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Poster Abstracts*)


# Integration of Pharmacogenomics in clinical decision support

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# Pharmacogenetics of myelodysplastic syndromes: CDA status as a predictive marker of clinical outcome in patients undergoing cytarabine and azacytidine therapy

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Developing precision medicine is a rising trend in clinical oncology and several strategies are currently undertaken to improve the efficacy/toxicity balance of anticancer agents. Germinal pharmacogenetics aims at identifying genetic polymorphisms affecting ADME processes (i.e., metabolism and transport) and possibly impacting on drug exposure and clinical outcome eventually. Cytidine deaminase (CDA) is a ubiquitous enzyme involved in the disposition of both cytarabine and azacytidine, two cytotoxics widely prescribed to treat hematological disorders. CDA gene is highly polymorphic with a variety of impact on function (i.e., poor metabolizer, extensive metabolizer, ultra-rapid metabolizer). Screening for CDA genetic polymorphisms in patients scheduled for such therapy could help, through adaptive dosing strategy, to reduce the risk of overexposure, thus maintaining all patients in the right therapeutic window. In this proof-of-concept study, we monitored efficacy and toxicity in 60 adult patients treated for a variety of hematological disorders and treated with either high-dose cytarabine or azacytidine. CDA status was evaluated following two distinct strategies: screening for canonical 79A>C SNP and ex-vivo establishment of patient's CDA phenotype using a surrogate test. Results showed that CDA deficiency, a condition defined as 50% cut in CDA activity as compared with reference values, was associated with higher risk for severe toxicities, including life-threatening/lethal ones. Of note, search for the CDA*2 allelic variant was not conclusive. Conversely, patients displaying a UM phenotype were less likely to respond to the therapy. Results from this pilot study will have to be further confirmed in a prospective fashion. However, our clinical data strongly suggest that CDA status could be used as a covariate to tailor dosing in nucleosidic analogs-based regimen in patients with hematological disorders.

# The frequency of CYP2C19 genetic polymorphisms in Russian patients with peptic ulcer treated with proton pump inhibitors

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**Background**: Proton pump inhibitors, which are widely used as acid-inhibitory agents for the treatment of peptic ulcer, are mainly metabolized by 2C19 isoenzyme of cytochrome P450 (CYP2C19). CYP2C19 has genetic polymorphisms, associated with extensive, poor, intermediate or ultrarapid metabolism of proton pump inhibitors. Genetic polymorphism of CYP2C19 could be of clinical concern in the treatment of peptic ulcer with proton pump inhibitors.
**Objective:** To investigate the frequencies of CYP2C19*2, CYP2C19*3 and CYP2C19*17 alleles and genotypes in Russian patients with peptic ulcer.
**Design:** The study involved 971 patients with peptic ulcer from the European part of Russia (Moscow), 428 male (44%) and 543 female (56%). The mean age was 44.6±11.9 years (range 15-88 years). DNA isolated from blood samples was used for the analysis of CYP2C19 genetic polymorphisms (CYP2C19*2, *3, *17 alleles) by real-time polymerase chain reaction.
**Results**: Regarding CYP2C19 genotype, 317 patients (32.65%) out of 971 were CYP2C19*1/*1 carriers classified as extensive metabolizers. 386 (39.75%) with CYP2C19*1/*17 or CYP2C19*17/*17 genotype were ultrarapid metabolizers. 251 people (25.85%) were intermediate metabolizers with CYP2C19*1/*2, CYP2C19*2/*17, CYP2C19*1/*3, CYP2C19*3/*17 genotypes. 17 patients (1.75%) with CYP2C19*2/*2, CYP2C19*3/*3, CYP2C19*2/*3 genotypes were poor metabolizers. The allele frequencies were the following: CYP2C19*2 – 0.140, CYP2C19*3 – 0.006, CYP2C19*17 – 0.274.
**Conclusion**: There is a high frequency of CYP2C19 genotypes associated with modified response on proton pump inhibitors in Russian patients with peptic ulcer. Genotyping for CYP2C19 polymorphisms is suggested to be a useful tool for personalized dosing of proton pump inhibitors.
**Reference:** Gardiner SJ, Begg EJ. Pharmacogenetics, Drug-Metabolizing Enzymes, and Clinical Practice. Pharmacol. Rev. 2006;58:521-590.

# Advances in pharmacogenomics of methotrexate membrane transporters: are we ready for personalized medicine in rheumatoid arthritis?

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**Background**: Methotrexate (MTX) is currently the most widely used disease-modifying antirheumatic drug for rheumatoid arthritis (RA) treatment. Despite the advances in the therapeutic management of RA, significant variability in MTX therapeutic outcome remains. MTX membrane transporters (solute carriers – SLCs and ATP-binding cassette – ABCs) are responsible for modulating MTX pharmacokinetics,

thus becoming targets in the quest for pharmacogenetic predictors of MTX therapeutic outcome. Yet, translation of this novel knowledge into clinical practice aimed to personalize and optimize therapy in RA patients has been slow.

**Objective:** To systematically review the state of art regarding pharmacogenomic studies conducted to investigate whether genetic polymorphisms related with MTX membrane transport pathway, might modulate RA patients' clinical response profile to MTX therapy.

**Design:** A comprehensive search of MEDLINE through PubMed was conducted and studies were selected if were aimed for MTX membrane transporters, RA, therapeutic outcome and pharmacogenomics.

**Results**: Several single nucleotide polymorphisms (SNPs) in transporters' genes, including the SLC19A1/RFC1, SLC46A1/PCFT, SLCO1B1/OATP1B1, ABCB1/MDR1/P-GP, ABCC1/MRP1, ABCC2/MRP2 and ABCG2/BCRP, have been studied regarding their association with MTX therapeutic outcome in RA. The most extensively studied SNP was SLC19A1 G80A (rs1051266), where homozygotes AA have been associated with a better response profile to MTX and a reduced risk for MTX-related toxicity. Other less studied SNPs in SLCs were associated with a non-response profile: A allele for SLC19A1 G>A (rs7499), G allele for SLC19A1 A>G (rs2838956) and T allele for SLC22A11 T>A (rs11231809). SNPs in SLCs associated with an increased risk for MTX-related toxicity were: G carriers for SLC19A1 G>A (rs7499), T carriers for SLC19A1 T>C (rs1131596), A carriers for SLC19A1 G>A (rs2838956), GG for SLC46A1 A>G (rs2239907) and TT for SLCO1B1 T>C (rs4149056). Concerning the ABCs, the most studied SNP was ABCB1 C3435T (rs1045642), where homozygotes TT were associated with a better response profile to MTX and with an increased risk for MTX-related toxicity. Other less studied SNPs in ABCs were associated with a non-response profile: G carriers for ABCB1 G>A/T (rs2032582), ABCC1 A>G (rs246240) and ABCC1 G>A (rs3784864). There were also SNPs in ABCs associated with an increased risk for MTX-related toxicity: TT for ABCB1 C>T (rs1128503), AA for ABCC2 G>A (rs2273697), CC for ABCC2 T>C (rs4148396), AA for ABCC2 G>A (rs7080681) and AA for ABCG2 C>A (rs2231142). Several studies demonstrated inconsistent results regarding the associations of SNPs in both SLCs and ABCs with MTX therapeutic outcome.

**Conclusions**: Key SNPs in SLCs and ABCs seemed to be important in clinical practice for predicting which RA patients will not benefit from MTX treatment, although further studies will be required to validate these findings. The advances in the discovery of pharmacogenetic predictors of MTX therapeutic outcome attending to MTX membrane transport pathway have potential to sustain a breakthrough in the field of personalized medicine.

**Reference:** Lima A et al. Genetic polymorphisms in low-dose methotrexate transporters: current relevance as methotrexate therapeutic outcome biomarkers. Pharmacogenomics. 2014;15(12):1611-35.

## Genetic variants in vincristine pathway and neurotoxicity in childhood acute lymphoblastic leukemia

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**Background:** Acute lymphoblastic leukemia (B-ALL) is the most common pediatric malignancy. Therapeutic advances have increased survival, due in part to standardized treatment protocols. Vincristine is one of the most important drugs used in B-ALL treatment. However, some individuals experience neurotoxicity that can lead dose reduction or treatment discontinuation, which can in turn have an impact on survival.

In the last years, an effort has been done looking for genetic markers that can predict adverse effects in ALL treatment. Polymorphisms in genes involved in the drug transport (ABCC4, ABCC2, etc.) are acquiring relevance as markers of toxicity for different drugs (methotrexate). Those transporters are also involved in vincristine transport, but few studies have been done in this line.

**Aim:** In this study, we look for genetic markers of neurotoxicity induced by vincristine.

**Design:** For this aim, we have analyzed 133 genetic variations in 6 vincristine transport genes in a large cohort of 152 children with B-ALL homogeneously treated with LAL/SHOP protocol.

**Results:** We found that 3 SNPs in ABCC2 gene were significantly associated with neurotoxicity after correction by False Discovery Rate (FDR).

**Conclusion:** Genetic variants in transport genes could be useful markers in the prediction of neurotoxicity to personalized pediatric ALL treatment.

**Acknowledgements:** This project was supported by RETICS (RD/12/0036/0060 and RD/12/0036/0036) and Basque Government (IT661-13, S-PE13UN068 and 2012111053).

## Variations in micrornas could be useful markers of MTX clearance in childhood acute lymphoblastic leukemia

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**Background**: Methotrexate (MTX) is a key component in the treatment of childhood Acute Lymphoblastic Leukemia (ALL). Treatment with high-dose MTX often causes toxicity, requiring a dose reduction or cessation of treatment, which has been demonstrated to reduce survival. Therefore, it would be useful to identify a predictor of the adverse effect of MTX.

In the last years, several studies have investigated the relationship between genetic variations and MTX toxicity. Nevertheless, most of these studies have focused on coding regions. Nowadays, it is known that genes that do not codify proteins, like microRNAs (miRNAs), can regulate genes involved in drug transport. MiRNA related-SNPs have been already associated with MTX toxicity.

**Aim**: The aim of this study was to determine if SNPs in microRNAs could be useful as new MTX toxicity markers in pediatric B-ALL treatment.

**Design**: DNA from blood of 180 childhood B-cell ALL patients during complete remission treated with the LAL/SHOP protocol were analyzed. MTX plasma levels were used as an objective and quantifiable marker of toxicity. 235 SNPs in 222 miRNAs were studied. VeraCode GoldenGate platform was used.

**Results**: Interestingly, we found 2 SNPs in one miRNA targeting the trasnporter SLC46A1 gene, significantly associated with MTX clearance.

**Conclusion**: Our results suggest that polymorphisms in miRNA genes may affect the risk of MTX toxicity in childhood ALL.

**Acknowledgements:** This project was supported by RETICS (RD/12/0036/0060 and RD/12/0036/0036) and Basque Government (IT661-13, S-PE13UN068 and 2012111053).

**Reference**: Lopez-Lopez E, Gutiérrez-Camino A, Piñan MA, Sanchez-Toledo J, Uriz JJ, Ballesteros J, García-Miguel P, Navajas A, Garcia-Orad A. Pharmacogenetics of microRNAs and microRNAs biogenesis machinery in pediatric Acute Lymphoblastic Leukemia. PLoS One. 2014 Mar 10;9(3):e91261.

# The effects of cytotoxic agents over NIBAN gene expression and 3T3-L1 adipocyte proliferation

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**Background**: Adipocyte death is crucial in obesity development. Since NIBAN is described as antiapoptotic protein and has crucial roles in adipogenesis we aimed to determine the role of various cytotoxic agents over NIBAN gene expression together with cell viability and proliferation data analysis.

**Objectives**: The aim of the work was to study the cytotoxicity of agents on 3T3-L1 cell line, using iCELLigence real time monitoring system, for measuring cell viability and proliferation. Additionally function of NIBAN gene expression for adipocyte development as a response to cytotoxic agents has been examined.

**Design**: 3T3-L1 fibroblasts differentiated into mature adipocytes were real time monitored by using iCELLigence system. Hydrogen peroxide was applied throughout 4,5, and 24 hours; ethanol, stearic acid, and lineloic acid was applied throughout 24 hours to adipocyte cells NIBAN gene expression levels were determined with qPCR in nanocycler.

**Results**: The exposure times of oxidative stressors were determined according to the IC50 values obtained by real time analysis for various concentrations. 24 hr lineloic acid and 4 hr hydrogen peroxide exposure increased; whereas stearic acid, ethanol and 5h hydrogen peroxide exposure decreased NIBAN gene expression levels. 24 hr lineloic acid administration over 480 μM concentration was found to have antiproliferative effect.

**Conclusion**: Linoleic acid in long term (24h) was determined to have reducing effect on NIBAN gene expression. Hereby linoleic acid treatment may be used to prevent adipocyte apoptosis in obesity.

**Acknowledgements:** This work was supported by the Marmara University Scientific Research Projects Commission.

# Development and implementation of clinical pharmacogenetics service system

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**Background**: The implementation of pharmacogenetic data for effective and safe drug response reactions is becoming a reality in some clinical fields.

**Objective:** The development and implementation of a Clinical Pharmacogenetics service in PharmacoGenomics Research Center (PGRC, Inje University College of Medicine) is described.

**Design**: To be successful pharmacogenetics-based personalized therapy, we developed PGRC Biomedical Resource Bank, Database, validated genotyping technology platform, and web/mobile-based genotype result reporting system, and provided clinical pharmacogenetic test result accompanied by clinical interpretation with clinical information.

**Results**: PGRC Biomedical Resource Bank has 11,255 whole blood, 990 tissues and 175 immortal cell, etc. Based on the genetic results from these resources, we got the database including the Asian (mainly Korean)-specific genotype profile and clinical information. This database includes 68,723 genetic data from 13,547 subjects enrolled in 357 clinical studies. We developed and validated fast and cost-effective genotyping method (pyrosequencing, SNaPshot, PCR-RFLP, direct sequencing, real-time PCR and PCR-SBT (sequence-based typing) etc.) covering all genotypes observed in Asian based on our database. The genotyping result and general pharmacogenetic information were provided using web & mobile. We provided clinical pharmacogenetics services for valid biomarker-drug pairs. If requested (almost cases), we gave a clinical consultation including drug and dosage selection, and other information like drug interaction after reviewing patients' disease and drug history. From 2008 to 2014, we provided about 240 cases of genotype results with clinical consultation.

**Conclusions**: Clinical pharmacogenetics service system provides an opportunity to give pharmacogenetics-guided personalized pharmacotherapy, which will improve understanding and implementation of pharmacogenetic data into clinical practice.

# Loss of PTEN expression is associated with aggressive behavior and poor prognosis in Middle Eastern triple negative breast cancer

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**Introduction**: PTEN is a tumor suppressor that negatively regulates the PI3K-AKT signaling pathway which is involved in the pathogenesis of many different tumor types and serves as a prognostic marker in breast cancer. However, the significance of the role of PTEN in Middle Eastern ethnic breast cancer has not been explored especially with the fact that breast cancer originating from this ethnic population tend to behave more aggressively than breast cancer in the west.
**Methods**: We analyzed PTEN alteration in a tissue microarray format containing more than 1000 primary breast cancers with clinical follow up data. Tissue Microarray sections were analyzed for protein expression and copy number change using immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH).
**Results**: Loss of PTEN immunostaining was observed in 77% of the cases. PTEN loss was significantly associated with large tumor size (p=0.0030), high grade (p=0.0281), tumor recurrence (p=0.0333) and Triple negative breast cancers (p=0.0086). PTEN loss in Triple negative breast cancers was significantly associated with rapid tumor cell proliferation (p=0.0396) and poor prognosis (p=0.0408). PTEN deletion was found only in 60 cases (6.4%) of cases.
**Conclusion**: Loss of PTEN protein expression occurs at high frequency in Middle Eastern breast cancer. PTEN inactivation may potentially lead to an aggressive behavior of tumor cells through stimulation of tumor cell proliferation. Furthermore PTEN signaling pathway might be used as potential therapeutic target in Triple negative breast cancers since loss of its expression is shown to be significantly associated with this aggressive subtype of breast cancer.
**Keywords**: breast cancer, PTEN, triple negative.

# Sex hormones and nuclear appendages

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**Background**: Some nuclear neutrophils contain a small chromatin mass appended to one of their nucleus lobes. To date, their nature has remained uncertain. Some published data demonstrated that the frequencies and the distribution of these appendages were influenced by sex and by many other factors such as hormones, granulocytes metabolism, cell proliferation, and age.
**Objective**: This blind study was designed to check whether appendages are related to sex hormones and change with menstrual cycle phases or not.
**Design**: Nuclear appendages were studied in ten women during different phases of menstrual cycle. A written consent was obtained from each individual.

Ages of the individuals varied from 25 to 35 years old. None of them had history of malignancy, severe systemic infection, pregnancy, recent transfusions, malnutrition, consumption of oral contraceptives or any other medication that affects the menstrual cycle.

Peripheral blood samples were collected into EDTA tubes at different phases of the menstrual cycle (1st day, 7th, 14th and the 21st). At the time blood samples were taken, whole blood count were studied. Blood smears were preformed from each tube, stained then observed under immersion oil light microscope.

Two hundred polynuclear neutrophils were examined for nuclear appendages for each sample and classified into four groups: neutrophils with form A(drumstick), form B(sessile nodules) or form C appendages (tag and hook) and neutrophils without any appendages.
**Results**: The difference (A-C) was calculated for each slide. There were significant variations of the (A-C) during the menstrual cycle for each individual but these variations were not homogeneous for a woman to another.
**Conclusions and acknowledgements:** These results support the hypothesis that there is no relationship beteween oestrogen and appendages formation.

# Personalized therapeutic opportunities for aminoglycoside treatments

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**Introduction**: Aminoglycosides are commonly used antibiotics with unexpected side effects to the inner ear and kidney. In the general population the prevalence of these side effects is about 7%. As a result of their potent antimicrobial activities, many efforts have been undertaken to prevent aminoglycoside ototoxicity. The aminoglycoside induced hearing loss showing a strong association of mitochondrial genomic (mtDNA) single nucleotide variations (SNPs) such as m.1555 A>G and m.1494 C>T in the 12S rRNA subunit of mtDNA. In Caucasians the prevalence of these mutations are 0.4-1%. The mutation frequencies in the different haplotypes are quite varied (17-33%), with the highest occurrence being found in East-Asian B and D haplotypes.
**Aims**: To estimate the mutation frequencies of the mtDNA m.1555 A>G and m.1494 C>T substitution in Hungarian persons.
**Patients and Methods**: The prevalence of these substitutions was investigated by PCR-RFLP methodology in patients with non-syndromic hearing loss (N=181), and maternally inherited mitochondrial disorders with hypoacusis (N=119) and in healthy controls (N=180).
**Results**: In our examined population (480 individuals) the mtDNS 1555 A>G mutation was found in heteroplasmic form in 5 patient with

mitochondrial disorders and 8 control persons. The mutation frequency of these substitutions is 2.7% in the Hungarian population. The mt.1494 C>T mutation was not detected in any cases.
**Conclusion**: The carrier screening with the targeted investigation of these mtDNA mutations opens the possibility of preventing individuals from the unexpected side effects of aminoglycoside treatment.

## Valproate toxicity in Hungarian patients with mitochondrial disorders

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**Background**: In the background of mitochondrial disorders both mitochondrial (mt) DNA and nuclear DNA mutations can be detected. Mitochondrial polymerase gamma (POLG) is one of the most important genes responsible for the intergenomial communication of these two genomes. POLG gene mutations may cause a wide range of clinical symptoms, such as PEO, Alpers syndrome and psychiatric disorders. Seven POLG mutations (L304R, A467T, G588D, Q879H, T885S, E1143G and Q1236H) have been found to have pharmacogenetic significance to associate with valproic acid (VPA) induced liver toxicity. VPA is an anticonvulsant and mood-stabilizing drug using in the treatment of epilepsy, bipolar disorder and migraine.
**Objective**: To identify POLG mutations associated with VPA toxicity in patients with mitochondrial disorders.
**Design**: We have investigated 124 patients with mitochondrial disease (male:female 1:2, mean age: 39±24 years). 60% of the cases had single or multiple mtDNA deletions in their muscle tissue. The mtDNA deletion was investigated by long PCR, the coding regions of the POLG gene was sequenced bidirectionally by Sanger method (ABI Prism 3500).
**Results**: In our cohort 3 POLG non-synonymous variations (A467T, Q1143G, and Q1236H) have been found to be associated with VPA toxicity in 36 cases (29%). The c.1399 G>A (A467T) pathogenic mutation was found in heterozygous form in a 5 year-old boy with PEO, myopathy, chronic intestinal pseudoobstruction and myoclonus epilepsy. At the age of 14 month after viral infection he had severe myoclonus episodes and elevated liver enzymes. His sister had VPA induced fatal hepatotoxicity. The c.3428 A>G (E1143G) polymorphism, modifying factor was detected in 13 cases (male:female 2:5, mean age: 37.4±6, 70) in heterozygous form. The third found SNP is associated with VPA toxicity was c.3708 (Q1236H) polymorphism in 23 cases (male:female 1:2, mean age: 40±5, 75) in 2 cases homozygous, and in 21 cases heterozygous form.
**Conclusion**: In the POLG gene there is a high prevalence of VPA toxicity predicted variations. Before VPA treatment screening for the predisposing alterations to VPA toxicity should be very important to avoid fatal hepatoxicity.
**Reference:** Stewart JD, Horvath R, Baruffini E, Ferrero I, Bulst S, Watkins PB, Fontana RJ, Day CP, Chinnery Hepatology 2010;52:1791-1796.

## Genetic polymorphisms in thymidylate synthase and the risk for colorectal cancer in west algerian population

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**Background**: Thymidilate synthase (TS) is a key enzyme in the biosynthesis of the thymidine which is required for the DNA synthesis and repair. It is also involved in folates metabolism known for their protective role against the colorectal cancer (CRC).
**Objective**: The aim of this study was to search for a possible relationship between the polymorphisms of TS (2R>3R and del/ins 6pb) and the occurring of CRC in a population of 71 CRC and 48 healthy subjects from the west of Algeria.
**Design**: The polymorphisms TS 2R>3R and del/ins 6pb were assayed by a DNA fragment analysis (ABI PRISM 3700 DNA analysis – Applied Biosystems).
**Results**: An association between the polymorphism del/ins6pb of the TS gene and occurring of CRC was found in our population. Indeed, the individuals carrying insertion 6pb (allele 6pb) in TS gene had more risk to develop CRC than the individuals presenting the deletion of 6pb (allele 0pb) (OR=1.92, p=0.018). Moreover, a statistically significant difference was found between the cases and controls concerning the genotypes, suggesting that the two genotypes 0pb/6pb and 6pb/6pb are associated with a higher risk of CRC.
**Conclusion**: Our results suggest that the insertion of 6pb would be correlated with a lower activity of the TS increasing susceptibility to develop a CRC via a deficiency in the folates metabolism. Moreover, the deficit in thymine pool caused by the reduction of the rate of TS would probably lead the cell on a carcinogenesis way because of the uracil incorporation instead of the thymine in the DNA chain.

## Individualized treatment with metformin to avoid lactic acidosis

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**Background**: Type 2 diabetes mellitus (DM2) is a heterogegenous group usually due to both enviromental and genetic factors.

Maternally inherited diabetes and deafness (MIDD) is a well known genetically determined subtype of DM2 due to different mutations of the mitochondrial DNA (mtDNA). The mitochondrion is a key player among others in glucose metabolism. Some antidiabetics like metformin may damage the mitochondria through the reduction of Complex I activity. The side effect of metformin mostly occured in elderly patients with renal dysfunction. Genomic factors influencing the side effects of metformin treatment were not investigated previously.

**Findings:** We present the case of a 58 year-old man suffering from DM2 and having severe lactic acidosis (6.6 mmol/l), during metformin treatment. The patient presently symptoms were myalgia and muscle tension. In his muscle specimen abnormal accumulation of mitochondria and lipid vacuoles have been found. Genetic analysis of the whole mtDNA was detected several homoplasmic SNPs that have been associated previously MIDD. After discontinuation of the metformin his serum lactate level decreased significantly.

**Conclusions**: We conclude, that screening for MIDD-associated mtDNA SNPs is recommended for patients having side effects from metformin such as muscle pain and lactic acidosis. Metformin should be given carefully in patients with mitochondrial disorders.

**Keywords**: diabetes, individualized treatment, metformin, mtDNA, lactic acidosis, myalgia.

**Grant:** Hungarian Brain Research Program – Grant, No. KTIA_13_NAP-A-III/6.

## The prevalence of the most common statin induced myopathy associated SLCO1B SNPs in Hungarian patients

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**Background**: The first choice treatment of hypercholesterolemia and hyperlipidemia are the HMG-CoA reductase inhibitors (statins). One of the side effects of statins is the statin-induced myopathy (SIM) which occurs in 5-15% of patients based on the literature. In the genetic background of SIM the SLCO1B1 gene c. 521 T> C (p.Val174Ala, rs4149056) variation with the most significance association was described.

The aim of our investigation was the analysis of the two commonly SIM associated SLCO1B1 SNP's – c.521 T> C and c.388 A> G (p.Asn130Asp, rs2306283) – in Hungarian statin treated patients with SIM and without any side effects.

**Patients and Methods**: Sixty patients with SIM (male 23, female 37; mean age 64.3 ± 9.9 years) and 30 statin treated patients without SRM (male 11, female 19; mean age 53.2 ± 8.2 years) has been investigated. Specific TaqMan SNP Assays were used for the real-time PCR (ABI StepOnePlus System).

**Results**: The allele frequency of the mutant C allele (rs4149056) was 81.7% in the SIM cohort (homozygous: 39 cases, heterozygous: 20 cases) and 81.6% in the group without SIM (homozygous: 19 cases, heterozygous: 11 cases). The allele frequency of the mutant G allele (rs2306283) was 45.8%, in the SIM group (homozygous: 9 cases, heterozygous: 37 cases) and 40% in the group without SIM (homozygous: 5 cases, heterozygous: 14 cases).

**Discussion**: The presence of rs4149056 and rs2306283 major variants of SLCO1B1 gene did not show any significant association to SIM in Hungarian patients. Analyses of larger cohorts, and SNP's of further genes, like KIF6, COQ2 ATP2B1 are in progress.

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## Genetic polymorphism of SLCO1B1, involved in the development of statin-indused myopathy, level of vitamin D in Russian patients with hyperlipidemia

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**Background**: Statins are the most commonly prescribed medicines for treatment of hypercholesterolemia. At the same time up to 25% patients cannot tolerate or discontinue statin therapy due to statin-induced side effects. In majority of cases side-effects are attributed to SLCO1B1 gene polymorphism.

**Objective**: Our research was focused on the frequency of the SLCO1B1*5 genetic variant in the Russian population.

**Design**: 1071 patients with were included into the study. Genotypes of SLCO1B1*5 (c.521T>C, rs4149056) were determined with polymerase chain reaction (PCR) amplification. Our data was compared to admissible data from Brazil and China. In 18 patients receiving statins for 3 months or more, we determined the level of 25 (OH) D in the blood plasma by high performance liquid chromatography.

**Results**: 665(62%) patients had TT genotype of allelic variant SLCO1B1*5, 346 (32%) participants had TC, CC variant was found in 60 patients (6%). The "carrier" and "not carrier" C allele statistically significant differences in the levels of 25 (OH) D was not found: 32,3 ± 13,4 vs 40,3 ± 10,8 nmol / l, p = 0.299.

**Conclusions**: As compared with data from Brazil and China, allele C frequency which causes an increased risk of statin-induced myopathy in the Russian population was found significantly more often. In this regard, pharmaco genetic testing (genotyping SLCO1B1*5) can be used in Russian patients with hyperlipidemia for calculations of maximal tolerated dose in accordance with the recommendations of

ESF experts. We need a more data to assess the relationship between carriage of C allele and the level of 25 (OH) D.

**References**: Ramsey LB, Johnson SG, Caudle KE, Haidar CE, Voora D, et al. The clinical pharmacogenetics implementation consortium guideline for SLCO1B1 and simvastatin-induced myopathy: 2014 update. Clin Pharmacol Ther. 2014 Oct;96(4):423-8

## Statins modify the expression of intracellular cholesterol metabolism genes by modulating epigenetic mechanisms

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**Background**: Statins are HMG-CoA reductase inhibitors. Alongside enzymatic inhibition, statins induce intracellular activation of transcription factors, leading to an increase in low-density lipoprotein receptors (LDLRs) coupled to the plasma membrane of hepatocytes, enhancing clearance of plasma cholesterol. Several proteins with key functions, which are regulated by statins, are involved in this pathway. However, to date, there are no reports describing epigenetic mechanisms contributing to their regulation.
**Objective**: To evaluate the effect of DNA methylation and histone modification in the modulation of intracellular cholesterol metabolism genes in monocytic cells treated with statins.
**Design**: THP-1 cells in the absence and presence of 10 μM of atorvastatin or simvastatin were cultured. Gene expression of HMGCR, LDLR, SCAP, INSIG1, INSIG2, SREBF1, SREBF2, MBTPS1, KPNB1 and MBTPS2 was evaluated by real-time PCR. The overall state of DNA methylation and modifications in H3 and H4 histones was determined by colorimetric assays.
**Results**: Both statins induced overexpression of LDLR, HMGCR, SREBF2 and INSIG1 genes. Simvastatin also overexpressed SCAP, MBTPS1 and MBTPS2. DNA hypomethylation was detected after statin therapy ($6.85 \pm 0.73$ ng control vs. $2.83 \pm 0.17$ and $1.70 \pm 0.10$ ng with atorvastatin and simvastatin, respectively; $p<0.05$). Only atorvastatin induced an increase in H3K4 di- and trimethylation, H3K9 monomethylation and H3K36 di- and trimethylation. Simvastatin and atorvastatin increased monomethylation of H3K36 and H3K79 di- and trimethylation, along with increase H3K14 and H3K9 acetylation and H3Ser28 phosphorylation. Simvastatin increases monomethylation of H4K20 and H4Ser1 phosphorylation. Both treatments were associated with H4K5 acetylation and only atorvastatin to a decreased H4K20 trimethylation.
**Conclusions**: Statins investigated differentially overexpressed genes involved in intracellular metabolism of cholesterol, probably due to greater transcriptional activity as a consequence of the DNA hypomethylation and modifications observed in histones H3 and H4.
**Acknowledgements:** FONDECYT – Chile (Grant Number 1130675).
**Reference:** Lamon-Fava S. Curr Opin Lipidol 2013; 24(3):221-6.

## Differentially expressed microRNAs in human peripheral blood mononuclear cells are potential markers for statin response

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**Background**: During years, statins have been the lipid-lowering drug of choice to attain lower LDL-C levels and reduce cardiovascular risk. In spite of being a safe and tolerable therapy, an unfavorable feature has been the considerable response variability among patients, frequently determined by genetic factors. However, there are few evidence exists about epigenetic-regulated mechanisms involved.
**Objective**: To evaluate the differential expression of microRNAs in peripheral blood mononuclear cells of hypercholesterolemic subjects undergoing statin treatment.
**Design:** Forty individuals were evaluated before and after completion of atorvastatin (10 mg/day; n=20) and simvastatin (10 mg/day; n=20) therapy during 4 weeks. Results from both treatments were analyzed using a PCR array platform, including 84 microRNAs previously selected and linked to cholesterol homeostasis.
**Results**: From the 84 microRNAs selected, six (miR-29a-3p, miR-29b-3p, miR-300, miR-33a-5p, miR-33b-5p and miR-454-3p) were downregulated after atorvastatin treatment ($P<0.05$). Regulatory pathway examination showed that deregulated microRNAs interact with key genes of lipid metabolism (HMGCR, LDLR, ABCA1, SCAP, INSIG1, LPL and SREBP1). Moreover, after sub grouping LDL-C reduction into quartiles of response according to specific lipid-lowering therapy, quartile 1 – poor response to atorvastatin – showed reduced expression of miR-106b-5p, miR-17-3p and miR-590-5p, whereas in the quartile 4 – enhanced response to simvastatin- miR-106b-5p, miR-17-3p and miR-183-5p were overexpressed.
**Conclusions**: Our results show, for the very first time worldwide, that statins modulates the microRNA expression pattern in vivo. Also, miRNAs miR-106b-5p and miR-17-3p, together with miR-590-5p and miR-183-5p, can be markers of decreased response to atorvastatin and high response to simvastatin therapy, respectively.
**Acknowledgements:** FAPESP-Brazil (N° 2011/21967-1) & FONDECYT-Chile (N° 1130675).
**Reference**: Lamon-Fava S. Curr Opin Lipidol 2013; 24(3):221-6.

## Evaluation of pharmacotherapy based on pharmacogenetics in a tertiary referral hospital

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**Background**: Inter individual genetic variation could cause no/partial drug efficacy and drug toxicity. Clinical implementation of personalized therapy based on pharmacogenetic test has been sparse due to difficult interpretation. There are many efforts to overcome the problem such as establishing standard guidelines, Clinical Pharmacogenetics Implementation Consortium (CPIC) and Dutch.
**Objective**: Our hospital, a tertiary referral hospital, has adopted pharmacogenetic tests since 2008. We reviewed and evaluated the pharmacogenetic test requested cases to ascertain the characteristics of the cases.
**Design**: We evaluated 250 pharmacogenetic test requested cases by requested department, target drugs, requested reasons and tested genotypes.
**Results**: The requested departments were 7 including internal medicine, neurology, psychiatry and dermatology. The genotyping was decided by evaluating the cases, target drugs, requested reasons (adverse drug reaction (ADR) or treatment failure), co-medications and other clinical findings. Most of the cases were requested the genotype due to ADR or treatment failure. The class of target drugs were anticoagulants, antipsychotics, antiepileptics, antiplatelet, etc. Tested genotypes were CYP2C9, CYP2C19, CYP2D6, CYP3A4/5, UGT1A1, UGT1A4, UGT1A9, TPMT, VKORC1, HLA, etc. using pyrosequencing, SnaPshot, PCR-RFLP, direct sequencing, real-time PCR and PCR-SBT (sequence-based typing). The most common target drug was warfarin because of inadequate INR level (high or low). The antipsychotics cases were due to ADRs, extrapyramidal symptoms. HLA typing was performed in hypersensitivity cases such as Stevens-Johnson syndrome, DRESS, and maculopapular rash due to Allopurinol, antiepileptics, etc.
**Conclusions**: Frequently requested genotypes were related to clinical usefulness, information for safe and efficient pharmacotherapy with clear drug to genotype relationship, and physicians' knowledge on pharmacogenetics. According to the analysis, pharmacogenetic test will be popular with clinical interpretation based on individual characteristics, and education on pharmacogenetics for the clients, physicians.
**Reference**: Caudle KE, Klein TE, Hoffman JM, Muller DJ, Whirl-Carrillo M, Gong L, etc. Incorporation of pharmacogenomics into routine clinical practice: the Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline development process. Crr Drug Metab 2014;15:209-17.

## Prediction of Methotrexate gastro-intestinal toxicity in Algerian Rheumatoid Arthritis Patients: implication of MTHFR rs1801133, MTHFR rs1801131, ABCB1 rs1045642 and GGH rs11545078 polymorphisms

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**Background**: Methotrexate (MTX) is the basic treatment of rheumatoid arthritis (RA). Its effectiveness has been widely demonstrated, however, some patients may develop toxicity to the treatment. Gastrointestinal toxicity was the most observed. Several studies have investigated individual genetic variation responsible of MTX gastrointestinal toxicity.
**Objective**: The aim of the study was to evaluate the impact of MTHFR rs1801133 (c.677C>T), MTHFR rs1801131 (c.1298A>C), ABCB1 rs1045642 (c.3435C>T) and GGH rs11545078 (c.452C>T) polymorphisms on gastrointestinal toxicity.
**Design**: The sample consists of 110 RA patients from West Algerian. Side effects were researched along the recruitment. Genotyping was performed by the allelic discrimination in real time technique for MTHFR rs1801133, MTHFR rs1801131 and ABCB1 rs1045642 polymorphisms and by the PCR Risa/RFLP technique for GGH rs11545078 polymorphism.
**Results**: Our results suggest that the MTHFR rs1801133, MTHFR rs1801131 and ABCB1 rs1045642 polymorphisms do not affect the MTX gastro-intestinal toxicity. However, the genotype GGH 452TT seems to be correlated with the development of gastrointestinal toxicity (20% vs 2.43%, p=0.03, OR= 0.09 [0.001-1.09]). The allelic analyses confirm this correlation (30% vs 10.97%, p=0.008, OR=0.28 [0.1-0.75]).
**Conclusion**: We demonstrated for the first time in West Algerian population, that the GGH rs11545078 polymorphism has an impact on MTX gastrointestinal toxicity. It would be interesting to increase the number of the cases investigated to confirm our findings and explore other polymorphisms of genes involved in the pharmacogenetics of MTX in RA.
**Keywords**: Methotrexate, Rheumatoid arthritis, MTHFR, ABCB1, GGH.
**Reference**: Ranganathan P, McLeod HL. Methotrexate pharmacogenetics: the first step toward individualized therapy in rheumatoid arthritis. Arthritis Rheum. 2006; (54):1366–1377.
**Acknowledgements:** We are grateful to RA patients, their families and rheumatologists for participation in this study.
We thank Dr. Baba Ahmed Bey (Department of Biotechnology, Université d'Oran IGMO), for technical support.

## Study of genetic and non-genetic interindividual variability in response to acenocoumarol in a Tunisian population cohort

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**Background**: Acenocoumarol (AC) represent the therapy of choice for the longterm treatment and prevention of thromboembolic diseases. Many genetic, clinical and demographic factors have been shown to influence the anticoagulant dose [1].
**Objective**: Our aim is to investigate the contribution of genetic and non genetic factors to variability in response to AC in Tunisian patients.
**Design**: We recruited 128 patients treated for the first time with AC during their hospitalization in the cardiology and internal medecine department. CYP2C9*2, VKORC1*2 and VKORC1*4 polymorphisms were analyzed by restriction fragment length polymorphism polymerase chain reaction. Normalized maintenance dose of AC (NMD): ratio equilibrium dose to INR, was calculated.
**Results**: No significant deviation from Hardy-Weinberg equilibrium was observed for any polymorphisms studied (P>0.05). Genotypes frequencies were: CYP2C9*2 (*1/*1 (74.8%),*1/*2(20.8%) *2/*2(0.8%)), VKORC1*2(GG(18.1%),GA(43.3%)and AA(38.6%)) and VKORC1*4 (CC(78.7%), CT(18.1%) and TT(3.1%)). Among non genetic parameters we considered those which influence NMD with p<0.25 as confounding factors (age, renal failure, hypertension, BMI…). Univariate analysis showed that adjusted NMD is significantly lower in carriers of mutant allele GA (-13.7%, p=0.002) and AA(-22.8%, p=0.001) of VKORC1*2 than wild type patient GG and significantly higher in patient carrying VKORC1*4 variant allele (CT 5% p=0.048 and TT 36.4% p= 0.033) than wild type CC. Patients carrying at least one CYP2C9*2 variant allele required a lower dose of AC than wild type patients but without significance (P=0.6). Especially in patients carrying VKORC1*2 mutant allele overdose and instability of treatment seemed to be more frequent than wild type patients and they are more likely to have a higher INR compared to carriers of the wild allele
**Discussion**: The interaction between genetic and non genetic factors may largely explain the interindividual variability equilibrium dose of AC.
**Conclusion**: The response to AC is multifactorial. Variations in dose response may be due to differences in pharmacokinetic and genetic polymorphism especially in VKORC1*2 and VKORC1*4.
**Keywords:** Acenocoumarol, variability, INR, genetic, non-genetic.
**Reference:** Smires FZ, Moreau C, Habbal R, Siguret V, Fadili S, Golmard JL, Assaidi A, Beaune P, Loriot MA, Nadifi S. Influence of genetics and non-genetic factors on acenocoumarol maintenance dose requirement in Moroccan patients. J Clin Pharm Ther, 2012; 37: 594-598.

## The effect of cytochrome P450 3A5 and P-glycoprotein polymorphism on tacrolimus dose requirements and trough blood levels in Tunisian renal transplant patients

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**Background**: Many studies on solid organ transplant patients treated with tacrolimus showed that the polymorphism of CYP3A5 and ABCB1 (MDR1) influences the dose-adjusted trough blood levels of tacrolimus.
**Objective**: The aim of our study was to evaluate the effect of the polymorphism of CYP3A5 and ABCB1 in the results of therapeutic drug monitoring of tacrolimus in the Tunisian population.
**Design:** A retrospective study was conducted on 40 renal graft recipients treated with tacrolimus in nephrology department at the University Hospital Sahloul. Clinical informations were collected from patient records. The dosage of tacrolimus was performed by chemiluminescence and CYP3A5*3, CYP3A5*6, MDR1 2677G>T and MDR1 3435C>T genotyping by PCR-RFLP. Statistical analysis was performed by the SPSSv20 software.
**Results**: Tacrolimus initial daily dose required was higher for patients with CYP3A5*1/*3 genotype compared to CYP3A5*3/*3 genotype (0,2±0,08 versus 0,16±0,05 mg/kg/day, respectively). Patients with genotype CYP3A5*3/*3 had higher tacrolimus trough levels than those with genotype CYP3A5 *1/*3 in the first dosage post transplantation (8 [2,9-11,7]versus 4,6 [2,7-12,5] mg L-1 respectively, p= 0,048) and in the third month post transplantation (9,7 [2,2-24,8] versus 7,8 [2,4-15,8] mg L-1 respectively, p=0,022), similar results were found for dose-adjusted trough Tacrolimus levels. The frequency of tremors, side effect of tacrolimus, and duration of hospitalization were significantly greater in patients with genotype CYP3A5 *3/*3. There were no significant associations with CYP3A5*6, MDR1 2677G>T and MDR1 3435C>T polymorphism.
**Conclusions**: Our preliminary results demonstrated that the renal graft recipients with genotype CYP3A5*3/*3 require lower doses of tacrolimus, and have higher tacrolimus trough levels than those with CYP3A5*1/*3, but have more risk to develop side effects. Our study will be continued by increasing the sample to establish a protocol for individualization of immunosuppressive therapy according to pharmacogenetic profile of CYP3A5 for Tunisian population of renal graft recipients.
**Acknowledgements:** Nephrology and biochemistry departments staff especially Henda Falfoul.
**Reference:** Haufroid V, Mourad M, Van Kerckhove V, Wawrzyniak J, De Meyer M, Eddour DC, and al. The effect of CYP3A5 and MDR1 (ABCB1) polymorphisms on cyclosporine and tacrolimus dose requirements and trough blood levels in stable renal transplant patients. Pharmacogenetics. 2004;14:147-54.

## Implication of SLCO1B1 polymorphism in efficiency of the treatment by statins and occurrence of statin induced myopathy

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**Background**: Statins are widely prescribed worldwide, but muscular intolerance they induce is a significant limitation for their use. The SLCO1B1 gene encodes an anionic transporter OATP1B1 that regulates hepatic uptake of statins. SLCO1B1*5 variant has been involved in efficiency and myotoxicity of statins but results are controversial.
**Objective:** The aim of this study was to to evaluate the association of SLCO1B1*5 with efficiency of treatment and with statin induced myopathy.
**Design**: Eighty nine coronary patients newly treated with statines were recruited at the University Hospital Sahloul. The patients underwent a standardized questionnaire. The lipid parameters were measured or calculated and creatine kinase (CK) activity was determinated on admission, after two and 16 weeks. Genotyping variant SLCO1B1*5 was performed by PCR-RFLP. Statistical analysis was performed by on SPSSv17.
**Results**: The frequency of mutated allele*5(C) was 0,16%, similar to this estimated in the Caucasian population. The study of the impact of the genetic variant SLCO1B1*5 on the changes in lipid parameters found that the reduction percentages of LDL-cholesterol, total cholesterol, triglycerides, ApoB, and non HDL cholesterol appear to be more important in homozygous mutated patients but without significance. We identified 14 cases of myopathy (19,2%) and only two cases of myositis (myalgia with elevated CK). Carriage of the variant allele C seems to be significantly associated with statin myopathy even after adjustment for potential confounders (OR=5,7 [1,9 to 17,4]; 0,001). This association was statin dependent, indeed it was significant with atrovastatin (OR= 3,8 [1,048 to 17,03]; 0,037) but not significant with rosuvastatine (p=0,141).
**Conclusions**: Our preliminary results suggest that the statin induced myopathy is associated to the variant SLCO1B1*5 and that rosuvastatin would be more tolerated than atrovastatin by a holder of the SLCO1B1*5. This pharmacogenetic study will be continued by increasing the sample to provide data about the more tolerated statin according to the genetic profile.
**Acknowledgements:** Cardiology and biochemistry departments staff especially Henda Falfoul.
**Reference**: Niemi M, Pasanen MK, Neuvonen PJ. Clin Pharmacol Ther. 2006;80:356-66.

# Association between marker at chromosome 2 and acute coronary syndrome is modified by statins

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**Background**: Acute coronary syndrome (ACS) is among the most common causes of death in industrial countries and traditional risk factors explain only approximately 60% of the cases. Therefore, the attention is focused on the genetic variants not associated with these risk factors. One of them is the rs2943634 marker within the "gene-free" area on chromosome 2. Most commonly used drugs to prevent ACS are statins. It is unknown, how they interact with newly detected genetic risk variants.
**Objective**: This study aimed to determine whether statins modify risk of ACS associated with rs2943634 marker.
**Design**: Only males younger than 65 years were included. Rs2943634 (C → A) variant was successfully genotyped in 1 162 controls (post-MONICA study), 924 consecutive ACS patients (GENDEMIP study) without previous statin treatment and in 472 retrospective patients treated by statins at least one year before the ACS manifestation. ANOVA and chi-square were used or the statistical analysis.
**Results**: Within the controls, rs2943634 polymorphism was not associated with smoking, obesity, dyslipidemia, diabetes or hypertension. Frequency of the AA genotype was significantly lower among untreated patients than controls (11.7% vs. 15.9%, p = 0.005; OR for AA homozygotes vs. C allele carriers C 0.69; 95% CI 0.54 – 0.89). In contrast, protective effect of the AA genotype was not significant in statin treated patients (13.9% vs. 15.9%, p = 0.26; OR for AA vs. C allele carriers 0.83; 95% CI 0.62- 1.14).
**Conclusions**: Statins modify association between the rs2943634 marker at chromosome 2 and ACS. Mechanism of the modification is recently unclear, potentially attributed to epigenetic factors.
**Acknowledgment**: Supported by the project No. NT 12217-5 (IGA MZ, ČR).

# Blood levels of 8-Isoprostane F2 Alpha in chronic hepatitis C

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**Background/Purpose**: The mechanism by which HCV causes liver damage is mediated through immunological means, direct viral toxicity and induction of oxidation stress (OS). 8-Isoprostane F2 Alpha (8-Isop) is an important marker to assess the OS in vivo and used as extensively to qualify lipid peroxidation. The purpose of the study was to determine the relation of 8-Isop to the severity of HCV related liver diseases.
**Methods**: Fifty subjects were evaluated and divided into 5 groups, 10 in each group: G1: Chronic hepatitis C without cirrhosis. G2: Chronic hepatitis C with child A cirrhosis. G3: Chronic hepatitis C with child B cirrhosis. G4: Chronic hepatitis C with child C cirrhosis. G5: Volunteers with no evidence or history of liver disease. All subjected to: determination of 8-Isop and lipid profile.
**Results**: Statistical comparison between the mean value of 8-Isop in the studied groups using the F test showed significant increase in groups 2, 3, and 4 than in G5. Also there was significant increase of 8-Isop in G1 than G2, and in G2 than G3, also, in G3 than G4. On the other hand, there was significant association between the severity of HCV liver disease and low cholesterol, TG, LDL, and VLDL.
**Conclusions**: This results highlight the importance of OS, marked by 8-Isop in the pathogenesis and severity of HCV related liver disease.

Also, the findings reported the importance of lipid profile parameters and its relation to disease severity. Further studies are needed entailing the association between lipid levels, liver histopathological characters, and virological parameters to predict the response to the therapy.

**Reference:** Spengler U, Nattermann J. Immunopathogenesis in hepatitis C virus cirrhosis. Clin Sic (London) 2007; 112(3):141-155.

## Molecular analysis of CYP2B6 gene A785G polymorphism in a Turkish population

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The cytochrome P450 (CYP450) proteins are monooxygenases, which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids (1). CYP2B6 is a human CYP isoform found in variable amounts in the liver and many extrahepatic tissues including kidney, intestine, and lung. CYP2B6, plays a major role in the metabolism of several therapeutically important drugs, including the anticancer agents, cyclophosphamide and ifosfamide; the antiretrovirals, efavirenz and nevirapine; the narcotics, propofol and ketamine; the antidepressants bupropion, sertraline; the antiestrogen tamoxifen; the synthetic opioid methadone; the anti-Parkinsonian selegiline; the antimalarial artemisinin, and many more (2,3). The enzyme also metabolizes certain endogenous compounds such as testosterone, as well as recreational drugs, including nicotine and ecstasy/MDMA and some precarcinogens. CYP2B6 is known to be inducible and polymorphic; as a result, extensive differences in CYP2B6 activity are also clinically important as they affect drug metabolism and alter responses to certain drugs.

The present study aimed to determine the frequency of CYP2B6*4 allele in healthy Turkish individuals. Variation in exon 5 (A785G) of the CYP2B6 gene were conducted using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method. Molecular analysis revealed that of 172 healthy individuals tested for the CYP2B6*4 genotype, 83 (48.2%) were AA, 66 (38.4%) were AG 1-2, and 23 (13.4%) were GG. On the basis of these data, the allele frequency of A was 0.67 and the frequency for G was 0.33. The frequency of CYP2B6*4 allele was similar to European populations but significantly different from that reported some populations. This is the first study to document the frequency of the CYP2B6*4 allele in the healthy Turkish individuals and our result could provide clinically useful information on drug metabolism by CYP2B6 in Turkish population.

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## Management and pharmacogenetics of iatrogenically induced opioid dependence

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**Introduction**: Opioids are valuable analgesics, capable of providing pain relief and functional improvement also in chronic noncancer-related pain (NCP) patients. However, recent data have shown that the increasing prescription of opioids is associated with a rise in aberrant drug-related behaviour in which genotype profile could be involved.

**Methodology:** A prospective study was performed with 70 NCP outpatients diagnosed with opioid iatrogenic DSMIV dependence and severe pain intensity. Study focus on analgesic efficacy, opioid withdrawal syndrome prevention, adverse side effects, functional status and aberrant drug-related behaviour. We design a contractual agreement on opioid therapy including goals, side effects and criteria to finish the opioid therapy. Genotyping of the OPRM (rs1799971), COMT (rs4680) and ABCB (rs1045642) genes was performed.

**Results:** A low dependence prevalence of 0.08% was found in our ambulatory patients. Results from 73% (50/70) of the patients included are presented. After a structured and progressive opioid conversion to buprenorphine/tramadol, a significant reduction of 47% of the total daily dose (TDD) with no withdrawal symptoms (OWS reduction of 9 points) was achieved, maintaining a moderate relief and pain intensity score. Quality of life tends to improve, as do the number of adverse reactions reported by the patients throughout the visits. OPRM and COMT gene variant distribution but ABCB variants were higher prevalent (9% C/C, 65% C/T, 26% T/T) versus general population distribution (21% C/C, 49% C/T, 25% T/T). Psychosocial risk was associated to a high prevalence of opioid iatrogenic dependence.

**Conclusions:** Prevention opioid dependence and attenuation of prescription abuse should focus on interdisciplinary strategies. Genetic risk profile could decrease the risk for iatrogenically induced overdose and dependence

## Association of IL-1B polymorphism and aggressive periodontitis in the Algerian population

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Periodontitis is a pathological multifactorial. It is recognized that microbial factors cannot be held solely responsible for periodontitis. The two major causes are pathogens and the host's genetic heritage. Genetic factors in part explain the clinical variability in periodontitis; however, a genetic basis has not been clearly defined. Search of susceptibility to the disease genotype is the subject of several studies on different genes related to the immune system. Among these genes those of the IL-1 cytokine that plays an important role in the pathogenesis of periodontitis. Our approach is the "case-control" study of IL1B Snp: C + 3954T (rs1143634T) gene polymorphism. The purpose of this study is the investigation of a possible association between IL-1B gene polymorphism and the risk of aggressive periodontitis in Algerian. We have achieved real-time PCR using Taq Man technology. Our sample consists of 188 individuals, including 60 DNA belonging to patients with periodontitis (cases) and 128 without periodontitis (controls). Significant differences were found in the frequencies of the minor alleles of cases of aggressive periodontitis compared to healthy controls $p < 0.05$. The statistical analysis of genotype showed a probable association between this polymorphism and periodontitis in our sample study. Gene polymorphism studied seems to be associated with a predisposition to aggressive periodontitis in the Algerian population.

**Keywords:** Interleukin-1, aggressive periodontitis, Algerian population.

# Analysing the potential for incorrect haplotype calls with different pharmacogenomic assays in different populations: a simulation based on 1000 Genomes data

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**Background:** Pharmacogenomics can potentially improve safety and effectiveness of medications in individual patients, and specific guidelines regarding the relationship of pharmacogenetic haplotypes and drug dosage recommendations are already available. However, preliminary studies trying to leverage this knowledge gave contradicting results.
**Objective:** To evaluate potential problems in haplotype calling procedures by performing an in silico study on published genome sequencing data. We simulated the results existing pharmacogenomic assays could report, based on the variants that are interrogated and PharmGKB haplotype definitions.
**Design:** We analyzed genome sequencing data from 2504 samples. Based on the variants interrogated by four existing pharmacogenomics assays, we evaluated their theoretical performance in assigning haplotypes according to the PharmGKB definitions for 7 pharmacogenes: CYP2C19, CYP2C9, CYP3A5, DPYD, SLCO1B1, TPMT and VKORC1. We compared these results to those of a theoretical assay interrogating all variants in the PharmGKB haplotype definitions, to gain a better understanding of the implications of the "constrained views" on a subset of polymorphism offered by different assays.
**Results:** Our results indicate significant shortcomings of the existing assays in their ability to report the correct haplotypes. Averaged over all genes, about 20% to 60% of the alleles present in the data could not be called correctly. This effect was especially marked for CYP2C19, SLCO1B1 and TPMT. Furthermore, even when considering all variants used in the PharmGKB haplotype definitions, a considerable proportion of these alleles could not be assigned any defined haplotype.
**Conclusions:** Our results highlight the need for improved genotyping solutions and results reporting. For some genes, star-allele nomenclatures fail to depict the genetic variation present in real populations.
**Acknowledgements:** This work was supported in part by the Austrian Science Fund (FWF) [P 25608-N15] and the NIH/NIGMS (U19 GM61388; the Pharmacogenomics Research Network) (RRF).
**Reference:** Samwald M, Blagec K, Hofer S, Freimuth RR. Analysing the potential for incorrect haplotype calls with different pharmacogenomic assays in different populations: a simulation based on 1000 Genomes data. Pharmacogenomics J (under review).

# Pharmacogenomic predictors of graft-versus-host disease: potential markers to optimize immunosuppressive therapy

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Hematopoietic stem cell transplantation (HSCT) is a potentially curative therapy for several haematological malignancies. However, it is associated with major risk of graft-versus-host disease (GvHD), a frequent immunological complication associated with a high morbidity rate. The optimization of the immunosuppressive regimen is certainly among the leading factors determining the occurrence of acute GvHD and fatal complications. However, despite prophylactic measures, the fact that a significant proportion of patients still develop GvHD suggests that additional, uncharacterized, inter-individual genetic factors may contribute to the development of acute

and chronic forms of GvHD. In this study, we tested twenty candidate genes related to metabolic and transport pathways of methotrexate and cyclosporine, in a population of 420 donor-recipient pairs of HSCT using a haplotype-tagging single nucleotide polymorphisms approach. Data indicated that nine markers in genes encoding transporters and molecular targets of methotrexate exhibit a remarkable influence on severe acute GvHD prevalence (grade III and IV). The genetic status of the recipient for ABCC1 (rs17264736, rs4781712) was protective against GvHD (HR=0.35-0.36; p=0.003, q=0.11), while an increased risk of developing the disease was observed for carriers of ABCC2 rs3740065 and ATIC rs2177735 (HR=3.53; p=0.002, q=0.022 and HR=3.04; p=0.002, q=0.022, respectively). Donors SLC19A1 (rs1051266, rs4818789 and rs4818128) and DHFR rs34965641 status were associated with reduced risk of severe acute GvHD (HR=0.29-0.38; p=0.002-0.005, q=0.048 and HR=0.32; p=0.001, q=0.024). In addition, two variants found in recipient NFATC1 (rs8090560) and in donor NFATC2 (rs3787186) genes, encoding molecular targets of CsA, were also associated with worse prognosis (HR=2.69; p=0.004, q=0.030 and HR=3.85; p=0.0004, q=0.013). These associations remained significant after multiple testing. Findings support that germline biomarkers in biologically relevant pharmacogenes encoding transporters and targets may underlie part of the heterogeneity in GvHD development and predict the risk of severe acute GvHD, beyond HLA matching. Further investigations are warranted to improve our understanding of these relationships to personalize immunosuppressive therapy and optimize outcomes.

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## Novel predictive markers of irinotecan-induced severe toxicity in metastatic colorectal cancer patients

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Irinotecan is a cytotoxic agent widely used for the treatment of solid tumors most particularly for metastatic colorectal cancers (mCRC). Treatment with this drug frequently results in severe neutropenia and diarrhea that can seriously impact the course of treatment and patients' quality of life. Pharmacogenomic tailoring of irinotecan-based chemotherapy has been the subject of several investigations especially for the UGT1A1 gene but with limited data regarding transporter genes. In this study, we sought to discover toxicity-associated markers using a haplotype-tagging SNP (htSNP) strategy to maximize gene coverage. We examined the genetic association across the UGT1 locus, and in seven transporter genes participating in irinotecan pharmacokinetics involving the ABC transporter genes ABCC1, ABCC2, ABCC5, ABCG1 and the solute carrier organic anion transporter gene SLCO1B1. The profiles of 167 mCRC Canadian patients treated with FOLFIRI-based regimens were examined and findings were replicated in an independent cohort of 250 Italian patients. We found rs11563250G, located in the intergenic region downstream of UGT1, to be significantly associated with reduced risk of severe neutropenia (odds ratio (OR)=0.21; p=0.043 and OR=0.27; p=0.036, respectively, and OR=0.31 when combined; p=0.001), which remained significant upon correction for multiple testing in the combined cohort (p=0.041). For the two-marker haplotype rs11563250G and UGT1A1*1 (rs8175347 TA6), the OR was of 0.17 (p=0.0004). Genetic testing of this marker may identify patients who might benefit from increased irinotecan dosing. In combined cohorts, a two-marker ABCC5 rs3749438 and rs10937158 haplotype (T–C) predicted lower risk of severe diarrhea (odds ratio (OR) of 0.43; p=0.001). The co-occurrence of ABCG1 rs225440T and ABCC5 rs2292997A predicted risk of severe neutropenia (OR=5.93; p=0.0002), which was further improved when incorporating the well-known risk marker UGT1A1*28 rs8175347 (OR=7.68; p<0.0001). In contrast, carriers of one protective marker (UGT1 rs11563250G) but none of these risk alleles experienced significantly less severe neutropenia (8.2% vs. 34.0%; p<0.0001). This combination of predictive genetic markers could lead to better risk assessment and may thus enhance personalized treatment.

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## Impact of genetic polymorphisms on asparaginase hypersensitivity in pediatric acute lymphoblastic leukemia

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**Background:** Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy. Survival rates for patients have remarkably improved over the last decades with higher than 80% of children are cured. The intensive use of L-asparaginase (ASP) has a key role in this great achievement. However, hypersensitivity reactions to ASP are major challenges in pediatric patients, because these can lead to suboptimal treatment response. In addition, in serious cases these reactions can be potentially life-threatening requiring urgent interventions. **Objective:** Therefore, our aim was to identify genetic variants that predispose to ASP hypersensitivity.

**Design:** Samples and clinical data collection was carried out from 576 pediatric ALL patients who were treated between 1990 and 2012 in 9 Hungarian pediatric hematology centers according to four consecutive trials using protocols from the Berlin-Frankfurt-Münster Study Group (ALL-BFM 90, 95, ALL IC-BFM 2002 and 2009). A total of 20 single nucleotide polymorphisms (SNPs) in GRIA1 and GALNT10 genes were genotyped by using KASPar-on-Demand prevalidated assays (LGC Genomics, Berlin, Germany) on 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Multi-adjusted logistic regression was performed by using IBM SPSS Statistic software, version 20.0 to test for associations.
**Results:** We found statistically significant associations between rs4958351 and rs2055083 polymorphisms in GRIA1 gene and the development of hypersensitivity to Escherichia coli-derived ASP in subgroups of the investigated population.
**Conclusions:** Our results suggest that polymorphisms in GRIA1 gene can influence the risk to ASP hypersensitivity. Further replication is required before these markers can be considered as predictive.
**Reference:** Chen SH, Pei D, Yang W, Cheng C, Jeha S, Cox NJ, et al. Genetic variations in GRIA1 on chromosome 5q33 related to asparaginase hypersensitivity. Clinical pharmacology and therapeutics. 2010 Aug;88(2):191-6.

## Epigenetic regulation of androgen inactivation by UDP-glucuronosyltransferase (UGT) enzymes in prostate cancer

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Prostate cancer is the second most common malignancy among men worldwide. Androgen plays a primordial role in prostate carcinogenesis and its progression. Systemic and local androgen bioavailability is controlled by inactivation by UDP-glucuronosyltransferase conjugating enzymes (UGT). Here, we investigated the miRNA–mediated regulation of major androgen-inactivating pathways, namely UGT2B15, UGT2B17 and UGT2B28. We initially assembled a list of miRNAs predicted to regulate these UGTs by three computational bioinformatic algorithms in addition to few miRNAs suspected by previous studies. Reporter vector assays validated the in silico–predicted binding potential of miRNAs to their target UGT transcripts in HEK293 cells. For UGT2B17, three miRNAs, miR-376c, miR-409 and miR-494, were the most efficient and achieved a significantly reduction (39-82%; $p<0.001$) in reporter gene activity. For UGT2B15, miR-331-5p and miR-376c reached a significantly reduction (>80%; $p<0.001$), whereas none were efficient for UGT2B28. These miRNAs were shown to bind the 3' untranslated region of UGT2Bs while reporter gene expression was rescued by disrupting the putative seed sequences for the miRs. miR-376c was demonstrated as the most effective to bind UGT2B15 and UGT2B17 through a site conserved in both UGTs. Ectopic expression of miR-376c in the androgen-dependent prostate cancer cell line LNCaP significantly reduced UGT2B15 and UGT2B17 mRNA and protein expression (>55%; $p<0.001$) and subsequent dihydrotestosterone (DHT) inactivation (37%; $p<0.001$). Consistent with an alteration in potent androgen bioavailability, a change in the expression of androgen responsive gene loci such as PSA was observed with no effect on androgen receptor (AR) levels. In prostatic tissues, miR-376c was differentially expressed with a lower expression in tumors compared to normal tissues, which is further decreased in metastases. Furthermore, an inverse relationship between the mRNA expression of UGT2B15 and UGT2B17 and miR-376c was evidenced ($r = -0.747$; $p=0.003$) whereas PSA mRNA levels were positively correlated to those of miR-376c ($r = 0.577$; $p=0.039$) in metastatic tissues. This study reveals that miR-376c is a potential negative modulator of androgen-inactivating UGT2B15 and UGT2B17 in the prostate; modulating androgen response and with potential implication in disease progression. Supported by Canadian Institutes of Health Research and Canada Research Chairs Program.

## Impact of Pharmacogenetic markers of CYP2D6 and DRD2 on prolactin response in risperidone-treated Thai children and adolescent with autism spectrum disorders

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The main aim of this study was to identify the impact of pharmacogenetic markers associated with prolactin concentration in risperidone-treated children and adolescents with autism spectrum disorders. One hundred and forty-seven children and adolescents with autism, aged 3 to 19, received risperidone. The clinical data of patients were recorded from medical records. Prolactin levels were measured by chemiluminescence immunoassay. Three CYP2D6 single nucleotide polymorphisms (SNPs), CYP2D6*4 (1846G>A), *10 (100C>T), and *41 (2988G>A), one gene deletion (*5), and DRD2 Taq1A (rs1800497) polymorphism were genotyped by TaqMan real-time PCR. The three common allelic frequencies were CYP2D6*10 (55.10%), *1 (32.65%) and *5 (6.12%), respectively. Patients were grouped according to their CYP2D6 genotypes. There was no significant correlation between the concentrations of prolactin among the CYP2D6 genotypes. In addition, there were no statistical differences in the prolactin response among the CYP2D6 predicted phenotypes of EM and IM. The DRD2 genotype frequencies were Taq1A A2A2 (38.77%), A1A2 (41.50%), and

A1A1 (19.73%), respectively. There were statistically significant differences in prolactin level of patients among the three groups (P = 0.033). The median prolactin level in patients with DRD2 Taq1A A2A2 (17.80 ng/ml) was significantly higher than A1A2 (17.10 ng/ml) and A1A1 (12.70 ng/ml). This is the first study in an Asian autistic population to investigate the associations of prolactin level and CYP2D6 and DRD2 genetic polymorphisms. Patients with homozygous wild-type of DRD2 Taq1A (A2A2) may be an important factor that may influence the prolactin elevation in risperidone therapy.

**Keywords**: prolactin; autism spectrum disorders; risperidone; CYP2D6; DRD2; Thai; children and adolescent; pharmacogenetics.

## DNA repair gene polymorphisms and smoking intensity in healthy population. Are SNP-smoking association studies needed in controls?

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**Introduction**: Variations in tobacco-related cancers, incidence and prevalence reflect differences in tobacco consumption in addition to genetic factors. Besides, genes related to lung cancer risk could be related to smoking behavior. Polymorphisms altering DNA repair capacity may lead to synergistic effects with tobacco carcinogen-induced lung cancer risk. The main purpose of this study was to evaluate the independence assumption for selected SNPs and smoking behavior in a cohort of healthy Spanish smokers.

**Material and Methods:** Six genetic polymorphisms in CYP1A1 (Ile462Val), XRCC1 (Arg399Gln), APEX1 (Asp148Glu), XRCC3 (Thr241Met) and XPD (Asp312Asn; Lys751Gln) were analyzed in 320 healthy smokers. Our subjects were well defined in terms of phenotype assessment (cigarettes per day; packs year smoked; FTND score; CO expired and urine cotinine levels). Genetic markers were determined by RT-PCR in blood samples. A logistic regression analysis adjusted for age, smoking history and gender was performed.

**Results:** We found an association between the wild type allele of XRCC3 Thr241Met and greater smoking intensity (OR=2.86, 95% CI=1.31-6.17) higher FTND score (OR=2.22, 95% CI=1-4.76) or more years smoking (OR=2.66, 95% CI=1.23-5.78)). There were not association between the rest of polymorphisms analyzed and smoking habits.

**Conclusions:** Although preliminary, the results of our study provide evidence that genetic variations in DNA-repair genes may influence both smoking habits and the development of lung cancer. Population-specific G x E studies should be carried out when genetic and environmental factors interact to cause the disease. Stricter criteria for phenotypic measures should be considered for studies in smoking population.

This study was supported by Universidad Europea, Madrid (project number 2012/UEM17).

## Overcoming PCR inhibitors to achieve rapid, accurate results in a high throughput pharmacogenomics sample-to-answer solution

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**Background:** Advances in personalized medicine have led to a growing demand for simple, affordable, and rapid sample-to-answer workflows for pharmacogenomics studies that can accommodate testing various sets of gene variants across a large number of samples. Unfortunately, the workflow for traditional pharmacogenomics studies requires highly-purified sample DNA and sometimes an additional pre-amplification step to obtain accurate results. Sample purification involves a series of labor-intensive steps that add significant time, cost, as well as complexity to the workflow. Low purity DNA samples cannot be used in current workflows due to the effect of PCR inhibitors, such as polyphenols, that are carried over from human buccal samples. These inhibitors lead to poor amplification which consequently produces discordant genotypes and inaccurate copy number calculations.

**Objective:** To overcome this problem, we formulated a qPCR master mix that is compatible with crude extraction methods and can tolerate PCR inhibitors that are carried over from sample preparation. With this master mix, we were able to develop a comprehensive pharmacogenomics sample-to-answer solution that bypasses sample purification without sacrificing the accuracy of the results.

**Design:** SNP genotyping and copy number variation data were generated with the new master mix using both purified and unpurified human buccal swab samples. A control experiment was conducted using existing master mixes that are recommended for pharmacogenomics applications. These real-time qPCR experiments were conducted with TaqMan® SNP genotyping and copy number assays designed to target the CYP2D6 gene. They were run in OpenArray® and 384-well plate formats, respectively, on the QuantStudio™ 12K Flex system.

**Conclusions:** In contrast to the control experiment, the proposed workflow, which pairs the new master mix with an existing crude DNA extraction method, produced accurate pharmacogenomics results with a significantly faster sample-to-answer turnaround time.

**Reference:** Hartshorne T, Le F, Lang J, Leong H, Hayashibara K, et al. (2014) A High-throughput Real-time PCR Approach to Pharmacogenomics Studies. J Pharmacogenomics Pharmacoproteomics 5:133. doi: 10.4172/2153-0645.1000133.

# Identification of novel genetic variations in the organic cation transporter 2 gene (SLC22A2) in the Xhosa population of South Africa

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**Background:** The SLC22A2 gene that encodes the organic cation transporter 2 (OCT2) is relevant for the pharmacokinetic disposition of a number of clinically important drugs, including those used in the treatment of type II diabetes, cancer, and HIV. However, little or no information is available regarding genetic polymorphisms of the SLC22A2 gene in the Xhosa population or on the inter-ethnic differences among Sub-Saharan African populations with regards to genetic polymorphisms related to impaired functional activity of OCT2.
**Objective:** The aim of this study was to sequence the coding and flanking regions of SLC22A2 to assess the extent of genetic variation in this gene in the Xhosa population.
**Material and Methods:** Ninety six unrelated healthy Xhosa subjects were recruited for the study. The SLC22A2 exons and flanking regions were amplified using specifically designed PCR primers. The PCR products were sequenced using Sanger sequencing.
**Results:** Sequence analysis revealed 28 variations, of which 7 were novel mutations. Twenty-one of the SNPs identified in this study were already reported and listed in the dbSNP database. Furthermore, we have identified a novel promoter SNP at position -156, which can potentially alter transcription of the SLC22A2 gene. In addition, we also observed two promoter SNPs (rs59695691 and rs150063153) that are found only in African population groups. Basal promoter activity is an important determinant of SLCA22A2 expression in vivo, and may influence the transport function of hOCT2, which in turn may affect the uptake, disposition, and elimination of its substrates.
**Conclusion:** This study represents the first report of novel OCT2 polymorphisms in the Xhosa population, and shows that allele frequencies for these variants are different from those observed for other African as well as Asian and Caucasian populations. The data observed suggest that drugs which are substrates for these OCT2 variants are likely to have different response profiles among African populations and populations of either Asian or Caucasian origin, highlighting the need to genetically characterize more African populations in order to realize the aim of personalized medicine.
**Reference:** Asaka, J.-I., Terada, T., Ogasawara, K., Katsura, T. & Inui, K.-I. 2007. Characterization of the basal promoter element of human organic cation transporter 2 gene. Journal of Pharmacology and Experimental Therapeutics, 321, 684-689.

# Characterization of drug transporters involved in drug-drug interactions in human skin

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**Aims:** Most identified drug transporters belong to the ATP-binding Cassette (ABC) and Solute Carrier (SLC) families. Recent research indicates that some of these transporters play an important role in the absorption, distribution and excretion of drugs, and are involved in clinically relevant drug-drug interactions for systemic drugs. However, very little is known about the role of drug transporters in human skin in the disposition of topically applied drugs and their involvement in drug-drug interactions. The aim of this work was to compare the expression in human skin (vs human hepatocytes and kidney) of ABC and SLC transporters included in the EMA guidance as the most likely clinical sources of drug interactions.
**Methods:** Gene expression of eleven SLC transporters and four ABC transporters was measured in human liver, kidney and skin (from abdominal area) by TaqMan Real-time RT-PCR. The genes studied were SLCO1B1, SLCO1B3, SLC22A1, SLC22A2, SLC22A6, SLC22A8, SLC47A1, SLC47A2, SLCO3A1, SLCO4A1, SLCO2B1, ABCB1, ABCC1, ABCC2 and ABCG2.

Localization and functional analysis of MRP1 in human skin was analyzed by immunohistochemistry by using specific substrate and inhibitor.
**Results:** SLC and ABC transporters have a very specific expression profile in human skin with SLCO4A1 (OATPE), SLC47A1 (MATE1) and ABCC1 (MRP1) being the most expressed. SLCO4A1 and ABCC1 are about 70 times and 15 times more expressed in human skin than in hepatocytes, respectively. Moreover, MRP1 was mainly expressed in the hair follicle and sweat gland, and involved in drug uptake in human skin.
**Conclusions:** This work shows for the first time the expression of MATE transporters in human skin. In addition, the localization of MRP1 transporters in human skin was characterized for the first time.

The expression profile of drug transporters in human skin demonstrated in this study was very specific and may play an important role in drug-drug interaction.
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# Characterization of additional polymorphisms in the DPYD gene in cancer patients pre-screened for IVS14+1G>A who evidenced toxicity after 5 -fluorouracil treatment

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**Background:** Dihydropyrimidine dehydrogenase (DPD) activity is subjected to a wide functional variability due to a large number of

SNPs and this variability may be responsible of toxicity in cancer patients during the treatment with the widely applied anticancer drug 5-fluorouracil (5-FU). This results in a broad range of enzymatic from partial (3-5% of population) to complete loss (0.2%) activity. Among the large number of variants described in DPYD gene, the variant IVS14+1G>A is the one with the most serious adverse effects, consequently at least its routine detection is now strongly recommended.

**Methods:** Herein we report our experience in the use of a specific High Resolution Melting Analysis (HRMA) based approach, combined with sequencing, for the detection of three DPYD variants: IVS14+1G>A (rs67376798); c.1679T>G (rs55886092) and c.2846A>T (rs67376798) in 608 cancer patients to evidence the possible role of these SNPs to prevent chemotherapy induced toxicity.

**Results:** Analysis revealed the presence of DPYD variants in exon 14 in 35 samples; among them the intronic variant IVS14+1 was found in 6 samples while the other patients showed the presence of low level of evidence variants. Unfortunately, among the 573 subjects that resulted wild type for IVS14+1G>A, actually 80 (data under collection) of them evidenced toxicity after the first chemotherapy treatment. To identify a possible role of other SNPs in the same gene, a deeper characterization of c.1679T>G (rs55886092) and c.2846A>T (rs67376798) was performed.

**Conclusions:** Considering the large number of patients treated each year with 5-FU or other FPs, and the human and economical cost of grade 3 and 4 toxic side effects, genetic characterization at least of the most important polymorphisms should be considered mandatory. Furthermore, to maximize to the informative role of genetic testing in DPYD gene, more efforts are requested in order to define the most correct panel of SNPs that should be analyzed to prevent chemotherapy induced toxicity.

## Methodological and clinical issues of BRAF analysis in over 200 melanoma patients

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**Background:** The incorporation of molecular diagnostics in clinical laboratories requires the identification of the target molecule and the related clinical question. The interest in BRAF mutational status derives from the development of new therapeutic treatment targeted towards the mutated BRAF gene product and thus affecting only cancer cells by suppression of essential tumor-growth pathways. Contextually the methodological aspects need particular consideration in spite of the large availability of several techniques for the identification of BRAF somatic mutations. Selection of the appropriate assay for the detection of genetic alterations depends on several factors: firstly the type of mutation under study, the kind of sample to be assayed and then the sample preparation procedure.

**Methods:** To guarantee a qualified response we selected a multitechnique approach to overcome the limits, in terms of specificity and sensitivity, of a single-assay analysis. The developed workflow for BRAF mutation in exon 15 was based on the combination of three different methods: pre-screening by High Resolution Melting Analysis (HRMA), specific characterization using Sanger sequencing and exclusion of false negative results by Allele Specific (AS) Real Time PCR.

**Results:** Here we report the experience of our laboratory conducted on melanoma samples in assessing this molecular diagnostic test in routine clinical practice. For diagnostic use, a total of 205 melanoma FFPE samples received in our Unit have been studied. 193/205 were analysed for BRAF exon 15 mutations: sequence variants have been found in 84 out of 193 (43.5%) samples (72 samples p.Val600Glu, 6 samples p.Val600Lys, 2 sample p.Val600Arg, 4 samples with other rarer mutations). All results have been obtained by HRMA and then confirmed by direct sequencing. A third level of confirmatory was performed by Allele Specific Real Time PCR in approximately 20% of all the tested samples.

The clinical validity of the test (the ability to discern in the patient population those patients with the target conditions) and of the selected methods (the ability to identify the presence of a mutation) were evaluated.

## Role of oxysterol metabolism and transport genes in prognosis of estrogen receptor positive breast carcinoma patients

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**Background and Objective:** Patients with breast carcinomas expressing hormonal receptors (HR+) are treated by selective estrogen modulators (most often Tamoxifen) to reduce recurrence risk after surgery. Oxysterols have been shown to interfere with proliferation of different types of carcinomas and a significance of oxysterols in response to endocrine therapy has been increasingly discussed. Aim of this work is evaluate the prognostic significance of genes metabolizing and transporting main oxysterols in HR+ breast carcinoma patients expressing estrogen receptor (ER+).

**Design:** The expression profile of oxysterol metabolism (CYP, AKR, DHCR, EBP, CH25H, HMGCS, ACAT, and OXCT) and transport genes (ABC, SLC) was assessed by qPCR in ER+ and negative ER-samples of breast carcinoma. The genes with altered expression were then

analyzed in 50 ER+ breast carcinoma patients and associations of their transcript levels with clinico-pathological data were evaluated for prognostic significance.
**Results:** Eleven genes were downregulated in ER+ breast carcinomas in comparison to ER- tumors (CYP24A1, 39A1, 51A1, 7B1, DHCR7, EBP, HMGCS1, ACAT2, OXCT1, ABCC1, and SLCO1A2), and seven upregulated (CH25H, HMGCS2, ABCA2, 9, 10, ABCG1 and 2). Higher levels of ACAT2, EBP and HMGCS1 associated with negative clinical prognostic factors as higher stage of tumors, higher expression of progression marker Ki-67 or positive lymph node metastasis. Due to the short follow up of patients, the predictive role of these genes could not have been evaluated, however higher levels of ACAT2 and ABCG2 were found in tumors from patients with intolerance to endocrine therapy.
**Conclusions:** Several new changes in expression of genes metabolizing and transporting oxysterols in ER+ breast carcinomas were found in this work. Predictive role of these genes for endocrine therapy response should be evaluated in the following studies.
**Acknowledgment:** This study was supported by IGA MH CR grant no. NT14055-3, and GACR grant no. 13-25222J.

## Combined polymorphisms in GGCX and MDR1 genes in the dosing of phenindion in patients with valvular atrial fibrillation

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**Background:** The role of personal analyzing of farmacogenetical differences in patients with cardiovascular pathology is increasing due to the increasing frequency of surgical treatment in the cases of valvular diseases. It needs correct and individual dozing of anticoagulants. Genotype of MDR1 3435TT (Multi-Drug Resistance Gene) is known as risk factor of myocardium infarction versus genotype 3435CT. Mutations in gene GGCX (gamma-glutamyl carboxylase) is connected with the activity of blood clotting factors and hemorrhagic desease. The role of the main genetic factors in individual dozing of coumarin's anticoagulants is well known.
**Objective:** To analyze the influence of combined gen's GGCX and MDR1 polymorphisms on dosing of phenindion in patients with valvular atrial fibrillation.
**Design:** 40 patients, 27-80 years (64,5±8,1 mg), valvular atrial fibrillation were studied. The using of coumarin anticoagulants was impossible in all of them. Genotyping for polymorphism's marker were designed using the PCR (polymerase chain reaction) and RFLP (restriction fragment length polymorphism). Statistics were performed by X-2 statistical tests.
**Results:** The polymorphism in GGCX (heterozygote type CG and CT) and MDR1 (homozygote type GG and TT) were identified in the same patients together. The combined analyzes of patients genotypes having INR in the level 2-3 showed that all patients with «wild» genotype CC (MDR1) were found in the group of patients achieved targets levels of INR (2-3). The doze of phenindion in this group of patients (INR 2-3) with cooperative combination of homozygote genotype CC (GGCX) and homozygote genotype CC (MDR1) was 52,5±7,2 мg vs 82,8±9,2 мg in the heterozygote group ($p < 0.01$).
**Conclusion:** The farmacogenetical impact on phenindion dosing may depends on mutual effect of several genes polymotphisms. The cooperative analyzes of genes GGCX and MDR1 polymorphisms may be valuable in the predicting of the necessity doze of phenindion.

## Epigenetics in bone: microRNA profile in osteoblasts is affected by estrogen treatment

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**Background:** miRNAs are small non-coding RNA molecules which represent important epigenetic regulators of gene expression. It is estimated that miRNAs regulate around 60% of human protein-coding mRNAs by either translational inhibition or mRNA degradation. miRNAs deregulation is increasingly recognized as an important factor in several pathological processes and also in treatment response. The effects of estrogens on miRNAs have so far mainly been studied in breast cancer, where miRNAs are emerging as novel biomarkers that can improve diagnosis and prognosis. Moreover, miRNAs were shown to be involved in tamoxifen and aromatase inhibitors resistance. There is only one study in which the effects of estrogen on bone miRNAs have been evaluated so far. Differences in miRNA profiles between ovariectomized and sham-operated mice suggest the likely role of miRNAs in the pathogenesis of postmenopausal osteoprosis.
**Objective:** The study objective was to evaluate the effect of 17β-estradiol on miRNAs expression in cultured human osteoblasts.
**Design:** HOS TE-85 cell line was transfected with a plasmid containing estrogen receptor α and the successful transfection was confirmed by Western blotting. Following 24 and 72 hours of 17β-estradiol treatment total RNA was isolated. Simultaneous detection and quantification of 800 miRNAs was performed by using Nanostring nCounter technology. Gene expression of estrogen receptors: ERα, ERβ and GPER1 was measured by quantitative real-time PCR.
**Results:** There was a downregulation of miR-4516 following 24 hours of estradiol treatment and downregulation of miR-520d-5p, miR-376a-3p, miR-518b, miR-593-3p and miR-338-3p following 72 hours of estradiol treatment. Interestingly, miR-338-3p has already been shown to have an important role in osteoblast biology by decreasing osteoblast diferentiation through direct inhibition of transcription factor RUNX2.

**Conclusions:** miRNAs show great potential in personalised medicine as new biomarkes as well as therapeutic targets. The results of our in vitro study support the role of miRNAs in epigenetic estrogen response in osteoblasts, which could be important also in vivo for example in hormone replacement therapy.
**Acknowledgement:** This work was supported by grants ARMR19, P3-0298 and J3-5511.
**Reference:** Vrtačnik P, Ostanek B, Mencej-Bedrač S, Marc J. The many faces of estrogen signaling. Biochem Med 2014;24: 329-42.

## Optimization of the multiplex primer extension assay for simultaneous analysis of 11 polymorphisms of CYP2D6 gene

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**Background:** CYP2D6 enzyme plays an important role in the metabolism of approximately 25% of all clinically used drugs. Due to the highly polymorphic status of the CYP2D6 gene, with more than 70 allele variants described so far, the enzyme is very variable in activity, ranging from zero in poor metabolizers to high in ultra rapid metabolizers. Preemptive genotyping for the CYP2D6 allele variants enables identification of patients with impaired or increased metabolic phenotypes and can help with individualization of therapy. Conventional methods for analysis of that many allele variants can be time consuming and expensive and fast and reliable multiplex method is needed for simultaneous analysis of multiple polymorphisms.
**Objective:** The aim of our study was to optimize the primer extension method for simultaneous determination of 11 most clinically relevant CYP2D6 gene polymorphisms in order to minimize the time and cost of CYP2D6 genotyping.
**Design:** We used multiplex primer extension method to screen for the 11 most clinically relevant CYP2D6 polymorphisms, including 100C>T, 2850C>T, 1023C>T, 1661G>C, 1707delT, 1846G>A, 2549delA, 2613-15delAAG, 2988G>A, 3183G>A and 4180G>C. To avoid interference from pseudogenes, the entire CYP2D6 gene was amplified using previously described primers and 5.1 kb product was used in the primer extension reaction. 11 interrogation primers, with 4-5 nucleotides difference in length, were designed to anneal next to all the mutation sites. The method included single-base extension of interrogation primers by fluorescent dye labeled terminators, separation of products by capillary electrophoresis and detection of labeled terminators. The method was verified by amplification and sequencing of CYP2D6 gene fragments, containing polymorphic sites.
**Results:** Using multiplex primer extension method and specific separation conditions at capillary electrophoresis, we successfully genotyped all of the 11 selected polymorphisms simultaneously in one assay. Total time needed for analysis is less than 10 hours, with hands-on time less than 1.5 hours.
**Conclusions:** Primer extension method represents an efficient, fast and cost effective way for simultaneous determination of multiple gene polymorphisms (SNPs or short deletions or insertions). Our optimized method allows for screening for some of the most clinically relevant polymorphisms in the CYP2D6 gene in minimum amount of time and at much lower cost compared to traditional TaqMan method. With further optimization, we expect to also determine gene deletion and duplications using the same method.
**Reference:** Sistonen J, Fuselli S, Levo A, Sajantila A. Clinical chemistry 2005; 51(7): 1291-1295.

## The influence of CYP3A4*22 and CYP3A5*3 polymorphisms on the efficiency of finasteride and dutasteride therapy in patients with benign prostatic hyperplasia

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**Background:** Finasteride and dutasteride are commonly used medicines for therapy of benign prostatic hyperplasia. They are both inhibitors of 5α-reductase type II, which converts testosterone to dihydrotestosterone in the prostate. Finasteride is mainly metabolized with CYP3A4, whereas dutasteride is metabolized with CYP3A4 and CYP3A5. As a wide interindividual variation in response exists, we focused our study on these two key metabolizing enzymes CYP3A4 and CYP3A5.
**Objective:** The aim of our study was to investigate the association of CYP3A4*22 (rs35599367) and CYP3A5*3 (rs776746) with the efficiency of finasteride/dutasteride in the therapy of benign prostatic hyperplasia.
**Design:** Seventy patients with benign prostate hyperplasia were involved in the study. Thirty-five patients were treated with 5 mg of finasteride per day, and the rest with 0.5 mg of dutasteride per day. Prostate specific antigen (PSA), volume of prostate (VP) and International Prostate Symptom Score (IPSS) were determined at baseline, after 6 months and 1 year of therapy.
**Results:** In finasteride-treated patients, the frequencies of CYP3A4 alleles were 5.7% vs. 94.3% for CYP3A4*22 heterozygotes and CYP3A4*22 homozygotes, and CYP3A5 frequencies were 91.4% vs. 8.6% for CYP3A5*1 homozygotes and CYP3A5*3 heterozygotes. In dutasteride-treated patients, CYP3A4 frequencies were 8.6% vs. 91.4% for CYP3A4*22 heterozygotes and CYP3A4*22 homozygotes, whereas CYP3A5 frequencies were 85.7% vs. 14.3% for CYP3A5*1 homozygotes and CYP3A5*3 heterozygotes. In finasteride-treated patients, CYP3A4*22 polymorphism influenced the change in VP after 6 months of therapy, though the difference was not statistically significant (p=0.072). In dutasteride-treated patients, CYP3A4*22 polymorphism also showed influence on the change of PSA levels, however with no statiistical significance (p=0.062). In CYP3A5*3, no significant association with the changes of VP, IPSS or PSA levels was observed.
**Conclusions:** CYP3A4*22 polymorphism indicates a possible influence on the efficiency of finasteride therapy, as well as dutasteride therapy. Due to small number of studied subjects, these data should be further confirmed on a larger number of treated patients.

# Microsomes genotyped for UGT1A1*28 polymorphism - in vitro model for prediction of drugs' metabolism (bazedoxifene and raloxifene case)

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**Background:** Raloxifene and bazedoxifene, selective estrogen receptor modulators, exhibit quite large interindividual variability in pharmacokinetics and pharmacodynamics. In human, raloxifene and bazedoxifene are extensively metabolized by different isoforms of UDP-glucuronosyltransferase (UGT) to its glucuronides. UGT1A1 is the isoform that is involved in metabolism of both studied drugs and is also responsible for the metabolism of endogenous bilirubin to its glucuronides. UGT1A1*28 is the most studied polymorphism, which leads to reduction of UGT1A1 transcription.
**Objective:** The aim of our in vitro study was to explain the mechanism behind the observed influence of UGT1A1*28 polymorphism on raloxifene pharmacokinetics in a small-sized in vivo study (Trontelj et al). As pharmacokinetics of bazedoxifene and raloxifene are very similar, we investigated also the influence of UGT1A1*28 polymorphism on metabolism of bazedoxifene.
**Design:** In order to gain an insight into metabolism by UGT1A1 human liver microsomes genotyped for UGT1A1*28 polymorphism were used.
**Results:** In human liver microsomes raloxifene-6-β-glucuronide (M1) formation followed the Michaelis-Menten kinetics, meanwhile raloxifene-4'-β-glucuronide (M2) formation followed the substrate inhibition kinetics. Formation of both bazedoxifene metabolites, bazedoxifene-4'-glucuronide (M4) and bazedoxifene-5- glucuronide (M5), followed the substrate inhibition kinetics. Incubation of raloxifene with human liver microsomes genotyped for UGT1A1*28 showed a significantly reduced metabolic clearance towards M1 in microsomes from donors with *28 allele. On the contrary, no significant genotype influence was observed on the formation of M2 due to the high variability in estimated apparent kinetic parameters, although a clear trend towards lower glucuronidation activities was observed when UGT1A1*28 polymorphism was present. In case of bazedoxifene, *28/*28 microsomes showed a 7 to 10 – fold lower intrinsic metabolic clearance of bazedoxifene towards both metabolites.
**Conclusions:** Previously published in vivo observed UGT1A1*28 influence on raloxifene metabolism was confirmed in vitro showing that microsomes genotyped for UGT1A1*28 polymorphism are valuable in vitro model for prediction of drugs' metabolism. The significant in vitro UGT1A1*28 genotype effect on bazedoxifene intrinsic metabolic clearance found in our study strongly indicates that this subject is well worth further exploration in vivo.
**Reference:** Trontelj J, Marc J, Zavratnik A, Bogataj M, Mrhar A. Br J Clin Pharmacol 2009; 67(4): 437-44.

# Characterization of the single nucleotide polymorphism 497A>G in the drug metabolizing enzyme thiopurine methyltransferase

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**Background:** Thiopurine methyltransferase (TPMT) is a polymorphic enzyme that converts cytotoxic thiopurine drugs into both active and inactive metabolites. In Caucasians 10% carries a single nucleotide polymorphisms (SNP) causing a less functional enzyme leading to an increased risk of severe adverse reactions during treatment with normal thiopurine doses. Today about 40 SNPs are known and named by the TPMT nomenclature committee (www.imh.liu.se/tpmtalleles).

Recently, we discovered a SNP in the drug metabolizing enzyme TPMT, in a young female patient with acute lymphoblastic leukemia. It had earlier been reported from the genomic ClinSeq Project but has not been further characterized.
**Objective:** The objective of this study was to characterize the TPMT SNP 497A>G, to describe the properties and function of the resulting protein in comparison to the wild type TPMT.
**Design:** In our study the TPMT 497A>G was characterized in context of enzyme activity in RBC and WBC, cDNA sequencing and heredity. We also produced recombinant human TPMT 497A>G in Escherichia Coli bacteria and investigated the stability and structural changes of the protein structure with biophysical methods (1).
**Results:** The SNP consists of a 497A>G mutation which cause an amino acid shift of Y166C. Our results shows that 497A>G is a heritable variant of TPMT that decrease the patient's enzyme activity dramatically. The overall structure of the enzyme is normal but the amino acid shift causes a pronounced less stable enzyme.
**Conclusions:** We present a full characterization of the nonfunctional TPMT SNP 497A>G, a SNP that influence the metabolism of thiopurine drugs, widely used during treatment of childhood leukaemia.
**Acknowledgements:** The authors express their gratitude to Britt Sigfridsson, Monica Häger, Annette Molbaeck and Åsa Schippert for excellent technical assistance. This study was supported with grants from Stiftelsen Linköpings Läkaresällskaps Forskningsfond and östgötaregionens Cancerfond.
**Reference:** 1. Wennerstrand P, Dametto P, Hennig J, Klingstedt T, Skoglund K, Lindqvist Appell M, Mårtensson L-G. Structural Characteristics Determine the Cause of the Low Enzyme Activity of Two Thiopurine S-Methyltransferase Allelic Variants: A Biophysical Characterization of TPMT*2 and TPMT*5. Biochemistry. 2012;51:5912-20.

# Pharmacogenetic approach for irinotecan toxicity in Turkish colon cancer patients

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Irinotecan treatment in cancer in chemotherapy could be complicated because of the toxicity that occurs in 36% of patients who are treated with irinotecan due to the large interpatient variability in drug metabolism. (Fuchs et al. 2003).

UGTs catalyze the glucuronidation of chemotherapeutics by conjugating the metabolite which has anti-tumoral activity. UGT1A1 has the major enzyme responsible in the biotransformation of irinotecan.

Increased or decreased UGT1A1 enzyme expression depending on genetic or epigenetic regulation of UGT1A1 gene directly affects the glucuronidation activity at the same time indirectly affects the drugs' metabolic clearance which use the main metabolic way by glucuronidation. In this context, it has been suggested that accumulation of SN-38, the active metabolite of irinotecan, decrease UGT1A1 enzyme activity in the body causes induced glucuronidation activity which leads to high SN-38 concentration with outcome of adverse reaction, such as neutropenia, diarrhea. (Mathijssen et al. 2001).

The thymine-adenine (TA) repeats located in the promoter region of the UGT1A1 gene at the first exon were detected as the genetic polymorphisms which are responsible from the changes in the enzyme UGT1A1 activity (Iyer et al. 2002). According to these repeats, UGT1A1*1, UGT1A1*28, UGT1A1*37, and UGT1A1*38 genotypes has been identified as enzyme activity is normal, low, low, and high respectively for genotyping analysis which can be predictive in irinotecan treatment (Palomaki et al. 2009).

UGT1A1 allelic frequencies vary among ethnic groups; the lowest UGT1A1*28 homozygote allelic frequency is in Asian populations (16%), the most common Africa (43%) and Europe (36%) populations (Innocenti et al. 2003). In Turkey, there has not been a comprehensive study of allele frequencies of UGT1A1.

In this study the primary goal is to feature the effect of UGT1A1 gene polymorphism involved in irinotecan metabolism, clinical outcomes, and irinotecan-induced side effects for Turkish colon cancer patients, highlighting relation between genetic and non-genetic factors, such diet and co-medication for possible irinotecan toxicities during treatment.

# CYP2D6 allele specific copy number analysis using TaqMan® SNP Genotyping Assays and digital PCR

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The major drug metabolizing enzyme, CYP2D6, is encoded by a highly polymorphic gene. Over 100 star allele haplotypes are known, which can contain SNP, InDel, and copy number variants (CNVs) and which fall into 3 main functional categories (full, reduced, or none). The diploid star allele content is predictive of CYP2D6 drug metabolizer phenotype (ultrarapid, extensive, intermediate, or poor). We previously described a workflow whereby sample SNP genotype and CNV analysis results, generated using TaqMan® SNP assays and TaqMan® Copy Number assays, respectively, can be translated to star allele diplotypes using AlleleTyper™ software. However, for samples that carry CYP2D6 duplications and are heterozygous for key SNPs, the specific allele that is duplicated cannot always be identified. A phenotype can be assigned if all 3 alleles are of the same functional category, but if alleles are from different functional categories there may be 2 possible phenotypes. To address this issue, we developed a method to detect allele-specific copy number by digital PCR using the QuantStudio™ 3D Digital PCR System.

# TaqMan® rare mutation assays for Quantstudio® 3D digital PCR system

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Detection and quantification of mutant alleles in tumor tissue allow for research disease monitoring and the research of drug efficacy. Detection of emerging secondary mutations in the same tumor tissue causing resistance to potential treatment will help guide decisions on future treatment plans. Testing for the presence of mutations in cell free DNA (cfDNA) is a less invasive research method than using tumor tissue.

We created a research tool for mutation detection at a sensitivity level of 1% and below. This allows researchers to find correlation

between types of mutations and types of tumors and determination of potential secondary mutations.

The tool combines TaqMan® SNP Genotyping Assays with digital PCR. A set of assays was optimized for use in digital PCR with the QuantStudio® 3D Digital PCR System. In digital PCR, partitioning the sample into many individual reaction wells facilitates detection and quantification of rare mutant alleles. TaqMan® SNP Genotyping Assays ensure reliable discrimination of mutant and wild-type allele.

Our current set of 60 assays covers mutations commonly found in tumor tissues, such as: BRAF V600E, mutations in EGFR exons 19, 20 and 21, KRAS codons 12 and 13, PIK3CA exons 9 and 20, and the JAK2 V617F mutations.

All assays were wet-lab tested at a 10% mutation rate and a 1% mutation rate using mutant plasmid spiked into wild-type genomic DNA. Additionally, selected assays were tested at the 0.1% mutation rate using mutant cell lines spiked into wild-type genomic DNA.

Wet-lab results confirm that all assays showed superior performance discriminating mutant and wild-type alleles. Mutant alleles were successfully detected as low as 0.1%.

## Mismatch repair SNPs and thyroid cancer susceptibility: a potential role for the MSH6 rs1042821 (Gly39Glu) polymorphism

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**Background**: Exposure to ionizing radiation (IR) is the best-established risk factor for thyroid cancer (TC) but genetic variation could also play a role. The DNA mismatch repair (MMR) pathway counteracts carcinogenesis through the supression of genetic instability and several lines of evidence suggest that altered function or expression of MMR proteins may also be implicated in TC pathogenesis.

**Objective**: We aimed to evaluate the potential modifying role of a panel of eight MMR single nucleotide polymorphisms (SNPs) on the individual susceptibility to non-familial differentiated TC.

**Design**: A small-scale hospital-based case-control study was performed in a Caucasian Portuguese population, comprising 106 histologically confirmed differentiated TC patients and 212 age and gender matched controls. DNA mismatch repair SNPs rs1799977 (MLH1), rs26279 and rs184967 (MSH3), rs5745325 and rs5745549 (MSH4), rs5742933 (PMS1), rs175080 (MLH3) and rs1042821 (MSH6) were genotyped using the TaqMan allelic discrimination assay and the genotype-associated TC risk was estimated by multivariate logistic regression analysis.

**Results**: The homozygous variant genotype of rs1042821 (MSH6) was significantly associated with increased differentiated TC risk, both under a codominant model (Glu/Glu vs. Gly/Gly: adjusted OR=3.42; 95%CI=1.04-11.24; p=0.04) and a recessive model (Glu/Glu vs. [Gly/Glu + Gly/Gly]: adjusted OR=3.84; 95%CI=1.18-12.44; p=0.03). This association was also observed after histological and gender stratification, both in the follicular subset (adjusted OR=20.98; 95%CI=1.08-406.53; p=0.04 – codominant model; adjusted OR=23.70; 95%CI=1.25-449.32; p=0.04 – recessive model) and in the female subset (adjusted OR=4.78; 95%CI=1.17-19.56; p=0.03 – codominant model; adjusted OR=5.42; 95%CI=1.34-21.92; p=0.02 – recessive model). No significant associations were observed for the remaining SNPs.

**Conclusion**: Our data supports the idea that the MSH6 rs1042821 SNP may contribute to differentiated TC susceptibility, particularly of the follicular type. The risk increase is also apparent in women.

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## Genetic variants of cytochrome P450 2C19: just markers for clopidogrel therapy alteration or one of the reasons of thrombotic / bleeding disorder?

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Cytochromes P450 are main origin of the variability in human drug pharmacokinetics. The clinically most important enzymes belong to the families 1, 2 and 3; they are responsible for processing and transformation of many foreign compounds including 70-80% drugs in clinical use. Multiallelic genetic polymorphism, contributing to the wide range of the enzyme activity, was discovered in most of the genes. Determining the specific activity for each allele is a goal of many research projects. Less is meant that these genes may play an important role in the development of some diseases without the use of known drugs.

Cytochrome P450 2C19 is known gene having major impact on biotransformation of Clopidogrel. Clopidogrel is an oral, thienopyridine-class antiplatelet agent used to inhibit blood clots in cerebrovascular disease, peripheral vascular disease and coronary artery disease. Clopidogrel itself is a pro-drug transformed to the active circulating substance SR26334 just with gen CYP 2C19. The drug dosing depends on the particular genotype of this gene. In CYP 2C19 gene was found several dozen alleles of which only part is clinicaly significant. In Europe, the most important alleles with

altered activity are * 2, * 3 and * 17. On the basis of specific genotypes are patient sorted into 4 groups: Poor metabolizers (homozygote *2 or *3), intermediate metabolizers (*2 or *3 heterozygote with wild type allele *1), extensive metabolizers (wild type homozygote *1) and ultrarapid metabolizers (homozygote or heterozygote *17). Treatment is modified or changed on the basis of genotyping.

In October 2014, we began to investigate CYP 2C19 genotype in patients after cerebrovascular incident in order to detect the poor, intermediate and ultrarapid metabolizers and provide them a tailored treatment. To our surprise there were more than 50% of patients positive, it means more than 50% was not extensive metabolizers. This finding does not match the frequency distribution of the standard population – there should be the reason why the frequency of aberrant genotypes is higher in patients with cerebrovascular disease. Selected reasons will be discussed.

## DNA repair SNPs as modulators of response to cancer radiotherapy: potential implications for oral cancer management

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**Background**: Cancer radiotherapy takes advantage of the ability of ionizing radiation to induce DNA damage hence killing tumor cells. Non-tumor cells are also affected, giving rise to radiation-related toxicity. Single nucleotide polymorphisms (SNPs), through interference with the DNA repair efficiency in both tumor and normal cells, may alter, respectively, the efficacy and safety of radiotherapy. As such, DNA repair SNPs could ultimately affect both the short and long-term clinical benefit of such therapy.
**Objective**: We aimed to systematically review the impact of DNA repair SNPs on the response to therapy, clinical outcome and prognosis in cancer patients submitted to radiotherapy, so that genetic biomarkers potentially relevant for the personalization of oral cancer management may be identified.
**Design**: The PUBMED database was searched using the following MeSH terms: neoplasms AND Polymorphism, Single Nucleotide AND DNA repair AND radiotherapy. 92 articles, published up to March 14, 2015, were retrieved. On applying predefined inclusion/exclusion criteria through manual curation, 30 articles were excluded. 62 articles were thus eligible for further data extraction and systematic review.
**Results**: A high number of significant associations were observed, between SNPs across the most relevant DNA repair pathways – direct damage reversal, base excision repair, nucleotide excision repair, mismatch repair, homologous recombination, non-homologous end-joining – and multiple efficacy (e.g. respose rate), toxicity (e.g. radiation-related pneumonitis, oral mucositis, skin reaction) or long-term clinical benefit (e.g. overall or disease-specific survival) endpoints. The XRCC1 Gln399Arg substitution (rs25487) was the DNA repair SNP most frequently associated with response to radiotherapy, with the variant allele consistently being associated with decreased survival. An association between the XRCC1 rs25487 variant allele and a radiation-related toxicity risk increase is also frequently suggested, but conflicting results exist.
**Conclusions**: DNA repair SNPs across different DNA repair pathways appear to modulate the individual response to radiotherapy. Evidence is stronger for the XRCC1 Gln399Arg substitution (rs25487). Integration of these pharmacogenetic biomarkers into the clinical decision process may, in a near future, allow for the optimization of the therapeutic approach to several types of cancer, maximizing the benefit while minimizing the risk. Oral cancer management, where radiotherapy is considered a 2nd line therapeutic option, is one of many possible examples.

## Sodium channel mutations and generalized epilepsy with febrile seizures plus

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**Background**: GEFS plus is a purely genetic epilepsy disorder with profound heterogeneity. Inheritance is generally autosomal dominant with incomplete penetrance.
**Objective**: This work aims to present two cases of GEFS plus with genetic mutations not yet described in the literature.
**Design**: Clinical case description.
**Results**: Case 1: Female, 16 years old, with an inaugural episode of febrile seizures at 4 months of age, after diphtheria-tetanus-pertussis vaccination. Febrile seizures continued up to 5 years of age, then she had several seizures with no fever and others triggered by physical exercise. She had no relevant personal history or obvious familial history of epilepsy. Neurological examination was normal with no cognitive impairment. The routine EEG reveal right, sometimes bilateral parieto-occipital paroxysmal activity and the MRI showed left peritrigonal white matter T2-weighted hyperintensity. Testing the possibility of GEFS plus syndrome, the genetic investigation confirmed a SCN1A gene mutation, in exon 13, which results in a premature STOP codon, resulting in a non functional, truncated protein, consistent with GEFS plus type 2.
Case 2: Female, 13 years old, with an inaugural episode of febrile seizures at 2 months of age. She had febrile seizures up to age 6. She was asymptomatic until age 12, when she evolved with 3 dialeptic seizures, without fever, two of them followed by tonic-clonic movements. There is a familial history of consanguinity and maternal lineage febrile seizures. Neurological examination was normal and EEG and MRI studies were unremarkable. Genetic studies revealed a mutation in SCN9A gene, which results in substitution of a serine for arginine at position 1181 making the diagnosis of GEFS plus type 7 more likely.
**Conclusions**: There are hundreds of sodium channel mutations linked to epilepsy, almost all in the SCN1A gene. Mutations in SCN9A gene have been associated with other neurological diseases and rarely with epilepsy. Thus, we find on these cases relevant scientific knowledge. Although currently the classification of epilepsy is mainly based on clinical data, nowadays, genetic studies support the clinical information and open pathophysiological and therapeutic horizons.

**Abbreviations and nomenclature:**
GEFS plus – Generalized Epilepsy with Febrile Seizures plus
EEG – Electroencephalogram
MRI – Magnetic resonance imaging
SCN1A – Sodium channel, voltage gated, type I alpha subunit
SCN9A – Sodium channel, voltage gated, type IX alpha subunit
**Reference:** Catterall WA. Sodium channels, inherited epilepsy, and antiepileptic drugs. Annu Rev Pharmacol Toxicol. 2014;54:317-38.

## Association of CTH variant with sinusoidal obstruction syndrome in children receiving intravenous busulfan and cyclophosphamide before hematopoietic stem cell transplantation

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Sinusoidal obstruction syndrome (SOS) is a frequent complication of hematopoietic stem cell transplantation (HSCT) and could be fatal, often attributed to the conditioning regimen prior to HSCT. We evaluated the association of SOS risk with gene variants in cystathionase (CTH), an enzyme involved in the metabolic fate of busulfan (Bu) and glutathione synthesis, in 76 children receiving intravenous Bu before HSCT. The overall incidence of SOS was 11.5%. Our results indicated an association with CTH c.1364 G>T ($p < 0.001$, OR = 10.6, 95% CI = 2.16, 51.54) and SOS risk, which was sex dependent, whereby the association was more apparent in the female patients ($p < 0.00001$), OR = 21.82, 95% CI = 3.6, 132.6). A Multifactor dimensionality reduction (MDR) analysis was then performed to investigate any gene-gene interactions between CTHc.1364 G>T, and GSTA1*B, a previously associated SOS risk variant in this cohort (1). A recessive model with the use of GSTA1*B*B and CTH c.1364 TT genotypes either individually or in combination proved to be useful for predicting SOS occurrence. This study indicates the possibility of using these gene variants as markers of SOS occurrence and to further individualize pre-emptive treatment aimed at reducing SOS incidence.
**Reference:** 1. M Ansari MR, Y Théoret, CRS Uppugunduri, S Mettiani, M-F Vachon, C Desjean, J Rousseau, M Labuda, C Przybyla, M Duval, M Champagne, C Peters, H Bittencourt and M Krajinovic. Glutatione S-Transferase gene variations influence BU pharmacokinetics and outcome of hematopoietic SCT in pediatric patients. Bone marrow transplantation. 2013;1-8.

## MicroRNA-212 & ABCG2: potential regulators of imatinib-sensitivity?

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**Background**: The hematopoetic disorder chronic myeloid leukemia (CML) is one of the most extensively studied neoplasms. It is caused by translocation between chromosomes 9 and 22 leading to the formation of the Philadelphia chromosome and the BCR-ABL fusion-gene. First-line therapy is still the tyrosine-kinase inhibitor imatinib (IM), which led to tremendous success in treatment. However, the amount of therapeutic resistances is increasing, caused either by BCR-ABL-dependent mechanisms (e.g. BCR-ABL amplification/over-expression, point mutations) or BCR-ABL-independent mechanism, which can be linked to altered expression of drug transporters or particularly, microRNA-expression levels.
**Objective**: In our previous study, we analyzed the alterations of microRNA expression profiles during the development of IM-resistances in the leukemic cell line K562. We found a relation of miR-212 to IM-resistance, indicated by an inverse correlation of miR-212 expression and protein levels of the efflux transporter ATP-binding cassette transporter G2 (ABCG2) in cells resistant to different IM-concentrations. In this study, we investigated whether miR-212 has a direct effect on IM-sensitivity.
**Design**: We transfected K562 cells either with miR-mimic pre-miR-212 or inhibitory anti-miR-212, challenged them with IM and analyzed effects on cell viability, activation of apoptosis and cell death using WST-1-, Caspase Glo 9-assay and cell counting. Furthermore, we analyzed alternations in IM-efflux using HPLC and Hoechst efflux assay.
**Results**: We found that under IM-treatment, inhibition of endogenous miR-212 using anti-miR-212 significantly promotes cell survival apparent on the level of respiratory chain function ($p<0.01$) and cell membrane integrity and reduced capase-9 activity ($p<0.05$). Furthermore, these miRNA-effects are dose-dependent as confirmed in concentration row-experiments.
**Conclusions**: Overall, these experiments indicate that miR-212 does not only affect ABCG2-expression, but does also influence cell sensitivity to IM in a more direct manner. Further analysis could now reveal, which mRNAs are targeted by miR-212, which pathways are influenced by this and how cell sensitivity to IM is altered. These findings could be relevant in CML-therapy, overcoming IM-resistances with a better understanding of miRNA-alteration in CML.
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## Roux-en-Y gastric bypass surgery leads to intestinal adaptation processes on mRNA and microRNA level

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**Background**: For morbid obese patients (BMI ≥ 40 kg/m$^2$) bariatric surgery, in particular the most commonly used Roux-en-Y gastric bypass (RYGB), is a chance to achieve long-term weight loss and substantial improvement or remission of obesity related comorbidity. The mechanisms underlying the metabolic benefit of RYGB are only poorly understood. However, several studies gave some evidence that changes in intestinal mucosal might be involved.
The aim of our study was to elucidate the molecular background of mucosal adaptation on transcriptional and posttranscriptional level and confirm the role of microRNA-target gene interactions as well as the role of bile acids for adaptation processes.
**Design**: Biopsies from twelve obese patients were obtained during RYGB (duodenum, jejunum) and one year later (Roux limb, former jejunum). Genome-wide mRNA and microRNA analysis was done using Affymetrix Human Gene 1.0 ST chip and TaqMan Human microRNA Arrays Pool A+B. Overrepresented pathways were determined, mRNA-microRNA pairs were identify through correlation analysis, subjected to in-silico target prediction and were confirmed using luciferase assays and western blot analysis. The impact of bile acids was verified using cell culture based experiments.
**Results**: In Roux limb one year after RYGB in particular genes associated with cholesterol and vitamin metabolism showed differential expression levels compared to Jejunum and Duodenum. Interestingly differentially expressed microRNAs were only found when comparing Jejunum with Roux limb and Duodenum. As example for the posttranscriptional regulation during intestinal adaptation, β-carotene 15,15'-monooxygenase 1 (BCMO1) was identified as target of miR-301a. Additionally, the regulatory effect of bile acids in adaptation was confirmed by 16% down-regulation of BCMO1 protein level after treatment with cholic acid (p=0.014).
**Conclusion**: The RYGB leads to strong changes of the mucosal gene expression profile in the Roux limb during one year possibly due to altered external influences, while microRNA expression level adapted to former duodenal microRNA level. The impact of microRNAs during adaptation was analysed for the pair BCMO1/miR-301a and bile acid was identified as potential regulatory factor for gene expressionin adaptation.

## The role of digital tools in translation of pharmacogenetics: a global prospective

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**Background:** Pharmacogenomics has the promise of delivering treatment, which is tailored to the individual. There are global initiatives underway to integrate pharmacogenetic data about a patient into clinical practice, to avoid adverse drug reactions and deliver more effective treatments. The Vanderbilt PREDICT, CLIP-MERGE, Eu-PIC and Saudi Human Genome Project are examples of such initiatives. Implementation projects differ in their emphasis on the importance of digital tools in translation and work needs to be carried out in understanding what will the role be to promote successful integration across health systems to promote safer prescribing practices.
**Objective:** This study aims to understand how implementation of pharmacogenetics relies on digital solutions by analysing different approaches. Recommendations will be made as to where to use digital tools during the process and limitations which have been observed.
**Design:** Secondary research into initiatives will be carried out and interviews conducted with stakeholders in the PREDICT and Eu-PIC projects. A quantitative study will be conducted to assess physicians' confidence in this area and approaches they believe will aid them in integrating pharmacogenetic data into their clinical care.
**Results:** Preliminary investigations reveal a need for further education amongst prescribers with regard to pharmacogenetics and also the promise of digital tools to integrate into physician practice. We reserve further detail of the trial outcomes until a complete analysis has been conducted.
**Conclusion:** Digital tools hold much promise for the integration of pharmacogenetics into clinical practice. However, limitations need to be understood and communicated. Such tools are not suitable in geographies where there is a lack of infrastructure and also have a danger of promoting digital exclusion alienating prescribers who are not comfortable with technology.

Findings from this study will assess in more depth the challenges faced by integration projects and highlight areas the community need to address to enable this approach in their own geography.
**Reference:** Bartlett MJ, Green D, Shephard EA, The Lancet, 2013; 381; 9881: 1903.

## The case of genetic determination of leukocyte telomere length in childhood

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**Background**: Telomeres are responsible for the protection of genomic integrity. The loss of several base pairs from telomeres during each mitotic division leads to telomere shortening which after a critical

limit results in telomere dysfunction that triggers cell cycle arrest, apoptosis and/or cellular senescence. Leukocyte telomere length (LTL) has been linked with a variety of human diseases, ranging from rare monogenic disorders to age-related complex disease such as cancer, cardiovascular diseases (CVDs) and CVD risk factors such as obesity, hypertension and diabetes (1). LTL is a highly heritable trait, as indicated by previous studies (40-80%). Genome-wide association studies (GWAS) have identified genetic variants which are associated with LTL, however, these studies are limited to adult populations. Nevertheless, childhood is an extremely crucial period for the determination of LTL and the assessment of age-specific genetic determinants, although neglected, could be of great importance.

**Objective**: The objective was to provide insights and preliminary results on the neglected field of genetic determinants of LTL in children compared to adults.

**Design**: In a population of healthy children (n=322, age range=6.75-17 years) with available GWAS data (Illumina Human CNV370-Duo array), the LTL was measured using multiplex quantitative real-time PCR. Linear regression models adjusted for age, gender, parental age at child's birth and body mass index were used to test the associations of LTL with polymorphisms identified in adult GWAS and to perform a discovery-only GWAS.

**Results**: Among the 21 polymorphisms previously shown to be associated with adults LTL through GWAS methodologies none was associated with LTL in our paediatric sample. Furthermore, our GWAS approach demonstrated 6 novel variants that reached the level of suggestive association ($P \leq 5 \times 10\text{-}5$) and explain a high percentage of children's LTL.

**Conclusions**: It appears that the study of genetic determinants of LTL in children may identify novel variants not previously identified in adults. Studies in large-scale children populations are needed for the confirmation of these results, possibly through a childhood consortium that could better handle the methodological challenges of LTL genetic epidemiology field.

**Acknowledgements**: This work was funded through the Collaborative BioIntelligence Program.

**Reference**: 1. Calado RT, Young NS. Telomere diseases. N Engl J Med 2009;361:2353-65.

## PACSIN2 revisited: rs2413739 TT genotype is a risk factor for 6-MP induced bone marrow suppression in TPMT wild-type ALL patients

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**Background**: Thiopurine S-methyltransferase (TPMT) is a major determinant of 6-mercaptopurine (6-MP) toxicity, however in some patients treatment related toxicities can't be predicted solely based on TPMT genotype. Recently, Stocco et al. (1) found the association of a PACSIN2 polymorphism rs2413739 with the incidence of severe gastrointestinal (GI) toxicity during consolidation therapy containing 6-MP, but this finding was not replicated by other researchers so far. PACSIN2 is a member of the 'protein kinase C and casein kinase substrate in neurons' family of proteins which interacts with RAC1, the primary target of thiopurine drugs and was found to modulate TPMT activity.

**Objective**: The objective of the study was to identify the influence of PACSIN2 polymorphism rs2413739 on safety and effectiveness of 6-MP therapy in children with ALL.

**Design**: To identify the influence of rs2413739 on 6-MP response, we systematically investigated its individual and combined effects with other genetic and environmental risk factors on 6-MP induced toxicities as well as relapse rates in a retrospective study including 308 pediatric ALL patients undergoing maintenance therapy. Patients' characteristics, such as gender, age at diagnosis, therapy protocol, adverse effects (bone marrow suppression, stomatitis, recurrent infections, febrile neutropenia, hepatotoxicity, osteonecrosis and secondary tumors), 6-MP dose reductions and relapses were obtained from their medical records. Data were analyzed using simple and complex statistical models.

**Results**: In addition to TPMT genotype, which was confirmed to be the major determinant of drug related toxicities, PACSIN2 rs2413739 TT genotype was identified as a significant risk factor for 6-MP induced bone marrow suppression and 6-MP dose reduction in wild-type TPMT patients.

**Conclusions**: To our knowledge, this is the first study showing PACSIN2 genotype association with bone marrow suppression in ALL patients undergoing maintenance therapy.

**Acknowledgements:** The work was supported by Slovenian research agency grant No. J3-6792.

**Reference:** 1. Stocco G, Yang W, Crews KR, Thierfelder WE, Decorti G, Londero M, Franca R, Rabusin M, Valsecchi MG, Pei D, Cheng C, Paugh SW, Ramsey LB, Diouf B, McCorkle JR, Jones TS, Pui CH, Relling MV, Evans WE. Hum Mol Genet 2012; 21(21): 4793-804.

## The first time identification of CYP2C19*2 polymorphisms in the three native ethnic groups living in Dagestan Republic: Laks, Dargins and Avars

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**Background**: Inter-ethnic differences in pharmacokinetics and pharmacodynamics and the resulting differences in efficacy and safety have been widely described for several drugs [1]. The presence of CYP2C19*2 polymorphism have been found to play a role in different individual responses to some therapeutic agents (clopidogrel, omeprazole, nelfinavir, clonazepam and etc.).

**Objective**: Since the frequency of CYP2C19*2 notoriously to differ from one population to another, the aim of this study was to examine their prevalence in the Laks, Dargins and Avars.
**Design**: Frequencies of CYP2C19*2 (G681A, rs4244285) alleles and genotypes have been evaluated in 122 unrelated healthy volunteers living in Dagestan Republic: 55 Avars (14 males and 41 females, mean age 21,4±2,8 years), 35 Laks (10 males and 25 females, mean age 21,7±4,0 years) and 32 Dargins (9 males and 23 females, mean age 22,4±6,0 years) subjects. Genotyping has been carried out on peripheral leukocytes DNA by Real-time PCR.
**Results**: Distribution of CYP2C19*2 allele and genotypes in healthy volunteers: Avars (*1/*1 – 76,4%, *1/*2 – 23,6%, *2/*2 – 0% and *2 – 11,8%); Laks (*1/*1 – 68,6%, *1/*2 – 28,6%, *2/*2 – 2,9% and *2 – 17,2%); and Dargins (*1/*1 – 96,9%, *1/*2 – 3,1%, *2/*2 – 0% and *2 – 1,6%). Significant differences in CYP2C19*2 allele frequencies were found comparing the Avars and Dargins (11,8% vs. 1,6% accordingly; p=0,01) and the Laks and Dargins subjects (17,2% vs. 1,6% accordingly; p=0,003). The observed genotype distribution did not deviate from Hardy–Weinberg equilibrium.
**Conclusions**: Our results indicate the existence of inter-ethnic differences in the CYP2C19*2 allele frequencies in the different native ethnic groups living in Dagestan Republic: Laks, Dargins and Avars. The frequencies of the CYP2C19*2 alleles determined in this study in the Laks and Avars (but no Dargins) are similar to those of other Caucasian (White) subjects.
**Reference:** 1. Yasuda SU, Zhang L, Huang SM. The role of ethnicity in variability in response to drugs: focus on clinical pharmacology studies. Clin Pharmacol Ther. 2008 Sep;84(3):417-23. doi:10.1038/clpt.2008.141.

## PXR (NR1I2): a new star in the sky of irinogenetics

**Litaty Mbatchi**

The nuclear receptors PXR (Pregnane X Receptor, gene NR1I2) and CAR (Constitutive Androstane Receptor, gene NR1I3) play critical parts in xenobiotic detoxification by regulating the transcription of many genes involved in drugs metabolism and transport. Irinotecan is a drug broadly used in the treatment of colorectal cancer. The toxicity of irinotecan can differ between patients, and these differences may be due to an inter-individual variability of its metabolism. Irinotecan displays a complex metabolism leading to one active metabolite (SN38), and 3 inactive compounds (SN38G, APC and NPC). Most of the enzymes and transporters involved in its metabolism (CYP3A4, UGT1A1, CEs, ABCB1…) are target genes of PXR and CAR. It has been demonstrated that extrinsic modulation of NR1I2 and NR1I3 activity by anticonvulsive agents (phenobarbital, phenytoin, etc.) or hyperforin (in Saint John's wort) could affect irinotecan pharmacokinetic. Then we hypothesize that intrinsic variability of NR1I2 and NR1I3, supported by SNPs (Single Nucleotide Polymorphisms), could explain a part of the variability of irinotecan pharmacokinetics and toxicity. We studied the data of 109 colorectal cancer patients treated by FOLFIRI (Leuvocorin, 5FU, Irinotecan). We performed a non-compartimental analysis (WinNonLin™) to determine individual pharmacokinetic parameters of these patients: AUC for irinotecan and metabolites; metabolic ratio (MR) for each transformation (AUCMETABOLITE/AUCPARENT); and biliary index of Gugpta, regarded as a predictor of digestive toxicity ([AUCSN38* AUCIRINOTECAN]/AUCSN38G). Thirteen SNP (Single Nucleotide Polymorphism) of NR1I2, 7 SNP of NR1I3 and UGT1A1*28 were genotyped. Association tests were conducted between candidates SNP and pharmacokinetic parameters of irinotecan and metabolites according to 2 approaches: a single locus analysis (software R, package SNPassoc) and a haplotype analysis (software R, pakage haplo.stats). NR1I2-rs10934498 was associated with AUCSN38, with biliary index and with hematologic toxicity at cycle 1; this associations were significant even after adjustment on UGT1A1*28 genotype. NR1I2-rs1523127, NR1I2-rs3814055 were associated with APC metabolic ratio (AUCAPC/AUCIRINOTECAN) and with hematologic toxicity at cycle 1. NR1I2-rs2472677 was associated with toxicity at cycle 1, but not with any pharmacokinetic parameters, suggesting a specific effect on irinotecan metabolism on target tissues. Our results revealed that NR1I2 polymorphisms are part of irinogenetics. A larger validation cohort is needed to get more statistical power and to confirm our findings.

## Interaction between CYP2C19 polymorphisms and smoking modifies the CRP level of post-percutaneous coronary intervention patients treated with thienopyridine

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**Background**: Cytochrome P450 (CYP) 2C19 loss- and gain-of-function polymorphisms (CYP2C19*2 and CYP2C19*17) influence the response to the anti-platelet effects of both clopidogrel and prasugrel. Also, some, but not all, studies suggested that smoking positively modify response to clopidogrel, but not prasugrel. Moreover, post-procedural CRP level is a reliable predictor of post-PCI complications like in-stent restenosis.
**Objective**: This study sought to investigate whether the interactions of *2 and *17 with smoking are associated with the levels of P2Y12 receptor inhibition and C-reactive protein (CRP), in patients undergoing percutaneous coronary intervention (PCI) treated with aspirin and either clopidogrel or prasugrel.
**Design**: In total, 1128 patients with acute coronary syndrome who underwent a successful PCI were recruited. At one-month clinical follow-up, the interactions of smoking and CYP2C19 polymorphisms on the levels of on-treatment platelet reactivity, assessed by vasodilator-stimulated phosphoprotein platelet reactivity index (VASP PRI)

and CRP were explored in three metabolizing groups as follow: poor metabolizers (PM) (*2 carriers/*17 non-carriers); intermediate metabolizers (IM) (*2 carriers/*17 carriers or *2 non-carriers/*17 non-carriers); and ultrametabolizers (UM) (*2 allele non-carriers/*17 carriers).
**Results**: There was a significant difference in VASP PRI, but not CRP, levels between the three metabolizing groups ($p < 0.001$ and $p = 0.412$, respectively). The interactions of metabolizing status and smoking was significant for CRP (p=0.004) but not for VASP PRI (p=0.720).
**Conclusion:** Interaction between CYP2C19 polymorphisms and smoking modifies the on-treatment CRP level of post-PCI, on-thienopyridine patients. This effect seems to be independent to the level of P2Y12 receptor inhibition.