

osmolar agents among high-risk patients, defined as those with preexisting renal dysfunction; the highest risk associated with high-osmolar contrast agents was found among patients with both preexisting azotemia and diabetes. Barrett and Carlisle¹² performed a meta-analysis that pooled data from 31 different trials and concluded that a statistically significant benefit of low-osmolar contrast agents in terms of renal function could be shown only among patients with preexisting renal dysfunction in whom the contrast material was administered intraarterially. In contrast, no benefit was found among patients with normal renal function (with or without diabetes) or among those in whom the contrast material was administered intravenously.

The study by Aspelin et al. suggests that iodixanol is of significant benefit in a group of patients known to be at high risk for the development of contrast-agent-induced acute renal dysfunction—patients with diabetes who have preexisting abnormal renal function and are undergoing arteriography. The authors have attempted to control some of the variables by excluding patients whose renal function before angiography was not stable and patients who had received any potentially nephrotoxic drugs or contrast agents less than seven days before angiography.

Although the results of the study by Aspelin et al. are encouraging, one should not conclude that iodixanol is the answer to contrast-agent-induced acute renal dysfunction. The precise mechanism by which such renal dysfunction occurs remains unknown, but experimental data suggest that the primary mechanism is arteriolar vasoconstriction mediated by endothelin-1 in response to the delivery of a large hyperosmotic load (i.e., the contrast material) to the juxtaglomerular apparatus.¹³ Since there is no satisfactory animal model of contrast-agent-induced acute renal dysfunction, it is likely

that we will have to depend on clinical studies such as the current one to further elucidate this important problem. Yet, since previous clinical studies have yielded conflicting results, there is reason to believe that future studies of iodixanol may provide conflicting data as well.

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Pharmacogenetics in the Laboratory and the Clinic

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One of the most striking features of modern medicines is how often they fail to work. Even when they do work, they are often associated with serious adverse reactions. Indeed, adverse reactions to drugs rank as one of the leading causes of death and illness in the developed world.¹ How can we improve the success rate?

The Human Genome Project and other advances have generated expectations that medicines can be customized to match the genetic makeup of patients, thereby dramatically improving efficacy and safety. These issues are examined in two review articles in this issue of the *Journal*.^{2,3} Although the prospects for basic research in pharmacogenetics

look very promising, the incorporation into clinical practice of the data it generates presents considerable challenges.

Polymorphism of the genes encoding drug-metabolizing enzymes has been the core focus of pharmacogenetics since its beginnings in the 1950s, but other classes of genes influencing responses to drugs are receiving increasing attention.^{2,3} In addition to the many well-known variants of drug-metabolizing enzymes,² pharmacogenetic variants have now been documented in drug transporters and targets, as well as in their associated pathways.³

Many of the genes that have been studied have variants that occur with moderate-to-high frequency and are known to influence the response to drugs or appear likely to do so (for example, because they change the activity of a relevant enzyme). These findings are important not only because of the specific variants that have been identified, but also because they suggest that it will be possible to find many of the variants that we do not yet know about. Much of the excitement about personalizing medicines is fueled by association studies, which seek to relate genetic variants to drug response. Genetic association studies will be more successful in finding common variants than rare variants, unless the variant has very high penetrance.

In a case-control study, genotypes of persons with and without a particular response of interest — either a therapeutic response or an adverse event — are compared. The key question is which genetic variants to consider. There are approximately 10 million single-nucleotide polymorphisms, or SNPs (variants with frequencies of more than 1 percent), in the human genome, of which as many as 4 million have been catalogued.⁴ Because our knowledge in this area is incomplete and because of cost constraints, it is not possible to evaluate all polymorphic SNPs in the genome directly through association studies. Instead, the aim is to use associations among variants, or linkage disequilibrium,⁵ to leverage the information provided by typing a subgroup of polymorphic markers.

Current thinking about the design of association studies has been greatly influenced by data showing that linkage disequilibrium in humans is composed of regions of low diversity of haplotypes, where the common haplotypes can be represented by knowledge of only a few genotypes in the block, and stretches of more rapid breakdown of linkage disequilibrium, where a few sentinel SNPs cannot reliably represent haplotype diversity; in many cases,

the latter regions correspond to hot spots of meiotic recombination.^{6,7} This structure of linkage disequilibrium inspired the idea of haplotype tagging, in which a set of SNPs is identified that tags each of the common haplotypes within a block of linkage disequilibrium. The general algorithm is straightforward: determine the haplotype structures of genes or genomic regions of interest in controls, identify tagging SNPs, and analyze the tagging SNPs in patients whose drug-response phenotype is known. By some estimates, an average of only five to seven SNPs per gene would be required to represent all the common polymorphisms in candidate genes. This means that it is now economical to conduct exhaustive studies of candidate genes once the haplotype structure of the genes has been determined.

It must be understood, however, that the tagging SNPs must be carefully selected and validated — not just any five to seven SNPs will do. Thus, many previous pharmacogenetic association studies have been incomplete, in that they did not fully represent the common variation in the genes studied, even when multiple polymorphisms within a gene were considered. For clarity, this approach should be referred to as the study of “candidate polymorphisms,” to distinguish it from a systematic study of candidate genes using tagging SNPs for each common haplotype. Failure of a candidate-polymorphism study does not rule out involvement of the gene or genes, whereas a negative candidate-gene study can provide a statistical limit to the importance of any common variant in the gene. Given the economy provided by tagging SNPs, there is little justification now for not conducting a candidate-gene study, especially since the haplotype structures of genes need only be determined once in the population of interest. The tags can then be used for any clinical study. It should be noted, however, that haplotype tagging will often fail to capture rare variants, which are also known to be important in variable responses to a drug.⁸

There are several implications of this approach, termed “haplotype mapping,” that are worth emphasizing. Limited diversity of the haplotypes of genes increases the prospects of identifying interactions between the haplotypes found in different genes — for example, elements of a common pathway. This opportunity argues strongly that potentially interacting genes are best analyzed as integrated sets (e.g., the genes encoding a receptor complex, the renin-angiotensin-aldosterone pathway, or drug-metabolizing enzymes).

Systematic association studies will very soon be feasible for large sets of candidate genes (i.e., sets numbering in the hundreds) within the context of academic laboratories, but systematic genome-wide analyses will not be possible for quite some time. The National Institutes of Health is spearheading a global effort to find an appropriate set of tagging SNPs for the entire genome and estimates that it will require 300,000 to 600,000 SNPs.⁹ If it takes 450,000 SNPs at the (currently reasonable) cost of 10 cents per sample per genotype, the cost of analyzing 1000 cases and 1000 controls will be \$90 million. On the other hand, a systematic candidate-gene study of the key elements of the renin-angiotensin pathway would cost several hundred thousand dollars at most, once the haplotype structure of the genes had been determined.

In short, much of the enthusiasm surrounding pharmacogenetics appears to be justified. Haplotype mapping is a powerful framework for finding common variants that influence drug response, and there is clear evidence that common variants play an important part in variable drug response. It does not follow, however, that pharmacogenetics will revolutionize health care overnight. The pharmacogenetics of asthma illustrates the reasons both for optimism about progress in research and for caution about translation into clinical practice. The β_2 -adrenergic receptor is the target of the most commonly used medicines for asthma and is therefore a natural candidate for influencing the response to inhaled β -agonists. Indeed, the Arg-Arg genotype at position 16 in the β_2 -adrenergic-receptor gene has been positively associated with the acute response to treatment.¹⁰ Dynamic analyses, however, revealed that the Arg-Arg genotype is also associated with a significant decrease in response after regular use of β -agonists, whereas the Gly-Gly genotype was unaffected by regular use.¹¹ Combining these data with functional analyses, Liggett¹² explained the results with a model of receptor regulation, but the model has not been verified and the clinical implications remain unclear,¹¹ despite the extremely effective combination of association and functional data.

Similarly, P-glycoprotein is a nonspecific drug-efflux pump that is active at the blood-brain barrier and is known to act on antiepileptic drugs. My colleagues and I showed that a previously known high-activity variant in exon 26 of the encoding ABCB1 gene is significantly associated with resistance to drug treatment in patients with epilepsy (unpub-

lished data), suggesting the possibility that inhibition of P-glycoprotein might improve the response to treatment in some patients with refractory epilepsy. Previous clinical experience with P-glycoprotein inhibitors, however, provides a strong cautionary note concerning the clinical exploitation of this result. Clinical trials of P-glycoprotein inhibitors as a means of reducing the frequency of multidrug resistance in patients undergoing antitumor therapy have been generally disappointing, because of inadequate knowledge about the relevance of P-glycoprotein in individual patients, drug-drug interactions, the importance of other transporters, and perhaps unaccounted-for variation in the ABCB1 gene itself.¹³

These and other examples suggest that, with only rare exceptions, the translation of pharmacogenetic research into clinical practice will be intellectually challenging, time-consuming, and expensive. It will usually require explicit clinical evaluation, meaning that translation will lag years behind basic research. There are steps, however, that the research community can take to improve the efficiency of translation.

Reported associations between genetic variants and drug responses should be as secure as possible, and every effort should be made to determine the causal variant or variants. It would be helpful to define minimal standards for reported associations. All reports should include an assessment of the structure of linkage disequilibrium surrounding the associated polymorphism in order to delimit an associated interval—that is, the boundaries on either side of the associated polymorphism that delimit an area of sufficiently high linkage disequilibrium that the causal variant responsible for the observed association could reside within it. In addition, all reports should include a check and, if necessary, correction for stratification of the population,¹⁴ which can create spurious association, in order to reduce the amount of effort wasted on spurious associations.

Finally, the biologic function of causal variants must be assessed in order to aid in the interpretation of the associations. Particular care would be required in the use of diagnostic associations in the absence of identified causal variants, in part because patterns of linkage disequilibrium will differ among populations, thereby changing the associations between causal variants and the associated markers. Basic research in pharmacogenetics deserves the support and the excitement that it has generated, but this excitement should not lead to unrealistic

expectations about the rate at which medicines can be personalized according to genotype.

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