Perspective

Will Pharmacogenomic Discoveries Improve HIV Therapeutics?

Pharmacogenomic studies are contributing to our understanding of interindividual differences in response to antiretroviral drugs. Genetic polymorphisms in major histocompatibility complex genes predict likelihood of hypersensitivity reactions in persons prescribed abacavir, and perhaps nevirapine. Recent studies have shown that a polymorphism in the CYP2B6 gene is associated with higher plasma efavirenz concentrations and increased efavirenz central nervous system side effects. Polymorphisms in the MDR1 gene encoding the drug pump, P-glycoprotein, may predict nevirapine-associated hepatotoxicity and long-term virologic response to efavirenz. CYP2C19 polymorphisms predict nelfinavir plasma levels and, possibly, risk of virologic failure on this drug. A European mitochondrial haplogroup may predict increased risk of peripheral neuropathy associated with nucleoside reverse transcriptase inhibitors. Expansion and refinement of knowledge regarding associations between human genetics and response to antiretroviral drugs may ultimately permit individualization of therapy based on genotyping. This article summarizes a presentation on HIV therapeutics and pharmacogenomics by David W. Haas, MD, at the International AIDS Society–USA course in Atlanta in March 2005.

Genetic differences among individuals can affect ways in which drugs are metabolized, distributed to cells and tissues, and eliminated from the body, and how the body responds to these drugs. Pharmacogenomic studies involving HIV-infected patients have begun to identify genetic differences and profiles that affect responses to antiretroviral drugs. However, there is much work to be done in this area before application of such knowledge can help to guide therapy. Ongoing studies need to determine the extent to which treatment efficacy and toxicities can be predicted by human genetic differences, and the underlying mechanisms involved. Furthermore, genetic analysis for use in clinical practice is somewhat expensive. The human genome consists of about 3 billion nucleotide base pairs that include approximately 1 single nucleotide polymorphism (SNP; a single nucleotide substitution that has the potential to affect gene expression or protein function) per 1000 base pairs. There are thus about 3 million SNPs in each individual. Currently, at the lowest possible assay cost of $.05 to $.10 per SNP, whole genome SNP scanning would theoretically cost about $150,000 to $300,000 per person. Cost reductions are being realized through advances in technology and also through such strategies as scanning only coding regions of genes, scanning only nonsynonymous SNPs (ie, those that alter the amino acids), and taking advantage of linkage disequilibrium (ie, the tendency of groups of SNPs to be inherited together).

Selected associations between genetic profiles and responses to antiretroviral drugs that have been reported in the literature include those between abacavir hypersensitivity and *HLA-B*5701* and *hsp70-hom* (Mallal et al, *Lancet*, 2002; Martin et al, *Proc Natl Acad Sci,* 2004); indinavir- and atazanavir-associated hyperbilirubinemia and UGT-1A1 (Gilbert's syndrome gene; Zucker et al, *Proc Natl Acad Sci*, 2001 and O'Mara et al, 42nd ICAAC, 2002); nucleoside reverse transcriptase inhibitor (nRTI)-associated lipoatrophy and a TNF-α gene promoter polymorphism (Maher, *AIDS*,

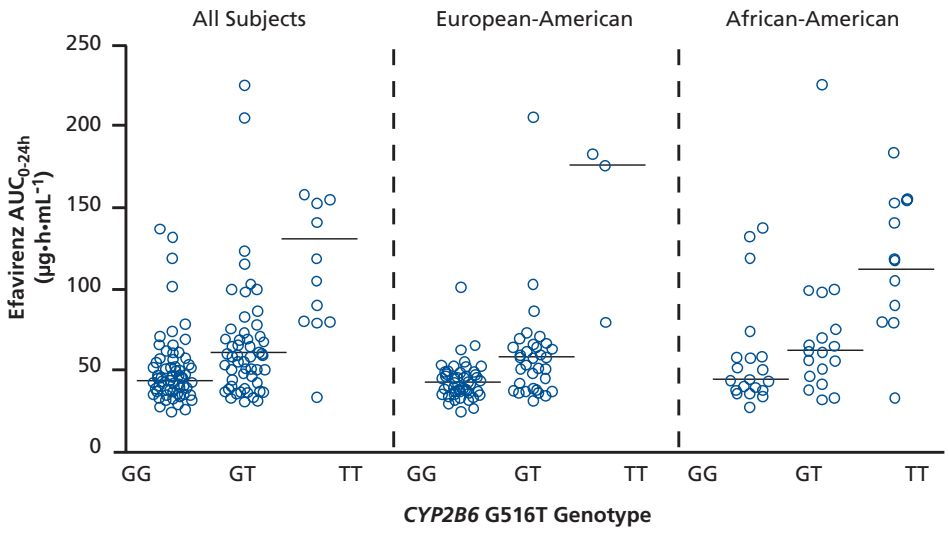

Figure **1.** Relationship between efavirenz area under the concentration-time curve (AUC) and the *CYP2B6* G516T single nucleotide polymorphisms in AIDS Clinical Trials Group A5095 and A5097s study participants. The T allele was associated with significantly greater plasma AUC values in all subjects (left panel), and in European-American (middle panel) and African-American (right panel) populations analyzed separately. Adapted with permission from Haas et al, *AIDS*, 2004.

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2002; Nolan, *AIDS*, 2003); and nevirapine-related rash/fever/hepatitis and *HLA-DRB1*01* (Martin, *AIDS*, 2005). The finding of a correlation between HLA type *HLA-B*5701* and the likelihood of hypersensitivity reaction to abacavir is the best example of the potential utility of pharmacogenomic analysis thus far. Approximately 3% of patients receiving abacavir have a hypersensitivity reaction to the drug, which can be life threatening. Since identifying the very strong association between HLA type and risk for this reaction, Mallal and colleagues routinely assess patients for HLA type prior to using abacavir in treatment, a practice that has markedly decreased abacavir hypersensitivity in their patient population (Mallal, personal communication).

To improve the pharmacogenomic database in HIV therapeutics, the Adult AIDS Clinical Trials Group (ACTG) instituted a program in which patients participating in ACTG clinical trials are offered the opportunity to contribute a DNA specimen to a repository for use in pharmacogenomic studies. Findings from initial studies in this program are summarized below.

Can Genetics Predict Efavirenz Central Nervous System Side Effects and Drug Levels?

Efavirenz is a commonly prescribed drug used in initial therapy for HIV infection in the United States. Central nervous system (CNS) side effects are common during the initial days of efavirenz treatment. Several studies have shown that efavirenz plasma clearance is slower in black patients than in white patients (Barrett et al, *Int J Clin Pharmacol Ther*, 2002; Pfister et al, *Antimicrob Agents Chemother*, 2003). Although one report suggested earlier virologic failure on efavirenz in blacks than in whites (Wegner et al, 9th CROI, 2002), this was not confirmed in a subsequent study (Lupo, 6th Int Congr Drug Ther HIV Infect, 2002). Many drugs are metabolized by cytochrome P450 (CYP450) isoforms, with the greatest proportion of these being metabolized by CYP3A isoenzymes. In contrast, efavirenz is one of a much smaller group of drugs, including the antiretroviral drug nevirapine, that are metabolized primarily via CYP2B6. An initial study examining the potential influence of genetic factors in CNS toxicity and drug clearance of efavirenz therefore included analysis of polymorphisms in *CYP2B6* and several other candidate genes.

This analysis involved antiretroviral-naive patients who had been randomized to receive efavirenz in ACTG study A5095 and its substudy A5097s. In study A5095, patients had received efavirenz 600 mg once daily, abacavir, or both, in combination with zidovudine and lamivudine. The efavirenz and abacavir were double-blinded. The A5097s substudy focused on characterizing efavirenz CNS adverse effects and pharmacokinetics, and 202 of the 303 subjects were randomized to efavirenz. Data from the pharmacokinetics study showed that efavirenz plasma levels were greater in both Hispanic patients and black patients than in white patients, although there were relatively few Hispanics in this study (Ribaudo et al, 11th CROI, 2004). The median values for area under the concentration-time curve from 0 to 24 hours ($AUC_{0\text{-}24}$) were 66, 58, and 46 µg•hr/mL, respectively ($P \geq .001$ for all), although there was considerable overlap in pharmacokinetic parameters among the groups.

A *CYP2B6* G-to-T polymorphism at position 516 was strongly associated with efavirenz plasma levels. As shown in Figure 1, the TT genotype predicted greater efavirenz AUC values among all subjects, as well as in white patients and in black patients separately (Haas et al, *AIDS*, 2004). This polymorphism was significantly more frequent in black patients (20% TT homozygotes) than in white patients (3% TT homozygotes), which may explain the finding of higher plasma efavirenz concentrations in the black patient population. Other genetic analysis from this study suggested that *CYP3A4* and *CYP3A5* SNPs may also be weakly associated with efavirenz levels. This association between *CYP2B6* G516T and plasma efavirenz exposure was subsequently validated in a larger group of patients

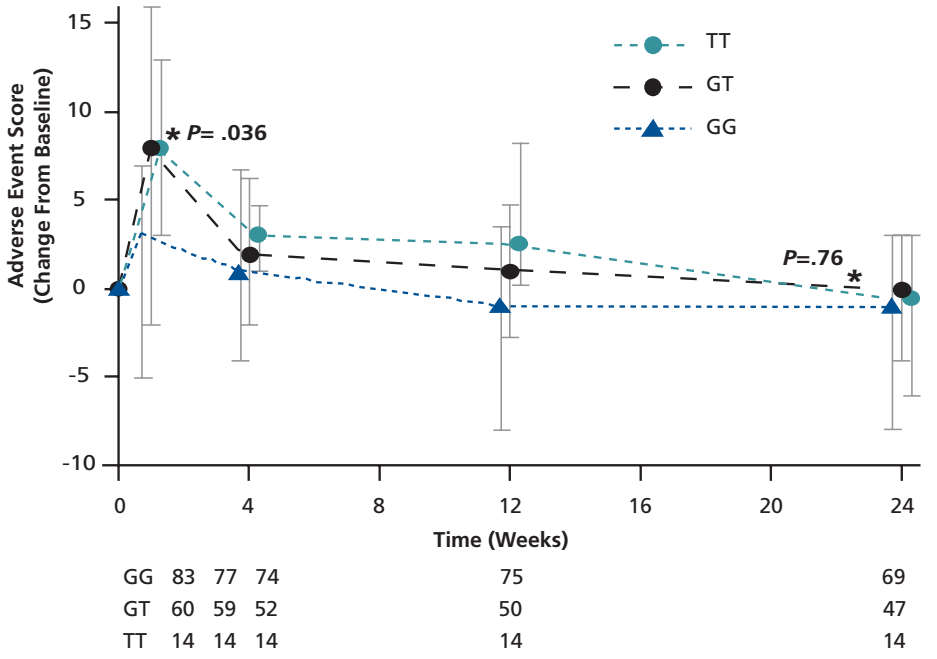


Figure **2.** Relationship between *CYP2B6* position 516 genotypes and risk of central nervous system adverse experiences in the AIDS Clinical Trials Group A5097s study population. Adapted with permission from Haas et al, *AIDS*, 2004.

from ACTG study 384, including in Hispanic, black, and white patients analyzed separately (Haas et al, 12th CROI, 2005).

Careful assessment of CNS side effects by questionnaires in study A5097s showed that the T allele at *CYP2B6* position 516 was associated with significantly greater adverse CNS experiences during the first week of efavirenz therapy, although this difference disappeared at later time points (Figure 2; Haas et al, *AIDS*, 2004). Thus the *CYP2B6* T516T variant, which is more common in black than in white individuals, is associated with higher efavirenz plasma levels and greater frequency of CNS adverse events. It must be emphasized that black patients did not have more CNS side effects in the ACTG substudy.

Can Genetics Predict Risk for Efavirenz Resistance During Treatment Interruption?

Efavirenz has a relatively long half-life and a low genetic barrier to viral drug resistance. These factors have raised concern that when a regimen containing efavirenz is completely interrupted, the persistence of the drug beyond clearance of the other drugs in the regimen may pose the risk of developing efavirenz resistance—that is, through exposure of virus to what is essentially efavirenz monotherapy. Researchers thus used modeled data from the study described above to predict how long efavirenz concentrations would persist above the protein-adjusted 95% inhibitory concentration (IC_{95}) according to *CYP2B6* genotype (Haas et al, 12th CROI, 2005). Greater time above the inhibitory level during drug clearance in the absence of the viral suppression provided by antiretroviral therapy is likely to increase the risk for emergence of efavirenz-resistant HIV variants. Elevated efavirenz concentrations were predicted to persist for prolonged durations in most patients with the TT genotype and to remain above the IC_{95} for at least 21 days in half of these patients (Figure 3). Studies examining actual levels of efavirenz according to genotype in patients stopping therapy will need to be determined to confirm this prediction.

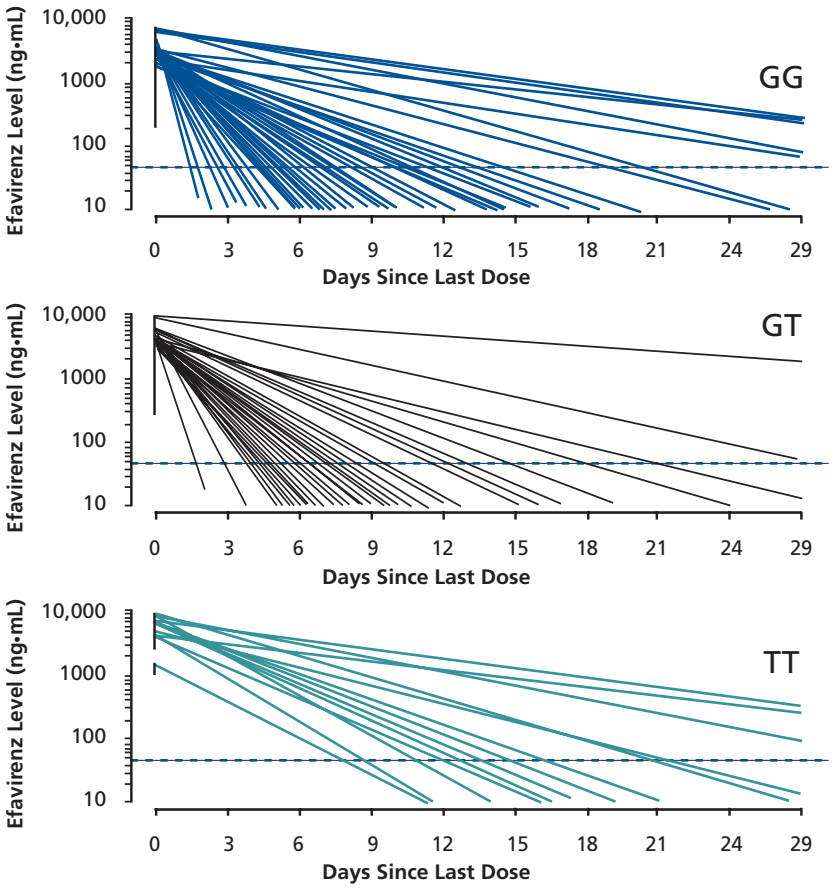


Figure **3.** Predicted plasma efavirenz levels according to the *CYP2B6* position 516 polymorphism (GG [top], GT [middle], or TT [bottom]) in the AIDS Clinical Trials Group A5097s study population. Dashed horizontal lines indicate the protein-adjusted efavirenz 95% inhibitory concentration. Adapted with permission from Haas et al, 12th CROI, 2005.

Can Genetics Predict Liver Toxicity Associated With Nevirapine?

Nevirapine can cause hepatotoxicity. As noted above, a recent report suggested an association between *HLA-DRB1*01* and nevirapine-associated hypersensitivity, which included hepatitis, in many patients (Martin, *AIDS*, 2005). Additional studies were conducted to assess the potential role of drug metabolism and transporter genes. As with efavirenz, nevirapine is primarily metabolized by CYP2B6; it has not been recognized as a substrate for P-glycoprotein. In a study performed in South Africa (FTC-302), grade 3 or 4 liver enzyme elevations occurred in 17% of nevirapine recipients. Researchers thus explored the potential association of *MDR1*, *CYP2B6*, *CYP3A4*, and *CYP3A5* SNPs and nevirapine hepatotoxicity in a case-control study using data and specimens from this study (Haas et al, 12th CROI, 2005). Cases and controls were matched for age, race, sex, pretreatment CD4+ cell count, and pretreatment plasma viral load. Patients with the *MDR1* position 3435 CT or TT genotypes were significantly less likely to have liver toxicity than those with the CC genotype, suggesting a potential protective effect of the T allele at this position.

The researchers examined this potential association with nonnucleoside reverse transcriptase inhibitor (NNRTI) hepatotoxicity in their own clinical population at Vanderbilt University and observed a similar pro-

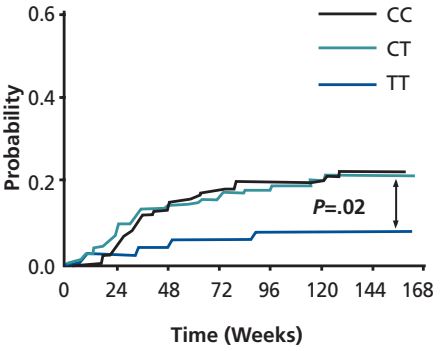


Figure **4.** Effect of *MDR1* single nucleotide polymorphism at position 3435 on the time to efavirenz virologic failure in patients in the ACTG 384 study. Adapted with permission from Haas et al, 12th CROI, 2005.

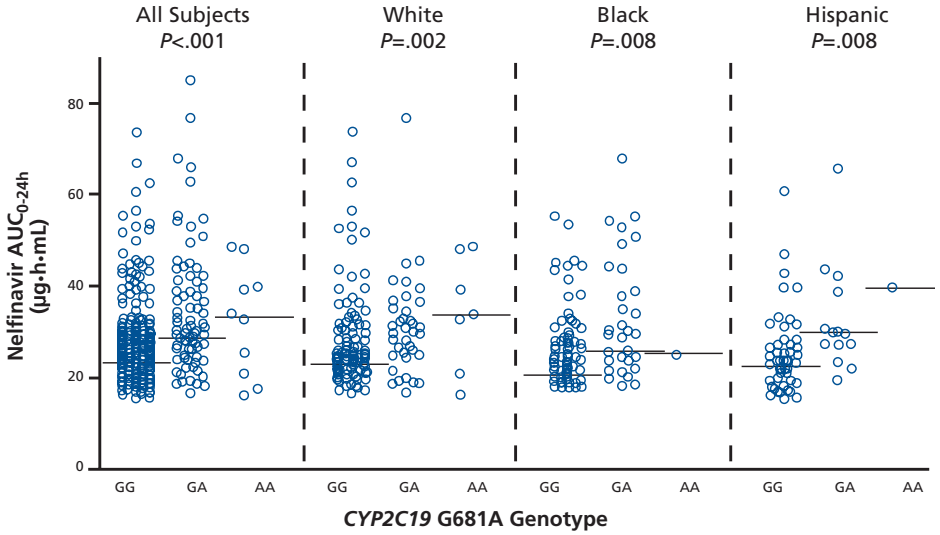
tective effect of the T allele. As noted, the P-glycoprotein pump encoded by *MDR1* is found at many sites in the body, including the brain, where it functions to keep drugs from crossing the blood-brain barrier; it is also present in the liver. However, nevirapine is known to achieve high concentrations in the brain and the placenta and thus had not been thought to be a substrate for P-glycoprotein. Cell culture studies have since shown that P-glycoprotein does in fact pump nevirapine out of cells, albeit in a less efficient manner than is observed with other drugs (eg, protease inhibitors, digoxin) known to be P-glycoprotein substrates. These findings suggest that SNPs in the *MDR1* gene may indeed affect the risk for nevirapine liver toxicity and suggest a possible mechanism.

Can Genetics Predict Long-term Response to Efavirenz?

Haas and colleagues subsequently examined the potential genetic correlates of long-term response to efavirenz in patients enrolled in ACTG study 384 (Haas et al, 12th CROI, 2005). In this study, treatment-naive patients were randomized to receive either efavirenz 600 mg once daily, nelfinavir 1250 mg twice daily, or both, in combination with either zidovudine/lamivudine or didanosine/stavudine. The efavirenz and nelfinavir were double-blinded. Data from patients followed up for as long as 3 years were available from this study. Somewhat surprisingly, no associations were observed between *CYP2B6* SNPs and long-term responses to efavirenz. In contrast, as shown in Figure 4, *MDR1* position 3435 TT genotype was associated with significantly less virologic failure on efavirenz. No differences among genotypes were observed for treatment failure due to toxicity or all-cause treatment failure. The *MDR1* T allele at position 3435 was also a significant predictor of decreased likelihood of virologic failure with emergence of efavirenz resistance ($P = 0.05$), with an odds ratio (OR) of 0.6 per T allele, whereas age, race, sex, baseline CD4+ cell count, and baseline plasma viral load were not significant predictors. This provocative finding needs to be validated in other studies.

Can Genetics Predict Nelfinavir Drug Levels and Treatment Response?

Nelfinavir is metabolized via CYP2C19, and it is known that genetic variants of the *CYP2C19* gene can result in a poor drug metabolism phenotype. This is most frequent among Asian populations. Analysis of nelfinavir levels among participants from ACTG 384 showed that those with GA or AA genotypes at *CYP2C19* position 681 had higher AUC values than GG homozygotes (Figure 5, left; Haas et al, 12th CROI, 2005). There was also a trend toward more favorable virologic responses on nelfinavir in subjects with the GA genotype (Figure 5, right).



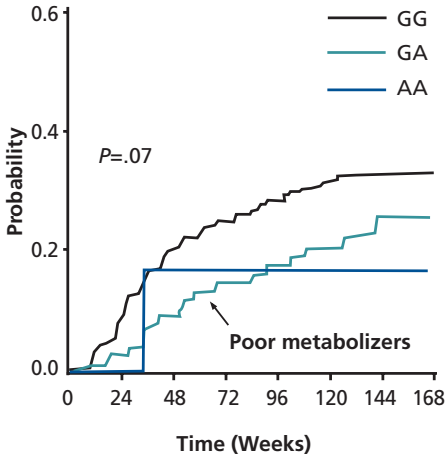


Figure **5.** Left: nelfinavir area under the concentration-time curve (AUC) in racial/ethnic groups by *CYP2C19* position 681 genotype in patients from the AIDS Clinical Trials Group 384 study. The A allele was associated with significantly greater plasma AUC values in all subjects, and in white, black, and Hispanic populations analyzed separately. Right: Effect of genotype on time to nelfinavir virologic failure. Adapted with permission from Haas et al, 12th CROI, 2005.

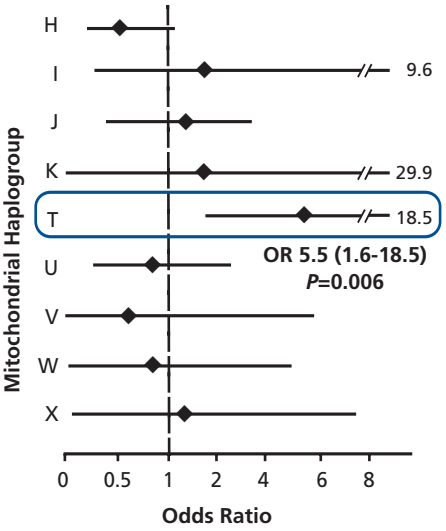


Figure **6.** Odds ratio (OR) for peripheral neuropathy according to mitochondrial haplogroup in white patients randomized to receive didanosine/stavudine in the AIDS Clinical Trials Group 384 study. Adapted with permission from Hulgan et al, 12th CROI, 2005.

Can Genetics Predict nRTI-Associated Peripheral Neuropathy?

In the ACTG 384 study, peripheral neuropathy was much more common among patients randomized to receive didanosine/stavudine than among those who received zidovudine/lamivudine. Peripheral neuropathy associated with nRTIs is the result of toxicity to mitochondria. Mitochondrial DNA from different individuals can be classified according to distinct haplogroups, which are specific heritable patterns of polymorphisms in the mitochondrial genome. There are 9 mitochondrial haplogroups that have been identified in persons of European ancestry. Analysis of risk for peripheral neuropathy by mitochondrial haplogroup among 137 white (European ancestry) patients in the ACTG 384 study who received didanosine/stavudine showed that patients with haplogroup T, who constituted 9% of study participants of European ancestry, had a significantly increased risk of developing neuropathy (Figure 6; Hulgan et al, 12th CROI, 2005). By multivariate analysis, independent predictors of peripheral neuropathy were randomization to didanosine/stavudine (OR, 2.57; $P = 0.003$), age at randomization (OR, 1.05 per year; $P = 0.005$), and haplogroup T (OR, 2.89; $P = 0.02$).

Conclusion

Associations between human genetics and HIV treatment responses are increasingly being described. Refining and expanding our knowledge of genotypes and their interaction with drug therapies will ultimately enable us to tailor drug treatments to individuals, whether the treatment be antiretroviral, antihypertensive, antidepressant, or otherwise. Since an individual's genetic profile does not change over time, genetic testing performed at one time point may help inform both current and future choices of pharmacologic agents for the individual. On a global level, the pharmacogenomic knowledge base is likely to be of considerable importance in helping to anticipate complications of antiretroviral drug treatment in ethnic populations that may not have been well represented in drug registration trials.

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