



Genome-wide association study reveals genetic variants associated with HIV-1C infection in a Botswana study population

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Although there have been many studies of gene variant association with different stages of HIV/AIDS progression in United States and European cohorts, few gene-association studies have assessed genetic determinants in sub-Saharan African populations, which have the highest density of HIV infections worldwide. We carried out genome-wide association studies on 766 study participants at risk for HIV-1 subtype C (HIV-1C) infection in Botswana. Three gene associations (*AP3B1*, *PTPRA*, and *NEO1*) were shown to have significant association with HIV-1C acquisition. Each gene association was replicated within Botswana or in the United States–African American or United States–European American AIDS cohorts or in both. Each associated gene has a prior reported influence on HIV/AIDS pathogenesis. Thirteen previously discovered AIDS restriction genes were further replicated in the Botswana cohorts, extending our confidence in these prior AIDS restriction gene reports. This work presents an early step toward the identification of genetic variants associated with and affecting HIV acquisition or AIDS progression in the understudied HIV-1C afflicted Botswana population.

AIDS | HIV-C | GWAS | Botswana | GWATCH

HIV infection is a fatal chronic disease that has spread across all continents since its emergence in the early 1980s. Important progress has been made against the epidemic in the last two decades. The global incidence–prevalence ratio has declined from 11.2% in 2000 to 4.6% in 2018. Despite this, the world is not yet on track to end HIV/AIDS as a public health threat (1). There are 38 million (31.6 million to 44.5 million) people in the world living with HIV; 32.7 million (24.8 million to 42.2 million) people who died from AIDS-related conditions since the onset of the pandemic. An estimated 690,000 (500,000 to 970,000) died of HIV/AIDS in 2019 (1). Despite the important breakthroughs in therapeutic approaches, as well as efforts and resources invested in the fight against the HIV epidemic for about four decades, no efficient vaccine or cure exists.

Sub-Saharan Africa is the region most affected by the HIV pandemic. Botswana falls within the top three countries with the highest HIV prevalence in the world, with an HIV prevalence rate of 20.3% (17.3 to 21.8) among adults (15 to 49 y of age) (1).

Currently HIV-1 subtype C (HIV-1C), which is widespread in the Botswana population, accounts for more than half of all

global HIV-1 infections and several times more than any other HIV-1 subtype. Despite this fact, HIV-1C infection remains understudied as prior research largely focused on HIV-1 subtypes (mostly B) more prevalent in Europe and in the United States (2–8).

HIV/AIDS exhibits considerable epidemiological heterogeneity (strength of the innate, humoral, and cell-mediated immune responses, variation in response to antiretroviral therapy, ART), much of which may be attributed to host genetic factors

Significance

The search for genetic variants associated with resistance or susceptibility to HIV/AIDS already yielded insights that allowed developing therapeutics that contribute to dramatic reduction in AIDS-related comorbidities. Unfortunately, nearly all studies focused on European-descent populations, even though most infections happen in Africa. In this genome-wide association study we explored the genetic background of several hundred individuals from Botswana in search of associations with HIV infection. We discovered several genetic variant associations in genes implicated in HIV/AIDS pathogenesis. We also confirmed several associations reported in previous research. This study provides valuable data on the influence of human genetic variation on HIV-1C infection and pathogenesis in southern Africa, a region with the world's largest number of HIV-1 infections.

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(3–8). African populations in general have the highest overall genetic variation, and it has been hypothesized that treatment-naïve Botswana people exhibit noticeable heterogeneity in natural disease progression (varying ability to maintain high CD4 counts and low viral load) in part due to the variable host genetic makeup (2, 9). Complex gene and gene–environment interactions affect HIV-1 infection and AIDS progression in major ways (6, 10). Much remains to be discovered about the genetic basis of individual host genetic differences in progression to AIDS in this geographic region.

Identification of genetic factors is important for several reasons. For example, the mechanism of HIV/AIDS restriction by genetic variants has previously encouraged the development of salvage pathway antiretroviral medications (e.g., enfuvirtide, maraviroc, which block HIV-1 binding to CCR-5 receptors and membrane fusion), while predicting progression to AIDS for a given genotype can be used to inform clinical trials for new drugs (4, 11–14). In this study, we searched for genetic factors associated with HIV-1C infection/acquisition and disease progression in Botswana.

Genome-wide association studies (GWAS) are a standard approach to scan for novel genetic variants that are associated with resistance or susceptibility to a particular disease (15–18). Here, we employed a GWAS approach to discover gene variants that regulate HIV-1C acquisition and are also related to HIV-1 pathogenesis. In this GWAS, we identified several novel genetic variants that demonstrated statistically significant association with HIV-1C infection in a Botswana cohort using single nucleotide polymorphisms (SNPs) determined by Illumina microarray (MA; $n = 809$) and by whole-genome sequencing (WGS; $n = 364$). We report gene associations for three HIV-1C associated loci: *AP3B1*, *PTPRA*, and *NEO1*. The discoveries were verified by independent cohort replication and by looking for previously reported implications in HIV-1 host-pathogenesis. We further replicated prior AIDS restriction gene (ARG) association signals from analyses with Botswana HIV-1C cohorts, adding confidence to the existing knowledge about gene influences in HIV/AIDS.

Results

We developed a cohort of 838 young African women living in Botswana who were exposed to or infected with HIV-1C. Study participants, each enrolled in one or more Botswana-AIDS Partnership studies (Table 1), were examined in a GWAS to search for genetic variants that influenced HIV-1C acquisition. The cohort was parsed into a case-controlled design with 447 HIV infected individuals and 315 HIV exposed but uninfected controls (Table 2). We genotyped these samples using two methods. First, DNA from 476 individuals were genotyped with the Illumina Human Omni 2.5 M BeadChip array (MA cohort), which resulted in genotypes for 1,374,256 SNPs after filtering, as described in Table 1. Second, the complete genomes of 364 individuals (nonoverlapping with the MA study participants) were sequenced (WGS cohort). SNPs annotated from the WGS samples were genotyped as described in *Methods* for a total of 8,636,400 SNPs that passed filters for discordant SNPs, missing genotypes, failure to conform to Hardy–Weinberg equilibrium (HWE), and minor allele frequency (MAF) < 0.05 (Table 1).

Genetic-association analysis was carried out with filtered study participants of three datasets, allowing us to perform both a discovery and a replication study. Three discovery datasets (Table 2) were considered: 1) the MA group of 430 females (194 HIV⁺ and 236 HIV⁻, but HIV-exposed); 2) the WGS group of 336 females (256 HIV⁺ and 80 of HIV⁻, but HIV-exposed); 3) shared SNPs from the MA and WGS study participants were then combined and analyzed for 1,333,944 shared SNP variants determined for a MA-WGS study group of 762 females (447 HIV⁺ and 315 HIV⁻, but HIV-exposed).

We performed a GWAS using logistic regression in PLINK on the MA, WGS, and combined MA-WGS groups, all exposed to HIV-1C, looking for genetic variants that influence HIV-1C acquisition. Manhattan plots for each group were obtained (Fig. 1 and Table 3). WGS Manhattan plots for HIV infection, using tests for allele and genotype frequency distribution in HIV⁺ vs. HIV⁻ people, revealed significant associations of multiple SNPs with HIV acquisition (Fig. 1, Table 3, *SI Appendix*, Table S1, and *Dataset S1*), including a single SNP, rs572880838 on chromosome 5 (position 77,590,422; GRCh37) within the *AP3B1* (*Adaptor Related Protein Complex 3 Subunit Beta 1*) gene, that reached the standard GWAS threshold for genome-wide significance after Bonferroni correction (*Methods*) for both the allele frequency and dominant genotype models ($P = 7.6 \times 10^{-9}$; odds ratio [OR] = 6.711) (Figs. 1A and 2A and Table 3). The *AP3B1* gene product, AP-3, is well known to influence HIV-1 gag assembly and AIDS pathogenesis (*Discussion*).

Replication and validation of associated variants were performed for the exact implicated SNP association in the non-discovery cohorts as well as in previously interrogated American AIDS cohorts that included actively gay male participants, recipients of HIV-1 contaminated clotting factors (hemophiliacs), contaminated donor blood transfusions, and persons who inject drugs and who exchanged HIV-contaminated syringes in urban settings (Table 2). In cases where a discovered associated SNP was not present in the replication cohort, we tested proxy SNPs with $r^2 > 0.8$ when feasible. The *AP3B1* SNP-rs572880838 had but one proxy to in the WGS SNP in the Botswana WGS dataset (rs777424597; $r^2 > 0.8$) (*SI Appendix*, Fig. S1); however, neither *AP3B1*-SNP was included among the genotyped MA SNPs, making exact SNP replication (or nonreplication) impossible.

A less stringent but informative “gene region” replication approach involved the loading of the GWAS results (WGS and MA) for discovery and replication cohorts into the GWATCH program (9, 18). GWATCH includes a suite of GWAS analytical programs that allow discovered SNP association signals to be quantified, viewed, and compared for gene region associations with multiple AIDS association test hypotheses and statistical models (i.e., HIV infection, Cox regression for survival analysis, case-control categorical analyses of AIDS progression, various AIDS defining pathologies, and progression to AIDS after ART treatment) (*Methods*). We used GWATCH to explore and replicate AIDS associations in AIDS progression tests in the replication African American (AA) cohort-ALIVE ($n = 1,460$ study participants genotyped with 700,022 SNPs) (Fig. 2B and Table 2) (<https://botswana.gen-watch.org/>). In addition, the *AP3B1* gene showed a series of SNPs that were highly associated with AIDS progression ($P = 1.7 \times 10^{-5}$ for Cox regression for survival analysis), including various AIDS defining end point analyses of the United States-European American (EA) cohort ($n = 1,527$ study participants genotyped with 700,022 SNPs; $P = 2.1 \times 10^{-3}$) (Table 2 and *SI Appendix*, Fig. S2) (<https://botswana.gen-watch.org/>).

The associated SNP (rs572880838) is located in the promoter (regulatory 5'UTR region) of the *AP3B1* gene, which comprises 27 exons encoding a 140-kD, 194-amino acid protein AP3B1. This promoter region is a binding site for multiple different transcription factors (19); rs572880838 could potentially influence the regulation of AP3B1 gene transcription or affect the secondary structure of the transcript itself (*SI Appendix*, Fig. S3). The rs572880838 has a noticeably high MAF (MAF = 0.379) in the Botswana population (based on our dataset of 336 WGS), where HIV-1C infection is prevalent. This Botswana MAF is higher than in any of the human populations studied to date. In the 1000 Genomes Project the highest detected population MAF of an *AP3B1* SNP (rs572880838) is 0.2654 (Gambian) (20). Two (rs572880838) SNP alleles are distributed in TT and TG

Table 1. Botswana study participants and SNP filtering

Cohort	Step	Dropped	Included	
Study participant filtering steps				
WGS	Total subjects		364	
	Discordant genotypes (WGS/MA)	3	361	
	No phenotype	1	360	
	low genotyping rate	2	358	
	IBD	2	356	
	PCA outliers	0	356	
	Males	20	336	
	Total used		336	
	MA	Total subjects		809
		MA/WGS duplicates	333	476
PCA outliers		6	470	
Males		40	430	
Total used			430	
MA-WGS (combined- shared SNPs)	Total subjects		1,173	
	MA/WGS duplicates	335	838	
	Discordant genotypes (WGS/MA)	1	837	
	No phenotype	1	836	
	low genotyping rate	1	835	
	IBD	2	833	
	PCA outliers	10	823	
	Males	61	762	
	Total used		762	
	Annotated SNPs and filtering steps			
WGS	Loaded from .bim file		19,715,426	
	Discordant SNPs (>5%)	66,218	19,649,208	
	Missing genotype data (0.05)	869,548	18,779,660	
	HWE	164,425	18,615,235	
	Minor allele threshold (0.05)	9,978,835	8,636,400	
	Total used		8,636,400	
	MA	Loaded from .bim file		1,822,601
Discordant SNPs (>5%)		66,218	1,756,383	
Missing genotype data (0.05)		8,250	1,748,133	
HWE		134	1,747,999	
Minor allele threshold (0.05)		373,743	1,374,256	
Total used			1,374,256	
MA-WGS (combined-shared SNPs)	Loaded from .bim file		1,705,582	
	Missing genotype data (0.05)	5,981	1,699,601	
	HWE	2,066	1,697,535	
	Minor allele threshold (0.05)	363,591	1,333,944	
	Total used		1,333,944	

In the top half are steps for filtering of study participants as described in *Methods*. Each row includes the number of individuals dropped (filtered) and retained in each step. Total indicates the final count of individuals with genotypes actually used in the analyses of MA, WGS, and combined MA-WGS cohorts from Botswana. In the bottom half are the same for filtering steps for SNPs. IBD, identity by descent test for close relative; PCS, principal component analysis; HWE, Hardy–Weinberg departue at $P > 0.0001$.

genotypes in the Botswana population, while the expected homozygous GG genotype is remarkably absent. The same *AP3BI* SNP (rs572880838) GG genotype is also completely absent among all (African, European, and Asian) populations sequenced in the 1000 Genomes Project ($n = 2,504$ people), potentially indicating an adverse (perhaps lethal) phenotype for the *AP3BI* SNP (rs572880838) GG genotypes worldwide. Several mutations, including a 63-bp deletion of *AP3BI* Exon 15, can cause Hermansky–Pudlak syndrome, a rare inherited disorder characterized by decreased pigmentation (albinism), visual impairment, and blood platelet dysfunction that is sometimes fatal (21–24). To date there are no clinical pathologies reported in association with the SNP rs572880838 in the *AP3BI* gene, here associated with HIV-1C acquisition in Botswana.

A second gene-association signal that approached (but did not exceed) genome-wide significance for the WGS dataset involves

SNPs within the *PTPRA* (protein tyrosine phosphatase receptor type A) locus on chromosome 20 (rs6076463; $P = 8.3 \times 10^{-7}$; OR = 0.172 for allele and dominant genotype models) (Figs. 1A and 3A, Table 3, *SI Appendix*, Table S1, and Dataset S1). This SNP has multiple proxy SNPs that are all associated with HIV acquisition (Fig. 3A). The strongest *PTPRA* SNP (rs6076463; $P = 8.3 \times 10^{-7}$; OR = 0.172) was not genotyped in the MA cohort nor was any informative linkage disequilibrium (LD) proxy SNP included in the MA genotyped SNPs. The *PTPRA* gene region showed multiple SNP associations with progression to AIDS endpoints revealed in the GWATCH snapshot of AAs (Fig. 3B) ($P = 8.3 \times 10^{-4}$; <https://botswana.gen-watch.org/>). *PTPRA* gene variants were also significantly associated with the development of certain AIDS defining conditions (sequelae; namely Kaposi's sarcoma, PJP [*Pneumocystis jirovecii pneumonia*], B cell lymphoma) in the EA cohorts screened by GWATCH (Fig. 3C) ($P =$

Table 2. Cohorts interrogated in this study

Application	Cohort	Genotyping platform	<i>n</i> individuals	HIV ⁺	HIV ⁻	Phenotype	SNPs after filtering (see Table 1)	Source (if published)
Discovery	Botswana	WGS	336	256	80	HIV acquisition	8,636,400	Present study
Discovery	Botswana	MA	430	194	236	HIV acquisition	1,374,256	Present study
Combined cohort	Botswana	MA+WGS	762	447	315	HIV acquisition	1,333,944	Present study
Replication	United States-EA	MA	1,527			HIV acquisition; AIDS progression; sequelae; ART	700,022	(8, 18)
Replication	United States-AA	MA	1,460			HIV acquisition; AIDS progression; sequelae; ART	700,022	(8, 18)

AA, African Americans; EA, European Americans.

3.2×10^{-4}). The *PTPRA* gene has also been reported as having a role in HIV/AIDS pathogenesis (25–27) (*Discussion*)

A third genetic association, initially detected as genome-wide significant in the GWAS on MA (Fig. 1B), involves multiple SNPs within the promoter region of the *NEO1* gene associated with HIV infection (chromosome band 15q24.1; rs9920504; $P = 1.03 \times 10^{-5}$; OR = 0.465) (Fig. 4A, Table 3, *SI Appendix, Table S1*, and *Dataset S1*). A GWATCH screen shot showed multiple SNPs within the *NEO1* gene in the AA cohort that were associated with AIDS progression using Cox regression for survival analysis ($P = 7.94 \times 10^{-5}$) (Fig. 4B). Furthermore, *NEO1* gene variants were also associated with the development of specific AIDS-defining conditions (sequelae: Kaposi's sarcoma, PCP, B cell lymphoma) in the EA cohorts screened by GWATCH (*SI Appendix, Fig. S4*) ($P = 9.59 \times 10^{-5}$). Finally, the identical *NEO1* SNP of the discovery cohort analysis (rs9920504) (Fig. 4A and Table 3) was also significantly associated in an HIV infection GWAS using the MA-WGS Botswana combined cohort ($P = 1.09 \times 10^{-5}$) (Fig. 4C). The *NEO1* gene encodes a cell surface protein member of the immunoglobulin superfamily that functions in multiple tissues and in chronic inflammation of HIV infected patients (28–30) (*Discussion*). The combined MA-WGS

cohort analyses for allele and genotyping effect did not yield any SNPs that exceeded genome-wide significance in allele or genotype association tests (<https://botswana.gen-watch.org/>).

We further searched across the three Botswana discovery cohorts (Table 2) for possible SNP and gene replications in HIV-1 acquisition of previously reported ARGs. We found 13 genes of the 41 tested that showed $P < 0.005$ for one or more stages of HIV infection and AIDS pathogenesis (Table 4 and *SI Appendix, Table S2*) (4, 7, 8). These include several loci within the HLA gene complex, as seen previously in EA-based GWAS but not reported to date in African cohorts. The *CCR2-64I* allele was associated with HIV infection in a Kenya cohort of sex workers (41), while *PROX1* has not been replicated to our knowledge prior to this study (32). The failure to replicate some of the additional associated ARGs might be explained in some cases by differences in ethnic populations used for GWAS and in HIV subtypes. For example, *CCR5-Δ32* is not present among African ethnic groups. Despite these differences, we were able to replicate the loci in such genes as *NCOR2*, *TRIM5*, *CXCL12*, and several *HLA* genes (Table 4). The present replication increases our confidence in the validity of these prior genetic associations.

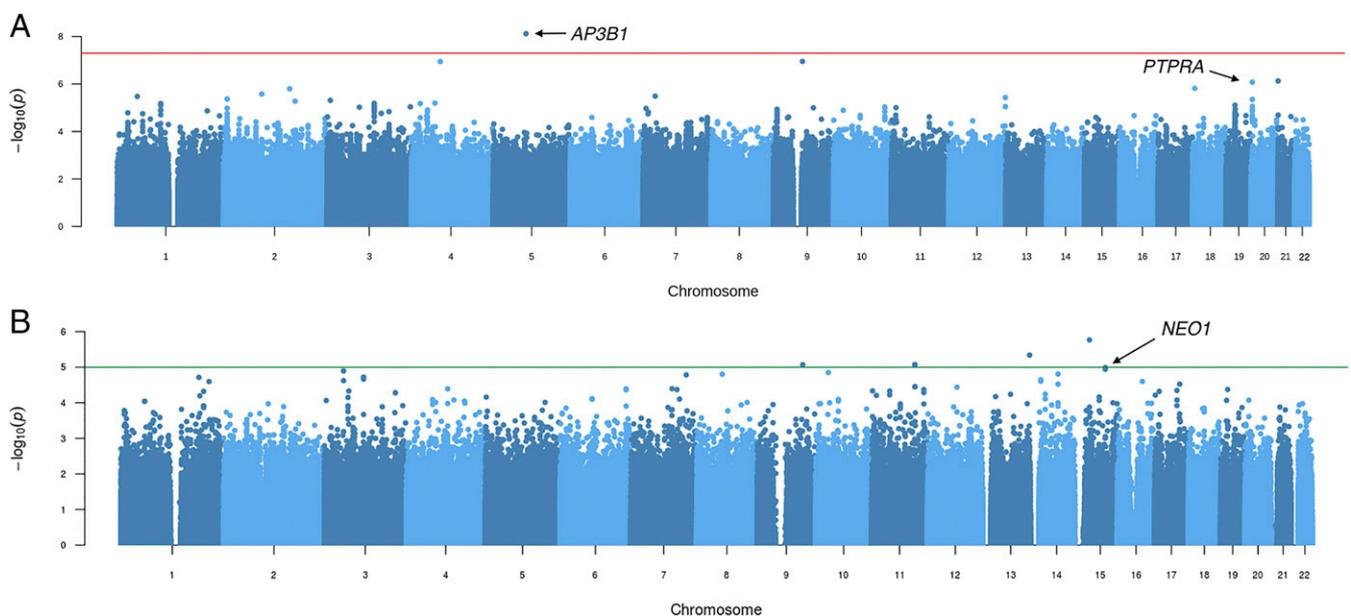


Fig. 1. Manhattan plots for associations with HIV infection using tests for allele and genotype frequency distribution in HIV⁺ vs. HIV⁻ people. (A) WGS dataset of 336 people under the allelic model revealed departure of 8,636,400 SNPs from expectation, including associations within the *AP3B1* (rs572880838; position 5:77,590,422; $P = 7.6 \times 10^{-9}$; OR = 6.711) and *PTPRA* (rs6076463; position 20:3012815; $P = 8.33E-07$; OR = 0.172) genes. (B) MA dataset: 430 people (1,374,256 SNP) under the allelic model revealed 1,374,256 SNPs. *NEO1* (rs9920504; position 15:73284181; $P = 1.03 \times 10^{-5}$; OR = 0.465) (Table 3). Manhattan plots for the combined MA-WGS cohort ($n = 762$) did not reveal any SNPs that exceeded genome-wide significance ($P < 3.75 \times 10^{-8}$).

Table 3. Gene variants associated with HIV-1C infection

Cohort	CHR	SNP	Coordinates	Allele (minor)	Test	NMISS	OR	STAT	P value	Gene	Distance	RR	AR	EF
WGS	5	rs572880838	77590422	G	ALLELIC	333	6.711	5.78	7.62E-09	<i>AP3B1</i>	0	1.210	0.074	0.029
	5	rs572880838	77590422	G	DOM	333	6.711	5.78	7.62E-09	<i>AP3B1</i>	0	NaN	NaN	NaN
	20	rs6076463	3012815	T	ALLELIC	336	0.172	-4.93	8.33E-07	<i>PTPRA</i>	0	0.564	-0.029	0.030
	20	rs6076463	3012815	T	DOM	336	0.172	-4.93	8.33E-07	<i>PTPRA</i>	0	NaN	NaN	NaN
	20	rs3787480	3016895	A	ALLELIC	336	0.179	-4.59	4.46E-06	<i>PTPRA</i>	0	0.573	-0.025	0.025
	20	rs3787480	3016895	A	DOM	336	0.179	-4.59	4.46E-06	<i>PTPRA</i>	0	NaN	NaN	NaN
MA	15	rs9920504	73284181	C	ALLELIC	422	0.465	-4.41	1.03E-05	<i>NEO1</i>	59870	0.648	-0.102	0.018
	15	rs7172316	73287152	G	ALLELIC	430	0.470	-4.39	1.14E-05	<i>NEO1</i>	56899	0.654	-0.100	0.017
	15	rs9920504	73284181	C	DOM	422	0.437	-4.06	4.94E-05	<i>NEO1</i>	59870	0.398	-0.040	0.015
	15	rs7172316	73287152	G	DOM	430	0.445	-4.01	6.05E-05	<i>NEO1</i>	56899	0.395	-0.039	0.015

AR, attributable risk; EF, explained fraction; Distance, distance to the closest gene (see column Gene); NaN, not a valid number; NMISS, number of nonmissing individuals included; RR, relative risk; STAT, coefficient *t*-statistic.

Discussion

In this study we performed a GWAS analysis using a combination of WGS and MA data obtained from 766 individuals (after filtering) (Table 1) from Botswana, either infected or exposed to HIV-1C, in order to identify genetic factors associated with HIV-1C acquisition in a Botswana population. The results presented reveal candidate gene associations with HIV-1C acquisition and were replicated in independent AIDS cohorts (Table 2). This is one of the few studies of the impact of human genetic variation on HIV-1C infection and pathogenesis in Botswana or in southern Africa, a locale with the world’s majority of HIV-1 infections. We report here several genes with significant association with HIV-1C acquisition: *AP3B1*, *PTPRA*, and *NEO1* (Table 3). Evidence of replication in independent datasets (listed in Table 2) including AA AIDS cohorts is presented for each associated locus (*Results*). We researched the recently discovered loci associated with HIV-1C infection and found that each gene plays an important functional role in HIV/AIDS disease processes.

AP3B1 encodes a clathrin-associated protein complex required for HIV-1 assembly and release (42–44). The matrix region of HIV-1 Gag interacts directly with the δ -subunit of the AP-3 complex (coded by the *AP3B1* gene), and this interaction plays an important functional role in viral particle assembly. Disruption of this interaction eliminates Gag trafficking to multivesicular bodies and diminishes HIV particle formation (43). In cultures of AP-3-deficient fibroblasts, induced by mutations in the *AP3B1* gene, HIV-1 particle assembly and release are diminished, while both transient and stable expression of the full-length wild-type β 3A subunit in these cells restores the impaired virus assembly and release (44). Intact and stable AP-3 complex is required for HIV-1 assembly and release, and the involvement of the AP-3 complex in late stages of the HIV-1 replication cycle is independent of clathrin-mediated endocytosis (44). The β -subunit of AP-3 is a target of IP7-mediated pyrophosphorylation, which modulates its interaction with Kif3A (a motor protein of the kinesin superfamily, which is also involved in an intracellular process required for HIV-1 Gag release) and,

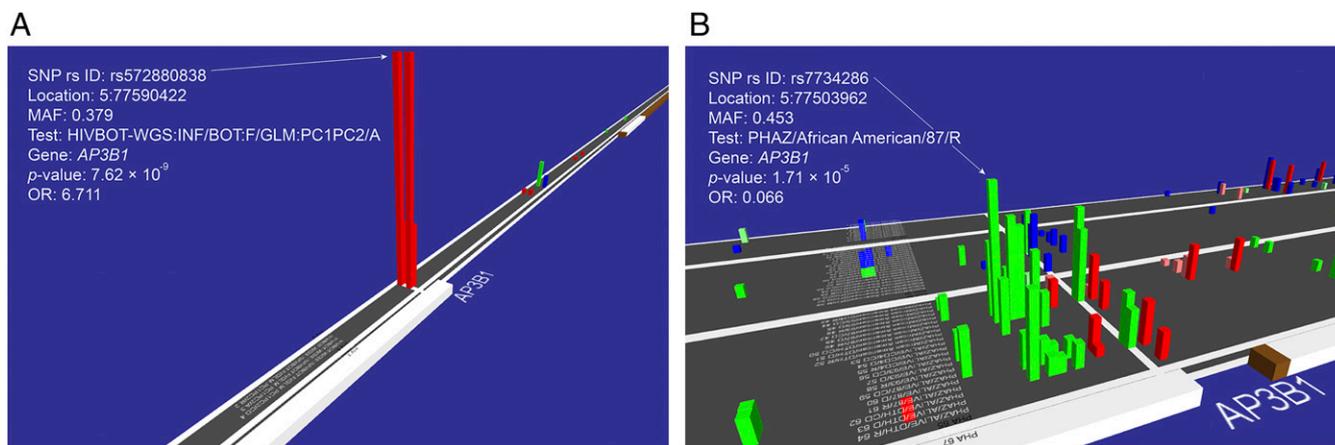


Fig. 2. GWATCH snapshots of associations in the *AP3B1* gene region for HIV infection and AIDS progression. GWATCH is a web-based dynamic real-time genome browser designed to discover, view, and assess hits from GWAS and WGS association studies (<https://botswana.gen-watch.org/>) (9, 18). The platform displays a three-dimensional viewer above and moving along human chromosomes with baseline x axis listing all association tests performed in the GWAS. Adjacent SNPs genotyped along each chromosome are the indices of the y axis. The rising blocks illustrate the resulting log of *P* value obtained (blocks are shown only for $P < 10^{-3}$ to avoid clutter of noise) for each SNP–test combination, with color showing direction of association (red for susceptible vs. green for protective) and color intensity indicating the hazard ratio/OR (unified under the term QAS for Quantitative Association Statistic) (18) of each association. The combination of nonindependent proxy SNPs (which track the operative SNP by LD) and the nonindependent SNP association tests produce clusters of *P* value peaks in associated regions. Nonassociated regions are generally flat with random false signals appearing infrequently. (A) HIV infection tests in the Botswana WGS discovery dataset ($n = 336$ study participants [256 HIV⁺ and 80 HIV⁻] genotyped with 8,636,400 SNPs). (B) AIDS progression tests in the replication AA cohort ($n = 1,460$ study participants genotyped with 700,022 SNPs) (Table 2). The American replication cohorts consist of multiple tests with different numbers of individuals in each. The individuals’ numbers for each test are listed in GWATCH (<https://gen-watch.org/#/option0>) under the “List of tests & Manhattan plots” tab for each cohort. The elevated *P* values reflect a series of different case-control hypothesis tests for AIDS progression among HIV infected AA study participants (<https://botswana.gen-watch.org/>) (9, 18).

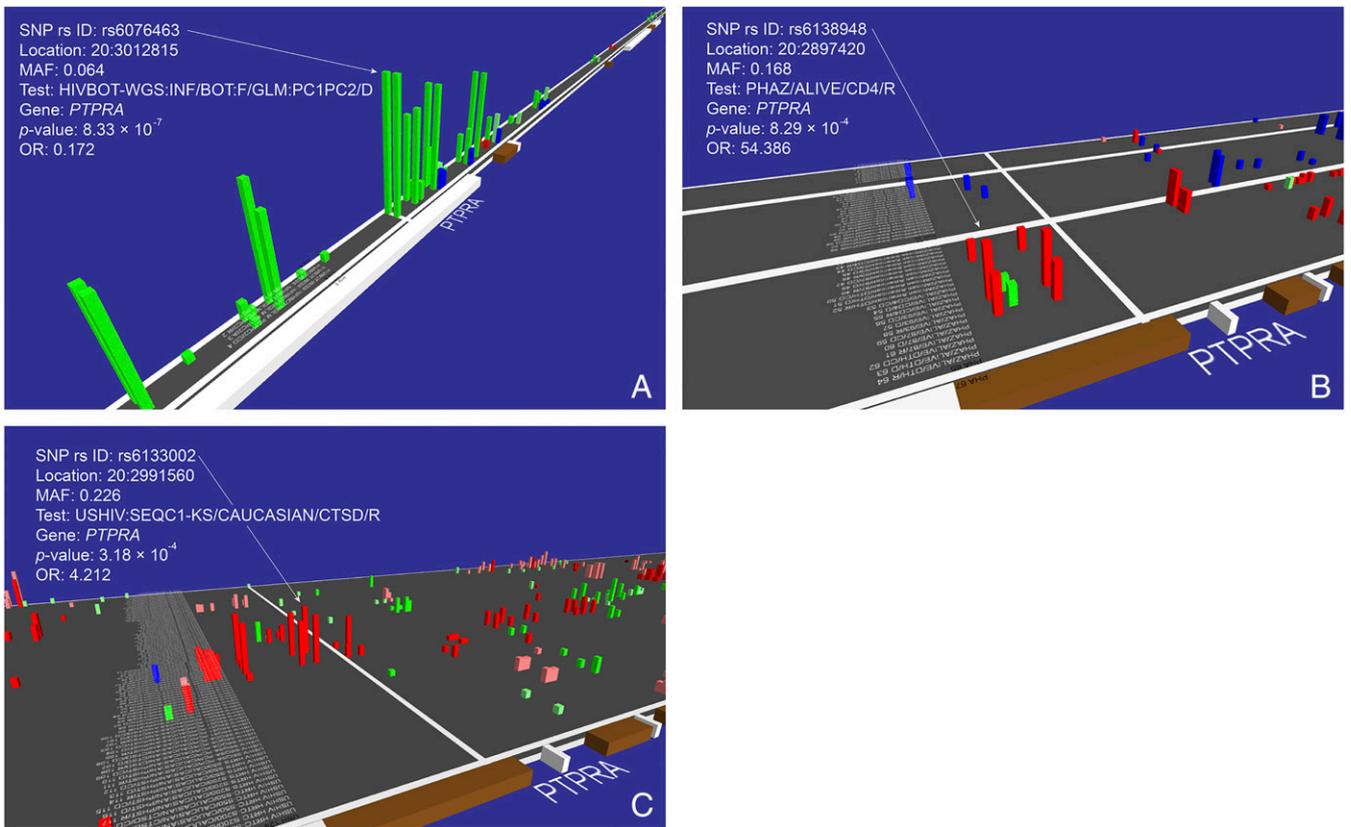


Fig. 3. GWATCH snapshots of associations in the *PTPRA* gene region. (A) HIV infection tests in the Botswana WGS discovery cohort ($n = 336$ study participants [256 HIV⁺ and 80 HIV⁻] genotyped with 8,636,400 SNPs). (B) AIDS progression tests in the replication AA cohort (also called ALIVE cohort, $n = 1,460$ study participants genotyped with 700,022 SNPs). (C) HIV/AIDS progression (sequelae) tests in the Botswana replication EA Mod A cohort ($n = 1,527$ study participants genotyped with 700,022 SNPs) (<https://botswana.gen-watch.org/>) (9, 18).

as a consequence, affects the release of HIV-1 virus-like particles (42).

PTPRA encodes a membrane receptor, member of the protein tyrosine phosphatase (PTP) family, which regulates a variety of cellular processes, including cell growth, differentiation, mitotic cycle, and oncogenic transformation. The *PTPRA* gene was shown to be up-regulated in CD4⁺ cells from HIV-1 patients (25, 26). It has also been detected among exosomal proteins found in urinary extracellular vesicles from HIV-1⁺ patients (27).

The human Neogenin 1 (*NEO1*) gene encodes a multifunctional cell surface protein that is a member of the immunoglobulin superfamily, implicated in tissue morphogenesis, angiogenesis, myoblast differentiation, and axon guidance (28). Plasma membrane expression of *NEO1* is down-regulated in CEMT4 T cells infected with VSV-G pseudotyped HIV-1 (29). Neogenin also demonstrates a greater than twofold increased relative abundance in HIV-1⁺ blood plasma and some studies suggest that it may play an important role in chronic inflammation induced by HIV infection. It was proposed that functional regulation of neogenin plasma level may have a therapeutic value in HIV⁺ patients (30).

It seems notable that all three important associations discovered in the African Botswana population showed gene replications in the United States-AA AIDS cohort (Figs. 2B, 3B, and 4B and Table 2). By contrast, over 30 ARGs were discovered in the United States Caucasian cohort (4, 7, 8) with very few previously described ARGs found in the same United States-AA cohort (Table 2). This difference may actually be attributable to ethnic genetic background. Furthermore, we were able to replicate the reported associations for 13 previously found ARGs, such as

NCOR2, *TRIM5*, *CXCL12*, and several *HLA* genes (Table 4 and *SI Appendix*, Table S2). These replications provide additional evidence in support of the previously reported associations and strengthen our confidence in the newly detected ones.

Our study was limited by rather small sample sizes and a gender emphasis on females, though previous ARG studies in AIDS cohorts from Western countries were biased toward male participants. More expansive studies in the future are certainly needed in this population, as well as in others, particularly in other African regions, ethnic groups, in men and in children in order to assess the findings more thoroughly. Multiple kinds of genome-level data (e.g., genomic DNA, epigenetic data, transcriptome data, siRNA screens) used in conjunction with resequencing strategies identifying rare causal variants are necessary to improve our comprehension of the role of host genome variability on HIV-1 acquisition, progression, and transmission at regional and global scales (6, 17). Finally, nongenetic factors are unaccounted for in present-day GWAS studies, such as host microbiome, epigenome, environmental, and social factors, which surely contribute to HIV-1 transmission and progression. These components should be assessed by the future study designs in this important search for the host genetic determinants of HIV/AIDS transmission and pathogenesis (6).

Methods

Populations Studied. Participants from two earlier Botswana Harvard AIDS Institute Partnership studies, Mashi and Tshedimoso, were enrolled in GWAS study. The Mashi study (clinicaltrials.gov identifier NCT00197587; NIH R01 HD37793) was a mother-to-child prevention 2 × 2 factorial randomized clinical trial with peripartum (single-dose nevirapine vs. placebo) and postpartum infant feeding formula vs. breastfeeding with

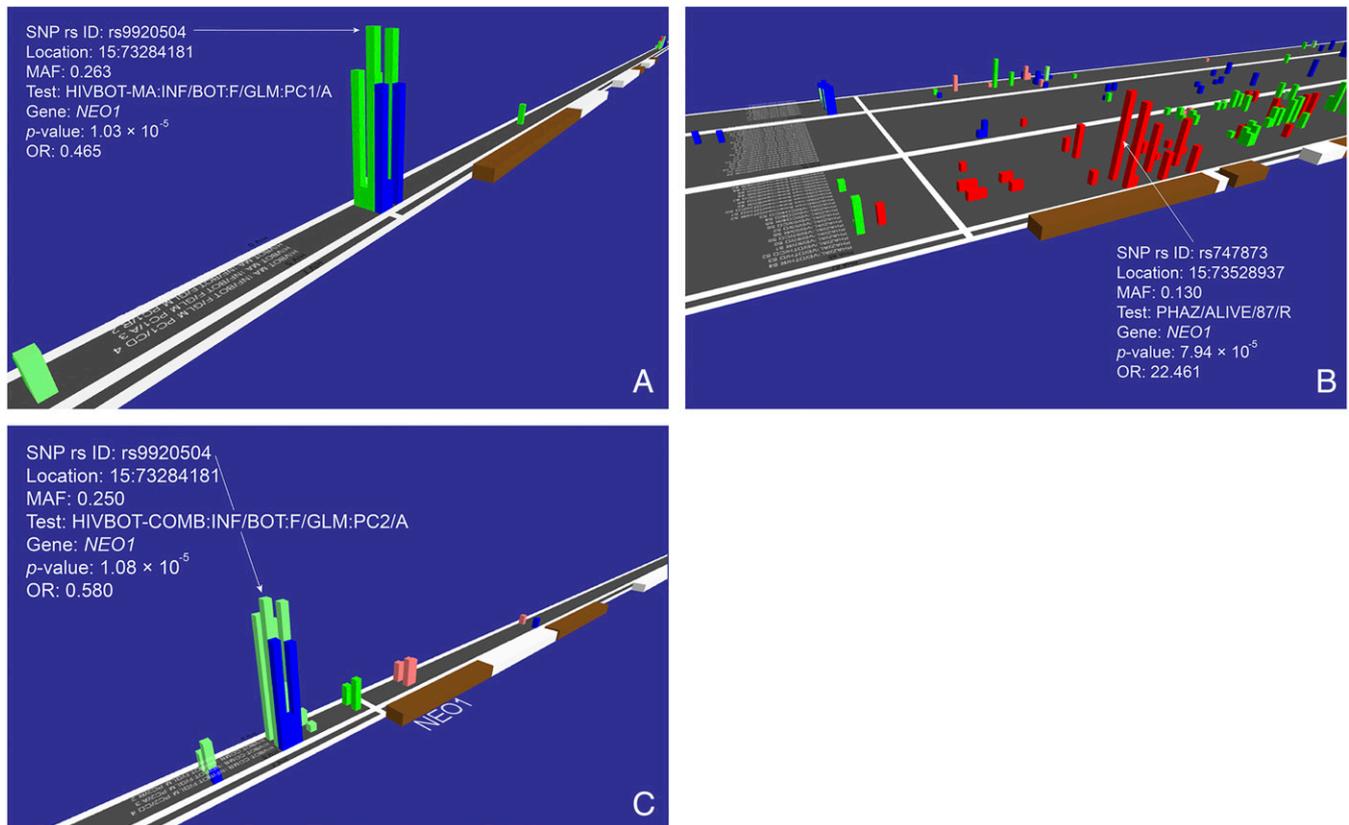


Fig. 4. GWATCH snapshots of associations in the *NEO1* gene region. (A) HIV infection tests in the discovery MA cohort ($n = 430$ study participants genotyped [194 HIV⁺ and 236 HIV⁻] with 1,374,256 SNPs). (B) AIDS progression tests in the replication United States-AA cohort ($n = 1,460$ study participants genotyped with 700,022 SNPs). (C) HIV infection tests in the replication combined MA-WGS cohort ($n = 762$ study participants genotyped with 1,333,944 SNPs) (<https://botswana.gen-watch.org/>) (9, 18).

infant zidovudine prophylaxis) interventions presented elsewhere (45–49). A subset of 436 individuals who participated in Mashi study were enrolled in this study. The Tshedimoso study (NIH AI057027 Markers of Viral Set Point in Primary HIV-1C Infection) was presented elsewhere (50–70).

A subset of 373 individuals who were screened for the Tshedimoso study were enrolled in the GWAS study. The samples all came from the two cohorts

that were collected/enrolled for the original clinical studies of largely Tswana ethnicity study participants.

The largest cohort was recruited only for HIV⁺ pregnant women to test interventions to prevent mother-to-child transmission. Thus, no males were ever involved in that cohort. As this study was performed in Botswana, a country with one of the highest HIV-1 prevalence (20.3%), a high proportion of citizens could be considered as likely exposed to HIV-1 infection.

Table 4. Replications of previously discovered AIDS restriction genes in the cohorts of this study

Dataset	Gene	Chr	SNP	Position	test	P value	Odds ratio	Citation (original source)
WGS	<i>NCOR2</i>	12	rs701025	125042336	DOM	8.0E-05	3.48	(31)
	<i>PROX1</i>	1	rs366684	214187262	DOM	5.1E-04	0.33	(32)
	<i>PROX1-AS1</i>	1	rs853757	214051639	REC	5.6E-04	0.24	(32)
	<i>TRIM5</i>	11	rs71490145	5691528	DOM	5.9E-04	0.38	(33)
	<i>CXCL12 (SDF1)</i>	10	rs1898318	44825430	DOM	6.6E-04	0.33	(34)
	<i>HCP5</i>	6	rs7746061	31419216	ALLELIC	1.2E-03	0.31	(35)
	<i>MYH9</i>	22	rs28478212	36734593	ALLELIC	2.1E-03	0.38	(36)
	<i>HLA-DRB1</i>	6	rs9270209	32556443	ALLELIC	2.5E-03	2.19	(37)
	<i>TSG101</i>	11	rs867590536	18508258	ALLELIC	2.8E-03	0.39	(38)
	<i>HLA-DMB</i>	6	6:32908833	32908833	ALLELIC	3.4E-03	0.36	(39)
	<i>HLA-C</i>	6	rs1049709	31236854	DOM	5.5E-03	0.37	(35)
	<i>HLA-A</i>	6	rs1059514	29911190	REC	5.8E-03	0.34	(40)
	<i>CCR2*</i>	3	rs62242985	46385638	ALLELIC	8.3E-03	1.79	(41)
	MA	<i>TRIM5</i>	11	rs73404271	5739483	ALLELIC	4.2E-04	2.17
<i>CXCL12 (SDF1)</i>		10	rs800323	44801673	REC	1.8E-03	0.39	(34)
<i>NCOR2</i>		12	rs12814949	124976612	DOM	5.3E-03	0.47	(31)
<i>HLA-DQB1-AS1</i>		6	rs6689	32627700	ALLELIC	8.7E-03	0.54	(37)

See *SI Appendix, Table S2* for more information on the original associations.

*In LD; 9,587 bp.

Individual exposure was not quantified. The data were obtained in collaboration with Harvard T. H. Chan School of Public Health AIDS Initiative (Boston, MA) and the Botswana/Harvard AIDS Institute (Gaborone, Botswana).

This study was conducted by the Botswana Harvard AIDS Institute Partnership and approved according to The Declaration of Helsinki. All participants consented to participate in the study. Institutional Review Board (IRB) approval was from Botswana Ministry of Health and Wellness–Health Research Development Committee and Harvard School of Public Health IRB (reference no.: HPDME 13/18/1).

Whole-Genome Sequencing. WGS was performed for 364 samples at 30× coverage (Table 1). Quality control of raw WGS reads was performed using FastQC v0.11.5 (71) and 23-mer counts distribution with KraTER (72). Trimmomatic (73) with default parameters was used to remove adapters and trim low-quality read ends. Trimmed reads were mapped to the human reference genome GRCh38 (top-level main chromosomes) using BWA-MEM v0.7.15 (74) with default parameters and the read alignments were sorted with SAMtools sort v0.1.19 (75, 76). These alignments were then used for genotyping by SAMtools and BCFtools toolchain (SAMtools mpileup with -Q 30 -q 30 -m, and BCFtools call) (76). After checking the F1-score between genotypes in WGS and microarray data with the DP (the variant read depth) range from 1 to 10 and for QUAL (the Phred-scaled quality score of alternative alleles of a variable site) from the set 15, 20, 25, 30, 35, and 40 variants with DP < 5 and QUAL < 20, and singletons with single called genotype were filtered out. The resulting variants were annotated using Ensembl (19). VEP WGS genotypes were lifted down to genome build hg19 using UCSC liftOver tool to make them compatible with MA data.

WGS Sample Filtering. All genotype quality control was performed using PLINK 1.9 (77) and custom scripts. First, for the samples with both Illumina MA genotypes and WGS genotypes, we calculated the fraction of concordant genotypes between the two methods. WGS samples with genotype concordance below 90% were removed. All males were removed, as there were no HIV-infected samples among them. In addition, we removed principal component analysis (PCA) outliers based on visual inspection of the plot of PC1 and PC2, samples with missing genotype rate >5% and cryptic relatives (PI_HAT > 0.25) (Table 1 and *SI Appendix, Fig. S5A*). These steps resulted in 336 study participants independent of the microarray individuals (Tables 1 and 2).

MA Sample Filtering. For MA sample filtering, 809 individual samples were genotyped using the Illumina Infinium Omni2.5-8 BeadChip genotyping MA [333 samples which overlap with WGS were excluded from the MA dataset (Table 1)]. All males were removed, as were PCA outliers, samples with missing genotype rate > 1%, and cryptic relatives (PI_HAT > 0.01) based on PLINK 1.9 variant statistics (Table 1). Sample filtering resulted in 430 individuals genotyped by microarray that were independent of the WGS cohort (Tables 1 and 2 and *SI Appendix, Fig. S5B*). Recently, additional MA chips have been developed for specifically evaluating African populations, namely the Infinium H3Africa Consortium Array (v2) and African Diaspora Power Chip, but these were unavailable at the time of our genotyping.

Association Tests. Prior to testing genetic variants for association with HIV, we performed SNP filtering and excluded SNPs with call rate < 95%, MAF < 5% or HWE *P* value < 1e-4 (Table 1). The resulting genotype data were used for

the association tests. Association tests were performed using PLINK v1.9 logistic regression: we applied additive allelic (–logistic) models for alleles, dominant (–logistic dominant), recessive (–logistic recessive), and codominant (–logistic genotypic) models for genotypes. PCs 1 and 2 were used as covariates (–covar) in the WGS dataset, and PC1 was used as a covariate in the MA dataset to account for potential batch effects based on visual inspection of PCA plots. To avoid inadequate *P* values in the codominant model, we calculated the *P* value only for the variants having enough observations (≥ 3) for each sample group, thus excluding those variants for which it is not possible to test the codominant model.

The GWAS *P* value threshold for genome-wide significance for WGS and MA-based GWAS follows the corrected *P* value threshold convention ($P < 5 \times 10^{-8}$ and $P < 10^{-5}$, respectively) (78). These thresholds are corrected from the standard Bonferroni application that assumes every genetic variant tested is independent of the others (i.e., ignoring LD) and considering false-discovery rate procedures, permutation-based approaches, and Bayesian approaches (78). Relative risk, attributable risk, and explained fraction were calculated as defined in refs. 8 and 79.

The numbers of HIV⁺ and HIV[−] study participants used for each test for Botswana analyses are presented in Table 2 and also in Figs. 2–4. The American replication cohorts consist of multiple tests with different numbers of individuals in each. The individuals' numbers for each test are listed in GWATCH (<https://gen-watch.org/#/option0>) under the "List of tests & Manhattan plots" tab for each cohort.

Visualization of the Results Using GWATCH. Genotyping and clinical data were loaded into the GWATCH analytical suite (9, 18) for further analysis and visualization. GWATCH is a web-based dynamic real-time genome browser designed to discover, view, and assess genetic association hits and genomic environment using multiple association test designs from GWASs ([botswana.gen-watch.org/](https://gen-watch.org/)). GWATCH displays a moving three-dimensional viewer above along human chromosomes with baseline *x* axis listing all association tests performed in the GWAS (<https://www.youtube.com/watch?v=vFHRCb4bUGs>). Adjacent SNPs genotyped along each chromosome are the indices of the *y* axis. The rising blocks (Figs. 2–4) illustrate the resulting negative logarithm with base 10 of *P* values obtained for each SNP–test combination, with color showing the direction of association (green indicates allele or genotype resistance to infection or disease OR < 1.0; red indicates susceptibility OR > 1.0). Color intensity of the value bars indicate the hazard ratio/OR of each association listed in Table 3. The *P* values for the gene-associated SNPs in replication cohorts were corrected by the number of SNPs in each gene interrogated in *SI Appendix, Table S3*.

Data Availability. SNP genotype data have been deposited in <https://botswana.gen-watch.org/> (Botswana GWATCH database database). All other study data are included in the article and supporting information. Previously published data were used for this work (18).

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