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APOBEC3B Deletion and Risk of HIV-1 Acquisition

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Abstract

The human APOBEC3 family of cytidine deaminases provides intrinsic immunity to retroviral infection. A naturally occurring 29.5-kb deletion removes the entire *APOBEC3B* gene. We examined the impact of the *APOBEC3B* gene deletion in >4000 individuals from five HIV-1 natural history cohorts. The hemizygous genotype had no effect on either infection or progression. However, the homozygous deletion was significantly associated with unfavorable outcomes for HIV-1 acquisition (OR=7.37, $P=0.024$), progression to AIDS (RH = 4.01, $P=0.03$), and viral set-point ($P=0.04$). These findings suggest that the loss of *APOBEC3B* may increase host susceptibility to HIV-1/AIDS and warrant further study.

Introduction

Many members of the APOBEC3 family have cellular cytidine deaminase activity that provide immunity against retrovirus infection [1, 2]. APOBEC3G, APOBEC3F, (APOBEC3G/F), APOBEC3B, and APOBEC3DE each contain a catalytically active cytidine deaminase (CDA) domain as well as a catalytically inactive CDA domain that binds RNA that is required for viral encapsidation [3]. APOBEC3G/F suppress HIV-infection by deamination of viral minus-strand DNA during reverse transcription, resulting in G-to-A hypermutation [1, 4], but this occurs only in the absence of HIV-1 virion infectivity factor (Vif), which blocks the activity of APOBEC3G /F via Vif-mediated proteosomal degradation [1, 5].

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The antiviral activity of APOBEC3B on HIV-1 *in vitro* has been demonstrated: several studies reported modest inhibitory effect on HIV-1 [2, 6], while others reported a strong inhibition [3, 7]. Bogerd et al. in extended earlier studies showing that APOBEC3B inhibited HIV-1 infectivity ~40-fold, whereas APOBEC3G inhibited HIV-1 by ~30-fold [3]. Spontaneous C to T editing of plasmids containing the *APOBEC3B* gene in bacteria may have contributed to the differences among studies [3]. Expression of *APOBEC3B* mRNA was detected in peripheral blood lymphocytes, activated CD4+ T cells and macrophages [6]. Although APOBEC3G/F are potent inhibitors of the replication of Vif-deficient HIV-1, the antiviral function of both APOBEC3G/F is blocked by the Vif protein expressed by wild-type HIV-1. In contrast, APOBEC3B is resistant to Vif-mediated degradation [2] and is able to suppress the infectivity of both Vif-deficient and wild-type HIV-1 with equal efficiency [7]. These lines of *in vitro* evidence suggest APOBEC3B as a potentially strong inhibitor of HIV-1 *in vivo*.

A 29.5-kb deletion occurs between the fifth exon of *APOBEC3A* and the eighth exon of *APOBEC3B*, leading to the complete removal of the *APOBEC3B* coding region [8]. The frequency of the deletion allele is stratified among continental and island populations—the allele frequency is 37% in East Asians, 6% in Europeans, and less than 1% in Africans. In some oceanic groups the deletion approaches fixation [8]. Here we report the effects of the *APOBEC3B* deletion on HIV-1 infection and progression to disease in African American and European American HIV-1 natural history cohorts.

Subjects, materials, and methods

Study participants (N = 4216) were enrolled in five USA-based, longitudinal natural history HIV/AIDS cohorts (Multicenter AIDS Cohort Study [MACS], AIDS Link to the Intravenous Experiences [ALIVE], Hemophilia Growth and Development Study [HGDS], Multicenter Hemophilia Cohort Study [MHCS], and San Francisco City Clinic Cohort [SFCC]), as previously described in An et al.[9, 10]. Review Boards of participating institutions approved the study protocols, and informed consent was obtained from all study participants.

Genotyping of the *APOBEC3B* deletion (D) and insertion (I) alleles was performed by a PCR assay as previously reported [8].

Analyses were conducted using SAS version 9.13 (SAS Institute, Cary, NC) and R version 2.8.1 (Vienna, Austria). European American and African American groups were analyzed separately because allele frequencies for the deletion were different between the two groups. As the hemizygous and homozygous deletion of the gene may have distinct functional consequences, model-free genotypic association was assessed comparing the hemizygous (D/I) and homozygous deletion (D/D) state to the reference group composed of homozygotes for the insertion (I/I).

The genetic effects of the *APOBEC3B* deletion on HIV-1 infection susceptibility were assessed by comparing allelic and genotypic frequencies between the HIV-1–infected group, comprising persons without HIV-1 infection at study entry (the seroconversion group) and persons with HIV-1 infection at study entry (the seroprevalence group), and the HIV-1–uninfected group, comprising hemophiliacs who received pooled plasma factors VIII or IX before 31 December 1984, men who have sex with men, and injection drug users. Odds ratios (ORs) and *P* values (determined by the exact test) for hemizygotes were obtained using a conditional logistic regression test. The exact test, based on Monte Carlo resampling, is considered suitable and valid for use with sparse data or in a “separation” situation in the presence of “0” in one of the cells [11]. The OR estimates presented are median unbiased estimates.

The genetic effects of the *APOBEC3B* deletion on the rate of progression to AIDS were evaluated by Kaplan-Meier survival statistics and the Cox proportional hazards model (Cox model) using only the seroconverter group for the following endpoints: 1987 CDC defined AIDS (AIDS): HIV-1 infection plus AIDS-defining illness or AIDS-related death. We compared the deletion genotypes (D/D and D/I) to the reference group comprising individuals homozygous for the insertion homozygous genotype (I/I). Genetic factors previously shown to affect progression to AIDS were included as confounding covariates in the adjusted Cox model: *CCR5* Δ32, *CCR2*-64I, *CCR5*-59029 (*CCR5*-2459, rs1799987), *HLA*-B*27, *HLA*-B*57, *HLA*-B*35Px group (including *HLA*-B*3502, B*3503, B*3504, and B*5301), *HLA* Class I homozygosity for European American (reviewed in [12]; *HLA*-B*57, and *HLA* Class I homozygosity for African American. Participants were stratified by sex and by age at seroconversion: 0–20, >20–40, and > 40 years. P values for Cox model analysis were from the Wald test. For genotype D/D, a likelihood ratio test was also performed as it is considered more reliable for small sample sizes. The genetic effect of the *APOBEC3B* deletion on plasma HIV-1 viral load setpoint levels (determined using the 2nd generation Roche Amplicor HIV-1 assay (Indianapolis, IN)) was estimated using a linear mixed effects regression model. Viral load setpoint measurements from 344 individuals were sampled between 12 and 36 months after seroconversion (n = 1184) to avoid confounding by peak viremia during the acute phase of infection. Random effects terms were included for each individual, and log₁₀ viral load measurements in individuals with and without the *APOBEC3B* deletion were compared. All P values were 2-tailed.

Population stratification was corrected by adjusting for the first eigenvector of a principal components analysis of 70 ancestry informative markers using the modified EIGENSTRAT method (EIGENSOFT) [13].

RESULTS

The genotype and allele frequencies for the *APOBEC3B* insertion and deletion alleles in HIV-1 positive and HIV-1 negative groups in European Americans and African Americans are presented in table 1. The deletion (D) allele was more frequent in European Americans compared to African Americans. In the European American HIV-1 negative group, a significant departure from Hardy-Weinberg genotype expectation was noted, as no homozygotes were observed but 4 were expected ($P = 0.026$, exact test with 50,000 permutations). All other groups conformed to Hardy-Weinberg genotype expectation ($P \geq 0.2$).

We compared genotype and allele distributions between HIV-1 infected and uninfected groups for the *APOBEC3B* deletion. In European Americans, the D allele was similarly distributed in the HIV-1 positive (8.2%) and negative (7.5%) groups. Homozygotes for the deletion (D/D) were more frequent in the HIV-1 positive (n=1950) than the negative group (n=724) (OR = 7.37, 95% CI, 1.24-infinity, $P = 0.024$, Table 1) in a conditional logistic exact test. There were 14 D/D homozygotes among the HIV-1 positive group and none in the HIV-1 negative group.

In African Americans, the deletion allele and hemizygous genotype (I/D) were only slightly higher in the HIV-1 positive (4.9%, 9.5%, respectively) compared to the negative (3.9%, 7.7%, respectively) groups ($p > 0.20$). One D/D genotype was observed in the HIV-1 positive group and none were observed in the HIV-1 negative group, consistent with the lower allele frequency for the deletion in African Americans (Table 1).

When both European American and African American groups were combined, the exact conditional logistic analysis stratified by the racial groups indicated that the D/D genotype

remained significantly associated with increased risk for infection (OR = 7.96, 95% CI, 1.35-infinity, $P = 0.017$).

Only 4 D/D homozygotes were observed among the European American seroconverter group. Compared to the I/I reference group, I/D hemizygotes showed no differential influence on AIDS progression (log-rank test, $P = 0.71$). However, the D/D homozygotes progressed significantly faster to AIDS compared to the I/I group in the Cox model analysis (adjusted for covariates and population substructure: RH = 4.01, 95% CI, 1.45–11.07, $P_{\text{WALD}} = 0.007$ and $P = 0.03$ from a likelihood ratio test, Table 2) and in the survival analysis (log-rank test, $P = 0.006$). When CD4<200 was used as an outcome, there was a non-significant trend towards more rapid loss of CD4 (RH = 2.1, $P_{\text{WALD}} = 0.22$) for the D/D group (data not shown). Results before and after adjusting for ancestry to control for population substructure were similar (Table 2).

Only a single African American carried the D/D genotype; however in 288 African Americans, the impact of the I/D genotype on AIDS progression was not significantly different from that of the I/I genotype (Table 2).

Among 340 European American seroconverters with at least one viral load measurement between 12 and 36 months post-seroconversion we identified only 4 homozygotes for the *APOBEC3B* deletion. The plasma HIV-1 copies/ml for these participants were 3,050,806 for patient A, 157,220 for patient B, 86,848 for patient C, and 5,612 for patient D. Of note, viral loads in 3 of these participants fall within the highest quintile, stratified by viral load at cohort entry, in the MACS and are much higher than the median set point of 28,000 copies/mL among MACS seroconverters [14]. The viral load for patient D was in the third quintile. He was a heterozygote for *CCR5* $\Delta 32$, whereas none of the other three patients carried a protective allele or genotype for *CCR5* or *HLA*.

We further evaluated the effect of the *APOBEC3B* deletion genotypes on HIV-1 viral load set point using the mixed effects model (Table 3). The four patients carrying two copies of the deletion allele (D/D) had a significant higher mean log₁₀ HIV-1 viral copy number than the reference group (I/I) (+0.45 log₁₀ copies/ml, $P = 0.039$).

Discussion

APOBEC3B is the only human APOBEC3 protein with intrinsic anti-HIV-1 activity that is resistant to HIV-1 Vif [2, 7]. Our findings suggest that the absence of *APOBEC3B* gene is significantly associated with increased susceptibility to HIV-1 infection. We also noted that the null genotype (D/D) was associated with a more rapid progression to AIDS and a higher HIV-1 load. The consistency of the associations between the null genotype and acquisition of HIV-1 infection, progression to AIDS, and plasma viral load supports an active role for APOBEC3B against HIV-1 in vivo. Because the null genotype is uncommon among both African Americans and European Americans, our results await further validation from studies with expanded sample sizes and other populations.

Studies have shown that APOBEC3G/F, and APOBEC3B function in a collaborative or additive manner to inhibit HIV-1 infectivity [15]. Our observation that the hemizygous state has no differential impact on HIV-1 infection and progression suggests that the presence of one copy of the *APOBEC3B* gene may be sufficient to confer partial restriction of HIV-1 and maintain overall APOBEC3's anti-HIV-1 activity. In contrast, the total loss of APOBEC3B protein increased risk to HIV-1 acquisition and progression suggesting that the loss of anti-HIV-1 function of APOBEC3B can not be fully compensated by either vif sensitive APOBEC3G or APOBEC3F [5]. This finding provides additional insights into the *in vivo* role of APOBEC3 proteins and their interplay. Similar dosage effect is also observed

for CCR5, in which homozygosity for *CCR5* $\Delta 32$ affords near-absolute protection against HIV-1 infection while heterozygosity for CCR5 $\Delta 32$ has no measurable impact on HIV-1 acquisition [12].

The frequencies of the *APOBEC3B* deletion show considerable range and population stratification. In addition to findings from this study, Kidd et al [8] observed the highest deletion allele frequencies among Eastern Asian ($\approx 37\%$), Amerindian populations ($\approx 58\%$) and Oceanic ($\approx 90\%$), and the lowest among Africans ($<1\%$), Europeans ($\approx 6\%$), Africa Americans ($\approx 4\%$) and European Americans ($\approx 8\%$) [8]. The fixation index statistic (F_{st}) measuring the genetic variability within and between populations of the *APOBEC3B* deletion among major continental groups suggests that the frequency differences are likely due to differential selection [8].

This study provides the first epidemiologic evidence that APOBEC3B may mediate host innate resistance to HIV-1 *in vivo*. As our data suggest, the absence of APOBEC3B antiretroviral activity may be a risk factor for HIV acquisition. These data also suggest that once infected, homozygotes for the deletion have decreased ability to control HIV replication and correspondingly faster progression to AIDS; however these latter observations were based on only 4 individuals and must be viewed with caution. If these results are replicated, the null genotype may have considerable impact on the spread of HIV and HIV progression in populations (e.g., East Asia) where the deletion allele is very common.

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Table 1

Frequency Distribution of the *APOBEC3B* Deletion among Persons with HIV-1(HIV-1⁺) and Persons without HIV-1(HIV-1⁻) Infection

Genotype or allele	HIV-1 ⁺ group			HIV-1 ⁻ group vs HIV-1 ⁺ group		
	Seroconverter	Seroprevalent	All HIV-1 ⁺	HIV-1 ⁻ group	OR (95% CI)	P
European American	n = 656 (%)	n = 1294 (%)	n = 1950 (%)	n = 724 (%)		
Genotype						
I/I	547 (83.4)	1097 (84.8)	1644 (84.3)	616 (85.1)	Reference	
I/D	105 (16.0)	187 (14.5)	292 (15.0)	108 (14.9)	0.89 (0.68–1.17)	0.40
D/D	4 (0.61)	10 (0.77)	14 (0.72)	0 (0)	7.37 (1.24–infinity) ^a	0.024 ^a
Allele						
I	1199 (91.4)	2381 (92.0)	3580 (91.8)	1340 (92.5)	Reference	
D	113 (8.6)	207 (8.0)	320 (8.2)	108 (7.5)	1.23 (0.78–1.95) ^b	0.37 ^b
Africa American	n = 296 (%)	n = 806 (%)	n = 1102 (%)	n = 440 (%)		
Genotype						
I/I	273 (92.2)	723 (89.7)	996 (90.4)	406 (92.3)	Reference	
I/D	23 (7.8)	82 (10.2)	105 (9.5)	34 (7.7)	1.22 (0.63–2.33)	0.55
D/D	0	1 (0.12)	1 (0.09)	0	NA	
Allele						
I	569 (96.1)	1528 (94.8)	2097 (95.1)	789 (96.1)	Reference	
D	23 (3.9)	84 (5.2)	107 (4.9)	34 (3.9)	1.64 (0.74–3.64) ^c	0.23 ^c

NOTE. See “Subjects, materials, and methods” for a description of the HIV-1⁺ and HIV-1⁻ groups. CI, confidence interval; I, *APOBEC3B* deletion allele; D, *APOBEC3B* insertion allele; OR, odds ratio.

^aData were obtained from a conditional logistic regression test. All results were adjusted for the first eigenvector determined by a principal components analysis, unless otherwise indicated.

^bDetermined using a conditional logistic exact test.

^cAdditive model.

Table 2

Impact of *APOBEC3B* Deletion on Progression to AIDS and Death

Genotype	Progression to AIDS			Death		
	RH (95% CI) ^a	P _{Wald}	P _{LRT}	RH (95% CI) ^a	P _{Wald}	P _{LRT}
European American (n=645)						
I/I vs D/D						
Unadjusted	3.69 (1.36–9.97)	0.01	0.03	3.26 (1.04–10.26)	0.043	0.09
Adjusted	4.01 (1.45–11.07)	0.007	0.03	3.88 (1.20–12.48)	0.023	0.01
I/I vs I/D						
Unadjusted	1.09 (0.81–1.47)	0.58		1.13 (0.83–1.54)	0.44	
Adjusted	1.07 (0.80–1.45)	0.64		1.14 (0.83–1.56)	0.41	
Africa American (n=288)						
II vs I/D						
Unadjusted	1.08 (0.43–2.72)	0.86		1.80 (0.63–5.13)	0.27	
Adjusted	0.95 (0.35–2.63)	0.93		1.65 (0.45–6.01)	0.45	

NOTE. HIV-1 load set points were analyzed by a mixed-effects model. CI, confidence interval; D, *APOBEC3B* deletion allele; I, *APOBEC3B* insertion allele.

^a Adjusted for ancestry, using the first eigenvector value from a principal components analysis.

Impact of the *APOBEC3B* Deletion on Human Immunodeficient Virus Type 1 (HIV-1) Load among European Americans

Table 3

Genotype	Persons, no. (n = 344)	Measurements, no. (n = 1184)	Log ₁₀ viral load difference			
			Unadjusted (95%CI)	P	Adjusted (95% CI) ^a	P
I/I	285	954	Reference			
I/D	55	211	0.03 (−0.09–0.16)	0.60	0.03 (−0.09–0.16)	0.60
D/D	4	19	0.44 (0.02–0.87)	0.042	0.45 (0.02–0.89)	0.039

NOTE. HIV-1 load set points were analyzed by a mixed-effects model. CI, confidence interval; D, *APOBEC3B* deletion allele; I, *APOBEC3B* insertion allele.

^aAdjusted for ancestry, using the first eigenvector value from a principal components analysis.