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Booiman, T.

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CHAPTER 8

Genetic variation in *Trex1* affects HIV-1 disease progression

Thijs Booiman, Laurentia C. Setiawan
and Neeltje A. Kootstra

AIDS, 2014

ABSTRACT

Objective

Three prime repair exonuclease 1 (TREX1) plays a pivotal role in HIV-1 infection. *In vitro* studies have shown that TREX1 degrades excess HIV-1 DNA thereby shielding HIV-1 from recognition by innate immune receptors and preventing a type 1 interferon response. To determine whether TREX1 plays a role in HIV-1 pathogenesis, we analyzed whether genetic variation in *Trex1* is associated with the clinical course of HIV-1 infection.

Design/Methods

Two tagging SNPs in *Trex1* were genotyped in a cohort of 304 HIV-1 infected men who have sex with men and a cohort of 66 high-risk sero-negative individuals. Kaplan-Meier and Cox regression survival analysis were used to analyze the effect of the SNPs on HIV-1 disease progression. *In vitro* HIV-1 infection assays and *Trex1* mRNA analysis were performed in peripheral blood mononuclear cells obtained from donors that were genotyped for the tag SNP in *Trex1.*

Results

We observed that the minor allele of SNP rs3135941 in *Trex1* is associated with faster HIV-1 disease progression. This association was independent of the CCR5-Δ32 genotype and HLA alleles that were previously found to be predictive for disease progression. In addition, we observed an increased HIV-1 replication in PBMC positive for the minor allele of SNP rs3135941.

Conclusions

Our data emphasizes the important role of TREX1 in HIV-1 pathogenesis. The association of SNP rs3135941 with accelerated disease progression that we observed might be explained by the increased HIV-1 replication observed in PBMC positive for the minor allele of the SNP.

INTRODUCTION

Infection of macrophages and $CD4^+$ T cells with HIV-1 does not evoke a strong antiviral interferon response[1;2]. Recently, several mechanisms have been described explaining how HIV-1 evades innate detection[3;4]. One of the cellular factors that plays a crucial role in shielding HIV-1 from innate detection is the cytosolic exonuclease three prime repair exonuclease 1 (TREX1)[2]. TREX1 degrades cytosolic HIV-1 DNA that is generated during the reverse transcription process. Knockdown of TREX1 from macrophages and CD4+ lymphocytes resulted in accumulation of cytosolic HIV-1 DNA and induction of type I interferons (IFNs) that inhibited replication and spreading of the virus[2]. Thus, by limiting the accumulation of viral DNA, TREX1 enables HIV-1 to escape from recognition by innate immune receptors[2]. While these studies have focused on the effect of TREX1 on *in vitro* HIV-1 infection, little is known about its role in the clinical course of HIV-1 infection. We hypothesize that genetic variation in *Trex1* could alter expression levels or function of TREX1 and thereby influence detection of HIV-1 by the innate immune system. These differences in HIV-1 induced innate signaling during the course of infection may therefore impact disease outcome.

METHODS

The effect of single nucleotide polymorphisms (SNPs) in the *Trex1* gene region on HIV-1 disease progression was studied in 365 HIV-1 infected men who have sex with men (MSM) that participate in the Amsterdam cohort studies (ACS) on HIV-1 and AIDS[5]. From these men, 243 did not receive any early treatment, 70 received zidovudine monotherapy, 10 received didanosine monotherapy and 42 received other ineffective antiretroviral therapy. A DNA sample was available from 335 of the 365 participants for genotyping analysis. To correct for the confounding effects of population stratification, genotyping data available from a previous study was used to remove outliers in the population (Eigenstrat, implemented in Eigensoft)[5]. As a result, DNA from 304 individuals was used for genotyping and subsequent analysis. The high-risk sero-negative (HRSN) cohort consists of 66 individuals that have a HIV-1 seronegative follow-up of more than 5 years despite unprotective receptive anogenital sex with at least 2 different non-steady partners and/or reported episode of syphilis[6].

Kaplan Meier and Cox proportional hazard analysis were performed to study the relation between polymorphisms in the *Trex1* gene and disease

progression. The following endpoints were considered for analysis: CD4 T cell counts below 200 cells/µl, AIDS according to the 1987 Centers for Disease Control and Prevention (CDC) definition, AIDS according to the 1993 CDC definition and AIDS related death. Individuals that started effective cART or that were lost to follow-up were censored. Statistical analyses were performed using IBM SPSS (version 20).

In vitro HIV-1 infection assays were performed in peripheral blood mononuclear cells (PBMC) obtained from buffy coats from 31 healthy blood donors. Cells were isolated by Ficoll-Isopaque density gradient centrifugation and stimulated for 3 days in Iscove's modified Dulbecco medium supplemented with 10% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 U/ml), ciproxin (5 µg/ml), and recombinant interleukin-2 (20 U/ml; Chiron Benelux, Amsterdam, the Netherlands) at a cell concentration of 5×10^6/ml. After 3 days, RNA was isolated to determine *Trex1* mRNA expression and in parallel the cells were infected with HIV-1 NL4-3 at a multiplicity of infection (MOI) of 0.005 and cultured for 8 days after which Gag p24 production in the culture supernatant was analyzed as a measure for viral replication by an in house ELISA.

RNA was extracted from IL-2 stimulated PBMC by TriPure Isolation Reagent (Roche, Basel, Switzerland). cDNA was prepared using the Roche Transcriptor First Strand cDNA Synthesis kit (Roche) with oligo(dT) primers. Resulting cDNA was used for quantitative PCR (qPCR) analysis using SYBR Green I Master or Probes Master (Roche) and the following primers: fwGAPDH 5′-CGAGCCACATCGCTCAGACACC-3′, revGAPDH 5′-CAAATGAGCCCCAGCCTTCTCCAT G-3′, fwTREX1 5′-GCCAAGACCATCTGCTG TCAC-3′ and revTREX1 5′-CAGGGTCCT TCACTGGAGGAA-3′. DNA of study participants was genotyped with ABI TaqMan® SNP assays (Applied Biosystems, Foster City, CA, USA): rs11797 (C__11537906_20) and rs3135941 (Custom design: rs3135941_F: 5′-TGCTGCC AGCGAGAGC-3′, rs3135941_R: 5′CAGGCAGGC AGTAGTGACT-3′ rs3135941_VIC: 5′-CTGCACGCTCTCC-3′ and rs3135941_FAM: 5′-CTGCACACTCTCC-3′).

RESULTS

We selected two tagging SNPs in the *Trex1* gene region that cover all genetic variation in the Caucasian population at a minor allele frequency of more than 5% and a r^2 of at least 0.8[7]. To determine the effect of these tag SNPs rs11797 and rs3135941 on HIV-1 disease progression and acquisition of HIV-1 infection we genotyped these SNPs in 304 HIV-1 infected MSM and 66 HRSN individuals. First we studied the effect of these SNPs on HIV-1 disease progression by using

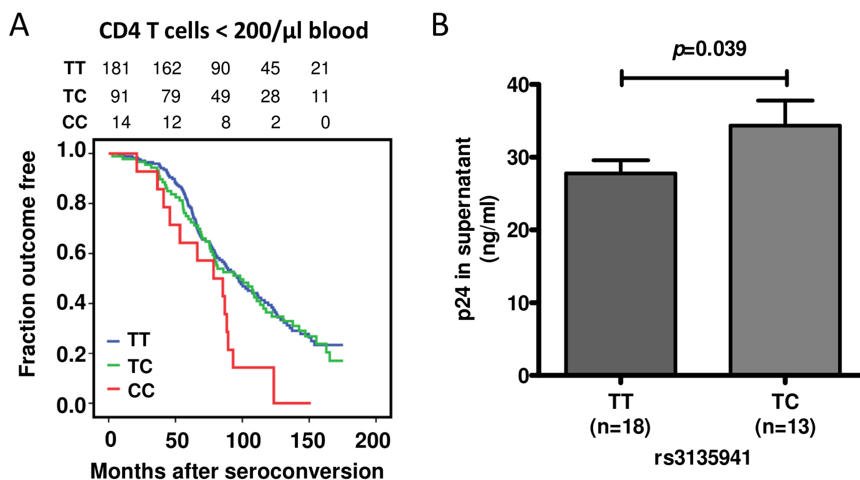

Figure 1: The effect of SNP rs3135941 on HIV-1 disease progression and *in vitro* HIV-1 replication in PBMC. (A) Kaplan-Meier survival analysis for SNP rs3135941 in *Trex1* with time in months from seroconversion to progression to $CD4^+$ T cell count below 200 cells/µl blood. (B) *In vitro* HIV-1 replication at 8 days post infection in PBMC from donors major or heterozygous for SNP rs3135941 (one-tailed independent sample T-test). Statistical analyses were performed using IBM SPSS (version 20).

Kaplan-Meier and Cox Proportional Hazard survival analysis with CD4 T cell counts below 200 cells per µl blood as an endpoint. We observed an accelerated disease progression in individuals homozygous for the minor allele of SNP rs3135941 (p=0.029) (Figure 1A and Table 1). SNP rs11797 was not associated with disease progression. Because the *Trex1* gene is located on the same chromosome as *CCR5* (chromosome 3), we determined whether the predictive value of the minor allele of SNP rs3135941 was independent of the CCR5-Δ32 genotype. In addition, we included the human leukocyte antigen (HLA)-B*27, HLA-B*35, HLA-B*57 and HLA-Cw*04 alleles, which were also found to be predictive for disease progression, in a multivariate analysis[8-13]. Indeed, the univariate analysis confirmed that heterozygosity for the CCR5-Δ32 genotype, the HLA-B*27 and HLA-B*57 alleles had a protective effect, whereas the HLA-B*35 allele, the HLA-Cw*04 allele and homozygosity for the minor allele of SNP rs3135941 were predictive for more rapid progression (Table 1). Multivariate analysis indicated that homozygosity for the minor allele of SNP rs3135941 predicts progression to CD4 T cell counts below 200 cells/µl blood independently from the other genetic markers, with a relative hazard of 2.2 (p=0.011) (Table 1). In addition, when treatment with monotherapy or inefficient therapy was included in the multivariate analysis, still an independent effect of the SNP

rs3135941 on disease progression was observed, showing that this is not a confounding factor in our study (data not shown). We also analyzed the effect of SNP rs3135941 on other endpoints for HIV-1 disease progression. In the multivariate analysis, homozygosity for SNP rs3135941 was predictive for progression from seroconversion to AIDS (CDC ’93), AIDS (CDC ’87) and to AIDS related death (Table S1).

To analyze whether these SNPs in *Trex1* affect the HIV-1 susceptibility of individuals, we compared genotype and allele frequencies between HIV-1 infected individuals and high-risk sero-negative individuals (HRSN). No significant differences in genotype or allele distribution was observed for either of the two SNPs (Table S2).

Next, we analyzed whether the difference in disease progression of individuals carrying the minor allele for SNP rs3135941 is the result of the differential ability of their macrophages and CD4+ T lymphocytes to support HIV-1 replication. First we analyzed whether there is an association between the SNP and HIV-1 replication in monocyte derived macrophages (MDM). We genotyped 388 healthy donors from our previously established MDM replication cohort for SNP rs3135941[14]. Previously, we observed that heterozygosity for the CCR5-Δ32 genotype was highly associated with *in vitro* HIV-1 replication in macrophages to HIV-1[14] and therefore donors heterozygous for the CCR5-Δ32 genotype were excluded from this analysis. No association was observed between the genotype of SNP rs3135941 and *in vitro* HIV-1 replication in macrophages (Figure S1A). To test the effect of SNP rs3135941 on *in vitro* HIV-1 replication in PBMC, we infected IL-2 stimulated PBMC from 31 healthy donors that were genotyped for the SNP with HIV-1 NL4-3. Interestingly, we observed an association between the minor allele of SNP rs3135941 and a higher HIV-1 replication (p=0.039) (Figure 1B). To test whether the minor allele of the SNP was also associated with a difference in *Trex1* expression, we determined *Trex1* mRNA expression at the moment of infection. However, no association between *Trex1* mRNA expression and SNP rs3135941 genotype was observed (Figure S1B).

Table 1: Univariate and multivariate analysis for progression to CD4 T cell counts below 200 cells/µl blood for the rs3135941 CC genotype, CCR5-Δ32 genotype, HLA-B*57 allele, HLA-B*27 allele, HLA-B*35 allele and the HLA-Cw*04 allele.

	Univariate					Multivariate		
	n	**events**	**P-value***	**RH**	**95%CI**	**P-value***	**RH**	**95%CI**
rs3135941 CC genotype	286	170	0.030	1.9	1.1-3.4	0.011	2.2	1.2-3.9
CCR5-Δ32 genotype	299	180	0.0003	0.5	0.3-0.7	0.0001	0.4	0.3-0.7
HLA-B*57	299	180	0.003	0.3	0.1-0.7	0.002	0.3	0.1-0.6
HLA-B*27	296	179	0.011	0.5	0.3-0.8	0.026	0.5	0.3-0.9
HLA-B*35	296	179	0.007	1.6	1.1-2.3	0.254	1.3	0.8-2.3
HLA-Cw*04	292	173	0.003	1.6	1.2-2.2	0.943	1.0	0.6-1.6

CI, Confidence interval; RH, relative hazard
* Cox regression survival analysis

DISCUSSION

TREX1 plays a crucial role in HIV-1 infection by inhibiting HIV-1 mediated activation of interferons in macrophages and CD4+ T cells[2]. Polymorphisms that alter TREX1 function or expression could therefore impact HIV-1 disease progression. Here we analyzed the effect of tagging SNPs rs11797 and rs3135941 on HIV-1 disease progression and acquisition of HIV-1 infection. We observed an association between the minor allele of SNP rs3135941 in *Trex1* and faster disease progression, whereas no effect was observed for SNP rs11797. The effect of SNP rs3135941 was independent of the CCR5-Δ32 genotype and HLA alleles that were previously found to be predictive for disease progression[8-13]. Genetic variation in *Trex1* had no effect on the acquisition of HIV-1 infection as demonstrated in our cohort of HRSN. Although these findings confirm previous observations, it should be noted that a relative low number of HRSN individuals was tested and that the inclusion criteria differ between the different HRSN cohorts[15].

When we analyzed the effect of SNP rs3135941 on *in vitro* HIV-1 infection in macrophages and CD4+ lymphocytes, we observed that the minor

allele was associated with increased *in vitro* HIV-1 replication in PBMC but not in macrophages. This increased replication was not explained by differential *Trex1* mRNA expression in PBMC of donors carrying the minor allele at the moment of infection. Due to its location in the 5′ untranslated region of the *Trex1* gene it might be that SNP rs3135941 is associated with altered protein expression[7]. The increased viral replication observed in PBMC from donors carrying the minor allele of the SNP, might be caused by higher TREX1 protein levels, which results in decreased induction of type I IFNs upon HIV-1 infection[2]. However, it has also been described that TREX1 regulates the activation of antiviral molecules[16], and therefore higher TREX1 protein levels might also decrease the antiviral state of the cell thereby supporting HIV-1 replication.

Here we show that the minor allele of SNP rs3135941 is associated with faster HIV-1 disease progression and in *in vitro* cultures we observed that this SNP is associated with increased HIV-1 replication in PBMC. These data suggests that the increased replication of HIV-1 in PBMC positive for the minor allele of SNP rs3135941 might explain the *in vivo* association of this SNP with the accelerated disease progression.

Ethics statement

The ACS have been conducted in accordance with the ethical principles set out in the declaration of Helsinki and all participants provided written informed consent. The study was approved by the Academic Medical Center institutional Medical ethical Committee of the University of Amsterdam.

ACKNOWLEDGEMENTS

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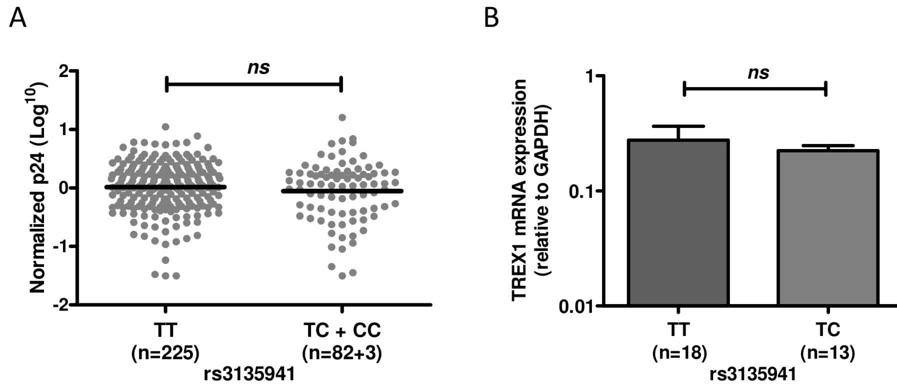
SUPPLEMENTARY MATERIALS



Supplementary figure 1: (A) Viral replication as determined by p24 production in the culture supernatant 14-days post-infection of MDMs isolated from 310 healthy donors genotyped for SNP rs3135941. (B) *Trex1* mRNA expression in IL-2 stimulated PBMC from 31 donors genotyped for SNP rs3135941.

Supplementary Table 1: Effect of SNP rs3135941 on HIV-1 disease progression

	Univariate					Multivariate		
Endpoints	n	events	P-value*	RH	95%CI	P-value⁺	RH	95%CI
CD4<200	286	170	0.029	1.9	1.1-3.4	0.003	2.4	1.3-4.3
AIDS (CDC '93)	286	199	0.16	1.5	0.9-2.7	0.033	1.9	1.1-3.4
AIDS (CDC '87)	288	144	0.29	1.4	0.7-2.8	0.049	2.0	1.0-3.8
AIDS related death	282	152	0.24	1.5	0.8-2.9	0.031	2.1	1.1-4.0

95%CI: 95% Confidence interval; RH: relative hazard

* Cox regression survival analysis for the rs3135941 CC genotype

⁺ Cox regression survival analysis for the rs3135941 CC genotype adjusted for the CCR5-Δ32 genotype