APOBEC3G expression is dysregulated in primary HIV-1 infection and polymorphic variants influence CD4^{+} T-cell counts and plasma viral load

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Objectives: In the absence of HIV-1 virion infectivity factor (Vif), cellular cytosine deaminases such as apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3G (APOBEC3G) inhibit the virus by inducing hypermutations on viral DNA, among other mechanisms of action. We investigated the association of *APOBEC3G* mRNA levels and genetic variants on HIV-1 susceptibility, and early disease pathogenesis using viral load and CD4^{+} T-cell counts as outcomes.

Methods: Study participants were 250 South African women at high risk for HIV-1 subtype C infection. We used real-time PCR to measure the expression of *APOBEC3G* in HIV-negative and HIV-positive primary infection samples. *APOBEC3G* variants were identified by DNA re-sequencing and TaqMan genotyping.

Results: We found no correlation between *APOBEC3G* expression levels and plasma viral loads ($r = 0.053$, $P = 0.596$) or CD4^{+} T-cell counts ($r = 0.030$, $P = 0.762$) in 32 seroconverters. *APOBEC3G* expression levels were higher in HIV-negative individuals as compared with HIV-positive individuals ($P < 0.0001$), including matched pre and postinfection samples from the same individuals ($n = 13$, $P < 0.0001$). Twenty-four single nucleotide polymorphisms, including eight novel, were identified within *APOBEC3G* by re-sequencing and genotyping. The *H186R* mutation, a codon-changing variant in exon 4, and a 3′ extragenic mutation (*rs35228531*) were associated with high viral loads ($P = 0.0097$ and $P < 0.0001$) and decreased CD4^{+} T-cell levels ($P = 0.0081$ and $P < 0.0001$), respectively.

Conclusion: These data suggest that *APOBEC3G* transcription is rapidly downregulated upon HIV-1 infection. During primary infection, *APOBEC3G* expression levels in peripheral blood mononuclear cells do not correlate with viral loads or CD4^{+} T-cell counts. Genetic variation of *APOBEC3G* may significantly affect early HIV-1 pathogenesis, although the mechanism remains unclear and warrants further investigation.

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Introduction

Apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3G (APOBEC3G), a human cytidine deaminase, has potent antiviral activity [1–4], and its polymorphic variants may modulate resistance to infection or disease progression among those infected [5]. In the absence of the HIV-1 accessory protein, virion infectivity factor (Vif), APOBEC3G, is packaged into budding virions, and subsequently deaminates dC to dU in the retroviral minus strand reverse transcripts in target cells. These substitutions register as dG (guanidine) to dA (adenine) transitions in retroviral plus stranded DNA [6]. Excessive G-to-A substitutions, known as hypermutation, are common among lentiviruses and introduce multiple termination codons across their genomes [6–9]. APOBEC3G and other cytidine deaminases may also inactivate lentiviruses by other mechanisms apart from hypermutation [10,11]. Hypermutated viral sequences have been identified in long-term nonprogressors and were predominant over time, suggesting that cytidine deaminases may play a role in viral control *in vivo* [12,13]. HIV-1 Vif counteracts APOBEC3G by blocking its encapsulation into virions, targeting the host protein to the ubiquitin pathway for proteasome-mediated degradation, resulting in the eradication of APOBEC3G and the loss of its anti-HIV activity [14–19].

Increased expression of *APOBEC3G* may overcome the effects of Vif by providing a competitive advantage over time and may eventually incapacitate the virus and suppress viremia [20]. Additionally, genetic variants of *APOBEC3G* may alter its function or level of expression, thereby enhancing or diminishing its anti-HIV activity [21–23].

Given that APOBEC3G is a key intrinsic antiretroviral host factor that possesses significant anti-HIV-1 activity *in vitro*, we reasoned that its antiviral effects *in vivo* might be particularly pronounced during the primary infection phase before adaptive immune responses become established. We therefore, investigated the hypothesis that high mRNA levels of *APOBEC3G* in peripheral blood mononuclear cells (PBMCs) of seroconverters are associated with low viral setpoint and high $CD4^+$ T-cell counts during primary HIV-1 subtype C (HIV-1C) infection. We also investigated the effects of *APOBEC3G* genetic polymorphisms on HIV-1C pathogenesis in a South African cohort, in a population where the HIV-1 epidemic is severest.

Participants and methods

Study participants

The Centre for the AIDS Programme of Research In South Africa (CAPRISA) acute infection study [24] is an observational natural history study of HIV-1C infection established in Durban, South Africa, in 2004. Two hundred and forty-five women at high risk for HIV infection were enrolled into phase 1 of the study. Participants were screened monthly and seroconverters were identified by two HIV-1 rapid antibody tests, Determine (Abbott Laboratories, Tokyo, Japan) and Capillus (Trinity Biotech, Jamestown, New York, USA). Antibody-negative samples underwent pooled PCR testing for HIV-1 RNA (Ampliscreen v1.5; Roche Diagnostics, Rotkreuz, Switzerland). HIV-1 RNA-positive samples were subsequently confirmed by quantitative RNA (Amplicor v2.0; Roche Diagnostics) and HIV enzyme immunoassay test (BEP 2000; Dade Behring, Marburg, Germany). Participants with acute HIV infection and those from other seroincidence cohorts were recruited into phase 2 on the basis of a reactive HIV antibody test within 3 months of a previously negative result or PCR positive in the absence of antibodies. The estimated time of seroconversion was determined as the midpoint between the last antibody-negative and first antibody-positive test or 14 days before the participant was PCR positive and antibody negative. Acutely infected participants are followed weekly for 3 weeks, fortnightly until 3 months after infection, monthly until 12 months after infection and thereafter quarterly for a maximum of 5.5 years. A flow diagram summarizing the study cohort and experiments is available in Fig. 1. Ethical approval was obtained from the University of KwaZulu-Natal's Biomedical Research Ethics Committee and all participants provided written informed consent.

Sample collection, measurement of CD4 cell counts and plasma viral load

Blood was obtained by venipuncture and PBMCs were isolated by Ficoll–Histopaque (Sigma, St Louis, Missouri, USA) density gradient centrifugation and frozen until use. Viral load was determined using the automated COBAS AMPLICOR HIV-1 Monitor Test v1.5 (Roche Diagnostics). CD4 cells were enumerated by using the Multitest kit (CD4/CD3/CD8/CD45) on a four-parameter FACS Calibur flow cytometer (Becton Dickinson, Franklin Lakes, New Jersey, USA).

mRNA expression analysis

APOBEC3G mRNA expression was quantified in 30 HIV-negative participants and in longitudinal samples of 32 HIV-positive participants. Additionally, 13 of the 32 HIV-positive participants had preinfection (baseline) samples available. RNA was isolated from cryopreserved PBMCs immediately after thawing using Trizol reagent (Invitrogen, Carlsbad, California, USA) according to the manufacturer's protocol. RNA was reverse transcribed to synthesize cDNA using the Quantitect Reverse Transcription Kit (Qiagen, Venlo, The Netherlands).

APOBEC3G mRNA expression was quantified by real-time PCR using SYBR Green chemistry (Roche

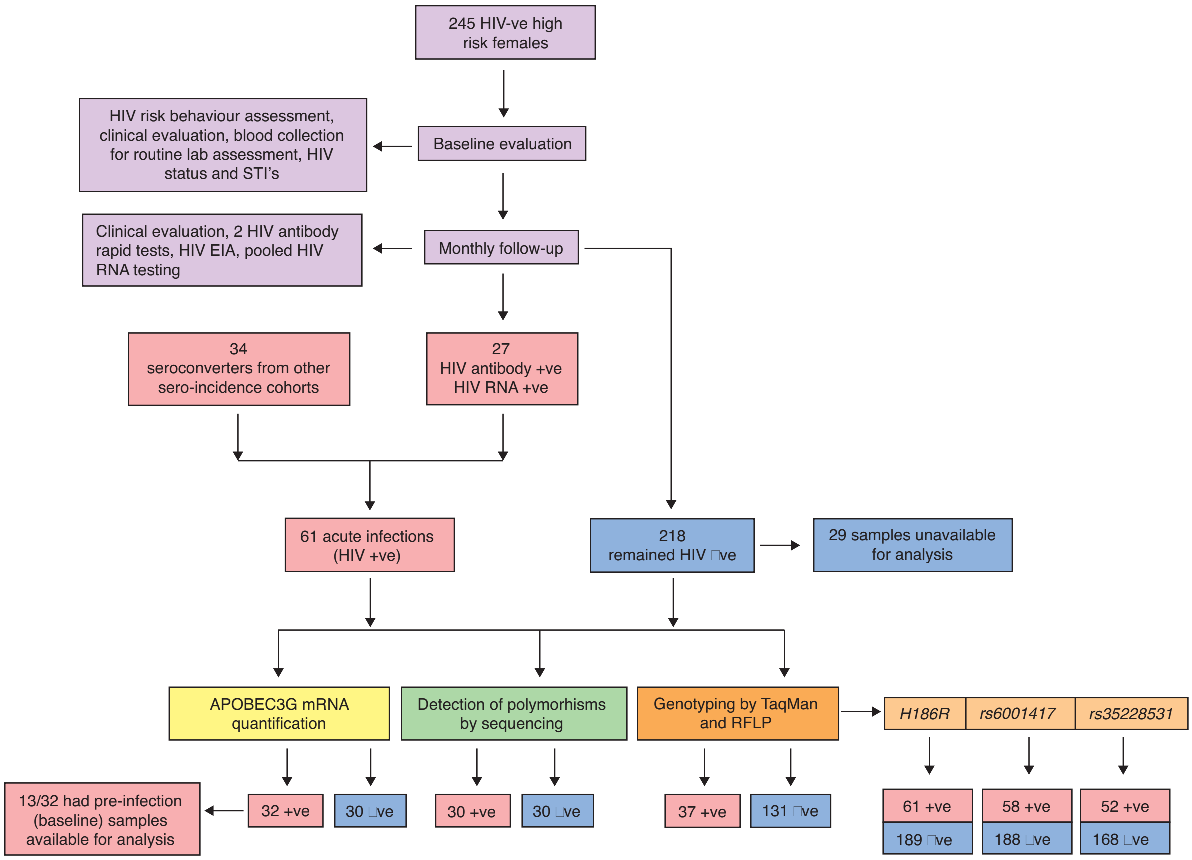

Fig. 1. Outline of study cohort and experiments. HIV-positive samples are indicated by pink blocks and HIV-negative samples are indicated by blue blocks.

Diagnostics). Target-specific primers, used to amplify *APOBEC3G*, were previously published [20]. The housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (NM_002046), was used to normalize for variations in cell count or differences in nucleic acid extraction. GAPDH primers were: forward, 5′-AAGGTCGGAGTCAACGGATT-3′ (nucleotides 115–134); reverse, 5′-CTCCTGGAAGATGGTGATGG-3′ (nucleotides 320–339). Each optimized 10 μl PCR reaction contained 1–2 μl of 25 mmol/l $MgCl_2$ (primer set dependent), 1 μl of 10× LightCycler FastStart DNA Master SYBR Green I (Roche Diagnostics), 0.2 μl of each 10 μmol/l *APOBEC3G* primer or 0.5 μl of each 10 μmol/l *GAPDH* primer and 2 μl of complementary DNA (cDNA) template. Reactions were run on the LightCycler Instrument Version 1.5 (Roche Diagnostics). PCR cycling conditions were one cycle at 95°C for 10 min, 40 cycles of 95°C for 5 s, 55°C (*APOBEC3G*) or 65°C (*GAPDH*) for 15 s and 72°C for 5 s. Standard curves were generated for *APOBEC3G* and *GAPDH* from 10-fold serial dilutions of cDNA of known concentration. Standard curves were imported into each PCR run and was used by LightCycler software (Roche Diagnostics) to quantify each gene in a sample by extrapolation. Samples and standards were run in duplicate and average values were used to compute *APOBEC3G* and *GAPDH* copy number. Relative expression levels of *APOBEC3G* to *GAPDH* in each sample were determined by dividing the concentration of *APOBEC3G* by the concentration of *GAPDH*.

Detection of *APOBEC3G* polymorphisms

A DNA panel of 30 HIV-positive and 30 HIV-negative samples was resequenced to identify single-nucleotide polymorphisms (SNPs) in *APOBEC3G*. Sequencing primers and protocols used were previously published [22]. Primers covered the putative 5′ regulatory region, eight exons, exon–intron junctions, intron 1 and the 3′ untranslated region of the *APOBEC3G* gene (GenBank sequences AL022318 and AL078641). These regions were amplified separately. Each 25 μl PCR reaction contained 10× PCR buffer, 1.5 or 2.5 mmol/l $MgCl_2$ (primer set dependent), 2.4 mmol/l deoxynucleoside triphosphate mix, 0.15 μl TaqGold and 5 μmol/l of each forward and reverse primer. This was amplified at 95°C for 10 min, 35 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 45 s and a final 10-min extension step at 72°C. PCR products were purified using exonuclease and shrimp alkaline phosphatase (Amersham Pharmacia, Uppsala, Sweden) and sequenced using overlapping primers and a BigDye Terminator Kit (Applied Biosystems, Foster City, California, USA). Sequencing primers, regions amplified and PCR conditions are available in supplementary material (Table S1).

Genotyping of variants

Five SNPs, identified by resequencing *APOBEC3G*, were further genotyped in 168 samples (37 HIV-positive and 131 HIV-negative). TaqMan SNP genotyping assays and PCR restriction fragment length polymorphism (RFLP) were used to determine genotypes for these polymorphisms. TaqMan assays were carried out according to the manufacturer's protocol (Applied Biosystems). PCR primers, conditions and restriction enzymes used for the RFLP assay and details of TaqMan genotyping assays are available in supplementary material (Table S2). After preliminary statistical analysis, the *H186R*, *rs6001417* and *rs35228531* SNPs were selected for further genotyping on the basis of their strong association with viral load and CD4 cell count. H186R was genotyped in 250 samples (61 HIV-positive and 189 HIV-negative), *rs6001417* was genotyped in 246 samples (58 HIV-positive and 188 HIV-negative) and *rs35228531* was genotyped in 220 samples (52 HIV-positive and 168 HIV-negative).

Statistical analysis

APOBEC3G mRNA expression levels were compared between HIV-negative and HIV-positive individuals using a generalized estimating equation (GEE) model [25–27]. This analysis takes into account longitudinal (repeated) measures for each participant. The association between *APOBEC3G* mRNA levels and viral loads and CD4 cell counts was determined using rank correlation tests. Fisher's exact test was used to test the association between HIV status and *H186R* (*rs8177832*), *rs6001417* and *rs35228531* genotypes. The genetic effects of these mutations on viral loads and CD4 cell counts were also determined by a GEE model taking into account longitudinal measures for each participant. The data were represented by a locally weighted scatterplot smoothing model, which was used to plot smooth curves over the data points. A Kaplan–Meier survival analysis was performed to assess the difference in CD4 decline between the H186R, *rs6001417* and *rs35228531* genotype groups, and Cox regression was used to acquire hazard ratios. All statistical analysis was performed using SAS version 9.1 (SAS Institute, Cary, North Carolina, USA) and graphs were generated using GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, California, USA).

Results

APOBEC3G expression

There is paucity of data regarding the interplay between HIV-1 and *APOBEC3G* expression *in vivo*, particularly, during primary infection when rapid viral replication occurs, followed by resolution of viremia and establishment of steady-state equilibrium between the virus and the body's immune responses. We therefore, investigated whether primary HIV-1C infection is associated with changes of *APOBEC3G* expression in PBMCs as compared with HIV-negative samples. Comparison of *APOBEC3G* mRNA levels between HIV-negative and HIV-positive individuals within 12 months of infection (primary infection) showed that *APOBEC3G* levels were significantly higher in HIV-negative individuals than in HIV-positive individuals ($P < 0.0001$) (Fig. 2a). Additionally, comparison of *APOBEC3G* expression levels in matched pre and postinfection samples of seroconverters also showed that *APOBEC3G* expression was significantly higher before seroconversion ($P < 0.0001$) (Fig. 2b). Further, there was no significant difference in APOBEC3G levels when compared between individuals who are persistently seronegative and preinfection samples of seroconverters (Fig. 2c). Comparison of *APOBEC3G* mRNA levels at various time points after infection (Fig. 2d) showed no significant change in expression levels over time.

There is conflicting data on the relationship between *APOBEC3G* mRNA levels in PBMCs versus plasma viral load and CD4 cell counts in chronic HIV-1 infection [20,28,29]. We thus next investigated whether there is a correlation between *APOBEC3G* mRNA levels and HIV-1 plasma viral load and CD4 cell counts during primary HIV-1 infection and found no association between these factors (data not shown).

APOBEC3G variants

APOBEC3G genetic variants have not been described in African populations. By resequencing and genotyping, we identified 24 SNPs within *APOBEC3G* in our cohort (Table 1). Sixteen of these SNPs were described previously and eight were novel. Further, An *et al.* [22] described seven SNPs within *APOBEC3G* in a USA-based study cohort, four of which were identified in our study cohort. Frequencies of these SNPs in our cohort were similar to those of the African–American group in the USA-based cohort (Table 1).

The codon-changing variant, *H186R* (*rs8177832*), in exon 4, had a frequency of 0.307 and was analyzed further, as the 186R allele was previously shown to have AIDS-accelerating effects [22]. Two other APOBEC3G variants, *rs6001417* in intron 3 and *rs35228531*, an extragenic mutation located 3′ near the gene, having allele frequencies of 0.303 and 0.207, respectively, were also further analyzed after preliminary analysis indicated that they also had AIDS-accelerating effects.

Effects of *H186R*, *rs6001417* and *rs35228531* mutations on primary HIV pathogenesis

H186R genotypes were determined for 250 individuals (61 HIV-positive and 189 HIV-negative). We tested the association between HIV status and *H186R* genotypes

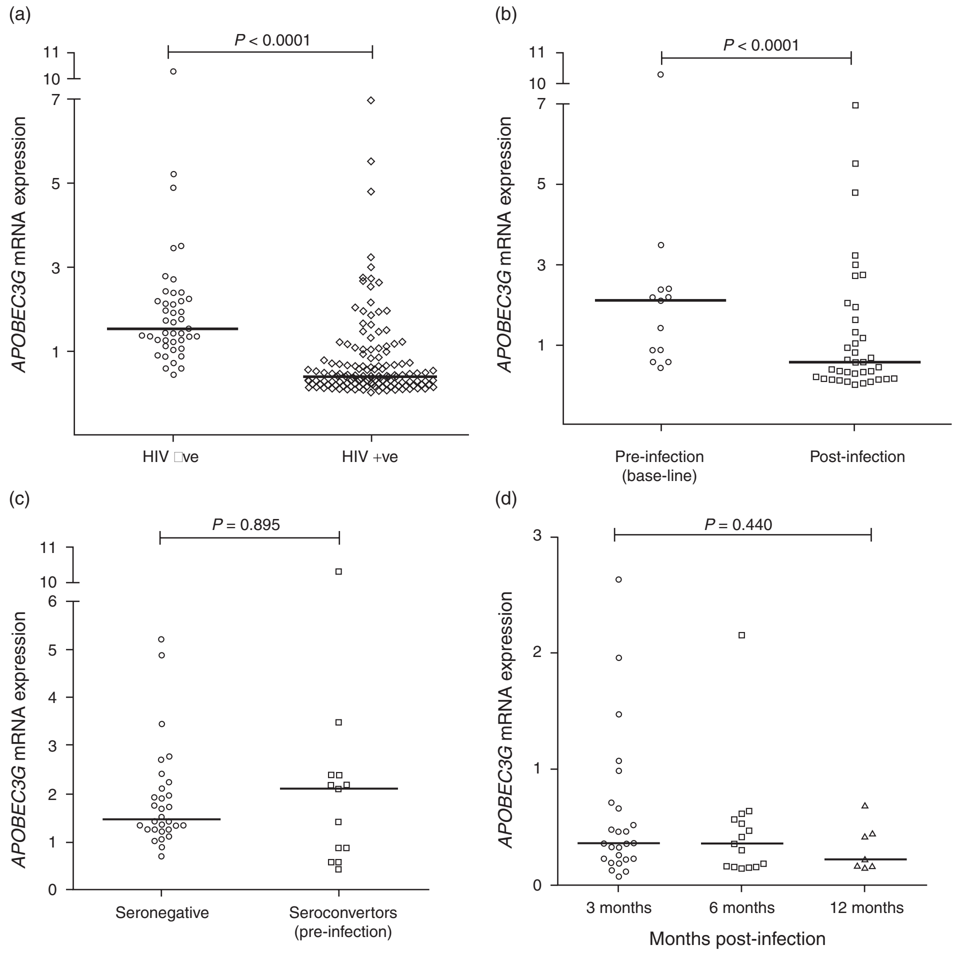

Fig. 2. Comparison of *APOBEC3G* mRNA levels between (a) HIV-uninfected and HIV-infected participants, (b) preinfection and postinfection samples of HIV-infected individuals, (c) persistently seronegative individuals and preinfection samples of seroconverters and (d) longitudinal postinfection samples. In (b), the postinfection column represents postinfection samples from three time points for each individual. These time points are at 3, 6 and 12 months after infection. A generalized estimating equation analysis model was used to analyze these data, as this model takes into account longitudinal (repeated) measures for each participant.

and found no significant difference in the distribution of *H186R* genotypes between HIV-negative and HIV-positive individuals ($P = 0.5838$) (data not shown).

We also compared viral loads (Fig. 3a) and $CD4^+$ T-cell counts (Fig. 3b) between genotypes. Viral load and CD4 measurements were classified into 0–3 months after infection and 3–12 months after infection time intervals to identify possible differences between genotypes during acute infection (0–3 months) and early chronic infection (3–12 months). There was an overall significant difference in viral loads between genotypes ($P = 0.0097$) across both time periods (Fig. 3a). In addition, during the first 3 months of infection, there was a significant difference in viral loads between the wild-type reference group (AA) and those homozygous for the mutation (GG) ($P = 0.0362$), with the GG genotype having higher viral loads than the AA genotype. There was also an overall significant difference in CD4 cell counts between the AA reference group and the GG genotypes ($P = 0.0081$) (Fig. 3b). Further, during the first 3 months of infection, there was a significant difference in CD4 cell counts between AA and GG genotypes ($P = 0.0006$), with GG genotype having lower CD4 cell counts than the AA genotype. The association with $CD4^+$ T-cell count is consistent with observed genotype effects on viral load. At 3–12 months after infection, the GG genotype maintained its association with higher viral loads and lower CD4 cell counts when compared with the other genotypes. Additionally, the heterozygous AG genotype also displays significantly higher viral loads ($P = 0.0005$) and lower CD4 cell counts ($P = 0.0078$) when compared with the reference AA genotype at this later time period. Kaplan–Meier survival analysis (Fig. 3c) shows that those who have the GG genotype have a significantly shorter time to CD4 cell count less than 350 cells/μl. The hazard ratio for the GG group as compared with the AA or AG group is 3.84 [95% confidence interval (CI) 1.43–10.35, $P = 0.0078$].

Table 1. ***APOBEC3G*** **single-nucleotide polymorphisms and their minor allele frequencies.**

						Minor allele frequency from the work of An *et al.* [22][a]			
NCBI dbSNP ID	Location	Nucleotide change	Amino acid change	Hardy–Weinberg	Minor allele frequency	SNP #	AA	EA	CA
rs5757463	5′ Regulatory region	C/G/T		0.419	0.089	*571G/C*	0.091	0.063	0.116
rs7291971	5′ Regulatory region	C/G		0.858	0.016				
rs6519166	5′ Regulatory region	A/C		0.185	0.111				
rs8142124	5′ Regulatory region	C/T		0.574	0.246				
rs5750743[b]	5′ Regulatory region	C/G		0.966	0.327	*90C/G*	0.319	0.340	0.263
rs8177832[c]	Exon 4	A/G	*H186R*	0.040	0.307	*H186R*	0.368	0.029	0.074
rs17496046[c]	Exon 6	C/G	*Q275E*	0.028	0.169				
rs11545130	Exon 7	C/T	*L371L*	0.846	0.019				
rs34300092	Intron 2	A/G		0.300	0.089				
rs6001417[c]	Intron 3	C/G		0.030	0.303	*197193T/C*[d]	0.368	0.029	0.086
rs3736685[c]	Intron 3	C/T		0.004	0.151				
rs17537581	Intron 6	A/G		0.112	0.454				
rs17537574	Intron 6	A/G		0.030	0.033				
rs17537588	Intron 7	A/G		0.722	0.031				
rs35342554	3′ near gene	C/T		0.155	0.290				
rs35228531	3′ near gene	C/T		0.519	0.207				
cem15-ex1-snp1-R[e]	Intron 1	A/G		0.357	0.078				
cem15-ex3-snp1-R[e]	Exon 4	A/G		0.060	0.164				
cem15-ex6-snp1-R[e]	Intron 7	A/G		0.846	0.019				
cem15-In13-snp3-R[e]	Intron 2	A/G		0.300	0.089				
cem15-In13-snp1-Y[e]	Intron 2	C/T		0.401	0.073				
cem15-pm1-snp1-Y[e]	5′ regulatory region	C/T		0.344	0.082				
cem15-3u-snp1-R[e]	Intron 7	A/G		0.581	0.048				
cem15-3u-snp2-W[e]	Intron 7	A/T		0.518	0.056				

NCBI dbSNP ID, National Center for Biotechnology Information SNP database reference number; RFLP, restriction fragment length polymorphism; SNP, single-nucleotide polymorphism.
[a]Comparisons with results from a published study.
[b]SNPs genotyped by RFLP assays.
[c]SNPs genotyped by TaqMan assays.
[d]T/C denotes complementary alleles.
[e]Novel SNPs.

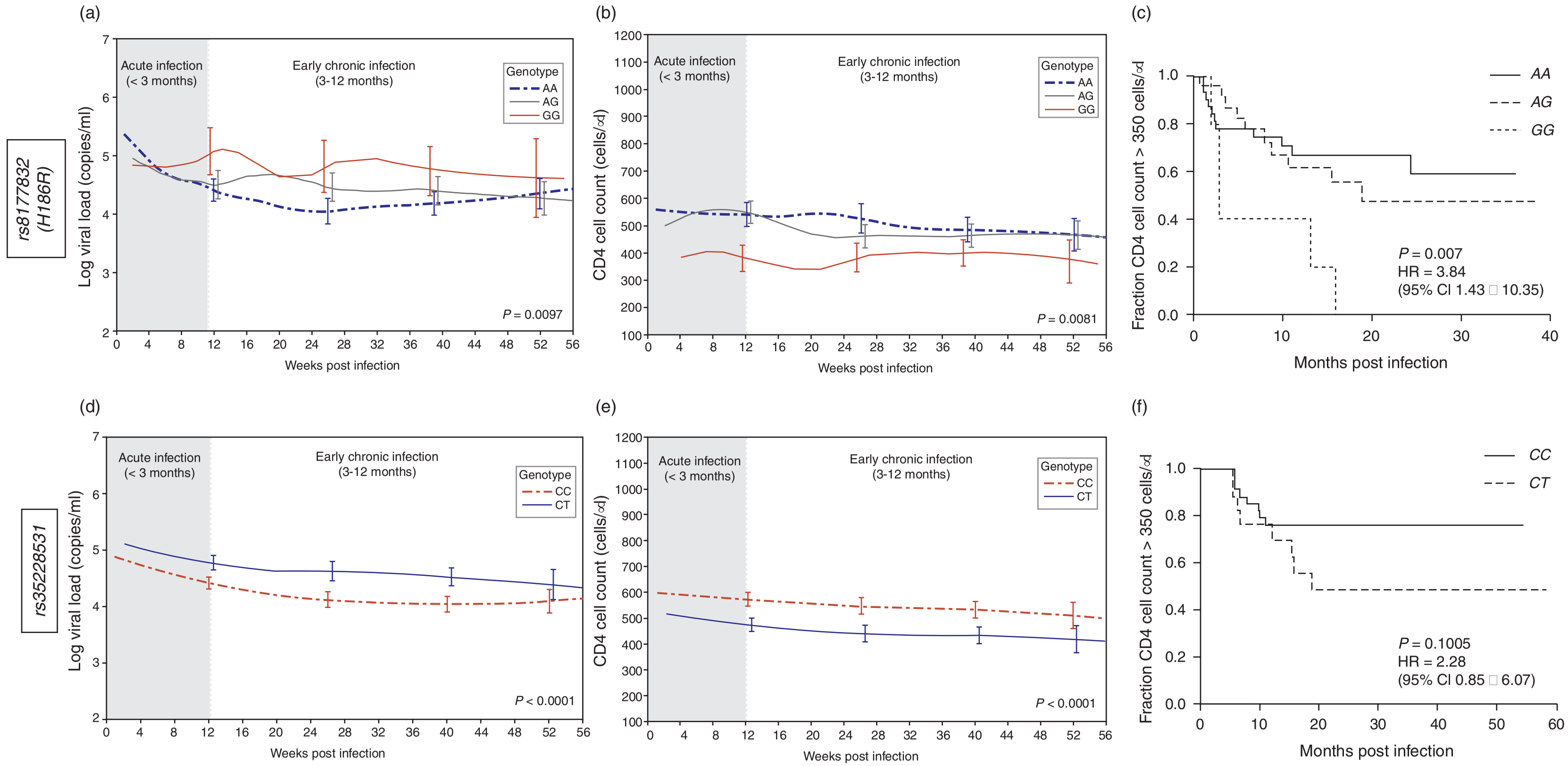

Fig. 3. Comparison of viral loads and CD4$^+$ T-cell counts between *H186R* (*rs8177832*) genotypes (a and b) and *rs35228531* genotypes (d and e) over 0–12 months after infection using a locally weighted scatterplot smoothing model. Measurements were classified into 0–3 months after infection and 3–12 months after infection time intervals to identify differences between the genotypes during acute infection and early chronic infection. Confidence intervals are shown by the vertical lines and overall P values are indicated. Kaplan–Meier survival curves were performed to assess the difference between the *H186R* (c) and *rs35228531* (f) genotype groups in the event of a CD4$^+$ T-cell count less than 350 cells/µl for more than two consecutive visits. Cox regression was used to acquire hazard ratios.

In addition, we tested the association of the *rs6001417* mutation with HIV status, CD4 cell count and viral load (data not shown). This polymorphism was in near-perfect linkage disequilibrium with H186R, had no significant association with HIV status but the mutant genotype (GG) was associated with significantly higher viral loads and lower CD4 cell counts ($P < 0.0001$).

Analysis of the *rs35228531* mutation indicated that individuals who are heterozygous for the mutation (CT) had significantly higher viral loads and lower CD4 levels over 12 months of infection as compared with the wild-type group (Fig. 3d and e). A Kaplan–Meier survival analysis (Fig. 3f) shows that those who have the CT genotype progress faster to a CD4 cell count below 350 cells/μl. The hazard ratio for the CT group as compared with the CC group is 2.28 (95% CI 0.85–6.07, $P = 0.1005$). Although this was not statistically significant, a trend is observable, as only 22.9% of the CC group progressed to a CD4 cell count below 350 cells/μl, whereas 47.1% of individuals with the CT genotype progressed to a CD4 cell count below 350 cells/μl.

Discussion

In this study, we were interested in describing the contribution of *APOBEC3G* to viral control during the critical primary infection phase, as well as understanding the kinetics of *APOBEC3G* expression before and after infection. Early HIV-1 infection has been associated with immobilization or profound dysregulation of antiviral immune responses [30,31]. Therefore, it is unclear whether HIV-1 infection would result in mobilization or dysregulation of intrinsic immunity factors such as APOBEC3G, which appear to be a key component of innate immunity. We hypothesized that at the critical phase of primary HIV-1 infection, *APOBEC3G* expression levels might correlate negatively with viremia and positively with $CD4^+$ T-cell counts.

Our data show that *APOBEC3G* mRNA levels are lower in HIV-positive primary infection PBMCs as compared with HIV-negative PBMCs. Furthermore, in matched pre and postinfection samples of seroconverters, *APOBEC3G* mRNA levels declined, suggesting a dysregulation of *APOBEC3G* mRNA during primary HIV-1 infection. Thus, our results are consistent with most of the earlier studies [20,28,29,32] showing that *APOBEC3G* expression levels were higher in HIV-uninfected individuals when compared with HIV-infected individuals, although contrary findings have also been reported [33]. Our study extends the findings from the earlier studies to the primary HIV-1 infection phase. We also show that in matched pre and postinfection samples, *APOBEC3G* levels declined, suggesting an active mechanism of *APOBEC3G* dysregulation rather than increased susceptibility to infection among those with low baseline (preinfection) levels of *APOBEC3G*. We found no significant differences in preinfection *APOBEC3G* mRNA levels between seroconverters and nonseroconverters followed longitudinally, suggesting that *APOBEC3G* mRNA levels *per se* are not associated with protection against HIV-1 infection.

Our results, therefore, lend support to previous observations that HIV-1 has specific mechanisms for counteracting APOBEC3G as a possible immune evasion strategy. We cannot also rule out the possibility that HIV-1 infection is associated with redistribution away from peripheral blood of cellular components that are enriched for *APOBEC3G* or that HIV-1 specifically targets such cells. Further studies will be needed to address how APOBEC levels change in different cell subsets following infection. Recent data from HIV-1-uninfected individuals reveal that APOBEC3G is broadly expressed in different hematopoietic subsets and that mRNA levels may not always be concordant with protein levels [34]. Thus future studies will need to address expression at both mRNA and protein levels in HIV-positive individuals.

Studies show that immune evasion against APOBEC3G is mediated by HIV-1 Vif, and that it is two-fold, involving translational and posttranslational inhibitory effects on *APOBEC3G* [17]. There have been no previous reports showing that HIV-1 inhibits *APOBEC3G* expression at the transcriptional level. Whether the *APOBEC3G* mRNA reduction seen in HIV-positive samples in this study is tied to or independent of ubiquitin proteasome degradation mediated via HIV-1 Vif may require further investigation, but our results suggest that a third mode of HIV-1 APOBEC3G inhibition may involve downregulation of mRNA expression. This observation is in contrast to in-vitro studies in which HIV-1 infection does not appear to affect *APOBEC3G* mRNA levels [17,35,36]. In agreement with two previous studies [28,29] performed in chronically infected patients, we found no correlation between *APOBEC3G* mRNA levels and viral load or CD4 cell counts in this primary infection cohort. In one study [20] that found a significant inverse correlation between *APOBEC3G* mRNA levels and viral load and a positive correlation with $CD4^+$ T-cell count, the investigators stimulated PBMCs with antibodies before RNA isolation, which may explain their contrary findings.

We also investigated the extent of genetic variation within *APOBEC3G* in a South African study cohort in which HIV-1C predominates. Sub-Saharan Africa is worst affected by the HIV-1 epidemic, and despite this situation, relatively few studies [5,22,37] have attempted to define genes that may affect HIV/AIDS outcomes in the local populations and yet their frequencies may vary according to ethnic background. In this cohort, we describe the frequencies of several SNPs and identify several novel SNPs that have not been described before.

Recently, it was reported that a genetic variant of *APOBEC3G*, the *H186R* mutation, is associated with an AIDS-accelerating effect in African–Americans infected with HIV-1B [22]. This mutation, which occurred at a frequency of approximately 30% in our HIV-1C-infected African cohort, was associated with significantly increased viral loads and decreased $CD4^+$ T-cell counts, consistent with the earlier findings. The *rs6001417* mutation is in linkage disequilibrium with *H186R* and, therefore, showed highly similar associations. The detrimental effects of this mutation are, therefore, observable during primary infection and become more prominent with progression to early chronic infection. It remains unclear how this polymorphism may affect the antiviral activity of APOBEC3G [22,23,38,39], and our findings emphasize the need for further studies to address the possible mechanisms.

Our data, however, indicate that this SNP is out of Hardy–Weinberg equilibrium (HWE) ($P = 0.04$) in our study population. We have eliminated genotyping error as a reason for this, as we have both resequencing and TaqMan genotyping confirmation. Further, genotypes that were obtained were consistent between duplicates and free of contamination, as negative controls did not amplify. When HWE was calculated separately for HIV-positive and HIV-negative individuals, we found that the HIV-positive group conformed to HWE ($P > 0.05$), whereas individuals in the HIV-negative group were out of HWE ($P = 0.03$). Therefore, the distortion seems to be due to the excess of the GG genotype in the negative group (26/31), which may suggest that the minor allele is protective against infection in homozygotes, although this was not significant in our cohort (odds ratio = 0.54, $P = 0.23$) using the AA as the reference group. This may be the reason for the distortion in HWE in the negative group in which we see an excess of GG genotypes. This suggests that this mutation may reduce susceptibility to HIV infection, but upon infection becomes detrimental and accelerates disease progression. A larger sample size will be required to resolve this issue.

Further, the *rs35228531* mutation, which occurred at an allele frequency of approximately 20% in our cohort, was associated with significantly high viral loads and low CD4 cell counts. This mutation, however, is extragenic, located 3′ near the gene and may, therefore, affect transcriptional regulation or posttranscriptional modifications of this antiviral factor.

Conclusion

We have shown that HIV-1 infection is associated with rapid downregulation of *APOBEC3G* expression at the transcriptional level. Studies to decipher the mechanisms involved and to possibly develop means for counteracting this are needed. During primary infection, *APOBEC3G* expression levels in PBMCs do not correlate with viral loads or $CD4^+$ T-cell counts. Importantly, this is the first study to describe *APOBEC3G* genetic polymorphisms in an African setting, where HIV-1C prevalence and incidence rates are extremely high. This is the first study that indicates that *APOBEC3G* may be an important HIV/AIDS-modifying gene during primary HIV-1 infection. Genetic variants of *APOBEC3G* significantly affect early and late HIV-1 pathogenesis, although the mechanism remains unclear and warrants further investigation.

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K.R., T.N. and C.W. conceived the study, designed the experiments and interpreted the data. K.R. performed the experiments. K.R. and L.W. analyzed the data. K.R. and T.N. wrote the paper and all coauthors reviewed the manuscript. S.A.K., K.M. and members of the CAPRISA acute infection study team designed, established and maintained the study cohort and provided the samples.

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Table S1. PCR sequencing primers, regions amplified and PCR conditions.

Primer Name	Amplified Regions	Forward primer	Reverse primer	T (°C)	Mg++
CEM15-Ex1	Exon 1, intron 1, exon 2, intron 2	gacggaatttcgctcttgtc	gggagagaaggacacactgg	60	1.5
CEM15-Ex3	Exon 3, Intron 3, exon 4, intron 4	ggtgagaagtgggaggttca	gacctggtctggaacagagg	60	1.5
CEM15-Ex4	Intron 4, exon 5	aggtttggaggctctagcaa	cactgaagccgaagtctcc	60	1.5
CEM15-Ex5	Exon 5, Intron 5, exon 6, intron 6	cctcatggcttgctttcttt	gtcgaccccaaagtcaggt	60	1.5
CEM15-Ex6	Intron 6, exon 7, intron 7,	gctggaagtggaagcagaac	agtgacaatgatcggagagga	60	1.5
CEM15-In12	Intron 2	ctgcgtgggtcacgtaca	gcctcgtgtgtgaattctagc	60	1.5
CEM15-In13	Intron 2, exon 3, intron 3	gctgggaaaacttccaaactc	tttccctccatccccctgt	60	1.5
CEM15-pm1	5′ region, exon 1	acgcctggccatttactct	aagtgaggcttcatccttgg	60	2.5
CEM15-pm2	5′ region	ctcctcctgtagcctgttcaa	gacagggagggagagtaa	60	1.5
CEM15-pm3	5′ region	ggcggtggaaagttacagtc	tttagaagcaggaggggttg	60	2.5
CEM15-3U	Intron 7, exon 8, 3′ region	cctcctctccgatcattgtc	cctcctctccgatcattgtc	60	1.5

Table S2. PCR primers, PCR conditions and restriction enzymes used for the RFLP assay, and details of TaqMan Genotyping Assays.

NCBI dbSNP ID	Method	Forward primer	Reverse primer	T (°C)	Mg++	Enzyme
rs5750743	RFLP	acgcctggccatttactct	gccctccctaaagtgacctc	62	2.0	AvaI
NCBI dbSNP ID	Method	ABI Assay ID				
rs8177832	TaqMan Assay	C_2189646_10				
rs17496046	TaqMan Assay	C_25649193_10				
rs6001417	TaqMan Assay	C_30089175_10				
rs3736685	TaqMan Assay	C_27489853_10				
rs35228531	TaqMan Assay	C_61215563_10				