Pharmacogenetics in Epilepsy Treatment-A Predictor of Therapeutic Efficacy

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ABSTRACT

Inter-individual differences in response to antiepileptic drugs in the form of reduced efficacy or drug toxicity are the major problems in the treatment of epilepsy. Moreover, adverse drug reactions are a major cause of concern because of fatal risk to patient’s life accompanied with loss of substantial amount of money. Considerable interest has been developed among researchers in digging out the association of genetic variants with a particular treatment/drug variation. A few candidate gene studies and genome wide association studies have focused on gene variants involved in metabolism, transport and target receptors leading to antiepileptic drug variation. Pharmacogenetics promises to predict and prescribe the right drug to the right patient in right doses to maximize the therapeutic benefit and to minimize the adverse effects of antiepileptic drugs. Translation of pharmacogenetics into clinical practice would result into development of an exclusive system of prescribing antiepileptic drugs based on genetic analysis. The concept of ‘one dose fits for all’ has to be replaced with ‘Individual genotype responsible for drug response’. The diagnosis and decision making process in the form of personalized medicine in epilepsy would precise the drug dosage in epileptic patients with optimization and rationalization of epilepsy therapy.

Keywords: Epilepsy, pharmacogenetics, gene variants, polymorphism, antiepileptic drugs

INTRODUCTION

Epilepsy is a chronic neurological disorder, characterized by recurrent seizures of cerebral origin. Seizure is a sudden, transient disturbance of brain functions, manifested by involuntary motor, sensory, autonomic phenomena accompanied with or without loss of consciousness (Shorvon, 2004). Epilepsy affects almost 60 million people worldwide with distinct symptoms, etiology and treatment (Loscher et al., 2009). The overall prevalence of disease approximately lies between 5-10 per 1000 persons, which is usually higher in developing countries (Kumari et al., 2010). A number of underlying mechanisms of cellular and molecular origin have been recognized to be responsible for epileptiform phenomena (Murray et al., 2009; Guberman and Bruni, 1999). The etiology of epilepsy is multifactorial with many causes ranging from age, sex, congenital abnormalities, cerebrovascular diseases, brain tumor, head trauma, severe head injury, central nervous system infection, metabolic derangements, alcoholism and stroke (Guberman and Bruni, 1999). Other factors such as drug abuse, hypoxia, asthma and hypertension independent of cerebrovascular disease have also been implicated in epileptogenesis (Sridharan, 2002).

An inherited contribution to the etiology of epilepsy has been suggested for centuries (Ottman et al., 1996). The Indian traditional medicine system, Ayurveda has also emphasized the role of hereditary component in epilepsy. However, little progress has been made in determining the specific genetic influences on seizure susceptibility in epilepsy. The epilepsies are etiologically and clinically heterogeneous in origin and genetic influences have appeared to be of primary importance in a subset of patients. A variety of genetic mechanisms have been identified contributing towards epileptogenesis. Some of these mechanisms may involve single genes resulting in simple patterns of inheritance in families e.g. autosomal or X linked and dominant or recessive. Other mechanisms responsible for seizure susceptibility in epilepsy, may involve the combined effects of multiple genes and environmental factors (Ottman et al., 1996).

The risk of developing epilepsy has been found to be higher in relatives of affected people as compared with general population. The best example of the extent of familial aggregation is from Rochester-Olmsted County Record Linkage project (Annegers et al., 1992). In this study, in the families of probands with idiopathic and cryptogenic epilepsy and with onset before age 16, the risk of developing epilepsy by the age group 40 was found to be 10.6% in offspring and 3.6% in siblings in comparison with 1.7 percent in the Rochester population. However, the risk of epilepsy did not increase in more distant relatives, such as nieces, nephews and grandchildren. The familial aggregation does not necessarily support a genetic etiology to epilepsy, instead of this epilepsy may be the result of shared environmental exposures in members of the same family. There are four major lines of evidences suggesting clearly a genetic contribution to the familial aggregation of epilepsy. First of all the twin studies have clearly indicated that there are higher concordance rates for epilepsy among monozygotic twin pairs as compared to dizygotic twin pairs (Sridharan, 2002; Berkovic and Scheffer, 1999). Secondly seizures may be a part of phenotype of some other genetic disorders resulting from chromosomal abnormalities and single gene mutations, indicating that a wide variety of genetic mechanisms can result in susceptibility to seizures (Andersen and Hauser, 1985). Thirdly, several genes have been identified in animal models which may have homology to human epilepsy susceptibility genes. Fourth line of evidence has been provided by positional cloning techniques, used to chromosomally localize and to identify susceptible genes leading to human epilepsy syndromes (Noebels, 1996). Linkage studies have also provided strong evidence suggesting that susceptibility to epilepsy is influenced by genetic factors. The epilepsy with well-established genetic causes constitutes only a small proportion of all types of epilepsies.

Recently, Channelopathies studies have revealed that mutations in ion channel genes play an important role in the development of monogenic forms of epilepsy (Depondt and Shrovo, 2006). Two main types of epilepsies e.g.
benign familial neonatal convulsions (BFNC) and generalized epileptic febrile syndrome (GEFS) are due to mutations in sodium ion channels and potassium ion channels (Tripathi and Jain, 2002). Spontaneous epileptic mutants have also been identified among diverse species of animals from rodents to primates. It has been suggested that some of these involve a single gene (tottering and lethargic) or multiple genes e.g. genetically epilepsy prone rats (Prasad et al., 1999).

Current medical therapy consists of mainly antiepileptic drug treatment depending on the type of epilepsy, clinical history, with brain surgery and vagal nerve stimulation reserved for selected refractory cases (Devinsky, 1999). Refractory epilepsy is the type of epilepsy in which seizures persist despite the use of several AEDs, even if polytherapy given at in maximum tolerated dose (Berg and Kelly, 2006). It is encountered in almost one third of patients diagnosed with epilepsy. More than 20 antiepileptic drugs (AEDs) with several different mechanisms of action are available, which are prescribed as the mainstay of epilepsy treatment (Depondt, 2008). The commonly prescribed antiepileptic drugs are carbamazepine, phenytoin, valproate, gabapentin, topiramate, tiagabine etc. with many more being added to this list. Despite the availability of more than 20 antiepileptic drugs and other therapeutic procedures there are many problems associated with epilepsy treatment such as unpredictability of efficacy, severe adverse drug reactions, inter-individual variation in drug dosage and pharmacoresistance, which are of major concern (Xie and Frueh, 2005). The characteristic of pharmacoresistant epilepsy is that patients with refractory epilepsy are resistant to most or often all AEDs (Regesta and Tanganelli, 1999).

Each individual responds differently to the drugs prescribed. Drugs proven to be effective for an individual may not be effective in another individual. A drug safe and effective for a person may cause fatal reactions to another person, given in same dosage (Islam, 2008). The common methods of drug prescribing involve consideration of weight, age, comorbid conditions, disease severity and body functions (including liver and kidney status). These are no more successful because of adverse effects associated with AEDs. Further the dose and efficacy of antiepileptic drugs are all influenced by multiple factors which comprise comorbid medical, neurological and psychiatric disorders, concomitant medications and factors related to epilepsy itself (Islam, 2008).

The patients receiving treatment can be divided into two main categories as responders and non-responders on the basis of therapeutic efficacy. The individuals, showing less therapeutic efficacy/toxic effects of a drug prescribed are known as non-responders, while as individuals showing desired therapeutic efficacy towards the drug prescribed, are known as responders (McLeod, 2007).

Although many factors may contribute towards the variability of clinical outcome in epileptic patients, unpredictability of drug response may at least in part, result from variation in genetic factors (Loscher et al., 2009). Genetic variability has been found to contribute towards both the susceptibility of occurrence of the diseases well as to the variability in therapeutic response (Bu佐ia et al., 2010). According to Kalow (2006) genetics can account for 20-95% of clinical variability in drug disposition and effects (Kalow, 2006). However, most of the drug responses are influenced by the interactions of genetic and environmental factors.

The term pharmacogenetics was first coined by Friedrich Vogel in 1959. Vogel defined ‘Pharmacogenetics’ as “clinically important hereditary variations” (Vogel, 1959). Pharmacogenetics investigates the way in which the genetic variations influence the specific responses to medicine and it permits to tailor the personalized treatment which increases the efficacy and safety. Although, we are 99.9% similar at the genetic level i.e. DNA, the 0.1% difference in the form of single nucleotide polymorphisms (SNPs) are accountable for genetic variation from individual to individual. SNPs are frequently occurring variations in the human genome with 1 per 1000 bases and account for approximately 90% of genetic variability. SNPs have been reported to have a major impact on the pharmacokinetic as well as pharmacodynamic profile of the antiepileptic drugs (Loscher et al., 2009). The completion of Human Genome project in 2003 (HUGO, 2005) has provided critical insights into the information regarding genetic variation (Depondt, 2008). Variations or mutations can occur at the level of genes encoding for AED metabolizing enzymes such as cytochrome P450 (CYP2C9, CYP2C19), drug transporting enzymes e.g. ATP cassette binding protein (MDR1 gene) and at the target binding receptor sites e.g. sodium channel neurons (SCN). Clinician can prescribe the best possible and appropriate dose for patients depending on their genetic makeup. This can be achieved by translating the pharmacogenetic information into clinical practice, so that the right drug can be administered in right dose (Islam, 2008). A phenomenal example of pharmacogenetics is the identification of human leukocyte antigen (HLA) as a biomarker (genetic marker), which is associated with life threatening fatal, skin reactions such as SJS and TEN following the treatment with AEDs carbamazepine. US Food and Drug Administration has recommended to label the carbamazepine to screen the patients for HLAB*1502 before starting the treatment with carbamazepine (FDA, 2008). Patients, if found positive for this test should not be treated with carbamazepine unless the expected benefit outweighs the increased risk of SJS/TEN. The present review provides an overview of the clinical significance of pharmacogenetics for reducing the ADRs of AEDs and for increasing their efficiency based on genetic variation.

GENETIC VARIANTS OF DRUG METABOLIZING ENZYMES

The metabolism of a drug into its active metabolites is carried out mainly in the liver with the help of specific enzymes. In the past few years cytochrome (CYP) enzymes have attracted the researchers and studies have shown that most of the AEDs except gabapentin, lamotrigine and levetiracetam are metabolized at least partially by CYP enzymes (Saruwaturi et al., 2010). The CYPs are encoded by 57 genes whose products are involved in oxidative drug metabolism and synthesis of cholesterol, steroids, prostacyclin and thromboxanes. The three families of CYP genes are CYP1, CYP2 and CYP3. The liver contains abundance of CYP enzymes, followed by gastrointestinal tract, kidney and central nervous system of brain. Seven primary isozymes that have been found to be involved in the hepatic metabolism of most of the AEDs are CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 (Szeoke et al., 2006). The allelic frequency of CYP genes varies considerably with race and ethnicity. The oxidation of AEDs by enzymes of CYP family results in the formation of reactive epoxide - an intermediate metabolite (Saruwaturi et al., 2010). This intermediate metabolite is further metabolized to more water soluble derivatives by micosomal epoxide hydrolase (mEH) enzymes encoded by EPHX1 gene (Decker et al., 2009). Other enzymes involved in metabolism of AEDs using conjugation process are uranyl glucuronosyl transferase (UGT) and glutathione S- transferase (GST).

Studies have indicated that genetic polymorphisms in CYP genes metabolizing AEDs can lead to inter-individual variability in the pharmacokinetics of AEDs. The variations in EPHX1 and UGT genes in the coding region have also been found to alter the enzyme stability thus affecting variability in metabolism of AEDs (Decker et al., 2009; Azzato et al., 2010; Dennery, 2007). A few examples of genetic polymorphisms affecting the metabolism and pharmacokinetics of commonly prescribed AEDs have been described in the following section.

GENETIC VARIANTS AFFECTING PHENYTOIN METABOLISM

The chemical name of the drug is 5, 5 diphenyl-2, 4 imidazolidinedione. The primary site of action is motor cortex where spread of seizure activity is inhibited. Phenytoin has a stabilizing effect on neuronal membrane and prevents repetitive detonation of normal brain cells during depolarization shift that occurs in epileptic patients. This is achieved by prolonging the inactivated state of voltage sensitive neuronal Na+ channels which governs the refractory period of the neuron (Tripathi, 2003). Phenytoin exhibits a non
Phenytoin shows a wide range of inter-individual variation in dosage which has been attributed to some genetic variants of CYP2C9 gene. Phenytoin is oxidized by CYP2C9 (90%) and CYP2C19 (10%) to yield 5-(para-hydroxyphenyl)-5-phenylhydantoin pHPH (Desta and Kaminsky, 2002; Tate et al., 2005). CYP2C19 might be important for metabolism when CYP2C9 is fully saturated. The major allelic variants of CYP2C9 which are responsible for inter-individual and interethnic variations in disposition of its substrates are CYP2C9*2 (Arg144Cys) and CYP2C9*3 (Ile359Leu). CYP2C9*1 is the reference allele (Brandoleso et al., 2001). These SNPs have been reported to influence the catalytic function of encoded enzymes. The frequencies of CYP2C9*2 and CYP2C9*3 are higher in Caucasians (18.9%) than in Asians (2.5%-3.5%) (Myrand et al., 2008; Makeeva et al., 2008).

A study in Turkish population revealed higher plasma concentration of phenytoin and reduced ratio of its metabolite (pHPH/phenytoin) in individuals bearing the variants CYP2C9*1*2, *2*2 and *1*3 as compared to CYP2C9*1*1 (wild type allele) (Aynacioglu et al., 1999). The maximum elimination rate (Vmax) of phenytoin was 33% lower in patients heterozygous for CYP2C9*1*3 when compared to patients homozygous for CYP2C9*1*1. Further the elimination rate was found to be slightly decreased in patients with CYP2C9*2 or CYP2C9*3 compared with homozygous normal CYP2C9*1*1. A significant association has also been suggested between genotype CYP2C9*3 allele and maximum dose of phenytoin by Tate and collaborators (Tate et al., 2005). As far as the variants of CYP2C19 are concerned, a variant allele, CYP2C19*2 (G681A) in exon 5 has been reported to create a cryptic site and form a truncated defective protein with altered functions of encoded enzyme. The splicing effect is a major genetic defect which accounts for 75-85% poor metabolizers (de Morais et al., 1994). Recently, other allelic variant CYP2C19*17 (c.C806T; 5 flanking region of the gene) has been associated with increased functional activity of enzyme. However, the role of genetic variants of CYP2C19 gene is still under investigation. The frequencies of CYP2C19 alleles (CYP2C19*2 and CYP2C19*3) have been reported to be higher among Asians (33%-43.5%) in comparison with Caucasians (13.6%) (Yasui-Furukori et al., 2007).

Therefore, genotyping of CYP2C9 and CYP2C19 is of clinical relevance along with consideration of other factors in treatment of epilepsy. Ramasamy et al. (2005) reported a case study of an Indian adult female with epilepsy who developed severe hypersensitivity and high serum concentration even in therapeutic doses of phenytoin. The genotypic analysis revealed that the patient was homozygous mutant for CYP2C9*3*3 allele (Ramasamy et al., 2007). The mutation resulted in marked decrease in enzyme activity with zero order pharmacokinetics and reduced clearance of drug leading to severe toxicity. The patient was found to have nystagmus, ataxia, sedation, lymphenadopathy and gum hypertrophy (Grade III). After replacing phenytoin with another antiepileptic drug, toxic symptoms were disappeared slowly (Ramasamy et al., 2005). This was an excellent example depicting the need for translation of pharmacogenetic knowledge into clinical practice in epilepsy treatment.

**GENETIC VARIANTS AFFECTING CARBAMAZEPINE METABOLISM**

Carbamazepine is an anticonvulsant and specific analgesic for trigeminal neuralgia, available for oral administration. Its chemical name is 5,5'-dibenz (b,f) azepine-5-carboxamide. It has narrow therapeutic index in the range of 3-12 µg/mL. Carbamazepine is predominantly metabolized by CYP3A5, CYP3A4 and partially by CYP2C8 and CYP1A2. The principal metabolite of carbamazepine, carbamazepine-10, 11-epoxide, has anticonvulsant activity as demonstrated in several in vivo animal models of seizures. Park et al. (2009) have also reported higher serum levels of drug with mutant allele of CYP3A5*3 in Korean epileptic patients on carbamazepine monotherapy (Park et al., 2009). Patients with CYP3A5*3*3 genotype showed 31% higher serum levels of carbamazepine as compared to those carrying CYP3A5*1*1 (wild type) and CYP3A5*1*3 (heterozygous) genotypes (Park et al., 2009). In addition to this oral clearance of carbamazepine in patients with CYP3A5*3*3 genotype was 29% lower as compared with patients bearing normal allele and carrier allele. The CYP3A5*3 has a high frequency of 65-85% in Asians, 84-95% in Caucasians and 27-55% in Africans-Americans (Myrand et al., 2008; Makeeva et al., 2008; Kuehl et al., 2001; Lin et al., 2002; Yamaori et al., 2004).

The influence of CYP3A5 polymorphisms has also been studied in a Japanese population (144 patients) treated with carbamazepine polytherapy in combination with phenytoin and phenobarbital as well as with carbamazepine monotherapy (Seo et al., 2006). There was an 8% increase in serum concentration of carbamazepine in patients with CYP3A5*3*3 when compared with CYP3A5*1*1and CYP3A5*1*3 variants indicating that CYP3A5*3 genotypes may not be very important in Japanese epileptic patients on carbamazepine therapy.

In addition to CYP enzymes, epoxide hydrolase (EPHX1) also contributes towards metabolism of primary metabolite of carbamazepine. Several polymorphisms in EPHX1 gene have been studied in context with altered carbamazepine-10, 11-epoxide metabolism. The two commonly occurring nonsynonymous polymorphisms, Try113His and His139Arg, have been reported in Caucasians as well as Asians (Graziano et al., 2009). The allele frequency of Try113His (337T>G) is reported to be 22-31% in Caucasians and approximately 14% in Asians (Graziano et al., 2009). In vitro expression studies have demonstrated that Try113His variant confers about 40% decrease in hydrolysis activity, whereas the His139Arg confers an increase in activity at least 25% (Hassett et al., 1994). These variations in EPHX1 could affect the pharmacokinetics of AEDs and can also influence the fetal exposure to the reactive oxide intermediates in pregnant epileptic women with these polymorphisms (Decker et al., 2009; Azzato et al., 2010; Denney, 2007). Makmor-Bakry et al. (2009) in a study of 70 patients from Scotland reported that EPHX1 (337T>C) polymorphism may be significantly associated with maintenance dose of carbamazepine (Makmor-Bakry et al., 2009). The genetic polymorphism in genes encoding for antioxidant enzyme glutathione S transferase (GST) has been found to be related with carbamazepine induced adverse reactions (Kasperaviciute and Sisodiya, 2009; Zaccara et al., 2007). In a retrospective study, genetic variant of GSTM1 null genotype has been implicated as a major risk factor for carbamazepine-induced mild hepatoxicity in Japanese epileptic patients (Ueda et al., 2007). The GSTM1 null genotype frequencies have been reported in Caucasians and Asians about 40-60% and 20-25% in Africans –Americans (Ginsberg et al., 2009).

**GENETIC VARIANTS AFFECTING LAMOTRIGINE METABOLISM**

Lamotrigine is an antiepileptic drug used in the treatment of partial seizures and myoclonic seizures in epilepsy. The chemical name of antiepileptic drug, lamotrigine (lamicital) is 3, 5-diamo-6-(2,3-dichlorophenyl)-1,3-triazine. It
is also used as an adjunctive therapy in epilepsy. Lamotrigine is metabolized predominantly by glucurononic acid conjugation (UGT enzyme), which are known to induce or inhibit glucuronidation may affect the apparent clearance of lamotrigine and doses of lamotilic may require adjustment based on clinical response. The metabolism of lamotrigine has been found to be affected by SNPs in UGT1A4 gene, which is mainly involved in lamotrigine metabolism (Saruwatari et al., 2010).

In a study involving Turkish epileptic patients, UGT1A4 gene polymorphisms L48V (142T>G) and P24T have been investigated by comparing serum levels of lamotrigine of wild type and polymorphic subjects (López et al., 2011). The serum concentration of lamotrigine was measured by high performance liquid chromatography. The L48V polymorphism was reported to decrease the serum concentration of lamotrigine in epileptic patients with monotherapy or polytherapy.

In addition to UGT1A4, UGT2B7 also contributes towards metabolism of lamotrigine. Recently in a study using Spanish epileptic patients a significant association was observed between lamotrigine concentration/dose ratio and UGT2B7 -161C>T polymorphisms. It was also found that co-medication with valproate and other AEDs were responsible for most of the inter-individual variation in lamotrigine concentration to drug ratio (70%), followed by patient age (24%) and UGT2B7 -161C>T polymorphism (12%) (Blanca et al., 2010). Therefore, genetic polymorphisms in UGT genes should be taken in to consideration for adjusting the dose of lamotrigine in epileptic patients.

Clobazam
Clobazam also known as Urbanyl or Frisium is used as an antiepileptic drug and as adjuvant therapy in refractory epilepsy. The chemical name of clobazam is 7-Chloro-1-methyl-5-phenyl-1,5-benzodiazepine-2,4(3H)-dione. More than 70% of clobazam is demethylated to yield N-desmethylclobazam, an active metabolite contributing to efficacy. The demethylation is carried out by CYP2C19, CYP3A4 and CYP2B6 enzymes. Among these enzymes CYP2C19 is primarily involved in metabolism of clobazam. The CYP2C19 gene polymorphism has been found to affect the metabolism of clobazam in a significant manner. See et al. (2008) demonstrated in a study including Japanese epileptic patients, that mean serum concentration of N-desethylclobazam metabolite was 9 times higher in individuals with CYP2C19*1*2 genotype (poor metabolizers) in comparison with genotype bearer CYP2C19*1*1(homozygous extensive metabolizers) (Seo et al., 2008). In this study, the responder rate was found to be significantly higher in CYP2C19 (poor metabolizers) and heterozygous extensive metabolizers as compared to CYP2C19 homozygous extensive metabolizers with a gene dose effect of 65.2%, 47.6% and 33.3% respectively. The incidence of adverse drug effects including drowsiness, dizziness associated with clobazam, was reported to be more in case of CYP2C19 PMs as compared to CYP2C19 homozygous EMs. The frequency of CYP2C19 PMs varies across races e.g. 13.23 % of Asians, 1-8% of Caucasians have been reported to be PMs (Myrand et al., 2008; Makeeva et al., 2008). Therefore, CYP2C19 polymorphisms might affect the metabolism of clobazam and incidence of drug related adverse reactions in epileptic patients (Seo et al., 2008).

Valproic acid
The antiepileptic drug valproic acid is available as sodium valproate or Depakote. Valproic acid has narrow therapeutic index therefore, plasma levels are to be monitored carefully during the course of treatment. The chemical name of valproic acid is 2-propylpentanoic acid. The metabolic pathways comprise CYP mediated oxidation (10%), glucuronidation (50%) and beta oxidation (40%). The main enzymes involved in the oxidation of valproic acid are CYP2C9, CYP2C19 and UGT2B7. SNPs have been identified in genes affecting pharmacokinetics of valproic acid (Jiang et al., 2009).

A recent population study including 287 Chinese epileptic patients showed that CYP2C9*3 and CYP2C19*2*3 genotypes significantly affect the pharmacokinetic variability of valproic acid (Jiang et al., 2009). UGTB7 gene also contributes to glucuronidation of valproic acid (Jin et al., 1993). UGT2B7*2 allele has been reported to have nonsignificant tendency to increase area under the curve (AUC) of serum concentration of valproic acid (Chung et al., 2008). In rare cases, valproic acid can cause liver damage partially due to one of its major hepatotoxic metabolite i.e. 4-enevalproic acid (Ferraro and Buono, 2005). However, it remains unclear as to whether CYP2C9 polymorphisms are responsible for hepatoxical potential of valproic acid.

Zonisamide
Zonisamide is a sulfonamide anticonvulsant approved as adjunctive therapy in partial seizures and tonic clonic seizures. The chemical name of zonisamide is benzol[d]isoxazol-3-yl methane sulfonamide. The drug is metabolized by CYP3A4 and to a minor extent by CYP3A5 and CYP2C19. Therapeutically effective serum concentration lies in between 10-38 μg/mL. The influence of genetic variants of CYP2C19 and CYP3A5, were studied on the pharmacokinetics of zonisamide in 99 Japanese epileptic patients. The clearance estimate of zonisamide was found to be 16% and 30% in CYP2C19 heterozygous EMs and PMs respectively (Okada et al., 2008). However, in contrast with CYP2C19 the CYP3A5*3 allele has not been found to influence the pharmacokinetics of zonisamide (Aarons, 1991). Therefore, CYP2C19 genotypes may play a critical role in pharmacokinetics of zonisamide and may also influence the development of drug associated adverse effects.

GENETIC VARIANTS AFFECTING TRANSPORTER PROTEINS OF AEDS
Majority of AEDs act on target receptor proteins, located in the brain. Therefore, sufficient penetration through blood brain barrier is a prerequisite for initiating the desired therapeutic effect in patients with epilepsy. The blood brain barrier (BBB) is a physical and metabolic membrane which serves as a barrier between the brain and systemic circulation (Huber et al., 2001). BBB protects and regulates the microenvironment of the brain. AEDs are quite lipophilic and can easily penetrate the brain capillary endothelial membranes which form BBB. The AEDs are transported across the blood brain barrier via transporters (e.g. ATP cassette binding proteins). Genetic polymorphisms in genes encoding for drug transporters or proteins have been reported to play an integral role in influencing the pharmacological response of AEDs (Loscher et al., 2009).

ATP cassette binding protein (MDR1)
The two principal families of AED transporters are multidrug resistance (MDR or ABCB) and multidrug associated proteins (MRP/ABCC). They are expressed in endothelial cells of the blood-brain barrier and in choroidal plexus epithelial cells of the blood cerebrospinal fluid barrier. They may limit brain accumulation of AEDs by reversing the transport of AEDs from brain
to blood. P-glycoprotein (P-gp) is encoded by MDR1/ABC1 gene. Human P-gp is a membrane protein containing 1280 amino acids. P-gp has increased transport capacity for certain drugs e.g. antiepileptic drugs. Many drugs including AEDs are the substrates of P-gp.

Functional polymorphisms in ABCB1 gene can lead to inter-individual differences in the rate of drug uptake, distribution or efflux, resulting in altered drug concentrations and therapeutic effectiveness (Depondt and Shrovon, 2006). Research studies have indicated that polymorphisms or genetic variation in ABCB1 gene may determine altered substrate specificity. MDR1 3435C>T, a silent mutation in exon 26 results into change in substrate specificity through alteration of conformation and insertion of P-gp into membrane. Kerb et al. (2001) studied the significant association of 3435C>T polymorphism in ABCB1 with phenytoin levels (Kerb et al., 2001). Simon et al. (2007) reported the positive influence of two SNPs 3435C>T and 2677G>T of ABCB1 on phenytoin and carbamazepine dose requirements (Simon et al., 2007). Parallel results were obtained by Ebid et al. (2007) where C3435T polymorphism affected phenytoin levels in epileptic patients. The individuals with CC genotype were more likely to have decreased phenytoin levels as compared to patients with TT genotype (Ebid et al., 2007).

Researchers have studied SNPs in ABCB1 gene at different nucleotide positions such as T129C, T1236C in exon12, G2677T in exon 21 and C3435T in exon 26 in relation to refractory epilepsy (Maleki et al., 2010). Accumulating evidences suggest that genetic variation in ABCB1 accounting over expression of P-gp might be associated with reduction in amount of drug reaching to epileptic/ target neuron thus leading to development of resistance against AEDs. The association between MDR1 3435C>T and pharmacoresistance in epilepsy is unclear with three of the published studies showing positive association whereas other studies were unable to establish an association (Siddiqui et al., 2003; Sills et al., 2005). Maleki et al. (2010) found an association between ABCB1-T1236C polymorphism and AED resistance in Iranian epileptic female patients (Maleki et al., 2010). In this study, the risk of drug resistance was found significantly more in female patients with CC genotype and CT genotype than the patients bearing wild type or homozygous normal genotype, TT (Maleki et al., 2010).

In a retrospective case control study by Siddiqui et al. (2003) the frequencies of ABCB1, 3435C>T variant were compared in AED responders (patients), AED resistant (patients) and control individuals (nonepileptic). It was found that patients with multidrug resistance epilepsy were more likely to be homozygous for the C allele. CC genotype has been found to be associated with over expression of p-glycoprotein and may lead to decreased AED levels at brain target sites (Sills et al., 2005; Hoffmeyer et al., 2000).

Another transport protein reported to be involved in AED transport is RLIP/RALBP1. RAL interacting protein or RAL binding protein (RLIP76 (RALBP1)), a non ABC drug transporter has been reported to be a major transporter of AEDs such as phenytoin and carbamazepine across the blood brain barrier (Awasthi et al., 2005). RLIP was found to be present in the luminal capillary endothelial surface in patients and has been found to be associated with drug resistant epilepsy (Soranzo et al., 2007). RLIP has greater effect in mediating resistance to AEDs in patients with drug resistant epilepsy than P-gp (Awasthi et al., 2005). In a study carried out by Soranzo et al. (2007) six tagging SNPs in the RLIP76 gene were not found to be associated with AED response in epileptic patients. In addition to this, polymorphisms in other transporter proteins such as Multidrug resistant P1K2 and organic cationic transporter protein have been reported to have a potential role in AED transport (Soranzo et al., 2007). However, the knowledge regarding their potential implication in AED transport is half way and requires further investigations.

With improved understanding of AED transport influenced by genetic variation, it is possible to identify patients for non AED treatments such as surgery and brain stimulation and to develop novel AEDs with improved access to critical sites of action in the brain.

**GENETIC VARIANT AFFECTING AED TARGETS**

After transport of AEDs across blood brain barrier, the next step is to reach the specific target in the brain to initiate desired therapeutic effect. Target protein can be a receptor or an enzyme encoded by a specific gene. Genetic polymorphisms in these target receptor genes e.g. variation in gamma amino butyric acid (GABA) and sodium channel neurons (SCN) can modify the pharmacological response of AEDs.

The sodium channels are the primary targets of AEDs. Other major AED targets are potassium ion channels, calcium channels, GABA and glutamate receptors (Depondt and Shrovon, 2006). Recently, a new target site for an antiepileptic drug levetiracetam has been identified to initiate its response via acting on synaptic vesicle protein (SV2A), suggesting a new mechanism of AEDs (Lynch et al., 2004). Most of the AEDs including carbamazepine, lamotrigine, valproic acid, topiramate and phenytoin are thought to exert their antiepileptic effect by blocking sodium channels. Voltage gated sodium channels are essential for generation of action potential and play an important role in membrane excitability and nerve conduction. The genes encoding for sodium channel neurons (SCN) have been found to be play a central role in various types of epilepsy phenotypes (Lakhan et al., 2009). The voltage gated sodium ion channels consist of α and β subunits. Each a subunit is associated with one or more β subunits to form functional voltage gated ion channels. Defects in subunits of sodium channels results in slow inactivation (membrane depolarization is delayed) resulting in epileptogenesis and seizure generation (Alekov et al., 2000; Villin and Ruben, 2001). Mutations in the α subunit of sodium channel gene (SCN1) have been reported to be associated with familial and sporadic epilepsies (Lakhan et al., 2009). It is well accepted fact that patients response to drug treatment, may differ according to the underlying molecular pathogenesis of disease. Therefore, any gene in which mutations or variations predisposing to disease have been identified is also a potential candidate for variation in drug response (Depondt and Shrovon, 2006). In case of SCN gene, the SNPs may lead to epileptogenesis as well as variation in AED response.

Several SNPs have been recognized in SCN genes but only few including SCN1A Thr1067Ala and SCN2A Arg19Lys have been reported to be of functional significance in neurological disorders including epilepsy (Lossin, 2008). Lakhan and co-workers (2009) reported the association of genetic polymorphism of SCN1A c. 3184A>G with drug resistance in North Indian epileptic patients (Lakhan et al., 2009). In this study, AG genotype was found to be associated with increased risk of epilepsy. However, no association of this polymorphism was found with drug response. Authors reported SCN2A c.56G>A polymorphism to be related with multidrug resistance phenotype in epileptic patients (Lakhan et al., 2009). The proposed mechanism for this polymorphism affecting AED response is that amino acid change from arginine to lysine somehow interferes with stabilization of biological membranes by AEDs thus altering the therapeutic response. A significant association was reported in an intronic SNP SCN1A IVS5-91G>A and maximum doses in regular usage of carbamazepine and phenytoin. In this study, patients with AA genotype had to be prescribed higher maximum doses of carbamazepine and phenytoin as compared to patients having GG genotype 9 Szooke et al., 2006; Tate et al., 2005). However, this polymorphism was not found to be associated with carbamazepine doses in Austrian epileptic patients, suggesting ethnic variation (Tate et al., 2005).

In addition to genetic variants of SCN genes, other AED targets, where functional polymorphisms could possibly influence the therapeutic response of AEDs are GABA receptors. GABA is the major inhibitory neurotransmitter which regulates neuronal excitability and network interactions in the cerebral cortex region of the brain. There are three types of GABA receptor gene classes in brain, these are: GABA_A, GABA_B, and GABA_C (Kumari et al., 2010). Recent findings have suggested that among the three receptors, GABA_A...
are of high importance because of receptor heterogeneity for excitation/inhibition properties and in relevance with various epilepsy phenotypes (Fritschy, 2008). GABA receptors are pentameric chloride ion channel combinations of proteins encoded by α (α1-66), β1-3, γ1-3, δ, ε, π, θ and ρ (1-3) subunit gene families. The α2y2β2 subunit of GABAα is most widely found in all the regions of brain (Reid et al., 2009). Dysfunction of gene encoding for this subunit may affect ion channel gating, expression and trafficking of the GABA receptor to the cell surface. These genes are believed to affect major antiepileptic drug targets required for the regulation of normal activity in brain (Chou et al., 2007). AEDs such as benzodiazepines, phenobarbital, gabapentin and topiramate are the important targets of GABA receptors (Moshe, 2000). Recent, studies involving rat model of epilepsy have suggested that AED resistant rats differ from drug responsive rats in GABA receptor subunit expression. It was also found that alterations in GABAα subunit might be involved in AEDs resistance or nonresponsiveness (Kumari et al., 2010).

A number of SNPs have been described in GABAα receptor gene so far but only few including intrinsic GABAR1 polymorphism IVS11+15A>G and exonic GABARG2 588C>T polymorphism, have been found to be functional significance in neurological disorders including epilepsy (febrile seizures) (Kumari et al., 2010). Data from research studies have indicated that GABAR1 IVS11+15A>G polymorphism in GABA receptor gene was associated with susceptibility of epileptogenesis and AED resistance. This intrinsic polymorphism does not lead to any amino acid change but may alter the conformation of mature protein by influencing alternative splicing. The exonic polymorphism GABARG2 588C>T results in synonymous or silent change Asn196Asn, which does not affect the sequence of the encoded protein, suggesting that this SNPs exists in linkage equilibrium with other disease causing variants. An association of this variant was reported with febrile seizures in Taiwanese children (Chou et al., 2007). However, it was not associated with epilepsy susceptibility or drug resistance.

In another study, Kumari et al. (2010) analyzed genotype and allelic frequencies of GABAR1 IVS11+15A>G in 395 sporadic epilepsy patients and 199 controls (Kumari et al., 2010). It was reported that frequencies of AG and GG genotypes were significantly higher in epilepsy patients versus control population, and the polymorphism was associated with increasing risk of epilepsy and modulating drug response in AED therapy. However, in same study group, another polymorphism GABARG2 588C>T was not found to be associated with epilepsy as well as with AED nonresponsiveness (Kumari et al., 2010).

GENETIC VARIANTS ASSOCIATED WITH AED EXCRETION

Few AEDs are eliminated via kidney involving glomerular filtration, tubular secretion and reabsorption. Genetic polymorphism in renal transporter protein has been found to interfere with normal excretion of antiepileptic drugs. Organic cation transporter (OCTN1) is a multispecific transporter expressed at apical membrane of intestine and kidney and facilitates sodium ion dependent transport of gabapentin. A genetic variant of OCTN1, L503F has been reported to alter the pharmacokinetic profile of antiepileptic drug, gabapentin. This polymorphism has been found to be prevalent in individuals of European descent (42%) as compared to other populations. It has been found that the individuals carrying OCTN1, 105F mutant allele have lower renal clearance rate as compared to individuals carrying wild type allele (Loscher et al., 2009; Szeoke et al., 2006).

GENETIC VARIANTS ASSOCIATED WITH ADVERSE DRUG REACTIONS

In epilepsy treatment most common adverse drug effects are dose related, which are mild and transient and consist of mainly neurological symptoms e.g. somnolence, dizziness accompanied with gastrointestinal symptoms, maculopapular exanthema and severe adverse reactions such as SJS and TEN. ADRs are specific to certain AEDs such as visual field constriction with vigabatrin and special pattern of cognitive impairment and language impairment with topiramate. Idiosyncratic ADRs due to AED treatment are rare but well recognized. The idiosyncratic adverse drug reactions are unpredictable and supposed to have underlying genetic etiology (Pirohamed et al., 2001). Idiosyncratic reactions are type B reactions, where the clinical symptoms are different from desired pharmacological effects of a drug. The proposed mechanism is immune mediated toxicity. Idiosyncratic reactions such as SJS/TEN have been found to be associated with mortality rate of 10% in epileptic patients on certain AEDs. These reactions are characterized by a blistering rash affecting a variable range of body surface area. The death rate is directly proportional to the degree of epidermal detachment.

The influence of genetic polymorphism on dose related ADRs has been found to be associated with drug metabolizing enzymes e.g. CYP450. ADRs may occur due to over accumulation of a drug in patients carrying defective allele (variant allele) in metabolizing genes. A case report has established the association of variant alleles CYP2C9*2 and CYP2C9*3 with slow activity of the encoded enzyme as well as phenytoin toxicity and developing ADRs such as gingival hyperplasia and other symptoms (Depondt and Shrovon, 2006; Brandolese et al., 2001).

Since the physiological basis of idiosyncratic reactions is not entirely elucidated, it has been hypothesized that ADRs such as hypersensitivity reactions may be immunogenetic in origin. Studies have suggested that life threatening ADRs associated with AED treatment are due to immunological mediated response restricted to few genetically predisposed individuals. A number of studies have revealed that genetic association between variants of genes encoding components of immune system such as tumor necrosis factor (TNF) and human leukocyte antigen (HLA) with idiosyncratic adverse drug reactions of AED treatment. HLA are important part of immune system (Major histocompatibility complex), which has been identified to be associated with AEDs induced ADRs.

Predisposition to carbamazepine hypersensitivity is likely to be genetically determined and genes within the major histocompatibility (MHC) e.g. HLA, have been implicated in AEDs (carbamazepine) induced ADRs. Szeoke et al. (2006) reported carbamazepine, lamotrigine and phenytoin induced SJS and involvement of a common risk allele HLAB*1502 (Szeoke et al., 2006). Authors recommended that HLAB*1502 carriers should not be treated with carbamazepine, oxcarbamazepine and phenytoin and lamotrigine should be prescribed with caution. The frequency of SJS and TEN is more among people from Southeast Asian lineage particularly Han Chinese than European and Japanese. Clinically, cross reactivity has been found to exist between structurally similar drugs such as carbamazepine, lamotrigine, phenytoin and oxcarbamazepine.

An association study in a small independent cohort of Han Chinese patients confirmed the strong association between HLA-B*1502 allele with SJS/TEN on carbamazepine therapy with 100% of patients carrying the allele versus 9% of controls (Chung et al., 2008). HLAB*1502 allele was also identified in two patients with SJS/TEN treated with phenytoin and lamotrigine, respectively. HLAB*1502 allele is found to be prevalent in Asian and Han Chinese population but not in European population (McCormack et al., 2011). Therefore, HLAB*1502 allele appears to be a population and phenotype specific marker for predicting life threatening ADRs associated with AEDs. However, HLAB*1502 is not a universal marker for AEDs induced hypersensitivity reactions and maculopapular eruption.

Another variant of HLA gene HLA-A*3101 has been identified to be related with AEDs induced hypersensitivity reactions. The presence of the HLA-A*3101 (rs1061235) allele has been identified to be strongly associated with carbamazepine-induced hypersensitivity reactions in Northern European population (McCormack et al., 2011). It was found that the presence of this allele increased the risk of AEDs induced hypersensitivity reactions from 5.0% to 26.0%, whereas its absence reduced the risk from 5.0% to 3.8%. All the patients carrying HLAB*1502 allele appeared to have Asian ancestry.
supporting that the HLA B*1502 is a population specific marker for AED induced hypersensitivity reactions (Depondt and Shrovon, 2006). Pirmohamed et al. (2001) identified an association between TNF2 allele of TNF α gene and severe carbamazepine hypersensitivity (Pirmohamed et al., 2001). The TNF α gene is in linkage disequilibrium with human leukocyte genes (HLA-DR3 and HLA-DQ2), and TNF-DR3-DQ2 haplotype appeared to be associated with severe carbamazepine toxicity.

The heat shock proteins (Hsp70) gene cluster located on MHC class III region, has also been found to be associated with drug induced hypersensitivity reactions. In a case control study involving European population, authors studied 25 SNPs across the three HSP70 genes. It has alleles G, T and C at the SNPs HSPA1A +1911 C/G, HSPA1A +438 C/T and HSPA1L +2437 T/C, respectively, were associated with protection from serious hypersensitivity reactions to CBZ, with the associated alleles falling on a common haplotype (Alfirevic et al., 2006).

**PROMISE OF PHARMACOGENETICS IN EPILEPSY TREATMENT**

Pharmacogenetics has been gaining attention as the key emerging area leading to the development of Individualized or Personalized medicine. The treatment in epilepsy with antiepileptic drugs is unpredictable in terms of drug associated adverse effects and dose optimization in individual patients and may be one of the consequences of genetic variation. Genetic polymorphisms have been found to play an integral role in the variability of AEDs therapeutic efficacy. SNPs can significantly influence the substrate specificity by altering the conformation of the encoded protein leading to AED toxicity and reduced efficacy.

The understanding of genetic variation and underlying molecular mechanism affecting the drug response in epilepsy will provide a new approach of individualized medicine with better and targeted drug therapy. This will help in predicting the patient’s response prior to initialization of treatment. The antiepileptic drug felbamate has been withdrawn because of rare occurrences of potentially fatal aplastic anemia and liver failure. If some genetic markers or biomarkers can predict the risk of these adverse effects in patients before prescribing such drugs, the drugs could be used safely in selected epileptic patients. Pharmacogenetics can provide an invaluable instrument for better drug design, optimization and rationalization of AED therapy with tailored approach based on patient’s genotypic profile. Although Pharmacogenetics would create a new revolution in the field of clinical sciences the associated ethical, legal and social issues also need to be considered. Legal liabilities may arise, if the patients at risk will be tested and treated with AEDs without prior genetic consent. Genetic information can lead to discrimination by insurers. This discrimination can also occur at workplace. Social issues regarding personalized treatment include public fears of refusal to genetic testing. Illicit mining of genetic information from insurers, diagnostic centers, employers or from researchers represents another major problem. Despite of all these roadblocks the potential benefit of translating Pharmacogenetics in epilepsy treatment hold the promise of optimization of drug treatment (Fig 1).
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