Points-to-consider Documents: Scientific Information on the Evaluation of Genetic Polymorphisms During Non-Clinical Studies and Phase I Clinical Trials in the Japanese Population

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Abstract

Pharmacotherapy shows striking individual differences in pharmacokinetics and pharmacodynamics, involving drug efficacy and adverse reactions. Recent genetic research has revealed that genetic polymorphisms are important intrinsic factors for these inter-individual differences. This pharmacogenomic information could help develop safer and more effective precision pharmacotherapies and thus, regulatory guidance/guidelines were developed in this area, especially in the EU and US. The Project for the Promotion of Progressive Medicine, Medical Devices, and Regenerative Medicine by the Ministry of Health, Labour and Welfare, performed by Tohoku University, reported scientific information on the evaluation of genetic polymorphisms, mainly on drug metabolizing enzymes and transporters, during non-clinical studies and phase I clinical trials in Japanese subjects/patients. We anticipate that this paper will be helpful in drug development for the regulatory usage of pharmacogenomic information, most notably pharmacokinetics.

Keywords: genetic polymorphism; pharmacogenomics; non-clinical study; phase I clinical trial; precision pharmacotherapy

Background

The Project for the Promotion of Progressive Medicine, Medical Devices, and Regenerative Medicine by the Ministry of Health, Labour and Welfare was established in 2012. One of the projects that was adopted, "evaluation procedures for the efficacy and safety of pharmaceuticals that use pharmacogenomics," conducted a non-clinical study and early-phase clinical trials on investigational drugs, and evaluated the effects of genetic polymorphisms of drug-metabolizing enzymes and transporters primarily involved in those trials. This work was conducted at the Graduate School of Pharmaceutical Sciences and Faculty of Pharmaceutical Sciences, Tohoku University, in cooperation with the National Institute of Health Sciences and Pharmaceuticals and Medical Devices Agency.

As a main achievement of this project, we compiled the current scientific information on genetic polymorphisms, which had been evaluated during these non-clinical studies and phase I clinical drug trials in Japanese subjects/patients, obtained through this project and scientific papers. This report focuses on the genetic polymorphisms of drug metabolizing enzymes (cytochrome P450 enzymes among others) and transporters with reported functional significance and allele frequencies of 1% or higher in the Japanese population, and outlines *in vitro* procedures for the evaluation of their functional effects on investigational drugs. This report also

67 provides examples of genetic polymorphisms and somatic mutations of proteins
68 involving drug efficacy or adverse drug reactions. We believe that this information
69 will be helpful to accelerate future research and continued discussions on drug
70 development using pharmacogenomic information (e.g., dose adjustment and
71 avoidance of adverse reactions) in Japan and possibly other East Asian countries.
72 Furthermore, a guideline from European Medicines Agency describes that 1) a
73 relevant involvement of a known polymorphic enzyme cannot be excluded (i.e., non-
74 clinical in vitro data predict >50% to be cleared by a single polymorphic enzyme in
75 vivo), it is advised to genotype the first time substance exposure in phase I clinical
76 trial, 2) if phase I studies indicate that pharmacogenetics influences the
77 pharmacokinetics of a drug to a possible clinically relevant extent (i.e., >25% of the
78 drug is metabolized by a single polymorphic enzyme), this should be reflected in the
79 design of the Phase II studies. No such a guideline is there in Japan. Since multi-
80 regional clinical trials including Japan have been common nowadays, clear
81 information on frequent and functional genetic polymorphisms in Japanese population
82 should be useful to promote the safer attendance of Japanese patients into the trials
83 (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/
84 02/WC500121954.pdf). The Graduate School of Pharmaceutical Sciences and Faculty
85 of Pharmaceutical Sciences, Tohoku University anticipated that this information will

serve as a scientific basis for discussion to make regulatory guidelines in the future.

1. Introduction

1.1 Purpose

Individual differences in efficacy and safety of drugs are well known, and factors affecting these differences can be classified into intrinsic and extrinsic factors. As listed in the ICH-E5 guideline, extrinsic factors include climate, culture, degree of drug compliance, and method of conducting or endpoint of the clinical trial, while intrinsic factors include gender, body weight and height, and polymorphisms of genes involved in drug metabolism. In recent years, many studies on genetic polymorphisms in drug-metabolizing enzymes and transporters have demonstrated associations with drug efficacy and/or adverse drug reactions [1-3]. The use of genetic information could lead to increased efficacy and decreased adverse reactions.

Drug efficacy can be efficiently demonstrated in clinical trials by excluding specific patient groups with certain types of genetic backgrounds that are associated with no or very low efficacy of the drug. For example, some studies showed a linkage between reduced drug efficacy and decreased activity of metabolizing enzymes due to genetic polymorphisms [4, 5]. As another example, drug toxicity can be caused by an elevation in blood/tissue concentrations by a reduction in drug metabolizing enzyme

105 or transporter activities by genetic polymorphisms, leading to decreased excretion or
106 decreased elimination of reactive metabolites [6]. Therefore, the use of information on
107 genetic polymorphisms related to pharmacokinetics/drug efficacy/adverse drug
108 reactions could potentially prevent delays in drug development and reduce costs
109 associated with the onset of serious adverse reactions.
110 This report aims to present scientific information for evaluation of genetic
111 polymorphisms (mainly on drug-metabolizing enzymes and transporters) and their
112 functional effects that can be taken into consideration during non-clinical studies (*in*
113 *vitro* studies using human samples) and phase I clinical trials in the Japanese
114 population.
115
116 **1.2 Scope of Application**
117 This report will present the methodology and important considerations for
118 referral when pharmaceutical companies obtain information on genetic
119 polymorphisms and/or assess their functional significance for investigational drugs in
120 non-clinical studies. These evaluations will be helpful to design phase I clinical trials
121 in the Japanese population. The scope of this document is limited to exploratory
122 evaluations of the effects of genetic polymorphisms. The methodologies and
123 information presented here are based solely on current knowledge, and thus, future

strategies for drug development should not be limited to this document.

2. Analysis Methods and Examples of Target Genes

2.1 Analytical Methods for Genetic Polymorphisms

Analytical methods for genetic polymorphisms can be divided into 1) candidate gene analysis and 2) comprehensive gene analysis. This section briefly outlines the advantages and disadvantages of the two methods.

2.1.1 Analysis of Genetic Polymorphisms of Candidate Genes

Enzymes that contribute to investigational drug metabolism can be identified by non-clinical studies using human hepatocytes, their microsomal, cytosolic, or S9 fractions, or expression systems of each drug-metabolizing enzyme. Cytochrome P450 and glucuronyl transferase are present in these samples (except for the cytosolic fraction). In addition, a growing number of studies have revealed the involvement of drug transporters that contribute to the uptake/excretion of drugs into/from the small intestine, liver, kidney, and other organs. When known drug metabolizing enzymes/transporters are involved in pharmacokinetics, efficacy, and the safety of the drug, it is possible to explore novel, related genetic polymorphisms and to evaluate the effects of the polymorphisms on the metabolism of the investigational drug in

143 non-clinical studies; this will allow the importance of these polymorphisms to be
144 considered in subsequent clinical phase I trials. Genetic polymorphisms associated
145 with functional changes have previously been reported, especially for major
146 cytochrome P450s, several conjugating enzymes, and transporters. We can use this
147 information when selecting genes and their polymorphisms for analysis.
148 For candidate gene analysis, we can use several methods, including
149 conventional sequencing (Sanger method) and novel sequencing methods using
150 highly-efficient sequencers (next generation). For analysis of known genetic
151 polymorphisms, a number of methods can be used, including the PCR-restriction
152 fragment length polymorphism (RFLP) method, the allele-specific PCR method, DNA
153 microarrays (products specialized for pharmacokinetics-related genes), and other
154 sequencing/PCR/hybridization-based methods [7, 8]. These methods can be selected
155 based on factors such as the number of samples, adjacent sequences of genetic
156 polymorphisms, instrument availability, and measurement duration.
157 Advantages of candidate gene analysis generally include its relatively low
158 cost, low multiplicity corrections in statistics since it measures limited numbers of
159 polymorphisms, and the need for a relatively small number of patients. A
160 disadvantage of this method is that it cannot find associated polymorphisms when
161 unknown molecules are involved in drug responsiveness. Blood or a buccal swab

could be used as a source of DNA, for example.

2.1.2 Comprehensive Analysis of Genetic Polymorphisms

Genetic polymorphisms that are involved in drug responsiveness can sometimes be difficult to determine, such as when unknown drug-metabolizing enzymes and transporters contribute to pharmacokinetics, efficacy, and adverse drug reactions. In these cases, it is necessity to search for related genetic polymorphisms by sequencing exon and/or transcriptional regulatory regions of several candidate genes or even performing genome-wide association studies (GWAS) for a large number of genetic polymorphisms.

An advantage of this approach is the possibility of finding genetic polymorphisms in unknown molecules related to drug responsiveness. The disadvantages include the high cost and the simultaneous detection of many genetic polymorphisms that may not cause functional changes.

At the stage of phase I clinical trial, it would be generally difficult to clarify the gene related to the pharmacokinetics and adverse drug reaction by the comprehensive analysis of genetic polymorphisms because of a small size of samples. However, if there is a gene associated with interindividual differences of pharmacokinetics, the comprehensive analysis can extract the candidates of relevant SNPs. Then, the in vitro functional analysis can be done as a non-clinical studies. So,

181 comprehensive analysis of genetic polymorphisms may be useful in phase I clinical
182 trial.

183 **2.2 Information on Known Genetic Polymorphisms and Their *In Vitro* Functional**
184 **Analysis**

185 This section describes reported genetic polymorphisms (allele frequencies of
186 ≥1% in the Japanese population) with functional changes (by *in vivo* or *in vitro*
187 studies) and how to evaluate the effects of these genetic polymorphisms on the
188 metabolic or transport activities of the investigational drug (Table 1). It is noteworthy
189 that several reports on cytochrome P450s have shown variable effects of genetic
190 polymorphisms with amino acid changes on enzymatic activities, depending on the
191 substrate structures [9, 10].

192

193 **2.2.1 Cytochrome P450**

194 Cytochrome P450 is a superfamily of metabolizing enzymes, and functional
195 effects have been widely reported with genetic polymorphisms. The primary enzymes
196 that contribute to drug metabolism and are expressed in the liver are CYP1A2,
197 CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5.
198 Isoforms with known functional polymorphisms with allele frequencies ≥1% in the
199 Japanese population, and thus could be examined in clinical trials, are CYP2A6,

200 CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5 [9, 11-16]. The
201 enzymes are anchored on the cytosolic side of the endoplasmic reticulum membrane,
202 and P450 oxidoreductase (POR) is required for their enzymatic activity. It is known
203 that the activities of some isoforms, such as CYP2C9 and CYP3A4, are elevated by
204 co-expression of cytochrome b_5. This co-expression could be important as it
205 facilitates the evaluation of enzymatic activity [17]. Functional analysis can be
206 performed using human liver microsomal fractions, in which genotype was
207 determined or microsomal fractions expressing recombinant enzymes bearing amino
208 acid alterations involved in genetic polymorphisms. Peptide sequences of the
209 membrane-anchored region are usually removed to allow soluble expression in *E. coli*
210 [18, 19]. When expressed in yeast [20, 21], insect [13, 22], or mammalian cells [23,
211 24], the enzymes are usually the membrane-anchored forms. It is important to co-
212 express or add POR in the assay system to ensure enzymatic activity, if cytochrome
213 P450s are expressed in *E. coli* or insect cells [13, 25, 26]. Although endogenous POR
214 can be used in yeast or mammal cell assays, some studies use elevated activity system
215 by co-expression or addition of POR [21]. Storing microsomal fractions at −80°C or
216 below is important to sustain enzyme activity of cytochrome P450s [27]. However, it
217 is known that storage of liver tissues at −80°C can decrease their enzymatic activity
218 [28]. In addition, an appropriate amount of NADPH or its regenerating system

(including NADP, glucose-6-phosphate dehydrogenase, and glucose-6-phosphate) must be present, taking the reaction time into account. Substrates used as positive controls for the measurement of enzymatic activity would be selected as described in the following section for each isoform. Omission of NADPH or a component of the regenerating system is often used as a negative control. Given that other metabolic pathways can potentially contribute to the reduction of the investigational drug, it is generally preferable to measure the metabolite production rate by the target metabolic pathways. However, an assessment of the reduction rate of the investigational drug is beneficial when evaluating the presence of alternate metabolic pathways, or when understanding the contribution of the target metabolic pathway to all of the metabolic pathways of the investigational drug.

In the section below, minor allele frequencies (MAFs) are the approximate reported values in the Japanese population.

2.2.1.1 CYP2A6

CYP2A6 contributes to 1–2% of the metabolism of marketed drugs [29] and there are marked ethnic differences in the frequency of *CYP2A6* alleles [30]. Known functional polymorphisms with allele frequencies of ≥1% in the Japanese population are as follows: *CYP2A6*4* (whole gene deletion, loss of activity, approximately 19%

238 in MAF), *CYP2A6*7* (1412T>C, I471T and gene conversion of 3'-flanking region,
239 decreased activity due to reduced protein stability, ~10% in MAF), and *CYP2A6*9* (-
240 1013A>G, -48T>G, decreased activity due to reduced gene expression level, ~19% in
241 MAF) [14, 15]. *CYP2A6*4*, **7*, and **9* are prevalent (10-19%) in Asian populations
242 but have a lower frequency (0-8%) in Caucasian populations [15, 30, 96]. Nicotine
243 (cotinine generation) and coumarin (7-hydroxylation) are frequently used as positive
244 controls for functional analysis of this isoform [31, 32].
245
246 **2.2.1.2 CYP2B6**
247 CYP2B6 contributes to 1–2% of the metabolism of marketed drugs [29].
248 Known functional polymorphisms with allele frequencies of ≥1% in the Japanese
249 population are as follows: *CYP2B6*4* (785A>G, K262R, ~9% in MAF), *CYP2B6*5*
250 (1459C>T, R487C, ~1% in MAF), and *CYP2B6*6* (516G>T, 785A>G, Q172H,
251 K262R, ~16% in MAF) [33]. In Caucasians, *CYP2B6*2*, **3*, **4*, **5*, **6*, and **7* were
252 found at variant frequencies of up to 30% [34]. There are reports of elevated protein
253 expression in *CYP2B6*4* and decreased protein expression in *CYP2B6*5* and
254 *CYP2B6*6* by *in vitro* analyses. For *CYP2B6*6*, decreased activity was observed
255 against efavirenz, while increased activity was observed for the metabolism of
256 cyclophosphamide and selegiline [16, 35]. Therefore, it is thought that the functional

257 effect of variation depends on the substrate. Efavirenz (8-hydroxylation) and
258 bupropion (hydroxylation), are frequently used as positive controls for functional
259 analysis [35].
260
261 **2.2.1.3 CYP2C8**
262 Since the year 2000, *CYP2C8* gene polymorphism has been identified in
263 various races. The *CYP2C8*3* allele is the most common in Caucasians
264 (approximately 13% in MAF) after the wild type *CYP2C8*1* allele [36]. Although
265 genetic polymorphisms that cause functional changes and have allele frequencies of
266 ≥1% were not reported in the Japanese population, *CYP2C8*2* (805A>T, I269F,
267 approximately 16% in MAF among black populations), *CYP2C8*3* (416G>A;
268 1196A>G, R139K; K399R, ~14% in MAF among Caucasians and 2% among black
269 populations), and *CYP2C8*4* (792C>G, I264M, ~6% in MAF in Caucasians) are
270 known to be associated with decreased metabolic activities [37, 38]. Substrates such
271 as paclitaxel (6α-hydroxylation) are often used as positive controls for functional
272 analysis of this isoform [38].
273
274 **2.2.1.3 CYP2C9**
275 CYP2C9 contributes to 15–25% of the metabolism of marketed drugs [29].

There are significant ethnic differences in the frequencies of *CYP2C9* variants [36]. *CYP2C9*3* (1075A>C, I359L, decreased activity), is a known functional polymorphism with an allele frequencies of 3% and 6% in the Japanese and Caucasian populations, respectively [12]. In contrast, *CYP2C9*2* (430C>T, R144C), which has approximately 14% in MAF among Caucasians, is not detected in Japanese. Diclofenac (4'-hydroxylation) and S-warfarin (7-hydroxylation) are frequently used as positive controls in the functional analysis [39-41].

2.2.1.4 CYP2C19

CYP2C19 contributes to 4–8% of the metabolism of marketed drugs [29]. Approximately 15-25% of the Japanese population are poor metabolizers of CYP2C19, whereas the poor metabolizer frequency in Caucasians is less than 5% [36]. Known functional polymorphisms with allele frequencies of ≥1% in the Japanese population are as follows: *CYP2C19*2* (681G>A, splicing defect, loss of activity, ~29% in MAF), *CYP2C19*3* (636G>A, W212Stop, loss of activity, ~12.4% in MAF), and *CYP2C19*17* (-806C>T, increased activity due to increased gene expression, 1% in MAF) [12]. S-mephenytoin (4'-hydroxylation) is an example of a positive control for the functional analysis [39-41]. However, the functional effects of these three genetic polymorphisms result from changes in protein expression levels. Therefore,

they are not considered to have significantly different effects on metabolism among substrates.

2.2.1.5 CYP2D6

CYP2D6 contributes to 20–30% of the metabolism of marketed drugs [29]. The frequency of CYP2D6 PMs is 5 to 10% in the Caucasian population and less than 1% in the Asian population [12]. Known functional polymorphisms with allele frequencies of ≥1% in the Japanese population are as follows: *CYP2D6*5* (whole gene deletion, loss of activity, ~6% in MAF), *CYP2D6*10* (100C>T and others, P34S and others, decreased activity, ~37.9% in MAF), and *CYP2D6*41* (2988G>A and others, splicing defect, decreased activity, ~1% in MAF) [12]. The decreased activity associated with *CYP2D6*10* is attributed to a decrease in both expression level and enzyme activity [9, 42-44]. Although the majority of these polymorphisms has been detected as a haplotype with *CYP2D6*10* (~30% in MAF), sole *CYP2D6*36* (gene conversion with *CYP2D7* in exon 9, loss of activity, ~1% in MAF) should also be taken into consideration [12, 45]. As for *CYP2D6*10*, it has been reported that ~5% are independent alleles and 26% are haplotypes as *CYP2D6*36-CYP2D6*10* [45]. In addition, although the frequency is low, it is noteworthy that there are many reported alleles with functional changes in this isoform: *CYP2D6*4* (1846G>A and others,

splicing defect, loss of activity, ~0.3% in MAF); *CYP2D6*21* (2573_2574insC, 267 frame-shift, loss of activity, ~0.6% in MAF), *CYP2D6*14* (1758G>A and others, G169R and others, decreased activity, ~0.3% in MAF), and *CYP2D6*18* (4125_4133dupGTGCCCACT, VPT duplication, decreased activity, ~0.5% in MAF) [9, 12, 46]. Please refer to a review paper for detailed descriptions of these low-frequency CYP2D6 genetic polymorphisms [44]. Bufuralol (1'-hydroxylation) and dextromethorphan (*o*-demethylation) are examples of substrates that are often used as positive controls in functional analysis [39-41]. Furthermore, *CYP2D6*3* (2549delA, frame shift, approximately 1.8% in MAF among Caucasians), *CYP2D6*4* (~20.5% in MAF among Caucasians and 5.7% among black populations), and *CYP2D6*6* (1707delT, frame shift, ~1.1% in MAF among Caucasians) are known polymorphisms associated with loss of enzymatic activity that occur at a frequency of ≥1% among Caucasians and black populations, but very low frequencies have been reported in Japanese populations [12]. In addition, a duplication polymorphism, *CYP2D6*2* × *CYP2D6*2* (~1.7% in MAF among black populations), leading to an ultra-rapid metabolizer phenotype has been reported.

2.2.1.6 CYP3A4

CYP3A4 contributes to 40–50% of the metabolism of marketed drugs [29].

Screening in different ethnic populations identified a number of *CYP3A4* variant alleles. Overall, variation of the *CYP3A4* genotype may contribute only to a minor extent or only in rare cases, to the interindividual differences in the CYP3A4 phenotypes. However, there is some evidence that *CYP3A4*16* displays reduced activity, which allele seems to be Asian-specific [47]. *CYP3A4*16* (554C>G, T185S, decreased activity, ~1.4% in MAF), is a known functional polymorphism with an allele frequency of ≥1% in Japanese [11]. Midazolam (1'-hydroxylation) and testosterone (6β-hydroxylation) are usually used as positive controls in the functional analysis [39-41]. Furthermore, since CYP3A4 and CYP3A5 have similar substrate specificity, it is recommended to use an expression system for each recombinant enzyme (cells or microsomes).

2.2.1.7 CYP3A5

*CYP3A5*3* (6986A>G, splicing defect, markedly decreased activity, ~76% in MAF) is a known functional polymorphism with an allele frequency of ≥1% in the Japanese population [12, 13]. There are significant ethnic differences in the frequency of *CYP3A5*3*, with allele frequencies ranging from 0.14 among sub-Saharan Africans to >0.95 in European populations [48]. Positive controls for the functional analysis of this isoform are similar to those used for CYP3A4. However, the effect of this genetic

polymorphism is a marked reduction in protein expression level, so its functional effect is similar among substrates.

2.2.2 Glucuronyl Transferase and Other Drug-Metabolizing Enzymes

This section describes genetic polymorphisms with functional changes of ≥1% in MAF in the Japanese population for drug-metabolizing enzymes other than cytochrome P450. Please see individual references for the functional analysis methods of each enzyme.

2.2.2.1 UGT1A1

Glucuronyl transferase, which transfers glucuronic acid to the substrate using a UDP-glucuronic acid, is a membrane-anchored enzyme that is expressed on the luminal side of the endoplasmic reticulum. Therefore, its activity generally increases after adding membrane pore-forming reagents, such as alamethicin, but the concentration used should be carefully titered. Recombinant UGT proteins are usually expressed in mammalian cells (e.g., COS-1 or HEK293), and by adding the substrate and UDP-glucuronic acid, functional studies are performed using microsomal fractions [49, 50]. Specific reactions of each isoform can be measured using recombinant UGT protein. However, other metabolic pathways can also contribute to

371 the loss of the investigational drug in assays using microsomes from human liver
372 cells, and thus, it is generally preferable to measure metabolite generation as the
373 metabolic activity. As a negative control, UDP-glucuronic acid is usually omitted.
374 UGT1A1 is one of nine types of UGT1A, and is expressed at high levels in the liver.
375 This enzyme is involved in the metabolism of bilirubin and is known to be a causative
376 gene for Gilbert's syndrome [51].
377 *UGT1A1*6* (211G>A, G71R, decreased activity, ~19% in MAF), *UGT1A1*28*
378 (-54_-39A(TA)$_6$TAA>A(TA)$_7$TAA, decreased activity due to reduced gene expression
379 level, ~13% in MAF); and *UGT1A1*60* (-3279T>G, decreased activity due to reduced
380 gene expression level, 26% in MAF [however, about half are linked to *UGT1A1*28*])
381 are known polymorphisms with an allele frequency of 1% or higher in Japanese [12,
382 52]. *UGT1A1*93* (-3156G>A) is known to be mostly in linkage disequilibrium with
383 *UGT1A1*28*. The frequencies of *UGT1A1*6* and **28* in Caucasians are up to 3% and
384 39%, respectively [12]. SN-38 (generation of 4-deoxy conjugate), a metabolite for
385 irinotecan, and estradiol (3-conjugation, also mediated by UGT2B7) are often used as
386 positive controls in the functional analysis of this enzyme [50, 53]. The functional
387 influence of both *UGT1A1*28* and *UGT1A1*60* is derived from altered protein
388 expression levels, and thus, their functional effects are considered to be similar among
389 substrates.

390

391 **2.2.2.2 NAT2**

392 N-acetyltransferase 2 transfers the acetyl group from acetyl-CoA to the
393 substrates and is expressed in the cytosol [54]. The antituberculosis drug, isoniazid,
394 can be used as a positive control substrate for the functional analysis of this enzyme.
395 However, although there are reports using environmental chemicals as substrates [55],
396 *in vitro* functional analyses with recombinant NAT2 enzyme using isoniazid as a
397 substrate have not been reported to date. Therefore, in non-clinical studies, the
398 influence of genetic polymorphisms of NAT2 can be evaluated by assessing the
399 relationship between the blood concentration ratio of the investigational drug and its
400 NAT2 metabolite (e.g., area under the curve [AUC] ratio) using blood samples from
401 dosed patients, and the results of a genetic polymorphism screening of the patients in
402 the clinical trials. For example, functional changes of NAT2 by genetic
403 polymorphisms can be evaluated from the ratio of isoniazid and its metabolite
404 acetylisoniazid, 3 h after a single dose [56] [57]. The genetic polymorphisms show
405 ethnic differences in enzyme activities; almost 50% of Caucasian are slow acetylators,
406 whereas the frequency of slow acetylators in Japanese populations is only 10% [58].
407 Of all the NAT2 allelic variants that had been identified, 3 variants (*NAT2*5*,
408 *NAT2*6*, and *NAT2*7*) have been shown to account for the majority of the slow

409 acetylator genotypes in Japanese subjects [59]. *NAT2*4* is a wildtype allele. *NAT2*5*
410 (341T>C, I114T, approximately 1% in MAF), *NAT2*6* (590G>A, R197Q, ~21% in
411 MAF), and *NAT2*7* (857G>A, G286E, ~9% in MAF) are decreased activity alleles
412 with known MAF ≥1% in the Japanese population [12]. Furthermore, the frequency of
413 isoniazid-induced liver injury is reported to be significantly higher in the low activity
414 genotypes (homozygotes or compound heterozygotes of *NAT2*5*, *NAT2*6*, and
415 *NAT2*7*) [60, 61].
416
417 **2.2.2.3 GSTM1/GSTT1**
418 These enzymes belong to the glutathione transferase group. The reduced form
419 of glutathione is transferred to the electrophilic compounds (e.g., reactive
420 metabolites), which contributes to reductions in, for example, oxidative stress [62].
421 *GSTM1*0/GSTT1*0* (both are whole-gene deletions), which cause loss of
422 activities together with GSTM1 and GSTT1, are known polymorphisms with an allele
423 frequency of ≥1% in Japanese populations [12]. The allele frequencies in Japanese
424 subjects are approximately 50% for both *GSTM1*0* and *GSTT1*0*. In contrast, the
425 frequencies of *GSTM1*0* and *GSTT1*0* in Caucasians are 53% and 20%, respectively
426 [12]. An *in vitro* functional analysis for metabolic activities of these alleles is
427 considered unnecessary because of the gene deletions.

2.2.2.4 TPMT

Thiopurine methyltransferase (TPMT) is a cytosolic enzyme that transfers the methyl group from S-adenosylmethionine to substrates [63]. As a functional analysis method, wildtype and variant-type enzymes are expressed in mammalian cells (COS-1 or COS-7). Then, substrate and S-adenosylmethionine are added to the cytosolic fraction, and activity is measured [64, 65]. In addition, red blood cell lysates from peripheral blood, as an enzyme source, are also frequently used with 6-mercaptopurine as a substrate to determine the metabolic production of 6-mercaptopurine using high-performance liquid chromatography (HPLC)-ultraviolet (UV) detection [66].

*TPMT*3A* (460A>G and 719A>G) was found in European Caucasians, African-Americans, and Southwest Asians and is the most prevalent allele in European Caucasians (approximately 5% in MAF), but not detected in Japanese [67]. *TPMT*3C* (719A>G, Y240C, ~1% in MAF), which causes decreased activity, is the most prevalent allele with an frequency of ≥1% in Japanese populations [68]. Compounds, including 6-mercaptopurine (S-methylation) and 6-thioguanine (S-methylation), are often used as positive controls in the functional analysis of this enzyme [64, 65].

2.2.2.5 ALDH2

Aldehyde dehydrogenase (ALDH) is an enzyme that metabolizes aldehyde to carboxylic acid and is known to metabolize acetaldehyde (an ethanol metabolite) into acetic acid. It is known to metabolize nitroglycerin [69, 70]. Although few functional analyses have been reported, some have used recombinant wildtype or variant-type enzymes expressed in *E. coli* or insect cells (Sf9) [71, 72]. Approximately 50% of Japanese have inactive ALDH2 whereas none of the Caucasian show this isozyme abnormality [73].

*ALDH2*2* (1510G>A, E504K, decreased activity, ~27% in MAF) is a known polymorphism with an allele frequency of ≥1% in Japanese subjects [73, 74]. Nitroglycerin (1, 2-dinitro glycerol generation) and acetaldehyde (acetic acid generation) are commonly used as positive controls in the functional analysis of this enzyme [71, 72].

2.2.3 Drug Transporters

Drug transporters are responsible for the uptake/excretion of drugs into/from tissues such as small intestine, liver, and kidney. Generally, a drug can be a substrate of multiple transporters, and in many cases, one transporter is not rate-limiting for

466 drug clearance. In addition, transporter functions sometimes affect tissue
467 concentrations of the drug more strongly than they do blood concentrations.
468 Therefore, the functional significance of transporter genetic polymorphisms is often
469 difficult to assess, and thus, many transporter polymorphisms await establishment of
470 their clinical significance, including ABCB1 (P-gp) polymorphisms. Therefore,
471 smaller numbers of functionally significant genetic polymorphisms have been
472 established compared with those of metabolizing enzymes. However, evaluation of the
473 investigational drugs that become substrates for the following two transporters is
474 worthwhile, since highly frequent and functionally significant genetic polymorphisms
475 were reported in the Japanese population [75].
476
477 **2.2.3.1 OATP1B1 (*SLCO1B1*)**
478 Organic anion transporting polypeptide (OATP)1B1, encoded by *SLCO1B1*,
479 is primarily expressed in the basolateral (basal) membrane of hepatocytes and
480 facilitates the transport from the blood into cells of organic compounds with an
481 anionic state at physiological pH [76]. Wild and variant-type transporters can be
482 expressed in mammalian cells (e.g., HEK293 and HeLa), and are used to determine
483 the initial velocity of transporting substrates into cells as an *in vitro* functional
484 analysis system [77, 78].

The alleles 521T>C (V174A, decreased expression and activity, approximately 13.9% in MAF, named *OATP1B1*5*) [12] and 388A>G (N130D, increased activity, ~66.7% in MAF, *OATP1B1*1b*) [79] are known polymorphisms with an allele frequency of ≥1% among Japanese populations. The 521T>C is in linkage disequilibrium with 388A>G, and they form haplotypes (*OATP1B1*15* and *OATP1B1*17*), especially in Japanese subjects [79]. Many clinical studies have reported significantly increased blood concentrations of most OATP1B1 substrates in 521T>C (*OATP1B1*5, OATP1B1*15, OATP1B1*17*) carriers. However, a significant decrease or tendency toward decrease in the blood concentrations of some OATP1B1 substrates was observed in *OATP1B1*1b* (bearing only 388A>G, 46.9% in MAF) carriers [80]. The allele frequencies of "*OATP1B1*5, OATP1B1*15, OATP1B1*17*" and *OATP1B1*1b* vary between races; approximately 16% and 25% in Caucasians and 5% and 76% in African populations, respectively [12]. The 388A>G is assumed to cause elevated transporting function via increased OATP1B1 protein expression, because increased protein expression was detected with an increased number of 388A>G alleles in human liver, but no change was observed in the net transporting activity. [81]. Estrone 3-sulfate and pitavastatin, for example, are often used as positive controls in functional analysis [82]. The substrate selectivity of OATP1B1 is known to be similar to that of OATP1B3 and much overlap is observed for both

transporter substrates. In addition, as mentioned previously, the functional effect of the 388A>G could be derived from increased protein expression levels, so that its functional effect is probably similar among substrates.

2.2.3.2 *ABCG2* (BCRP)

The ATP-binding cassette transporters transport substrates from cells using ATP as an energy source. Breast cancer resistance protein (BCRP), encoded by *ABCG2*, is a multi-drug resistant transporter expressed in the canalicular (apical) membrane of the bile duct, small intestine, and rectum [83]. As *in vitro* functional assay systems, wild and variant-type transporters are expressed in mammalian cells (e.g., HEK293), and the velocities of substrate accumulation into the cells are compared. Another method utilizes the expression of recombinant wild and variant-type transporters in polarized cells (such as Caco-2), and the flux ratios [i.e., the permeability of the investigational drug from the apical side (A) to the basal side (B) and the permeability of the drug in the opposite direction (from B to A) are measured and expressed as a ratio (B to A/A to B)] are compared between the expressed cells. In this method, a correction using the flux ratio of non-expressing cells is applied to calculate the net flux ratio [(flux ratio of expressing cells)/(flux ratio of non-expressing cells)]. The third method uses assays that compare the velocity of substrate

accumulation into vesicles expressing wild and variant-type transporters [84].

The allele 376C>T（Q126X, loss of activity, ~1% in MAF) and 421C>A (Q141K, decreased expression and activity, ~31% in MAF) are known polymorphisms with an allele frequency of ≥1% among Japanese populations [12, 85]. The allele frequency of 421C>A in Japanese subjects is about 3-fold higher than that in Caucasians (11%) [12]. Estrone 3-sulfate and sulfasalazine, for example, often used as positive controls in the functional analysis. Estrone 3-sulfate is also a substrate of OATP1B1. The functional effect of the 376C>T polymorphism is based on the loss of protein expression, so its functional effect is considered similar among substrates.

2.2.4 Efficacy or Adverse Drug Reaction-Related Molecules

Genetic polymorphisms can potentially have an influence on the efficacy or safety of drugs, where amino acid substitutions or altered expression levels of molecules related to efficacy or adverse drug reactions are observed. Please refer to the appropriate papers for specific functional analyses, since the methods vary for each molecule. As examples, two cases are described in the following section.

2.2.4.1 EGFR/K-RAS

Recently, tyrosine kinase inhibitors and antibodies against epidermal growth

542 factor receptor (EGFR) have been developed for the treatment of various cancers, and
543 somatic mutations of EGFR and its downstream molecules are reported to be
544 associated with the drug's efficacy. For example, patients with L858R or deletion of
545 exon 19 (variations of the tyrosine kinase domain, collectively reported to account for
546 85% of mutations) of EGFR, showed higher response rates to gefitinib [3, 86]. K-
547 RAS, an intracellular signal transduction molecule of EGFR, is also known to have
548 somatic mutations that alter the glycine at codon 12 or 13, which is associated with
549 low efficacy of cetuximab and panitumumab (low response rates and short overall
550 survival time) [87, 88]. These acquired mutations are only detected in tumor cells (in
551 somatic cell lineages), and thus are not detected in leukocyte-derived genomic DNA
552 in the blood. It is noteworthy that use of a highly sensitive and specific detection
553 method is important, because the percentage of the cells bearing the mutations are
554 sometimes very low [89].
555
556 **2.2.4.2 HLA**
557 Some types of human leukocyte antigens (HLAs) are known to be related to
558 specific drug-induced severe cutaneous adverse reactions (SCARs) and hepatotoxicity,
559 which are believed to be idiosyncratic. For example, *HLA-B*58:01* is associated with
560 allopurinol-induced SCARs, and HLA-B75 (for example, *HLA-B75*15:02* or *HLA-*

561 *B75*15:11*) and *HLA-A*31:01* are associated with carbamazepine-induced SCARs
562 [90]. These data suggest that HLA molecules are off-targets of these drugs. In fact,
563 carbamazepine and oxypurinol, an allopurinol metabolite, were suggested to bind to
564 HLA-B75 and HLA-B*58:01 proteins, respectively; these bindings are known to
565 induce cytotoxic T cell activation [91, 92].
566
567 **3. Points to Consider when Designing Phase I Clinical Trials, Based on the**
568 **Results of the Non-Clinical Studies**
569 ● When the drug is metabolized by a single enzyme, the metabolism will be greatly
570 influenced by its genetic polymorphisms. In such cases, functional evaluation of
571 the genetic polymorphisms for the investigational drug through the methods
572 described in this paper is of general importance. However, in the case of multiple
573 enzyme involvement in the investigational drug metabolism, the effect of genetic
574 polymorphisms in one metabolizing enzyme will be limited.
575 ● In some cases, care must be exercised when other drugs are co-administered.
576 Where one metabolic pathway is affected by genetic polymorphisms and the other
577 is inhibited by a co-administered drug (i.e., drug interaction), safety issues need
578 to be considered [93, 94]. In such cases, functional evaluation of genetic
579 polymorphisms using the methods described in this report will be important.

- The frequencies of several genetic polymorphisms are known to show ethnic differences. During drug development, it is important to consider the allele frequency of genetic polymorphisms in each ethnicity when such genetic polymorphisms are considered to have an effect on pharmacokinetics, efficacy, or the safety of the investigational drug. In general, however, the differences in allele frequencies between adjacent populations, such as within Europeans or East Asians, are not large for drug-metabolizing enzymes and transporters [12].

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Conflict of interest

The authors declare no conflict of interest.

References

[1] International Warfarin Pharmacogenetics C, Klein TE, Altman RB, Eriksson N, Gage BF, Kimmel SE, Lee MT, Limdi NA, Page D, Roden DM, Wagner MJ, Caldwell MD, Johnson JA. Estimation of the warfarin dose with clinical and pharmacogenetic data. *N Engl J Med* 2009;360: 753-64.

[2] Sai K, Sawada J, Minami H. [Irinotecan pharmacogenetics in Japanese cancer patients: roles of UGT1A1*6 and *28]. *Yakugaku Zasshi* 2008;128: 575-84.

[3] Shigematsu H, Gazdar AF. Somatic mutations of epidermal growth factor receptor signaling pathway in lung cancers. *Int J Cancer* 2006;118: 257-62.

[4] Hulot JS, Collet JP, Silvain J, Pena A, Bellemain-Appaix A, Barthelemy O, Cayla G, Beygui F, Montalescot G. Cardiovascular risk in clopidogrel-treated patients according to cytochrome P450 2C19*2 loss-of-function allele or proton pump inhibitor coadministration: a systematic meta-analysis. *J Am Coll Cardiol* 2010;56: 134-43.

[5] Zabalza M, Subirana I, Sala J, Lluis-Ganella C, Lucas G, Tomas M, Masia R, Marrugat J, Brugada R, Elosua R. Meta-analyses of the association between cytochrome CYP2C19 loss- and gain-of-function polymorphisms and cardiovascular outcomes in patients with coronary artery disease treated with clopidogrel. *Heart* 2012;98: 100-8.

[6] Watanabe I, Tomita A, Shimizu M, Sugawara M, Yasumo H, Koishi R, Takahashi T, Miyoshi K, Nakamura K, Izumi T, Matsushita Y, Furukawa H, Haruyama H, Koga T. A study to survey susceptible genetic factors responsible for troglitazone-associated hepatotoxicity in Japanese patients with type 2 diabetes mellitus. *Clin Pharmacol Ther* 2003;73: 435-55.

[7] Deeken J. The Affymetrix DMET platform and pharmacogenetics in drug development. *Curr Opin Mol Ther* 2009;11: 260-8.

[8] Hiratsuka M. [Development of simplified and rapid detection assay for genetic polymorphisms influencing drug response and its clinical applications]. *Yakugaku Zasshi* 2002;122: 451-63.

[9] Hiratsuka M. In vitro assessment of the allelic variants of cytochrome P450. *Drug Metab Pharmacokinet* 2012;27: 68-84.

[10] Maekawa K, Harakawa N, Yoshimura T, Kim SR, Fujimura Y, Aohara F, Sai K, Katori N, Tohkin M, Naito M, Hasegawa R, Okuda H, Sawada J, Niwa T, Saito Y. CYP3A4*16 and CYP3A4*18 alleles found in East Asians exhibit differential catalytic activities for seven CYP3A4 substrate drugs. *Drug Metab Dispos* 2010;38: 2100-4.

[11] Fukushima-Uesaka H, Saito Y, Watanabe H, Shiseki K, Saeki M, Nakamura T, Kurose K, Sai K, Komamura K, Ueno K, Kamakura S, Kitakaze M, Hanai S, Nakajima T, Matsumoto K, Saito H, Goto Y, Kimura H, Katoh M, Sugai K, Minami N, Shirao K, Tamura T, Yamamoto N, Minami H, Ohtsu A, Yoshida T, Saijo N, Kitamura Y, Kamatani N, Ozawa S, Sawada J. Haplotypes of CYP3A4 and their close linkage with CYP3A5 haplotypes in a Japanese population. *Hum Mutat* 2004;23: 100.

[12] Kurose K, Sugiyama E, Saito Y. Population differences in major functional polymorphisms of pharmacokinetics/pharmacodynamics-related genes in Eastern Asians and Europeans: implications in the clinical trials for novel drug development. *Drug Metab Pharmacokinet* 2012;27: 9-54.

[13] Maekawa K, Yoshimura T, Saito Y, Fujimura Y, Aohara F, Emoto C, Iwasaki K, Hanioka N, Narimatsu S, Niwa T, Sawada J. Functional characterization of CYP3A4.16: catalytic activities toward midazolam and carbamazepine. *Xenobiotica* 2009;39: 140-7.

[14] Minematsu N, Nakamura H, Furuuchi M, Nakajima T, Takahashi S, Tateno H, Ishizaka A. Limitation of cigarette consumption by CYP2A6*4, *7 and *9 polymorphisms. *Eur Respir J* 2006;27: 289-92.

[15] Nakajima M, Fukami T, Yamanaka H, Higashi E, Sakai H, Yoshida R, Kwon JT, McLeod HL, Yokoi T. Comprehensive evaluation of variability in nicotine metabolism and CYP2A6 polymorphic alleles in four ethnic populations. *Clin Pharmacol Ther* 2006;80: 282-97.

[16] Watanabe T, Sakuyama K, Sasaki T, Ishii Y, Ishikawa M, Hirasawa N, Hiratsuka M. Functional characterization of 26 CYP2B6 allelic variants (CYP2B6.2-

CYP2B6.28, except CYP2B6.22). *Pharmacogenet Genomics* 2010;20: 459-62.

[17] Peyronneau MA, Renaud JP, Truan G, Urban P, Pompon D, Mansuy D. Optimization of yeast-expressed human liver cytochrome P450 3A4 catalytic activities by coexpressing NADPH-cytochrome P450 reductase and cytochrome b5. *Eur J Biochem* 1992;207: 109-16.

[18] Iwata H, Fujita K, Kushida H, Suzuki A, Konno Y, Nakamura K, Fujino A, Kamataki T. High catalytic activity of human cytochrome P450 co-expressed with human NADPH-cytochrome P450 reductase in Escherichia coli. *Biochem Pharmacol* 1998;55: 1315-25.

[19] Yamazaki H, Nakamura M, Komatsu T, Ohyama K, Hatanaka N, Asahi S, Shimada N, Guengerich FP, Shimada T, Nakajima M, Yokoi T. Roles of NADPH-P450 reductase and apo- and holo-cytochrome b5 on xenobiotic oxidations catalyzed by 12 recombinant human cytochrome P450s expressed in membranes of Escherichia coli. *Protein Expr Purif* 2002;24: 329-37.

[20] Imaoka S, Yamada T, Hiroi T, Hayashi K, Sakaki T, Yabusaki Y, Funae Y. Multiple forms of human P450 expressed in Saccharomyces cerevisiae. Systematic characterization and comparison with those of the rat. *Biochem Pharmacol* 1996;51: 1041-50.

[21] Rao KN, Suman SK, Kiran YR, Kuanr AR, Gupta AK, Bhalla K, Kumar V, Kundu P, Arora K, Soni R. Co-expression of recombinant human CYP2C9 with human cytochrome P450 reductase in protease deficient S. cerevisiae strain at a higher scale yields an enzyme of higher specific activity. *Drug Metab Lett* 2010;4: 246-53.

[22] Yu A, Kneller BM, Rettie AE, Haining RL. Expression, purification, biochemical characterization, and comparative function of human cytochrome P450 2D6.1, 2D6.2, 2D6.10, and 2D6.17 allelic isoforms. *J Pharmacol Exp Ther* 2002;303: 1291-300.

[23] Saito Y, Hanioka N, Maekawa K, Isobe T, Tsuneto Y, Nakamura R, Soyama A, Ozawa S, Tanaka-Kagawa T, Jinno H, Narimatsu S, Sawada J. Functional analysis of three CYP1A2 variants found in a Japanese population. *Drug Metab Dispos* 2005;33: 1905-10.

712 [24] Sakuyama K, Sasaki T, Ujiie S, Obata K, Mizugaki M, Ishikawa M, Hiratsuka M.
713 Functional characterization of 17 CYP2D6 allelic variants (CYP2D6.2, 10, 14A-B,
714 18, 27, 36, 39, 47-51, 53-55, and 57). *Drug Metab Dispos* 2008;36: 2460-7.
715
716 [25] Lee CA, Kadwell SH, Kost TA, Serabjit-Singh CJ. CYP3A4 expressed by insect cells
717 infected with a recombinant baculovirus containing both CYP3A4 and human
718 NADPH-cytochrome P450 reductase is catalytically similar to human liver
719 microsomal CYP3A4. *Arch Biochem Biophys* 1995;319: 157-67.
720
721 [26] Pritchard MP, Glancey MJ, Blake JA, Gilham DE, Burchell B, Wolf CR, Friedberg
722 T. Functional co-expression of CYP2D6 and human NADPH-cytochrome P450
723 reductase in Escherichia coli. *Pharmacogenetics* 1998;8: 33-42.
724
725 [27] Yamazaki H, Inoue K, Turvy CG, Guengerich FP, Shimada T. Effects of freezing,
726 thawing, and storage of human liver samples on the microsomal contents and
727 activities of cytochrome P450 enzymes. *Drug Metab Dispos* 1997;25: 168-74.
728
729 [28] Pearce RE, McIntyre CJ, Madan A, Sanzgiri U, Draper AJ, Bullock PL, Cook DC,
730 Burton LA, Latham J, Nevins C, Parkinson A. Effects of freezing, thawing, and
731 storing human liver microsomes on cytochrome P450 activity. *Arch Biochem*
732 *Biophys* 1996;331: 145-69.
733
734 [29] Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into
735 rational therapeutics. *Science* 1999;286: 487-91.
736
737 [30] Di YM, Chow VD, Yang LP, Zhou SF. Structure, function, regulation and
738 polymorphism of human cytochrome P450 2A6. *Curr Drug Metab* 2009;10: 754-80.
739
740 [31] Higashi E, Nakajima M, Katoh M, Tokudome S, Yokoi T. Inhibitory effects of
741 neurotransmitters and steroids on human CYP2A6. *Drug Metab Dispos* 2007;35:
742 508-14.
743
744 [32] Yoo HH, Lee MW, Kim YC, Yun CH, Kim DH. Mechanism-based inactivation of
745 cytochrome P450 2A6 by decursinol angelate isolated from Angelica Gigas. *Drug*
746 *Metab Dispos* 2007;35: 1759-65.
747
748 [33] Hiratsuka M, Takekuma Y, Endo N, Narahara K, Hamdy SI, Kishikawa Y, Matsuura
749 M, Agatsuma Y, Inoue T, Mizugaki M. Allele and genotype frequencies of CYP2B6

and CYP3A5 in the Japanese population. *Eur J Clin Pharmacol* 2002;58: 417-21.

[34] Mo SL, Liu YH, Duan W, Wei MQ, Kanwar JR, Zhou SF. Substrate specificity, regulation, and polymorphism of human cytochrome P450 2B6. *Curr Drug Metab* 2009;10: 730-53.

[35] Zanger UM, Klein K. Pharmacogenetics of cytochrome P450 2B6 (CYP2B6): advances on polymorphisms, mechanisms, and clinical relevance. *Front Genet* 2013;4: 24.

[36] Hiratsuka M. Genetic Polymorphisms and in Vitro Functional Characterization of CYP2C8, CYP2C9, and CYP2C19 Allelic Variants. *Biol Pharm Bull* 2016;39: 1748-59.

[37] Pechandova K, Buzkova H, Matouskova O, Perlik F, Slanar O. Genetic polymorphisms of CYP2C8 in the Czech Republic. *Genet Test Mol Biomarkers* 2012;16: 812-6.

[38] Soyama A, Saito Y, Hanioka N, Murayama N, Nakajima O, Katori N, Ishida S, Sai K, Ozawa S, Sawada JI. Non-synonymous single nucleotide alterations found in the CYP2C8 gene result in reduced in vitro paclitaxel metabolism. *Biol Pharm Bull* 2001;24: 1427-30.

[39] Tucker GT, Houston JB, Huang SM. Optimizing drug development: strategies to assess drug metabolism/transporter interaction potential--toward a consensus. *Pharm Res* 2001;18: 1071-80.

[40] Walsky RL, Obach RS. Validated assays for human cytochrome P450 activities. *Drug Metab Dispos* 2004;32: 647-60.

[41] Yuan R, Madani S, Wei XX, Reynolds K, Huang SM. Evaluation of cytochrome P450 probe substrates commonly used by the pharmaceutical industry to study in vitro drug interactions. *Drug Metab Dispos* 2002;30: 1311-9.

[42] Hanioka N, Okumura Y, Saito Y, Hichiya H, Soyama A, Saito K, Ueno K, Sawada J, Narimatsu S. Catalytic roles of CYP2D6.10 and CYP2D6.36 enzymes in mexiletine metabolism: in vitro functional analysis of recombinant proteins expressed in Saccharomyces cerevisiae. *Biochem Pharmacol* 2006;71: 1386-95.

[43] Muroi Y, Saito T, Takahashi M, Sakuyama K, Niinuma Y, Ito M, Tsukada C, Ohta K, Endo Y, Oda A, Hirasawa N, Hiratsuka M. Functional characterization of wild-type and 49 CYP2D6 allelic variants for N-desmethyltamoxifen 4-hydroxylation activity. *Drug Metab Pharmacokinet* 2014;29: 360-6.

[44] Nakamura K, Ariyoshi N, Yokoi T, Ohgiya S, Chida M, Nagashima K, Inoue K, Kodama T, Shimada N, Kamataki T. CYP2D6.10 present in human liver microsomes shows low catalytic activity and thermal stability. *Biochem Biophys Res Commun* 2002;293: 969-73.

[45] Soyama A, Saito Y, Kubo T, Miyajima A, Ohno Y, Komamura K, Ueno K, Kamakura S, Kitakaze M, Tomoike H, Ozawa S, Sawada J. Sequence-based analysis of the CYP2D6*36-CYP2D6*10 tandem-type arrangement, a major CYP2D6*10 haplotype in the Japanese population. *Drug Metab Pharmacokinet* 2006;21: 208-16.

[46] Chida M, Yokoi T, Kosaka Y, Chiba K, Nakamura H, Ishizaki T, Yokota J, Kinoshita M, Sato K, Inaba M, Aoki Y, Gonzalez FJ, Kamataki T. Genetic polymorphism of CYP2D6 in the Japanese population. *Pharmacogenetics* 1999;9: 601-5.

[47] Werk AN, Cascorbi I. Functional gene variants of CYP3A4. *Clin Pharmacol Ther* 2014;96: 340-8.

[48] Suarez-Kurtz G, Vargens DD, Santoro AB, Hutz MH, de Moraes ME, Pena SD, Ribeiro-dos-Santos A, Romano-Silva MA, Struchiner CJ. Global pharmacogenomics: distribution of CYP3A5 polymorphisms and phenotypes in the Brazilian population. *PLoS One* 2014;9: e83472.

[49] Gagne JF, Montminy V, Belanger P, Journault K, Gaucher G, Guillemette C. Common human UGT1A polymorphisms and the altered metabolism of irinotecan active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38). *Mol Pharmacol* 2002;62: 608-17.

[50] Jinno H, Tanaka-Kagawa T, Hanioka N, Saeki M, Ishida S, Nishimura T, Ando M, Saito Y, Ozawa S, Sawada J. Glucuronidation of 7-ethyl-10-hydroxycamptothecin (SN-38), an active metabolite of irinotecan (CPT-11), by human UGT1A1 variants, G71R, P229Q, and Y486D. *Drug Metab Dispos* 2003;31: 108-13.

826 [51] Guillemette C. Pharmacogenomics of human UDP-glucuronosyltransferase
827 enzymes. *Pharmacogenomics J* 2003;3: 136-58.
828
829 [52] Saeki M, Saito Y, Jinno H, Sai K, Ozawa S, Kurose K, Kaniwa N, Komamura K,
830 Kotake T, Morishita H, Kamakura S, Kitakaze M, Tomoike H, Shirao K, Tamura T,
831 Yamamoto N, Kunitoh H, Hamaguchi T, Yoshida T, Kubota K, Ohtsu A, Muto M,
832 Minami H, Saijo N, Kamatani N, Sawada JI. Haplotype structures of the UGT1A
833 gene complex in a Japanese population. *Pharmacogenomics J* 2006;6: 63-75.
834
835 [53] Soars MG, Ring BJ, Wrighton SA. The effect of incubation conditions on the enzyme
836 kinetics of udp-glucuronosyltransferases. *Drug Metab Dispos* 2003;31: 762-7.
837
838 [54] Hein DW, Doll MA, Fretland AJ, Leff MA, Webb SJ, Xiao GH, Devanaboyina US,
839 Nangju NA, Feng Y. Molecular genetics and epidemiology of the NAT1 and NAT2
840 acetylation polymorphisms. *Cancer Epidemiol Biomarkers Prev* 2000;9: 29-42.
841
842 [55] Ferguson RJ, Doll MA, Rustan TD, Gray K, Hein DW. Cloning, expression, and
843 functional characterization of two mutant (NAT2(191) and NAT2(341/803)) and
844 wild-type human polymorphic N-acetyltransferase (NAT2) alleles. *Drug Metab*
845 *Dispos* 1994;22: 371-6.
846
847 [56] Seifart HI, Parkin DP, Botha FJ, Donald PR, Van Der Walt BJ. Population screening
848 for isoniazid acetylator phenotype. *Pharmacoepidemiol Drug Saf* 2001;10: 127-34.
849
850 [57] Hiratsuka M, Kishikawa Y, Takekuma Y, Matsuura M, Narahara K, Inoue T, Hamdy
851 SI, Endo N, Goto J, Mizugaki M. Genotyping of the N-acetyltransferase2
852 polymorphism in the prediction of adverse drug reactions to isoniazid in Japanese
853 patients. *Drug Metab Pharmacokinet* 2002;17: 357-62.
854
855 [58] Cascorbi I, Drakoulis N, Brockmoller J, Maurer A, Sperling K, Roots I. Arylamine
856 N-acetyltransferase (NAT2) mutations and their allelic linkage in unrelated
857 Caucasian individuals: correlation with phenotypic activity. *Am J Hum Genet*
858 1995;57: 581-92.
859
860 [59] Mashimo M, Suzuki T, Abe M, Deguchi T. Molecular genotyping of N-acetylation
861 polymorphism to predict phenotype. *Hum Genet* 1992;90: 139-43.
862
863 [60] Huang YS, Chern HD, Su WJ, Wu JC, Lai SL, Yang SY, Chang FY, Lee SD.

Polymorphism of the N-acetyltransferase 2 gene as a susceptibility risk factor for antituberculosis drug-induced hepatitis. *Hepatology* 2002;35: 883-9.

[61] Possuelo LG, Castelan JA, de Brito TC, Ribeiro AW, Cafrune PI, Picon PD, Santos AR, Teixeira RL, Gregianini TS, Hutz MH, Rossetti ML, Zaha A. Association of slow N-acetyltransferase 2 profile and anti-TB drug-induced hepatotoxicity in patients from Southern Brazil. *Eur J Clin Pharmacol* 2008;64: 673-81.

[62] Strange RC, Spiteri MA, Ramachandran S, Fryer AA. Glutathione-S-transferase family of enzymes. *Mutat Res* 2001;482: 21-6.

[63] Wang L, Weinshilboum R. Thiopurine S-methyltransferase pharmacogenetics: insights, challenges and future directions. *Oncogene* 2006;25: 1629-38.

[64] Salavaggione OE, Wang L, Wiepert M, Yee VC, Weinshilboum RM. Thiopurine S-methyltransferase pharmacogenetics: variant allele functional and comparative genomics. *Pharmacogenet Genomics* 2005;15: 801-15.

[65] Ujiie S, Sasaki T, Mizugaki M, Ishikawa M, Hiratsuka M. Functional characterization of 23 allelic variants of thiopurine S-methyltransferase gene (TPMT*2 - *24). *Pharmacogenet Genomics* 2008;18: 887-93.

[66] Chouchana L, Narjoz C, Roche D, Golmard JL, Pineau B, Chatellier G, Beaune P, Loriot MA. Interindividual variability in TPMT enzyme activity: 10 years of experience with thiopurine pharmacogenetics and therapeutic drug monitoring. *Pharmacogenomics* 2014;15: 745-57.

[67] Tai HL, Krynetski EY, Yates CR, Loennechen T, Fessing MY, Krynetskaia NF, Evans WE. Thiopurine S-methyltransferase deficiency: two nucleotide transitions define the most prevalent mutant allele associated with loss of catalytic activity in Caucasians. *Am J Hum Genet* 1996;58: 694-702.

[68] Hiratsuka M, Inoue T, Omori F, Agatsuma Y, Mizugaki M. Genetic analysis of thiopurine methyltransferase polymorphism in a Japanese population. *Mutat Res* 2000;448: 91-5.

[69] Kollau A, Hofer A, Russwurm M, Koesling D, Keung WM, Schmidt K, Brunner F, Mayer B. Contribution of aldehyde dehydrogenase to mitochondrial bioactivation of

nitroglycerin: evidence for the activation of purified soluble guanylate cyclase through direct formation of nitric oxide. *Biochem J* 2005;385: 769-77.

[70] Mackenzie IS, Maki-Petaja KM, McEniery CM, Bao YP, Wallace SM, Cheriyan J, Monteith S, Brown MJ, Wilkinson IB. Aldehyde dehydrogenase 2 plays a role in the bioactivation of nitroglycerin in humans. *Arterioscler Thromb Vasc Biol* 2005;25: 1891-5.

[71] Beretta M, Gruber K, Kollau A, Russwurm M, Koesling D, Goessler W, Keung WM, Schmidt K, Mayer B. Bioactivation of nitroglycerin by purified mitochondrial and cytosolic aldehyde dehydrogenases. *J Biol Chem* 2008;283: 17873-80.

[72] Li Y, Zhang D, Jin W, Shao C, Yan P, Xu C, Sheng H, Liu Y, Yu J, Xie Y, Zhao Y, Lu D, Nebert DW, Harrison DC, Huang W, Jin L. Mitochondrial aldehyde dehydrogenase-2 (ALDH2) Glu504Lys polymorphism contributes to the variation in efficacy of sublingual nitroglycerin. *J Clin Invest* 2006;116: 506-11.

[73] Maezawa Y, Yamauchi M, Toda G, Suzuki H, Sakurai S. Alcohol-metabolizing enzyme polymorphisms and alcoholism in Japan. *Alcohol Clin Exp Res* 1995;19: 951-4.

[74] Goedde HW, Agarwal DP, Fritze G, Meier-Tackmann D, Singh S, Beckmann G, Bhatia K, Chen LZ, Fang B, Lisker R, et al. Distribution of ADH2 and ALDH2 genotypes in different populations. *Hum Genet* 1992;88: 344-6.

[75] Maeda K, Sugiyama Y. Impact of genetic polymorphisms of transporters on the pharmacokinetic, pharmacodynamic and toxicological properties of anionic drugs. *Drug Metab Pharmacokinet* 2008;23: 223-35.

[76] Nakanishi T, Tamai I. Genetic polymorphisms of OATP transporters and their impact on intestinal absorption and hepatic disposition of drugs. *Drug Metab Pharmacokinet* 2012;27: 106-21.

[77] Kameyama Y, Yamashita K, Kobayashi K, Hosokawa M, Chiba K. Functional characterization of SLCO1B1 (OATP-C) variants, SLCO1B1*5, SLCO1B1*15 and SLCO1B1*15+C1007G, by using transient expression systems of HeLa and HEK293 cells. *Pharmacogenet Genomics* 2005;15: 513-22.

[78] Nozawa T, Minami H, Sugiura S, Tsuji A, Tamai I. Role of organic anion transporter OATP1B1 (OATP-C) in hepatic uptake of irinotecan and its active metabolite, 7-ethyl-10-hydroxycamptothecin: in vitro evidence and effect of single nucleotide polymorphisms. *Drug Metab Dispos* 2005;33: 434-9.

[79] Kim SR, Saito Y, Sai K, Kurose K, Maekawa K, Kaniwa N, Ozawa S, Kamatani N, Shirao K, Yamamoto N, Hamaguchi T, Kunitoh H, Ohe Y, Yamada Y, Tamura T, Yoshida T, Minami H, Ohtsu A, Saijo N, Sawada J. Genetic variations and frequencies of major haplotypes in SLCO1B1 encoding the transporter OATP1B1 in Japanese subjects: SLCO1B1*17 is more prevalent than *15. *Drug Metab Pharmacokinet* 2007;22: 456-61.

[80] Maeda K. Organic anion transporting polypeptide (OATP)1B1 and OATP1B3 as important regulators of the pharmacokinetics of substrate drugs. *Biol Pharm Bull* 2015;38: 155-68.

[81] Peng KW, Bacon J, Zheng M, Guo Y, Wang MZ. Ethnic variability in the expression of hepatic drug transporters: absolute quantification by an optimized targeted quantitative proteomic approach. *Drug Metab Dispos* 2015;43: 1045-55.

[82] Yoshida K, Maeda K, Sugiyama Y. Transporter-mediated drug--drug interactions involving OATP substrates: predictions based on in vitro inhibition studies. *Clin Pharmacol Ther* 2012;91: 1053-64.

[83] Ieiri I. Functional significance of genetic polymorphisms in P-glycoprotein (MDR1, ABCB1) and breast cancer resistance protein (BCRP, ABCG2). *Drug Metab Pharmacokinet* 2012;27: 85-105.

[84] Kondo C, Suzuki H, Itoda M, Ozawa S, Sawada J, Kobayashi D, Ieiri I, Mine K, Ohtsubo K, Sugiyama Y. Functional analysis of SNPs variants of BCRP/ABCG2. *Pharm Res* 2004;21: 1895-903.

[85] Maekawa K, Itoda M, Sai K, Saito Y, Kaniwa N, Shirao K, Hamaguchi T, Kunitoh H, Yamamoto N, Tamura T, Minami H, Kubota K, Ohtsu A, Yoshida T, Saijo N, Kamatani N, Ozawa S, Sawada J. Genetic variation and haplotype structure of the ABC transporter gene ABCG2 in a Japanese population. *Drug Metab Pharmacokinet* 2006;21: 109-21.

978 [86] Janne PA, Johnson BE. Effect of epidermal growth factor receptor tyrosine kinase
979 domain mutations on the outcome of patients with non-small cell lung cancer
980 treated with epidermal growth factor receptor tyrosine kinase inhibitors. *Clin*
981 *Cancer Res* 2006;12: 4416s-20s.
982
983 [87] Karapetis CS, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC,
984 Simes RJ, Chalchal H, Shapiro JD, Robitaille S, Price TJ, Shepherd L, Au HJ,
985 Langer C, Moore MJ, Zalcberg JR. K-ras mutations and benefit from cetuximab in
986 advanced colorectal cancer. *N Engl J Med* 2008;359: 1757-65.
987
988 [88] Siddiqui AD, Piperdi B. KRAS mutation in colon cancer: a marker of resistance to
989 EGFR-I therapy. *Ann Surg Oncol* 2010;17: 1168-76.
990
991 [89] Milbury CA, Li J, Makrigiorgos GM. PCR-based methods for the enrichment of
992 minority alleles and mutations. *Clin Chem* 2009;55: 632-40.
993
994 [90] Kaniwa N, Saito Y. Pharmacogenomics of severe cutaneous adverse reactions and
995 drug-induced liver injury. *J Hum Genet* 2013;58: 317-26.
996
997 [91] Wei CY, Chung WH, Huang HW, Chen YT, Hung SI. Direct interaction between
998 HLA-B and carbamazepine activates T cells in patients with Stevens-Johnson
999 syndrome. *J Allergy Clin Immunol* 2012;129: 1562-9 e5.
1000
1001 [92] Yun J, Marcaida MJ, Eriksson KK, Jamin H, Fontana S, Pichler WJ, Yerly D.
1002 Oxypurinol directly and immediately activates the drug-specific T cells via the
1003 preferential use of HLA-B*58:01. *J Immunol* 2014;192: 2984-93.
1004
1005 [93] Mikus G, Schowel V, Drzewinska M, Rengelshausen J, Ding R, Riedel KD, Burhenne
1006 J, Weiss J, Thomsen T, Haefeli WE. Potent cytochrome P450 2C19 genotype-related
1007 interaction between voriconazole and the cytochrome P450 3A4 inhibitor ritonavir.
1008 *Clin Pharmacol Ther* 2006;80: 126-35.
1009
1010 [94] Shi HY, Yan J, Zhu WH, Yang GP, Tan ZR, Wu WH, Zhou G, Chen XP, Ouyang DS.
1011 Effects of erythromycin on voriconazole pharmacokinetics and association with
1012 CYP2C19 polymorphism. *Eur J Clin Pharmacol* 2010;66: 1131-6.
1013
1014 [95] Piliguian M, Zhu AZ, Zhou Q, Benowitz NL, Ahluwalia JS, Sanderson Cox L,
1015 Tyndale RF. Novel CYP2A6 variants identified in African Americans are associated

with slow nicotine metabolism in vitro and in vivo. *Pharmacogenet Genomics* 2014;24: 118-28.

[96] Yoshida R, Nakajima M, Nishimura K, Tokudome S, Kwon JT, Yokoi T. Effects of polymorphism in promoter region of human CYP2A6 gene (CYP2A6*9) on expression level of messenger ribonucleic acid and enzymatic activity in vivo and in vitro. *Clin Pharmacol Ther* 2003;74: 69-76.

[97] Niinuma Y, Saito T, Takahashi M, Tsukada C, Ito M, Hirasawa N, Hiratsuka M. Functional characterization of 32 CYP2C9 allelic variants. *Pharmacogenomics J* 2014;14: 107-14.

[98] Drogemoller BI, Wright GE, Niehaus DJ, Koen L, Malan S, Da Silva DM, Hillermann-Rebello R, La Grange AM, Venter M, Warnich L. Characterization of the genetic profile of CYP2C19 in two South African populations. *Pharmacogenomics* 2010;11: 1095-103.

[99] Scott SA, Martis S, Peter I, Kasai Y, Kornreich R, Desnick RJ. Identification of CYP2C19*4B: pharmacogenetic implications for drug metabolism including clopidogrel responsiveness. *Pharmacogenomics J* 2012;12: 297-305.

[100] Ragia G, Arvanitidis KI, Tavridou A, Manolopoulos VG. Need for reassessment of reported CYP2C19 allele frequencies in various populations in view of CYP2C19*17 discovery: the case of Greece. *Pharmacogenomics* 2009;10: 43-9.

[101] Bradford LD. CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics* 2002;3: 229-43.

[102] Dodgen TM, Hochfeld WE, Fickl H, Asfaha SM, Durandt C, Rheeder P, Drogemoller BI, Wright GE, Warnich L, Labuschagne CD, van Schalkwyk A, Gaedigk A, Pepper MS. Introduction of the AmpliChip CYP450 Test to a South African cohort: a platform comparative prospective cohort study. *BMC Med Genet* 2013;14: 20.

[103] Gaedigk A, Twist GP, Leeder JS. CYP2D6, SULT1A1 and UGT2B17 copy number variation: quantitative detection by multiplex PCR. *Pharmacogenomics* 2012;13: 91-111.

[104] Montane Jaime LK, Lalla A, Steimer W, Gaedigk A. Characterization of the

CYP2D6 gene locus and metabolic activity in Indo- and Afro-Trinidadians: discovery of novel allelic variants. *Pharmacogenomics* 2013;14: 261-76.

[105] Elens L, van Gelder T, Hesselink DA, Haufroid V, van Schaik RH. CYP3A4*22: promising newly identified CYP3A4 variant allele for personalizing pharmacotherapy. *Pharmacogenomics* 2013;14: 47-62.

[106] Keshava C, McCanlies EC, Weston A. CYP3A4 polymorphisms--potential risk factors for breast and prostate cancer: a HuGE review. *Am J Epidemiol* 2004;160: 825-41.

[107] Lamba JK, Lin YS, Thummel K, Daly A, Watkins PB, Strom S, Zhang J, Schuetz EG. Common allelic variants of cytochrome P4503A4 and their prevalence in different populations. *Pharmacogenetics* 2002;12: 121-32.

[108] Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, Watkins PB, Daly A, Wrighton SA, Hall SD, Maurel P, Relling M, Brimer C, Yasuda K, Venkataramanan R, Strom S, Thummel K, Boguski MS, Schuetz E. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet* 2001;27: 383-91.

[109] Tseng E, Walsky RL, Luzietti RA, Jr., Harris JJ, Kosa RE, Goosen TC, Zientek MA, Obach RS. Relative contributions of cytochrome CYP3A4 versus CYP3A5 for CYP3A-cleared drugs assessed in vitro using a CYP3A4-selective inactivator (CYP3cide). *Drug Metab Dispos* 2014;42: 1163-73.

[110] Uesugi M, Kikuchi M, Shinke H, Omura T, Yonezawa A, Matsubara K, Fujimoto Y, Okamoto S, Kaido T, Uemoto S, Masuda S. Impact of cytochrome P450 3A5 polymorphism in graft livers on the frequency of acute cellular rejection in living-donor liver transplantation. *Pharmacogenet Genomics* 2014;24: 356-66.

[111] Palomaki GE, Bradley LA, Douglas MP, Kolor K, Dotson WD. Can UGT1A1 genotyping reduce morbidity and mortality in patients with metastatic colorectal cancer treated with irinotecan? An evidence-based review. *Genet Med* 2009;11: 21-34.

[112] Stingl JC, Bartels H, Viviani R, Lehmann ML, Brockmoller J. Relevance of UDP-glucuronosyltransferase polymorphisms for drug dosing: A quantitative systematic

review. *Pharmacol Ther* 2014;141: 92-116.

[113] Innocenti F, Grimsley C, Das S, Ramirez J, Cheng C, Kuttab-Boulos H, Ratain MJ, Di Rienzo A. Haplotype structure of the UDP-glucuronosyltransferase 1A1 promoter in different ethnic groups. *Pharmacogenetics* 2002;12: 725-33.

[114] Sugatani J, Yamakawa K, Yoshinari K, Machida T, Takagi H, Mori M, Kakizaki S, Sueyoshi T, Negishi M, Miwa M. Identification of a defect in the UGT1A1 gene promoter and its association with hyperbilirubinemia. *Biochem Biophys Res Commun* 2002;292: 492-7.

[115] Maeda H, Hazama S, Shavkat A, Okamoto K, Oba K, Sakamoto J, Takahashi K, Oka M, Nakamura D, Tsunedomi R, Okayama N, Mishima H, Kobayashi M. Differences in UGT1A1, UGT1A7, and UGT1A9 polymorphisms between Uzbek and Japanese populations. *Mol Diagn Ther* 2014;18: 333-42.

[116] Karas-Kuzelicki N, Mlinaric-Rascan I. Individualization of thiopurine therapy: thiopurine S-methyltransferase and beyond. *Pharmacogenomics* 2009;10: 1309-22.

[117] Ho RH, Choi L, Lee W, Mayo G, Schwarz UI, Tirona RG, Bailey DG, Stein CM, Kim RB. Effect of drug transporter genotypes on pravastatin disposition in European- and African-American participants. *Pharmacogenet Genomics* 2007;17: 647-56.

[118] Kim HS, Sunwoo YE, Ryu JY, Kang HJ, Jung HE, Song IS, Kim EY, Shim JC, Shon JH, Shin JG. The effect of ABCG2 V12M, Q141K and Q126X, known functional variants in vitro, on the disposition of lamivudine. *Br J Clin Pharmacol* 2007;64: 645-54.

[119] Tamura A, Onishi Y, An R, Koshiba S, Wakabayashi K, Hoshijima K, Priebe W, Yoshida T, Kometani S, Matsubara T, Mikuriya K, Ishikawa T. In vitro evaluation of photosensitivity risk related to genetic polymorphisms of human ABC transporter ABCG2 and inhibition by drugs. *Drug Metab Pharmacokinet* 2007;22: 428-40.

1127 **Table 1. Major genetic polymorphisms of drug-metabolizing enzymes and transporters in Japanese populations**

Gene	Allele	Locations		Activity	Allele frequencies			References
		Nucleotide changes	Amino acid changes		Japanese	Caucasian	African American	
CYP2A6	**4*	gene deleted		None	0.19	0	0.009	[15, 95, 96]
	**7*	1412T>C, gene conversion in the 3' flanking region	I471T	Decrease	0.098	0	0	[15, 95, 96]
	**9*	-1013A>G, -48T>G	TATA box	Decrease	0.19	0.08	0.085	[15, 95, 96]
CYP2B6	**4*	785A>G	K262R	Change	0.093	0.040	0.000	[33, 35]
	**5*	1459C>T	R487C	Decrease?	0.011	0.109	0.01-0.04	[33, 35]
	**6*	516G>T, 785A>G	Q172H, K262R	Change	0.164	0.256	0.33-0.50	[33, 35]

CYP2C9	**3*	1075A>C	I359L	Decrease	0.029	0.064	0.018	[12, 97]
	**2*	681G>A, 991A>G	Splicing defect, I331V	None	0.267-0.29	0.050-0.250	0-0.330	[12, 98, 99]
CYP2C19	**3*	636G>A, 991A>G	W212Stop, I331V	None	0.108-0.128	0-0.004	0-0.060	[12, 98, 100]
	**17*	-806C>T; 99C>T	I331V	Increase	0.011	0.188	0.235	[12}
	**5*	gene deleted		None	0.041-0.072	0.016-0.082	0.028-0.107	[101-103]
	**10*	100C>T, 1661G>C, 4180G>C	P34S, S486T	Decrease	0.333-0.408	0.001-0.080	0.019-0.086	[101-103]
CYP2D6	**41*	-1584C, -1235A>G, -740C>T, -678G>A, CYP2D7 gene conversion in intron 1, 1661G>C, 2850C>T,	R296C, Splicing defect, S486T	Decrease	0-0.016	0.031-0.150	0.004-0.149	[102-104]

		2988G>A, 4180G>C						
CYP3A4	**16*	554C>G	T185S	Decrease	0.014	0	0	[105-107]
CYP3A5	**3*	219-237A>G	Splicing defect	Decrease	0.71-0.85	0.9	0.27-0.5	[108-110]
UGT1A1	**6*	211G>A	G71R	Decrease	0.13-0.19	0.001-0.03	0	[12, 111, 112]
	**28*	-54_-39A(TA)$_6$TAA>A(TA)$_7$TAA		Decrease	0.09-0.13	0.30-0.39	0.36-0.45	[12, 111, 112]
	**60*	-3279T>G		Decrease	0.14-0.26	0.45-0.55	0.79-0.85	[112-114]
	**93*	-3156G>A		Decrease	0.12	0.28-0.33	0.28-0.32	[112, 113, 115]
NAT2	**5*	341T>C	I114T	Decrease	0.014	0.448	0.342	[12, 60, 61]

	**6*	590G>A	R197Q	Decrease	0.205	0.283	0.213	[12, 60, 61]
	**7*	857G>A	G286E	Decrease	0.088	0.018	0.063	[12, 60, 61]
GSTM1	**0 (null)*	gene deleted		None	0.501	0.529	0.266	[6, 12]
GSTT1	**0 (null)*	gene deleted		None	0.496	0.197	0.231	[6, 12]
TPMT	**3C*	719A>G	Y240C	Decrease	0.008-0.028	0.017-0.080	0.024-0.076	[65, 68, 116]
ALDH2	**2*	1510G>A	E504K	None	0.267	0	0.002	[69, 72, 73]
SLCO1B1	**1b*	388A>G	N130D	Increase	0.469	0.246	0.763	[79, 117]
	**5, *15, *17*	521T>C	V174A	Decrease	0.139	0.161	0.048	[12, 77, 78]
ABCG2(BCRP)		421C>A	Q141K	Decrease	0.313	0.105	0.027	[12, 118, 119]
		376C>T	Gln126Stop	None	0.009-0.017	0	0	[83, 118]

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