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The Promises of Quantitative Proteomics in Precision Medicine

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Abstract

Precision medicine approach has a potential to ensure optimum efficacy and safety of drugs at individual patient level. Physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) models could play a significant role in precision medicine by predicting interindividual variability in drug disposition and response. In order to develop robust PBPK/PD models, it is imperative that the critical physiological parameters affecting drug disposition and response are precisely characterized along with their variability. Currently used PBPK/PD modeling software, e.g., Simcyp and Gastroplus, encompass information such as organ volumes, blood flows to organs, body fat composition, glomerular filtration rate, etc. However, the information on the interindividual variability of the majority of the proteins associated with PK and PD, e.g., drug metabolizing enzymes (DMEs), transporters and receptors, are not fully incorporated into these software. Such information is significant because the population factors (e.g., age, genotype, disease and gender), can affect abundance or activity of these proteins. To fill this critical knowledge gap, mass spectrometry (MS)-based quantitative proteomics has emerged as an important technique to characterize protein abundance of DMEs, transporters and receptors across the population. Integration of these quantitative proteomics data into *in silico* PBPK/PD modeling tools will be crucial toward precision medicine.

Introduction

In 2013 alone, the US FDA Adverse Event Reporting System (FAERS) database documented a total of 711,232 adverse drug events in the United States including 117,752 instances of death (<http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Surveillance/AdverseDrugEffects/>). Many of these cases are associated with medication error; however, under-predicted and uncharacterized interindividual variability in drug disposition, i.e., absorption, distribution, metabolism, excretion (ADME) and response has been an important reason for the adverse drug events. The President, Barack Obama recently launched the Precision Medicine Initiative to encourage research that takes into account interindividual variability in drug response to ensure optimum drug safety and effectiveness¹. The variability in drug disposition due to the effect of age, genetics/epigenetics, disease condition and gender (Fig. 1), could be a cause of either adverse drug reactions or a lack of therapeutic effect. However, it is neither ethically nor logistically

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feasible to perform clinical trials to determine drug pharmacokinetic (PK) and pharmacodynamic (PD) at individual patient level. Therefore, to achieve the goals of precision medicine, it is essential to predict interindividual differences in drug disposition and response using alternate approaches. The physiologically based PK and PD (PBPK/PD) modeling that relies on the use of activity or abundance of specific proteins associated with drug disposition and the response has a great potential to predict these processes. For example, PBPK modeling has been recently used to successfully predict PK of drugs in the populations where clinical trials are not often feasible, e.g., children, pregnant women, diseased population, etc. (Table 1)²⁻⁹. To this end, determination of variability in the fraction of a drug metabolized or transported by individual pathways (i.e., f_m or f_t) is important for the development of generic population-based PBPK models.

The conventional *in vitro* methods to characterize f_m and f_t are based on low throughput activity or protein quantification (Western blotting) methods. While these methods are specific for the major phase I drug metabolizing enzymes (DMEs), the probe substrates or antibodies used for other DMEs and transporters are non-selective. In order to resolve this limitation, targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) proteomics is now recognized as a gold-standard protein quantification technique⁹⁻²⁰. In general, the methodology and applications of targeted proteomics in ADME research are discussed in elsewhere¹⁸⁻²¹. For example, Ohtsuki et al. presented the technical features of quantitative proteomics, summarized its advantages and discussed the importance of the technique in the evaluation of species differences of blood-brain barrier (BBB) protein levels in human, monkey, and mouse²². Uchida et al. also discussed quantitative proteomics method and its application in determining BBB transporters and inter-strain differences^{18,21}. Further, translation of quantitative protein data for *in vitro-in vivo* extrapolation (IVIVE) of drug disposition is also demonstrated recently²³. Therefore, the scope of the present commentary is to specifically highlight the importance of quantitative proteomics to characterizing the impact of factors affecting interindividual variability (e.g., age, genetics, epigenetics, disease condition and gender), relevant to the implementation of precision medicine concept. The quantitative proteomics is applicable for this purpose because of the following reasons: 1) protein quantification in banked human tissues provides a non-invasive option of predicting interindividual variability without conducting clinical trials; 2) the approach allows fast, selective and reproducible estimation of variability which can be readily assimilated into PBPK models; and, 3) the need of small sample size makes this technique applicable to small biopsy samples.

Quantitative proteomics data can be used as a critical predictor of protein activity

While the mechanism of drug binding with DME or transporter could be complex^{24,25}, such interaction is often presented by a simple clearance (CL) equation shown below (Eq. 1). K_m in the equation indicates affinity of the drug for the target and V_{max} is the maximum velocity of the kinetic reaction. Generally, V_{max} is the main variable in the CL equation that determines interindividual variability primarily because of its dependence on the protein expression [E] (Eq. 1). This implies that protein expression based inter-system scaling

factors (ISEF) can be used as a surrogate of differences in the protein activity in populations (Eq. 2).

$$CL = \frac{V_{max}}{K_m} = \frac{[E] \cdot K_{cat}}{K_m} \quad (\text{Eq. 1})$$

$$CL_{\text{population 1}} = \frac{E_{\text{population 1}}}{E_{\text{population 2}}} \times CL_{\text{population 2}} \quad (\text{Eq. 2})$$

K_{cat} (Eq. 1) is the turnover number which is defined as the number of substrate molecule each enzyme or transporter site converts to product or transport per unit time. The above model is based on the assumption that protein activity directly correlates with the expression. In the case of drug metabolism, correlation of activity of drug metabolizing enzymes (DMEs) with protein expression is well established^{26–30}. However, whether drug transporter expression correlates with activity depends on the proper localization of the protein in the plasma membrane. Such correlation is not well characterized except for a few key transporter proteins summarized below. We recently demonstrated using gene knockdown of OATP1B1 and BCRP *in vitro* that transporter protein expression correlates with protein activity¹¹. At *in vivo* level, an impact of genetic polymorphism of OATP1B1 on the PK of its substrate, repaglinide, was accurately predicted using proteomics data⁹. Recently, Sakamoto et al. also reported a significant correlation between the kinetic parameter V_{max} for OCTN1 and MRP1 substrates with the protein expression in the plasma membrane of tracheal, bronchial, and alveolar cells^{15,16}. While the above simplistic model to predict functional activity does not consider other catalytical factors such as cooperative binding for the drug substrate²⁵ and protein localization (for transporters)¹¹, the above examples support the hypothesis that protein expression of both DMEs and transporters is one of the most critical predictors of variability in the functional activity.

Conventionally, immunoaffinity methods have been used to quantify DMEs, transporters and receptors^{31,32}. But since some of these proteins are homologous - e.g., CYP3A4, CYP3A5, and CYP3A7 in humans, and Mdr1a and Mdr1b in rat - it is often not possible to distinguish them by antibody-based methods. In addition, some of these proteins (especially transporters) have transmembrane structures, which makes it challenging to develop selective antibodies for these proteins. Therefore, alternative methods are needed for the reproducible quantification of proteins associated with drug disposition. LC-MS/MS, also referred as selective reaction monitoring (SRM) proteomics, is one such novel approach which was recently recognized as the method of the year³³. SRM protein quantification relies on selective quantification of surrogate peptide(s) in a digested protein sample (Fig. 2), where two stages of mass selection, i.e., selection of a precursor ion and monitoring of specific production(s), provides the highest specificity. Multiple proteins can be quantified from a small volume of sample. The latter makes this method suitable for the characterization of inter- and intra-individual variability of multiple proteins simultaneously. A detailed and optimized approach of protein quantification by SRM proteomics is reported elsewhere¹⁴.

With respect to pharmacodynamics (PD), precision medicine approach is often applied in the management of cancer because of the narrow therapeutic window of anti-cancer drugs. Transcript^{34,35} and protein^{36,37} levels of human equilibrative nucleoside transporter (hENT1) to predict gemcitabine efficacy against pancreatic cancer are used as a clinical marker of the drug response. While mRNA quantification is simple, poor correlation between protein expression/activity and mRNA expression is often observed because of the poor mRNA stability in tissues or posttranscriptional variability. This means that the technical variability associated with mRNA quantification could be a major confounding factor in the determination of interindividual variability. Moreover, non-synonymous SNPs can lead to differences in the protein stability leading to poor transcript and protein correlation. Therefore, protein quantification is a better surrogate of the *in vivo* activity. Correlation of ^{99m}Tc-Sestamibi uptake with Western blot analysis has been shown to demonstrate P-glycoprotein (P-gp) mediated mechanism of drug resistance *in vitro*³⁸. Nie et al. used quantitative proteomics to discover potential serum glycoprotein biomarkers that distinguish pancreatic cancer from other pancreas related conditions (diabetes, cyst, chronic pancreatitis, obstructive jaundice) and healthy controls³⁹. While applications of MS proteomics to characterize interindividual differences in drug efficacy and resistance are also becoming increasingly available in the literature, the following section primarily focuses on the application of the technique to characterize PK related interindividual variability.

Effect of age on the expression of DMEs and transporters

It is practically challenging to conduct clinical studies on children. Therefore, it is desirable to use protein expression data to predict differences in drug disposition amongst neonates, infants, children, adolescents and adults. In this direction, while ontogeny data on protein expression are available for the major Cytochrome P450 (CYP) enzymes, data for many other DMEs and drug transporters are scarce^{40–42}. While these *in vitro* ontogeny data are valuable, much of the data were obtained with non- (or partially)-selective antibodies^{40–42}. Further, only mRNA data are available for few phase II enzymes such as UGTs^{43,44}. The corresponding data for other DMEs (e.g., AOXs, CYP1As^{45,46}) are based on non-selective substrates. Most of these *in vivo* and *in vitro* studies have not considered the potential confounding effect of genetic polymorphism. Transporter ontogeny data are available at mRNA and protein levels but limited to hepatic transporters^{40,47,48}. In the liver, OCT1, OATP1B3 and P-gp are the main transporters affected by the age⁴⁸. Considering these limitations, it is critical for the development of pediatric PBPK models that robust, selective and absolute ontogeny data on DMEs and transporters are obtained. SRM proteomics would allow simultaneous quantification of multiple proteins using small initial tissue sample from pediatric donors. Such information would be valuable for the development of fully mechanistic pediatric PBPK models that will allow integration of age-dependent dynamic profiles of multiple pathways responsible for drug disposition.

SRM proteomics can be used to further understand mechanisms underlying ontogenic expression. The technique was recently used to simultaneously quantify the influence of gut microbiome on the ontogeny of mice hepatic ADME proteins, Cyp2b9, Cyp3a11, Cyp4a10, Ntcp, Abcg5, and Abcg8⁴⁹. The proteomics data showed good correlation with the Western

blot data and the activity, and confirmed the impact of the microbiome on the regulation of ontogeny of these proteins⁴⁹.

Genetic polymorphism affecting protein expression

Genetic polymorphism can either affect protein expression, substrate affinity (K_m) or protein localization^{12,27,50–53}. However, the genetic mechanisms frequently affect protein expression as illustrated in Fig 3, by the promoter mutation, insertion of a new stop codon in an exon, gene-deletion and duplication, loss of start codon and mRNA splicing or protein degradation. We recently utilized SRM proteomics to determine the magnitude of the effect of the genetic polymorphism on CYP2C19 protein expression with a robust correlation ($r^2=0.984$) between CYP2C19 protein expression and the (S)-mephenytoin hydroxylase activity²⁷. Using a linear trend analysis, the rank order of enzyme protein level and activity for the common diplotypes (CYP2C19*17/*17 > *1B/*17 > *1B/*1B > *2A/*17 > *1B/*2A > *2A/*2A) was highly significant ($P<0.0001$). Similarly, the utility of SRM proteomics to characterize the effect of genotype/haplotype on OATP1B1⁹, BCRP¹² and MRP2¹⁰ was demonstrated. Further, the haplotype-dependent OATP1B1 protein expression data successfully predicted clinical PK of repaglinide when integrated into the PBPK model⁹.

Expression quantitative trait loci (eQTLs) are a type of genetic variation that are associated with the mRNA expression levels of a gene. eQTLs are classified as i) cis-eQTLs, which are found near the gene they regulate; and ii) trans-eQTLs, which are located some distance away from the affected gene (Supplementary Table 1)^{54,55}. eQTL analyses can be used to predict differences in function for DMEs and transporters. The minor alleles of particular eSNPs can be associated with different expression levels of a gene, which can result in a change in protein expression based on the mRNA transcripts. Therefore, it is important to characterize eQTLs as biomarkers of interindividual variability in the drug disposition and response. For example, rs10871454, an SNP associated with VKORC1 and located about 50kb upstream of the gene, influences the gene expression levels^{56–58}. The minor allele of rs10871454 is associated with decreased expression of VKORC1. VKORC1 is the target gene of warfarin, so the decrease in expression of the gene due to the eSNP provides an explanation for how that particular eQTL is associated with a phenotypic difference in warfarin response^{56,58}. Using proteomics approach transthyretin, a plasma transport protein, is shown to be associated with VKORC1 genotype, and can be used to predict warfarin response and dosing⁵⁹. Therefore, eQTL studies along with SRM proteomics can determine the effect of eSNPs on the protein expression of DMEs and transporters. Application of such approaches is emerging, e.g., Johansson et al. used genome-wide SNP data with high-throughput MS to identify individual cis-acting SNPs influencing 11 peptides from 5 individual proteins⁶⁰.

Epigenetic regulation of DMEs and transporters

Epigenetics involves any process that alters gene activity without changing the DNA sequence and leads to modifications that may be transmitted to daughter cells. Epigenetic processes are natural and critical for multiple organism functions. However, an abnormality in epigenetic processes could lead to significant adverse health and behavioral effects⁶¹.

There is increasing evidence supporting that the transcriptional up- or down-regulation of DMEs and transporters is also mediated by various epigenetic regulatory mechanisms^{62–65}. Processes such as DNA methyltransferases, histone deacetylases, histone acetylases, histone methyltransferases and nucleosomal remodeling can affect DME and transporter expression^{66–68}. For example, the expression of *CYP1A2*, *CYP2C19*, *CYP2D6*, *GSTA4*, *GSTM5*, *GSTT1*, and *SULT1A1* is inversely correlated with the DNA methylation⁶⁹. The tissue-specific expression of UGT1A1 in the liver and intestine is mediated by both histone hyperacetylation and DNA hypomethylation⁷⁰. Similarly, the induction of CYP1A1 involves increased active histone mark of H3K4me3 at the gene promoter and increased histone acetylation at both the promoter and enhancer region⁷¹. Non-coding microRNAs (miRNAs) can also lead to changes at the protein level. For example, *CYP3A4* is down-regulated by miR-27b through a post-transcriptional mechanism⁷². miR-328, -519c, and -520h are known to affect ABCG2 protein expression probably through accelerated mRNA degradation mechanism⁷³. A list of miRNA shown to affect ADME proteins is given in Supplementary Table 2^{62,63,74–81}. Changes in protein abundances due to these different epigenetic mechanisms can be accurately quantified using SRM proteomics approach. The applications of MS proteomics in studying various aspects of chromatin biology is discussed elsewhere in great detail^{82,83}.

Impact of diseases in drug metabolism and transport

Effect of diseases on drug PK and PD are well investigated and summarized in Table 2. Diseases such as liver or kidney failure, inflammation or diabetes can influence DME and transporter expression, activity or localization in the liver^{84–92}. For example, during cholestasis and nonalcoholic steatohepatitis, several liver-specific adaptations occur that serve to limit hepatic exposure to bile acids in response to the increased bile acid levels⁹³. Kidney failure alters drug metabolism and transport not only in the kidney but also in the liver^{94–97}. Similarly, diseases such as diabetes mellitus can alter clearance of drugs⁹⁸. Several studies have shown that systemic inflammation due to acute or chronic diseases can lead to an impairment of the expression and activity of DMEs⁹⁹. The proinflammatory cytokines IL-6, INF- γ , TNF- α , and IL-1 β are the most potent mediators of reduced enzyme activity and expression. Reduced CYP3A4 activity in cancer is linked to inflammation-induced changes in CYP3A4 gene expression, with a concurrent rise in IL-6 concentrations¹⁰⁰. Such alteration in activity of multiple DMEs and transporters by diseases can be accurately characterized by SRM proteomics when these changes are mediated by the expression. SRM proteomic analysis of subcellular fractions can also be used to study the effect of disease on the protein localization⁹². However, while protein quantification is an important surrogate of activity, factors such as posttranslational modification by diseases can only affect substrate affinity (Km) without changes in the protein expression.

Conclusions and future directions

SRM proteomics can determine the interindividual variability in the protein expression of DMEs, transporters and receptors in a reproducible and high-throughput manner. Such protein expression data can be used to develop better PBPK/PD models for predicting drug disposition and response. The commercially-available PBPK modeling software such as

Simcyp and Gastroplus have already integrated basic physiological information such as organ volumes, blood flows, body fat composition, etc. with the information on their variability (including genotype effect for the major DMEs). These software are now able to accept protein quantification results. Integration of SRM proteomics data in the PBPK/PD models to predict inter-individual variability will be a crucial milestone in the field of precision medicine. One of the current challenges to tissue proteomics approach in precision medicine research is the lack of well-characterized tissue repositories. Therefore, it is important to create well-characterized tissue repositories to capitalize the advantages of SRM proteomics toward the goal of precision medicine¹⁰¹. There is also scope to further improve high throughput-ness of SRM approach. In this direction, global proteomics tools such as data-independent SWATH-MS are emerging as a new generation proteomics tools and could be used complementary to SRM proteomics¹⁰².

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Intrinsic and extrinsic factors affecting drug disposition. Effect of these factors on activity or abundance of ADME proteins will be crucial in developing PBPK models to predict interindividual variability in drug disposition

Fig. 2.

Steps involved in tissue protein quantification by SRM proteomics. The tissue sample is homogenized and relevant protein fraction (e.g., microsomes, cytosol or plasma membrane), is isolated. The protein is digested using trypsin and a surrogate peptide unique to the protein of interest is quantified using triple quadrupole LC-MS/MS instrument

Fig. 3.

Genetic and epigenetic regulation of protein expression. The genetic mechanisms frequently affecting protein expression are the promoter mutation, insertion of a new stop codon in an exon, gene deletion or duplication, loss of start codon, mRNA splicing or protein degradation. DNA methylation, histone modification and miRNA are the major epigenetic mechanisms. Sites shown in orange, green and red colors indicate codon, promoter and miRNA binding regions of DNA, respectively.

Table 1Examples of PBPK modeling used in prediction of disposition of drugs²⁻⁹

Drug(s)	Aim of the study
Acetaminophen	A pediatric PBPK model was applied to predict acetaminophen metabolism and pharmacokinetics in infants, children and adolescents
Midazolam, nifedipine and indinavir	A PBPK Model was developed and applied to predict gestational age-dependent changes in hepatic CYP3A activity during pregnancy
Metformin, digoxin, midazolam, and emtricitabine	A model was developed based on physiological changes that occur during pregnancy to predict disposition of renally excreted and CYP3A metabolized compounds during pregnancy
Bosentan, repaglinide, telmisartan, valsartan, olmesartan	A mechanistic PBPK model was developed to predict drug hepatic transport under liver cirrhosis conditions
Indomethacin	Development of PBPK model for indomethacin disposition in pregnancy
Simvastatin, theophylline and (S)-warfarin	PBPK model assessed the influence of blinatumomab-mediated cytokine elevations on CYP3A4, CYP1A2 and CYP2C9
Sirolimus	The impact of CYP3A5*3 polymorphism on sirolimus PK was assessed with a PBPK model
Repaglinide	<ul style="list-style-type: none"> Impact of OATP1B1 and CYP2C8 genotype on repaglinide PK was predicted Physiological modeling accurately predicted the impact of OATP1B1 genotypes on repaglinide PK

Table 2Examples of effect of diseases on hepatic drug disposition pharmacokinetics^{79–92}

Drugs	Change in CL/AUC/Cmax
Effect of liver impairment	
S-Mephenytoin	↓ Oral CL by 20%
Carvedilol	↑ AUC by 4.4 fold
Labetalol	↑ AUC by 1.9 fold
Meperidine	↑ AUC by 1.8 fold
Metoprolol	↑AUC by 1.6 fold
Midazolam	↑ AUC by 2.0 fold
Morphine	↑ AUC by 2.1 fold
Nifedipine	↑ AUC by 1.8 fold
Nisoldipine	↑ AUC by 3.8 fold
Propranolol	↑ AUC by 1.7 fold
Effect of kidney impairment	
Duloxetine	↑ AUC by 2.0-fold
Tadalafil	↑AUC by 2.7- to 4.1-fold
Rosuvastatin	↑ C _{plasma} by 3-fold
Telithromycin	↑ AUC by 1.9-fold
Solifenacin	↑ AUC by 2.1-fold
Effect of anti-inflammatory treatment	
Simvastatin	↑CL by 2-fold with a ↓ AUC by 4-fold