The Influence of host genetic factors on HIV-1 infection

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ASSOCIATION OF HLA-C AND HCP5 GENE REGIONS WITH THE CLINICAL COURSE OF HIV-1 INFECTION

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Department of Experimental Immunology, Sanquin Research, Landsteiner Laboratory, Center for Infectious Diseases and Immunity Amsterdam (CINIMA), Academic Medical Center of the University of Amsterdam, the Netherlands

ABSTRACT

Recently, a genome-wide association analysis revealed single-nucleotide polymorphisms (SNPs) in the gene regions of *HLA-C* and *HCP5* to be associated with viral load at set point and SNPs in the *RNF39/ZNRD1* gene region to be associated with HIV-1 disease course. We studied whether the association of these SNPs with viral load at set point could be replicated and whether these SNPs also associated with other clinical outcomes of HIV-1 infection in 335 HIV-1-infected homosexual participants from the Amsterdam Cohort Studies on HIV infection and AIDS (ACS).

Significant associations between the minor allele variants of SNPs *HLA-C* rs9264942 and *HCP5* rs2395029 and a lower viral load at set point could be replicated in the ACS. Moreover, these SNPs were significantly associated with delayed progression to AIDS, AIDS-related death, and a CD4$^+$ T-cell count below 400 cells/µl. Both minor allele variants were independent predictors of disease progression, also when a *CCR5* Δ32 heterozygous genotype was included in the analysis. However, predictive value was not independent from viral load and CD4$^+$ T-cell count at set point. The SNPs in the *RNF39/ZNRD1* gene region were associated with set point CD4$^+$ T-cell count but not with disease course in the ACS.

The minor allele variants of SNPs in the *HLA-C* and *HCP5* gene regions are also in the ACS associated with a lower viral load at set point and additionally with delayed HIV-1 disease progression. The association of these SNPs with the relatively early course of infection may help unravel their mode of action.
INTRODUCTION

Humans display a large variability in their susceptibility to HIV-1 infection and in subsequent disease course. In the absence of antiviral therapy, AIDS develops typically within 7-11 years after infection through homosexual contact [1-3], though very rapid (<2 years) or virtually no disease progression are observed as well [4]. The variable clinical course is determined by both viral and host factors. The emergence of HIV-1 variants that use coreceptor CXCR4 in the course of infection is associated with an accelerated CD4+ T-cell decline and more rapid progression to AIDS [5,6]. Other evidence for viral factors that may influence the clinical course of HIV-1 infection comes from a cohort of long-term nonprogressors (LTNP) who were all infected with attenuated HIV-1 caused by a deletion in the viral nef gene [7]. The human leukocyte antigen (HLA) type is a strong example of a host factor that is associated with HIV-1 disease course. HLA-B*5701 and HLA-B27 are more prevalent among LTNP whereas HLA-B35 is associated with an accelerated progression to AIDS [8-10]. The HIV-1 coreceptors and the retroviral restriction factor Trim5α are other examples of host factors in which polymorphisms have been associated with the clinical course of HIV-1 infection [11-14].

Recently, Fellay et al. [15] published the first genome-wide association (GWA) study in HIV-1-infected individuals. They reported that the minor allelic variants of single-nucleotide polymorphisms (SNPs) rs9264942 and rs2395029 in the gene regions of the HLA-C and HLA complex P5 (HCP5), respectively, were associated with a significantly lower viral load at set point. In addition, they reported a significant association between seven polymorphisms (rs9261174, rs3869068, rs2074480, rs7758512, rs9261129, rs2301753, rs2074479) located in and near the ring finger protein 39 (RNF39) and zinc ribbon domain-containing 1 (ZNRD1) genes, and HIV-1 disease progression as defined by CD4 T-cell depletion.

Here we determined whether the association of these SNPs with viral load at set point could be replicated in the Amsterdam Cohort Studies on HIV infection and AIDS (ACS) in homosexual men with an accurately imputed seroconversion date. In addition, we studied in the same ACS whether these SNPs were associated with the clinical course of infection, also in relation to known progression markers.

METHODS

Study population

We studied HIV-1-infected homosexual men who participate in the Amsterdam Cohort Studies on HIV infection and AIDS (ACS), were enrolled in the cohort between October 1984 and March 1986, and from whom long-term follow-up data is available (every 3 months: collection of clinical and epidemiological data and cryopreservation of serum and peripheral blood mononuclear cells). In the first serum sample taken at entry in the cohort, 728 men tested negative for HIV-1 antibodies and 238 men tested
positive for HIV antibodies of whom four refused to participate further; 131 of the negative men subsequently seroconverted during active follow-up (until May 1996). For seroprevalent individuals, an imputed seroconversion date (on average, 18 months before entry into the ACS) was used [16]. AIDS-free survival was similar for persons who seroconverted during the cohort study and seroprevalent persons at entry (Log Rank P value > 0.2), suggesting a good estimation of the seroconversion date in the latter group. The seroconverter cohort and the seroprevalent cohort both consisted of Caucasian men with homosexual contact as risk factor for HIV-1 infection. The mean age at (imputed) seroconversion, as well as viral load and CD4+ T-cell count at set point, was not different between both groups. Finally, heterozygosity for a 32 base-pair deletion in the CCR5 gene had a similar effect on AIDS-free survival in the two cohorts [12]. Therefore, we here used the two cohorts as one study sample (n = 365).

Most seropositive men (n = 243 [67%]) did not receive any early treatment, 70 (19%) received zidovudine monotherapy, 10 (3%) received didanosine monotherapy, and 42 (11%) received other ineffective antiretroviral therapy before AIDS diagnosis. The mean age of participants at the time of (imputed) seroconversion was 34.5 years (range 19.5 - 57.7 years).

From 335 of these 365 cohort participants a DNA sample was available for genotyping analysis and consequently these 335 individuals (205 seroprevalent cases and 130 seroconverters) were included in further analyses.

When AIDS according to the Centers for Disease Control and Prevention (CDC) 1993 definition [17] was used as an end point in Kaplan-Meier survival analysis, 235 individuals had an event, 57 were censored due to loss to follow-up, and 43 were censored because of initiation of HAART. When AIDS-related death, defined as death with AIDS-related malignancy, death with AIDS-opportunistic infections, or death with AIDS-related cause not specified by the treating physician, was used as an end point, 180 individuals had an event, 81 were censored due to loss to follow-up, and 74 were censored at initiation of HAART. When a CD4+ T-cell count of 400 cells/µl was used as an end point, 281 had an event, 49 were censored due to loss to follow-up, and five were censored at initiation of HAART. For survival analysis after AIDS diagnosis, 235 individuals were included of whom 171 had an event, 31 were censored due to loss to follow-up, and 33 were censored at initiation of HAART.

The ACS has been conducted in accordance with the ethical principles set out in the declaration of Helsinki, and written informed consent is obtained prior to data collection. The study was approved by the Academic Medical Center institutional medical ethics committee.

**Genotyping**

We used SNP data for rs2395029, rs3869068, rs2074480, rs7758512, rs9261129, rs2301753 and rs2074479 that we generated in a GWA study in the ACS (van Manen et al. manuscript in preparation) using the Illumina Infinium HumanHap300 BeadChip (Illumina, San Diego, California, USA) [18]. SNP rs9264942 (HLA-C) was absent
on this chip and determined by a predeveloped assay based on ABI TaqMan allelic discrimination-based technology and an ABI7900 Sequence Detection System (ABI, Foster City, California, USA).

**Virological assays**

Serum viral load was measured by using a quantitative HIV-1 RNA nucleic acid-based sequence amplification (Organon Teknika, Boxtel, the Netherlands) with electrochemiluminescently labeled probes [19]. Set point viral load data were available for 332 of 335 (99%) patients. For one participant, the RNA copy number in plasma was below the test threshold of quantification of the assay (50 copies/ml) and was therefore arbitrarily set at 50 copies/ml plasma. Viral load data were analyzed after log10 transformation.

**Immunologic Assays**

Enumeration of CD4⁺ T-cells was done using flow cytometry. CD4⁺ T-cell count was first measured at the first visit after entry in the ACS (for seroprevalent patients this is ~18 months after the imputed seroconversion date). Set point CD4⁺ T-cell count data were available for 325 of 335 (97%) patients.

**Statistical analysis**

For each of the three SNPs under study, the cohort was divided in homozygotes for the major allele (MAJ), heterozygotes (HZ), and homozygotes for the minor allele (MIN). The association of SNP genotypes with the clinical course of HIV-1 infection was tested in Kaplan-Meier survival analyses using AIDS according to the CDC 1993 definition [17], AIDS-related death, and CD4⁺ T-cell count below 400 cells/µl blood as end points. In addition, we studied time to death from the moment of AIDS diagnosis onwards. Log rank $P$ value was used to determine significant differences in the clinical course of infection between genotypic groups for each SNP. Univariate and multivariate analysis in a model that included the minor allele of $HLA-C$ rs9264942, the minor allele for $HCP5$ rs2395029, and the $CCR5$ Δ32 heterozygous genotype were done from seroconversion onwards. Multivariate analyses in a model that included, CD4⁺ T-cells more than 500 cells/µl blood at approximately 2 years after imputed seroconversion and a viral RNA load less 10⁴.5 copies/ml plasma at approximately 2 years after imputed seroconversion were performed from 2 years after seroconversion onwards. For Kaplan-Meier survival analysis and the univariate and multivariate analyses, left truncation of follow-up time was performed for time between imputed seroconversion date and first seropositive visit using S-PLUS 8 (Insightful Corporation, Seattle, Washington, USA).

The association of each of the three SNPs with viral load or CD4⁺ T-cell count at set point was tested using one-way analysis of variation (ANOVA) for SNPs with a MAJ, HZ, and MIN group (rs9264942 and rs2074479) or Student’s $t$-test for the SNP with only a MAJ and HZ group (rs2395029) as implemented in GraphPad Prism 5 (GraphPad Software, La Jolla, California, USA).
RESULTS

Genotype distribution for rs9264942 in the HLA-C gene region, rs2395029 in the HCP5 gene region, and rs2074479 in the RNF39/ZNRD1 gene region in the Amsterdam Cohort Studies on HIV infection and AIDS

In our study population of 335 HIV-1-infected men who participate in the ACS, we first analyzed the prevalence of eight of the nine SNPs previously described to be associated with viral load at set point or the clinical course of infection [15]. The eight SNPs under study are rs9264942 in the HLA-C gene region, rs2395029 in the HCP5 gene region, and rs3869068, rs2074480, rs7758512, rs9261129, rs2301753, rs2074479 in the RNF39/ZNRD1 gene region. Considering the high linkage disequilibrium between these latter SNPs in the ACS ($r^2 = 0.87-1$), only the results for rs2074479 are shown. Table 1 shows the allele frequencies and the percentage of individuals who were homozygous for the major allele (MAJ), heterozygous (HZ), or homozygous for the minor allele (MIN). No genotype data deviated from Hardy-Weinberg equilibrium. There was no difference in mean age at the imputed seroconversion date between individuals in the MAJ, HZ, and MIN groups for the 3 SNPs under study (data not shown).

Replication of the association of HLA-C rs9264942 and HCP5 rs2395029 genotypes with viral load at set point

The viral load at set point is considered to be established 18-24 months post seroconversion and is highly predictive for the clinical course of infection [20,21]. Viral load set point was one of the phenotypes used in the genome-wide association analysis performed by Fellay et al. [15]. To replicate their findings, we first evaluated the association between the selected SNPs and viral load at set point in the ACS. Indeed, the two SNPs reported to be associated with viral load at set point (HLA-C rs9264942 and HCP5 rs2395029) were also significantly associated with viral load at set point in the ACS (Figure 1a), with $P$ values of $2.10 \times 10^{-2}$ and $5.59 \times 10^{-3}$, respectively. The progression SNP rs2074479 in the RNF39/ZNRD1 gene region, for which a weaker association with viral load at set point was reported by Fellay et al. [15] ($P = 7.11 \times 10^{-3}$), was not associated with viral load at set point in ACS participants (Figure 1a).

Table 1. Distribution of HLA-C rs9264942, HCP5 rs2395029, and RNF39/ZNRD1 rs2074479 genotypes

<table>
<thead>
<tr>
<th>SNP</th>
<th>Change</th>
<th>Gene</th>
<th>Chr</th>
<th>MAJ (%)</th>
<th>HZ (%)</th>
<th>MIN (%)</th>
<th>Ancestral allele (%)</th>
<th>Minor allele (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs9264942</td>
<td>T&gt;C</td>
<td>HLA-C</td>
<td>6</td>
<td>164 (49.0%)</td>
<td>139 (41.5%)</td>
<td>32 (9.6%)</td>
<td>69.7</td>
<td>30.3</td>
</tr>
<tr>
<td>rs2395029</td>
<td>T&gt;G</td>
<td>HCP5</td>
<td>6</td>
<td>315 (94.0%)</td>
<td>20 (6.0%)</td>
<td>0</td>
<td>97.0</td>
<td>3.0</td>
</tr>
<tr>
<td>rs2074479</td>
<td>T&gt;C</td>
<td>RNF39/</td>
<td>6</td>
<td>273 (81.5%)</td>
<td>58 (17.3%)</td>
<td>4 (1.2%)</td>
<td>90.1</td>
<td>9.9</td>
</tr>
</tbody>
</table>

SNP, single-nucleotide polymorphism; Chr, chromosome; MAJ, homozygotes for the major allele; HZ, heterozygotes; MIN, homozygotes for the minor allele
Next, we analyzed whether these selected SNPs associated with the CD4+ T-cell count at 2 years after the imputed seroconversion date. In agreement with the association with viral load at set point, we observed significant associations between the minor alleles of HLA-C rs9264942 and HCP5 rs2395029 and a higher CD4+ T-cell count at 2 years after seroconversion, with \( P \) values of \( 1.86 \times 10^{-7} \) and \( 6.35 \times 10^{-3} \) (Figure 1b). In line with the association of SNP rs2074479 with protection from HIV-1 disease progression as defined by CD4+ T-cell depletion, there was also a strong association between the minor allele of SNP rs2074479 in the RNF39/ZNRD1 gene and a higher CD4+ T-cell count at 2 years after seroconversion with a \( P \) value of \( 9.82 \times 10^{-3} \) (Figure 1b).

Figure 1. Set point HIV viral load and set point CD4+ T-cell count per genotypic group. (a) HIV viral load at set point is correlated with the HLA-C rs9264942 genotype (left panel), where T is the major allele and C is the minor allele, and with the HCP5 rs2395029 genotype (middle panel), where T is the major allele and G is the minor allele, but not with the RNF39/ZNRD1 rs2074479 genotype (right panel), where T is the major allele and C is the minor allele. (b) CD4+ T-cell count at set point is correlated with the HLA-C rs9264942 genotype (left panel), the HCP5 rs2395029 genotype (middle panel), and with the RNF39/ZNRD1 rs2074479 genotype (right panel). MAJ: homozygotes for the major allele, HZ: heterozygotes, MIN: homozygotes for the minor allele. All comparisons were analyzed for statistical significance using ANOVA or Student’s t-test as implemented in GraphPad Prism 5 software. Mean and SEM (error bars) are represented for the respective genotypes. Number of participants per genotype is indicated below each bar. MAJ, homozygotes for the major allele; HZ, heterozygotes; MIN, homozygotes for the minor allele. \( P \) values for significance are shown above each figure.
Association of HLA-C rs9264942 and HCP5 rs2395029 genotypes with clinical course of infection

Kaplan-Meier survival analysis in all 335 HIV-1-seropositive participants showed a significantly prolonged AIDS-free survival in carriers of the MIN and HZ genotype compared with carriers of the MAJ genotype of HLA-C rs9264942 (log rank $P = 7.55 \times 10^{-4}$; median survival time in years: MAJ = 6.2 ± 0.3, HZ = 7.9 ± 0.6 and MIN = 8.7 ± 3.0; Figure 2a). A similar effect was observed when AIDS-related death was used as an endpoint in survival analysis (log rank $P = 2.78 \times 10^{-3}$; median survival time in years: MAJ = 9.2 ± 0.4, HZ = 11.7 ± 0.8 and MIN = 13.3 ± 1.9; Figure 2a). When the first CD4+ T-cell count below 400 cells/µl blood was used as an endpoint, an even stronger prolonged survival was observed for individuals who carried the MIN or HZ genotype (log rank $P = 2.03 \times 10^{-5}$; median survival time in years: MAJ = 4.0 ± 0.3, HZ = 5.3 ± 0.5 and MIN = 6.3 ± 0.9; Figure 2a), suggesting that this SNP genotype may have an effect mainly early in the clinical course of HIV infection. The minor allele of the SNP was not associated with prolonged survival after AIDS diagnosis (Figure 2a), though this may be due to insufficient power due to the small risk group for this analysis.

![Figure 2. Kaplan-Meier Survival Analysis for the HLA-C rs9264942 and HCP5 rs2395029 genotype.](image-url)
None of the ACS participants was homozygous for the minor allele of SNP rs2395029 in the HCP5 locus. Therefore, we compared individuals homozygous for the major allele with individuals heterozygous for this genotype in Kaplan-Meier survival analysis. Carriers of minor allele of SNP rs2395029 in the HCP5 gene had a prolonged AIDS-free survival and a prolonged time to AIDS-related death (log rank \( P = 3.17 \times 10^{-3} \) and \( P = 3.91 \times 10^{-3} \) respectively; median AIDS free survival time in years: MAJ= 6.6 ± 0.3, HZ= 13.4 ± 3.2, median survival time in years MAJ= 10.2 ± 0.6, HZ >15; Figure 2b). The minor allele was also associated with a prolonged time to the first moment that the CD4+ T-cell count dropped below 400 cells/µl blood (log rank \( P = 1.97 \times 10^{-4} \); median survival time in years: MAJ= 4.5 ± 0.2, HZ= 8.8 ± 2.6; Figure 2b), suggesting that also this SNP genotype may have an effect early in the course of infection. The minor allele of this SNP was not associated with prolonged survival after AIDS diagnosis (Figure 2b) though here also this may be due to insufficient power.

SNP rs2074479 in the RNF39/ZNRD1 gene region was not associated with the clinical course of HIV-1 infection in the ACS (data not shown).

Finally, none of the SNPs were associated with time to the first appearance of CXCR4-using (X4) HIV-1 variants or the prevalence of X4 variants (data not shown).

**Predictive value of HLA-C rs9264942 and HCP5 rs2395029 genotypes for HIV-1 disease course**

To study the predictive value of the SNP genotypes, we performed univariate Cox proportional-hazard analyses at the time of seroconversion for HLA-C rs9264942, and separately for HCP5 rs2395029, as only these SNPs were significantly associated with disease course in the ACS. We observed a relative hazard for progression to AIDS of 0.80 [95% confidence interval (CI) 0.70 - 0.91] for carriers of the minor allele of HLA-C rs9264942 (Table 2). The minor allele of HCP5 rs2395029 provided a relative hazard of 0.38 (95% CI 0.20-0.74) for progression to AIDS. A similar protective effect of both minor alleles was observed when AIDS-related death was used as end point in the analysis (Table 2).

Multivariate analysis indicated that HLA-C rs9264942 and HCP5 rs2395029 were both independent predictors for delayed progression to AIDS or AIDS-related death (Table 2) from seroconversion onwards. Moreover, this predictive effect was also independent of the CCR5 Δ32 heterozygous genotype that has previously been demonstrated to be associated with protection from disease progression (Table 3) [11-13].

However, independent predictive values of both HLA-C rs9264942 and HCP5 rs2395029 were lost when viral load or CD4+ T-cell count or both at set point were included in a model analyzing the time from 2 years after seroconversion, in other words after the set points for CD4+ T cell count and viral load were established (Table 4).
### Table 2. Predictive value of HLA-C rs9264942 and HCP5 rs2395029 genotype for progression to AIDS or AIDS-related death in the Amsterdam Cohort Studies on HIV infection and AIDS.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Comparison</th>
<th>n</th>
<th>Univariate</th>
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<th></th>
<th>Multivariate</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>P value</td>
<td>RH (95% CI)</td>
<td>P value</td>
<td>RH (95% CI)</td>
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<tr>
<td>rs9264942</td>
<td>MIN+HZ vs MAJ</td>
<td>335</td>
<td>6.38 x 10^{-4}</td>
<td>0.80 (0.70-0.91)</td>
<td>6.48 x 10^{-3}</td>
<td>0.83 (0.73-0.95)</td>
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<tr>
<td>rs2395029</td>
<td>HZ vs MAJ</td>
<td>335</td>
<td>4.54 x 10^{-3}</td>
<td>0.38 (0.20-0.74)</td>
<td>2.06 x 10^{-2}</td>
<td>0.45 (0.23-0.88)</td>
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</tr>
</tbody>
</table>

CI, confidence interval; MAJ, homozygotes for the major allele; HZ, heterozygotes; MIN, homozygotes for the minor allele; n, number of individuals included in analysis; RH, relative hazard; SNP, single-nucleotide polymorphism. P value from univariate and multivariate Cox proportional hazard analyses.

### Table 3. Predictive value of HLA-C rs9264942, HCP5 rs2395029, and CCR5 genotype for progression to AIDS or AIDS-related death in the Amsterdam Cohort Studies on HIV infection and AIDS.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Comparison</th>
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<th>Univariate</th>
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<td>P value</td>
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<tr>
<td>rs9264942</td>
<td>MIN+HZ vs MAJ</td>
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<td>6.38 x 10^{-4}</td>
<td>0.80 (0.70-0.91)</td>
<td>1.47 x 10^{-2}</td>
<td>0.85 (0.75-0.94)</td>
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<td>rs2395029</td>
<td>HZ vs MAJ</td>
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<td>4.54 x 10^{-3}</td>
<td>0.38 (0.20-0.74)</td>
<td>1.33 x 10^{-2}</td>
<td>0.42 (0.22-0.84)</td>
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<tr>
<td>CCR5 Δ32</td>
<td>vs WT</td>
<td>335</td>
<td>1.95 x 10^{-4}</td>
<td>0.52 (0.36-0.73)</td>
<td>2.30 x 10^{-2}</td>
<td>0.52 (0.37-0.74)</td>
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</table>

CI, confidence interval; MAJ, homozygotes for the major allele; HZ, heterozygotes; MIN, homozygotes for the minor allele; n, number of individuals included in analysis; RH, relative hazard; SNP, single-nucleotide polymorphism; WT, wild type. P value from univariate and multivariate Cox proportional hazard analyses.

### Table 4. Predictive value of HLA-C rs9264942, HCP5 rs2395029 genotype, CCR5 genotype, set point CD4+ T-cell count and set point HIV-1 viral load for progression to AIDS or AIDS-related death in the Amsterdam Cohort Studies on HIV infection and AIDS.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Comparison</th>
<th>n</th>
<th>Univariate</th>
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<td>P value</td>
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<td>P value</td>
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<td>MIN+HZ vs MAJ</td>
<td>315</td>
<td>1.43 x 10^{-3}</td>
<td>0.81 (0.71-0.92)</td>
<td>2.34 x 10^{-1}</td>
<td>0.92 (0.80-1.05)</td>
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<td>rs2395029</td>
<td>HZ vs MAJ</td>
<td>315</td>
<td>5.49 x 10^{-3}</td>
<td>0.39 (0.20-0.76)</td>
<td>1.18 x 10^{-1}</td>
<td>0.56 (0.27-1.16)</td>
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<tr>
<td>CCR5 Δ32</td>
<td>vs WT</td>
<td>315</td>
<td>1.70 x 10^{-4}</td>
<td>0.51 (0.36-0.72)</td>
<td>7.08 x 10^{-4}</td>
<td>0.54 (0.38-0.75)</td>
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<tr>
<td>CD4 &gt;500</td>
<td>cells/µl</td>
<td>307</td>
<td>3.40 x 10^{-8}</td>
<td>0.47 (0.36-0.62)</td>
<td>1.75 x 10^{-5}</td>
<td>0.55 (0.42-0.72)</td>
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<tr>
<td>HIV RNA &lt;1</td>
<td>10^{4.5}</td>
<td>log cp/ml</td>
<td>312</td>
<td>7.52 x 10^{-13}</td>
<td>0.37 (0.28-0.48)</td>
<td>9.06 x 10^{-10}</td>
<td>0.41 (0.31-0.55)</td>
<td></td>
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</tbody>
</table>

CI, confidence interval; MAJ, homozygotes for the major allele; HZ, heterozygotes; MIN, homozygotes for the minor allele; n, number of individuals included in analysis; RH, relative hazard; SNP, single-nucleotide polymorphism; WT, wild type. * Univariate and multivariate analysis in a model analyzing the time from 2 years after seroconversion until AIDS diagnosis or AIDS-related death. P value from univariate and multivariate Cox proportional hazard analyses.
<table>
<thead>
<tr>
<th>SNP Comparison</th>
<th>n</th>
<th>Univariate P value</th>
<th>RH (95% CI)</th>
<th>Multivariate P value</th>
<th>RH (95% CI)</th>
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<tr>
<td>rs9264942 MIN+HZ vs. MAJ</td>
<td>335</td>
<td>2.66 x 10^{-3}</td>
<td>0.80 (0.69-0.92)</td>
<td>2.03 x 10^{-2}</td>
<td>0.84 (0.72-0.97)</td>
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<td>rs2395029 HZ vs. MAJ</td>
<td>335</td>
<td>4.54 x 10^{-3}</td>
<td>0.38 (0.20-0.74)</td>
<td>1.33 x 10^{-2}</td>
<td>0.42 (0.22-0.84)</td>
</tr>
<tr>
<td>CCR5 Δ32 vs. WT</td>
<td>335</td>
<td>1.70 x 10^{-4}</td>
<td>0.51 (0.36-0.72)</td>
<td>7.08 x 10^{-4}</td>
<td>0.54 (0.38-0.75)</td>
</tr>
<tr>
<td>CD4 &gt;500 cells/µl</td>
<td>307</td>
<td>3.40 x 10^{-8}</td>
<td>0.47 (0.36-0.62)</td>
<td>1.75 x 10^{-5}</td>
<td>0.55 (0.42-0.72)</td>
</tr>
<tr>
<td>HIV RNA &lt;1 x 10^{4.5} log cp/ml</td>
<td>312</td>
<td>7.52 x 10^{-13}</td>
<td>0.37 (0.28-0.48)</td>
<td>9.06 x 10^{-10}</td>
<td>0.41 (0.31-0.55)</td>
</tr>
</tbody>
</table>

CI, confidence interval; MAJ, homozygotes for the major allele; HZ, heterozygotes; MIN, homozygotes for the minor allele; n, number of individuals included in analysis; RH, relative hazard; SNP, single-nucleotide polymorphism; WT, wild type.
Cumulative effect of HLA-C rs9264942 and HCP5 rs2395029 genotypes on HIV-1 disease course

Using the Kaplan-Meier product-limit method, we analyzed whether clinical progression to AIDS was further delayed with an accumulating number of independent protective genotypes fulfilled (Figure 3). Interestingly, 19 of 20 individuals who had the minor allele for HCP5 rs2395029, also had the minor allele of HLA-C rs9264942.

Figure 3. Cumulative effect of single-nucleotide polymorphism genotypes on time to AIDS or AIDS-related death in the Amsterdam Cohort Studies on HIV infection and AIDS of homosexual men. Kaplan-Meier survival analysis for progression to AIDS (left panel) or AIDS-related death (right panel) with an accumulating number of independent SNP genotypes fulfilled. (a) Absence or presence of any of the protection SNP genotypes: the minor allele of rs9264942 (homozygous and heterozygous genotype; n = 131) and heterozygous genotype of rs2395029 (n = 20). Three hundred and thirty-five individuals were included in the analysis. Black line indicates 0 progression genotypes fulfilled; gray line indicates one progression genotype fulfilled; black dashed line indicates two progression genotypes fulfilled. (b) Same as panel (a) but now including the CCR5 Δ32 heterozygous genotype (n = 64; relative to a homozygous CCR5 wild-type genotype). Black line indicates 0 progression genotypes fulfilled; gray line indicates one progression genotype fulfilled; black dashed line indicates two or three progression genotypes fulfilled. Numbers at the top of each figure represent the number of participants at risk per group, for different time points. P value from log rank test, as implemented in S-PLUS software, is denoted above each figure.
Therefore, the group of individuals with only one protective SNP genotype consisted of 152 individuals who had the protective SNP genotype in the HLA-C region and only 1 individual who had the minor allele for rs2395029 in the HCP5 gene region. This group had a relative hazard of 0.68 (95% CI 0.52-0.89) for progression to AIDS and a relative hazard of 0.70 (95% CI 0.52-0.94) for progression to AIDS-related death. This risk was even further decreased for individuals who had the minor alleles of both HLA-C rs9264942 and HCP5 rs2395029 [relative hazard 0.33 (95% CI 0.17-0.65) and 0.22 (95% CI 0.08-0.61) for AIDS and AIDS-related death, respectively. Addition of the CCR5 Δ32 heterozygous genotype only slightly influenced the relative hazard of combined genotypes. As only four individuals had the minor allele for HLA-C rs9264942 and HCP5 rs2395029 in combination with a heterozygous CCR5 Δ32 genotype these four individuals were included in the group who had two out of three protective genotypes. For this combined group, the relative hazard for AIDS and AIDS-related death were 0.31 (95% CI 0.20-0.48) and 0.21 (95% CI 0.15-0.44), respectively.

**DISCUSSION**

GWA studies allow assessment of genetic variability in relation to a phenotype without a priori assumptions about phenotype-related genes. The results of the HIV-1 host control study performed by Fellay *et al.* [15] is the first such example in the HIV-1 field. In this GWA study, SNPs in the gene regions of HLA-C and HCP5 were found to be significantly associated with viral load at set point. In addition, they observed SNPs in the RNF39/ZNRD1 gene region to be significantly associated with disease progression as defined by CD4+ T-cell depletion.

In our present study, we were able to confirm a significant association between viral load at set point for SNPs HLA-C rs9264942 and HCP5 rs2395029, respectively. In addition, we observed a strong association between these SNPs and other clinical end points. However, we did not observe an effect of rs3869068, rs2074480, rs7758512, rs9261129, rs2301753 and rs2074479 in RNF39/ZNRD1 on any of the clinical end points analyzed, except for CD4+ T-cell count at set point. Taking into account the relatively small number of study samples, the possibility remains that this is due to type 2 error. Additional studies with larger sample sizes may be necessary to confirm the association between the RNF39/ZNRD1 gene region and HIV-1 disease course.

Moreover, our cohort consists of Caucasian men who are all infected with subtype B HIV-1 most likely transmitted via homosexual contact. It remains to be established whether the association of the SNPs in the RNF39/ZNRD1 gene region and HIV-1 disease course may be replicated in cohorts with other ethnicities, HIV-1 subtypes, and HIV-1 risk factors.

The minor alleles of rs9264942 and rs2395029 in the HLA-C and HCP5 gene regions, respectively, were independent predictors of disease progression. However, their effect was noticeably reduced when viral load at set point was included as a covariate in multivariate analysis. This may imply that the effect of these two gene loci on HIV-1...
disease course is partly mediated by influencing viral load, in line with their strong association with viral load at set point, though it cannot be excluded that a lack of power is responsible for the reduction of their effect in multivariate analysis.

It remains to be established how rs9264942 in the HLA-C gene region and rs2395029 in the HCP5 gene region influence HIV-1 disease course. The SNP in HLA-C is associated with HLA-C expression levels and may thus facilitate the host immune response to viral peptides. The SNP in HCP5 is in high linkage disequilibrium with HLA-B*5701 ($r^2=1$ in our study population), which has been strongly associated with prolonged AIDS-free survival [10]. Similar to our observation for HCP5 rs2395029, HLA-B*5701 has an effect early in the course of HIV-1 infection but not in the phase after AIDS has developed [10]. It seems likely that the association of rs2395029 in the HCP5 gene region is in fact the already known effect of HLA-B*5701. However, further investigation is warranted to identify the most likely functional variants.

It may seem rather disappointing that a GWA study on HIV-1 disease course has delivered so few new host factors involved in the clinical course of disease. Indeed, the likely possibility that the effect of HCP5 rs2395029 is related to the effect of HLA-B*5701, and the absent replication of the effect of rs2074479 in RNF39/ZNRD1 in our cohort, may imply that the HLA-C gene region is the only novel host factor involved in HIV-1 disease course. However, the GWA study performed by Fellay et al. [15] was performed in a relatively small study group. Increasing the power of GWA studies by combining different cohorts of HIV-1-infected individuals may definitely reveal new host factors involved in the clinical course of infection.

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