The clinical impact of cytochrome P450 polymorphisms on anti-epileptic drug therapy

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Summary

The goal of pharmacogenetics is to deliver safe and effective drug therapy. Genetic polymorphisms in cytochrome P450 (CYP) enzyme genes are implicated in the inter-individual variability in pharmacokinetics of anti-epileptic drugs (AEDs). However, the clinical impact of CYP polymorphisms on AED therapy remains controversial. Previous studies have shown that the defective CYP2C9 alleles affect the required dose of phenytoin and the risk of its toxicity. We have reported that the CYP2C19-deficient genotype is associated with the serum concentration of an active metabolite of clobazam, N-desmethylclobazam, and with the clinical efficacy of clobazam therapy. We determined also the influence of polymorphisms in CYP genes on the population pharmacokinetic parameters of AEDs us-
ing a non-linear mixed effect modeling pro-
gram, which enables us to define relevant ge-
netic factors together with other factors, and
the magnitude of the effect on variation in
pharmacokinetics in patients. The defective
alleles of CYP2C9 and CYP2C19 were found
to have significant effects on the inter-
individual differences in clearance of pheno-
barbital and zonisamide, respectively. Based
on these recent findings, we discuss the cli-
nical significance of AED dose adjustment ac-
cording to both genetic and non-genetic fac-
tors that affect CYP activity.

**Introduction**

Pharmacogenetics encompasses the princi-
ple of how genetic variation among individu-
als affects variation in pharmacokinetics and
pharmacodynamics of drugs [1-3]. It provides
the ability to identify potential adverse drug
reactions or lack of therapeutic effectiveness
before administration [1-3]. Therefore, the
goal of pharmacogenetics is to deliver safe
and effective drug therapy based on patient
genotype, but so far it has been implemented
in clinical practice in only a few isolated ex-
amples [1-3]. Substantial evidence from phar-
cmacogenetic studies of anti-epileptic drug
(AED) therapy indicates that genetic poly-
morphisms probably affect the variation in
pharmacokinetics and pharmacodynamics of
AEDs in any given patient [4-7, 10]. A recent
review article [10] pointed out that the genetic
variation of drug metabolizing enzymes such
as cytochrome P450 (CYP) 2C9 or CYP2C19
has limited clinical impact on AED therapy so
far. The aim of this article is thus to discuss the
clinical impact of CYP polymorphisms on
AED therapy. Based on the recent findings,
we discuss the clinical significance of AED
dose adjustment according to both genetic and
non-genetic factors that affect CYP activity.

**Pharmacokinetics of AEDS**

In the past, there were attempts to optimize
the clinical outcome by individualization of
AED dosage regimens using measurement of
blood concentrations, so-called therapeutic
drug monitoring [6]. Inter-individual variabil-
ity in pharmacokinetics of AEDs is the pri-
mary cause of variability in blood concentra-
tions or even response to the AEDs [5, 6].
Variability in pharmacokinetics affects ab-
sorption, protein binding, distribution at re-
ceptor site, metabolism and excretion [5, 6, 9,
10]. As many AEDs are cleared from the body
by hepatic metabolism (Table 1), the in-
dividual’s metabolic capacity is one of the
key determinants of drug response [5].

**CYP polymorphisms and AED therapy**

In the last few years, CYPs have attracted
much attention, and genetic polymorphisms
have been recognized in several of the CYPs
[1-3]. Most AEDs, except gabapentin, lam-
otrigine and levetiracetam, are metabolized
mainly or partially by CYP enzymes (Table 1)
[5]. Therefore, genetic polymorphisms of
CYP enzymes are implicated in the inter-
individual variability of the pharmacokinetics
of AEDs [4-7, 10].

CYP2C9 and CYP2C19 have well-
characterized functional variants. The fre-
quencies of defective CYP2C9 alleles
(CYP2C9*2 and CYP2C9*3) are higher in Caucasians (18.9%) than in Asians (2.5-3.5%) [11, 12]. Conversely, the frequencies of defective CYP2C19 alleles (CYP2C19*2 and CYP2C19*3) are higher in Asians (33-43.5%) compared to Caucasians (13.6%) [11-13]. Population studies have shown that individuals can be classified into three subgroups: namely, homozygous extensive metabolizers (homo EMs), heterozygous EMs (hetero EMs) and poor metabolizers (PMs) according to the number (i.e. 0, 1 and 2, respectively) of the defective allele(s) of each CYP2C gene [11-13]. Although individuals with defective alleles of CYP2C9 or CYP2C19 genes were shown to have reduced metabolism of some AEDs such as phenytoin, compared to those with wild-type (normal) alleles, the clinical impact of CYP2C9/2C19 polymorphisms on AED therapy remains controversial [4-6, 10, 14-21]. In addition to CYP2C9/2C19, CYP3A5 is polymorphically expressed in the liver, small intestine and kidney, and represents 5 to 85% of the total hepatic and intestinal CYP3A content [22-24]. The most common CYP3A5 polymorphism is CYP3A5*3, which has a frequency of 65-85% in Asians, 84-95% in Caucasians and 27-55% in African-Americans [11, 12, 22-24]. This allele contains a splice variant, which encodes a truncated nonfunctional protein [22-24]. Regardless of the race, in vitro studies using human

Table 1. Elimination patterns of anti-epileptic drugs (AEDs)

<table>
<thead>
<tr>
<th>AED</th>
<th>Involved enzymes/pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>CYP3A; microsomal epoxide hydrase 1 for carbamazepine-10,11-epoxide</td>
</tr>
<tr>
<td>Clobazam</td>
<td>CYP3A and CYP2C19; CYP2C19 (for N-desmethyloclobazam)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>CYP3A (?); acetylation, reduction</td>
</tr>
<tr>
<td>Diazepam</td>
<td>CYP2C19, CYP3A</td>
</tr>
<tr>
<td>Ethosuximide</td>
<td>CYP3A (?); 10%-20% renal</td>
</tr>
<tr>
<td>Felbamate</td>
<td>40-60% renal; CYP2C19, glucuronidation</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>Renal elimination</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>Glucuronidation; 8% renal</td>
</tr>
<tr>
<td>Levetiracetam</td>
<td>66% renal; nonhepatic hydrolysis</td>
</tr>
<tr>
<td>Oxcarbamazepine</td>
<td>CYP3A, glucuronidation</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>CYP2C19, CYP2C9</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>CYP2C9, CYP2C19</td>
</tr>
<tr>
<td>Topiramate</td>
<td>60%-80% renal; CYP3A, glucuronidation</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>CYP2C9; glucuronidation; β- and ω-oxidation</td>
</tr>
</tbody>
</table>

CYP, cytochrome P450. This table is adopted from a recent review [5] with slight modifications.
liver and intestine have demonstrated the homozygous CYP3A5*3 allele to express lower or insignificant levels of CYP3A5 mRNA and protein in comparison with heterozygous or homozygous wild-type (CYP3A5*1) allele [22-24]. Hence, the clinical importance of CYP3A5*3 polymorphisms in the pharmacokinetics of AEDs such as carbamazepine is not yet clear [19, 20, 25, 26].

**Clinical impacts of CYP2C9/2C19 polymorphisms on AED therapy**

**Phenytoin therapy**

Phenytoin exhibits a non-linear relationship between doses and serum concentrations, and the therapeutic window is narrow, with a range usually between 10 and 20 μg/mL. Seventy to 90% of orally administrated phenytoin is oxidized mainly by CYP2C9, and to a minor extent by CYP2C19, to yield S-5-(4p-hydroxyphenyl)-5-phenylhydantoin in humans [27, 28]. The relative contribution of CYP2C19 in phenytoin metabolism increases as phenytoin concentrations increase, leading to saturation of CYP2C9 [28]. Several studies of phenytoin have shown that genetic polymorphisms of CYP2C9 and CYP2C19 correlate with the dose needed by patients to control seizures [14, 15, 21, 29, 30]. In a recent analyses of 169 epileptic patients from Taiwan, a clinically relevant decrease in maximal rate of metabolism (Vmax) and intrinsic clearance was observed when the patients were carriers of defective alleles for both CYP2C9 and CYP2C19 (hetero EM<sub>CYP2C9</sub>/hetero EM<sub>CYP2C19</sub> or hetero EM<sub>CYP2C9</sub>/PM<sub>CYP2C19</sub>) [15]. Based on the calculated pharmacokinetic parameters, the authors recommended reducing the normal dosage of phenytoin (5-7 mg/kg/day) to 2-4 mg/kg/day for carriers of these genotypes [15]. In another retrospective analysis of 269 epileptic patients from the UK, the maximal dose of phenytoin was also differentiated according to the CYP2C9 genotypes of patients [21]. Carriers of one or two defective CYP2C9 alleles (i.e. CYP2C9 hetero EMs or PMs) apparently needed 13% and 30% lower dosages, respectively [21]. Several case studies reported that phenytoin-induced central nervous system (CNS) toxicities were observed when the patients were carriers of defective CYP2C9 and/or CYP2C19 allele(s) (Table 2) [31-34]. Concerning cutaneous drug reactions or gingival hyperplasia in phenytoin therapy, it is not yet clear whether there is an association with CYP2C9/2C19 polymorphisms [35-37].

**Clobazam therapy**

More than 70% of administered clobazam is demethylated to yield N-desmethylclobazam (N-clobazam), a pharmacologically active metabolite that reaches higher plasma concentrations than clobazam and may substantially contribute to the efficacy and safety of long-term clobazam therapy [38, 39]. This demethylation is facilitated by CYP3A4, CYP2C19 and CYP2B6, and the subsequent inactivation of N-clobazam to 4’-hydroxynorclobazam is catalyzed primarily by CYP2C19 [40]. Therefore, our group retrospectively evaluated the association between the CYP2C19 genotypes and the pharmacokinetics of clobazam and N-clobazam, as
Table 2. Case reports relating *CYP2C9/2C19* genotypes to central nervous system (CNS) toxicities of phenytoin

<table>
<thead>
<tr>
<th>Adverse reactions</th>
<th>CYP2C9/2C19 genotypes</th>
<th>Descriptions of cases</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenytoin intoxication (diplopia and ataxia)</td>
<td><em>CYP2C9</em> hetero EMs and <em>CYP2C19</em> hetero EMs</td>
<td>Phenytoin intoxication was observed in a 40-year-old Japanese male patient, with a serum phenytoin concentration of 32.6 μg/mL at a dose of 187.5 mg/day. His V$<em>{\text{max}}$ and K$</em>{m}$ values were 18.4% lower and 3 times higher, respectively, than in homo EMs of both <em>CYP2C9</em> and <em>CYP2C19</em>.</td>
<td>[31]</td>
</tr>
<tr>
<td>Severe phenytoin intoxication (e.g. dysarthria, nystagmus)</td>
<td><em>CYP2C9</em> PMs and <em>CYP2C19</em> hetero EMs</td>
<td>Severe intoxication was observed in a 31-year-old female patient in Italy treated with oral phenytoin (300 mg/day) for 10 days. She had high serum phenytoin concentration (&gt; 100 μg/mL), with an elimination half-life of 103 hours.</td>
<td>[32]</td>
</tr>
<tr>
<td>CNS toxicities (e.g. mental confusion, slurred speech)</td>
<td><em>CYP2C9</em> PMs</td>
<td>CNS toxicities were observed 13 days after starting phenytoin treatment (300 mg/day) in a 64-year-old female African-American patient. Her plasma phenytoin concentration was 49.5 μg/mL, and the AUC was 5.8 times that of <em>CYP2C9</em> homo EMs.</td>
<td>[33]</td>
</tr>
<tr>
<td>Severe CNS toxicities (e.g. nystagmus, ataxia and excessive sedation)</td>
<td><em>CYP2C9</em> PMs</td>
<td>Severe features of CNS toxicities were observed 12 month after starting phenytoin therapy in a 22-year-old female patient in India. Administration of 300 mg/day of phenytoin in this patient resulted in toxic symptoms associated with an excessive serum phenytoin concentration of 33.2 μg/mL.</td>
<td>[34]</td>
</tr>
</tbody>
</table>

Homo EMs, homozygous extensive metabolizers; Hetero EMs, heterozygous extensive metabolizers; PMs, poor metabolizers; V$_{\text{max}}$, maximal elimination rate; K$_{m}$, Michaelis-Menten constants; AUC, area under the concentration-time curve.
well as the efficacy, tolerance and adverse reaction of clobazam therapy in 110 Japanese epileptic patients [16]. The mean serum concentration of $N$-clobazam was 9 times higher in PMs than in homo EMs, and the degree of elevation in serum $N$-clobazam/clobazam concentration ratio was dependent on the number of defective alleles of $CYP2C19$ (Table 3). The responder rate was also significantly greater in PMs and hetero EMs than in homo EMs, showing a gene-dose effect (65.2, 47.6 and 33.3%, respectively), and the adjusted odds ratio (95% confidence interval) of PMs versus homo EMs was 9.88 (2.47-39.56). In a Kaplan-Meier analysis, the cumulative incidence for treatment failure was significantly lower in PMs than hetero EMs and homo EMs ($P = 0.02$, Figure 1). The adverse reactions, including drowsiness and dizziness, tended to be more frequent in PMs (64.0%) than in hetero EMs or homo EMs (43.2 and 39.0%, respectively, $P = 0.07$), and the incidence in homo EMs was closer to that in Canadian or European studies [41-43]. The frequency of PMs varies across races, for example, 13-23% in Asians and 1-8% in Caucasians [11, 12]. Therefore, the variety of $CYP2C19$ polymorphism may affect the incidence of the adverse effects of clobazam. On the other hand, the $CYP2C19$ genotypes were not associated with the frequencies of tolerance. These results suggest that $CYP2C19$ polymorphisms are associated with serum $N$-clobazam concentrations and with the clinical efficacy of clobazam therapy, thus indicating a gene-dose effect, whereas they are not associated with tolerance [16].

Population pharmacokinetics of AEDS

Because population pharmacokinetics (PPK) is the optimal methodology for identifying the possibly relevant factors affecting the pharmacokinetic parameters and the magnitude of the effect on variation in pharmacokinetics in patients, a PPK approach allows the determination of dosing regimen to achieve plasma concentrations within a given target range. Table 4 summarizes the evidence for $CYP$ genotypes associated with the PPK of AEDs. Our group determined the influences of $CYP$ polymorphisms on the PPK of phenobarbital, zonisamide and carbamazepine in Japanese epileptic patients [17, 19, 20, 25]. Associations between the genotypes of $CYP2C9$, $CYP2C19$ and/or $CYP3A5$ and population clearance estimates were evaluated using a non-linear mixed effect model program, because of the advantage that the PPK parameters can be assessed even when only a limited number of blood concentrations are available for each patient. Moreover, with the goal to assess the clinical acceptability of the predictions [20, 25], we assessed the accuracy and robustness of final PPK models by calculating the prediction errors using methods as previously reported [44, 45].

PPK model of phenobarbital

Phenobarbital is eliminated by a combination of renal excretion of unchanged drug (25%), $N$-glucoside formation (25%) and $CYP2C9$- and/or $CYP2C19$-dependent oxidation ($\leq 25\%$) [46, 47]. However, independent
**Table 3. Association between the CYP2C19 genotypes and clobazam pharmacokinetics [16]**

<table>
<thead>
<tr>
<th></th>
<th>Homo EMs (n = 16)</th>
<th>Hetero EMs (n = 22)</th>
<th>PMs (n = 12)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLB dose (mg/kg/day)</td>
<td>0.49 ± 0.20</td>
<td>0.49 ± 0.23</td>
<td>0.43 ± 0.30</td>
<td>0.72</td>
</tr>
<tr>
<td>CLB concentration (µg/mL)</td>
<td>0.14 ± 0.08</td>
<td>0.24 ± 0.13</td>
<td>0.21 ± 0.15</td>
<td>0.04</td>
</tr>
<tr>
<td>N-CLB concentration (µg/mL)</td>
<td>0.92 ± 0.61</td>
<td>2.14 ± 1.69</td>
<td>7.70 ± 6.04</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CLB C/D ratio [(µg/mL)/(mg/kg)]</td>
<td>0.27 ± 0.10</td>
<td>0.57 ± 0.34</td>
<td>0.55 ± 0.30</td>
<td>0.005</td>
</tr>
<tr>
<td>N-CLB C/D ratio [(µg/mL)/(mg/kg)]</td>
<td>1.99 ± 1.27</td>
<td>4.93 ± 3.72</td>
<td>18.57 ± 9.87</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>N-CLB/CLB ratio</td>
<td>7.57 ± 4.57</td>
<td>9.77 ± 7.94</td>
<td>35.66 ± 16.73</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Homo EMs, homozygous extensive metabolizers; Hetero EMs, heterozygous extensive metabolizers; PMs, poor metabolizers; CLB, clobazam; N-CLB, N-desmethyl clobazam; C/D, concentrations to dose ratio. Presented are mean ± SD. P-values were determined by ANOVA and Games-Howell post-hoc test. *P < 0.05 versus homo EMs. †P < 0.001 versus homo EMs. ‡P < 0.05 versus hetero EMs. §P < 0.001 versus hetero EMs.

**Table 4. Overviews of evidence for CYP genotypes associated with the population pharmacokinetics (PPK) of AEDs**

<table>
<thead>
<tr>
<th>AEDs</th>
<th>CYP genotypes</th>
<th>Differences in PPK parameters compared to the homo EMs of each CYP enzyme</th>
<th>Population</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenytoin</td>
<td>CYP2C9 hetero EMs</td>
<td>V_{max}: 42%↓; K_m: 22%↑; K_m: 54%↑</td>
<td>Japanese</td>
<td>[14]</td>
</tr>
<tr>
<td></td>
<td>CYP2C19 hetero EMs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CYP2C19 PMs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CYP2C9 hetero EMs and CYP2C19 hetero EMs</td>
<td>V_{max}: 37%↓; K_m: 27.4%↑</td>
<td>Taiwanese</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td>CYP2C9 hetero EMs and CYP2C19 PMs</td>
<td>V_{max}: 45.7%↓; K_m: 91.7%↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenytoin</td>
<td>CYP2C9 hetero EMs</td>
<td>CL: 48%↓</td>
<td>Japanese</td>
<td>[17, 20]</td>
</tr>
<tr>
<td></td>
<td>CYP2C19 PMs</td>
<td>CL: 18.8%↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenytoin</td>
<td>CYP2C19 hetero EMs</td>
<td>CL: 16%↓</td>
<td>Japanese</td>
<td>[19-20]</td>
</tr>
<tr>
<td></td>
<td>CYP2C19 PMs</td>
<td>CL: 30%↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zonisamide</td>
<td>CYP2C19 hetero EMs</td>
<td>CL: 8%↑</td>
<td>Japanese</td>
<td>[20, 25]</td>
</tr>
<tr>
<td></td>
<td>CYP2C19 PMs</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Homo EMs, homozygous extensive metabolizers; Hetero EMs, heterozygous extensive metabolizers; PMs, poor metabolizers; V_{max}, maximal elimination rate; K_m, Michaelis-Menten constants; CL, apparent clearance; ↓, decrease; ↑, increase; †, versus carriers of both CYP2C9 and CYP2C19 homo EMs; ‡versus CYP3A5*1/*1 or *1/*3 genotypes.
valid data showing a substantial contribution of these CYPs on phenobarbital metabolism are currently missing. We evaluated the influences of CYP2C9 and CYP2C19 genotypes on the PPK of phenobarbital in 79 Japanese epileptic patients [17, 20]. The final model of phenobarbital apparent clearance was as follows:

$$CL = 0.23 \times \left( \frac{\text{body weight}}{40} \right)^{0.21} \times 0.53^{CYP2C9\text{hetero EM}} \times 0.68^{VPA} \times 0.85^{PHT} \times 0.85^{SMID} \times (1+\eta_{CL})$$

where $CL$ is apparent clearance of phenobarbital; $CYP2C9$ hetero EMs = 1, $CYP2C9$ homo EMs = 0; VPA = 1 if valproic acid is co-administered, otherwise 0; PHT = 1 if phenytoin is co-administered, otherwise 0; SMID = 1 if complications of severe or profound mental retardation with a significant behavior impairment are presented, otherwise 0; and $\eta_{CL}$ = the independent random error distributed normally with the mean zero. In 47 other Japanese epileptic patients, the performance of the final population model was better than that of the basic model without covariates [20].

The phenobarbital clearance in CYP2C9 hetero EMs was 48% lower than in homo EMs ($P < 0.001$). Valproic acid, a known inhibitor of phenobarbital metabolisms, and phenytoin, a substrate of CYP2C9 and CYP2C19, were also incorporated into the PPK models. Although the CYP2C19 genotypes have been shown to affect the clearance of phenobarbital in Japanese patients in a previous study [48], in which carriers of the defective CYP2C9 allele(s) were excluded, we observed no effect of CYP2C19 genotype on phenobarbital metabolism.
clearance ($P > 0.05$). Individuals with reduced CYP2C9 activity; that is, carriers of one or two defective $CYP2C9$ allele(s), are more prevalent in Caucasians than in African-Americans and Asians (Figure 2) [11, 12]. The incidence of phenobarbital-related adverse reactions was reported to be higher in Caucasians than in African-Americans and Asians (Figure 2) [49-52]. The lower frequency of individuals with reduced CYP2C9 activity among Asians and African-Americans might be a possible explanation for the higher tolerability of phenobarbital in these races than in Caucasians. It should be noted that the number of $CYP2C9$ hetero EMs was small ($n = 10$ in total), and the effects of $CYP2C9$ genotypes on the pharmacokinetics of phenobarbital were model-based and hence indirect proof. These results, however, suggest that the $CYP2C9$ genotypes could have an important role in the pharmacokinetics of phenobarbital in Japanese epileptic patients, and might affect the ethnic differences in tolerability of phenobarbital therapy.

**PPK model of zonisamide**

Zonisamide is eliminated via renal excretion of the 2-sulfamoylacetyl-phenol (SMAP)-glucuronide (50%), unchanged form (35%) and $N$-acetyl zonisamide (15%) [53, 54]. The formation of SMAP is catalyzed mainly by CYP3A4 and to a minor extent by CYP3A5 and CYP2C19 in an *in vitro* study [55]. Our group determined the influences of $CYP2C19$ and $CYP3A5$ genotypes on the PPK of zonisamide in 99 Japanese epileptic patients [19, 20].

The final model of zonisamide apparent clearance was as follows:

$$CL = 1.22 \times \left(\frac{\text{body weight}}{44}\right)^{0.77} \times \text{Dose}^{-0.17} \times$$

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**Figure 2.** The frequencies of individuals with reduced $CYP2C9$ activity and the incidence of adverse reactions of phenobarbital in Asians, African-Americans and Caucasians, based on previous reports [11, 12, 49-52].
0.84^{CYP2C19\text{hetero EM}} \times 0.70^{CYP2C19\text{PM}} \times 1.24^{CBZ} \times 1.28^{PHT} \times 1.29^{PB} \times \eta_{CL}

where CL is apparent clearance of zonisamide; Dose is daily dose of zonisamide; CYP2C19 hetero EM or CYP2C19 PM = 1 if one or two CYP2C19-defective alleles are carried, respectively, otherwise 0; CBZ = 1 if carbamazepine is co-administered, otherwise 0; PHT = 1 if phenytoin is co-administered, otherwise 0; PB = 1 if phenobarbital is co-administered, otherwise 0; and \eta_{CL} = the independent random error distributed normally with the mean zero. In 71 other Japanese epileptic patients, the performance of the final population model was better than that of the basic model without covariates [20].

The zonisamide clearance in CYP2C19 hetero EMs and PMs was 16% and 30%, respectively, lower than in homo EMs (P < 0.001). A gene-dose effect was observed for the number of defective CYP2C19 allele(s), which lends further credence to the involvement of CYP2C19 in zonisamide metabolism in patients. CYP inducers comprising carbamazepine, phenytoin and phenobarbital were also incorporated into the PPK models. Conversely, the CYP3A5*3 genotype did not affect the clearance of zonisamide, thus indicating little contribution of CYP3A5*3 genotypes to the zonisamide pharmacokinetics in Japanese epileptic patients.

Based on these results, we verified the clinical impact of CYP2C19 genotype on zonisamide therapy. The median concentration to dose (C/D) ratios of zonisamide in PMs tended to be higher than in homo EMs (Figure 3). The differences in the C/D ratios between the CYP2C19 genotypes were more pro-

**Figure 3.** The median zonisamide serum concentration to dose (C/D) ratios with respect to the CYP2C19 genotypes in patients treated with zonisamide alone (monotherapy) or combined with CYP inducers; carbamazepine, phenytoin and phenobarbital (polytherapy). Homo EMs (n = 20), hetero EMs (n = 23) and poor metabolizers (PMs, n = 10) in monotherapy; homo EMs (n = 34), hetero EMs (n = 52) and PMs (n = 16) in polytherapy.
nounced in polytherapy with CYP inducers than in zonisamide monotherapy (Figure 3). Two of 27 PMs (7.4%) and 5 of 79 hetero EMs (6.3%), but none of 64 homo EMs (0%), developed zonisamide-specific adverse reactions of fever and/or hypohidrosis. These results suggest that the CYP2C19 genotypes may have an important role in the pharmacokinetics of zonisamide and may affect the development of some adverse reactions.

**PPK model of carbamazepine**

Carbamazepine is extensively metabolized in the liver, with less than 5% of an oral dose excreted unchanged in urine [56]. Carbamazepine is predominantly metabolized to carbamazepine-10,11-epoxide by CYP3A, and carbamazepine-10,11-epoxide and carbamazepine-10,11-trans-diol are primary (≤60%) metabolites in urine [57, 58]. Our group thus determined the influences of CYP3A5 genotype on the PPK of carbamazepine in 144 Japanese epileptic patients [20, 25]. The final model of carbamazepine apparent clearance was as follows:

\[
CL = 0.17 \times \frac{\text{body weight}}{40}^{0.11} \times \text{Dose}^{0.45} \times 1.08^{CYP3A5*3/^3} \times 1.40^{\text{PHT}} \times 1.21^{\text{PB}} \times e^{\eta_{CL}}
\]

where CL is apparent clearance of carbamazepine; Dose is daily dose of carbamazepine; CYP3A5*3/^3 = 1, otherwise 0; PHT = 1 if phenytoin is co-administered, otherwise 0; PB = 1 if phenobarbital is co-administered, otherwise 0; and \( \eta_{CL} \) is the independent random error distributed normally with the mean zero. In 32 other Japanese epileptic patients, the performance of the final population model was better than that of the basic model without covariates [20, 25].

The carbamazepine clearance was 8% higher in the CYP3A5*3/^3 genotype than in the CYP3A5*1/^1 or *1/^1 genotype in Japanese epileptic patients. The CYP3A5*3/^3 genotype was associated with low expression of CYP3A5 in the liver of Japanese and Caucasians [22-24]. Conversely, a large interindividual difference (around 50%-fold) has been reported for the expression of CYP3A4 [59], which cannot be explained by the genetic polymorphisms per se [60, 61]. The in vitro metabolic activity of CYP3A5 for carbamazepine 10,11-epoxide formation is 40-90% that of CYP3A4 [62, 63]. Our results may not apply to the drugs preferably metabolized by CYP3A5.

The mechanism(s) regulating the hepatic CYP3A expressions may be different among races, because the level of CYP3A4 mRNA does not correlate with the level of CYP3A5 mRNA in the liver of Japanese, in contrast to the co-regulatory expression of CYP3A5 and CYP3A4 in the liver of Caucasians [23, 24]. Carriers of the CYP3A4*1B and CYP3A5*3 haplotypes had a 46% higher clearance of midazolam than non-carriers in Caucasian patients with cancer [64]. Multigene haplotypes may also be attributed to the effects of CYP3A5*3 genotypes on the pharmacokinetics of CYP3A substrates; however, the CYP3A4*1B allele is scarce or nil in Japanese [11]. Rifampin, a potent inducer of CYP3A, increases the oral clearance of midazolam, a substrate of CYP3A, by 50% in the CYP3A5*3/^3 genotype compared to the CYP3A5*1/^1 genotype in Caucasians and
African-Americans [61]. The CYP3A expression may thus be induced more extensively in individuals with the CYP3A5*3/*3 genotype than with the CYP3A5*1/*1 genotype. The carbamazepine clearance was 29% lower in the CYP3A5*3/*3 genotype than in the CYP3A5*1/*1 or *1/*3 genotypes in Korean patients treated with carbamazepine mono-therapy [26]. Carbamazepine is known to be an inducer of CYP3A, which is the major metab-olizing enzyme of carbamazepine itself [65]. Our PPK model included carbamazepine polytherapy with CYP3A inducers, phenytoin and phenobarbital, as well as carbamazepine monotherapy. The higher inducibility of CYP3A in the CYP3A5*3/*3 genotype is a possible explanation for higher clearance among subjects with the CYP3A5*3/*3 genotype in this study than that in Korean study.

Our result of an 8% difference in carbamazepine clearance indicates that CYP3A5*3 genotypes could not have an important role in the pharmacokinetics of carbamazepine in Japanese epileptic patients, although further studies are needed to elucidate underlying mechanisms of these findings in larger and different ethnic populations.

**Conclusion**

In conclusion, these recent findings indicate that: (1) the defective alleles of CYP2C9 and/or CYP2C19 may affect the pharmacokinetics and the incidence of CNS toxicity in pheny-toin therapy; (2) CYP2C19-deficient geno-types are associated with the serum concentra-tion of an active metabolite of clobazam, N-clobazam, and with the clinical efficacy of clobazam therapy; (3) the defective alleles of CYP2C9 and CYP2C19 have clinically sig-nificant effects on the inter-individual vari-abilities in pharmacokinetics of phenobarbital and zonisamide, respectively; and (4) the CYP3A5*3 genotypes appear to have little contribution to zonisamide and carbamazepine therapies in Japanese epileptic patients. These results suggest that the defective CYP2C9/2C19 polymorphisms could have a potential clinical impact on AED therapy. Additionally, PPK modeling in patients allows assessment of substantial contribution of CYP enzymes to AED metabolisms, and even the AED adverse reactions.

**Future Perspective**

Despite the abundance of information relating CYP polymorphisms to AED therapy, genotype is rarely a crucial determinant in setting drug dose [10]. We should obtain more clinical integration of CYP pharmacogenetic information including: firstly, replication of the observed associations; secondly, careful analysis of confounding factors such as drug-drug interactions and population stratification; and thirdly, analysis of combination of other genetic factors affecting CYP activity (e.g. transcriptional nuclear factors). Based on our recent findings, the PPK modeling incorporating the CYP genotypes may be one of the most useful tools to facilitate determination of individualized dose regimen in AED therapy, and genetic and non-genetic factors affecting CYP activity can be used reliably by clinicians in making decision of AED dose-adjustment.
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References
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