CYP2D6:
Genotypes, Phenotypes, and Genetic Testing

In 1975, several laboratory scientists at St. Mary’s Hospital Medical School in London each ingested a 40 mg dose of debrisoquine, an antihypertensive drug then in clinical use. While the majority of the researchers reported no adverse side effects, Robert Smith, the laboratory director, experienced dizziness and suffered from a bout of orthostatic hypotension that lasted several days. In addition to his physical ailments, Smith’s urine contained unusually low levels of 4-hydroxy-debrisoquine, a metabolite of debrisoquine. This discovery led him and his colleagues to suspect that his peculiar reaction to the drug was a result of a metabolic deficiency. A follow-up study identified additional individuals who also appeared to be “poor metabolizers” of debrisoquine and went onto to characterize the two observed phenotypes. Smith’s research was not the only drug study at the time to report such findings. Indeed, eight years earlier in Sweden, the Sjöqvist lab had observed considerable inter-individual variation in the amount of circulating substrate after study subjects were given standardized doses of nortriptyline and desimipramine, two anti-depressants. They later established that this variation was indeed genetic by conducting a twin study. Similarly, research carried out by Michel Eichelbaum and Hans Dengler, examining sparteine, an anti-arrhythmic, revealed that some individuals suffered from nausea, diplopia, blurred vision, and headaches after taking the drug, side affects accompanied by circulating levels of sparteine that were 4 to 5 times the normal concentration. Ten years later, the work of F. P. Guengerich and Urs Meyer tied the curious results of all three of these studies to an enzyme of the cytochrome P450 enzyme family. Cloning experiments subsequently identified the sequence and chromosomal location of the culprit protein, now known as CYP2D6.

CYP2D6 is one of 57 cytochrome P450s, a class of metabolic enzymes found primarily in the human liver. Many of these enzymes, including CYP2D6, play an instrumental role in the breakdown and clearance of clinically prescribed drugs. It is now known that CYP2D6 is a hydroxylase, which specifically acts by adding protons to amenable nitrogen atoms found on lipophilic bases. The body maintains CYP2D6 at low concentrations relative to other cytochrome P450s, a homeostatic condition which
may compensate for the enzyme’s hyperactivity. Indeed, along with CYP2C9 and CYP2C19, CYP2D6 is responsible for 40% of first pass hepatic metabolism and is thought to be active in the enzymatic breakdown of 20-25% of all medicines now prescribed, a fraction that consists of approximately 100 identified drugs. Included among these medications are many tricyclic antidepressants, like clomipramine and desipramine, serotonin reuptake inhibitors, like paxil and prozac, neuroleptics, like codeine, beta-blockers, like propranolol and atenolol, and antiarrhythmics, like quinidine. In mice, CYP2D6 is part of the extensive CYP2D family, which includes a total of nine active genes. This family has been reduced to just three members in humans, CYP2D6 and its neighbors, the pseudogenes CYP2D7P and CYP2D8P, which are all located in tandem on chromosome 22. It is thought that the drastic reduction of the CYP2D family in humans relative to mice and many other mammals may be attributable to selective pressures raised by dietary alterations, since cytochrome P450s are also capable of breaking down plant alkaloids.

The structure of the CYP2D locus in humans makes it a hot spot for mutations arising from unbalanced recombination. As a result, insertions and deletions involving the CYP2D6 gene are commonly observed, including deletion of the entire gene, which is referred to as the CYP2D6*5 allele. Tandem duplication of CYP2D6 is also known to occur and individuals have been observed that carry as many as 12 active copies of the gene. In addition to large indels, CYP2D6 is also the site of numerous point mutations, including synonymous and non-synonymous SNPs and small insertions/deletions leading to frameshifts and premature stop codons. Overall, about 91 unique haplotypes have been described for the CYP2D6 locus, a number that is still regularly increasing as new mutations are discovered. Most of these haplotypes differ from the CYP2D6 *1A haplotype, considered to be the wild-type allele, at multiple positions and as a result the haplotypes are arranged into 52 groups based on shared mutations. Although the majority of CYP2D6 haplotypes have not yet been evaluated for enzymatic activity, a few have been identified as being either deficient (e.g. *10, *17, and *41) or totally lacking (i.e. *4, *5) in function.

The polymorphic nature of the CYP2D6 locus and the functional inequalities associated with its variants are ultimately responsible for the variable drug responses
observed by Smith, Sjöqvist, Eichelbaum, and Dengler. Today, phenotypic classification of CYP2D6 activity has formally been extended from the two functional categories characterized in those early studies (essentially normal and abnormal) to four- PMs, poor metabolizers, IMs, intermediate metabolizers, EMs, extensive metabolizers, and UMs, ultrametabolizers. Individuals are placed into one of these categories based on a direct assay of their enzymatic activity rather than their genotype for CYP2D6. However, it has been recognized that CYP2D6 genotype generally correlates with metabolic classification in the following manner: PMs typically carry two CYP2D6 null haplotypes, IMs have either two functionally deficient haplotypes or one deficient and one null haplotype, EMs carry either one or two fully functional haplotypes, and UMs usually have more than two copies of CYP2D6, although some haplotypes (e.g. *35) have been associated with ultrametabolism independent of gene duplication. Overall, PMs and, to a lesser extent, IMs are prone to exaggerated side effects from drugs metabolized by CYP2D6, whereas normal doses of the same drugs tend to be ineffectual for UMs.

Although EMs are typically considered to have the “normal” CYP2D6 phenotype (and indeed their metabolic rate of pertinent medications forms the basis for what is considered a “normal” dose), this phenotype does not dominate in all human populations. Indeed, the most common CYP2D6 allele in Asia (frequency ~50%) and, perhaps in the world, is *10. This haplotype carries a particular non-synonymous SNP, which seriously impairs the stability of the functional enzyme and may reduce its affinity for substrates. Thus, as a result of this allele alone, approximately 25% of all Asians are classed as intermediate metabolizers. IMs are also common among Black Africans and African-Americans due to the high frequency (~25-30%) in these populations of the *17 haplotype, a variant which carries two non-synonymous SNPs that significantly alter the CYP2D6 binding site. Conversely, UMs are rare in both Black Africans and Asians, but are frequently found in North Africa and the Middle East, where whole gene duplication of CYP2D6 is commonly observed. Extraneous copies of CYP2D6 are also often found in neighboring parts of Mediterranean Europe, where the frequency of UMs reaches ~10% in Greek, Italian, Spanish, and Portuguese populations. This region represents one extreme of a CYP2D6 allelic cline across the European continent. At the northern end of this cline, UMs become rare, “replaced” by PMs, most of whom are
homozygous for the null *4 allele (frequency ~12-21%)\textsuperscript{8,9,13}. Indeed, because their studies were all conducted in Northern Europe on primarily Northern European subjects, the *4/*4 genotype was probably responsible for the poor metabolizers characterized in the work of Smith, Sjöqvist, Eichelbaum, and Dengler\textsuperscript{6,8,9}.

Genotypic and phenotypic frequency information for CYP2D6 has now been ascertained by researchers for most of the world’s populations\textsuperscript{9}. Chronologically, the test to determine an individual’s phenotypic status (either PM, IM, EM, or UM) was developed first and involves establishing an individual’s metabolic ratio (or MR, where MR is equal to the concentration of a substance over the concentration of a selected metabolite) for a given drug metabolized by CYP2D6\textsuperscript{6}. Discreet cut-off values for MR are used to unambiguously distinguish between the four phenotypic groups\textsuperscript{6}. Genotyping an individual for the CYP2D6 locus is somewhat more complicated and involves two independent steps: determining the number of copies of CYP2D6 that a person carries and determining which mutations (SNPs and indels) are present within the gene\textsuperscript{11,14,15}. The latter step is now relatively straightforward and can either be done by sequencing the entire gene, using flanking primers, or can be expedited by genotyping particular mutations individually, using, for instance, a Taqman Assay\textsuperscript{11}. The former step has proved more challenging and some techniques have been questioned with respect to their reliability and accuracy\textsuperscript{11}. Originally, gene copy number was determined by long-range PCR across and gel electrophoresis\textsuperscript{11}. Now this assay has been replaced by other procedures like RT-PCR and more specialized techniques like pyrosequencing, where amplification of a small region of CYP2D6 is coupled to an enzymatic reaction that can be quantified\textsuperscript{11,14,15}.

Despite the fact that phenotyping and genotyping tests exist for the CYP2D6 enzyme, their use in clinical practice remains rare\textsuperscript{16}. Indeed, one survey of laboratories providing genetic testing services in Australia and New Zealand determined that out of the 507 facilities sampled, just six provided clinical phenotyping for CYP2D6 while only three provided clinical genotyping\textsuperscript{16}. Multiple factors contribute to the limitation of CYP2D6 testing in practice. First of all, as long as testing remains relatively rare in the clinical setting, the cost of electing to test an individual patient will be high for the individual, his insurance, or his state-provided health care coverage\textsuperscript{16}. Secondly, it is
unclear whether phenotyping or genotyping is the best avenue for estimation of an individual’s CYP2D6 enzymatic activity\cite{16}. Direct ascertainment of MR is vulnerable to quantification errors and day-to-day variations in CYP2D6 function, whereas genotyping provides a static assessment of the unchanging basis for CYP2D6 activity\cite{16}. However, many alleles of CYP2D6, particularly rare alleles, have not yet been characterized with respect to enzymatic function, and so provide no phenotypic information\cite{6,13}. Lastly, since the 1970’s, drug companies have been actively avoiding the development of drugs that are metabolized by CYP2D6 precisely because of its polymorphic nature\cite{9,17}. Thus, the total number of such drugs on the market has steadily declined\cite{9,17}. However, with the development of tests that can reliably genotype and phenotype patients for CYP2D6, this trend may ultimately reverse itself, given the number of potential drugs that would become available for development if no restrictions were placed based on CYP2D6 metabolism\cite{17}. The fact that genetic sequencing and genotyping assays are, in general, continually decreasing in cost will only add to this imputus\cite{17}. Thus, one day, genotyping (or phenotyping) for CYP2D6 may be one of a host of genetic tests performed on a patient over the course of his or her lifetime as part of standard health care procedures\cite{17}.

References


