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Research paper

Impact of polymorphisms in the *HCP5* and *HLA-C, and ZNRD1* genes on HIV viral load

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ABSTRACT

AIMS: Single nucleotide polymorphisms (SNPs) in the human leucocyte antigen (HLA) complex P5 (*HCP5*), *HLA-C*, and near the zinc ribbon domain containing 1 (*ZNRD1*) have been shown to influence viral load (VL) set point in HIV-infected individuals with a known seroconversion onset. We aimed to determine the influence of *HCP5* rs2395029, *HLA-C* rs9264942, and *ZNRD1* rs3869068 on VL in antiretroviral-naïve individuals and on time to the first VL < 51 copies/ml and on $CD4^+$ T-cell recovery after initiation of combination antiretroviral therapy (cART).

Material and methods: We genotyped the rs2395029 (A > C), rs9264942 (T > C), and rs3869068 (C > T) SNPs in 1897 Caucasians from The Danish HIV Cohort Study — a prospective, nationwide, population-based study of HIV-infected individuals in Denmark. General linear models evaluated the effect of SNPs on VL in antiretroviral-naïve individuals 0–18 months after diagnosis and on $CD4^+$ T-cell recovery during cART. Cox proportional hazard regression analysis assessed the association with time to first VL < 51 copies/ml. All models were assuming additive genetic effects.

Results: The rs2395029, rs9264942, and rs3869068 minor alleles were associated with lower VL in antiretroviral-naïve individuals (rs2395029: [mean VL (copies/ml)], A/A: 70,795 [61,660–79,433], A/C: 33,884 [19,498–58,884], P = 0.002; rs9264942: TT: 81,283 [67,608–97,724], T/C: 63,096 [54,954–75,858], CC: 38,905 [25,119–58,884], P < 0.0001; rs3869068, CC: 72,444 [63,096–83,176], C/T: 45,709 [33,113–64,565], TT: 58,884 [20,417–169,824], P = 0.01). Moreover, the C-alleles of rs2395029 and rs9264942 were associated with shorter time to VL < 51 copies/ml: (HR [95% confidence interval], 1.67 [1.09–1.72], P = 0.008; 1.16 [1.06–1.28], P = 0.002; 1.30 [1.08–1.53], P = 0.005, respectively, adjusted for last VL before cART). None of the SNPs predicted $CD4^+$ T-cell recovery during cART.

Conclusions: The minor alleles of rs2395029, rs9264942, and rs3689068 associate with lower VL among antiretroviral-naïve individuals and with shorter time to first VL < 51 copies/ml during cART even after adjustment for VL before cART.

1. Introduction

There is interindividual variation in HIV disease susceptibility and viral replication control. The factors contributing to this variation include environmental exposures, viral factors, and HIV host genetics (O'Brien and Nelson, 2004; An and Winkler, 2010; Shearer and Clerici, 2010; Piacentini et al., 2009). In recent years, genome-wide association studies (GWAS) have reported novel associations between common polymorphisms and infectious diseases (Chapman and Hill, 2012; Fellay et al., 2007; Dalmasso et al., 2008; Limou et al., 2009; Le et al., 2009; Herbeck et al., 2010; Pelak et al., 2010; Troyer et al., 2011; van Manen et al., 2009; Lingappa et al., 2011).The first GWAS of HIV-related phenotypes was performed in individuals of European ancestry and revealed 3 top single nucleotide polymorphisms (SNPs) (Fellay et al., 2007). The first SNP, rs2395029 (A > C) located in HLA complex P5 (*HCP5*), explained close to 10% of the total variation in the HIV viral load (VL) set point with the minor C-allele being associated with lower viral load. In European individuals rs2395029 is an almost perfect

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proxy for the HLA-B*5701 allele, which is strongly associated with long-term non-progression (Haynes et al., 1996; Kaslow et al., 1996; Kiepiela et al., 2004). The second SNP, rs9264942 (T > C) located upstream of the human leucocyte antigen (*HLA-C*) region, explained close to 7% of HIV VL set point, and the minor C-allele was associated with higher *HLA-C* expression levels [6]. Subsequently, it has been shown that rs9264942 is a marker for a SNP in the 3′UTR of *HLA-C*, rs67384697, which changes a miRNA binding site resulting in higher surface expression of HLA-C and, consequently, a slower disease progression. The mechanism is possibly through improved antigen presentation to cytotoxic T lymphocytes or recognition and killing of infected cells by natural killer cells (Kulkarni et al., 2011). The third SNP, rs3869068 (C > T), is located in the regulatory region of the zinc ribbon domain containing 1 (*ZNRD1*) and ring finger protein 39 (*RNF39*). The minor T-allele of rs3869068 was primarily associated with progression and was associated with lower VL at the set point. This region included rs9261174, rs3869068, rs2074480, rs7758512, rs9261129, rs2301753, and rs2074479, which all were in high linkage disequilibrium with an R^2 above 0.8. From small interfering RNA (siRNA)-based functional analysis it has been suggested that *ZNRD1* down-regulation by siRNA or small hairpin RNA (shRNA) causes an impairment of HIV-1 replication at the transcriptional level in both lymphoid and non-lymphoid tissue, suggesting an involvement of this gene in the control of HIV infection (Ballana et al., 2010). Recently, SNPs in the regulatory region of *ZNRD1* have been shown to be associated with HIV-1 disease progression and host resistance to HIV-1 acquisition (An et al., 2014). The above SNP associations have been replicated in most studies (Limou et al., 2009; Pelak et al., 2010; van Manen et al., 2009; Van et al., 2011); however, to our knowledge, the effects of these SNPs have primarily been evaluated in antiretroviral-naïve HIV-infected individuals and not during combination antiretroviral treatment (cART).

Although treatment with cART is efficient at reducing HIV VL, some patients have a low $CD4^+$ T-cell recovery despite a complete virologic response to cART (VL < 51 copies/ml) (Nicastri et al., 2005; Tan et al., 2008; Gutierrez et al., 2008). Most studies of rs2395029, rs9264942, and rs3869068 (or of SNPs in almost perfect linkage disequilibrium with rs3869068) have been conducted during early or establishing stages of HIV infection, whereas the importance of these SNPs in the later stages of HIV infection and during cART remains to be explored. As these SNPs are some of the most important HIV-related genetic variants to date, we aimed to genotype the rs2395029, rs9264942, and rs3869068 (due to complete linkage disequilibrium, a marker for rs9261174, rs2074480, rs7758512, rs9261129, rs2301753, and rs2074479) in 1700 HIV-infected Caucasians (of European descent) from the Danish HIV cohort study (DHCS). We aimed to determine the influence of rs2395029, rs9264942, and rs3869068 on VL during the antiretroviral-naïve period and during cART and on $CD4^+$ T-cell recovery during the first year with cART.

Table 1
The values are medians with IQR. cART, combination antiretroviral therapy; IDU, injection drug use; IQR, interquartile range.

HIV-infected patients	1700
Men, *N* (%)	1457 (86)
Age at initiation of cART, years, median (IQR)	40 (34–48)
Route of transmission:	
Homosexual/Heterosexual/IDU/other (%)	62/27/6/5
$CD4^+$ nadir, median (IQR)	149 (60–231)
$CD4^+$ T-cell count at start of cART, (cells/μl), median (IQR)	290 (135–450)
Viral load at baseline, copies/ml, median (IQR)	71,289 (15,400–259,726)

2. Material and methods

2.1. Study population

The Danish HIV Cohort Study (DHCS) is a prospective, nationwide, population-based cohort study of all HIV-infected individuals treated in Danish HIV clinics since 1 January 1995 (Lohse et al., 2005; Obel et al., 2008) (Table 1). The study is on-going with continuous enrollment. HIV treatment in Denmark is restricted to 8 specialized centers, and the Danish health care system provides free medical care, including antiretroviral treatment. Danish HIV-infected individuals are monitored according to local guidelines at intended intervals of 12–24 weeks. Updates of the study cohort are performed annually. The complete data on all of the patients seen in any of the centers since 1 January 1995 include: date of birth, sex, route of infection, race, place of birth, treatment centers, date of first consultation in the center, dates of most recent HIV-1 negative and positive tests, date of immigration and emigration, height, and weight. HIV VL and $CD4^+$ T-cell count were measured at least once a year. Since 2007, blood samples from individuals from the DHCS have been collected for DNA extraction. At the time of initiation of the present study, DNA was available from 1897 HIV-infected individuals (Fig. 1). For the study of $CD4^+$ T-cell change from initiation of cART up to 1 year with cART we included all Caucasians ($N = 1700$) registered in the DHCS with an available DNA sample who were at least 18 years of age, initiated cART, achieved viral suppression, and had available $CD4^+$ T-cell counts (Fig. 1). Individuals who had received one-drug or two-drug treatment prior to cART were excluded. For the study of VL in antiretroviral-naïve HIV-infected individuals, we included patients who were antiretroviral-naïve for at least 18 months and had VL measurements available. Patients

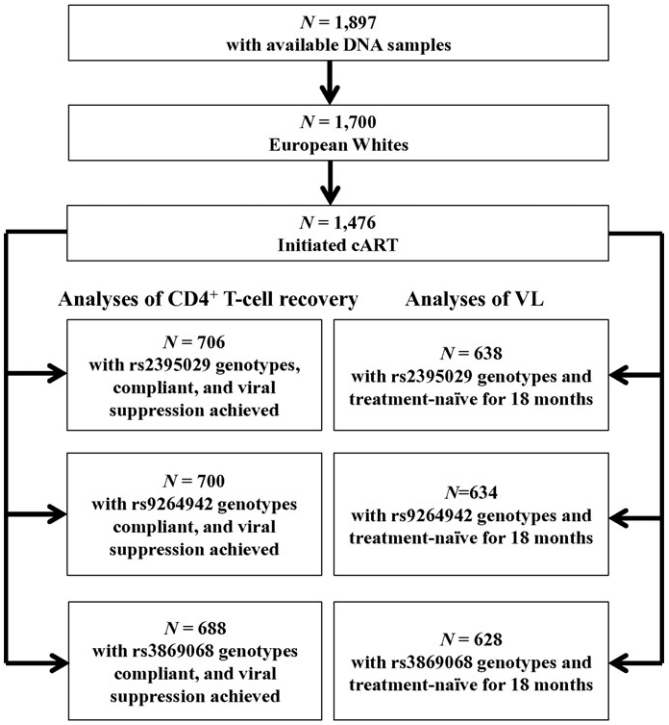

Fig. 1. Study population. At initiation of the study, 1897 DNA samples were available. A total of 197 nonwhite individuals were excluded. For the study of $CD4^+$ T-cell recovery only individuals initiating combination antiretroviral therapy (cART) and having a baseline $CD4^+$ T-cell count were included. Furthermore, only individuals achieving viral suppression within the time window and with $CD4^+$ T-cell counts within a time window of 6 months to 1 year were accepted. Furthermore, all individuals who have received one-drug or two-drug treatment prior to cART were excluded. The study of mean viral load before cART was performed within a time window of 18 months after diagnosis.