Association between AIDS disease progression rates and the \textit{Fok-I} polymorphism of the VDR gene in a cohort of HIV-1 seropositive patients

Gema Nieto\textsuperscript{a}, Yolanda Barber\textsuperscript{a}, Maria Carmen Rubio\textsuperscript{b}, Manuel Rubio\textsuperscript{b}, Joan Fibla\textsuperscript{a,\ast}

\textsuperscript{a} Unidad de Genética Humana del Departamento de Ciències Mèdiques Bàsiques de la Facultat de Medicina de la Universitat de Lleida, C/ Montserrat Reig 2, 25199 Lleida, Catalonia, Spain
\textsuperscript{b} Servei de Medicina Interna de l’Hospital Universitari Arnau de Vilanova, Av. Reina Reixa 80, 25199 Lleida, Catalunya, Spain

Abstract

In addition to its role in mineral metabolism, 1,25-dihydroxyvitamin D\textsubscript{3} (1,25(OH)\textsubscript{2}D\textsubscript{3}) also has immunomodulatory effects. Vitamin D receptor (VDR) mediates genomic actions of 1,25(OH)\textsubscript{2}D\textsubscript{3}, by acting as a transcription factor that modulates the expression of several 1,25(OH)\textsubscript{2}D\textsubscript{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 \textit{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 \textmu L\textsuperscript{-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 \textit{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, \textit{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 \textmu L\textsuperscript{-1} (52\% of progressors versus 36\% of non-progressors, \textit{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 \textit{P} = 0.035; Cox hazard ratio (HR) for FF versus non-FF = 1.53 (95\% CI: 1.0–2.33), \textit{P} = 0.047). In addition the drop in CD4 cell count to below 200 \textmu L\textsuperscript{-1} was reached faster in Ff carriers than in non-Ff patients (log rank test \textit{P} = 0.015; HR for FF versus non-FF = 1.77 (95\% CI: 1.12–2.8), \textit{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.

© 2004 Elsevier Ltd. All rights reserved.

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\textsubscript{3} (1,25(OH)\textsubscript{2}D\textsubscript{3}), is also involved in the regulation of cell-mediated immunity [1]. 1,25(OH)\textsubscript{2}D\textsubscript{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(OH)\textsubscript{2}D\textsubscript{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)\textsubscript{2}D\textsubscript{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 \textit{\textsuperscript{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

\textsuperscript{\ast} Corresponding author. Tel.: +34-973-702441; fax: +34-973-702426. E-mail addresses: joan.fibla@cnb.ub.edu (J. Fibla).
described. Three SNP polymorphisms that modify endonuclease restriction sites of Bsm-I, Apg-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 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 serorevalent 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 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...
(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/\mu L 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 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/\mu L 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/Bb), one genotype risk factor associated with Fok-I (Ff/Bb) and Bsm-I (non-Bb/bb) polymorphisms, and the absence of any genotype risk factors (non-Ff/non-Bb). The genotypes considered in the non-FfBB group were: FFBB and FfBB. 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/\mu L (panel B) was lower than in those who remained outcome-free. The percentage of patients who only exhibited risk factors attributable to Fok-I polymorphism (non-FfBB) 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.)
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/L (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.

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/L as survival endpoints (Fig. 3). Hazard ratios for progression to AIDS or to a fall in CD4 cell count below 200/L 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/L. 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 (logrank 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).
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 and to a fall in CD4 cell count. Hemozygosity for the Fok-I allele [32] and heterozygosity for the Fok-I polymorphism of the VDR gene 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 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)₂ D₃ [38]. The effect of the f allele was translated into an increase in ED₅₀ and not into the maximal inhibition generated by 1,25(OH)₂ D₃. Thus, the effect of the polymorphism would seem to depend on the 1,25(OH)₂ D₃ status of the subject. Almost normal levels of circulating 1,25-(OH)₂ D₃ 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)₂ D₃ 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 Rom-1 VDR alleles differentially mediate 1,25(OH)₂ D₃ 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)₂ D₃. 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 Rom-1 polymorphism is included in a 17 kb region with a strong linkage disequilibrium [41]. This region expands from VDR

---

**Table 1**

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Outcomes considered</th>
<th>AIDS CDC-1993</th>
<th>Ff/Bb</th>
<th>p value</th>
<th>HR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fok-I genotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;200/CD4µL</td>
<td></td>
</tr>
<tr>
<td>Ff</td>
<td>1.53 (1.2–2.3)</td>
<td>0.047</td>
<td>1.77 (1.1–2.8)</td>
<td>0.014</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Ff(*)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Fok-I and Rom-I combined genotypes
| Ff/FB                | 1.7 (1.0–2.6)       | 0.114        | 2.5 (1.2–5.2) | 0.014 |
| Non-Ff/FF(*)         | 3.0 (1.4–6.0)       | 0.003        | 3.7 (1.8–7.8) | <0.001 |
| Ff/non-BB(*)         | 1.8 (1.1–2.9)       | 0.014        | 2.2 (1.3–3.4) | 0.005 |

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.

* Genotypes used as references for comparisons.

1 Non-Ff genotypes were: FF and ff.

2 Genotypes were grouped according to the presence of two genotype risk factors conferred by Fok-I and Rom-I polymorphisms (FF/ff) one genotype risk factor due to Fok-I (Ff/FF or Ff/ff) and Rom-I (non-Ff/non-BB) polymorphisms and the absence of any genotype risk factor (non-Ff/non-BB).

3 Genotypes considered in this group were: FF/BB and ff/BB.

4 Genotypes considered in this group were: Ff/BB and Ff/ff.

5 Genotypes considered in this group were: Ff/Bb, Ff/bb, FF/BB and ff/BB.

6 Genotypes considered in this group were: Ff/Bb, Ff/bb, FF/BB and ff/BB.
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/L−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-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 pleiotropic role of 1,25(OH)2D3 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)2D3 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 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.

Acknowledgements

We are indebted to all the patients who participated in this study. This work was supported by grants from
FLSss-P021476 and the “Fundación Marató TV3” to JF. GN received a scholarship from the Spanish Ministry of Science and Technology. This study was performed within the framework of the FLSss’s “Red Temática Cooperativa de Investigación en SIDA (Red de grupos 173).”

References


