Pharmacogenetics in neuropsychiatric diseases : epilepsy as a model

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Abstract

Individual drug responses are the result of interactions of multiple environmental and genetic factors. Pharmacogenetics studies the influence of genetic variation on interindividual differences in drug efficacy, adverse events and dosing.

This article discusses general strategies for candidate gene selection and pharmacogenetic association studies. A summary of the major pharmacogenetic associations reported in neuropsychiatric disease is presented. The example of epilepsy pharmacogenetics will be covered in more detail, including an overview of epilepsy pharmacogenetic candidate genes and results of association studies reported so far.

With the advent of large-scale, rigorously designed association studies, it is hoped that genetic factors will be identified that will lead to a targeted, more efficacious and safer treatment, and perhaps to the development of new and more efficacious drugs for neuropsychiatric diseases.

Key words : Pharmacogenetics ; neuropsychiatric disease ; epilepsy ; gene ; association ; drug response.

Introduction

Neuropsychiatric diseases are estimated to affect 450 million people worldwide and account for almost 11 per cent of the global disease burden. The prevalence of neurodegenerative diseases in particular is likely to increase as life expectancy increases throughout the world. Most neuropsychiatric diseases are chronic and progressive. Although numerous medical treatments for neuropsychiatric diseases are available, the large majority of these are symptomatic rather than curative. Moreover, these treatments show no or insufficient effect in a substantial number of patients. Even if they work, they often do so at the cost of adverse drug reactions (ADRs). In the UK for instance, approximately 7% of all hospital patients are affected by ADRs and the annual costs of ADRs are estimated at 706 million euros (Pirmohamed *et al.* 2004).

Thus, what we need is increased efficacy and safety of existing drugs on the one hand and development of new, more efficacious and safer treatments on the other hand. Pharmacogenetics can contribute to both these needs.

Treatment efficacy and tolerability are influenced by an interaction of multiple factors, including environmental, patient-related, disease-related and genetic factors. The aim of pharmacogenetics is to identify genetic variants that contribute to individual drug responses. This may allow for prediction of efficacy and tolerability based on genotype in subgroups of patients and even in individual patients. This is the principle of "personalized or tailored therapy", which is expected to result in more efficacious, safer and ultimately cheaper medical therapy. Identification of genetic variants contributing to drug response will also improve our understanding of disease pathophysiology. This in turn may lead the development of new, more targeted and more efficacious therapies. Finally, pharmacogenetics may also enhance insight in human biology in general by unravelling the physiological processes underlying development of ADRs.

This article first discusses the major candidate gene classes in pharmacogenetics and the main strategies to study these genes. Then, a brief overview is given of the current knowledge in pharmacogenetics of neuropsychiatric diseases. Finally, the example of epilepsy pharmacogenetics is discussed in more detail.

Candidate genes for pharmacogenetic studies

Knowledge of pharmacokinetic and pharmacodynamic factors involved in drug disposition and action allows for drafting a list of candidate genes for pharmacogenetic studies.

1. GENETIC FACTORS AFFECTING PHARMACOKINETICS

Pharmacokinetic candidate genes comprise two major gene classes : those encoding drug transporters and those encoding drug metabolizing enzymes (DMEs).

Drug transporters belong to the extended family of membrane transport proteins, of which the major members are the superfamilies of the ATP-binding cassette (ABC) proteins, the ATPases and the

solute carriers (SLC). Functional polymorphisms in these genes can be expected to result in interindividual differences in the rate of drug uptake, distribution or efflux, leading to interindividual differences in drug concentration, effectiveness and/or occurrence of side effects.

The most important class of DMEs is the cytochrome P450 (CYP450) superfamily. There are four main enzyme families (CYP1-4), encoded by at least 25 different genes (Park *et al.* 1995, Patsalos *et al.* 2002), involved in the metabolism of drugs. They are responsible for most of the phase I biotransformation of drugs in the liver. 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 then undergo renal clearance either with or without a subsequent phase II biotransformation, which may consist of glucuronidation, methylation, sulfation or acetylation. Toxicity may be caused by the parent drug in some cases and by a metabolite in other cases. In the former case, toxicity will be increased by over-accumulation of the drug in poor metabolizers, in the latter toxicity will occur if the balance between biotransformation and detoxication is perturbed. The functional polymorphisms underlying alleles with variable metabolization rates are known for several of the CYP450 genes (Daly 2003). One study reported that 59% of the 27 drugs most frequently cited in ADR studies were metabolized by at least one enzyme with a reported variant allele known to cause altered drug metabolism (Phillips *et al.* 2001). It has long been acknowledged that such genetic variations may lead to interindividual differences in drug concentration, effectiveness and/or occurrence of side effects. Moreover, it is well known that there are substantial variations in the frequency of the different CYP450 alleles between populations, which may explain differences in metabolization rates among ethnic groups (Xie *et al.* 2001).

2. Genetic factors affecting pharmacodynamics

Pharmacodynamics is the interaction of a drug with its target at the cellular level, for example binding to a receptor or inhibition of an enzyme. Pharmacodynamic candidate genes include those genes encoding drug targets, as well as effector genes downstream in the pathway of drug target and action. Drug targets obviously include a very wide variety of molecules, depending on the disease they are intended to treat. The main candidate gene categories with regards to neuropsychiatric disease comprise those encoding central nervous system neurotransmitter transporters, receptors and metabolizers. Functional polymorphisms in these genes may result in structural or functional alterations of the encoded proteins, leading to altered pharmacosensitivity of the target, which in turn may explain some of the interindividual variation in AED response.

3. Genetic factors relating to the disease itself

It is well known that a patient's response to drug treatment may differ according to the underlying molecular disease pathogenesis. Thus, any gene in which mutations or variations predisposing to disease have been identified is also a potential candidate for variation in drug response. This class of candidate genes will obviously overlap with those genes encoding drug targets, as drugs usually act on those mechanisms thought to be involved in the generation of the disease they are intended to treat. Thus, for neuropsychiatric diseases, genes encoding proteins involved in neurotransmitter disposition and action are major candidates in this category. However, disease susceptibility genes that are not encoding actual drug targets can also predispose to drug response. For example, the E4 allele of the APOE gene, a risk factor for developing Alzheimer's disease, has been shown to be a predictor of poor response to the cholinesterase inhibitor tacrine in one study (Poirier *et al.* 1995), although this has been contradicted in later studies (Rigaud *et al.* 2000, Almkvist *et al.* 2001).

4. Genetic factors related to idiosyncratic drug reactions

Although relatively rare, idiosyncratic drug reactions are important because they pose the patient at a significant, potentially life-threatening risk. Although the physiological basis of idiosyncratic drug reactions is not entirely elucidated yet, it is thought that they are immune-mediated, probably involving the formation of reactive metabolites (Uetrecht 2003).

It is likely that genetic factors play a role in an individual's predisposition to develop an idiosyncratic drug reaction. Candidate genes in this category are those encoding the enzymes involved in the generation of toxic metabolites (mainly CYP450 iso-enzymes), genes encoding enzymes involved in the detoxification of reactive metabolites (for instance microsomal epoxide hydrolase or mEH), and genes encoding components of the immune system, such as HLA- and TNF-encoding genes.

5. Genes with indirect influence

It is worth mentioning that besides genetic polymorphisms resulting in a direct alteration of the encoded protein, genetic variants may also exert their functional effects indirectly, such as through transcriptional effects, differential splicing or posttranslational influences. Therefore, candidate genes

also include genes encoding for instance transcription factors, regulators, kinases, phosphatases etc.

Strategies in pharmacogenetics

1. GENETIC ASSOCIATION STUDIES

Several different approaches can be used to study the role of genes in drug response. Currently the best established approach is that of genetic association studies, in which correlations between genetic variants and phenotypical differences are assessed on a population scale, in order to identify those genetic variations contributing to the condition of interest. A pharmacogenetic association study can for instance compare a group of patients that respond to a given treatment with a group of non-responders, or a group of patients with a particular ADR with patients that do not exhibit that ADR on the same therapy.

Most association studies reported so far have looked at specific candidate genes. However, thanks to improved knowledge of the structure of the human genome, ever decreasing costs of high-throughput genotyping and development of sophisticated statistical methods, it is likely that studies including all common genetic variation in an entire pathway or even in the entire human genome will soon become available.

Genetic association studies can further be subdivided in those using a direct or sequence-based approach and those using an indirect or map-based approach (Goldstein *et al.* 2003). The former concentrate on known or putative functional variants, for instance those located in gene exons, splice sites or promoter regions. The latter use genomic patterns of linkage disequilibrium (LD) to select a set of markers that are statistically associated with many other variants in the genetic region of interest. This 'map' of markers, often called tagging single nucleotide polymorphisms (SNPs) or tSNPs, is then typed in order to economically represent genomic variation. Association studies are notorious because they often produce contradictory results. Much of this non-replicability is probably due to a multitude of methodological problems. It is beyond the purpose of this review to address these in detail, and excellent reviews on this topic have been published elsewhere (Cardon and Bell 2001, Healy 2006).

2. OTHER STRATEGIES

Direct functional assays of known mutations or polymorphisms can be used to compare drug sensitivity of encoded proteins with and without a particular genetic variant in vitro.

Another approach to study differential drug responses is to study mRNA expression profiles using micro-arrays (also called "transcriptomics").

For instance, gene expression levels could be compared between drug-responsive and drug-refractory patients, or between patients with and without ADRs. Aberrantly expressed genes could provide insight into the pathophysiology of drug resistance or development of ADRs, and the encoded proteins are plausible targets for new drugs. mRNA expression studies have already resulted in clinical applications in oncology. For example, micro-array analysis of certain tumors has lead to correlating particular expression patterns with patients' prognosis (Macgregor 2003).

Finally, proteomics, although currently rather unexplored in drug response in neuropsychiatric disease, could lead to the identification of certain protein profiles, which might be useful for predicting drug response or ADRs. A potential obstacle to both proteomics and transcriptomics is the availability of the appropriate source of sampling, namely brain tissue in the case of neuropsychiatric disease. Other potential problems are the interpretation of the large amount of data and confirmation of functional relevance. Nevertheless, it is likely that these techniques will prove a successful complementation to pharmacogenetics in the near future.

It must be noted that although retrospective studies are valuable, prospective studies are superior in assessing potential clinical significance, i.e. ideally, patients should be characterized on the basis of their DNA-, RNA- or protein signatures before or at the time they start a specific drug, and then have their response studied over time and correlated to those signatures.

Pharmacogenetics in neuropsychiatric disease

Thanks to recent major advances in genetics, there is currently a great interest in pharmacogenetics in several disease domains. Numerous pharmacogenetic associations have been published and some of these have led to clinical applications. However, despite the availability of a number of obvious candidate genes, pharmacogenetic studies in neuropsychiatric disease have not yielded any major results leading to clinical applications so far. Some positive associations have been reported and replicated in psychiatric disease, but hardly any firm pharmacogenetic associations in neurological disease have been reported so far. Moreover, as in other fields, many reported associations have been subsequently contradicted, while others await replication. With the advent of high-throughput genotyping and the collection of large patient cohorts, it is now possible to conduct large, high-quality association studies, which will hopefully lead to new insights and applications in the near future.

Table 1 gives an overview of the major pharmacogenetic associations in neuropsychiatric disease known at present.

Table 1

Major pharmacogenetic associations in neuropsychiatric disease

Phenotype	Drug	Gene	Allele	Findings	Main references
Psychiatric disease					
Schizophrenia	Atypical antipsychotics	DRD3 (dopamine D3 receptor)	Ser9Gly	Carriers of Gly show better response	(Scharfetter *et al.* 1999, Szekeres *et al.* 2004)
	Clozapine	HTR2A (serotonin receptor 2A)	T102C and His452Tyr	Carriers of C and Tyr carriers show reduced response	(Arranz *et al.* 1998)
Tardive dyskinesia (TD)	Antipsychotics	DRD3 (dopamine D3 receptor)	Ser9Gly	Carriers of Gly have increased risk of TD	(Lerer *et al.* 2002)
		HTR2A (serotonin receptor 2A)	T102C	Carriers of C or CC have increased risk of TD	(Segman *et al.* 2001)
		CYP2D6 (cytochrome P450 2D6)	Poor metabolizer alleles	Poor metabolizers have increased risk of TD	(Patsopoulos *et al.* 2005)
Depression	SSRIs	GNB3 (G protein β-polypeptide 3)	Exon 10 C825T	Association between TT homozygosity and response	(Zill *et al.* 2000)
		SLC6A4 (serotonin transporter)	Promoter ins/del	Carriers of L-allele show better response	(Smeraldi *et al.* 1998)
	Lithium, SSRIs	TPH (tryptophan hydroxylase)	A218C	Carriers of AA and AC respond worse	(Serretti *et al.* 1999, Serretti *et al.* 2001, Serretti *et al.* 2001)
Neurological disease					
Alzheimer's disease	Acetylcholinesterase inhibitors	CHAT (choline acetyltransferase)	SNP rs733722	rs733722 accounts for 6% of the variance in response	(Harold *et al.* 2006)
Parkinson's disease	Levodopa	DAT (dopamine transporter)	nine copy allele of 40–bp VNTR	Carriers have higher risk of ADRs	(Kaiser *et al.* 2003)
Narcolepsy	Modafinil	COMT (catechol-O-methyltransferase)	Low activity allele	Association with response and dose	(Dauvilliers *et al.* 2002)

SSRI = selective serotonine reuptake inhibitor, SNP = single nucleotide polymorphism, VNTR = variable number tandem repeat.

Pharmacogenetics in epilepsy

Epilepsy lends itself well to pharmacogenetic studies because of a number of reasons : it has one the highest prevalence rates amongst neuropsychiatric diseases ; there is a wide variety of individual responses to AEDs ; the outcome measure of seizure control is readily quantifiable ; validated scales to classify seizures and ADRs are available and the pathways involved in drug metabolism and action are known for most antiepileptic drugs (AEDs).

I will first give an overview of candidate genes in epilepsy pharmacogenetics and then discuss pharmacogenetic associations that have been reported so far.

1. CANDIDATE GENES IN EPILEPSY PHARMACOGENETICS

In accordance with pharmacogenetics in general, the major classes of candidate genes for pharmaco-genetic studies in epilepsy include : a) genes encoding drug transporters of which AEDs are known substrates ; b) genes encoding DMEs involved in the breakdown of AEDs ; c) genes encoding AED targets and their related pathways ; d) genes involved in the pathogenesis of epilepsy ; e) immune-related genes.

a. *Genes encoding AED transporters*

The two principal families of AED transporters are the multidrug-resistance proteins (MDR or ABCB) and the multidrug-resistance associated proteins (MRP or ABCC). They are expressed in endothelial cells of the blood-brain barrier and in choroid plexus epithelial cells of the blood-CSF barrier, where they act as active defense mechanisms, transferring substances from the inside of cells to the outside (Schinkel 1998, Seetharaman *et al.* 1998). They may pump AEDs back from brain into blood, thus limiting brain accumulation of AEDs.

Table 2

Presumed drug transporters for AEDs

AED	Drug transporters
CBZ	MDR1, MRP2
FBM	MDR1
GBP	MDR1, LNAA
LTG	MDR1
PGB	LNAA
PHB	MDR1
PHT	MDR1, MRP2
TPM	MDR1
VPA	MRP

Adapted from (Jezyk *et al.* 1999, Loscher and Potschka 2002).

MDR1 = multidrug-resistance protein 1, MRP = multidrug-resistance associated protein, LNAA = large neutral amino acid transporter.

Expression studies on resected brain tissue from patients with drug-refractory epilepsy of different aetiologies have demonstrated upregulation of several drug transporter proteins, including MDR1, MRP1, MRP2, and MRP5 (Sisodiya *et al.* 2002, Sisodiya *et al.* 2003). This may be an important factor in drug resistance, although definite proof of a causal relation in humans is currently lacking. For example, confounding effects may arise from seizures and AED treatment themselves.

Several AEDs are thought to be substrates of more than one drug transporter and conversely most drug transporter proteins can transport different AEDs. 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 (Sisodiya 2003).

Table 2 shows the currently available data on drug transporters for AEDs.

b. *Genes encoding AED metabolizing enzymes*

AEDs of the first generation (e.g. phenobarbitone, phenytoin, carbamazepine and valproate) are metabolized via biotransformation in the liver, while many of the newer AEDs are directly eliminated through the kidneys without liver biotransformation. The metabolic pathways and specific DMEs for most AEDs are known (see Table 3). Functional variants in the encoding genes are expected to result in interindividual differences in the rate of AED metabolism, leading to differences in concentration, effectiveness and/or occurrence of ADRs. It is unlikely that these functional variants would also contribute significantly to true drug resistance, as their effects are expected to be reflected in drug plasma levels, which can be readily monitored for most AEDs.

At least eight isoenzymes are known to be involved in the metabolism of AEDs (see Table 3). The most important are CYP2C9 and CYP3A4. Although several polymorphisms in CYP3A4 are known, current evidence suggests that genetic variation in CYP3A4 is not a major factor in interindividual variability in drug clearance (Lamba *et al.* 2002).

The gene encoding microsomal epoxide hydrolase (mEH), which is responsible for detoxification of epoxide intermediates, is a candidate for variation in response to carbamazepine, phenobarbitone and phenytoin.

The role of phase II enzymes in AED metabolism is currently less well defined. The principal enzyme family in this category is that of the UGTs (UDP-glucuronosyltransferases), which conjugate their substrates through addition of a glycosyl group or glucuronidation. The two major sub-families are UGT1 and UGT2 (Burchell *et al.* 1995, King *et al.* 2000). The UGT isoenzymes have few specific substrates and show wide degrees of overlapping substrate specificity. Other phase II enzymes with a role in AED metabolism include the N-acetyltransferases (NAT1 and NAT2) and glutathione S-transferase (GST).

c. *Genes encoding AED targets*

The main candidates in this category are genes encoding ion channel subunits and elements of neurotransmitter pathways. Several first-line AEDs act through binding to and modulation of voltage-gated sodium channel α-subunits (see Table 4). Therefore, the genes encoding voltage-gated neuronal sodium channels are the prime 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 (Lynch *et al.* 2004). Other candidates in this category include genes coding for effector components downstream in the pathway of AED action and target. Table 4 lists the (presumed) targets for the most commonly used AEDs. The mechanism of action of some AEDS is not entirely understood.

A few in vitro studies have looked at AED sensitivity of ion channels carrying mutations identified in rare monogenic forms of epilepsy. One group compared sensitivity to valproate and carbamazepine of wild-type nicotinic acetylcholine receptors (nAchR) versus those with mutations in the CHRNA4 gene causing autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) in Xenopus oocytes (Picard *et al.* 1999). They showed that carbamazepine acts as a non-competitive

Table 3

Metabolism of the most commonly used AEDs

AED	Metabolism
CBZ	Epoxidation (CYP3A4 > CYP1A2, CYP2C8), hydrolysis (mEH) ; glucuronidation (UGT2B7)
CLB	Oxidation (CYP3A4) ; conjugation
CZP	Acetylation ; reduction and nitration
ESX	Oxidation (CYP3A4 > CYP2B, CYP2C9, CYP2E1) ; conjugation
FBM	– 60% hydroxylation (CYP3A4, CYP2E1 > CYP2C19) ; conjugation – 40% unchanged renal excretion
GBP	> 95% unchanged renal excretion
LTG	Glucuronidation (UGT1A4)
LEV	Hydrolysis in blood and other tissues + unchanged renal excretion
OXC	Hydroxylation (limited) ; glucuronidation
PGB	98% unchanged renal excretion
PHB	– 8-34% hydroxylation (CYP2C9, CYP2C19 > CYP2E1) ; glucuronidation – N-glucosidation ; – epoxidation, hydrolysis (mEH)
PHT	Hydroxylation (~90% CYP2C9, ~10% CYP2C19), hydrolyse (mEH), or GSH and GST ; glucuronidation
TGB	Oxidation (> 90% CYP3A4) ; glucuronidation
TPM	– 80% unchanged renal excretion – 20% hydroxylation (CYP2C19) and glucuronidation
VPA	– β-oxidation ; glucuronidation – CYP2A6, CYP2C9, CYP2C19
VGB	> 95% unchanged renal excretion
ZNS	Acetylation (CYP3A4), isoxazole ring cleavage ; glucuronidation

Adapted from (Patsalos *et al.* 2002, Shorvon 2004, Staines *et al.* 2004). Specific (iso)enzymes are mentioned if known.
GSH = glutathion ; GST = glutathione S-transferase.

inhibitor of acetylcholine currents and that this effect was enhanced in mutant α4b2 nAchR compared to wild-type receptors. Similarly, another group recently demonstrated that neuronal sodium channels expressing a mutant auxiliary β1-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 (Lucas *et al.* 2005). These results suggest that mutations in an AED target can affect drug response. This may happen through a direct change, i.e. a structural change of the target affecting AED binding, or indirectly, e.g. through altered gating resulting in differential AED action. As a consequence, common polymorphisms in genes encoding AED targets may also be expected to alter AED pharmacodynamics, thus accounting for some of the interindividual variation in AED response.

d. *Genes related to epilepsy itself*

As explained above, any gene with a role in the molecular pathology of epilepsy is also a potential candidate for variation in AED response. In recent years at least a dozen causative genes have been identified in rare forms of monogenic epilepsies. The large majority of these genes encode brain-expressed ion channels or neurotransmitter receptors, thus overlapping with those genes encoding AED targets. However, some of those genes do not directly encode ion channels or neurotransmitter receptors (Kalachikov *et al.* 2002, Suzuki *et al.* 2004). Moreover, mutations have been identified in a variety of different genes in syndromic epilepsies. These include for instance genes encoding phosphatases, kinases and proteins involved in central nervous system development. Variants in any of these genes could potentially also contribute to AED responsiveness.

e. *Genetic factors related to idiosyncratic drug reactions*

Although rare, idiosyncratic ADRs are an important problem in AED treatment. The best known examples are the hypersensitivity syndrome induced by aromatic AEDs (carbamazepine, phenobarbital, phenytoin) and lamotrigine, and aplastic anemia induced by felbamate. Identification of genetic factors contributing to these severe ADRs could have important clinical implications.

Table 4

Proposed targets of AEDs

AED	Main target	Other targets
CBZ	VG Na$^+$ channels	NMDA, adenosine, monoamine, serotonine, Ach receptors
CLB	GABA$_A$ α-subunit	VG Na$^+$ and Ca^{2+} channels
CZP	GABA$_A$ α-subunit	VG Na$^+$ channels
ESX	T-type Ca^{2+} channel	GABA receptor ?
FBM	NMDA receptors	VG Na$^+$ and Ca^{2+} channels, GABA$_A$ receptor ?
GBP	?	GABA synthesis and metabolism ?, VG Na$^+$ channels ?, α2δ-subunit of L-type Ca^{2+} channel ?, monaoamine release ?, serotonin ?
LTG	VG Na$^+$ channels	N- and P-type Ca^{2+} channel ?
LEV	SV2A	
OXC	VG Na$^+$ channels	K$^+$ and Ca^{2+} channels
PGB	VG Ca^{2+} channel α2δ-subunit	
PHB/PRM	GABA$_A$ receptor	Ca^{2+}, Na$^+$ and K$^+$ channels, AMPA/kainate receptors
PHT	VG Na$^+$ channels	Ca^{2+} channels, K$^+$ channels ?, GABA$_A$ receptor ?, calmodulin ?, second messenger systems ?
TGB	GAT-1	
TPM	VG Na$^+$ channels	GABA$_A$ receptor, AMPA/kainate receptors, L-type Ca^{2+} channel ?, carbonic anhydrase
VPA	?	GABA synthesis and metabolism ?, aspartate and Glu inhibition ?, Ca^{2+}, Na$^+$ and K$^+$ channels ?
VGB	GABAT	
ZNS	VG Na+ channels	T-type Ca^{2+} channel, carbonic anhydrase, GABA receptor ?, glutamate release ?, acetylcholine release and metabolism ?, dopamine accumulation ?

Adapted from : (Kwan *et al.* 2001, Shorvon 2004)
VG = voltage gated ; SV2A = synaptic vesicle protein 2A ; GAT-1 = GABA transporter 1 ; GABAT = GABA transaminase.

2. Reported associations in epilepsy pharmacogenetics

Epilepsy pharmacogenetics is a relatively new and undiscovered field. Nevertheless, some interesting results have emerged during recent years. An overview of the main results reported in the literature is given in Table 5.

One polymorphism in the MDR1 drug transporter (also called PGP or ABCB1) gene has been the subject of several genetic association studies in patients with drug refractory epilepsy. SNP C3435T in exon 26 is significantly correlated with expression levels and function of MDR1 in Caucasians (Hoffmeyer *et al.* 2000). In 2003, an association of the C3435T polymorphism with multidrug resistance in patients with different types of epilepsy was reported (Siddiqui *et al.* 2003). This is the only genetic polymorphism that has been associated with multidrug resistance in epilepsy to date. Several groups have attempted to confirm this association since (Tan *et al.* 2004, Zimprich *et al.* 2004, Hung *et al.* 2005, Sills *et al.* 2005, Kim *et al.* 2006, Kim *et al.* 2006, Leschziner *et al.* 2006, Seo *et al.* 2006). Although three of these were reported as positive, none of them succeeded in exactly replicating the original associa-

tion. Therefore, the role of genetic variation in the MDR1 gene in drug refractory epilepsy remains uncertain at present.

A number of studies have reported correlations of variants in genes encoding DMEs and AED response, most of them relating to drug toxicity. Low activity alleles of CYP2C9, which accounts for up to 90% of the metabolism of phenytoin, are associated with decreased phenytoin clearance, higher plasma levels and increased toxicity (Ninomiya *et al.* 2000, Brandolese *et al.* 2001). A small study identified an association between the low activity alleles CYP2C9*2 and CYP2C9*3 and lower dose requirement of phenytoin (van der Weide *et al.* 2001). We recently typed the CYP2C9*2 and CYP2C9*3 alleles in 281 patients treated with phenytoin and identified a significant correlation (p = 0.0066) between the *3 allele and the maximum daily dose of phenytoin that patients took, with patients carrying the *3 allele taking lower doses (Tate *et al.* 2005).

In the same study, we also related genetic variation in SCN1A, one of the genes encoding the main target of phenytoin and carbamazepine, to clinical dosing of these two AEDs. We found that one SNP (rs3812718) is highly associated with maximum dose of both AEDs (p = 0.0014 and p = 0.0045,

Table 5

Reported pharmacogenetic associations in epilepsy

Gene category	Gene	Phenotype	Main references
Transporter	MDR1	Drug refractory epilepsy	(Siddiqui *et al.* 2003, Tan *et al.* 2004, Zimprich *et al.* 2004, Hung *et al.* 2005, Sills *et al.* 2005, Kim *et al.* 2006, Kim *et al.* 2006, Leschziner *et al.* 2006, Seo *et al.* 2006)[a]
DME	CYP2C9	PHT toxicity	(Ninomiya *et al.* 2000[b], Brandolese *et al.* 2001[b], Tate *et al.* 2005)
		PHT dose	(Ninomiya *et al.* 2000[b], Brandolese *et al.* 2001[b], van der Weide *et al.* 2001, Tate *et al.* 2005)
Target	SCN1A	PHT and CBZ dose	(Tate *et al.* 2005)
		PHT levels	(Tate *et al.* 2006)
Immune response	TNFα	CBZ hypersensitivity	(Pirmohamed *et al.* 2001)
	HLA-B (*1502 allele)	Stevens-Johnson syndrome on CBZ	(Chung *et al.* 2004, Lonjou *et al.* 2006)

[a] not exact replications
[b] single case reports.

respectively) (Tate *et al.* 2005). This polymorphism is predicted to disrupt the consensus sequence of the 5' splice donor site of a highly conserved alternative exon ("exon 5N"). We also demonstrated that this polymorphism affects the proportions of alternative transcripts in brain tissue from individuals with a history of refractory epilepsy. We subsequently assessed the correlation of this SCN1A variant with serum levels of phenytoin in a different patient cohort (Tate *et al.* 2006). We found that the polymorphism was significantly associated with phenytoin serum levels at maintenance dose (p = 0.03). These results provide the first evidence of a drug target polymorphism associated with the clinical use of AEDs.

Two genetic associations with idiosyncratic drug reactions on AED treatment have been published to date. The first identified an association between the TNF2 allele of the tumour necrosis factor α (TNFα) gene, resulting in elevated expression of TNFα, and carbamazepine hypersensitivity (Pirmohamed *et al.* 2001). The TNFα gene is in linkage disequilibrium (LD) with the HLA-DR3 and -DQ2 genes, and the TNF-DR3-DQ2 haplotype appeared also to be associated with severe drug toxicity. The second report identified an exceptionally strong association of the HLA-B*1502 allele in Chinese patients who developed Stevens-Johnson syndrome on carbamazepine therapy (Chung *et al.* 2004). The strength of the association in this population is such that identification of the polymorphism might allow a predictive test to be developed. A small study in Caucasian patients with Stevens-Johnson syndrome on carbamazepine found that only 33% of patients carried the HLA-B*1502 allele (Lonjou *et al.* 2006). However, all patients carrying the allele appeared to have Asian ancestry, suggesting that the HLA-B*1502 allele is a population-specific marker for Stevens-Johnson syndrome.

Conclusions

Pharmacogenetics offers the potential to predict drug responses in individual patients and to contribute to the discovery of new drug targets. There are numerous candidate genes for pharmacogenetic studies in neuropsychiatric disease. Nevertheless, hardly any firm pharmacogenetic associations have been identified at present and clinical applications still seem far away. One of the main explanations is that drug response is a complex trait, influenced by interactions of multiple genetic and environmental factors. As a consequence, the effect size of any single genetic variant is likely to be small. Therefore, looking at interactions between multiple variants – and possibly exogenic factors – is likely to be more fruitful, though complicated by statistical challenges. Another explanation for the lack of consistent results, especially with older studies, lies in the failure to adhere to stringent methodological quality standards for genetic association studies (Cardon and Bell 2001, Goldstein 2003).

Efforts in other disease domains have already yielded some robust results, and the first clinical pharmacogenetic applications are now available (Jain 2005, Woelderink *et al.* 2006).

It is now up to the neuropsychiatric research community to join efforts and set up large-scale, rigorously designed pharmacogenetic association studies in large, well-phenotyped patient cohorts. If this can be achieved, it is likely that robust and potentially clinically relevant results will ultimately emerge, offering hope for more efficacious treatment for the millions of patients affected by neuropsychiatric diseases.

Acknowledgements

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