GENOMIC AIDED PHARMACOTHERAPY (GAP): A PILOT STUDY IN PATIENTS SUFFERING FROM CHRONIC NON-CANCER PAIN
Houlind, M. B.; Treldal, C.; Vagn-Hansen, L.; Hansen, R. W.; Chrstrup, L. L.

Publication date:
2018

Document version
Publisher's PDF, also known as Version of record

Document license:
Unspecified

Citation for published version (APA):
ABSTRACTS FROM FIRST NORDIC CONFERENCE ON PERSONALIZED MEDICINE 2018 IN NYBORG, DENMARK

NorPM-P1  
EFFECT OF RARE GENETIC VARIANTS ON ORGANIC ANION TRANSPORTING POLYPEPTIDE 1B1  
Kiander W.1, Kidron H.1, Niemi M.2  
1Faculty of Pharmacy, University of Helsinki, Helsinki, Finland; 2Faculty of Medicine, University of Helsinki, Helsinki, Finland

Aims: To examine the effect of single-nucleotide polymorphisms in the transmembrane domains of Organic Anion Transporting Polypeptide 1B1 (OATP1B1, SLCO1B1) on its activity.

Methods: 8 naturally occurring variants in the transmembrane domains of OATP1B1 and a common variant (c.521T>C) which served as a control were selected. The mutations were inserted in the wildtype OATP1B1 gene by site-directed mutagenesis. The Bac-to-Bac baculovirus system was used to transiently express the variant proteins in mammalian cells (HEK293). Dichlorofluorescein and estrone-3-sulfate were used as substrates in cellular uptake studies to test the activity of the variants. The effects of the polymorphisms on expression levels and localization of the variants were studied with Western blotting and immunofluorescence microscopy.

Results: The transport activity of some of the variants in the transmembrane domain was significantly impaired compared to wildtype OATP1B1, while others exhibited normal or even enhanced uptake of the study substrates. Immunofluorescence studies in cells indicate impaired localization for some of the variants, which could at least partly explain the decrease in activity.

Conclusion: The activity of OATP1B1 may be altered due to rare single nucleotide polymorphisms in the transmembrane domains.

NorPM-P2  
CLOPIDOGREL MARKEDLY INCREASES DASABUVIR EXPOSURE, AND RITONAVIR INHIBITS THE BIOACTIVATION OF CLOPIDOGREL  
Ritonen M.K., Tornio A., Lapatto-Reiniluoto O., Neuvonen M., Neuvonen P.J., Niemi M., Backman J.  
Clinical Pharmacology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

Aims: The purpose of this clinical study was to characterize the effect of the CYP2C8 inhibitor clopidogrel on the pharmacokinetics of the CYP2C8 substrate dasabuvir. Because dasabuvir is routinely used in an antiviral drug regimen containing ritonavir, a strong CYP3A4 inhibitor, the secondary aims were to explore how ritonavir modifies clopidogrel-dasabuvir interaction and whether ritonavir affects the bioactivation of clopidogrel, which is partly CYP3A4-mediated.

Methods: In a four-phase cross-over study, twelve healthy volunteers were administered clinical doses of clopidogrel, ritonavir, their combination, or placebo as pretreatments, and a single dose of dasabuvir. The plasma concentrations of dasabuvir, clopidogrel, their metabolites, and ritonavir were determined with liquid chromatography/mass spectrometry. The antiplatelet effect of clopidogrel was measured with a turbidimetric optical detection system.

Results: Clopidogrel increased the geometric mean AUC$_{0-\infty}$ of dasabuvir 4.7-fold (90% CI of geometric mean ratio 3.2–6.7-fold; $p = 8 \times 10^{-3}$), compared with placebo. When clopidogrel and ritonavir were co-administered, dasabuvir AUC$_{0-\infty}$ was 3.9-fold (90% CI 2.8–5.5-fold; $p = 2 \times 10^{-9}$) of that during the ritonavir phase. Ritonavir decreased the AUC$_{0-\infty}$ of clopidogrel active metabolite by 51% (90% CI 39–61%; $p = 0.0001$), and average platelet inhibition diminished from 51% to 31% by ritonavir (mean difference 90% CI $-27$% to $-12$%; $p = 0.0007$).

Conclusion: Clinical doses of clopidogrel markedly augment dasabuvir exposure and inhibit the CYP2C8-mediated formation of the dasabuvir metabolite, M1. Therefore, the risk for dasabuvir adverse effects, i.e. QTc prolongation, may increase if clopidogrel is used concomitantly with dasabuvir, irrespective of ritonavir. In addition, dasabuvir can serve as a CYP2C8 probe substrate for drug interaction studies. Lastly, ritonavir can reduce the antithrombotic efficacy of clopidogrel. Thus, concurrent use of clopidogrel and dasabuvir is best avoided.

NorPM-P3  
UNDERSTANDING DRUG REACTIONS USING GENOME SEQUENCING (UDRUGS) STUDY: A NEW ZEALAND INITIATIVE TO INVESTIGATE THE GENETICS OF ADRS  
Maggo S.1, Liau Y.1, Doogue M.2, Kennedy M.1  
1Pathology & Biomedical Science, University of Otago, Christchurch, New Zealand; 2Department of Medicine, University of Otago, Christchurch, New Zealand

Aims: UDRUGS is a New Zealand initiative to systematically collect samples from people who have had adverse drug reactions (ADRs) to characterize known or identify novel genetic variants associated with rare, unusual, and severe ADRs.

Methods: Participants for the UDRUGS study are largely recruited via referral through clinicians working in Christchurch hospital, but also from clinicians across New Zealand. Written consent is sought from all patients referred to the UDRUGS study. Genetic analysis largely involves Sanger sequencing of candidate genes. We have also used whole exome sequencing (WES) or whole genome sequencing (WGS) for a subset of cases. We communicate relevant research findings back to the participants and/or the referring clinician citing guidelines from the Clinical Pharmacogenetics Implementation Consortium (CPIC).

Results: To date we have collected ~100 samples across 12 general drug classes. The majority of cases recruited have been for ADRs to antidepressants, drugs affecting immune response (azathioprine) and cardiovascular medication (statins and medication used for hypertension). For antidepressants, we have genotyped 37 patients, and observed that 65% of the cohort had at least one non-functional CYP2D6 allele ($p < 0.01$, compared with CYP2D6 EXAC NFE). In addition we have carried out WES on participants with recurrent statin-myalgia, and participants with ACEi induced angioedema. For both cohorts our analyses have identified rare variants which require further validation.

Conclusion: We have developed a programme for studying severe, rare or unusual ADR cases resulting from pharmacological treatment. Genomic analyses should eventually identify most genetic variants that predispose to ADRs, enabling a priori detection of such variants with high throughput DNA tests. Establishing UDRUGS, a New Zealand DNA bank that can receive ADR samples and associated clinical data, will ensure we contribute to and benefit from such research.
**NorPM-P4**  
DIFFERENCES IN SENSITIVITY TO DETECT TIME-DEPENDENT INACTIVATION OF CYP2C8 BETWEEN RECOMBINANT EXPRESSED ENZYMES AND HUMAN LIVER MICROSOMES  

Kahma H. 1, Filippulla A.M. 1, Launiainen T. 1, Vinanäki J. 1, Evangelista E.A. 2, Tohat R.A. 2, Backman J.T. 1

1Clinical Pharmacology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland; 2Medicinal Chemistry, University of Washington, Seattle, United States

**Aims:** Clopidogrel acetyl-β-D-glucuronide causes strong time-dependent inactivation of CYP2C8 in human liver microsomes (HLMs). However, in preliminary studies on the inactivation mechanism, we were unable to detect inactivation of CYP2C8 by clopidogrel glucuronide in Supersomes. Therefore, we investigated the performance of different CYP2C8 sources in detection of time-dependent inactivation.

**Methods:** Time-dependent inactivation of CYP2C8 by clopidogrel acetyl-β-D-glucuronide (100 µM) and gemfibrozil I-OH-β-glucuronide (100 µM) and amiodarone (100 µM) was studied in HLMs and three recombinant human CYP2C8 preparations (Supersomes, Bactosomes and EasyCYP Bactosomes) using amodiaquine N-demethylation as the marker reaction. The inactivation kinetics of CYP2C8 by clopidogrel acetyl-β-D-glucuronide (5–250 µM) was studied in Supersomes and Bactosomes.

**Results:** Time-dependent inactivation of CYP2C8 by amiodarone and gemfibrozil glucuronide was observed in all enzyme preparations, with moderate variability between preparations. However, the extent of inactivation by clopidogrel glucuronide varied markedly between different preparations, as well as between different Supersome lots. While clopidogrel glucuronide (100 µM) caused strong inactivation of CYP2C8 in HLMs (72% and 16% inhibition with and without 30-min preincubation, respectively) and in one Supersome lot (54% and 0% inhibition with and without preincubation, respectively), significant inactivation could not be reliably detected in two other lots. In Bactosomes, the K_i and k_inact of clopidogrel glucuronide (14 µM and 0.054 min^{-1}) were close to those determined previously in HLMs (9.9 µM and 0.047 min^{-1}).

**Conclusion:** The results show that there are differences between enzyme sources and between lots of recombinant enzymes in sensitivity to detect inactivation of CYP2C8. The risk of false negatives should be taken into account when recombinant CYP enzymes are used to identify time-dependent inactivators of CYP enzymes.

**NorPM-P5**  
NEW CONCEPTS IN DISEASE CLASSIFICATION, TOWARD GENE BASED DIAGNOSIS, SUBTYPES AND PREDICTION OF DRUG RESISTANCE IN MALIGNANT B-CELL DISEASES


Research Section, Department of Haematology, Clinical Cancer Research Center and Department of Clinical Medicine, Aalborg University Hospital and Aalborg University, Aalborg, Denmark

**Aims:** We perceive the future of cancer treatment as being in the development of “personalised medicine” strategies and malignant B-cell diseases (MBCD) is an excellent example, where this approach can be clinically validated.

Our concept is to take individual gene expression variability and new classifications into account by new strategies of disease diagnosis, new subtyping and new drug specific prediction. Here we present a summary of our work with the primary aims to give useful information to clinical decision making.

**Methods:** Tumor biopsies from 4,226 patients with Affymetrix U133 plus 2.0 microarray mRNA expression datasets, in collaborations and/or on line from 21 newly diagnosed/unreared cohorts with lymphoid leukemia, non-Hodgkin lymphoma or multiple myeloma. Powerful methods for phenotyping by multi omics, diverse preclinical cell line models and bioinformatic tools for large data set analysis allowed us to address new classifications exemplified by diagnostic analysis gene signature (DAGS), B-cell associated gene signature (BAGS) and resistant gene signature (REGS) assignment of individual tumors.

**Results:** The resultant tumor assignments in the available clinical datasets exhibited the following:

1. Individual DAGS have a significant diagnostic accuracy; (Table 1).
2. Individual BAGS disease subtypes have prognostic impact, and therefore reflect pathogenesis; (Figure 1).
3. Individual REGS for specific drug resistance have predictive information documented by its prognostic impact; (Figure 2).

Consequently, we have prepared an R-package based tool: http://www.hemaclass.org/ relevant for implementation of PM in the clinical setting.

**Conclusion:** The results “proves our concept” that individual variability and new classification by gene expression may allow more precise diagnostics, subtyping and identify drug specific resistance that will reduce overtreatment, increase the “Value in Health Care” and validate the PM strategy and goals.

**NorPM-P6**  
HEMATOLOGICAL CANCER PATIENTS’ POSITION ON PRECISION MEDICINE – A QUESTIONNAIRE SURVEY

Sommer M. 1, Nielsen M.M. 1, Vesteghem C. 2, Bøgsted M. 2, Dybkjær K. 2, Johnsen H.E. 1, El-Galaly T.C. 1, Bryndum R.F. 1

1Department of Hematology, Aalborg University Hospital, Aalborg, Denmark; 2Department of Clinical Medicine, Aalborg University, Aalborg, Denmark

**Aims:** Precision Medicine has gained increased medical and political attention in recent years. In Denmark, this has resulted in The National Strategy for Precision Medicine 2017–2020 and development of a national genome center. In 2016, the Danish Ministry of Health and Danish Regions performed a population-based survey of the public’s position on precision medicine. However, to our knowledge no study has investigated patients’ position on this area. Therefore, the aim of this study was to investigate hematological cancer patients’ position on precision medicine compared to the healthy population.

**Methods:** Data was collected using a questionnaire developed by ADVICE Communication Agency for the population-based survey and on behalf of the Danish Ministry of Health and Danish Regions. Eligible participants were hematological cancer patients affiliated to the Department of Hematology, Aalborg University Hospital. The questionnaire was administered electronically through REDCap or on paper. Data was collected anonymously.

**Results:** Between October 2017 and January 2018, we enrolled 286 patients (median age; men 66 years, women 67 years). The study population had a higher median age compared to the public (n = 1005) (median age; men 60.5 years, women 53 years) and a lower education level. We found that 84% of the patients had none or limited knowledge about gene testing compared to 55% of the public (p = 0.0001). 97% of the patients found gene testing and precision medicine interesting compared to 52% of the public (p = 0.0001). Furthermore, 92% of the patients were positive towards genetic research compared to 40% of the public (p = 0.0001).

**Conclusion:** We have identified a difference in the knowledge level between hematological cancer patients and the public. A difference, which should be addressed in order to optimize and support patient dialogue in a precision medicine context.
NorPM-P7
MEDICATIONS DURING PREGNANCY: EFFECTS ON NEURODEVELOPMENTAL DISORDERS IN CHILDHOOD AND EPIGENETIC OUTCOMES


1Pharmacoepidemiology and Drug Safety, School of Pharmacy, University of Oslo, Oslo, Norway; 2Pharmacoepidemiology and Drug Safety, School of Pharmacy, University of Oslo, Oslo, Norway; 3Department of Biosciences, University of Oslo, Oslo, Norway; 4Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo, Oslo, Norway; 5Department of Mathematics, University of Oslo, Oslo, Norway; 6Department of Informatics, University of Oslo, Oslo, Norway; 7Department of Medical Genetics, Oslo University Hospital and School of Pharmacy, University of Oslo, Oslo, Norway

Aims: To present the PharmaTox initiative and our on-going studies to assess effects of pharmaceuticals on human neurodevelopment and neurotoxicity.

Rationale: The fetal period is believed to be one of the most sensitive periods for brain development. During this period, neurons are created, differentiate and migrate to form the various parts of the brain. The theoretical potential of neurotoxicity of pharmaceuticals is related to their ability to pass the blood-brain barrier and impact on the developing fetal nervous system. The PharmaTox Strategic Research Initiative was established in 2015 uniting strong research groups in pharmacoepidemiology, toxicology, epigenetics, bioinformatics and statistics at the University of Oslo, Norway.

Methods: A primary data source is epidemiological data from the Norwegian Mother and Child (MoBa) cohort, along with corresponding genetic and epigenetic data derived from blood samples of infants in MoBa. Moreover, to give biological plausible molecular and cellular mechanisms of neurotoxicity, a CNS pharmacology safety model (chicken embryo) and human embryonic stem cells are used. Advanced statistical methods, medical bioinformatics and microarray technology are an integrated part of this research.

Results: Several results have been published in relation to paracetamol use in pregnancy. An association between long-term use of paracetamol with increased risk of ADHD was recently published, as well as findings of altered DNA-methylation patterns among children with ADHD diagnosis prenatally exposed to long-term use of paracetamol. Preliminary results from the chicken embryo model suggests that paracetamol and other medications can change the expression of specific microRNAs.

Conclusion: Novel insight into neurotoxicity of pharmaceuticals can be obtained by using a translational approach, advanced statistical methods, medical bioinformatics and microarray technology on epidemiological and human genetic data in combination with experimental in vivo and intro models.

NorPM-P9
EFFECTS OF GENETIC VARIANTS OF HUMAN NICOTINIC RECEPTOR SUBUNITS CHRNA3, CHRNA4, CHRNA5 ON NICOTINE DEPENDENCE AND SMOKING CESSATION

Muderrisoglu A.1, Babaoglu E.2, Korkmaz E.T.2, Kalkisim S.1, Karabulut E.3, Emri S.3, Babaoglu M.O.3

1Department of Pharmacology, Hacettepe University Faculty of Medicine, Ankara, Turkey; 2Department of Chest Diseases, Hacettepe University Faculty of Medicine, Ankara, Turkey; 3Department of Biostatistics, Hacettepe University Faculty of Medicine, Ankara, Turkey; 4Department of Chest Diseases, Atatürk University Faculty of Medicine, Istanbul, Turkey

Aims: To investigate the effects of genetic variants in nicotinic receptor subunits of CHRNA3, CHRNA4, CHRNA5 on nicotine dependence and outcomes of smoking cessation treatments at the 12th week in a Turkish population.

Methods: We recruited 130 smokers and 130 non-smokers for this study. The heaviness of nicotine dependence was determined by Fagerstrom Test for Nicotine Dependence and CO levels. Scores ≤7 were considered as low nicotine dependence (n = 60), scores >7 were considered as medium or low nicotine dependence (n = 70). Treatment groups were nicotine replacement therapy (NRT, n = 40), bupropion (n = 47), bupropion+NRT (n = 15) and varenicline (n = 28). PCR-RFLP was used for genotyping.

Results: We found an association between the heaviness of nicotine dependence and AA genotype for the CHRNA4 rs1044396 variant (p = 0.02) and, with A allele for the CHRNA5 rs16969968 variant (p = 0.003). Frequencies for the CHRNA4 rs1044396 GG, GA, and AA genotypes were 30%, 53.3%, 16.7% in high nicotine-dependent smokers; 22.9%, 52.9%, 24.3% in medium or low nicotine-dependent smokers; 30%, 34.6%, 35.4% in non-smokers, respectively. Frequencies for the CHRNA5 rs16969968 G and A alleles were 58.3%, 41.7% in high nicotine-dependent smokers; 42.1%, 57.9% in medium or low nicotine-dependent smokers; 40%, 60% in non-smokers, respectively. We also found an association between T allele for the CHRNA3 rs578776 genetic variant and failure of bupropion+NRT treatment (p = 0.033). C and T allele frequencies in the bupropion+NRT group were: 83.3% and, 16.7% in quitters; 44.4% and, 55.6% in non-quitters, respectively.

Conclusion: AA genotype for the CHRNA4 rs1044396 variant and A allele for the CHRNA5 rs16969968 variant was associated with a lower level of nicotine dependence. The T allele for the CHRNA3 rs578776 genetic variant was associated with failure of smoking cessation therapy in patients treated with bupropion+NRT. Supported by Hacettepe University grant TSA-2017-12810.

© 2018 The Authors

Basic & Clinical Pharmacology & Toxicology © 2018 Nordic Association for the Publication of BCPT (former Nordic Pharmacological Society).

Basic & Clinical Pharmacology & Toxicology, 123 (Suppl. 1), 3–14
Aims: CYP2B6 is responsible for the metabolism of nicotine and bupro- pion. The aim of this study was to investigate the effects of CYP2B6 rs2279343 genetic polymorphism on the heaviness of nicotine dependence and smoking cessation after 12 weeks of treatment in a Turkish population.

Methods: A total of 260 volunteers; 130 smokers and 130 non-smokers were recruited. Determination of the heaviness of nicotine dependence in the smokers was performed by Fagerström Test for Nicotine Dependence and verified by breath CO measurements. Scores ≥7 were considered as high nicotine dependence (n = 60), scores <7 were considered as medium or low nicotine dependence (n = 70). There were four treatment groups: nicotine replacement therapy (NRT, n = 40), bupropion (n = 47), bupropion+NRT (n = 15) and varenicline (n = 28). PCR-RFLP was used for genotyping.

Results: Frequencies for the AA, AG and GG genotypes were similar in all groups: 43.3%, 48.3%, 8.3% in high nicotine-dependent smokers; 45.7%, 44.3%, 9.6% in medium or low nicotine-dependent smokers; 46.2%, 40.8%, 13.1% in non-smokers, respectively (p ≥ 0.05). Also, we found no association between CYP2B6 rs2279343 polymorphism and cessation rates of smokers after 12 weeks of treatment (p ≥ 0.05).

Conclusion: This study showed no association of CYP2B6 rs2279343 polymorphism with the heaviness of nicotine dependence and with smoking cessation after 12 weeks of treatment.

Supported by Hacettepe University grant, TSA-2017-12810.
(MR) O-desmethylvenlafaxine/N-desmethylvenlafaxine in two dose groups of BUP (≤150 mg and >150 mg) and LEV (≤50 mg and >50 mg) were compared with a reference group of patients receiving the potent CYP2D6 inhibitors fluoxetine (FLU) or paroxetine (PAR). Groups were compared using a multiple linear regression model.

Results: A total of 158 patients were included in this study. All patients were treated with venlafaxine in combination with one of the following CYP2D6 inhibitors: BUP ≤150 mg/day (n = 54), BUP >150 mg/day (n = 35), LEV ≤50 mg/day (n = 20), LEV >50 mg/day (n = 32) or FLU/PAR (n = 17). MR in patients receiving high doses of BUP or LEV were not significantly different from the reference group (p > 0.15), while patients receiving low doses BUP or LEV had higher MR than the reference groups (+147% p < 0.001 and +297% p < 0.001 respectively).

Conclusion: Doses in the higher therapeutic range of bupropion and levomepromazine show a similar CYP2D6 inhibition as the potent inhibitors fluoxetine and paroxetine. The dose-dependent CYP2D6-inhibitory effect of bupropion and levomepromazine is important to consider regarding interaction risk in clinical practice.

NorPM-P14
ENANTIOSPECIFIC PHARMACOGENOMICS OF FLUVASTATIN

Hirvensalo P.1, Tornio A.1, Neuvonen M.1, Käänder W.2, Kidron H.2, Palle-Hyvarinen M.1, Tapaninen T.1, Backman J.1, Niemi M.1
1Department of Clinical Pharmacology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland; 2Division of Pharmaceutical Biosciences, University of Helsinki, Helsinki, Finland

Aims: Large interindividual variability exists in the pharmacokinetics and effects of fluvastatin. Our aim was to investigate how sequence variations in pharmacokinetic genes affect fluvastatin exposure.

Methods: A single 40 mg oral dose of racemic fluvastatin was administered to 200 healthy volunteers. Plasma 3R,5S- and 3S,5R-fluvastatin enantiomer concentrations were measured using liquid chromatography-tandem mass spectrometry and 379 genes related to pharmacokinetics were fully sequenced using massive parallel sequencing.

Results: The CYP2C9 decreased-function allele CYP2C9*3 (rs1057910; c.T075A>C; p.Ile359Leu) was associated with increased area under the plasma concentration-time curve (AUC) of both fluvastatin enantiomers. The AUCl of 3R,5S-fluvastatin was 67% (p = 3.44 x 10^-7) and that of 3S,5R-fluvastatin was 94% (p = 2.80 x 10^-12) larger per copy of the variant allele. In addition, the SLCO1B1 decreased-function rs4149056 (c.521T>C; p.Val174Ala) variant increased the AUCl of 3R,5S-fluvastatin by 34% per minor allele (p = 7.55 x 10^-3), but had no effect on 3S,5R-fluvastatin. In vitro, however, the two enantiomers were similarly transported by OATP1B1 (encoded by SLCO1B1).

Conclusion: These results indicate that CYP2C9*3 plays an important role in determining interindividual variability in fluvastatin pharmacokinetics. Furthermore, SLCO1B1 genotype affects the pharmacokinetics of the pharmacologically more active 3R,5S-enantiomer of fluvastatin. This knowledge may aid in individualizing cholesterol-lowering therapy.

NorPM-P16
MECHANISM OF IMATINIB-RESISTANCE – MICRONNA-212-ABC2-AXIS CONTRIBUTES TO DEVELOPMENT OF IMATINIB-RESISTANCE IN LEUKEMIC CELLS

Kätler M.1, Ruememann I.1, Gommermann D.2, Nagel U.1, Braun D.1, Haensisch S.1, Annerpohl O.3, Wesch D.2, Cascorbi I.1, Bruckmüller H.1
1Institute of Experimental and Clinical Pharmacology, University hospital Schleswig-Holstein, Kiel University, Kiel, Germany; 2Institute of Immunology, University hospital Schleswig-Holstein, Kiel University, Kiel, Germany; 3Institute of Human Genetics, University hospital Schleswig-Holstein, Kiel University, Kiel, Germany

Aims: The hematopoietic malignancy chronic myeloid leukemia (CML) is caused by translocation between chromosomes 9 and 22 leading to formation of the Philadelphia chromosome and the BCR-ABL fusion gene. Although the tyrosine-kinase inhibitor (TKI) imatinib led to tremendous treatment success, the development of resistances against imatinib is an emerging problem. Beside BCR-ABL-dependent mechanisms (e.g. BCR-ABL amplification/overexpression, point mutations), BCR-ABL-independent mechanisms occur, which can be linked in part to dysregulation of ATP-binding cassette (ABC)-transporters leading to increased TKI efflux, potentially caused by changes in microRNA expression or DNA-methylation. In an in vitro-imatinib-resistance model using K-562 cells, microRNA-212 was identified to be deregulated and inversely correlated to ABC-transporter ABCG2 expression, targeting its 3’-UTR.

Methods: To analyze the functional impact on drug sensitivity, transfection experiments with microRNA-mimics and –inhibitors were performed to investigate their effect on imatinib-susceptibility in sensitive and resistant leukemic cell lines. In addition the methylation status of miR-212 and ABCG2 promoter regions were analyzed.

Results: Under imatinib treatment, miR-212 inhibition led to enhanced cell viability (p = 0.01), reduced apoptosis (p = 0.01) and cytotoxicity (p = 0.03). These effects were only observed in treatment-naïve cells but not in cells resistant to various imatinib-concentrations (0.1 μM to 2 μM). In treatment-naïve cells inhibition of miR-212 resulted in ABCG2 upregulation and increased ABCG2-dependent efflux. In addition, in 0.5 and 2 μM IM-resistant sublines were observed a hypermethylation of the miR-212 promoter region, whereas the ABCG2 gene methylation status was not altered.

Conclusion: In summary, the miR-212/ABCG2-axis influences imatinib-susceptibility and contributes to development of imatinib-resistance. Our data reveal new insights into mechanisms initiating imatinib-resistance in leukemic cells.

NorPM-P18
IMMUNOGENETICS AND PROGNOSIS OF PROSTATE CANCER: FIRST RESULTS

Dresi E.1, Zanussu C.1, Ronauldi C.2, Gagno S.1, Mattia E.D.1, Romano L.1, Sartor F.1, Bartolus R.1, Quarantucci L.1, Cecchin E.1, Toffoli L.1
1Experimental and Clinical Pharmacology, National Cancer Institute, Aviano (Pordenone), Italy; 2Department of Biology, University of Padova, Padova, Italy; 3Department of Radiation Oncology, National Cancer Institute, Aviano (Pordenone), Italy; 4Rheumatology Clinic, Department of Medical and Biological Sciences, University Hospital ‘Santa Maria della Misericordia’, Udine, Italy

Aims: Many efforts are still necessary to determine robust pharmacogenetics (PGx) biomarkers to be implemented in the clinical practice to advance the field of personalized medicine in cancer treatment. Regarding prostate cancer (PCa). The primary aim of this study is the identification of new biomarkers of biochemical recurrence (BCR) analyzing polymorphisms (SNPs) of genes involved in immune system. The secondary aim is the determination of biomarkers of overall survival (OS). These new biomarkers, if validated, could be implemented in the electronic clinical record to personalize patients’ treatment.

Methods: 418 Caucasian patients affected by clinically localized PCa treated with RT as primary therapy were enrolled. The genetic analyses of 447 SNPs in genes of immune system were determined in germline DNA samples using a Veracode (Illumina) platform. Multivariate COX regression was applied to identify the prognostic role of SNPs in terms of both BCR and OS. Significant associations were determined according to p-value (<0.05) and q-value (<0.15), defined according to the False discovery rate (FDR) method.

Results: 19 SNPs were significantly associated with BCR. Interestingly, these SNPs addressed in an independent way the key role of PD1, VEGFR, FOXO1, SMAD3, SMAD2, and IL4R, that are involved in angiogenesis and TGFB pathway.
The rs3918262-MMP9, rs7692791-VEGFR, and rs2034967-VEGFR can predict OS, reinforcing the importance of angiogenesis in this clinical setting.

**Conclusion:** This study identified the key role of SNPs impacting immune system activity in the prediction of BCR and OS, highlighting the relevance of angiogenesis in this setting. The prognostic role of these SNPs is going to be tested in a replication set of 131 PCa patients. These analyses are needed to hopefully offer new biomarkers to optimize PCa patients' management through the development of innovative and user-friendly IT tools.

**NorPM-P19**

DEVELOPMENT OF AN INTEGRATED HEALTH IT PLATFORM FOR A PRECISE AND COST-EFFECTIVE THERAPY IN ONCOLOGY

Roncato R., Cin L.D., Mezzalira S., Comello F., Bignucolo A., Gioletti L., D’Errico 3, Emili L., Carboni V., Guardascione M., Mattia E.D., Toffoli G., Cecchin E.

1Experimental and Clinical Pharmacology, Aviano (PN), Italy; 2National Cancer Institute – CRO Aviano, Aviano (PN), Italy; 3Department of Pharmaceutical and Pharmacological Science, University of Padova, Padova, Italy; 4Insiel Mercato, Trieste, Italy; 5Premeditec, Trieste, Italy

**Aims:** The integration of the pharmacogenetic data in the electronic clinical record and the development of a decision support system could be helpful for prescribing the most efficacious and cost-effective pharmacological therapy available. This is particularly relevant in oncology, where the therapeutic index of drugs is limited and the cost of therapies is high. The aim of the project is to radically innovate the prescriptive process of oncological medications by providing the prescriber an IT infrastructure for the automated management of the patient’s molecular data that will be translated into specific indications.

**Methods:** The most up-to-date literature data will be mined to extract a list of gene-drug interactions based on the most recent published guidelines to provide a list of the most relevant genetic information to be included in the patients clinical health record together with the corresponding pharmacogenetic guidelines.

**Results:** Public available guidelines were reviewed and compared to reach a consensus in terms of gene-drug pairs considered, their different level and classification of evidence rating based on a scoring system, the therapeutic strategy recommended and the clinical impact of pre-emptive PGx test. Information on the strength of the recommendation (classification of recommendation, level of evidence and clinical impact) were considered necessary to be integrated in the prescribing system along with the therapeutic recommendation. A prototype of a decision support system is being developed through the partnership with two high tech companies active in Italy the healthcare system computerization.

**Conclusion:** Coordinated efforts through partnership are able to put together scientific and clinical expertise in the oncologic pharmacogenomic field, to implement the pre-emptive pharmacogenomic approach in the clinical practice in Italy, and to demonstrate its benefit in both patients clinical outcome and quality of life, with an economic advantage for the healthcare system.

**NorPM-P20**

MAJOR INFLUENCE OF THE CYP2D6*41 ALLELE ON CYP2D6 METABOLIZER PHENOTYPE – A STUDY ON 1,021 VENLAFAXINE-TREATED PATIENTS

Haslamo T.1, Eliasson E.2, Jukic M.3, Ingelman-Sundberg M.3, Milden E.1

1Center for Psychopharmacology, Diakonhjemmet Hospital, Oslo, Norway; 2Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden; 3Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

**Aims:** To compare the in vivo CYP2D6 metabolism encoded by the *9*, *10* and *41* alleles, and assess the phenotype overlap between carriers of these variants with non-coding (null) alleles and null/null carriers (poor metabolizers, PMs).

**Methods:** The study included 1,021 patients from a therapeutic drug monitoring service performing both drug concentration analyses and genotyping. Absence of comedication with CYP2D6 inhibitors or antiepileptic enzyme inducers was confirmed for all patients. Metabolic rate (MR) of O/N-desmethyl venlafaxine (MR) of O/N-desmethyl venlafaxine for the study was genotyped using the TaqMan genotyping test. Genotyping analysis was carried out in three different laboratories: Reagenz, Oslo, Norway; 2Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden; 3Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

**Results:** The MR of O/N-desmethyl venlafaxine was lower in *41* than *9* or *10* carriers combined with *1* and null alleles (p < 0.002). In *41/null carriers (n = 30), MRs of 26 patients (86.7%) were in the range of null/null carriers (n = 95) compared to three out of 17 carriers of *9/null or *10/null (17.4%). The multiple linear regression analysis provided MR estimates of 0.47, 1.33, 3.55, and 9.54, respectively, in carriers of null/null, *9* or *10* combined with wild type (*1) or null alleles were compared by Mann-Whitney tests. In addition, the degree of subgroup overlaps in MR was calculated from direct observations, and a multiple linear regression analysis performed to provide MR subgroup estimates adjusting for covariates.

**Conclusion:** CYP2D6*41 encodes significantly lower CYP2D6 metabolism in vivo than *9* and *10*. The majority of *41/null carriers express a CYP2D6 phenotype within the range of null/null carriers. Thus, pharmacogenetic assays for clinical routine and research should include CYP2D6*41 to capture most functional PMs.

**NorPM-P21**

PRELIMINARY RESULTS OF THE CLINICAL TRIAL GENOTYPE-PHENOTYPE CORRELATION OF DRUG-METABOLIZING ENZYMES

Böhm R.1, Bruckmüller H.1, Hiibenthal M.2, Siegmund W.3, Oswald S.3, Jücker J.4, Franke A.2, Cascomb i.1

1Institut für Experimentelle und Klinische Pharmakologie, Campus Kiel, Kiel, Germany; 2IKMB, UKSH, Campus Kiel, Kiel, Germany; 3Institut für Pharmakogenomik und Pharmakogenetik, Universität Greifswald, Germany; 4Klinik für Anästhesiologie und operative Intensivmedizin, Friedrich-Ebert-Krankenhaus, Neumünster, Germany

**Aims:** To detect new genetic variants with impact on drug metabolism and to test the performance of genotyping and phenotype-prediction methods.

**Methods:** 200 healthy volunteers were exposed to a drug cocktail containing caffeine, losartan, omeprazole, dextromethorphan and midazolam. A blood sample was obtained after 5 h, urine was collected for 8 h. Subsequent measurement of the above mentioned drugs and their metabolites allowed calculation of metabolic ratios which were considered to show the activity of CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5 respectively. For genotyping, BFLP, qRT-PCR, pyrosequencing, Affymetrix DMET chips and Illumina exome SNP-chips were employed.

© 2018 The Authors

Basic & Clinical Pharmacology & Toxicology © 2018 Nordic Association for the Publication of BCPT (former Nordic Pharmacological Society).

Basic & Clinical Pharmacology & Toxicology, 123 (Suppl. 1), 3–14
Methods: Since January 1, 2016, relapsing haematologic cancer patients at Aalborg University Hospital have been offered to participate in the ProSeq protocol, where RNA- and DNA-sequencing are performed on the cancer cells to identify drug targets. Injectable (IJ) drugs dosages and prices were obtained from the hospital pharmacy combined with clinical data and dosages for non-IJ drugs from electronic health records. Data recordings end at death or lost to follow-up. The drugs were grouped by ATC codes and the price was estimated by a mg price based on the total number and price of packages and doses sold to the department. The total drug related treatment cost (DRTC) over the inclusion period was divided by the total days at risk to get an estimate of cost per day per patient.

Results: After 25 months, 208 patients have been included. Of these, 111 (myeloid leukemias = 11, lymphoid leukemias = 25, multiple myeloma = 16, lymphoma = 59) have received drug related treatment. The included patients have a median follow-up time (95% CI) of 11 months (9.6 months, 13 months) and one-yr survival rate of 76% (70%, 82%). Assuming 1 year stays in ProSeq the DRTC of relapsing patients was estimated to be 29 million DKK/year, which is 1/2 of the DRTC of all haematologic patients.

Conclusion: We have formulated a methodology to estimate DRTC. This will e.g. be used to 1) identify costly patients, 2) evaluate the benefit of implementing of PM and 3) aid in health care planning.
evidence and lack of competence among medical professionals. There is little evidence that taking GTs gives health benefits. Transmission of personal genetic data to private companies is worrisome. Among the EU countries legislation is diverse from virtually banning GTs to fully permitting them. In Norway the sale of GTs is not regulated by law, but testing of children is forbidden.

Conclusion: Most published studies deal with validity of GTs, bioethics, and legislation. Major challenges with GTs are deficient scientific evidence, low public and professional literacy in genomic health and vague regulations of GTs. GTs may contribute to early diagnostics and better health awareness, but progress is necessary in validating GTs and elaborating regulations.

**NorPM-P25**

**VALPROATE AND PROTEIN BINDING – THE IMPORTANCE OF MEASURING FREE CONCENTRATIONS**

Baffiu A.1,2, Hege K.1,3, Burns M.L.4, Gottius A.4, Johannessen S.I.1,4,5, Landmark C.J.1,4,5

1Programme for Pharmacy, Metropolitan University, Oslo, Norway; 2Norwegian Medicines Agency, Oslo, Norway; 3The Norwegian University of Science and Technology, Trondheim, Norway; 4Department of Pharmacology, Oslo University Hospital, Oslo, Norway; 5The National Center for Epilepsy, Oslo University Hospital, Sandvika, Norway

**Aims:** Routine use of therapeutic drug monitoring (TDM) of antiepileptics drugs by measurement of total concentrations is well established in epilepsy. In cases where changes in protein binding affect highly bound drugs (≥90% for valproate), measurement of the unbound concentration will be more accurate for exposure of active drug in the body. The aim of this study was to describe current practice where unbound serum concentrations of valproate were measured and to discuss the clinical impact in patients with epilepsy.

**Methods:** Retrospective, anonymous TDM-data from patients with measurements of unbound valproate concentrations were collected from the National Center for Epilepsy (2012–2017). The samples were taken drug-fasting in the morning at assumed steady-state. The study was approved by the Regional Ethics Committee.

**Results:** Data from 132 measurements in 81 patients using valproate were included, 40/60% women/men, average age was 39 (1–88) years. Hypoalbuminemia, reduced renal function, intensive care monitoring, adverse effects, displacement interaction with striptentol, young/old age and pregnancy (5 pregnancies in 3 women) were relevant reasons for the request. Total serum concentration of valproate was 82–908 µmol/L (reference range 300–700), average unbound concentration 20% (5–93, reference ≈10%), and average dose was 1245 mg/day (72–3,000).

Unbound concentrations of valproate increased up to 5-fold in two pregnancies and decreased post-partum, reflecting an increased exposure where use of total concentrations would be misleading.

**Conclusion:** Measurement of total and unbound concentrations of valproate showed increased free fraction in most of these vulnerable patients, and total concentrations may then underestimate drug exposure. Implementation of TDM with total and unbound concentrations may contribute to improved and tailored treatment for the individual patient.

**NorPM-P26**

**GENOMIC AIDED PHARMACOTHERAPY (GAP): A PILOT STUDY IN PATIENTS SUFFERING FROM CHRONIC NON-CANCER PAIN**

Houbløn M.B.1, Tredal C.1, Vagn-Hansen L.2, Hansen R.W.3, Christrup L.T.4

1Department of Drug Design and Pharmacology, University of Copenhagen, København Ø, Denmark; 2Allévia, Private Hospital Moelholm, Aarhus, Denmark; 3Kysthosiptalet, Skodsborg, Denmark; 4GeneTelligence, Høersholm, Denmark

**Aims:** Efficacy and safety of drugs vary significantly between individuals. The CYP450 enzyme system is responsible for the metabolism of most drug substances. During the recent years genetic testing has become more widely available. In Denmark the genetic test “Personalised Medicine Profile” (PMP) was recently made available by GeneTelligence as a tool to optimise and individualise pharmacotherapy. PMP is a genotyping test, which translates data on genetic variation and generates a personalised and evidence-based report on the influence of genetic variation on drug transport and metabolism by the CYP450 system. The report includes evidence based recommendations for individual therapy of 158 commonly prescribed drugs and identify the drugs that may cause significant side-effects or have reduced efficacy at standard doses. The patients’ metabolic status is categorised in four classes: poor-, intermediate-, normal – and ultra rapid metabolisers.

The aim’s of this pilot study was to evaluate the practical issues using the PMP test and the interpretation of the resulting report in a small selected population of patients suffering from chronic non-cancer pain and further to assess the number and types of therapy related challenges identified by the test.

**Methods:** Patients were recruited from two private Pain Centres in Denmark: Allévia and Kysthosiptalet during January and February 2018. Eligible patients received a buccal swab kit. After performing the buccal swab, the kit was sent to GeneTelligence for DNA analysis. After genomic data translation the personalised evidence-based report is generated, using the PMP software platform. Based on this PMP report and the patients’ current medication a pharmacist identified clinically significant drug therapy challenges and created a report containing recommendations for medication optimisation.

**Results:** Ten patients were included in this pilot study; the data analyses are still ongoing. The results will be presented at the 1.NORPM conference.

**Conclusion:** Conclusions will be presented at the meeting.

**NorPM-P27**

**A CLINICALLY EFFECT OF UGT1A4*3 ON LAMOTRIGINE CONCENTRATION IS PREDOMINATELY OBSERVED IN POSTMENOPAUSAL FEMALES – A STUDY OF 534 PATIENTS**

Smith R.L., Haslø T., Chan H., Refsum H., Molden E.

Center for Psychopharmacology, Diakonhjemmet Hospital, Oslo, Norway

**Aims:** Studies on therapeutic drug monitoring (TDM) of lamotrigine (LTG) have provided conflicting results regarding the impact of the UDP-glucuronosyltransferase (UGT) 1A4 genotype on the pharmacokinetics of LTG. As multiple factors may influence LTG pharmacokinetics, the present study aimed to investigate the impact of the UGT1A4*3 variant on LTG serum concentration accounting for age, sex and valproic acid (VPA) comedication in a large patient population.

**Methods:** Matched TDM data on LTG and UGT1A4 genotype of patients with information about VPA comedication were included retrospectively from a TDM service. Linear mixed model analysis allowing multiple measurements per patients was used to evaluate the impact of UGT1A4*3, as well as age, sex and VPA comedication, on dose-adjusted serum concentrations (C0D ratio) of LTG.

**Results:** Totally, 1735 LTG serum concentration from 534 patients were included. In the population, UGT1A4*3 carriers had 10–15%
lower LTG C/D ratio compared to non-carriers ($p = 0.01$), but the quantitative impact of $UGT1A4^{*3}$ depended on age and sex. The difference was greatest in postmenopausal females ($p = 0.001$), where $UGT1A4^{*3}$ carriers obtained $\sim40\%$ lower LTG C/D ratio than $UGT1A4^{*3}$ non-carriers ($p = 0.001$). The $UGT1A4^{*3}$ variant had only minor effect on C/D ratio in younger females ($p \geq 0.1$) and males ($p > 0.1$). Regardless of genotype, patients with concomitant use of VPA had the most pronounced effect by increasing the LTG C/D ratio about 2.5-fold ($p < 0.001$).

**Conclusion:** This study shows that $UGT1A4^{*3}$ has a modest impact on LTG exposure, but it could lead to clinically relevant lowering in serum concentration among postmenopausal females. Thus, genotyping of $UGT1A4$ could be valuable for dose individualization when initiating LTG treatment in postmenopausal females.

**NorPM-P29**

**P-GP INHIBITION INCREASES NEURONAL TOXICITY OF PACLITAXEL IN SH-SYSY-DERIVED NEURONS**

*Stage T.B., Khalaf S., Svenningsen A.F., Kroetz D.*

1Clinical Pharmacology and Pharmacy, Department of Public Health, University of Southern Denmark, Odense, Denmark; 2Neurobiology, University of Southern Denmark, Odense, Denmark; 3Department of Bioengineering and Therapeutic Sciences, University of California San Francisco, San Francisco, United States

**Aims:** Paclitaxel is an anticancer agent efficacious in the treatment of breast, ovarian and lung cancer. $ABCB1$ polymorphisms have been associated with increased risk of paclitaxel-induced peripheral neuropathy. The aim of this study was to investigate the link between the drug efflux transporter P-glycoprotein (P-gp, encoded by $ABCB1$) and the neuropathy caused by paclitaxel.

**Methods:** Neuroblastoma SH-SYSY cells were differentiated to neurons and treated with $0.1–10 \mu M$ paclitaxel for $24$ h. Cytotoxicity of paclitaxel was assessed using the CellTiter-Glo® luminescence assay. The influence of P-gp inhibition during paclitaxel treatment was investigated by selectively inhibiting P-gp using $4 \mu M$ valspodar. Neurons treated with paclitaxel were immunolabeled with the neuronal marker tubulin beta III (TUBB3) and the number of neurites/cells were counted and neurite length measured using ImageJ.

**Results:** Paclitaxel caused a dose-dependent decrease in both number and length of neurites in SH-SYSY-derived neurons, but did not cause cell death. In SH-SYSY-derived neurons, $70\%$ had more than two neurites after $0.1 \mu M$ paclitaxel exposure, while only $5\%$ of the neurons had more than two neurites after $10 \mu M$ paclitaxel exposure. Neurite length was also significantly decreased in a dose-dependent manner ($p < 0.01$). Inhibiting P-gp led to a marked increased toxicity of paclitaxel when assessing both number and length of neurites ($p < 0.01$).

**Conclusion:** Paclitaxel caused a clear dose-dependent effect on number and length of neurites in SH-SYSY-derived neurons that was exacerbated by inhibition of the efflux transporter P-gp. This may contribute to the understanding of the association of genetic variation in $ABCB1$ and paclitaxel-induced peripheral neuropathy.

**NorPM-P30**

**CLINICAL APPLICATIONS OF CYP450 GENOTYPING. SEVEN YEARS’ EXPERIENCE IN A SWEDISH CLINICAL SETTING**

*Scordo M.G.*

1Department of Medical Sciences, Clinical Pharmacology, Uppsala University, Uppsala, Sweden; 2Section for Clinical Pharmacology, Uppsala University Hospital, Uppsala, Sweden

**Aims:** Genetics is a known source of interindividual variability in drug response. Polymorphic enzymes such as $CYP2D6$, $CYP2C19$ or $CYP2C9$ catalyse the oxidative metabolism of numerous commonly prescribed drugs, such as antipsychotics, antiepileptics, antidepressants and cardiovascular drugs. Evaluation of the metabolic capacity of an individual by genotyping may therefore help the clinicians to select the right dose and/or the best drug for a patient, thereby minimizing the risk of side-effects or therapeutic failure.

**Methods:** In the period 2011–2017 over 700 genotyping analyses were performed at our Department, with a constant yearly increase in the number of tests performed. The most requested test was $CYP2D6$ ($47\%$), followed by $CYP2C19$ ($33\%$) and $CYP2C9$ ($20\%$). Most patients were treated with psychoactive drugs ($80\%$), such as antidepressants and classical antiepileptics, followed by cardiovascular, anticoagulant and antipilet drugs ($15\%$). The principal reason for genotyping was lack of optimal drug response/therapeutic failure ($60\%$), followed by side-effects ($35\%$), while a few subjects were genotyped before starting therapy with drugs such as warfarin, clopidogrel, propafenone or tamoxifen.

**Results:** Ultrarapid metabolism (caused either by $CYP2D6$ gene duplication or homozygosity for $CYP2C19^{*17}$) was detected in a handful of subjects with therapeutic failure, suggesting that non-compliance plays a major role in therapeutic failure. Conversely, among subjects who had experienced side-effects, the frequency of detrimental alleles was higher than the background incidence. Furthermore, in some cases extensive metabolisers with side effects were phenotypically poor metabolisers, due to co-medication with potent $CYP2D6$ inhibitors such as paroxetine and bupropion.

**Conclusion:** Based on our experience, we conclude that genotyping is a valuable complement to plasma concentration determination when poor or ultrarapid drug metabolism is suspected.

**NorPM-O1**

**EFFECT OF RARE SINGLE-NUCLEOTIDE VARIANTS ON THE BREAST CANCER RESISTANCE PROTEIN**


1Faculty of Pharmacy, University of Helsinki, Helsinki, Finland; 2Radboud University Medical Centre, Nijmegen, Netherlands

**Aims:** To study the effect of single-nucleotide variations in transmembrane regions and the first intracellular loop of the breast cancer resistance protein (BCRP, ABCG2).

**Methods:** Nine naturally occurring variants in the transmembrane region of BCRP (G406R, F431L, S441N, P480L, F489L, M515R, L525R, A528T, and T542A) and five in the first intracellular loop (K453R, 1456V, H457R, G462R and G462V) were selected. In addition, two common variants (V12M and Q141K) in the binding domain were included as controls. The variant proteins were expressed using a baculovirus expression system in mammalian cells (HEK293). Crude membrane fractions of the cells were extracted to prepare membrane vesicles. Western blotting and immunofluorescence microscopy were used to study the expression and localization of the variants. The activity of the variants was tested using the vesicular transport assay with BCRP substrates Lucifer yellow and estrone sulfate. The data was used to predict in vivo effects of the BCRP variants on rosuvastatin pharmacokinetics in two PBPK models using Simcyp and MATLAB SimBiology.

**Results:** The transport activity of all variants in the transmembrane region was significantly impaired compared to wt BCRP, but two of the variants (K453R, 1456V) in the intracellular loop retained comparable activity to wt BCRP. Decreased expression levels could explain the low activity for some, but not all, of the variants. Immunofluorescence studies in cells indicated impaired localization for some of the variants, in line with the activity and Western blot results. Based on the simulations, variants with impaired activity may increase the Cmax of rosuvastatin by two-fold and the AUC over three-fold compared to the wt BCRP.

**Conclusion:** Single nucleotide variations in the transmembrane region or the first intracellular loop may have destructive effects on BCRP activity, which may lead to unexpected pharmacokinetic events in patients carrying these variants.
NorPM-O2
DOXORUBICIN UPTAKE IN CARDIOMYOCYTES IS DEPENDENT ON ORGANIC CATION TRANSPORTER 3
Huang K.1, Magdy T.2, Bonilla I.3, Hu S.1, Gibson A.1, Carnes C.3, Sparreboom A.1, Burridge P.2
1Pharmaceuticals, The Ohio State University, Columbus, OH, United States; 2Pharmacology, Northwestern University, Chicago, OH, United States; 3Pharmacology, The Ohio State University, Columbus, IL, United States

Aims: The anthracycline doxorubicin is among the most effective and commonly used anticancer agents, but its use is associated with a potentially life-threatening cardiotoxicity. We hypothesized that doxorubicin-induced cardiotoxicity (DIC) is initiated by transporter-mediated mechanism in which cells of the myocardium are selectively damaged, and that identification of the involved solute carriers can provide insights into the development of preventative strategies.

Methods: Transporter gene expression studies were conducted on human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) from patients that did or did not experience DIC. Determination of transport kinetics and mechanism of transport inhibition were performed in stably transfected HEK293 using TEA and metformin as prototypical substrates. Furthermore, doxorubicin disposition in ex vivo isolated cardiomyocytes and in vivo hearts were determined in wild-type and age-matched transporter-knockout mice.

Results: Multiple solute carrier genes were overexpressed by ≥2-fold in hiPSC-CMs from patients experiencing DIC, including OCTN1, OCT1, and OCT3. Among these transporters, deficiency of OCT3 in mouse cardiomyocytes was associated with the most significant decrease in doxorubicin uptake ex vivo (p < 0.0001). Accumulation of doxorubicin in cardiomyocytes of wild-type mice could be inhibited by known OCT3 inhibitors, including the tyrosine kinase inhibitors dasatinib and nilotinib, through a non-competitive mechanism. Consistently, heart levels were reduced in OCT3-knockout mice after doxorubicin administration in vivo.

Conclusion: We identified a previously unrecognized pathway of DIC that is initiated by an organic cation transporter system and highly sensitive to pharmacological inhibition. These findings shed further light on the etiology of DIC, and provide a rationale for the future development of new targeted interventions to mitigate this debilitating side effect.

NorPM-O3
MICRORNA-155 CONTROLS VINCRISTINE SENSITIVITY AND PREDICTS SUPERIOR CLINICAL OUTCOME IN DIFFUSE LARGE B-CELL LYMPHOMA
Due H.1, Schönherr AA.1, Ryo LB.2, Roug AS.1, Johnsen HE.1, Børgsted K.1, Mikkelsen JG.2, Dybkær K.1
1Department of Hematology, Aalborg University Hospital, Aalborg, Denmark; 2Department of Biomedicine, Aarhus University, Aarhus, Denmark

Aims: A major clinical challenge of diffuse large B-cell lymphoma (DLBCL) is that 30–40% of patients have refractory disease or relapse after initial response to therapy, due to drug-specific molecular resistance. The purpose of this study was to investigate miRNA involvement in vincristine resistance in DLBCL.

Methods: By analyses of miRNA microarrays from DLBCL cell lines with different vincristine sensitivity, we identified lower miR-155 expression in resistant cells. This association was further examined by functional in vitro analysis, studying the direct impact of miR-155 in vincristine response, and by target gene analysis. Secondly, prognostic impact of miR-155 expression was evaluated in two independent DLBCL cohorts.

Results: Exogenous upregulation of miR-155 sensitized GCB-DLBCL cells to vincristine, and consistently, downregulation and knock-out of miR-155 induced vincristine resistance, documenting that miR-155 functionally controls vincristine sensitivity. Target gene analysis identified Wee1, a cell-cycle checkpoint gene, as a target of miR-155 in DLBCL. Chemical inhibition of Wee1 sensitized GCB-DLBCL cells to vincristine, suggesting that miR-155 controls vincristine response through Wee1. Clinical outcome analysis of DLBCL patients revealed that high miR-155 expression level was significantly associated with superior survival for R-CHOP treated patients of the GCB subclass, independent of the well-established international prognostic index (IPI), an observation challenging the commonly accepted perception of miR-155 as an oncomiR.

Conclusion: We experimentally confirmed a direct link between high miR-155 expression and vincristine sensitivity in DLBCL and documented an improved clinical outcome of GCB classified patients with high miR-155 expression level. This suggests that the role of miR-155 in vincristine response is important enough to affect overall survival.

NorPM-O4
THE PROTEIN KINASE INHIBITORS MIDOSTAURIN AND NINTEDANIB ARE TIME-DEPENDENT INHIBITORS OF CYP3A4
Filippula A., Mustonen T., Backman J.
Department of Clinical Pharmacology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

Aims: Protein kinase inhibitors display a tendency to affect cytochrome P450 (CYP) 3A4 by time-dependent inhibition. As CYP2C8 and CYP3A4 share overlapping substrate specificity, we tested six novel kinase inhibitors for time-dependent inhibition of these enzymes.

Methods: The inhibitory effects of dovitinib, masitinib, midostaurin, nintedanib, trametinib and vatalanib on amodiaquine N-deethylation (CYP2C8) and midazolam 1’-hydroxylation (CYP3A4) were evaluated in human liver microsomes. Static predictions were used to estimate the clinical significance of the observed inhibition.

Results: Dovitinib, midostaurin and nintedanib exhibited time-dependent inhibition of CYP3A4 (IC50 shift >1.5), whereas masitinib, trametinib and vatalanib did not affect CYP2C8 or CYP3A4 by time-dependent inhibition (IC50 shift <1.5). Further experiments identified midostaurin and nintedanib as mechanism-based inhibitors of CYP3A4, with maximal inactivation rate (kinact) and inhibitor concentration supporting half of kinact (Ki) values of 0.052 1/min and 2.72 µM, and 0.025 1/min and 17.3 µM, respectively. Predictions indicated that standard doses of nintedanib are unlikely to cause drug interactions with CYP3A4-dependent substrates, whereas midostaurin and vatalanib could increase the plasma exposure to such substrates several-fold. Furthermore, based on reversible inhibition, masitinib and trametinib were predicted to increase the plasma exposure to sensitive CYP2C8 and CYP3A4 substrates by ≥2-fold.

Conclusion: Our data identifies two additional kinase inhibitors as time-dependent inhibitors of CYP3A4, and detects a risk for drug interactions between several of the tested inhibitors and CYP2C8 and CYP3A4 substrates.

NorPM-O5
GENOME-WIDE ASSOCIATION STUDY OF PANDEMRIX-INDUCED NARCOLEPSY IN SWEDEN – A POSSIBLE ROLE FOR GLIAL DERIVED NEUROTROPHIC FACTOR (GDNF)
Wadelius M.1, Eriksson N.2, Smedje H.3, Yue Q.-Y.4, Magnusson P.K.E.5, Hallberg P.1
1Medical Sciences and Science for Life Laboratory, Uppsala University, Uppsala, Sweden; 2Uppsala Clinical Research Center, Uppsala University, Uppsala, Sweden; 3Division of Child and Adolescent Psychiatry, Karolinska Institutet, Stockholm, Sweden; 4Medical Products Agency, Uppsala, Sweden; 5Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden

Aims: Narcolepsy is an autoimmune disease characterized by an inability to control sleep and wakefulness. The number of young
diagnosed with narcolepsy rose sharply following immunization with the A(H1N1)pdm09 influenza virus strain vaccine Pandemrix in Sweden 2009–2010. The most frequent form, narcolepsy type I, is known to be caused by a loss of hypocretin neurons, and is strongly associated with HLA-DQB1*0602. However, only 0.02% of HLA-DQB1*0602 carriers developed narcolepsy following vaccination. We sought to determine whether other genetic factors contribute to the risk of narcolepsy associated with Pandemrix.

Methods: 43 adjudicated cases of Pandemrix-associated narcolepsy from the Swedish adverse drug reaction biobank Swedegene were compared with 4891 Swedish population controls from TwinGene. Genotyping was performed on Illumina arrays. The merged dataset contained 600 K single nucleotide polymorphisms (SNPs), and after phasing and imputation 8.6 million SNPs. We corrected for principal components 1–4. The genome-wide significance p-value threshold was set to \( p \leq 5 \times 10^{-8} \).

Results: Narcolepsy was significantly associated with HLA-DQB1*0602, odds ratio (OR) 6.4 [95% confidence interval (CI) 4.2, 9.8], \( p = 1.4 \times 10^{-17} \). After correction for HLA-DQB1*0602, the strongest association was with the glial cell line-derived neurotrophic factor antisense gene (GDNF-AS1), OR=8.6 [95% CI 4.2, 17.6], \( p = 2.6 \times 10^{-9} \).

Conclusion: We found an association with an antisense RNA that is transcribed from the opposite stand of the glial cell line-derived neurotrophic factor gene (GDNF). The antisense transcript has been shown to regulate the expression of GDNF, which encodes an essential neurotrophic factor that supports neuronal survival. Dysregulation of GDNF has been seen in brain cells from patients with Alzheimer’s and Parkinson’s disease. This finding may increase the understanding of mechanisms behind vulnerability to narcolepsy.

NorPM-O7
SELECTING PHARMACOGENETIC TEST FOR CLOPIDOGREL PATIENTS
Forström J.¹, Tulikki M.²
¹Abomic Oy, University of Turku, Turku, Finland; ²Department of Biochemistry, University of Turku, Turku, Finland

Aims: Clopidogrel is a pro-drug which is metabolized by CYP2C19 to an active substance. About 30% of European population are poor or intermediate CYP2C19 metabolizers. For them the effect of clopidogrel may be absent or poor and testing of CYP2C19 is therefore recommended. Patients using clopidogrel often use other drugs as well. Based on epidemiological data we evaluated, how often the patient would benefit from a larger pharmacogenetic profile compared to CYP2C19 definition alone.

Methods: From Finnish prescription statistics we collected all prescriptions from over 30 cities in Finland. It covered 20% of the Finnish population. All patients who had received clopidogrel prescription in 2015–2017 were selected. From these patients we calculated, how many patients had received in the same period a drug where defining CYP2D6 or SLCO1B1 is recommended. According to Finnish database the substances where CYP2D6 is recommended are: amitriptyline, codeine, metoprolol, nortriptyline, paroxetine, risperidone and tamoxifen. SLCO1B1 is recommended for simvastatin patients.

Results: In the material we found 17,373 patient who had a clopidogrel prescription. 6,547 patients (37.7%) had only clopidogrel prescription. 5,105 patients (29.4%) had at least one prescription of drugs where CYP2D6 is recommended. The four most commonly used CYP2D6 drugs in the clopidogrel population were: metoprolol (2,089), codeine (1,876), amitriptyline (1,037) and risperidone (674). In the population we had 5,503 patients (31.7%) who had received simvastatin.

Conclusion: Clopidogrel patients often have other drugs where pharmacogenetics testing would be beneficial. Based on our data it is evident, that the population would benefit from larger set of pharmacogenetic tests. At least CYP2D6 and SLCO1B1 are relevant in this patient population. When the test is performed from the same sample, the additional testing cost is minimal compared to the benefit for the patient and health care payers.
yet the mechanism for this variation is uncertain and only short-term glycaemic response can predict who will have a merked mid-long-term response and who will respond less. Common missense variants in the GLP-1R have previously been reported to alter GLP-1 mediated insulin secretion in non-diabetic individuals. We aimed to investigate how GLP-1R variants alter glycaemic response to the GLP-1RA.

**Methods:** We performed a meta-analysis across the DIRECT, PRIBA and GoDARTS cohorts. A total of 1,156 T2D subjects were followed-up for 6 months after initiation of GLP-1RA treatment. The association of GLP-1R variants, rs6923761 (Gly168Ser) and rs10305420 (Pro7-Leu), with reduction in glycated haemoglobin (HbA1c) after treatment were assessed using multiple linear regression in an additive model.

**Results:** Gly168Ser and Pro7Leu variants were independently associated with reduced efficacy of GLP-1RA to lower HbA1c (Gly168Ser $\beta$ (HbA1c change per allele) = $-0.18\%$, $p = 0.001$, Pro7Leu $\beta = -0.14\%$, $p = 0.01$). We then derived a genetic risk score, summing up these two variants. The 22% of the population who carry no variant allele in either of the variants had a mean HbA1c reduction of (1.39%[1.21–1.58] (15.22 mmol/mol [13.17–17.27])). In contrast the 17% percent of the population who carry 3 or more risk alleles had a much lower glycaemic response (0.86% [0.66–1.13] (9.4 mmol/mol [7.00–11.92])), a difference of 0.53% [5.8 mmol/mol] ($p < 0.001$). There was no significant impact of these SNPs on weight change in response to GLP-1RA suggesting the variants impact on the glycaemic lowering mechanisms of GLP-1RA rather than weight lowering mechanisms.

**Conclusion:** Missense variants in the GLP-1R have a large clinical impact on glycaemic response to GLP-1RA. The frequencies of these variants are not rare, making this the largest, most common pharmacogenetic effect described to date in T2D.