Current antiepileptic drug (AED) therapy requires trial and error in determining the most effective AED and dosage for a patient, and almost one-fourth of patients are resistant to AED therapy. The introduction of individualized medicine for epilepsy based on genetic information is a new avenue to improve current AED therapy. However, several crucial issues remain to be resolved before the development of individualized medicine for epilepsy can proceed further. The epilepsy genes responsible for common phenotypes have not yet been identified, and ongoing pharmacogenetic studies continue to search for an explanation of why 25 to 30% of patients do not respond to AEDs. There is no convincing clinical evidence indicating the impact of P-glycoprotein on prediction of clinical response. This
article provides a critical review of the status and perspectives for the development of individualized medicine for epilepsy based on genetic polymorphisms/mutations in relation to core elements such as pharmacodynamic pathway, pharmacokinetic pathway, prediction of idiosyncratic reaction to AED, and mechanisms of action of AEDs.

1. Introduction

Epilepsy, defined by the recurrence of unprovoked seizures, is a complex disorder that occurs when the electrical signals in the brain are disrupted, leading to repetitive seizures. Epilepsy is one of the most common neurological disorders, affecting about 0.6–0.8% of the general population [1]. It is estimated that 25-30 % of patients with epilepsy fail to achieve good control with antiepileptic drugs (AEDs) [2]. None of the currently available AEDs can eliminate epileptogenesis or ictogenesis. Surgical treatment—resection of focal epileptogenic brain tissue—is considered to be the only curative treatment available for patients with epilepsy, but patients are still required to continue AED treatment after surgery to ensure that they remain seizure-free [3]. AED treatment will thus continue to be the major therapy for epilepsy until more successful curative measures become available.

Currently, AED is selected according to seizure phenotype rather than epilepsy genotype, and the optimal dosage is determined by trial and error. However, the molecular revolution in epilepsy research during the last two decades is beginning to have a significant impact on diagnosis and treatment of epilepsy. In the clinical setting, genetic information can be used pretherapeutically to optimize the AED dosage, and in some cases, selection of an AED may be possible even at present.

For individualized medicine to become a reality, it is necessary to develop tools that could assess the degree of variability with regard to both pharmacokinetics and pharmacodynamics, as well as establish a well-defined model of the mechanism of action of AEDs. In this review, we discuss the core elements of individualized medicine for epilepsy based on genetic information.

2. Core elements of individualized medicine for epilepsy

2-1 Mutations of epilepsy genes as biomarkers for the selection of AED

The key elements for the development of individualized medicine for epilepsy are pharmacokinetic and pharmacodynamic pathways, mechanisms of action of AEDs, and prediction of adverse effects of AEDs (Figure 1). By combining epilepsy phenotype (seizure type) of a patient with the information of pharmacodynamic pathway, mechanisms of action of AED, and prediction of untoward effects of AEDs related to genetic makeup, a suitable AED can be selected. Then, the optimal dose can be decided according to pharmacokinetic information of the patient. Among these, pharmacodynamic analysis is the key for evaluating therapeutic response. Recently, epilepsy genes that are responsible for several rare forms of epilepsy phenotype have been uncovered (Table 1) [4-5]. It has
Table 1. A list of epilepsy genes and epilepsy phenotypes

<table>
<thead>
<tr>
<th>Epilepsy gene</th>
<th>Epilepsy phenotypes[^1]</th>
<th>Epilepsy gene</th>
<th>Epilepsy phenotypes[^1]</th>
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<td>MASS1</td>
<td>FS</td>
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<td>GABRG2</td>
<td>FS, CAE, ADEFS⁺</td>
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ADNFLE: Autosomal dominant nocturnal frontal lobe epilepsy; BFNC: Benign familial neonatal convulsion; JME: Juvenile myoclonic epilepsy; CAE: Child absence epilepsy; ADEFS⁺: Autosomal dominant epilepsy with febrile seizure plus; SMEI: severe myoclonic epilepsy of infancy; BFNIS: benign familial neonatal-infantile seizures; FS: febrile seizure; IGE: Idiopathic generalized epilepsy; IS: infantile spasms; ICEGTC: intractable childhood epilepsy with generalized tonic-clonic seizures; SMEB: severe myoclonic epilepsy in infancy borderline; CPE: Cryptogenic partial epilepsy; GEPSE: Generalized epilepsy Praxis-induced seizures Episodic ataxia; ADPEAF: Autosomal Dominant Partial Epilepsy with Auditory Features
been also reported that polymorphisms of AED targets in the brain may affect drug response and polymorphisms in the HLA region may be implicated in the occurrence of severe skin reactions [6-7]. Mechanisms of action and pharmacokinetic aspects of AEDs have been extensively studied in the past two decades [4].

2-2 Epilepsy genes

While several epilepsy genes with different mutations have been identified to date (Table 1), these account only for less than 1% of all epilepsy cases, with the exception of severe myoclonic epilepsy in infancy (SMEI), benign familial neonatal convulsions (BFNC), juvenile myoclonic epilepsy (JME), and autosomal dominant epilepsy with febrile seizure plus (ADEFs+; previously termed generalized epilepsy febrile seizure plus). The alpha-subunit of Na+ channel (SCN1A) mutations account for more than 60% of SMEI [8] and 10% of ADEFs+ [9], while K+ channel (KCNQ2, KCNQ3) mutations account for approximately 60% of BFNC [10], and myoclonin1/EFHC1 mutations account for 3-9% of JME [11]. The genes responsible for the common phenotypes of epilepsies are yet to be identified, although novel susceptibility loci associated with febrile seizure (FS), ADEFs+ and SMEI have been found [12]. New technologies such as multiplex ligation-dependent probe amplification (MLPA) [8] and array-based comparative genomic hybridization [5] may uncover new genes, as demonstrated by the identification of a de novo microdeletion and heterozygous missense mutations in the gene coding syntaxin binding protein 1 (STXBPI) in a girl with early infantile epileptic encephalopathy and suppression-burst, also known as Ohtahara syndrome [5]. The question arises as to why mutations in the same gene show different responses to AED treatment. For example, for the SCN1A gene, the differences can range from no need of AED treatment in patients with FS to high drug resistance in those with SMEI. These differences may in part be explained by the locations of the mutations in the gene coding SCN1A [13] and the physicochemical properties of amino acid residues [14], but other factors such as the role of the modifier genes may also be implicated. However, details of patients who may be haplotype carriers, either for the same epilepsy gene, or the level of significance of each (Table 1) cannot be determined at present. To better understand the genetic basis of epilepsy phenotypes showing complex inheritance pattern and genetic heterogeneity, genetic polymorphisms offer a convenient avenue. The Epilepsy Genetics Consortium in Europe examined the common variations among 279 prime candidate genes in 2721 cases and 1118 control samples to search for genetic susceptibility loci in sporadic epilepsy syndrome. They found that seizure types did not identify clear common genetic risk factors that contribute to selected epilepsy sub-phenotypes across multiple populations, and they were not able to identify risk factors for the general all-epilepsy phenotype. However, they found that numerous single nucleotide polymorphisms (SNPs)
contributed to disease predisposition in a population-specific manner, and that variations in KCNAB1, GABRR2, KCNMB4, SYN2, and ALDH5A were significant and warranted further study [15]. SNPs in ion channel-related genes in generalized epilepsy, which encode ubiquitous enzymes have been comprehensively reviewed elsewhere [16].

Better understanding of the genetic and molecular basis of epilepsies has raised hopes for finding effective preventions and cures, and improvements in pharmacotherapy along with individualized medicine hold great promise in this regard. Readers are referred to the reviews by Lucarini et al. [16], Heron et al. [17], and Dube et al. [18] that address the role of genetics in epilepsy and the molecular targets reported thus far.

3. Mechanisms of action of AEDs on ion channels and receptors

AEDs exhibit a number of pharmacological effects on molecular targets (Table 2) [4], but the mechanisms of action that account for their antiepileptic properties are not fully understood. In Table 2, three pluses indicate well-documented action believed to account for a major part of the drug’s antiepileptic action; two pluses indicate effect probably of clinical significance; and one plus indicates effect only tentatively characterized or seen only at supra-therapeutic AED concentrations. Currently available AEDs predominantly target voltage-gated cation channels, or influence GABA-mediated inhibition, glutamate-mediated excitation or calcium ion channels. Voltage-gated Na⁺ channels are essential for

<table>
<thead>
<tr>
<th>AED</th>
<th>Na⁺ channel blockade</th>
<th>T-type Ca²⁺ channel blockade</th>
<th>Non-T-type Ca²⁺ channel blockade</th>
<th>K⁺ channel</th>
<th>GABA mimics</th>
<th>Anti-glutamate</th>
<th>Muscarine release</th>
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1) L-type Ca²⁺ channel (α2δ subunit); 2) N-type Ca²⁺ channel; 3) P/Q-type Ca²⁺ channel; 4) L-type Ca²⁺ channel; 5) R-type Ca²⁺ channel; 6) GABAA receptor allosteric modulator; 7) increase brain GABA turnover, GABA excitatory; 8) GABA uptake inhibitor; 9) GABA-T block; 10) AMPA/kainate receptor; 11) NMDA receptor

+++ : possible major effects observed at therapeutic levels; ++ : effects observed at therapeutic levels; + : effects observed at supratherapeutic levels
action potentials, and their mutations form the substrate for ADEFS+ and benign familial neonatal-infantile seizures (BFNIS). Carbamazepine (CBZ), oxcarbazepine (OXC), phenytoin (PHT), lamotrigine (LTG), and felbamate (FBM) limit sustained repetitive firing through acting on the voltage-sensitive Na⁺ channels, and other AEDs such as topiramate (TPM), zonisamide (ZNS), and valproate (VPA) have a similar effect on Na⁺ channels [19-20].

Voltage-gated K⁺ channels are essential in the repolarization and hyperpolarization that follow paroxysmal depolarization shifts (PDSs), and their mutations form the substrate for BFNC and episodic ataxia type 1. Retigabine acts by opening KCNQ2/KCNQ3 potassium channels [21-22], which mediate the M-type potassium current [23], leading to neuronal hyperpolarization without affecting the cardiac KCNQ1 channel [24-25]. TPM may also exert an action at the K⁺ channels [26]. Voltage-gated Ca²⁺ channels are involved in neurotransmitter release in the sustained depolarization phase of PDSs and in the generation of absence seizures. Their mutations may be a substrate for JME. Ethosuximide (ESM) inhibits thalamic T-type Ca²⁺ channels [27]. By preventing Ca²⁺-dependent depolarization of thalamocortical neurons, ESM is thought to block the synchronized firing associated with spike-wave discharges. ZNS and VPA also reduce voltage-dependent T-type calcium currents in cultured neurons and primary afferent neurons, respectively [28-29]. Gabapentin (GBP) binds to the two regulatory subunits of the voltage-sensitive Ca²⁺ channel [30]. FBM inhibits dihydropyridine-sensitive, high-threshold, voltage-sensitive Ca²⁺ currents [31]. LTG also inhibits dose-dependent high-voltage activation of Ca²⁺ currents, possibly through inhibition of presynaptic N and P/Q-type Ca²⁺ channels [31-32]. Levetiracetam (LEV) modulates high-voltage activated calcium current [33], and has been found to be a selective blocker of N-type Ca²⁺ channels [34]. This compound also promotes inhibitory neurotransmission by reducing the negative allosteric effects of zinc and betacarbolines on GABA_A and glycine receptors [35].

Voltage-gated Cl⁻ channels are implicated in GABA_A transmission, and mutations in these channels have been found in some families with JME, in epilepsy with grand mal seizures on awakening (EGMA), and juvenile absence epilepsy (JAE). The Cl⁻ ionophore of the GABA_A receptor is responsible for the rapid post-PDS hyperpolarisation and has been involved in epileptogenesis in both animals and humans. Mutations in these receptors have been found in families with JME and ADEFS+. Enhancement of GABA_A inhibitory transmission is the primary mechanism of benzodiazepines (BZDs) and phenobarbital (PB). Barbiturates act mainly by increasing the mean open duration of Na⁺ channels without affecting channel conductance or opening frequency, whereas the binding of a BZD to its allosterically coupled GABA_A binding sites increases opening frequency without affecting open or burst duration [36-37]. TPM, tiagabine (TGB) [38], and vigabatrin (VGB) [39-40] enhance GABAergic transmission by dif-
ferent mechanisms. Since TPM modulates GABA-evoked currents in cortical neurons by a nonbenzodiazepine mechanism [41], it may be effective in cases of FS evolving to childhood absence epilepsy (CAE) or FS requiring AED treatment, in which BZP binding sites are dysfunctional due to mutations of the alpha 1 or delta 2 subunit of the GABA receptor gene [42].

Ionotropic glutamate receptors are implicated in the sustained depolarization phase of PDS and in epileptogenesis. FBM, PB, and TPM block these receptors [43-45]. FBM reduces NMDA receptor-modulated cationic conductance [46-47]. LTG is twice as effective as GABA in inhibiting the release of glutamate [48].

Mutations in the nAChR are the substrates for nocturnal frontal lobe epilepsy (NFLE). CBZ, ZNS, and BDZ are efficacious for controlling the seizures of NFLE and autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE). A therapeutic dose of CBZ was found to enhance acetylcholine release and synthesis in rat striatum and hippocampus [49].

Some AEDs such as CBZ, ZNS [50-51], VPA [50, 52], GBP [53], and TPM [54] increase monoamine (MA) release within therapeutic concentrations, which may explain their neuroprotective action, but the degree to which they contribute to antiepileptic action is yet to be clarified. The binding site of LEV is highly homologous to synaptic vesicle protein 2A [55] which is thought to contribute to docking and release of neurotransmitters, suggesting that LEV modifies neurotransmitter release. Understanding of the roles of ion channels and receptors in the context of the mechanisms of action of AEDs is essential for the development of individualized treatment regimens for patients with specific mutations [56-57].

4. Selection of AEDs based on genetic information

The selection of an AED is bound to be far more effective when it is based on the mutation identified in epilepsy genes in conjunction with the clinical phenotypes. Drug resistance has been found in most mutations of SCN1A (with the exception of del2528G) and SCN2A identified in SMEI, SMEI-borderline (SMEB), severe idiopathic generalized epilepsy of infancy (SIGEI), infantile spasms (IS), intractable childhood epilepsy with generalized tonic-clonic seizures (ICEGTC), and generalized tonic-clonic seizures (GTCS) associated with mesial temporal sclerosis (MTS). Na+ channels and GABA_A receptors are major targets of PHT, CBZ, LTG, VPA, BDZs, and PB. All mutations of SCN1A and SCN2A identified in patients with SMEI are highly drug resistant to all AEDs except one. The identification of such exception will be the basis for individualized medicine. The combination of clobazam (CLB) and stiripentol (CYP 3A4, 1A2, 2C9, 2C19, and 2D6 inhibitor) has been proven to be effective in patients with SMEI [58], possibly because of the enhanced action of the active metabolite nor-CLB. In general, patients with KCNQ mutations respond to AEDs, but in a rare case of mutation in KCNQ2 (K526N), drug resistance
has been reported, which might be influenced by acquired factors [59].

Various voltage-gated Ca\(^{2+}\) channel subunits and auxiliary proteins are targets of ESM, VPA, LTG, ZNS, LEV, CBZ, OXC, GBP, and TPM, which are AEDs selected for patients with mutations in the Ca\(^{2+}\) channels. Since barbiturates, BDZs, VPA, GBP, TGB, TPM, and VGB show GABA-mimetic actions, these AEDs can be prescribed for idiopathic generalized epilepsy (IGE), JME, and CAE. However, R43Q mutation in the gene coding GABRG2 results in disturbance of BDZ binding sites of the GABA receptor, leading to insensitivity to BZDs [42, 60-63]. In such case, TPM may be selected for treatment because TPM enhances GABA-evoked currents in clonazepam-insensitive cortical neurons, indicating that GABA\(_A\)-receptor sensitivity to TPM is not dependent on the presence of BZD sensitivity [41]. Three missense mutations (R43Q, K289M, and R139G) and one truncation mutation (Q351X) of GABRG2 were identified in patients with FS evolving to CAE, FS or FS plus, and SMEI, respectively [42, 60-63]. One missense mutation (A322D) of GABRA1 was reported to be associated with JME [60]. For patients with K289M, R139G or A322D, VPA or AEDs with GABA\(_A\)-mimetic activity should be selected. Patients with Q351X are drug resistant, and stiripentol may be useful (see above). AEDs that act as GABA agonists, but not BZDs, are recommended for patients with R34Q.

In general, CBZ, BZD, and ZNS are options for patients with CHRNA4 or CHRN2 mutations, but CBZ is preferred in most cases because ADNFLE mutations are sensitive to CBZ [64]. However, in patients with S284F mutation in CHRNA4, which was recently identified in ADNFLE and isolated cases of NFLE, ZNS was more effective than CBZ [64]. Patients with I279N mutation in CHRNA2 are reported to be drug resistant [65]. Most often, patients with FS do not require AED treatment, but in extreme cases—possibly due to frequent seizures with high fever caused by various etiologies—barbiturates or BDZs are selected, except in patients with R43Q mutation lacking BDZ binding sites (see above).

In addition to mutations in epilepsy genes, polymorphisms of SCN1A such as IVS5-91 associated with molecular targets in the brain are likely to be involved in drug response. AA genotype of SCN1A IVS5-91 was associated with patients who were prescribed higher maximum doses of CBZ and PHT than those with GG genotype [66]. Likewise, a significant association between AA genotype and CBZ-resistance has been confirmed in Japanese patients with epilepsy [67]. The C121W SCN1B mutation identified in patients with ADEFS+ may change voltage-dependent gating but does not directly affect drug receptors, suggesting that a sodium channel mutation responsible for epilepsy can also alter channel response to AEDs [68]. In a study using human hippocampal slices, a loss of Na\(^+\) channel drug sensitivity to CBZ has been reported to contribute to drug resistance in epilepsy treatment, indicating the importance of this channel in pharmacoresistance [69]. These find-
ings are interesting, but needs confirmation.

5. Prediction of unwanted effect

Some AEDs including CBZ often result in cutaneous adverse drug reactions such as Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). Chung et al. [6] reported that SJS/TEN caused by CBZ was strongly associated with HLA-B*1502 in Han Chinese. They further studied the implication of this polymorphism for cutaneous adverse drug reactions including maculopapular eruption (MPE) and hypersensitivity syndrome (HSS), and found that HLA-B*1502 was not associated with MRE or HSS. However, MPE was associated with SNPs in the HLA-E region and a nearby allele HLA-A+3101, and HSS with SNPs in the motilin gene located in major histocompatibility complex class II. These evidences strongly suggest the importance of HLA genotyping.

However, replication of the Chung et al. study in Europe found that in 12 patients with CBZ-induced SJS, only 4 patients had the HLA-B*1502 allele. These 4 patients had an Asian ancestry [70]. Another study in Europe reported that HLA-B*1502 was not a marker for all forms of CBZ-induced hypersensitivity in Caucasians [71]. Recently, Ikeda et al.[7] suggested that HLA-B*5901 was one of the candidate markers for CBZ-induced SJS in Japanese. These results suggest that the HLA region, which may contain genes relevant to SJS, is not a universal marker but rather is linked to ethnicity. Therefore, further investigation is required to delineate the exact role of the HLA region in adverse reactions.

6. Relevance of CYP and transporter genotypes to optimizing dosage

Our understanding of the mechanisms of AED concentrations at target sites in the brain has progressed greatly in the last two decades. Analysis of phenotype–genotype relationships that regulate drug concentration at target sites provides crucial data for the creation of personalized medicine. CYP is the collective term for a superfamily of heme-containing membrane proteins responsible for the metabolism of approximately 70–80% of clinically used drugs. The multidrug resistance protein (MDR1) gene product P-glycoprotein (Pgp), or ATP-binding cassette (ABC) transporter ABCB1, is an ATP-driven efflux pump contributing to the pharmacokinetics of drugs that are Pgp substrates. This mechanism has also been studied extensively. Table 3 summarizes the commonly used AEDs [4] which are substrates for various CYPs and transporters. Among these, CYP3A4/5, CYP2C9, CYP2C19 and MDR1 are most relevant to AED pharmacokinetics, and to a less extent, pharmacodynamics.

6-1. AED-related CYPs

Most AEDs, except GBP [72] and LEV [73], are metabolized by at least one CYP enzyme. TPM is a partial substrate for CYP2C19, and 60% of TPM is excreted in urine. Likewise, TGB, VGB, and ZNS are also partially excreted in urine [74]. Analysis of CYP2C9, CYP2C19 and CYP3A4/5 variants is most relevant for the understanding of AED metabolism.
CYP2C9 and CYP2C19 variants affect the hydroxylation capacity of PHT, and mutations of these genes reduce the dose of PHT needed to achieve the therapeutic drug concentration range, and this has been observed in a wide range of ethnic populations studied [4]. Compared to CYP2C19, CYP2C9 variants have greater impact on PHT metabolism [4]. Patients with two poor metabolizer alleles of CYP2C19*2 or *3 or a combination of the two showed reduced clearance of PB compared with those without mutation [75]. The total clearance of PB decreased by 48% in patients with CYP2C9*1*3 genotype compared with those with CYP2C9*1*1 genotype; however, no effect of CYP2C19 was reported [76]. VPA and CBZ metabolisms are influenced by CYP2C9 mutations [77]. VPA competitively inhibits CYP2C9, and inhibits CYP2C19 and CYP3A4 activities slightly [78]. CYP2C9*1 is the predominant catalyst in the formation of 4-ene-VPA (a toxic metabolite of VPA), 4-OH-VPA, and 5-OH-VPA. CYP2A6 contributes partially to 3-OH-VPA formation [79]. CBZ and OXC are inducers of drug metabolism via CYP3A4. The inductive effect of CBZ is about 46% higher than that of OXC [80], and both compounds have clinical significance. The clearance of ZNS is affected by CYP2C19 genotypes, and was lower in heterozygous extensive metabolizers (EMs) and poor metabolizers (PMs) by 16% and 30%, respectively. A combination of enzyme-inducing AEDs such as CBZ, PHT and PB

Table 3. Anti-epileptic drug substrates for presumed cytochrome P450s and drug efflux transporters.

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<tr>
<th>AEDs</th>
<th>CYPs</th>
<th>Drug efflux transporter genes</th>
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<td>Carbamazepine</td>
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<tr>
<td>Valproate</td>
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<td>Gabaertin</td>
<td>-</td>
<td>MDR1, LNAA</td>
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</tr>
<tr>
<td>Phenytoin</td>
<td>3A4, 2C8, 2C9, 2C10, 2C19</td>
<td>MDR1, MRP2, RLIP76</td>
</tr>
<tr>
<td>Topiramate</td>
<td>2C19</td>
<td>MDR1</td>
</tr>
<tr>
<td>Levetiracetam</td>
<td>-</td>
<td>MDR1</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Zonisamide</td>
<td>3A4, 2D6</td>
<td>-</td>
</tr>
<tr>
<td>Diazepam</td>
<td>3A4, 2C19, 1A2</td>
<td>-</td>
</tr>
<tr>
<td>Clobazam</td>
<td>3A4</td>
<td>-</td>
</tr>
<tr>
<td>Felbamate</td>
<td>2C19</td>
<td>-</td>
</tr>
<tr>
<td>Mephitobarbital</td>
<td>2C19</td>
<td>-</td>
</tr>
<tr>
<td>Oxcarbazepine</td>
<td>3A4, 2C19</td>
<td>-</td>
</tr>
<tr>
<td>N-desmethyl clobazam</td>
<td>2C19</td>
<td>-</td>
</tr>
</tbody>
</table>
increased the clearance of ZNS by 24–29% [81].

*N*-desmethyl-clobazam (N-CLB), the major metabolite of CLB with long half-life, exerts great influence on therapeutic and adverse effects of CLB. The degree of elevation in serum N-CLB/CLB concentration ratio is dependent on the number of mutated alleles of CYP2C19 (gene-dose effect). The ratio in patients with two mutated alleles was more than six-fold higher than that in patients with wild-type alleles [82]. Although the development of tolerance was not affected by genotype, the responder rate was greater in PMs and heterozygous EMs than in homozygous EMs, with a gene-dose effect [83]. Thus, gene testing of CYP2C19 is a valuable parameter for screening patients at risk of adverse effects and for predicting the effects of drugs at various dosages. This is particularly important in Asian populations with a high mutant allele frequency. The overall effect of CYP3A4 inducer on N-CLB metabolism is small, indicating that the CYP2C19 genotype is the major determinant of N-CLB concentration.

CBZ, PB, and PHT are inducers of CYP3A (Table 3) and are in turn metabolized by the induced CYP3A enzymes. This feedback regulation influences drug response. CYP3A induction affects the efficacy of co-administered AEDs and inhibition, for instance by ingestion of pomegranate juice, may cause unwanted effects from the CYP3A substrate CBZ [84].

The mechanism(s) regulating hepatic CYP3A expressions may be different among races because the level of CYP3A4 mRNA did not correlate with the level of CYP3A5 mRNA in the liver of Japanese subjects, in contrast to the co-regulatory expression of CYP3A5 and CYP3A4 in the liver of Caucasians [85-86]. The carriers of *CYP3A4*1B and *CYP3A5*3 haplotype had a 46% higher clearance of midazolam than non-carriers in Caucasian patients with cancer [87]. Multigene haplotypes may also be responsible for the effects of *CYP3A5*3 genotypes on the pharmacokinetics of CYP3A substrates. However, the *CYP3A4*1B allele is scarce or nil in Japanese [88].

Rifampin, a potent inducer of CYP3A, increased the oral clearance of midazolam, a substrate of CYP3A, by 50% in the *CYP3A5*3/*3 genotype than in the *CYP3A5*1/*1 genotype in Caucasians and African-Americans [11]. CYP3A expression may thus be induced more extensively in the *CYP3A5*3/*3 genotype than in the *CYP3A5*1/*1 genotype.

After identification of the genes that code for CYPs or other enzymes responsible for metabolism, the genetic variation that distinguishes the poor metabolizer from the rapid metabolizer will become a significant factor in adjusting AED dose to a level taking into account the predicted metabolism. Drug monitoring may be especially useful in personalizing pharmacotherapy for the intermediate metabolizers (IM), since it is unclear how the IM phenotype affects drug and dose selection.

6-2. Ethnic differences in frequency of CYP variants
The data accumulated to date clearly im-
plicates ethnicity as a factor that influences drug response and disease state. Myrand et al. [88] reported that the mean metabolic ratios (MRs) for Japanese, Korean, Chinese, and Caucasian populations were similar, except for a lower activity of CYP2D6 in Caucasians and CYP2C19 in Asians. Their study did not find any subjects with CYP3A4*1B in Asian populations, but did find CYP3A4*1B in Caucasian populations. This was a comprehensive and informative study, but the major setback was that the study population was small (82 to 143). Further studies with a larger number of subjects are needed to identify the precise frequency of genetic variance and metabolic profile [80-89].

Regarding CYP3A5, *1B, *1C, or *6 were found in Caucasians, but not in Asians, and unreported variants of CYP3A4/5 may exist (unpublished data). Differences in the hepatic expression levels of CYP3A4, CYP3A5 and CYP3A7 mRNA were observed in Japanese and Caucasian subjects. Moreover, these levels were higher in the Japanese population compared to Caucasians. The levels of CYP3A4 mRNA in the livers of Caucasians did not correlate with CYP3A5 mRNA levels in the liver of Japanese, in contrast to co-regulatory expression of CYP3A4, CYP3A5 and CYP3A7 in the liver of Caucasians, indicating the existence of ethnic differences in the expression levels of liver CYP3A mRNAs between Japanese and Caucasians adults. It is possible that the mechanism(s) regulating hepatic CYP3A expression may be different between these ethnic groups [90]. The significance of CYP3A5 coding variants has been reported, but they occur at relatively low allelic frequencies and their functional significance has not yet been established.

The allele frequency of CYP2C9 *2 and that of CYP2C9*3 are higher in Caucasians than in Asians. Conversely, the allele frequencies of CYP2C19*2 and CYP2C19*3 are high in Asian populations than in Caucasians [4].

Recently, genotyping of CYPs and transporters has been conducted to determine whether there is a difference in frequency among Japanese people (n = 1002) sampled from four different regions in Japan. No substantial difference in the frequency of genotypes was found that would justify a nationwide collection of samples. The frequencies of CYP alleles conferring homozygote PM status in Japanese are less than 1% (*2) and 2–5% (*3) for CYP2C9, 32% (*2) and 5–10% (*3) for 2C19, and 75.6% for CYP3A4/5*3 [88]. Such prospective studies involving a large number of subjects and genotype–phenotype analysis in each ethnic group would establish a firm basis for the development of individualized medicine.

Identification of the genotypes of patients prior to AED treatment can prevent adverse effects of AEDs such as SJS [6, 7] and shorten titration period. Unfortunately, no large-scale clinical association studies have been conducted that can provide relevant data, and only a few reports are available on the newer AEDs. Most published reports on epilepsy have limited usefulness for epilepsy, due to the lack of information about the concomitant drugs used by the subjects, co-
morbidities, ethnic origin, medications and seizure phenotype, as well as inadequate information on the doses of AEDs.

6-3 Population pharmacokinetic model of AEDs

Although the impact of CYP genotype on AED pharmacokinetics based on genetic make-up in individual patient has not yet been fully evaluated, prediction of preferable doses for some AEDs has become possible.

(1) Phenobarbital

PB is eliminated by a combination of renal excretion of unchanged drug (25%), N-glucoside formation (25%) and CYP2C9- and/or CYP2C19-dependent oxidation (≤25%) [91, 92]. Nakagawa et al. [76, 93] evaluated the influences of CYP2C9 and CYP2C19 genotypes on the pharmacokinetics of PB in 79 Japanese epileptic patients and reported the final model of apparent clearance of PB as follows:

\[
CL = 0.23 \times \left(\frac{\text{body weight}}{40}\right)^{0.21} \times 0.53^{\text{CYP2C9 hetero EM}} \times 0.68^{\text{VPA}} \times 0.85^{\text{PHT}} \times 0.85^{\text{SMID}} \times (1+\eta_{CL})
\]

where CL is apparent clearance of PB; CYP2C9 hetero EM = 1, CYP2C9 homo EM = 0; VPA = 1 if VPA is co-administered, otherwise 0; PHT = 1 if PHT is co-administered, otherwise 0; SMID = 1 if complications of severe or profound mental retardation with a significant behavior impairment are presented, otherwise 0; and \(\eta_{CL}\) = the independent random error distributed normally with the mean zero. In another 47 Japanese epileptic patients, the performance of the new population model was better than that of the basic model without covariates [93].

PB clearance in CYP2C9 heterozygous EMs was 48% lower than in homozygous EMs (\(P < 0.001\)). VPA, a known inhibitor of PB metabolisms, and PHT, a substrate of CYP2C9 and CYP2C19, were also incorporated into the pharmacokinetic model. Although the CYP2C19 genotypes were shown to affect the clearance of PB in Japanese patients in a previous study [75] in which carriers of the defective CYP2C9 allele(s) were excluded, CYP2C19 genotype had no effect on PB clearance (\(P > 0.05\)). Individuals with reduced CYP2C9 activity; i.e. carriers of one or two defective CYP2C9 allele(s), are more prevalent in Caucasians than in African-Americans and Asians [88, 94]. The incidence of PB-related adverse reactions was reported to be higher in Caucasians than in African-Americans and Asians [95-98]. The lower frequency of individuals with reduced CYP2C9 activity among Asians and African-Americans is a possible explanation for the higher tolerability of PB in these races than in Caucasians.

It should be noted that the number of CYP2C9 hetero EMs was small (n = 10 in total), and the effects of CYP2C9 genotypes on the pharmacokinetics of PB were model-based and therefore indirect proof. These results, however, suggest that the CYP2C9 genotypes could play an important role in the pharmacokinetics of PB in Japanese epileptic patients, and might account for the ethnic differences in tolerability of PB therapy.
(2) Zonisamide

ZNS is eliminated via renal excretion of the 2-sulfamoylacetyl-phenol (SMAP)-glucuronide (50%), unchanged form (35%) and N-acetyl zonisamide (15%) [99-100]. The formation of SMAP is catalyzed mainly by CYP3A4 and to a minor extent by CYP3A5 and CYP2C19 in an in vitro study [101]. The influence of CYP2C19 and CYP3A5 genotypes on the pharmacokinetics of ZNS was determined in 99 Japanese epileptic patients [81, 93], and the final model of apparent clearance of ZNS was as follows:

$$CL = 1.22 \times \left(\frac{\text{body weight}}{44}\right)^{0.77} \times \text{Dose}^{-0.17} \times 0.84^{\text{CYP2C19 hetero EM}} \times 0.70^{\text{CYP2C19 PM}} \times 1.24^{\text{CBZ}} \times 1.28^{\text{PHT}} \times 1.29^{\text{PB}} \times e^{\eta_{CL}}$$

where $CL$ is apparent clearance of ZNS; Dose is daily ZNS dose; $CYP2C19$ hetero EM or $CYP2C19$ PM = 1 if one or two $CYP2C19$-defective alleles, respectively, are carried, otherwise 0; $CBZ = 1$ if CBZ is co-administered, otherwise 0; $PHT = 1$ if PHT is co-administered, otherwise 0; $PB = 1$ if PB is co-administered, otherwise 0; and $\eta_{CL}$ = the independent random error distributed normally with the mean zero. The performance of the new population model was better than that of the basic model without covariates [93].

ZNS clearance in $CYP2C19$ hetero EMs and PMs was 16% and 30%, respectively, lower than in homo EMs ($P < 0.001$). A gene-dose effect was observed for the number of defective $CYP2C19$ allele(s); and CBZ, PHT and PB were also incorporated into the pharmacokinetic model. The $CYP3A5*3$ genotype did not affect the clearance of ZNS, indicating little contribution of $CYP3A5*3$ genotype to ZNS pharmacokinetics in Japanese epileptic patients.

The median concentration to dose (C/D) ratios of ZNS in $CYP2C19$ PMs tended to be higher than in homozygous EMs. The differences in the C/D ratios between the $CYP2C19$ genotypes were more pronounced in polytherapy of ZNS with CYP inducers than in ZNS monotherapy. Two of 27 PMs (7.4%) and 5 of 79 heterozygous EMs (6.3%) but none of 64 homozygous EMs (0%) developed ZNS-specific adverse reactions of fever and/or hypohidrosis.

These results suggest that the $CYP2C19$ genotypes play an important role in the pharmacokinetics of ZNS and may affect the development of some adverse reactions.

(3) Carbamazepine

CBZ is extensively metabolized in the liver, with less than 5% of an oral dose excreted unchanged in urine [102]. CBZ is predominantly metabolized to CBZ-10,11-epoxide by CYP3A, and CBZ-10,11-epoxide and CBZ-10,11-trans-diol are primary ($\leq 60\%$) metabolites in urine [103-104]. In 144 Japanese epileptic patients, the influences of $CYP3A5$ genotype on the pharmacokinetics of CBZ was determined and the final model of apparent CBZ clearance was as follows [88, 105]:

$$CL = 0.17 \times \left(\frac{\text{body weight}}{40}\right)^{0.11} \times \text{Dose}^{-0.45} \times 1.08^{\text{CYP3A5*3/*3}} \times 1.40^{\text{PHT}} \times 1.21^{\text{PB}} \times e^{\eta_{CL}}$$

where $CL$ is apparent clearance of CBZ; Dose is daily CBZ dose; $CYP3A5*3/*3 = 1$, otherwise 0; $PHT = 1$ if PHT is co-administered, otherwise 0; $PB = 1$ if PB is co-administered,
otherwise 0; and \( \eta_{cL} \) = the independent random error distributed normally with the mean zero. In another 32 Japanese epileptic patients, the performance of the new population model was better than that of the basic model without covariates [88, 105].

CBZ clearance was 8% higher in the CYP3A5*3/*3 genotype than in the CYP3A5*1/*1 or *1/*3 genotype in Japanese epileptic patients. The CYP3A5*3/*3 genotype was associated with low expression of CYP3A5 in the liver of Japanese and Caucasians [104-106]. Conversely, a large interindividual difference (approximately 50%) has been reported for the expression of CYP3A4 [107], which cannot be explained by its genetic polymorphisms [108-109]. The in vitro metabolic activity of CYP3A5 for CBZ 10,11-epoxide formation was 40-90% of CYP3A4 [110-111]. These results may imply that drugs are not preferably metabolized by CYP3A5.

CBZ clearance was 29% lower in the CYP3A5*3/*3 genotype than in the CYP3A5*1/*1 or *1/*3 genotype in Korean patients on CBZ monotherapy [26]. CBZ is known to be an inducer of CYP3A, which is the major metabolizing enzyme of CBZ itself [112]. This pharmacokinetic model included CBZ polytherapy with CYP3A inducers, PHT and PB, as well as CBZ monotherapy. The higher inducibility of CYP3A in the CYP3A5*3/*3 genotype might be a possible explanation for higher clearance among subjects with the CYP3A5*3/*3 genotype in this study than that in the Korean study.

An 8% difference in CBZ clearance indicates that CYP3A5*3 genotypes could not have an important role in the pharmacokinetics of CBZ in Japanese epileptic patients, although further studies are needed to elucidate underlying mechanism of these findings in larger and different ethnic populations.

6-4. Transporters

A number of AED efflux transporters such as Pgp and multidrug resistance-associated proteins (MRPs), have been identified (Table 3). In the brain, they are located in the apical membrane of capillary endothelial cells that form the blood–brain barrier (BBB). Pathologically elevated expression of Pgp has been found in the region of experimentally induced seizure foci and in spatial association with a number of clinical neuropathologies associated with uncontrolled seizures [113]. This was supported by Vogelgesang’s study [114], in which Pgp expression was predominantly observed in the endothelial cells of brain capillaries and in process-bearing astrocytes in dysembryoplastic neuroepithelial tumor samples, which are often associated with AED-resistant epilepsy. MRP2, MRP5, and BCRP were also over-expressed and localized in the endothelium of brain capillaries together with reactive astrocytic processes and epileptogenic tissue (tumor and peritumoral tissues).

Numerous studies have investigated the relationships between phenotypes (drug resistant or not) and genotypes (polymorphism of transporters) that regulate AED concentrations at target sites in the brain, based on the premise that the majority of AEDs are substrates for active efflux by one or more drug
transport proteins. However, there is significant disagreement in the literature as to whether all AEDs are substrates of transporters. For example, PHT and LEV are directionally transported by mouse but not by human Pgp, and CBZ is not transported by Pgp [114]. None of these three AEDs are transported by MRP2 [115]. VPA is not a substrate for Pgp, MRP1 or MRP2 in several in vivo and in vitro transport assays [116].

Japanese patients with CBZ-resistant epilepsy (n = 210) are more likely to have the T allele and the TT genotype at C3435T, and a high frequency of TT genotype at G2677T/A (odds ratio vs. the GG genotype). The frequency of the T-T-T haplotype at C1236T and C3435T is significantly higher, and the CC-GG-CC diplotype is lower in drug-resistant patients than in drug-responsive patients. None of the MDR1 influence the plasma CBZ levels [117]. These results are the inverse of previous findings in European patients with epilepsy [118-119], which suggests that the influence of MDR1 polymorphisms on Pgp activity may differ among races. However, no evidence has been presented that explains these discrepancies. Simon et al. [120] observed that low CBZ plasma levels tended to be associated with higher intestinal MDR1 expression and that CBZ dose correlated positively with MRP2 expression. MDR1 expression and CBZ and PHT dose requirement were found to be influenced by the genotype at positions 2677 and 3435 of MDR1. Based on these results, the authors suggested that the difference in intestinal MDR1 and MRP2 expression might influence CBZ and PHT disposition and account for inter-individual pharmacokinetic variability. In a study using mdr1a knockout mice, no increase in brain uptake of PB, PHT, CBZ, VGP, LTG, and GBP was observed in the knockout mice compared to wild-type mice. Among the AEDs studied, only TPM showed a twofold increase [121]. A recent study failed to find a significant association between MRP2 haplotype and drug resistance in 279 Japanese epilepsy patients, although the delGCGC haplotype of G-a774delG, C-24T, G1249A, and C3972T was over-represented among patients with mental retardation in comparison with patients who did not have this condition [122]. Despite the great efforts devoted to research, the association of MRPs and MDR1 genetic variations with clinical phenotype has not yet been elucidated. In specific experimental models, both seizures and AED treatment can induce MDR1 expression, thus amplifying the effect of MDR1 genotype on the quantity and function of Pgp [123]. Genetic variation within MDR1 affects the structure and transport function of the encoded Pgp [124], indicating not only that the transport function of Pgp depends on genotype at specific loci within MDR1, but also that differences in transport function are exaggerated when the transcription machinery is put under stress such as increased gene expression [125]. Therefore, to evaluate the impact of polymorphisms of transporters on individualized medicine for epilepsy, a definition of the degree of drug response should be established and whether the epilepsy phenotype affects the drug response has to be determined. Only
then can the following issues be resolved: (1) whether the major AEDs are substrates for Pgp or MRPs or both, (2) the relative impact of allelic variants of transporters on efflux efficacy, (3) the degree of ethnic difference in the effects of transporters on efflux efficacy, and (4) the difference between species and tissue in the expression of transporters at the BBB. Better understanding of the properties of these transporters will do much to further the development of individualized medicine.

Recently, a novel mechanism of epilepsy multidrug resistance has been described. Ral-binding protein 1 (RLIP76), a protein that catalyzes the ATP-dependent transport of glutathione conjugates, has been proposed as an alternative mechanism of drug resistance in epilepsy [126-128]. However, in a retrospective cohort study, Soranzo et al. [129] demonstrated no association between common genetic variants in the RLIP76 gene and drug resistance. Leschziner et al. [130] were also unable to find evidence supporting the suggestion that RLIP76 polymorphisms have a role in drug resistance in epilepsy. Thus, it is unlikely that common variants in RLIP76 contribute to drug response in epilepsy.

7. Tools to assist analysis of drug response

7-1 Model animals

In the context of true individualized medicine, pharmacodynamic analysis is the key to evaluate therapeutic response, and the pathomechanisms of epileptogenesis must be considered. Clinical data that allow prediction/estimation of drug response for each mutation of a responsible gene are required. However, this is almost impossible to realize since numerous unknown responsible genes and/or susceptible genes may exist. Animal models can help speed up the data collection, provided that they resemble clinical cases in terms of etiology, biochemistry, symptomatology, and treatment. An epilepsy model animal must conform to three validation criteria: face validity, construct validity, and predictive validity. Face validity is the ability to fundamentally mimic the seizure characteristics of epilepsy. Construct validity is the conformation to a theoretical rationale for epilepsy. Predictive validity is the ability to predict unknown aspects of behavior, genetics, and neurobiology of epilepsy from the model [131]. Recently, a series of transgenic animal models bearing human epilepsy genes, such as ADNFLE (S284L-TG of CHRNA4), has been generated for use as an evaluative tool for determining the therapeutic efficacy of AEDs [132]. The S284L-TG rats fulfills the criteria for animal models, and the therapeutic responses of the rats to ZNS, CBZ, and diazepam are quite similar to those of epileptic patients with this mutation; namely, S284L-TG rats respond to ZNS and diazepam, but not to CBZ [64]. The effects of mutations of epilepsy genes on response to AEDs will be evaluated more effectively by developing this kind of animal models, which when used in combination with clinical data may lead to more accurate selection of AEDs.
7-2 DNA chip for genetic diagnosis

Determination of genetic background of individual patients is crucial for the development of individualized medicine. A prototype DNA chip (based on re-sequence array) has been developed for genetic diagnosis of epilepsy, in which 14 epilepsy genes have been tiled [133]. The data determined by this DNA chips accord well with those determined by conventional technique (the accordance rate was 92 %). Improvement of this kind of testing tool will likely escalate the development of a comprehensive system leading to truly individualized medicine for epilepsy, a part of which may be used in the clinical setting [133].

8. Conclusions

Pharmacogenetic studies are yet to explain why 25–30% of patients do not respond to AEDs. There is no convincing clinical evidence that Pgp at the BBB limits the uptake of AEDs into the brains of epileptic patients and contributes to drug-resistance phenotype. The definition of drug responsiveness should take into account the current discrepancies in association studies between genetic variations in transporters or CYPs and the clinical phenotype of drug response. The pharmacokinetics and clinical outcomes for most AEDs still need to be evaluated. As most of the data currently available in the literature are based on observations of epileptic patients treated with polypharmacy without stratification by epilepsy seizure type or epilepsy syndrome, future studies should examine the phenotype–genotype relationship in monopharmacy cases and conduct stratification by epilepsy phenotype to exclude confounding factors.

The inclusion of animal data and the introduction of tools such as DNA chip for genetic testing are essential for rapid development of a system of individualized medicine. The reasons are: (1) many new drugs await introduction into the market; (2) there are profound ethnic differences in genotype frequencies of CYP and transporters and even in polymorphisms at target sites of AEDs; (3) the response to AEDs differs greatly depending on mutations in epilepsy patients and even within the same epilepsy phenotype; and (4) for better understanding of drug response, monopharmacy cases must be analyzed with a large number of subjects in each ethnic group where stratification by seizure type or epilepsy syndrome is crucial. The collaboration of research institutions would do much to make individualized medicine a reality in the treatment of epilepsy. In the coming years, new genes and susceptible genes for epilepsy will be identified at a rapid pace in keeping with the continual advances in mutation detection technologies such as the combination of genome-wide case-controlled studies using microsatellite markers and genome-wide DNA screening with high-density oligonucleotide microarrays that exclude false-positive candidate/susceptible genes. Ultimately, by combining these innovative technologies, selection of the most effective AEDs and optimal dosage for patients can be established based on the mechanisms of action of AEDs.
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