Molecular Genetics of Drug-resistance in Epilepsies

Kurupath Radhakrishnan
Professor & Head and Director, Department of Neurology
Sree Chitra Tirunal Institute for Medical Sciences and Technology
Thiruvananthapuram-695011, Kerala.

SUMMARY

Nearly one-third of newly diagnosed patients with epilepsy remain unresponsive to antiepileptic drugs (AEDs), etiopathogenesis of which is poorly understood. The genes encoding the proteins that regulate the pharmacokinetics such as P-glycoprotein *[ABCBI]*, major vault protein *[MVP gene]* and drug metabolizing enzymes *[ABCB1, ABCG2, MVP, CYP2C9, CYP2C19, CYP3A4, CYP3A5, EPHX1, UGT1A1, UGT2B7]*, and pharmacodynamics such as sodium channels *[SCN1A, SCN2A]* and GABA receptors *[GABRA1, GABRA6, GABRB2, GABRG2]* of AEDs are under intense investigation to unravel the mysteries of AED-resistance. However, till today, a consistent and reliable result that could help the clinician either to predict drug-resistance or to overcome it has not been forthcoming. The discrepant results may be related to variations in the definition of drug-resistance, heterogeneous patient populations, ethnic variations in the frequency distribution of single nucleotide polymorphisms (SNPs) and the selection of SNPs. Understanding of these limitations of existing studies, hopefully, will help in designing better studies.

Correspondence : Dr. Kurupath Radhakrishnan, Professor & Head and Director, Department of Neurology, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram-695011, Kerala. Telephone: 91-471-2524282, Email: krk@sctimst.ac.in .

Introduction

Epilepsy is a common neurological disorder and is a major public health concern, directly affecting an estimated 50 million people worldwide and involving an additional 500 million people as family members and caregivers of patients (1). It constitutes a heterogeneous group of disorders characterized by recurrent unprovoked epileptic seizures due to widely different etiologies with a prevalence rate of about 5 per 1,000 and an annual incidence rate of about 50 per 100,000 (2). Although, a majority of patients with epilepsy are responsive to presently available antiepileptic drugs (AEDs), 20% to 30% of them continue to exhibit recurrent seizures, despite optimal AED therapy (3). Based on a conservative estimate, there will be more than five million people with active epilepsy in India, and of them, at least one million will be drug-resistant. Resource-poor countries are ill-equipped to tackle the enormous medical, social and economic challenges posed by drug-resistant epilepsies (4)

Definition of drug-resistance

It is generally agreed that an adequate trial of appropriate AEDs should be given before labeling the epilepsy as drug-resistant. However, the concept of adequate trial of AEDs is highly arbitrary. To overcome some of the ambiguity involved in defining drug-resistant epilepsy, International League Against Epilepsy (ILAE) has proposed a consensus definition of drug-resistant epilepsy (5). According to this definition, drug-resistant epilepsy is defined as "failure of adequate trials of two tolerated and appropriately chosen and used AED schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom". Sustained seizure freedom is defined as seizure freedom for a minimum of 12 months or for a period three times the previous longest seizure-free period, whichever is longer. The ILAE definition places a greater emphasis on seizure freedom, as this is the only meaningful outcome which can lead to improved quality of life of persons with epilepsy. This definition equally emphasizes the importance of appropriate and tolerated treatment schedules as treatment failures due to inappropriately chosen or non-tolerated drugs, non-compliance to drugs or due to the unknown drug schedules cannot be classified as drug-resistant. Failure of two AEDs has been included in the definition with the recognition of the fact that once the patient fails two AED trials, subsequent chances of sustained seizure freedom from further AED trials are very unlikely (3).

Patients with drug-resistant epilepsy have considerable impairments in activities of daily living, education, employment and social interaction due to continuing seizures and AED side effects. These patients are at higher risk of developing various psychological problems like depression, anxiety and psychosis (6). Additional morbidity and mortality of continued seizures include accidental injury, cognitive decline and sudden death (7). Rates for employment,

marriage and fertility are considerably lower in patients with poorly controlled seizures (8,9). Patients with drug-resistant epilepsies account for nearly 80 percent of the annual cost attributable to epilepsy (10). A select group of patients with drug-resistant epilepsy has a chance of becoming seizure-free with epilepsy surgery. However, a majority of them are not candidates for epilepsy surgery and will have to be continued on AED therapy with the hope of achieving seizure control.

Causes of drug-resistance

Several clinical factors have been found to be associated with the drug-resistance in patients with epilepsy, which include early seizure onset, number of seizures before the initiation of AED, high seizure burden within first few months of starting AED, seizure clustering, family history of epilepsy, febrile seizures, electroencephalographic abnormalities, history of status epilepticus, demonstrable brain lesion on magnetic resonance imaging, remote symptomatic etiology, abnormal neurological status, traumatic brain injury, and psychiatric comorbidity (11,12). However, a significant proportion of patients may not have any one of the above attributes to associate with drug-resistance. Perhaps, the most important determinant of AED response/resistance is the epilepsy syndromic diagnosis. While an idiopathic epilepsy syndrome like juvenile myoclonic epilepsy responds very well to AED therapy, the syndrome of mesial temporal lobe epilepsy with hippocampal sclerosis (MTLE-HS) is very resistant to AED. Moreover, the pattern of the drug-resistance observed also varies; *de novo*, where patients might be resistant right from the starting of AED therapy, progressive drug resistance, where patients become resistant in due course of disease progression and a third type, waxing and waning resistance, where active epilepsy is interrupted by periods of remission (13). Conceptually, the variable response to AEDs can be attributed to factors related to altered pharmacokinetic factors and pharmacodynamic factors influencing their efficacy and tolerability.

Putative mechanisms of drug-resistance

The clinical efficacy of an AED depends on its absorption, distribution and elimination, which in turn is influenced by the physicochemical properties of the drug **(Figure 1).** Most of the AEDs are lipophilic and penetrate the biomembranes by passive diffusion. The activity of the efflux transporters in the gastrointestinal tract and blood-brain-barrier influence the absorption and brain uptake of AEDs, respectively. Furthermore, the altered targets of AEDs may affect the clinical efficacy. In addition, the differential activity of the drug metabolizing enzymes, which are substrates for AEDs, influences drug efficacy and tolerability. To understand the genetics of drug-resistance, three major hypotheses have been proposed **(Figure 1).**These mechanisms are not mutually exclusive; they can occur together in an individual patient and can collectively contribute to drug-resistance.
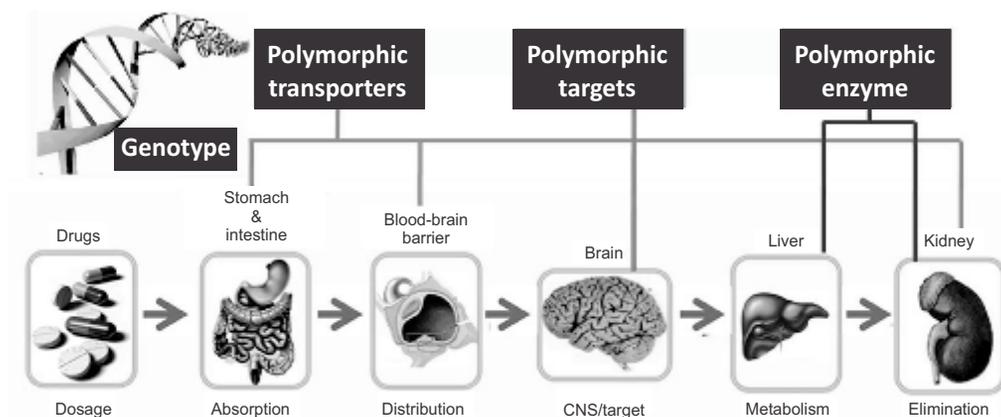
Figure 1: A schematic illustration of the mechanisms involved in inter-individual variability in drug-response.

Transporter hypothesis

According to transporter hypothesis, drug-resistance is a consequence of increased expression or function of multidrug transporter proteins, so that sufficient intraparenchymal AED concentrations are not attained at their targets, even in the presence of adequate serum AED levels. This notion has been supported by the characterization of different efflux transporter proteins that function as drug efflux pumps at the blood-brain-barrier, gastrointestinal tract and other privileged environments (14,15). The genes that encode efflux transporters are highly conserved and the vast majority of them belong to the super family of adenosine triphosphate-binding cassette (ABC) proteins. Single nucleotide polymorphisms (SNPs) of the encoding genes of efflux transporters, including P-glycoprotein (p-gp) (*ABCB1*), and other members of the multidrug resistance-associated protein family (ABCC/MRP family) and breast cancer resistance protein (BCRP) (*ABCG2/BCRP*) can theoretically affect the function of the blood–brain barrier and for this reason been widely studied in context of AED-resistance (14,15).

Target hypothesis

To exert its pharmacological effect, AED after successfully crossing the blood-brain-barrier, should reach its target. Currently available AEDs act on a relatively smaller number of targets. The major targets of the AEDs are voltage-gated sodium channels, calcium channels and neurotransmitter systems (GABA and glutamate). Voltage-gated sodium channels form the targets for a majority of first-line AEDs. According to target hypothesis, drug-resistance can be caused by the modification of one or more AED target molecules resulting in reduced efficacy of a given AED (16).
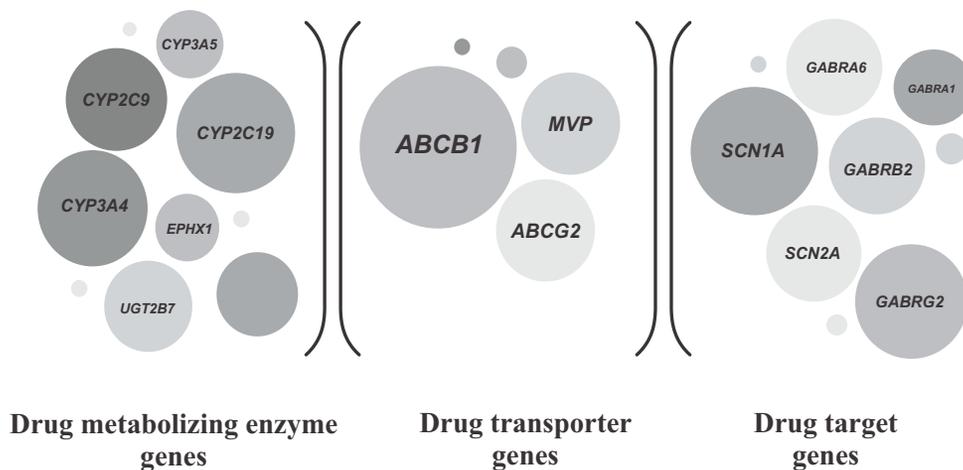
Role of drug metabolizing enzymes and other factors in drug-resistance

An array of enzymes is involved in the biotransformation of AEDs. Drug

metabolism is accomplished by two phases, where the most common reaction involving phase I enzymes (cytochrome P450 family of mixed function oxidases) is hydroxylation. Phase 2 metabolism involves various conjugation reactions that increase hydrophilicity and facilitate renal excretion of drug. A genetic variation with a very high enzymatic activity may be associated with poor drug response in conventional dosages (13,16).

Molecular Genetics of Drug-resistance

The molecular targets with respect to above described three hypotheses are depicted in **Figure 2.** Although several of these targets have come under intense investigations, it should be admitted that, till today, a consistent and reliable result that could help the clinician either to predict drug-resistance or to overcome it has not been forthcoming. What follows is a critical appraisal of the molecular genetic studies on AED-resistance, including my own. These studies can be broadly grouped into those inquiring genetic variations in the pharmacokinetic and pharmacodynamic

Drug metabolizing enzyme genes

Drug transporter genes

Drug target genes

Figure 2 : A schematic illustration of the candidate genes for studies on drug-resistance.

aspects of AEDs **(Table 1).**

Over the last five years, the author and his colleagues at the R. Madhavan Nayar Center for Comprehensive Epilepsy Care, in collaboration with the Rajiv Gandhi Center for Biotechnology, Trivandrum, has undertaken a series of molecular genetic studies with respect to AED-responsiveness/resistance. We

studied several genes related to AED pharmacokinetics and pharmacodynamics among three homogeneous groups of subjects: 200 drug-resistant epilepsy (MTLE-HS), 200 drug-responsive epilepsy (juvenile myoclonic epilepsy) and 200 non-epilepsy controls from south Indian population of Kerala. We screened 29 SNPs from 10 genes involved in AED

pharmacokinetics (*ABCB1, ABCG2, MVP, CYP2C9, CYP2C19, CYP3A4, CYP3A5, EPHX1, UGT1A1, UGT2B7*) and 13 SNPs from 6 genes involved in pharmacodynamics of AED (*SCN1A, SCN2A, GABRA1, GABRA6, GABRB2, GABRG2*), totaling 42 SNPs from 16 genes. We selected these SNPs based on their functional significance or by the tagging status to uncover maximum variation in the genes of interest.

Table 1 : Classification of phamacogenetics of drug-body interaction with regard to antiepileptic drug-resistance

Protein / Receptor	Gene
Pharmacokinetic Interaction	
P-glycoprotein	ABCB1
Breast cancer resistance protein	ABCG2
Major vault protein	MVP
Metabolizing enzymes	
Phase I	CYP2C9, CYP2C19, CYP3A4, CYP3A5, EPHX1
Phase II	UGT1A1, UGT2B7
Pharmacodynamic interaction	
Voltage gated sodium channel	SCN1A, SCN2A
GABA receptor	GABRA1, GABRA6, GABRB2, GABRG2

Genes involved in AED pharmacokinetics P-glycoprotein (*ABCB1*)

The gene encoding p-gp, *ABCB1,* is the maximally investigated gene with respect to AED-resistance (Aronica, 2012). An initial study in 2003 showed a significant association of a synonymous variant (C3435T) of *ABCB1* with multidrug resistant epilepsy (17). However, a series of studies that followed, including ours, failed to consistently replicate such an association. We found that subjects carrying ABCB1 rs1922242 polymorphism had five times higher risk of developing corpora amylacea accumulation in the hippocampus, compared to those MTLE-HS patients without this polymorphism (18). In addition to its function as an efflux transporter, the role of CoA in sequestration of toxic cellular metabolites is being increasingly recognized (19). Therefore, upregulation of p-gp function in patients with uncontrolled seizures might be a consequence rather than the cause of seizures (20). Two recent meta-analyses on AED-resistance and *ABCB1* genotype, both having over 3000 drug-resistant epilepsy patients and controls across multiple populations, revealed no

significant association between the *ABCB1* C3435T genotype and resistance to AEDs (21,22).

Breast cancer resistance protein (ABCG2)

A limited number of studies have investigated genetic variations in *ABCG2,* which codes for the BCRP. The non-synonymous polymorphisms, rs2231142 (C421A) and rs2231137 (G34A) have been associated with decreased BCRP expression and decreased BCRP transporter activity, respectively (23,24). In our study, three functional variants of *ABCG2* were screened and the data did not identify any significant association between these polymorphisms and response to AED treatment. Similarly, a Korean study and a Chinese study on multidrug-resistant epilepsy patients revealed no association of drug-resistance with *ABCG2* gene (23,24).

Major vault protein (MVP gene)

Recent studies have associated an intracellular organelle called vaults (named so because of their resemblance to vaulted ceilings in cathedrals) with multidrug resistance in cancer cells (25,26). Vaults are localized mainly in the cytoplasm, but a small fraction also resides at the nuclear membrane and the nuclear pore complex. Although the exact function of vaults remains unknown, several lines of evidence indicate that they are involved in intracellular vesicular and bidirectional nucleo-cytoplasmic

transports (25,26). Over expression of MVP has been reported in brain tissue samples from rat model of drug-resistant temporal lobe epilepsy, AED-resistant human MTLE-HS, frontal lobe epilepsy, and focal epilepsies due to benign neoplasm (27,28). We compared the distribution of three SNPs of the *MVP gene,* rs4788187, rs3815824 and rs3815823, among cohorts of AED-resistant and responsive patients and non-epileptic controls (29). To our knowledge, ours is the first study that inquired the association of genetic variants of *MVP* gene with AED resistance. However, the results revealed that rs4788187, rs3815824, rs3815823 variants of *MVP gene* were associated neither with predisposition for epilepsy nor with AED-resistance in the population we studied (29).

Drug metabolizing enzymes

The Phase I and Phase II drug metabolizing enzymes are major players in determining AED pharmacokinetics. The author studied the functional polymorphisms in Phase I drug metabolizing enzymes (*CYP2C9, CYP2C19, CYP3A4, CYP3A5, EPHX1*) and Phase II (*UGT1A1, UGT2B7*) drug metabolizing enzymes. Studies have reported the association of genetic variations in drug metabolizing enzymes with dosage requirement of commonly prescribed AEDs (30). It can be hypothesized that patients with over-expression of metabolizing enzymes are more likely to become drug-resistant during the course of AED treatment than

with slow metabolizer phenotype, which require lesser drug doses to control seizures. However, we could not observe any significant association of functional variants pertaining to AED response/resistance. The frequency distribution of the variants among the MTLE-HS and juvenile myoclonic epilepsy cohorts was similar to that of the normal Kerala population. In a study from North India, the frequency of a variant genotype (*CYP2C9*1/*3*), which is known to result in slow metabolizer phenotype, was found significantly lower in drug-resistant group as compared to drug-responsive group (31). With respect to *EPHX1*, involved in the biotransformation of carbamazepine, we did not find a significant difference in the frequency of the alleles among the AED-resistant and responsive epilepsy patients. The distribution of the SNP frequencies in the present study differed from those reported in other Indian studies, which can be attributed to the differences in the phenotypic classification of the drug-resistant and responsive epilepsies. The frequency of the extensive metabolizer (EM) TT genotype, intermediate metabolizer (IM) CT genotype and poor metabolizer (PM) CC genotype of rs1051740 in epilepsy patient groups pooled together in our study were 41%, 42.2% and 16.8%, respectively, which is different from the frequency distribution of North Indian epilepsy patients of 43.5% TT, 41.6% CT and 14.9% CC genotypes (32). However, the distribution of EM and IM genotypes, when pooled together, were similar in both populations. The frequency of EM, IM and PM of

rs1051740, in AED-resistant group were 41.1%, 44.7% and 14.2% and in AED-responsive group, the distribution was 40.9%, 39.4% and 19.7%, respectively. In case of rs2234922, frequency of EM GG genotype is higher (8.8%) in the pooled epilepsy samples, AED resistant (7.3%) and AED responsive (10.5%), when compared to the data from the North Indian epilepsy samples (32).

Genes involved in AED pharmacodynamics

Studies that have investigated the role of target genes have concentrated on genes encoding voltage-gated sodium channels and GABA$_A$ receptors.

Voltage-gated sodium channel genes

Voltage-gated sodium channels are primarily involved in the generation of action potentials and also the high frequency firing in epileptic discharges. With the notion that nucleotide variations in the genomic region coding AED binding domains, DIIIS6 and DIVS6 segments of the NaV1.1 channel, can affect the drug binding and thereby drug response, we selected SNPs from *SCN1A*. Additionally, we genotyped three SNPs of SCN2A that were found to be associated with AED-resistance in the Chinese population (33). The results showed that there was no association of the studied SNPs with drug-resistance. Our results were contradictory to the initial report and were aligning with other studies (33,36), with the exception of a study from Japan which demonstrated a significant

association between the rs3812718 AA genotype and carbamazepine-resistant epilepsy (34). Additionally our study also showed, the variant rs3812718 increases the susceptibility to MTLE-HS, independently without increasing the susceptibility to the febrile seizures (35).

GABA receptor genes

In a rodent model of drug-resistant temporal lobe epilepsy, altered GABA receptor subunit expression and differential drug response has been observed (36**).** We probed the role of functional variants in *GABRA1, GABRA6, GABRB2, GABRG2* receptor subtype genes for its contribution to the AED-resistance in Kerala population. Although we found no association of these variants with respect to AED-resistance, the variant rs211037 in *GABRG2* showed an association with the increased predisposition to develop epilepsy. Previous reports from North Indian epilepsy patients (37) and in Caucasians epilepsy patients (38,39) did not show any involvement of *GABRG2* rs211037 synonymous variant with epilepsy.

Putative causes for the discrepant results

Despite the large volume research undertaken during last one decade in epilepsy pharmcogenetics, translation to clinical utility has been very limited, to date, with the exception of a strong association between HLA-B*1502 and carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in Han Chinese people (40). The discrepant results can be accounted for a variety of factors such as variations in the definition of the drug resistance, heterogeneous patient populations, ethnic variations in the distribution of the SNPs and the selection of SNPs.

Variation in the definition of AED-resistant epilepsies

The lack of consensus among the different study groups in defining the drug-resistance might have resulted in the inclusion of the patients defined as drug-resistant in one study as drug-responsive in another, and thereby influencing the results of the genetic association. Generally, in clinical practice, AED-resistance is defined as seizure recurrence despite the trial of two to three AEDs or surgical intervention for seizure control, and drug responsiveness as seizure freedom on AEDs for a certain period of time. In studies reviewed here, the number of seizures and duration have varied widely from no seizures for one year, one seizure in 6 months, ≥1 seizure in a month, ≥1 seizure in a year, ≥4 seizures in 6 months to one year, >10 seizures in a year, > 2 seizures in 2years, and 50% reduction of seizures in a year. The AED-responsive cohort has been defined more or less uniform throughout the studies mainly as seizure freedom for >1 years, although certain studies have defined >6 months to 2 years of seizure freedom. Hopefully, future studies are likely to adhere to the recent ILAE definition of AED-resistant epilepsy (5).

Heterogeneity of epilepsy phenotype

A majority of the pharmacogenetic studies in epilepsies conducted till now have included a variety of epilepsy syndromes together by ignoring the underlying disease pathobiology (41). Since epilepsy syndromes are highly heterogeneous with respect to age at onset, seizure type, pathogenesis and AED responsiveness, comparison of molecular genetic results from heterogeneous samples becomes difficult (42).

Population stratification

Population stratification can results in the non-replicability of genetic association studies, which can be defined as occurrence of subpopulations for the studied populations that have different allele frequencies for the candidate gene studied. This disparity in frequencies arises because each population has a unique genetic and social history, and thus ancestral patterns of geographical migration, mating practices, reproductive expansions and bottlenecks, and stochastic variation all yield differences in allele frequencies between individuals (43). Thus the differential risk of a trait for the subpopulation and its inclusion in a study population can confound the association between the candidate gene and the disease. The issue of the population stratification and admixture effects can be addressed by matching geographical region (at study design stage) and by ancestry information markers (at analysis stage).

Biased selection of functional variants

The lack of association can also be attributed to the biased selection of functional variants, which may not be tagging the true causal variants. Since the selection of tagged SNPs to uncover maximum variation are based on the HapMap database with reference to Caucasian and Gujarati Indians, subtle difference may occur in the linkage disequilibrium pattern with respect to studied population of Kerala, which affects the tagging status and information content of the selected SNPs. Further, the small effect size of the common variants in explaining a small fraction of variability and heritability of the complex traits makes then clinically negligible.

Future perspectives

Future studies should attempt to rectify the problems of existing literature by assembling uniform patient populations, especially with respect to drug-resistance and epilepsy syndromic diagnosis. With the slashing of the cost of genotyping technologies, studies should undertake high density genotyping to identify the common variants and whole genome sequencing to identify the rare variants with high effect size (44). Further, better statistical analysis methods, which accounts for all potential confounding factors, have to be developed to enhance the accuracy of the results. With the rapid progress being made in the field of pharmacogenetics, hopefully, we will in the near future be able to identify patients with drug-resistant epilepsy early and

consider alternate treatment options. Further, genomic information will enable the clinician to prescribe syndrome specific AED therapy with optimum dosage for efficacy and with minimum adverse drug reaction, thereby paving way to individualized management of persons with epilepsy.

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