DETECTING POLYMORPHISMS IN DRUG METABOLISM GENES – TAQMAN® DRUG METABOLISM ASSAY (DME)

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Abstract

Drug metabolizing enzymes (DMEs) are proteins involved in the biotransformation, metabolism, and/or detoxification of endogeneous and foreign compounds (for example, naturally occurring compounds like prostaglandins, drugs and environmental agents). Genes that code for these enzymes are often referred to as »DME genes«. Polymorphisms in the DME genes may influence the rate of foreign compound metabolism and/or excretion among individuals, thereby potentially affecting foreign compound efficacy and/or toxicity. Many polymorphisms within the genes of drug-metabolizing enzymes (DME) have been shown to alter drug responses in individuals. Pharmacogenetics, (understanding the affect of drug response in individuals), has become an area of focus in both basic research and drug development. Polymorphisms within drug metabolism pathways have been difficult to study due to the complexity of genetic information associated with these genes. Many of the genes within these pathways are part of large gene families that include several pseudogenes, thus generating large homology barriers that are difficult for most technologies to overcome. To overcome these barriers, Applied Biosystems has developed an extensive collection of TaqMan® Genotyping Assays to detect single nucleotide polymorphisms (SNPs), multiple nucleotide polymorphisms (MNPs), and insertion/deletions (in/dels) within 220 drug metabolism genes. A comprehensive catalog of > 3,000 protein-coding SNPs for 220 DME-related genes was created using the data from a variety of proprietary and public sources, including several prominent allele nomenclature. The results enable the design of a DME genotyping platform for researchers to screen for these polymorphisms SNPs, MNPs, in/dels and STRs. Gene deletion and gene duplication are genomic variations that can also affect protein function or phenotype. Deletions and duplications in drug metabolism genes have been associated with phenotypic variation, for example, they can characterize a phenotype as poor (PM), intermediary (IM), extensive (EM), and ultra-rapid metabolizer (UM). Each assay was wet-lab tested on 180 DNA samples from 4 human populations, (Caucasian, African American, Chinese, and Japanese) to estimate the minor allele frequency in each of these populations and all assays were checked for Hardy-Weinberg Equilibrium. During the performance testing of the assays, many of the genes in this collection were identified as having unique characteristics, for example, gene deletions or amplifications (GSTM1 and CYP2D6) that can affect the interpretation of results from SNP genotyping assays. It is important to understand the underlying biology of particular genes when interpreting results from genotyping experiments and when evaluating how well a particular genotyping technology performs. The examples of drug metabolism enzymes are the cytochrome P450 gene super-family and alcohol dehydrogenase gene family. Four members of the P450 super-family CYP3A, CYP2D6, CYP2C19 and CYP2C9, account for almost 50 % of metabolism of commonly used drugs. A large number of commonly prescribed drugs like antidepressants and cardiovascular drugs are metabolized by the well-studied cytochrome P450 2D6 (CYP2D6) enzyme. For example there are 78 known variants of the CYP2D6 gene. Some of the variations result in a complete loss of enzymatic activity while some of the variants only reduce activity. The gene exhibits polymorphism and frequencies of the alleles in various populations vary widely. Depending on the combinations of CYP2D6 alleles present, individuals may be classified as poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM) and ultra-rapid metabolizer (UM) of CYP2D6 substrates. PMs carry enzyme-inactivating polymorphisms, resulting in high concentrations of parent drug in plasma. In the case of UM, gene duplications may lead to increased enzyme activity, resulting in reduced parent drug concentration in blood stream. Dosage recommendations, to avoid both side effects and therapeutic failure therefore depend on CYP2D6 genotype for CYP2D6 substrates. In the case of study CYP2D6 genotype Applied Biosystems has developed a 26 different TaqMan® Genotyping Assays to detect different variants of the CYP2D6 gene.

Finally a DME genotyping platform would allow researchers to screen for these polymorphisms and may serve as an aid to determine individualizing treatment choice.