Brief Report: CYP27B1 rs10877012 T Allele Was Linked to Non-AIDS Progression in ART-Naïve HIV-Infected Patients: A Retrospective Study

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Abstract

Background: HIV/AIDS progression is linked to vitamin D, which is regulated by several key cytochromes P450 (CYP). Single nucleotide polymorphisms (SNPs) in *CYP* genes influence vitamin D metabolism and serum levels. The objective of this study was to evaluate the association between *CYP* SNPs and the clinical AIDS progression in antiretroviral treatment (ART)-naïve HIV-infected patients.

Methods: We performed a retrospective study in 661 ART-naïve HIV-infected patients who were stratified by their AIDS progression pattern (181 long-term non-progressors (LTNPs), 332 moderate progressors (MPs), and 148 rapid progressors (RPs)). Four *CYP* SNPs (*CYP2R1* rs10500804, *CYP2R1* rs1993116, *CYP27B1* rs10877012, and *CYP24A1* rs6013897) were genotyped using Agena Bioscience's MassARRAY platform. Correction for multiple testing was performed using the false discovery rate (FDR; Benjamini-Hochberg procedure).

Results: The adjusted regression showed a significant association only for *CYP27B1* rs10877012 SNP. When analyzing all HIV patients, the rs10877012 T allele was protective against AIDS progression (ordinal outcome) under the dominant (adjusted OR (aOR)=0.69; p=0.021) and additive (aOR)=0.75; p=0.025) inheritance models. When analyzing LTNPs versus RPs, the rs10877012 T allele also showed a significant protective association under the dominant (aOR=0.45; p=0.004) and additive (aOR=0.54; p=0.008) inheritance models. P-values remained significant after correcting by multiple comparisons only for the comparison of LTNPs versus RPs (extreme phenotypes).
Conclusions: The *CYP27B1* rs10877012 T allele was linked to non-AIDS progression in ART-naïve HIV-infected patients. The rs10877012 SNP seems to have an impact on the clinical AIDS progression, possibly modifying vitamin D levels, which could be relevant for the pathogenesis of HIV infection.

Keywords

Single nucleotide polymorphisms; cytochrome P450; LTNPs; AIDS; non-progression

Background

Vitamin D (VitD) is a hormone that mainly regulates calcium homeostasis, as well as other functions of the organism, such as the immune response [1]. VitD activates genes and antimicrobial pathways in the host that enhance the immunity [1-3]. Besides, VitD deficiency is linked to skeletal and non-skeletal diseases, among which are the infectious diseases [4] like such as influenza, sepsis, tuberculosis, fungal infections, and HIV infection [5]. In fact, VitD deficiency in human immunodeficiency virus (HIV)-infected patients is very prevalent (detected in around 70-85% of the cases) [5, 6] and is related to an increased level of viral load, inflammation, and immune activation, as well as to decreased levels of CD4+ T-cells and progression to acquired immunodeficiency syndrome (AIDS). Conversely, increased levels of VitD provide natural resistance to HIV infection [1].

The first genome-wide association study (GWAS) in untreated HIV-infected patients found two single nucleotide polymorphisms (SNPs) located at human leukocyte antigen (HLA) -B

and -C associated with set-point viral load (spVL) [7, 8], which correlate with the rate of AIDS [9]. Next, a large number of GWAS have been carried out in which susceptibility to HIV infection, spVL, AIDS progression, and rate of CD4 T-cell decline is mainly analyzed [10]. In all these studies, associations with a large number of SNPs have been found, which are mainly located at *HLA* and *C-C motif chemokine receptor 5* [10].

The main circulating VitD metabolite is 25-hydroxycholecalciferol (25(OH)D). The active metabolite of VitD in several tissues, including immune system cells, is 1, 25-dihydroxycholecalciferol ($1,25(OH)_2D$), which binds to the vitamin D receptor (VDR) on target cells and promotes gene transcription [1]. VitD metabolism is regulated at the level of several key cytochromes P450 (CYP), such as VitD 25-hydroxylase (CYP2R1), responsible for converting vitamin D to 25(OH)D; 25-hydroxyvitamin D-1-alpha-hydroxylase (CYP27B1), which activates 25(OH)D to $1,25(OH)_2D$; and 25-hydroxyvitamin D-24-hydroxylase (CYP24A1), which inactivates 25(OH)D and $1,25(OH)_2D$ [1]. The *CYP* genes have high variability, and single nucleotide polymorphisms (SNPs) within them are involved in variations in the levels of 25(OH)D and $1,25(OH)_2D$ [11]. *CYP* SNPs are related to tenofovir concentration and kidney-associated urinary and serum abnormalities in antiretroviral treatment (ART)-treated HIV-infected patients [12]. However, there is no information about the influence of *CYP* SNPs on AIDS progression in people living with HIV (PLWH) withot ART.

ART-naïve PLWH have a high AIDS progression variability [13, 14], which is linked to a complex interaction among genetic background, immune system, and viral characteristics, among other factors [15, 16].

Objective

We aimed to evaluate the association among genetic variants in three *CYP* genes (*CYP2R1*, *CYP27B1*, and *CYP24A1*) and the clinical AIDS progression in ART-naïve PLWH from two big Spanish cohorts.

Methods

Design and Patients

A retrospective study was carried out in 661 ART-naïve HIV-infected patients, who were included in the Cohort of LTNPs (**Appendix 1**) and the Cohort of the Spanish AIDS Research Network (CoRIS, **Appendix 2**). The LTNP Cohort was formed from the CoRIS Cohort data, which has been previously described [17].

PLWH were classified in three groups according to their clinical AIDS progression [18]: a) long-term non-progressors (LTNPs): 181 asymptomatic patients over ten years after HIV seroconversion, CD4+ ≥500 cells/mm^3, and RNA-HIV load ≤10,000 copies/ml; b) moderate progressors (MPs): 332 patients with a moderate decrease in CD4+ T cells (50-100 CD4+/mm^3 per year) for at least two years after HIV diagnosis; c) rapid progressors (RPs): 148 patients with rapid immunological and clinical progression within three years following HIV seroconversion, two or more counts of CD4+ T-cell ≤350 CD4+/mm^3 or an AIDS-related event (including death). The three PLWH groups did not involve ART. Data and samples were collected retrospectively and correspond to a time when ART criteria were more conservative, and the fall of CD4 + to ≤350 CD4+/mm^3 was expected before starting ART. Subsequently, patients who progressed were treated according to relevant clinical guidelines. Besides, 111 healthy blood donors were used as a Control-group, who were negative for HIV, hepatitis B virus, and hepatitis C virus antibodies. This study was carried out on individuals of European origin, as we previously selected subjects belonging to the European mtDNA haplogroup N [18].

The Institutional Review Boards of each center approved the study protocol. Besides, the study was approved by the Research Ethics Committee of the Instituto de Salud Carlos III (CEI PI_2010-v3). The study was conducted in accordance with the Declaration of Helsinki Ethical Principles and Good Clinical Practices.

Samples

Blood samples collected by venous puncture were sent to the Spanish HIV HGM Biobank integrated into the Spanish AIDS Research Network (RIS). Samples were processed following current procedures and frozen immediately after their reception. All patients participating in the study gave their informed consent, and protocols were approved by institutional ethical committees.

DNA Genotyping

We selected four SNPs arbitrarily (*CYP2R1* rs10500804, *CYP2R1* rs1993116, *CYP27B1* rs10877012, and *CYP24A1* rs6013897 polymorphisms), which have been previously related to circulating VitD levels and non-skeletal diseases [11]. Total DNA was isolated from whole blood with Wizard® SV Genomic DNA Purification System (Promega, Madison, WI, USA). DNA genotyping was performed by the Agena Bioscience's MassARRAY platform (San Diego, CA, USA) using the iPLEX® Gold assay design system, at the Spanish National Genotyping Center (http://www.cegen.org/ - CeGen).

Statistical Analysis

Stata 15.0 (StataCorp, Texas, USA) and Statistical Package for the Social Sciences (SPSS) 24.0 software (IBM Corp., Chicago, USA) were used to perform the statistical analyses. The statistical significance was defined as $p < 0.05$, and all p-values were two-tailed. The false discovery rate (FDR), with the Benjamini and Hochberg procedure, was used for the correction of multiple testing.

The chi-square or Fisher´s exact tests were used to analyze categorical data. The Mann-Whitney U and Kruskal-Wallis tests were used to analyze continuous variables.

The genetic association between *CYP* SNPs and the clinical patterns of AIDS progression was evaluated according to dominant, recessive, and additive models. The unadjusted statistical association was performed by the Mantel-Haenszel linear-by-linear association test, and we selected the SNPs that showed *p*-values <0.05 and FDR <0.2.

For the multivariate association analysis, we used binary logistic regression and ordinal logistic regression tests, in which the specific SNP and other adjusted variables (age, gender, risk category, and *VDR* rs2228570, *DBP* rs16846876 and *DBP* rs12512631 polymorphisms) were included. Both *VDR* and *DPB* genes are related to vitD effect [1], and we have previously found that *VDR* rs2228570 [19], *DBP* rs16846876 [20], and *DBP* rs12512631 [20] polymorphisms are related to AIDS progression in this cohort.

Results

Study population

The Control-group showed similar age and gender distribution to that observed for the HIV-groups (**Supplementary Table 1**). LTNPs were older at the time of HIV diagnosis (*p*-value <0.001) and at the time of study inclusion (*p*-value <0.001), and there was a higher percentage of intravenous drug users (*p*-value <0.001). At the same time, LTNPs showed the lowest percentage of male patients (*p*-value <0.001), acquired HIV related to men who have sex with men (p-value <0.001), and were diagnosed with HIV infection before the year 2000

(**Supplementary Table 1**). Moreover, we also found 88 elite controllers within LTNP group (at least three consecutive undetectable viral loads for at least 12 months of follow-up, in the absence of ART). These elite controllers were around 47 years old, 58.6% were men, 74.7% were infected with HIV by intravenous drug infection, and 17.2% by men who have sex with men.

Characteristics of *CYP* polymorphisms

All *CYP* SNPs had minor allelic frequency values higher than 5%. DNA genotyping call-rates success was over 95% for all SNPs. **Supplementary Table 2** shows the characteristics of the four *CYP* SNPs in the Control-group and the HIV-group. All *CYP* SNPs were in Hardy-Weinberg equilibrium (*p*-value >0.05) and had similar genotypic frequencies, in the Control-group and HIV-group, except for rs6013897 (p= 0.028).

CYP polymorphisms and AIDS progression

Figure 1 shows the unadjusted genetic association of the *CYP* SNPs with the three clinical patterns of AIDS progression (LTNPs, MPs, and RPs). We found a significant association between *CYP2R1* rs1993116 and AIDS progression under a dominant inheritance model (*p*= 0.040), while *CYP27B1* rs10877012 showed significant values under dominant inheritance (*p*= 0.044) and additive (*p*= 0.040) models. All these significant p-values disappeared after correcting by multiple comparisons (FDR -Benjamini & Hochberg). However, we decided to continue the analysis with SNPs that had p<0.05 and FDR <0.2 (rs1993116 and rs10877012). The adjusted regression model for all PLWH showed only a significant association for the *CYP27B1* rs10877012 SNP (**Figure 2**). The rs10877012 T allele was protective against AIDS progression (ordinal outcome) under the dominant (adjusted odds ratio (aOR)= 0.69; p= 0.021) and additive (aOR= 0.75; p= 0.025) inheritance models. When analyzing LTNPs versus RPs, the rs10877012 T allele also showed a protective significant association under the dominant (aOR= 0.45; p= 0.004) and additive (aOR= 0.54; p= 0.008) inheritance models. *P*-values remained significant after correcting by multiple comparisons only for the comparison of LTNPs versus RPs (extreme phenotypes).

Discussion

In this study, the *CYP27B1* rs10877012 T allele was linked to non-progression of HIV infection to AIDS in ART-naïve PLWH from two large Spanish cohorts (CoRIS and LTNPs cohorts) collected from different regions of Spain. To our knowledge, this is the first report that shows the association between a genetic variant in *CYP27B1*, which is a CYP linked to VitD metabolism, and the nonclinical AIDS progression.

CYP27B1 is a mitochondrial enzyme, whose gene is located at 12q14.1 and contains nine exons [21]. There are many *CYP27B1* SNPs showing different effects on VitD levels and chronic diseases such as cancer, multiple sclerosis, type 1 diabetes, Addison's disease, hepatitis C, and congestive heart failure [21]. The *CYP27B1* rs10877012 T allele is linked to lower VitD deficiency than G allele in Caucasians [22, 23], which is also related to lower AIDS progression [1], although controversial results have been published in the literature [11]. This is concordant with our results, where the presence of the T allele was associated with non-progression of HIV infection. Moreover, higher mRNA levels of *CYP27B1* have been found in HIV-1-exposed seronegative individuals compared to non-exposed controls [24]. Additionally, Laplana et al. [25] did not find a significant association between *CYP27B1* rs10877012 polymorphism and AIDS progression in a similar population. However, our study had a larger sample size, and the study design was different, with well-defined extreme AIDS progression profiles.

The rs10877012 SNP is located in an intergenic region upstream from the *CYP27B1* gene. We performed an *in silico* analysis to investigate the regulatory role of this SNP. By using

rVarBase database [26], we observed that rs10877012 SNP is located within a transcription factor (TF)-binding region (POLR2A) and is implicated in chromatin remodeling of the surrounding areas in different cell lines and tissues. This finding could influence the DNA accessibility to the RNA polymerase and transcription factors, and thus, it could lead to changes in gene expression. Additionally, it is important to note that the rs10877012 polymorphism is in very high linkage disequilibrium (LD) with other SNPs, which could be the cause or jointly contribute to the effect on AIDS progression observed in this study. By using HaploReg v4.1 software (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php), a bioinformatics tool designed for developing mechanistic hypotheses of the impact of non-coding variants within haplotype blocks on clinical phenotypes, we found that the *CYP27B1* rs10877012 SNP is in high LD with numerous SNPs implicated in promotion and enhancement of histone marks, protein binding, and motifs changes. In this regard, further studies would be needed to corroborate the functional role of rs10877012 SNP itself or indirectly infer it by studying the effect of other SNPs in high LD.
Another point to discuss is the adjustment for multiple comparisons. We selected as significant those SNPs with $p<0.05$ and FDR <0.2, discarding the FDR <0.05 due to excessive stringency, but this could inflate the possibility of Type I errors. However, this study is a clinical-orientated study (not a random search of a meaningful result) because we had a hypothesis supported by theory and previous reports in HIV-infected patients, as we discussed above. Thus, we think that the association initially found between *CYP* SNPs (rs1993116 and rs10877012) and AIDS progression could not be a false positive since the FDR correction is very strict and can also rule out associations that exist [27, 28]. Additionally, we observed that the distribution of genotypes in each of the three categories of AIDS progression showed a prominent "effect size" of both the *CYP2R1* rs1993116 A allele and the *CYP27B1* rs10877012 T allele (see **Figure 1**).

Other limitations of our study must also be taken into account to interpret our results correctly. Firstly, this is a retrospective study which may have some bias, such as the absence of substantial information and the idiosyncrasy of each group. Secondly, a low sample size per group could limit the statistical power of the tests performed, particularly after FDR controlling multiple testing. Thirdly, the three groups of patients (LTNPs, MPs, and RPs) showed differences in clinical and epidemiological characteristics, although these variables were accounted for in the logistic regression analysis. Fourthly, we did not have data on plasma VitD because the adequate sample was not available for most patients. However, it should also be noted that our study included PLWH with difficult-to-obtain AIDS progression profiles (LTNPs, MPs, and RPs), which makes our results more relevant for the study of the natural history of HIV infection.

Conclusions

The *CYP27B1* rs10877012 T allele was linked to non-AIDS progression in ART-naïve HIV-infected patients. The rs10877012 SNP seems to have an impact on the clinical AIDS progression, possibly modifying VitD levels, which could be relevant for the pathogenesis of HIV infection.

List of abbreviations

VitD, Vitamin D

HIV, human immunodeficiency virus

AIDS, acquired immunodeficiency syndrome

25(OH)D, 25-hydroxyvitamin D

1,25(OH)2D, 1,25-dihydroxy vitamin D

VDR, vitamin D receptor

CYP, cytochrome P450

CYP2R1, Vitamin D 25-hydroxylase

CYP27B1, 25-hydroxyvitamin D-1-alpha-hydroxylase

CYP24A1, 25-hydroxyvitamin D-24-hydroxylase

SNPs, single nucleotide polymorphisms

ART, antiretroviral treatment

PLWH, people living with HIV

CoRIS, Cohort of the Spanish AIDS Research Network

MPs, moderate progressors

LTNPs, long-term non-progressors

RPs, rapid progressors

FDR, false discovery rate

SPSS, Statistical Package for the Social Sciences

aOR, adjusted odds ratio

LD, linkage disequilibrium

Declarations

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of the Instituto de Salud Carlos III (CEI PI_2010-v3).

Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed during the current study may be available upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Project Administration: JLJ and SR.

Supervision and visualization: SR.

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Not applicable.

References

1. Jimenez-Sousa MA, Martinez I, Medrano LM, Fernandez-Rodriguez A, Resino S. **Vitamin D in Human Immunodeficiency Virus Infection: Influence on Immunity and Disease**. *Front Immunol* 2018; 9:458.

2. Lucas RM, Gorman S, Geldenhuys S, Hart PH. **Vitamin D and immunity**. *F1000Prime Rep* 2014; 6:118.

3. Sassi F, Tamone C, D'Amelio P. **Vitamin D: Nutrient, Hormone, and Immunomodulator**. *Nutrients* 2018; 10(11).

4. Autier P, Boniol M, Pizot C, Mullie P. **Vitamin D status and ill health: a systematic review**. *Lancet Diabetes Endocrinol* 2014; 2(1):76-89.

5. Gois PHF, Ferreira D, Olenski S, Seguro AC. **Vitamin D and Infectious Diseases: Simple Bystander or Contributing Factor?** *Nutrients* 2017; 9(7).

6. Mansueto P, Seidita A, Vitale G, Gangemi S, Iaria C, Cascio A. **Vitamin D Deficiency in HIV Infection: Not Only a Bone Disorder**. *Biomed Res Int* 2015; 2015:735615.

7. Fellay J, Ge D, Shianna KV, Colombo S, Ledergerber B, Cirulli ET, et al. **Common genetic variation and the control of HIV-1 in humans**. *PLoS genetics* 2009; 5(12):e1000791.

8. Fellay J, Shianna KV, Ge D, Colombo S, Ledergerber B, Weale M, et al. **A whole-genome association study of major determinants for host control of HIV-1**. *Science* 2007; 317(5840):944-947.

9. Mellors JW, Kingsley LA, Rinaldo CR, Jr., Todd JA, Hoo BS, Kokka RP, et al. **Quantitation of HIV-1 RNA in plasma predicts outcome after seroconversion**. *Annals of internal medicine* 1995; 122(8):573-579.

10. Gingras SN, Tang D, Tuff J, McLaren PJ. **Minding the gap in HIV host genetics: opportunities and challenges**. *Hum Genet* 2020; 139(6-7):865-875.

11. Jolliffe DA, Walton RT, Griffiths CJ, Martineau AR. **Single nucleotide polymorphisms in the vitamin D pathway associating with circulating concentrations of vitamin D metabolites and non-skeletal health outcomes: Review of genetic association studies**. *J Steroid Biochem Mol Biol* 2016; 164:18-29.

12. Cusato J, Calcagno A, Marinaro L, Avataneo V, D'Avolio A, Di Perri G, et al. **Pharmacogenetic determinants of kidney-associated urinary and serum abnormalities in antiretroviral-treated HIV-positive patients**. *The pharmacogenomics journal* 2019.

13. Gurdasani D, Iles L, Dillon DG, Young EH, Olson AD, Naranbhai V, et al. **A systematic review of definitions of extreme phenotypes of HIV control and progression**. *AIDS* 2014; 28(2):149-162.

14. Munoz A, Sabin CA, Phillips AN. **The incubation period of AIDS**. *AIDS* 1997; 11 Suppl A:S69-76.

15. Estes JD, LeGrand R, Petrovas C. **Visualizing the Immune System: Providing Key Insights into HIV/SIV Infections**. *Front Immunol* 2018; 9:423.

16. Biasin M, De Luca M, Gnudi F, Clerici M. **The genetic basis of resistance to HIV infection and disease progression**. *Expert review of clinical immunology* 2013; 9(4):319-334.

17. Sobrino-Vegas P, Gutierrez F, Berenguer J, Labarga P, Garcia F, Alejos-Ferreras B, et al. **[The Cohort of the Spanish HIV Research Network (CoRIS) and its associated biobank;**

organizational issues, main findings and losses to follow-up]. *Enferm Infecc Microbiol Clin* 2011; 29(9):645-653.

18. Guzman-Fulgencio M, Jimenez JL, Garcia-Alvarez M, Bellon JM, Fernandez-Rodriguez A, Campos Y, et al. **Mitochondrial Haplogroups Are Associated With Clinical Pattern of AIDS Progression in HIV-Infected Patients**. *J Acquir Immune Defic Syndr* 2013; 63(2):178-183.

19. Jimenez-Sousa MA, Jimenez JL, Fernandez-Rodriguez A, Brochado-Kith O, Bellon JM, Gutierrez F, et al. **VDR rs2228570 Polymorphism Is Related to Non-Progression to AIDS in Antiretroviral Therapy Naive HIV-Infected Patients**. *Journal of clinical medicine* 2019; 8(3).

20. Jimenez-Sousa MA, Jimenez JL, Fernandez-Rodriguez A, Bellon JM, Rodriguez C, Riera M, et al. **DBP rs16846876 and rs12512631 polymorphisms are associated with progression to AIDS naive HIV-infected patients: a retrospective study**. *Journal of biomedical science* 2019; 26(1):83.

21. Latacz M, Snarska J, Kostyra E, Fiedorowicz E, Savelkoul HF, Grzybowski R, et al. **Single Nucleotide Polymorphisms in 25-Hydroxyvitamin D3 1-Alpha-Hydroxylase (CYP27B1) Gene: The Risk of Malignant Tumors and Other Chronic Diseases**. *Nutrients* 2020; 12(3).

22. Ramos-Lopez E, Kahles H, Weber S, Kukic A, Penna-Martinez M, Badenhoop K, et al. **Gestational diabetes mellitus and vitamin D deficiency: genetic contribution of CYP27B1 and CYP2R1 polymorphisms**. *Diabetes, obesity & metabolism* 2008; 10(8):683-685.

23. Lange CM, Bojunga J, Ramos-Lopez E, von Wagner M, Hassler A, Vermehren J, et al. **Vitamin D deficiency and a CYP27B1-1260 promoter polymorphism are associated with chronic hepatitis C and poor response to interferon-alfa based therapy**. *Journal of hepatology* 2011; 54(5):887-893.

24. Aguilar-Jimenez W, Saulle I, Trabattoni D, Vichi F, Lo Caputo S, Mazzotta F, et al. **High Expression of Antiviral and Vitamin D Pathway Genes Are a Natural Characteristic of a Small Cohort of HIV-1-Exposed Seronegative Individuals**. *Front Immunol* 2017; 8:136.

25. Laplana M, Sanchez-de-la-Torre M, Puig T, Caruz A, Fibla J. **Vitamin-D pathway genes and HIV-1 disease progression in injection drug users**. *Gene* 2014; 545(1):163-169.

26. Guo L, Du Y, Qu S, Wang J. **rVarBase: an updated database for regulatory features of human variants**. *Nucleic Acids Res* 2016; 44(D1):D888-893.

27. Perneger TV. **What's wrong with Bonferroni adjustments**. *BMJ* 1998; 316(7139):1236-1238.

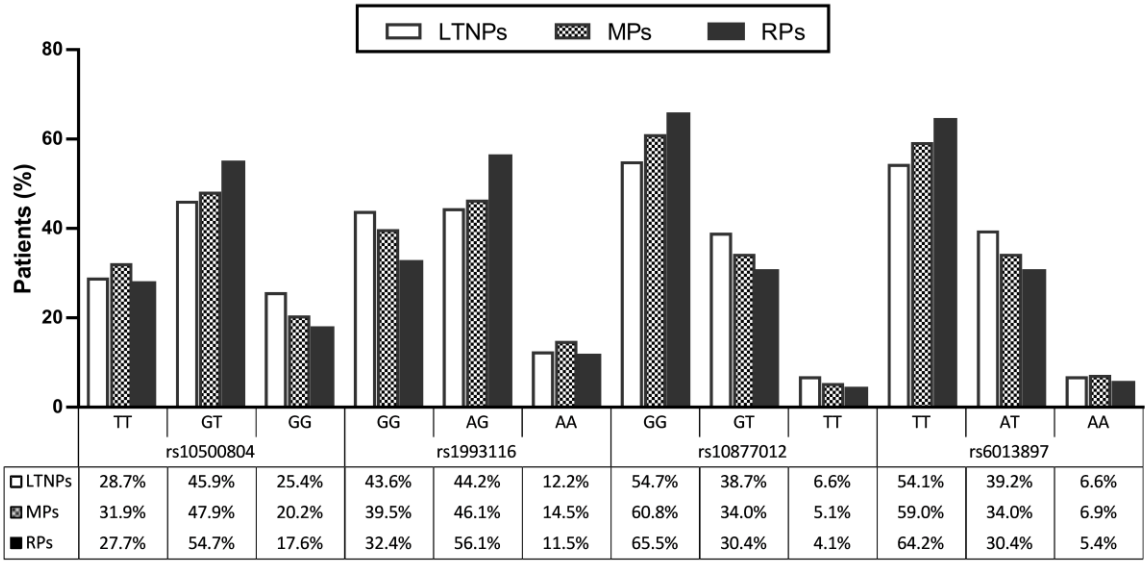
28. Sterne JA, Davey Smith G. **Sifting the evidence-what's wrong with significance tests?** *BMJ* 2001; 322(7280):226-231.

Figures

Figure 1. Univariate ordinal association of *CYP2R1*, *CYP27B1*, and *CYP24A1* polymorphisms with distinct patterns of AIDS progression in HIV infected patients.

Statistics: *p*-values were calculated by the Mantel-Haenszel linear-by-linear association test. *P*-values were corrected for multiple testing using the false discovery rate (FDR) with Benjamini and Hochberg procedure (n=12 multiple comparisons (4 SNPs * 3 inheritance models)).

Abbreviations: CYP2R1, cytochrome P450 family 2 subfamily R member 1; CYP27B1, cytochrome P450 family 27 subfamily B member 1; CYP24A1, cytochrome P450 family 24 subfamily A member 1; LTNPs, Long Term Non-Progressors; MPs, Moderate Progressor; RPs, Rapid Progressor.



	rs10500804			rs1993116			rs10877012			rs6013897		
	TT	GT	GG	GG	AG	AA	GG	GT	TT	TT	AT	AA
LTNPs	28.7%	45.9%	25.4%	43.6%	44.2%	12.2%	54.7%	38.7%	6.6%	54.1%	39.2%	6.6%
MPs	31.9%	47.9%	20.2%	39.5%	46.1%	14.5%	60.8%	34.0%	5.1%	59.0%	34.0%	6.9%
RPs	27.7%	54.7%	17.6%	32.4%	56.1%	11.5%	65.5%	30.4%	4.1%	64.2%	30.4%	5.4%

	p-value	q-value (FDR)	p-value	q-value (FDR)	p-value	q-value (FDR)	p-value	q-value (FDR)
Dominant	0.898	0.915	**0.040**	0.175	**0.044**	0.175	0.065	0.184
Recessive	0.077	0.184	0.915	0.915	0.294	0.441	0.678	0.814
Additive	0.350	0.467	0.153	0.262	**0.040**	0.175	0.101	0.203

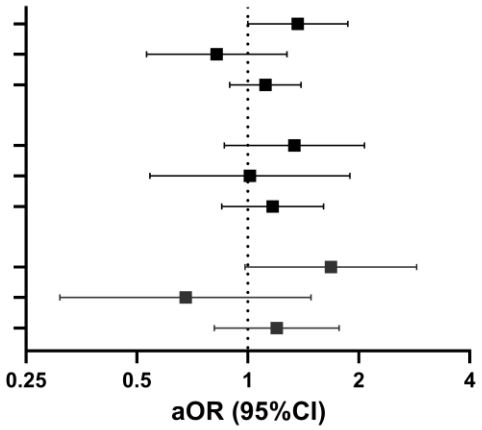
Figure 2. Adjusted genetic association of *CYP2R1*, *CYP27B1*, and *CYP24A1* polymorphisms with distinct patterns of AIDS progression in HIV infected patients.

Statistics: *p*-values were calculated by logistic regression analysis or ordinal regression analysis adjusted for age, gender, risk category, *VDR* rs2228570 polymorphism, *DBP* rs16846876 polymorphism, and *DBP* rs12512631 polymorphism. *P*-values were corrected for multiple testing using the false discovery rate (FDR) with Benjamini and Hochberg procedure (n= 9 multiple comparisons (3 comparisons * 3 inheritance models for each SNP)).

Abbreviations: CYP2R1, cytochrome P450 family 2 subfamily R member 1; CYP27B1, cytochrome P450 family 27 subfamily B member 1; CYP24A1, cytochrome P450 family 24 subfamily A member 1; LTNPs, Long Term Non-Progressors; MPs, Moderate Progressor; RPs, Rapid Progressor; VDR, vitamin D3 Receptor; DBP, vitamin D-binding protein.

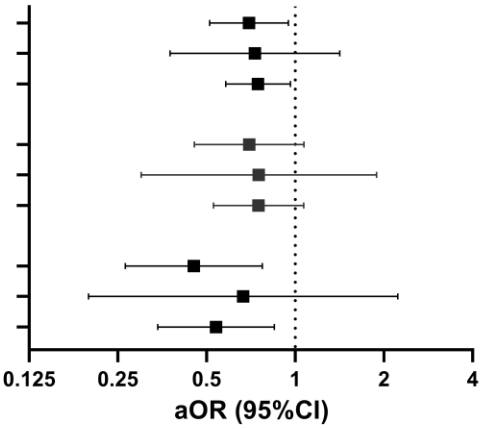
A) *CYP2R1* rs1993116 polymorphism

			OR (95%CI)	p-value	FDR
LTNPs vs RPs vs MPs					
	Dominant	AG/AA	1.37 (1.00 - 1.87)	0.050	0.261
	Recessive	AA	0.82 (0.531 - 1.28)	0.385	0.433
	Additive	A	1.12 (0.893 - 1.39)	0.336	0.433
LTNPs vs MPs					
	Dominant	AG/AA	1.34 (0.863 - 2.07)	0.193	0.433
	Recessive	AA	1.01 (0.542 - 1.89)	0.969	0.969
	Additive	A	1.17 (0.85 - 1.60)	0.339	0.433
LTNPs vs RPs					
	Dominant	AG/AA	1.68 (0.983 - 2.87)	0.058	0.261
	Recessive	AA	0.68 (0.309 - 1.48)	0.330	0.433
	Additive	A	1.19 (0.811 - 1.77)	0.365	0.433



B) *CYP27B1* rs10877012 polymorphism

			OR (95%CI)	p-value	FDR
LTNPs vs RPs vs MPs					
	Dominant	GT/TT	0.69 (0.51 - 0.95)	**0.021**	0.056
	Recessive	TT	0.73 (0.37 - 1.41)	0.351	0.451
	Additive	T	0.75 (0.58 - 0.96)	**0.025**	0.056
LTNPs vs MPs					
	Dominant	GT/TT	0.69 (0.45 - 1.07)	0.100	0.168
	Recessive	TT	0.75 (0.30 - 1.89)	0.546	0.546
	Additive	T	0.75 (0.53 - 1.07)	0.112	0.168
LTNPs vs RPs					
	Dominant	GT/TT	0.45 (0.26 - 0.77)	**0.004**	**0.036**
	Recessive	TT	0.66 (0.19 - 2.23)	0.510	0.546
	Additive	T	0.54 (0.34 - 0.85)	**0.008**	**0.036**



Supplementary Tables

Supplementary Table 1. Clinical and epidemiological characteristics of HIV infected patients and healthy donors.

	Controls vs. all HIV patients			HIV patient groups			
Characteristics	**Control**	**All HIV (*)**	**p-value**	**LTNPs-group**	**MPs-group**	**RPs-group**	**p-value**
No.	111	661		181	332	148	
Male	91 (82.0%)	534 (81.3%)	0.860	113 (63.8%)	281 (84.6%)	140 (94.6%)	**<0.001**
Age (study inclusion), years	42 (37 - 49)	41 (35 - 48)	0.534	49 (46 - 52)	38 (33 - 45)	38 (33 - 43)	**<0.001**
Age (HIV diagnosis), years	-	34 (29 - 40)	-	40 (34 - 44)	32 (27 - 38)	34 (29 - 38)	**<0.001**
Year at HIV diagnosis	-	2006 (1999 - 2008)	-	1993 (1990 - 1997)	2006 (2005 - 2008)	2009 (2007 - 2010)	**<0.001**
HIV acquired							
IDU	-	164 (25.0%)	-	128 (72.3%)	29 (8.7%)	7 (4.7%)	**<0.001**
Homosexual (MSM)	-	356 (54.2%)	-	13 (7.3%)	219 (66.0%)	124 (83.8%)	
Heterosexual	-	117 (17.8%)	-	27 (15.3%)	75 (22.6%)	15 (10.1%)	
Others	-	20 (3.0%)	-	9 (5.1%)	9 (2.7%)	2 (1.4%)	

Statistics: P-values were calculated by Chi-square or Fisher's exact test, Mann-Whitney, and Kruskal-Wallis tests. Significant differences are shown in bold.

Abbreviations: MSM, men who have sex with men; IDU, intravenous drug users; HIV, Human immunodeficiency virus; LTNPs, Long Term Non-Progressors; MPs, Moderate Progressors; RPs, Rapid Progressors.

Supplementary Table 2. Characteristics of *CYP2R1*, *CYP27B1*, and *CYP24A1* polymorphisms in HIV infected patients and healthy donors.

***CYP* genotypes**			**Study groups**			**HWE**	
Gene	**SNPs**	**Genotype**	**Control group**	**HIV group**	**p-value**	**Control group**	**HIV group**
CYP2R1	**rs10500804**	TT	38 (34.2%)	199 (30.1%)	0.666	0.698	0.695
		GT	52 (46.8%)	323 (48.9%)			
		GG	21 (18.9%)	139 (21.0%)			
	rs1993116	GG	41 (36.9%)	258 (39.0%)	0.059	0.120	0.559
		AG	46 (41.4%)	316 (47.8%)			
		AA	24 (21.6%)	87 (13.2%)			
CYP27B1	**rs10877012**	GG	62 (55.9%)	398 (60.2%)	0.430	0.466	0.738
		GT	40 (36.0%)	228 (34.5%)			
		TT	9 (8.1%)	35 (5.3%)			
CYP24A1	**rs6013897**	TT	76 (68.5%)	389 (58.9%)	**0.028**	0.296	0.239
		AT	34 (30.6%)	229 (34.6%)			
		AA	1 (0.9%)	43 (6.5%)			

Statistics: P-values were calculated by the Chi-square test.
Abbreviations: HIV, human immunodeficiency virus; CYP2R1, cytochrome P450 family 2 subfamily R member 1; CYP27B1, cytochrome P450 family 27 subfamily B member 1; CYP24A1, cytochrome P450 family 24 subfamily A member 1; HWE, Hardy-Weinberg equilibrium.

Appendix 1: Centers involved in Long Term Non-Progressors (LTNP) chort:
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H. 12 de Octubre - Madrid
H. Arnau de Vilanova - Lleida
H. Asturias
H. Bellvitge - Barcelona
H. Castellón
H. Clínic - Barcelona
H. Donostia - San Sebastián
H. Elche - Alicante
H. Germans Trias i Pujol - Badalona
H. Gregorio Marañón - Madrid
H. Joan XXIII - Tarragona
H. La Fe - Valencia
H. La Paz/Carlos III - Madrid
H. La Princesa - Madrid
H. Navarra - Pamplona
H. Parc Taulí- Sabadell
H. Ramón y Cajal - Madrid
H. San Cecilio - Granada
H. San Pedro - Logroño
H. Son Dureta - Mallorca
H. Virgen del Rocío - Sevilla

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