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Vitamin-D pathway genes and HIV-1 disease progression in injection drug users ☆

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ABSTRACT

Vitamin-D has pleiotropic effects on calcium and bone metabolism, cellular growth control, cell differentiation and modulation of both innate and acquired immune response. Previous studies revealed the association of *vitamin-D receptor* gene (VDR) polymorphism with infection diseases including HIV-1 infection. To assess for association between polymorphisms of vitamin-D pathway genes *CYP27B1*, vitamin-D binding protein (*VDBP*) and *VDR* with HIV-1 infection, disease progression to acquired immunodeficiency syndrome (AIDS) was analysed according to CDC93 criteria in a cohort of 185 HIV-1 seroprevalent patients belonging to the injection drug users. Genotype data was obtained from rs10877012, rs3782130 and rs4646536 markers at *CYP27B1 locus*; rs7041 and rs4588 at *VDBP locus*; and rs11568820, rs4516035, rs2228570, rs1544410 and rs17878969 at *VDR locus*. Distribution of genotypes between patients grouped by outcome was compared by contingency table analysis. Marker–marker interaction was assessed by a MDR analysis. Assuming an additive model for *VDR markers*, a Kaplan–Meier survival analysis was employed to evaluate association with disease progression. Among vitamin-D pathway genes, *VDR locus* reveals specific 5′UTR and 3′UTR diplotype combinations associated with both, slower and faster progression to AIDS. Marker–marker interaction analysis indicates a strong interaction between *VDR* markers and a redundant effect for *CYP27B1* markers. According to our results, *VDR locus* association follows an additive model in which increased genetic risk score for the *VDR* is directly correlated with AIDS progression rates. Our data supports a role of vitamin-D pathway gene variability on HIV-1 disease progression.

1. Introduction

In addition to its role on mineral metabolism, 1α,25-dihydroxyvitamin-D_3 (henceforth vitamin-D) has pleiotropic effects on cellular growth control, cell differentiation and modulation of the immune response. A growing body of experimental evidence has been obtained in the last decade that supports a key role of vitamin-D in the control of both innate and acquired immune responses (Adams, 2006; Hewison, 1992, 2008; White, 2008). Following the activation of vitamin-D precursors in the skin by sunlight exposure and their biochemical transformation in the liver, vitamin-D acquires its full active form after being converted to vitamin-D by the kidney enzyme 25-hydroxyvitamin-D_3 1-α-hydroxylase (CYP27B1) (Lips, 2006). Under normal physiological conditions, nearly all circulating vitamin-D compounds are bound to the vitamin-D-binding protein (VDBP) that transports vitamin-D metabolites to the target tissues (Daiger et al., 1975). At the molecular level vitamin-D excerpt its action by interacting with the nuclear vitamin-D receptor (VDR), that acts as a transcription factor activating or repressing specific genes (Pike, 1991).

Vitamin-D pathway genes have been largely evaluated in disease by their implication in the immune response regulation. *CYP27B1* and *VDR* are expressed in several immune cells, such as macrophages, dendritic cells (DCs) and lymphocytes. Identification of extra-renal sites for *CYP27B1* and *VDR* expression has led to hypothesize that local production of vitamin-D could play an important autocrine or paracrine role

Abbreviations: SNP, single nucleotide polymorphisms; Vitamin-D, 1α,25-dihydroxyvitamin-D_3; *VDBP*, vitamin-D binding protein; VDR, *vitamin-D receptor*; *CYP27B1*, 25-hydroxyvitamin-D_3 1-α-hydroxylase; GcMAF, Macrophage Activating Factor; DCs, dendritic cells; UTR, untranslated region; AIDS, acquired immunodeficiency syndrome; HIV-1, human immunodeficiency virus Type 1; CDC, Center for Disease Control; CVC, cross-validation consistency; MDR, multifactor dimensionality reduction.

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in the differentiation and function of these cells (Bouillon et al., 1995a, 1995b; Haussler et al., 1998; Walters, 1992). Macrophages and DCs, as they differentiate towards a mature antigen-presenting phenotype, show simultaneously an increase on *CYP27B1* expression and a decrease on *VDR* expression (Hewison et al., 2003). As a consequence, immature DCs are able to respond to vitamin-D, suppressing their differentiation by a negative feedback control (Hewison et al., 2004). Moreover, in addition to its role on vitamin-D transport, vitamin-D-binding protein derived Macrophage Activating Factor (GcMAF), is also involved in the immune response acting as a chemotactic factor in the recruitment of neutrophil leucocytes (Kew and Webster, 1988).

A potential role of vitamin-D on human immunodeficiency virus Type 1 (HIV-1) infection has been previously considered (Villamor, 2006; Vincek, 1995) and comprehensively reviewed in Fibla and Caruz (2010). Two processes are crucial against HIV-1 infection in which vitamin-D plays opposite effects, the synthesis of antimicrobial peptides and the role of T-helper mediated response. On one hand the hormone promotes the synthesis of peptides that exert antimicrobial effect to the virus entry (Quiñones-Mateu et al., 2003), whereas on the other hand, induces the polarization of the immune response towards a less effective T-helper response against viral infections. Noteworthy, vitamin-D is known to alter both, Th1/Th2 and Th17/Treg balances, to Th2 and Treg responses, that in the context of viral infections should be considered detrimental (Bruce et al., 2010).

Almost normal levels of circulating vitamin-D have been described in HIV-1 infected patients without acquired immunodeficiency syndrome (AIDS) events. In contrast these levels were strongly reduced during disease progression and directly correlated with survival (Haug et al., 1994). Although vitamin-D deficiency in AIDS progression has not been related to 1α-hydroxylase dysfunction, it has been described that protease inhibitors used in the treatment of HIV-1 infected patients interfere with vitamin-D metabolism by inhibiting 25-hydroxylase, 24-hydroxylase and 1α-hydroxylase activities (Cozzolino et al., 2003).

Association of *VDR* gene polymorphisms have been reported with disease progression rates in patients infected by HIV-1 (Barber et al., 2001; Moodley et al., 2013; Nieto et al., 2004). Several studies revealed the association between *CYP27B1* polymorphisms and immune related diseases, such as Hashimoto's thyroiditis, Graves' disease and type 1/2 diabetes (Lopez et al., 2004) and Addison's disease (Jennings et al., 2005). *VDBP* polymorphisms have been associated with HIV-1 infection (Eales et al., 1987) but these findings have not been replicated (Alonso et al., 1990; Cleve et al., 1988). In addition, *VDBP* variants have been associated with susceptibility to tuberculosis infection (Hewison, 2008; Martineau et al., 2010; White, 2008) and asthma (Li et al., 2011; Lips, 2006) and VDBP has been proposed as a multiple sclerosis biomarker (Disanto et al., 2010).

In the present study we have evaluated vitamin-D pathway genes *CYP27B1*, *VDBP* and *VDR* as candidate genes involved in HIV-1 disease progression.

2. Materials and methods

2.1. Study participants

The main characteristics of the HIV-1 infected cohort have been previously described (Barber et al., 2001; Nieto et al., 2004). Briefly, the Lleida AIDS Cohort is a prospective seroprevalent cohort of HIV-1 infected patients belonging to the intravenous drug users risk group drawn from all HIV-1 seropositive adults enrolled in the AIDS Service of the University Hospital Arnau de Vilanova. Only Caucasian patients recruited between 1982 and 1991 were included in the cohort. All patients selected were in follow-up for more than 7 years (median, 127.7 months; range 84–198 months). Progression criteria have been established according to the 1993 Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS Among Adolescents and Adults of the Center for Disease Control classification (CDC93) that considers both clinical and immunological parameters (Anonymous, 1993). In addition to AIDS-defining illness considered in the 1983-CDC AIDS-defining criteria, the 1993-CDC AIDS-defining criteria expanded the AIDS surveillance case definition to include all HIV-infected people with CD4+ T-lymphocyte counts of less than 200 cells/μL. Epidemiological and clinical characteristics of the HIV-1 seropositive patients have been presented in Barber et al. (2001), Nieto et al. (2004) and Laplana et al. (2013) and are summarized according to CDC93 progression status in Supplementary Table 1. The observation period for progression status ended in December 1999. All participants gave written informed consent. The Ethics committee from our institution approved the study.

2.2. DNA sources and genotyping

Genomic DNA was extracted from peripheral blood lymphocytes using a KG-Midi extraction kit (Camgen, Cambridge, UK). Selected single nucleotide polymorphisms (SNP) were; rs4646536, rs3782130 and rs10877012 from the *CYP27B1* gene; rs7041 (*Glu416Asp*) and rs4588 (*Thr420Lys*) from *VDBP* gene and rs17878969 (*PolyA*), rs1544410 (*Bsm-I*), rs2228570 (*Fok-I*), rs4516035 (*A1012G*) and rs11568820 (*Cdx*) from the *VDR* gene. Polymerase Chain Reaction (PCR) protocols were developed to genotype the 10 SNP, the main characteristics of the genotyping methods are described in Supplementary Table 2. For each SNP, assay validation was performed using representative individuals whose genotypes were previously determined by sequencing.

2.3. Statistical analysis

We examined the relationship between single SNP at *CYP27B1*, *VDBP and VDR loci* with AIDS disease status by conducting a cohort-based study. Hardy–Weinberg equilibrium was tested comparing expected and observed genotype frequencies by Chi-square test. We used Haploview 4.2 software (Barrett et al., 2005) to estimate linkage disequilibrium among the analysed markers and pLink software (Purcell et al., 2007) to infer haplotype and diplotype frequencies from our sample data.

Allele, genotype, haplotype and diplotype frequencies were compared among patients categorized as progressors and non-progressors according to the CDC93 that considers both clinical and immunological parameters. Single-marker association P-values were corrected for multiple testing following the SNP spectral decomposition approach, a modified Bonferroni-corrected nominal threshold of $P = 0.05/N$, where N is the "effective number of independent marker *loci*" after consideration of linkage disequilibrium between markers. N was calculated using the web-based program SNPSpD (http://gump.qimr.edu.au/general/daleN/SNPSpD/). Following this, the experiment-wide significance threshold required to keep Type I error rate at 5% is $P < 0.0056$. Differences among genotypes in median values for numerical variables were tested by non-parametric Kruskal–Wallis test.

In addition, we studied HIV-1 disease progression rates by a Kaplan–Meier survival analysis in patients stratified according to genotype/diplotype groups. Differences between groups were tested by Log-Rank test. Hazard ratios were estimated using a Cox proportional hazard model (HRu) and adjusting for sex, age at first HIV positive test and *CCR5Δ32* genotype (as previously reported for this cohort in Barber et al. (2001) (HRa). Survival time ranges from date of the first HIV-1 positive test to outcome or censoring date (last clinic examination date or date of death if not caused by HIV-1 infection). Five patients died after reaching the outcome and 1 patient died before reaching outcome by heroine overdose. This patient was assumed as outcome free as remained more than seven years with CD4 > 200/μL and free of antiretroviral therapy. A $P = 0.05$ was considered as statistically significant. Statistical analyses were performed by the SPSS 20.0 package.