PHARMACOGENETICS OF EPILEPSY: ONE STEP FORWARD?

Genetic Predictors of the Maximum Doses Patients Receive during Clinical Use of the Antieileptic Drugs Carbamazepine and Phenytoin

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Phenytoin and carbamazepine are effective and inexpensive antiepileptic drugs (AEDs). As with many AEDs, a broad range of doses is used, with the final "maintenance" dose normally determined by trial and error. Although many genes could influence response to these medicines, obvious candidates are known. Both drugs target the α-subunit of the sodium channel, encoded by the *SCN* family of genes. Phenytoin is principally metabolized by CYP2C9, and both are probable substrates of the drug transporter P-glycoprotein. We therefore assessed whether variation in these genes associates with the clinical use of carbamazepine and phenytoin in cohorts of 425 and 281 patients, respectively. We report that a known functional polymorphism in *CYP2C9* is highly associated with the maximum dose of phenytoin ($P = 0.0066$). We also show that an intronic polymorphism in the *SCN1A* gene shows significant association with maximum doses in regular use of both carbamazepine and phenytoin ($P = 0.0051$, and $P = 0.014$, respectively). This polymorphism disrupts the consensus sequence of the 5′ splice donor site of a highly conserved alternative exon (5N), and it significantly affects the proportions of the alternative transcripts in individuals with a history of epilepsy. These results provide evidence of a drug-target polymorphism associated with the clinical use of AEDs and set the stage for a prospective evaluation of how pharmacogenetic diagnostics can be used to improve dosing decisions in the use of phenytoin and carbamazepine. Although the case made here is compelling, our results cannot be considered definitive or ready for clinical application until they are confirmed by independent replication.

COMMENTARY

Interindividual variability in the susceptibility to disease and the outcome of drug treatment is dependent on the complex interplay of genetic and environmental factors. The study of genetic influences on the response to drug treatment is termed pharmacogenetics (1). It focuses primarily on single nucleotide polymorphisms (SNPs), which are single base variations in the sequence of genes, observed in at least 1% of individuals, with the potential to influence the structure and/or function or both of the proteins those genes encode. SNPs are the most frequently occurring form of variation in the human genome and account for more than 90% of all genetic variability (2). SNPs are observed at a rate of approximately 1 per 1,000 bases, although the frequency may be higher in coding sequences (exons) than in untranscribed regions (introns) of the genome. Whereas many SNPs are silent, functional consequences can be observed when coding SNPs result in amino acid substitutions in physiologically sensitive regions of the encoded protein. Intronic and promoter region SNPs also can affect functionality by influencing alternative splicing and the control of transcription, respectively.

Pharmacogenetics has recently arrived in the epilepsy field, and a number of recent articles have described how genetic variants may (or may not) influence the drug treatment of seizure disorders. Candidate genes for pharmacogenetic study in epilepsy are those that encode proteins directly involved in the pharmacokinetics (hepatic drug–metabolizing enzymes, membrane drug transporters) and pharmacodynamics (sodium channels, GABA receptors, etc.) of antiepileptic drug (AED) action (3). This principal has been applied to a recent investigation of maximally tolerated doses of phenytoin and carbamazepine and the prevalence of SNPs in genes encoding the drug-metabolizing enzyme CYP2C9, the multidrug transporter P-glycoprotein (*ABCB1*), and a neuronal sodium channel α-subunit (*SCN1A*). The authors report significant associations between maximally tolerated doses of phenytoin and specific polymorphisms in *CYP2C9* and *SCN1A*. Likewise, the maximal doses of carbamazepine appear to correlate with a reported variation in the *SCN1A* gene. No association was found for either drug in relation to a well-characterized polymorphism in *ABCB1*.

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It is perhaps not surprising that tolerability to phenytoin is related to a known functional polymorphism in the gene encoding CYP2C9, given that this enzyme is primarily responsible for its hepatic metabolism (4). One could easily envisage the effect of compromised (or indeed enhanced) metabolic capacity on drug pharmacokinetics and thereby dosing requirements. However, it is interesting that only the *3 allele of *CYP2C9* was associated with maximal phenytoin dose, and even then, it accounted for a relatively modest reduction in the limit of tolerability, despite the fact that this variant is known to reduce drug clearance by up to 95% (5). In the absence of serum drug concentrations, the basis for this apparent anomaly is unclear, although it may point to a differential metabolism of phenytoin in patients carrying the *CYP2C9*3* allele, with a potentially more prominent role of the CYP2C19 isoenzyme.

A more intriguing observation is the association between maximally tolerated doses of both phenytoin and carbamazepine and a previously unreported intronic polymorphism in *SCN1A*. By its nature, this SNP should not influence the amino acid sequence of the encoded protein. However, its position in the 5′ splice-donor site of a highly conserved, alternatively spliced exon, predominantly expressed in fetuses, led the authors to speculate that it may be responsible for the differential incorporation of the fetal exon into the encoded protein. This hypothesis was tentatively confirmed in a small number of temporal lobe specimens obtained from patients who had undergone epilepsy surgery but did not hold in a similarly limited number of "nonepileptic" samples from a Parkinson's disease brain bank, with no apparent association between genotype and the relative expression of the fetal exon. These findings raise the possibility that seizures, in combination with a splice-donor site polymorphism in *SCN1A*, might result in a localized increase in the incorporation of a predominantly fetal exon into *SCN1A*-derived sodium channels, leading to an increase in their sensitivity to AEDs—manifested in this case as a reduction in the maximally tolerated dose of sodium channel–blocking agents.

This hypothesis is based on the assumption that voltage-gated sodium channels are the primary determinants of tolerability to sodium channel–blocking drugs, yet no direct evidence supports this premise. If it were the case, then it would be reasonable to suggest that epilepsy patients with the variant form of this particular *SCN1A* polymorphism would have a better response, in terms of efficacy, to sodium channel–blocking agents. Unfortunately, the authors do not report any efficacy data in their article. They do, however, validate their conclusions on the basis of functional replication, that is, that similar effects are independently observed with phenytoin and carbamazepine, both of which block voltage-gated sodium channels. This argument and the question of whether sodium channels are involved in tolerability of phenytoin and carbamazepine would have been significantly strengthened by the failure to demonstrate an association in a third cohort of patients treated with non–sodium channel-blocking AEDs.

One of the great challenges of pharmacogenetic studies, particularly in the epilepsy field, is the ability to identify a clear and unequivocal phenotype. In choosing to investigate maximally tolerated doses of commonly used AEDs, Tate and colleagues may have avoided many of the issues that surround the subjective assessment of efficacy, but they fail to address other areas of potential heterogeneity. The study of maintenance doses of phenytoin and carbamazepine might have been a more accurate representation of clinical effect. Many patients, even in a tertiary referral setting, become seizure free on a modest dose and may never reach their personal limit of tolerability (6). In addition, the effect of age and the unique drug-handling capacities of children and the elderly have not been given sufficient consideration. Concomitant medication (antiepileptic or otherwise) can also have a significant influence on the pharmacokinetics and, thereby, dosing requirements of phenytoin and carbamazepine. The authors acknowledge this potential confounder but fail to afford it the importance that it undoubtedly deserves. Finally, the collection of exquisitely detailed clinical information in such studies is paramount, and yet, some of the most basic data in this study, such as age at onset of treatment, are incomplete. Such are the limitations of investigations based on the retrospective review of clinical case notes.

Previous pharmacogenetic investigations in epilepsy have revealed a number of positive associations. The first publication of note reported a correlation between the C3435T polymorphism in the *ABCB1* gene and response to drug treatment (7). Subsequent studies, however, have failed to confirm this finding (8,9), and the current investigation, which presumably included some patients from the original report, did not reveal any association with maximally tolerated doses of AEDs. Other initially positive findings have, likewise, not been reproduced (10). The ability to confirm such data is essential, and the continuing failure to do so might bring into question the pharmacogenetic approaches that are currently used. Issues of ethnicity, population size, heterogeneity of epilepsy, gradations of response, likelihood of chance observation, and the undoubtedly limited contribution of a single SNP to outcome have all been mooted as potential contributory factors. There is little doubt that the current study needs to be reproduced for its findings to be validated. Even then, the authors acknowledge that the true effect of variant genotypes might become apparent only with a prospective evaluation of dosage, clinical effects, and serum drug concentrations in a large cohort of patients receiving AED monotherapy.

It is easy to adopt the role of devil's advocate and be dismissive about the quality of this investigation. However, conducting a suitably comprehensive pharmacogenetic study in a traditional clinical environment is inherently difficult. Patients

are not closely monitored laboratory animals, heterogeneity is a common characteristic of epilepsy, and case notes are often the only means of data collection for necessarily large cohorts. The study reported by Tate and colleagues is an important investigation, which demonstrates both impressive scientific application and intriguing findings. It is the first full publication of an association between a polymorphism in a gene encoding a primary AED target and the clinical use of first-line antiepileptic agents. The limitations of this study are evident and potentially confounding, the data and its interpretation should be considered with caution, and yet, in spite of these issues, the study represents another small step in the effort to unravel the pharmacogenetics of epilepsy.

by Graeme J. Sills, PhD

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