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Association of Pharmacogenetic Markers With Atazanavir Exposure in HIV-Infected Women

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Abstract

SORCS2 rs73208473 was recently associated with decreased atazanavir (ATV) concentration in the hair of women with seropositive HIV. Herein, we report on a pharmacogenetic study of women with seropositive HIV demonstrating a similar association between rs73208473 and dose-adjusted plasma ATV concentration in African Americans.

Drug exposure is a major determinant of therapeutic or toxic response. A variety of environmental (e.g., diet, smoking, and concurrent medications), physiologic (e.g., age, disease, sex, and pregnancy), as well as enzyme and transporter phenotypes influence the absorption, distribution, metabolism, and excretion of a medication determining exposure.

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

In a recent genomewide association study (GWAS), we reported a statistically significant association between rs73208473 ($P = 1.71 \times 10^{-8}$) in intron 1 of *SORCS2* and ATV hair concentration where a single copy of the A allele was associated with a 0.46-fold change (95% confidence interval (CI) = 0.35–0.61) in ATV hair concentration.[1] Although discussed previously,[1] in intron 1 of *SORCS2* lies the MIR4798 microRNA, which among its many targets includes NR1I2 mRNA, the protein product of which is known to regulate both *CYP3A* and *ABCB1* expression that are involved in metabolism of ATV. We, therefore, investigated whether rs73208473, along with previously reported *ABCB1*,[2–4] *NR1I2*,[5,6] and *CYP3A5*[7,8] variants associated with oral clearance and plasma concentration of ATV, influence ATV exposure in plasma under conditions of routine clinical use in the Women's Interagency HIV Study, a multicenter longitudinal observational cohort study of women with and at risk for HIV infection. In the current study, over a period of 5 years (2003–2008), 123 ATV recipients participated in an intensive pharmacokinetic (iPK) study of serial plasma ATV measurements over a 24-hour period (i.e., 0, 4, 8, 16, and 24 hours). Of these 123 participants, only 3 individuals did not contribute to our original hair GWAS for ATV. Plasma ATV levels were determined by high-performance liquid chromatography tandem mass spectrometry with exposure estimated by area under the concentration-time curve (AUC) using the trapezoidal rule. The primary outcome was dose adjusted AUC normalized using a natural log transformation. Genotyping, which was coupled with imputation,[1] was performed by microarray (i.e., Illumina Omni2.5, San Diego, CA) in 116 of 123 participants. In addition to genotypes (coded as the number of rare alleles: 0–2), we assessed a variety of nongenetic factors that could influence ATV concentration in plasma (Table 1). A list of medications known to impact plasma ATV concentration was constructed using the HIV InSite database (http://hivinsite.ucsf.edu/) and assessed for association with plasma ATV concentration.

The first three principal components (PCs), calculated from genomewide genotype data using PLINK 1.9 (https://www.cog-genomics.org/plink2), were included as covariates in the multiple regression models to control for population admixture and stratification. We used the same approach to statistical modeling as was described previously.[1] Briefly, association analyses were performed in three strata (i.e., African American (AA), white (WT), and Hispanic (HIS) participants) with correction for the first three PCs and following nongenetic predictors: ritonavir, diarrhea, heroin use (AA only), and orange juice consumption. Given the existence of linkage disequilibrium between *ABCB1* rs1045642 and rs2032582 (r^2 = 0.31) and between *SORCS2* rs73208473 and rs73208470 (r^2 = 0.77), a Bonferroni-adjusted significance threshold of 0.05/6 = 0.008 was applied.

The majority of the participants were self-reported AA participants (64.2%), followed by HIS (18.7%), then WT (13%) with < 5% identifying themselves as Asians, Pacific Islanders, or others. The median AUC, peak plasma concentration (C_{max}), trough plasma concentration (C_{trough}), and time of maximum plasma concentration (T_{max}) for plasma ATV from the iPK study were 32,272 ng hour/mL, 2,300 ng/mL, and 549 ng/mL, 4 hours, respectively. Of 123 individuals who participated in the iPK, 73.8% reported a 300 mg and 18.9% reported a 400 mg daily dose of ATV. The dose range for ATV in the sample was 150–800 mg. The final multivariable models fit for nongenetic predictors, unstratified and stratified, are presented in Table 1. There are 123 samples that contributed data to the unstratified model,

but stratification based on PCs resulted in three major subgroups with smaller sample sizes (n_{AA} = 69, n_{HIS} = 14, and n_{WT} = 14). The use of ritonavir was associated with higher ATV exposure (fold effect: 2.3; $P = 5.4 \times 10^{-8}$) and this effect held true in all strata. Consumption of orange juice during the 5 days that preceded the iPK visit was associated with increased ATV exposure (fold effect: 1.37; $P = 0.0055$) and the increase in exposure was also observed in the AA participant (fold effect: 1.30) and HIS participant (fold effect: 1.65) groups, although the effect did not reach statistical significance in the subgroups. Other covariates included in the model were history of diarrhea (fold effect: 0.64; 95% CI = 0.47–0.88; $P = 0.045$) and use of heroin (fold effect: 3.00; 95% CI = 1.35–6.7; $P = 0.007$). The association of decreased ATV concentration with recent diarrhea was statistically significant in AA and HIS participants ($P < 0.05$), and heroin use was too rare to evaluate except in AA participants ($P = 0.0070$).

Of the two variants in the *SORCS2*, only rs73208473 had a statistically significant association with decrease in ATV exposure in plasma in AA participants (fold effect: 0.54; $P = 0.0015$; Table 2). The median oral clearances in AA participants' homozygous and heterozygous for the reference allele of rs73208473 were 8.73 and 14.3 L/hour, respectively. Although rs73208470 was in high linkage disequilibrium with rs73208473 ($r^2 = 0.77$ across the entire multi-ethnic sample) and was also associated with a decrease in ATV exposure among AA participants (fold effect: 0.73; $P = 0.028$), it did not meet our *a priori* criterion ($P < 0.008$). Meta-analysis did not identify associations between ATV plasma levels and the single nucleotide polymorphisms evaluated that met the $P < 0.008$ criterion.

Although impractical for routine clinical application, iPK protocols for plasma remain the "gold standard" for determining drug PK and AUC, the measure used in this study, is a more robust estimate of exposure to medications than single plasma levels commonly used in ATV studies. In this study, we demonstrate that ATV plasma exposure under conditions of actual use in women representative of US women living with HIV is associated with novel genetic and nongenetic predictors. Our analysis provides evidence for the first time that *SORCS2* rs73208473 is associated with decreased plasma concentration of ATV in AA participants. Additionally, ATV combination with ritonavir, diarrhea, heroin use, and recent orange juice consumption were also associated with plasma exposure of ATV.

In a GWAS of ATV concentration measured in hair, combining the data across three different strata, our group identified an association between *SORCS2* rs73208473 ($P = 1.71 \times 10^{-8}$) and ATV hair concentration where a single copy of the A allele was associated with a 0.46-fold change (95% CI = 0.35–0.61) in ATV hair concentration.[1] Whereas the effect size resulting from combining the three strata for this variant in plasma was small and not statistically significant (fold effect: 0.96; 95% CI = 0.92–1; $P = 0.91$), this variant was associated with 0.54-fold change (95% CI = 0.38–0.78; $P = 0.0015$) of ATV concentration in plasma among AAs, a nearly identical estimate to what we observed with ATV concentrations in hair (fold effect in hair: 0.54; 95% CI = 0.37–0.80; $P = 0.0025$). Lack of an association among HIS participants between *SORCS2* rs73208473 and ATV in plasma (fold effect: 1.02; 95% CI = 0.52–1.99; $P = 0.95$) is comparable to our findings in hair (fold effect: 0.91; 95% CI = 0.34–2.37; $P = 0.84$). However, the plasma findings for WT

participants (1.91; 95% CI = 0.89–4.09; $P = 0.15$) are different from our findings in hair for this variant (fold effect: 0.34; 95% CI = 0.24–0.51; $P = 3.09 \times 10^{-6}$).

Notably, with the exception of our *SORCS2* findings, all other genetic variants reported in previous ATV pharmacogenetic studies for *ABCB1*,[2–4] *NR1I2*,[5,6] and *CYP3A5*[7,8] to impact oral clearance or plasma concentrations did not reach statistical significance for their association with ATV exposure in plasma, but CIs were too wide to rule out the possibility of an effect. We observed similar trends as in prior studies[7,8] where CYP3A5 expressers (i.e., *1/*1, *1/*3, *1/*6, and *1/*7) had an increase in oral clearance of ATV compared with nonexpressers (i.e., *3/*6, *3/*7, *6/*6, and *7/*7), and the difference was more pronounced in WT participants (fold effect change in oral clearance for WT: 1.39; 95% CI = 0.38–5.1), AA participants 1.02 (95% CI = 0.73–1.44), and HIS participants: 0.92 (95% CI = 0.40–2.12) but the results did not reach statistical significance. In addition to demographic and clinical differences between participants of published studies, our study used dose-adjusted AUC as the primary outcome for ATV exposure (oral clearance,[6,7] steady-state concentration,[4] or trough concentration[2,5,8]) adjusted for novel predictors (e.g., consumption of orange juice and diarrhea).

Among the nongenetic predictors, the effects of concurrent ritonavir use and diarrhea on higher and lower plasma exposure of ATV, respectively, were as expected and intuitive. Orange juice consumption has previously been associated with increased efavirenz[9] or sildenafil[10] exposures, and its consumption was also associated with increased ATV exposure, presumably due to inhibition of intestinal first pass (inhibition of intestinal CYP3A enzymes) or pH effects. The mechanism for association of heroin with increased plasma concentration of ATV remains unknown. Heroin use and plasma ATV exposure were inversely associated in this study compared with previous results from hair[1] (fold effect: 3.0 in plasma vs. 0.23 in hair). Opiates are not known to affect any metabolizing enzymes or drug transporters in general or those relevant to ATV kinetics, specifically. Rather than a direct kinetic effect, nonphysiological factors are likely to confound the relationship between heroin use and ATV exposure, such as heroin effect on behavior, and socioeconomic or demographic factors.

There are potential limitations that need to be acknowledged. First, given that the observed associations are novel, they should be treated as preliminary until replicated in another independent study. Second, our results are based on a small sample, which is particularly true for the smaller ethnic strata in our analysis. All of the power from this analysis is derived from the individuals of African ancestry. Third, the analysis depends on accurate self-reporting of participants on factors such as diet, use of illicit drugs, adherence, other prescribed and nonprescribed medications, alcohol consumption, and diarrhea, which are subject to recall bias.

In summary, this is the first iPK analysis of ATV exposure in plasma of women who are representative of HIV patients in the United States. The observation of an *SORCS2* effect on hair concentration of ATV and now in plasma in the AA population is exciting given the plausible mechanistic basis for this effect, which warrants evaluation. To better understand the relationship between plasma and hair exposure for ATV, a population PK model is

necessary. Although specific genetic polymorphisms hitherto reported to impact ATV PKs exist, the effect of these polymorphisms on ATV exposure in our population did not result in a statistically significant effect.

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Table 1

Nongenetic predictors associated with atazanavir concentration in plasma

Predictor	Total (*N* = 123)		AA (*N* = 69)		WT (*N* = 14)		HIS (*N* = 14)	
	Estimated fold effect (95% CI)	*P* value[a]	Estimated fold effect (95% CI)	*P* value[a]	Estimated fold effect (95% CI)	*P* value[a]	Estimated fold effect (95% CI)	*P* value[a]
Ritonavir	2.29 (1.73–3.03)	5.4×10^{-8}	2.36 (1.53–3.63)	0.0002	1.84 (0.67–5.04)	0.26	2.33 (1.32–4.10)	0.01
Diarrheaa	0.64 (0.47–0.88)	0.0058	0.64 (0.43–0.96)	0.034	0.50 (0.12–2.02)	0.35	0.24 (0.09–0.67)	0.02
Heroin use	3.08 (1.38–6.87)	0.0070	3.13 (1.32–7.45)	0.012	—	—	—	—
Orange juice consumption (past 5 days)	1.37 (1.10–1.70)	0.0055	1.30 (0.97–1.76)	0.089	0.98 (0.45–2.16)	0.97	1.65 (0.89–3.07)	0.14

Additional covariates evaluated that were not significantly associated with atazanavir (ATV) concentration in plasma: age, alanine aminotransferase, aspartate aminotransferase, body mass index, creatinine clearance, dietary fat content, and drug interaction variables (i.e., medications that decrease ATV exposure, ATV and antacid use, and ATV and ritonavir), estimated glomerular filtration rate, gamma-glutamyl transferase, grapefruit juice consumption in the past 5 days, hepatitis B virus antigen positive, hepatitis C virus, RNA positive, self-reported ethnicity, self-reported adherence, smoking status, substance use (i.e., crack, cocaine, alcohol use), and total bilirubin.

AA, African American; CI, confidence interval; HIS, Hispanic; WT, White.

[a]Diarrhea is defined as having three or more soft stools per day in the 30 days prior to intensive pharmacokinetic study.

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Table 2

Results from candidate gene analysis for association with ATV plasma levels

Gene	rsID	Genotyped or imputed	Chr:Position[a]	A1/A2 allele	MAF	Ethnic group (*n*)	Fold difference (95% CI)	*P* [b]
SORCS2	rs73208473	Imputed	4:7324596	A/C	0.09	AA (66)	0.54 (0.38–0.78)	**0.0015**
					0.36	WT (14)	1.91 (0.89–4.09)	0.15
					0.14	HIS (14)	1.02 (0.52–1.99)	0.95
						Meta-analysis	0.96 (0.92–1.00)	0.91
	rs73208470	Genotyped	4:7323913	G/C	0.14	AA (66)	0.73 (0.55–0.96)	**0.028**
					0.36	WT (14)	1.91 (0.89–4.09)	0.15
					0.18	HIS (14)	0.95 (0.46–1.95)	0.89
						Meta-analysis	1.01 (1.0–1.02)	0.98
CYP3A5	rs776746	Genotyped	7:99672916	T/C	0.75	AA (66)	0.79 (0.63–0.99)	**0.049**
					0.1	WT (14)	0.90 (0.43–1.90)	0.79
					0.32	HIS (14)	0.88 (0.19–4)	0.87
						Meta-analysis	0.8 (0.63–1.02)	**0.045**
	rs10264272	Genotyped	7:99665212	T/C	0.16	AA (66)	0.89 (0.68–1.17)	0.42
					0	WT (14)	NA	NA
					0	HIS (14)	NA	NA
						Meta-analysis	NA	NA
	rs41303343	Imputed	7:99652770	-/A	0.09	AA (66)	0.87 (0.61–1.24)	0.45
					0	WT (14)	NA	NA
					0	HIS (14)	NA	NA
						Meta-analysis	NA	NA
NR1I2	rs2472677	Imputed	3:11979957	T/C	0.47	AA (66)	1.05 (0.86–1.29)	0.62
					0.5	WT (14)	0.68 (0.29–1.57)	0.40
					0.5	HIS (14)	1.02 (0.58–1.79)	0.95
						Meta-analysis	1.03 (0.64–1.64)	0.78
ABCB1	rs1045642	Genotyped	7:87509329	A/G	0.18	AA (66)	1.11 (0.86–1.43)	0.41
					0.39	WT (14)	0.79 (0.38–1.64)	0.55

Gene	rsID	Genotyped or imputed	Chr:Position[a]	A1/A2 allele	MAF	Ethnic group (*n*)	Fold difference (95% CI)	*P* [b]
					0.32	HIS (14)	0.57 (0.36–0.89)	**0.049**
						Meta-analysis	0.82 (0.23–2.96)	0.40
	rs2032582	Genotyped	7:87531302	A/C	0.04	AA (66)	1.49 (0.90–2.47)	0.13
					0.25	WT (14)	1.31 (0.48–3.58)	0.61
					0.18	HIS (14)	0.51 (0.31–0.82)	**0.030**
						Meta-analysis	0.96 (0.85–1.09)	0.92

A1/A2, minor/major alleles; AA, African American; ATV, atazanavir; Chr, chromosome; CI, confidence interval; HIS, Hispanic; MAF, minor allele frequency; NA, not available because minor allele not detected; WT, white.

[a] Position based on GRCh37/hg19 assembly.

[b] The *a priori* threshold for statistical significance is 0.008. *P* values < 0.05 are bolded.