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Pharmacogenomics of *CYP3A*: considerations for HIV treatment

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Abstract

The understanding of the cytochrome P450 3A SNP in antiretroviral therapy is important, because it is highly inducible, extremely polymorphic and metabolizes many of the drugs that are key components of highly active antiretroviral therapy regimens. This enzyme is prolific and promiscuous towards drug and xenobiotic substrate selection and it is also unpredictable among individuals, having a 5- to 20-fold variability in its ability to contribute to drug clearance. The importance of human *CYP3A* pharmacogenetics is also gaining attention in other established areas of pharmacotherapy as it may contribute to the goal of predicting efficacy and/or toxicity, specifically with the discovery of null allele *CYP3A4*20*. This review summarizes the current understanding, implications of genetic variation in the *CYP3A* enzymes, the central role of *CYP3A* in linking human genetics, the pharmacokinetics and resulting pharmacodynamic responses to certain antiretroviral drugs, and their eventual place in applied clinical pharmacotherapy.

Keywords

cytochrome P450 3A; drug metabolism; HIV-treatment; protease inhibitors; SNPs

In 1996, the introduction of nonnucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) and their subsequent use in combination regimens with nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) resulted in potent viral suppression as well as a new period of highly active antiretroviral therapy (HAART) for patients with HIV infection. However, the complex pharmacokinetic properties of individual antiretrovirals in HAART regimens also stimulated new research into the gastrointestinal and hepatic drug-metabolizing capacity of the isoforms of cytochrome P450 (CYP) oxidative enzymes and their contribution to drug interactions. In the case of the NNRTIs, nevirapine and efavirenz, their inductive characteristics created concern for the enhanced metabolism of concurrently administered medications, while the use of PIs with their inhibitory effects on *CYP3A*, led to a challenging scenario for clinicians who were aware of the viral suppression potential of HAART but also the unpredictable nature of these drug interactions. The pharmacokinetic variation of NNRTIs and PIs when used in combination regimens led to an increased interest

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in the use of therapeutic drug monitoring (TDM) in an attempt to determine individual patient drug exposure and avoid under- or over-dosing [1,2]. The role of TDM in addressing the interindividual pharmacokinetic variation and complex pharmacologic factors that result from HIV disease, co-morbidities and drug interactions [3] have been suggested as a clinical approach that may allow prescribers to optimize HAART outcomes. TDM has been implemented in certain countries to assist with the individualization of antiretroviral therapy [4–8]. Subsequently, an evolving appreciation of pharmacogenomics and the potential for genetic variation to be a contributing factor to pharmacokinetic variability prompted clinical research to investigate CYP SNPs in HIV-infected patients and to examine their relationship with pharmacokinetics and treatment outcomes. However, the enzymatic activity of *CYP3A* SNPs has only been partially elucidated. Additional research remains to be conducted to define the contribution of CYP activity in the context of genetic variation in cell membrane transporters and to evaluate genetic influences on treatment outcomes. This review provides a comprehensive summary of our knowledge of *in vitro* and clinical pharmacogenomics of *CYP3A* as well as the molecular genomics approaches to investigate the activity of *CYP3A* SNPs.

In addition to influencing the pharmacokinetics of individual NNRTIs and PIs, the impact of drug interactions in HIV-infected individuals continues to be of utmost significance for both the US epidemic and on a global basis [4,9–22]. In both geographic settings, drug interactions contribute to clinical dilemmas making successful antiretroviral therapy, as well as the management of long-term complications, a formidable challenge. Concurrent infection with hepatitis B or C, the recreational use and abuse of addictive drugs, changes in body size owing to disease and poor nutrition as well as altered immunologic status also contributes to individual pharmacokinetic characteristics displayed by NRTIs, NNRTIs, PIs and potential drug interactions [10,23,24]. Therefore, the central role of *CYP3A* in determining NNRTI and PI pharmacokinetics, drug–drug interactions and the contribution of genetic variation is an important consideration for current and future HAART.

CYP3A SNPs

Humans have 57 genes and more than 59 pseudogenes divided among 18 families of CYP genes and 43 subfamilies. The *CYP3A* subfamily is the most abundant of the CYPs [25–27] and consists of four functional members – *CYP3A4*, *CYP3A5*, *CYP3A7* and *CYP3A43* [26, 28]. The corresponding genes for these subfamilies are located on chromosome 7 [29]. *CYP3A4* and *CYP3A5* are most abundant in the liver [28]. In addition, they are also expressed in some other tissues and organs including the gut lumen [27] and kidney [30]. Furthermore, *CYP3A4* and *CYP3A5* account for 40–80% of total CYP in human liver and intestine, respectively [31]. It is also known that *CYP3A4* and *CYP3A5* are major drug-metabolizing enzymes in humans and mediate biotransformation of more than half of the marketed drugs that are eliminated by enzyme-catalyzed processes [32]. *CYP3A* is also a major isoform that contributes to a large first-pass metabolism of *CYP3A* substrates [33]. Thus, *CYP3A4* and *CYP3A5* are the predominant forms of the *CYP3A* subfamily. There is up to a 40-fold interindividual variation, specifically on substrates such as triazolam, midazolam, or ciclosporin, in the hepatic *CYP3A4* level. Elucidation of the determinants of *CYP3A* activity is important because of the interindividual variability present in the activity and its involvement in the metabolism of a large number of drugs [34]. The prevalence of *CYP3A* gene SNPs and contribution to the metabolism of *CYP3A* substrates are summarized below.

CYP3A4

Of the total of 16 5'-UTR mutations found to date, one allelic variant of *CYP3A* has been identified [35–37], *CYP3A4*1B*, involves an A>G transition at -293 position from the transcription start site. It has been hypothesized that individuals with the *CYP3A4*1B* allele

have decreased CYP3A4 protein activity and, thus, decreased 2 β , 6 β - and 15 β -testosterone oxidation [35]. Furthermore, the *CYP3A4*1B* allele is also associated with a decrease in nifedipine activity, possibly owing to disruption of the nifedipine specific element. The *CYP3A4*1B* allelic frequency varies among different ethnic groups: 0% in Chinese and Taiwanese [38,39], 2–9.6% in Caucasians [36,38,40,41], 9.3–11% in Hispanic Americans, and 35–67% in African-Americans [28,42,43]. Other allelic variants among Caucasians reported were *CYP3A4*1C* (1%), *CYP3A4*1D* (1%), *CYP3A4*1E* (<1%), with exception of *CYP3A4*1F* (20%) [36,44] and were in lower frequency. Although there are clinical data that indicate that there is a connection between the *CYP3A4*1B* polymorphism and disease risk/treatment toxicity, however, direct *in vivo* evidence is lacking. This suggests a possible linkage disequilibrium between *CYP3A4*1B* and another genetic mutation that may be the true cause of the clinical phenotype. The possibility of missing polymorphic allele *CYP3A4*1B* is less than 5%, even if the sample size is as small as 10 subjects. The genotypic frequency, 9.6% Caucasians and 6% in Iranians, of this variant (*CYP3A4*1B*) was further confirmed by Hamzeily *et al.* [44]. They also identified three new mutations in the 5' regulatory region. The *CYP3A4*1E* -369 T>A transversion, which was found in one Caucasian subject, might have some impact on enzyme activity owing to its presence near the CAAT box and other regulatory regions. The *CYP3A4*1F* -747 C>G transversion is a very high frequency mutation, with an incidence of 20% in Caucasians and 18% in Iranians, but may not have an effect on gene expression owing to its absence in the vicinity of any transcription regulatory element. In another study of 416 Japanese subjects, the distal enhancer region, promoter region, all exon and surrounding intrinsic regions of *CYP3A4* were sequenced. A total of 24 SNPs were detected including 17 novel ones, two in the distal enhancer region, 4 in the proximal promoter region, one in the 5'-UTR, seven in introns and three in 3'-UTR. These ethnic differences also imply haplotype differences were also evaluated. In addition, a major combination between *CYP3A4*–*CYP3A5* SNPs is *CYP3A4*1A*–*CYP3A5*3* and *CYP3A4*1G*–*CYP3A5*1E*. It has been previously shown that *CYP3A5*3* is a defective allele in the Japanese population [45]. According to the haplotype combination described earlier, *CYP3A4* haplotypes containing allele **1A* are linked to *CYP3A5*3* with a 97% probability. On the other hand, 88% of the *CYP3A4* haplotypes with **1G* are linked to *CYP3A5*1*. In addition, haplotype *CYP3A4*1G* is completely linked with *CYP3A5*1E*, but not **3* [46], suggesting that resulting expression of *CYP3A5* can compensate for decreased *CYP3A4* activity. *CYP3A4* SNPs in the 5'- and 3'-UTR are provided in Table 1.

The *CYP3A4* gene spans 13 exons covering 27 kb sequence [47,48], and encodes 502 amino acids with a molecular weight of 57 kDa. Large ethnic differences have been reported in *CYP3A4*, with a total of 20 unique SNPs discovered so far in the coding region. However, many of the variant alleles have not been verified and validated, and some of them may be sequence artifacts. The incidence of *CYP3A4*2* was 5.26% in Russian–Caucasians and these individuals with *CYP3A4*2/*2* genotype are considered a 'slow metabolizer' [36,49]. Also *CYP3A4*2* allele, which encodes a Ser222Pro change, has an allelic frequency of 2.7% in the white (Finnish) population but was absent in African-Americans and Chinese subjects. The recombinant protein had lower intrinsic clearance for the *CYP3A4* substrate nifedipine compared with the wild-type enzyme, but was not significantly different from the wild-type enzyme for testosterone 6 β -hydroxylation [43]. Since proline is a helix breaker, serine to proline changes can affect the 3D structure of the protein.

In a recent study conducted on the frequency of the *CYP3A4*1B*, **2*, **4*, **5* and **18* alleles among both Tepehuan Amerindians, a native group that has inhabited northern Mexico for thousands of years, and Mestizo Mexicans. The data was compared with those of other populations. Genotyping revealed that 8.8 and 8.0% of the Mestizo and Tepehuano subjects carried the *CYP3A4*1B* allele, respectively. Only one Mestizo subject was heterozygous for the *CYP3A4*2* variant, while *CYP3A4*4*, **5* and **18* allelic variants were not detected in either

group. On the other hand, the frequencies of the *CYP3A4*1B* variant in Mestizos and Tepehuanos were similar to those reported for Caucasians, but different from those observed for African and Asian populations [50].

In another study of 500 white Dutch subjects, the *CYP3A4*3* allele was found at a frequency of 1.1% in Caucasians [51]. The *CYP3A4*3* allele encodes a mutation leading to M445T at the conserved heme-binding region, just two amino acids C-terminal to the conserved Cysteine at position 442. This variant *CYP3A4*3* allele is not rare as was considered earlier, but instead represents a genetic polymorphism that can be found in a substantial part of the population. Furthermore, in another report, two point mutations in *CYP3A4* allele were found; *CYP3A4*4* (I118V) and *CYP3A4*5* (P218R), and an insertion that causes a frameshift mutation (*CYP3A4*6*) that results in stop codon in exon 9 [39]. Eiselt *et al.* identified 18 *CYP3A4* variants, and eight protein variants including *CYP3A4*3* (M445T), *CYP3A4*7* (G56D), *CYP3A4*8* (R130Q), *CYP3A4*9* (V170I), *CYP3A4*10* (D174H), *CYP3A4*11* (T363M), *CYP3A4*12* (L373F) and *CYP3A4*13* (416) [52]. However only *CYP3A4*12* (L373F) exhibits an altered testosterone metabolite profile and a fourfold increase in K_m values for 1-OH midazolam production compared with the wild-type protein. *CYP3A4*11* (T363M) and *CYP3A4*16* (T185S) have reduced *in vitro* catalytic activity for testosterone with lesser protein expression levels [52]. *CYP3A4*15* in which the amino acid R-162 position changed to Q-162 was identified only among African-Americans population with an allelic frequency of 4%. Murayama *et al.* reported that *CYP3A4*16* exhibited a 60% decrease in testosterone 6 β -, 2 β - and 15 β -hydroxylation as compared with the wild-type allele [53]. *CYP3A4*17* and *CYP3A4*18* were found in Caucasians and Asians at an allelic frequency of 2%, respectively. Later, when testosterone and the insecticide chlorpyrifos were used to assess the catalytic activity of wild-type (*CYP3A4*1*) and its variants *3, *15, *17, *18 and *19, *CYP3A4*17* led to lower turnover numbers for testosterone and chlorpyrifos, while *CYP3A4*18* had higher turnover numbers for both substrates. However there was no difference in the turnover number for the *CYP3A4*3* and *CYP3A4*19* with respect to the wild-type protein [28,54]. *CYP3A4*17* allele is present in a ratio of 1:5 among eastern Europeans (Adygei ethnic group). The recombinant *CYP3A4*17* is markedly defective in nifedipine metabolism and exhibits over 99% decrease in the V_{max} and CL_{max} [55].

In a recent report, a novel *CYP3A4* allele, *CYP3A4*20* was identified, which is devoid of any catalytic activity as a result of a premature stop codon yielding a truncated protein [56]. *CYP3A4*20* represents the first *CYP3A4* allele that has no functional activity. The allelic frequency of *CYP3A4*20* allele among white, black and Chinese subjects are 6%, 26% and 22%, respectively. The SNP information in the coding region of *CYP3A4* is tabulated in Table 2. From these data it is reasonable to speculate that SNPs contribute to the variability in the NNRTI and PI pharmacotherapy. However, there remains a paucity of data on the contribution of *CYP3A* SNPs to HIV treatment outcomes and future research investigating the contribution of these SNPs to drug metabolism determine correlations between dosing regimens and outcome according to genotypes.

CYP3A5

The 5'-upstream region of *CYP3A5*1A* was first described by Jounaidi *et al.* [57]. Later, Paulussen *et al.* reported two linked mutations in the promoter, -45A>G and -368T>G, which were associated with the expression and activity of the protein [58]. G-45A occurs in the basic transcription binding (BTE) element of *CYP3A5*. This mutation may disturb Sp1 binding, reducing gene transcription and resulting in low protein expression in the variant allele A-45. Another common SNP, *CYP3A5*1C*, in the promoter -74C>T has been identified in 3–4% in Caucasians and 0–7% in Africans [36,59]. It was also demonstrated that livers obtained from Caucasian and African-Americans that had at least one copy of the *CYP3A5*1A* allele

exhibited a mean midazolam hydroxylation activity that was approximately twofold higher than that observed from the corresponding *CYP3A5**3/*3 allele. This variant is in a strong linkage disequilibrium with *CYP3A5**3. Lee *et al.* identified several SNPs in the 5'-UTR of *CYP3A5* gene [59]. The majority of these SNPs occurred in African-Americans, only one or two SNPs occurred in Asians and Caucasians. The -7995T>A was more common in Asians (17%) than Africans and Caucasians (2% each). A common SNP (*CYP3A5**1D) in the 3'-UTR, that is highly linked to *CYP3A5**3 where 31611C>T (*CYP3A5**1D), has a wide distribution of T allele among different ethnic groups (Caucasians, 86%, Asians, 60% and Africans, 40%) [59]. There were no 5'-UTR SNPs located in any transcription factor elements [36,59].

The first genetic variant reported in the coding region of the *CYP3A5* gene was the *CYP3A5**2 allele, which was found in heterozygous form at a frequency of 3 in 10 among French Caucasians [60] (Table 3). Among the other variants found in *CYP3A5*, *CYP3A5**3 (g. 6986A>G) is the only one reported in all ethnic groups and is therefore considered the most ancient allele. The allelic frequency of *CYP3A5**3 among east Asian populations (Chinese, Japanese, Koreans) is 71–75%, south Asians (Malay and Indians) 55–65% and African-Americans 29–35%, Caucasian-Americans 89–91%, European-Caucasians 92–94% and Hispanics 60–66%. In this allele a guanine at position 6986 creates a cryptic splice site in intron 3, which leads to aberrant splicing of the transcript and leads to very low amount of *CYP3A5* protein [61,62]. The *CYP3A5* allele 6986A>G (*CYP3A5**3) is associated with reduced drug clearance such as simvastatin, lovastatin, midazolam and tacrolimus [63–65]. The expression of *CYP3A5* can be reduced by quite a few other alleles in addition to *CYP3A5**3. The *CYP3A5**6 allele leads to the skipping of exon 7 in *CYP3A5* transcripts in 17% African-Americans [66] and is absent in Asians [67]. Furthermore, a single base insertion into exon 11 leads to premature termination of the reading frame at amino acid 348 in *CYP3A5**7 allele (null allele). This allele is only reported to be present in the population of African ancestry with a allele frequency of 8.5%. It is absent in Caucasians or east and south Asians [68]. *CYP3A5**4 is a coding SNP in the exon 7 due to a nucleotide change, A to G, which produces an amino acid substitution from Glu to Arg at position 200 (Q200R) [69]. The allele *CYP3A5**4 and *CYP3A5**5 are rare mutations present only in East Asians at less than 1% frequency [70]. The *CYP3A5**8 variant caused by the substitution of nucleotide C to T leads to an arginine to cysteine change at codon 28 [59].

*CYP3A5**9 is another coding variant, resulting from G>A transition in exon 10, that leads to a change in the amino acid alanine to threonine at codon 337 [59]. *CYP3A5**10 is a functionally important SNP that results in a coding change within the heme-binding region of *CYP3A5*. *CYP3A5**10 is caused by a point mutation, T>C in exon 12, resulting in an amino acid substitution at residue 446 from phenylalanine to serine [59]. The *CYP3A5**8 was found in blacks (4%), *CYP3A5**9 in Asians (2%), and *CYP3A5**10 in Caucasians (2%). A recent study performed with cancer patients in Australian subjects reported the mean midazolam clearance was significantly higher in *CYP3A5**1/*3 individuals than *CYP3A5**3/*3 individuals [71], thus indicating that *CYP3A5* expression was reduced and the *CYP3A5* genotype is an important determinant of overall CYP3A activity. In another study, the total CYP3A activity determined by quinine was higher in Swedes than in Tanzanians. This difference was attributed to higher CYP3A4 enzyme activity in Swedes because this was present also when Swedes and Tanzanians lacking the wild-type *CYP3A5**1 allele were compared [72]. The wild-type *CYP3A5* had the highest catalytic activity towards testosterone followed by A337T and R28C allele. However, the *CYP3A5* F446S (2% Caucasians) had minimal activity towards testosterone [59]. The kinetic parameters for nifedipine metabolism exhibits the highest $V_{\max}$ in the wild-type allele (*CYP3A5**1), however, *CYP3A5**9 (A337T) and *CYP3A5**8 (R28C) exhibited a modest decrease in $V_{\max}$ for nifedipine. In a recent report, kinetic parameters for nifedipine metabolism by the *CYP3A5**11 protein exhibited a 2.7-fold increase in K_m , a 39% decrease in $V_{\max}$, and a 4.3-fold decrease in intrinsic clearance compared with

the wild-type protein [73]. Since CYP3A7 is a predominant form in the fetal liver, it will not be discussed in this review.

CYP3A43

CYP3A43 is expressed in several tissues that metabolize steroid hormones, carcinogens and many other drugs [74]. There has been a wide interindividual variation in its enzymatic activity and the content of CYP3A reported in general [75]. Domanski *et al.* cloned and characterized CYP3A43, while a relatively low level of testosterone 6 β -hydroxylase activity was obtained [74]. The report suggested that the substrate specificity of *CYP3A43* may be different from other *CYP3A* forms and that the appropriate CYP3A43 substrates may not have been tested in the prior investigation. There is a single report on CYP3A43 allelic distribution. Because of the lack of mono-oxygenase activity observed with the standard CYP3A4 reconstitution conditions, a more thorough investigation of optimal conditions for CYP3A43 may be required. Analysis of the mutation distribution reported five different *CYP3A43** alleles (Table 4). The most frequent allele is the wild-type allele *CYP3A43**1A. A silent mutation 1047C>T leads to a functional allele and was named *CYP3A43**1B. A deletion mutation, 74delA, and a missense 1018C>G mutation are harbored in *CYP3A43**2A and *CYP3A43**3 alleles, respectively [76]. The frameshift caused by a deletion, 74delA, leads to a premature stop codon at nucleotides 265–267 in the *CYP3A43**2A allele. In the third allele, *CYP3A43**3, the C>G substitution result in an amino acid exchange P340A. However, allele *CYP3A43**2B harbors two mutations, 1018C>G and 74delA. The functional significance of these alleles remains to be determined. *CYP3A43* is reported to be associated with prostate cancer [77]. There are controversial reports regarding the function of the *CYP3A4**1B allele. When CYP3A43 and CYP3A4 were considered in a pairwise interaction to determine effect of genotypes with at least one *CYP3A43**3 and one *CYP3A4**1B allele, the effects become stronger, specifically with respect to the onset of prostate cancer. This is a more common haplotype observed in African-Americans (35%) than Caucasians (4%). However, this data could not be explained clinically because *CYP3A43**3 and *CYP3A4**1B allele were in linkage equilibrium [77].

Regulation of CYP3A expression

CYP3A enzymes have long been known to be inducible and are regulated via the pregnane X receptor (PXR). CYP gene families like *CYP3A* and *CYP2A* have a similar mechanism of gene activation through PXR and/or constitutive androstane receptor (CAR) [78]. The interest in the regulation of *CYP3A* increased after the nuclear receptor PXR (also called SXR or PAR1) was recognized as a major transcriptional regulator of *CYP3A* [78–81]. Though *CYP3A4* is more inducible than *CYP3A5*, both are inducible by PXR and CAR agonists [82]. *CYP3A5* contains proximal PXR elements only where *CYP3A4* contains both proximal as well as distal PXR elements [82,83]. The activation of gene expression in CYP3A7 is also regulated via PXR and CAR. The xenobiotic response elements (XRE) motifs within *CYP3A7* are conserved and have been shown to mediate PXR- and CAR-dependent induction [80]. In comparison with CYP3A4, the ER6 of *CYP3A7* and its vicinity differs in several nucleotides, which reduces binding of PXR and CAR and thus the induction of *CYP3A7* [84]. However, the 5'-regulatory regions of *CYP3A5* shows only a limited sequence identity to *CYP3A4*. There is 90% identity up to -8800 bp, in the 5'-upstream regions of *CYP3A4* and *CYP3A7*. The XRE motifs within *CYP3A7* are conserved and have been shown to mediate PXR- and CAR-dependent induction [80].

Pregnane X receptor is a promiscuous nuclear receptor, which can be activated by numerous structurally diverse xenobiotics and drugs [85,86]. An important concern in HIV pharmacotherapy is the potential involvement of multidrug-mediated CYP induction. The CYP induction may cause reduction in therapeutic efficacy, by an increase in drug elimination,

resulting in lower plasma concentrations and thus a decreased antiviral effect [87,88]. Also, induction may create an undesirable imbalance between detoxification and activation as a result of increased formation of reactive metabolites, leading to an increase in the risk of metabolite-induced toxicity [89,90]. In HIV treatment one of the drugs of main concern is ritonavir, which is used as a pharmacokinetic enhancer for other protease inhibitors through inhibition of CYP3A4. When given in combination with other PIs allows the second PI to maintain prolonged plasma concentrations, allowing for less frequent dosing [91].

Molecular genetics approaches to assess CYP3A activity

Until a decade ago, the clinical importance of genetic polymorphisms in drug metabolism was based primarily on phenotypic differences among individuals in a population [92,93]. With the development of sophisticated genetic tools, such as molecular sequencing technology, the sequencing of the whole human genome (the human genome project) was conducted. This, in turn, led to the discovery of SNPs. As summarized above, SNPs have a major impact on the functionality of the gene specifically in the coding or regulatory regions. Several clinical studies have been designed to assess phenotypic consequences in patients. One example is that of thiopurine *S*-methyltransferase (TPMT) and its effect on thiopurine drugs. A considerable degree of interindividual variability has been documented, presenting the challenge of using genotype-to-phenotype approaches to recognize which of the more than 10 million human genetic polymorphisms control interindividual differences in drug response. The international HapMap project has currently genotyped more than 3.3 million SNPs in four diverse human populations in an effort to provide a framework for genotype-to-phenotype research (International HapMap consortium, 2004).

There is a wide variety of genetic approaches to assess CYP3A activity, such as the assessment of the mRNA transcript level, protein activity and the regulation of the enzyme/gene activity. All *CYP3A* genes undergo induction by many drugs, environmental pollutants and dietary substances [92]. Also, since *CYP3A* genes appear to be regulated typically at the transcriptional level [94,95], mRNA quantitation would give a fair estimate of the variability of their expression. Determining CYP contribution is not only useful in the prediction of potential drug–drug interactions but also highlights the potential for metabolic contribution from polymorphically expressed CYP, a factor leading to large interindividual variability in the clinical setting and a complication to individual dose estimation [96].

The search for genetic variants that result in clinically relevant phenotypes has been driven by the central dogma of genetics, that DNA encodes genes as distinct units of inheritance, which in turn are transcribed to RNA and subsequent translation of mRNA into proteins. With technology advances, the genetic analysis of the entire genome of an individual can be screened for the genetic variation at a series of preselected locations, termed as markers. If not more, there are as many markers an individual can have as genes. The most widely used method to assess genetic polymorphism is the PCR-RFLP. In this method, the substitution or deletion of a base pair resulting in generation of a restriction endonuclease site or elimination of a restriction site can be used as a measure of one specific genotype [84,97–99]. SNP discoveries are also performed by ‘assay by design’, which provides customized and quality-validated high throughput assays [100,101]. Also, this technique provides a substantial benefit to the users of the ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems, CA, USA). This is important for the 7900HT system users in enabling high-throughput SNP genotyping, approximately 250,000 genotypes per day can be analyzed, with only a small amount of DNA sample [102]. Recently, a novel method has also been used for typing hundreds of thousands of SNPs by fluorescence resonance energy transfer (FRET) and has been well established for the rapid assessment of SNPs and deletions [103].

CYP3A substrates

General

The CYP3A enzymes have very broad substrate specificity and an extremely large number of structurally diverse chemicals are metabolized by a variety of different pathways [31,104]. Since CYP3A enzymes metabolize more than 50% of prescribed drugs, it is clear that CYP3A is of major importance in clinical pharmacokinetics. As described above, some drugs can increase CYP3A activity via transcriptional activation (induction) mediated by nuclear receptors such as CAR and PXR [82,105]. *CYP3A* induction and its inhibition are not relevant to the clinical management of HIV infection [83]. Some of the important *CYP3A* inducers and the data derived from *in vitro* studies are summarized in Table 5. Rifampicin, an antibacterial agent, is the most commonly used *CYP3A* inducer. The oligonucleotide expression arrays along with cDNA studies have shown that rifampicin can induce the expression of a large number of phase I and phase II enzymes, with CYP3A4 being induced 55-fold comparative to CYP3A5 and CYP3A7 by 5- and 28-fold, respectively [106].

Phenobarbital and similar compounds have a considerable hepatic inducing effect [107]. The level of *CYP3A4* mRNA in cultured HepG2 cells was induced threefold higher than under the constitutive condition, whereas *CYP3A5* induction was twofold [108]. However, in another study with human lung, *CYP3A5* was the major *CYP3A* form and was induced 11-fold by phenobarbital, but *CYP3A5* was not induced by any *CYP3A4* inducers [109]. Phenobarbital is a CAR mediated and to some extent, PXR activator. Dexamethasone is another *CYP3A* inducer, though it is less potent than rifampicin and phenobarbital. These agents are commonly used in early drug-development phases to evaluate the metabolism and drug interactions of antiretrovirals. Some drugs are used as model substrates of CYP3A4 when assessing *in vitro* and *in vivo* phenotyping activity and drug interactions. Testosterone is the most acceptable CYP3A4 probe recommended to be used in addition to midazolam, whereas nifedipine, felodipine and erythromycin have also been studied. Midazolam is considered one of the best *in vivo* probe drugs for the study of CYP3A4 [110]. Some of the commonly used probes are given in Table 6.

Antiretrovirals

There are currently six different classes of antiretroviral drugs including NRTIs, NNRTIs, PIs, entry inhibitors that include fusion inhibitors and co-receptor antagonists, integrase inhibitors and maturation inhibitors. Each drug class interferes with the HIV lifecycle at a different stage.

The CYPs that are primarily involved in the metabolism of NNRTIs and NRTIs are CYP2B6, and to a lesser extent CYP3A4. By contrast to the NNRTIs, PIs for the most part are metabolized by the CYP3A enzyme system, the expression level and enzyme activity of which decreases with age [111]. CYP enzymes in human liver, primarily CYP3A4, play a pivotal role in PI biotransformation, converting these agents to largely inactive metabolites. *In vitro* studies with recombinant human CYPs and/or human liver microsomes have demonstrated that CYP3A4 has the largest capacity (lowest K_m and highest V_{max}) to hydroxylate and/or dealkylate indinavir [112,113], ritonavir [112,114] and saquinavir. PI metabolism is affected both by induction and/or inhibition of CYP3A. Drugs that are more potent inhibitors of CYP3A may reduce the metabolism of protease inhibitors. Ritonavir and amprenavir are mechanism-based potent inhibitors of human CYP3A, both *in vitro* and *in vivo* [114–118]. Ritonavir is also a potent inhibitor of CYP2D6, CYP2C9 and CYP2C19. Saquinavir, indinavir and ritonavir were administered in high doses and in combination with other antiretrovirals [114,119]. However, PI competition for the same enzyme (CYP3A4), results in a complicated metabolism scenario [120]. The decrease in microsomal CYP3A activity for PIs, other than indinavir, is time and concentration dependent [118].

Lopinavir is another PI that when co-administered with low-dose ritonavir, has a significantly improved pharmacokinetic profile and hence the significant antiretroviral activity. Both lopinavir and ritonavir undergo extensive and rapid first-pass hepatic metabolism [114,121]. The enzymatic inactivation of lopinavir is also mediated by CYP3A4. Another, HIV-1 PI, atazanavir, is an azapeptide PI that is metabolized by hepatic CYP3A. Further, atazanavir and its metabolites undergo biliary and urinary elimination [122,123]. The drug is 86% bound to human serum protein and has a half-life of 6–7 h. Nelfinavir is metabolized into three distinct products, namely, 2-methoxy-3-hydroxy nelfinavir (M1), 3,4-dihydroxy nelfinavir (M3) and nelfinavir hydroxyl-t-butylamide (M8). Previous *in vitro* studies have shown that M1 and M3 are formed by CYP3A4, whereas the predominant M8 metabolite is produced by CYP2C19 [124]. However, after oral administration of nelfinavir only M1 and M8 have been documented in the plasma. Since M8 is as potent as nelfinavir with regards to its antiviral properties, thus it is more important than M1 or M3 [125]. Some of the HIV protease inhibitors that are metabolized by CYP3A are tabulated with their K_m values in Table 7.

Clinical pharmacogenomics: HIV-infected patient studies of CYP3A SNPs

The significance of certain SNPs associated with pharmacokinetic factors, such as metabolism (CYP system), absorption and tissue distribution (efflux transporter; e.g., P-gp), have continued to increase [126–129]. Pharmacogenomic investigations have examined the relationship between CYP2B6 and ABCB1 (efflux transporter) and efavirenz, CYP2C9 and nelfinavir, and CYP3A4 and indinavir/atazanavir [130–139]. The relative contribution to the first-pass metabolism of pharmacologic agents for gastrointestinal 3A versus hepatic 3A varies for different compounds and varies across individuals [116]. This contributes another layer of complexity to the relationship between CYP3A and antiretroviral disposition. Factors contributing to intersubject variation in 3A include gender, age, ethnicity and diet. Important SNPs may involve *CYP3A4*1B* (thought to be in the promoter region, mutation upstream to *CYP3A4* gene) [100]. ABC efflux transporters are cell membrane proteins present in epithelial cells ‘guarding’ access to tissue compartments. As a factor in drug absorption, P-glycoprotein (P-gp) in the gastrointestinal epithelium is also linked to gut CYP3A activity [36,101,140–143]. Drug interactions between antiretroviral therapy and other medications may be related to both CYP3A interactions and P-gp interactions [144,145]. Allelic variations in genes coding for P-gp have been reported previously. Homozygosity for CC at this codon in the P-gp encoding gene was noted in 44% in subjects with higher antiretroviral drug concentrations compared with 8% with lower, and with greater efavirenz exposure, respectively [146,147]. The underlying mechanisms for this paradox are largely unknown. A related hypothesis is that CYP3A plays a more important pharmacokinetic role than P-gp for certain antiretroviral drugs because TT has been associated with higher CYP3A expression [2].

Despite different chemical structures and pharmacokinetics, NNRTIs share a similar metabolism and both efavirenz and nevirapine are converted to inactive metabolites by CYP2B6 and CYP3A4. Several studies have investigated the relationship between SNPs in the *CYP2B6* and *CYP3A4* genes, and their effects on efavirenz pharmacokinetics, toxicity and patient response. The *CYP2B6* gene is highly polymorphic and a number of novel functional variants have been identified in different ethnic populations. Among the multiple SNPs linked with the expression of *CYP2B6*, the 516G>T variant has been associated with differences in plasma exposure and CNS toxicity of efavirenz in adult HIV patients [130]. A significant decrease in the clearance of efavirenz and a corresponding increase in plasma concentrations were observed in patients with the T/T genotype [138,139]. However, such association has not been revealed between *CYP3A4* SNPs and efavirenz pharmacokinetics in either association studies or more sophisticated gene interaction studies suggesting a minor involvement of CYP3A4 in the efavirenz metabolism [137,139]. Efavirenz induces hepatic CYP3A4 activity in a dose-dependent manner [148].

With the wide-scale use of nevirapine in developing countries, it has become important to determine functional genetic markers for nevirapine containing regimens, particularly in certain subpopulations. As nevirapine is metabolized by both *CYP2B6* and *CYP3A4*, the polymorphisms associated with an altered expression of these enzymes may theoretically have an influence on nevirapine pharmacokinetics and patient response. There are few studies published since Rotger *et al.* found a significant association between the *CYP2B6* 516G>T allele and greater plasma exposure of nevirapine [130]. Penzak *et al.* investigated 23 HIV-infected Ugandan patients treated with nevirapine and found a markedly higher trough concentration for patients with the *CYP2B6* 516 T/T genotype than that for those with G/G or G/T genotypes (7607, 4181 and 5559 ng/ml for T/T, G/G and G/T, respectively, $p = 0.011$) [149]. Although this study was limited by its small sample size, it represented one of the first few attempts to investigate the influence of *CYP2B6* genotype on nevirapine pharmacokinetics in the developing world. In a recent study by Haas *et al.* of 385 patients in South Africa exploring associations between *CYP2B6*, *CYP3A* and *ABCB1* polymorphisms and nevirapine hepatotoxicity, interestingly, it was found that the T allele in the *ABCB1* gene at position 3435 was significantly related with a decreased risk of liver toxicity from nevirapine ($p = 0.016$) [150]. The underlying mechanism of this association is unclear, as nevirapine appears to be a weak P-glycoprotein substrate. Additional studies are necessary to further define the clinical relevance of this preliminary finding. No association between *CYP3A4* SNPs and nevirapine pharmacokinetics was noted in these studies, however, this likely reflects a minor role for *CYP3A4* in nevirapine metabolism [150].

HIV-1 PIs play a major role in the chronic management of HIV infection [8,22]. Unlike efavirenz or nevirapine, which are predominantly metabolized by *CYP2B6*, the phase I metabolism of PIs is mainly carried out by *CYP3A4/CYP3A5* enzymes. The genes coding for these enzymes have attracted a fair amount of attention as candidates for clinical pharmacogenomic investigations. Indeed, several recent studies have suggested that the disposition of certain PIs might be predicted by the *CYP3A5**1 genotype. A report published by Mouly *et al.* analyzed the association between saquinavir oral clearance and different allelic variants of genes that encode *CYP3A4* and *CYP3A5* enzymes in 20 healthy volunteers [151]. In this study, the *CYP3A5**1 genotype was associated with increased saquinavir clearance that was almost twice as high as for the *CYP3A5**3 carriers (mean 36.7 vs 19.3 l/h, $p = 0.03$). This association has been recently confirmed by an international study in 16 black Tanzanian healthy volunteers in which the median saquinavir exposure among individuals with two functional *CYP3A5* alleles (*1/*1) was 34% lower than among those without functional alleles [152]. However, the clinical relevance of the *CYP3A5* genotype as a pharmacokinetic predictor should be clarified in further studies conducted on saquinavir at steady-state and in the presence of ritonavir enhancement among HIV patients. The *CYP3A5**1 genotype has also been related to 44% faster indinavir oral clearance in 11 HIV patients expressing *CYP3A5* ($p = 0.002$) [153]. It is important to note, however, that the sample size in all these studies was small and that further prospective investigation in a large cohort of HIV-infected patients is warranted. Perhaps of more clinical relevance, a recent study in 118 Spanish HIV+ patients has demonstrated that the presence of at least one T allele at *ABCB1* 3435C>T independently predicts lower atazanavir plasma concentrations [154]. Since the C to T change is significantly associated with decreased P-gp activity, this finding contradicts the overall hypothesis that high plasma concentrations would be linked with T allele. A possible explanation for this paradox has been proposed in a recent study showing individuals homozygous for the *ABCB1* 2677T allele have enhanced *CYP3A4* expression in the liver and intestine that metabolizes atazanavir with decreased plasma concentrations [155]. This finding also suggests that genetic variation in metabolic enzymes might play a more important role than those in transporters with respect to pharmacokinetics of certain PIs.

Future perspective

The molecular basis of inherited traits began to be elucidated in the late 1980s, with the initial cloning and characterization of a polymorphic human gene encoding the drug-metabolizing enzyme CYP2D6 [37]. Genes are considered functionally polymorphic when allelic variants exist firmly in the population, and when one or more of them alters the activity of the encoded protein in relation to the wild-type allele. In many cases the genetic variability is associated with reduced activity of the encoded protein, but there are also instances where the allelic variant encodes for proteins with increased activity. Despite the potential importance of the clinical variables in determining drug effects, it is now recognized that innate differences in the metabolism and disposition of drugs, and genetic polymorphism in the targets of drug therapy can have an important influence on the efficacy and toxicity of medications. The overall pharmacologic effects of drugs are typically not monogenic traits; rather, effects are determined by the interaction of several genes encoding different proteins involved in different pathways of drug metabolism, disposition and effects. CYP3A4 has received increasing attention as a candidate for pharmacogenetic investigation since the discovery of the SNP *CYP3A4*20*, because *CYP3A4*20* represents the first *CYP3A4* allele, which has no functional activity. Atazanavir and indinavir are metabolized via CYP3A, although these drugs can also be substrates for UGT. The *CYP3A4*20* allele may have an important impact on the clinical use of these and other PIs that are substrate of CYP3A enzyme. Several recent studies have shown that *CYP3A5*1* also contributes in the disposition of PIs [156]. A recent report by Mouly *et al.*, has shown that *CYP3A5*1* genotype is associated with increased saquinavir clearance, which was twice as high as among the individuals carrying the *CYP3A5*3* allele [151]. This study was later confirmed among 16 black Tanzanian healthy volunteers. In addition, Fukushima-Uesaka *et al.*, found 25 haplotypes, including 16 unambiguous ones among *CYP3A4* and *CYP3A5* [46]. There is no detailed report on the haplotypes discovery in ethnic groups other than Japanese. Since *CYP3A4* and *CYP3A5* largely metabolize the same substrates it is worth analyzing the possible haplotype combinations.

Also, despite the fact that pharmacogenetics has gained immense importance, the use of pharmacogenetics has remained limited to investigation in only a few clinical fields such as oncology, psychiatry, abacavir treatment in HIV-1 and warfarin therapy and so on [157–161]. The main reason is the paucity of scientific evidence supporting pharmacogenetic analysis and improved clinical outcome [162,163]. That may be compounded by an increasing lack of detailed knowledge of clinical pharmacology. Both physicians and pharmacists might find it difficult to interpret pharmacogenetic results and apply them to the clinical setting. The major goal of this review is to decipher the important pharmacogenetic markers in antiretroviral therapy and drug interactions that involve *CYP3A4* [164]. The significance of drug interactions increases when a compound has a high affinity for a single metabolizing enzyme compared with affinity for a number of different enzymes. Because CYP activities can be measured by multiple *in vivo* and *in vitro* drug assays, *CYP3A* genotype to drug-metabolism phenotype correlations can be determined for antiretrovirals. Clinically relevant *CYP3A* alleles may guide more rational use of drugs and appropriate drug dosage to achieve maximum therapeutic efficacy for the antiretroviral therapy.

There have been a number of reports examining the relationship of CYP or P-gp SNPs with PI or NNRTI pharmacokinetics, and in some studies virologic response [23,133,139,147,165–168]. Overall, the predictive value of these SNPs has been variable and in some cases predictive of pharmacokinetics but not response and in others predictive of response but not pharmacokinetics [130,131,138,165,169–171]. In considering chronic antiretroviral drug dosing and the likelihood that a SNP(s) would be related to pharmacokinetics, it may be important to consider metabolism and efflux transporters, rather than only one of these. In a recent analysis, SNPs for CYP2B6 and P-gp were more predictive of virologic failure than

either SNP alone [172]. Continued research is needed at the molecular biology level as well as in translational research studies to determine the optimal approach to provide treatment for HIV-infected patients receiving multiple medications that are substrates and/or inducers of CYP3A.

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Table 1
SNPs in *CYP3A4* 3'- and 5'-UTRs

Study	Allele	Gene	Ref.
Gonzalez <i>et al.</i> (1988)	<i>CYP3A4*1A</i>	Wild-type	[37]
Rebbeck <i>et al.</i> (1998)	<i>CYP3A*1B</i>	-392A>G	[35]
Kuehl <i>et al.</i> (2001)	<i>CYP3A4*1C</i> <i>CYP3A4*1D</i>	-444T>G -62C>A	[36]
Hamzeiy <i>et al.</i> (2002)	<i>CYP3A4*1E</i> <i>CYP3A4*1F</i>	-369T>A -747C>G	[44]
Fukushima-Uesaka <i>et al.</i> (2004)	<i>CYP3A4*1G</i> <i>CYP3A4*1H</i> <i>CYP3A4*1J</i> <i>CYP3A4*1K</i> <i>CYP3A4*1L</i> <i>CYP3A4*1M</i> <i>CYP3A4*1N</i> <i>CYP3A4*1P</i> <i>CYP3A4*1Q</i> <i>CYP3A4*1R</i> <i>CYP3A4*1S</i> <i>CYP3A4*1T</i>	20230G>A 20230G>A; 26206C>A 6077A>G -655A>G -630A>G -156C>A 14200T>G 15727G>A 15809T>C 16775A>G 17815_17816delAT 26013T>C	[46]

Table 2
***CYP3A4* SNPs in the coding region**

<i>Allele</i>	Nucleotide changes		Effect	Ref.
	<i>cDNA</i>	<i>Gene</i>		
<i>CYP3A4</i> *2	664T>C	5713T>C	S222P	[43]
<i>CYP3A4</i> *3	1334T>C	23171T>C	M445T	[43]
<i>CYP3A4</i> *4	352A>G	13871A>G	I118V	[39]
<i>CYP3A4</i> *5	653C>G	15702C>G	P218R	[39]
<i>CYP3A4</i> *6	830_831insA	17661_17662insA	277 Frameshift	[39]
<i>CYP3A4</i> *7	167G>A	6004G>A	G56D	[52]
<i>CYP3A4</i> *8	389G>A	13908G>A	R130Q	[52]
<i>CYP3A4</i> *9	508G>A	14292G>A	V170I	[52]
<i>CYP3A4</i> *10520G>C		14304G>C	D174H	[52]
<i>CYP3A4</i> *111088C>T		21867C>T	T363M	[52,53]
<i>CYP3A4</i> *121117C>T		21896C>T	L373F	[52]
<i>CYP3A4</i> *131247C>T		22026C>T	P416L	[52]
<i>CYP3A4</i> *1444T>C		44T>C	L15P	[28]
<i>CYP3A4</i> *15485G>A		14269G>A	R162Q	[28,44]
<i>CYP3A4</i> *16554C>G		15603C>G	T185S	[28,46,53]
<i>CYP3A4</i> *17566T>C		15615T>C	F189S	[54]
<i>CYP3A4</i> *18878T>C		20070T>C	L293P	[46,54]
<i>CYP3A4</i> *191399C>T		23237C>T; 20230G>A	P467S	[54]
<i>CYP3A4</i> *201461	1462insA	25889_25890insA	488 Frameshift	[56]

Table 3

CYP3A5 SNPs in noncoding and coding regions

Allele	UTR	Gene	Nucleotide change	Effect (enzyme activity)	Ref.
CYP3A5*1A-	-	Wild-type	-	-	[173]
CYP3A5*1B-86G>A	-	-	-	-	[36]
CYP3A5*1C-74C>T	-	-	-	-	[36]
CYP3A5*1D31611C>T	-	-	-	-	[61]
CYP3A5*1E17163G>T	-	-	-	-	[45]
CYP3A5*2	-	27289C>A	T398N	-	[60]
CYP3A5*3	-	6986A>G	Splicing defect	Severely decreased	[45,61,71]
CYP3A5*4	-	14665A>G	Q200R	-	[69]
CYP3A5*5	-	12952T>C	Splicing defect-	-	[69]
CYP3A5*6	-	14690G>A	Splicing defect	None or severely decreased	[36]
CYP3A5*7	-	27131_27132insT346	Frameshift	Null	[61]
CYP3A5*8	-	3699C>T	R28C	Decreased	[59]
CYP3A5*9	-	19386G>A	A337T	Decreased	[59]
CYP3A5*10-	-	31611T>C	F446S	Decreased	[59]
CYP3A5811-	-	3775A>G	Y53C	Decreased	[59]

Table 4
***CYP3A43* SNPs in noncoding and coding regions**

Study	Allele	Nucleotide change	Effect	Ref.
Domanski <i>et al.</i> (2001)	<i>CYP3A43*1A</i>	None	Normal	[74]
Cauffiez <i>et al.</i> (2004)	<i>CYP3A43*1</i>	33518C>T		[76]
	<i>CYP3A43*2</i>	8340delA	25 Frameshift	
	<i>CYP3A43*3</i>	31867C>G	P340A	

Table 5
CYP3A inducers data from *in vitro* studies

Drug (inducer)	Fold induction		Method used	Ref.
	<i>CYP3A4</i>	<i>CYP3A5</i>		
Rifampicin	55.1	5	cDNA expression array	[173]
	4.5	2	HepG2 cells	[175]
Phenobarbital	>10	–	Reporter gene assay	[174]
	0	11	Alveolar cells	[176]
	3	1.2	HepG2 cells	[174]
Dexamethysone	5	1.5	HepG2 cells	[177]
	–	4.0	Human adenocarcinoma	[176]
Carbamazepine	4.5	1.5	HepG2 cells	[174]
Sulfinpyrazone	2–3	–	Hepatocytes	[175]
	>10	–	Reporter gene assay	[175]
Phenytoin	7	–	Hepatocytes	[178]
	5.2	–	Reporter gene assay	[178]
	4.5	2	HepG2 cells	[174]

Table 6
Validated probe drugs for CYP3A4 and/or CYP3A5

Probes	Metabolized by	
	CYP3A4	CYP3A5
Alfentanil	Yes	Yes
Alprazolam (α -hydroxylation)	No	Yes
Erythromycin	Yes	No
Cortisol	Yes	Yes
Midazolam	Yes	Yes
Quinidine	Yes	No
Tacrolimus	No	Yes
Triazolam	Yes	Yes
Vincristine	No	Yes

Table 7
Some of the HIV-protease inhibitors metabolized by CYP3A

HIV-protease inhibitors	K_m (μ M)
Amprenavir	25.50
Atazanavir	–
Indinavir/lopinavir	1.30
Nelfinavir	40.00
Ritonavir	20.00
Saquinavir	00.35