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Commentary

Pharmacogenetics: The slow, the rapid, and the ultrarapid

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Pharmacogenetics, the study of genetically determined variations in drug response, has its origins in discoveries made in the 1950s. In 1957, Motulsky (1) pointed out that certain adverse drug reactions could be caused by genetically determined variations in enzyme activity. It was recognized that variants of glucose-6-phosphate dehydrogenase caused primaquine-induced hemolysis (2) and that pseudocholinesterase variants caused sensitivity to suxamethonium (3). At the same time, genetic variation in the capacity to perform acetylation reactions was found to be related to adverse effects of isoniazid (4). In 1959, Vogel (5) first proposed the term "pharmacogenetics", and in 1962, Kalow (6) wrote the first monograph on the subject. Numerous additional examples of pharmacogenetic traits have been described since.

The field of pharmacogenetics was further stimulated in the 1970s when Vesell and his colleagues (7) demonstrated that identical twins were more similar than were fraternal twins in regard to the plasma half-lives of numerous drugs. The implication was that multiple genes may determine individual drug biotransformation (multigenic inheritance). More recently, genetic polymorphisms of drug-metabolizing enzymes with monogenic inheritance such as debrisoquine polymorphism, mephenytoin polymorphism, and acetylation polymorphism have received much interest because they affect the metabolism of numerous clinically useful drugs and concern a sizeable proportion of patients (8). As emphasized at a recent workshop on enzymes of drug metabolism at the Institute of Medicine of the National Academy of Sciences (Enzymes of Drug Metabolism: Importance to Drug Safety and Efficacy, Jan. 25–26, 1993, Washington, DC) pharmacogenetics has become relevant for the development and use of most drugs and not just for the relatively rare occurrences of enzyme variants that can cause a dramatic drug response.

Interethnic differences in reactions to drugs and chemicals, sometimes called "pharmacoanthropology," represent another recent branch of pharmacogenetics. As an example, the frequency of "slow acetylators" of arylamine drugs and carcinogens, a recessively inherited deficiency of the *N*-acetyltransferase arylamine *N*-acetyltransferase 2 (9), is high in Caucasians (40–70%) but lower in Asians (10–20%). By contrast, inefficient or slow metabolism of the anticonvulsant mephenytoin and some other drugs, another recessively inherited deficiency of a drug-metabolizing enzyme (8), is infrequent in Caucasians (3–5%) and more common (15–20%) in Asians. The importance of these population differences in the incidence of enzyme variants has only recently been the subject of epidemiological and clinical investigations (10).

Like most areas of biomedical science, pharmacogenetics has been profoundly transformed by molecular biology. Many pharmacogenetic conditions are now being studied at the gene level. Simple PCR-based DNA tests for the debrisoquine and the acetylation polymorphism were developed in my laboratory in 1990 (11) and 1991 (9), respectively. Similarly, the many variants of pseudocholinesterase (12) or of glucose-6-phosphate dehydrogenase (13) can now be assessed by DNA technology, rather than by the difficult characterization of the enzyme itself.

The best-studied example of a genetic variation in drug response is the debrisoquine polymorphism. Five to 10% of individuals in Caucasian populations are so-called "poor metabolizers" and are homozygous for two recessive loss-of-function alleles of the *CYP2D6* gene encoding the microsomal cytochrome P450 monooxygenase CYP2D6 (8, 14). These poor metabolizers of debrisoquine are inefficient in the metabolism of >30 clinically used drugs, including important antidepressants, neuroleptics, opioids, and cardiovascular drugs. Moreover, a higher frequency of some of the mutant alleles is observed in patients with Parkinson disease, and this suggests a role of this enzyme in the pathogenesis of this neurodegenerative disorder (15, 16).

In the last 5 years, >90% of the mutations of the *CYP2D6* gene that cause absence of the CYP2D6 protein and result in the debrisoquine poor-metabolizer phenotype have been identified (17–19). As for other human enzyme deficiencies, many different mutations can cause a defective enzyme. The most common mutant allele (>70% of poor-metabolizer alleles) is characterized by a point mutation at a splice-site recognition sequence that leads to a frameshift. Another mutant allele (5%) consists of a 1-bp deletion in the coding sequence, causing a frameshift, and yet another loss of enzyme activity (10–15%) is caused by the deletion of the entire *CYP2D6* gene (20).

The mutations described provide a molecular explanation for the subgroup of poor metabolizers. However, the enormous variation among the individuals of the much larger group of so-called "extensive metabolizers," remained unexplained. These individuals presumably carry one or two normal (or wild-type) alleles of *CYP2D6*. But extensive (or rapid) metabolizers with a known mutant allele of *CYP2D6* (heterozygotes) were on the average only slightly "slower" and not clearly distinguishable from those with two normal *CYP2D6* genes (19). Additional genetic or epigenetic variation was suspected. Environmental factors causing enzyme induction or repression appeared of negligible importance because in the same individual the debrisoquine urinary metabolic ratios are constant over years. Recent studies have shed some light on this problem in that several *CYP2D6* alleles that result in only slightly decreased metabolism of debrisoquine have been observed (21, 22). These mutations explain the so-called "intermediate metabolizer" phenotype—i.e., individuals that are on the "slow side" of the extensive metabolizer distribution curve but are not really poor metabolizers.

In this journal, Johansson *et al.* (23) added another dimension to our understanding of the *CYP2D6* locus and to pharmacogenetic variation in general. In two families of extremely rapid metabolizers of debrisoquine, designated by the authors as "ultrarapid metabolizers," they discovered amplification of functional *CYP2D6* genes. With studies in two families, they provide convincing genetic evidence that this amplification causes ultrarapid metabolism.

The *CYP2D6* gene was amplified 12-fold on one allele in three members of one family, and two gene copies were present on one allele in another family of ultrarapid metabolizers. The duplicated or amplified *CYP2D6* is a common variant of the initially described *CYP2D6* wild-

type gene and was named *CYP2D6L* by the authors. As liver tissue could not be obtained from these individuals, the authors could not provide biochemical evidence for expression of increased amounts of CYP2D6 protein, but they found an excellent correlation between debrisoquine metabolic rate and the presence of amplified *CYP2D6L* genes in family members. The amplification is dominantly inherited. Data in unrelated individuals carrying one copy of *CYP2D6L* revealed that the enzyme activity of the CYP2D6L product is identical to that of the CYP2D6 wild-type enzyme.

These are new and exciting findings, providing a molecular explanation for the "rapid" corner of the distribution curve of metabolic ratios. Furthermore, this is the first observation of gene amplification that results in the gain-of-function alleles in healthy individuals. Clinical studies with polymorphically metabolized drugs have hitherto predominantly emphasized the therapeutic importance of the poor metabolizer phenotype, where inefficient metabolism predisposes to adverse drug effects. The ultrarapid metabolizer, on the other hand, will not respond to standard doses of drugs because of his extremely rapid elimination of the active agent. Johansson *et al.* (23) also describe two additional unrelated patients who in previous clinical studies required very high doses of antidepressant drugs to achieve therapeutic concentrations. These drugs are known substrates of the CYP2D6 enzyme. In the DNA of these patients, evidence for two amplified *CYP2D6L* genes was indeed obtained, establishing the clinical relevance of the amplification.

As Johansson *et al.* (23) point out, the *CYP2D6* amplification is different from an inheritable amplification event described by Prody *et al.* (24). These investigators observed a 100-fold amplification of parts of the DNA of a nonfunctional or "silent" cholinesterase gene in a farmer and his son. The family of the farmer had been exposed for generations to the organophosphorous insecticide parathion, an irreversible inhibitor of cholinesterases. By contrast, the amplification described by Johansson *et al.* (23) involves an intact functional gene, and the two families were not exposed to known substrates or inhibitors of the CYP2D6 enzyme.

The discovery of amplification of a functionally active gene for drug and xenobiotic metabolism in healthy individuals raises numerous questions of scientific and medical interest. First, DNA amplification is an extremely rare event in normal human cells (25) and has been described almost exclusively with oncogenes and genes mediating drug resistance in tumor cells and certain mammalian cell lines (25–27). One reason for this is that the genetic instability observed in karyotypes of tumor cells and cell lines is not seen in normal cells (27). The tendency to rearrangement of the *CYP2D6* gene locus on chromosome 22 with gene deletions, gene conversions, and variable numbers of two or three pseudogenes may indicate some instability of this particular chromosomal area (28). Chromosome 22, indeed, has several regions noted for nonrandom chromosomal rearrangements (for review, see ref. 29). We also have identified a number of repetitive DNA sequences in the vicinity of the *CYP2D6* deletion (20). These repetitive elements were described earlier and thought to predispose to large deletions in the β-globin gene cluster (for references, see ref. 20).

Amplification events may occur during germ-line cell development and early embryogenesis and be selected for when the gene product has a protective function against processes interfering with normal spermatogenesis or oogenesis (26). CYP2D6 metabolizes numerous xenobiotic drugs and alkaloids and conceivably may react to environmental perturbations. One would like to know, of course, if the *CYP2D6* amplification event is accompanied by other chromosomal rearrangements or by amplifications of other genes and if drug-metabolizing enzymes are preferred targets for amplification in situations of xenobiotic or toxic exposure. A case in point may be that a 250-fold amplification of an esterase gene can be the cause of insecticide resistance in mosquitos (30). Understanding the mechanisms and consequences of amplification in normal cells remains a major challenge for the future.

At this time, the findings of Johansson *et al.* (23) provide a molecular explanation for extremely rapid drug metabolism in individuals that require much higher than usual doses to reach therapeutic drug concentrations. Gene amplification in the described individuals represents a predictable pharmacogenetic trait complicating drug therapy and a novel source of interindividual variation in drug response.

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