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CYP1A1 GENOTYPE MODIFIES THE IMPACT OF SMOKING ON EFFECTIVENESS OF HAART AMONG WOMEN

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Abstract

We have recently shown that cigarette smoking is associated with lesser responses to potent antiretroviral therapies. Certain Cytochrome P-450 enzymes activate compounds derived from tobacco smoke into toxic forms that may promote HIV-1 gene expression through promotion of DNA-adduct formation by the oxidation of chemical constituents of cigarette smoke, such as polycyclic aromatic hydrocarbons and dioxins. To explore the association between environmental and genetic factors to viral replication in women who smoke and receive highly active anti-retroviral therapy (HAART), we assessed the impact of polymorphisms in a panel of four Cytochrome P-450 genes (CYP1A1, CYP2A6, CYP2D6, and CYP2E1) and two Glutathione S-transferase genes (GSTM1 and GSTT1) in 924 participants of the Women's Interagency HIV Study (WIHS). Our findings showed that GSTM1 and GSTT1 deletions were not associated with HAART effectiveness. By contrast, homozygosity for the CYP1A1-m1 polymorphism, was associated with impaired viral response to treatment among smokers (relative hazard (RH) = 0.54; 95% confidence interval = 0.31-0.94) after adjustment for pretreatment viral load, CD4 count, age, hepatitis C infection, prior HAART therapy and race, although it had no effect among nonsmokers. We conclude that the

association of the CYP1A1-m1 variant with a reduced response to HAART therapy in HIV infected smokers is consistent with this enzyme's role in the metabolic conversion of environmental toxins to DNA adducts, which may directly promote HIV-1 gene expression.

We have reported that cigarette smoking may impede optimal responses to highly active antiretroviral therapies (HAARTs) (Feldman et al., 2006). In the United States, HIV-positive women tend to be from lower socioeconomic strata in which smoking is common and where its adverse effects add to those of HIV disease and poverty (Sepowitz, 2001). The reduced benefit from HAART among women smokers could be caused by either an effect of smoking or increased high risk behavior, or lack of health seeking behavior among smokers.

In our previous work, self-reported adherence to HAART was significantly lower among smokers than nonsmokers with 30% of smokers reporting taking their medications all the time compared with 43% of nonsmokers ($p = .001$). However, even after adjusting for adherence levels, poorer responses to HAART were still more frequent among smokers indicating that smoking may have effects on treatment outcome that are independent of adherence.

To investigate the potential biological effect of smoking on the effectiveness of HAART therapy we determined the prevalence of polymorphisms in Cytochrome P-450 and Glutathione S transferase genes (CYP1A1, CYP2A6, CYP2D6, CYP2E1, GSTT1, and GSTM1) because these molecules are involved in the metabolism of cigarette smoke constituents (Sellers, 1998). CYP2A6 is responsible for inactivating Nicotine. CYP2A6, as well as CYP2D6 and CYP2E1, metabolize certain drugs and they also transform smoke derived compounds into DNA-damaging agents. The CYP2A6*2 and CYP2D6*4 alleles are relatively common inactivating polymorphisms that were assessed. For the CYP2E1 gene, the c2 polymorphism was assessed, which is upstream from the coding region and it increases gene transcription.

The CYP1A1 gene product is known to convert polycyclic aromatic hydrocarbons (PAHs) and aromatic amines to more toxic compounds in smokers (4). More specifically, CYP1A1 catalyzes the formation of epoxy and diol modifications of PAHs to form products, such as benzo[a]pyrene. One common CYP1A1 gene variant, designated as "m1", corresponds to a single nucleotide polymorphism (T to C transition) located 450 bases downstream from the coding region and it is a MspI restriction fragment length polymorphism (RFLP) site. The CYP1A1-m1 variant has increased catalytic activity and it has been associated with elevated benzo[a]pyrene-derived DNA adduct formation in leukocytes. The underlying mechanism of this effect is uncertain, although it has been postulated that the m1 polymorphism sequence change might enhance stability of the CYP1A1 RNA transcript. Another mechanism may be due to linkage with the m2 polymorphism, which results in an Isoleucine to Valine substitution at codon 462, in exon 7. This results in a structural change to the heme-binding domain (Hamada et al., 1995; Landi et al., 1994; Mooney et al., 1997). The m4 polymorphism (Thr461Asp) occurs in the same region of the CYP1A1 protein.

The enzymes produced by the GSTM1 and GSTT1 genes are responsible for the metabolism of the epoxide and diol modified PAHs. Inactivating deletions of the GSTM1 and GSTT1 are common variants; homozygotes for these deletions would be expected to have increased exposure to PAHs from tobacco smoke (Sobti, Sharma, Joshi, Jindal, & Janmeja).

The PAHs have been implicated in the formation of DNA adducts, which may stimulate HIV replication. Yao and colleagues have shown that two PAHs, benzo[a]pyrene and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) significantly activate the expression of genes linked to the long terminal repeat (LTR) sequences of HIV-1 and they postulated the formation of PAH derived DNA adducts, which may alter transcription factor binding site sequences that stimulate HIV replication (Yao, Hoffer, Chang, & Puga, 1995). In this study we investigated

the relationship of several polymorphisms in genes for metabolic enzymes, smoking and the outcomes of HAART among women.

METHODS

STUDY DESIGN

The Women's Interagency HIV Study (WIHS) is a multisite longitudinal cohort study of HIV infection among women enrolled between 1994 and 1995 at six sites in the United States (Bronx/Manhattan, NY; Brooklyn, NY; Washington, DC; San Francisco Bay Area, CA; Los Angeles/Southern California/Hawaii and Chicago). Details of study objectives, design and methodology have been published previously (Barkan et al., 1998). The study was designed to investigate virologic, immunologic, clinical and behavioral factors associated with disease progression, and to assess the effects of treatment on the clinical course of HIV disease.

Study participants were screened and enrolled if they were at least 13 years of age, agreed to be tested for HIV, could complete assessments in either English or Spanish, and were able to travel to and from the study site. During their initial visit, participants were required to complete a series of structured core assessments on sociodemographic characteristics, mental and physical health history, alcohol and drug use, sexual behavior, medication, and health care utilization. A physical exam and routine laboratory tests were also required. Follow-up visits were scheduled semiannually and included similar assessment tools, repeat physical exams, and additional laboratory tests. All participants were offered remuneration for baseline and follow-up interviews consistent with local institutional review board and community advisory board guidelines. Written informed consent was obtained at the time of initial WIHS enrollment. After five years, approximately 80% of the participants remained in the study (Hessol et al., 2001).

During each subsequent study visit, HAART compliance was reassessed. Definitions of HAART and usage patterns in the WIHS have been described previously (Cook et al., 2002; Kirstein et al., 2002; Wilson et al., 2002).

LABORATORY METHODS

Quantification of HIV-1 RNA viral load in plasma was measured using the iso-thermal nucleic acid sequence based amplification (Nuclisens) method (bioMérieux, Boxtel, NL) and was restricted to laboratories participating in the National Institutes of Health/National Institute of Allergy and Infectious Disease (NIH/NIAID) Virology Quality Assurance Laboratory proficiency testing program. Viral load levels less than 80 copies (cps)/ml were reported as undetectable. Lymphocyte subsets were quantified using standard flow cytometric methods, and again, restricted to laboratories participating in the NIH/NIAID Flow Cytometry Quality Assessment Program (Calvelli, Denny, Paxton, Gelman, & Kagan, 1993).

HOST DNA EXTRACTION

Genomic DNA was isolated from peripheral blood leukocytes using the Pure-gene DNA Isolation Kit (Gentra Systems., Minneapolis, MN). The DNA concentration of all of the samples was quantitated using the PicoGreen dsDNA quantitation kit (Molecular Probes, Eugene, OR) in a microplate format using a Perkin Elmer HTS7000 BioAssay Reader. The samples were stored at -20 C.

DETECTION OF *GSTT1* AND *GSTM1* DELETIONS

To detect the null alleles for the *GSTT1* and *GSTM1* genes, a modified multiplex PCR method was used as described by Teixeira et al. (2002). Amplification of the *GSTT1* and *GSTM1* genes produced 459 bp and 219 bp fragments, respectively, whereas the null alleles do not amplify

and thus are not detected. A 268 bp albumin DNA fragment was also amplified in the multiplex assay as a control.

DETECTION OF CYP1A1-M1, M2, M3, AND M4 VARIANT ALLELES

The DNA samples were screened for the presence of the polymorphisms that characterize the CYP1A1-m1, m2, m3 and m4 variant alleles by PCR-restriction fragment length polymorphism (PCR-RFLP) analysis as described by Li et al. (2004) with some modifications. The National Center for Biotechnology Information reference SNP identification numbers are: CYP1A1-m1, rs4646903; CYP1A1-m2, rs1048943; CYP1A1-m3, rs1800031; CYP1A1-m4, rs1799814. The following primer sequences were used: For *CYP1A1-m1*, forward 5' ACTCACCTGAACCCCATTC -3' and reverse 5'- GGCCCCAACTACTCAGAGGCT-3', which produced a 240 bp product. For *CYP1A1-m3*, forward 5' GGCTGAGCAATCTGACCCTA -3' and reverse 5'-AGTCCTGGTGCCTGGATATG -3', which produced a 198 bp product.

For CYP1A1-m2 and m4, forward 5' CTGTCTCCCTCTGGTTACAGGAAGC -3' and reverse 5'-TTCCACCCGTTGCAGCAGGATAGCC -3', which produced a 204 bp product that included both the m2 and m4 variants.

PCR was performed in a final volume of 20 ul, consisting of DNA (20 ng) dNTP (0.2 mM each) (Invitrogen, Carlsbad, CA) MgCl₂ (2.5 mM), 1uM each primer (Invitrogen, Carlsbad, CA), AmplitaqGold polymerase (1.25 U) (Applied Biosystems, Foster City, CA), and 1× reaction buffer. Amplification was performed with an initial denaturation at 94°C for 10 minutes, followed by 30 cycles of amplification were performed at 94°C for 30 seconds, 60°C for 1 minute and 72°C for 1 minute 30 seconds, and a final extension at 72°C for 5 minutes, using a GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, CA).

The restriction enzyme MSPI (New England BioLabs, Beverly, MA) was used to distinguish the CYP1A1-m1 and m3 variants. Both polymorphisms of the m1 and m3 variants contain MSPI restriction sites resulting in two fragments each (194 and 46 bp for m1 and 98 and 100 bp for m3). The wild-type allele remained as a single band representing the original undigested PCR fragments. The restriction enzymes BsrDI and BsaI (New England BioLabs, Beverly, MA) were used to distinguish the polymorphisms characteristic of the CYP1A1-m2 and m4 variant alleles, respectively, from the same 204 bp PCR fragment. The BsrDI and BsaI digests were performed individually. The variant allele resulted in two fragments each (151 and 53 bp for m2 or 149 and 55 bp for m4), whereas the wild-type allele resulted in a single band representing the original undigested PCR fragment. Differences in RFLP patterns were visualized using an ethidium bromide stained 3% agarose gel.

DETECTION OF THE CYP2E1-C2 VARIANT ALLELE

The DNA samples were screened for the presence of two polymorphisms of the CYP2E1 gene. PCR-RFLP analysis was performed 412 by fragment, as described by Liu, Park, Schantz, Stern, Lazarus (2001). The designation of the genotypes are: c1, RsaI(+)/PstI(-) and c2, RsaI(-)/PstI(+); The PstI and RsaI RFLPs together distinguish the wild-type allele (c1) from a variant allele (c2). The NCBI reference SNP identification numbers are CYP2E1-c1, rs2031920 and CYP2E1-c2, rs3813867.

DETECTION OF THE CYP2A6*2 ALLELE

Pyrosequencing was used to screened for the presence of the CYP2A6*2 allele (Paschke et al., 2001). The assay was made available on the Pyrosequencing AB Web site (www.pyrosequencing.com). The following PCR primer sequences were used: forward 5'

TCTCTCTCTCTACCTCGACATC -3' and reverse 5'-GGCGCCTGCGGGTA-3', which generates a 580 bp product. The reverse primer was biotinylated at the 5' end.

PCR was performed in a final volume of 20 μ l, consisting of DNA (20 ng) dNTP (0.2 mM each) (Invitrogen, Carlsbad, CA) MgCl₂ (1.5 mM), 1 μ M each primer (Invitrogen, Carlsbad, CA), AmplitaqGold polymerase (1.25 U) (Applied Biosystems, Foster City, CA), and 1 $\times$ reaction buffer. Amplification was performed with an initial denaturation at 94°C for 10 min, followed by 40 cycles of amplification were performed at 94°C for 30 seconds, 58°C for 1 min and 72°C for 1 minutes 30 seconds, and a final extension at 72°C for 5 min, using a GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, CA). The pyrosequencing primer sequence used for the CYP2A6*2 variant was 5'-CTTCCTCATCGACGC-3'. The samples were submitted to the Albert Einstein College of Medicine Sequencing Facility where pyrosequencing was performed. The NCBI reference SNP identification number is: CYP2A6*2, rs180127.

DETECTION OF THE CYP2D6*4 ALLELE

The DNA samples were screened for the presence of the CYP2D6 *2 and *4 alleles using Pyrosequencing as per the methods described by Zackrisson and Lindblom (2003). The samples were submitted to the Albert Einstein College of Medicine Sequencing Facility where pyrosequencing was performed. The NCBI reference SNP identification number is CYP2D6*4, rs3892097.

SMOKING VARIABLE

Participants were asked about smoking habits and anyone reporting smoking since the last study visit (i.e., past 6 months) was considered a smoker. A positive response, since the last 6-month visit, to use of either cocaine/crack or heroin was regarded as evidence for current illicit drug use.

INDICES OF HAART EFFECTIVENESS

We estimated the time from HAART initiation (defined as the midpoint between the last visit without HAART and the first visit with HAART use) to both virologic and immunologic response to HAART and to onset of clinical events, including the incidence of AIDS-defining conditions (ADCs) all cause of death and death from AIDS. Additionally, we estimated the time from viral response (the first time HIV-1 RNA dropped to ≤ 80 cps/ml) to subsequent viral failure (the first time HIV-1 RNA increased to $> 1,000$ cps/ml after achieving initial viral response). We also tracked the time from immunologic response (defined as the first time CD4 + lymphocyte counts increased by at least 100 cells/mm³ beyond the preHAART CD4+ nadir levels) to subsequent immunologic failure (defined as the first time CD4+ lymphocyte counts dropped below the preHAART nadir level after attaining immunologic response). Data were censored at the last interview date for those failing to attain the event of interest. All deaths from any cause, including death due to AIDS based on a prior algorithm, were considered events of interest in the survival analyses (Cohen et al., 2002). Data were analyzed on an intent-to-treat basis, ignoring treatment changes and interruptions.

As established by the Centers for Disease Control and Prevention (CDC) in 1993, ADCs (excluding CD4+ lymphocyte counts < 200 cells/mm³) were ascertained through self-report or confirmed through AIDS, cancer, and tuberculosis registries (CDC, 1992). In the models measuring HAART efficacy, any ADC was recorded as incident, except for preexisting diagnoses of cervical cancer, Kaposi's sarcoma, non-Hodgkin's lymphoma, tuberculosis, or wasting syndrome prior to HAART initiation. An ADC reported during the same study visit when HAART was first reported was not classified as incident.

Information regarding deaths was obtained from study participants' friends, relatives, medical providers, and through medical and death registries. Methods for determining cause and date of death have been described previously (CDC, 1992).

STATISTICAL METHODS

Survival time was modeled as a function of single ("fixed") values of smoking and the prevalence of selected polymorphisms and of the other covariates collected at the visit prior to the start of HAART. Each genotype class was tested against a reference wild type. The proportional hazard models estimated the interaction between preHAART smoking status and the prevalence of selected polymorphisms on the outcome variables described earlier, adjusting for age, race, serologic reactivity to hepatitis C antigens at baseline, preHAART CD4+ lymphocyte count, and preHAART viral load. A significant interaction indicated a different outcome of HAART in smokers and nonsmokers. Models stratified by smoking status were estimated for those outcomes for which there were significant interactions between the polymorphism and smoking. Tables show the relative hazards (RHs) of the outcome and its 95% confidence interval (CI). The survival curves displayed for the categories of CYP1A1-m1 were adjusted for the mean values of the other covariates. SPSS software (Version 13.0 Chicago) was used to conduct all study analyses. Trend tests were based on a log linear model.

RESULTS

CIGARETTE SMOKING IN THE WIHS

As previously reported, smoking was very common among WIHS participants. At time of enrollment, 56.7% of the women "currently smoked" and an additional 16% had smoked previously (Feldman et al., 2006). Smokers were relatively consistent in their smoking habits nine years after enrollment. Of the 909 women who reported smoking at baseline or at the visit prior to HAART, 93.2% (466/500) smoked at both times.

HAART USE IN THE WIHS

Of the 2,059 HIV-1 seropositive women enrolled in WIHS, 1,277 (62.0%) initiated HAART between July 1, 1995, and September 30, 2003. For inclusion in this analysis women must have had detectable viral loads and CD4 cell count and anti-retroviral treatment data available from visits prior to and after HAART initiation. In addition, only women with either viral load assays run with a detection threshold of 80 copies/ml or who had detectable viral RNA after initiation of HAART were included. Analysis was restricted to the 961 (88.7%) eligible women, for whom the date of HAART initiation could be estimated accurately to within one year. Of the 961, 924 (96%) had reported their smoking status. The median follow-up after HAART initiation for the 924 women with complete data was 5.2 years (range = 0.2-7.9; Interquartile range = 3.2-6.3) during which time 164 deaths occurred among HAART initiators.

Table 1 shows the demographic characteristics of the 924 women included in this analysis by smoking status. Smokers were disproportionately African American, drug users, hepatitis C infected, and previously diagnosed with AIDS. Nonetheless, smokers had higher CD4 counts than nonsmokers. Table 2 shows the prevalence of the eight studied single nucleotide polymorphisms and the two deletions. The Hardy-Weinberg Equilibrium was checked by racial/ethnic group for each polymorphism and no significant deviations ($p < .05$) were observed. Genotyping concordance between repeated samples was >99%. The CYP1A1-m1 variant was the only polymorphism that had a sufficiently high prevalence of the homozygote type (5.8%) to provide adequate power (80%) to detect an association with smoking, assuming a hazard ratio of 0.75 over the 7.5-year study period and a 15% attrition rate. Table 3 shows the prevalence of the CYP1A1-m1 variant by ethnicity, smoking status, hepatitis C infection and drug use. Prevalence differed only by ethnicity with Caucasians having a much higher

prevalence of the wild type. The next most common variant, *CYP2D6**4 (1.8% homozygous) had a power of only 48% under similar conditions. For heterozygous variants of the *CYP2A6*, *CYP2D6*, and *CYP2E1* genes, no interactions with smoking and HAART were found.

Table 4 shows an interaction between the prevalence of the *CYP1A1*-m1 variants and smoking with the time to HIV-1 virologic response. Homozygotes for the *CYP1A1*-m1 variant who smoked had longer time to virologic response than nonsmokers ($P < .026$). Smokers who were homozygous for the *CYP1A1*-m1 variant were less likely to experience maximal viral suppression while on HAART. For nonsmokers there was no difference in viral response regardless of whether or not they carried the polymorphism. Furthermore, smokers who were heterozygous for *CYP1A1*-m1 were 82% (95% CI 63%-106%) as likely as wild types to experience a viral response, while smokers who were homozygous for *CYP1A1*-m1 were only 54% (31%-94%) as likely to experience a viral response compared with wild type individuals (Table 5).

Figure 1 demonstrates the small difference in time to viral response among non-smoking women irrespective of *CYP1A1*-m1 variant status. Figure 2 shows a clear trend in time to viral response over the 7-year study period in women who smoked. *CYP1A1*-m1 homozygotes had the poorest responses. *CYP1A1* wild type individuals had the best response and *CYP1A1*-m1 heterozygotes had intermediate responses ($P_{\text{trend}} = .01$; RH = 0.78 95% CI .64-.95). Smokers without the *CYP1A1*-m1 variant had a 5-year cumulative probability of no viral response of 33% which was similar to the probability for nonsmokers of 29%. This rate was markedly lower than the probability of 50% for smokers who were homozygous for the *CYP1A1*-m1 variant and it was marginally different from the 37% nonresponse rate of *CYP1A1*-m1 heterozygous smokers.

DISCUSSION

It is likely that the effect of cigarette smoking on HAART responses is a product of both behavioral factors and genetics, which is consistent with our previous observation that smokers had poorer results for six of the seven indices of HAART effectiveness (Feldman et al., 2006). A panel of CYP gene polymorphisms was evaluated to look for associations which might have a pathophysiologic role. There is previous evidence to suggest that smoking decreases the effectiveness of HAART by up regulating HIV replication (Ohata, Tetsuka, Hayashi, Onozaki, & Okamoto, 2003 Yao et al., 1995). Although unlikely, it is also possible that there could be an influence via shared metabolic pathways for degradation of the drugs used in HAART, but our study did not have adequate statistical power to examine such potential pharmacogenetic associations.

We have shown that the *CYP1A1*-m1 variant is associated with impaired viral suppression during HAART with a greater impact in homozygotes than heterozygotes among women who smoke cigarettes, an effect that does not extend to non-smokers. The likelihood of viral response was similar in nonsmoking homozygotes and heterozygotes to that of smokers with the wild type. The absence of association of the *CYP1A1*-m1 variant with the other outcomes of HAART effectiveness may be due to the low prevalence of the *CYP1A1*-m1 variant, which resulted in a relatively small number of homozygotes and the length of time necessary for some of the outcomes to develop. Because all the outcomes are ultimately dependent on viral response, the impaired viral response of smokers who had the *CYP1A1*-m1 variant has important biological and clinical implications. A possible mechanism involves the interaction of specific Cytochrome P-450 catalytic products that may produce DNA adducts and stimulate HIV replication. Further evaluation of molecular pathways related to *CYP1A1* may identify additive genetic associations. In light of our data, it would be intriguing to conduct follow-up

studies to assess the potential impact of the polymorphism within the Aryl-hydrocarbon Receptor gene, which encodes the principle transcriptional activator of the CYP1A1 gene. The Aryl-hydrocarbon Receptor has been implicated as being essential for the stimulation of HIV-1 expression by PAHs (Ohata et al., 2003).

Although the mechanistic pathways have been only partially defined, there is evidence suggesting that Cytochrome P-450 1A1-activated tobacco derived chemicals can promote HIV expression in latently infected cells (Hayashi, Ueno, & Okamoto, 1993; Ohata et al., 2003; Yao et al., 1995). These studies provide a biological basis for at least a moderate prior probability that the association between the *CYP1A1-m1* variant and viral suppression is real. With a prior probability ranging from 0.05 to 0.1, the false positive report probability of the above association ($RH = .75$) would range from 28% to less than 16% (Wacholder, Chanock, Garcia-Glosas, Elghormli, & Rotheman, 2004). This level of false positive report probability suggests that additional studies are needed to verify the association. If our findings are confirmed, there may potentially be a role for the CYP1A1 genotype test in the counseling of HIV patients to assess their smoking risk and treatment needs.

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FIGURE 1. Cumulative Probability of Viral Response Among Non-Smokers by CYP1A1-M1-MSP1 Status for Covariance

FIGURE 2.
Cumulative Probability of Viral Response Among Smokers by CYP1A1-M1-MSP1 Status
Adjusted for Covariates

TABLE 1

Selected Characteristics in WIHS Patients by Smoking Status Prior to HAART

	Smoking Status ^a Prior to HAART		<i>p</i> -value
	Non-Smoker (<i>N</i> = 400) %	Smoker (<i>N</i> = 524) %	
Race			
African-American	36.4	63.6	
Caucasian	48.9	51.1	.001
Hispanic	57.7	42.4	
Current use Crack/Cocaine/Heroin	4	24.4	.001
Hepatitis C infection	25.7	74.3	.001
Risk Category			
IDU	21.5	78.5	
Heterosexual	52.8	47.2	.001
Transfusion	46.2	53.9	
Unknown	58.3	41.7	
Previously diagnosed with AIDS defining condition	37.3	52.1	.001
ART naïve	41.8	58.2	.69
Smoker at baseline	6.8	93.2	.001
CD4+ Median (range)	248(2-1896)	274(2-1909)	.02
Log ₍₁₀₎ Viral load (mean ±se)	4.13(.054)	4.08(.048)	.42

^aSmoking status at the visit prior to HAART was missing for 37 women who are excluded.

TABLE 2
Overall Prevalence of Genetic Polymorphisms in 924 Women in WIHS

Gene Variants Assessed	Wild Type Sequence for Each Polymorphic Region Assessed		Heterozygous for Polymorphism		Homozygous for Polymorphism	
	Number	%	Number	%	Number	%
CYP1A1-m1	523	56.6	285	30.5	54	5.8
CYP1A1-m2	847	91.7	34	3.7	1	0.1
CYP1A1-m3	765	82.8	77	8.3	4	0.4
CYP1A1-m4	773	83.7	92	10.0	12	1.3
CYP2A6*2	859	93.0	23	2.5	—	—
CYP2D6*4	653	70.7	161	17.4	15	1.6
CYP2E1-c2(c1 corre- sponds to the wild type)	801	86.7	43	5.1	2	0.2
GSTM1 deletion	587	66.2	—	—	300	33.8
GSTT1 deletion	725	81.7	—	—	162	18.3

Note. Percents do not total to 100 because some specimens did not amplify. For the GSTM1 and the GSTT1 gene deletions, only homozygous deletions were detected.

TABLE 3
Prevalence of the CYP2A1-m1 Variant by Race, Smoking Status and Drug Use

	Wild Type		Heterozygous for Polymorphism		Homozygous for Polymorphism		p
	#	%	#	%	#	%	
Race/Ethnicity							
African American	307	59.8	176	34.3	30	5.8	
White	114	74	35	22.7	5	3.2	0.001
Hispanic	120	53.8	83	37.2	20	9	
Smoker							
Yes	229	60.8	163	33.1	30	6.1	0.97
No	224	60.5	122	33	24	6.5	
Art naive							
Yes	228	62.1	117	31.9	22	6	0.76
No	296	59.7	169	34.1	31	6.3	
Current use crack, cocaine, heroin							
Yes	78	56.9	51	37.2	8	5.8	0.52
No	465	61.6	243	32.2	47	6.2	

TABLE 4

Interaction of the Homozygous CYP1A1-m1 Variant With Smoking on Outcomes of Highly Active Antiretroviral Therapy

Outcome	Hazard Ratio of Interaction with Smoking	<i>p</i> -Value
Viral response	0.67	.026
Viral rebound	1.10	.54
Immunologic response	1.04	.83
Immunologic failure	0.74	.28
AIDS incidence	1.12	.65
Total death	0.76	.26
Death from AIDS	1.15	.73

TABLE 5
The Association Between the CYP1A1-m1 Variant and Virologic Response Stratified by Smoking and Adjusted for Covariates.

	Non-Smoker				Smoker			
	RH	95% CI	p		RH	95% CI	p	
Age at HAART	1.01	(1.00-1.02)	0.03		1.01	(.99-1.02)	0.11	
Log(10) viral load at visit prior to HAART	0.67	(.58-.77)	0.001		0.72	(.63-.81)	0.001	
CD4 at visit prior to HAART	1	(.99-1.01)	0.21		1	(.99-1.01)	0.83	
Race								
White vs. African American	1.23	(.88-1.73)	0.25		1.35	(.98-1.86)	0.07	
Hispanic vs. African American	1.26	(.94-1.69)	0.12		0.87	(.63-1.19)	0.38	
CYP1A1 m1 Variant								
Heterozygous polymorphism vs. wild type	1.1	(.83-1.46)	0.49		0.82	(.63-1.06)	0.13	
Homozygous polymorphism vs. wild type	0.98	(.58-1.65)	0.94		0.54	(.31-.94)	0.03	
ART naïve (Yes vs. No)	2.23	(1.55-3.19)	0.001		1.94	(1.44-2.63)	0.001	
Hepatitis C infection (Yes vs. No)	0.95	(.71-1.28)	0.72		1.19	(.92-1.52)	0.18	

Note. HAART = highly active antiretroviral therapy; ART = active retroviral therapy.