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The importance of drug transporter characterization to precision medicine

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1. Introduction

The aim of precision medicine is to improve health care by tailoring drug treatment to patients’ individual characteristics through the integration of knowledge explaining interindividual variability of drug response caused by an interaction of genetic and epigenetic factors as well as environmental and lifestyle factors. Desired drug effects are essentially dependent on the processes of drug absorption, distribution metabolism, and excretion (ADME) of therapeutic compounds. In order to understand the underlying mechanisms of interindividual variability in drug efficacy and safety, major research efforts have been spent on characterizing proteins involved in ADME processes, such as drug-metabolizing enzymes or drug transporters, during the last decades.

Based on well-established and continually expanding scientific evidence, members of the two major superfamilies of membrane transport proteins, the solute carrier (SLC) and the ATP-binding cassette (ABC) transporter families, mediate the translocation of endogenous and xenobiotic compounds across cell membranes and are therefore crucially involved in the absorption, disposition, and excretion of clinically relevant drugs [1]. The SLC superfamily comprises uptake as well as efflux transporters [2]. In contrast, members of the ABC transporter family are predominantly efflux transporters [3]. Interindividual variability of SLC and ABC transporter expression and function thus contributes to differences in treatment efficacy or the occurrence of adverse drug reactions (ADRs). In consequence, the precision medicine concept aims to identify biomarkers for better prediction of transporter function to enable the desired individual drug response.

Apart from translocation of drugs, membrane transporters are involved in normal cell physiology and homeostasis, by mediating the movement of endogenous compounds, such as nutrients, hormones, or signaling molecules, across cell membranes [1]. Accordingly, their role in pathophysiology is increasingly acknowledged as well. In this regard, understanding the endogenous function can help to uncover mechanisms of disease and exploit membrane transporters as targets for therapeutic intervention. The identification of endogenous substrates might support the development of new drug candidates.

2. Clinical consequences of interindividual variability of transporter expression and function

Many studies, using in vitro cell models or transporter knockout mice, but also in vivo data derived from genome-wide association studies or clinical trials, were conducted demonstrating that transporter proteins expressed, e.g. in the intestine, liver, kidney, or blood–brain barrier, have an influence on the absorption, distribution, and excretion of drug agents [1]. Along with the understanding of the role and function of transporters, the highly complex interplay of regulatory pathways determining transporter expression/function is getting elucidated. The interindividual variability arises from the interaction of (a) nongenetic factors, such as age, sex, or concomitant medication; (b) genetic variation, including common (minor allele frequency [MAF] >1%), rare (MAF <1%), and very rare (MAF <0.1%) genetic variants; (c) epigenetic mechanisms, i.e. DNA methylation, histone modifications, or miRNAs; and (d) regulatory factors, e.g. transcriptional, posttranscriptional, or posttranslational regulation [4].

One very well-characterized drug transporter is the human organic cation transporter OCT1, encoded by SLC22A1. OCT1 is a primarily hepatic polyspecific transporter for hydrophilic organic cations, weak bases, and small neutral compounds mediating, for instance, the uptake of antidiabetic (e.g. metformin), antiviral (e.g. acyclovir), or anticancer drugs (e.g. platinum-based drugs). Interindividual variability of hepatic OCT1 expression in humans is large [5], most likely contributing to the variability of pharmacokinetics of the antidiabetic drug metformin. The SLC22A1 gene is highly polymorphic, and its expression and function are considerably influenced by various common genetic variants (Figure 1). Exemplarily, the missense variant rs12208357 (R61C) is associated with reduced hepatic OCT1 membrane expression and with considerably lower uptake of metformin into the liver. Results regarding the clinical consequence of this variant are contradictory. While there are studies suggesting a reduced glucose-lowering effect of metformin treatment in type 2 diabetes mellitus (T2DM) patients carrying this variant, other studies could not confirm an effect on metformin responses [6].
Several rare variants within the *SLC22A1* gene region with most likely deleterious effects on OCT1 function have been identified recently [7]. Although they are supposed to have substantial clinical relevance for the individual patient, actual functional consequences of rare genetic variants need further experimental validation. In addition to genetic variation, cholestasis as a nongenetic factor was linked to reduced OCT1 mRNA and protein expression in liver [5]. In hepatocellular carcinoma, *SLC22A1* gene expression was shown to be downregulated epigenetically by promoter DNA hypermethylation [8]. As a consequence of the reduced expression in cancer tissue, the efficacy of administered anticancer

![Diagram](image-url)
compounds which are OCT1 substrates, such as oxaliplatin, might be limited. Moreover, concomitant administration of commonly prescribed drugs which act as OCT1 inhibitors, such as proton-pump inhibitors or calcium channel blockers, with metformin, can provoke clinically relevant drug–drug interactions (DDIs), e.g., altered metformin treatment response in T2DM patients (Figure 1). This is supported by a large study of T2DM patients, indicating that the combination of factors leading to reduced OCT1 transport, such as concomitant medication with OCT1-inhibiting drugs in patients carrying at least two nonfunctional OCT1 alleles, predisposes to metformin intolerance. The clinical consequence is to prescribe alternative medications, not interacting with OCT1, for T2DM patients with inactive OCT1 alleles receiving metformin [9].

Endogenously, OCT1 is responsible for the translocation of e.g. serotonin or thiamine across the plasma membrane [10]. Impairment of this endogenous transport might also play a role in disease susceptibility. First evidence was provided by Ohishi et al. who suggested a connection between reduced choline transport capacity due to OCT1 variants and altered phosphatidylcholine synthesis, causing insufficient protection of bile ducts from bile acids, eventually leading to bile duct damage and progression of primary biliary cirrhosis in Japanese patients [11]. Chen et al. found a link between OCT1 deficiency and inhibition in the liver with reduced risk to develop hepatic steatosis due to reduced thiamine uptake. In fact, the authors propose altered thiamine disposition as a mode of metformin action [10]. However, the exact role and involvement of OCT1 in disease susceptibility need further evaluation.

Similar as exemplified in detail for the SLC uptake transporter OCT1, ABC transporters underlie significant interindividual variability of expression and function supporting the concept of precision medicine. One of the first and so far most extensively studied efflux transporters of the ABC transporter family is ATP binding cassette subfamily B member 1 (ABCB1)/multi-drug resistance protein 1 (MDR1)/P-glycoprotein. The ABCB1 expression profile, e.g., in hepatocytes, enterocytes, renal proximal tubule cells, or endothelial cells of the blood–brain barrier, suggests an excretion and barrier function. ABCB1 has broad substrate specificity and mediates the efflux of a variety of structurally diverse drugs, including anticancer (e.g., paclitaxel), antidepressant (e.g., citalopram), or antiviral (e.g., efavirenz) agents. Its expression undergoes large variability, which is expected to contribute to the interindividual variability in response to ABCB1 substrate drugs. Overexpression of ABCB1 has repeatedly been shown to contribute to multidrug resistance or ADRs. The interindividual variability in ABCB1 expression/function has been demonstrated to be caused by multiple factors, including genetic variants or epigenetic mechanisms. Epigenetically, the ABCB1 gene is regulated, e.g., by DNA methylation. DNA methylation levels in the ABCB1 promoter have been shown to be associated with ABCB1 expression in vitro and in vivo [12]. In breast cancer patients treated with doxorubicin, ABCB1 together with GSTP1 promoter hypomethylation was associated with poor patient survival [12]. In addition, ABCB1 activity is substantially altered by concomitantly administered compounds, which can act as ABCB1 inducers or repressors, leading to clinically relevant DDIs with undesired as well as beneficial effects. Inhibition of ABCB1, for example, can on the one hand lead to serious neurotoxic side effects, since ABCB1 protects the central nervous system (CNS) from drug exposure. On the other hand, ABCB1 inhibition can increase the bioavailability of a competing substrate drug and therefore improve its efficacy. Respective warnings are included in labels of several drugs, which are ABCB1 substrates. To what extent genetic variants in the ABCB1 gene affect clinical drug response is still inconclusive because study results are in part controversial and/or do not provide sufficient evidence to affect treatment decisions [3]. Therefore, further large-scale studies are warranted to determine the relevance of ABCB1 genetics to precision medicine.

3. Integrative technologies in precision medicine

The advent of next-generation sequencing (NGS) technologies as one example of ‘-omics’-technologies (Figure 2) enabled whole-genome sequencing in large-scale projects and led, e.g., to the identification of rare and very rare variants with substantial impact on drug transporter expression. In addition, causative genetic variants for drug response or toxicity, but also for genetic diseases, could be identified. The recent years have shown that the search for clinically relevant biomarkers to predict drug response or ADRs also derives benefit from collaborations and large international consortia to provide large study cohorts, but also combined expertise to advance research in the field of precision medicine [13].

Similar to NGS in the early 2000s, the field of metabolomics is gaining particular attention in recent years, as metabolites represent the final downstream product of gene expression and are therefore suggested to very closely reflect the actual phenotype. Targeted/untargeted metabolomics approaches or state-of-the-art imaging techniques, e.g., matrix-assisted laser desorption/ionisation (MALDI)-imaging, which allows label-free assessment of drugs or their metabolites or endogenous substrates within tissue samples, are likely to substantially boost the identification of transporter substrates, elucidating the importance of selected transporters for drug response [14]. A recent study by Yee et al. demonstrated that the combination of results from genomic studies and metabolomic approaches enables the identification of novel endogenous transporter substrates with potential application as metabolite biomarkers or probes to determine clinically relevant DDIs [15].

Apart from state-of-the-art ‘-omics’-technologies, a key component of precision medicine is the analysis and documentation of the individual phenotype, i.e., the entirety of phenotypic traits. In order to uncover molecular biomarkers for disease or drug response to guide treatment decisions, detailed description and documentation of patient cohorts and precise description of phenotypes of ideally large patient groups are necessary. Hence, there is an urgent need for electronic medical record systems and databases that integrate, e.g., family history, comorbidities, or medical records. Up to now, generally accepted and standardized approaches
to record the data from ‘-omics’-technologies as well as individual clinical information are missing. A key requirement for such medical databases is the consistent inclusion of the relevant clinical parameters to guarantee comparability and applicability of collected data.

The overall aim is to integrate the multiple ‘-omics’-levels to get a complete picture of individual phenotypes in order to estimate the contribution of, for example, drug transporters to the complex processes leading to interindividual variability of drug response (Figure 2).

4. Expert opinion

Personalized medicine primarily focused on pharmacogenetics as a cause for interindividual variability of drug response, and the extensive research in this field gave rise to a number of validated helpful common genetic variants as biomarkers to support treatment decisions. Although as yet barely taken into account in clinical practice, in addition to common genetic variants, rare and very rare genetic variants, epigenetic as well as transcriptional and posttranslational regulatory mechanisms are well accepted as key determinants of heterogeneous expression and function of drug transporters. As exemplified for OCT1 and ABCB1, systematic characterization of membrane transporters, considering all sources of interindividual variability, helps to better understand drug safety or efficacy and corroborates consideration of drug transporters during drug development. Based on increasing scientific evidence, the International Transporter Consortium provides continually updated recommendations for the consideration of selected transporters with clinically significant influence on drug pharmacokinetics and suggests model systems to evaluate the involvement of transporters and their impact on drug pharmacokinetics during drug development (http://www.ascpt.org/Membership/My-ASCPT/Networks-and-Communities/International-Transporter-Consortium-ITC). Still, there are numerous poorly characterized ABC and SLC transporters with unknown function. A major goal is on the one hand to deorphanize these transporters and evaluate their contribution to interindividual variability of drug response, but on the other hand to understand the involvement in pathophysiology and exploit their potential as drug targets [2].

With the development and implementation of novel high-throughput technologies, the so-called ‘-omics’-technologies, scientific research has experienced an enormous technical progress for the evaluation and integration of all sources of interindividual variability to characterize drug transporters and other enzymes involved in ADME processes. In contrast to conventional single candidate gene/molecule approaches, the novel ‘-omics’-technologies enable cost-effective and high-quality analysis of the entirety of different components in a biological system. As yet, the major challenge of the ‘-omics’-technologies is to cope with the enormous amount of data generated. Processing, analysis, and interpretation of the acquired data demand close cooperation of bioinformaticians, biostatisticians, biologists, and physicians.

The integration of the emerging innovative and sophisticated technologies will help to further elucidate the relevance of interindividual variability of membrane transporters for drug response and precision medicine, providing the basis for translation of the most valuable findings with benefit for patients into clinical practice. However, the impact of the...
interindividual variability of transporter expression/function on drug response appears to be clinically less important compared to drug-metabolizing enzymes, which may be explained by the fact that altered transporter function is substantially compensated by overlapping substrate specificity of membrane transporters. Still, characterization of drug transporters to understand the factors contributing to interindividual variability of specific transporter expression/function is essential, since particularly the combination of impaired or reduced function of redundant transporters and coadministered drugs or concomitant diseases is likely to critically affect response to treatment and need to be considered in the future in the clinical setting to maximize the benefit of drug treatment and minimize ADRs.

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**Declaration of interest**

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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7. Very recent large-scale meta-analysis summarizing the effects of selected genetic variants in nine candidate genes on metformin response in T2DM patients.
9. DNA methylation in human hepatic OCT1 expression/function.