HAART-Associated Dyslipidemia Varies by Biogeographical Ancestry in the Multicenter AIDS Cohort Study

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Abstract

Highly active antiretroviral therapy (HAART) has been successful in delaying the progression to AIDS in HIV-1-infected individuals. Exposure to HAART can result in metabolic side effects, such as dyslipidemia, in a subset of recipients. Longitudinal data and frozen peripheral blood mononuclear cell pellets were obtained from 1,945 men enrolled in the Multicenter AIDS Cohort. Individuals were genotyped for ancestry informative markers (AIMs) and stratified by biogeographical ancestry (BGA). Then serum levels of total cholesterol (TCHOL), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TRIG) were examined controlling for a number of HIV and HAART-related covariates using multivariate mixed-effects linear regression. HIV-1 infection, in the absence of HAART, was associated with altered lipid levels for all phenotypes tested when compared to HIV-negative men. HIV-1-infected men receiving HAART also had significantly different lipid levels compared to HIV-negative men, except for LDL-C. There were statistically significant interactions between BGA and HIV/HAART status for all lipids tested. BGA remained significantly associated with lipid levels after controlling for other HIV and HAART-related covariates. There was low concordance between self-reported race (SRR) and BGA in admixed populations. BGA performed better than SRR in our statistical models. Lipid profiles in untreated HIV-1-positive men and HIV-1-positive men receiving HAART differ from HIV-negative men and this effect varies by BGA. BGA performed better in our statistical analysis as a racial classifier but SRR remains a good clinical surrogate for BGA.

Introduction

DYSLIPIDEMIA, CHARACTERIZED BY hypocholesterolemia and hypertriglyceridemia, is common in patients infected with human immunodeficiency virus (HIV).^{1–4} Treatment of HIV-1 infection with highly active antiretroviral therapy (HAART) has prolonged the progression to AIDS in these patients.⁵ Yet drug-specific metabolic side effects such as lipodystrophy and dyslipidemia have been reported in HIV-1-seropositive patients receiving HAART.^{6–15} These drug-related side effects augment the aberrant metabolic state associated with HIV-1 infection. The overriding clinical concern is that these metabolic alterations may be associated with an increased risk of cardiovascular disease (CVD) and myocardial infarction in this population.^{16–24}

Previous studies associating dyslipidemia with specific drug combinations used in HAART have also shown that the extent of dyslipidemia tends to vary between self-reported racial populations.^{25–28} Serum lipid levels, atherosclerosis, and CVD risk are all known to vary by race in the general population and polymorphisms in genes involved in cholesterol transport and metabolism may play a role in this variation.^{29–36} Investigating lipid patterns by self-reported race may be complicated by the heterogeneous genetic profiles of individuals in those self-classified populations.^{37–39} A more sensitive approach would be to use specific genetic markers that identify ancestrally unique populations. We designed a custom genotyping array containing polymorphisms associated with dyslipidemia and CVD in the general population, but that also included a panel of ancestry

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informative marker (AIM) single nucleotide polymorphisms that have previously been shown to define biogeographical ancestry (BGA) well in samples from mixed population groups.^{37,38} Here we present a detailed characterization of serum lipid measures in participants from the Multicenter AIDS Cohort Study (MACS) and factors that influence total cholesterol (TCHOL), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TRIG) profiles.

Materials and Methods

Study population

The MACS is a prospective multiethnic cohort study of the natural history of HIV-1 in homosexual and bisexual men from four major metropolitan areas: Los Angeles, CA; Chicago, IL; Baltimore, MD; and Pittsburgh, PA.⁴⁰ MACS participants are seen semiannually with each visit consisting of interviews, mental and physical examinations, and biological sample collections. Permission was obtained from the MACS to receive peripheral blood mononuclear cell (PBMC) pellets for our study investigating possible genetic susceptibilities to HAART-associated dyslipidemia. PBMC pellets were obtained from 1,945 active MACS participants who were seen in 2005 and had serum lipid data recorded in that year. The baseline visit demographics of the 1,779 MACS individuals who were selected for this study (exclusion criteria described below) are shown in Supplementary Table S1 (Supplementary Data are available online at www.liebertpub.com/aid).

Biogeographical ancestry: sample processing, genotyping, and definition

Frozen PBMC pellets were obtained and genomic DNA was extracted using the Qiagen QIAmp DNA Blood Mini Kit using the Blood or Body Fluid Spin Protocol (Qiagen Inc., Valencia, CA). The extracted genomic DNA was then stored at 4°C at a total volume of 400 μ l. To obtain the DNA quantities needed for the Illumina GoldenGate assay, a whole-genome amplification (WGA) was performed on all of the samples using the Illustra Genomphi V2 DNA Amplification Kit (GE Healthcare Biosciences, Pittsburgh, PA). The WGA DNA was quantified using the Quant-iT PicoGreen dsDNA Reagent Kit (Invitrogen Ltd., Carlsbad, CA) and diluted to a concentration of 50 ng/ μ l for use in the genotyping assay.

A panel of 128 AIM single nucleotide polymorphisms (SNPs) was used to determine BGA in our samples.^{37,38} Samples were genotyped using a custom designed GoldenGate array (Illumina Inc., San Diego, CA) at the University of Pittsburgh Genomics and Proteomics Core Laboratories. Genotypes were called using the standard clustering algorithm in the Illumina BeadStudio software. Each SNP's cluster graph was examined manually and genotype calls were adjusted following visual inspection. Samples were excluded ($n = 31$) if the sample call rate was <90%. Loci were excluded if the locus call rate was <80% and the minor allele frequency was <0.05. Using these quality control (QC) metrics 113 of the 124 (91%) AIMS used were found to be suitable for defining BGA in the MACS. The AIMS were a component of a custom GoldenGate array consisting of 1,536 SNPs reported to have established associations with CVD and dyslipidemia in the general population.

To define BGA a multidimensional scaling (MDS) procedure was performed using the 113 AIM panel. The Multidimensional Scaling Population Stratification procedure was performed using the PLINK software package.^{41,42} The first two principal components were used to cluster the MACS population graphically, and reference populations downloaded from the HapMap database were used to define the BGA origin of each cluster. To define clear boundaries between BGA clusters, a k -means clustering procedure was performed, using the R software package, on the first two components of the MDS.⁴³ Three possible clusters ($k = 3$) were defined, comprising individuals of primarily European ancestry (EA), African/European admixed ancestry (AEA), and Asian/European (AsEA) admixed ancestry. Self-reported race (SRR) in the MACS consists of eight possible categories. For simplicity we created a three-level variable consisting of white non-Hispanic (WnH), black non-Hispanic (BnH), and other (Oth). Figure 1 shows the first two principal components of the MDS with BGA cluster boundaries and colors reflecting SRR. The Asian/European admixed population ($n = 135$) was excluded from the analysis due to small sample size. The EA ($n = 1,291$) and AEA ($n = 488$) populations comprised the majority of the sample and were used for our analyses.

Lipid measurements and covariate data

Longitudinal patient visit (PV) data on relevant covariates were captured including age, body mass index (BMI), SRR, HIV-1 serostatus, specific HAART regime, HAART

FIG. 1. Self-reported race varies by genetically defined biogeographical ancestry. The scatter plot shows the first two principal components from the multidimensional scaling (MDS) procedure, performed on the ancestry informative markers (AIMs) data. Each dot represents an individual who was genotyped in the Multicenter AIDS Cohort Study (MACS) cohort ($n = 1,914$). Colors represent self-reported race (SRR) (red = BnH, blue = WnH, green = other) and boundaries of biogeographical ancestry (BGA) populations (EA, AEA, and AsEA) were defined by a k -means clustering procedure.

adherence, HAART duration, HIV-1 viral load, CD4 T cell count, lipid-lowering medication use, and hours fasting. The PV data were restricted to visits for which individuals reported fasting for greater than 8 h and not taking lipid-lowering medication. Additionally, we limited the analysis to individuals who we defined as being either of EA or AEA, and that had at least one of the four lipid phenotypes recorded at a particular visit. TCHOL, LDL-C, HDL-C, and TRIG were determined by standard methodologies.^{44–47} The LDL-C was calculated using the Friedewald equation [LDL-C = TC – (HDL-C + (TRIG/5))] or by direct measurement if the triglyceride level was above 400 mg/dl.

HAART use in the MACS was defined in accordance with the DHHS/Kaiser Panel guidelines.⁴⁸ Three treatment groups were defined based on HIV-1 serostatus and HAART status at a specific visit; HIV-1 seronegative = HIV[−], HIV-1 seropositive not receiving HAART = HIV/HAART naive, and HIV-1 seropositive receiving HAART = HIV/HAART. The specific HAART regime in use at each PV was dichotomized to drug combinations that contained a protease inhibitor (PI/HAART) and combinations that did not contain a PI (non-PI/HAART). The duration of HAART was assessed at each PV by calculating years since the first visit at which HAART use was reported. Adherence to HAART was defined as either being 100% adherent since the last visit or less than 100% adherent since the last visit. HAART treatment response and clinical progression

were assessed using plasma HIV-1 RNA viral load (VL). VL was dichotomized according to the current clinical definition of virologic/treatment failure, as a VL of >200 copies/ml, or successful viral suppression, with a VL ≤200 copies/ml.⁴⁹ Calendar time was categorized as pre-2001 and 2001–2010 to coincide with recruitment waves in the MACS study. This categorical variable also controls for any effects of exposure to different drug types used early in the development of HAART.

Statistical analysis

Mixed-effects linear regression analysis was used to assess the association between lipid levels and BGA. The correlation between repeated lipid measurements for each individual was accounted for in the analysis using a random intercept term. The variance–covariance structure of the random effect was treated as a multiple of the identity matrix that assumes equal variances and that all covariances are zero. The identity covariance matrix is the default for factor variables in STATA 12. The parameter estimates did not change significantly when sensitivity analysis was performed by substituting an exchangeable covariance matrix for the identity matrix. Hypothesis testing was performed using Wald tests and parameters were estimated using maximum likelihood. The effects of each covariate were assessed in univariate and multivariate models. Continuous variables including age, BMI, and

TABLE 1. COVARIATES BY HIV SEROSTATUS AND BIOGEOGRAPHICAL ANCESTRY

% (N = Pt. visits)	HIV/HAART		HIV/HAART naive		HIV [−]	
	AEA N = 1,319	EA N = 2,710	AEA N = 781	EA N = 964	AEA N = 1,457	EA N = 5,291
Age						
<45	53 (701)	29 (775)	59 (462)	45 (432)	54 (779)	24 (1,284)
45–49	25 (332)	28 (746)	20 (158)	20 (197)	17 (257)	21 (1,098)
50–54	14 (181)	24 (647)	12 (94)	20 (196)	14 (211)	21 (1,098)
55–59	5 (72)	12 (347)	8 (62)	11 (100)	10 (143)	15 (820)
≥60	3 (33)	7 (195)	1 (5)	4 (39)	5 (67)	19 (991)
BMI, kg/m ²						
<25	49 (643)	55 (1,489)	42 (328)	53 (508)	39 (563)	38 (1,997)
25–29	34 (450)	30 (808)	33 (258)	30 (293)	35 (514)	37 (1,948)
≥30	17 (226)	15 (413)	25 (195)	17 (163)	26 (380)	25 (1,346)
Years on HAART						
<3	22 (294)	17 (460)	—	—	—	—
3–10	65 (853)	65 (1,766)	—	—	—	—
>10	13 (172)	18 (484)	—	—	—	—
HAART adherence						
100%	35 (455)	41 (1,101)	—	—	—	—
<100%	65 (851)	59 (1,587)	—	—	—	—
Type of HAART						
Non-PI/HAART	51 (672)	52 (1,402)	—	—	—	—
PI/HAART	49 (647)	48 (1,308)	—	—	—	—
Viral load, copies/ml						
≤200	71 (942)	83 (2,261)	—	—	—	—
>200	29 (377)	17 (449)	—	—	—	—
Visit years						
<2001	3 (38)	12 (331)	3 (24)	13 (122)	5 (78)	9 (470)
2001–2010	97 (1,281)	88 (2,379)	97 (757)	87 (842)	95 (1,379)	91 (4,821)

Ns represent the number of patient visits for each covariate from individual subpopulations. AEA, African/European ancestry; EA, European ancestry; BMI, body mass index; HAART, highly active antiretroviral therapy; non-PI/HAART, HAART containing no protease inhibitors; PI/HAART, HAART containing at least one protease inhibitor.

duration of HAART were centered to make interpretation of the model intercepts meaningful. Triglyceride levels were log transformed before analysis. Statistical analysis was performed using the STATA 12 and R software packages.^{43,50}

Results

MACS demographics

AIMs were used to define BGA in the MACS and then compared to SRR. The SRR of white non-Hispanic generally agreed with the BGA defined group of primarily EA with a concordance of 85%. There was substantial disagreement between the SRR black non-Hispanic and the AEA with a concordance of 69%. There was also disagreement between SRR-other and the AsEA with a concordance of 66%. This discordance between BGA and SRR in admixed populations is consistent with previous reports.^{39,51} Figure 1 shows a scatter plot of the first two principal components from the multidimensional scaling (MDS) analysis of the AIMs. These data demonstrate the distribution of SRR by the BGA-defined population clusters, and the heterogeneity seen in the admixed populations is represented clearly in this plot.

Summaries of MACS PV demographic data are shown in Table 1. The AEA population had a younger age distribution with a larger number of PVs coming from individuals who were less than 45 years of age for all treatment groups. The HIV⁺ individuals had more PVs with a recorded BMI <25

compared to the HIV⁻ group. Years on HAART and type of HAART for HIV⁺ men were similar between both BGA populations in HIV⁺ men. In HIV⁺ men, the AEA population had poorer HAART adherence and virologic control (VL <200 copies/ml) compared to the EA population. There were fewer PVs prior to 2001 in AEA men compared to EA men for all treatment groups, which may reflect exposure to more lipid unfriendly drug regimes in the EA men

Lipid levels vary by HIV/HAART status and BGA

To characterize the lipid profiles in our population the mean lipid level was calculated across all visits for each individual and plotted by BGA and treatment status (Fig. 2). The mean lipid levels for each phenotype tested appeared to vary depending on treatment status and BGA. Results from the multivariate mixed-effects linear regression analyzing the effects of treatment status and BGA on serum lipids using the longitudinal data are shown in Table 2. AEA was associated with lower serum levels of LDL-C and higher levels of HDL-C compared to the EA population. HIV-1 infection alone was associated with lower levels of TCHOL, LDL-C, and HDL-C compared to HIV⁻. TRIG levels were higher in HIV-infected men compared to HIV⁻. HIV/HAART was associated with lower levels of LDL-C and HDL-C while being associated with higher levels of TCHOL and TRIG compared to HIV⁻. Importantly, the terms comparing the interaction of BGA and HIV/HAART status on lipid levels show statistically

FIG. 2. Mean serum lipids vary by biogeographical ancestry and HIV/highly active antiretroviral therapy (HAART) status (-/- = HIV negative, +/- = HIV/HAART naive, +/+ = HIV/HAART). (A) Mean total cholesterol (TCHOL) by HIV/HAART status. (B) Mean low-density lipoprotein cholesterol (LDL-C) by HIV/HAART status. (C) Mean high-density lipoprotein cholesterol (HDL-C) by HIV/HAART status. (D) Mean triglyceride (TRIG) by HIV/HAART status. The means of the European ancestral population (EA) are shown in the right panels and the means of the African/European ancestral population (AEA) are shown in the left panels. Raw calculated means are shown in blue and adjusted means are shown in red. Bars indicate 95% confidence intervals of the mean.

TABLE 2. SERUM LIPID LEVELS VARY BY HIV/HAART STATUS AND BIOGEOGRAPHICAL ANCESTRY

Parameter	Mean difference (mg/dl)	95% CI	p
TCHOL			
Intercept ^a	199.9	(197.0, 202.7)	—
AEA (vs. EA)	−3.7	(−9.5, 2.1)	NS
HIV/HAART naive (vs. HIV [−])	−13.9	(−18.7, −9.2)	<0.001
HIV/HAART (vs. HIV [−])	5.3	(1.0, 9.7)	0.015
Effect of AEA on HIV/HAART naive (vs. HIV [−])	−5.5	(−13.8, 2.8)	NS
Effect of AEA on HIV/HAART (vs. HIV [−])	−12.3	(−20.2, −4.4)	0.002
LDL-C			
Intercept ^a	124.5	(122.0, 126.9)	—
AEA (vs. EA)	−5.8	(−10.8, −0.8)	0.023
HIV/HAART naive (vs. HIV [−])	−9.1	(−13.2, −4.9)	<0.001
HIV/HAART (vs. HIV [−])	−3.1	(−6.9, 0.7)	NS
Effect of AEA on HIV/HAART naive (vs. HIV [−])	−6.7	(−13.9, 0.5)	NS
Effect of AEA on HIV/HAART (vs. HIV [−])	−7.3	(−14.1, −0.4)	0.037
HDL-C			
Intercept ^a	50.1	(49.2, 51.1)	—
AEA (vs. EA)	2.5	(0.5, 4.4)	0.012
HIV/HAART naive (vs. HIV [−])	−9.5	(−11.1, −7.9)	<0.001
HIV/HAART (vs. HIV [−])	−5.7	(−7.2, −4.3)	<0.001
Effect of AEA on HIV/HAART naive (vs. HIV [−])	2.7	(−0.1, 5.5)	NS
Effect of AEA on HIV/HAART (vs. HIV [−])	4.1	(1.4, 6.7)	0.002
TRIG			
Intercept ^a	111.5	(107.2, 115.9)	—
AEA (vs. EA)	−4.5	(−12.3, 5.6)	NS
HIV/HAART naive (vs. HIV [−])	28.9	(20.1, 39.0)	<0.001
HIV/HAART (vs. HIV [−])	63.6	(53.5, 74.7)	<0.001
Effect of AEA on HIV/HAART naive (vs. HIV [−])	−14.5	(−25.6, −2.2)	0.017
Effect of AEA on HIV/HAART (vs. HIV [−])	−24.5	(−33.5, −14.5)	<0.001

^aModel intercepts represent HIV-seronegative men of European ancestry with an age of 51 years and a BMI of 26.8.

Regression coefficients (mean change) and 95% confidence intervals from a multivariate mixed-effects linear regression analyses on longitudinal repeated measures. Lipid phenotypes tested; total cholesterol (TCHOL), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TRIG). Each model contained all the parameters listed as well as centered age and BMI. Data represent 11,702–11,214 patient visits from 1,506–1,535 individuals depending on the lipid phenotype. Parameter *p*-values were calculated using Wald tests, NS=*p*>0.05.

significant effects of AEA on HIV/HAART versus HIV[−] for all lipid phenotypes. This suggests that lipid level alterations associated with HAART differ depending on BGA.

BGA effects on serum lipids after controlling for other HIV/HAART covariates

The results from the mixed-effects linear regression analysis investigating the effect of BGA on lipid levels in HIV/HAART men, controlling for other HIV and HAART-related covariates that may be contributing to or confounding the observed BGA effect, are shown in Table 3. BGA was consistently statistically significant for all lipid phenotypes tested even after controlling for the other covariates in the model. In HIV/HAART men the AEA population had lower mean lipid levels for TCHOL, LDL-C, and TRIG, and higher mean levels for HDL-C. Comparing PI-containing HAART regimes to non-PI/HAART showed that PI/HAART was associated with higher levels of TRIG, but was associated with lower levels of HDL-C. The type of HAART used was not significantly associated with LDL-C levels either alone or controlling for the other covariates. Virological failure, as measured by a VL >200 copies/ml, was significantly associated with lower TCHOL, LDL-C, and HDL-C levels compared to a VL ≤200 copies/ml, but did not have significant effects associated with

TRIG levels when controlling for other covariates in the model. HAART adherence and duration were not associated with any of the lipid phenotypes tested when controlling for the other covariates. The adjusted lipid values from the models used in Tables 2 and 3 were calculated and plotted in a fashion similar to the raw mean values shown in Fig. 2. With the exception of TRIG levels, the adjusted means and 95% CIs overlap with the raw values. This shows that the initial observed differences in lipid levels between BGA populations persist after adjusting for a number of HIV and HAART-related covariates.

Biogeographical ancestry versus self-reported race

To investigate the potential differences between using BGA or SRR as a racial classifier the multivariate mixed-effects linear regression analysis on HIV/HAART men was repeated substituting SRR for BGA. Then, to determine which factor had the best goodness of fit, the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) for each of the models containing BGA, SRR, or both were compared (Supplementary Table S2). The parameter estimates for each model changed slightly with the addition or replacement of SRR, but the statistical associations for each covariate did not change (data not shown). Finally, we performed residual

TABLE 3. VARIABLES THAT AFFECT SERUM LIPID LEVELS IN HIV-SEROPOSITIVE INDIVIDUALS RECEIVING HAART

	Univariate model		Full model	
	Mean difference (95% CI)	p	Mean difference (95% CI)	p
TCHOL (mg/dl)				
AEA (vs. EA)	-17.7 (-24.1, -11.3)	<0.001	-13.6 (-20.4, -6.8)	<0.001
PI/HAART (vs. non-PI/HAART)	4.9 (1.8, 7.9)	0.002	2.1 (-1.2, 5.4)	NS
VL ^a >200 (vs. VL ≤200)	-11.2 (-14.2, -8.3)	<0.001	-9.4 (-12.7, -6.1)	<0.001
<100% ADR (vs. 100% ADR)	-2.9 (-5.3, -0.6)	0.015	-2.4 (-4.9, 0.1)	NS
Duration of HAART	0.3 (-0.1, 0.7)	NS	-0.3 (-0.9, 0.3)	NS
Intercept ^b	—	—	209.2 (203.6, 214.9)	—
LDL-C (mg/dl)				
AEA (vs. EA)	-13.9 (-19.2, -8.5)	<0.001	-11.4 (-17.1, -5.8)	<0.001
PI/HAART (vs. non-PI/HAART)	1.5 (-1.1, 4.1)	NS	-0.5 (-3.3, 2.4)	NS
VL ^a >200 (vs. VL ≤200)	-7.5 (-10.0, -4.9)	<0.001	-5.9 (-8.7, -2.9)	<0.001
<100% ADR (vs. 100% ADR)	-1.8 (-3.8, 0.2)	NS	-1.6 (-3.7, 0.6)	NS
Duration of HAART	0.1 (-0.2, 0.5)	NS	-0.4 (-0.9, 0.1)	NS
Intercept ^b	—	—	122.5 (117.5, 127.4)	—
HDL-C (mg/dl)				
AEA (vs. EA)	5.5 (3.2, 7.7)	<0.001	6.6 (4.3, 8.9)	<0.001
PI/HAART (vs. non-PI/HAART)	-2.7 (-3.6, -1.7)	<0.001	-3.3 (-4.3, -2.3)	<0.001
VL ^a >200 (vs. VL ≤200)	-1.9 (-2.9, -1.1)	<0.001	-2.1 (-3.2, -1.1)	<0.001
<100% ADR (vs. 100% ADR)	0.4 (-0.3, 1.2)	NS	0.6 (-0.2, 1.3)	NS
Duration of HAART	0.1 (-0.1, 0.3)	NS	0.1 (-0.1, 0.3)	NS
Intercept ^b	—	—	47.4 (45.6, 49.3)	—
TRIG (mg/dl)				
AEA (vs. EA)	-39.5 (-51.6, -27.5)	<0.001	-38.8 (-50.8, -25.5)	<0.001
PI/HAART (vs. non-PI/HAART)	28.8 (21.6, 37.5)	<0.001	34.2 (24.6, 44.3)	<0.001
VL ^a >200 (vs. VL ≤200)	-3.2 (-11.1, 3.1)	NS	-1.9 (-9.8, 6.4)	NS
<100% ADR (vs. 100% ADR)	-6.5 (-12.9, -1.6)	0.011	-5.9 (-11.7, 0.1)	NS
Duration of HAART	-1.6 (-3.2, 1.6)	NS	-1.4 (-2.8, 0.1)	NS
Intercept ^b	—	—	167.8 (154.5, 182.6)	—

^aViral load (VL) measured in copies/ml indicates virological control.

^bThe full model intercepts represent HIV-seropositive men of European ancestry who are 47.5 years old, have a BMI of 25, have received HAART that does not contain a PI for 5.8 years, have a viral load ≤200 copies/ml, are 100% adherent to their therapy, and whose PV data came from visits before 2001.

Regression coefficients (mean change) and 95% confidence intervals from univariate and multivariate mixed-effects linear regression analyses on longitudinal repeated measures. Lipid phenotypes tested; total cholesterol (TCHOL), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TRIG). Parameter *p*-values were calculated using Wald tests. The multivariate analysis was adjusted for all parameters listed as well as age, BMI, and visit years at which patient data were obtained. EA, European ancestry; AEA, African/European ancestry; VL, viral load; ADR, adherence. Data represent 3,515–3,523 patient visits on 564–588 individuals depending on the model and lipid phenotype.

analysis on these factors by regressing SRR on the residuals from the univariate analysis of BGA and the four lipid phenotypes to see if SRR had any additional explanatory power. This analysis showed that SRR is not significantly associated with the BGA residuals and the *R*-squared value for the models was close to zero (data not shown). These results show that for our PV data, BGA alone performs better than SRR in the models we tested, having the lowest BIC for all phenotypes and the lowest AIC for all lipids with the exception of HDL-C and TRIG. Also, SRR does not add any additional explanatory power beyond that of BGA.

Discussion

Hitherto our study is the most extensive investigation of long-term HAART use on serum lipids in HIV-positive men. After examining 10 plus years of longitudinal data on over a thousand individuals we found that BGA had the most profound effects on determining the dyslipidemic response to HAART. The effects of BGA remain significant after

controlling for numerous known predictors of lipid levels in HIV/HAART individuals. Importantly, it appears that the dyslipidemic response to HIV infection and HAART is more atherogenic in EA than AEA men, having higher TRIG levels with lower HDL-C levels. Exposure to HAART can have a variety of metabolic side effects. The dyslipidemia observed is well documented with varying degrees and prevalence in any given population. The extent to which these metabolic side effects, like dyslipidemia, vary in different ethnic or ancestral populations has been demonstrated by a number of studies, but the racial effects seen are not necessarily consistent across studies.^{25–27} In general, these studies found that whites had more atherogenic lipid profiles in response to HAART than blacks or hispanics. Overall our results agree with these findings, but the HAART-associated change in specific lipid phenotypes does vary from previous reports.

Initially we investigated the mean levels of four lipid phenotypes by HIV/HAART status and BGA. When examined graphically there were lower levels of TCHOL, LDL-C, and HDL-C in the HIV/HAART-naïve men compared to the

HIV⁻ men for both BGA populations but the extent of lowering appears to differ by BGA. Also there are higher levels of TRIG in both BGA populations of HIV/HAART-naïve men compared to HIV⁻. This reflects the metabolic impact that HIV-1 infection alone has on serum lipid levels and suggests that these effects are more pronounced depending on the BGA and particular lipid phenotype. In the HAART group there are different patterns of response between BGA populations for each lipid phenotype. This is most markedly observed in TRIG levels, which are higher in the HIV/HAART groups for both populations, but to a greater extent in the EA men. These data show that EA and AEA HIV-infected men have different lipid levels when receiving HAART, and that EA men, particularly with regard to TRIG levels, had a more atherogenic lipid profile. This may suggest a genetic or shared susceptibility present in the EA men compared to the AEA men.

For the second major set of analyses we focused our attention on HIV/HAART men. There were a number of variables that differed between the BGA populations that may have explained the observed differences in mean lipid levels. We formally tested the effects of BGA controlling for these other covariates using mixed-effects linear regression analysis. Our results revealed that BGA not only remained associated with mean lipid levels after controlling for other HIV and HAART covariates, but that this effect was larger than the other covariates in our models. Other factors, such as type of regime, adherence, and duration of HAART were significant predictors of lipid levels in HIV/HAART men but were often associated with only a marginal change in the mean (mg/dl) level. Contrary to previous findings, HAART that contained a PI had significant effects only on TRIG and HDL-C levels.^{6,8,9,15,52} Also, while these were statistically significant, only TRIG levels seemed to be substantially affected by PI/HAART, with an associated higher level of 35.3 mg/dl. In contrast, the effects of PI/HAART were associated with a 3.2 mg/dl lower HDL-C level. BGA consistently was associated with larger changes in the mean lipid levels than the other covariates tested.

In the final stage of analysis we compared BGA and SRR. We found that BGA consistently performed better as a predictor of lipid levels in the models we tested, but that SRR is a viable surrogate for BGA in the clinical setting. What is of most importance is the information that these two factors are capturing and how they differ. BGA is defining the genetically determined ancestry of an individual. SRR does this to a lesser extent though it also is reflecting shared cultural/environment factors that are associated with self-identifying to a particular ethnic population. While both have the potential to be informative, if the primary interest is determining possible genetic susceptibilities associated with a particular disease phenotype, BGA is clearly favored over SRR.

How BGA mechanistically is affecting serum lipids is difficult to predict. We hypothesize that BGA represents shared genetic susceptibilities, but whether this susceptibility is related to metabolism of HAART drugs, cholesterol, or both is difficult to hypothesize. Previous findings have shown that certain HLA types are associated with adverse HAART drug reactions and that these HLA variants do vary in frequency across racial populations.^{53,54}

Our study has a number of limitations. The MACS is a multiethnic cohort of men only and thus our analysis and

conclusions are not directly applicable to dyslipidemic responses in women on HAART. We also limited our analysis to men of European and African/European ancestry and our results may not apply to men of other admixed ancestries such as European/Asian. There is potential for bias in our data because our HAART drug regime and adherence data are self-reported by individuals. We also were unable to account for specific drug type in our analyses. Our study would be improved by having reliable clinical data reporting the exact drug regimen and specific drug doses that were being administered at each visit. Another limitation of our study is that we did not consider the effects of hepatitis C virus (HCV) coinfection in our population. HCV infection has been shown to be associated with metabolic alterations seen in HIV-positive patients receiving HAART.^{13,55,56} The strength of our study is the extensive lipid and phenotype data we have analyzed on a large cohort of HIV⁺ and HIV⁻ men. This enabled us to analyze and compare lipid levels in HIV/HAART-naïve and HIV/HAART men to HIV⁻ controls across thousands of patient visits while controlling for numerous covariates.

Our data show that BGA plays an important role in the dyslipidemic responses to HAART therapy in HIV-1-infected men. These differences persist even after controlling for many HIV/HAART-related covariates, suggesting previously unexplained risk factors associated with BGA populations. We hypothesize that this variation could be explained by shared genetic susceptibilities present within each population. This genetic variation could be associated with genes related to lipid metabolism, HIV-1 infection, HAART drug metabolism, inflammatory response, or some additional unknown pathway. The major clinical implications of our findings are that the possible CVD risks attributed to altered serum lipid levels associated with HAART are mostly restricted to EA men. It does appear that AEA men receiving HAART do not possess a very atherogenic lipid profile, although these findings do not suggest any definitive alteration to current treatment practices for these populations. Additionally, while BGA performed statistically better in our models as a racial classifier and is superior in studies investigating genetic variants, SRR still serves as a good surrogate for BGA in the clinical setting, despite the discordance between the two.

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Author Disclosure Statement

No competing financial interests exist.

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