Genetic association studies in epilepsy pharmacogenomics: lessons learnt and potential applications

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Although epilepsy is one of the most common neurological disorders and genetic factors are well known to play a role in response to antiepileptic drug (AED) treatment, the study of the pharmacogenetics of epilepsy has received relatively little attention and has not resulted in clinical applications to date. Our improved understanding of the pathogenesis of epilepsy and the mechanism of action of AEDs, together with recent advances in genetics and decreasing genotyping costs, have now paved the way for a more systematic application of pharmacogenetics in the field of epilepsy. It is hoped that the resulting knowledge will lead to a more rational treatment of epilepsy, development of more efficacious AEDs, and facilitation of clinical trials of new AEDs. However, there are formidable practical, methodological and theoretical hurdles to overcome before pharmacogenomic information will have any major utility in the clinical setting. Here, we discuss the evidence for a genetic contribution to AED response, review current knowledge in epilepsy pharmacogenetics and discuss potential future avenues with their implications, both for the clinical treatment of epilepsy and new AED development.

Epilepsy is the most common serious chronic neurological disorder, affecting an estimated 42 million people worldwide [1]. There are approximately 15 antiepileptic drugs (AEDs) currently widely available for the treatment of epilepsy, and several new drugs in the pipeline. All have been shown to be efficacious in trials, but their efficacy and adverse drug reaction (ADR) profiles are generally unpredictable in an individual patient. Furthermore, it is currently impossible to predict the optimum dose for individual patients that best balances seizure control against ADRs. Most AEDs are characterized by wide dose range distributions. For example, in a recent retrospective study of 448 patients with epilepsy treated with the AED phenytoin and 533 patients treated with carbamazepine, we recorded maximum daily doses ranging from 75–900 mg and from 200–3000 mg, respectively [2]. An even more important problem is that up to a third of all patients with epilepsy are drug-refractory, despite optimal AED treatment [3]. Although selected subgroups of these refractory patients may become seizure free with surgery or other specialized treatments, many of them will have ongoing seizures.

AED efficacy, toxicity and resistance are all multifactorially determined, i.e., influenced by interactions of multiple genetic, environmental, disease-related and drug-related factors.

If it were possible to identify factors that predict AED response – in terms of efficacy and/or ADRs – then the current practice of trial and error in the treatment of epilepsy could evolve toward a more targeted, efficacious and less harmful treatment. The identification of genetic factors that predict AED response could also lead to the development of new, more efficacious AEDs and the genetic identification of potential responders/nonresponders would have important implications for the conduct of new AED trials.

In this review we discuss the evidence for the role of genetic factors in AED response, currently known associations in epilepsy pharmacogenetics, and the potential implications of current and future studies both for the clinical treatment of epilepsy and AED development.

The role of genetic factors in AED response

Genetic factors have long been known to play a role in drug response in many areas of medicine. Well-established examples, such as the N-acetyltransferase-2 (NAT2) [4,5] or thiopurine-S-methyltransferase (TPMT) [6] polymorphisms, are nearly all common and/or highly penetrant variants and thus relatively straightforward to identify. However, in reality most drug responses are influenced by interactions of multiple genes and additional environmental factors. Most drug responses can thus be considered ‘complex’ traits, comparable in this regard to most common sporadic diseases. However, some have suggested that the genetic unraveling of drug responses could be more straightforward than that of complex diseases,
Genetic factors affecting pharmacokinetics. These include genetic influence on, for instance, drug absorption, distribution, metabolism and elimination. Drug absorption is dependent both on the properties of the drug (e.g., formulation and lipid solubility) and biological properties of the individual, which are both exogenic (e.g., intake of food or concurrent medication) and endogenic. AEDs are distributed throughout the body partly by passive, concentration-driven transfer and partly through active transport mechanisms. Again, this process is influenced by drug-dependent (e.g., lipid solubility) and patient-dependent (e.g., altered protein binding) factors. Drug elimination from the body is usually through metabolism or biotransformation in the liver, followed by excretion in the kidney. Some drugs, including many of the newer AEDs, are eliminated through the kidneys without liver biotransformation. Most biotransformation occurs via hepatocyte microsomal enzymes in two phases. Phase I is usually a process of oxidation, reduction or hydroxylation, mostly carried out by enzymes of the cytochrome P450 (CYP) family. In Phase II, the resulting metabolite is conjugated, usually by glucuronidation. The resulting conjugate is then excreted.

Genetic factors influencing pharmacodynamics. Pharmacodynamics is the interaction of a drug with its target(s) at the cellular level, for example, binding to a receptor or inhibition of an enzyme. Except for levetiracetam, which acts on synaptic vesicle protein SV2A [10], all currently-licensed AEDs of which the mechanism of action is known act through one or several of the following three mechanisms: modulation of voltage-dependent ion channels (Na⁺, Ca²⁺ and K⁺), enhancement of γ-aminobutyric acid (GABA)-mediated inhibitory neurotransmission, and attenuation of excitatory (particularly glutamate-mediated) transmission [11]. The mechanism of action of some AEDs is not fully understood.

Genetic factors relating to the epilepsy itself. The type of epilepsy is an important determinant in response to treatment, with idiopathic (i.e., genetically determined) epilepsies showing a higher response rate than symptomatic and cryptogenic epilepsies (i.e., those with a defined or presumed underlying cerebral abnormality) [8]. Effective drugs in one seizure type can worsen others - seizure-exacerbation due to carbamazepine, tiagabine or gabapentin in idiopathic generalized epilepsy are examples of this, although the molecular basis is obscure. Patients with multiple seizure types and certain seizure syndromes also show a less favorable outcome [9]. The etiology of the epilepsy, the presence of comorbid brain disorder and other developmental factors also influence treatment response. Within categories, there are also undoubtedly largely unknown genetic factors affecting the inherent severity of the disease which will also affect the response to therapy.

Currently, mutations in ion channel genes have been found to be the most important in monogenic forms of epilepsy. However, the situation is complicated by the very wide phenotypic variation observed in the form of epilepsy caused by many of the currently identified causal mutations. Furthermore, similar forms of epilepsy have been shown to be associated with widely varying genetic mutations. Presumably, this reflects polygenic and environmental/developmental influences, and there is no reason to suspect that a similar variation and lack of specificity may apply in relation to the genes underpinning drug response; however, data is currently lacking on this point.

In this review, we will further consider only the first two points above (pharmacokinetics and pharmacodynamics); however, it is worth mentioning that genetic factors in the third group (relating to the epilepsy itself) will overlap with genetic factors influencing pharmacodynamics, as AEDs are acting on those mechanisms thought to be involved in the generation of seizures/epilepsy.

Furthermore, environmental factors are numerous and include, for instance, lifestyle factors, such as alcohol use and sleep deprivation, stress, noncompliance, and the presence of comorbid disease. In addition, there are factors relating to drug formulation, dose, intake with food, concurrent medication and dosing regimens which may have major influences on drug response. The mode of pharmacological action of
the drug may also be more or less appropriate for different types of epilepsy, and the appropriate choice of drug is also obviously important. Although all such factors undoubtedly have important influences on AED response, they are also beyond the topic of this review.

Genetic influences on AED pharmacokinetics & pharmacodynamics
To date, three main categories of candidate genes underlying AED response in relation to pharmacokinetics and pharmacodynamics have been the subject of study. These three categories are:

- Genes encoding drug transporters of which AEDs are known substrates
- Genes encoding drug-metabolizing enzymes (DMEs) involved in the breakdown of AEDs
- Genes encoding AED targets

Drug transporters
Functional polymorphisms in genes encoding drug transporters, of which AEDs are known substrates, can be expected to alter AED uptake, cerebral distribution or efflux, and thus result in interindividual differences in AED concentration, effectiveness and/or occurrence of ADRs. Most drug transporters show a broad substrate specificity and several AEDs are known to be transported by more than one transporter protein. Thus, one may expect that a functional polymorphism in one of the encoding genes would affect the kinetics of several AEDs, which might explain the clinical observation that patients with refractory epilepsy are usually resistant to a broad range of AEDs with different mechanisms of action [12]. Table 1 shows the currently available data on drug transporters for different AEDs.

Drug transporters are members of the extended family of membrane transport proteins, which consists of several categories, of which the superfamily of ATP-binding cassette (ABC) proteins have been most studied in relation to AED transport.

The two principal families within the ABC superfamily are the multidrug resistance proteins (MDR or ABCB) and the multidrug resistance associated proteins (MRP or ABCC). They act as active efflux pumps, transferring substances from the inside of cells to the outside [13,14]. They may pump AEDs back from the brain into the blood, and perhaps from blood into the gut, thus lowering the concentration of AEDs and contributing to AED resistance.

The MDR family consists of at least three members: MDR1 (P-glycoprotein [PGP], ABCB1), MDR3 (MDR2) and sister of P-GP (SPGP) [15]. Of these, MDR1 has been most extensively studied. MDR1 has a wide tissue distribution, including the gut and brain [101]. Recently, several groups have studied the role of MDR1 in AED responsiveness. Several AEDs are thought to be substrates for MDR1 [16] (Table 1). Resistance to AEDs is associated with increased expression of MDR1 in resected brain tissue in symptomatic epilepsies of different etiologies [17–21]. MDR3 and SPGP are almost exclusively expressed in liver [15].

The MRP family consists of at least 13 members (MRP1–13), most of which are ubiquitously expressed [101]. MRP1, -2 and -5 were demonstrated to be upregulated in the brain of drug refractory epilepsy patients [19,21,22].

A recent report showed that the extracellular brain concentration of the AED levetiracetam is not affected by inhibition of PGP or MRP1/2 [23]. This was thought to be a possible explanation for the efficacy of levetiracetam that is seen in some patients with pharmacoresistant epilepsy.

Other multidrug resistance proteins shown to be upregulated in human refractory epileptic tissue are the cisplatin resistance-associated protein (hCRA-α) [21] and major vault protein (MVP) [24,25].

In conclusion, overexpression of multidrug transporter proteins appears to be a common finding in brain tissue of patients with drug-resistant epilepsy of different origins, and this may be an important influence in determining drug resistance.

However, a too simplistic view of the role of an individual brain transporter should be avoided. Various cautions are in order. Firstly, there are many transporter systems in the brain, and it remains highly likely that other transporters, not yet identified, are also important in AED transport. For example, a recent abstract suggested that a interacting protein 1 (RLIP76), a non-ABC drug transporter, plays a major role in the
transport of phenytoin (PHT) at the blood–brain barrier [26]. To date, only efflux systems have been studied, and it is possible that drug-influx systems are also important. Furthermore, most drug transportation systems are capable of handling multiple drugs, and genetically-determined underexpression of one system may lead to compensatory changes in another system – reducing the effect of genetic variation. Secondly, the observed overexpression of drug-resistant proteins in resistant cases may not be a causal association. Seizures themselves have been shown in animal experimentation [27,28] to influence levels of Pgp (amongst their many other effects on protein expression) and it remains possible that some of the changes seen in resistant cases are simply the result of increased seizure activity. In some of these experiments, there was no Pgp upregulation following AED treatment [27,29]. Conversely, in human colon carcinoma cell lines, phenobarbital and, to a lesser extent, phenytoin were found to upregulate PGP [30]. Thirdly, human tissue has only been studied from resistant cases, and it is not known whether upregulation of drug-resistant proteins occurs in drug-responsive cases also. In a rat model of self-sustaining status epilepticus, Pgp activity was found to be greater in resistant than in responsive cases, but there are no human data on this point [31]. Finally, pharmacogenetics of drug transporters is a novel field and, at present, few data are available on functional variation in the encoding genes.

Drug-metabolizing enzymes
The metabolic pathways and specific metabolizing enzymes for most AEDs are known, and functional variants in the encoding genes are expected to result in interindividual differences in the rate of AED metabolism, again leading to differences in concentration, effectiveness and/or occurrence of ADRs.

The main candidate genes in this category are those encoding the different enzymes of the CYP superfamily. Each individual enzyme may have several different substrates and can effect several types of biotransformation, and each biotransformation can be catalyzed by more than one enzyme. Oxidative biotransformation results in the formation of metabolites that subsequently undergo renal clearance either with or without a subsequent Phase II biotransformation. Toxicity may be caused by the parent drug in some cases and by a metabolite in other cases (for example, valproate). In the former case, toxicity will be increased by overaccumulation of the drug in poor metabolizers, in the latter toxicity will occur if the balance between biotransformation and detoxication is perturbed.

There are four main enzyme families (CYP1–4), encoded by at least 25 different genes [32,33], involved in the metabolism of drugs. At least eight isoenzymes are known to be involved in the metabolism of AEDs (Table 2). The functional polymorphisms underlying alleles with variable metabolism rates are known for several of these genes [34].

Much less is known about the Phase II enzymes, which are responsible for the conjugation and detoxication of reactive metabolites, in relation to AEDs. The most important enzyme family in this category is that of the UDP-glucuronosyltransferases (UGTs), which conjugate their substrates through the addition of a glycosyl group or glucuronidation. The UGT family comprises two major subfamilies, UGT1 and UGT2 [35,36]. The UGT1 subfamily is encoded by a single gene on chromosome 2q37, which gives rise to at least 12 isoforms, the substrate specificity of each of which is thought to arise from differential splicing. The main substrates of UGT1 are amines, phenols and bilirubin. The UGT2 subfamily comprises at least eight isoenzymes, probably encoded by several independent genes on chromosome 4q13. The UGT2 family is further subdivided on the basis of tissue-specific expression patterns into the UGT2A subfamily (olfactory specific, odorant-metabolizing isoforms) and the UGT2B subfamily (liver, steroid-metabolizing isoforms). The UGT isoenzymes have few specific substrates and show wide degrees of overlapping substrate specificity.

<table>
<thead>
<tr>
<th>AED</th>
<th>Drug transporters</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBZ</td>
<td>PGP, MRP2</td>
</tr>
<tr>
<td>FBM</td>
<td>PGP</td>
</tr>
<tr>
<td>GBP</td>
<td>PGP, LNAA</td>
</tr>
<tr>
<td>LTG</td>
<td>PGP</td>
</tr>
<tr>
<td>PGB</td>
<td>LNAA</td>
</tr>
<tr>
<td>PHB</td>
<td>PGP</td>
</tr>
<tr>
<td>PHT</td>
<td>PGP, MRP2, RLIP76</td>
</tr>
<tr>
<td>TPM</td>
<td>PGP</td>
</tr>
<tr>
<td>VPA</td>
<td>MRP</td>
</tr>
</tbody>
</table>

Adapted from [16,26,83].
AED: Antiepileptic drug; CBZ: Carbamazepine; FBM: Felbamate; GBP: Gabapentin; LNAA: Large neutral amino acid transporter; LTG: Lamotrigine; MRP2: Multidrug resistance-associated protein 2; PGB: Pregabalin; PGP: P-glycoprotein; PHB: Phenobarbital; PHT: Phenytoin; RLIP76: Ra1-interacting protein 1; TPM: Topiramate; VPA: Valproic acid.
Other Phase II enzymes with a role in AED metabolism include the N-acetyltransferases (\textit{NAT1} and \textit{NAT2}) and glutathione S-transferase (\textit{GST}). However, whether genetic variation in DM Es will ever be clinically important is unclear for a number of reasons. First, although these genetic effects can influence drug plasma levels, blood level changes can be circumvented simply by changing dose. Levels of most AEDs can be readily monitored, and for most clinical purposes blood level monitoring may prove simpler than genetic profiling. Second, the functional expression of the enzyme systems is highly variable across populations and also within individuals. Induction and inhibition have a marked effect on metabolic profile, and AEDs themselves can greatly affect these processes. These processes will apply whatever the genomic composition and therefore can diminish the influence of genetic variation. Thirdly, the systems overlap and many drugs are subject to metabolism via more than one pathway, a compensatory factor which mitigates the effects of change in any one DME. Finally, for many drugs, there appears to be only a loose relationship between efficacy (or toxicity) and blood level - suggesting that pharmacodynamic factors are of importance irrespective of blood level.

### AED targets

The genetic determinants influencing drug targets have only recently become the focus of attention. Altered pharmacosensitivity of drug targets due to polymorphisms in the encoding genes may explain some of the interindividual variation in AED response.

As several first-line AEDs are known to act through binding to the sodium channel $\alpha$-subunit, genes encoding sodium channels are the most obvious candidates in this category. Other major AED targets include potassium channels.
calcium channels, GABA and glutamate receptors, GABA transporters and GABA transaminase. Levetiracetam was recently shown to act through binding to synaptic vesicle protein 2A (SV2A), suggesting a novel mechanism of action for AEDs [10].

Besides the genes encoding the actual AED target, this category also includes effector genes downstream in the pathway of AED action and target.

Others
Although relatively rare, idiosyncratic drug reactions are a well known problem with AED treatment and are important because they pose the patient at a significant, potentially life-threatening risk. The best known examples are the hypersensitivity syndrome induced by aromatic AEDs (phenytoin, phenobarbital and carbamazepine) and lamotrigine [27], and felbamate-induced aplastic anemia [38]. Although the physiological basis of idiosyncratic drug reactions is yet not entirely elucidated, it is thought that they are immune-mediated, probably involving the formation of reactive metabolites [39].

It is likely that genetic factors play a role in an individual’s predisposition to develop an idiosyncratic drug reaction. Candidate genes are those encoding the enzymes involved in the generation of toxic metabolites (mainly CYP isoenzymes), genes encoding enzymes involved in the detoxification of reactive metabolites (for instance microsomal epoxide hydrolase (mEH)), and genes encoding components of the immune system.

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**Table 3. Proposed targets of antiepileptic drugs.**

<table>
<thead>
<tr>
<th>AED</th>
<th>Main target</th>
<th>Other targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBZ</td>
<td>VG Na(^+) channels</td>
<td>NM DA, adenosine, monoamine, serotonin, Ach receptors</td>
</tr>
<tr>
<td>CLB</td>
<td>GABA(_A) (\alpha)-subunit</td>
<td>VG Na(^+) and Ca(^{2+}) channels</td>
</tr>
<tr>
<td>CZP</td>
<td>GABA(_A) (\alpha)-subunit</td>
<td>VG Na(^+) channels</td>
</tr>
<tr>
<td>ESX</td>
<td>T-type Ca(^{2+}) channel</td>
<td>GABA receptor?</td>
</tr>
<tr>
<td>FBM</td>
<td>NM DA receptors</td>
<td>VG Na(^+) and Ca(^{2+}) channels, GABA(_A) receptor?</td>
</tr>
<tr>
<td>GBP</td>
<td>?</td>
<td>GABA synthesis and metabolism?, VG Na(^+) channels? (\alpha2)-subunit of L-type Ca(^{2+}) channel? Monoamine release? Serotonin?</td>
</tr>
<tr>
<td>LTG</td>
<td>VG Na(^+) channels</td>
<td>N- and P-type Ca(^{2+}) channel?</td>
</tr>
<tr>
<td>LEV</td>
<td>SV2A</td>
<td></td>
</tr>
<tr>
<td>OXC</td>
<td>VG Na(^+) channels</td>
<td>K(^+) and Ca(^{2+}) channels</td>
</tr>
<tr>
<td>PGB</td>
<td>VG Ca(^{2+}) channel (\alpha2)-subunit</td>
<td></td>
</tr>
<tr>
<td>PHB/PRM</td>
<td>GABA(_A) receptor</td>
<td>Ca(^{2+}), Na(^+) and K(^+) channels, AMPA/kainate receptors</td>
</tr>
<tr>
<td>PHT</td>
<td>VG Na(^+) channels</td>
<td>Ca(^{2+}) channels, K(^+) channels? GABA(_A) receptor? Calmodulin? Second messenger systems?</td>
</tr>
<tr>
<td>TGB</td>
<td>GAT-1</td>
<td></td>
</tr>
<tr>
<td>TPM</td>
<td>VG Na(^+) channels</td>
<td>GABA(_A) receptor, AMPA/kainate receptors, L-type Ca(^{2+}) channel? Carbonic anhydrase</td>
</tr>
<tr>
<td>VPA</td>
<td>?</td>
<td>GABA synthesis and metabolism? Aspartate and Glu inhibition? Ca(^{2+}), Na(^+) and K(^+) channels?</td>
</tr>
<tr>
<td>VGB</td>
<td>GABAT</td>
<td></td>
</tr>
<tr>
<td>ZNS</td>
<td>VG Na(^+) channels</td>
<td>T-type Ca(^{2+}) channel, carbonic anhydrase, GABA receptor? Glutamate release? Acetylcholine release and metabolism? Dopamine accumulation?</td>
</tr>
</tbody>
</table>

Adapted from [1,84].

?: Mechanism unknown or unsure.

Ach: Acetylcholine; AMPA: \(\alpha\)-amino-5-hydroxy-3-methyl-4-isoxazole propionic acid; CBZ: Carbamazepine; CLB: Clobazam; CZP: Clobazam; ESX: Ethosuximide; FBM: Felbamate; GABA: \(\gamma\)-aminobutyric acid; GABAT: \(\gamma\)-aminobutyric acid transaminase; GBP: Gabapentin; LEV: Levetiracetam; LTG: Lamotrigine; NM DA: N-methyl \(\alpha\)-aspartate; OXC: Oxcarbazepine; PGB: Pregabalin; PHB: Phenobarbital; PHT: Phenytoin; SV2A: Synaptic vesicle protein 2A; TGB: Tiagabine; TPM: Topiramate; VG: Voltage gated; VGB: Vigabatrin; VPA: Valproic acid; ZNS: Zonisamide.
What have we learnt?
This section reviews currently known associations in epilepsy pharmacogenetics.

Drug transporters
A single nucleotide polymorphism (SNP) (C3435T) in exon 26 of the MDR1 gene is significantly correlated with expression levels and function of MDR1 in Caucasians [40]. Individuals with the TT genotype have lower levels of MDR1 expression and higher plasma concentrations of the substrate digoxin. As the polymorphism itself is silent, the true functional variant remains to be identified. Our group reported an association of the C3435T polymorphism with multidrug resistance in patients with different types of epilepsy, with an overrepresentation of the CC genotype in drug-resistant patients [41]. This is the only genetic polymorphism that has been associated with multidrug resistance in epilepsy to date.

Recently, three groups attempted to replicate the association. One group studied the association of 3-SNP MDR1 haplotypes in drug refractory patients with temporal lobe epilepsy. Although their overall analysis was negative, they reported an association of a common MDR1 haplotype with a subgroup of patients with the highest degree of pharmacoresistance [42]. The second group performed an exact replication of the original study, using approximately twice as many patients. They failed to detect any association [43]. In addition, the third group, which used slightly different AED response definitions, could not confirm the association [44]. We recently failed to find any significant correlation between the MDR1 C3435T polymorphism and dosage of phenytoin or carbamazepine in 281 and 425 patients, respectively [2]. In conclusion, the role of genetic variation in the MDR1 gene in drug refractory epilepsy remains uncertain at present.

Drug-metabolizing enzymes
Several studies have addressed the relation of genetic variants in genes encoding DMEs to AED response, mostly with regard to drug toxicity. Probably the most studied in this category is CYP2C9, which accounts for up to 90% of the metabolism of phenytoin [45]. The encoding

<table>
<thead>
<tr>
<th>Gene category</th>
<th>Gene</th>
<th>Phenotype</th>
<th>Positive associations</th>
<th>Negative associations</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transporter</td>
<td>MDR1</td>
<td>Drug refractory epilepsy</td>
<td>2*</td>
<td>2*</td>
<td>[41–44]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PHT and CBZ dose</td>
<td>0</td>
<td>1</td>
<td>[2]</td>
</tr>
<tr>
<td>DME</td>
<td>CYP2C9</td>
<td>PHT toxicity</td>
<td>2</td>
<td>1</td>
<td>[2,46,47]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PHT dose</td>
<td>2</td>
<td>0</td>
<td>[2,48]</td>
</tr>
<tr>
<td></td>
<td>mEH</td>
<td>ADRs on aromatic AEDs</td>
<td>0</td>
<td>2</td>
<td>[52,53]</td>
</tr>
<tr>
<td>Target</td>
<td>CHRNA4</td>
<td>CBZ sensitivity</td>
<td>1</td>
<td>0</td>
<td>[54]</td>
</tr>
<tr>
<td></td>
<td>SCN1B</td>
<td>PHT sensitivity</td>
<td>1</td>
<td>0</td>
<td>[55]</td>
</tr>
<tr>
<td></td>
<td>SCN1A</td>
<td>AED response</td>
<td>0</td>
<td>1</td>
<td>Depondt et al.†</td>
</tr>
<tr>
<td></td>
<td>SCN2A, 3A, 8A, 1B, 2B</td>
<td>PHT and CBZ dose</td>
<td>1</td>
<td>0</td>
<td>[2]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AED response</td>
<td>0</td>
<td>1</td>
<td>Cavalleri et al.†</td>
</tr>
<tr>
<td>Immune response</td>
<td>TNFα</td>
<td>CBZ hypersensitivity</td>
<td>1</td>
<td>0</td>
<td>[58]</td>
</tr>
<tr>
<td></td>
<td>HLA-B (*1502 allele)</td>
<td>Stevens-Johnson syndrome on CBZ</td>
<td>1</td>
<td>0</td>
<td>[59]</td>
</tr>
<tr>
<td>Other enzyme</td>
<td>DBH</td>
<td>AED response</td>
<td>0</td>
<td>1</td>
<td>[63]</td>
</tr>
</tbody>
</table>

*Different phenotype definitions.
†Manuscript in preparation.
§Single case reports.
ADR: Adverse drug reaction; AED: Antiepileptic drug; CBZ: Carbamazepine; CHRNA4: Cholinergic receptor, nicotinic, α4-subunit; CYP: Cytochrome P450; DBH: Dopamine β-hydroxylase; HLA-B: Major histocompatibility complex, class I, B; MDR1: Multidrug resistance protein 1; mEH: Microsomal epoxide hydrolase; PHT: Phenytoin; SCN: Sodium channel, neuronal, voltage-gated; TNFα: Tumor necrosis factor α.
gene has at least 12 different alleles (CYP2C9*1–CYP2C9*12) [102], with CYP2C9*1 being the wild-type allele and the others variants resulting in impaired metabolic activity or altered substrate specificity. CYP2C9 low-activity alleles are associated with decreased phenytoin clearance, and thus higher plasma levels and increased toxicity \[46,47\]. A small study identified an association between the low activity alleles CYP2C9*2 and CYP2C9*3 and lower dose requirement of phenytoin \[48\]. We recently typed the CYP2C9*2 and CYP2C9*3 alleles in 281 patients treated with phenytoin \[2\]. We identified a significant correlation \(p = 0.0066\) between the *3 allele and the maximum dose of phenytoin that patients took. Mean daily phenytoin doses for individuals with zero, one or two copies of the *3 allele were 354 mg, 309 mg and 250 mg, respectively. CYP2C9*2 did not show a significant association with dose. We could not identify any associations between *2 or *3 alleles and the presence of phenytoin ADRs.

The genes encoding the different CYP3A isoforms are clustered on chromosome 7q, with CYP3A4, 3A5 and 3A7 being the most important isoforms in adults. They metabolize several AEDs (Table 2). Although several polymorphisms in CYP3A4 are known, coding variants generally occur at low frequencies and result in limited alteration of enzyme expression and function \[49\]. The most common CYP3A4 variant is the promoter variant CYP3A4*1B. Although this variant shows marked differences in frequency amongst different populations, it has only a modest effect on enzyme activity and does not result in any significant alteration of metabolism of drug substrates \[50\]. Overall, current evidence suggests that genetic variation in CYP3A4 is not a major factor in interindividual variability in drug clearance.

The gene encoding mEH, which is responsible for detoxification of epoxide intermediates, is a candidate for variation in response to carbamazepine, phenobarbital and phenytoin. An early study proposed a correlation between a genetic defect in arone oxide detoxification and major birth defects induced by phenytoin \[51\]. However, two small studies found no correlation between mutations in the mEH encoding gene and AED toxicity \[52,53\].

No genetic association studies of genes encoding Phase II metabolism enzymes have been reported in epilepsy.

AED targets
One study compared sensitivity to valproate and carbamazepine of wild-type nicotinic acetylcholine receptors (nAchR) versus those with mutations in the nAchR \(\alpha\)-subunit gene (CHRNA4) gene causing autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) in Xenopus laevis oocytes \[54\]. The results showed that carbamazepine acts as a noncompetitive inhibitor of acetylcholine currents and that this effect was greater in mutant \(\alpha\)4β2 nicotinic acetylcholine receptor (nAchR) compared with wild-type receptors. Similarly, it was recently demonstrated that voltage-gated neuronal sodium channels expressing a mutant auxiliary \(\beta\)-subunit, encoded by the SCN1B gene and responsible for the monogenic epilepsy syndrome GEFS+ (generalized epilepsy with febrile seizures plus), display a reduced sensitivity to phenytoin \[55\]. These results suggest that mutations in an AED target can affect drug response.

We recently examined the role of genetic variation in the SCN1A gene, encoding the \(\alpha\)-subunit of the voltage-gated neuronal sodium channel, in AED response in patients with epilepsy \[56\]. As the SCN1A gene contained no known functional variants, we used a haplotype tagging approach \[57\] to represent common variation in the gene. We typed four tagging SNPs (tSNPs) in 439 patients with epilepsy and compared genotypic, allelic and haplotypic frequencies between drug refractory and drug-sensitive patients. Although several results were nominally significant, no statistically significant results remained after correction for multiple testing. The strongest association identified was that of one tSNP (rs2126152) with response to phenytoin, carbamazepine, lamotrigine and oxcarbazepine (genotypic \(p = 0.004\)). We retested this association in a second, independent patient cohort, comprising 186 patients. Although the association could not be confirmed, the genotype distribution showed a similar trend. We believe that replication of this result in a large, independent cohort is warranted.

We also related genetic variation in SCN1A to clinical dosing of phenytoin and carbamazepine, two AEDs that bind to the SCN1A product \[58\]. We analyzed the four tSNPs in 425 patients treated with carbamazepine and 281 patients treated with phenytoin \[2\]. We found that one of the tSNPs (IVS5–91 G>A, or rs3812718) is highly associated with maximum dose of both carbamazepine and phenytoin \(p = 0.0014\) and
We further demonstrated that the IVS5–91 G→A polymorphism disrupts the consensus sequence of the 5′ splice donor site of a highly conserved alternative exon (exon 5N) and that it significantly affects the proportions of the alternative transcripts in the brain of individuals with a history of epilepsy. Analysis of transcript levels in individuals without a history of epilepsy showed no such effect. These results provide the first clear evidence of a drug target polymorphism associated with the clinical use of AEDs.

We also examined the role of genetic variation in five other sodium channel genes in AED response [56]. We typed 25 tSNPs representing common variation in the genes SCN2A, SCN3A, SCN8A, SCN1B and SCN2B in our patient cohort of 439 individuals, and assessed genotypic, allelic and haplotypic association with AED response. Although several test statistics were nominally significant, none reached statistical significance after correction for multiple testing. Therefore, it seems unlikely that common variation in these genes plays a significant role in AED response.

Others

Two associations of immune response genes with severe AD Rs in patients on AEDs have been reported. An association was identified between the TNF2 allele of the tumor necrosis factor α (TNFα) gene, resulting in elevated expression of TNFα, and CBZ hypersensitivity [59]. The TNFα gene is in linkage disequilibrium (LD) with the human leukocyte antigen (HLA)-DR3 and -DQ2 genes, and the TNF-DR3-DQ2 haplotype was also shown to be associated with severe drug toxicity. An exceptionally strong association has been found between the HLA-B*1502 allele in Han Chinese patients in Taiwan who developed Stevens-Johnson syndrome on carbamazepine therapy [59]. This allele occurs in 8% of Han Chinese and in 1–2% of Caucasians and is in LD with a causative polymorphism which has not been identified. The strength of this association is such that identification of the polymorphism might allow a predictive test to be developed.

We performed an association study in 673 patients with epilepsy of a putative functional variant (-1021C→T) in the DBH gene, which encodes dopamine β-hydroxylase, the enzyme catalyzing the conversion of dopamine to norepinephrine. Norepinephrine is a potent endogenous anticonvulsant neurotransmitter [61], and DA β-hydroxylase knockout (dbh/-) mice exhibit a reduced response to certain antiepileptic treatments [62,63]. We compared genotypic and allelic frequencies of the -1021C→T variant in AED refractory and sensitive patients [64]. None of the associations reached statistical significance, suggesting that variation in the DBH gene does not contribute significantly to AED response.

The potential of genetic association studies in epilepsy pharmacogenomics

Due to improved insights in the pathogenesis of epilepsy and the mechanism of action of AEDs on the one hand, and major advances in the field of genetics on the other hand, the scope of association studies in epilepsy and epilepsy pharmacogenetics in general is changing rapidly. Major international efforts, such as the Human Genome Project [65] and the HapMap project [66], together with the availability of large numbers of SNP markers (for example [103]), have made systematic screening of almost any gene possible. Moreover, costs for high-throughput genotyping have decreased and statistical tools have improved, such that it has now become possible to study all common variation in an entire pathway, or even in the entire genome [67]. Such large-scale projects will also allow researchers to look at interactions between different variants in the same gene or variants in different genes. Strategies to look at such genetic interactions are now becoming available [68].

However, case-control association studies have methodological problems that are probably responsible for the numerous contradictory results between studies, and the importance of these problems must not be underestimated. Therefore, and in order to maintain the confidence of the clinical world, it is essential to respect the quality criteria that have been issued for association studies [69,70] (Box 1). Although retrospective pharmacogenetic studies are valuable, prospective studies are superior in assessing potential clinical significance, i.e., ideally, patients should be genotyped before or at the time they begin treatment with a specific drug, and then have their response studied over time and correlated to their genotype. Extreme care must be taken when phenotyping, as errors in attribution in only a few cases can greatly reduce the statistical sensitivity of investigation. There are currently no generally accepted definitions for AED response or refractoriness of epilepsy. Defining AD Rs may be easier, but confounding
Box 1. Quality standards for association studies in pharmacogenetics.

- Ideally, a prospective study design.
- Robust, preferably standardized phenotype definition.
- Large sample size.
- Biological plausibility of candidate gene.
- Use of appropriate statistics; reporting of all association tests conducted; stringent corrections for multiple testing.
- Careful choice of control group; check for and correction of stratification.
- Assessment of linkage disequilibrium (LD) structure in candidate region.
- Validation of association by functional assays.
- Use of appropriate statistics; reporting of all association tests conducted; stringent corrections for multiple testing.
- Biological plausibility of candidate gene.

may still arise from other factors, such as comedication or intercurrent disease. One option may be to look at extreme phenotypes, for example comparing a group of highly drug refractory patients vs a group of patients who have been in complete remission for years. However, such strict definitions will reduce patient numbers. Standardization of phenotype definitions should facilitate multicenter collaboration and could significantly improve the replicability of association results. A plausible biological role for the relevant gene increases the likelihood that an identified association is true. Ideally, the identified polymorphism should represent a functional change in physiology, although it is recognized that identification of the causal variant is often not straightforward. Associations should be validated by functional assays whenever possible. In order to avoid false positive results due to multiple testing, either with multiple markers or independent phenotypes, all tests conducted should be reported, even if nonsignificant, and stringent statistical corrections should be applied. An additional major problem is the matching of patient and control populations - population differences can be subtle, yet responsible for false positive and negative results. Reports should include a check and, if necessary, correction for population stratification. For instance, it is well known that there are substantial variations in the frequency of the different CYP alleles between populations. Stratification seems to be more important in large studies assessing variants of weak effect than in smaller studies searching for variants of modest to high effect. Ideally, all reports should include an assessment of the structure of LD surrounding the associated polymorphism, in order to delimit the associated interval, i.e., the boundaries delimiting an area of sufficiently high LD that the causal variant could reside within it. Efforts should be made to identify all variants in high LD with the associated variant within this interval. Replication of results in an independent population should be aimed for whenever possible. To date, very few research groups have attempted to replicate their own findings. Results of replication by other groups are often confounded by differences in methodology and characteristics of the population studied. The need for large sample sizes and independent replication of results will require collaboration between different centers, and perhaps between the academic world and the pharmaceutical industry.

Broadly speaking, pharmacogenetic and pharmacogenomic studies in epilepsy are expected to affect two domains: the clinical treatment of epilepsy and AED development.

Clinical practice

The ultimate goal is to replace the current practice of trial and error with a more rationalized and perhaps personalized treatment. Nowadays, clinicians wanting to initiate AED treatment for a patient take into account factors, such as epilepsy type, concomitant disease and comedication when choosing between the approximately 15 different AEDs currently available. Other factors influencing their choice are restrictions by local regulatory instances, such as reimbursement of newer AEDs. Guided by these variables, they will choose the AED that they think is most suitable for the patient, i.e., the AED with the highest chance of rendering the patient seizure free while causing the least possible ADRs. The optimal dose of an AED is determined in the same empirical way, judging by seizure frequency and the occurrence of ADRs. In approximately 50% of patients treated in this way, the first AED will be effective. In the remaining cases, the clinician will either replace the AED or add a second AED, of which the choice will be made along the same lines. At present, genetic factors play no role whatsoever in the choice of AED treatment.

However, one could imagine that the clinician could avail of a set of genetic tests to aid his choice of AED. These tests could, for instance, include genotyping of a few polymorphisms each in one or more drug transporter genes, DMEs, AED target genes and immune-related genes. The outcome of these tests could then be converted into an individualized ranking order of AEDs. Additionally, the results could help predict which dose should be aimed for to control seizures without causing ADRs, and perhaps how quickly the dose can be increased. Furthermore,
The multifactorial nature of drug response is significant, and the clinical effect of any genetic variation may be small. Factors such as environment, lifestyle, disease characteristics, and drug formulation can override the importance of individual genetic variants. Pharmacogenetics may aid in drug development by identifying new drug targets and improving the efficiency of existing drugs.

Box 2. Factors which reduce clinical utility of knowledge of pharmacogenetic variation in any individual patient.

- Polygenic nature of drug response (important, especially if individual effect is small).
- Environment and lifestyle (e.g., alcohol, stress, sleep deprivation and compliance).
- Disease (e.g., severity, seizure type, etiology, comorbidity and degree of brain damage).
- Drug (e.g., dosing regimen, drug formulation, food intake, comedication and mode of action) - variable protein/enzyme levels due to enzyme induction/upregulation/gene expression over time due to medication (e.g., drug-metabolizing enzymes, transporters and receptors).
- Variable protein expression over time due to seizures and underlying disease processes.

These factors are likely to override the importance of any individual single nucleotide polymorphism/mutation in any gene involved in drug response, unless the clinical effect of this variation is large in magnitude.

If genetic testing could help predict which patients are more likely to be AED refractory, the delay to surgical treatment or other second-line treatment could be shortened. Ultimately, all these factors should significantly improve quality of life for epilepsy patients.

Experience from pharmacogenetics in other domains has taught us that translating laboratory findings to clinical practice is usually a slow process. For example, the many genetic variants currently known to influence drug response, only a limited number has found more or less common application in clinical practice. Recently, the US FDA approved a DNA microarray-based test for diagnostic genotyping of CYP2D6 and CYP2C19 variants. These examples illustrate that research findings are slowly starting to influence clinical practice.

Any genetic test battery should have sufficient predictive value to be clinically useful. As most AEDs have several mechanisms of action and AED response is likely to be a complex, rather than a monogenic trait, a genetic test battery for AED treatment would probably need to include several genes. Furthermore, ideally the functional variant(s) underlying the association should be known, rather than a (set of) SNP(s) in LD with an unidentified causal variant.

At a more fundamental level, the multifactorial nature of drug response may prove too great a hurdle for the knowledge of any individual genetic variation to be of any wide clinical utility in epilepsy treatment. Listed in Box 2 are some of the factors that could diminish the importance of specific genetic variation. Currently, the effect of genetic variation in any one factor has been small, especially in relation to the wide population variation - and too small to be of clinical value. This is undoubtedly due to the complex interaction of environmental and genetic factors, the fact that multiple genetic factors probably play a part in determining overall drug response and the fact that gene expression is highly variable. To date, the only genetic variation that appears strong enough to warrant clinical application is the association of carbamazepine hypersensitivity with the HLA-B*1502 allele in Han Chinese patients in Taiwan.

Drug development
Pharmacogenetics may aid AED development in two ways: to identify new drug targets and as a tool during clinical trials of new AEDs.

A total of 70-80% of patients with epilepsy achieve seizure control with currently available AEDs [9]. The remaining 20-30% of patients develop drug refractory epilepsy. Despite the advent of around ten new AEDs in recent years, none has succeeded in rendering a substantial fraction of those patients seizure free [74]. This illustrates the need for novel AEDs with mechanisms of action that are different from the mechanism of action of the currently available AEDs, i.e., modulation of ion channels or CNS neurotransmission. It is hoped that pharmacogenetics will contribute to a better understanding of the molecular mechanisms underlying drug resistance, which may lead to the development of new, more efficient drugs. For example, elucidation of the role of multidrug resistance proteins in cancer has led to clinical trials with MDR inhibitors [75]. Similarly, co-administration of MDR inhibitors with AEDs could be a novel therapeutic approach in epilepsy [76,77].

Identification of drug-target polymorphisms associated with AED response could guide drug designers to develop AEDs that are more efficacious and/or produce fewer adverse events. For example, some drug designers are attempting to create compounds that will bind to a target regardless of mutations.

Another promising avenue is the use of gene expression microarrays. For instance, gene expression levels could be compared between AED-sensitive and AED-refractory patients. The products of aberrantly expressed genes are plausible targets for new AEDs, specifically aimed at the drug refractory population. This approach is illustrated by another example from oncology. Overexpression of the human epidermal growth factor receptor 2 (HER2/ERBB2) oncogene in patients with breast cancer is associated with an
unfavourable prognosis. This subgroup of patients exhibits an enhanced response to trastuzumab (Herceptin®), a monoclonal antibody against the HER2 receptor [78]. A potential obstacle to this strategy in epilepsy is the availability of human brain mRNA, especially from patients with drug-sensitive epilepsy. Other problems are the interpretation of the large amount of data and confirmation of functional relevance. Nevertheless, a small number of studies in human tissue have already hinted at some interesting gene categories, involved in apoptosis [79], gene transcription control and calcium homeostasis [80].

More recently, pharmacogenetics is also becoming a tool during trials of new drugs [71,81,82]. Several of the larger pharmaceutical companies are now systematically collecting DNA from patients participating in Phase II clinical trials.

The purpose is to identify:

- A set of genetic variants that predict response to the drug (efficacy pharmacogenetics)
- Genetic variants associated with toxicity (safety pharmacogenetics)

Those variants are then typed prospectively both during later-stage trials and as a part of post-marketing surveillance. Drug trials may thus become faster and more targeted and clinical drug use safer and more efficient. Furthermore, drugs that are efficacious, but cause severe toxicity in a relatively small subset of people, and thus would not normally obtain approval, could be rescued. An example in the field of epilepsy is felbamate, an efficacious drug that had to be withdrawn due to rare occurrences of potentially fatal aplastic anemia and hepatic failure. If a (set of) genetic polymorphism(s) could be identified that reliably predict the risk for these serious ADRs, then felbamate could be used safely in selected patients.

Expert commentary & outlook
The pharmacogenetics of epilepsy is a promising, but relatively undiscovered field. There is ample evidence that response to AEDs is influenced by genetic factors, and the first reports of genetic associations in AED response are now becoming available. Thanks to major advances in genetics, large-scale projects screening entire drug-related pathways, rather than looking at individual genes, are now becoming a reality. Other strategies, such as mRNA profiling and proteomics, will complement findings of genetic association studies. However, several methodological issues remain unresolved, such as rigorous and standardized phenotype definitions and correction for multiple comparisons. Ideally, pharmacogenetic studies should be prospectively designed. The need for large sample sizes and independent replication of results warrants collaboration between multiple centers. Moreover, due to the multifactorial influences on drug response, the impact of any single genetic variant is likely to be small; hence, looking at interactions between multiple

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**Highlights**

- Antiepileptic drug (AED) efficacy, toxicity and resistance are largely unpredictable in individual patients.
- AED efficacy, toxicity and resistance are influenced by interactions of multiple genetic, environmental, disease-related and drug-related factors. This is likely to reduce the clinical value of any single genetic variant in an individual patient.
- Thanks to improved insights in the pathogenesis of epilepsy and the mechanism of action of AEDs and major advances in the field of genetics, large-scale pharmacogenetic studies – including large numbers of patients and studying several genes at the time – are now possible.
- The main candidate gene categories in epilepsy pharmacogenetics are: genes affecting pharmacokinetics, e.g., drug transporter and drug-metabolizing enzyme-encoding genes; genes influencing pharmacodynamics, e.g., drug target-encoding genes; genetic factors relating to the epilepsy itself; and others, e.g., genes encoding immune factors implicated in idiosyncratic drug reactions.
- Established genetic associations in epilepsy pharmacogenetics include cytochrome P450 (CYP)2C9 alleles and doses and levels of the AED phenytoin; a functional polymorphism in the voltage-gated neuronal sodium channel gene SCN1A and doses of phenytoin and carbamazepine; and the human leukocyte antigen (HLA)-B*1502 allele and Stevens-Johnson syndrome on carbamazepine.
- Pharmacogenetic association studies should adhere to published quality standards with regards to methodology, in order to avoid false positive and false negative results.
- Potential implications of epilepsy pharmacogenetics include a more rational treatment of epilepsy, development of more efficacious AEDs and facilitation of clinical trials of new AEDs.
- Multicenter collaboration should facilitate epilepsy pharmacogenetic studies.
variants – and possibly exogenic factors – is likely to be more fruitful, though complicated by statistical challenges.

Epilepsy pharmacogenetic studies will probably lead to the discovery of a number of genetic variants and genes implicated in AED response and resistance. However, although there is reason for optimism, there is also need for moderation. In 2000, Allen Roses predicted that it would take “3–5 years before widespread application” of pharmacogenetics [81]. Experience from pharmacogenetics in other domains has shown that translating laboratory findings to clinical practice is often a long and slow process.

Nevertheless, it is hoped that, ultimately, advances in pharmacogenomics will lead to an improved, more efficacious and less harmful therapy for patients with epilepsy, to faster and more efficient evaluation and approval of new AEDs, and to the advent of novel AEDs, particularly targeting the approximately 33% of the epileptic population with drug refractory epilepsy.

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• Provides the first evidence for association of a drug transporter gene polymorphism with drug-refractory epilepsy.


• Good review of the current understanding of mechanisms underlying idiosyncratic drug reactions.


Genetic association studies in epilepsy pharmacogenomics - REVIEW


Websites