Getting a grip on drug side-effects

Dr Gerd-Achim Kullak-Ublick provides an inside look into his work to understand the regulation and genetic variability of drug and organic anion transporter genes, which has revealed answers to important questions about drug elimination for liver and intestinal disease sufferers

Could you provide an overview of the aims of your current research?

My current research objectives are focused on studying the mechanisms that regulate the expression and function of drug transporters in human tissues. Drug transporters are a large family of different genes and gene products that mediate the uptake and efflux of drugs into and out of epithelial cells. Our research group in Zurich first cloned the family of organic anion transporting polypeptides (OATPs) that mediate the uptake of a wide variety of drugs into tissues such as the liver. We are studying the genetic regulation of OATPs and other transporters, for instance the role of nuclear receptors and other transcription factors in the transactivation or suppression of gene transcription. The elucidation of these mechanisms is pivotal to understanding factors that affect the important process of drug elimination from the body.

How have you used your finding of the novel BSEP mutation to help patients with a juvenile-onset form of progressive familial intrahepatic cholestasis type 2?

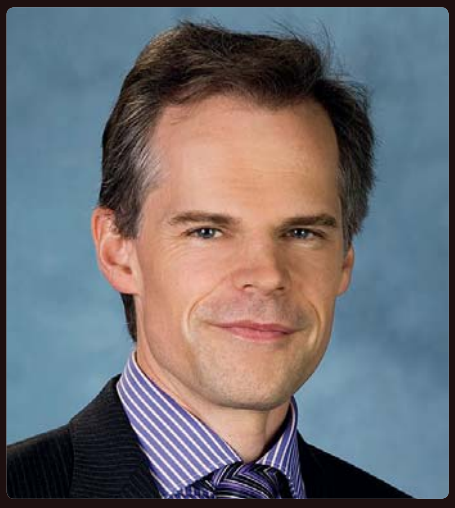
Our lab offers routine sequencing of the BSEP gene to identify established or novel mutations in the ABCB11 gene that can cause cholestatic liver disease. Of all the cases identified to date, one case is particularly illustrative of the value of this diagnostic service: a 16-year-old male patient was diagnosed as having benign recurrent intrahepatic cholestasis (BRIC) and we found compound heterozygosity for an E297G mutation (inherited from the mother) and an R432T mutation (inherited from the father). Liver disease worsened in the patient and the phenotype of the disease switched to progressive familial intrahepatic cholestasis (PFIC type 2). The patient required liver transplantation, but the shortage of donor organs presented a severe risk to survival. The patient's brother was then evaluated as a potential living-related liver donor and his BSEP gene was sequenced. Luckily, the brother had inherited the healthy allele from both the mother and the father (a 1:4 chance) and was thus found to be suitable as an organ donor. He donated part of his liver to his brother and today both brothers are in excellent condition.

To what extent does your research make use of a multidisciplinary approach?

Various clinical partners have been involved, notably from the fields of gastroenterology and hepatology, as well as basic science partners that have helped to generate and characterise antibodies, supply expression plasmids and cell lines, carry out imaging studies and many other collaborative efforts. Research in Zurich is strongly network-orientated, both within the university itself, as well as in national research consortia that aim to join forces to solve more global research aims, such as the National Centres of Competence in Research (NCCRs), funded by the Swiss National Science Foundation.

How has your collaboration with the International Serious Adverse Event Consortium contributed to your work?

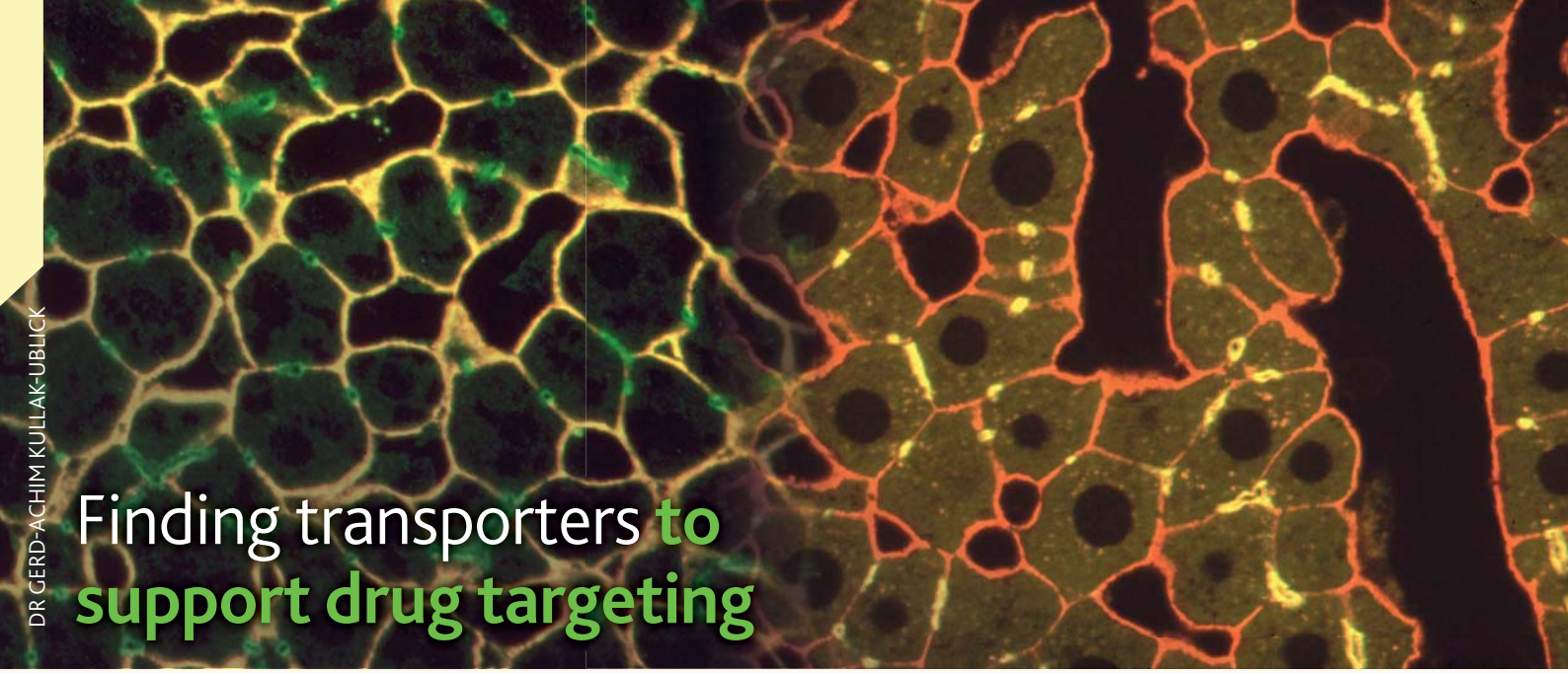
We are still at the beginning of this highly interesting collaboration, mainly within the international Drug-induced Liver Injury Consortium (iDILIC). This consortium will carry out genome-wide association studies in patients with DILI to identify novel genetic risk factors that could be used diagnostically for individual risk prediction. From a pharmacoepidemiological perspective, we have established collaborations with other safety networks such as the Swiss pharmacovigilance network, the General Practitioner Research



database and other epidemiological projects that focus on drug safety in the field of neurological and psychiatric disorders.

How will you direct your research focus over the next year? What aspects have you pinpointed for further investigation?

The next era in our research will be directed towards studying the epigenetic regulation of drug transporters, for instance the role of microRNAs. We will try to assess the usefulness of microRNAs as biomarkers that allow early diagnosis of DILI. We will also carry out phase I/IV studies in healthy human volunteers, to assess the effect that the selective induction of individual transporters, for example in the intestine, has on the pharmacokinetics and disposition of model substrate drugs. Finally, new genetic markers of DILI will be assessed in selected populations, such as patients that develop liver injury during treatment with tyrosine kinase inhibitors that are used to treat malignant tumours.



DR GERD-ACHIM KULLAK-UBLICK

Finding transporters to support drug targeting

Researchers at the **University Hospital Zurich** are using *in vivo* models to study human organic anion transporting polypeptides and give way to major breakthroughs in the field of clinical pharmacology and liver disease

ALTHOUGH DRUG-INDUCED liver disease (DILI) is a rare phenomenon, occurring at an incidence rate of about 1:10,000 in patients receiving medications, the problem lies in the severity of the adverse drug reactions and the poor predictability in an individual. Fatal cases of liver failure caused by widely available drugs are not uncommon. Finding answers to this dangerous side-effect is the focus of a team at the University Hospital Zurich who are studying the role of drug transporters in the overall pathogenesis of DILI. Research leader, Dr Gerd-Achim Kullak-Ublick, says they have also studied the role of transporters in cholestatic liver diseases, particularly pregnancy-associated liver disorders and inherited forms of cholestatic liver disease caused by genetic variants in transporter genes. "These inherited cholestatic syndromes are rare but can have devastating consequences for the affected infants," he points out. In addition to these areas of study, the research group has been investigating the pharmacogenetics of inflammatory bowel diseases (Crohn's disease and ulcerative colitis), two chronic inflammatory disorders that act on the small and large intestine and occur at a reasonably high incidence rate.

THE BENEFIT OF *IN VIVO* LOOPING

An in situ loop is a technique that is used for studying the uptake of a given substrate into the intestinal wall. Defined segments of the intestine are ligated to form loops during anaesthesia. Kullak-Ublick says that transport substrates can be then injected into these loops, after which their concentration in blood or within the intestinal loop itself can be measured at desired time points following the *in vivo* treatments. To support the *in vitro* studies on the regulation of intestinal transporter genes, the team has established a novel technique of short-term tissue culture. "This technique allows determination of changes in gene expression and protein phosphorylation in human intestinal biopsies in response to defined compounds added to the culture media," Kullak-Ublick comments. Changes in mRNA expression in the genes of interest are measured by real-time PCR, and changes in protein expression and modification are analysed by immunoblotting. Other techniques employed include the isolation of primary hepatocytes directly from rat or human liver (the latter is obtained from surgically resected specimens in collaboration with surgical units) and the isolation of primary proximal renal tubular cells from the kidney.

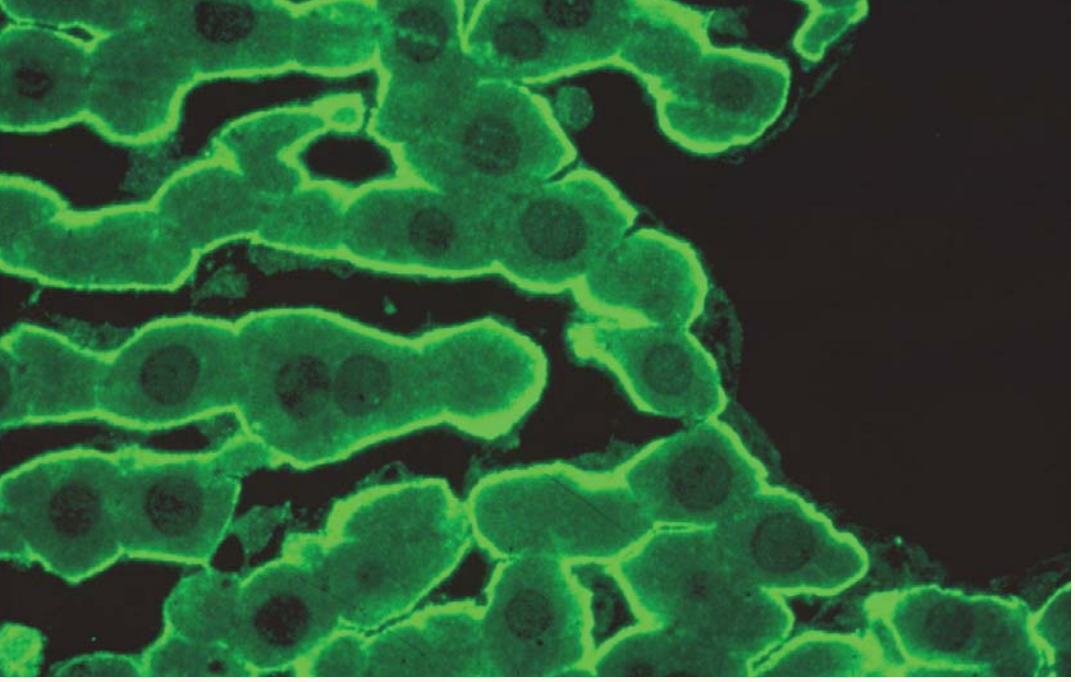
One of the most significant parts of their work is the cloning of two human organic anion transporting polypeptides (OATP1A2 and OATP2B1). The human OATP1A2 was the first human OATP to be cloned. Before OATP1A2 was identified, the first rat OATP, now called OATP1A1, was cloned in their laboratory in Zurich. Using the rat OATP1A1 as a probe, human OATP1A2 was cloned from a human liver cDNA library by homology to rat OATP1A1. It later turned out human OATP1A2 is predominantly expressed in the brain, with additional expression in cholangiocytes in the liver and in enterocytes in the intestine. "OATP1A2 has the broadest spectrum of transport substrates known to date and thus probably plays an important role in drug disposition in the tissues where it is expressed," he affirms, and as such it has played an important role in their research. Human OATP2B1 was later cloned using an expressed sequence tag approach and was found in several tissues, with strongest presence in human liver (hepatocytes). OATP2B1 is an uptake system for organic anions and also statins, and in the intestine both OATP1A2 and OATP2B1 mediate the uptake of drugs such as fexofenadine.

DRUG TRANSPORTING PROTEINS

One of the biggest obstacles the research team had to overcome was attempting to assess the importance of an individual transporter for overall pharmacokinetics of the drug that serves as a substrate of the transporter in question. Kullak-Ublick describes: "The multidrug resistance gene product, MDR1, is important in the elimination of drugs such as digoxin from the human body. Induction of MDR1 by compounds such as rifampicin or St John's wort can have deleterious effects on the plasma levels of drugs with a narrow therapeutic index such as digoxin or cyclosporine A". Thus induction or inhibition of a transporter can severely influence PK of a drug substrate. Another example of an obstacle the group needed to overcome was the genetic variation of the OATP1B1 transporter (SLCO1B1 gene), that is expressed at the basolateral surface of hepatocytes and mediates the uptake of statins into the liver. A reduction of OATP1B1 transport function, caused by a genetic variant (c521T>C variant) increases plasma concentrations of simvastatin. As a result, the risk for myopathy or even rhabdomyolysis caused by statins also increases quite dramatically.

BSEP TRANSPORT FUNCTION

An area in which this research has managed to deliver some major results is the cloning of

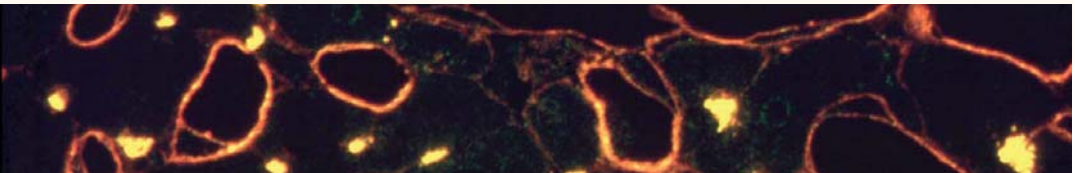


the first human OATP – now called OATP1A2 – from human liver. The group consortium has shown that the human bile acid transporter NTCP shows reduced expression in human hepatocellular carcinomas, which Kullak-Ublick believes is an important finding for assessing the usefulness of a drug targeting approach that employs cytostatic agents covalently linked to bile acids: "We showed that nuclear receptors are involved in the regulation of various drug uptake systems, notably that FXR induces expression of OATP1B3 and the transporter heterodimer OST-alpha/OST-beta," he elucidates. that the glucocorticoid receptor transactivates the sodium-dependent bile acid transporters, NTCP and ASBT, and that the ASBT expression was shown to be induced in ileal biopsies of healthy volunteers that underwent intestinal biopsy before and during intake of the glucocorticoid budesonide.

In addition, the organic cation transporter OCT1, the organic anion transporter OAT2 and the concentrative nucleoside transporter CNT1 are all expressed mainly in human hepatocytes and were all shown to be transactivated by the nuclear receptor HNF4-alpha (hepatocyte nuclear factor 4-alpha). This, according to Kullak-Ublick, is important because changes in the expression levels of these transporters may be caused by altered gene activation via HNF4-alpha. Also, in several genetic studies, they have found an important polymorphism in the BSEP gene (the V444A variant) that predisposes to cholestatic liver damage of various etiologies. Establishing an *in vitro* assay to assess BSEP transport function in the presence of potential inhibitors was one of the most successful outcomes from this research, as this assay is now widely used throughout pharmaceutical industry to test new compounds with respect to their inhibitory potential towards BSEP, which is a risk factor for DILI.

> OATP1A2 has the broadest spectrum of transport substrates known to date and thus probably plays an important role in drug disposition in the tissues where it is expressed

Ultimately, the goal of the team's subspecialty is to translate their *in vitro* findings that concern drug efficacy and safety into the clinical situation. 20 years ago nobody believed that transporters play any role in mediating drug movement across epithelial membrane barriers. Today it is a different story. Research on two classes of transporters, OATPs and BSEP, is extremely active both in academia and the pharmaceutics industry, he says: "The signalling networks that operate to fine-tune the regulation and expression of drug transporters are being elucidated step-by-step, and it is our hope that our achievements to date and our future progress will help to advance this field of research into a new era of optimised and individualised drug therapy for patients".



INTELLIGENCE

REGULATION AND GENETIC VARIABILITY OF DRUG AND ORGANIC ANION TRANSPORTER GENES: IMPLICATIONS FOR LIVER AND INTESTINAL DISEASES

OBJECTIVES

This project analyses (i) the transcriptional regulation of drug transporter genes, (ii) the importance of changes in transporter expression for the pharmacokinetics of drugs, (iii) genetic variations of nuclear receptor and drug transporter genes, and (iv) the role of transporters and nuclear receptors in the pathogenesis of drug-induced liver injury.

KEY COLLABORATORS

Bruno Stieger, PhD, Department of Clinical Pharmacology and Toxicology, University Hospital Zurich • **Swiss IBD Cohort Study** (http://ibdcohort.ch/) • **International Serious Adverse Event Consortium** (www.saeconsortium.org)

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