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Association between AIDS disease progression rates and the *Fok*-I polymorphism of the *VDR* gene in a cohort of HIV-1 seropositive patients☆

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Abstract

In addition to its role in mineral metabolism, 1,25-dihydroxivitamin D_3 ($1,25(OH)_2D_3$) also has immunomodulatory effects. Vitamin D receptor (VDR) mediates genomic actions of $1,25(OH)_2D_3$, by acting as a transcription factor that modulates the expression of several $1,25(OH)_2D_3$ response genes. Variations at the *VDR* locus have been associated with susceptibility and progression to several immune diseases. We investigated the association between rates of progression to acquired immunodeficiency syndrome (AIDS) and the *Fok*-I polymorphism, which is located at the initiation codon of the *VDR* gene. The study was performed with a cohort of 185 patients infected with human immunodeficiency virus type 1 (HIV-1): all belonged to the intravenous drug abuse risk group. Progression to AIDS was according to the Centers for Disease Control 1993 criterion (CDC-1993). In addition, a first drop in CD4 cell count to below 200 μL^{-1} was considered as outcome. Patients who reached outcomes during follow-up were considered progressors. Non-progressors were those patients remaining outcome-free after a minimum follow-up of 8 years. Heterozygous at the *Fok*-I polymorphism were over-represented in the group of patients that progressed to AIDS CDC-1993 (50% of progressors versus 36% of non-progressors, $P = 0.061$; risk ratio (RR) $= 1.38$ (95% confidence interval (CI): 0.98–1.96)) and in the group of patients that showed a drop in CD4 cell count to below 200 μL^{-1} (52% of progressors versus 36% of non-progressors, $P = 0.037$; RR $= 1.44$ (95% CI: 1.02–2.03)). Mean time to AIDS CDC-1993 was shorter for those with Ff genotype than for those with FF and ff genotypes (non-Ff genotype patients), (log rank test $P = 0.035$; Cox hazard ratio (HR) for Ff versus non-Ff $= 1.53$ (95% CI: 1.0–2.33), $P = 0.047$). In addition the drop in CD4 cell count to below 200 μL^{-1} was reached faster in Ff carriers than in non-Ff patients (log rank test $P = 0.015$; HR for Ff versus non-Ff $= 1.77$ (95% CI: 1.12–2.8), $P = 0.014$). According to these results, HIV-1 seropositive patients carrying the Ff genotype could be considered prone to a faster progression to AIDS.

Keywords: Vitamin D receptor polymorphisms; Human immunodeficiency virus type-1; Acquired immunodeficiency syndrome; Disease progression rates; Association studies

1. Introduction

In addition to its role in calcium and skeletal homeostasis, the active metabolite of 1,25-dihydroxyvitamin D_3 ($1,25\text{-}(OH)_2D_3$), is also involved in the regulation of cell-mediated immunity [1]. $1,25\text{-}(OH)_2D_3$ promotes the differentiation of monocytes and suppresses lymphocyte proliferation by inhibiting the secretion of a number of cytokines, including IL-2, granulocyte-macrophage colony-stimulating factor and IFN-gamma, in T cells, and IL-12, in macrophages and B cells [2–5]. According to the pattern of cytokine inhibition the regulatory effect of $1,25\text{-}(OH)_2D_3$ on immune response appears to target Th1 cells by preventing their activation and the subsequent production of lymphokine [2].

At the cellular level $1,25(OH)_2D_3$ exerts its actions by binding to its specific intracellular receptor (Vitamin D receptor, VDR), which promotes the formation of functional heterodimers with retinoid X receptor (RXR). These heterodimers interact with target sequences, the Vitamin D responsive elements (VDREs), which are located in the 5′ region of Vitamin D responsive genes, and either promote or inhibit their expression. The VDR is expressed in peripheral blood monocytes and in rested and activated lymphocytes [6]. Polymorphisms of the *VDR* gene have been previously

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described. Three SNP polymorphisms that modify endonuclease restriction sites of *Bsm*-I, *Apa*-I and *Taq*-I, and a poly (A) microsatellite polymorphism, have been described in the 3′-UTR region of the *VDR* gene. These 3′-UTR polymorphisms exhibit strong linkage disequilibrium in Caucasian populations [7,8].

Associations have been reported between *Bsm*-I polymorphism and bone mineral density and osteoporosis [8–10], primary hyperparathyroidism [11] and the incidence and aggressiveness of prostate and breast cancer [7,12,13]. Recent findings have extended this association to several autoimmune diseases such as primary biliary cirrhosis and autoimmune hepatitis [14], Graves' disease [15] multiple sclerosis [16] and insulin-dependent diabetes mellitus [17] and to susceptibility to, and disease progression rates in, infectious diseases such as tuberculosis and leprosy [18,19].

In addition to the 3′-UTR variants, a SNP polymorphism that modifies the endonuclease restriction site of *Fok*-I has also been described in the translation initiation codon of the *VDR* gene [20]. This polymorphism corresponds to a T to C transition that eliminates the first translation initiation codon of the *VDR* gene. In homozygous individuals of the *ACG* sequence (referred to as *F* allele, as they lack the *Fok*-I restriction site), translation of the *VDR* gene initiates from a second potential start site located two codons away. In contrast to 3′-UTR polymorphisms of the *VDR* gene, which do not alter the VDR amino acid sequence, the start site polymorphism gives rise to a three-amino-acid difference in VDR length, which may affect the protein function. *Fok*-I polymorphism has been reported to influence bone mineral density [20], contribute to prostate cancer risk [21] and increase predisposition to several autoimmune diseases [22]. 3′-UTR polymorphisms are in linkage equilibrium with the *Fok*-I polymorphic site and several studies have showed that their effects on the associated diseases are independent [17,23–25]. We recently reported that the *Bsm*-I polymorphism is associated with disease progression rates in patients infected by the human immunodeficiency virus type 1 (HIV-1) [26]. Progression to the acquired immunodeficiency syndrome (AIDS) is faster in *B* allele homozygotes than in those not belonging to this genotype. These results allowed us to propose that homozygosity for the *Bsm*-I *B* allele of the *VDR* locus could be considered a risk factor for a less favorable progression in HIV-1 disease. In the present work we have extended our analysis to test for association between the *Fok*-I polymorphism and disease progression rates in our cohort of HIV-1 infected patients. We have also looked for interactions between these two polymorphic *VDR* sites.

2. Methods

2.1. Study population

The main characteristics of the population studied have been previously described [26]. Briefly stated, the Lleida AIDS Cohort is a prospective seroprevalent cohort of HIV-1 infected patients, belonging to the intravenous drug users (IVDU) risk group, drawn from the total cohort of HIV-1 seropositive adults enrolled in the AIDS Service of the Hospital Arnau de Vilanova. Only white Caucasian patients recruited between 1982 and 1991 were included in the study. All patients selected were in follow-up for more than 7 years. The median follow-up time for the patients selected was 127.7 months with a minimum of 85 months and a maximum of 198 months. A total of 185 patients met the inclusion criteria. The cohort study was approved by the local medical ethics committee.

At the times of registration and subsequent periodic visits, a complete clinical examination was undertaken that emphasized HIV-1 related signs and symptoms. Information on all HIV-1 clinical events, the use of antiretroviral therapy, and routine measurements were obtained by reviewing clinical records.

HIV-1 disease progression was analyzed according to the 1993 criteria for defining AIDS established by the Centers for Disease Control and Prevention [27]. A first drop in CD4 cell count below the 200 cells/μL level was also used as a separate indicator of outcome [28].

HIV-1 antibodies were detected in serum using a commercial Micro particle Enzyme Immunoassay (MEIA, Abbott Laboratories, IL, USA) and were confirmed by Western Blotting assay (INNO-LIA HIV Confirmation, Innogenetics, Zwijnaarde, Belgium). Serum virus load was measured by the Amplicor HIV-1 Monitor test (Roche Molecular Systems, Somerville, NJ, USA) following the manufacturer's instructions. Viral load was analyzed on a base 10 logarithmic scale; values below the level of detection (500 copies/mL) were recorded as 499 copies/mL. CD4 cell counts were investigated using a fluorescence-activated cell sorter analyzer (Becton Dickinson, San José, CA, USA). A control population of 224 healthy Caucasian HIV-1 negative individuals was recruited for this study from voluntary blood donors.

2.2. Genotyping

Genomic DNA was extracted from peripheral blood lymphocytes using a KG-Midi extraction kit (Camgen, Cambridge, UK). PCR protocols were developed to amplify the *Fok*-I polymorphism located in the 5′ region of the *VDR* gene. *Fok*-I polymorphism was detected by single strand conformation polymorphism (SSCP) analysis [29]. All the patients included in this study had been previously genotyped for *Bsm*-I polymorphism [26].

2.3. Statistical analysis

Hardy–Weinberg equilibrium was tested by comparing expected and observed genotype frequencies using a Chi-square test. Distribution of genotypes between patient groups and controls were compared by Chi-square test.

We explored the relationship between VDR *Fok*-I polymorphism and HIV-1 disease status by conducting a nested case-control study among IVDU patients with more than 8 years of follow-up. Cases were defined as IVDU patients who developed AIDS according to Centers for Disease Control 1993 criterion (CDC-1993) or exhibited a drop in CD4 cell count below 200 cells/μL. Controls were CDC-1993 AIDS-free patients and patients with CD4 cell counts >200 cells/μL. We also studied HIV-1 disease progression profiles for patients grouped according to genotypes by Kaplan–Meier survival analysis. Differences between groups were compared by log rank test. Hazard ratios (HR) were estimated using a Cox proportional hazard model adjusted for sex and age at first HIV-1 positive test. Patients were censored at either their last clinic examination date or date of death (not caused by AIDS). The observation period ended in March 1999. A P value $= 0.05$ was considered as statistically significant.

3. Results

The distribution of *Fok*-I genotypes among healthy controls (FF 43%, Ff 48% and ff 9%) and HIV-1 seropositive patients (FF 44%, Ff 42% and ff 14%) were in Hardy–Weinberg equilibrium ($P = 0.307$ and 0.125, respectively). There were no significant differences in *Fok*-I genotype frequencies between these two populations. This suggested that this polymorphism of the *VDR* locus had no effect on susceptibility to HIV-1 infection (Chi square value $= 3.5$; $P = 0.173$). *Fok*-I and *Bsm*-I polymorphisms were independently distributed in healthy controls and in IVDU seropositive patients (Chi square value $= 0.737$; $P = 0.947$ and 3.61; $P = 0.461$, respectively).

To analyze differences in disease progression between genotypes, IVDU seropositive patients were categorized into two main progression groups according to their clinical status. The 174 IVDU seropositive patients considered

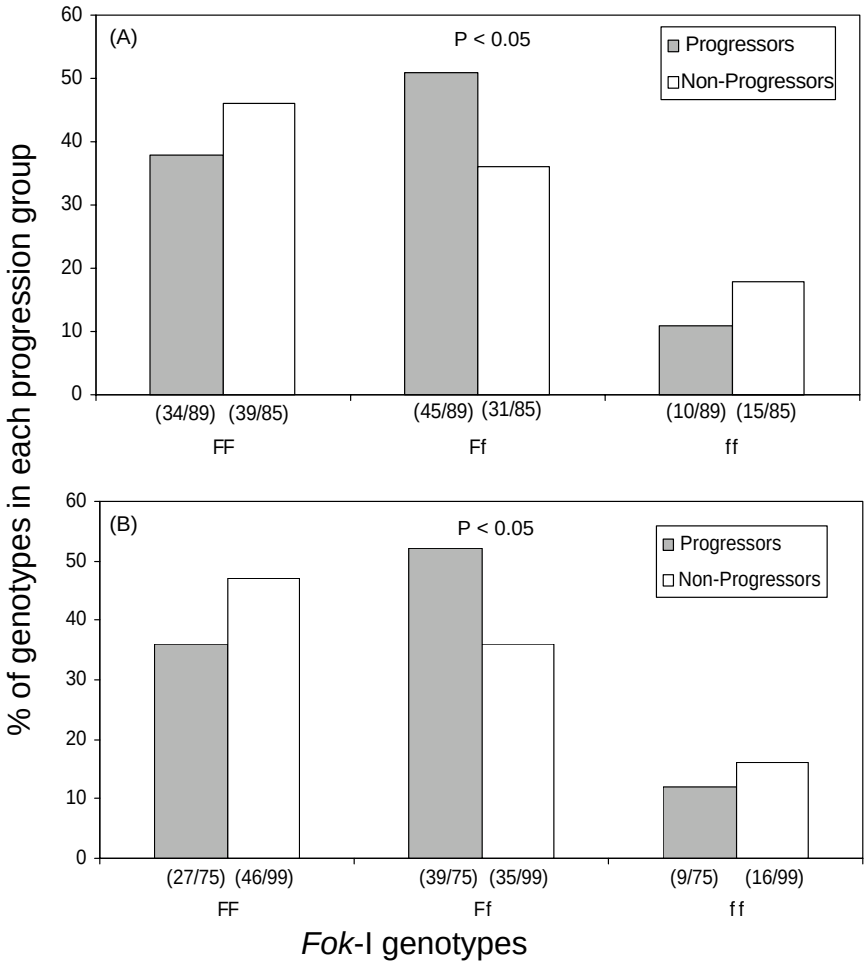


Fig. 1. Distribution of *Fok*-I genotypes in relation to progression group. We compared the distribution of *Fok*-I genotypes between HIV-positive/IVDU patients who progressed to outcome (shaded bars) and those who remained outcome-free (unshaded bars). The percentage of Ff heterozygotes among patients who progressed to both AIDS CDC-1993 (panel A) and CD4 cell counts of below 200 cells/μL (panel B) was higher than among those who remained outcome-free. Differences between the proportions were statistically significant ($P < 0.05$). The number of individuals in each genotype/total subjects in each outcome category is expressed at the bottom of each column.

(11 patients were in follow-up for less than 8 years), progressors included 89 (51%) subjects who developed AIDS CDC-1993 during follow-up and 75 (44%) patients who dropped below 200 cells/μL. Non–progressors were those patients who maintained AIDS-free status or with CD4 cell counts above 200 cells/μL at the end of the study. Fig. 1 shows the distribution of VDR genotypes among patients arranged according to disease status. The prevalence of FF and ff homozygotes among non-progressors was not statistically different from that observed among progressors. In contrast, a higher frequency of Ff heterozygotes was observed among progressors (50 and 52% for CDC-1993 and CD4 cell count, respectively) than in non-progressors (36% for both CDC-1993 and CD4 cell count). When compared to non-Ff heterozygotes (FF and ff homozygotes considered together), Ff heterozygosity was associated with progression to AIDS CDC-1993 and to a fall in CD4 cell count below 200 cells/μL with a risk ratio (RR) of 1.38 (95% confidence interval (CI): 0.98–1.96; $P = 0.061$) and 1.44 (95% CI: 1.02–2.03; $P = 0.037$), respectively. These results seem to indicate a worse disease progression for Ff heterozygotes.

In our previous work [26] we showed that BB homozygosity could be considered a risk factor for progression to AIDS in this cohort. To test for interactions between these two *VDR* polymorphic loci, patients were grouped according to the presence of two, one and no risk factors for progression to AIDS attributable to both *Fok*-I and *Bsm*-I polymorphism. Those that exhibited no risk factors were non-Ff and non-BB genotypes (those with genotypes FFBb, FFbb, ffBb

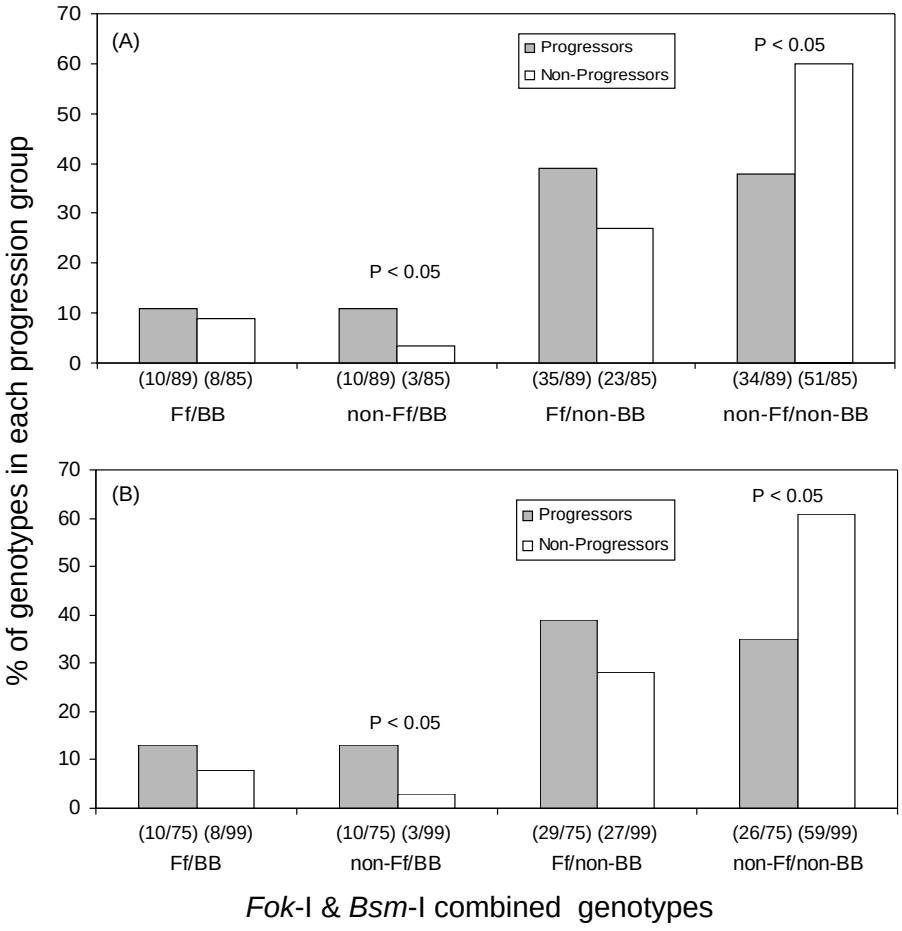
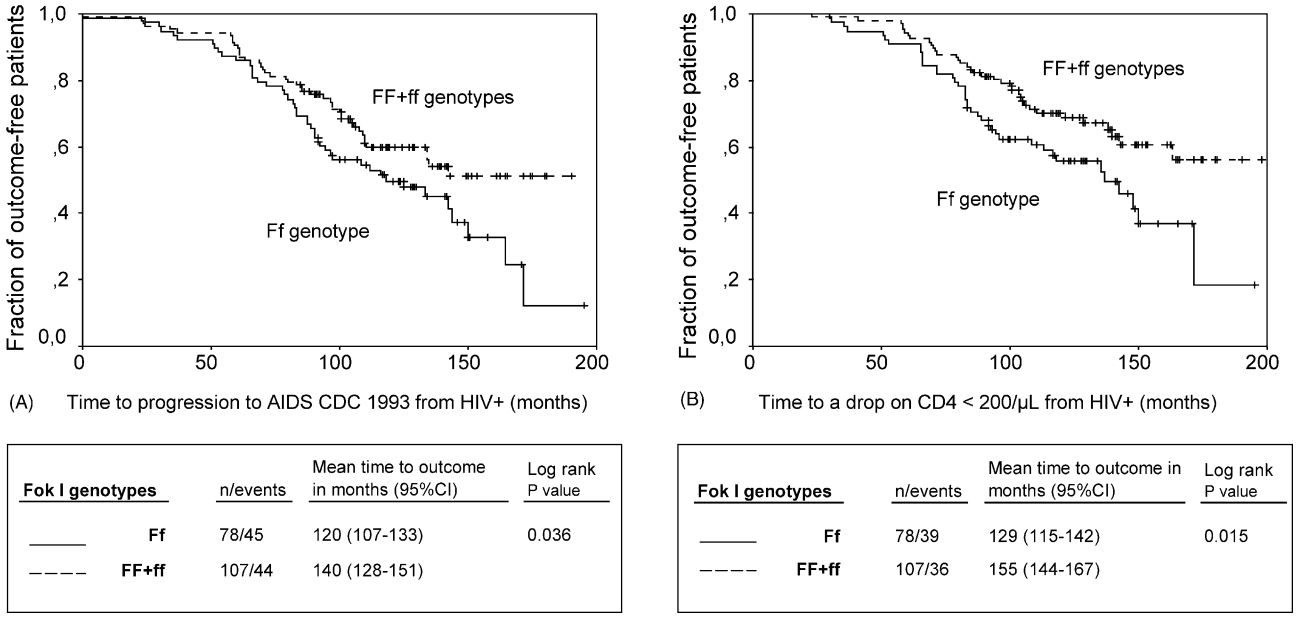


Fig. 2. Distribution of combined *Fok*-I and *Bsm*-I genotypes in relation to the progression group. Patient genotypes were respectively grouped according to the presence of two genotype risk factors conferred by *Fok*-I and *Bsm*-I polymorphisms (BB/Ff), one genotype risk factor associated with *Fok*-I (Ff/non-BB) and *Bsm*-I (non-Ff/BB) polymorphisms, and the absence of any genotype risk factors (non-Ff/non-BB). The genotypes considered in the non-Ff/BB group were: FF/BB and ff/BB. The genotypes considered in the non-BB/Ff group were: Ff/Bb and Ff/bb. The genotypes considered in the non-BB/non-Ff group were: FF/Bb, FF/bb, ff/Bb and ff/bb. The distribution of combined genotypes was compared between HIV-positive/IVDU patients who progressed to outcome (shaded bars) and HIV-positive/IVDU patients who remained outcome-free (unshaded bars). The percentage of patients who exhibited no risk factors attributable to *Fok*-I and *Bsm*-I polymorphisms (non-Ff/non-BB) among those who progressed to both AIDS CDC-1993 (panel A) and CD4 cell counts below 200 cells/μL (panel B) was lower than in those who remained outcome-free. The percentage of patients who only exhibited risk factors attributable to *Bsm*-I polymorphism (non-Ff/BB) among those who progressed to outcomes was higher than in those who remained outcome-free. Differences between the proportions were statistically significant ($P < 0.05$). The number of individuals in each genotype/total subjects in each outcome category is expressed at the bottom of each column.



Fok I genotypes		n/events	Mean time to outcome in months (95%CI)	Log rank P value
———	Ff	78/45	120 (107-133)	0.036
– – – –	FF+ff	107/44	140 (128-151)	

Fok I genotypes		n/events	Mean time to outcome in months (95%CI)	Log rank P value
———	Ff	78/39	129 (115-142)	0.015
– – – –	FF+ff	107/36	155 (144-167)	

Fig. 3. Disease progression rates in relation to *Fok*-I genotypes in HIV-positive/IVDU patients. Kaplan–Meier analysis is presented for both AIDS CDC-1993 (panel A) and outcomes involving a fall in CD4 cell counts below 200 μL^{-1} (panel B). For all 185 HIV-positive/IVDU patients, Kaplan–Meier curves were plotted for Ff heterozygotes (Ff) and FF and ff homozygotes were considered together (FF + ff). The mean time to outcome (in months) and the log rank test *P* value for comparisons between genotypes are indicated in the lower box of each panel. AIDS, acquired immunodeficiency syndrome; CDC, centers for disease control and prevention; HIV+, human immunodeficiency virus type-1 seropositive; CI, confidence interval.

and ffbb). This group of patients was more prevalent in the group of non-progressors than in that of progressors (60% versus 38% for progression to AIDS CDC-1993 and 69% versus 35% for a drop below 200 CD4/μL) (Fig. 2). The risk ratio for progression to AIDS CDC-1993 for patients with non-Ff/non-BB genotype was 0.64 (95% CI: 0.46–0.87) and 0.57 (95% CI: 0.4–0.8) for a drop below 200 CD4/μL. These groups of patients, who lacked any of the risk factors attributable to *Fok*-I and *Bsm*-I polymorphism, were protected from progression to AIDS and a drop in CD4 cell count. We also showed that those that exhibited a risk factor that was solely attributable to the BB genotype (those with genotypes FFBB and ffBB) were less prevalent in the group of non-progressors than in that of the progressors (3.5% versus 11% for progression to AIDS CDC-1993 and 3.1% versus 13.3% for a fall below 200 CD4/μL) (Fig. 2). When we analyzed the distribution of the *Bsm*-I genotypes in the 98 patients with the non-Ff genotype, we observed that individuals with BB genotype were less prevalent in the group of non-progressors than in that of the progressors (6% versus 23% for progression to AIDS CDC-1993 and 3% versus 28% for a fall below 200 CD4/μL). In individuals with the non-Ff genotype, the risk ratios for progression to AIDS CDC-1993 in patients with BB genotype was 4.0 (95% CI: 1.2–14) and it was 5.7 (95% CI: 1.7–19.5) for a fall below 200 CD4/μL. The risk conferred by the BB genotype was subsequently more pronounced in individuals who were safeguarded from risks attributable to the *Fok*-I polymorphism.

We also analyzed disease progression rates by Kaplan–Meier analysis using time to AIDS CDC-1993 and time to a first drop in CD4 cell count below 200 μL^{-1} as survival endpoints (Fig. 3). Hazard ratios for progression to AIDS or to a fall in CD4 cell count below 200 μL^{-1} were calculated by Cox proportional models (Table 1).

The mean time to outcome for Ff heterozygotes was 119 months for AIDS CDC-1993 and 128 months for CD4 cell count below 200 μL^{-1}. In spite of this, time to progression was slower in those with non-Ff genotypes (FF and ff genotypes considered together) (140 and 155 months for CDC-1993 and CD4 cell count, respectively). These differences were statistically significant (log rank test $P = 0.035$, and $P = 0.015$ for CDC-1993 and CD4 cell count, respectively). Cox proportional hazard ratios for progression to outcome adjusted for sex and age at the time of first HIV positive test were statistically significant (Table 1).

We also compared disease progression rates of patients grouped according to both *Fok*-I and *Bsm*-I polymorphism. Time to progression was longer for those without any risk factors attributable to *Fok*-I and *Bsm*-I polymorphism (non-FF/non-BB genotype) than for those who exhibited one or two risk factors (Fig. 4). Cox proportional hazard ratios for progression to outcome adjusted for sex and age at the time of first HIV positive test were statistically significant when non-FF/non-BB patients were compared with any other combination of genotypes (Table 1).

Table 1
Cox proportional hazard ratios for progression to outcomes in the cohort of HIV-1 seropositive intravenous drug user patients grouped according to *VDR* genotypes

Genotypes	Outcomes considered			
	AIDS CDC-1993		<200 CD4/μL	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Fok-I genotypes				
Ff	1.53 (1–2.3)	0.047	1.77 (1.1–2.8)	0.014
Non-Ff[a,b]	1		1	
Fok-I and *Bsm*-I combined genotypes[c]				
Ff/BB	1.7 (0.9–3.6)	0.114	2.5 (1.2–5.2)	0.014
Non-Ff/BB[d]	3.0 (1.4–6.0)	0.003	3.7 (1.8–7.8)	<0.0001
Ff/non-BB[e]	1.8 (1.1–2.9)	0.014	2.2 (1.3–3.4)	0.005
NonFf/non-Bb[a,f]	1		1	

Abbreviations: HIV-1, human immunodeficiency virus type-1; AIDS, acquired immunodeficiency syndrome; CDC, centers for disease control and prevention; HR, hazard ratio; CI, confidence interval.

[a] Genotypes used as references for comparisons.

[b] Non-Ff genotypes were: FF and ff.

[c] Genotypes were grouped according to the presence of two genotype risk factors conferred by *Fok*-I and *Bsm*-I polymorphisms (BB/Ff) one genotype risk factor due to *Fok*-I (Ff/non-BB) and *Bsm*-I (non-Ff/BB) polymorphisms and the absence of any genotype risk factor (non-Ff/non-BB).

[d] Genotypes considered in this group were: FF/BB and ff/BB.

[e] Genotypes considered in this group were: Ff/Bb and Ff/bb.

[f] Genotypes considered in this group were: FF/Bb, FF/bb, ff/Bb and ff/bb.

4. Discussion

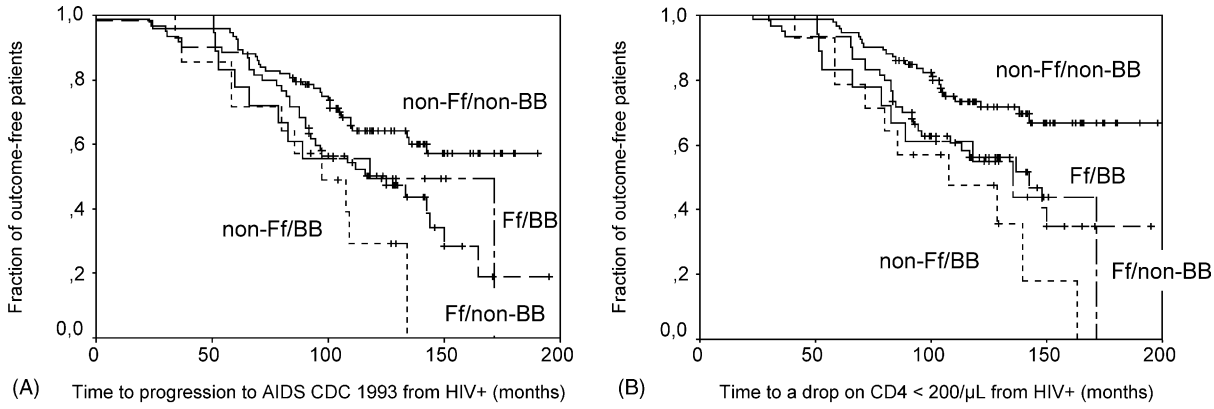
The apparent resistance to HIV-1 infection found in some people at risk and the variability observed in the rate of disease progression after infection are likely to be multifactorial and to involve both acquired and inherited factors [30]. To date, most attention has focused on the human leukocyte antigen (HLA) system [31], however there is now growing evidence that non-HLA loci may also play an important role. Several genes have been studied in relation with their possible roles in determining both the susceptibility of CD4 cells to HIV infection and the subsequent rate of disease progression to AIDS. The *CCR5* chemokine receptor gene has been associated with susceptibility to HIV-1 infection as it acts as a co-receptor for virus entry into CD4 cells. Individuals who are homozygous for the inactivating delta-32 deletion allele are resistant to infection by R5 strains of the HIV-1 virus [32]. Furthermore, HIV-1 infected patients who are heterozygous for this allele show a delayed disease progression to AIDS [33]. Many other genes have been related with HIV-1 infection and disease progression; these include other chemokine receptor alleles [34] and genes [35] and other genes involved in the modulation of the immune response [36].

In a previous work [26] we showed how the *Bsm*-I polymorphism of the *VDR* gene influenced disease progression rates in a cohort of HIV-1 infected patients belonging to the IVDU risk group. We have now evaluated the role of the *Fok*-I polymorphism of the *VDR* gene in the same cohort and tested for interactions between these two *VDR* polymorphisms.

A less favorable disease progression could be attributed to Ff heterozygotes as reflected by the higher prevalence of this genotype in patients who reached outcome and their faster progression to AIDS and to a fall in CD4 cell count. Homozygosity for the *Bsm*-I *B* allele [26] and heterozygosity for the *Fok*-I polymorphism of the *VDR* locus could be considered risk factors for less favorable progression in HIV-1 disease. When interactions between these two polymorphic sites were tested we found that carriers of non-risk genotypes (non-Ff/non-BB subjects) were protected from progression to AIDS CDC-1993 and from reaching CD4 cell counts of less than 200 μL^{-1}. In addition, the less favorable disease progression observed in BB homozygotes was more evident in individuals carrying the non-Ff genotype. Taken together, these findings provide strong evidence to suggest that genetic variations at the *VDR* locus may affect the rate of progression to HIV-1 infection. The complex network of interactions that seems to operate between these to polymorphisms requires further study.

Recent reports have presented data that suggests a functional difference between *Fok*-I variants. The VDR protein coded by the *F* allele interacted most efficiently with TFIIB and showed greater transcriptional activity than the full-length VDR protein coded by the *f* allele [37]. The growth of phytohaemaglutinin-stimulated PBMC in individuals who were homozygous for the *f* allele was not efficiently inhibited by $1,25(OH)_2D_3$ [38]. The effect of the *f* allele was translated into an increase in ED_{50} and not into the maximal inhibition generated by $1,25(OH)_2D_3$. Thus, the effect of the polymorphism would seem to depend on the $1,25(OH)_2D_3$ status of the subject. Almost normal levels of circulating $1,25\text{-}(OH)_2D_3$ have been reported in HIV-1 infected patients who did not exhibit AIDS related events. These levels declined sharply during disease progression and correlated directly with survival [39,40]. In advanced stages of HIV-1 infection, limiting amounts of $1,25(OH)_2D_3$ would result in abnormal VDR mediated immunomodulation; in carriers of less efficient VDR alleles we could expect a more pronounced dysfunction.

In contrast, there is no definitive evidence to show how *Bsm*-I *VDR* alleles differentially mediate $1,25(OH)_2D_3$ response. Allelic variations in the 3′-UTR of the *VDR* gene may have affected message stability and translation efficiency and could have subsequently led to variations in the responsiveness of cells to $1,25(OH)_2D_3$. Another alternative is that genetic variation in the 3′-UTR region could be a marker for a hitherto unknown locus that is in linkage disequilibrium with the 3′-UTR polymorphisms. The haplotype structure of the *VDR* genomic region shows that the *Bsm*-I polymorphism is included in a 17 kb region with a strong linkage disequilibrium [41]. This region expands from *VDR*



Fok I & Bsm I combined genotypes		n/events	Mean time to outcome in months (95%CI)	Log rank P value
–·–·–	Ff/BB	18/10	121 (96-146)	0.115
------	non-Ff/BB	14/10	93 (74-113)	0.003
– – – –	Ff/non-BB	60/35	120 (105-135)	0.011
———	nonFf/non-BB	93/34	146 (134-158)	

Fok I & Bsm I combined genotypes		n/events	Mean time to outcome in months (95%CI)	Log rank P value
–·–·–	Ff/BB	18/10	123 (99-147)	0.017
------	non-Ff/BB	14/10	107 (84-131)	0.0004
– – – –	Ff/non-BB	60/29	133 (117-148)	0.006
———	nonFf/non-BB	93/26	163 (152-174)	

Fig. 4. Disease progression rates in relation to combined *Fok*-I and *Bsm*-I genotypes in HIV-positive/IVDU patients. Kaplan–Meier analysis is presented for both AIDS CDC-1993 (panel A) and outcomes involving a fall in CD4 cell count below 200 μL^{-1} (panel B). Patient genotypes were, respectively grouped according to the presence of two genotype risk factors conferred by *Fok*-I and *Bsm*-I polymorphisms (BB/Ff), one genotype risk factor due to *Fok*-I (Ff/non-BB) and *Bsm*-I (non-Ff/BB) polymorphisms, and the absence of any genotype risk factors (non-Ff/non-BB). The genotypes considered in the non-Ff/BB group were: FF/BB and ff/BB. The genotypes considered in the non-BB/Ff group were: Ff/Bb and Ff/bb. The genotypes considered in the non-BB/non-Ff group were: FF/Bb, FF/bb, ff/Bb and ff/bb. Kaplan–Meier curves were plotted for each genotype combination for all 185 HIV-positive/IVDU patients,. The mean time to outcome (in months) and the log rank test *P* value for the comparisons between non-BB/non-Ff patients with the other genotype combinations are indicated in the lower box of each panel. AIDS, acquired immunodeficiency syndrome; CDC, centers for disease control and prevention; HIV+, human immunodeficiency virus type-1 seropositive; CI, confidence interval.

intron 3 to the end of *VDR* exon 9 and exhibits linkage equilibrium with both the upstream *VDR* region and with two downstream genes: the predicted *LOC341566* gene and the *HDAC7* gene. Thus, if the disease-causing variations do in fact exist, they should be sought in the 17 kb block marked by the *Bsm*-I polymorphism.

The pleiothropic role of $1,25(OH)_2D_3$ allows us to consider several hypotheses that could explain the described association between *VDR* polymorphisms and disease progression rates in HIV-1 infected patients. $1,25(OH)_2D_3$ exerts its immunomodulatory effects at the level of the T lymphocytes, shifting in cytokine profile from Th1 to Th2 response [1,2]. The cell-mediated immune response against HIV-1 virus infection plays a critical role in the control of this disease. A switch from a Th1 to a Th2 response has been described that parallels with disease progression to AIDS [42,43]. The decision of a naive T cell to differentiate into Th1 or Th2 is crucial, since in a first approximation it determines whether a cell-mediated or humoral immune response is triggered against a particular pathogen, and this profoundly influences disease outcome. On the other hand, chemokine receptor expression could be influenced by changes in the cytokine milieu [44]. This may contribute to the emergence of tropism-specific strains that facilitate HIV transmission and disease progression.

The extent of the genetic contribution in the modulation of immune response is not easy to ascertain, but the individual contribution of each particular gene is likely to be relatively modest. Future studies will focus not only on identifying all of the genes involved, but also on defining the interactions of these genes with each other and with environmental factors. In the light of our data, the *VDR* gene emerges as a non-HLA-linked gene that confers susceptibility to AIDS disease progression. Further studies will be required with additional cohorts and other risk groups in order to clarify the role of *VDR* polymorphisms in HIV-1 pathogenesis and exact also the nature of their interactions.

In summary, *VDR* gene polymorphisms seem to contribute to the rate of HIV-1 disease progression in IVDU seropositive patients. Heterozygosity for the *Fok*-I polymorphism of the *VDR locus* could be considered a risk factor for less favorable progression in HIV-1 disease. In addition, combined non-risk genotypes for *Fok*-I and *Bsm*-I polymorphisms seem to provide major protection against progression to AIDS.

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References

[1] J.M. Lemire, Immunomodulatory actions of 1,25-dihydroxyvitamin D3, J. Steroid. Biochem. Mol. Biol. 53 (1–6) (1995) 599–602.
[2] J.M. Lemire, D.C. Archer, L. Beck, H.L. Spiegelberg, Immunosuppressive actions of 1,25-dihydroxyvitamin D3: preferential inhibition of Th1 functions, J. Nutr. 125 (6 Suppl) (1995) 1704S–1708S.
[3] W.F. Rigby, S. Denome, M.W. Fanger, Regulation of lymphokine production and human T lymphocyte activation by 1,25-dihydroxyvitamin D3. Specific inhibition at the level of messenger RNA, J. Clin. Invest. 79 (6) (1987) 1659–1664.
[4] B. Hodler, V. Evequoz, U. Trechsel, H. Fleisch, B. Stadler, Influence of Vitamin D3 metabolites on the production of interleukins 1,2 and 3, Immunobiology 170 (4) (1985) 256–269.
[5] M. Willheim, R. Thien, K. Schrattbauer, E. Bajna, M. Holub, R. Gruber, K. Baier, P. Pietschmann, W. Reinisch, O. Scheiner, M. Peterlik, Regulatory effects of 1alpha,25-dihydroxyvitamin D3 on the cytokine production of human peripheral blood lymphocytes, J. Clin. Endocrinol. Metab. 84 (10) (1999) 3739–3744.
[6] C.M. Veldman, M.T. Cantorna, H.F. DeLuca, Expression of 1,25-dihydroxyvitamin D(3) receptor in the immune system, Arch. Biochem. Biophys. 374 (2) (2000) 334–338.
[7] S.A. Ingles, R.K. Ross, M.C. Yu, R.A. Irvine, P.G. La, R.W. Haile, G.A. Coetzee, Association of prostate cancer risk with genetic polymorphisms in Vitamin D receptor and androgen receptor, J. Natl. Cancer Inst. 89 (2) (1997) 166–170.
[8] N.A. Morrison, J.C. Qi, A. Tokita, P.J. Kelly, L. Crofts, T.V. Nguyen, P.N. Sambrook, J.A. Eisman, Prediction of bone density from Vitamin D receptor alleles, Nature 367 (6460) (1994) 284–287.
[9] A. Tokita, H. Matsumoto, N.A. Morrison, T. Tawa, Y. Miura, K. Fukamauchi, N. Mitsuhashi, M. Irimoto, S. Yamamori, M. Miura, T. Watanabe, Y. Kuwabara, K. Yabuta, J.A. Eisman, Vitamin D receptor alleles, bone mineral density and turnover in premenopausal Japanese women, J. Bone Miner. Res. 11 (7) (1996) 1003–1009.
[10] S. Ferrari, R. Rizzoli, T. Chevalley, D. Slosman, J.A. Eisman, J.P. Bonjour, Vitamin D receptor gene polymorphisms and change in lumbar-spine bone mineral density, Lancet 345 (8947) (1995) 423–424.
[11] T. Carling, A. Kindmark, P. Hellman, E. Lundgren, S. Ljunghall, J. Rastad, G. Akerstrom, H. Melhus, Vitamin D receptor genotypes in primary hyperparathyroidism, Nat. Med. 1 (12) (1995) 1309–1311.
[12] A.C. Lundin, P. Soderkvist, B. Eriksson, M. Bergman-Jungestrom, S. Wingren, Association of breast cancer progression with a Vitamin D receptor gene polymorphism. South-East Sweden Breast Cancer Group (in process citation), Cancer Res. 59 (10) (1999) 2332–2334.
[13] L. Correa-Cerro, P. Berthon, J. Haussler, S. Bochum, E. Drelon, P. Mangin, G. Fournier, T. Paiss, O. Cussenot, W. Vogel, Vitamin D receptor polymorphisms as markers in prostate cancer, Hum. Genet. 105 (3) (1999) 281–287.
[14] A. Vogel, C.P. Strassburg, M.P. Manns, Genetic association of Vitamin D receptor polymorphisms with primary biliary cirrhosis and autoimmune hepatitis, Hepatology 35 (1) (2002) 126–131.
[15] Y. Ban, M. Taniyama, Vitamin D receptor gene polymorphism is associated with Graves' disease in the Japanese population, J. Clin. Endocrinol. Metab. 85 (12) (2000) 4639–4643.
[16] T. Fukazawa, I. Yabe, S. Kikuchi, H. Sasaki, T. Hamada, K. Miyasaka, K. Tashiro, Association of Vitamin D receptor gene polymorphism with multiple sclerosis in Japanese, J. Neurol. Sci. 166 (1) (1999) 47–52.
[17] M.A. Pani, M. Knapp, H. Donner, J. Braun, M.P. Baur, K.H. Usadel, K. Badenhoop, Vitamin D receptor allele combinations influence genetic susceptibility to type 1 diabetes in Germans, Diabetes 49 (3) (2000) 504–507.
[18] R. Bellamy, C. Ruwende, T. Corrah, K.P. McAdam, M. Thursz, H.C. Whittle, A.V. Hill, Tuberculosis and chronic hepatitis B virus infection in Africans and variation in the Vitamin D receptor gene, J. Infect. Dis. 179 (3) (1999) 721–724.
[19] S. Roy, A. Frodsham, B. Saha, S.K. Hazra, C.G. Mascie-Taylor, A.V. Hill, Association of Vitamin D receptor genotype with leprosy type, J. Infect. Dis. 179 (1) (1999) 187–191.
[20] C. Gross, T.R. Eccleshall, P.J. Malloy, M.L. Villa, R. Marcus, D. Feldman, The presence of a polymorphism at the translation initiation site of the Vitamin D receptor gene is associated with low bone mineral density in postmenopausal Mexican–American women, J. Bone Miner. Res. 11 (12) (1996) 1850–1855.
[21] A.P. Chokkalingam, K.A. McGlynn, Y.T. Gao, M. Pollak, J. Deng, I.A. Sesterhenn, F.K. Mostofi, J.F. Fraumeni, A.W. Hsing, Vitamin D receptor gene polymorphisms, insulin-like growth factors, and prostate cancer risk: a population-based case-control study in China, Cancer Res. 61 (11) (2001) 4333–4336.
[22] Y. Ban, M. Taniyama, T. Katagiri, Vitamin D receptor initiation codon polymorphism in Japanese patients with Graves' disease, Thyroid 10 (5) (2000) 375–380.
[23] S. Ferrari, R. Rizzoli, D. Manen, D. Slosman, J.P. Bonjour, Vitamin D receptor gene start codon polymorphisms (*Fok*-I) and bone mineral density: interaction with age, dietary calcium, and 3′-end region polymorphisms, J. Bone Miner. Res. 13 (6) (1998) 925–930.
[24] S.A. Ingles, D.G. Garcia, W. Wang, A. Nieters, B.E. Henderson, L.N. Kolonel, R.W. Haile, G.A. Coetzee, Vitamin D receptor genotype and breast cancer in Latinas (United States), Cancer Causes Control 11 (1) (2000) 25–30.
[25] D. Bretherton-Watt, R. Given-Wilson, J.L. Mansi, V. Thomas, N. Carter, K.W. Colston, Vitamin D receptor gene polymorphisms are associated with breast cancer risk in a UK Caucasian population, Br. J. Cancer 85 (2) (2001) 171–175.
[26] Y. Barber, C. Rubio, E. Fernández, M. Rubio, J. Fibla, Host genetic background at CCR5 and VDR loci and human immunodeficiency virus (HIV)-1 disease progression among HIV-1 seropositive intravenous drug users, J. Infect. Dis. 184 (10) (2001) 1279–1288.
[27] Centers For Disease Control, 1993 Revised classification system for HIV infectioan and expanded surveillance case definition for AIDS among adolescents and adults, Morb. Mort. Wkly. Rep. 41 (51) (1993) 961–962.
[28] J. Schinkel, M.W. Langendam, R.A. Coutinho, A. Krol, M. Brouwer, H. Schuitemaker, No evidence for an effect of the CCR5 delta32/+ and CCR2b 64I/+ mutations on human immunodeficiency virus (HIV)-1 disease progression among HIV-1-infected injecting drug users, J. Infect. Dis. 179 (4) (1999) 825–831.
[29] M.P. Marco, L. Craver, J. Fibla, E. Fernandez, Influence of Vitamin D receptor gene polymorphism on mortality risk in hemodialysis patients, Am. J. Kidney Dis. 38 (5) (2001) 965–974.
[30] M. Roger, Influence of host genes on HIV-1 disease progression, FASEB J. 12 (9) (1998) 625–632.
[31] C. Kaplan, J.Y. Muller, C. Doinel, J.J. Lefrere, F. Paquez, P. Rouger, D. Salmon, C. Salmon, HLA-associated susceptibility to acquired immune deficiency syndrome in HIV-1-seropositive subjects, Hum. Hered. 40 (5) (1990) 290–298.
[32] M. Samson, F. Libert, B.J. Doranz, J. Rucker, C. Liesnard, C.M. Farber, S. Saragosti, C. Lapoumeroulie, J. Cognaux, C. Forceille, G. Muyldermans, C. Verhofstede, G. Burtonboy, M. Georges, T. Imai, S. Rana, Y. Yi, R.J. Smyth, R.G. Collman, R.W. Doms, G. Vassart, M. Parmentier, Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR5 chemokine receptor gene, Nature 382 (6593) (1996) 722–725.
[33] Y. Huang, W.A. Paxton, S.M. Wolinsky, A.U. Neumann, L. Zhang, T. He, S. Kang, D. Ceradini, Z. Jin, K. Yazdanbakhsh, K. Kunstman,

D. Erickson, E. Dragon, N.R. Landau, J. Phair, D.D. Ho, R.A. Koup, The role of a mutant CCR5 allele in HIV-1 transmission and disease progression, Nat. Med. 2 (11) (1996) 1240–1243.

[34] C. Quillent, E. Oberlin, J. Braun, D. Rousset, G. Gonzalez-Canali, P. Metais, L. Montagnier, J.L. Virelizier, F. Arenzana-Seisdedos, A. Beretta, HIV-1-resistance phenotype conferred by combination of two separate inherited mutations of CCR5 gene, Lancet 351 (9095) (1997) 14–18.

[35] M.W. Smith, M. Dean, M. Carrington, C. Winkler, G.A. Huttley, D.A. Lomb, J.J. Goedert, T.R. O'Brien, L.P. Jacobson, R. Kaslow, S. Buchbinder, E. Vittinghoff, D. Vlahov, K. Hoots, M.W. Hilgartner, S.J. O'Brien, Contrasting genetic influence of CCR2 and CCR5 variants on HIV-1 infection and disease progression. Hemophilia growth and development study (HGDS), multicenter AIDS cohort study (MACS), multicenter hemophilia cohort study (MHCS), San Francisco City Cohort (SFCC), ALIVE study, Science 277 (5328) (1997) 959–965.

[36] V.I. Konenkov, M.V. Smolnikova, Polymorphism of promotor sites of interleukins-4 and -10 and tumor necrosis factor-alpha genes in HIV-infected patients, Bull Exp. Biol. Med. 133 (4) (2002) 389–391.

[37] P.W. Jurutka, L.S. Remus, G.K. Whitfield, P.D. Thompson, J.C. Hsieh, H. Zitzer, P. Tavakkoli, M.A. Galligan, H.T. Dang, C.A. Haussler, M.R. Haussler, The polymorphic N terminus in human Vitamin D receptor isoforms influences transcriptional activity by modulating interaction with transcription factor IIB, Mol. Endocrinol. 14 (3) (2000) 401–420.

[38] E.M. Colin, A.E. Weel, A.G. Uitterlinden, C.J. Buurman, J.C. Birkenhager, H.A. Pols, J.P. van Leeuwen, Consequences of Vitamin D receptor gene polymorphisms for growth inhibition of cultured human peripheral blood mononuclear cells by 1, 25-dihydroxyvitamin D3, Clin. Endocrinol. 52 (2) (2000) 211–216.

[39] C. Haug, F. Muller, P. Aukrust, S.S. Froland, Subnormal serum concentration of 1,25-Vitamin D in human immunodeficiency virus infection: correlation with degree of immune deficiency and survival, J. Infect. Dis. 169 (4) (1994) 889–893.

[40] C.J. Haug, P. Aukrust, E. Haug, L. Morkrid, F. Muller, S.S. Froland, Severe deficiency of 1,25-dihydroxyvitamin D3 in human immunodeficiency virus infection: association with immunological hyperactivity and only minor changes in calcium homeostasis, J. Clin. Endocrinol. Metab. 83 (11) (1998) 3832–3838.

[41] A. Velasco, J. Fibla, Haplotype structure of the genomic region surrounding Vitamin D receptor gene, in: Proceedings of the 12th Workshop on Vitamin D, Maastricht, 2003 (abstract 177).

[42] S.A. Klein, J.M. Dobmeyer, T.S. Dobmeyer, M. Pape, O.G. Ottmann, E.B. Helm, D. Hoelzer, R. Rossol, Demonstration of the Th1 to Th2 cytokine shift during the course of HIV-1 infection using cytoplasmic cytokine detection on single cell level by flow cytometry, AIDS 11 (9) (1997) 1111–1118.

[43] M. Clerici, G.M. Shearer, A TH1 → TH2 switch is a critical step in the etiology of HIV infection, Immunol. Today 14 (3) (1993) 107–111.

[44] B.K. Patterson, M. Czerniewski, J. Andersson, Y. Sullivan, F. Su, D. Jiyamapa, Z. Burki, A. Landay, Regulation of CCR5 and CXCR4 expression by type 1 and type 2 cytokines: CCR5 expression is downregulated by IL-10 in CD4-positive lymphocytes, Clin. Immunol. 91 (3) (1999) 254–262.