Linkage Disequilibrium between Polymorphisms of ABCB1 and ABCC2 to Predict the Treatment Outcome of Malaysians with Complex Partial Seizures on Treatment with Carbamazepine Mono-Therapy at the Kuala Lumpur Hospital

Soobitha Subenthiran¹*, Noor Rain Abdullah¹, Joyce Pauline Joseph², Prem Kumar Muniandy¹, Boon Teck Mok¹, Chee Cheong Kee³, Zakiah Ismail¹, Zahurin Mohamed⁴

¹Bioassay Unit, Herbal Medicine Research Center, Institute for Medical Research, Kuala Lumpur, Malaysia, ²Department of Neurology, Kuala Lumpur General Hospital, Kuala Lumpur, Malaysia, ³Medical Research Center, Institute for Medical Research, Kuala Lumpur, Malaysia, ⁴Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Abstract

Purpose: Carbamazepine (CBZ) is used as the first line of treatment of Complex Partial Seizures (CPS) in the Epilepsy Clinic, Neurology Department of Kuala Lumpur Hospital (KLH). More than 30% of the patients remain drug resistant to CBZ monotherapy. CBZ is transported by the P-glycoprotein (P-gp). The P-gp encoded by the ABCB1 and ABCC2 genes are expressed in drug resistant patients with epilepsy. A few studies have shown significant association between CBZ resistant epilepsy and Linkage Disequilibrium (LD) with adjacent polymorphisms of these genes. Our study is aimed at determining the correlation between patients’ response to CBZ mono-therapy to Single Nucleotide Polymorphisms G2677T and C3435T of the ABCB1 gene as well as G1249A and 24C>T of the ABCC2 gene.

Method: 314 patients with CPS were recruited from the Neurology Department of the KLH based on stringent inclusion and exclusion criteria, of whom 152 were responders and the other 162 were non-responders. DNA was extracted from their blood samples and Taqman technology for allelic discrimination was performed. Results were described as genotype frequencies. The SHEsis analysis platform was used to calculate linkage disequilibrium index and infer haplotype frequencies. Haploview was used to do permutation test to obtain a corrected p-value.

Results: Resistance to treatment with CBZ mono-therapy was significantly associated with the 2677TT and the 3435TT genotypes while it was not significantly associated with the G1249A and 24C>T polymorphisms. The GCGC haplotype combination of the 2677G>T, 3435C>T, 1249G>A and 24C>T respectively was found to be extremely significant (p = 1.10e-20) with good drug response to CBZ mono-therapy.

Conclusion: Linkage disequilibrium between the 2677G>T, 3435C>T, 1249G>A and 24C>T SNPs may be used as a reliable screening marker to determine the treatment outcome of CBZ mono-therapy with CPS irrespective of race or gender.

Citation: Subenthiran S, Abdullah NR, Joseph JP, Muniandy PK, Mok BT, et al. (2013) Linkage Disequilibrium between Polymorphisms of ABCB1 and ABCC2 to Predict the Treatment Outcome of Malaysians with Complex Partial Seizures on Treatment with Carbamazepine Mono-Therapy at the Kuala Lumpur Hospital. PLoS ONE 8(5): e64827. doi:10.1371/journal.pone.0064827

* E-mail: soobitha@imr.gov.my

Introduction

Epilepsy is a complex of seizures which arise from abnormal, excessive, synchronous and sustained discharge of a group of neurons. This in turn leads to persistent increase of neuronal excitability. 50% of all epilepsies are idiopathic while other causes of epilepsy are trauma, oxygen deprivation, tumors, infection, metabolic derangement, disorders of neuronal migration and monogenic epilepsies [1]. Complex Partial Seizures (CPS) start focally within the brain and causes impairment of consciousness [2]. It is the most common manifestation of Temporal Lobe Epilepsy (TLE) [3]. The incidence of epilepsy in Malaysia is 4–8 per 1000 population [4], while the percentage of CPS of idiopathic etiology is 5.7% [5].

Carbamazepine (CBZ) is used in the treatment of Generalized Tonic Clonic (Grand Mal), Partial and Generalized Secondary Seizures. CBZ is a derivative of Iminostilbene which is related to...
the tricyclic antidepressant family. This makes it suitable in the treatment of Bipolar Affective Disorder and Trigeminal Neuralgia [6].

In KLH, CBZ is used as the first line of treatment of CPS as it is known to control seizures effectively in most individuals and also because it is economical. CBZ is transported by binding to Albumin and 9α-acid Glycoprotein (AGP), an acute phase protein. In normal individuals 75–85% of the drug is bound to these plasma proteins while 20–25% of it remains in its free form [7]. CBZ exhibits significant dose response variability so, individualizing dosage and determining if a person is resistant to the drug is essential. Drug resistance is known to be influenced by failure of drug transport which is thought to be governed by genetic factors. Three genes known to be involved in the drug transport are ABCB1, ABCC2 and RALBP1.

Conflicting results have been reported regarding the contribution of ABCB1 polymorphisms to CBZ disposition. The most common single nucleotide polymorphisms (SNPs) identified on the ABCB1 gene were C3435T, G2677T and G1236T. A study conducted on the Japanese population with epilepsy showed that the TT genotype of the C3435T and that the TTT haplotype of the 3 SNPs were associated with CBZ-resistant epilepsy [8]. However, a meta-analysis done between drug resistant epilepsy in general showed no association to the C3435T SNP [9]. The same lack of association was also demonstrated in the Turkish population [10]. The correlation between the presence of “T” allele of the G2677T SNP and resistance to antiepileptic drugs (AEDs) were studied in a few countries such as Mosyagin I et al [11] in Germany, Grover S et al [12] and Vahab SA et al [13] in India and was previously thought to be non-significant. However, we did not include the G1236T SNP in this study as it has never shown any association with drug resistant epilepsy in any of the previous studies unlike the other SNPs.

The ABCC2 also known as the Multidrug resistance protein 2 (MRP2) is involved in the transport of antiepileptic drugs and was found to be up-regulated in the brain tissues of epileptic patients. It was hypothesized that genetic variations in the MRP2 gene would probably affect individual drug responses to CBZ. A study conducted in Korea showed that the “A” allele of the MRP2 single nucleotide polymorphism 1249G>A was associated with adverse neurological drug reactions to CBZ [14]. Another study which was conducted in Germany on 103 responders and 113 non-responders to first line antiepileptic drug therapy showed that the non-responders tended to carry the “T” allele of the −24C>T variant of the ABCC2 gene.

Malaysia is a multiracial country. Its peninsular comprises of three major ethnic groups namely, the Malays, Chinese and Indians who have lived in Malaysia for over five generations. They have been exposed to the same environmental factors such as diet and lifestyle. Recent association studies have shown no significant difference between the three ethnic groups in terms of drug response and disease susceptibility. The difference in findings of association studies among these SNPs which were conducted in other countries may be due to racial differences but after careful observation, it seems more likely due to the method in which the subjects were selected.

Methods and Methodology

This study was approved by the Research Review Committee, Institute for Medical Research and Medical Research Ethics Committee (MREC), Ministry of Health Malaysia. The research was conducted in accordance to the principles expressed in the Declaration of Helsinki.

2.1 Recruitment of patients for study

Malaysian patients of Malay, Chinese and Indian were recruited from the Epilepsy Clinic, Neurology Department of KLH, weekly. There were a total of 40 to 50 patients who are followed up every Monday as KLH serves as a tertiary referral hospital. The patient files were identified prior to the epilepsy clinic and written informed consent was obtained from suitable study volunteers. Study volunteers who fulfilled the inclusion criteria were enrolled into the study. They were provided with clear verbal as well as written information about the study. Those enrolled into the study gave their informed consent voluntarily.

A total of 314 patients were recruited and enrolled into the study. One hundred and fifty six (49.7%), 94 (26.8%) and 70 (23.5%) were Malays, Chinese and Indians respectively. The study volunteers were diagnosed with CPS and secondary causes such as trauma, metabolic disorders, tumors and infectious causes were ruled out. As per the study design, 152 were responders to CBZ, while 162 were non-responders to CBZ. The study volunteers were required to answer a few questions regarding their family background (family history of epilepsy) their disease history (onset of the disease, characteristics as well of evolution of the seizure, frequency of attacks and treatment history) and progression (change in frequency of seizures after the onset of treatment) before they were assigned to the respective groups.

The responders were found to have normal MRI findings and mostly normal scalp EEG except a few patients who showed left or right temporal spikes. They were men and women between the age group of 18 to 60 years with no other co-morb. They were already on an optimum dose of CBZ as a single drug therapy and were compliant to treatment. As they were being followed up every 3 months, we had chosen patients with no episodes of seizures in more than a year. We made sure that their serum CBZ levels were within the therapeutic range (17–51 μmol/L) to ensure compliance to treatment.

The non-responders were patients with CPS with similar MRI and EEG findings as the responders. They were men and women between the age group of 18 to 60 years with no other co-morb. They were on a poly-therapy which included CBZ, at the time of recruitment. They were drug resistant patients. Their compliance to treatment while they were on CBZ mono-therapy was supported by their serum CBZ level before other antiepileptic agents such as Lamotrigine and Sodium Valproate were introduced.

Patients with other types of seizures were excluded from the study. Patients who were never treated with CBZ as well as those who had abnormal renal and liver function tests were also excluded from the study. In addition to this, patients who were on drugs which interacted with CBZ such as Alcohol, Macrolides, Anti Tubercular Drugs, Antifungal agents, Benzodiazepines, Anti Psychotics, Antidepressants, Calcium Channel Blockers, Cisplatin, H2 blockers, Cyclosporine, Doxorubicin, Tetracycline, Hormone Contraceptives, Anti Retro-viral drugs and Theophylline, were excluded from the study, to rule out confounding factors which may have influenced the outcome of treatment.

2.2 DNA extraction from whole blood

DNA extraction was done from theuffy coat using the Qiagen DNA extraction blood kit. DNA concentration and purity was quantified using the Nanodrop 2000 Spectrophotometer by Thermo Scientific.

2.3 Real Time PCR

Real Time PCR was run using SNP Specific Predesigned Taqman Probes as shown in Table 1 as well as Vic and Fam
Results

Among the 314 patients recruited, 156 were males while 158 were females with a mean age of 37.5 ± 12.3 and 38.0 ± 10.3 years among responders and non-responders respectively. The responders were on a mean dose of 9.75 ± 5.1 mg/kg/day and a mean serum CBZ level of 27.5 ± 10.7 μmol/L.

The distribution of all genotypes (wild-type, heterozygous and homozygous mutant variants) and genotype frequencies were as shown in Table 3. Genotype frequencies were in accordance to Hardy-Weinberg equilibrium model. Linkage Disequilibrium (LD) between haplotypes of the rs2032582 (ABCB1), 1249G>T (ABCC2), 24C>T (ABCC1), 1249G>A (ABCC2), −24C>T (ABCC2) were determined using the SHEsis program platform, as shown in Table 4. The C1236T SNP was not included in this study as it failed to show any association with drug resistant epilepsy in any of the previous studies. Haplotypes with frequencies of less than 0.03 were omitted from the analysis. Permutation test was applied 1000 times to obtain the corrected p-value. The Linkage Disequilibrium test which includes D’ and R² is as shown in Figure 1.

Discussion

The patients were recruited irrespective of race or gender. The percentage distribution of the patients based on race was reflective of the Malaysian population in the peninsular region. They have been exposed to the same environmental factors such as diet and lifestyle. Previous findings of association studies among these SNPs which were conducted in other countries were found to be different. This was most likely due to the method in which the subjects were selected. The p value for each race in association to each polymorphism as well as to the treatment outcome was determined using Chi square statistics, to rule out genetic heterogeneity. The p values for all the above mentioned parameters were >0.05. Confounding factors such as patient compliance, drug interaction and presence of preexisting diseases were eliminated. In the end of the study, it was evident that neither race (p = 0.569) nor gender (p = 0.090) played a significant role in the outcome of treatment or had any genetic association with any of the polymorphisms. In addition to this, their renal profile and liver function test were determined to exclude the failure of therapy due to poor drug metabolism or elimination. 152 patients were responders while the other 162 were non-responders to CBZ mono-therapy. Patients were considered responders if they were free of unprovoked seizures for more than a year since the initiation of the optimum dose of CBZ as a single drug therapy.

Table 1. Step One Plus Real Time PCR setting.

| Stage            | Step                 | Temp | Time |
|------------------|----------------------|------|------|
| Holding          | DNA Polymerase Activation | 95°C | 20 sec |
| Cycling (40 cycles) | Denature            | 95°C | 3 sec |
|                  | Anneal/Extend        | 60°C | 30 sec |

doi:10.1371/journal.pone.0064827.t001

Table 2. Single Nucleotide Polymorphism (SNP) Probe sequence and Assay ID.

| SNP      | RS number | Probe Sequence | Assay code |
|----------|-----------|----------------|------------|
| G2677T   | rs2032582 | TATTTAGTTTGACTCACTTCCCCAG [C/T] ACCTTCTAGTTCTTTCTTATCTTTC | C_11711720D_40 |
| C3435T   | rs1045642 | TGTTGGCCTCTTTGCTGCCCTCAC [A/G] ATCTCTCCTGTCACACCCCGGGC | C_7586657_20 |
| c1249G>A | rs2273697 | CACCTGCGCAAGAGGAGTACACC [A/G] TGGAGAAAACTGGAAAGCTGTAGTC | C_2227980_20 |
| −24C>T   | rs717620  | ACAATCATATTTTAGAGAGTCTT [C/T] GTTCAGACGCGAGTCAGGAAATCAT | C_2814642_10 |

doi:10.1371/journal.pone.0064827.t002
Non-responders were patients who had poor seizure control with an optimum dose CBZ mono-therapy, requiring an addition of other AEDs. Failure in drug transport has been implicated in many of the studies involving resistant to treatment in numerous diseases. The ABCB1 gene has been found to be associated with treatment resistance in many of the neurological diseases which have been studied thus far. ABCB1 is a multidrug efflux pump known to be present in all excretory organs including the epithelial lining of the blood-brain barrier. This P-gp macromolecule is responsible for drug transport [18–23]. ABCC2 is a member of the MRP subfamily which is involved in multi-drug resistance [24]. This protein is expressed in the canalicular (apical) part of the hepatocyte and functions in biliary transport [25].

The G2677T was the first SNP discovered from the human P-gp. The association between the G2677T SNP in relation to the treatment outcome with CBZ among Europeans [11] and Indians from India [12] were found to be non-significant. In contrary to that, a similar study done on the Japanese patients with TLE [8] was found to be significant. A study done on the Chinese population of Beijing showed a high degree of linkage disequilibrium between G2677T and two other polymorphisms of the ABCB1 gene in relation to drug resistant epilepsy [26]. However the study we conducted on the Malaysian population showed that the 2677TT genotype was significantly (p = 0.001) associated with CBZ resistance in the treatment of CPS. This is similar to the study conducted on the Japanese population only recruited patients with Temporal Lobe epilepsy which is responsible for most CPS and managed to obtain significant association with the outcome of treatment.

We also found that patients with 3435TT genotype were significantly less (p = 0.007) likely to respond to CBZ mono-therapy. The 3435TT genotype was associated with decreased expression of P-gp among Caucasians [27] but increased expression in the Japanese patients [28–29]. Two other studies conducted on Japanese [8] and Turkish [10] patients showed evidence that the 3435TT genotype was associated with drug resistant epilepsy. A study was previously conducted by another

| SNP / Genotype | Genotype frequency (%) | *Permuted *Permuted |
|---------------|------------------------|---------------------|
|               | Responder | Non-responder | χ²  | p  |
| ABCB1/ C3435T |           |             |      | |
| C/C          | 35(0.22) | 51(0.34)    | 12.06 | 0.007 |
| T/T          | 68(0.42) | 36(0.24)    |       |     |
| C/T          | 59(0.36) | 65(0.43)    |       |     |
| ABCB1/ G2677T|           |             |      | |
| G/G          | 59(0.36) | 144(0.75)   | 61.70 | <0.001 |
| T/T          | 57(0.35) | 25(0.16)    |       |     |
| G/T          | 46(0.28) | 13(0.09)    |       |     |
| ABCC2/ G1249A|           |             |      | |
| G/G          | 120(0.74) | 124(0.82)  | 2.23  | 0.511 |
| A/A          | 0(0.00)  | 1(0.01)     |       |     |
| G/A          | 42(0.26) | 27(0.18)    |       |     |
| ABCC2/ −24C>T|           |             |      | |
| C/C          | 110(0.68) | 102(0.67)  | 0.14  | 0.986 |
| T/T          | 4(0.03)  | 6(0.04)     |       |     |
| C/T          | 48(0.30) | 44(0.29)    |       |     |

*1000 permutation applied.
doi:10.1371/journal.pone.0064827.t003

![Figure 1. Linkage Disequilibrium test.](doi:10.1371/journal.pone.0064827.g001)
team in Malaysia which showed no association between polymorphisms of the ABCB1 gene and drug resistant epilepsy [9]. However they had recruited patients without specifying the type of epilepsy or a specific drug used for treatment. Another important confounding factor we eliminated was known drug interaction with CBZ.

We found no correlation between the G1249A of the ABCC2 gene and the outcome of treatment of patients with CPS on CBZ monotherapy. This is similar to the findings of the study conducted on the Japanese patients [8] and the Korean population [32]. However, the −24C>T polymorphism was found to be significantly associated with drug resistant epilepsy among German patients [30].

The haplotypes of the four SNPs showed significant association with the outcome treatment CPS with CBZ monotherapy. The haplotype combination GCAC of the 2677G>A and −24C>T respectively was found to be extremely significant (p = 1.10e-20) with good drug response to CBZ monotherapy while the haplotype combination of TTGC was associated with poor response. Though screening the 2677GG genotype to determine good response to CBZ monotherapy was found to be significant, based on our findings, we found that people of different ethnic background may influence the differences in the frequencies of these haplotypes. These differences observed may also be due to the recruitment method of patients. Different types of seizures respond differently to different AEDs. It is very important to determine patient compliance to treatment as well as to rule out drug interaction. The findings of this study may be helpful in predicting the treatment outcome of patients with CPS of certain populations before CBZ is introduced so a more effective method of treatment may be advocated.

The inference drawn from this study would presumably have a tremendous impact on cost-benefit ratio. Though this would require a separate study to be conducted it is safe to say that screening patients by determining the presence of these polymorphisms would help in deciding the best treatment option to be chosen for the patient. This would in the long term reduce the morbidity among these patients.

Acknowledgments

We would like to thank Dr. Md Hanip Rafia and the staff of Neurology department of Kuala Lumpur General Hospital for allowing me to use their facilities to recruit patients for the study.

Author Contributions

Conceived and designed the experiments: SS CCK JPJ ZM. Performed the experiments: SS PKM BTM NRA. Analyzed the data: SS CCK. Contributed reagents/materials/analysis tools: SS NRA ZI JPJ. Wrote the paper: SS NRA ZM. Edited: NRA ZM ZI. Contributed reagents/materials/analysis tools: SS PKM BTM NRA. Analyzed the data: SS CCK. Prepared figures: BTM.

References

1. Engelborghs S, D’Hooge R, De Deyn PP (2000) Pathophysiology of epilepsy. Acta Neurol Belg 100: 201–213.
2. International League Against Epilepsy. (1981) Proposal for revised clinical and electroencephalographic classification of epileptic seizures. Epilepsia 22: 409–501. doi:10.1111/j.1528-1157.1981.tb0159.x.
3. Elizabeth C. (2011) Complex partial seizures. Available: http://emedicine.medscape.com/article/1183962-overview. Accessed 10 August 2012.
4. Zakaria AK. (1998) Principles of treatment of epilepsy. Mal J Med Sci 5: 11–17.
5. Pratap RC, Gururaj AK. (1989) Clinical and electroencephalographic features of complex partial seizures in infants. Acta Neurol Scand 79: 125–127.
6. Micromedex Healthcare Series. (2010) Drugdex evaluations: Carbamazepine. Available: http://www.thomsonhc.com/hcs/librarian/PFDefaultActionId/pf. Accessed 10 August 2012.
7. Miller JW (2007) Epilepsy surgery in the frontal lobe: terra incognita or new frontier? Epilepsy Curr 7: 98–99. doi: 10.1111/j.1535-7511.2007.00186.x.
8. Seo T, Ishitsu T, Ueda N, Nakada N, Yurube K, et al. (2006) ABCB1 polymorphisms influence the response to antiepileptic drugs in Japanese epilepsy patients. Pharmacogenomics 7: 531–561. doi: 10.2217/1470242416.7.4.531.

Table 4. ABCB1 and ABCC2 haplotypes and treatment response to Carbamazepine mono-therapy.

| Haplotypes* | Responders (Frequencies) | Non- responders (Frequencies) | p | OR (95% C.I.) |
|-------------|--------------------------|-------------------------------|---|--------------|
| GCAC        | 8.75(0.03)               | 11.55(0.04)                  | 0.43 | 1.43 [0.59–3.50] |
| GCAC        | 16.23(0.05)              | 103.38(0.34)                 | 1.10e–20 | 10.06 [5.78–17.53] |
| GCAC        | 9.26(0.03)               | 19.16(0.06)                  | 0.04 | 2.30 [1.03–5.14] |
| GTGC        | 17.19(0.05)              | 10.19(0.03)                  | 0.24 | 0.62 [0.28–1.37] |
| GTGC        | 84.82(0.26)              | 74.56(0.25)                  | 0.66 | 0.92 [0.64–1.32] |
| GTGC        | 27.68(0.09)              | 22.13 (0.07)                 | 0.57 | 0.84 [0.47–1.51] |
| TACG        | 10.32(0.03)              | 1.47(0.01)                   | 0.01 | 0.15[0.03–0.84] |
| TGCG        | 73.98(0.23)              | 26.72(0.09)                  | 1.69e–6 | 0.32 [0.20–0.52] |
| TCGT        | 10.30(0.03)              | 4.72(0.02)                   | 0.18 | 0.48 [0.16–1.44] |
| TGTC        | 51.04(0.16)              | 15.25(0.05)                  | 1.27e–5 | 0.28 [0.16–1.44] |

Order of polymorphisms: 2677G>T, 3435C>T, 1249G>A, −24C>T
*Global χ² = 120.11, df = 9, p = 1.27e–21
(Haplotype were omitted if the estimated haplotype probability was less than 3%)
doi:10.1371/journal.pone.0064827.t004
9. Haerian BS, Lim KS, Mohamed EH, Tan HJ, Tan CT, et al. (2011) Lack of association of ABCB1 and MRP2 polymorphisms with response to treatment in epilepsy. Seizure 20: 387–394. doi: 10.1016/j.seizure.2011.01.008.

10. Orhon GO, Bebek N, Gul G, Cine N (2000) Association of MDR1 (C3435T) polymorphism and resistance to carbamazepine in epileptic patients from Turkey. Eur Neurol 59: 67–70. doi: 10.1159/000109264.

11. Mosyagin I, Runge U, Schroeder HW, Dazert E, Vogelgesang S, et al. (2008) Association of ABCB1 genic variants 3435G>T and 2677G>T to ABCB1 mRNA and protein expression in brain tissue from refractory epilepsy patients. Epilepsia 49: 1555–1561. doi: 10.1111/j.1528-1167.2008.01661.x.

12. Grover S, Bala K, Sharma S, Gourie-Devi M, Baghel R, et al. (2010) Absence of a general association between ABCB1 genic variants and response to antiepileptic drugs in epilepsy patients. Biochimie 92: 1207–1212. doi: 10.1016/j.biochi.2010.04.008.

13. Vahab SA, Sen S, Ravindran N, Mony S, Mathew A, et al. (2009) Analysis of genotype and haplotype effects of ABCB1 (MDR1) polymorphisms in the risk of medically refractory epilepsy in an Indian population. Drug Metab Pharmacokinet 24: 253–260. doi: 10.2133/dmnpk.24.253.

14. Kim WJ, Lee JH, Yi J, Cho YJ, Heo K, et al. (2010) A nonsynonymous variation on MRP2/ABCC2 is associated with neurological adverse drug reactions of Carbamazepine in patients with epilepsy. Pharmacogenet Genomics 20: 249–256.

15. Yin Z, Su M, Li X, Li M, Ma R, et al. (2009) ERCC2, ERCC1 polymorphisms and haplotypes, cooking oil fume and adenocarcinoma risk in Chinese non-smoking females. J Exp Clin Cancer Res 28: 153. doi: 10.1186/1756-9966-28-153.

16. Shi YY, He L (2005) SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 15: 97–98. doi: 10.1038/sj.cr.7290272.

17. Li Z, Zhang Z, He Z, Tang W, Li T, et al. (2009) A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis [http://analysis.bio-x.cn]. Cell Res 19: 519–523. doi: 10.1038/cr.2009.33.

18. Chaudhary FM, Mechetner EB, Resnison IB. (1992) Expression and activity of the multidrug resistance P-glycoprotein in human peripheral blood lymphocytes. Blood 80: 2735–2739.

19. Eichelbaum M, Fromm MF, Schwab M. (2004) Clinical aspects of the MDR1 (ABCB1) gene polymorphism. Ther Drug Monit 26: 180–185.

20. Fromm MF. (2004) Importance of P-glycoprotein at blood-tissue barriers. Trends Pharmacol Sci 25: 423–429. doi: 10.1016/j.tips.2004.06.002.

21. Meissner K, Jellinckhgy G, Meyer zu Schwabedissen H, Dazert P, Eickel E, et al. (2004) Modulation of multidrug resistance P-glycoprotein 1 (ABCB1) expression in human heart by hereditary polymorphisms. Pharmacogenetics 14: 381–385.

22. Rao VN, Dahlheimer JL, Bardgett ME, Snyder AZ, Finch RA, et al. (1999) Choroid plexus epithelial expression of MDR1 P glycoprotein and multidrug resistance-associated protein contribute to the blood-cerebrospinal-fluid drug-permeability barrier. Proc Natl Acad Sci U S A 96: 3900–3905.

23. Saito T, Zhang ZJ, Ohtsubo T, Noda I, Shibamori Y, et al. (2001) Homozygous disruption of the mdrla P-glycoprotein gene affects blood-nerve barrier function in mice administered with neurotoxic drugs. Acta Otolaryngol 121: 735–742.

24. Glavinas H, Krajcsi P, Correjes J, Sarkadi B. (2004) The role of ABC transporters in drug resistance, metabolism and toxicity. Curr Drug Deliv 1: 27–42.

25. Nakano T, Sekine S, Ito K, Horie T. (2009) Correlation between apical localization of Abcc2/Mrp2 and phosphorylation status of ezrin in rat intestine. Drug Metab Dispos 37: 1521–1527. doi: 10.1124/dmd.108.024836.

26. Qiang L, Liwen W, Lizi J, Qi X, Yan S. (2007) Association analysis of a C2677G polymorphism of MDR1 gene and refractory temporal lobe epilepsy in a Chinese population. Neurology 12: 94–95.

27. Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, et al. (2000) Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in man. Proc Natl Acad Sci U S A 97: 3473–3478.

28. Nakamura T, Sakaeda T, Horinouchi M, Tamura T, Aoyama N, et al. (2002) Effect of the mutation (C3435T) at exon 26 of the MDR1 gene on expression level of MDR1 messenger ribonucleic acid in duodenal enterocytes of healthy Japanese subjects. Clin Pharmacol Ther 71: 297–303. doi: 10.1067/mcp.2002.122055.

29. Sakaeda T, Nakamura T, Horinouchi M, Kakumoto Y, Ohmoto M, Aoyama N, et al. (2001) MDR1 genotype-related pharmacokinetics of digoxin after single oral administration in healthy Japanese subjects. Pharm Res 18: 1400–1404.

30. Ufer M, Mosyagin I, Muhle H, Jacobsen T, Haenisch S, et al. (2009) Non-functional polymorphism of MDR1 gene and refractory temporal lobe epilepsy in an Indian population. Epilepsy Res 84: 86–90.

31. Qu J, Zhou BT, Yin JY, Xu XJ, Zhao YC, et al. (2012) ABCC2 polymorphisms and haplotypes, cooking oil fume and adenocarcinoma risk in Chinese non-smoking females. J Exp Clin Cancer Res 28: 153. doi: 10.1186/1756-9966-28-153.

32. Kim DW, Lee SK, Chu K, Jang IJ, Yu KS, et al. (2009) Lack of association between ABCG2 and ABCB1 genic variants and drug resistance in partial epilepsy. Epilepsy Res 84: 86–90.