Polymorphisms of the *SAMHD1* Gene Are Not Associated with the Infection and Natural Control of HIV Type 1 in Europeans and African-Americans

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Abstract

The HIV-1 restriction factor SAM domain and HD domain-containing protein 1 (*SAMHD1*) blocks HIV-1 infection in human myeloid cells. Mutations in the *SAMHD1* gene are associated with rare genetic diseases including Aicardi-Goutieres syndrome. However, it is unknown whether polymorphisms of *SAMHD1* are associated with infection and natural control of HIV-1 in humans. Our objective was to determine whether the expression of *SAMHD1* mRNA is affected by common single nucleotide polymorphisms (SNPs) in *SAMHD1* and whether the SNPs are associated with HIV-1 infection status. Using a tagging SNP approach, we determined the association between eight tagging SNPs in *SAMHD1* and the mRNA expression in B-lymphocyte cell lines from 70 healthy white donors. We identified one SNP (rs1291142) that was significantly associated with *SAMHD1* mRNA expression, with minor allele carriers having 30% less mRNA levels ($p=0.015$). However, after analyzing the published genome-wide association study data of 857 HIV-1 controllers and 2088 HIV-1 progressors from the European and African-American cohorts, we did not find a significant association between SNPs in *SAMHD1* and HIV-1 infection status, including SNP rs1291142 ($p>0.05$). We also observed 2- to 6-fold variations of *SAMHD1* mRNA levels in primary B-lymphocytes, CD4⁺ T-lymphocytes, and CD14⁺ monocytes from five healthy donors. Our results suggest that common regulatory polymorphism(s) exist in the *SAMHD1* gene that affects its mRNA expression in B-lymphocyte cell lines from healthy whites. However, polymorphisms of *SAMHD1* are unlikely to contribute to the infection and natural control of HIV-1 in European and African-American individuals.

Introduction

POLYMORPHISMS OF HUMAN GENES can profoundly affect the acquisition and progression of infectious diseases including HIV type 1 (HIV-1) infection and AIDS.¹ A small proportion of HIV-1-infected individuals (0.5% ~ 4%) are termed HIV-1 controllers, as they can achieve long-term control of HIV-1 viremia and avoid AIDS without the need for antiretroviral therapy.² A genome-wide association analysis in multiethnic cohorts of HIV-1 controllers in comparison with HIV-1 progressors identified more than 300 genome-wide significant single nucleotide polymorphisms (SNPs) within the gene encoding the major histocompatibility complex, but not other genes.³ This study suggested that the major genetic determinants of natural control of HIV-1 infection are

the polymorphisms of the genes encoding human leukocyte antigen (HLA) class I that may affect HLA class I-restricted peptide presentation.³

HIV-1 restriction factors are host proteins that potently block viral replication in the cell.⁴⁻⁶ The well-characterized HIV-1 restriction factors include APOBEC3G (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G),⁷ TRIM5 α (tripartite motif 5 α),⁸ and Tetherin (BST-2 or CD317).^{9,10} Polymorphisms in the genes encoding HIV-1 restriction factors have been shown to associate with HIV-1 infection and AIDS progression in humans. For example, polymorphisms in the *APOBEC3G* and *TRIM5 α* genes are associated with status and clinical course of HIV-1 infection,¹¹⁻¹⁴ suggesting that these HIV-1 restriction factors contribute to HIV-1 infection and AIDS progression *in vivo*.

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SAMHD1 is a cellular protein that restricts HIV-1 infection in human myeloid-lineage cells, such as monocytes, dendritic cells, and macrophages.^{15,16} As a dGTP-regulated deoxynucleoside triphosphate (dNTP) triphosphohydrolase, SAMHD1 degrades dNTPs^{17,18} and mediates HIV-1 restriction by decreasing the concentration of the cellular dNTP pool.¹⁹ Mutations in *SAMHD1* are associated with a rare genetic disorder known as Aicardi-Goutieres syndrome (AGS).²⁰ Patients with AGS have mutations in multiple exons of *SAMHD1* that affect protein expression or in an intron that alters the splicing acceptor site of *SAMHD1*.²⁰ SAMHD1-deficient monocytes from one AGS patient have been shown to be highly susceptible to HIV-1 infection *in vitro*,²¹ suggesting that SAMHD1 may block HIV-1 infection in myeloid cells *in vivo*. Moreover, a mutation in the splice acceptor site of an intron in *SAMHD1* results in a shorter mRNA and an aberrant protein with an in-frame deletion, which is associated with cerebral vasculopathy and early onset stroke in Amish individuals.²² However, it is unknown whether common regulatory polymorphisms exist in *SAMHD1* and whether such polymorphisms are associated with infection and natural control of HIV-1 in humans.

In this study, we hypothesized that common *SAMHD1* SNPs may affect its mRNA expression and such SNPs could be associated with HIV-1 infection status in humans. We identified an intron SNP in *SAMHD1* that significantly affects the mRNA expression of *SAMHD1* in B-lymphocytes from 70 healthy white donors. However, our analysis indicated that SNPs in *SAMHD1* are not associated with HIV-1 infection status among 857 HIV-1 controllers and 2088 HIV-1 progressors from the European and African-American cohorts.³ Our results suggest that polymorphisms of *SAMHD1* are unlikely to contribute to the infection and natural control of HIV-1 in European and African-American populations.

Materials and Methods

Selection of intron SNPs in the *SAMHD1* gene

Tagging SNPs in *SAMHD1* were selected from HapMap²³ setting parameters for an minor allele frequency over 10% and linkage disequilibrium of 0.8 in the white population (Table 1). Parameters yielded eight tagging SNPs: rs4810362, rs6030346, rs6065494, rs6072746, rs6016783, rs7268811, rs12481143, and

rs1291142. The sequence, reference genotype and variant, and allele frequency for each of these SNPs were determined using the SNP database on www.ncbi.nlm.nih.gov/snp/.

Isolation of primary B-lymphocytes, monocytes and CD4⁺ T-lymphocytes

Primary B-lymphocytes, monocytes, and resting CD4⁺ T-lymphocytes were isolated from fresh peripheral blood mononuclear cells (PBMCs) of five healthy blood donors using anti-CD19-, anti-CD14-, and anti-CD4-coated magnetic particles (BD Biosciences), respectively. The purity of the isolated primary cells (>98%) was confirmed by specific antibody staining and flow cytometry.

Genomic DNA and cDNA samples

Seventy Epstein-Barr virus (EBV)-transformed B-lymphocyte cell lines from healthy white donors were obtained from Coriell Cell Repositories as previously described.²⁴ Genomic DNA and total RNA were isolated from the B-lymphocytes and the RNA samples were treated with DNase I to avoid genomic DNA contamination as described.²⁴ Total cellular RNA samples from B cell lines, primary B-lymphocytes, monocytes, and CD4⁺ T-lymphocytes were used to synthesize cDNA using a reverse transcription kit as previously described.²⁴ All samples were tested by real-time PCR, showing no amplification after 40 cycles in cDNA preparation without reverse transcriptase.

Genotyping

We used allele-specific real-time PCR for SNP genotyping as previously described.²⁵ Three primers were designed for each SNP. A reverse primer was designed and then two separate forward primers were designed to recognize the reference genotype and allele variants of each SNP (Table 2). Real-time PCR was conducted with the CFX 96-Bio-Rad Real-time PCR Detection System using SYBR Green (Bio-Rad) and the following protocol: step 1 (initiation): 95°C for 3 min; step 2 (denature): 94°C for 30 s; step 3 (annealing): 60°C for 45 s; step 4 (extension): 72°C for 1 min. Steps 2–4 were repeated for 40 cycles. Genomic DNA was used as a template in real-time PCR. There were at least three cycle differences between primer pairs targeting reference or variant alleles.

TABLE 1. SELECTED TAGGING SINGLE NUCLEOTIDE POLYMORPHISMS OF THE *SAMHD1* GENE

SNP #	SNP ID (sequences)	Position (chromosome 20)	MAF	Captured SNPs
1	rs4810362 (A > G)	Intron 1	0.179	rs4812586, rs4810362, rs1291144, rs1291149, rs6130139
2	rs6030346 (G > T)	Intron 2	0.102	rs6030346
3	rs6065494 (A > T)	Intron 3	0.217	rs1935919, rs6030188, rs6072797, rs6065494
4	rs6072746 (A > C)	Intron 4	0.460	rs6072746
5	rs6016783 (T > G)	Intron 5	0.412	rs1891643, rs6130119, rs6030315, rs6016783, rs6030195
6	rs7268811 (C > A)	Intron 6	0.164	rs7268811
7	rs12481143 (G > A)	Intron 11	0.221	rs12481143
8	rs1291142 (G > A)	Intron 15	0.345	rs1291142

SNP, single nucleotide polymorphism; MAF, minor allele frequency. Used tagging SNPs, subset of SNPs designed to predict other SNPs in the region, to capture other SNPs. This table is based on the search of the HapMap database (the catalog of human genetic variants²³).

TABLE 2. PCR PRIMERS USED FOR *SAMHD1* mRNA QUANTIFICATION AND DNA GENOTYPING

Primer names	Sequences (5'-3')
For mRNA quantification	
SAMHD1-quant-F	GGA TTA CTA AAA ACC AGG TTT CAC AAC T
SAMHD1-quant-R	GCT CTG CAA ATT TCT CTG GCA G
For DNA genotyping	
SAMHD1 rs6016783 G-F	GAG CAG TCT CCA GTC ACC ACG G
SAMHD1 rs6016783 T-F	GAG CAG TCT CCA GTC ACC ACG T
SAMHD1 rs6016783 R	GCA AGA CTC TGT CTC TTC AAA AAA CAA
SAMHD1 rs6065494 C-F	GCA AGA AAA TAA TTT TGT ACA GCT GAT CC
SAMHD1 rs6065494 A-F	GCA AGA AAA TAA TTT TGT ACA GCT GAT CA
SAMHD1 rs6065494 R	GGT GAG AAA GCG AGA CCC TAT CTC
SAMHD1 rs7268811 G-R	GAT TAC TTG AAT CCA GGA GTT CAC GG
SAMHD1 rs7268811 T-R	GAT TAC TTG AAT CCA GGA GTT CAC GT
SAMHD1 rs7268811 F	GGC TCT TGC TCT ATC ACC CAG G
SAMHD1 rs1291142 G-F	GTG CTC CTC CCA CTG GCG
SAMHD1 rs1291142 A-F	GTG CTC CTC CCA CTG GCA
SAMHD1 rs1291142 R	GTT GTG TTT GGA TGA GTA GCA GGG
SAMHD1 rs6030346 G-F	GGA AAA CCT AGA AAT ATG CTC AAG ATC GG
SAMHD1 rs6030346 T-F	GGA AAA CCT AGA AAT ATG CTC AAG ATC GT
SAMHD1 rs6030346 R	GAA TTA TAC CAC TGG GAA ATA CAT AAG AAC C
SAMHD1 rs4810362 A-F	CAC TCC ACT TGT TGC TAG TCA AAG AAA CTA
SAMHD1 rs4810362 G-F	CAC TCC ACT TGT TGC TAG TCA AAG AAA CTG
SAMHD1 rs4810362 R	CCC AAA GAT AGC TTG AAT GGG TAT GGG
SAMHD1 rs6072746 A-F	AGT TCT GGT CCT CAA GAA CTG CA
SAMHD1 rs6072746 C-F	AGT TCT GGT CCT CAA GAA CTG CC
SAMHD1 rs6072746 R	CCA TGG CAG CTA CAC CAT AAT ACA TCC
SAMHD1 rs12481143 G-F	ATA GAA TCC AGC TGT GGT AGA GCG
SAMHD1 rs12481143 A-F	ATA GAA TCC AGC TGT GGT AGA GCA
SAMHD1 rs12481143 R	GTT GCG GTG AGC AGA GAT CGC

This table contains two primers for quantification of cDNA and three primers for each of the SNPs. Primers for genotyping contain a reference genotype primer, an SNP allele primer, and either a reverse/forward primer that concurs with the others.

SAMHD1 mRNA quantification

The amount of *SAMHD1* mRNA was quantified by real-time PCR using the primers shown in Table 2. *β-Actin* was used as a control (Forward primer: 5'-CCTGGCACCCAGC ACAAT-3'; Reverse primer: 5'-GCCGATCCACACGGAGT ACT-3') for normalization. The condition of real-time PCR reaction was as described above and cDNA was used as a template.

Statistical analysis

The amount of *SAMHD1* mRNA was calculated as the ratio of *SAMHD1*/*β-actin* amplified by 100,000. Since the data were not normally distributed, we transformed the data into a log₁₀ scale. The HelixTree program (Golden Helix, Bosman, MT) was used for initial screening of multiple SNPs. If any positive SNPs were identified, statistical analysis was performed using Minitab software by fitting single linear regression models as described.^{24,25} The goodness of fit was judged by residual plots and normal quartile plots, testing the fulfillment of linear regression assumptions, which included constant variance, normality, and independency.

Analysis of the datasets of HIV-1 controllers and progressors

Sample sizes of the analyzed data were 857 HIV-1 controllers and 2088 HIV progressors (total $n = 2945$, Table 3). The two available datasets contain data from European HIV-1

controllers and progressors (comparing 516 HIV-1 controllers to 1196 HIV-1 progressors) and African-American HIV-1 controllers and progressors (comparing 341 HIV-1 controllers to 892 HIV-1 progressors). The analysis was performed using the online Ricopili tool on <http://www.broadinstitute.org/mpg/ricopili/>. Genome-wide SNP imputation of HIV-1 progressors and controllers from the European and African-American cohorts were performed using BEAGLE (<http://faculty.washington.edu/browning/beagle/beagle.html>) and phased haplotype data from the HapMap resource as previously reported.³ Association testing and meta-analysis of the European and African-American cohorts were performed using MANTEL (www.broadinstitute.org/

TABLE 3. HIV-1 CONTROLLER AND PROGRESSOR NUMBERS FROM THE EUROPEAN AND AFRICAN-AMERICAN COHORTS AND GENOTYPED SINGLE NUCLEOTIDE POLYMORPHISM NUMBERS IN THE *SAMHD1* GENE

	HIV-1 controllers	HIV-1 progressors	SNP numbers of <i>SAMHD1</i>
African-Americans	341	892	29
Europeans	516	1196	23
Total subjects	857	2088	

This table is based on the International HIV Controllers Study³ and the datasets are available at www.broadinstitute.org/mpg/ricopili/

FIG. 1. Polymorphism rs1291142 of *SAMHD1* affects its mRNA expression levels in B-lymphocytes from healthy white donors ($n=70$). The mRNA levels of *SAMHD1* in B-lymphocytes from 70 healthy white donors were measured using real-time PCR methods. The GA and AA carriers of *SAMHD1* single nucleotide polymorphisms (SNPs) (rs1291142) show 0.67-fold lower *SAMHD1* mRNA than those of GG carriers ($p=0.015$, two-sided, linear regression).

~debakker/mantel.html) and inverse-variance weighting under a fixed-effects model²⁶ as previously described.³

Results

Polymorphism of *SAMHD1* affects mRNA expression levels in B-lymphocytes from healthy white donors

Human *SAMHD1* spans 59,532 bp of genomic sequence in 16 exons in chromosome 20 (position 34,954,059–35,013,590) and encodes a 626-amino acid protein.²⁰ Human peripheral blood leukocytes express high levels of *SAMHD1* mRNA.²⁷ EBV-mediated transformation of B-lymphocytes is a standard protocol to generate immortalized cells to preserve genetic materials of individuals.^{3,24} We used EBV-transformed B-lymphocytes from 70 healthy white donors as the source of *SAMHD1* mRNA and measured the mRNA levels of *SAMHD1* by real-time PCR methods. The mRNA levels of β -actin were also measured and used as a control. Our results indicated that *SAMHD1* mRNA was expressed in B-lymphocytes at the level of $\sim 0.01\%$ of β -actin mRNA. In 70 B-lymphocyte cell lines derived from different donors, the average level of *SAMHD1* mRNA was 69 ± 55 (mean $\pm$ SD, arbitrary unit), with 50-fold interindividual variability (ranging from 7 to 355). These re-

sults suggest the expression levels of *SAMHD1* mRNA in B-lymphocytes vary considerably among different individuals.

To identify frequent polymorphisms in the *SAMHD1* gene that may affect its mRNA expression, we selected eight tagging SNPs in *SAMHD1* introns with minor allele frequencies over 10% (Table 1) and associated the genotypes with *SAMHD1* mRNA expression. Interestingly, single SNP analysis revealed that one SNP (rs1291142) was significantly associated with *SAMHD1* mRNA expression (Fig. 1). SNP rs1291142 is located in intron 15 of *SAMHD1* and presents as GG for the reference genotype and GA or AA for variants. B-lymphocytes with a GA or AA genotype showed 0.67-fold ($p=0.015$) lower *SAMHD1* mRNA levels than the B-lymphocytes homozygous for reference (GG) genotype (Fig. 1). By contrast, there was no significant association between the other seven SNPs and *SAMHD1* mRNA levels. These results suggest that a regulatory polymorphism exists in the *SAMHD1* gene, which can significantly affect *SAMHD1* mRNA expression in B-lymphocytes from healthy whites.

Comparison of *SAMHD1* mRNA levels in primary B-lymphocytes, monocytes, and CD4⁺ T-lymphocytes

To compare *SAMHD1* mRNA levels in different types of immune cells, we measured *SAMHD1* mRNA levels in primary B-lymphocytes, monocytes, and CD4⁺ T-lymphocytes isolated from PBMCs of five healthy blood donors using real-time RT-PCR. We compared the mRNA levels of *SAMHD1* relative to β -actin controls in these samples. Our results indicated that the average *SAMHD1* mRNA levels in primary B-lymphocytes are lower ($p>0.1$) than that in CD4⁺ T-lymphocytes and CD14⁺ monocytes from five donors. The average *SAMHD1* mRNA level in primary CD14⁺ monocytes was the highest among these cell types (Table 4), although these differences are not statistically significant ($p \geq 0.19$). In addition, we observed 2- to 6-fold variations of *SAMHD1* mRNA levels among different donors and cell types (Table 4). The analysis does not allow us to conclude that there is a difference in *SAMHD1* mRNA levels among different cell types, although there may be a pairwise trend of lower *SAMHD1* mRNA expression in B-lymphocytes.

Polymorphisms of *SAMHD1* are not significantly associated with HIV-1 infection status of controllers and progressors from the European and African-American cohorts

A previous genome-wide association study using the samples of HIV-1 controllers and progressors from the European

TABLE 4. RELATIVE mRNA LEVELS OF DIFFERENT PRIMARY IMMUNE CELLS ISOLATED FROM FIVE HEALTHY INDIVIDUALS

Donor number	CD19 ⁺ B-lymphocytes	CD14 ⁺ monocytes	CD4 ⁺ T-lymphocytes
403	399.8	644.9	688.0
418	285.3	501.3	765.2
422	276.9	601.7	445.6
463	810.7	1770.1	1372.9
465	1741.7	1505.8	1084.6
Mean $\pm$ SD	702.9 $\pm$ 620.2	1004.8 $\pm$ 587.8	871.3 $\pm$ 361.6
<i>p</i> values	0.19	0.36	0.48
	(vs. monocytes)	(vs. T-lymphocytes)	(vs. B-lymphocytes)

Real-time RT-PCR was used to measure the mRNA levels of *SAMHD1* and β -actin. The relative mRNA levels of *SAMHD1* compared to β -actin controls are shown (arbitrary unit). Each sample was detected in triplicate.

FIG. 2. Polymorphisms of *SAMHD1* are not significantly associated with HIV-1 infection status of HIV-1 controllers and progressors from the European cohort. The results were derived from the analysis of 23 available SNPs in *SAMHD1* in 516 HIV-1 controllers and 1196 HIV-1 progressors. The color squares and the sizes of color diamonds represent the r^2 values of the linkage disequilibrium. The *SAMHD1* gene, SNP (rs1291142), and its p value (0.256) are labeled. The red and green dot line represents $p=0.05$ and genome-wide significance ($p<5\times 10^{-8}$), respectively. The x -axis indicates the location and organization of the genes. The blue line represents the gene recombination rate (y -axis on the right side).

and African-American cohorts has yielded two public data sets containing genome-wide SNPs of HIV-1 controllers³ and <http://www.broadinstitute.org/mpg/ricopili/>. In the published study, HIV-1 controllers were defined by at least three measurements of plasma virus load <2000 RNA copies/ml

over at least a 12-month period in the absence of antiviral therapy.³ These HIV-1 controllers had median virus load, CD4⁺ T cell count, and disease duration of 241 copies/ml, 699 cells/mm³, and 10 years, respectively.³ In contrast, HIV-1 progressors were treatment-naïve, chronically infected

FIG. 3. Polymorphisms of *SAMHD1* are not significantly associated with HIV-1 infection status of HIV-1 controllers and progressors from the African-American cohort. The results were derived from the analysis of 29 available SNPs in *SAMHD1* in 341 HIV-1 controllers and 892 HIV-1 progressors. The color squares and the sizes of color diamonds represent the r^2 values of the linkage disequilibrium. The *SAMHD1* gene, SNP (rs1291142), and its p value (0.321) are labeled. The red and green dot line represents $p=0.05$ and genome-wide significance ($p<5\times 10^{-8}$), respectively. The x -axis indicates the location and organization of the genes. The blue line represents the gene recombination rate (y -axis on the right side).

TABLE 5. *SAMHD1* POLYMORPHISMS GENOTYPED IN THE EUROPEAN COHORT OF 516 HIV-1 CONTROLLERS AND 1196 HIV-1 PROGRESSORS

SNP #	SNP ID (sequences)	Position (chromosome 20)	p value	Frequency (HapMap)	Odds ratio	Standard error
1	rs4810362 (A > G)	Intron 1 (35012094)	0.753811	0.1626	1.0361	0.217844
2	rs6030346 (G > T)	Intron 2 (35007258)	0.716925	0.0777	1.0485	0.249704
3	rs6065494 (A > T)	Intron 3 (34998103)	0.127526	0.7937	1.1499	0.166885
4	rs6072746 (A > C)	Intron 4 (34992766)	0.759989	0.4563	1.0247	0.154753
5	rs6016783 (T > G)	Intron 5 (34989606)	0.395236	0.4175	1.0710	0.152653
7	rs12481143 (G > A)	Intron 11 (34970620)	0.893253	0.7694	1.0126	0.181413
8	rs1291142 (G > A)	Intron 15 (34958776)	0.320922	0.3592	1.0852	0.154799

The results were derived from the analysis of the dataset at www.broadinstitute.org/mpg/ricopili/

individuals with advanced disease (median virus load and CD4⁺ T cell count of 61,698 copies/ml and 224 cells/mm³, respectively).³ To examine whether *SAMHD1* SNPs are associated with HIV-1 infection status, we analyzed all available *SAMHD1* SNPs in the published SNP datasets of 857 HIV-1 progressors and 2088 HIV-1 controllers from the European and African-American cohorts (Table 3).³

A total of 23 and 29 SNPs in *SAMHD1*, including seven of the eight SNPs that we tested in this study, were genotyped in the HIV-1 controllers and progressors from the European and African-American cohorts, respectively (Table 3 and Supplementary Tables S1 and S2; Supplementary Data are available online at www.liebertpub.com/aid). However, our analysis indicated that none of these SNPs in the *SAMHD1* gene, including SNP rs1291142, was significantly associated with the HIV-1 infection status of the individuals from the European and African-American cohorts ($p > 0.05$, Figs. 2 and 3, Tables 5 and 6, and Supplementary Tables S1 and S2). Overall, our results suggest that SNPs in *SAMHD1* are not significantly associated with HIV-1 infection and the natural control of HIV-1 infection in European and African-American individuals.

Polymorphisms of *TRIM22* are significantly associated with HIV-1 natural control in controllers from the African-American cohort

To generate a positive control for association with HIV-1 infection and progression, we analyzed polymorphisms of the *TRIM22* gene in the same datasets used for *SAMHD1* SNP analysis as a positive control. A recent study has shown a significant correlation of *TRIM22* mRNA levels associated with HIV-1 viral loads, suggesting that *TRIM22* likely acts as an antiviral effector *in vivo*.²⁸ Our analysis revealed that an SNP

rs11038741 in the *TRIM22* gene is significantly associated with natural control of HIV-1 infection in HIV-1 controllers compared to progressors (linkage disequilibrium = 1, $p = 0.00459$) in the African-American cohort (Fig. 4), but not in the European cohort (data not shown). These results validated our correlation analysis of *SAMHD1* SNPs and HIV-1 infection using the same dataset and methods.

Discussion

Given the important role of *SAMHD1* in HIV-1 infection *in vitro* and that several rare exon and intron mutations of *SAMHD1* are associated with AGS and other genetic disorders,^{20,22} it is conceivable that common regulatory polymorphisms of *SAMHD1* that affect its mRNA expression may play an important role in controlling HIV-1 infection *in vivo*. Indeed, our results indicated that SNP rs1291142 significantly affected *SAMHD1* mRNA expression in B-lymphocytes from 70 healthy white donors, suggesting that a common polymorphism exists in *SAMHD1* and can regulate its mRNA expression in B-lymphocytes. However, SNP rs1291142 and other SNPs in *SAMHD1* are not associated with HIV-1 infection status. The effect of this SNP on *SAMHD1* mRNA expression is limited with a 0.67-fold difference, which may not be significant enough to exert the effect on *SAMHD1* protein expression and HIV-1 restriction *in vivo*. Other host factors (such as microRNA) may also affect mRNA expression, since there were 50-fold interperson differences among 70 healthy white donors.

SNP rs1291142 itself is unlikely to be functional; it may be in high linkage disequilibrium with functional SNPs, because it is located in the middle of intron 15 of *SAMHD1* and is not a splicing acceptor or donor site. However, we cannot completely rule out the possibility that SNP rs1291142 itself is

TABLE 6. *SAMHD1* POLYMORPHISMS GENOTYPED IN THE AFRICAN-AMERICAN COHORT OF 341 HIV-1 CONTROLLERS AND 892 HIV-1 PROGRESSORS

SNP #	SNP ID (sequences)	Position (chromosome 20)	p value	Frequency (HapMap)	Odds ratio	Standard error
1	rs4810362 (A > G)	Intron 1 (35012094)	0.136558	0.3162	1.1630	0.183488
2	rs6030346 (G > T)	Intron 2 (35007258)	0.493459	0.0321	1.2306	0.531001
3	rs6065494 (A > T)	Intron 3 (34998103)	0.786723	0.8765	1.0415	0.288122
4	rs6072746 (A > C)	Intron 4 (34992766)	0.292418	0.3602	0.8862	0.238733
5	rs6016783 (T > G)	Intron 5 (34989606)	0.127702	0.5786	1.1845	0.199027
7	rs12481143 (G > A)	Intron 11 (34970620)	0.063401	0.9111	1.6791	0.402499
8	rs1291142 (G > A)	Intron 15 (34958776)	0.255835	0.5331	1.1228	0.187920

The results were derived from the analysis of the dataset at www.broadinstitute.org/mpg/ricopili/

FIG. 4. Polymorphisms of the *TRIM* genes are significantly associated with natural control of HIV-1 infection in HIV-1 controllers from the African-American cohort. The results were derived from the analysis of 133 available SNPs of the *TRIM* family genes in 341 HIV-1 controllers compared to 892 HIV-1 progressors. The color squares and the sizes of color diamonds represent the r^2 values of the linkage disequilibrium. The *TRIM* genes and SNP rs11038741 ($p=0.00459$) are labeled. The red and green dot line represents $p=0.05$ and genome-wide significance ($p < 5 \times 10^{-8}$), respectively. The x-axis indicates the location and organization of the genes. The blue line represents the gene recombination rate (y-axis on the right side).

functional, because SNPs in introns can modulate RNA levels of some human genes by affecting RNA elongation.^{24,25,29} It is unclear whether this SNP affects *SAMHD1* mRNA expression in primary B-lymphocytes or other cell types from healthy donors or from HIV-1 infected individuals, since the regulation of polymorphisms on the expression of other genes has been shown to be tissue specific.^{25,29} Given that *SAMHD1* is a myeloid-cell-specific HIV-1 restriction factor,^{15,16} it is interesting to test whether the same polymorphism affects *SAMHD1* mRNA expression in myeloid cells.

Previous studies indicated that *SAMHD1* mRNA expression is upregulated by the infections of adenovirus, rabies virus, and respiratory syncytial virus in human cells.^{30–32} It is unknown whether EBV infection of B-lymphocytes may affect the expression of *SAMHD1* mRNA in the cell. Our real-time PCR-based *SAMHD1* cDNA quantification was based on the relative levels of *SAMHD1* and β -actin mRNAs, which represented the relative levels of *SAMHD1* mRNA among all 70 individuals' samples. Furthermore, our real-time PCR data suggest that the average level of *SAMHD1* mRNA expression in EBV-transformed B-lymphocytes was lower than that in primary B-lymphocytes. Thus, our correlation analysis between the SNP genotypes and the mRNA expression levels of *SAMHD1* was valid. However, it is possible that the regulation of *SAMHD1* mRNA expression in EBV-transformed B-lymphocytes is different from that in other cell types. For example, HIV-1 target cells including myeloid cells *in vivo* may be subjected to certain regulations of *SAMHD1* expres-

sion after HIV-1 infection, which may override the effect from *SAMHD1* polymorphisms.

Our analyses based on available datasets suggest that SNPs in *SAMHD1* are not significantly associated with HIV-1 infection and the natural control of HIV-1 infection in European and African-American individuals. It would be interesting to examine whether SNPs in *SAMHD1* are associated with HIV-1 infection and natural controlling of AIDS in different ethnic populations, such as Asians and Hispanic populations. Of note, SNP rs7268811 in intron 6 of *SAMHD1* that we analyzed in the healthy white donors was not found in HIV-1 controllers and progressors from the published European and African-American cohorts (Tables 5 and 6 and Supplementary Tables S1 and S2). It is possible that this SNP has a different allele frequency and heterozygosity among different ethnic populations.

Polymorphisms of other genes in addition to the *HLA class I* genes can be associated with natural control of HIV-1 infection. For instance, our analysis revealed that an SNP (rs11038741) in the *TRIM22* gene is significantly associated with natural control of HIV-1 infection in HIV-1 controllers compared to progressors in the African-American cohort (Fig. 4). Of note, a recent *in vitro* study indicated that *TRIM22* significantly inhibits HIV-1 replication in cells, likely by interfering with viral transcription.³³ Additional studies on the association of *TRIM22* with the type 1 interferon response and viral control during primary HIV-1 infection indicate concordance between type 1 interferon and *TRIM22*, but not

human *TRIM5α* expression in PBMCs, suggesting that TRIM22 likely acts as an antiviral effector *in vivo*.²⁸ Collectively, these studies and our analyses suggest that TRIM22 is an HIV-1 restriction factor associated with HIV-1 natural controlling in African-Americans.

SAMHD1 is a novel HIV-1 restriction factor since its restriction function appears to be myeloid cell-type-specific and HIV-1 has not evolved a viral antagonist to counteract SAMHD1-mediated restriction.³⁴ In contrast, Vpx of less pathogenic HIV type 2 (HIV-2) and certain simian immunodeficiency viruses (such as SIVsm lineage) can induce proteolytic degradation of SAMHD1 and relieve SAMHD1-mediated viral restriction in human myeloid cells.^{15,16} The lack of Vpx in HIV-1 may benefit HIV-1 as an immune evasion strategy for the escape from immune surveillance initiated by the infected myeloid cells.^{35,36} Further studies of the function and mechanism of SAMHD1-mediated HIV-1 infection can help in understanding the pathogenesis of primate lentiviruses and may aid in the development of more effective interventions against HIV-1/AIDS.

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Author Disclosure Statement

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