/X genotype have already been correlated with a rapid rate of HIV infection progression [40], and homozygosity for the XA/XA genotype was proposed as an important genetic determinant of HIV-1 acquisition through vertical transmission [45].

Most studies with HIV patients did not analyze simultaneously exon 1 and promoter *MBL2* polymorphisms known to be in linkage disequilibrium: the analysis of *MBL2* haplotypes and combined genotypes, known to be correlated with MBL levels, is able to provide more complete information about the role of MBL in the susceptibility to HIV infection. The exon 1 variants disturb protein polymerization, not only resulting in reduced serum MBL levels, but also functionally altering its ligand binding and complement activation capacities. The SNPs in the promoter region can modulate the serum concentration of the protein [48,49]. Therefore, MBL concentrations may vary considerably, due to a combination of structural and promoter gene polymorphisms.

When we considered together promoter and exon 1 gene polymorphisms a significant difference between HIV-positive patients and controls among European-derived individuals was found. The haplotypic combinations related to high MBL levels (LYA/A and HYA/A) were more frequent in controls (0.57) than in HIV-positive individuals (0.46), and the combined genotypes related with deficient MBL levels (LXA/0 and 0/0) were more frequent in HIV-positive patients (0.17) when compared to controls (0.05). Similar data were observed in a study performed with Hispanic individuals, when HIV-positive children, under 2 years of age, carrying *MBL2* genetic variants resulting in lower MBL levels, were at a significantly greater risk of disease progression and central nervous system impairment when compared to the other patients tested [46]. Other studies found that HIV-positive pediatric patient carriers of the 'B' allele and/or with low MBL levels progress more rapidly to AIDS. Also, adult HIV-positive patients with this pattern exhibit a significantly shorter survival time after an AIDS diagnosis [44,50,51].

Among African-derived individuals, exon 1 polymorphisms (grouped as '0' allele) showed similar genotypic frequencies between patients and controls, although an increased frequency of the 'B' allele was observed among patients and an increased frequency of the 'C' allele was detected in controls. It is important to point out that the *MBL2* gene 'C' allele is relatively frequent among individuals of African ancestry, which can explain the higher frequency observed among our controls [52,53]. Another study, in a South African population, observed that in the absence of intervention, infants with *MBL2* genetic variants were more likely to acquire HIV through vertical transmission than infants with the wild-type genotype [54].

The analyses of promoter polymorphisms showed an increased frequency of the LY/HY and HY/HY haplotypic combinations in patients, when compared to controls, as well as a lower frequency of LY/LY. The analysis of *MBL2* haplotypes confirmed this higher frequency of the LY haplotype in controls, and of the HY haplotype in HIV-positive individuals. Since the LY haplotype is related to intermediate MBL serum levels and the HY haplotype is related with high MBL serum levels, the results indicate that African-derived patients have genotypes related to high MBL levels. Some studies with African-derived population suggested an association of MBL deficiency with resistance against leprosy and leishmaniasis [55,56]. The high prevalence of infections caused by intracellular pathogens in Africa may explain the differences in the *MBL2* allelic frequency observed between European and African populations [53]. Two studies carried out with sub-Saharan and Indian populations showed that low MBL levels were related with susceptibility to HIV infection, but high levels of MBL may be involved in the pathogenesis of tuberculosis in these individuals, indicating that MBL may exert opposing effects, suggesting the need of a balance, modulated by different pathogens [51,57].

In the present study we considered only asymptomatic HIV-infected individuals that have been on HAART for 1 year, with HIV RNA less than 50 copies/ml. It is true that these criteria restrict the analyses to a specific group of patients, since MBL levels and/or variant alleles have been associated with progression of HIV disease in infected individuals, nevertheless, our study was aimed to detect the eventual association between *MBL2* variants and the risk of being infected by HIV and no speculation about the role of *MBL2* polymorphisms in the modification of the disease progression can be drawn with the study group analyzed.

Actually, we observed a marked difference in the distribution of *MBL2* SNPs frequencies between European-derived and African-derived individuals. In European-derived individuals the majority of HIV-positive patients have genotypes related to low MBL serum levels. In African-derived individuals an opposite effect for *MBL2* polymorphisms in the susceptibility to HIV infection has been observed. Genetic factors, other than *MBL2*, reflecting the selection exercised by the exposure of the two ethnic groups to a different environment through time can explain these differences.

Finally, it has to be said that the ethnic classification used by our group (based on phenotypic characteristics of individuals and ethnicity data of parents/grandparents reported by the participants) is widely adopted in Brazil. However, we must admit that individuals classified as European-derived or African-derived can present a certain degree of admixture. A recent study by Santos *et al.* [36] assessed individual interethnic admixture using a 48-insertion-deletion Ancestry-Informative Marker panel. The authors identified a very high level of European contribution (94%) and fewer Native American (5%) and African (1%) genes in a sample of 81 European-derived individuals from southern Brazil [36]. Therefore, the subgrouping of our HIV-infected patients and controls seems to reflect the actual ethnic/genetic background of this human population.

In conclusion, since low serum MBL concentrations are associated with inefficient phagocytosis and opsonization, our data suggest that *MBL2* polymorphisms – and consequently low MBL levels – could lead to an inefficient response against viral infection in individuals with European ancestry. Thus, we suggest that individuals carrying *MBL2* variants being less efficient in the clearance of pathogens with mannose surface residues, such as HIV-1, would be more susceptible to HIV-1 infection.

Acknowledgements

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