

HLA-B*57 and IFNL4-related polymorphisms are associated with protection against HIV-1 disease progression in controllers

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[†]The clinical Centers which contribute to ECRIS are shown in the **Annex I**.

Running Title: Immunologic progression in controllers.

Summary

HIV-controllers group comprises patients with different clinical characteristics, including immunologic progression as defined by CD4⁺T-cells drop. We have defined two genetic markers able to segregate HIV-controllers with CD4⁺T-cell counts >500 mm³ along time, from those who experience CD4⁺T-cell decline.

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Abstract

Background: HIV-1-controllers maintain HIV-1 viremia at low levels (normally <2000 HIV-RNA copies/mL) without antiretroviral treatment. However, some HIV-1-controllers have evidence of immunologic progression with marked CD4⁺T-cell decline. We investigated host genetic factors associated with protection against CD4⁺T-cell loss in HIV-1-controllers.

Methods: We analysed the association of interferon lambda 4 (IFNL4)-related polymorphisms and HLA-B haplotypes within Long Term Non-Progressor HIV-1-controllers ((LTNP-C), defined by maintaining CD4⁺T-cells counts >500 cells/mm³ for more than 7 years after HIV-1 diagnosis) versus non-LTNP-C, who developed CD4⁺T-cells counts <500 cells/mm³. Both a Spanish study cohort (n=140) and an international validation cohort (n=914) were examined. Additionally, in a subgroup of individuals HIV-1-specific T-cell responses and soluble cytokines were analysed.

Results: HLA-B*57 was independently associated with the LTNP-C phenotype (OR=3.056 (1.029-9.069) p=0.044 and OR=1.924 (1.252-2.957) p=0.003) while IFNL4 genotypes represented independent factors for becoming non-LTNP-C (TT/TT, ss469415590, OR=0.401 (0.171-0.942) p=0.036 or A/A, rs12980275, OR=0.637 (0.434-0.934) p=0.021) in the Spanish and validation cohort, respectively, after adjusting for sex, age at HIV-1 diagnosis, IFNL4-related polymorphisms and different HLA-B haplotypes. LTNP-C showed lower plasma IP-10 (p= 0.019) and higher IFN- γ (p=0.02) levels than the HIV-1-controllers with diminished CD4⁺T-cell numbers. Moreover, LTNP-C exhibited higher

quantities of IL2⁺CD57⁻ and IFN- γ ⁺CD57⁻ HIV-1-specific CD8⁺T-cells ($p=0.002$ and 0.041, respectively) than non-LTNP-C.

Conclusions: We have defined genetic markers able to segregate stable HIV-1-controllers from those who experience CD4⁺T-cell decline. These findings allow for identification of HIV-1-controllers at risk for immunologic progression, and provide avenues for personalized therapeutic interventions and precision medicine for optimizing clinical care of these individuals.

Keywords: HIV-controllers, progression, HLA-B*57, IFNL4.

Introduction

HIV-1-controllers are a rare group of individuals who maintain HIV-1 viremia at extremely low (<2000 HIV-RNA copies/ml) or even undetectable levels in the absence of antiretroviral treatment [1]. This group constitutes an important field of study, since they have been proposed as a model for a functional cure, investigating immune defense mechanisms in these persons may reveal relevant information for HIV-1 vaccine development and eradication strategies [2,3]. However, HIV-1-controllers are a heterogeneous group composed of subsets with different clinical characteristics. Epidemiological studies showed that about 6-7% of these persons progressed to AIDS [4,5], 20% experienced at least one non-AIDS defining event (NADEs) [6], and 30% eventually experienced loss of spontaneous viral control [4,6]. Importantly, immunologic progression as defined by CD4⁺T-cells <500 cells/mm³ has been documented in some HIV-1-controllers [7–8], leading to institution of combination antiretroviral therapy (cART) [4], although treatment-related CD4⁺T-cell increases were frequently less pronounced than non-controllers [9]. Identifying biological markers of HIV-1-controllers who are at risk for HIV-1 disease progression as defined by declining CD4⁺T-cell counts is an important objective, both for improving clinical care of these persons and for extending the conceptual understanding of immune mechanisms involved in HIV spontaneous control.

Single-nucleotide polymorphisms (SNPs) located in the major histocompatibility complex, are the major genetic host factor associated with HIV-1 immune control [10]. HLA-I alleles, in particular HLA-B*57, have been associated with slower progression to AIDS and hence to lower CD4⁺T-cell drop

in natural history cohorts [11]. Moreover, we have reported an association between the CC variant of a SNP within IL-28B (rs12979860) and HIV-1 spontaneous control in Caucasian subjects [12], suggesting that polymorphisms at the Interferon-lambda (IFNL) gene locus may influence or modulate the dynamics of HIV-1 disease progression in controllers. Prokunina-Olsson et al. described the ss469415590 polymorphism within IFNL region, which is in linkage disequilibrium with rs12979860 and rs12980275 [13, 14]. This novel polymorphism seems to be the functional one of the family, leading to the IFNL4 production and modulation of Interferon-stimulated genes (ISGs) expression [13, 15].

Based on these observations, we hypothesized that these host genetic factors could segregate different HIV-1-controller phenotypes, and may be correlated with different patterns of HIV-1 disease progression within HIV-1-controllers. Consequently, in this study we tested associations between immunologic progression in HIV-1-controllers (defined by longitudinal evolution of CD4⁺T-cell counts) and the presence of HLA-B*57 alleles and IFNL4-related polymorphisms, first in a study cohort and then in a validation cohort of Caucasian subjects.

Patients and Methods

Study Cohort

HIV-1-controllers were defined exclusively based on virological criteria, as subjects with plasma HIV viral load <2000 HIV-RNA copies/ml plasma for at least one year in the absence of cART. Long Term Non-Progressors (LTNP) were defined exclusively based on immunological criteria, as subjects with CD4⁺T-cell counts >500 cell/mm³ for more than seven years after HIV diagnosis. HIV-1-controllers who met LTNP criteria were considered as LTNP-controllers (LTNP-C) and those who did not fulfill this criteria and progress to CD4⁺T cell counts <500 cells/mm³ in less than seven years were considered as non-LTNP-C. One hundred LTNP-C and 40 non-LTNP-C recruited within the multicenter Spanish AIDS Research Network HIV-Controllers Cohort (ECRIS) were included according to sample availability. HIV-2 infected patients were excluded. Viral load, CD4⁺ and CD8⁺ T-cell counts were routinely collected and data recorded in the ECRIS cohort by each collaborating center (Annex I). Samples were kindly provided by the HIV-1 Biobank integrated within the Spanish AIDS Research Network. Each participating subject signed an informed consent form. The program was approved by the Institutional Review Boards of the participating hospitals and centers.

Genotyping

HLA-B alleles and IFNL4 ss469415590 polymorphisms were genotyped, as previously described [12, 16].

Validation Cohort

As a validation cohort, 914 Caucasian HIV-1-controllers included in a prior genome wide association study (GWAS) from the International HIV Controllers Study [10] were analyzed. These HIV-controllers were split into LTNP-C and non-LTNP-C in accordance with the above-mentioned criteria. Data on HLA-B alleles and IFNL4-associated polymorphisms were extracted from the prior GWAS study. The IFNL4-associated polymorphism available in the validation cohort was rs12980275, which is in strong linkage disequilibrium with ss469415590 ($r^2=0.901$).

Analysis of HIV-1-specific T-cell responses and soluble cytokines

Frozen peripheral blood mononuclear cells (PBMCs) were *in vitro* stimulated with a pool of HIV-1 Gag peptides, stained and analyzed by flow cytometry as previously described [17].

Soluble plasma concentrations of interferon-gamma (IFN- γ) and IFN- γ induced protein 10 (IP-10) were assayed in duplicate with a bead-based immunoassay using Luminex technology (HCYTMAG-60K-PX29, Merck Millipore, Darmstadt, Germany).

Statistical analyses

For the bivariate and multivariate analyses, a logistic regression model was applied. Log-rank Mantel Cox test was used for univariate survival analysis for time-to-CD4⁺T-cell drop <500 cells/mm³, followed by stepwise Cox regression multivariate analyses. All variables with *p* values <0.1 were included in the multivariate analysis, and *p*<0.05 was considered significant. To compare HIV-specific T-cell responses and soluble cytokine levels, Mann-Whitney U tests were used. Statistical analyses were performed using the Statistical Package for Social Sciences software (SPSS 20.0; SPSS Inc., Chicago, IL, USA).

Results

LTNP-C association with HLA*B57 allele and ss469415590 TT/TT genotype

The ss469415590 polymorphisms (TT vs Δ G) at the IFN- λ gene locus has previously been found to be strongly associated with natural clearance and clinical responses to IFN- α treatment in patients with Hepatitis C Virus (HCV) infection [18, 13]. To investigate the impact of these polymorphisms on the dynamics of HIV-1 disease progression in HIV-1-controllers, we analyzed this polymorphism in a cohort of HIV-1-controllers recruited through a multi-center network in Spain. The characteristics of the study cohort are summarized in Table 1. Notably, LTNP-C, were enriched with men compared with non-LTNP-C, (69% Vs 50%, $p=0.037$) despite an overrepresentation of women in the entire ECRIS cohort [4, 6] and other cohorts [1]. There were no differences in viral load (VL) between the two study groups, expressed as the mean of the logarithm of VL determinations (Log mean VL, LTNP-C= 2.14 [1.79-2.61] Vs non-LTNP-C= 2.06 [1.70-2.64], $p= 0.583$). Interestingly, the proportion of persons carrying the homozygous IFNL4 (ss469415590) TT/TT genotype, previously associated with improved clinical responses to exogenous IFN- α treatment in the context of HCV infection, was significantly lower among LTNP-C than within non-LTNP-C (44% Vs 65% $p= 0.029$). In contrast, homozygous carriers of the Δ G/ Δ G genotype, associated with reduced natural clearance of HCV infection, were significantly more frequent in LTNP-C, while being completely absent in non-LTNP-C (12.8% Vs 0%, $p=0.019$). Notably, the protective HLA class I allele HLA-B*57 was also more frequent among LTNP-C than non-LTNP-C (29% Vs 13%, $p=0.054$). There were no differences in the proportions of HLA-B*27, -B*35 and -B*08 alleles between groups (Table 1).

We next determined whether the abovementioned genetic factors were independently associated with the LTNP-C phenotype. Taking LTNP-C as the reference variable, we performed a bi- and multivariate logistic regression analysis, shown in Table 1. After adjusting for sex, age at HIV-1 diagnosis, IFNL4 (ss469415590) TT/TT genotype, and carrier status for HLA-B*27, -B*57, -B*35 and -B*08 alleles, the protective factors independently associated with LTNP-C phenotype were male sex and HLA-B*57 allele carrier. On the other hand, the IFNL4 (ss469415590) TT/TT genotype was an independent factor associated with the non-LTNP-C phenotype (Table 1).

In addition, we also analyzed HLA-B*57 and IFNL4 (ss469415590) genotypes as a composite variable, categorizing individuals in HLA-B*57+/ IFNL4 nonTT (i.e.: TT/□G or □G/□G), HLA-B*57+/ IFNL4 TT/TT, HLA-B*57-/ IFNL4 nonTT and HLA-B*57-/ IFNL4 TT/TT (Supplementary table 1). The composite variable was associated with LTNP-C ($p=0.035$). The combined genotype HLA-B*57-/ IFNL4 TT/TT was independently associated with developing non-LTNP-C phenotype after adjusting for sex, age and Log mean VL (Supplementary Table 1).

Replication of the association between LTNP-C, HLA-B*57 allele and IFNL4-related genotype in a validation cohort

In order to validate our results, we next analyzed available data of 914 Caucasian HIV-1-controllers included in a GWAS from the International HIV Controllers Study [10]. The characteristics of these subjects are summarized in Table 2. We observed 52.8% of subjects with an LTNP-C phenotype within this

cohort. Of the 914 patients, 465 had available data for the rs12980275 genotype, which is in strong linkage disequilibrium with ss469415590 ($r^2=0.901$). The proportion of patients with the A/A genotype, was 48.2%; 42.2% of individuals had an A/G genotype and 9.7% were carriers of the G/G genotype (Table 2).

In this cohort, there was a strong association between younger age at HIV-1 diagnosis and development of an LTNP-C phenotype, suggesting that improved regenerative immunological capacities [19] may facilitate preservation of adequate CD4⁺T-cell counts in HIV-1-controllers. In contrast to the study cohort, there were no gender differences between LTNP-C and non-LTNP-C (Table 3). Similar to the observations in the study cohort, we observed an overrepresentation of HLA-B*57 alleles among LTNP-C in the validation cohort (35% Vs 22%, $p=0.002$). There were no differences in the proportions of HLA-B*27, -B*35 and -B*08 alleles between groups. The rs12980275 A/A genotype was found in elevated frequencies among non-LTNP-C in the validation cohort (43% Vs 54%, $p=0.012$) (Table 3), analogous to what was found with the corresponding SNP in the study cohort. After adjusting for age at HIV-1 diagnosis, rs12980275 A/A genotype, HLA-B*27, -B*57, -B*35 and -B*08 alleles, we replicated the genetic associations found in the study cohort: the protective factors independently associated with LTNP-C phenotype were age at HIV-1 diagnosis and frequency of HLA-B*57 alleles. The IFNL4 (rs12980275) A/A genotype was independently associated with the non-LTNP-C phenotype (Table 3).

Although we found differences in VL between the two groups in the validation cohort (Log mean VL, LTNP-C= 2.15 [1.70-2.64] Vs non-LTNP-C= 2.51 [1.88-

2.91], $p= 0.001$), the above-mentioned associations were maintained even after adjusting by Log mean VL. In this case the protective factors independently associated with LTNP-C phenotype were: lower Log mean VL (OR (CI95%) = 0.628 (0.447-0.883), $p= 0.007$), younger age at HIV-1 diagnosis (OR (CI95%) = 0.962 (0.941-0.983), $p< 0.0001$) and frequency of HLA-B*57 alleles (OR (CI95%) = 1.869 (1.212-2.883), $p= 0.005$); once more the frequency of IFNL4 (rs12980275) A/A genotype was independently associated with the non-LTNP-C phenotype (OR (CI95%) = 0.644 (0.438-0.948), $p= 0.026$).

Again, we analyzed HLA-B*57 and IFNL4 (ss469415590) genotypes as a composite variable, categorizing individuals in HLA-B*57+/ IFNL4 (rs12980275) A/A- (i.e.: A/G or G/G), HLA-B*57+/ IFNL4 (rs12980275) A/A+, HLA-B*57-/ IFNL4 (rs12980275) A/A- and HLA-B*57-/ IFNL4 (rs12980275) A/A+. The composite variable was associated with LTNP-C ($p=0.001$). The combined genotype HLA-B*57-/ IFNL4 A/A+ was independently associated with developing non-LTNP-C phenotype after adjusting for age and Log meanVL (Supplementary Table 2).

HLA-B*57 allele and IFNL4-related genotypes are independently associated with faster CD4⁺T-cell loss below 500 cell/mm³

We next analyzed variables associated with the time to CD4⁺T-cell loss below 500 cells/mm³. This analysis was only performed in the validation cohort; there was not enough statistical power to perform the analysis in the study cohort. HLA-B*57 was a protective factor independently associated with the time to CD4⁺T-cells below 500 cell/mm³ (Figure 1A), while the frequency of IFNL4

(rs12980275) A/A genotypes (Figure 1B) and age at HIV-1 diagnosis (Figure 1C) represented risks factors for accelerated CD4⁺T-cell decline (Table 4). After adjusting by Log mean VL, HLA-B*57 remained a protective factor (OR (CI95%) = 0.493 (0.259-0.941), $p= 0.032$) and higher Log mean VL was a risk factor (OR (CI95%) = 3.057 (1.694-5.518), $p= <0.0001$); in this analysis the frequency of IFNL4 (rs12980275) A/A genotype and age at diagnosis showed a trend toward being a risk factor for accelerated CD4⁺T-cell decline ($p= 0.093$ and $p= 0.059$, respectively).

We also analyzed HLA-B*57 and IFNL4 (rs12980275) genotypes as a composite variable, as mentioned above. The composite variable was associated with the time to event (Figure 1D). The combined genotype HLA-B*57-/ IFNL4 (rs12980275) A/A+ was a risk factor independently associated with the time to CD4⁺T-cell loss below 500 cell/mm³ after adjusting for gender, age and Log mean VL (Supplementary Table 3).

HIV-specific T-cell response and plasma soluble cytokines

We analyzed HIV-1-specific T-cell responses and plasma soluble cytokines in a subgroup of LTNP-C (n=18) and non-LTNP-C (n=5) patients with available plasma and PBMCs samples and undetectable plasma viral load. LTNP-C exhibited a higher percentage of IL2⁺CD8⁺CD57⁻ and IFN γ ⁺CD8⁺CD57⁻ HIV-1-specific T-cells ($p=0.002$ and 0.041 , respectively) than non-LTNP-C (Figure 2A). Moreover, LTNP-C showed higher IFN- γ and lower IP-10 plasma levels ($p= 0.004$ and $p<0.001$, respectively) than non-LTNP-C (Figure 2B).

Discussion

The present study shows that dynamics of CD4⁺T-cell decline among HIV-1-controllers is associated with HLA-B*57 and IFNL4-related genotypes. HIV-1-controllers with stable CD4⁺T-cell counts, termed here LTNP-C, were more likely to carry HLA-B*57 allele and less likely to express the IFNL4-related genotypes TT/TT (ss469415590) or A/A (rs12980275). The consistency of these results was further confirmed in an independent validation cohort with 3.5-fold larger patient numbers. Notably, these associations were true not only when taking the definition of LTNP-C as an endpoint but also in the subgroup longitudinally studied, when considering the time to event analysis, strengthening the findings of this study.

HLA-B*57 has been associated with delayed progression to AIDS and CD4⁺T-cell losses in several prior natural-history cohorts [11, 20]. This observation is consistent with our finding that LTNP-C were enriched with HLA-B*57 alleles. Interestingly, despite the known influence of HLA-B*57 on HIV viremia [10, 21], the associations between HLA-B*57 and reduced CD4⁺T-cell number remained independent even after adjusting for VL in our model. The precise mechanism by which HLA-B*57 protects against CD4⁺T-cell losses in HIV-1 patients is unknown, but likely includes the restriction of HIV-1-specific CD8⁺T-cell responses [22] with improved antiviral functions (e.g. IL2 and IFN- γ production among HLA-B*57-restricted HIV-specific T-cells [23]) as well as altered immunoregulatory interactions with HLA class I receptors expressed on innate immune cells [24].

We previously communicated that the CC genotype of rs12979860 polymorphisms was associated with spontaneous control of HIV-1 in Caucasian subjects recruited in Spain [12]. This SNP is in strong linkage disequilibrium with rs12980275 [14] and ss469415590 [13]. The Δ G allele of this polymorphism encodes for the IFNL4 protein [13], and seems to be associated with lower expression of IL28 mRNA and IP-10 than wild type alleles carriers [15]. Interestingly, IP-10, a proinflammatory chemokine related with immune activation, has been reported to negatively correlate with CD4⁺T-cell counts in HIV-1-controllers [25]. This observation is consistent with our findings, as LTNP-C were more likely to carry the Δ G mutant allele and have lower levels of soluble IP-10. These findings are also in accordance with the time to event analysis presented in this work using HLA-B*57 and IFNL4-related genotype as a composite variable. Together, these data raise the provocative hypothesis that subjects with IFNL4 TT/TT genotype may have higher antiviral capacity mediated by type I IFN responses [26] and IFN-stimulated genes upregulation [15], possibly through an enhanced plasmacytoid dendritic cell (pDCs) function [27]. This robust IFN response may have a role in supporting the ability to naturally control HIV-1 replication for a limited period of time, or clearing non-integrating viruses like HCV, but may be achieved at the expense of increased T-cell activation [25] and IFN-mediated thymic dysfunction [28] that eventually may contribute to CD4⁺T-cell drop and a loss of the HIV-1-controller status. This fact could explain the women overrepresentation in nonLTNP-C group, as it is known that women exhibit higher TLR-mediated pDCs responses and the subsequently higher CD8⁺T-cell activation [29].

Defining heterogeneous phenotypes among HIV-1-controllers could help to clarify some controversial results in literature. For example, the above-mentioned association between the CC genotype of rs12979860 and HIV-1 control in a Caucasian cohort [12] was not found by others authors [30]. Beside ethnic differences in the cohorts, these contradictory data could be due to the heterogeneity of controller cohorts being analyzed. We have noticed that in our previous work, the cohort was enriched with non-LTNP-C (about 75%). In contrast, other studies, in particular the cohort described by Salgado et al [30], consisted predominantly (>90%) of LTNP-C. Together, these data argue that investigating genetic polymorphisms associated with an HIV-1-controller status will require more robust definitions of clinical phenotypes that do not solely rely on viral load as the only criteria.

The segregation between LTNP-C and non-LTNP-C may have important implications. First, controllers who are at risk of disease progression [4–8] could possibly be identified at earlier stages, and may benefit from more rapid cART initiation or other immunotherapeutic interventions, while in HIV-1-controllers genetically predisposed to maintain an LTNP-C phenotype, the risk/benefit ratio for institution of cART may be less definitive. Secondly, HIV-1-controllers have been proposed as a model for functional cure and effective viral eradication strategies [2,3]. The subgroup of HIV-1-controllers with no detectable viremia, high CD4⁺T-cell counts and no signs of disease progression may represent the most informative patient populations in this regard. So, it is pivotal to accomplish a more precise definition of controller phenotypes in order to avoid spurious results and properly identify immune correlates of persistent

spontaneous viral control in the search of the right model of a functional cure or “spontaneous eradicators”.

This work has some limitations. First, both cohorts were composed of seroprevalent patients, for which precise times of HIV-1 seroconversion or viral transmission could not be firmly established. Consequently, follow-up times were calculated based on the time since HIV-1 diagnosis; hence initial VL and CD4⁺T-cell evolution prior to entry into the cohort were missed. However, the confirmation of the results in a large validation cohort supports the appropriateness of the LTNP-C classification criteria. Unfortunately we had few samples available to perform functional analyses. Although results were consistent with the literature, further analyzes should be performed in larger number of patients. Lastly, we have only analyzed HLA-B and IFNL4-related genotypes. It could be desirable to perform more comprehensive genetic analysis segregating HIV-1-controllers in different phenotypes and/or genotypes, in order to propose an algorithm with improved discriminatory capacity.

In conclusion, we have been able to segregate two HIV-1 controller phenotypes based on two genetic markers for which established laboratory tests are available and which are routinely used in HIV-1 clinical practice. The better characterization of distinct HIV-1-controllers phenotypes may allow improved clinical care of this specific patient population, and enhance our understanding of how long-term spontaneous control of HIV-1 infection can be naturally achieved.

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Figure Legends

Figure 1. Kaplan-Meier curves for factors associated with CD4⁺ T-cell drop <500 cell/mm³. Variables with a p value <0.1 in the Cox proportional-Hazards bivariate regression are represented. A) HLA-B*57, B) IFNL4-related genotype, C) Age at diagnosis and D) HLA-B*57 and IFNL4-related genotype composite variable expressed as the four possible combinations. Log rank-test bivariate analysis was performed to assess statistical differences among the curve.

Figure 2. HIV-specific T-cell response and plasma soluble cytokine levels in LTNP-C and non-LTNP-C subgroup. A) IL2⁺CD8⁺CD57⁻ (left panel) and IFN γ ⁺CD8⁺CD57⁻HIV-specific T-cell response (right panel), were assayed in 18 LTNP-C and 5 non-LTNP-C with peripheral blood mononuclear cell samples available. B) IP-10 (left panel) and IFN- γ (right panel) levels were assayed in 17 LTNP-C and 5 non-LTNP-C with plasma samples available.

Table 1. Characteristics and bi-and multivariate analyses of the study cohort

| | LTNP-C (n=100) | NonLTNP-C (n=40) | p; OR (CI 95%) | |
|-----------------------------------|-------------------|---------------------|-----------------------------------|-----------------------------------|
| | | | Unadjusted | Adjusted |
| Sex (male) | 69 (69) | 20 (50) | 0.037; 2.226 (1.054-4.716) | 0.040; 2.467 (1.040-5.852) |
| Age at HIV diagnosis (years) | 26.2 [22.1-31.8] | 29.9 [25.0-34.1] | 0.038; 0.950 (0.904-0.997) | 0.102; 0.957 (0.908-1.009) |
| IFNL4 TT/TT genotype [±] | 42 (44) | 26 (65) | 0.029; 0.427 (0.198-0.918) | 0.036; 0.401 (0.171-0.942) |
| HLA-B*27 ^{#‡} | 18 (18) | 7 (18) | 0.982; 0.989 (0.375-2.607) | |
| HLA-B*57 ^{#‡} | 29 (29) | 5 (13) | 0.054; 2.770 (0.981-7.824) | 0.044; 3.056 (1.029-9.069) |
| HLA-B*35 ^{#‡} | 10 (10) | 7 (18) | 0.194; 0.498 (0.174-1.426) | |
| HLA-B*08 ^{#‡} | 5 (5) | 2 (5) | 0.964; 0.962 (0.178-5.188) | |

Qualitative data are no. (%) of individuals and quantitative data are median (interquartile range). [±]Data were available for 135 patients (95 LTNP-C and 40 nonLTNP-C). [#]Data were available for 133 patients (96 LTNP-C and 37 nonLTNP-C). [‡]Dominant model was applied. There were not homozygous subjects for HLA-B57. Abbreviations: OR: Odds ratio; CI: confidence interval. Significant values are shown in bold.

Table 2. Characteristics of the validation cohort

| | Whole cohort (N=914) | Selected cohort (N=465) |
|---------------------------------|---------------------------------|------------------------------------|
| Sex (male) | 678 (74.3) | 392 (81.2) |
| Age at HIV diagnosis (years) | 33 [27-40] | 33 [27-40] |
| LTNP-C [‡] | 468 (52.8) | 241 (51.8) |
| IFNL4 A/A genotype [‡] | NA | 224 (48.2) |
| HLA-B*27 | 127 (14.2) | 94 (20) |
| HLA-B*57 | 268 (29.9) | 134 (28.6) |
| HLA-B*35 | 85 (9.5) | 46 (9.8) |
| HLA-B*08 | 74 (8.3) | 53 (11.3) |

Qualitative data are no. (%) of individuals. and quantitative data are median (interquartile range). [‡]LTNP-C classification was possible for 887 patients. [‡]Data were available for 465 individuals, for whom characteristics are listed in the right column. NA= Not available in the whole cohort.

Table 3. Bi- and multivariate analysis of validation cohort

| | LTNP-C (n=242) | NonLTNP-C (n=222) | p; OR (CI 95%) | |
|------------------------------|-------------------|----------------------|-----------------------------------|-----------------------------------|
| | | | Unadjusted | Adjusted |
| Sex (male) | 197 (81.4) | 183 (82.1) | 0.774; 0.933 (0.581-1.498) | |
| Age at HIV diagnosis (years) | 32 [26-38] | 35 [29-41] | 0.001; 0.965 (0.945-0.985) | 0.001; 0.962 (0.942-0.983) |
| IFNL4 A/A genotype | 103 (42.6) | 121 (54.3) | 0.012; 0.625 (0.433-0.901) | 0.021; 0.637 (0.434-0.934) |
| HLA-B*27 [‡] | 53 (22.3) | 38 (17.9) | 0.253; 1.312 (0.824-2.089) | |
| HLA-B*57 [‡] | 84 (35.3) | 47 (22.2) | 0.002; 1.915 (1.259-2.912) | 0.003; 1.924 (1.252-2.957) |
| HLA-B*35 [‡] | 23 (9.7) | 22 (10.4) | 0.801; 0.924 (0.499-1.711) | |
| HLA-B*08 [‡] | 23 (9.7) | 26 (12.3) | 0.378; 0.765 (0.422-1.387) | |

Qualitative data are no. (%) of individuals. and quantitative data are median (interquartile range) [‡]Dominant model was applied. There were only three homozygous subjects for HLA-B57. Abbreviations: OR: Odds ratio, CI: confidence interval. Significant values are shown in bold.

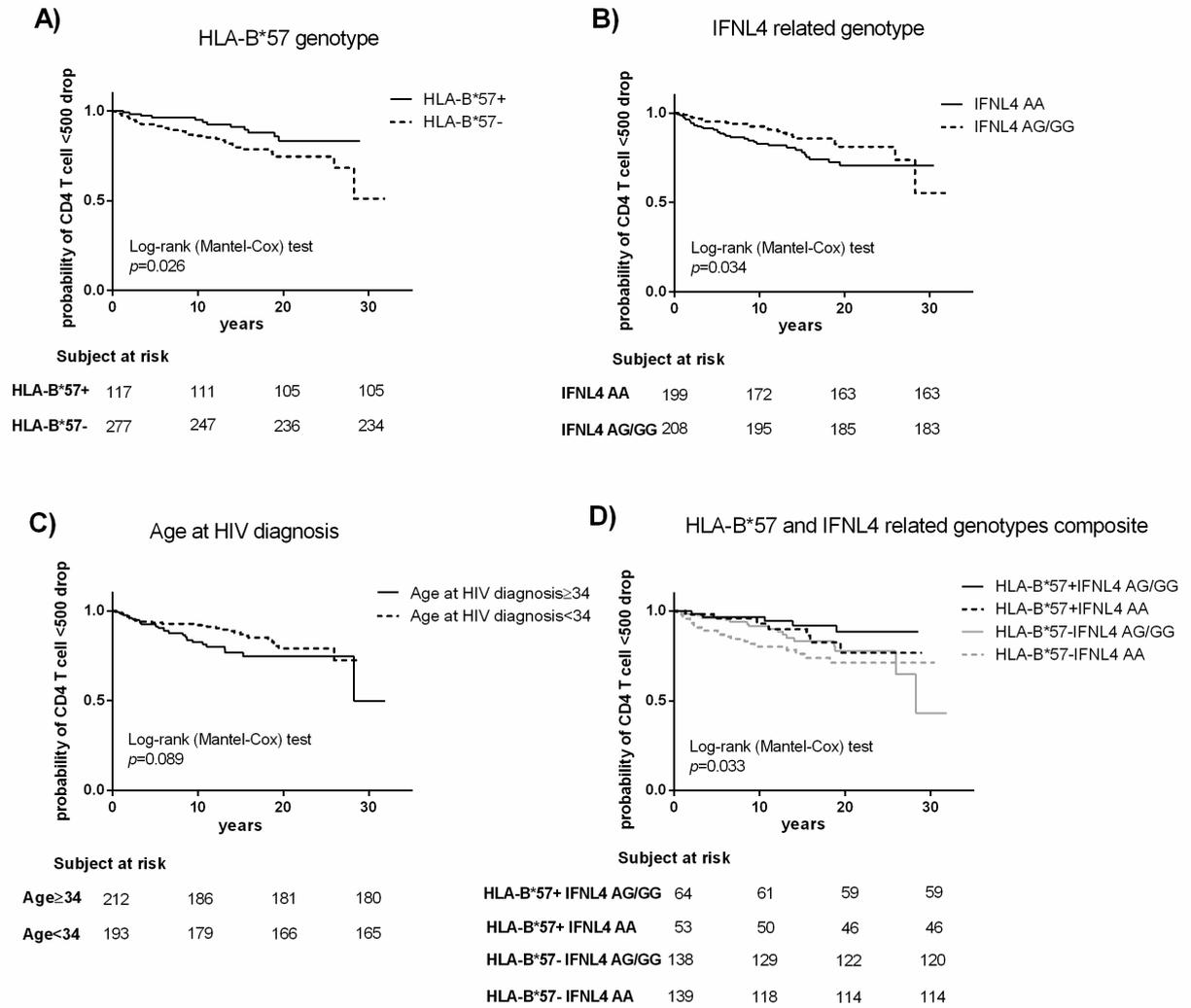
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Table 4. Time-to-CD4⁺<500 cell/mm³ drop analysis of validation cohort.

| | <i>p</i> ; HR (CI 95%) | |
|------------------------------|-----------------------------------|-----------------------------------|
| | Unadjusted | Adjusted |
| Sex (male) | 0.066; 0.585 (0.330-1.036) | |
| Age at HIV diagnosis (years) | 0.091; 1.562 (0.931-2.621) | 0.043; 1.770 (1.019-3.076) |
| IFNL4 A/A genotype | 0.038; 1.717 (1.030-2.865) | 0.037; 1.790 (1.035-3.096) |
| HLA-B*27 | 0.972; 1.041 (0.540-1.894) | |
| HLA-B*57 | 0.029; 0.490 (0.257-0.935) | 0.045; 0.510 (0.271-0.985) |
| HLA-B*35 | 0.425; 0.661 (0.258-1.835) | |
| HLA-B*08 | 0.157; 1.725 (0.811-3.662) | |

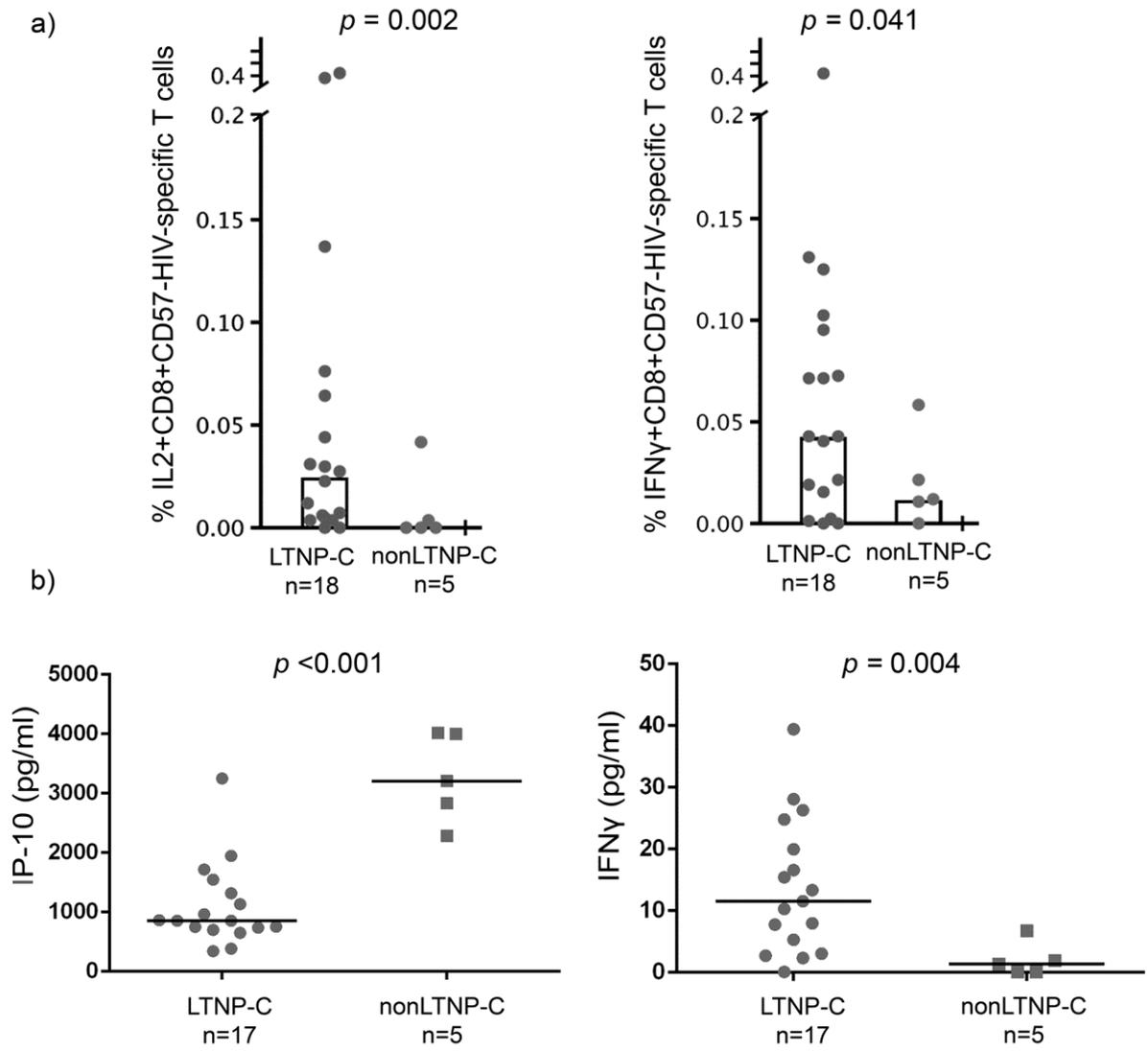
Abbreviations: HR: Hazard ratio, CI: confidence interval. Significant values are shown in bold.

Figure 1



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Figure 2



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