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ESHG

ESHG Pharmacogenetics Course

Book of Abstracts

Portorož, Slovenia
22. – 24. September 2022

ESHG Pharmacogenetics Course

Book of Abstracts
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WELCOME ADDRESS

Dear colleagues,

We are pleased to welcome you to the **ESHG Pharmacogenetics Course**. The event will take place in Portorož, Slovenia, from September 22nd to September 24th 2022 at Grand Hotel Bernardin.

This course aims at delivering up-to-date knowledge on pharmacogenetics to clinicians, pharmacists, clinical pharmacologists, genetic counsellors, clinical and molecular geneticists in training or certified as well as PhD students and researchers.

The programme includes invited lectures that will give the introduction to the field as well as present the latest breakthrough research, tutorials, discussions as well as short oral and poster presentations. The programme is designed to facilitate productive discussions with experts in the field and to provide ample opportunities for the participants to present and discuss their work.

The faculty combines experts from many fields of pharmacogenetics known for their teaching skills. Participants will co-create the programme with on-site presentations of clinical or genetic cases and studies in a poster format or as a short presentation.

The course is organized by the Pharmacogenetics laboratory, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana as a collaborative action with the European Society of Human Genetics (ESHG).

The course is also endorsed and supported by the HORIZON-WIDERA-action: PharmGenHUB (GA No. 101059870) and H2020 MSC ITN-action: TranSYS (GA No. 860895). The course is endorsed by ERA Chair CONI, funded from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 951851.

Such a collaborative action will maximize interactions and encourage the exchange of knowledge and experience and possibly stimulate new collaborations that will facilitate the translation of novel findings into preventive, personalized curative, promotional and rehabilitative health care services to improve health outcomes, reduce health inequalities and to promote wellbeing.

We are delighted to welcome you to what promises to be an exciting course with a high scientific standard in one of the most beautiful parts of the Slovenian coast.

Prof. Dr. Vita Dolžan
Chair of the International Scientific Committee and Organizing Committee

Assist. Prof. Dr. Katja Goričar
Co-chair of the Organizing Committee

ORGANIZATION

Organized by

Pharmacogenetics Laboratory, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana

International Scientific Committee

- Vita Dolžan (Faculty of Medicine, University of Ljubljana, Slovenia)
- William Newman (Manchester Centre for Genomic Medicine, Manchester University NHS Foundation Trust, United Kingdom)
- Sir Munir Pirmohamed (MRC Centre for Drug Safety Science and Wolfson Centre for Personalised Medicine and Institute of Translational Medicine, University of Liverpool, United Kingdom)
- Cristina Rodriguez-Antona (Spanish National Cancer Research Centre, Madrid, Spain)
- Ron van Schaik (Erasmus University Medical Centre, Rotterdam, The Netherlands)

Organizing Committee

- Vita Dolžan, chair
- Katja Goričar, co-chair
- Sara Redenšek Trampuž
- Tanja Blagus
- David Vogrinc

Endorsed and supported by

- HORIZON-WIDERA-action: PharmGenHUB (GA No. 101059870);
- H2020 MSC ITN-action: TranSYS (GA No. 860895);
- H2020 research and innovation programme: ERA Chair CONI (GA No. 951851)

GENERAL INFORMATION

CONGRESS VENUE
The event will take place at the **Grand Hotel Bernardin**, Obala 2, Portorož, Slovenia.

REGISTRATION AND INFORMATION DESK
Lobby of the lecture hall Mediteranea:
Thursday, 22. 09. 2022 8:00 – 10:00
Friday, 23. 09. 2022 8:00 – 10:00
Saturday, 24. 09. 2022 8:00 – 10:00

The certificate of attendance will be issued at the registration desk.

NAME BADGES
All participants will receive name badges upon registration and are kindly requested to wear badges during all sessions and events of the course.

ESHG FELLOWSHIP RECIPIENTS
Participants that received the ESHG fellowship have to sign the appropriate attendance list every day. ESHG fellowship recipients also have to submit the "Information for remittances" form.

PRESENTATION PREVIEW AND DEPOSITION
Presentation preview point where speakers can check and load their presentations will be available in the lecture hall Mediteranea, where all lectures will take place. Speakers are kindly requested to upload their presentations during breaks before sessions.

POSTER DISPLAY AREA
Poster session will be held in the lecture hall Adria.
Presenters are kindly asked to mount their posters during the lunch break on Thursday, 22. 09. 2022, and remove them on Saturday, 24. 09. 2022 by 16:30.
Presenters are responsible for setting and removing the posters. Material for mounting the posters will be available at the venue. Presenters are kindly requested to be present at their poster board for the duration of their allocated poster session.

INTERNET ACCESS
Internet access will be available during the course.

COFFEE BREAKS AND LUNCHES
Coffee breaks will be arranged in the lobby in front of the lecture hall Adria or in the lecture hall Adria during the poster sessions. Lunches will be served at the restaurant of the Grand Hotel Bernardin.

SOCIAL ACTIVITIES FOR ALL REGISTERED PARTICIPANTS
Thursday, 22. 09. 2022
19:00 Welcome reception, Grand Hotel Bernardin

Friday, 23. 09. 2022
19:00 Conference dinner, Grand Hotel Bernardin

Saturday, 24. 09. 2022
18:15 A guided tour of Piran
19:30 Dinner in Piran

PROGRAMME OUTLINE

	22. 09. 2022	23. 09. 2022	24. 09. 2022
Morning session	Course welcome Introduction to Pharmacogenetics	Clinical topics in Pharmacogenetics	Clinical Implementation of Pharmacogenetics I
	Pharmacogenomics of Adverse Drug Reactions	Cancer Pharmacogenomics I	Clinical Implementation of Pharmacogenetics II Short talks
Afternoon session	Pharmacogenomics Resources	Cancer Pharmacogenomics II	Extracting Pharmacogenetic Information from NGS data
	Poster viewing	Poster viewing	Poster viewing
	Short Talks	Novel Biomarkers of Treatment Response	Closing lecture
Evening event	Welcome reception	Conference dinner	A guided tour of Piran and farewell dinner

DETAILED PROGRAMME

THURSDAY, 22. 09. 2022

8:00 – 8:30 **Registration**

Introduction to Pharmacogenetics

08:30 – 08:45 **Course Welcome: Vita Dolžan** (Slovenia)

08:45 – 09:30 **Opening lecture:** Improved individualized drug treatment using novel pharmacogenomic biomarkers; an update - **Magnus Ingelman-Sundberg** (Sweden)

09:30 – 10:15 Pharmacogenetics and drug-drug-gene interactions for precision dosing - **Julia C. Stingl** (Germany)

10:15 – 11:00 Coffee break

Pharmacogenomics of Adverse Drug Reactions

11:00 – 11:45 Pharmacogenomics of drug adverse events - **Sir Munir Pirmohamed** (United Kingdom)

11:45 – 12:15 Drug-drug-gene interactions as mediators of adverse drug reactions in statin treatment - **Nada Božina** (Croatia)

12:15 – 12:30 Hepatotoxicity and rhabdomyolysis in kidney transplant patient with COVID-19: possible role of remdesivir and atorvastatin drug-drug-gene interactions - **Nada Božina** (Croatia)

12:30 – 14:00 Lunch

Pharmacogenomics Resources

14:00 – 15:30 **Workshop:** Interpreting pharmacogenetic data in clinical practice: databases and resources - **John McDermott, William Newman** (United Kingdom)

15:30 – 16:30 Coffee break and poster viewing

Short Talks

16:30 – 16:45 Genotyping paediatric obesity – an argument for early genetic screening - **Jernej Kovač** (Slovenia)

16:45 – 17:00 Cytokine expression in asthmatic primary cell model as an element of response to biological therapy - **Larisa Goričan** (Slovenia)

17:00 – 17:15 Polymorphisms in oxidative stress response genes as biomarkers in Alzheimer's disease - **David Vogrinc** (Slovenia)

17:15 – 17:30 Pharmacogenetics of ADME genes to predict differences in CDK4/6 inhibitors exposure and toxicity - **Rossana Roncato** (Italy)

17:30 – 17:45 EGFR and KRAS genetic testing enhances personal treatment selection in lung cancer patients - **Jelle Vlaeminck** (Belgium)

17:45 – 18:00 Genetic variability and expression of miR-21 and miR-34a as biomarkers of cardiotoxicity after radiotherapy in breast cancer - **Sara Redenšek Trampuž** (Slovenia)

19:00 **Welcome reception**

FRIDAY, 23. 09. 2022

Clinical Topics in Pharmacogenetics

08:30 – 09:10	Pharmacogenetics and the brain - **Julia C. Stingl** (Germany)
09:10 – 09:50	Pharmacogenetics in treatment of cardiovascular diseases - is it cost-effective? - **Lana Ganoci** (Croatia)
09:50 – 10:30	Pharmacogenetics lessons from clinical genetics - **William Newman** (United Kingdom)
10:30 – 11:00	Coffee break

Cancer Pharmacogenomics I

11:00 – 11:30	From somatic variants to targeted cancer treatment - **Cristina Rodriguez-Antona** (Spain)
11:30 – 12:00	Cancer pharmacogenetics in the era of chronic treatment with oral anticancer drugs - **Erika Cecchin** (Italy)
12:00 – 12:30	Pharmacogenomics and pharmacotranscriptomics of acute leukemia in children: a path to personalized medicine - **Sonja Pavlović** (Serbia)
12:30 – 14:00	Lunch

Cancer Pharmacogenomics II

14:00 – 15:00	**Workshop**: Detection of somatic mutations: CAST vs. dPCR - **Nataša Toplak** (Slovenia)
15:00 – 15:30	Pharmacogenomic and epigenomic biomarkers in radiotherapy - **Katja Goričar** (Slovenia)
15:30 – 16:30	Coffee break and poster viewing

Novel Biomarkers of Treatment Response

16:30 – 17:00	Extracellular vesicles as biomarkers (of treatment response) - **Metka Lenassi / Marija Holcar** (Slovenia)
17:00 – 17:30	Extracellular vesicles as innovative tools for assessing adverse effects of immunosuppressant drugs - **Gabriele Stocco** (Italy)
17:30 – 18:00	Peptide biosensors for diagnosis and monitoring of genetic-driven oncohaematological diseases - **Raffaella Franca** (Italy)
18:00 – 18:30	Pharmacogenomics landscape of COVID-19 therapy response in Serbian population - **Branka Zukić** (Serbia)
19:00	**Conference dinner**

SATURDAY, 24. 09. 2022

Clinical Implementation of Pharmacogenetics I

08:30 – 09:10	How to make pharmacogenomic information accessible to healthcare: the UK experience - **William Newman** (United Kingdom)
09:10 – 09:50	Clinical implementation of pharmacogenetics for routine drug prescription: what are the unmet needs? - **Ron van Schaik** (The Netherlands)
09:50 – 10:30	Clinical implementation of germline variants in cancer therapy - **Erika Cecchin** (Italy)
10:30 – 11:00	Coffee break

Clinical Implementation of Pharmacogenetics II

11:00 – 11:30	Pharmacogenetic testing in routine clinical practice: it's about time - **John McDermott** (United Kingdom)
11:30 – 12:00	Implementation of panel pharmacogenomics testing: lessons learnt from the PREPARE study - **Vita Dolžan** (Slovenia)
12:00 – 12:15	*CYP2C19* genetic polymorphism and clopidogrel antiplatelet therapy - **Maria Gabriella Scordo** (Sweden)
12:15 – 12:30	Active pharmacovigilance as a tool for value-based prescribing through implementation of Pharmacogenetics, TDM and DDi - **Elena Peruzzi** (Italy)
12:30 – 14:00	Lunch

Workshop: Extracting Pharmacogenetic Information from NGS Data

14:00 – 14:20	Next Generation Sequencing (NGS) Technology - **Branka Zukić** (Serbia)
14:20 – 14:50	Bioinformatic preprocessing of NGS data: from raw data to genetic variants - **Biljana Stanković** (Serbia)
14:50 – 15:10	Interpretation of NGS Results: analysis of pharmacogenomic variants - **Vladimir Gasić** (Serbia)
15:10 – 15:30	Bioinformatics resources in pharmacogenomics research - **Nikola Kotur** (Serbia)
15:30 – 16:30	Coffee break and poster viewing
16:30 – 17:00	Clinical pharmacogenetic analysis from diagnostic exome sequencing data - **Cristina Rodriguez-Antona** (Spain)
17:00 – 17:45	**Closing lecture:** Pharmacogenetics – future directions - **Sir Munir Pirmohamed** (United Kingdom)
18:15	**A guided tour of Piran**
19:30	**Farewell dinner in Piran**

LECTURE ABSTRACTS

THURSDAY, 22. 09. 2022

INTRODUCTION TO PHARMACOGENETICS

OPENING LECTURE

Improved individualized drug treatment using novel pharmacogenomic biomarkers; an update

Magnus Ingelman-Sundberg[1]

[1]*Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden*

Genetic factors as well as drug-drug interactions, dietary, pathophysiological and environmental factors contribute to interindividual variability in drug response. Overall, it can be estimated that 20-30% of this variability is caused by genetic factors. Much emphasis has recently been placed to evaluate the role of ADME gene variation for interindividual variability in drug pharmacokinetics, adverse reactions and drug response. The implementation into the clinics has however been slow and been mostly successful in the area of oncology. Recently the increased power and better design of pharmacogenomics studies, including many randomized clinical trials, has brought more firm knowledge into the field. However, much work remains before valuable conclusions of clinical importance of pharmacogenomics in many therapeutic areas can be provided.

One major obstacle is the missing heritability. Whereas twin studies indicate a heritability in drug pharmacokinetics of 80% in some cases, the known genetic variants sometimes only explain a fraction of this variation. Recent results shed light on some of the reasons why, whereas much remains unknown. The major putative factors of the missing pharmacogenomic information in drug pharmacokinetics include **i)** the contribution of rare variants where for e.g. CYP2D6 and CYP2C19, 12 and 7% of the variability, respectively, can be explained by rare variants, **ii)** incomplete NGS sequencing in genetically complex loci, which requires long read based sequencing or special bioinformatic tools, **iii)** the occurrence of functionally different haplotypes of alleles traditionally classified as e.g. *1, **iv)** the global inheritance of genetic variants indirectly affecting the level of enzyme/transporter expression and **v)** the direct regulation of ADME genes by polymorphic nuclear factors like NFIB. The lecture will provide an update of the field, discuss several examples, and suggest ways forward.

Pharmacogenetics and drug-drug-gene interactions for precision dosing

Julia C. Stingl[1]

[1]*Institute of Clinical Pharmacology, University Hospital of RWTH University Aachen, Germany*

Introduction: Systematic quantitative adjustments of drug dose based on pharmacogene alleles focused on highly polymorphic enzymes including CYP2C9, CYP2C19 and CYP2D6 relevant especially in the metabolism of psychotropic drugs have been issued about 20 years ago (Kirchheiner et al. 2004). In that approach, dose adjustments estimated from each clinical study identified from the literature, were averaged to provide a final estimate, weighted by the size of participants in each study.

Methods: Drug-drug-gene interactions are additionally modifying the clearance which may be considered for precision dose adjustments in patients. We analysed drug interactions together with pharmacogenetic profiles in a cohort study on adverse drug reactions leading to emergency visits (ADRED study with n=2939 cases).

Results: For CYP2D6, the number of substrates and inhibitors prevalent in comedication was assessed in patients presenting at emergency hospitals due to adverse drug reactions. The adverse effect dizziness was associated with the number of concomitantly taken CYP2D6 substrates and inhibitors, indicating a drug-drug interaction effect on the CYP2D6 phenotype. However, the number of subjects genotyped in this cohort was too low to show additional gene-drug-drug interactions.

For CYP2C19, higher activity predicted from genotype was associated with bleeding and no additional effect of drug interactions in polytherapy-patients with adverse drug reactions was observed. Similar, anticoagulant activity determined by the pharmacogenomic profiles of CYP2C9 combined with VKORC1 prevailed any effect of drug interactions in the polytherapy treated patients of the ADRED study.

Conclusions: In this large cohort study in polymedicated patients, no strong drug-drug-gene interactions were detected. Despite polypharmacy with median seven drugs taken concomitantly, there was no additional modification of the pharmacogenetic predicted clearance detected, showing no strong evidence for phenocopying by drug interactions. The assessment of drug-drug-gene interactions for precision dosing may be based on larger datasets and more sophisticated methods like random modelling approaches and the integration of drug interaction knowledge graphs.

PHARMACOGENOMICS OF ADVERSE DRUG REACTIONS

Pharmacogenomics of drug adverse events

Sir Munir Pirmohamed[1]

[1]*Wolfson Centre for Personalised Medicine, Department of Pharmacology and Therapeutics, University of Liverpool, Liverpool, United Kingdom*

Adverse drug events or reactions (ADRs) are a major clinical problem accounting for a great deal of morbidity, mortality and are a drain on healthcare resources. ADRs can generally be divided into on-target and off target reactions. Both types of ADRs have a genetic predisposition, but the quantitative contribution of genetic *vs*. non-genetic factors varies with the type of reaction, the drug implicated and the patient's clinical co-morbidities. My talk will focus on genetic factors predisposing to ADRs, and how advances over the last 20 years have led not only to discovery, but also to some genetic tests becoming incorporated into clinical practice. There are some well-known polymorphisms in genes such as glucose-6-phosphate dehydrogenase and butyrylcholinesterase which have been known about for decades, and testing can be undertaken in healthcare systems. More recently, the role of HLA and predisposition to immune mediated adverse reactions has been particularly fertile in identifying new associations, often through genome wide technologies. Indeed, since 2001, at least 30 new HLA-ADR associations have been reported. Two of these are in clinical practice (*HLA-B*57:01* for abacavir hypersensitivity, and *HLA-B*15:02* for carbamazepine-induced Stevens-Johnson Syndrome). Genetic factors can also determine dose - for example, for warfarin, polymorphisms in *VKORC1* and *CYP2C9* account for almost 50% of the variance in individual dose requirements. Investigation of the genomic basis of ADRs is not only important for development of predictive genetic testing but can also provide insights into the mechanisms of ADRs.

Drug-drug-gene interactions as mediators of adverse drug reactions in statin treatment

Nada Božina[1]

[1]*University of Zagreb School of Medicine, Department of Pharmacology, Zagreb, Croatia*

Inter-individual variability in drug response is a major clinical challenge, as it can result in adverse drug reactions (ADRs) or treatment failure. It is estimated that 80 % of all ADRs depend on the dose and could be prevented. Hydroxymethylglutaryl-coenzyme A reductase inhibitors (statins) are considered effective and safe, but they have side effects, and skeletal muscle toxicity is the most common. Myotoxicity is considered a statin exposure-dependent ADR, ranging from mild myalgia to potentially lethal rhabdomyolysis with myoglobinuria and acute renal failure. Statins are often used in combination with other drugs that affect statin pharmacokinetics and the subsequent accumulation of statins or their metabolites, which increases the risk of ADRs. Simvastatin, lovastatin, and atorvastatin are metabolized by CYP3A4, and concomitant therapy with inhibitors of this enzyme can significantly prolong their bioavailability and ADRs risk. Fluvastatin is metabolized by CYP2C9, whereas pravastatin, rosuvastatin, and pitavastatin are not significantly affected by inhibition by either CYP. Statins also have different affinities for ABC and SLC membrane transporters involved in intestinal and hepatic absorption, and biliary and renal excretion. Considering different affinity of individual statins for CYPs and drug transporters, gene variants have different significance for their pharmacokinetics and drug-drug-gene interactions. The functional SNP, *SLCO1B1 c.521T>C*, had a strong effect on the AUC of atorvastatin and simvastatin acid, while the effect of *ABCG2 c.421C>A* is more important for rosuvastatin. Our data showed that carriers of *CYP2C9* and *ABCG2* variant alleles had an increased risk of developing fluvastatin ADRs in kidney transplant patients. This risk was even higher in patients who were additionally treated with enzyme inhibitors. Data also indicate a role of the *ABCG2* variants in the development of statin-induced hepatotoxicity. Understanding the mechanisms underlying statin interactions along with pharmacogenetics predisposition can help minimize drug-drug- gene interactions and reduce ADRs caused by statins.

WORKSHOP: PHARMACOGENOMICS RESOURCES

Interpreting pharmacogenetic data in clinical practice: databases and resources

John H. McDermott[1,2], William Newman[1,2]

[1]Manchester Centre for Genomic Medicine, St Mary's Hospital, Manchester University NHS Foundation Trust, Manchester, UK; [2]The Division of Evolution, Infection and Genomics, School of Biological Sciences, University of Manchester, Manchester, UK

For pharmacogenetic data to guide prescribing decisions, genetic data first must be converted into a clinically meaningful format. This workshop outlines the databases available to support interpretation of single nucleotide polymorphism (SNP) genotype data and will explore the various strategies to scale and automate this process. The workshop will provide an opportunity to interact with pharmacogenetic data and, through utilisation of the available databases, appreciate how pharmacogenetic reports might be developed. The potential barriers involved in the manual processing of these datasets will be discussed and technological solutions to overcome these will be highlighted.

Delegates will require access to a laptop with Microsoft Excel for this workshop.

FRIDAY, 23. 09. 2022
CLINICAL TOPICS IN PHARMACOGENETICS

Pharmacogenetics and the brain

Julia C. Stingl[1]

[1]*Institute of Clinical Pharmacology, University Hospital of RWTH University Aachen, Germany*

Introduction: Central nervous system (CNS) drugs are often substrates of the phase-I enzyme cytochrome P450 (CYP) 2D6 or 2C19. In addition to drug metabolism, there may be also a constitutive role of these enzymes in transforming endogenous neuroactive substrates or transforming drugs locally in the CNS. CYP2D6 is widely expressed in neuronal cells throughout the brain, and CYP2C19, while not expressed in the adult brain, relevant expression levels during neurodevelopment has been reported.

Methods: We performed pharmacogenetic studies in healthy volunteers using psychological paradigms in fMRI and brain perfusion studies. For the analysis of side effects that may be associated with psychoactive substrate metabolism in the brain, we analysed a large cohort study in patients on adverse drug reactions leading to emergency visits (ADRED).

Results: Functional MRI studies in healthy volunteers and observational studies in patients on the occurrence of severe adverse drug effects leading to emergency admissions have resulted showing pharmacogenetic influences on psychological and functional brain phenotypes. We detected an association of the CYP2D6 activity score predicted by pharmacogenetics with resting brain perfusion localized in regions that are connected to alertness and sustained attention. In addition, certain reports in patients report higher suicidality ideation in patients with CYP2D6 fast metabolizer genotypes. In patients taking drugs metabolized by CYP2D6, we observed higher rates of side effects in the CNS (with neurological or psychological symptoms), than in drugs not metabolized by CYP2D6. For CYP2C19 we found a role in structural phenotypes of subcortical brain volume with structural imaging.

Conclusions: These findings support the role of pharmacogenetic traits affecting not only the safety and efficacy of psychoactive drugs, but also leading to a constitutive phenotype which - as shown by Jukic et al – may be associated with a mitigated anxious-depressed phenotype for CYP2C19, and with sustained attention in CYP2D6 metabolizer groups.

Pharmacogenetics in the treatment of cardiovascular diseases - is it cost-effective?

Nada Božina[1], Lana Ganoci[2]

[1]University of Zagreb School of Medicine, Department of Pharmacology, Zagreb, Croatia; [2]University Hospital Centre Zagreb, Department of Laboratory Diagnostics, Division for Pharmacogenomics and Therapy Individualization, Zagreb, Croatia

Pharmacogenomics testing is applied in personalizing drug treatment of various diseases, including cardiovascular disease (CVD), to individualize the therapy with the aim of achieving maximum efficacy while reducing the adverse drug reactions (ADRs) that attributes to a high economic burden, high mortality and morbidity, and higher hospitalization costs globally.

Pharmacogenomics-guided therapy is considered cost-effective if it is superior compared to the standard therapy. Health outcomes can be measured according to life years gained, lives saved and avoidance of incidences and hospitalizations. To be cost-effective, pharmacogenomics-guided therapy must be cost saving and give higher quality-adjusted life per year (QALY) than standard therapy. However, pharmacogenomics-guided therapy can also be considered cost-effective if the quality of life that results from the guided treatment gives significantly better quality of life although the cost is more expensive than that of standard care.

Genotyping cost and frequency of risk alleles in the populations influence the cost-effectiveness outcome. Most studies used a single gene, rather than a gene panel for pharmacogenomics testing. The majority of studies on the cost-effectiveness of CV drugs focused on warfarin and clopidogrel, and only a few investigated statins and antihypertensive drugs. Data on the cost-effectiveness of pharmacogenomics-guided treatment in CVD reveals that most studies performed were cost-effective. However, some studies observed questionable the cost-effectiveness of PGx vs standard treatment, while some studies were inconclusive. The data were specifically supportive of pharmacogenomics-guided (CYP2C19) clopidogrel treatment in patients with Acute Coronary Syndrome (ACS) and Atrial Fibrillation. Besides, data showed that the cost-effectiveness of multigene testing (CYP2C19, SLCO1B1, CYP2C9, VKORC1) compared with single-gene testing (CYP2C19) and standard of care (no genotyping) in ACS patients undergoing PCI has a higher probability of being cost-effective. Further studies are warranted to examine the clinical and economic validity of pharmacogenomics testing for CVD.

Pharmacogenetics lessons from clinical genetics

William Newman[1]

[1]*Manchester Centre for Genomic Medicine, Manchester, United Kingdom*

The disciplines of clinical genetics and pharmacogenetics have developed in parallel, with few clinical geneticists taking an active role in research or implementation of pharmacogenetics. This likely reflects the lack of prescribing of medication by most clinical geneticists.

However, there are important lessons that can be learned from consideration of rare monogenic disorders e.g. long QT syndrome, porphyria, where there exists an increased risk of adverse drug reactions and appropriate advice to patients and at risk family members is vital. Ethical considerations arise when pharmacogenetics data potentially reveals susceptibility to diseases. Sharing knowledge around presentation of complex issues including incomplete penetrance, variable expression and information regarding risks are skills that clinical geneticists can contribute to the development and integration of pharmacogenetics into routine healthcare.

CANCER PHARMACOGENOMICS I

From somatic variants to targeted cancer treatment

Cristina Rodriguez-Antona[1]

[1]*Spanish National Cancer Research Centre, Madrid, Spain*

The effectiveness and precision of oncology therapies have greatly increased over the past few decades, helping to lower the overall cancer death rate. Understanding the molecular processes behind cancer development, growth, and metastasis has been essential for the development of innovative treatments that result in long-lasting responses in a variety of tumor types. There are already more than 100 targeted cancer therapy drugs on the market, many of which have multiple indications for various tumor types. This achievement has been fueled by the explosion of the novel genomic technologies.

At the forefront of recent cancer research is the success of immunotherapy, the growing role of precision medicine, the development of liquid biopsies and the integration of "omic" data for tumor classification and for prognostic and drug response prediction. Currently, treatment selection based on the genomic information of each person's specific cancer is a routine procedure for several tumor types, most notably for lung cancer.

The pioneer studies that resulted in the discovery of EGFR mutation as a biomarker to identify lung cancer patients who responded favourably to inhibitors of this route, cleared the path for the rapid development of tailored drugs and predictive biomarkers. In this way, the use of targeted therapies, designed to inhibit cancer cell growth by blocking a specific tumor molecular pathway, usually requires molecular testing to confirm that the tumor harbours the molecular biomarker that the drug targets and is predictive of treatment benefit. In most cases, the biomarker detection—which dictates the selection of the drug—is restricted to a specific tumor type, but for some treatments the approved indication is independent of the tissue type from which the cancer originated, defining the so-called "tissue-agnostic" biomarkers.

Unfortunately, there are many challenges to overcome, as cancer cells frequently evolve to become treatment-resistant. Furthermore, only a small subset of molecular targets—many of which are rare in solid tumors—are currently inhibited by targeted therapies, and many common tumor genomic alterations remain undruggable.

Cancer pharmacogenetics in the era of chronic treatment with oral anticancer drugs

Erika Cecchin[1]

[1]*Experimental and Clinical Pharmacology Unit, CRO-Centro di Riferimento Oncologico di Aviano- IRCCS, Aviano, Italy*

Introduction: In the era of precision medicine, the dose and schedule of targeted oral anticancer drugs are still based on the "one-size-fits-all" paradigm, with dose adjustments driven by the occurrence of toxicity or lack of efficacy. There is growing evidence of the clinical benefits of an intensified pharmacological care in terms of pharmacogenetics (PGx), therapeutic drug monitoring (TDM) and co-medication management.

Methods: Based on the evidence from the literature and our long-term experience in the field of PGx and TDM of anticancer drugs, we will discuss the current state of the art and report original data on the subject.

Results: In the literature and in the collection of our patients treated with different drugs such as imatinib, palbociclib, ribociclib, etc., enormous inter-individual variability in plasma exposure to targeted oral anticancer drugs has been reported. PGx profile of the patient, concomitant medications or food intake, comorbidities (e.g. obesity, COVID-19) and lifestyle habits (e.g. smoking) could contribute to this variability. Frontline use of PGx may be effective in lowering interindividual variability in both plasma exposure and patient clinical outcome. Specifically genetic polymorphisms in CYP metabolizing enzymes and ABC transporters were evaluated as potential PGx markers for oral anticancer drugs. Furthermore, drug-drug interactions play a key role in this context, particularly in specific PGx settings, and active drug interaction monitoring has been shown to improve patient quality of life and safety profiles.

Conclusions: The application of PGx to the personalization of treatment with targeted oral anticancer drugs is still lagging behind, despite huge inter-individual variability in treatment outcome and drug exposure. An integrated pharmacological approach including PGX and TDM and monitoring of drug-drug interaction could be useful for optimizing cancer therapy.

Acknowledgment: All the researchers of Experimental and Clinical Pharmacology Unit directed by Dr Giuseppe Toffoli at CRO-Aviano and all the oncologists of the Institute.

Pharmacogenomics and pharmacotranscriptomics of acute leukemia in children: a path to personalized medicine

Sonja Pavlović[1]

[1]*Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Laboratory for Molecular Biomedicine, Belgrade, Serbia*

Introduction: Personalized medicine is focused on research disciplines which contribute to the individualization of therapy, like pharmacogenomics and pharmacotranscriptomics. Acute lymphoblastic leukemia (ALL) is the most common malignancy of childhood. It is one of the pediatric malignancies with the highest cure rate, but still a lethal outcome due to therapy accounts for 1–3% of deaths. Further improvement of treatment protocols is needed through the implementation of pharmacogenomics and pharmacotranscriptomics.

Methods: Emerging high-throughput technologies, including microarrays and next-generation sequencing, have provided an enormous amount of molecular data with the potential to be implemented in childhood ALL treatment protocols.

Results: Numerous molecular markers responsible for the efficacy, side effects, and toxicity of the drugs commonly used for childhood ALL treatment, i.e., glucocorticoids, vincristine, asparaginase, anthracyclines, thiopurines, and methotrexate are identified. Big data was generated using high-throughput technologies, but their implementation in clinical practice is poor.

Conclusions: Research efforts should be focused on data analysis and designing prediction models using machine learning algorithms. Bioinformatics tools and the implementation of artificial intelligence are expected to open the door wide for personalized medicine in the clinical practice of childhood ALL.

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CANCER PHARMACOGENOMICS II

Workshop: Detection of somatic mutations: CAST vs. dPCR

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Quantitative PCR (qPCR) is one of the most powerful and sensitive nucleic acid analysis methods and it is used for a broad range of applications. A very challenging application is the detection of a rare low-frequency mutation, where the method needs to discriminate between two highly similar sequences, one of which is significantly more abundant than the other. One of the variations of qPCR for detection of somatic mutation (sequence variant that is present at a very low frequency in a pool of wild-type background) is competitive allele specific TaqMan PCR (Cast-PCR) technology, which allows not only the selective amplification of minor alleles, but it also blocks the amplification of non-mutant allele. Another approach is the next generation of PCR technology called digital PCR (dPCR). The main principle of dPCR also utilizes quantitative PCR and it is based on the separation of the reaction mixture into thousands of partitions, which is followed by a real time PCR or end point detection of the amplification in each of the partitions. The distribution of target sequences into partitions allows for accurate and absolute quantification and detection of the rare target sequence. dPCR has proven to be particularly useful for accurate detection and quantification of low-abundance nucleic acids, highlighting its advantages in cancer diagnostics.

The comparison of CAST-PCR and dPCR performed with the new Applied Biosystems QuantStudio Absolute Q Digital PCR System is presented.

Pharmacogenomic and epigenomic biomarkers in radiotherapy

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Radiotherapy is one of the three main modalities of oncological treatment, used in various cancer types. Radiotherapy improves patients' survival and local disease control, but also leads to acute and late adverse events that can influence patients' quality of life. Several clinical and molecular parameters can affect the interindividual variability in the risk for occurrence of adverse events, including genetic and epigenetic factors.

Radiogenomics is the research field trying to identify the genetic determinants of radiotherapy outcome. Within the last decade, several studies observed the contribution of genetic variability of genes involved in DNA repair, inflammation and response to oxidative stress to the occurrence of adverse events. Particularly DNA repair genes were also implicated in treatment efficacy and patients' survival. In genome-wide association studies and meta-analyses, *XRCC3* rs861539, *XRCC1* rs2682585, *XRCC1* rs25487 and *TGFB1* rs1800469 were among the key single nucleotide polymorphisms (SNPs) significantly associated with adverse events of radiotherapy. Good predictors were especially multi-SNP models combining DNA repair and transforming growth factor beta (TGF-β) signaling genes.

Epigenetic factors may also play a role in radiotherapy outcome. miRNAs, small non-coding RNAs involved in the posttranscriptional regulation of gene expression, are often differentially expressed in cancer. They can affect numerous processes, including cell cycle, apoptosis, cell proliferation and differentiation. Radiotherapy also affects the expression of miRNAs that target mRNAs involved in pathways associated with radiation response. In different model systems, several miRNAs were proposed as potential biomarkers of radiation exposure. Among the most commonly identified miRNAs that increase after radiation exposure are hsa-miR-21 and hsa-miR-34a. However, the association of miRNA expression with adverse events of radiotherapy is not well understood.

In conclusion, genetic and epigenetic changes could serve as additional biomarkers of treatment outcome in cancer patients treated with radiotherapy and could enable a more personalized treatment approach.

NOVEL BIOMARKERS OF TREATMENT RESPONSE

Extracellular vesicles as biomarkers (of treatment response)

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Extracellular vesicles (EVs) are a promising new source of disease biomarkers. They are nanometer-to micrometer-sized spherical particles, enclosed by a phospholipid bilayer which are secreted by all cell types. EVs reflect the physiological state of their cell of origin in terms of their molecular composition (e.g., proteins, miRNAs) and biophysical characteristics (e.g., size, concentration). They accumulate in all body fluids, even when released from remote organs or tissues while protecting their cargo from degradation, making them ideal candidates to be used as biomarkers.

The most frequently used body fluid for EV biomarker research is blood, as its minimally invasive collection procedure and its dynamic composition closely relate to the (pato)physiological changes in the organism. However, biomarker studies of blood EVs have to address the challenges of the complexity of source material, interpersonal variability of tested individuals, as well as the influence of pre-analytic variables and limitations of chosen downstream analytic methods which can all impact the outcomes of the biomarker study.

EVs are most frequently studied as biomarkers in cancer, whereas disease groups like infectious, metabolic, and cardiovascular diseases are studied less often. It has been shown that EVs released from cancer cells can promote cancer progression through the delivery of accumulated oncogenes, tumor suppressor genes and their products, signature proteins and RNAs, and mutated genomic DNA to recipient cells. However, EVs also reflect alterations in the state of diseases during therapy and are promising biomarkers for therapeutic response evaluation, especially resistance to therapy. Future potential clinical significance of EVs can therefore be in guiding therapeutic strategy adjustments for better-stratified therapy.

Extracellular vesicles as innovative tools for assessing adverse effects of immunosuppressant drugs

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Introduction: Extracellular vesicles (EVs) are a diversified group of small vesicles released by donor cells and absorbed by recipient cells, which represent key mediators with important roles in both healthy tissues and disease. EVs are present in a many biological fluids and have a significant diagnostic and prognostic potential. Among candidate genes potentially involved in regulating the release and content of EVs we evaluated PACSIN2. Variants regulating the expression of this gene are associated with the biotransformation and gastrointestinal effects of thiopurines with an unknown mechanism that my involve modulation of EVs release.

Methods: The main reciprocal interactions between EVs and a panel of immunosuppressive drugs will be presented. In particular studies evaluating the effect of PACSIN2 knock down on EVs released by cell lines representative of the tissues targeted by thiopurines will be presented.

Results: The known interactions between EVs and a panel of immunosuppressive drugs will be presented. Preliminary analysis of the contribution of EVs as a mechanism of the association between interindividual variability in response to the immunosuppressive agent mercaptopurine and variation in the concentration of a candidate gene involved in EVs release, PACSIN2, will be provided.

Conclusions: This presentation will provide insights on the associations between EVs and immunosuppressive drugs with a focus on EVs' role as tools to assess the effects of immunosuppressants, suggesting innovative properties and new possible therapeutic uses.

Peptide biosensors for diagnosis and monitoring of genetic-driven oncohematological diseases

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Introduction: The pathogenic role of overactivated ABL1 or JAK2 tyrosine kinase (TK) pathway is well recognized in some oncohematological diseases; overactivation is genetically-driven by a plethora of mutations in key genes of the pathway. Aim of the study is to set up a peptide biosensor *in vitro* assay to investigate the aberrant TK activity in tumor cells, as an integrative information to cytogenetics, and to screen *in vitro* the efficacy of TK inhibitors (TKIs) to timely assess primary and acquired drug resistance.

Methods: The biosensor for ABL1 (P_{ABL}) or JAK2 (P_{JAK2}) is a biotinylated peptide, whose sequence is derived from literature and comprises a tyrosine (Y) phosphorylation site for the TK of interest. The biosensor phosphorylation after cell lysates incubation, in the presence or absence of TKI, is quantified by an ELISA assay as fluorescent signal; immortalized cell lines with different genetic background and patient tumoral cells are used.

Results: After incubation of P_{ABL} with whole cell lysates, a significant increase in the fluorescence signal over lysate background is observed (p-value<0.0001, two-way ANOVA, Bonferroni post-test). Cell lines expressing ABL1-chimeric proteins (i.e., K562 and ALL-SIL) present comparable TK activity on P_{ABL}, with higher signals than cells with wild type ABL1 (i.e., NALM6 and REH, p<0.001 and p<0.05 *versus* K562, respectively). P_{ABL} phosphorylation is inhibited by ABL1-inhibitors (e.g, imatinib, p-value<0.01 in cell lines) but not by the JAK2-inhibitor ruxolitinib. Similarly, preliminary analysis on patients' primary malignant cells (with and without chimeric BCR-ABL1) show that P_{ABL} phosphorylation is specifically inhibited after the incubation of BCR-ABL1 positive cell lysates with imatinib, but not with ruxolitinib. The P_{JAK2}- based ELISA assay is still under optimization.

Conclusions: While requiring further optimization, the P_{ABL}-based ELISA assay paves the way to a point-of-care device that might improve the diagnosis and treatment of oncohematological patients with aberrant ABL1 activity.

Pharmacogenomics landscape of COVID-19 therapy response in Serbian population

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Introduction: Treating COVID-19 patients after two years of pandemics is still a challenge. It has been evident that with the same cause of the disease, the clinical presentation and the course of the disease in COVID-19 patients vary from asymptomatic to extremely severe. With lack of time to test individual pharmacogenomics markers, population pharmacogenomics could be helpful in predicting a higher risk of developing adverse reactions and treatment failure in COVID-19 patients. We identified pharmacogenes and pharmacogenomics markers associated with drugs recommended for the first line of COVID-19 treatment in 2020, namely chloroquine/hydroxychloroquine, azithromycin, lopinavir and ritonavir, in population of Serbia and other world populations.

Methods: Genotype information of 143 individuals of Serbian origin was extracted from database previously obtained using TruSight One Gene Panel (Illumina). Genotype data of individuals from different world populations were extracted from the 1000 Genome Project. Fisher's exact test was used for comparison of allele frequencies.

Results: We have identified 11 potential pharmacogenomics markers in 7 pharmacogenes relevant for COVID-19 treatment. Based on high alterative allele frequencies in population and the functional effect of the variants, ABCB1 rs1045642 and rs2032582 could be relevant for reduced clearance of azithromycin, lopinavir and ritonavir drugs and UGT1A7 rs17868323 for hyperbilirubinemia in ritonavir treated COVID-19 patients in Serbian population. SLCO1B1 rs4149056 is a potential marker of lopinavir response, especially in Italian population. Our results confirmed that pharmacogenomics profile of African population is different from the rest of the world.

Conclusions: Considering population specific pharmacogenomics landscape, preemptive testing for pharmacogenes relevant for drugs used in COVID-19 treatment could contribute to better understanding of the inconsistency in therapy response and could be applied to improve the outcome of the COVID-19 patients.

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SATURDAY, 24. 09. 2022

CLINICAL IMPLEMENTATION OF PHARMACOGENETICS I

Clinical implementation of pharmacogenetics for routine drug prescription: what are the unmet needs?

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The discovery that the metabolism of drugs is highly variable between patients, and can be predicted (to a certain level) by DNA analysis of drug metabolizing enzyme genes, paved the way for translating pharmacogenetics into clinical care. Important in this are the cytochrome P450 enzymes, with CYP2D6 (involved in the metabolism of 20% of all drugs) and CYP2C19 as major players. However, for CYP2D6, 5-10% of the population is deficient, making that standard doses prescribed to these patients increase the risk of adverse drug reactions.

There are currently 15-30 genes which can be used clinically for optimizing personalization of drug therapy. The clinical implementation is, however, differs between countries. In the Netherlands, every pharmacist can provide dosing advice based on pharmacogenetic information. But also there, the use and uptake of pharmacogenetics differs beaten hospitals.

In this presentations, successes and challenges in implementing pharmacogenetics into routine health care will be highlighted, with a focus on unmet needs.

Clinical implementation of germline variants in cancer therapy

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Introduction: The analysis of germline genetic polymorphisms can be useful for the early identification of patients at risk of toxicity. We contributed to the clinical development of pre-therapy tests of *UGT1A1* and *DPYD* polymorphisms to increase the safety of irinotecan (IRI) and fluoropyrimidines (FL). More recently, regulatory agencies across Europe have recommended frontline genotyping for the first time to prevent adverse events associated with IRI and FL. However, a systematic application of *UGT1A1* and *DPYD* pretreatment genotyping is lagging behind due to several obstacles.

Methods: The state of the art and the most recent results of our group on these issues will be presented.

Results: Despite the improvement in the clinical application of *DPYD* and *UGT1A1* genotyping in cancer practice, still the lack of convincing data on cost-effectiveness, the knowledge gap for clinical practitioners in addressing genetic information of patients, the lack of adequate infrastructures and clinical decisions support systems represent the main obstacles to the implementation of pharmacogenomics. The result of large implementation studies such as Ubiquitous Pharmacogenomics could provide definitive proof of the utility of germline genotyping in cancer to prevent adverse drug reactions. Among the limitations of the application of *DPYD* genotyping is the low specificity of the test which leaves many toxic events unexplained by known variants. We demonstrated that an NGS approach coupled with an in silico functional assay could be applied to detect rare and novel genetic variants in *DPYD* significantly related to a higher risk of toxicity.

Conclusions: The recent publication of the EMA's genotyping recommendation for *DPYD* and *UGT1A1* significantly improved the clinical application of pharmacogenetics in oncology. However, some barriers still need to be addressed. A future implementation of NGS could improve the ability to prevent drug-related adverse events.

Acknowledgment: All the researchers of Experimental and Clinical Pharmacology Unit directed by Dr Giuseppe Toffoli at CRO-Aviano and all the oncologists of the Institute.

CLINICAL IMPLEMENTATION OF PHARMACOGENETICS II

Pharmacogenetic testing in routine clinical practice: it's about time

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If pharmacogenetics is to be adopted into routine clinical practice, results need to be available in a clinically interpretable format and a clinically relevant timeframe. However, standard genetic testing pathways and reporting structures are not sufficiently rapid to achieve this. As such, novel approaches are required to achieve widespread implementation. Emerging technologies offer opportunities for rapid point of care genotyping tests which can be deployed in near-patient settings, whilst interoperable informatic solutions could facilitate pre-emptive pharmacogenetics. This seminar outlines the development of these technological solutions and highlights how they can be integrated into clinical practice without disrupting existing care pathways.

Implementation of panel pharmacogenomics testing: lessons learnt from the PREPARE study

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Pharmacogenetic (PGx) testing has the potential to contribute to personalized pharmacotherapy by utilizing patient's genetic data to optimize the selection of the right drug and dose, thus making the treatment safer and more effective. However, application into routine care is slow and the evidence supporting PGx testing prior to prescribing is mostly limited to individual drug-gene pairs. Therefore, one of the aims of the The Ubiquitous Pharmacogenomics (U-PGx) Consortium was to provide evidence about the collective clinical utility of implementing a panel of PGx-markers into routine care.

With that aim, a prospective, block-randomized, controlled clinical study PREPARE (PREemptive Pharmacogenomic testing for prevention of Adverse drug REactions), was conducted to implement pre-emptive genotyping of a panel of clinically relevant PGx-markers with actionable guidelines in seven European countries.

Patients with first prescription of index drugs with drug-gene based treatment guidelines by the Dutch Pharmacogenetics Working Group were invited to participate in the study. Slovenia, Spain and Greece were randomized to start with PGx-guided prescribing (study arm), while The Netherlands, United Kingdom, Austria, and Italy stated with standard of care (control arm). After 19 months, a new set of patients was recruited to the other arm. Patients in the study arm were pre-emptively genotyped for 46 clinically relevant PGx markers in 12 pharmacogenes and received treatment recommendations within 7 days. Patients in the control arm received standard of care treatment and were genotyped at the end of the study. Patients' reported ADRs and treatment changes were monitored at 4 and 12 weeks and at the end of each arm. Clinical decision support (CDS) tools were developed and implemented to generate PGx reports in respective countries' languages and to make them available to patients and healthcare providers.

Within the PREPARE study, panel-based pre-emptive pharmacogenetic testing was successfully introduced within diverse European healthcare systems. The lessons learnt may facilitate further implementation of pre-emptive panel-based PGx testing within patient care.

Acknowledgement: Horizon 2020 UPGx Project - grant No 668353.

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WORKSHOP: EXTRACTING PHARMACOGENETIC INFORMATION FROM NGS DATA

Next Generation Sequencing (NGS) Technology

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Precision or personalized medicine relies to individual genomic information. Advances in Next Generation Sequencing (NGS) Technologies have improved our knowledge in genomics, enabling better understanding of rare diseases, cancer and other diseases. Deep genomic analysis drives treatment decisions based on cell type and pathway to matched therapies thus facilitating application of personalized medicine.

Several Next Generation Sequencing and Third Generation Sequencing approaches will be presented. Advantages and disadvantages of NGS in pharmacogenomics use namely; whole-genome sequencing (WGS), whole-exome sequencing (WES), targeting sequencing (TS) or custom gene panels will be discussed.

MiSeq Ilumina platform has been used at Institute of Molecular Genetics and Genetic Engineering, University of Belgrade for over eight years and hundreds of patients have been analyzed using TruSight One clinical exome panel. The Illumina sequencing technology and the TruSight One platform covering more than 4,800 genes with known clinical phenotypes, including pharmacogenes will be explained in more details.

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Bioinformatic preprocessing of NGS data: from raw data to genetic variants

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Next-generation sequencing (NGS) technologies enable analysis of whole genomes in just a few days. NGS analysis of the human genomes allows detecting causative variants of rare and chronic diseases as well as variants associated with variable drug response.

To turn NGS datasets into meaningful information, complex bioinformatic analyses have to be performed. NGS produces hundreds of giga base pair information per one sample of whole human genomic data in the form of scattered sequence reads. In order to interpret these data, a bioinformatic analysis entails a multistep process that uses different software that lines up the reads with the reference genome and looks for variations in the genetic code.

This workshop aims to introduce basic concepts of NGS bioinformatic analysis of genomic data. Also, the workshop will cover different tools that turn the raw, unprocessed data into genetic variation that could be further interpreted as disease causing, having a modifying effect on phenotypic traits or influence a person's response to therapy.

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Interpretation of NGS results: analysis of pharmacogenomic variants

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Introduction: Advances in next-generation sequencing (NGS) have contributed to better molecular diagnosis of diseases with genetic origin as well as development of various tests, including pharmacogenetic tests. Targeted sequencing, which uses panels with selected genes specific for the pathology or pharmacogenomics profiling, is especially suitable for diagnostic purposes.

Methods: TruSight One Gene Panel (Illumina) is used for the sequencing of the samples. The Variant Studio software is used for detection and variant filtering.

Results: Relevant pharmacogenomics variants are checked using PharmGKB, PubMed and in-house databases. They are further processed using the InterVar software tool and ACMG classification, ClinVar, and Varsome databases. The novel potential pharmacogenomics variants are further analyzed with *in silico* tools, such as EIGEN, FATHMM-MKL, MutationTaster, SIFT, BayesDel_addAF, Polyphen2-HVAR and PrimateAI. The *in silico* relevance prediction precedes the experimental functional analyses.

Conclusions: The high-throughput sequencing technology (NGS) provides a large pool of data. However, further increase of pharmacogenomics knowledge is needed to build valid and comprehensive pharmacogenomics databases. Only then NGS will enable patient-specific pharmacogenomics profile, leading to valuable information for therapy choice and good patient management.

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Bioinformatics resources in pharmacogenomics research

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The last part of the workshop will cover bioinformatics and web-based resources to search for pharmacogenes and genetic variants associated with drug response. Genome wide and candidate gene approach will be employed to pinpoint most suitable candidate pharmacogenes. Genetic variants will be searched and analyzed taking into account effect prediction and population pharmacogenomics aspects using resources such as the GWAS Catalog, PharmGKB, and tools incorporated into Ensembl genome browser.

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Clinical pharmacogenetic analysis from diagnostic exome sequencing data

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Therapeutic failure and adverse drug reactions are major public health care problems. However, despite the fact that more than 95% of people have genetic variants that affect drug response and that pharmacogenomic information is included in drug labels and clinical guidelines, the implementation of pharmacogenetics in the clinics is still low.

While genotyping-based technologies are useful strategies for reactive pharmacogenetics, the recent explosion of next-generation sequencing (NGS) techniques creates a unique opportunity for preemptive pharmacogenetics. In recent years, the use of whole exome sequencing (WES) for the diagnosis of genetic disorders has increased dramatically. Repurposing this data to report pharmacogenetic actionable alleles has the potential to transform this field. In addition, NGS has the capacity to uncover novel pharmacogenetic variants of clinical relevance.

Several studies have demonstrated the robustness of repurposing large-scale NGS data for pharmacogenetics, encouraging the use of this strategy to increase preemptive pharmacogenetics. However, the different characteristics of the pharmacogenes (e.g. CYPs, HLAs, mitochondrial genes) and of their genetic variation have to be considered, together with the limitations imposed by the exome capture panels routinely-used in genetic diagnosis (e.g. Whole Exome Sequencing versus the so-called Clinical Exome Sequencing, of typically 60 Mb and 17 Mb, respectively) and of copy number prediction based on short read sequencing. In addition, quality control for single nucleotide variant and indel retrieval, genotype to haplotype conversion and pharmacogenetic phenotype classification has to be performed. A recent study established that diagnostic exome data can be used to inform of the pharmacogenetic phenotypes associated to 11 genes (CACNA1S, CYP2B6, CYP2C9, CYP4F2, DPYD, G6PD, NUDT15, RYR1, SLCO1B1, TPMT, and UGT1A1).

In summary, the exponential growth in large-scale NGS diagnostics, with exomes being the most widely used platforms, argue for repurposing these data for clinical pharmacogenetics. However, there are limitations for this strategy that have to be taken into account.

CLOSING LECTURE
Pharmacogenetics – future directions

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Pharmacogenetics or pharmacogenomics, as an area of study, has been around for a long time. Indeed, since the completion of the human genome project, the amount of research being undertaken in pharmacogenomics has increased substantially. As we look to the future, the scope of pharmacogenomics, the amount of research being undertaken, and the interest from industry (including diagnostics and data science industries) is likely to increase. To fully embrace the opportunities for pharmacogenomics, there are some key areas which I think are important for the future. These include:

- Broaden the definition of pharmacogenomics to leverage knowledge of genomic variation in drug discovery and drug development.
- Improve the pathways from discovery to implementation with greater focus on implementation science.
- Develop novel study designs to rapidly increase evidence base to enable uptake into clinical practice.
- Integrate pharmacogenomic biomarkers into multimodal algorithms which include clinical factors such as renal function, drug-drug interactions, and other host/clinical variables.
- Identify factors that account for missing heritability including rare variants, but also common polygenic variants leading to the development of polygenic scores.
- Make increasing use of population biobanks to undertake large scale pharmacogenomic studies including for the development of polygenic scores.

All these areas have their own challenges, but collaboration across borders and working in multi-disciplinary teams will help turn these challenges into opportunities.

SHORT TALK ABSTRACTS

THURSDAY, 22. 09. 2022

Hepatotoxicity and rhabdomyolysis in kidney transplant patient with COVID-19: possible role of remdesivir and atorvastatin drug-drug-gene interactions

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Introduction: Because of very variable clinical presentation of COVID-19 and polypharmacy in elderly, sometimes is difficult to distinguish between the drug-drug, disease-drug or drug-drug-gene induced side effects.

Description: A 63–year old Caucasian woman with kidney transplant, was hospitalized due to COVID-19 infection. She was treated with remdesivir for 10 days along with meropenem and methylprednisolone. Mycophenolate was excluded for 10 days. Tacrolimus, atorvastatin, ramipril and ezetimibe were continued and furosemide and pantoprazole were added. After discharge, she started to feel muscle weakness in her extremities and laboratory results at admission showed elevated value of creatinine kinase (CK-MM 6975 U/L), AST (455 U/L), ALT (516 U/L). CK returned to the normal range and liver damage was resolved in two weeks following the cessation of atorvastatin and ezetimibe.

Discussion: In this case atorvastatin and remdesivir were the most prominent candidates for drug-drug and drug-drug-gene interactions resulting in elevated CK and rhabdomyolysis as well as liver damage. Pharmacogenetic analysis showed that patient was a carrier of inactivating alleles of *CYP2D6*1/*4*, *CYP3A4*1/*22*, *SLCO1B1 *5/*5*. Remdesivir is substrate of CES1, CYP2D6, CYP3A4, OATP1B1(SLCO1B1) and inhibitor of CYP3A4 and SLCO1B1. Atorvastatin is substrate of CYP3A4 and OATP1B1 and can moderately inhibit the CES1 enzyme, the main metabolic pathway of remdesivir. Other concomitantly prescribed medicines, such as ezetimibe, furosemide and proton pump inhibitors could have added to the drug-drug-gene interactions.

Conclusions: The pharmacogenetic profiling along with the assessment of drug interactions and pharmacokinetics in polypharmacy can significantly contribute to the minimization of the risk of developing side effects especially in a vulnerable subpopulation of patients such are the kidney transplant patient.

Genotyping paediatric obesity – an argument for early genetic screening

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Introduction: Monogenic obesity is a severe, genetically driven disorder that affects up to 1/1000 newborns. Novel therapeutics and innovative clinical approaches have highlighted the need for early identification of children with rare genetic variants that affect the leptin-melanocortin signalling pathway, in order to improve clinical intervention and reduce the risk of chronic complications.

Methods: NGS sequencing of central genes in the leptin-melanocortin pathway (*AGRP, LEP, LEPR, MC3R, MC4R, NPY, NPY1R, NPY5R, PCSK1, PCSK2, POMC*) was performed in 1508 children and adolescents with and without obesity, aged 2-19 years. The recruited cohort comprised approximately 5% of the national paediatric population with obesity. The estimated effect size of rare variants in the leptin-melanocortin signalling pathway on longitudinal weight gain between carriers and non-carriers was derived and approximate body mass growth curves were generated to simulate potential weight gain in individuals with rare potentially gene function altering genetic variants. Multiple iterations of effect size calculations were run to reduce the sampling effect and bias of the analysis.

Results: Causative genetic variant was identified in 1.4% (N=21) individuals. Additionally, 4.1% (N=62) were carriers of variants of unknown clinical significance. Estimated incidence of obesity associated genetic variants in analysed population was between 1/150 and 1/850. On average, weight gain of identified variants, at the age of 18 years, was estimated at ~7.5 kg. The weight gain effect was identified in autosomal recessive genes with a single heterozygous genetic variant as well.

Conclusions: Approximately 6% of all obese children have strong genetic predisposition for obesity encoded in genes of LEP-MCH signalling pathway. Early identification of population at risk could reduce the societal burden and improve the clinical management of early severe childhood obesity and should be further investigated.

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Cytokine expression in asthmatic primary cell model as an element of response to biological therapy

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Introduction: Asthma is one of the most common chronic non-communicable diseases in children. 5-10% of patients respond poorly to therapy despite high doses of inhaled corticosteroids and are thus eligible for biologic treatment such as omalizumab. Unfortunately, 40% of patients does not respond to it. There is therefore a need to discover markers of non-responsiveness and to further our understanding of omalizumab's mechanism of action. Omalizumab is well known to affect cytokine expression, so in this study, we focused on the impact of pre-therapy cytokine expression on the response to omalizumab.

Methods: We have adopted an *in vitro* cell model using primary blood cells from paediatric asthma patients to assess basophil cell-specific gene expression and immune cell response to omalizumab treatment. Briefly, white blood cells from paediatric patients obtained by gradient centrifugation were collected. Part of cells was used for basophil ($CD45^+$ $CD123^+$ $HLA\text{-}DR^{NEG}$) isolation via fluorescence-activated cell sorting, followed by gene expression analysis of selected anti-inflammatory (i.e. *IL4, IL13*) and pro-inflammatory (i.e. *IL1B, IL6, TNF*) cytokines. The other part of cells was subjected to pre-incubation with/without omalizumab, followed by an allergen challenge with a patient-specific allergen (i.e. grass or dust mite allergen). The proportion of activated basophils was determined by the basophil activation test using anti-CD63 antibody and the percentage of reduced activation in the omalizumab-treated samples was estimated.

Results: The response of individuals to biological therapy was different, and we found altered gene expression patterns of pro- and anti-inflammatory cytokines associated with response to omalizumab.

Conclusions: The data suggest that the expression status of cytokines reflects an altered response to biological therapy. Further study of selected markers and biological pathways could additionally elucidate the role of cytokines on the response to biological treatment and identify potential biomarkers of non-responsiveness.

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Polymorphisms in oxidative stress response genes as biomarkers in Alzheimer's disease

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Introduction: Oxidative stress is an important process of neurodegeneration. Elevated levels of oxidative stress biomarkers, followed by activation of reactive oxygen species scavenging enzymes were reported in Alzheimer's disease (AD). Polymorphisms in genes involved in oxidative stress response can affect the antioxidative mechanisms of the brain. It remains unclear whether that can promote neurodegeneration from mild cognitive impairment (MCI) to AD. Our aim was to evaluate the association of common polymorphisms in oxidative stress response with cerebrospinal fluid (CSF) biomarkers and mini-mental state exam (MMSE) scores in patients with MCI and AD.

Methods: Our study included 54 AD patients, 14 MCI patients with pathological CSF biomarker levels and 20 MCI patients with normal CSF biomarker levels. Genomic DNA was genotyped for polymorphisms in *SOD2*, *CAT*, *GPX1*, *KEAP1*, *NFE2L2*, *HMOX1* and *HMOX2* using competitive allele-specific PCR. Their association with CSF biomarker levels and MMSE scores was evaluated using nonparametric tests.

Results: In the whole cohort, carriers of at least one polymorphic *NFE2L2* rs35652124 allele had lower CSF $A\beta_{1-42}$ levels (p=0.031), while carriers of at least one *NFE2L2* rs6721961 polymorphic allele had lower total tau levels (p=0.020). In the AD group, carriers of at least one polymorphic *HMOX2* rs1051308 allele had lower $A\beta_{42/40}$ ratio (p=0.013). Significant associations with MMSE score were observed for *CAT* rs1001179 (p=0.022), *NFE2L2* rs35652124 (p=0.030), *KEAP1* rs1048290 and rs9676881 (both p=0.019) in the whole cohort. In AD group, only *KEAP1* rs1048290 and rs9676881 (both p=0.035) were associated with MMSE score.

Conclusions: Observed associations with CSF biomarker levels and cognitive test results suggest that genetic variability in studied oxidative stress response genes could play a role in AD and MCI. These results might contribute to the search of additional biomarkers contributing for early diagnosis of dementia.

Pharmacogenetics of ADME genes to predict differences in CDK4/6 inhibitors exposure and toxicity

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Introduction: A wide inter-individual variability in therapeutic response to cyclin-dependent kinases 4 and 6 inhibitors (palbociclib, ribociclib and abemaciclib, CDKis) has been reported. An exposure-toxicity relationship was demonstrated for all CDKis only regarding neutropenia. Regardless, other dose-limiting toxicities have been described to be associated with treatment suspension in about 70% of patients and with early dose reduction in 40-50% of patients in treatment with CDKis.

Methods: Metastatic breast cancer patients on treatment with a CDKi were considered eligible for enrolment. A set of 47 polymorphisms in CYP3A4; CYP3A5; ABCB1 and ABCG2 were analysed in genomic DNA. Patients' plasma samples for therapeutic drug monitoring analysis (TDM) were collected at steady state and analyzed by a LC-MS/MS method for minimum plasma concentration (C_{min}) evaluation in a sub-group of patients. For the evaluation of dose-limiting toxicities, treatment suspension was considered only when superior to 7 days and dose reduction was considered early when occurring within the end of cycle 2. All patients signed an informed consent.

Results: Pharmacogenetic analysis and clinical data collection for early events were completed for 200 advanced breast cancer patients. Of the 200 patients enrolled: 36% underwent an early dose reduction and 34% a treatment suspension (> 7 days) within the second cycle of treatment. 28% of patients carried polymorphisms in at least one ADME gene. Patients' carriers of polymorphisms in at least one ADME gene, as *ABCB1* and *CYP3A4*, were at higher risk of developing dose-limiting toxicities (p=0.008), experience dose reduction (p=0.026). C_{min} was successfully evaluated in 80 patients out of 200. Investigation of the association between pharmacogenetic variants and plasma exposure to CDKis (C_{min}) is ongoing.

Conclusions: These findings highlight those genetic polymorphisms in *ABCB1* and *CYP3A4* significantly impact the development dose-limiting toxicities in breast cancer patients treated with CDKis. Although further evidence is needed for the definition of their clinical validity, these polymorphisms appear to be promising biomarkers that can be used in clinical practice to help clinicians in decision-making in the context of metastatic breast cancer with CDKis.

EGFR and KRAS genetic testing enhances personal treatment selection in lung cancer patients

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Introduction: Tyrosine kinase inhibitors (TKIs) are frequently utilised drugs in lung cancer (LC) treatment, especially those targeting Epidermal Growth Factor Receptor (EGFR). Over time, genetic variants conferring better or worse TKI response have evolved, as well as Kirsten Rat Sarcoma virus (KRAS) activating variants that circumvent the need for EGFR stimulation to activate downstream signalling for cell growth/proliferation. Genetic screening could enhance selection of personal treatment options based on identified somatic EGFR/KRAS variants in biopsy samples for these patients.

Methods: Genomic DNA was extracted from clinical LC-related formalin-fixed, paraffin-embedded biopsies (n=339) and sequenced using a capture-based assay towards a 54 LC-related gene panel. Obtained data was processed, aligned to GRCh37-hg19, annotated and variants called using an in-house bioinformatics pipeline. Only EGFR and KRAS variants with allele frequency >5% were considered.

Results: EGFR variants were identified in 16.2% (n=55) of samples with 3.8% (n=13) harbouring L858R, increasing EGFR-TKI response. Other increased EGFR-TKI response linked variants (in-frame, L861, G719) were identified in 7.1% (n=24) of samples. However, 0.59% (n=2) harboured T790M, conferring resistance towards 1st/2nd generation EGFR-TKI (gefitinib, erlotinib). The worse EGFR-TKI response linked N770delinsGY variant was observed in one sample. Third generation EGFR-TKI (osimertinib) resistance conferring C797S was not observed and EGFR variants with unknown effect were identified in 4.4% (n=15) of samples. Lastly, KRAS activating variants (G12, G13, Q61) were identified in 31.3% (n=106) of samples, activating downstream signalling and nullifying EGFR-TKI efficacy. In total, variants linked to increased EGFR-TKI response were identified in 10.9% (n=37) of samples while decreased EGFR-TKI response variants were observed in 32.2% (n=109).

Conclusions: Genetic testing of EGFR and KRAS enables identification of variants linked to better or worse EGFR-TKI response and could be of substantial added clinical value in LC treatment.

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Genetic variability and expression of miR-21 and miR-34a as biomarkers of cardiotoxicity after radiotherapy in breast cancer

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Introduction: Ductal carcinoma *in situ* (DCIS) is a non-invasive form of breast cancer treated with surgery and radiotherapy. Radiation reduces the chance of disease recurrence, but also causes side effects, such as cardiotoxicity. There is large inter-individual variability in the incidence of cardiotoxicity after radiotherapy, possibly due to different molecular factors. We investigated whether genetic variability and expression of miR-21 and miR-34a are associated with the occurrence of cardiotoxicity after radiotherapy in DCIS.

Methods: The prospective study included 119 patients with DCIS treated with radiotherapy. Cardiotoxicity was evaluated using the New York Heart Association (NYHA) score and measurement of the N-terminal pro-B-type Natriuretic Peptide (NT-proBNP) levels immediately after radiotherapy and six months after radiotherapy. DNA was isolated from peripheral blood. MiRNA was isolated from plasma before and after radiotherapy. Quantitative PCR was used to determine miR-21 and miR-34a expression, and allele-specific PCR was used for genotyping of *MIR21* rs1292037 and *MIR34A* rs2666433 polymorphisms. We used logistic regression to assess the association of studied miRNAs with the occurrence of cardiotoxicity.

Results: Patients with NYHA class 2 after radiotherapy had a greater change in miR-21 expression after radiotherapy in both univariable (OR=3.44; 95% CI=1.44-8.21; p=0.005) and multivariable analyses (OR_{adj}=3.24; 95% CI_{adj}=1.34-7.87; p_{adj}=0.009). No association with NYHA was observed six months after treatment. In regards to NT-proBNP levels after radiotherapy, the change in miR-21 expression was greater in patients with higher NT-proBNP after adjustment for heart diseases and sample haemolysis (OR_{adj}=2.04; 95% CI_{adj}=1.02-4.09; p_{adj}=0.044). Patients with higher expression of miR-21 (OR=1.76, 95% CI=1.15-2.67; p=0.009) and miR-34a (OR=1.26; 95% CI=1.04-1.54; p=0.021) after radiotherapy had higher NT-proBNP levels after six months. Both associations remained significant even after adjustment for heart diseases, age, and sample haemolysis (OR_{adj}=1.69, 95% CI_{adj}=1.08-2.66; p_{adj}=0.023 and OR_{adj}=1.27; 95% CI_{adj}=1.03-1.56; padj=0.028, respectively). We did not observe any associations of selected polymorphisms with cardiotoxicity after radiotherapy.

Conclusions: Our results suggest that the miR-21 and miR-34a expression after radiotherapy or the change of their expression might be indicative of cardiac adverse events immediately after radiotherapy as well as after six months.

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CYP2C19 genetic polymorphism and clopidogrel antiplatelet therapy

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Introduction: Clopidogrel is an atiplatelet prodrug that needs to be converted into an active drug. CYP2C19 plays a major role in the drug's metabolism, directly participating in the conversion of clopidogrel to its active metabolite.

Methods: To improve the chances of a good therapeutic antiplatelet effect, 28 patients (14 males and 14 females, median age 52 years) candidate to clopidogrel therapy, were referred to us from different clinics from our hospital and genotyped for three single-nucleotide polymorphisms (*2, *4, *17) that define the major CYP2C19 alleles.

Results: The patients could be classified into 3 groups according to their CYP2C19 genotype: 11 subjects with increased CYP2C19 activity (n.2 CYP2C19*17/*17, n.9 CYP2C19*1/*17); 6 subjects with normal activity (CYP2C19*1/*1) and 11 subjects with impaired CYP2C19 metabolic activity (n.7 CYP2C19*1/*2, n.4 CYP2C19*2/*17). Antiplatelet therapies (loading doses, switch to alternative drugs such as prasugrel or ticagrelor) were chosen according to the patient's genotype, and in most cases their efficacy was confirmed by testing the platelet reactivity index (PRI).

Conclusions: Genotyping patients for CYP2C19 prior starting antiplatelet therapy with clopidogrel could result in enhanced therapeutic efficacy and reduced risk of therapeutic failure/side effects.

Active pharmacovigilance as a tool for value-based prescribing through implementation of Pharmacogenetics, TDM and DDi

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Introduction: Interindividual variability in response to oral target therapies is greater than for standard cytotoxic agents.[1] In this context, we have designed an active pharmacovigilance approach, incorporating pharmacogenetics, therapeutic drug monitoring (TDM), and drug-drug interactions (DDIs) evaluations. The study aim is to improve appropriateness of prescribing, minimizing risk for adverse events and related costs of CDK4/6 inhibitors, PARP inhibitors, imatinib and sunitinib.[2,3]

Methods: Patients candidate to this approach undergo blood sampling from which plasma is separated for TDM evaluation by LC-MS/MS. For pharmacogenetic analyses, DNA extraction is performed, and selected variants in genes encoding enzymes related to ADME of the selected drugs (including CYP3A4, CYP3A5, ABCB1, ABCG2, SLCO1B1) are typed by real-time PCR or pyrosequencing. For the study of DDIs, specific databases (as Lexicomp, Medscape, Cyclib-tool) are used to develop interaction profiles for the patient's polypharmacy. Finally, all of this information are implemented in the Naranjo tool for calculating the "Causality Assessment" of reports of suspected adverse reactions.

Results: The study will involve 350 patients who will be compared with a historical cohort treated without this integrated approach. LC-MS/MS methods for Cmin evaluation have been developed for the drugs under study. The analysis platform with a panel of 62 ADME related polymorphisms has been selected and validated. Structured questionnaires were designed and approved by the ethics committee for the collection of clinical and demographic data and monitoring of patients' quality of life. A workflow for clinician reporting was established to provide timely suggestions for more personalized pharmacologic treatment.

Conclusions: In the context of oral target therapies, the proposed "active" pharmacovigilance approach has the potential to identify major drug interactions or inappropriate exposure that would result in toxicity risk or poor efficacy.[4] In addition, it can help rationalize the costs associated with prescribing these expensive drugs under the most appropriate conditions to ensure greater efficacy.

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POSTER ABSTRACTS

HLA variants associated with azathioprine-induced pancreatitis in patients with Crohn's disease

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Introduction: The immunosuppressant drug azathioprine is associated with a 4% risk of acute pancreatitis in patients with inflammatory bowel disease (IBD). Studies have demonstrated an increased risk of adverse drug reaction in carriers of HLA-DQA1*02:01 and HLA-DRB1*07:01.

Methods: We investigated whether these human leukocyte antigen (HLA) types were associated with azathioprine-induced pancreatitis also in Swedish patients with IBD, and whether the type of disease affected the association.

Results: Nineteen individuals with IBD who developed acute pancreatitis after initiation of azathioprine were genotyped and compared with a population control cohort (n=4891) and a control group matched for disease (n=81). HLA-DQA1*02:01 and HLA-DRB1*07:01 were in full linkage disequilibrium and were significantly associated with acute pancreatitis both when cases were compared with population controls (OR 3.97 [95% CI 1.57-9.97], p=0.0035) and matched controls (OR 3.55 [95% CI 1.23-10.98], p=0.0275). In a disease-specific analysis, the correlation was positive in patients with Crohn's disease versus matched controls (OR 9.27 [95% CI 1.86-46.19], p=0.0066), but not in those with ulcerative colitis versus matched controls (OR 0.69 [95% CI 0.07-6.74], p=0.749). In patients with Crohn's disease, we estimated the conditional risk of carriers of HLA-DQA1*02:01-HLA-DRB1*07:01 to 7.3%, and the conditional risk of a non-carrier to 2.2%.

Conclusions: We conclude that HLA-DQA1*02:01-HLA-DRB1*07:01 is a marker for increased risk of acute pancreatitis in individuals of Swedish genetic origin, treated with azathioprine for Crohn's disease.

Inflammation and oxidative stress related biomarkers of response to treatment with selective laser trabeculoplasty in primary open-angle glaucoma and ocular hypertension

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Introduction: Reduction of intraocular pressure (IOP) with eye drops or with selective laser trabeculoplasty (SLT) are effective treatments of primary open-angle glaucoma (POAG), however sufficient reduction of IOP cannot be achieved in all patients. The aim of our study was to identify pharmacogenetic biomarkers of treatment response to SLT in patients with ocular hypertension (OH) and POAG.

Methods: Our pilot study included 51 treatment naive patients with OH or mild to advanced POAG. Response to SLT was evaluated as a reduction of IOP at 6 weeks after STL treatment. All patients were genotyped for polymorphisms in genes involved in inflammatory pathway (*TNF* rs1800629; *IL1B* rs16944, rs1143623; *IL6* rs1800795) and oxidative stress pathway (*GSTM1**0; *GSTT1**0; *GSTP1* rs1695, rs1138272; *SOD2* rs4880; *CAT* rs1001179; *GPX1* rs1050450). Logistic regression and ROC curve analysis were used for statistical analysis.

Results: At 6 weeks after SLT treatment IOP was reduced for >30% (good treatment outcome) in 39.2%, for 15-30% in 47.1% and for <15% in 13.7% of patients. Among clinical parameters, family history of glaucoma and central corneal thickness tended to be associated with less effective treatment outcome (p=0.033 and p=0.057, respectively). Carriers of at least one polymorphic *IL6* rs1800795 allele were less likely to achieve good treatment outcome (OR=0.24, 95% CI=0.07-0.81, p=0.021). The same trend was observed for carriers of at least one polymorphic *GSTP1* rs1138272 allele (OR=0.13, 95% CI=0.02-1.11, p=0.062). Clinical and pharmacogenetic data were used to build clinical and clinical-pharmacogenetic models for prediction of treatment outcome. IOP reduction was more successfully predicted with clinical-pharmacogenetic model (area under the curve (AUC)=0.85, p<0.001) than clinical model alone (AUC=0.74, p=0.005).

Conclusions: Pharmacogenetic biomarkers may be associated with treatment response to SLT. Clinical-pharmacogenetic models may enable better prediction of SLT treatment efficacy than clinical model, and could support the selection of the most effective first line treatment of POAG and OH patients.

Healthy Aging Pharmacogenomics and Polypharmacy (HAPPY): implementing PGx in primary care

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Introduction: Pharmacogenetic (PGx) testing is an effective strategy for optimising medication, however several challenges have been identified preventing widespread adoption. There is potential to apply PGx testing in the ageing population on polypharmacy to impact adverse drug reactions which are associated with this population.

The HAPPY project has two key aims, 1) to develop an implementation plan for PGx in polypharmacy patients via a clinical study providing PGx reports to general practitioners 2) to develop an innovative, scalable, integrated clinical decision support platform to support the implementation of PGx into primary care. We focus here on aim 1.

Methods: 500 patients over the age of 50 years on three or more medications (with at least one medication being a known PGx drug) are being recruited at two general practices in England. Patients consent to donate a saliva sample for DNA extraction. Samples will be processed in batches of 96 via two methods for confirmation: Illumina Global Diversity Array with enhanced PGx and Agena Veridose Core, *CYP2D6* CNV and custom panels.

PGx reports will be generated by Abomics (Finland). Results are reviewed by GP and pharmacist, and recommended medication changes documented and actioned where agreed. Patient interaction with healthcare providers (eg. A&E admissions) 12mnths prior to recruitment and 12mnths post-medication review will be reviewed. Participants also complete a baseline, 1mnth, 3mnth and 12mnth questionnaire on adverse drug reactions and frailty.

Results: To date 88 patients have been recruited. Updated figures will be presented.

Conclusions: The HAPPY study will generate evidence to inform utility of PGx testing in primary care and develop the analytical solutions to support large scale implementation.

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Drug-gut-microbiota triad in rheumatic diseases: a neglected association

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Introduction: Rheumatic diseases (RDs) comprise over 200 inflammatory and autoimmune diseases that can affect articular (eg. rheumatoid arthritis and gout) and extra-articular organs (eg. inflammatory bowel diseases). Evidence has accumulated to reveal that interaction between genetics, environment and microbiota is a hallmark of rheumatic diseases, yet the nonclassical triad—gene, drug, and microbiota is overlooked.

Importantly, imbalanced microbial community of the gut ecosystem—referred to as dysbiosis—is associated with the development, progression and treatment outcome of several RDs. Furthermore, experimental research emphasizes a direct role for microbiota in driving inflammation and autoimmunity in the pre-disease stage in conventional transgenic animals. However, germ-free mice failed to display clinical symptoms.

The most commonly used drugs for RDs are disease modifying anti-rheumatic drugs (DMARDs) and non-steroidal anti-inflammatory drugs (NSAIDs). Methotrexate and ibuprofen are showcased as anchor DMARDs and NSAIDs for treating rheumatoid arthritis, respectively. These drugs have a bi-directional interaction with gut microbiota. Multiple independent lines of evidence indicate either a direct repressive role on the growth of gut microbiota or an indirect effect on the microbiota community composition and functionality. On the other hand, microbiota is also reported to modulate drug bioavailability and pharmacokinetics either directly *via* drug metabolism or indirectly *via* hepatic modulation of xenobiotic metabolism.

In fact, NSAIDs *per se* can compromise the integrity of the intestinal barrier. As such, translocation of gut bacteria-derived lipopolysaccharide into the systemic circulation could potentially elicit NLRP3 inflammasome activation. Alarmingly, the usual coadministration of NSAIDs and proton-pump inhibitors (eg. omeprazole) adds another layer of complex drug-drug-gut-microbiota interactions with subsequent worsening of disease conditions and therapeutic response.

Conclusions: This proposed drug-gut-microbiota triad should be adopted by clinicians in tailoring drug therapy with minimal adverse drug-drug and microbe-microbe interactions in the gut environment. Also, microbiome-based biomarkers should be integrated with PGx findings in future clinical trials of precision medicine in rheumatic diseases.

Pharmacogenomic markers of glucocorticoid response in the remission induction therapy in childhood acute lymphoblastic leukemia

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Introduction: Response to glucocorticoid (GC) monotherapy in the initial phase of remission induction treatment in childhood acute lymphoblastic leukemia (ALL) represents important biomarker of prognosis and outcome. We aimed to study variants in several pharmacogenes (NR3C1, GSTs and ABCB1) that could contribute to improvement of GC response through personalization of GC therapy. The marker of GC response is blast count per microliter of peripheral blood on treatment day 8. We carried out analysis in which cut-off value for GC response was 1000 (according to BFM protocol), as well as 100 or 0 blasts per microliter.

Methods: Retrospective study enrolling 122 ALL patients was carried out to analyze variants of NR3C1 (rs33389, rs33388 and rs6198), GSTT1 (null genotype), GSTM1 (null genotype), GSTP1 (rs1695 and rs1138272) and ABCB1 (rs1128503, rs2032582 and rs1045642) genes using PCR-based methodology.

Results: Carriers of rare NR3C1 rs6198 GG genotype were more likely to have blast count over 1000, than the non-carriers (p=0.030). NR3C1 CAA (rs33389-rs33388-rs6198) haplotype was associated with blast number below 1000 (p=0.030). GSTP1 GC haplotype carriers were more likely to have blast number below 1000 (p=0.036), below 100 (p=0.028) and to be blast negative (p=0.054), while GSTP1 GT haplotype and rs1138272 T allele carriers were more likely to be blasts positive (p=0.034 and p=0.024, respectively). ABCB1 CGT (rs1128503-rs2032582-rs1045642) haplotype carriers were more likely to be blast positive (p=0.018).

Conclusions: Our results have shown that NR3C1 rs6198 variant and GSTP1 rs1695-rs1138272 haplotype are the most promising pharmacogenomic markers of GC response in ALL patients.

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Inflammatory bowel disease patient-derived organoids for the evaluation of thiopurine effects on intestinal epithelium

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Introduction: Thiopurines, azathioprine (AZA) and mercaptopurine (MP), are immunomodulatory drugs used to maintain remission in patients with inflammatory bowel disease (IBD). As the therapeutic effects of these drugs could also involve a direct action on epithelial cells, the aim of the project is to establish an in vitro IBD model using organoids to investigate thiopurine effects on the intestinal epithelium.

Methods: 53 pediatric patients with IBD were enrolled. Intestinal biopsies were used to isolate crypts and generate organoids. The cytotoxicity of thiopurines was evaluated by CellTiter-Glo 3D assay. Gene expression was evaluated using TaqMan® technology. The quantification of thiopurine metabolites was performed by LC-MS/MS analysis. Proteomic profiles were analyzed by Q-Exactive Plus mass spectrometer and REACTOME pathway software.

Results: A dose dependent cytotoxic response was demonstrated in organoids treated with AZA and MP (Two-way ANOVA $p<0.0001$ for both drugs). Correlation analysis between expression levels of candidate genes involved in thiopurine pharmacokinetics and pharmacodynamics and the percentage of cytotoxicity on organoids treated with thiopurines (2 μM) showed a significant negative correlation for ITPA ($p=0.004$), TPMT ($p=0.003$) and PACSIN2 ($p=0.02$) and AZA treatment. The most abundant thiopurine metabolites on organoids exposed for 48 h to AZA and MP (2 μM) were MeTIMP and TGMP; the association with cytotoxicity is still ongoing. Proteomic analysis on organoids treated for 72 h with thiopurines (0.2 μM) showed 194 proteins differentially expressed for AZA and 231 for MP ($p<0.05$). REACTOME enrichment analysis revealed that the most involved pathways are related to vesicular traffic and autophagy

Conclusions: Organoid cultures are a valid cellular model to investigate the mechanisms of action of immunomodulators on intestinal epithelium.

Drug-gene interactions of EMA-authorised and potential COVID-19 treatments: current data

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Introduction: The pandemic of coronavirus disease (COVID-19) caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), declared in 2019, is not abating and continues to spread, leaving serious health and socioeconomic consequences. The SARS-CoV-2 contains ribonucleic acid (RNA) as genetic material and belongs to the genus *Betacoronavirus*, using a spike glycoprotein to bind to receptors for angiotensin-converting enzyme-2 (ACE-2), through which a connection with the host cell is established. This research aims to provide information on current data regarding drug-gene interactions of already authorised COVID-19 treatments, but also treatments under development and review.

Methods: The screening of COVID-19 treatments either currently being under rolling review, with a marketing authorisation application submitted or authorised for use by the European Medicines Agency (EMA) was done. The EMA authorised eight drugs to treat COVID-19, 103 drugs received EMA-advice (guidance to assist drug developers to prepare the application for marketing authorisation), one drug is now being under review while reviews have been completed for seven drugs, two drugs are also being under marketing authorisation evaluation with one drug withdrawn from rolling review. All these drugs were checked in *The Drug Gene Interaction Database* (DGIdb) for potential drug-gene interactions and the interaction scores were analysed.

Results: Out of all these drugs, 39 were detected in DGIdb with 327 drug-gene interactions found. Colchicine (received EMA-advice) and dexamethasone (endorsed after Article 5(3) review for patients on oxygen or mechanical ventilation) showed the most interactions with genes, 46 and 83, respectively. The most common genes that interacted with the checked drugs were C5, CSF2, CSF2RA, IL6, and TFF2. The highest interaction score was 61.83 (plitidepsin-PPT1 and remdesivir-NUCB1).

Conclusions: With more potential COVID-19 treatments being evaluated and authorised for use, the importance of researching their interactions with genes will elevate, which is crucial for the personalised treatment of COVID-19 patients.

PGx-CardioDrug - The role of pharmacogenomics in the prediction of cardiovascular ADRs

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Introduction: This study aims to investigate pharmacogenomic variants, drug-drug-gene interactions and their relevance for predicting cardiovascular drugs' adverse reactions (ADRs). Preliminary data from our prospective nested case-control study is presented here.

Methods: The primary cohort consists of cardiovascular patients with new indications for direct oral anticoagulants (DOACs: apixaban, dabigatran etexilate, edoxaban, rivaroxaban); platelet aggregation inhibitors (PAI: clopidogrel, prasugrel, ticagrelor), statins (atorvastatin, fluvastatin, rosuvastatin, simvastatin). Patients have been recruited for 18 months. The cases represent subjects who developed ADRs during the follow-up period: bleeding/inefficiency from DOACs and PAIs, myotoxicity/hepatotoxicity from statins, and other serious ADRs. Controls are subjects with no ADRs presented during the follow-up period recruited from the same cohort. The relevant ADME gene variants are continuously genotyped: *CYP2C9*2*3*, *CYP2C19*2*3*17*, *CYP2D6*3*4*5*6*9*10*41*, *CYP2J2*7*, *CES1* (rs2244613, rs8192935), *CYP3A4*1B*22*, *CYP3A5*3*, *ABCB1* (*c.1236C>T*, *c.2677G>T/A*, *c.3435C>T*, *rs4148738*), *ABCG2 c.421C>A*, *SLCO1B1 c.521T>C*, depending on the subjects' therapy. Clinical and laboratory parameters are also monitored. The Lexicomp® Clinical Decision Support System is used for drug-drug interactions (DDI) analysis.

Results: Currently 660 patients are recruited (female=312, male=348), with cardiovascular drugs prescribed as follows: DOACs (n=318), PAIs (n=117), statins (n=386). 450 samples are genotyped according to prescribed drug-substrates: CYP2C9 (n=280; 42%), CYP2C19 (n=354; 54%), CYP3A4*22 (n=548; 83%), CYP3A5 (n=421; 64%), CYP2D6 (n=114; 17%), CYP2J2*7 (n=109; 17%), CES1 (n=31; 4.7%), ABCB1 (n=344; 52%), ABCG2 (n=525; 80%), and SLCO1B1 (n=366; 55%). 300 subjects are evaluated for potential DDIs with increased risk for ADRs, and found in group of statins (n=39/182; DDI level C=15%; D=2%), DOACs (n=133/135, DDI level C=21%, D=26%) and PAIs (n=68/76, DDI level C=71%, D=2.6%).

Conclusions: The preliminary data of the study points out that drug-drug-gene interactions and genetic polymorphisms may be important risk factors for cardiovascular ADRs.

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Expression pattern of lncRNA GAS5 in the remission induction therapy in childhood acute lymphoblastic leukemia

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Introduction: Long non-coding RNA GAS5 (growth arrest specific 5) is deregulated in many cancers because of its role in cell growth arrest and apoptosis. Additionally, GAS5 interacts with glucocorticoid receptor, making it a potential pharmacotranscriptomic marker of glucocorticoid therapy. In this study we aimed at analyzing GAS5 expression in the remission induction therapy phase of childhood acute lymphoblastic leukemia (ALL), in which glucocorticoids are mandatory used, and to correlate it with therapy response.

Methods: GAS5 expression was measured in peripheral blood mononuclear cells taken from 29 childhood ALL patients at diagnosis, on day 15 and day 33 of remission induction therapy using RT-qPCR methodology.

Results: Our results have shown noted interindividual differences in GAS5 expression at all time points. For each ALL patient, GAS5 expression was higher on day 15 in comparison to its level at diagnosis ($p<0.0005$). On day 33, the level of GAS5 expression was decreased in comparison with day 15 ($p<0.0005$), but it was still significantly higher than at diagnosis for the majority of patients ($p=0.001$). Patients whose number of blasts on day 8 was below 100 per microL of peripheral blood had higher GAS5 expression at diagnosis ($p=0.016$), and lower ratio day 15/diagnosis ($p=0.009$).

Conclusions: Our results suggest that expression level of GAS5 could be a potential marker of therapy response in remission induction therapy of childhood ALL.

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Paraoxonase 1 in Parkinson's disease dementia: associations between genetics, enzyme kinetics, and disease

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Introduction: Paraoxonase 1 (PON1) is an antioxidative enzyme in human blood serum/plasma which has been implicated in several neurodegenerative disorders, including Alzheimer's and Parkinson's disease (PD). Potential associations between some of the symptoms of PD and PON1 status have already been investigated, but Parkinson's disease dementia (PDD) has not yet been investigated in this context.

Methods: We have collected data from 231 PD patients, divided into a group with and a group without dementia, and determined their genotype for four SNPs in the *PON1* gene: rs662 (Q192R), rs854560 (L55M), rs705379 (-108 C/T) and rs705381 (−162 A/G). We have also calculated the kinetic parameters K_m and V_{max} for the enzymatic reaction of dihydrocoumarin (DHC) with blood plasma samples, using a recently developed approach for time-concentration progress curve analysis (iFIT).

Results: We have found no statistically significant associations between *PON1* genotypes or enzyme kinetics and cognitive status. However, we have shown a strong correlation between rs662 (Q192R) genotype and PON1 K_m and V_{max}, and a strong correlation between rs705379 (-108 C/T) genotype and V_{max}.

Conclusions: Our results suggest that PON1 does not play a notable role in the development of cognitive impairment in the context of PD. While the influence of the genotypes rs662 and rs705379 on enzyme concentration and activity has been shown before for other substrates, e.g. phenylacetate, our study has confirmed them using DHC as a substrate in a large number of subjects.

The pharmacogenomics of vincristine-induced peripheral neuropathy in pediatric acute lymphoblastic leukemia patients in Serbia

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Introduction: Vincristine (VCR) is one of the key drugs in current treatment protocols for pediatric acute lymphoblastic leukemia (ALL). By destabilization of microtubules, VCR arrests cells in metaphase, inducing apoptosis of malignant cells. VCR also causes axonal degradation and impairment of axonal transport, which leads to vincristine-induced peripheral neuropathy (VIPN). The aim of this study was to determine if the selected genetic variants are associated with the development of VIPN in ALL children treated with VCR in Serbia. This study also aimed to discover candidate pharmacogenomic markers of VIPN in Serbian population.

Methods: PCR and sequencing-based methodology was used to detect variants in following genes: *CYP3A5* (rs776746), *CEP72* (rs924607), *ACTG1* (rs1135989), *MIR3117* (rs12402181) and *MIR4481* (rs7896283). Statistical analyses were performed for investigation of their association with VIPN in 56 pediatric ALL patients. Population VCR pharmacogenomics analysis of 17 pharmacogenes from in-house next-generation sequencing data was also done. Data on allele frequency distribution for European population were extracted from public databases.

Results: During the treatment, 17.86% of patients developed VIPN. Association analyses have shown that none of the investigated genetic variants contributed to the occurrence of VIPN in our study group. Population pharmacogenomics study didn't reveal valid candidate pharmacovariants for the occurrence of VIPN. Our results suggested that pre-emptive pharmacogenetic testing for VCR is not applicable.

Conclusions: More comprehensive approaches are needed to identify panel of genes that could explain the VIPN development after VCR administration in ALL patients. Utilizing better designed GWAS studies and more robust artificial intelligence-based tools would provide a panel of pharmacogenes for pre-emptive tests of VIPN to individualize therapy for ALL in children.

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Impact of genetic variability in glucocorticoid pathway in COVID-19 patients on treatment outcome

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Introduction: Glucocorticoids are the first choice in treatment of severe COVID-19, as they attenuate excessive immune response with their anti-inflammatory properties. The aim of this study was to evaluate the associations of glucocorticoid pathway polymorphisms with disease severity, duration of dexamethasone treatment and hospital care, requirement for oxygen supplementation and treatment at ICU.

Methods: This study included 107 hospitalized COVID-19 patients, treated with dexamethasone. All patients were genotyped for *NR3C1* (rs6198, rs33388, rs33389), *CYP3A4* (rs35599367, rs2740574), *CYP3A5* (rs776746), *GSTP1* (rs1695, rs1138272), *GSTM1/GSTT1* deletions and *ABCB1* (1045642, rs1128503, rs2032582) polymorphisms. Logistic regression, Mann-Whitney and Fisher's test were used in statistical analysis.

Results: Among patients, 69.2% were male and 30.8% female, with median (range) age 62 (26–85) years. *CYP3A4* rs35599367 carriers had higher odds for critical disease (OR=6.69, 95% CI=1.22–36.75, p=0.029) and need for ICU treatment (OR=10.22, 95% CI=1.79–58.27, p=0.009). Odds for ICU treatment were also higher in *GSTP1* rs113827 carriers (OR=4.88, 95% CI=1.33–17.87, p=0.017), but lower in *NR3C1* rs33388 carriers (OR=0.15, 95% CI=0.03–0.79, p=0.025). On the contrary, heterozygous carriers of *GSTP1* rs1695 and also *ABCB1* rs1128503, *ABCB1* rs2032582 required shorter hospitalization and less oxygen supplementation in comparison with homozygotes for either allele. *NR3C1* rs6198 (p=0.048) *ABCB1*1045642 (p=0.047), *ABCB1* rs1128503 (p=0.024), *GSTP1* rs1695 (p=0.022) polymorphisms were associated with shorter dexamethasone treatment, where polymorphic homozygotes had shorter treatment in comparison with heterozygotes or normal homozygotes. However, shorter dexamethasone treatment was mostly due to drug ineffectiveness that resulted in its replacement with methylprednisolone.

Conclusions: Glucocorticoid pathway polymorphisms are associated with disease severity and treatment response in COVID-19 patients. The associations of *NR3C1* rs6198, *ABCB* rs11045642, *ABCB1* rs1128503, and *GSTP1* rs1695 with treatment outcome may suggest lower efficacy of dexamethasone treatment in patients with these polymorphisms.

Comprehensive somatic testing of KRAS-G12C-positive colorectal cancers enhances personal treatment selection of patients

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Introduction: Kirsten Rat Sarcoma virus (KRAS)-G12C-variants occur in approximately 13% of non-small-cell lung cancers (NSCLC) and 3% of colorectal cancers (CRC). Early-phase clinical trials with two selective KRAS-G12C-inhibitors (adagrasib and sotorasib), have shown promising results in NSCLC (mean response rate 41%) and more modest efficacy in CRC (mean response rate 12%). Still acquired resistance to single-agent therapy eventually occurred in most patients, which is mainly due to alterations activating RAS-signaling pathways. Comprehensive somatic testing of KRAS-G12C-positive NSCLC/CRC might unravel these modified pathways not only as acquired mechanisms but also as co-drivers in CRC, conferring primary resistance to KRAS-G12C-inhibitors, leading to lower response rates in CRC compared to NSCLC.

Methods: Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissues from NSCLC (n=457) and CRC (n=405) patients without previous KRAS-G12C-inhibitor-treatment, and sequenced using a capture-based assay towards a comprehensive gene panel. Obtained data was processed, aligned to GRCh37-hg19 and variants called using an in-house bioinformatics pipeline. Variants related to RAS-signaling pathways and allele frequency ≥5% were considered.

Results: KRAS-G12C-variants were identified in 14% (64/457) of NSCLC and 3.5% (14/405) of CRC patients. Of those 14% KRAS-G12C-NSCLC samples, only 9.4% (6/64) revealed extra variants activating RAS-signaling; two PTEN loss of function (LoF), one NF1 LoF, two PIK3CA activation and one PIK3R1 LoF. On the contrary, of 3.5% KRAS-G12C-CRC samples, 50% (7/14) harbored RAS-pathway activating variants, including six PIK3CA activation and one BRAF activation. These data suggest the possible existence of co-drivers in KRAS-G12C-CRC, which might be responsible for the limited response rate in CRC treated with KRAS-G12C-inhibitors. Identification of this unique molecular subtype of KRAS-G12C-CRC creates the possibility to overcome primary resistance to KRAS-G12C-inbibitor by considering combination-targeted therapy.

Conclusions: Comprehensive somatic testing of KRAS-G12C-positive CRC is of substantial benefit in CRC-treatment decision-making.

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Case report: Patient ultrarapid metabolizer (UR) for CYP2D6 and CYP2C19

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Introduction: This case report highlights the importance of the correct interpretation of the haplotypes in Pharmacogenetics to help in the correct treatment to avoid the toxicity that some drugs may cause.

Methods: The patient in this case report undergoes MassArray analysis and MLPA technique for the CNV of the *CYP2D6* and then the results are entered in the MyPGx® interpretation algorithm to make a final report with several genes haplotype involved in well-known pharmacological pathways.

Results: MassArray analysis revealed CYP2C19 *1/*17, which could explain the therapeutic failure with antidepressants, and CYP2D6 *1/*41 haplotypes while the MLPA technique detected 5 copies of *CYP2D6*. MLPA doesn't allow a determination of the phase of the 5 copies of *CYP2D6*. The segregation study is mandatory to establish the number of copies of the *CYP2D6* for both alleles but there are difficulties in interpreting the attribution of how many copies are present in each allele.

Conclusions: Considering that duplications are not so frequent, the patient has two options: low activity if he has 4 copies from *41, he is UR but low levels, if he has 4 copies from *1, he is UR but high levels. Most importantly, the patient has 5 copies of CYP2D6, with at least 1x *1 and 1x *41 (*1 is more likely based on population frequency). He is therefore an ultra-rapid metabolizer with a predicted CYP2D6 activity between 3 and 4.5. Interestingly, the MyPGx® interpretation algorithm had trouble with predictions, because of the (*1 and/or *41) X5 uncertainty. In the end, this is a life-changing result for the patient, positively: physicians should be able to find an effective treatment and protect him from potentially serious side effects.

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