Association of polymorphisms in the LEDGF/p75 gene (*PSIP1*) with susceptibility to HIV-1 infection and disease progression

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Objective: LEDGF/p75, encoded by the *PSIP1* gene, interacts with HIV-1 integrase and targets HIV-1 integration into active genes. We investigated the influence of polymorphisms in *PSIP1* on HIV-1 acquisition and disease progression in black South Africans.

Methods: Integrase binding domain of LEDGF/p75 was sequenced in 126 participants. Four haplotype tagging SNPs rs2277191, rs1033056, rs12339417 and rs10283923 referred to as SNP1, SNP2, SNP3 and SNP4, respectively, and one exonic SNP rs61744944 (SNP5, Q472L) were genotyped in 195 HIV-1 seronegative, 52 primary and 403 chronically infected individuals using TaqMan assays. LEDGF/p75 expression was quantified by real-time RT-PCR. The impact of Q472L mutation on the interaction with HIV_1 IN was measured by AlphaScreen.

Results: rs2277191 (SNP1) A was more frequent among seropositives ($P = 0.06$, Fisher's exact test). Among individuals followed longitudinally SNP1A trended towards association with higher likelihood of HIV-1 acquisition [relative hazard (RH) = 2.21, $P = 0.08$; Cox model] and it was also associated with rapid disease progression (RH = 5.98, $P = 0.04$; Cox model) in the recently infected (primary infection) cohort. rs12339417 (SNP3)C was associated with slower decline of $CD4^+$ T cells ($P = 0.02$) and lower messenger RNA (mRNA) levels of LEDGF/p75 ($P < 0.01$). Seroconverters had higher preinfection mRNA levels of LEDGF/p75 ($P < 0.01$) and these levels decreased after HIV-1 infection ($P = 0.02$).

Conclusions: Genetic variants of *PSIP1* may affect HIV-1 outcomes. Further studies are needed to confirm the effect of genetic variation of *PSIP1* on HIV-1 pathogenesis in different cohorts. © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins

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Introduction

Human immunodeficiency virus type 1 (HIV-1) requires host cell factors for productive infection [1]. Lens epithelium-derived growth factor p75 (LEDGF/p75) also known as proprotein convertase 4 (PC4) or splicing factor, arginine/serine-rich 1 (SFRS1)-interacting protein 1 (PSIP1), is ubiquitously expressed in all tissues and cell types (by convention, the gene and its protein are referred to as *PSIP1* and LEDGF/p75, respectively). LEDGF/p75 is a member of the hepatoma-derived growth factor (HDGF)-related protein family (HRP-family) whose members are involved in chromosomal replication, transcription and chromatin structure formation [2–7]. LEDGF/p75 interacts with HIV-1 integrase through specific binding of the integrase-binding domain (IBD) of LEDGF/p75 to the catalytic core domain of integrase to tether HIV-1 to the chromosome and target HIV-1 integration into active genes [8–15]. Disruption of the interaction between LEDGF/p75 and integrase, either by integrase mutations or LEDGF/p75 knockdown, inhibits HIV-1 replication [16–19], confirming the important role of host LEDGF/p75 as an HIV-1 replication cofactor.

Association studies of the influence of human genetic variation on HIV-1 replication may reveal the essential in-vivo host factors that interact with HIV-1 and their epidemiologic importance at the population level [20]. This approach has been used mostly in studies conducted on populations from developed countries [20–23]. However, there are differences in allele frequencies among potential disease-influencing gene variants between ethnic groups and geographically separated populations [24]. Therefore, host genetic studies of HIV-1 infection need to be extended to developing world populations heavily burdened with HIV/AIDS. We investigated the influence of genetic variation in *PSIP1* on HIV-1 infection and disease progression in black South Africans.

Methods

Study participants

The Center for the AIDS Program of Research in South Africa Acute Infection 002 (CAPRISA AI 002) [25,26] and the Sinikithemba [27,28] cohorts were used for this study. The CAPRISA AI 002 cohort is an ongoing observational natural history study of HIV-1 subtype C infection established in Durban, KwaZulu-Natal, South Africa in 2004. HIV-negative women ($n = 245$) at high risk for HIV infection were enrolled into phase I of the study. Participants in this cohort were screened monthly for recent HIV-1 infection by two rapid HIV-1 antibody tests (Abbott Laboratories, Tokyo, Japan) and Capillus (Trinity Biotech, Jamestown, New York, USA). HIV-1 antibody-negative samples were tested for HIV-1 RNA in batches of 10 plasma samples per pool using the Ampliscreen v1.5 assay (Roche Diagnostics, Rotkreuz, Switzerland), which has a detection limit of 10 copies/ml. Samples that tested positive in pooled plasma were individually tested by quantitative RNA (Amplicor v2.0, Roche Diagnostics) and HIV enzyme immunoassay (BEP 2000; Dade Behring, Marburg, Germany) to identify HIV-1 infection. $CD4^+$ T-cell counts were determined by a four-parameter FACSCalibur flow cytometer (Becton Dickinson). Participants with acute HIV-1 infection were enrolled into phase II of the study on the basis of a reactive HIV antibody test within 3 months of previously negative results or positive HIV RNA PCR in the absence of antibodies. Date of infection was estimated by taking the midpoint between the last HIV antibody-negative result and the first HIV antibody-positive result or 14 days before the first positive HIV RNA PCR assay result for those identified as antibody-negative but HIV RNA-positive. An additional 34 acutely infected participants (who met the criteria for acute infection, as aforementioned) were recruited from other ongoing CAPRISA cohorts. Participants in phase II were monitored weekly for 3 weeks, fortnightly for 2 months then monthly for 9 months and quarterly thereafter.

The Sinikithemba cohort comprises 450 antiretroviral naïve, HIV-1 subtype C chronically infected adults enrolled from McCord Hospital (Durban, South Africa) from August 2003 to 2008 and followed longitudinally [27,28]. Sociodemographic characteristics, plasma viral load and CD4 cell count measurements were obtained at baseline. CD4 cell counts and viral loads were measured every 3 and 6 months, respectively, from enrollment. Viral loads were determined using the automated Cobas Amplicor HIV-1 Monitor test (version 1.5; Roche Diagnostics). $CD4^+$ T cells were enumerated using the Multitest kit (CD4/CD3/CD8/CD45) on a FACSCalibur flow cytometer (Becton Dickinson).

Ethical approval for this study was obtained from Biomedical Research Ethics Committee of the University of KwaZulu-Natal. All participants provided written informed consent.

Identification of polymorphisms in the C terminal region of *PSIP1*

Single-nucleotide polymorphisms (SNPs) in the C terminal region of *PSIP1* were screened in a panel of 83 seronegative and 43 seropositive black South African women from the CAPRISA AI 002 cohort by resequencing (Table 1).

Genotyping of single-nucleotide polymorphisms in *PSIP1* gene from the CAPRISA AI 002 participants

Four haplotype tagging (tag) SNPs rs2277191, rs1033056, rs12339417 and rs10283923 in this manuscript designated SNP1, SNP2, SNP3 and SNP4,

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Table 1. PCR, sequencing and real time PCR primers and cycling conditions used in this study.

	Gene name, accession and SNP number	Primer/probe name and rs numbers	PCR primer sequence 5′→3′, TaqMan assay ID or assay sequences	Cycling conditions: denaturation, annealing and extension
PCR	*PSIP1* AF_ 199339	LEDGF1	F: TGG GCT CAA AGC ATTA ATC C	(95°C, 10 min), 35 cycles of (94°C, 30 s; 60°C , 30 s; 72°C , 45 s) and (72°C, 10 min)
		Seq5	R: TCT GTG GCG TAT ACA CAG TG	
Sequencing		Seq1	F: GCC AGA TATGAT TTA ATC TAG	(96°C, 3 min), 25 cycles of (96°C, 15 s; 50°C, 15 s; 60°C, 2 s), (72°C, 5 min)
		Seq6	R: GTA GAC TTT TCC ATG ATT CCT GAC	
		Seq2	F: GCC TGT ATA TAG AAA TAC TGG	
		Seq5	R: TCT GTG GCG TAT ACA CAG TG	
		S9	F: CTT CAA AGG ATA CAT GC	
		R1	R: GTA GAC TTT TCC ATG ATT CCT GAC	
TaqMan Assay	SNP1	rs2277191	C_15883595_10	
	SNP2	rs10283923	C_29529242_10	
	SNP3	rs12339417	C_31936110_10	
	SNP4	rs1033056	C_2757693_20	
	SNP5	PSIP1_Q472L	PSIP1_Q472L_s AAAACCAAAGATCAAGG GAAGAAA Psip1_q472l_a TGTGAAATTGTTGGCTTTTT ACCA	
Real-time PCR		PTZ1	F: GTC AAC CCC ACC GTG TTC TTC	(95°C, 6 s), (60°C, 6 s) and (72°C, 10 s)
		PTZ2	R: TTT CTG CTG TCT TTG GGA CCT TG	
	GAPDH NM_ 002046	GAPDH1	F: AAG GTC GGA GTC AAC GGA TT	(95°C, 6 s), (60°C, 6 s) and (72°C, 10 s)
		GAPDH2	R: CTC CTG GAA GAT GGT GAT GG	

F, forward primer; R, reverse primer; SNP, single-nucleotide polymorphism.

respectively, available from NCBI dbSNP (http://www.ncbi.nlm.nih.gov/SNP) and HapMap databases (http://www.hapmap.org) selected by considering location, spacing, and allele frequency of at least 10% and one exonic SNP rs61744944 (Q472L) designated, SNP5 also available from the aforementioned databases (Fig. 1a and Table 1) were genotyped in 247 patient samples (195 seronegative and 52 seropositive individuals) from the CAPRISA AI 002 cohort. The tag SNPs were chosen because they are tag SNPs for *PSIP1* in the Yoruba from Nigeria (http://www.snp.cshl.org) and SNP5 based on the preliminary analysis of the sequencing data that suggested an association between SNP5 mutation and lower CD4 cell count and higher viral load ($P < 0.01$ for both, data not shown). Genotyping was performed by TaqMan SNP assay as per manufacturer's protocol (Applied Biosystems). TaqMan assays were obtained from the Assay-by-Demand service of Applied Biosystems (http://www.appliedbiosystems.com). Eight negative water controls were included in each run to rule out PCR contamination. Samples were genotyped in duplicate and genotypes were accepted after confirmation of no contamination or inconsistencies between duplicates.

Genotyping of single-nucleotide polymorphisms in *PSIP1* gene from the Sinikithemba participants

PSIP1 SNPs associated with HIV-1 outcomes in the CAPRISA AI 002 cohort were further analyzed in the larger Sinikithemba cohort of 450 seropositive individuals, in order to confirm the results obtained from the CAPRISA AI 002. Genotyping assays were performed as described above.

LEDGF/p75 mRNA expression analysis

Peripheral blood mononuclear cells (PBMCs) were isolated from 57 seronegative and 38 seropositive individuals from the CAPRISA AI 002 cohort. RNA was extracted from 2×10^6 PBMCs immediately after thawing using Trizol LS reagent (Invitrogen, Carlsbad, California, USA) according to the manufacturer's protocol and the integrity of RNA was confirmed using 3-(N-morpholino) propanesulfonic acid (MOPS) gels. RNA was reverse transcribed using the iScript complementary DNA (cDNA) synthesis kit (Bio-Rad). LEDGF/p75 mRNA expression was quantified by real-time PCR using SYBR Green Chemistry on a Roche Lightcycler version 1.5 (Roche Diagnostics) (Table 1).

Expression and purification of recombinant proteins

His_6-tagged HIV-1 integrase, 3xflag-tagged LEDGF/p75, MBP-JPO2 and MBP-pogZ were purified for AlphaScreen applications as described previously [29,30].

AlphaScreen

The AlphaScreen assay is a technique used to measure protein–protein affinity interactions. To measure the

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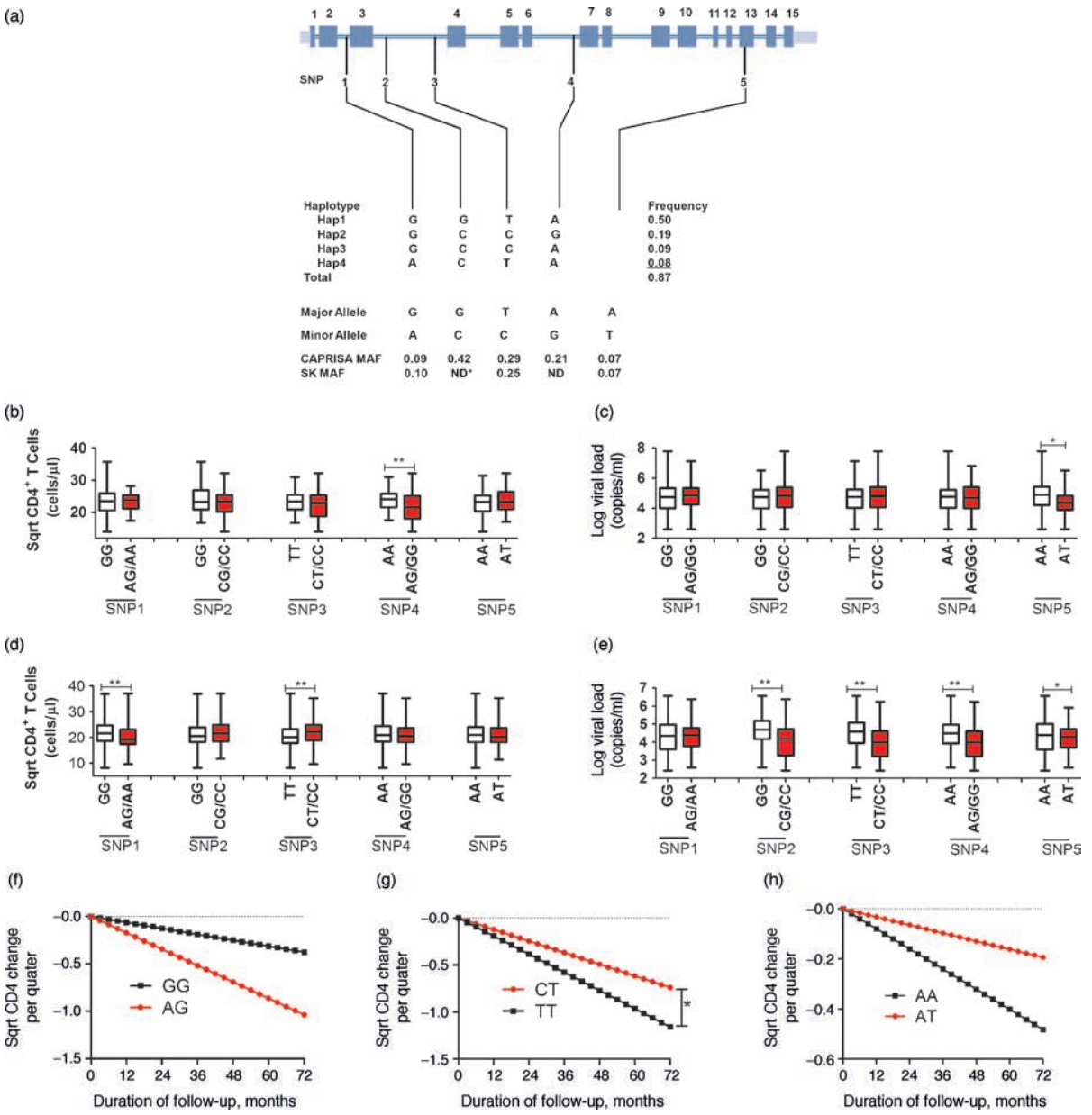

Fig. 1. Locations of *PSIP1* single-nucleotide polymorphisms on chromosome 9p22 and single-nucleotide polymorphisms influence on disease outcome. (a) *PSIP1* gene: coding exons are marked with solid blue boxes. Minor allele frequencies (MAFs) of the single-nucleotide polymorphisms (SNPs) are shown for the CAPRISA and Sinikithemba (SK) cohorts. (b and c) Association of individual SNPs with differential $CD4^+$ T-cell counts and viral loads, respectively, during the acute infection phase ($\leq$3months post infection) of the CAPRISA 002 study cohort. (d and e) Association during the early chronic phase of infection (3–12 months) of the CAPRISA 002 cohort. Rates of CD4 cell count decline stratified by genotypes for (f) SNP1, (g) SNP3 and (h) SNP5 for the Sinikithemba seroprevalent cohort. Individuals homozygous for the major allele reference genotype are indicated in black, heterozygous and homozygous for the minor allele are shown in red. MAF, minor allele frequencies for CAPRISA and Sinikithemba cohorts are shown at the bottom of panel A. ND, not done. *, An asterisk represents *P* values that remain significant after correction for multiple comparison (Bonferroni), with a single asterisk representing $P < 0.05$ and double asterisks $P < 0.01$.

influence of SNP5T (Q472L) on the binding affinity of LEDGF/p75 we performed AlphaScreen assay according to the manufacturer's protocol (Perkin Elmer, Benelux). Reactions were performed in 25 μl volume in 384-well Optiwell microtiter plates (Perkin Elmer). The reaction buffer contained 25 mmol/l Tris–HCl (pH 7.4), 150 mmol/l NaCl, 1 mmol/l $MgCl_2$, 0.01% (v/v) Tween-20 and 0.1% (w/v) bovine serum albumin. Three-hundred mmol/l His_6-tagged integrase was incubated with 100 nmol/l flag-LEDGF/p75 for an hour at 4°C. Subsequently 5 μl of Ni-chelate-coated acceptor beads and 5 μl antiflag donor beads were added to a final

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concentration of 20 μg/ml of both beads. Proteins and beads were incubated for 1 h at 30°C. Exposure of the reaction to direct light was avoided and the emission of light from the acceptor beads was measured in the EnVision plate reader (Perkin Elmer, Benelux) and analyzed using the EnVision manager software. Assays with JPO2 or pogZ, respectively, were essentially performed as described previously [29,30].

Complemented cell lines

Complemented HeLaP4/CCR5 knockdown cells (A3 clone) were generated and grown as described earlier [31]. Briefly, the Q472L mutation was introduced in pLNC_LEDGF BC-Ires-Bsd and MLV-based vectors were generated [31]. Following transduction, cells were selected with 3 μg/ml blasticidin (Invitrogen, Merelbeke, Belgium). Protein expression was verified by Western blot analysis and immunocytochemistry (data not shown).

Virus strains

The molecular clone pNL4.3 was obtained through the NIH AIDS Research and Reference Reagent Program. Virus stock ($HIV_{NL4.3}$) was produced as described earlier [31].

HIV-1 breakthrough assay

Cells were seeded at 30 000 cells per well in a six-well dish and infected as described earlier with minor modifications [19]. Briefly, cells were infected with 56 000 pg p24 $HIV_{NL4.3}$ in a total volume of 2 ml (MOI 0.01). Twenty-four hours later, cells were washed twice with 1× PBS prior to addition of 4 ml of fresh medium. HIV replication was monitored by sampling the culture medium for p24 ELISA (HIV-1 p24 ELISA kit, Perkin Elmer).

Quantitative PCR

Integrated proviral copies were quantified by real-time quantitative PCR (Q-PCR) on genomic DNA using the iQ5 Multicolor RT PCR detection system (BioRad, Nazareth, Belgium). In order to allow quantification of integrated proviral copies in HIV-$1_{NL4.3}$ infected cells, cells were subcultured at day 6 and grown under zidovudine/ritonavir treatment for 10 days, 0.5 and 1.5 μmol/l, respectively, that is 25-fold IC_{50} as determined in MT4/MTT assay [32] to eliminate all nonintegrated viral DNA. Genomic DNA was extracted using the GenElute mammalian genomic DNA miniprep kit (Sigma, Bornem, Belgium); for each reaction 100 ng was used for Q-PCR. Integrated copies for HIV-$1_{NL4.3}$ were quantified using a Gag-derived primer-probe set. Each 25 μl reaction contained 12.5 μl 2× iQ Supermix (BioRad), 40 nmol/l primer and 40 nmol/l probe. RNaseP was used as house-keeping gene control (TaqMan RNaseP control reagent; Applied Biosystems, the Netherlands). All samples were run in quadruplet and subjected to 3 min at 95°C, 50 cycles of 95°C for 10 s and 55°C for 30 s. Data were analyzed with iQ5 Optical System software (BioRad, Nazareth, Belgium).

Statistical analysis

The difference in allele frequency distribution between the HIV-1-positive and HIV-1-negative group was determined by Fisher's exact test (FET) for each SNP to test the null hypothesis that allele frequencies were the same in the two groups.

The effect of each SNP on HIV-1 viral load and $CD4^+$ T-cell count was determined using a Generalized Estimating Equation (GEE) model [33] taking into account longitudinal measures for each participant. Viral loads were log-transformed and the square root of $CD4^+$ T-cell count was used to normalize their measurements.

Kaplan–Meier survival statistics and the Cox proportional hazards model (Cox model) were used to assess the effect of each SNP on time to HIV-1 infection after enrollment and on the rate of progression to AIDS defined as $CD4^+$ T-cell decline to less than 350 cells/μl (CD4 <350 cells/μl). Decline in CD4 levels was determined and compared for the group with one or two copies of the minor allele to a reference group with two copies of the major frequency allele (dominant genetic model), for each SNP. The significance of genotypic associations and relative hazard was determined by unadjusted Cox regression analysis for the dominant genetic model.

LEDGF/p75 mRNA expression levels were compared between seronegative and seropositive individuals by performing dot plot graphical representation, nonparametric statistical analysis, and correlation (Pearson). Values were expressed as median values. Differences between the two groups were evaluated using Dunn's multiple comparison test, whereas the Mann–Whitney *U*-test was used for any two-group comparisons. The software used for the analysis was SAS version 9.1.3 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Study design and selection of patient samples

One hundred and ninety-five seronegative and 52 seropositive individuals from the CAPRISA AI 002 cohort and 403 seropositive individuals from the Sinikithemba cohort were genotyped. Thirty-four samples from the CAPRISA AI cohort and 48 samples from the Sinikithemba cohort were excluded from all analysis due to sample unavailability or poor quality genotype data.

Identification of Q472L as an exonic polymorphism in the C-terminus of LEDGF/p75

The *PSIP1* gene is 46.9 kb long and consists of 15 exons (Fig. 1a). We sequenced part of the C-terminal region to

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Table 2. Association of *PSIP1* single-nucleotide polymorphisms with HIV-1 acquisition in the CAPRISA AI 002 cohort, dominant model.

SNP, risk group	*n*	Genotype number (frequency)			22 and 12 versus 11 (reference group)*	
					RH (95% CI)	*P*
SNP1, G→A		GG	AG	AA		
SN	183	158 (0.86)	22 (0.12)	3 (0.02)		
SP	52	39 (0.75)	12 (0.23)	1 (0.01)	2.21 (0.92–5.28)	0.08
SNP2, G→C		GG	CG	CC		
SN	181	66 (0.36)	77 (0.43)	38 (0.21)		
SP	52	20 (0.38)	23 (0.44)	9 (0.17)	0.99 (0.44–2.24)	0.98
SNP3, T→C		TT	CT	CC		
SN	172	86 (0.49)	64 (0.36)	22 (0.13)		
SP	50	29 (0.58)	19 (0.38)	2 (0.04)	0.60 (0.26–1.37)	0.23
SNP4, A→G		AA	AG	GG		
SN	172	108 (0.63)	53 (0.31)	11 (0.06)		
SP	48	31 (0.65)	16 (0.33)	1 (0.02)	0.85 (0.36–2.01)	0.71
SNP5, A→T		AA	AT	TT		
SN	184	162 (0.88)	21 (0.11)	1 (0.005)		
SP	51	41 (0.80)	10 (0.20)	0 (0.00)	1.90 (0.71–5.09)	0.20

Kaplan–Meier survival statistics and the Cox proportional hazards model (Cox model) were used to assess the effect of each single-nucleotide polymorphism (SNP) on time to HIV-1 acquisition after enrollment. *P* values uncorrected for multiple comparisons are shown. CI, confidence interval; RH, relative hazard. *11 represents wild-type (homozygous) genotype, 12 heterozygous genotype, 22 mutant homozygous genotype.

screen for SNPs in 83 seronegative and 43 seropositive individuals from the CAPRISA AI 002 cohort. Fourteen previously described SNPs (dbSNP, www.ncbi.nlm.nih.gov) were discovered (data not shown). Only one SNP (rs61744944) was in the exon region and nonsynonymous (Q472L).

Effect of *PSIP1* single-nucleotide polymorphisms on susceptibility to HIV-1 infection in the CAPRISA AI cohort

The CAPRISA AI 002 cohort comprises high-risk individuals who were initially identified as seronegative and then followed longitudinally. This study design allowed us to test whether genetic variation in *PSIP1* was associated with susceptibility to HIV-1 infection. The minor allele frequencies (MAFs) of SNPs and haplotype frequencies studied are shown (Fig. 1a). Kaplan–Meier survival analysis of time to HIV-1 acquisition suggested a trend of association between SNP1A and HIV-1 acquisition (relative hazard 2.21, 95% CI 0.92–5.28; uncorrected $P = 0.08$, Cox model) (Table 2).

Association of *PSIP1* single-nucleotide polymorphisms with CD4$^+$ T-cell counts and viral load in the CAPRISA AI 002 cohort

The approximate time of infection was known for seropositive individuals in the CAPRISA AI 002 cohort [26]. Since viral loads and CD4$^+$ T-cell counts fluctuate significantly during acute HIV-1 infection, we analyzed the data in two intervals post infection, 0–3 months (acute) and 3–12 months (early chronic), to detect possible differences between genotypes during these phases of infection.

A dominant model analysis using GEE showed significant associations between SNP4G and lower CD4$^+$ T-cell count ($P<0.01$, uncorrected) (Fig. 1b) and between SNP5T and lower viral load ($P = 0.02$, uncorrected) during acute phase of infection (Fig. 1c). SNP1A was associated with lower CD4$^+$ T-cell count ($P < 0.01$, uncorrected) and SNP3C with higher CD4$^+$ T-cell count ($P < 0.01$, uncorrected) during early chronic phase of infection (Fig. 1d). The minor allele of all SNPs, except for SNP1, were associated with lower viral loads ($P < 0.05$, uncorrected) during early chronic phase of infection (Fig. 1e).

Effect of *PSIP1* single-nucleotide polymorphisms and haplotypes on CD4$^+$ T-cell decline in the CAPRISA AI 002 cohort

We tested association of genetic variation with the rate of CD4 decline to CD4 less than 350 in the CAPRISA cohort. SNP1A was associated with rapid CD4$^+$ T-cell decline ($P = 0.04$ uncorrected, Cox model) (Table 3). Haplotype 4 (HAP4) – the only haplotype carrying SNP1A – was also trending towards association with rapid CD4$^+$ T-cell decline (relative hazard 2.46, 95% CI 0.87–6.93; $P = 0.09$) (Table 3). However, the effect of SNP3C on CD4 decline could not be assessed in the CAPRISA AI 002 cohort as there were only three SNP3C participants whose CD4 cell count had dropped to 350 cells/μl and below, at the time of this analysis.

Effect of *PSIP1* single-nucleotide polymorphisms on CD4$^+$ T-cell decline in the Sinikithemba cohort

Interpretation of the data from the CAPRISA AI 002 cohort was complicated by small sample size of the cohort and fluctuations in CD4$^+$ T-cell counts and viral loads that characterize acute and early chronic phases of HIV-1 infection. To further elucidate the role of *PSIP1* genetic variants in HIV-1 pathogenesis, we extended our analysis

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Table 3. Effects of *PSIP1* single-nucleotide polymorphisms on disease progression in the CAPRISA AI 002 cohort, dominant model.

		SNPs on AIDS progression				Haplotypes on AIDS progression		
Endpoint	SNP number	RH	95% CI	*P*	Haplotype number	RH	95% CI	*P*
CD4 <350 cells/μl	SNP1	5.98	2.27–5.82	0.04	HAP1	1.84	0.55–6.12	0.77
CD4 <350 cells/μl	SNP2	2.68	0.99–7.24	0.70	HAP2	0.84	0.19–3.77	0.21
CD4 <350 cells/μl	SNP3	1.25	0.44–3.56	0.29	HAP3	0.00	0.00–0.00	0.99
CD4 <350 cells/μl	SNP4	2.05	0.70–6.02	0.90	HAP4	5.41	1.92–5.24	0.09
CD5 <350 cells/μl	SNP5	1.74	0.50–6.05	0.71	HAP5	1.58	0.36–7.49	0.66

Cox proportional hazards model (Cox model) was used to calculate the rate of CD4 decline to less that 350 cells/ml. *P* values uncorrected for multiple comparisons are shown. CI, confidence interval; SNP, single-nucleotide polymorphism.

to the larger Sinikithemba cohort from the same geographical area. SNP2 and SNP4 were excluded from this analysis because they were not associated with $CD4^+$ T-cell count during early chronic phase of infection in the CAPRISA AI 002 cohort. Since the Sinikithemba cohort is a seroprevalent cohort with unknown dates of infection, we assessed the influence of each of SNPs on disease progression by comparing the slopes of $CD4^+$ T-cell decline levels using the dominant model. SNP3C was associated with delayed disease progression as reflected by a slower rate of $CD4^+$ T cells decline to CD4 less than 350 ($P = 0.02$ uncorrected, Cox model) (Fig. 1g).

LEDGF/p75 mRNA expression levels in the CAPRISA AI 002 cohort

Quantitative RT-PCR on LEDGF/p75 mRNA levels was performed in 57 seronegative and 38 seropositive individuals. PBMCs obtained from seropositive individuals had reduced LEDGF/p75 expression compared to PBMCs obtained from seronegative individuals ($P = 0.02$) (Fig. 2a). For 13 of seropositive individuals, samples pre (baseline) and post-HIV-1 infection were available for analysis. Baseline PBMCs from seroconverters had higher LEDGF/p75 expression compared to PBMCs from nonseroconverters ($P < 0.01$) (Fig. 2b). SNP3C was significantly associated with reduced levels of LEDGF/p75 ($P < 0.01$) (Fig. 2c) in both the seronegative and seropositive groups and slower rate of $CD4^+$ T-cell decline (Fig. 1g) in the seropositive group.

Functional analysis of Q472L LEDGF/p75

To determine the impact of the Q472L mutation on the interaction with integrase – since this exonic mutation was located adjacent to the IBD (amino acids 347–429) – we measured LEDGF/p75-IN affinity in an AlphaScreen assay and evaluated the effect of the Q472L mutation on the interaction with JPO2 and pogZ, two cellular binding partners of LEDGF/p75 [29,30]. Potent RNAi-mediated knockdown of LEDGF/p75 also severely hampers HIV replication affecting the integration step of the provirus [19,34], a phenotype that is readily rescued upon re-introduction of RNAi resistant LEDGF/p75 (LEDGF BC) [31]. In a similar setting, we complemented potent LEDGF/p75 knockdown cells with the Q472L variant (LEDGF BC Q472L). Q472L did not alter the binding affinity of LEDGF/p75 for integrase, JPO2 and pogZ and complementation of LEDGF/p75-depleted cells with mutant LEDGF/p75 (Q472L) rescued HIV-1 replication to near wild-type levels (data not shown).

Discussion

LEDGF/p75 promotes HIV-1 replication *in vitro* [9–11, 13,15,34,35]. In this study, we investigated the association between genetic variation in *PSIP1* and susceptibility to HIV-1 infection and disease progression in two South African cohorts. We found that SNP1A was associated with higher likelihood of HIV-1 acquisition, lower $CD4^+$

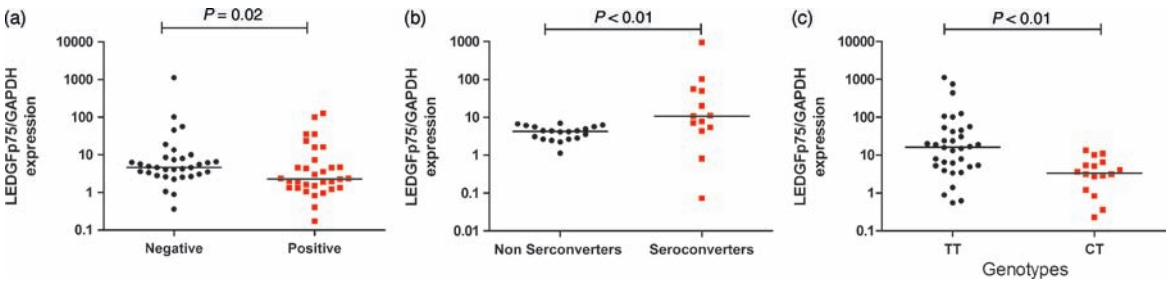

Fig. 2. Expression of LEDGF/p75 mRNA in peripheral blood mononuclear cells (PBMCs) represented as the normalized ratio of LEDGF/p75 to GAPDH. (a) Expression of LEDGF/p75 mRNA in PBMCs obtained from HIV-1-positive versus negative participants. (b) Expression of LEDGF/p75 mRNA in PBMCs obtained seroconverters versus nonseroconverters. (c) Expression of LEDGF/p75 mRNA as modulated by different genotypes of SNP3. TT, wild type genotype of SNP3. CT, heterozygous genotype of SNP3. (–) The horizontal line in the middle of the points denotes the median value.

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T-cell counts during the early chronic phase of infection and rapid $CD4^+$ T-cell depletion in CAPRISA 002 acute infection cohort. SNP3C was associated with higher $CD4^+$ T-cell count and lower viral load during the early chronic phase of infection in the CAPRISA AI 002 cohort.

The Sinikithemba cohort comprises chronically HIV-1-infected individuals with unknown date of infection and therefore we could examine the effect of SNPs on trajectory of $CD4^+$ T-cell decline over 6 years of follow-up. SNP3C was significantly associated with delayed disease progression as was reflected by a slower rate of $CD4^+$ T-cell decline in the Sinikithemba cohort. The protective effect of SNP3C was consistent between the two cohorts suggesting that SNP3C may be associated with reduced HIV-1 replication.

Next, we investigated the association between *PSIP1* genetic variation and expression levels of LEDGF/p75 and found that SNP3C was significantly associated with lower levels of LEDGF/p75 which corroborates the plausible role of SNP3C in reducing HIV-1 replication. These findings suggest that SNP3C reduces the mRNA levels of LEDGF/p75 thereby inhibiting HIV-1 replication, a finding that is consistent with in-vitro knockdown studies [19,36]. Preinfection PBMCs from seroconverters had significantly higher LEDGF/p75 mRNA levels compared to nonseroconverters. HIV-1 infection reduced LEDGF/p75 expression to lower levels compared to uninfected individuals. These findings suggested that high levels of LEDGF/p75 may increase the likelihood of HIV-1 acquisition and the rate of disease progression confirming that LEDGF/p75 is an important host factor for productive HIV-1 infection [10]. On the contrary, SNP1 associated with differential susceptibility and early HIV-1 infection outcomes in the CAPRISA acute infection cohort but not in the Sinikithemba chronic infection cohort. This SNP was not associated with differential LEDGF/p75 mRNA expression levels and future studies will need to address other possible contributing mechanisms.

Lastly, we analyzed the impact of the Q472L mutation on LEDGF/p75–integrase interaction and plausible effects on cellular binding factors of LEDGF/p75, JPO2 and pogZ. Q472L did not alter the binding affinity of LEDGF/p75 for integrase, JPO2 and pogZ and complementation of LEDGF/p75-depleted cells with mutant LEDGF/p75 (Q472L) rescued HIV-1 replication to near wild-type levels. It is not inconceivable that this mutation may affect other LEDGF/p75 functions such as integration site targeting.

Conclusion

These findings demonstrate that genetic variation in *PSIP1* may influence susceptibility to HIV-1 infection and disease progression, which provides in-vivo evidence that LEDGF/p75 is an important host cofactor for HIV-1 replication. However, due to small sample size and heterogeneous nature of our cohorts, our findings should be interpreted with caution and will need to be replicated in additional studies.

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Conflicts of interest

There are no conflicts of interest.

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