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Genetic Variants in the Host Restriction Factor *APOBEC3G* are Associated With HIV-1–Related Disease Progression and Central Nervous System Impairment in Children

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Abstract

Background—Apolipoprotein B mRNA editing catalytic polypeptide 3G (*APOBEC3G*) protein is incorporated into nascent virus particles and mediates cytidine deamination (C-to-U) of first-strand reverse transcripts of HIV-1 in target cells resulting in G-to-A hypermutation of the coding strand and premature degradation. We investigated the effects of *APOBEC3G* genetic variants on HIV-1–related disease in children.

Methods—*APOBEC3G* variants were detected using real-time polymerase chain reaction in HIV-1–infected children from Pediatric AIDS Clinical Trials Group (PACTG) protocols P152 and P300 that evaluated the effectiveness of 3 mono- or dual-nucleoside reverse transcriptase inhibitor treatments.

Results—Of the 1049 children evaluated, 60% were non-Hispanic black, 26% Hispanic, 13% non-Hispanic white, and 1% other or unknown race/ethnicity. Age ranged from 42 days to 18 years; 45% were males. *APOBEC3G*-H186R homozygous G/G genotype was associated with more rapid HIV-1 disease progression [hazard ratio (HR): 1.69; *P* = 0.01] and central nervous system (CNS) impairment (HR: 2.00; *P* = 0.02) compared with the wild-type A/A or heterozygous A/G genotype in a recessive model. In both additive and dominant models, *APOBEC3G*-F119F-C allele was associated with protection against disease progression (HR [additive]: 0.69; *P* = 0.002 and HR [dominant]: 0.60; *P* = 0.001, respectively) and CNS impairment (HR [additive]: 0.65; *P* = 0.02 and HR [dominant]: 0.54; *P* = 0.007, respectively). These associations remained significant in multivariate analyses controlling for baseline characteristics or previously identified genetic variants known to alter HIV-1–related disease in this cohort of children.

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The authors have no conflicts of interest to disclose.

Informed consent was obtained from study participants. This study followed the human experimentation guidelines of the US Department of Health and Human Services and the UCSD review board.

Conclusions—*APOBEC3G*-H186R and F119F variants are associated with altered HIV-1–related disease progression and CNS impairment in children.

Keywords

APOBEC3G; polymorphisms; HIV-1; children; disease progression; CNS impairment

INTRODUCTION

A new class of host restriction factors has been found to play an important role in restricting intracellular HIV-1 replication. Apolipoprotein B mRNA editing catalytic polypeptide 3G (*APOBEC3G*), formerly known as CEM15, is an endogenous inhibitor of HIV-1 replication.^{1–4} During permissive infection, *APOBEC3G* is incorporated into nascent virus particles and mediates extensive deoxycytidine-to-deoxyuridine deamination of minus (first-)strand reverse transcripts in target cells. This results in guanine-to-adenine hypermutation of the viral plus (sense) coding strand and is associated with incomplete cDNA synthesis leading to premature cDNA degradation.^{5,6} Thus, *APOBEC3G* limits the spread of HIV-1 infection by altering viral assembly in the producer cell^{7,8} and when present as a functional protein can provide protection against progression of HIV-1–related disease.

The antiviral activity of *APOBEC3G* can be successfully neutralized by wild-type HIV-1 through viral infectivity factor (vif),^{9–11} which mediates *APOBEC3G* polyubiquitination and subsequent rapid degradation through the proteasome.^{10–13} Hence, vif neutralizes the *APOBEC3G* activity by binding the enzyme and interfering with its incorporation into virions. In the HIV-1 replication cycle, vif is expressed late and is essential for viral replication in primary human CD4⁺ T cells and some transformed cell lines.¹

The presence of genetic variants in *APOBEC3G* has been shown to alter its antiviral response against HIV-1. An *APOBEC3G* variant containing a nonsynonymous substitution of Arg instead of His at amino acid position 186 in exon 4 has been identified in African Americans and is strongly associated with a more rapid decline of CD4⁺ lymphocytes and accelerated progression to AIDS.¹⁴ An extensive analysis of a French cohort failed to identify significant associations between 29 *APOBEC3G* polymorphisms and disease progression, although the discrepancies with previous studies may be explained by the different variables used to analyze disease progression.¹⁵ In an additional study, another *APOBEC3G* variant in intron 4 (40693-C/T) was also found to be strongly associated with an increased risk of infection in a cohort of white subjects.¹⁶ However, no study to our knowledge has examined the effects of *APOBEC3G* variants in children.

In this study, we evaluated the effects of *APOBEC3G* genetic variants on the HIV-1–related disease progression and CNS impairment in children. We hypothesized that the presence of *APOBEC3G* genetic variants that alter its expression and function would be associated with HIV-1–related disease progression in children.

SUBJECTS AND METHODS

Patient Populations

One thousand forty-nine children with symptomatic HIV-1 infection enrolled in Pediatric AIDS Clinical Trial Group (PACTG) protocols P152¹⁷ and P300,¹⁸ which enrolled before the availability of effective combination antiviral therapy, were genotyped for *APOBEC3G* variants¹⁴ and evaluated for HIV-1–related disease progression. The primary end points of the analyses were progression-free survival (PFS) and central nervous system (CNS)

impairment. PFS was defined as either time from randomization to progression to first clinical HIV-1–related disease outcome or death, whichever occurred earlier. The disease outcomes included weight growth failure, 2 opportunistic infections, malignancy, CDC clinical disease category C, and/or abnormality of the CNS (eg, neurological deterioration, decline in neurocognitive test scores, and/or brain growth failure). The CNS impairment end point, a subset of PFS, was defined as time from randomization to the deterioration in brain growth, psychological function, and/or neurological status.

Detailed characteristics of children, eligibility criteria, study end points, disease progression, and neuropsychological tests were described earlier.^{17,18} This study followed the human experimentation guidelines of the US Department of Health and Human Services and the University of California San Diego Institutional Review Board, and informed consent was obtained from study participants.

Genotyping

Genomic DNA extracts from peripheral blood mono-nuclear cells were genotyped for *APOBEC3G* polymorphisms by polymerase chain reaction and melting curve analysis (LightCycler; Roche Diagnostics, Indianapolis, IN). Studied *APOBEC3G* variants included those in the promoter region: -571-G/C (rs5757463), -199-G/A (rs34550797), -90-C/G (rs5750743), exon 3 F119F-T/C (rs5757465), intron 3 (197193-T/C), exon 4 H186R-A/G (rs8177832), intron 4 (199376-G/C, 40693-C/T), and exon 6 Q275E-C/T (rs17496046). We developed 9 real-time polymerase chain reaction genotyping assays (primer and probe sequences available on request) based on the melting curves; assays were confirmed by sequencing the amplicons.

Statistical Analyses

Analyses of the association between *APOBEC3G* genotype markers and the time to PFS or CNS impairment used Cox proportional hazards model. The analysis of identifying potentially significant associations of PFS and CNS with each of the 8 genotyped single nucleotide polymorphisms (SNPs) used a Wald-type test for trend (ie, additive genetic model). Multiple comparison adjustment used the Benjamini and Hochberg method.¹⁹ The distribution of PFS and CNS by genotype variants was estimated using Kaplan–Meier method. Once an SNP with the potential association was identified and the mode of genetic model (additive, recessive, or dominant) was determined, further investigation of the association was performed using multivariate Cox regression model that adjusted for baseline covariates including CD4⁺ lymphocyte count and percentage, log₁₀ HIV-1 RNA, treatments, age, gender, race, and weight for age z-score (WAZ score) as well as other genetic markers identified to significantly affect PFS or CNS impairment in this cohort of children (ie, *CCR5*-wt/Δ32, *CCR5*-59029-G/A, *CCR2*-wt/64I, *SDF-1*-180-G/A, *MCP-1*-2518-G/A, *CX₃CR1*-249-V/I, -280-T/M, *MBL2*-A/O, *MBL2*-X/Y, *MBL2*-P/Q, and *MBL2*-H/L).^{20–23}

RESULTS

Baseline Characteristics of Studied Children

Of the 1049 studied children, 60% were non-Hispanic black, 26% Hispanic, 13% non-Hispanic white, and 1% other or unknown race/ethnicity. Age ranged from 42 days to 18 years; 45% were males. Of the 1049 subjects, 228 (22%) experienced disease progression. The median follow-up time for the analytic cohort of 1049 patients was 18.6 months (95% CI: 17.8 to 19.5 months). Weight growth failure, deterioration of brain growth, and CNS event were the leading causes of disease progression (Table 1). Of the 7 children with opportunistic infections, none experienced any CNS event. At study entry, children who

experienced disease progression had significantly higher $\log_{10}$ RNA copies per milliliter and lower CD4⁺ lymphocyte counts and percentages, WAZ scores, and cognitive scores.

Distribution of *APOBEC3G* Genotypes by Race/Ethnicity

Distributions of *APOBEC3G* genotypes by race/ethnicity are presented in Table 2. For the F119F polymorphism, the heterozygous variant T/C and the homozygous variant C/C were least common in the non-Hispanic black children (15% and 1%, respectively), whereas Hispanics (39% and 16%, respectively) and non-Hispanic white children (50% and 12%, respectively) had higher frequencies of these genotypes ($P < 0.001$). For H186R-A/G polymorphism, the heterozygous variant A/G and the homozygous variant G/G were most common in the non-Hispanic black children (43% and 12%, respectively), whereas Hispanics (26% and 1%, respectively) and non-Hispanic white children (9% and 0%, respectively) had lower frequencies of these genotypes ($P < 0.001$).

At study entry, none of the *APOBEC3G* SNPs was significantly associated with CD4⁺ count, CD4⁺ percentage, plasma HIV-1 RNA, weight for age and gender z-score, or cognitive score (data not shown). Table 3A summarizes the analysis results from additive model of potential association of PFS and CNS with each of the 8 genotyped SNPs, with univariate Wald test P values and the Benjamini and Hochberg¹⁹ adjusted P values controlling for multiple comparisons. Of the SNPs evaluated, those identified as being significantly associated with disease progression, *APOBEC3G*-C/T [F119F (rs5757465)] and *APOBEC3G*-A/G [H186R (rs8177832)], are discussed below.

Effects of *APOBEC3G*-C/T (F119F) Genotypes on the HIV-1–related PFS and CNS Impairment

At study entry, none of the *APOBEC3G* genotypes was significantly associated with CD4⁺ count, CD4⁺ percentage, plasma HIV-1 RNA, weight for age and gender z-score, or cognitive score (data not shown). However, significant associations were identified with the rate of disease progression including the development of cognitive impairment. The presence of *APOBEC3G*-F119F-C or minor allele was shown to be associated with both reduced risk of disease progression [univariate hazard ratio (HR): 0.60; $P = 0.001$] (Fig. 1A and Table 3B) and CNS impairment (HR: 0.54; $P = 0.007$) (Fig. 1B and Table 3B). These associations remained significant after adjusting for baseline covariates (race, gender, age, baseline HIV-1 RNA log RNA, baseline CD4⁺ lymphocyte count and percentage, and baseline WAZ score) or when adjusted for other genotypes known to alter HIV-1 disease progression in children (ie, *CCR5*-wt/ Δ 32, *CCR5*-59029-G/A, *CCR2*-wt/64I, *CX3CR1*-249-V/I, -280-T/M, *SDF-1*-180-G/A, *MCP-1*-G/A, *MBL2*-A/O, *MBL2*-X/Y, *MBL2*-P/Q, and *MBL2*-H/L) (Table 3B).

Effects of *APOBEC3G*-A/G (H186R) genotypes on the HIV-1–related PFS and CNS impairment

The presence of *APOBEC3G*-H186R homozygous G/G genotype (or recessive model) was shown to be associated with both a more rapid disease progression (univariate HR: 1.69; $p = 0.01$) (Fig. 1C and Table 3B) and CNS impairment (HR: 2.00; $P = 0.02$) (Fig. 1D and Table 3B) compared with the wild-type A/A or heterozygous A/G genotype. However, each of these associations lost significance for disease progression and CNS impairment after adjusting for baseline covariates in Cox regression model (Table 3B). In contrast, these associations remained significant after adjusting for other genotypes known to alter HIV-1 disease progression in children (Table 3B).

Effects of *APOBEC3G* haplotypes on the HIV-1–related PFS and CNS Impairment

Pairwise tests of linkage disequilibrium were performed among the *APOBEC3G* SNPs except for -199-G/A SNP. Strong LD was only observed between H186R-A/G and 197193-T/C SNPs ($D' = 0.995$). Haplotypes were constructed using these two SNPs, and no association between the haplotypes and HIV-1–related disease progression or CNS impairment was identified (data not shown).

DISCUSSION

APOBEC3G alleles have been found to alter HIV-1–related disease progression in adults^{14,16}; however, there is little information on their association with HIV-1–related disease progression in children. In this study, we found that the presence of *APOBEC3G*-F119F and H186R genetic variants were associated with altered HIV-1 disease progression in children.

The presence of *APOBEC3G*-F119F-C allele was associated with protection against disease progression and CNS impairment. The observed associations remained significant in multivariate analyses controlling for baseline characteristics and other genetic variants known to alter HIV-1 disease in children. *APOBEC3G*-F119F is a nonsynonymous genetic variant that altered the rate of disease progression in children. The mechanism by which F119F polymorphism affects the HIV-1 disease progression is unclear; however, one possibility is that F119F variants are in linkage disequilibrium with another yet undiscovered variant that alters the *APOBEC3G* expression or function. Although synonymous variants do not change the amino acid code, their effects on gene expression are possible. It is shown that certain codons are translated more efficiently (faster or more accurately) than others and changing codons to suboptimal synonyms reduces the level of corresponding protein. Second, synonymous changes may not be always silent. Exon sequences close to exon–intron border act as RNA splicing signals, and if a splicing signal is destroyed by a synonymous variant, the exon does not appear in the final protein and it may lead to expression of a truncated protein. Also, the presence of synonymous variants can alter the secondary structure of RNA, which can affect translation efficiency.

In our study, the presence of *APOBEC3G*-H186R homozygous G/G genotype was significantly associated with more rapid disease progression and CNS impairment in children and remained significant in multivariate analyses. Effects of *APOBEC3G*-H186R polymorphisms could be due to the potential alteration of *APOBEC3G* protein expression or function due to change of histidine to arginine amino acid at the 186 position. In an earlier study of 290 African American adults, An et al¹⁴ found an association of 186-R/R variant with more rapid disease progression. We found a similar significant association of 186-G/G with more rapid disease progression in 625 HIV-1–infected African American children. We also observed comparable *APOBEC3G*-H186R-G allele frequency in the children (0.33 and 0.04) compared with adults (0.37 and 0.03) in African Americans and whites, respectively. In another study of 363 HIV-1–infected white Hispanic children in Argentina, *APOBEC3G*-186-H/R polymorphism had no effect on vertical transmission or progression to pediatric AIDS; however, *APOBEC3G* was reported to interact with HIV vif proteins.²⁴

A novel aspect of our study was the analyses of the association of *APOBEC3G* variants with the progression to CNS outcomes in the studied children, where the presence of either F119F or H186R variants led to more rapid CNS impairment. It has been shown that functional *APOBEC3G* is expressed in the neurons and astrocytes, and *APOBEC3G*-specific siRNA led to increase of pseudotyped HIV-1 replication in these cells.²⁵ Our findings combined with these reports provide additional support that there is intracellular

expression and regulation of functional APOBEC3G in neuronal cells, which may represent innate defense mechanisms involved in the neuronal protection of the CNS.

No significant effects of *APOBEC3G* haplotypes on HIV-1 disease were observed in the children compared with those reported in an adult cohort.¹⁴ Although this difference could be due to the difference in the race/ethnicity of 2 study groups, we did not observe any effects of race/ethnicity on the impact of *APOBEC3G* genotypes on disease progression in children. Of note, our pediatric cohort consisted of 61% black, 13% white, and 26% Hispanic children while the earlier report¹⁴ studied 61% whites and 39% blacks. However, we found no indication that race/ethnicity altered the effect of *APOBEC3G* genotypes.

APOBEC3G restricts HIV-1 replication in vitro through the induction of G/A hypermutation and is independently associated with reduced pretreatment viremia. An analysis of *APOBEC3G* polymorphisms revealed that the gene is highly conserved at the amino acid level suggesting that APOBEC3G-induced HIV-1 hypermutation represents a potent host antiviral factor in vivo and that the APOBEC3G-vif interaction may represent a valuable therapeutic target.²⁶

Other than the *APOBEC3G*, *APOBEC3H* variants encoding an SNP cluster (G105R, K121D and E178D, hapII-RDD) have been reported to restrict HIV-1 more efficiently than wild-type APOBEC3H (hapI-GKE).²⁷ Also, it has been demonstrated that APOBEC3G and APOBEC3F block HIV-1 replication by interfering with the proviral DNA formation. In HIV-1 virions, APOBEC3G can also interact with HIV-1 integrase and nucleocapsid, the key viral factors for reverse transcription and integration, respectively.²⁸

It has been shown that a small molecule, RN-18, that antagonizes vif function and inhibits HIV-1 replication only in the presence of APOBEC3G, increases cellular APO-BEC3G levels in a vif-dependent manner and increases APOBEC3G incorporation into virions without inhibiting general proteasome-mediated protein degradation.²⁹ RN-18 enhances vif degradation only in the presence of APO-BEC3G, reduces viral infectivity by increasing APOBEC3G incorporation into virions, and enhances cytidine deamination of the viral genome. These results provide further evidence that the HIV-1 vif-APOBEC3G axis is a suitable target for developing small molecule-based therapies for HIV infection.

A newly discovered single amino acid substitution mutant of human *APOBEC3G* (D128K) was found to interact with HIV-1 vif but was not depleted from cells; thus, it inhibited HIV-1 replication in a vif-resistant manner. This has the potential to provide a gene therapy approach to combat HIV-1 infection.³⁰ In another recent study, the expression of APOBEC3G was found to be significantly increased in CD14⁺ cells and in cervical tissues of HIV-exposed seronegative individuals.³¹ Higher APOBEC3G expression was associated with a reduced susceptibility of peripheral blood mononuclear cells (PBMCs) to in vitro infection with the HIV-1 Ba-L R5 strain. Thus, APOBEC3G could be important in modulating in vivo susceptibility to sexually transmitted HIV infection.

In a study of the rate of *APOBEC3G* gene evolution from 5 hominoids and 2 Old World monkeys averaged across the entire coding region, the rate of nonsynonymous nucleotide substitutions was 1.4 times the rate of synonymous substitutions,³² suggesting that *APOBEC3G* gene has been under positive Darwinian selection. Furthermore, the rate of charge-altering nonsynonymous substitution is 1.8 times that of a charge-conserving substitution, indicating that the selection is promoting the diversity of the protein charge profile. However, no difference in selective pressure on *APOBEC3G* is detected between hosts and non-hosts of HIV-1 or simian immunodeficiency virus (SIV). These results, together with recent findings that the antiviral activity of APOBEC3G is not limited to HIV/

SIV, suggest that the selective pressure on the *APOBEC3G* gene is not solely from HIV/SIV and APOBEC3G has broad antiviral effects.

In summary, our studies show that the presence *APOBEC3G*-H186R and F119F variants are important determinants of HIV-1–related disease progression and CNS impairment in children. These findings further support APOBEC3G as a potential cellular target for the control and possible elimination of HIV-1 infection.

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References

1. Simon JH, Gaddis NC, Fouchier RA, et al. Evidence for a newly discovered cellular anti-HIV-1 phenotype. *Nat Med*. 1998; 4:1397–1400. [PubMed: 9846577]
2. Harris RS, Liddament MT. Retroviral restriction by APOBEC proteins. *Nat Rev Immunol*. 2004; 4:868–877. [PubMed: 15516966]
3. Harris RS, Petersen-Mahrt SK, Neuberger MS. RNA editing enzyme APOBEC1 and some of its homologs can act as DNA mutators. *Mol Cell*. 2002; 10:1247–1253. [PubMed: 12453430]
4. Mangeat B, Turelli P, Caron G, et al. Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. *Nature*. 2003; 424:99–103. [PubMed: 12808466]
5. Mariani R, Chen D, Schrofelbauer B, et al. Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. *Cell*. 2003; 114:21–31. [PubMed: 12859895]
6. Klarmann GJ, Chen X, North TW, et al. Incorporation of uracil into minus strand DNA affects the specificity of plus strand synthesis initiation during lentiviral reverse transcription. *J Biol Chem*. 2003; 278:7902–7909. [PubMed: 12458216]
7. Goff SP. Retrovirus restriction factors. *Mol Cell*. 2004; 16:849–859. [PubMed: 15610729]
8. Goff SP. Death by deamination: a novel host restriction system for HIV-1. *Cell*. 2003; 114:281–283. [PubMed: 12914693]
9. Marin M, Rose KM, Kozak SL, et al. HIV-1 Vif protein binds the editing enzyme APOBEC3G and induces its degradation. *Nat Med*. 2003; 9:1398–1403. [PubMed: 14528301]
10. Sheehy AM, Gaddis NC, Malim MH. The antiretroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif. *Nat Med*. 2003; 9:1404–1407. [PubMed: 14528300]
11. Stopak K, de Noronha C, Yonemoto W, et al. HIV-1 Vif blocks the antiviral activity of APOBEC3G by impairing both its translation and intracellular stability. *Mol Cell*. 2003; 12:591–601. [PubMed: 14527406]
12. Yu X, Yu Y, Liu B, et al. Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex. *Science*. 2003; 302:1056–1060. [PubMed: 14564014]
13. Mehle A, Strack B, Ancuta P, et al. Vif overcomes the innate antiviral activity of APOBEC3G by promoting its degradation in the ubiquitin-proteasome pathway. *J Biol Chem*. 2004; 279:7792–7798. [PubMed: 14672928]
14. An P, Bleiber G, Duggal P, et al. APOBEC3G genetic variants and their influence on the progression to AIDS. *J Virol*. 2004; 78:11070–11076. [PubMed: 15452227]

15. Do H, Vasilescu A, Diop G, et al. Exhaustive genotyping of the CEM15 (APOBEC3G) gene and absence of association with AIDS progression in a French cohort. *J Infect Dis.* 2005; 191:159–163. [PubMed: 15609224]
16. Valcke HS, Bernard NF, Bruneau J, et al. APOBEC3G genetic variants and their association with risk of HIV infection in highly exposed Caucasians. *AIDS.* 2006; 20:1984–1986. [PubMed: 16988524]
17. Englund JA, Baker CJ, Raskino C, et al. Zidovudine, didanosine, or both as the initial treatment for symptomatic HIV-infected children. AIDS Clinical Trials Group (ACTG) Study 152 Team. *N Engl J Med.* 1997; 336:1704–1712. [PubMed: 9182213]
18. McKinney RE Jr, Johnson GM, Stanley K, et al. A randomized study of combined zidovudine-lamivudine versus didanosine monotherapy in children with symptomatic therapy-naïve HIV-1 infection. The Pediatric AIDS Clinical Trials Group Protocol 300 Study Team. *J Pediatr.* 1998; 133:500–508. [PubMed: 9787687]
19. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B Stat Methodol.* 1995; 57:289–300.
20. Singh KK, Barroga CF, Hughes MD, et al. Genetic influence of CCR5, CCR2, and SDF1 variants on human immunodeficiency virus 1 (HIV-1)-related disease progression and neurological impairment, in children with symptomatic HIV-1 infection. *J Infect Dis.* 2003; 188:1461–1472. [PubMed: 14624371]
21. Singh KK, Hughes MD, Chen J, et al. Genetic polymorphisms in CX3CR1 predict HIV-1 disease progression in children independently of CD4+ lymphocyte count and HIV-1 RNA load. *J Infect Dis.* 2005; 191:1971–1980. [PubMed: 15871132]
22. Singh KK, Hughes MD, Chen J, et al. Impact of MCP-1-2518-G allele on the HIV-1 disease of children in the United States. *AIDS.* 2006; 20:475–478. [PubMed: 16439891]
23. Singh KK, Lieser A, Ruan PK, et al. An age-dependent association of mannose-binding lectin-2 genetic variants on HIV-1-related disease in children. *J Allergy Clin Immunol.* 2008; 122:173–180. 180.e1–2. [PubMed: 18602571]
24. De Maio FA, Rocco CA, Aulicino PC, et al. Effect of HIV-1 Vif variability on progression to pediatric AIDS and its association with APOBEC3G and CUL5 polymorphisms. *Infect Genet Evol.* 2011; 11:1256–1262. [PubMed: 21571098]
25. Wang YJ, Wang X, Zhang H, et al. Expression and regulation of antiviral protein APOBEC3G in human neuronal cells. *J Neuroimmunol.* 2009; 206:14–21. [PubMed: 19027180]
26. Pace C, Keller J, Nolan D, et al. Population level analysis of human immunodeficiency virus type 1 hypermutation and its relationship with APOBEC3G and vif genetic variation. *J Virol.* 2006; 80:9259–9269. [PubMed: 16940537]
27. Harari A, Ooms M, Mulder LC, et al. Polymorphisms and splice variants influence the antiretroviral activity of human APOBEC3H. *J Virol.* 2009; 83:295–303. [PubMed: 18945781]
28. Luo K, Wang T, Liu B, et al. Cytidine deaminases APOBEC3G and APOBEC3F interact with human immunodeficiency virus type 1 integrase and inhibit proviral DNA formation. *J Virol.* 2007; 81:7238–7248. [PubMed: 17428847]
29. Nathans R, Cao H, Sharova N, et al. Small-molecule inhibition of HIV-1 Vif. *Nat Biotechnol.* 2008; 26:1187–1192. [PubMed: 18806783]
30. Xu H, Svarovskaia ES, Barr R, et al. A single amino acid substitution in human APOBEC3G antiretroviral enzyme confers resistance to HIV-1 virion infectivity factor-induced depletion. *Proc Natl Acad Sci U S A.* 2004; 101:5652–5657. [PubMed: 15054139]
31. Biasin M, Piacentini L, Lo Caputo S, et al. Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G: a possible role in the resistance to HIV of HIV-exposed seronegative individuals. *J Infect Dis.* 2007; 195:960–964. [PubMed: 17330785]
32. Zhang J, Webb DM. Rapid evolution of primate antiviral enzyme APOBEC3G. *Hum Mol Genet.* 2004; 13:1785–1791. [PubMed: 15198990]

FIGURE 1. Association of *APOBEC3G*-F119F-C/T and -H186R-A/G variants with HIV-1–related PFS and progression to CNS end points.

TABLE 1

Baseline Characteristics.

Characteristic	PFS events		
	Total (N = 1049)	No (N = 821)	Yes (N = 228)
Follow-up time (mo)			
Median (95% CI)	18.60 (17.80 to 19.50)	—	—
Gender			
Male	475 (45%)	372 (45%)	103 (45%)
Female	574 (55%)	449 (55%)	125 (55%)
Race/Ethnicity			
Black	625 (60%)	496 (60%)	129 (57%)
Hispanic	270 (26%)	197 (24%)	73 (32%)
White	137 (13%)	114 (14%)	23 (10%)
Unknown	14 (1%)	11 (1%)	3 (1%)
Others	3 (0%)	3 (0%)	0 (0%)
Age (y) at randomization			
N	1,049	821	228
Median (Min–Max)	2.35 (0.12–17.98)	2.79 (0.12–17.98)	0.99 (0.20–17.42)
<2 y	483 (46%)	330 (40%)	153 (67%)
2 y	566 (54%)	491 (60%)	75 (33%)
Disease progression outcome			
No event	821 (78%)	821 (100%)	0 (0%)
Death	25 (2%)	0 (0%)	25 (11%)
2 opportunistic infections	7 (1%)	0 (0%)	7 (3%)
Weight growth failure	74 (7%)	0 (0%)	74 (32%)
Malignancy	1 (0%)	0 (0%)	1 (0%)
CDC clinical disease category C	12 (1%)	0 (0%)	12 (5%)
All CNS events:	109 (10%)	0 (0%)	109 (48%)
Deterioration of brain growth	67 (6%)	0 (0%)	67 (29%)
Deterioration of physical function	13 (1%)	0 (0%)	13 (6%)
CNS deterioration	29 (3%)	0 (0%)	29 (13%)
Baseline CD4 percentage			
N	1041	815	226
Median (Q1–Q3)	24.00 (16.49–31.00)	24.92 (18.00–31.50)	21.00 (9.90–29.39)
Baseline CD4 count/mm ³			
N	1041	815	226
Median (Q1–Q3)	783.00 (419.45–1,320.00)	816.00 (462.00–1,313.76)	582.75 (197.00–1,412.94)
Baseline log ₁₀ RNA copies/mL			
N	868	688	180
Median (Q1–Q3)	5.12 (4.49–5.71)	4.99 (4.40–5.54)	5.84 (5.11–6.32)
Baseline cognitive score			
N	989	781	208

Characteristic	PFS events		
	Total (N = 1049)	No (N = 821)	Yes (N = 228)
Median (Q1–Q3)	83 (71–93)	84 (74–94)	78.00 (60.50–92.00)
Baseline WAZ score			
N	1,049	821	228
Median (Q1–Q3)	−0.68 (−1.53–0.25)	−0.42 (−1.29–0.37)	−1.40 (−2.06–0.50)

TABLE 2

Distribution of *APOBEC3G* Genotypes: Overall and by Race/Ethnicity

Genotype	Total (N = 1049)	Black (N = 625)	Hispanic (N = 270)	White (N = 137)	Unknown (N = 14)	Others (N = 3)	P (χ^2 test)
F119F-C/T							
C/C	69 (7%)	9 (1%)	43 (16%)	16 (12%)	0 (0%)	1 (33%)	<0.001
C/T	273 (26%)	94 (15%)	106 (39%)	68 (50%)	5 (36%)	0 (0%)	
T/T	706 (67%)	522 (84%)	120 (44%)	53 (39%)	9 (64%)	2 (67%)	
Missing	1 (0%)	0 (0%)	1 (0%)	0 (0%)	0 (0%)	0 (0%)	
H186R-A/G							
A/A	613 (58%)	279 (45%)	195 (72%)	125 (91%)	11 (79%)	3 (100%)	<0.001
A/G	355 (34%)	269 (43%)	71 (26%)	12 (9%)	3 (21%)	0 (0%)	
G/G	80 (8%)	77 (12%)	3 (1%)	0 (0%)	0 (0%)	0 (0%)	
-90-C/G							
G/G	830 (79%)	500 (80%)	207 (77%)	108 (79%)	12 (86%)	3 (100%)	0.725
C/G	160 (15%)	87 (14%)	48 (18%)	24 (18%)	1 (7%)	0 (0%)	
C/C	59 (6%)	38 (6%)	15 (6%)	5 (4%)	1 (7%)	0 (0%)	
-571-G/C							
G/G	4 (0%)	4 (1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.561
G/C	68 (6%)	46 (7%)	12 (4%)	10 (7%)	0 (0%)	0 (0%)	
C/C	977 (93%)	575 (92%)	258 (96%)	127 (93%)	14 (100%)	3 (100%)	
-199-G/A							
G/G	1049 (100%)	625 (100%)	270 (100%)	137 (100%)	14 (100%)	3 (100%)	
199376-G/C							
C/C	496 (47%)	367 (59%)	78 (29%)	48 (35%)	2 (14%)	1 (33%)	<0.001
C/G	370 (35%)	200 (32%)	111 (41%)	48 (35%)	11 (79%)	0 (0%)	
G/G	181 (17%)	57 (9%)	80 (30%)	41 (30%)	1 (7%)	2 (67%)	
197193-T/C							
C/C	16 (2%)	15 (2%)	0 (0%)	1 (1%)	0 (0%)	0 (0%)	0.003
T/C	109 (10%)	82 (13%)	21 (8%)	6 (4%)	0 (0%)	0 (0%)	
T/T	923 (88%)	528 (84%)	248 (92%)	130 (95%)	14 (100%)	3 (100%)	
Q275E-C/T							
C/C	851 (81%)	484 (77%)	233 (86%)	119 (87%)	12 (86%)	3 (100%)	0.019

Genotype	Total (N = 1049)	Black (N = 625)	Hispanic (N = 270)	White (N = 137)	Unknown (N = 14)	Others (N = 3)	P (χ^2 test)
C/T	182 (17%)	127 (20%)	36 (13%)	17 (12%)	2 (14%)	0 (0%)	
T/T	15 (1%)	14 (2%)	0 (0%)	1 (1%)	0 (0%)	0 (0%)	
40693-C/T							
C/C	933 (89%)	553 (88%)	243 (90%)	123 (90%)	11 (79%)	3 (100%)	0.816
C/T	111 (11%)	69 (11%)	26 (10%)	13 (9%)	3 (21%)	0 (0%)	
T/T	3 (0%)	2 (0%)	0 (0%)	1 (1%)	0 (0%)	0 (0%)	

TABLE 3

Association of PFS and CNS With the 8 *APOBEC3G* SNPs Using Cox Regression Model

A. Additive Model for Genetic Association									
<i>APOBEC3G</i> Genotype	HR (95% CI)	PFS			CNS			Adjusted [‡] HR (95% CI)	P
		Univariate P	Multiple Comparison Adjusted P*	HR (95% CI)	Univariate P	Multiple Comparison Adjusted P*	Adjusted [‡] HR (95% CI)		
F119F	0.69 (0.54 to 0.88)	0.002	0.016	0.65 (0.45 to 0.92)	0.020	0.160	0.54 (0.39 to 0.75)	<0.001	
H186R	1.17 (0.96 to 1.43)	0.120	0.480	1.32 (1.00 to 1.75)	0.050	0.200	1.8 (1.17 to 2.79)	0.008	
-90-C/G	0.93 (0.74 to 1.18)	0.560	0.590	0.92 (0.66 to 1.28)	0.610	0.770	0.49 (0.3 to 0.81)	0.006	
-571-G/C	0.70 (0.39 to 1.26)	0.230	0.590	0.53 (0.20 to 1.42)	0.210	0.560	2.25 (1.25 to 4.07)	0.007	
199376- C/G	0.95 (0.80 to 1.13)	0.540	0.590	0.96 (0.74 to 1.23)	0.730	0.770			
197193-T/C	1.13 (0.80 to 1.59)	0.500	0.590	1.08 (0.65 to 1.81)	0.770				
Q275E-C/T	1.07 (0.91 to 1.26)	0.420	0.590	1.13 (0.90 to 1.42)	0.300	0.600			
40693-C/T	0.89 (0.59 to 1.35)	0.590	0.590	0.76 (0.40 to 1.44)	0.400	0.640			

B. Dominant and Recessive Models for Genetic Association									
End Point	<i>APOBEC3G</i> Genotype	Model	Comparison	Unadjusted HR (95% CI)	P	Adjusted [‡] HR (95% CI)	P	Adjusted [‡] HR (95% CI)	P
PFS	F119F	Dominant	C/C + C/T vs. T/T	0.60 (0.44 to 0.81)	0.001	0.65 (0.45 to 0.94)	0.021	0.54 (0.39 to 0.75)	<0.001
	H186R	Recessive	G/G vs. A/A + A/G	1.69 (1.13 to 2.55)	0.01	1.49 (0.91 to 2.45)	0.113	1.8 (1.17 to 2.79)	0.008
CNS	F119F	Dominant	C/C + C/T vs. T/T	0.54 (0.35 to 0.85)	0.007	0.56 (0.32 to 0.96)	0.034	0.49 (0.3 to 0.81)	0.006
	H186R	Recessive	G/G vs. A/A + A/G	2.00 (1.14 to 3.50)	0.02	1.82 (0.89 to 3.71)	0.099	2.25 (1.25 to 4.07)	0.007

* Adjusted for multiple comparisons using the Benjamini and Hochberg¹⁹ method.[‡] Adjusted for baseline covariates: race, gender, age, baseline HIV-1 RNA log RNA, baseline CD4⁺ lymphocyte count and percentage, and baseline WAZ score.[‡] Adjusted for other genotypes: *CCR5*-wt/Δ32, *CCR5*59029-G/A, *CCR2*-wt/64I, *CX3CR1*-249-V/I, -280-T/M, *SDF1*-180-G/A, *MCP1*-I-G/A, *MBL2*-A/O, *MBL2*-X/Y, *MBL2*-P/Q, and *MBL2*-H/L.