REVIEW ARTICLE

Role of ABCB1 C3435T Variant in Response to Antiepileptic Drugs in Epilepsy: A Review

Haerian BS[1], Mohamed Z[1], Mohamed EHM[1], Lim KS[2], Tan HJ[3], Tan CT[2], Raymond AA[3]

Departments of [1]Pharmacology and [2]Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur
[3]Department of Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur

ABSTRAK

Pengekspresan terlampau P-glikoprotin (P-gp), hasil bagi gen untuk kaset pengikatan ATP (ABC), subfamili B, ahli 1 (ABCB1/MDR1), memain peranan penting dalam memperantarai ketahanan *multidrug* terhadap dadah antiepilepsi (DAE), pada lebih kurang 30% daripada pesakit epilepsi. Variasi genetik mungkin merupakan sebahagian daripada sebab kenapa terdapatnya perbezaan inter-individu dalam perhubungan fenotip-genotip bagi respons farmakologikal pesakit epilepsi terhadap DAE. Sinonim polymorfisme C3435T adalah satu daripada varian alel yang paling kerap berlaku pada gen ABCB1/MDR1, dan ini dicadangkan sebagai penyebab epilepsi refraktori. Banyak kajian telah menunjukkan hubungan antara polimorfisme C3435T dan refraktori terhadap DAE bagi penyakit epilepsi. Walaupun begitu, terdapat kontroversi antara hasil penemuan dari pelbagai kajian, iaitu, sama ada polimorfisme gen ABCB1/MDR1 C3435T berkait dengan respons terhadap DAE bagi pesakit epilepsi. Kertas ulasan ini memberikan latarbelakang dan membincangkan hasil penyiasatan pada faktor-faktor yang bercanggah yang mempengaruhi interpretasi dan implementasi kajian-kajian asosiasi dalam bidang ini.

Kata kunci: epilepsi, dadah antiepileptik, ketahanan dadah, respons terhadap dadah, ABCB1, P-gp, polimorfisme nukleotid tunggal

ABSTRACT

Over-expression of P-glycoprotein (P-gp), the encoded product of the ATP-binding cassette (ABC), sub-family B, member 1 (ABCB1/MDR1) gene, plays an important role in mediating multidrug resistance to antiepileptic drugs (AEDs) in about 30% of patients with epilepsy. Genetic variation may in part explain inter-individual differences in phenotype-genotype relationships in the pharmacological response of epilepsy patients to AEDs. The synonymous C3435T polymorphism is one of the most common allelic variants in the ABCB1/MDR1 gene, proposed in the causation of refractory epilepsy. Many studies have shown the relationship between C3435T polymorphism

Address for correspondence and reprint requests: Prof Dr Raymond Azman Ali, Department of Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur. Email: raymond@ppukm.ukm.my

and refractoriness to AEDs in epilepsy. However, there is controversy between the findings of various studies, that is, whether ABCB1/MDR1 C3435T gene polymorphism is associated with response to AEDs in epilepsy patients. This review provides a background and discusses the results of investigations on possible confounding factors affecting the interpretation and implementation of association studies in this area.

Key words: epilepsy, antiepileptic drugs, pharmacoresistance, drug responsiveness, ABCB1, P-gp, single nucleotide polymorphism

INTRODUCTION

Epilepsy is a chronic disorder characterized by a predisposition to recurrent unprovoked seizures. Seizures are caused by abnormal neuronal discharges in the brain. Despite appropriate pharmacotherapy, about one third of newly treated epilepsy patients have refractoriness to medical treatment (Elger & Schmidt 2008). The most prominent hypothesis for explaining refractoriness to antiepileptic drugs (AEDs) in epilepsy is the multidrug-transporter hypothesis, which was first explored in chemotherapy-resistant cancer. Excess efflux of AEDs across the blood-brain barrier (BBB) caused by overexpression of efflux transporters suggests that alterations in entry and extrusion of drugs into and out of cells may be an important mechanism of pharmacoresistance (Schmidt & Löscher 2005). P-glycoprotein 170 (P-gp) is the most studied protein among the ATP-binding cassette (ABC) efflux transporters (Schinkel et al. 1994), encoded by ABC subfamily B member 1 transporter or multidrug resistance (ABCB1/MDR1) gene. Evidence has shown that ABCB1/MDR1 expression was elevated in the epileptic foci of drug resistant epilepsy up to ten times indicating its main role in pharmacoresistance (Tishler et al. 1995; Gottesman et al. 2002).

Different responses to pharmacotherapy among patients with similar clinical phenotypes and taking the same doses of medication may be explained, in part, by single-nucleotide polymorphisms (SNPs) as the most frequent type of genetic variation (Schmidt & Löscher 2005). Numerous SNPs have been identified in ABCB1/MDR1 gene among which the C3435T variant is the most common polymorphism (Fung & Gottesman 2009). The key question of this review is "is the C3435T variant of the ABCB1/MDR1 gene associated with outcomes of AEDs treatment in epilepsy?" The initial pharmacogenetic study demonstrated an association of C3435T polymorphism with response to AEDs while the replication of this study has been controversial with consistent and conflicting studies. Herein, the sources of bias in association studies for the C3435T variant and refractoriness to antiepileptic drug (AED) therapy are briefly assessed.

P-gp Structure

P-gp is the first discovered human ABC transporter more than 30 years ago in drug-resistant ovarian cells obtained from Chinese hamsters (Juliano & Ling 1976). This protein is the product of the ABCB1/MDR1 gene, located at 7q21 and containing 28 exons with two transcription start sites. It is a phosphorylated and glycosylated trans-membrane protein consisting of approximately 1280 amino acids and with a molecular mass of

around 170 kDa. P-gp contains two ho-mologous halves in which a short linker region joining the symmetric sequences together (Figure 1). Each half is com-posed of one trans-membrane domain (TMD) containing six α-helix segments and one ATP-binding motif or ABC unit located at the cytoplasmic site. The two ATP-binding domains generate energy for interaction of drugs with drug-binding sites and for efflux transport function. P-gp mutations in TMDs 5, 6, 11 and 12 affect substrate specificity and indicate the presence of a drug-binding site in this region (Fung & Gottesman 2009).

P-gp function

P-gp functions as a transmembrane ef-flux pump, by moving drugs from the in-tracellular to the extracellular domain. It may also interact with drug molecules trapped within the cell membrane lipid bilayer. This protein is expressed in many normal tissues such as the liver, gastro-intestinal tract, kidney, adrenal glands and the blood–brain barrier (BBB) (Löscher & Potschka 2005a). Anatomic localization and ability of P-gp in trans-porting a broad range of compounds

suggest that this transporter acts as an effective cellular protector against toxic xenobiotics, drugs and metabolites that are its substrates. It does this by secret-ing these compounds into the bile, urine and intestinal lumen, thereby preventing their accumulation in critical organs such as the brain. Altered P-gp expression and activity may influence an individual's sus-ceptibility to drugs (Löscher & Potschka 2005b). P-gp transports a broad spec-trum of substrates in the plasma mem-brane ranging in size from 250 to almost 4000 Da including many classes of drugs such as calcium channel blockers, lipid-lowering statins, opioids, chemothera-peutic agents, HIV protease inhibitors, antibiotics, immunosuppressive agents and β-adrenergic antagonists. Most AEDs are planar lipophilic agents and thus substrates for the P-gp transporter (Kwan & Brodie 2005).

ABCB1/MDR1 polymorphisms

Any two unrelated humans have millions of genetic differences caused by inser-tion/deletions or SNPs. SNPs are the most abundant form of genetic variation. About 0.1% of an individual's DNA se-
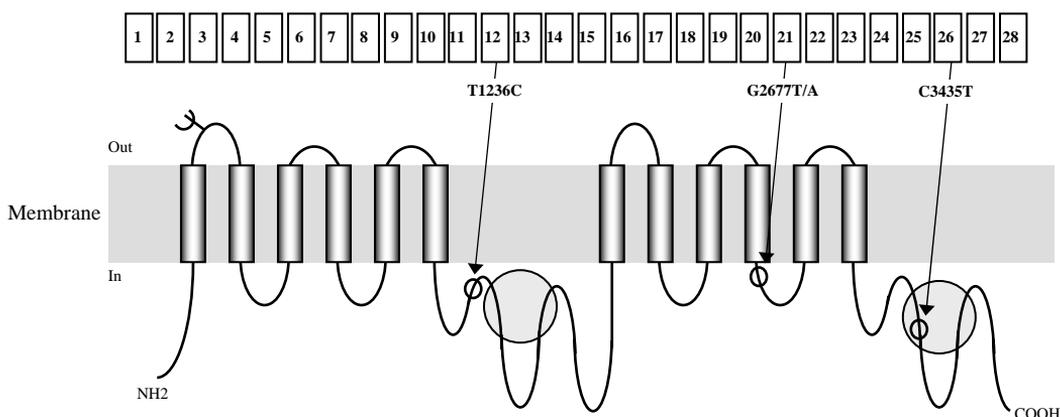

Figure 1: Schematic diagram of human P-gp showing the 12 transmembrane segments fold together to form a three dimensional barrel-structure in the membrane. N-linked phosphrylation and glycosylation sites are found in the first extracellular loop. The location of common polymorphisms are shown with small circles (arrows) and ATP-binding motifs with gray circles (modified from Tanabe et al. 2001).

quences are different among which approximately 80% is represented by SNPs. SNPs may be the causal factor of about 40% of the variation of the most common diseases and 70% of schizophrenia (Goldstein & Cavalleri 2005). Do the ABCB1/MDR1 variants account for the inter-individual variability in the pharmacokinetics and pharmacodynamics of drugs? The level of P-gp expression is highly variable between different individuals. Inter-individual variability of P-gp activity might affect blood levels and drug distribution to the specific target compartment. Insufficient or excessive drug penetration could cause adverse side effects or inactivity of drugs, respectively (Löscher & Potschka 2005a). The ABCB1/MDR1 gene is highly polymorphic. Numerous SNPs have been found in the ABCB1/MDR1, the majority of which involve noncoding regions thus not affecting the P-gp amino acid sequence. More than 50 variants reside in the coding region which can possibly cause altered function (Fung & Gottesman 2009).

Allele frequency for most of the coding region SNPs is less than 8% in different ethnic populations with the exception of 3 SNPs in exons 12 (C1236CT), 21 (G 2677T/A), and 26 (C3435T) in ABCB1/MDR1 gene. Both synonymous C1236T and C3435T polymorphisms change a GGC codon to GGT (Gly412 Gly) and ATC to ATT (Ile1145Ile), respectively (Table 1). So, what is the role of C3435T polymorphism in pharmacoresistance in epilepsy? An original report suggested a strong and significant association (P=0.006) between the CC geno-

type at 3435 in ABCB1/MDR1 gene and drug-resistant epilepsy (Siddiqui et al. 2003). Zimprich et al. (2004) confirmed the results of the first report (p = 0.035), but Tan et al. (2004a), with the exact replication of the first study and almost twice the sample size, failed to confirm the original findings (Zimprich et al. 2004; Tan et al. 2004a). Furthermore, Leschziner et al. (2008) in a comprehensive genome wide approach failed to confirm the association of C3435T polymorphism and response to AEDs (Leschziner et al. 2008). In total, 26 studies attempted to examine this hypothesis in epilepsy, but only 8 found a positive association (Table 2). The studies are divided into two groups: positive and negative associations, some of which are replications of the initial positive study (Table 2). It is unclear why these reports have found such contradictory results. How can such contradictory results be interpreted? Is there any obvious effect of C3435T polymorphism on response to AEDs even if the phenotypes are almost similar? There may be several reasons for such discordant findings which are discussed below.

Publication bias

Authors and journal editors prefer to publish papers with positive findings which can lead to publication bias. Methodologic weaknesses and consequent bias or even robust study designs and analyses with significant positive results at the 5% level in association studies may lead to false positive data probably arising by

Table 1: Common polymorphisms in ABCB1/MDR1 gene

Exon no.	Ref SNP	mRNA position	Wild- type allele	Mutant allele	a.a. position	a.a. residue	a.a. change	Function
12	Rs1128503	1236	C	C	412	Gly	Gly	Synonymous
21	Rs2032583	2677	G	G	893	Ala	Ser	Missense
21	Rs2032583	2677	G	G	893	Ala	Thr	Missense
26	Rs1045642	3435	C	T	1145	Ile	Ile	Synonymous

a.a.: amino acid

Table 2: Association studies for ABCB1/MDR1 C3435T polymorphism and response to AEDs treatment in epilepsy patients

Author	Year	Origin	Association	Definition Non-respondent	Definition Respondent	Sample size NR	R	C	Non-respondent CC (%)	CT (%)	TT (%)	C	T	Respondent CC (%)	CT (%)	TT (%)	C	T
Positive association																		
Siddiqui et al.	2003	UK	+	>4 seizures in year with >3 AEDs or surgery	>1 year seizure free With AEDs	200	115	200	27.50	53.00	19.50	0.54	0.46	15.65 / 18.50*	54.78 / 58.00*	29.57 / 23.50*	0.43 / 0.47*	0.57 / 0.53*
Hajnsek et al. **	2004	Croatia	+	?	?	30	30	-	40.00	46.67	13.33	0.63	0.37	13.33	50.00	36.67	0.38	0.62
Hung et al.	2005	Taiwan	+	>10 seizures in year	>2 year seizure free	108	223	287	43.52	42.59	13.89	0.65	0.35	13.90 / 18.12*	52.91 / 42.51*	33.18 / 39.37*	0.40 / 0.39*	0.60 / 0.61*
Seo et al.	2006	Japan	+ (reverse)	≥1 seizure/month with ≥ 2 AEDs or surgery	>1 year seizure free with AEDs	126	84	-	26.98	46.03	26.98	0.50	0.50	42.86	40.48	16.67	0.63	0.39
Kwan et al.	2007	China	+ (reverse)	≥1 seizure/month with ≥ 2 AEDs or surgery	>1 year seizure free with AEDs	221	297	179	36.20	47.06	16.74	0.60	0.40	38.38 / 37.99*	54.21 / 51.40*	7.41 / 10.61*	0.63 / 0.64*	0.37 / 0.36*
Soranzo et al.	2004	UK	+	-	-	286	135	-	26.07	51.79	22.14	0.52	0.48	14.71	58.82	26.47	0.44	0.56
Zimpnch et al.	2004	Austria	+	≥6 seizures/month	≤2 seizures/month	66	83	44										
Ebid et al.	2007	Egypt	+	Seizure in 3 month with PHT	3 month seizure free with PHT	63	37	50	55.56	38.10	6.35	0.75	0.25	13.51 / 24.00*	45.95 / 48.00*	40.54 / 28.00*	0.35 / 0.48*	0.65 / 0.52*
Hung et al.	2007	Taiwan	+	>10 seizures in year	>2 year seizure free	114	213	-	35.09	48.25	16.67	0.59	0.41	18.31 / 18.12*	50.23 / 42.51*	31.46 / 39.37*	0.43 / 0.39*	0.57 / 0.61*
Negative association																		
Tan et al.	2004	Australia	-	>4 seizures in year with >3 AEDs or surgery	>1 year seizure free With AEDs	401	208	-	18.70	48.13	33.17	0.43	0.57	17.79	55.29	26.92	0.45	0.55
Sills et al.	2005	Scotland	-	Seizure in year with ≥2 AEDs	>1 year seizure free with AED	230	170	-	17.83	48.70	33.48	0.42	0.58	18.82	48.24	32.94	0.43	0.57
Kim et al.	2006a	Korea	-	>4 seizures in year with >3 AEDs or surgery	>1 year seizure free	99	108	-	47.47	46.46	6.06	0.71	0.29	45.00	48.00	7.00	0.69	0.31
Kim et al.	2006b	Korea	-	>4 seizures in year with >3 AEDs	>1 year seizure free	59	101	213	32.20	45.76	22.03	0.55	0.45	47.52 / 41.51*	29.70 / 43.40*	22.77 / 15.09*	0.62 / 0.63*	0.38 / 0.37*
Kim et al.	2009	Korea	-	>4 seizures in year with >3 AEDs	>1 year seizure free with 1 or 2 AEDs	198	193	-	36.87	48.99	14.14	0.61	0.39	41.97	46.63	11.40	0.65	0.35
Shahwan et al.	2007	Ireland	-	No change or <50% reduction in seizures with >2 AEDs	>1 year seizure free or ≥50% reduction in seizures	198	242	-	16.39	52.46	31.15	0.43	0.57	15.88	51.07	33.05	0.41	0.59
Chen et al. **	2007	China	-	?	?	50	164	-	30.00	50.00	20.00	0.55	0.45	38.41	48.17	13.41	0.62	0.38
Leschziner et al.	2006	UK	-	-	-	-	503	-										
Leschziner et al.	2007	UK	-	>4 seizures in year with >3 AEDs or surgery	The rest of epilepsy patients	73	76	-										
Lakhan et al.	2008	India	-	>4 seizures in year with >3 AEDs or surgery	>1 year seizure free	94	231	-	9.57	55.32	35.11	0.37	0.63	16.45	45.02	38.53	0.39	0.61
Saadi et al.	2009	India	-	< 6 month terminal remission with ≥2 AEDs	>1 year seizure free	113	129	256	2.65	53.98	43.36	0.30	0.70	3.10	63.57	33.33	0.35	0.65
Ozgon et al.	2008	Turkey	-	≥4 seizures in 6 months with CBZ	>1 year seizure free with CBZ	44	53	174	29.55	59.09	11.36	0.59	0.41	30.30 / 28.20*	54.70 / 46.00*	15.00 / 25.80*	0.58 / 0.51*	0.42 / 0.49*
Dericioglu et al.	2008	Turkey	-	Surgery	-	89	-	100	29.21	38.20	32.58	0.48	0.52	27.40*	38.36*	34.25*	0.47*	0.53*
Szoeke C et al.	2009	Scotland	-	Seizure in 1 year with AEDs	>1 year seizure free	133	152	-	15.04	51.88	33.08	0.41	0.59	22.37	47.37	30.26	0.46	0.54
		Australia	-			64	148	-	32.81	42.19	25.00	0.54	0.46	22.97	45.27	31.76	0.46	0.54
		Hong Kong	-			11	34	-	9.09	72.73	18.18	0.45	0.55	38.24	58.82	2.94	0.68	0.32
		Total				208	334	-	20.19	50.00	29.81	0.45	0.55	24.25	47.60	28.14	0.48	0.52

NR: non-respondent; R: respondent; C: control (in sample size column); ?: no information; *: control; **: as cited in Bournissen et al. 2009

chance (Cardon & Palmer 2003; Tan et al. 2004b). The initial positive association study between C3435T polymorphism and multidrug resistance in epilepsy (Siddiqui et al. 2003) is a publication bias and may be a false positive result. Hence 17 subsequent studies could not confirm the first finding by improving the statistical power through increasing sample size (Colhoun et al. 2003). To avoid such a false positive association report, it is recommended to publish the original study only with α = 0.005 or lower. Even at this significance level and 50% power, the chance of true positive findings is about 10%, and so the findings should be interpreted with caution. In association studies between C3435T polymorphism and multidrug resistance in epilepsy, most authors have pointed to low power due to small sample size as a limitation. The total sample size in the 26 association studies is 7352 (282.77 ± 31.29), ranging from 45 to 609 epilepsy cases (Table 2). In order to have a power of 80%, these studies require a large sample size or collaboration between multi-centres and countries (Otto 2004).

Definition of drug-responsiveness and drug-resistant epilepsy

There have been a number of definitions for drug-resistant epilepsy. Selection of the most appropriate definition for a particular study depends on the type of seizure and epilepsy syndrome and the purpose of using the definition. According to the National Association of Epilepsy Centres (NAEC), pharmacoresistance in epilepsy is defined when seizures do not come under control after 9 months of treatment under the care of a neurologist or when a patient does not become seizure-free for 12 months during long-term state-of-the-art treatment with several suitable AEDs at maximal tolerated doses. Surgery, as an alternative to pharmacotherapy for about 5–10% of

newly diagnosed patients can be used to define drug resistance because it would be assumed that the seizures remained uncontrolled after treatment with various drugs (Elger 2003; Schmidt & Löscher 2005). Most association studies for C3435T and multidrug resistance epilepsy have divided patients into "drug-resistant" and "drug-responsive" groups. The definition of drug-responsiveness and drug-resistance varies amongst these studies. In summary, drug-responsiveness is defined as complete seizure freedom over three months to one year and/or more or 50% reduction or more in seizure frequency within one year, while drug-resistance is defined as having one or more seizures per month or four to 10 or more seizures in six months or one year, or less than 50% reduction of seizures per year taking at least two AEDs, and also surgery for seizure control (Table 2). Additionally, some studies have used healthy individuals instead of drug-responsive patients as controls (Kasperaviciute & Sisodiya 2009). Since the definition of drug-responsiveness and drug-resistance is different between studies, patients who are classified as drug resistant in some studies may be drug-responsive in others. Therefore such variation in patients' classification may lead to wrong conclusions. To have reliable findings, there need to be an agreement on the definition of drug responsiveness and drug resistance in epilepsy.

AED-therapy

a. AEDs–P-gp interactions

There is discrepancy in the transporter hypothesis as a mechanism for AED-resistant epilepsy. Some reports suggest that P-gp plays a significant role in mediating resistance to AEDs. This includes drugs such as phenytoin (PHT), carbamazepine (CBZ), phenobarbitone

(PHB), and lamotrigine (LTG) which, as P-gp substrates induce upregulation of P-gp expression in the brain. However, valproic acid (VPA) and vigabatrin do not produce the same effects (Sills et al. 2005). Therefore, based on this hypothesis, in some association studies for ABCB1/MDR1 gene polymorphism and response to CBZ, VPA or other drugs that may not be substrates for P-gp, this polymorphism probably may not play any role in determining drug resistance (Kimura et al. 2007) and the patients, classified as drug resistant, may not really be pharmacoresistant. However, in-vitro observations do not support this hypothesis as CBZ, PHT, and VPA (Baltes et al. 2007a, Baltes et al. 2007b, Rivers et al. 2008), possibly belong to a class of "weak substrates" of human P-gp and may compete for drug-binding sites on P-gp and inhibit the transport of other substrates (Luna-Tortós et al. 2008). Determination of which AEDs are real substrates of P-gp requires in vivo experiments.

b. AED–AED and AED-nonAED interactions

In patients who are on multiple drug therapy, drug interactions may occur in which one drug may modify the activity of another by enhancing or reducing its pharmacologic effects with beneficial or harmful outcomes. AED–AED or AEDs and non-AED interactions in polytherapy regimen can significantly influence the clinical management in patients with epilepsy, pharmacokinetically and pharmacodynamically. For example, phenobarbitone (PHB), PHT, and CBZ as enzyme inducers, can increase the level of activities of various cytochrome P450 and UGT isoenzymes. Therefore, a combination of PHB, PHT, and CBZ with VPA, LTG, topiramate, and tiagabine in polytherapy increases and decreases their metabolism and half-lives, respectively

(Patsalos et al. 2002). In association studies for C3435T polymorphism and refractory epilepsy, some authors have not differentiated between monotherapy and polytherapy treatments which could have influenced the results of their study. To prevent such a problem, the possible effect of ABCB1/MDR1 polymorphism on refractoriness to each AED monotherapy must be established.

Haplotype

SNPs are often associated with particular variants existing on the same chromosome known as a 'haplotype' which may play a role in response to drugs and disease susceptibility (Goldstein & Cavalleri 2005). Such a non-random association of SNPs is called linkage disequilibrium (LD) (Goldstein & Cavalleri 2005). Some reports have demonstrated significant LD between synonymous 3435C>T and 1236C>T and nonsynonymous 2677G>T/A (Ala893Ser or Ala893Thr), suggesting that C3435T and 2677G>T/A may influence P-gp function in epilepsy patients in response to AEDs (Kim et al. 2001). In association studies for C3435T polymorphism and refractory epilepsy with negative results, some authors have suggested that since the C3435T variant is silent, its effect is possibly caused by specific haplotypes (CGC, TGC and TTT) of C1236T and G2677T/A (Hung et al. 2005). It was hypothesized that the presence of the C3435T variant within a haplotype block can affect the timing of co-translational folding and P-gp structure which could influence its insertion into the membrane and subsequently change the structure of substrate and inhibitor interaction sites (Kimchi-Sarfaty et al. 2007). Haplotypes may potentially improve the statistical power under certain circumstances. However, the debate still exists whether SNPs or haplotypes are more appropriate for genetic association studies (Tan et al. 2004b). Finally,

many genes including drug-transporters, drug-metabolizing enzymes, and drug targets are responsible for drug responsiveness in epilepsy (Löscher et al. 2008) Therefore, one need to investigate the entire genetic effect of these genes and their SNPs together, rather than study a particular variant or haplotype in a single gene.

Population heterogeneity

a. Epilepsy types

Epilepsy is heterogenous in nature, influenced by different genes and environment. Drug resistance differ amongst patients with different types of seizures and epilepsy syndromes, and the population (Duncan et al. 2006). A study on Finnish epilepsy cases performed by Sillanpää et al. (1999) showed that pharmacoresistance to AEDs was seen in about 78% of patients with symptomatic partial or symptomatic generalised epilepsies and 13% of patients with idiopathic generalised epilepsies but not in patients with idiopathic partial epilepsies (Sillanpää et al. 1999). Such a high frequency of drug resistance in symptomatic epilepsy (for example structural brain lesions) could be caused by overexpression of P-gp due to the effect of frequent seizures or AEDs therapy (Löscher 2005c). This will release excitotoxic metabolites from the altered tissue and inhibit the efficacy of AEDs for treatment, irrespective of the C3434T gene polymorphism involvement (Schmidt & Löscher 2005). Most association studies for C3434T gene polymorphism and refractoriness have been conducted on either a specific subset of epilepsy types/syndromes or all the various types of epilepsies which could therefore confound the results. To avoid such a bias, the relationship of pharmacoresistance with C3434T gene polymorphism should be separately calculated for different epilepsy types including idiopathic, symptomatic and cryptogenic.

b. Ethnicity effect

The distribution of allele and haplotype frequency is different among populations, reflected by differing ancestral histories. This difference may be caused by responses to natural selection, migration patterns, stochastic effects and founder events. Therefore, different ethnic groups may have various allele frequencies in both disease and non-disease genes (Cardon & Palmer 2003). Genotyping of the C3435T polymorphism in different normal populations have shown that the C-variant is more frequent than T-variant and is the dominant ancestral allele, carried by more than 74% Africans (Figure 2) (Ameyaw et al. 2001). The wild haplotype (CGC) frequency in South American and African populations is more than the mutated type (TTT) while the opposite is true for the Asians (Fung & Gottesman 2009). The prevalence of the C-variant in the initial report was more in the drug-resistant than the drug-responsive Caucasian epilepsy patients (Siddiqui et al. 2003). However, in most of the studies with Asian cases (Seo et al. 2006; Kwan et al. 2007; Kim et al. 2008a, Kim et al. 2008b, Chen et al. 2007; Lakhan et al. 2009; Vahab et al. 2009; Szoeke et al. 2009) the opposite was seen, which was similar to the normal population (Figure 2), probably reflecting the complex role of C3435T polymorphism in the response of AEDs in different ethnic populations. In summary, based on the C allele and LD differences between various epilepsy and normal populations, inclusion of patients with various ethnicities in the association studies for C3435T variant and refractoriness in epilepsy can potentially introduce heterogeneity which would
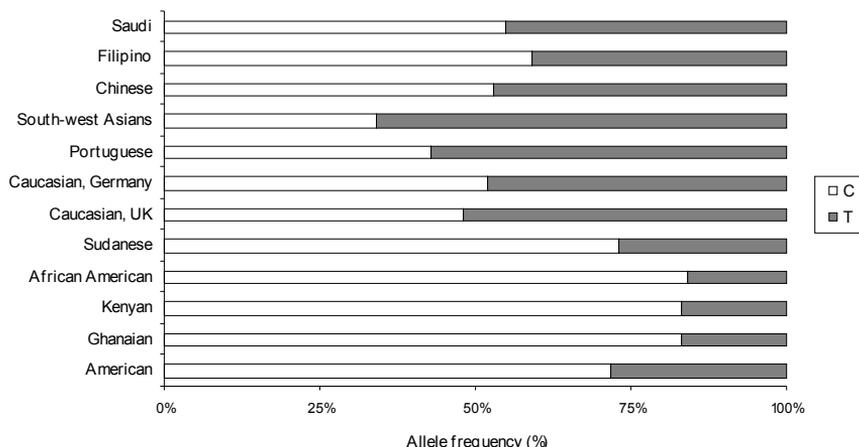
Figure 2: The C3435T allele frequency amongst normal populations with different ethnicities

confound the findings (Tan et al. 2004a, Shahwan et al. 2007).

CONCLUSION

Despite more than 26 association studies in epilepsy involving the common ABCB1/MDR1 C3435T variant and refractoriness, 16 of the replicated studies failed to confirm the original report. This review has provided an opportunity for better understanding of the methodological problems in these association studies by highlighting five sources of bias which may confound the results. First, publication bias which can be caused by false positive results, found by chance in the initial report as a result of insufficient sample size, and low statistical power. Second, varying definitions of drug-responsive and drug-resistant epilepsy in the different studies could lead to different results. Third, for some AEDs, it is not clear whether they are substrates of P-gp; hence justification of the results is difficult. Moreover, the interaction between AED–AED and AED-nonAED in polytherapy regimen can influence response to AEDs. Fourth, certain haplotypes, formed by C3435T with specific variants in ABCB1/MDR1 or other genes and SNPs may affect the response to

AEDs. Finally, the studies may involve a heterogeneous population made up of combinations of various cases with different epilepsy syndromes or ethnicities. In order to obtain reliable results in the genetic association studies for C3435T polymorphism and refractoriness, these factors need to be controlled.

REFERENCES

Ameyaw, M.M., Regateiro, F., Li, T., Liu, X., Tariq, M., Mobarek, A., Thornton, N., Folayana, G.O., Githang'a, J., Indalo, A., Ofori-Adjei, D., Price-Evans, D.A. & McLeod, H.L. 2001. MDR1 pharmacogenetics: frequency of the C3435T mutation in exon 26 is significantly influenced by ethnicity. *Pharmacogenetics J.* **11**:217-221.

Baltes, S., Gastens, A.M., Fedrowitz, M., Potschka, H., Kaever, V. & Löscher, W. 2007a. Differences in the transport of the antiepileptic drugs phenytoin, levetiracetam and carbamazepine by human and mouse P-glycoprotein. *Neuropharmacology.* **52**:333–346.

Baltes, S., Fedrowitz, M., Tortos, C.L., Potschka, H. & Loscher, W. 2007b. Valproic acid is not a substrate for P glycoprotein. *J Phar.* **320**:3331-343.

Bournissen, F.G., Moretti, M.E., Juurlink, D.N., Koren, G., Walker, M. & Finkelstein, Y. 2009. Polymorphism of the MDR1/ABCB1 C3435T drug-transporter and resistance to anticonvulsant drugs: A meta-analysis. *Epilepsia.* **50**(4):898-903.

Cardon, L.R. & Palmer, L.J. 2003. Population stratification and spurious allelic association. *Lancet.* **361**:598–604.

Chen, L., Liu, C.Q., Hu, Y., Xiao, Z.T., Chen, Y. & Liao, J.X. 2007. Association of a polymorphism in MDR1 C3435T with response to antiepileptic drug treatment in ethic Han Chinese children with epilepsy Zhongguo. Dang. Dai. Er. Ke. Za. Zhi. **9**(1):11-14.

Colhoun, H.M., McKeigue, P.M. & Davey, S.G. 2003. Problems of reporting genetic associations with complex outcomes. *Lancet.* **361**:865–872.

Dericioglu, N., Babaoglu, M.O., Yasar, U., Bal, I.B., Bozkurt, A. & Saygi, S. 2008. Multidrug resistance in patients undergoing resective epilepsy surgery is not associated with C3435T polymorphism in the ABCB1 (MDR1) gene. *Epilepsy Res.* **80**(1):42-6.

Duncan, J.S, Sander, J.W., Sisodiya, S.M. & Walker, M.C. 2006. Adult epilepsy. *Lancet.* **367**:1087–1100.

Ebid, A.H., Ahmed, M.M. & Mohammed, S.A. 2007. Therapeutic drug monitoring and clinical outcomes in epileptic Egyptian patients: a gene polymorphism perspective study. *Ther Drug Monit.* **29**:305–312.

Elger, C.E. & Schmidt, D. 2008. Modern management of epilepsy: A practical approach. *Epilepsy Behav.* **12**:501–539.

Elger, C.E. 2003. Pharmacoresistance: modern concept and basic data derived from human brain tissue. *Epilepsia.* **44**(suppl 5):9–15.

Fung, K.L. & Gottesman M.M. 2009. A synonymous polymorphism in a common MDR1 (ABCB1) haplotype shapes 3 protein function. *Biochim Biophys Acta.* **1794**(5):860-871.

Goldstein, D.B. & Cavalleri, G.L. 2005 Understanding human diversity. *Nature.* **437**;1241-1242.

Gottesman, M.M., Fojo T. & Bates S.E. 2002. Multidrug resistance in cancer: role of ATP-dependent transporters. *Nat. Rev. Cancer* **2**:48–58.

Hung, C.C., Jen, T.J., Kao, P.J., Lin, M.S. & Liou, H.H. 2007. Association of polymorphisms in NR1I2 and ABCB1 genes with epilepsy treatment responses. *Pharmacogenomics J.* **8**:1151–1158.

Hung, C.C., Tai, J.J., Lin, C.J., Lee, M.J. & Liou, H.H. 2005. Complex haplotypic effects of the ABCB1 gene on epilepsy treatment response. *Pharmacogenomics J.* **6**:411–417.

Juliano, R.L. & Ling, V. 1976. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim. Biophys. Acta.* **455**:152–162

Kasperaviciute, D. & Sisodiya, S.M. 2009. Epilepsy parmacohenetics: *Pharmacogenomics J.* **10**(5), 817-836.

Kim, D.W, Lee, S.K., Chu, K., Jang, I.J., Yu, K.S., Cho, J.Y. & Kim, S.J. 2009. Lack of association between ABCB1, ABCG2, and ABCC2 genetic polymorphisms and multidrug resistance in partial epilepsy. *Epilepsy Res.* **84**(1):86-90.

Kim, Y.O., Kim, M.K., Woo, Y.J., Lee, M.C., Kim, J.H., Park, K.W., Kim, E.Y., Roh, Y.I. & Kim, C.J. 2006a. Single nucleotide polymorphisms in the multidrug resistance 1 gene in Korean epileptics. *Seizure.* **15**:67–72.

Kim, D.W., Kim, M., Lee, S.K., Kang, R. & Lee, S.Y. 2006b. Lack of association between C3435T nucleotide MDR1 genetic polymorphism and multidrug-resistant epilepsy. *Seizure.* **15**:344–347.

Kim, R.B., Leake, B.F., Choo, E.F., Dresser, G.K., Kubba, S.V., Schwarz, U.I, Taylor, A., Xie, H.G., McKinsey, J., Zhou, S., Lan, L.B., Schuetz, J.D., Schuetz, E.G. & Wilkinson, G.R.. 2001. Identification of functionally variant MDR1 alleles among European Americans and African Americans. *Clin Pharmacol Ther.* **70**:189-199.

Kimchi-Sarfaty, C, Oh, J.M., Kim, I.W., Sauna, Z.E., Calcagno, A.M., Ambudkar, S.V. & Gottesman, M.M. 2007. A "silent" polymorphism in the MDR1 gene changes substrate specificity. *Science.* **315**(5811):525-8.

Kimura, Y., Morita, S.Y., Matsuo, M. & Ueda, K., 2007. Mechanism of multidrug recognition by MDR1/ABCB1. *Cancer Sci.* **98**:1303–1310.

Kwan, P. & Brodie, M.J. 2005. Potential Role of Drug Transporters in the Pathogenesis of Medically Intractable Epilepsy. *Epilepsia.* **46**(2):224–235.

Kwan, P., Baum, L., Wong, V., Ng, P.W., Lui, C.H., Sin, N.C., Hui, A.C., Yu, E. & Wong, L.K. 2007. Association between ABCB1 C3435T polymorphism and drug-resistant epilepsy in Han Chinese. *Epilepsy Behav.* **11**:112–117.

Lakhan, R., Misra, U.K., Kalita, J., Pradhan, S., Gogtay, N.J., Singh, M.K. & Mittal, B. 2009. No association of ABCB1 polymorphisms with drug-refractory epilepsy in a north Indian population. *Epilepsy Behav.* **14**(1):78-82.

Leschziner, G.D., Jorgensen, A.L., Andrew, T., Pirmohamed, M., Williamson, P.R., Marson, A.G., Coffey, A.J., Middleditch, C., Rogers, J., Bentley, D.R., Chadwick, D.W., Balding, D.J. & Johnson, M.R. 2006. Clinical factors and ABCB1 polymorphisms in prediction of antiepileptic drug response: a prospective cohort study. *Lancet Neurol.* **5**:668–676.

Löscher, W. & Potschka, H. 2005a. Blood-brain barrier active efflux transporters: ATP-binding cassette gene family. *Epilepsia.* 1–23.

Löscher, W. & Potschka, H. 2005b. Role of drug efflux transporters in the brain for drug disposition and treatment of brain diseases. *Prog Neurobiol.* **76**(1):22-76.

Löscher, W. 2005c. Mechanisms of drug resistance. Epileptic Disord. **7**(Suppl) 1:S3-9.

Löscher, W., Klotz U., Zimprich F. & Schmidt D. 2008. The clinical impact of pharmacogenetics on the Treatment of epilepsy. *Epilepsia.* 1–23.

Luna-Tortos, C., Fedrowitz, M. & Löscher, W. 2008. Several major antiepileptic drugs are substrates for human P-glycoprotein. *Neuropharmacology* **55**(8):1364-1375.

Otto, J. 2004. Association of genetic loci Replication or not, that is the question. *Neurology.* **63**:955–958.

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

Patsalos, P.N., Früscher, W., Pisani, F. & van Rijn, C.M. 2002. The importance of drug interactions in epilepsy therapy. *Epilepsia.* **43**(4):365–385.

Rivers, F., O'Brien, T.J. & Callaghan, R. 2008. Exploring the possible interaction between anti-epilepsy drugs and multidrug efflux pumps; in vitro observations. *Eur J of Pharmacol.* **598**:1-8.

Schinkel, A.H., Smit, J.J.M., Beijnen, J.H., Wagenaar, E., van Deemter, L., Mol C.A.A.M., van der Valk M.A., Robanus Maandag E.C., te Riele H.P.J., Berns A.J.M. & Borst P. 1994. Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood–brain barrier and to increased sensitivity to drugs. *Cell.* **77**:491–502.

Schmidt, D. & Löscher, W. 2005. Drug resistance in epilepsy: Putative neurobiologic and clinical mechanisms. *Epilepsia.* **46**(6):858–877.

Seo, T., Ishitsu, T., Ueda, N., Nakada, N., Yurube, K., Ueda, K., & Nakagawa, K. 2006. ABCB1 polymorphisms influence the response to antiepileptic drugs in Japanese epilepsy patients. *Pharmacogenomics J.* **7**:551–561.

Shahwan, A., Murphya, K., Doherty, C., Cavalleri, G.L., Muckiane, C., Dickere, P., McCarthya, M., Kinironsa, P. & Goldsteind, D. 2007. The controversial association of ABCB1 polymorphisms in refractory epilepsy: An analysis of multiple SNPs in an Irish population. *Epilepsy Res.* **73**:192-198.

Siddiqui, A., Kerb, R., Weale, M.E., Brinkmann, U., Smith, A., Goldstein, D.B., Wood, N.W. & Sisodiya, S.M. 2003. Association of multidrug resistance in epilepsy with a polymorphism in the drug-transporter gene ABCB1. *N Engl J Med.* **348**:1442–1448.

Sillanpää, M., Jalava, M. & Shinnar, S. 1999. Epilepsy syndromes in patients with childhood-onset seizures in Finland. *Pediatr Neurol.* **21**:533–7

Sills, G.J., Mohanraj, R., Butler, E., McCrindle, S., Collier, L., Wilson, E.A. & Brodie, M.J. 2005. Lack of association between the C3435T polymorphism in the human multidrug resistance (MDR1) gene and response to antiepileptic drug treatment. *Epilepsia.* **46**:643–647.

Soranzo, N., Cavalleri, G.L., Weale, M.E., Wood, N.W., Depondt, C., Marguerie, R., Sisodiya, S.M. & Goldstein, D.B. 2004. Identifying candidate causal variants responsible for altered activity of the ABCB1 multidrug resistance gene. *Genome Res.* **14**(7):1333-44.

Szoeke, C., Sills, G.J., Kwan, P., Petrovski, S., Newton, M., Hitiris, N., Baum, L., Berkovic, S.F., Brodie, M.J., Sheffield, L.J. & O'Brien, T.J. 2009. Multidrug-resistant genotype (ABCB1) and seizure recurrence in newly treated epilepsy: Data from International Pharmacogenetic Cohorts. *Epilepsia.* 2009 May 9.

Tan, N.C.K., Heron, S.E., Scheffer, I.E., Pelekanos, J.T., Mc Mahon, J.M., Vears, D.F., Mulley, J.C. & Berkovic, S.F. 2004a. Failure to confirm association of a polymorphism in ABCB1 with multidrug-resistant epilepsy. *Neurology.* **63**:1090–1092.

Tan, N.C.K., Mulley, J.C. & Berkovic, S.F. 2004b. Genetic association studies in epilepsy: "The truth is out there" *Epilepsia.* **45**(11):1429–1442.

Tanabe, M., Ieiri, I., Nagata, N., Inoue, K., Ito, S., Kanamori, Y., Takahashi, M., Kurata, Y., Kigawa, J., Higuchi, S., Terakawa, N. & Otsubo, K. 2001. Expression of P-glycoprotein in human placenta: Relation to Genetic Polymorphism of the Multidrug Resistance (MDR)-1 gene. *J Pharmacol Exp Ther.* **297**(3):1137–1143..

Tishler, D.M., Weinberg, K.I., Hinton, D.R., Barbaro, N., Annett, G.M. & Raffel, C. 1995. MDR1 gene expression in brain of patients with medically intractable epilepsy. *Epilepsia.* **36**(1):1-6.

Vahab, S.A., Sen, S., Ravindran, N. Mony, S., Mathew, A., Vijayan, N, Nayak, G, Bhaskaranand, N., Banerjee, M. & Satyamoorthy, K. 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**(3): 255–260.

Zimprich, F., Sunder-Plassmann, R., Stogmann, E., Gleiss, A., Dal, B.A., Zimprich, A., Plumer, S., Baumgartner, C. & Mannhalter, C. 2004. Association of an ABCB1 gene haplotype with pharmacoresistance in temporal lobe epilepsy. *Neurology.* **63**:1087–1089.