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Can personalized drug therapy be achieved? A closer look at pharmaco-metabonomics

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Between 1930 and 1990, several dozen high-penetrance, predominantly monogenic disorders were identified and characterized, which led some investigators to speculate that individualized drug treatment was just around the corner. Informative DNA tests were sought to determine genetic predisposition to toxicity and cancer, thereby identifying individuals in which a drug was likely to be effective and those at increased risk of drug toxicity. These assays represent the leading edge of phenotype–genotype association studies, which are a major goal of clinical pharmacology and pharmacogenomics. Because of the complexity of the genome, however, the task is more challenging than anticipated originally. In the past decade we have come to appreciate how difficult it is to determine unequivocally either an exact phenotype or genotype. In the near future it seems unlikely that, by themselves, either transcriptomics or proteomics will be particularly helpful in achieving individualized drug therapy. However, recent advances in metabonomics are exciting and show promise. In the future, and perhaps in combination with proteomics, metabonomics might complement genomics in achieving personalized drug therapy.

The quest for individualized drug therapy: pharmacogenomics

In the best of all possible worlds, before administering a drug, clinicians would ascertain what dose in each and every patient would provide maximum efficacy and what dose would cause toxicity. Because this ideal is presently unattainable, however, the general rule for dosing patients in clinical pharmacology remains: 'start low, go slow.'

Previously, we have described shortcomings of various pharmacogenomic tests in attaining individualized drug therapy [1,2]. For example, thiopurine methyltransferase (TPMT) is a phase II enzyme that detoxifies purine antimetabolites. The frequency of the strong 'monogenic high-penetrance' genotype (see Glossary) in a Caucasian population is ~89% *TPMT(high/high)* homozygotes, ~11% *TPMT(high/low)* heterozygotes and ~0.36% *TPMT(low/low)* homozygotes; it is now known that the commonly prescribed dose of 6-mercaptopurine leads to a cure in *TPMT(high/low)* children with acute lymphocytic leukemia, whereas *TPMT(high/high)* patients require ~4-fold higher doses and *TPMT(low/low)* patients are best treated with 10–15-times less drug [3]. However, in a retrospective study in 12 hospitals that correlated thiopurine-related drug toxicity with the *TPMT* genotype [4], >78% of adverse drug reactions were associated with factors other than the *TPMT*-gene polymorphism.

Another example is the polymorphism in the gene that encodes cytochrome P450 2D6 (CYP2D6) [1]. The human genome contains 57, putatively functional, *CYP* genes [5] that encode phase I enzymes involved in oxidative and reductive reactions. However, <12 CYP enzymes, mostly members of the CYP2 and CYP3 families, are responsible for the metabolism of virtually all drugs [6]. The CYP2D6 monooxygenase, which has been studied thoroughly, metabolizes, to some degree, 25–33% of all commonly prescribed drugs [7]. Over 75 variant alleles of the *CYP2D6* gene have been detected (http://www.imm.ki.se/CYPalleles/). Therefore, unless every variant site in the genome that affects *CYP2D6* expression is tested, it is difficult to conclude that a patient is a poor, intermediate, efficient, or ultra-rapid metabolizer. In summary, it is virtually impossible at the present time to ascertain an unequivocal genotype in phenotype–genotype association studies of human populations because of the complexity of the genome [1,2]. Studying groups of subjects can determine trends, odds ratios and relative risks; however, a DNA test cannot provide absolute certainty in predicting drug responses for individual patients.

A recent example of a shortcoming in pharmacogenomic testing involves *CYP1A2*, which encodes an enzyme that participates in the metabolism of almost two dozen drugs (e.g. tacrine, caffeine, theophylline, erythromycin, propranolol, naproxen and verapamil) and many environmental procarcinogens. In the human populations studied, there is a >60-fold variability in activity of CYP1A2 in the liver [8,9]. At least 33 allelic variants have been reported, which have different levels of either basal activity or inducibility (http://www.imm.ki.se/CYPalleles/). One DNA-variant site in intron 1 of the *CYP1A2* gene is presumed to be responsible for differences in CYP1A2 activity in several clinical

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Glossary

Correlation coefficient (*r*): sometimes called 'coefficient of correlation'. A measure of the interdependence between two random variables that ranges from −1.0 (perfect negative correlation) to +1.0 (perfect positive correlation). $r = 0$ indicates an absence of correlation. r^2 gives the predictive value of a correlation between two random variables. For example, a correlation coefficient r of 0.80 gives a predictive value (r^2) of 0.64 (64% success rate).
Electrospray ionization MS (ESI-MS): one of the two most popular ionization techniques in the MS field. The electrospray is produced by applying a strong electric field to a capillary with liquid flowing through it, resulting in small charged droplets that are eventually decreased to singly charged droplets. ESI is a soft ionization technique that produces multiply charged ions from large molecules.
Genotype: any change in DNA. The genetic constitution, either at one specific locus or more generally. In the general sense, genotype is essentially synonymous with genome.
Mass spectrometry (MS): analytical method in which chemical substances are identified by using electric and magnetic fields to sort gaseous ions by mass. MS is used widely to measure the masses and the relative abundance of different isotopes, and to analyze products of separation by liquid (LC) or gas (GC) chromatography. MS is also used to measure the geological age of minerals.
Matrix-assisted laser desorption ionization MS (MALDI-MS): one of the two most popular ionization techniques in the MS field. The solid sample of interest is ablated with a laser, creating a plume of ions and neutral molecules. MALDI is a pulsed ionization technique that produces singly charged ions and is widely used for the analysis of proteins.
Monogenic: pertaining to a single gene.
Odds ratio (OR): a measure of the degree of association. For example, the odds of having an adverse drug reaction among the cases, compared with that among the controls. The OR can be either <1.0 or >1.0. An OR > 1.0 indicates a positive relationship.
Orthogonal acceleration tof-MS (oa-tof-MS): a refinement of tof-MS in which acceleration of the material occurs at right angles. This provides a mass-to-charge range $\leq$12 000. Many compounds acquire multiple charges, however, enabling analysis of high-mass proteins (100 kDa).
Penetrance: in a given population, the proportion of individuals with a defined genotype who manifest a particular trait.
Phenotype: a trait. Any observable (biochemical, physiological, morphological and behavioral) characteristic of an organism. In clinical pharmacology this might include a specified plasma level of drug within X hours after taking a dose of Y mg kg^{-1} body weight.
Phenotype–genotype association studies: an attempt to correlate a given phenotype with a specific DNA change in a defined population.
Procarcinogens: chemicals that require metabolic activation to cause cancer.
Projection-to-latent-structure (PLS): a model of control strategy in which several linear and nonlinear steady-state techniques are used to build empirical models to determine the most relevant variables.
Proton-coupled nuclear magnetic resonance (^{1}H-NMR): the resonance of protons to radiation in a magnetic field. The nuclei of atoms absorb electromagnetic radiation in the radio frequency region (~4–900 MHz) when placed in an intense magnetic field; this technique enables the elucidation of the structures of chemical species and the quantitative determination of absorbing species.
Relative risk (RR): the ratio of the risk of developing, within a specified period of time, an adverse drug reaction in individuals taking the drug, compared with those not taking the drug. An individual with an RR value of 1.6 indicates a 60% increased risk of drug reaction (or other disease or trait) compared with someone not taking the drug or without the disease or trait.
Tandem MS (MS/MS): an MS method involving (at least) two stages of mass analysis; this can be in conjunction with a dissociation process or a chemical reaction that causes a change in the mass or charge of the ion. Most commonly, a precursor ion is isolated by a first mass analyzer and subsequently fragmented to yield products and neutral fragments that are then analyzed by a second spectrometer. MS/MS can exist either in space (using two distinct instruments) or in time (using a sequence of events in an ion storage device). This method is useful for the determination of structures, fragmentation mechanisms and composition determinations.
Time-of-flight MS (tof-MS): a mass spectrometer that makes use of the fact that ions of different mass:charge ratios (*m/z*), and all with the same initial translational energy, require different times to traverse a given distance in a field-free region (i.e. no electric or magnetic fields for accelerating or directing the beam). This time will depend on the mass of the particle (i.e. heavier particles reach lower speeds).

studies, but transcription factors do not bind to this segment of DNA and the mechanism responsible for this difference in activity is unknown. A recent study of 280 non-smokers [10] that analyzed DNA samples for informative single-nucleotide polymorphisms in ~40 kb of the *CYP1A1_CYP1A2* locus concludes that, currently, DNA testing to predict the CYP1A2 extensive-metabolizer or poor-metabolizer phenotype in individual patients is not informative. The unequivocal prediction of drug response is also not possible for other genes that encode either drug-metabolizing enzymes or transporters [1,2].

The quest for individualized drug therapy: transcriptomics

Transcriptomics, the study of gene transcripts, is analyzed usually using cDNA expression microarrays. Such cDNA expression studies have led to several breakthroughs. For example, microarray analyses of some tumors have correlated a particular gene-expression pattern with patient prognosis, responsiveness to either hormones or therapy, and beneficial effects of other drugs [11]. Advances in data mining and statistical analysis of expression microarrays [12,13] continue to increase the accuracy of this methodology. However, in the foreseeable future, transcriptomics alone is not expected to have a major role in individualizing drug therapy, principally because of the major limitation of sources for performing microarray analyses, such as blood, excreta (e.g. urine and feces) and tissue that contains the relevant cDNA (e.g. surgical biopsy of tumor, other tissue biopsies, placenta and foreskin). For example, in healthy individuals it is both unreasonable and unethical to attempt to predict drug responses from biopsies of either brain or liver.

The quest for individualized drug therapy: proteomics

Proteomics is the study of all the proteins that are encoded by the genome [14–16]. In humans, it is estimated that, on average, each gene encodes three proteins [17] but the true number might be considerably higher. Proteomics has the same limitations of assay sources as transcriptomics. In the future, proteomics, like metabonomics, might enable investigators to identify certain protein profiles that predict either efficacy or adverse drug reactions. During the next decade, we expect pharmacogenomics to be complemented by both transcriptomics and proteomics.

Pharmaco-metabonomic phenotyping and personalized drug treatment

A recent study [18] shows that a combination of pre-dose metabolite profiling and chemometrics can predict the outcome for individual subjects. The study compared 65 adult male Sprague-Dawley rats, orally intubated with one large dose (600 mg kg^{-1}) of paracetamol (acetaminophen), with ten control male Sprague-Dawley rats dosed orally with vehicle only. Individual urine samples were collected pre-dose (−48 h to −24 h) and post-dose (0 h to +24 h) (Figure 1), and blood plasma samples were drawn at +24 h for clinical chemistry. Ten representative slices of each liver were given a 'mean histology score' of the severity of damage (least severe, class 1; intermediate, class 2; most severe, class 3). Urinary metabolite profiles were measured by proton-coupled ^{1}H-nuclear magnetic resonance (NMR) and analyzed by multivariate modeling.

The hypothesis [18] was that 'the pre-dose metabolite profile of an individual rat contains sufficient information