The role of pharmacometabonomics in predicting drug pharmacokinetics

“...pharmacometabonomics is highly complementary to pharmacogenomics and therefore the integration of the two technologies will be both powerful and could provide more insight into mechanisms underlying individual variation in drug response.”

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Personalized or stratified therapy is a key goal for 21st century medicine in order to maximize therapeutic efficacy and minimize the likelihood of adverse drug reactions for groups of patients. In addition, personalized medicine has the potential to substantially enhance the process of drug discovery and development [1]. Thus far, personalized medicine has been mainly based on pharmacogenomics (PG), where an individual's genetic profile is used to predict the clinical outcome of drug treatment [2]. There are now numerous PG studies associating human genetic polymorphisms with drug effects. The best recognized examples are genetic polymorphisms of drug-metabolizing enzymes such as CYP450 isoenzymes, *N*-acetyl transferases, sulfotransferases and glucuronosyltransferases [3]. Although genetic variation is a well-recognized factor contributing to drug-response variability, the achievement of 'individualized drug therapy' for a wide range of diseases is unlikely using genomic knowledge alone. This is because interindividual variation in drug response is influenced by the complex interplay between genetic and environmental factors including nutritional status, lifestyle, age, gender, diseases, gut microbiota, the exposome [4] and co- or preadministration of other drugs. These factors can significantly impact the pharmacokinetic (PK) characteristics of a drug including the processes of absorption, distribution, metabolism and excretion and thereby cause interindividual variation in drug effects.

In an alternative but complementary approach, pharmacometabonomics has now been applied to predict the efficacy, safety, metabolism and PK of drugs. Pharmacometabonomics is an extension of metabolomics [5] and is defined as *“the prediction of the outcome (for example, efficacy or toxicity) of a drug or xenobiotic intervention in an individual based on a mathematical model of preintervention metabolite signatures”* [6]. In a pharmacometabonomics study, the metabolite profiles in predose biofluid samples (typically urine or blood plasma) from a group of subjects are analyzed by technologies such as nuclear magnetic resonance spectroscopy or mass spectrometry (MS), the latter typically hyphenated with a separation technology. Multivariate statistical analysis methods, such as principal components analysis or partial least squares (PLS), are then used to analyze the predose metabolite profiles to discover statistically significantly different subgroups of subjects whose postdose outcomes, such as levels of efficacy or degree of drug metabolism, are different from those of other subgroups [7]. Preferably, the predictive model is created on a training set of subjects and then tested in an independent, 'external' validation set.

Pharmacometabonomics was initially discovered in a study on a group of 75 Sprague–Dawley rats [6]. It was shown that predose urinary metabolite profiles could be used to predict the metabolism and the hepatotoxic

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effects of the analgesic paracetamol. Subsequently, this approach was demonstrated in humans in a study of the metabolism of the same drug paracetamol [8] and a clear relationship was found between the volunteers' predose, urinary, endogenous metabolite profiles and the postdose metabolism of the drug. Nuclear magnetic resonance-based analysis revealed that human volunteers excreting relatively large amounts of the microbial co-metabolite *para*-cresol sulfate in their predose urines tended to have a lower ratio of paracetamol sulfate to paracetamol glucuronide in their postdose urines than individuals with low amounts of predose urinary *para*-cresol sulfate [8]. *Para*-cresol-sulfate is a metabolite produced from the hepatic sulfonation of *para*-cresol, which itself is generated by gut bacteria, particularly *Clostridium* species. Paracetamol and *para*-cresol have similar molecular structures and both compete for sulfonation via the same human sulfotransferase enzymes, particularly SULT1A1 [9,10]. This study demonstrated that the sulfonation of paracetamol (and potentially any other drug) can be restricted by competition from *para*-cresol, and also demonstrated the critical role of the gut microbiome in human drug metabolism.

Since its initial discovery, pharmacometabonomics has been increasingly applied in both preclinical and clinical studies to predict drug safety, efficacy, metabolism and PKs [11]. The potential of pharmacometabonomics in predicting the PK profile of a drug was first demonstrated in a study by Yoon *et al.* for the commonly used immunosuppressive drug, tacrolimus [12]. The efficacy of this drug is associated with a narrow therapeutic index combined with a large degree of variability in patient blood levels. Therefore, it is important to predict the PK of tacrolimus in order to minimize adverse drug reactions. Tacrolimus was administered to 29 healthy, Korean males (75 µg/kg, oral) while controlling food intake and environmental conditions. LC–MS analysis of the predose urines of these volunteers resulted in detection of 1256 ions, among which 42 key metabolic features were shown to be closely correlated with the drug's PK in terms of the AUC. Using LC–MS/MS along with database searching, 28 metabolites were identified and subsequently used to reconstruct a hypothetical metabolic network. To generate a more clinically applicable model, four metabolites (cortisol, acetyl-arginine, phosphoethanolamine and 1-methylguanosine), with high contributions to the PLS model and representing four major metabolic pathways, were selected for predicting the AUC of tacrolimus. The new model successfully classified individuals into high, medium and low AUC groups. This study demonstrated the potential of pharmacometabonomics to predict PK. The authors also anticipated that this approach could be used in

clinical settings to predict PK of tacrolimus in transplantation patients.

Kaddurah-Daouk *et al.* used an LC–MS approach to show that predose plasma levels of the bile acids, chenodeoxycholic acid and deoxycholic acid, were correlated to post-treatment simvastatin levels in a broader study using pharmacometabonomics methodology to predict statin efficacy [13]. These authors have also developed a pharmacometabonomics-informed PG approach that is anticipated to be important in these predictive studies in the future [14].

Liu *et al.* applied pharmacometabonomics to predict the PK characteristics of triptolide in male Sprague–Dawley rats [15]. Triptolide is a major bioactive diterpenoid triepoxide that possesses a variety of anti-inflammatory, immunosuppressive and antitumor properties and has been used for centuries in traditional Chinese medicine for the treatment of immune-related diseases [15]. The clinical application of triptolide, however, is restricted by its narrow therapeutic index and high toxicity. Groups of rats were treated with one of three diets: normal, calorie-restricted or high fat diet, and then administered triptolide (0.60 or 1.80 mg/kg oral). GC–MS analysis of the predose serum detected 267 metabolite ions, of which 85 were identified. Multivariate regression analysis showed that the predose serum concentrations of creatinine and glutamate were linearly negatively correlated to postdose triptolide plasma maximal concentration (C_{max}) and AUC values. This study hypothesized that glutamate was significant as it is a critical precursor of glutathione, which is involved in conjugation and detoxification of triptolide and the levels of which are known to be affected by diet. It was concluded that the study results may also have translational value for human PK prediction, as creatinine and glutamate are readily measured.

The same group employed GC–MS analysis of predose plasma to predict the PKs of atorvastatin (oral, 20 mg/kg) in 48 healthy volunteers hospitalized at clinical research units with strict control over diet and environment [16]. Atorvastatin is an HMG–CoA reductase inhibitor that is generally used to lower levels of low-density lipoprotein cholesterol in plasma and reduce the risk for coronary artery disease. The PKs of atorvastatin vary considerably between individuals and hence its therapeutic efficacy is also variable [17]. The initial PLS multivariate analysis, conducted on 181 measured metabolite ions and 16 physiological and biochemical parameters from individuals in a training set ($n = 36$), revealed 63 and 57 variables, which were correlated with atorvastatin C_{max} and AUC, respectively. Subsequently, sets of 17 and 12 key metabolites with high contributions to the initial PLS model and significant correlation to PK parameters, were selected to construct a refined

model that could predict individualized $C_{\max}$ and AUC, respectively. This refined model allowed the prediction of the PK parameters of 12 other healthy volunteers in a validation set (with correlation coefficients of $r = 0.83$ for $C_{\max}$ and $r = 0.87$ for AUC) and could also successfully classify individual PK responses into subgroups, which is a promising development for the field.

The group of Barin-Le Guellec and co-authors recently reported the use of GC–MS-based pharmacometabonomics to predict the clearance of methotrexate (MTX) in a cohort of 62 adult patients being treated for lymphoid malignancies [18]. Variable PK of MTX is known to be responsible for serious patient toxicities, even death, and overexposure can occur even in the same patient between MTX treatment courses, thus indicating that genetic factors *per se* are not responsible for all of the variability observed [18]. In a well-designed study utilizing internal and external validation of the models, the predose urine levels of 28 metabolites were shown to be predictive of MTX clearance with mean prediction error and precision of 0.4 and 21%, respectively. An orthogonal PLS discriminant analysis model showed a partial separation between patients with normal or delayed MTX elimination and while the specificity was excellent (93%), sensitivity was poor (42%) and model improvements would be required for clinical utility of this element. The model for the prediction of MTX clearance is, however, expected to have clinical utility and also gave insights into the underlying mechanisms of MTX excretion, including the role of organic anion transporters [18].

Pharmacometabonomics, though still an emerging technology, has shown significant promise in the prediction of drug efficacy, safety and metabolism, in addition to the prediction of PK. Around 20 studies of pharmacometabonomics in humans have now been reported [19] in addition to preclinical studies. The advantages of this technology are several:

- It inherently takes into account both genetic and environmental influences in its predictive models;
- The predose metabolic profiles that are used to predict drug response reflect the actual physiological status of the patient or subject at that point in time, in contrast to PG, where a patient's static genetic profile may or may not translate into differences in drug response; and
- Longitudinal measurements of metabolic profiles are also possible, enabling monitoring of patients over time and using this information to make improved drug response predictions [20].

The existing studies clearly demonstrate the potential of pharmacometabonomics to help predict variation in PK and thereby facilitate the delivery of personalized drug therapy. Further investigations will be required to validate these early, pioneering findings and demonstrate the broader utility of this approach for the general patient population and also to monitor and predict optimal longer term treatments [20]. It is also clear that pharmacometabonomics is highly complementary to PG, and therefore the integration of the two technologies will be both powerful and could provide more insight into mechanisms underlying individual variation in drug response [14].

Financial & competing interests disclosure

JR Everett is a co-inventor on a granted patent on pharmacometabonomics. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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