Title: Association of polymorphisms in *EPHX1, UGT2B7, ABCB1, ABCC2, SCN1A* and *SCN2A* genes with carbamazepine pharmacotherapy optimization in Han Chinese epileptic patients

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Abstract

Objective Carbamazepine (CBZ) is one of the most widely used antiepileptic drugs. The aim of the present study is to investigate the impacts of polymorphisms in genes related to pharmacokinetic and pharmacodynamic pathways of CBZ on the large interindividual variability in dosages and concentrations.

Methods and results Genetic polymorphisms in the candidate genes were detected in 234 epileptic patients under maintenance CBZ monotherapy by realtime-PCR and PCR-RFLP. Results of statistical analysis demonstrated that carriers of the variant *SCN1A* IVS5-91G>A and *EPHX1* c.337T>C allele tended to require higher CBZ dosages and lower lnCDRs than noncarriers ($p<0.0001$) and the homozygous carriers also more likely to require higher CBZ dosages and lower lnCDRs ($p<0.0001$). In addition, the multiple regression model of concentration-dose-ratio of CBZ also revealed that genetic variants in *SCN1A*, *EPHX1* and *UGT2B7* genes interactively affect concentration-dose-ratio of CBZ (adjusted $r^2=55\%$).

Conclusion The present study identified genetic factors associated with CBZ therapy optimization and provided useful information for individualized CBZ therapy in epileptic patients. Further studies in larger populations need to confirm our results.

Keywords carbamazepine, *SCN1A*, *EPHX1*, *UGT2B7*, polymorphism, pharmacogenomics

Introduction

Carbamazepine (CBZ) is one of the most widely prescribed antiepileptic drugs (AEDs) and has been used as a first-line therapy in treatment of partial and generalized tonic-clonic seizures [1]. However, large interindividual variability in dosages and concentrations of CBZ has been observed in clinical practice [2]. During the process of dosage adjustment, the quality of life of epileptic patients may be compromised by ongoing seizure attacks.

The large variability in treatment efficacy of CBZ may be associated with interindividual differences in pharmacokinetics and pharmacodynamics. CBZ is primarily metabolized in the liver to form the active metabolite, carbamazepine 10,11-epoxide, which is subsequently transformed by microsomal epoxide hydrolase (mEH) to the inactive carbamazepine-10,11-diol [3]. The human mEH, encoded by *EPHX1* gene, is expressed polymorphically [4]. The two most common variants, c.337T>C and c.416A>G, were demonstrated to alter the enzymatic expression levels and activity both *in vitro* and *in vivo* [5-7]. Glucuronidation is another important detoxification pathway for CBZ. The CBZ N-glucuronide and glucuronides of the hydroxylated metabolites are major urinary metabolites [3]. Among the human uridine diphosphate glucuronosyl-transferase (UGT) isoforms, UGT2B7 has been demonstrated to be the major isoform glucuronidating CBZ [8]. The *UGT2B7* gene

has been reported to be polymorphic in the proximal promoter region, such as -327A>G, -161T>C, -138G>A, and -125T>C, in addition, in the coding region, 211G>T and 802C>T were identified as non-synonymous polymorphisms [9-10]. Although these genetic variants have been suggested to affect gene expression, the clinical effects on CBZ maintenance doses, concentrations and CDRs were unclear.

CBZ has been demonstrated to be an active substrate of human P-glycoprotein (P-gp) in several *in vitro* transport studies [11-12]. P-glycoprotein (P-gp), encoded by the highly polymorphic *ABCB1* gene, is a major efflux transporter in human blood-brain-barrier associated with AEDs transport [13-14]. The *ABCB1* 1236C>T, 2677G>T/A and 3435C>T were extensively studied in respect to the influences on the disposition of CBZ with conflicting results [15-18]. Another important efflux transporter, MRP2 (multidrug resistant associated protein 2), encoded by the *ABCC2* gene, is also mediated the transport of AEDs in the brain [19]. The *ABCC2* -24T variant were significantly over-represented in the non-responders of AEDs [20]. Another genetic polymorphism, *ABCC2* c.1249G>A, was in linkage with c.-24C>T and was showed to be associated with the neurological adverse drug reactions of carbamazepine in patients with epilepsy [21-22]. Whether genetic polymorphisms in these efflux transporters associated with CBZ maintenance doses, concentrations and CDRs were important issues to be elucidated.

Blocking voltage-gated sodium channels in the brain was the primary mechanism that many AEDs exerted their anti-epileptic effects, such as CBZ, felbamate, lamotrigine, oxcarbazepine, phenytoin, topiramate and zonisamide [23]. The brain voltage-gated sodium channels comprise a central large α-subunit and two auxiliary small β-subunits [24]. The α-subunit was the major binding site of many AEDs, therefore, the interest in these genes lied not only in the possible causal roles in epilepsy but also in the potential effects on the efficacy of AEDs. A common polymorphism in *SCN1A* (IVS5-91 G>A) was demonstrated to correlate with the maximum doses of phenytoin or CBZ [25-27]. Therefore, genetic polymorphisms on these genes could be involved in the interindividual variability of treatment efficacy of AEDs.

In order to evaluate the genetic variants associated with CBZ maintenence dosages and steady-state concentrations, we included candidate genes with biological plausibility with CBZ treatment efficacy. By comprehensive analyses of genetic polymorphisms related to the pharmacokinetic and pharmacodynamic pathways of CBZ, the present study aimed to identify the multiple genetic effect and the interactions between genes regarding influences on CBZ dosages, concentrations and CDRs in epileptic patients.

Methods

Subjects

The study was approved by the Ethics Committee of the National Taiwan University Hospital. Blood samples for genotyping were collected after informed consents were obtained from all subjects. All patients had electroencephalogram and magnetic resonance imaging (MRI) brain scans. The classifications of epilepsies and epileptic syndromes were conducted according to the guidelines of ILAE 1989 [28]. For each patient the following clinical information was recorded: gender, weight (kg), age, epilepsy classification, etiology, carbamazepine maintenance dose (mg/kg/day), carbamazepine serum concentration at maintenance dose (mg/L). The inclued subjects did not use any other AED or non-AED that may interact with CBZ. The maintenance dose of carbamazepine was defined as the dosage which has not been changed for at least one year under good compliance and good seizure control. Drug responsiveness (seizure free/ good seizure control) was defined as freedom from seizures for a minimum of three times the longest preintervention interseizure interval (determined from seizures occurring within the past 12 months) or 12 months, whichever is longer [29]. Concentration-dose ratios (CDRs) were calculated by dividing the mean steady state CBZ serum concentration by the CBZ daily dose. The steady-state CBZ serum concentration was measured by the homogeneous particle enhanced turbidimetric

inhibition immunoassay (PETINIA; CRBM Carbamazepine Flex®, Dade Behring Inc., USA) according to the manufacture protocol.

A total of 234 patients with epilepsy under carbamazepine monotherapy treatment (111 men, 123 women, age 39.23 ± 11.88 (mean ± SD)) were included and each of them reached a maintenance dose for at least one year (CBZ dose: 848.72 ± 229.68 mg/day; concentration: 7.80 ± 2.28 mg/L). Of these patients, 197 subjects (84.18 %) were localization-related epilepsies (Table 1). The patient compliance to the medication was checked by counting the pills remain in the drug bag. All of the recuited patients were achieved over 95% compliance. As normal controls, 189 healthy volunteers were genotyped for comparison (97 men, 92 women, age 39.1± 1.52). All patients and controls were recruited from the same center, of the same ethnic background and unrelated. The ethnic background of patients and controls was Han Chinese and it was ascertained by patient self-identity. The healthy control group was used as a evidence of the identical genetic background and provide information of whether the genotypic distributions were all consistent with Hardy-Weinberg equilibrium proportions in patients and healthy controls.

Genotyping

It is known that several variants in *SCN1A, SCN2A, EPHX1, UGT2B7, ABCB1,* and *ABCC2* genes would affect the basic expression or induction function of the target

protein [9-10, 15-18, 21-22, 25-27]. Among these variants, it has been shown that *SCN1A* IVS5-91 G>A (rs3812718), c.3184A>G (rs2298771), *SCN2A* c.56G>A (rs17183814), *EPHX1* c.337T>C (rs1051740), c.416A>G (rs2234922), *UGT2B7* -161C>T (rs7668258), -842A>G (rs7438135), c.735A>G (rs28365062), c.802T>C (rs7439366), *ABCB1* c.1236C>T (rs1128503), c.2677G>T/A (rs2032582), c.3435C>T (rs1045642), *ABCC2* c.-24C>T (rs717620) and c.1249G>A (rs2273697) were the fourteen polymorphisms with higher allele frequency of variant in Han Chinese according to NCBI database. Hence, these fourteen variants were selected for investigation in our study. Genomic DNA was isolated from peripheral blood sample by use of a QIAamp DNA Mini Kit. Genotyping of *SCN1A* IVS5-91 G>A (rs3812718), c.3184A>G (rs2298771), *SCN2A* c.56G>A (rs17183814), *EPHX1* c.337T>C (rs1051740), *UGT2B7* -161C>T (rs7668258), -842A>G (rs7438135), c.735A>G (rs28365062), *ABCB1* c.1236C>T (rs1128503), c.2677G>T/A (rs2032582), *ABCC2* c.-24C>T (rs717620) and c.1249G>A (rs2273697) were carried out using the Applied Biosystem Assay on Demand reagents (Applied Biosystem, Foster City, Calif.). PCRs were performed in 20 μl volume, containing allele-specific probes, assay-specific primers, TaqMan Universal PCR Master Mix, and genomic DNA (50 ng). Genotypes analyses were estimated by SDS 2.2 software (Applied Biosystems).

Genotyping of *EPHX1* c.416A>G (rs2234922), *UGT2B7* c.802T>C (rs7439366),

and *ABCB1* c.3435C>T (rs1045642) were conducted by using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCRs were performed in 25 ul volumes containing 50 ng genomic DNA, 2.5 μM/μL dNTPs, 10 μM/μL each primers, 1× reaction Buffer, and 5 unit Taq DNA polymerase (Fermentas, Inc.). PCR conditions were denaturation at 94 °C for 5 min followed by 35 cycles at 94 °C for 20 s, annealing at 60°C for 20 s, 72 °C for 20 s with a final elongation step at 72 °C for 5 min. The restriction enzymes used in the genotyping of *EPHX1* c.416A>G (rs2234922), *UGT2B7* c.802T>C (rs7439366), and *ABCB1* c.3435C>T (rs1045642) were *Rsa* I, *Bse* GI and *Mbo* I, respectively [30-32]. The genotyping methods were validated by direct sequencing.

Statistical Analysis

Prior to statistical analysis, the normality of data set, such as CBZ dosage, concentration and CDR, were tested. The CDR of CBZ was not demonstrated normal distribution before natural logarithm transformation. Therefore, lnCDR of CBZ was applied in the statistical analysis. For the association between dosages, concentrations and lnCDRs with each genetic polymorphism were analyzed by one way ANOVA followed by Bonferroni post hoc test. Identification of haplotypes was performed using EM algorithm [33]. The standardized linkage disequilibrium values were calculated for measurement of the linkage disequilibrium among these loci [34-35]. A

p-value less than 0.05 was considered to indicate statistical significance. Multiple comparisons were corrected using Bonferroni's method. Analysis of association between a genotype and the CBZ dosage, concentration or lnCDR, the adjusted α level was prescribed at 0.0012 (0.05/42) for the 42 comparisons among the fourteen genetic polymorphisms and the three genotype groups. Similar association analysis was also carried out for alleles. The adjusted α level was set at 0.0018 (0.05/28) for the 28 comparisons among the fourteen genetic polymorphisms and two allele groups. As for haplotype analysis, the adjusted α level was prescribed at 0.0125 (0.05/4) for haplotypes composed of *SCN1A* IVS5-91 G>A (rs3812718) and c.3184A>G (rs2298771). Further analysis of diplotypes was similar, the adjusted α level was prescribed at 0.005 (0.05/9) for diplotypes composed of *SCN1A* IVS5-91 G>A (rs3812718) and c.3184A>G (rs2298771).

Further multivariate regression models were conducted to detect the joint effect of *SCN1A*, *SCN2A*, *EPHX1*, *UGT2B7*, *ABCB1*, and *ABCC2* polymorphisms on lnCDR of CBZ with the adjustment for the epilepsy syndromes, age and weight. Epilepsy syndromes may not influence the doses of antiepileptic drugs directly, however, it may have indirect effect. Since different epileptic syndromes may associated with treatment outcomes, as we alrealdy know that patients with temporal lobe epilepsy may be less responsive to antiepileptic drugs. Therefore, patients with

temporal lobe epilepsy may require higher dose than others to control their seizures. To identify whether epileptic syndromes may show their effect on CBZ dosage or concentration-to-dose ratio, we took into account the epilepsy syndrome as a cofactor in the regression model. The temporal lobe epilepsy was included as a dummy varible. Therefore, the number of patients in the other group was enough for statistical analysis. The model selection procedures were undergone based on the backward elimination method [36]. All data analyses were performed using SAS version 9.1.3 (SAS Inc, Cary, NC, USA).

Results

The allele and genotype frequencies of the *SCN1A*, *SCN2A*, *EPHX1*, *UGT2B7*, *ABCB1*, and *ABCC2* polymorphic loci for patients under CBZ maintenance mono-therapy and healthy controls were listed in Table 2 and Table 3. The genotypic distributions were all consistent with Hardy-Weinberg equilibrium proportions and genotype frequencies were not significantly different between patients and healthy controls. Significant linkage disequilibrium was detected between *SCN1A* IVS5-91 G>A (rs3812718) and c.3184A>G (rs2298771), among *UGT2B7* -161C>T (rs7668258), -842A>G (rs7438135), and 802T>C (rs7439366), and among *ABCB1* c.1236C>T (rs1128503), c.2677G>T/A (rs2032582), and c.3435C>T (rs1045642) as indicated by high values of D' (>0.8; all p-values < 0.0001).

Association of genetic variants with CBZ maintenance Dosage

The statistical analysis revealed that among the tested fourteen SNPs, only *SCN1A* IVS5-91 G>A (rs3812718) and *EPHX1* c.337T>C (rs1051740) showed significant associations with the CBZ maintenance dosages (mg/kg/day) (Table 2 and Table 3). Carriers of the variant *SCN1A* IVS5-91 G>A allele tended to require higher CBZ maintenance dosage than noncarriers ($p<0.0001$, uncorrected) and the homozygous variant carriers also seemed to require higher CBZ maintenance dosage ($p<0.0001$, uncorrected). Carriers of *EPHX1* c.337T>C revealed similar results.

Patients with the variant *EPHX1* c.337T>C allele were more likely to require higher maintenance dosage of CBZ than patients with wild types ($p<0.0001$, uncorrected) and the homozygous variants carriers seemed to require higher maintenance dosage as well ($p<0.0001$, uncorrected). These genetic associations remained significant after Bonferroni's correction as described in the statistical methods.

Haplotype analysis demonstrated that only haplotypes composed of *SCN1A* IVS5-91 G>A (rs3812718) and c.3184A>G (rs2298771) demonstrated significant association with maintenance dosages of CBZ, whereas the haplotypes composed of *UGT2B7* -161C>T (rs7668258), -842A>G (rs7438135), and 802T>C (rs7439366), or *ABCB1* c.1236C>T (rs1128503), c.2677G>T/A (rs2032582), and c.3435C>T (rs1045642) showed no effect on CBZ maintenance dosages. The comparisons of haplotype pattern distributions revealed that patients with AA and AG haplotypes composed of *SCN1A* IVS5-91 G>A (rs3812718) and c.3184A>G (rs2298771) were more likely to require higher maintenance dosages of CBZ (both $p<0.0001$, uncorrected; Table 4). These haplotype associations remained significant after Bonferroni's correction as described in the statistical methods. Since diplotypes were the genetic composition of human, we further analyzed the association between diplotypes of *SCN1A* IVS5-91 G>A (rs3812718) and c.3184A>G (rs2298771) and CBZ maintenance dosages. The comparisons of genotype combination distributions

showed that patients with GA/AA, AA/AA, GA/AG or AA/AG diplotypes were more likely to require higher CBZ maintenance dosages (all $p \leqq 0.0002$, uncorrected; Table 4). These diplotype associations remained significant after Bonferroni's correction as described in the statistical methods.

Association of genetic variants with CBZ Concentration-Dose Ratio

Among the fourteen candidate SNPs, only *SCN1A* IVS5-91 G>A (rs3812718) and *EPHX1* c.337T>C (rs1051740) were significantly associated with lnCDRs of CBZ (Table 2 and Table 3). Carriers of the variant *SCN1A* IVS5-91 G>A allele tended to have lower lnCDRs than noncarriers ($p<0.0001$, uncorrected) and the homozygous carriers also seemed to have lower lnCDRs ($p<0.0001$, uncorrected). Similar results were observed in carriers of *EPHX1* c.337T>C. Carriers of the variant *EPHX1* c.337T>C allele were more likely to have lower lnCDRs than noncarriers ($p<0.0001$, uncorrected) and the homozygous carriers seemed to have lower lnCDRs as well ($p<0.0001$, uncorrected). These genetic associations remained significant after Bonferroni's correction as described in the statistical methods.

Haplotype analysis demonstrated that only haplotypes composed of *SCN1A* IVS5-91 G>A (rs3812718) and c.3184A>G (rs2298771) demonstrated significant association with lnCDRs of CBZ, whereas the haplotypes composed of *UGT2B7* -161C>T (rs7668258), -842A>G (rs7438135), and 802T>C (rs7439366), or *ABCB1*

c.1236C>T (rs1128503), c.2677G>T/A (rs2032582), and c.3435C>T (rs1045642) showed no effect on lnCDRs of CBZ. The comparisons of haplotype pattern distributions revealed that patients with AA haplotype composed of *SCN1A* IVS5-91 G>A (rs3812718) and c.3184A>G (rs2298771) were more likely to have lower lnCDRs of CBZ (p=0.0003, uncorrected; Table 4). This haplotype association remained significant after Bonferroni's correction as described in the statistical methods. Since diplotypes were the genetic composition of human, we further analyzed the association between diplotypes of *SCN1A* IVS5-91 G>A (rs3812718) and c.3184A>G (rs2298771) and ln CDRs of CBZ. The comparisons of diplotype distributions showed that patients with GA/AA, AA/AA, GA/AG or AA/AG diplotypes were more likely to have lower lnCDRs of CBZ (all $p<0.001$, uncorrected; Table 4). These diplotype associations remained significant after Bonferroni's correction as described in the statistical methods.

Regression model analysis

Multivariate regression analysis was applied to evaluate the combined effect of pharmacokinetic and pharmacodynamic related genes on lnCDRs of CBZ under adjustment of cofactors, such as epilepsy syndromes, weight, and age. Among the included factors, genetic variants in *SCN1A*, *EPHX1* and *UGT2B7* genes demonstrated significant effect on lnCDR of CBZ. The most fitted model indicated

that *SCN1A* IVS5-91 G>A (rs3812718), *EPHX1* c.337T>C (rs1051740) and *UGT2B7* c.802T>C (rs7439366) work synergistically on the effect of lnCDRs of CBZ (adjusted r^2=55.92 %; Table 5).

Discussion

The present study demonstrated that the *SCN1A* IVS5-91 G>A and *EPHX1* c.337T>C were significantly associated with maintenance dosages and lnCDRs of CBZ in single SNP analyses. With adjustment of cofactors, *SCN1A* IVS5-91 G>A, *EPHX1* c.337T>C and *UGT2B7* c.802T>C showed significant effect on lnCDRs of CBZ in the multivariate model. This was a proof-of-principle study and the polygenic effect on CDRs of CBZ has not been reported before.

The effect of *SCN1A* IVS5-91 G>A on the dosages of sodium channel-blocking AEDs or drug resistance has been evaluated in several studies. Carriers of the variant A allele were demonstrated to require higher CBZ maximum dosages, higher phenytoin steady-state concentrations or more likely to be CBZ-resistant [25-27]. However, inconsistent results have been reported in other studies as well [37-38]. The molecular mechanism of the possible association between *SCN1A* IVS5-91 G>A and the efficacy of sodium channel-blocking AEDs has been explored. The variant A allele has been demonstrated to generate an alternative splicing and resulted in altered proportions of neonate and adult exon 5 transcripts in adult brain tissue [26, 39]. Individuals with the wild-type G allele expressed 30-40% neonatal form in the SCN1A transcripts, whereas those with the variant A allele expressed less than 1%. The effect of this polymorphism seemed to be modified by neuro-oncological ventral

antigen 2 (Nova2) expression levels, with higher Nova2 expression increasing the proportion of the neonate form and larger Nova2-mediated effect was identified in the AA genotype which was associated with increased dosage requirements [39]. Our results confirmed the previous findings and further demonstrated the association between *SCN1A* IVS5-91 and CDRs of CBZ. Since the efficacy to AEDs is theoretically polygenic in respect to inherited component [40], the impact of a single gene was likely to be confounded by effects of other genes and disease related factors. Therefore, we included genes related to pharmacokinetic and pharmacodynamic of CBZ and the synergistic effect of *SCN1A*, *EPHX1* and *UGT2B7* genes on CBZ treatment optimization were revealed by our results.

The nonsynonymous SNPs, *EPHX1* c.337T>C and c.416A>G, were suggested to influence the stability of human mEH and affect enzyme activity in a substrate specific way [5, 7, 41-42]. Using benzo(α)pyrene-4,5-epoxide and *cis*-stilbene oxide as substrates, the 337C allele demonstrated decreased human mEH activity whereas the 416G allele was associated with enhanced hydrolysis activity [5, 41]. In studies using carbamazepine-10,11-epoxide as the substrate, the 337C allele demonstrated higher human mEH activity and the 416A>G allele was associated with decreased hydrolysis activity [7, 42]. The effects of *EPHX1* c.337T>C and c.416A>G on the dosage of CBZ have been demonstrated in a previous study in the multivariate model

incorporating patient age [31]. Patients with the 337C allele and the 416G allele were associated with higher CBZ dosages [31]. However, the effects of these two SNPs on CBZ steady-state concentrations were not evaluated. The present study not only demonstrated that patients with the 337C allele were more likely to require higher CBZ maintenance dose and also further revealed that patients with the 337C allele tended to have significantly lower CBZ CDRs. In the multivariate model, *SCN1A* IVS5-91, *EPHX1* c.337T>C and *UGT2B7* c.802T>C were synergistically affect CDRs of CBZ under adjustment for the epilepsy syndromes, age and weight.

Although the effect of *UGT2B7* c.802T>C on CDRs of CBZ did not detected in the single gene analysis, it was demonstrated to be significantly associated with the CDRs of CBZ in the multivariate model. These findings suggested that multivariate analysis may be more informative than single variant in clinical studies. The UGT2B7 enzyme predominately mediated the N-glucuronidation of CBZ which accounted for 15% of parent CBZ metabolism [8]. The functional significance of *UGT2B7* genetic polymorphisms was controversial. Several studies have demonstrated that the activities of UGT2B7 enzymes may be higher, similar or lower in cells or subjects with variant *UGT2B7* -161C>T and 802T>C [43-46]. Both methodological and substrate factors may contribute to the differences. Our results indicated that subjects with variants of *UGT2B7* c.802T>C may have higher UGT2B7 enzyme activities

toward glucuronidation of CBZ and patients with the variant C allele tended to require higher CBZ maintenance doses.

There were inter-ethnic differences in the frequencies of genetic variants in *SCN1A*, *EPHX1* and *UGT2B7* genes. According to the HapMap database, the minor allele frequencies of *EPHX1* c.337T>C were lower in both Caucasians and Africans as compared with the present study. As for the minor allele frequencies of *UGT2B7* c.802T>C, the frequency of the variant C allele was lower in Caucasians and higher in Africans as compared with the present study. In addition, the minor allele frequency of *SCN1A* IVS5-91G>A was similar in Caucasians and lower in Africans as compared with the present study. Therefore, there might be the possibility of a population-specific effect regarding the genetic association of CBZ therapy optimization.

In conclusion, based on comprehensive analysis of pharmacokinetic and pharmacodynamic related genetic variants, the present study revealed that *EPHX1*, *UGT2B7* and *SCN1A* genetic polymorphisms simultaneously modulated the CBZ maintenance doses and CDRs. Population trough CDRs of antiepileptic drugs has been suggested to be a clinical reference to estimate the dose required to achieve a target concentration [47]. It has also been proposed for the pharmacokinetic monitoring of cyclosporine trough concentration [48]. Therefore, factors that

contributed to interindividual variability of CDRs may be important in clinical application. Although these results may provide information regarding personalized pharmacotherapy approaches to CBZ maintenance therapy, further larger population studies need to confirm our findings.

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References

1. Brodie MJ, Dichter MA: Established antiepileptic drugs. *Seizure* 6, 159-174 (1997).

2. Kwan P, Brodie MJ: Effectiveness of first antiepileptic drug. *Epilepsia* 42, 1255-1260 (2001).

3. Tomson T, Tybring G, Bertilsson L: Single-dose kinetics and metabolism of carbamazepine-10,11-epoxide. *Clin Pharmacol Ther* 33, 58-65 (1983).

4. Smith CA, Harrison DJ: Association between polymorphism in gene for microsomal epoxide hydrolase and susceptibility to emphysema. *Lancet* 350, 630-633 (1997).

5. Hassett C, Aicher L, Sidhu JS, Omiecinski CJ: Human microsomal epoxide hydrolase: genetic polymorphism and functional expression in vitro of amino acid variants. *Hum Mol Genet* 3, 421-428 (1994).

6. Maekawa K, Itoda M, Hanioka N *et al.*: Non-synonymous single nucleotide alterations in the microsomal epoxide hydrolase gene and their functional effects. *Xenobiotica* 33, 277-287 (2003).

7. Nakajima Y, Saito Y, Shiseki K *et al.*: Haplotype structures of EPHX1 and their effects on the metabolism of carbamazepine-10,11-epoxide in Japanese epileptic patients. *Eur J Clin Pharmacol* 61, 25-34 (2005).

8. Staines AG, Coughtrie MW, Burchell B: N-glucuronidation of carbamazepine in human tissues is mediated by UGT2B7. *J Pharmacol Exp Ther* 311, 1131-1137 (2004).

9. Bhasker CR, McKinnon W, Stone A *et al.*: Genetic polymorphism of UDP-glucuronosyltransferase 2B7 (UGT2B7) at amino acid 268: ethnic diversity of alleles and potential clinical significance. *Pharmacogenetics* 10, 679-685 (2000).

10. Hirota T, Ieiri I, Takane H *et al.*: Sequence variability and candidate gene analysis in two cancer patients with complex clinical outcomes during morphine therapy. *Drug Metab Dispos* 31, 677-680 (2003).

11. Luna-Tortos C, Fedrowitz M, Loscher W: Several major antiepileptic drugs are substrates for human P-glycoprotein. *Neuropharmacology* 55, 1364-1375 (2008).

12. Weiss J, Kerpen CJ, Lindenmaier H, Dormann SM, Haefeli WE: Interaction of antiepileptic drugs with human P-glycoprotein in vitro. *J Pharmacol Exp Ther* 307, 262-267 (2003).

13. Potschka H, Fedrowitz M, Loscher W: P-glycoprotein and multidrug resistance-associated protein are involved in the regulation of extracellular levels of the major antiepileptic drug carbamazepine in the brain. *Neuroreport*

12, 3557-3560 (2001).

14. Potschka H, Fedrowitz M, Loscher W: P-Glycoprotein-mediated efflux of phenobarbital, lamotrigine, and felbamate at the blood-brain barrier: evidence from microdialysis experiments in rats. *Neurosci Lett* 327, 173-176 (2002).

15. Meng H, Guo G, Ren J, Zhou H, Ge Y, Guo Y: Effects of ABCB1 polymorphisms on plasma carbamazepine concentrations and pharmacoresistance in Chinese patients with epilepsy. *Epilepsy Behav* 21, 27-30 (2011).

16. Seo T, Ishitsu T, Ueda N *et al.*: ABCB1 polymorphisms influence the response to antiepileptic drugs in Japanese epilepsy patients. *Pharmacogenomics* 7, 551-561 (2006).

17. Ozgon GO, Bebek N, Gul G, Cine N: Association of MDR1 (C3435T) polymorphism and resistance to carbamazepine in epileptic patients from Turkey. *Eur Neurol* 59, 67-70 (2008).

18. Simon C, Stieger B, Kullak-Ublick GA *et al.*: Intestinal expression of cytochrome P450 enzymes and ABC transporters and carbamazepine and phenytoin disposition. *Acta Neurol Scand* 115, 232-242 (2007).

19. Begley DJ: ABC transporters and the blood-brain barrier. *Curr Pharm Des* 10, 1295-1312 (2004).

20. Ufer M, Mosyagin I, Muhle H *et al.*: Non-response to antiepileptic pharmacotherapy is associated with the ABCC2 -24C>T polymorphism in young and adult patients with epilepsy. *Pharmacogenet Genomics* 19, 353-362 (2009).

21. Haenisch S, Zimmermann U, Dazert E *et al.*: Influence of polymorphisms of ABCB1 and ABCC2 on mRNA and protein expression in normal and cancerous kidney cortex. *Pharmacogenomics J* 7, 56-65 (2007).

22. Kim WJ, Lee JH, Yi J *et al.*: A nonsynonymous variation in MRP2/ABCC2 is associated with neurological adverse drug reactions of carbamazepine in patients with epilepsy. *Pharmacogenet Genomics* 20, 249-256 (2010).

23. Szoeke CE, Newton M, Wood JM *et al.*: Update on pharmacogenetics in epilepsy: a brief review. *Lancet Neurol* 5, 189-196 (2006).

24. Ferraro TN, Buono RJ: The relationship between the pharmacology of antiepileptic drugs and human gene variation: an overview. *Epilepsy Behav* 7, 18-36 (2005).

25. Abe T, Seo T, Ishitsu T, Nakagawa T, Hori M, Nakagawa K: Association between SCN1A polymorphism and carbamazepine-resistant epilepsy. *Br J Clin Pharmacol* 66, 304-307 (2008).

26. Tate SK, Depondt C, Sisodiya SM *et al.*: Genetic predictors of the maximum

doses patients receive during clinical use of the anti-epileptic drugs carbamazepine and phenytoin. *Proc Natl Acad Sci U S A* 102, 5507-5512 (2005).

27. Tate SK, Singh R, Hung CC *et al.*: A common polymorphism in the SCN1A gene associates with phenytoin serum levels at maintenance dose. *Pharmacogenet Genomics* 16, 721-726 (2006).

28. Proposal for revised classification of epilepsies and epileptic syndromes. Commission on Classification and Terminology of the International League Against Epilepsy. *Epilepsia* 30, 389-399 (1989).

29. Kwan P, Arzimanoglou A, Berg AT *et al.*: Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. *Epilepsia* 51, 1069-1077 (2010).

30. Siegmund W, Ludwig K, Giessmann T *et al.*: The effects of the human MDR1 genotype on the expression of duodenal P-glycoprotein and disposition of the probe drug talinolol. *Clin Pharmacol Ther* 72, 572-583 (2002).

31. Makmor-Bakry M, Sills GJ, Hitiris N, Butler E, Wilson EA, Brodie MJ: Genetic variants in microsomal epoxide hydrolase influence carbamazepine dosing. *Clin Neuropharmacol* 32, 205-212 (2009).

32. Zimmermann A, Blaszkewicz M, Roth G *et al.*: UDP-glucuronosyltransferase

2B7 C802T (His268Tyr) polymorphism in bladder cancer cases. *J Toxicol Environ Health A* 71, 911-914 (2008).

33. Excoffier L, Slatkin M: Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. *Mol Biol Evol* 12, 921-927 (1995).

34. Lewontin RC: The Interaction of Selection and Linkage. Ii. Optimum Models. *Genetics* 50, 757-782 (1964).

35. Lewontin RC: The Interaction of Selection and Linkage. I. General Considerations; Heterotic Models. *Genetics* 49, 49-67 (1964).

36. Kelly PJ, Stallard N, Whittaker JC: Statistical design and analysis of pharmacogenetic trials. *Stat Med* 24, 1495-1508 (2005).

37. Zimprich F, Stogmann E, Bonelli S *et al.*: A functional polymorphism in the SCN1A gene is not associated with carbamazepine dosages in Austrian patients with epilepsy. *Epilepsia* 49, 1108-1109 (2008).

38. Manna I, Gambardella A, Bianchi A *et al.*: A functional polymorphism in the SCN1A gene does not influence antiepileptic drug responsiveness in Italian patients with focal epilepsy. *Epilepsia* 52, e40-44 (2011).

39. Heinzen EL, Yoon W, Tate SK *et al.*: Nova2 interacts with a cis-acting polymorphism to influence the proportions of drug-responsive splice variants

of SCN1A. *Am J Hum Genet* 80, 876-883 (2007).

40. Loscher W, Klotz U, Zimprich F, Schmidt D: The clinical impact of pharmacogenetics on the treatment of epilepsy. *Epilepsia* 50, 1-23 (2009).

41. Hassett C, Lin J, Carty CL, Laurenzana EM, Omiecinski CJ: Human hepatic microsomal epoxide hydrolase: comparative analysis of polymorphic expression. *Arch Biochem Biophys* 337, 275-283 (1997).

42. Kitteringham NR, Davis C, Howard N, Pirmohamed M, Park BK: Interindividual and interspecies variation in hepatic microsomal epoxide hydrolase activity: studies with cis-stilbene oxide, carbamazepine 10, 11-epoxide and naphthalene. *J Pharmacol Exp Ther* 278, 1018-1027 (1996).

43. Daly AK, Aithal GP, Leathart JB, Swainsbury RA, Dang TS, Day CP: Genetic susceptibility to diclofenac-induced hepatotoxicity: contribution of UGT2B7, CYP2C8, and ABCC2 genotypes. *Gastroenterology* 132, 272-281 (2007).

44. Holthe M, Rakvag TN, Klepstad P *et al.*: Sequence variations in the UDP-glucuronosyltransferase 2B7 (UGT2B7) gene: identification of 10 novel single nucleotide polymorphisms (SNPs) and analysis of their relevance to morphine glucuronidation in cancer patients. *Pharmacogenomics J* 3, 17-26 (2003).

45. Levesque E, Delage R, Benoit-Biancamano MO *et al.*: The impact of

UGT1A8, UGT1A9, and UGT2B7 genetic polymorphisms on the pharmacokinetic profile of mycophenolic acid after a single oral dose in healthy volunteers. *Clin Pharmacol Ther* 81, 392-400 (2007).

46. Ross JR, Rutter D, Welsh K *et al.*: Clinical response to morphine in cancer patients and genetic variation in candidate genes. *Pharmacogenomics J* 5, 324-336 (2005).

47. Blanca Sanchez M, Herranz JL, Leno C *et al.*: UGT2B7_-161C>T polymorphism is associated with lamotrigine concentration-to-dose ratio in a multivariate study. *Ther Drug Monit* 32, 177-184 (2010).

48. Garcia-Saiz M, Lopez-Gil A, Alfonso I, Boada JN, Armijo JA: Factors influencing cyclosporine blood concentration-dose ratio. *Ann Pharmacother* 36, 193-199 (2002).