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APOBEC3G Expression is Dysregulated in Primary HIV-1 Infection and a Polymorphic Variant Influences CD4+ T Cell Counts and Plasma Viral Load

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Abstract

Objectives—In the absence of HIV-1 Vif, cellular cytosine deaminases such as APOBEC3G, inhibit the virus by inducing hypermutations on viral DNA, among other mechanisms of action. We investigated the association of *APOBEC3G* mRNA levels and *APOBEC3G* genetic variants on HIV-1 susceptibility, and early disease pathogenesis using viral load and CD4+ T cell counts as outcomes.

Methods—Study subjects were 250 South African females at high risk for HIV-1C infection. We used quantitative real-time PCR to measure the expression of *APOBEC3G* in HIV−ve and HIV +ve 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. However, *APOBEC3G* expression levels were significantly higher in HIV−ve individuals compared to HIV+ve individuals (p<0.0001), including matched pre- and post infection samples from the same individuals (n=13, p<0.0001). 25 single nucleotide polymorphisms (SNPs), nine of which were novel, were identified within *APOBEC3G* by re-sequencing followed by genotyping of 168 individuals. The *H186R* mutation, a codon changing variant in exon 4, was associated with high viral loads (p=0.0097) and decreased CD4+ T cell levels (p=0.0081).

Conclusions—These data suggest that *APOBEC3G* transcription is rapidly downregulated upon HIV-1 infection. During primary infection, *APOBEC3G* expression levels in PBMCs do not correlate with viral loads or CD4+ T cell counts. However, structural variation of *APOBEC3G* may significantly affect early HIV-1 pathogenesis, although the mechanism remains unclear and warrants further investigation.

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Author Contributions

KR, TN, CW conceived and designed the experiments. KR performed the experiments. LW analyzed the data. SAK, KM designed and established the study cohort and provided the samples.

Keywords

APOBEC3G; HIV-1 C; Primary Infection; mRNA Expression; Polymorphisms; Host proteins

Introduction

Apolipoprotein *B* mRNA-editing enzyme catalytic polypeptide-like 3G (APOBEC3G), a human cytidine deaminase, has potent antiviral activity [1–4], and is a novel candidate AIDS restriction gene, defined as a host gene with polymorphic variants that 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 besides hypermutation [10, 11]. Hypermutated viral sequences have been identified in long term non-progressors 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* mRNA may overcome the effects of Vif by providing a competitive advantage over time and the cumulative G-to-A hypermutations in the HIV genome, induced by APOBEC3G, 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 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.

Materials and Methods

Study Participants

The CAPRISA Acute Infection Study is an observational natural history study of HIV-1 subtype C infection established in Durban, South Africa in 2004 [24]. 245 females 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, NY, 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 (EIA) test (BEP 2000; Dade Behring, Marburg, Germany). Participants with acute HIV infection and those from other seroincidence cohorts were

recruited into Phase 2, based on 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 post-infection, monthly until 12 months post-infection and thereafter quarterly for a maximum of 5.5 years. A flow diagram summarizing the study cohort and experiments is available in Figure 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 counts and Plasma Viral Load

Blood was obtained by venipuncture and PBMCs were isolated by Ficoll-Histopaque (Sigma, St Louis, MO) density gradient centrifugation and frozen until use. Viral load was determined using the automated COBAS AMPLICOR HIV-1 Monitor Test v1.5 (Roche). 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−ve participants and in longitudinal samples of 32 HIV+ve participants. Additionally, 13 of the 32 HIV+ve participants had pre-infection (baseline) samples available. RNA was isolated from cryopreserved PBMCs immediately after thawing using Trizol reagent (Invitrogen) according to the manufacturer's protocol. RNA was reverse transcribed to synthesize cDNA using the Quantitect Reverse Transcription Kit (Qiagen).

APOBEC3G mRNA expression was quantified by real-time PCR using SYBR Green chemistry (Roche). 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 to 134); reverse, 5′ CTCCTGGAAGATGGTGATGG 3′ (nucleotides 320 to 339). Each optimized 10 μl PCR reaction contained 1–2 μl of 25 mM $MgCl_2$ (primer set dependent), 1 μl of 10X LightCycler FastStart DNA Master SYBR Green I (Roche), 0.2 μl of each 10 μM *APOBEC3G* primer or 0.5 μl of each 10 μM *GAPDH* primer and 2 μl of cDNA template. Reactions were run on the LightCycler Instrument Version 1.5 (Roche). PCR cycling conditions were 1 cycle at 95°C for 10 minutes, 40 cycles of 95°C for 5 seconds, 55°C (*APOBEC3G*) or 65°C (*GAPDH*) for 15 seconds and 72°C for 5 seconds. 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 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 the target gene (*APOBEC3G*) by the concentration of the housekeeping gene (*GAPDH*). The resulting target/reference ratio expressed the amount of *APOBEC3G* normalized to the level of *GAPDH*.

Detection of *APOBEC3G* polymorphisms

A DNA panel of 30 HIV+ve and 30 HIV−ve samples was resequenced to identify 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 10X PCR buffer, 1.5 or 2.5 mM $MgCl_2$ (primer set dependent), 2.4 mM deoxynucleoside triphosphate mix, 0.15 μl TaqGold, 5 μM of each forward and reverse primer. This was amplified at 95°C for 10 minutes, 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 45 seconds, and a final 10 minute extension step at 72°C. PCR products were purified using Exonuclease and Shrimp Alkaline Phospatase (SAP) (Amersham Pharmacia) and sequenced using overlapping primers and a BigDye Terminator Kit (Applied Biosystems). Sequencing primers, regions amplified and PCR conditions are available in supplementary material (Table S1).

Genotyping of Variants

5 SNPs, identified by resquencing *APOBEC3G*, were further genotyped in 168 samples (37 HIV+ve and 131 HIV−ve). 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* SNP was selected for further genotyping based on its strong association with viral load and CD4 count. This SNP was genotyped in 250 samples (61 HIV+ve and 189 HIV−ve).

Statistical Analysis

APOBEC3G mRNA expression levels were compared between HIV−ve and HIV+ve 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 counts was determined using rank correlation tests. Fisher's Exact Test was used to test the association between HIV status and *H186R* genotypes. The genetic effect of the *H186R* mutation on viral loads and CD4 counts was also determined by a GEE model taking into account longitudinal measures for each participant. This data was represented by a locally weighted scatterplot smoothing (LOWESS) 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 genotype groups and Cox Regression was used to acquire hazard ratios. All statistical analysis was performed using SAS version 9.1 (SAS Institute, Cary, NC, USA.) and graphs were generated using GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, California, USA).

Results

APOBEC3G Expression

A significant number of *in vitro* studies suggest that APOBEC3G and other cytidine deaminases may constitute an important mechanism of cellular defense against viruses. However, 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 compared to HIV −ve samples. Comparison of *APOBEC3G* mRNA levels between HIV−ve and HIV+ve subjects within 12 months of infection (primary infection) showed that *APOBEC3G* levels were significantly higher in HIV−ve individuals than in HIV+ve individuals ($p<0.0001$)

(Fig. 2a). Additionally, comparison of *APOBEC3G* expression levels in matched pre- and post-infection 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 pre-infection samples of seroconverters (Fig. 2c). Comparison of *APOBEC3G* mRNA levels at various time points post-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 re-sequencing and genotyping we identified 24 SNPs within *APOBEC3G* in our cohort (Table 1). Sixteen of these SNPs were described previously and 8 were novel. Further, An *et. al.* [22] described 7 SNPs within *APOBEC3G* in a United States based study cohort, 4 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 United States 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 allelle was previously shown to have AIDS accelerating effects [22].

Effects of *H186R* mutation on Primary HIV Pathogenesis

H186R genotypes were determined for 250 subjects (61 HIV+ve and 189 HIV−ve). We tested the association between HIV status and *H186R* genotypes and found no significant difference in the distribution of *H186R* genotypes between HIV−ve and HIV+ve subjects ($p=0.5838$) (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 post infection and 3–12 months post 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 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 counts between *AA* and *GG* genotypes ($p=0.0006$), with *GG* genotype having lower CD4 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 post-infection, the *GG* genotype maintained its association with higher viral loads and lower CD4 counts when compared to the other genotypes. Additionally, the heterozygous *AG* genotype also displays significantly higher viral loads ($p=0.0005$) and lower CD4 counts ($p=0.0078$) when compared to 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 count < 350 cells/3l. The hazard ratio (HR) for the *GG* group as compared to the *AA* or *AG* group is 3.84 (95% CI 1.43 – 10.35, p=0.0078).

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 pre- and post-infection. Studies have shown that natural killer cells frequencies rapidly expand during early HIV-1 infection and then decline as virus specific T-cells and antibody responses become established [30]. However, HIV-1 infection is also associated with profound immune dysregulation [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. Therefore, we investigated how primary HIV infection may affect *APOBEC3G* expression, and whether APOBEC3G levels contribute to viral control during this phase.

Our data show that *APOBEC3G* mRNA levels are lower in HIV+ve primary infection PBMCs compared to HIV−ve PBMCs. Furthermore, in matched pre-infection and post-infection samples of seroconverters, *APOBEC3G* mRNA levels declined suggesting a rapid and active dysregulation of *APOBEC3G* mRNA following HIV-1 infection. Thus, our results are consistent with earlier studies showing that *APOBEC3G* expression levels were higher in HIV-uninfected individuals when compared to HIV-infected individuals [20, 28, 29, 32]. However, our study extends the earlier findings by showing that *APOBEC3G* is downregulated even in primary HIV-1 infection, and that in matched pre- and post-infection samples, *APOBEC3G* levels declined suggesting an active mechanism of *APOBEC3G* dysregulation rather than increased susceptibility to infection among those with low baseline (pre-infection) levels of *APOBEC3G*. Indeed, we also tested the possibility that individuals with lower expression of *APOBEC3G* are more susceptible to HIV-1 infection by comparing *APOBEC3G* mRNA levels in pre-infection samples of seroconverters to levels in persistently seronegative participants. We found no significant differences between these two groups, 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. Although our study and the several previous studies of HIV-1 chronically infected participants suggest a downregulation of APOBEC3G, one striking contrary finding has been reported recently from a Senegalese cohort in which APOBEC3G and APOBEC3F levels were observed to increased following HIV-1 infection [33]. The latter results are hard to explain in light of our study and those of others [20, 28, 29, 32] and further investigations are warranted to explore whether HIV-1 subtype differences or human genetic variation might explain the unique findings from the Senegalese cohort.

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+ve 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 down-regulation of mRNA expression. This observation is in contrast to *in vitro* studies where HIV-1 infection does not appear to affect *APOBEC3G* mRNA levels [17, 34, 35]. We found no correlation between *APOBEC3G* mRNA levels and viral load or CD4 counts in this primary infection cohort. This is in agreement with at least two other studies, albeit performed in chronic infection settings [28, 29]. One study found a significant inverse correlation between *APOBEC3G* mRNA levels and viral load and a positive correlation with CD4+ T cell count in chronic HIV-1 infection [20] but it is worth noting that the investigators stimulated PBMCs with antibodies before RNA isolation. This difference in processing and handling of samples may explain the unique findings of that study. Given the significant downregulation of *APOBEC3G* mRNA in HIV-1 infected PBMCs compared to HIV uninfected PBMCs, it is perhaps not surprising that we found no correlation between *APOBEC3G* mRNA levels and viral load or CD4+ T cell counts. HIV appears to have evolved efficient mechanisms for counteracting APOBEC3G even at the transcriptional level, thus inhibiting its contribution to antiviral control in most instances. Further studies may be required to assess whether stabilization of *APOBEC3G* mRNA in HIV-1 infection could result in improved correlation with markers of disease progression such as viral load and CD4 counts. Further studies may also investigate *APOBEC3G* expression in specific subsets of cells within PBMCs that are specifically targeted by HIV-1.

We also investigated the extent of genetic variation within *APOBEC3G* in a South African study cohort where HIV-1 subtype C predominates. Sub-Saharan Africa is worst affected by the HIV-1 epidemic and despite this situation, relatively few studies have attempted to define AIDS restriction genes in the local populations and yet their frequencies may vary according to ethnic background [22, 36, 37]. 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-1 subtype B [22]. This mutation, which occurred at a frequency of approximately 30% in our HIV-1 subtype C infected African cohort, was associated with significantly increased viral loads and decreased CD4+ T cell counts, consistent with the earlier findings. The detrimental effects of this mutation are therefore observable during primary infection and become more prominent with progression to early chronic infection. The presence of this *APOBEC3G* polymorphism may affect its activity or levels of expression by altering its interaction with other proteins, or modifying its editing functions, thus influencing HIV-1 replication [22, 23, 38, 39]. In this study, the numbers were too small to link this polymorphism with expression levels. However, given the lack of correlation between *APOBEC3G* mRNA levels and viral load or mRNA levels, it is doubtful that this polymorphism acts by merely affecting *APOBEC3G* expression.

Our data however, indicates 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 re-sequencing 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+ve and HIV−ve individuals we found that the HIV+ve group conformed to HWE (p>0.05) while the HIV−ve 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 (OR=0.54; p=0.23) using the *AA* as the referent group. This may be the reason for the

distortion in HWE in the negative group where 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.

In 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 AIDS restriction 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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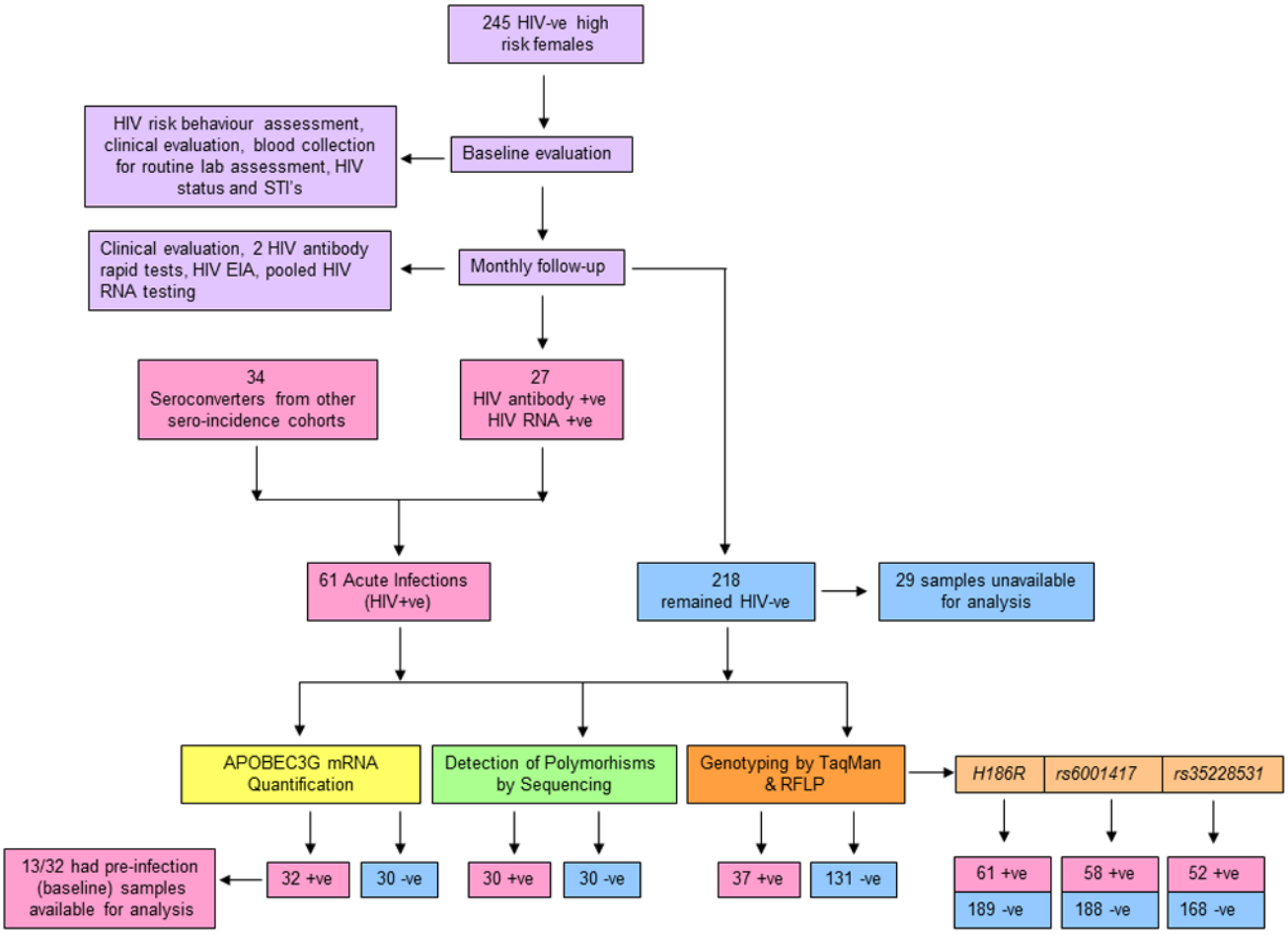

Figure 1.

Outline of study cohort and experiments. HIV+ve samples are indicated by pink blocks and HIV−ve samples are indicated by blue blocks

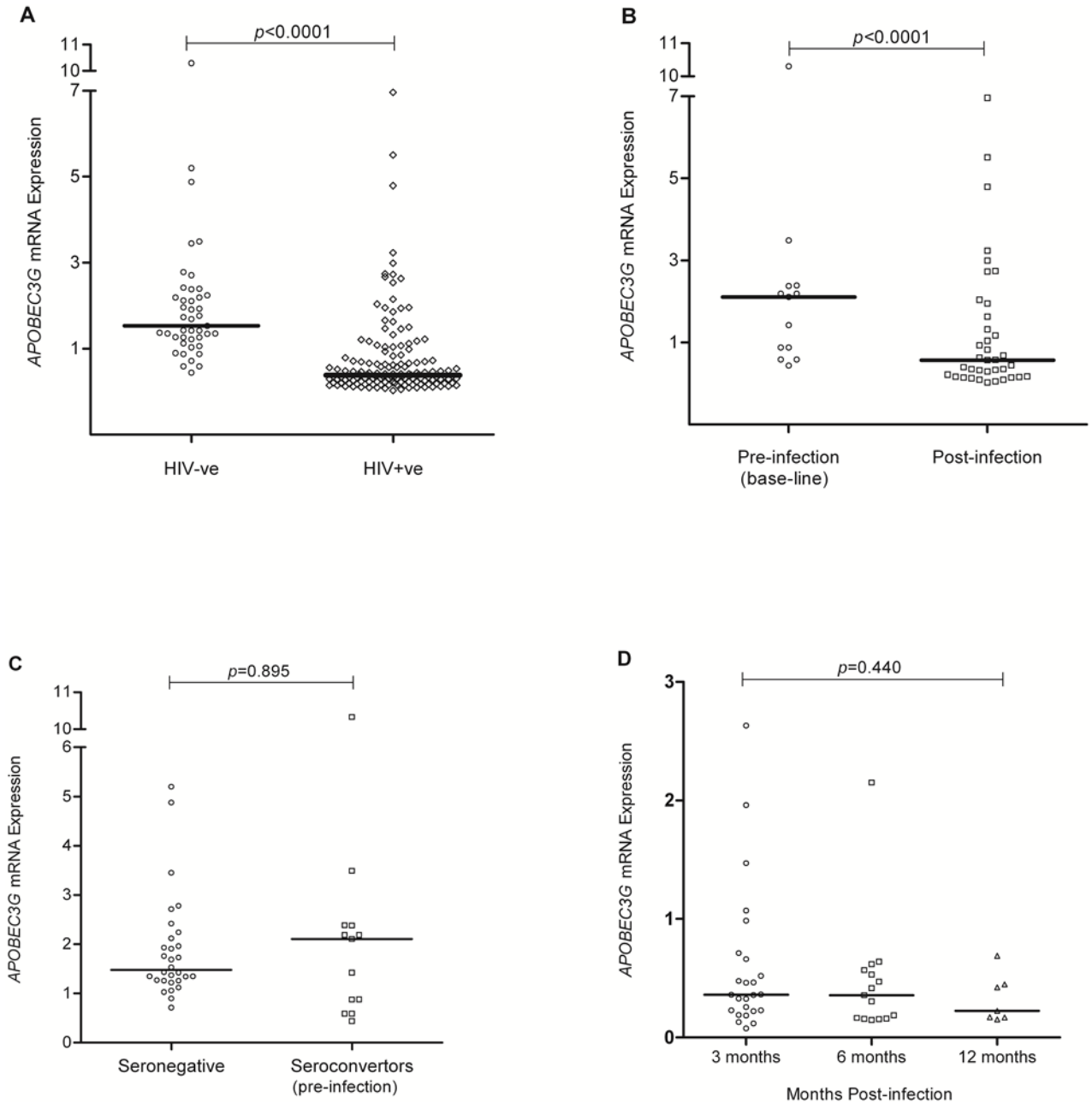

Figure 2.

Comparison of *APOBEC3G* mRNA levels between (a) HIV-uninfected and HIV-infected participants, (b) pre-infection and post-infection samples of HIV-infected individuals, (c) persistently seronegative subjects and pre-infection samples of seroconverters and (d) longitudinal post-infection samples.

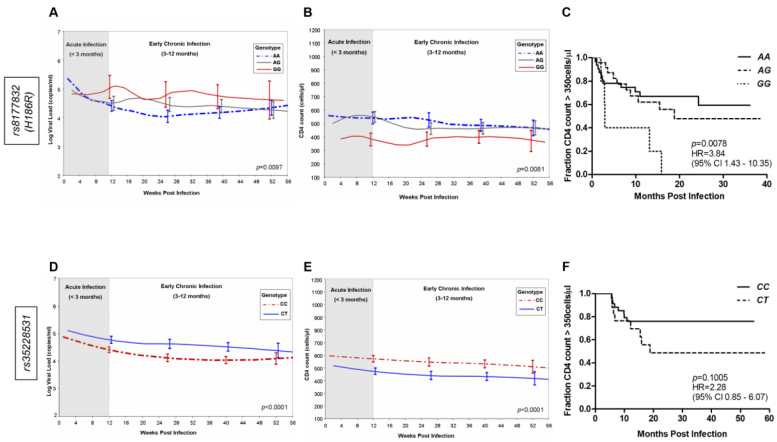

Figure 3.

Comparison of viral loads (a) and CD4+ T cell counts (b) between *H186R* genotypes over 0–12 months post infection using a LOWESS model. Measurements were classified into 0–3 months post infection and 3–12 months post 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. A Kaplan-Meier survival curve (c) performed to assess the difference between the genotype groups in the event of a CD4+ T cell count less than 350 cells/3l for more than two consecutive visits. Cox Regression was used to acquire Hazard Ratios.

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Table 1

APOBEC3G SNPs and their minor allele frequencies

NCBI dbSNP ID[a]	Location	Nucleotide Change	Amino Acid Change	Hardy Weinberg	Minor Allele Frequency	Minor Allele Frequency from the work of An. et. al (3)[b]			
						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[e]	5′ regulatory region	c/g		0.966	0.327	*90C/G*	0.319	0.340	0.263
rs8177832[d]	exon 4	a/g	*H186R*	0.040	0.307	*H186R*	0.368	0.029	0.074
rs17496046[d]	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[d]	intron 3	c/g		0.021	0.280				
rs3736685[d]	intron 3	c/t		0.004	0.151	*197193T/C*	0.368	0.029	0.086
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′ region	c/t		0.155	0.290				
rs35228531	3′ region	c/t		0.401	0.073				
cem15-ex1-snp1-R[c]	intron 1	a/g		0.357	0.078				
cem15-ex3-snp1-R[c]	exon 4	a/g		0.060	0.164				
cem15-ex6-snp1-R[c]	intron 7	a/g		0.846	0.019				
cem15-In13-snp3-R[c]	intron 2	a/g		0.300	0.089				
cem15-In13-snp1-Y[c]	intron 2	c/t		0.401	0.073				
cem15-pm1-snp1-Y[c]	5′ regulatory region	c/t		0.344	0.082				
cem15-3u-snp1-R[c]	intron 7	a/g		0.581	0.048				
cem15-3u-snp2-W[c]	intron 7	a/t		0.518	0.056				

[a] NCBI dbSNP ID, National Center for Biotechnology Information SNP database reference number.

[b] Comparisons with results from a published study.

[c] Novel SNPs

[d] SNPs genotyped by TaqMan assays

[e] SNPs genotyped by RFLP assays