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PharmGKB Summary - Very Important Pharmacogene Information for Cytochrome P-450, Family 2, Subfamily A, polypeptide 6 (CYP2A6)

Ellen M. McDonagh, Ph.D[a,*], **Catherine Wassenaar, B.Sc.**[b,*], **Sean P. David, M.D., S.M., D.Phil.**[a,c], **Rachel F. Tyndale, Ph.D.**[b], **Russ B. Altman, M.D., Ph.D.**[a,d], **Michelle Whirl-Carrillo, Ph.D.**[a], and **Teri E. Klein, Ph.D.**[a]

[a]Department of Genetics, Stanford University

[b]Center for Addiction and Mental Health, Departments of Psychiatry, Pharmacology and Toxicology, University of Toronto

[c]Department of Medicine, Stanford University

[d]Department of Bioengineering, Stanford University

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Introduction

Human cytochrome P450 (CYP-450), family 2, subfamily A, polypeptide 6 (CYP2A6) is a monooxygenase enzyme that metabolizes xenobiotic compounds and activates toxins [1–3]. In this review we focus on the effect of genetic variants on the role of CYP2A6 in drug metabolism, and provide a pharmacogenomic overview of *CYP2A6* in humans. This Very Important Pharmacogene summary is available with interactive links to gene variants, haplotypes and drugs on the PharmGKB website http://pharmgkb.org/vip/PA121 [4].

CYP2A6 expression and function

CYP2A6 represents approximately 4% of the total CYP-450 enzyme protein content of adult liver microsomes [5]. CYP2A6 is also expressed in the lung, trachea, nasal mucosa, and sex organs such as breast [2, 6]. Human CYP2A6 enzyme activity is determined by measuring coumarin 7-hydroxylation [7–10]. CYP2A6 expression, enzyme function and induction vary

Correspondence to Dr Teri E. Klein, PhD, Department of Genetics, 1501 California Avenue, Stanford University, Palo Alto, 94304, USA, Tel: +1 650 736 0156; fax: +1 650 725 3863; teri.klein@stanford.edu.
*co-first authors.

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Conflicts of Interest

Dr. Tyndale owns shares and participates in Nicogen Research Inc., a company focused on novel smoking cessation treatment approaches. No Nicogen funds were used in this work and no other Nicogen participants reviewed the manuscript. Dr. Tyndale has also participated in one day Novartis and McNeil meetings. S.P.D. is a consultant for Genophen and has participated in a one day Pfizer meeting. R.B.A. serves as a founder and consultant for Personalis.

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considerably between individuals [2, 5, 7–12], and an individual's CYP2A6 enzyme expression and activity may depend on a combination of environmental factors (such as xenobiotic compounds) and genetic factors (including polymorphic variants) [1, 13]. Modeling human CYP2A6 activity in rodents has limited utility due to species-specific CYP2A6 ortholog expression patterns and activity profiles – for example, in rats little or no coumarin is 7-hydroxylated, and a CYP2B enzyme metabolizes nicotine to cotinine [14–19]. The crystal structure of human CYP2A6 bound to coumarin was originally described in 2005, revealing a smaller active site than other CYP2 enzymes C8 and C9, composed of a hydrophobic cavity ideal for the oxidation of small planar compounds such as coumarin [20]. Several structures with the enzyme in complex with different substrates and/or mechanism based inhibitors have been described since (the Protein Data Bank [21]; [1]).

The *CYP2A6* gene

CYP2A6 was the first gene of the human CYP2A cytochrome subfamily to be cloned and mapped, and was previously known as *CYP2A3* (a name now used for the rat ortholog) [3, 22–26]. The *CYP2A6* gene sits within a cluster of *CYP2* genes on chromosome 19 q13.2, thought to have arisen through duplication events, and shares extensive homology with subfamily members *CYP2A7* and *CYP2A13* [1–3]. The gene is composed of 9 exons spanning around 6kb, encoding a protein of 494 amino acids [1, 7].

Genetic variation of CYP2A6

The *CYP2A6* gene is highly polymorphic, with over 35 different *CYP2A6* alleles described thus far, with additional subgroups (see [27, 28]) [3]. Star (*) nomenclature is used to describe different *CYP2A6* alleles, with the 'wildtype' reference allele defined as **1* [27, 28] [8]. Genetic variants in the *CYP2A6* locus include alleles with single nucleotide polymorphisms (SNPs) (for example **2, *9*), whole and partial gene deletions (alleles **4A-H*), a gene hybrid with *CYP2A7* (for example **12*) and gene conversions (for example **1B*) [28]. The complex genetic architecture of *CYP2A6* coupled with its significant homology with other *CYP2A* genes makes genotyping challenging, particularly when using SNP based arrays [3, 29]. Since single amplifications often cannot discriminate between the different *CYP2A* genes, special amplification and sequencing techniques have been developed for *CYP2A6* genotyping, revealing errors in allele characterization in older studies [3, 29–32].

CYP2A6 genotypes are often classified into predicted phenotype groups, describing the effect on enzyme activity, for example 'poor' metabolizer (no active *CYP2A6* alleles, homozygous for inactive alleles), 'slow' (one inactive or two decreased activity alleles), 'intermediate' metabolizer (heterozygous with one decreased activity allele and one active allele), 'normal'/ 'extensive' (2 active alleles), or 'ultrarapid'/ 'fast' (>2 active alleles) [29, 33–36]. Studies may also compare reduced (one or more inactive or decreased activity allele) to normal (two active alleles) metabolizers [37, 38]. These categories often overlap or differ between studies. In addition, the same polymorphism can have a different effect on the metabolism of different substrates. For example, *CYP2A6*18* activity against nicotine is similar to wildtype enzyme, but is reduced for coumarin and tegafur metabolism, *in vitro* [39], and conversely **17* activity against coumarin is similar to wildtype but significantly reduced for nicotine metabolism [40]. People with *CYP2A6*12* are slow metabolizers of letrozole, but intermediate metabolizers of nicotine [41]. Therefore the effect of *CYP2A6* polymorphisms on metabolism should be investigated and interpreted in the context of individual substrates [29].

Genetic variants in the *CYP2A6* gene can result in reduced expression by affecting transcriptional or translational processes [1]. For example, a SNP within the TATA box of the *CYP2A6* promoter (allele **9*) reduces gene transcription [42]. The *CYP2A6*1B* allele

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differs from **1A* by a *CYP2A7* gene conversion in the 3' untranslated region (UTR), and correlates with increased CYP2A6 protein expression and activity, likely through increased mRNA stability [43]. Individuals homozygous for the **4* gene deletion allele lack detectable *CYP2A6* mRNA expression and enzyme activity [44, 45].

Wide variation in the frequency of *CYP2A6* alleles across ancestral groups is observed. For example, the frequency of *CYP2A6*4* alleles ranges from 0–4% in White, 0–2% in Black, 5–15% in Chinese and 17–24% in Japanese populations [30, 36, 45–54]. The frequency of the *CYP2A6*9* allele ranges from around 5–8% in White, 6–9% in Black, up to 16% in Chinese and 19–21% in Japanese populations [36, 42, 46–51, 54, 55]. Other alleles are found predominantly in one ethnic group, for example *CYP2A6*17* (defined by the variant rs28399454, V365M) is found at a frequency of around 10% in Black individuals, but not identified in White, Korean or Japanese individuals, whereas **7* is found in Asian subjects at a frequency of around 10%, but not White or Black individuals [49, 56].

CYP2A6 as an important pharmacogene

Around 3% of the drugs metabolized by CYP-450 enzymes involve CYP2A6 (reviewed in [1]). In the sections below, we describe known pharmacogenetic associations between *CYP2A6* variants and drugs, further detailed in Table 1 and 2. When examining these associations, it should be taken into account that environmental factors, such as compounds found in food, cigarettes, hormones or therapeutic drugs, can affect CYP2A6 expression, which can therefore influence drug pharmacokinetics and responses [1, 13]. Xenobiotics in our diet, such as the flavonoid biochanin A (found in plants), can upregulate *CYP2A6* expression *in vitro* [57]. The hormone estrodiol induces CYP2A6 expression via direct binding of the transcription factor estrogen receptor 1 (*ESR1*) to a promoter element upstream of the *CYP2A6* gene *in vitro* [58], and may explain why CYP2A6 activity is higher in women than men, and higher in women taking oral contraceptives compared to those not taking them [59]. The anti-inflammatory drug dexamethasone, via the glucorticoid receptor (*NR3C1*), induces *CYP2A6* transcription in human hepatocytes *in vitro* by augmentation of Hepatocyte Nuclear Factor 4, alpha (*HNF4A*) [60], likely explaining the enhanced CYP2A6 activity seen with dexamethasone treatment [9]. The anticonvulsant phenobarbital also enhances CYP2A6 enzyme activity *in vitro* [9, 10]. Inhibitors of CYP2A6 enzyme activity include the antibacterial and antifungal agents isoniazid and ketoconazole [1, 13, 61], and traditional Chinese medicine [62]. Identifying environmental and therapeutic compounds that regulate CYP2A6 activity, as well as genetic polymorphisms, may be important for optimal therapeutic efficacy and avoiding adverse drug reactions [1].

Another factor is *CYP2A6* genotyping. Most drug-response studies group *CYP2A6* genotypes into predicted enzyme activity phenotype groups and assess associations compared to **1* homozygotes, because there are many variant alleles found at low frequencies. Some studies use the * allele name without screening for all variants in the allele as defined by the CYP-450 allele nomenclature committee [27, 28]. Therefore individuals may have variants not screened for, or not have all variants conferring an allele. As described previously, the complexity of *CYP2A6* in terms of polymorphisms and homology to other genes means that genotyping errors can occur; as knowledge about the gene and its variants increases, genotyping assays should improve, as was seen with *CYP2D6*. We therefore provide genotyping details in Table 2.

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CYP2A6 polymorphisms, nicotine metabolism and cigarette smoking behavior

The vast majority of published work describing the phenotypic effects of *CYP2A6* polymorphisms on enzyme activity has been carried out using nicotine as a substrate (Table 1). These studies have revealed important mechanistic consequences of *CYP2A6* alleles on nicotine metabolic inactivation and related smoking behaviors, and by characterizing the relationship between *CYP2A6* genotypes and enzyme phenotypes provide a starting point for how these polymorphisms may contribute to the observed inter-individual variability in the PK of other CYP2A6 substrates.

Nicotine is extensively metabolized and has a short plasma half-life of around 2 hours [63]. Approximately 80% of nicotine is inactivated *in vivo* into cotinine in a two-step process (see the PharmGKB Nicotine Pathway, Pharmacokinetics https://www.pharmgkb.org/pathway/PA2011) [63–65]. CYP2A6 has a predominant role in the oxidation of nicotine to form a nicotine iminium ion, which is subsequently converted to cotinine (COT) by aldehyde oxidase (AOX) [63, 65–67]. The majority of COT is metabolized to *trans*-3'-hydroxy-cotinine (3HC) in a reaction exclusively mediated by CYP2A6 [67–69]. The ratio of 3HC/COT is often used as a phenotypic marker of CYP2A6 metabolic activity among smokers due to the long half-life of COT and the *in vivo* formation dependent kinetics of 3HC [63, 65, 70].

Nicotine metabolism is subject to large inter-individual variation [67, 71], and seven *CYP2A6* polymorphisms explain most of the inter-individual variation in nicotine to COT metabolism, in European-Americans [72]. Nicotine dependence and cigarette smoking behaviors are closely related to the pharmacokinetics of nicotine, for example cigarette craving negatively correlates with blood levels of nicotine [73]. Polymorphisms in *CYP2A6* which effect nicotine metabolism have therefore been associated with smoking behaviors (see Table 1) [31, 53, 74], and are an important consideration in the efficacy of nicotine replacement based smoking cessation treatments [75–77]. For example, slow metabolizers (as determined by *CYP2A6*2, *4, *9* and **12* alleles), are less likely to be smokers, smoke fewer cigarettes per day, take smaller puff volumes, have lower levels of dependence, are more able to quit, and benefit more from regular and extended nicotine patch replacement therapy compared to normal metabolizers [31, 36, 37, 46, 78–81].

Evidence suggests that *CYP2A6* polymorphisms that confer decreased CYP2A6 enzyme activity result in reduced or deficient nicotine metabolism [31, 56, 71], and this is thought to lead to lower cigarette consumption [47]. On the other hand, alleles conferring increased enzyme activity (e.g. duplication or **1B*) result in enhanced rates of nicotine metabolism and thus are associated with increased cigarette consumption and depth of inhalation [29, 31, 47, 71, 82]. Inhibition of CYP2A6 has therefore been investigated as a smoking cessation treatment [29]. In Chronic Obstructive Pulmonary Disease (COPD) patients, *CYP2A6* is associated with number of cigarettes smoked per day (cpd) and age of initiation of smoking [83]. As smoking cessation is important in preventing COPD progression, identifying patients with risk genotypes for particular smoking behaviors may aid in treatments to help patients reduce smoking [83].

The *CYP2A6* and *CYP2B6* genes are closely localized within the *CYP2* cluster on chromosome 19, suggesting potential linkage disequilibrium [3, 84]. Human liver CYP2A6 and CYP2B6 enzyme expression is correlated and they share some inducers and substrates (as discussed in [85]). Examining the contribution of *CYP2B6* genotype on a potentially common substrate (i.e. nicotine, efavirenz) should take into consideration *CYP2A6* status,

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and vice-versa. For example, the association seen between CYP2B6 and nicotine C-oxidation *in vitro* is abrogated after controlling for CYP2A6 protein levels [85].

CYP2A6 and caffeine

CYP2A6 plays a part in caffeine metabolism, as the major enzyme required to convert paraxanthine (1,7-dimethylxanthine, 17X) into 1,7-dimethyluric acid (17U) via 8-hydroxylation [86] (see the PharmGKB Caffeine Pathway, Pharmacokinetics https://www.pharmgkb.org/pathway/PA165884757) [87]. Human liver microsomes (HLMs) with the *CYP2A6* genotype **1/*4, *4/*9* or **1/*9* display significantly reduced 8-hydroxylase enzyme activity against paraxanthine, and **4/*4* samples have undetectable activity, compared to **1/*1* wildtype samples [86]. Kinetic assays with CYP2A6 protein fractions demonstrate **7, *10* and **11* alleles confer reduced 8-hydroxylation activity [86]. Amongst non-smokers, *CYP2A6* intermediate and poor metabolizer genotypes have lower paraxanthine metabolism compared to 'normal metabolizers' (Table 2) [88]. In addition to *CYP2A6* genotype, cigarette smoking significantly reduces paraxanthine metabolism (17U/17X ratio in urine), and may be due to competition between paraxanthine and nicotine [88], or via the impact of current smoking status which decreases CYP2A6 activity [89]. Therefore, both smoking and *CYP2A6* genotype influences CYP2A6 8-hydroxylation activity against caffeine, contributing to the inter-individual variability observed [88]. By measuring 17U/17X ratios, caffeine has been proposed as a potentially more suitable substrate for studying the functional effects of *CYP2A6* polymorphisms *in vivo* than nicotine or coumarin [86], although further characterization of the timing, dose and association with genotype is required.

CYP2A6 and cancer therapeutics

CYP2A6 has a key role in the metabolism of several drugs involved in cancer treatment. Associations between *CYP2A6* genotype, rate of drug metabolism and treatment efficacy are detailed below and in Table 2. *CYP2A6* genotype is also associated with risk of developing cancer [90], see the '*CYP2A6* and toxicology' section.

Tegafur

The prodrug tegafur is initially metabolized into 5' hydroxytegafur, which rapidly breaks down into 5-fluorouracil (5 FU) and is further processed into active metabolites with anticancer properties (see the PharmGKB Fluoropyrimidine Pathway, Pharmacokinetics https://www.pharmgkb.org/pathway/PA150653776 and the Fluoropyrimidine (PD) Pathway https://www.pharmgkb.org/pathway/PA165291507) [91]. Although several CYP-450 proteins have a role in the biotransformation of tegafur into 5 FU, including CYP1A2, CYP2C8, CYP2C9 and CYP1A1, CYP2A6 has a principle role [92, 93]. In human liver microsomes, 5 FU formation correlates significantly with coumarin 7-hydroxylation and with CYP2A6 expression [92, 93]. Selective inhibition of CYP2A6 activity drastically attenuates 5 FU formation [92–94]. Genetic variants that affect CYP2A6 expression and function are associated with altered metabolism of tegafur and clinical outcome. The *CYP2A6*4* gene deletion allele significantly reduces *CYP2A6* mRNA and protein levels in human liver samples, which correlates with a reduced rate of tegafur metabolism *in vitro* [55] and *in vivo* the **4C* and **11* alleles confer poor tegafur metabolism [95]. Conversely, a novel **1B* allele is associated with increased CYP2A6 protein expression and significantly higher rates of 5 FU formation in human liver microsome (HLM) samples, compared to samples without the haplotype (Table 2) [55].

To optimize 5 FU efficacy and reduce the toxicity of side effects, tegafur is combined with 5-chloro-2,4-dihydroxypyridine (CDHP) (inhibits degradation of 5 FU) and potassium

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oxonate (prevents gastrointestinal toxicity), to form the oral drug S-1 [96]. In cancer patients treated with S-1, genotypes containing *CYP2A6*4, *7, *9* and **10* alleles are associated with reduced metabolism of tegafur compared to wildtype *CYP2A6* (Table 2) [97–100]. Those with two variant *CYP2A6* alleles (**4/*4, *4/*7* or **7/*7*) have significantly lower oral clearance of tegafur compared to wildtype homozygotes [100]. Those without the **4C* allele have significantly lower tegafur and higher 5 FU plasma concentrations compared to those with the allele [99]. However, other studies find no association between 5 FU plasma concentrations and *CYP2A6* genotype, with 5 FU levels correlating instead with CDHP concentrations [97, 100].

Examining clinical outcome rather than 5FU blood concentrations suggests that the influence of *CYP2A6* genetic variants on tegafur's pharmacokinetics (PK) may have clinical importance. S-1 and cisplatin-treated patients with two *CYP2A6* variant alleles **4, *7, *9, *10*, or the **1/*4* genotype, have significantly lower treatment response rates, increased risk of disease progression and increased likelihood of reduced overall survival time than other genotypes [101]. Similar findings are observed in a study treating patients with S-1 and docetaxel; those with two *CYP2A6* variant alleles (**4, *7, *9* or **10*) display a 5-fold increased risk of cancer progression [102]. However, in this study overall survival is not significantly associated with genotype, possibly due to switching to alternative chemotherapy upon tumor progression (as discussed in [102]). Whether the association between *CYP2A6* variants and reduced tegafur treatment efficacy is due to reduced formation of 5 FU cannot be concluded due to a lack of parallel PK studies [101, 102]. Significantly higher 5 FU plasma concentrations are found in responders to S-1 treatment compared to non-responders, and although no direct significant association between *CYP2A6* genotype and treatment response is found in this study, those with one variant *CYP2A6* allele have significantly higher 5 FU and significantly lower tegafur plasma concentrations compared to those with two variant alleles [103].

Combining these results suggests that a poor-metabolizer *CYP2A6* genotype is associated with reduced tegafur metabolism to 5 FU and thus reduced anti-tumor efficacy [101–103]. *CYP2A6* genotype does not seem to influence treatment side effects, such as hematological toxicity [101–103]. Although these associations remain to be investigated further in studies that combine PK and clinical outcome in large sample sizes, current findings suggest that *CYP2A6* genotype may be a useful addition to tegafur dosing guidelines to increase treatment efficacy.

Letrozole

Letrozole is a daily oral treatment for estrogen- or progesterone-receptor positive breast cancer in postmenopausal women, and suppresses estrogen synthesis by inhibiting the aromatase enzyme [104]. Letrozole plasma levels, elimination rate and clearance show high inter-individual variation, and may contribute to adverse drug reactions or differences in treatment efficacy [41, 105]. CYP2A6 has a major role in the breakdown of letrozole into its inactive carbinol metabolite [106, 107] (see also the letrozole (Femara) tablet drug label, Novartis Pharmaceuticals Corporation) [108]. *In vitro*, HLM samples from individuals with *CYP2A6* genotypes **1/*4, *4/*4, *4/*9* and **1/*7* have significantly reduced letrozole oxidation compared to **1/*1* wildtype samples [106]. Clinical studies in healthy postmenopausal women show that clearance of letrozole is lower in individuals with a *CYP2A6* variant allele *(*4, *9, *7*) compared to **1* (see Table 2) [105]. In a cohort of breast cancer patients, *CYP2A6* genotype is significantly related to letrozole plasma concentrations, explaining around 26% of the inter-individual variability observed [41]. *CYP2A6* genotypes defined as conferring slow and intermediate metabolism are associated with significantly higher plasma concentrations of letrozole compared to the wildtype genotype (**1/*1*) [41] (see Table 2). The ability to predict letrozole plasma concentration

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using *CYP2A6* genotype is further improved by integrating age and body mass index (BMI) variables, explaining 32.3% of inter-individual variation [41]. As both *CYP2A6* genotype and body weight influence letrozole levels, together they may explain some of the differences in letrozole PK parameters seen between Asian and Caucasian individuals [105]. Overall, these studies provide evidence to suggest that *CYP2A6* genotyping, along with BMI and age, could be useful for predicting exposure to letrozole in patients [41, 105, 106].

CYP2A6 and treatment of infectious diseases

CYP2A6 has a role in the metabolism of several drugs involved in the treatment of infectious diseases, as outlined below and detailed in Table 2. When treating individuals for co-infections, such as HIV and malaria infection, the added complication of drug-drug interactions and induction or inhibition of CYP-450 enzymes by these drugs should be considered when assessing the pharmacogenetic effect [109, 110].

Efavirenz

Efavirenz (EFV) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) that suppresses viral replication and is used as a component in highly active anti-retroviral therapy (HAART) regimens for HIV-infected patients [111]. High inter-individual variability of EFV plasma levels exists between patients receiving a fixed daily dose, and this has clinical implications; higher EFV plasma levels are associated with increased risk of central nervous system (CNS) side effects, whereas significantly lower levels are associated with failure to suppress viral replication [112–115]. Demographic factors sex, age, body mass index, or co-medication, cannot explain this variability [113–115], and instead genetic variants underlie a high proportion of the inter-individual variation in EFV plasma concentrations (discussed below). Genotyping may therefore aid EFV dosing, help avoid virolic failure and CNS related adverse reactions.

CYP2B6 has a key role in EFV metabolism, predominantly forming the major metabolite 8-hydroxyefavirenz (the product of over 90% of EFV oxidation) [116–118], and a high percentage of inter-individual variation in EFV PK is attributed to *CYP2B6* genetic variation [115, 118–123]. CYP2B6 and CYP2A6 contribute to the 7-hydroxylation of EFV (represents less than 10% of EFV oxidation) [116–118]. *CYP2A6* genetic variation therefore also plays a role in the variability of EFV PK seen in patients, the effects of which are particularly prominent in *CYP2B6* slow metabolizers [118, 121, 122]. These studies are detailed below and in Table 2.

In small studies of 50–65 individuals, there is no statistically significant association between EFV PK parameters and the SNPs rs8192726 (1836 G>T, **9b*) or rs28399454 (5065 G>A, **17*) [119, 122], though rs8192726 is associated with higher EFV plasma levels when the study size is increased to 94 individuals [123]. A trend for higher EFV plasma concentrations in rs28399433 T/G (*CYP2A6*1/*9)* heterozygotes compared to TT homozygotes does not reach significance after correcting for multiple comparisons in a small study of 45 individuals [115]. The lack of significant association in these studies may be due to low allele frequencies of the variants examined, small sample sizes, the relatively smaller contribution of CYP2A6 to EFV PK and/ or a weak association; for instance, in Kwara *et al.* a significant association between EFV PK parameters and *CYP2A6* was observed when individuals with one or more copies of *CYP2A6*9b* (rs8192726) and *CYP2A6*17* (rs28399454) were grouped together, but not when the SNPs were analyzed individually [122].

Individuals with two *CYP2A6* loss-of-function alleles, two decreased function *CYP2A6* alleles, or one of each (Table 2) have significantly higher EFV plasma concentrations,

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compared to those without these alleles, in individuals carrying *CYP2B6* reference alleles [118]. Stratifying for both *CYP2A6* and *CYP2B6* genotype, a trend for lower 7-hydroxy-EFV metabolite levels is seen in patients with two loss of function *CYP2B6* alleles and two *CYP2A6* loss or decrease function alleles, and higher levels of the CYP-450 independent metabolite *N*-glucuronide-EFV [118]. Individuals with *CYP2A6* loss-of-function alleles have lower clearance of EFV, and this is more pronounced in those who also have *CYP2B6* loss-of-function alleles [121]. In multiple regression analysis, incorporating multiple variants, *CYP2A6* rs8192726 and/ or rs28399454 status independently contributes to EFV inter-individual plasma concentrations, accounting for around 10% (8.6–12%), with *CYP2B6* rs3745274 genotype TT contributing 36–45.2%, and *UGT2B7 *1a* genotype also significantly contributing [122, 123]. To conclude, genetic variation in the *CYP2B6, CYP2A6, CYP3A4* and *UGT2B7* genes contributes to inter-individual variation of EFV clearance [121–123], and the effect of carrying *CYP2A6* loss-of-function alleles on EFV clearance is more pronounced in people who are also *CYP2B6* slow metabolizers [118, 121, 122]. The clinical consequence of *CYP2A6* genotypes is not reported in the studies above, although higher plasma concentrations of EFV are associated with both increased viral suppression and increased likelihood of CNS adverse reactions [112–114]. The CYP2A6 enzyme may have a role in the metabolism of other HAART drugs prescribed alongside EFV, for example Zidovudine (see the PharmGKB Zidovudine Pathway, PK/PD https://www.pharmgkb.org/pathway/PA165859361) [124], which could affect overall clinical outcome of viral suppression or adverse drug effects.

Artemisinin and derivatives

Artemisinin and its derivatives are drugs used to fight malaria infection [109, 125]. Recombinant CYP2A6 metabolizes artemisinin and CYP2A6 inhibition attenuates the rate of drug disappearance in human microsomes *in vitro* [126]. However, CYP2B6 and CYP3A4 enzymes are thought to have a greater role in artemisinin metabolism [126] (see the PharmGKB Artemisinin and Derivatives Pathway, Pharmacokinetics https://www.pharmgkb.org/pathway/PA165378192). Artemisinin derivatives (arteether, artemether, artesunate) were developed to enhance drug bioavailability, and are used in combination with a second unrelated slower acting drug, in order to initially rapidly eradicate malaria parasites within red blood cells, and then kill any residual parasites [109,125]. CYP2A6 is the major CYP450 enzyme involved in artesunate metabolism, forming dihydro-artemisinin, which is then inactivated by UGT enzymes (see the PharmGKB Artemisinin and Derivatives Pathway, Pharmacokinetics) [109, 127]. Therefore, *CYP2A6* alleles which confer loss-of-function or decreased function may affect metabolism of these anti-malarial drugs. However, studies investigating urinary metabolites after dosing with artemisinin or derivatives, and use concurrent coumarin or nicotine probe drugs, see no clear correlation between *CYP2A6* genotype and an effect on PK or enzyme activity [128, 129]. Artemisinin and derivatives induce CYP2B6 expression [130, 131], further complicating *CYP2A6* association studies. Studies controlling for CYP2B6 status and with larger numbers are therefore required to investigate the clinical implications of *CYP2A6* genotype on the metabolism of artemisinin and its derivatives.

CYP2A6 and other therapeutic drugs

Valproic acid

The antiepileptic drug valproic acid (VPA) is also used to treat migraines and schizophrenia, and could be a potential anti-cancer drug [132]. *In vitro* studies demonstrate CYP2C9 is the principle enzyme in VPA metabolism, however CYP2A6 contributes to around 50% of VPA 3-hydroxylation in human microsomes [133] and can contribute to 4-ene-VPA formation, a metabolite of VPA thought to cause hepatotoxicity [11, 133–135]. CYP2A6 activity against

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coumarin is inhibited by VPA treatment, therefore VPA may affect responses to drugs metabolized by CYP2A6 taken concurrently [136]. Individuals with the **4* gene deletion allele have significantly increased plasma levels of VPA, likely due to decreased CYP2A6 enzyme metabolic activity, and may result in increased drug exposure [137].

Pilocarpine

Pilocarpine is used for the treatment of glaucoma and xerostomia [138]. CYP2A6 is the principle enzyme involved in 3-hydroxypilocarpine formation from pilocarpine in HLMs [138]. Poor metabolizers with two inactive *CYP2A6* alleles have significantly increased pilocarpine plasma concentrations and a trend for higher excretion in the urine, with concurrent reductions of the metabolite 3-hydroxypilocarpine compared to non-poor metabolizers [139]. Despite these significant differences in metabolism, no severe adverse effects are observed, and the authors suggest *CYP2A6* poor metabolizers likely use alternative renal clearance pathways [139].

SM-12502

SM-12502 is a platelet activating factor (PAF) receptor antagonist and has potential for use in asthma therapy [140]. CYP2A6 is the major metabolizer enzyme of SM-12502 in human microsomes *in vitro* [141], and the compound has been used to identify *CYP2A6* polymorphisms that confer poor and extensive metabolizer phenotypes *in vivo* [142].

CYP2A6 is involved to a varying extent in the metabolism of numerous other therapeutic drugs (see [1] for an extensive list of substrates). These include the antitumour drugs ifosfamide (see the PharmGKB Ifosfamide Pathway, Pharmacokinetics https://www.pharmgkb.org/pathway/PA2037) and cyclophosphamide (see the PharmGKB cyclophosphamide Pathway, Pharmacokinetics https://www.pharmgkb.org/pathway/PA2034), the epilepsy treatments phenytoin (see the PharmGKB Phenytoin Pathway, Pharmacokinetics https://www.pharmgkb.org/pathway/PA145011115) [143], carbamazepine (see the PharmGKB Carbamazepine Pathway, Pharmacokinetics https://www.pharmgkb.org/pathway/PA165817070) [144, 145], and losigamone [146,147]. CYP2A6 also has a secondary role in metabolism of the anaesthesia halothane [148]. Therefore, *CYP2A6* polymorphisms may affect additional pharmaceuticals, however the extent of CYP2A6's role in the metabolism/ clearance of these drugs may be minor and/ or redundant, and other genetic variants including those in CYP-450 genes may play a more prominent role in the overall outcome.

CYP2A6 and toxicology

CYP2A6 polymorphisms have not only been associated with extent of nicotine metabolism and effect on smoking behaviors (as discussed previously and outlined in Table 1), but also with activation of carcinogens from tobacco and xenobiotics [1, 149]. Tobacco-specific nitrosamines, including nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N-nitrosonornicotine (NNN), are present in tobacco and cigarette smoke, and hydroxlylation by CYP2A6, CYP2A13 and other CYPs activates NNK and NNN into metabolites that can then react with DNA to form adducts [1, 149]. CYP2A6 is involved in the activation of herbicides and pollutants such as hexamethylphosphoramide, dichlorobenzonitrile, aflatoxin B1, naphthalene, methyl *tert*-butyl ether, and N-nitrosobenzylmethylamine (NBzMA) [1, 12], which are also procarcinogens.

CYP2A6 polymorphisms conferring lower enzyme activity are associated with reduced risk of lung, oral, head and neck, and upper aerodigestive cancers, particularly in tobacco users [150–156]. The association between *CYP2A6* and lung cancer is especially prominent

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among those who smoke 20 or fewer cigarettes per day [38]. Higher CYP2A6 enzyme activity is associated with increased risk of pancreatic cancer (adjusted for smoking status) [157]. The results of different association studies are mixed, often due to a lack of statistical power or failure to adjust for smoking status and behaviors [90, 149]. The relative contribution of *CYP2A6* polymorphisms to cancer risk through smoking behavior versus carcinogen activation is difficult to define [29, 90, 149], though studies controlling for the amount of cigarette exposure suggest that carcinogen bioactivation is a significant contributor [38, 152]. Inhibition of CYP2A6 could potentially reduce cancer risk [29, 90, 149].

Conclusions

CYP2A6 has a major role in the metabolism of nicotine and several commonly prescribed drugs, as well as a lesser role in the metabolism of a large variety of other pharmaceuticals and xenobiotics. The *CYP2A6* gene is highly polymorphic, and this has been associated with the high level of inter-individual and inter-ethnic variability in CYP2A6 enzyme activity and to variation in drug responses. *CYP2A6* alleles conferring reduced enzyme activity have been associated with reduced metabolism and drug clearance, and thus linked with altered treatment response, as well as protection from cancer. *CYP2A6* genotype may be important in determining the dosage for several treatments important in cancer therapy and HIV, in order to optimize efficacy and reduce the likelihood of adverse drug reactions. Further studies are required to confirm the role of individual genotypes in drug responses in larger cohorts and new technologies may help define *CYP2A6* alleles more accurately.

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Abbreviations

17U	1,7-dimethyluric acid
17X	1,7-dimethylxanthine, paraxanthine
3HC	*trans*-3'-hydroxy-cotinine
5FU	5-fluorouracil
AOX	aldehyde oxidase
CDHP	chloro-2,4-dihydroxypyridine
CNS	central nervous system
COPD	Chronic Obstructive Pulmonary Disease
COT	cotinine
Cpd	cigarettes per day
CYP-450	cytochrome P450
EFV	efavirenz
HAART	highly active anti-retroviral therapy
HLM	human liver microsomes
NBzMA	N-nitro-sobenzylmethylamine

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NNK	nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
NNN	N-nitrosonornicotine
PAF	platelet activating factor
PD	pharmacodynamic
PK	pharmacokinetic
SNP	single nucleotide polymorphism
VPA	valproic acid

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Table 1

CYP2A6 polymorphisms and nicotine associations

***CYP2A6* allele**	**Effect on nicotine metabolism**	**Association with smoking behavior and response to nicotine replacement therapy**
*CYP2A6*1B*	Greater nicotine clearance in individuals with alleles **1B1-15* compared to wildtype homozygotes **1A/*1A* [158].	Genotype **1B/*1B* genotype is associated with increased cpd compared to **1A/*1A*, but not significantly associated with smoking status or ability to quit [82]. Increased likelihood of being a smoker [81].
*CYP2A6*1X2*	***1X2A***: Higher levels of exhaled carbon monoxide compared to **1/*2, *2/*2, *1/*4*, and higher cotinine plasma levels compared to **1/*1 or *1/*2, *2/*2, *1/*4* [31] #. ***1X2B***: increased nicotine metabolism compared to **1/*1* (ns), measured by cotinine/nicotine ratios [159].	***1X2A***: Greater smoking intensity (CO per cpd, COT per cpd) compared to **1/*1 or *1/*2, *2/*2, *1/*4* [31] #.
*CYP2A6*2*	Longer half-life of nicotine and cotinine, and reduced nicotine metabolism [49], [34]#.	In adolescents, increased risk of becoming nicotine dependent, but slower progression and lower cigarette consumption once dependent [74], [47]#. Fewer compared to normal nicotine metabolizers [31, 36, 74] #, [160]. Fewer cpd and lower FTND [38]#. Increased ability to quit smoking (ns) [161]#. Better response to extended transdermal nicotine replacement therapy [37]#.
*CYP2A6*4*	Reduced nicotine metabolism and altered metabolite profile [49, 50,162–164].	In adolescents, increased risk of becoming nicotine dependent, but slower progression and lower cigarette consumption once dependent [74], [47]#. Reduced risk of being a smoker [81], reduced cpd [31, 36, 54, 74, 152]#, [165, 166], and increased ability to quit smoking (ns) [161]#. Though in other studies, not significantly associated with smoker status, age started smoking, cpd or ability to quit [82, 167–169]. Fewer cpd and lower FTND [38]#. Better response to extended transdermal nicotine replacement therapy [37]#.
*CYP2A6*7*	Reduced nicotine metabolism [39, 45, 49, 50, 164]. SNP T1412C (rs5031016): Reduced nicotine metabolism [170].	Fewer cpd, later onset of smoking, shorter smoking duration, but reduced likelihood of smoking cessation [54, 152]#.
*CYP2A6*9*	Reduced nicotine metabolism and clearance [44, 49], [34]#.	Fewer cpd [36, 38, 54]#, lower FTND [38]#, later onset of smoking, shorter smoking duration, but reduced likelihood of smoking cessation [54]#. Better response to extended transdermal nicotine replacement therapy [37]#.
*CYP2A6*10*	Reduced nicotine metabolism [45, 49, 164].	Reduced cpd [54, 152]#.
*CYP2A6*12*	**1/*12* genotype is associated with normal nicotine metabolism, but **9/*12* is associated with reduced metabolism [34]#.	Fewer cpd [36, 38]#, lower FTND [38]#. Better response to extended transdermal nicotine replacement therapy [37]#.
*CYP2A6*17*	Reduced nicotine metabolism and clearance [49, 56].	Increased ability to quit smoking (ns) [161]#.
*CYP2A6*35*	Reduced nicotine metabolism [171].	Increased ability to quit smoking (ns) [161]#.

Table Key:

= studies that analyze combined genotypes, or analyze this allele combined with other alleles in a phenotype category, e.g. reduced activity alleles.

CO = carbon monoxide

COT = cotinine
cpd = Cigarettes per day
FTND: Fagerström Test for Nicotine Dependence
ns = not significant

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Table 2

CYP2A6 polymorphisms and association with therapeutic drug response

CYP2A6 Allele or Genotype	Details of genotyping	Drug	Association	Reference and study details
***1/*4** or ***4/*4** or ***4/*9** or ***1/*9**	*§*	**Caffeine**	Significantly reduced metabolism of paraxanthine compared to **1/*1* (not detectable in **4/*4* samples)	[86] *in vitro* study, n=42 human liver microsomes
***7** or ***10** or ***11**	*§*	**Caffeine**	Reduced activity against paraxanthine	[86] *in vitro* study, *CYP2A6* transfected into *E. coli*, fractions then incubated with paraxanthine
Intermediate metabolizers: *1A/*4, *1A/*9, *1B1/*4, *1B1/*9 Poor metabolizers: *4/*9, *9/*9	**4*: gene deletion **9*: g.-48T>G (rs28399433) **1x2* gene duplication **1B1* gene conversion in 3' region	**Caffeine**	Reduced metabolism of paraxanthine into 17U compared to extensive metabolizers (**1A/*1A*, **1A/*1B1*, **1A1/*1B1*x2, **1B1/*1B1*).	[88] n=100 Serbian, healthy volunteers.
rs8192725 Genotype CC g.1620T>C Intron 2	Method: direct gene sequencing, provide details of primers and *§*. Reference sequences used: NG_00008.7 and NP_000753.3	**Tegafur**	Significantly increased mRNA expression and a trend for increased rate of 5 FU formation (ns), compared to genotype TT	[55] *In vitro*, (n=45) Chinese HLM and liver tissue samples
rs8192720 Genotype C/T or TT c.22C>T (NM_000762.5) Leu8Leu exon 1	Method: direct gene sequencing, provide details of primers and *§*. Reference sequences used: NG_00008.7 and NP_000753.3	**Tegafur**	Increased *CYP2A6* mRNA expression and an increased rate of 5 FU formation, compared to genotype CC.	[55] *In vitro*, (n=45) Chinese HLM and liver tissue samples
rs28399433 Genotype G/T or GG g. -48T>G	Method: direct gene sequencing, provide details of primers and *§*. Reference sequences used: NG_00008.7 and NP_000753.3	**Tegafur**	A trend for reduced *CYP2A6* mRNA and protein expression, and reduced rate of 5 FU formation (ns), compared to genotype TT.	[55] *In vitro*, (n=45) Chinese HLM and liver tissue samples
***4**	Allele defined by: *CYP2A6* gene deletion. Method: direct gene sequencing, provide details of primers and *§*. Reference sequences used: NG_00008.7 and NP_000753.3	**Tegafur**	Significantly reduced *CYP2A6* mRNA and protein expression, and significantly reduced rate of 5 FU formation, compared to those without the **4* allele.	[55] *In vitro*, (n=45) Chinese HLM and liver tissue samples
Two alleles with a gene conversion in the 3' UTR	Method: direct gene sequencing, provide details of primers and *§*. Reference sequences used: NG_00008.7 and NP_000753.3	**Tegafur**	Associated with a trend for reduced CYP2A6 expression and a trend for reduced rate of 5 FU formation, compared to those with no alleles with a gene conversion in the 3' UTR.	[55] *In vitro*, (n=45) Chinese HLM and liver tissue samples
***1B (haplotype 14)**	Haplotype defined by: gene conversion in the *CYP2A6* 3' UTR, and the SNPs 22C>T (rs8192720) and 1620T>C (rs8192725) Method: direct gene sequencing, provide details of primers and *§*.	**Tegafur**	significantly increased *CYP2A6* mRNA expression, and significantly increased rates of 5 FU formation, compared to those without the allele.	[55] *In vitro*, (n=45) Chinese HLM and liver tissue samples

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CYP2A6 **Allele or Genotype**	**Details of genotyping**	**Drug**	**Association**	**Reference and study details**
	Reference sequences used: NG_00008.7 and NP_000753.3			
Genotype **1/*1*	Sequenced for **9* at -48T>G (rs28399433), **10* at 6600G>T) rs28399468), **7* at 6558T>C (rs5031016). **4* gene deletion §. Therefore **1* was defined as wildtype with none of the above variants.	**S-1 and oxaliplatin**	Associated with increased tegafur metabolism and a trend for higher 5 FU plasma concentrations compared to individuals with one or two variant alleles *(*4, *7, *9, *10* combined) (p values were not given), but not associated with increased likelihood of diarrhea or neutropenia.	[98] Study: Biliary tract cancer patients, (n=48).
****4C* and **11* combined**	Alleles defined by: **4C*: Gene deletion (identical to **4A* according to [27, 28]). **11*: c.670T>C, g.3391T>C, Ser244Pro (rs111033610) Method: §, amplification of exon 8 to 3' UTR, restriction digest analysis. Amplification and sequencing of exon 5.	**Tegafur**	Reduced metabolism compared to four other patients.	[95] A case study of a gastric cancer patient subsequently found to have both alleles, and follow-up *in vitro* experiments.
Allele **7*	g.6558T>C (Ile471Thr) (rs5031016) and gene conversion with *CYP2A7* in the 3' UTR §	**Tegafur**	Reduced 5 FU formation rate compared to **1A/*1A*	[39] *In vitro* kinetic assays, using transformed *E. coli* membrane preparations (n=3)
Allele **18*	Single SNP rs1809810 (g. 5668A>T, Tyr392Phe). Allele specific primers used for genotyping.	**Tegafur**	Slightly reduced 5 FU formation rate compared to **1A/*1A*	[39] *In vitro* kinetic assays, using transformed *E. coli* membrane preparations (n=3)
Allele **19*	g.5668A>T (Tyr392Phe) (rs1809810) and g.6354T>C (intron 8), 6558T>C (Ile471Thr) (rs5031016) and gene conversion with *CYP2A7* in the 3' UTR. Allele specific primers used for genotyping.	**Tegafur**	Reduced 5 FU formation rate compared to **1A/*1A*	[39] *In vitro* kinetic assays, using transformed *E. coli* membrane preparations (n=3)
Individuals with two variant alleles (4/*4, *7/*7* or **4/*7*)**	**4A*: § **7*: § with some modifications, c.1412T>C, Ile471Thr (rs5031016)	**S-1**	Significantly reduced oral clearance of tegafur, compared to **1/*1*	[100] n=54 Japanese patients
****4C***	Alleles defined by: **4C, *7, *9*. Method: § amplification	**S-1**	Significantly reduced metabolism of tegafur, compared to individuals without the allele.	[99] n=46 Japanese patients with non-small-cell lung cancer.
Individuals with two variant alleles, (combined genotypes): **4/*4* **4/*7* **4/*9* **7/*9* **9/*9*	Alleles defined by: **4*: entire gene deletion §, Positions genotyped: g.-48T>G (for **9*) (rs28399433), g. 6558T>C (rs5031016 Ile471Thr) (for **7, *10*), and g.6600G>T (rs28399468, Arg485Leu) (for **10*)	**S-1**	Reduced treatment efficacy compared to individuals with one or two wildtype **1* alleles. Increased risk of disease progression and reduced progression-free survival, as measured by significantly reduced probability of tumor response,	[102] n= 50 Korean patients with metastatic gastric cancer

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CYP2A6 Allele or Genotype	Details of genotyping	Drug	Association	Reference and study details
Two variant alleles, (combined genotypes): ***4A/*4A*** ***4A/*7*** ***4A/*9*** ***7/*7*** ***7/*9*** ***9/*9***	*§* (introduction describes **7* as c.1412T>C (rs5031016 Ile471Thr), and **9* as g.-48T>G (rs28399433), and **4* as complete lack of activity.)	**S-1**	Associated with significantly reduced tegafur clearance, compared to **1/*1* or heterozygotes with one variant allele (tegafur plasma concentrations did not correlate with 5 FU concentrations)	[97] n=57 Japanese patients with solid tumors.
Individuals with two variant alleles, (combined genotypes): ***4A/*4A*** ***4A/*9*** ***4A/*10*** ***7/*9*** ***9/*9*** ***9/*10***	Alleles defined by: **4* gene deletion **7*: c.1412T>C , Ile471Thr (rs5031016) **9*: g.-48T>G (rs28399433)	**S-1**	Associated with higher tegafur and significantly lower 5 FU plasma concentrations compared to heterozygote patients (**1/*4A* **1/*7* **1/*9* **1/*11* combined genotypes). No significant association with adverse effects or treatment response were found.	[103] n=34 patients with solid tumors.
Individuals with one or two variant alleles, (combined genotypes): ***4/*7*** ***4/*9*** ***4/*10*** ***9/*9*** ***1/*4***	Alleles defined by: **4* entire gene deletion, positions genotyped: g.-48T>G (rs28399433) (for **9*), g.6558T>C (rs5031016 Ile471Thr) (for **7, *10*), and g.6600G>T (rs28399468, Arg485Leu) (for **10*). *§*	**S-1 and cisplatin**	Significantly associated with lower response rate, increased risk of disease progression and reduced overall survival time.	[101] n=106, Korean patients, with metastatic gastric cancer
1/*4* or** ***4/*4* or** ***4/*9* or** ***1/*7	*§*	**Letrozole**	Significantly associated with reduced metabolism compared with **1/*1* wildtype	[106] *In vitro* study using Japanese HLM samples (n=31)
1A/*1A ***1A/*1B*** ***1B/*1B***	*§*	**Letrozole**	Increased clearance compared to heterozyogous or homozygous individuals with variant alleles (**4, *9, *7*)	[105] n=22 healthy Japanese postmenopausal women
Slow metabolizers =2 copies of decrease-of-function alleles (**9, *12*), or 1 or 2 copies of loss-of-function alleles (**2, *4, *7, *10, *17, *20, *23-*27, *35*), or 1 decrease-of-function allele and 1 loss-of-function allele.	*§ *2*: 1799 T>A; **4E*: gene deletion (intron 7); **7*: 6558 T>C; **9*: -48 T>G; **10*: 6558 T>C, 6600 G>T; **12*: exon 1–2 CYP2A7, exon 3-9 CYP2A6; **17*: 5065 G>A; **23*: 2161 C>T; **24*: 594 G>C; (if **25* is positive then perform **26* & **27* assays*)* **26*: 1711 T>G; **27*: 2162–2163 GC>A frameshift; **35*: 6458 A>T.	**Letrozole**	Significantly higher plasma levels of drug compared to normal metabolizers (genotype **1/*1*)	[41] n=259 mixed population, postmenopausal women with hormone receptor positive breast cancer
Intermediate metabolizer = one copy of decrease-of-function alleles **9, *12*	*§ *2*: 1799 T>A; **4E*: gene deletion (intron 7); **7*: 6558 T>C; **9*: -48 T>G; **10*: 6558 T>C, 6600 G>T; **12*: exon 1–2 CYP2A7, exon 3–9 CYP2A6; **17*: 5065 G>A; **23*: 2161 C>T; **24*: 594 G>C; (if **25* is positive then perform **26* & **27* assays) **26*: 1711	**Letrozole**	Significantly higher plasma levels of drug compared to normal metabolizers (genotype **1/*1*)	[41] n=259 mixed population, postmenopausal women with hormone receptor positive breast cancer

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CYP2A6 Allele or Genotype	Details of genotyping	Drug	Association	Reference and study details
	T>G; **27*: 2162–2163 GC>A frameshift; **35*: 6458 A>T.			
Two loss-of-function alleles (2, *4A-F, *5, *34*) or two reduced function alleles (**1H, *1J, *7, *9, *10, *12, *13, *15, *17, *19*) or one of each**	‡: for novel variants. **1X2A, *1X2B*: gene duplication, **1H and *1J*: rs61663607 g.-745A>G, **2:* rs1801272 g.1799T>A **4A – *4F* intron 8 gene conversion with *CYP2A7* - gene deletion. **5*: rs5031017 g.6582G>T, **7, *10, *19*: rs5031016 g.6558T>C, **9, *13, *15*: rs28399433 g.-48T>G, **17*: rs28399454 g.5065G>A, **12*: intron 2 gene conversion with *CYP2A7, *34*: intron 4, gene conversion with *CYP2A7*.	**Efavirenz**	Reduced EFV metabolism – significantly higher EFV plasma AUC compared to those without variant alleles (in individuals with *CYP2B6* reference alleles – please note reference alleles were not stated in the study).	[118] n=169 mixed population, HIV -infected individuals, The Swiss HIV Cohort Study. (Please note, the phenotype categories included different allele groupings from those described in [121]).
loss-of-function alleles (2, *4*) and/ or diminished function alleles (**1H,*1J, *5, *7, *9,*10,*12,*13, *15, *17, *19, *34*)**	‡	**Efavirenz**	Reduced clearance of the drug irrespective of *CYP2B6* status, however more pronounced in homozygotes with *CYP2B6* loss-of-function alleles.	[121] n=169 mixed population, HIV-infected individuals, The Swiss HIV Cohort Study. (Please note, the phenotype categories included different allele groupings from those described in [118]).
****9B* allele**	rs8192726 g.1836G>T (genotyped only rs8192726 and no other SNPs which make up the **9B* allele) §	**Efavirenz**	Slow metabolism of EFV (significantly increased plasma concentrations) compared to those without the allele. *CYP2B6* genotype status was not accounted for in the initial analysis.	[123] n=94 Ghanaian patients with HIV-infection, some also with TB coinfection. Please note; this was the same study cohort as [122] but more patients
****17***	rs28399454 genotype AA+GA g. 5065G>A, Val365Met (genotyped only rs28399454 and no other SNPs which make up the **17* allele)	**Efavirenz**	Not associated with slow metabolism of EFV (not statistically significant higher plasma concentrations) compared to those without the allele. *CYP2B6* genotype status was not accounted for in the initial analysis.	[123] n=94 Ghanaian patients with HIV-infection, some also with TB coinfection. Please note; this was the same study cohort as [122] but more patients
****9B* and/ or **17***	rs8192726 or rs28399454 (genotyped only rs8192726 and rs28399454, and no other SNPs which make up the **9B* and **17* alleles)	**Efavirenz**	Slow metabolism of EFV (significantly increased plasma concentrations) compared to individuals without the alleles. *CYP2B6* genotype status was not accounted for in the initial analysis but in multiple regression analysis, *CYP2A6* genotype status independently contributed to EFV variation, along with *CYP2B6* and *UGT2B7* genotype.	[123] n=94 Ghanaian patients with HIV-infection, some also with TB coinfection. Please note; this was the same study cohort as [122] but more patients
****9B*** genotype TG vs GG	rs8192726 g.1836G>T (genotyped only this position and no other	**Efavirenz**	Not associated with EFV plasma or cell concentrations (*CYP2B6*	[119] n=50 a mixed population of patients

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CYP2A6 Allele or Genotype	Details of genotyping	Drug	Association	Reference and study details
	SNPs which make up the *9B* allele)		status not considered in this initial analysis)	with HIV-infection.
17 Genotype GA vs GG	rs28399454 g.5065 G>A, Val365 Met (genotyped only this position and no other SNPs which make up the *17* allele)	**Efavirenz**	Not associated with EFV plasma concentration, peripheral blood mononuclear cell concentrations or accumulation ratio. (*CYP2B6* status was not considered in this initial analysis)	[119] n=50 a mixed population of patients with HIV-infection.
*9	rs28399433 Genotype T/G	**Efavirenz**	A trend for higher EFV plasma concentrations compared to genotype T/T (not considering *CYP2B6* status), although this was not statistically significant after Bonferroni correction for multiple comparisons.	[115] n=45, Haitians of African decent, with HIV-infection.
***4**	§	**Valproic acid**	Significantly higher steady state plasma concentrations (reduced metabolism) in individuals with the *4 allele compared to those without the allele.	[137] n=179 Northern Han Chinese epilepsy patients
Genotype *7/*9 or ***4A/*7** or ***4A/*9** or ***4A/*10**	§ *4A (entire gene deletion) *7: Ile471Thr (rs5031016) *8: Arg485Leu (rs28399468) *9: g.-48T>G (rs28399433) *10: Ile471Thr (rs5031016) and Arg485Leu (rs28399468)	**Pilocarpine**	Poor metabolism and low clearance.	[139] study 1 n=20, study 2 n=8, healthy Japanese individuals administered with a single dose of pilocarpine hydrochloride.

Table Key:

5 FU = Fluorouracil

AUC = Area Under the Curve

EFV = efavirenz

HLM = Human Liver Microsomes

ns = not statistically significant

SNP = Single Nucleotide Polymorphism

§ = give reference to other studies for the method of genotyping.

‡ = refer to genotyping alleles in concordance with the CYP Allele Nomenclature Committee (www.cypalleles.ki.se) [27, 28].

g = gene nucleotide position according to NG_000008.7 (unless otherwise stated) as given by the CYP Allele Nomenclature Committee [27, 28] Please note that this NCBI reference sequence has been removed.

c = cDNA nucleotide position according to NM_000762.4 (unless otherwise stated) as given by the CYP Allele Nomenclature Committee [27, 28]. Please note that this NCBI reference sequence has been updated.

Where possible, dbSNP rsID have been provided for variants, according to links from [27, 28] or from the cited journal. Please note, due to the reference sequences on the NCBI having been updated from those given by the CYP Allele Nomenclature Committee (www.cypalleles.ki.se) [27, 28], the gene and cDNA nucleotide positions on dbSNP may differ, despite being consistent in amino acid position.

More details on these associations can be found at www.pharmgkb.org. Please note that the alleles reported throughout this summary are those on the minus chromosomal strand, where the CYP2A6 gene is found. On the PharmGKB all alleles are standardized to the plus strand, and therefore will differ in a complementary manner from those given here.