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Proceedings of the  
2009 Golden Helix  
Symposium<sup>®</sup>

"Pharmacogenomics:  
Paving the path to  
personalized medicine"

By  
Federico Innocenti  
and George P. Patrinos

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Pharmacogenomics:  
Paving the path to  
personalized medicine

**Proceedings of the 2009 Golden Helix Symposium®  
“Pharmacogenomics: Paving the path to  
Personalized Medicine”**

**Athens, Greece, October 15-17, 2009**

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# Proceedings of the 2009 Golden Helix Symposium® “Pharmacogenomics: Paving the path to Personalized Medicine”, Athens, Greece, October 15-17, 2009

Federico Innocenti and George P. Patrinos

## Introduction

The 2009 Golden Helix Symposium® “Pharmacogenomics: Paving the path to personalized Medicine” was held in Athens Greece. The Golden Helix Symposia® (<http://www.goldenhelixsymposia.org>) are 2-3 day scientific meetings that are organized every year on different topics in the field of human genomics and personalized medicine. These symposia aim to advance biomedical and life sciences by bringing together scientists within and across disciplines in the fields of human genomics and personalized medicine. This symposium series is named after the house of late Francis Crick (‘The Golden Helix’; 19/20 Portugal Place, Cambridge, UK) to emphasize their focus on human genomics and personalized medicine. Conference venues are usually major cities or summer retreats in the Southern Mediterranean or Middle East regions. Furthermore, these symposia aim to maximize information exchange and promote collaborative relationships between regional research institutes and research centers of excellence in the United States and Europe. Such information exchange and collaboration ties are strengthened by interactions between participants and lecturers, the latter being internationally renowned scientists, recognized leaders in their field.

The 2009 Golden Helix Symposium® consisted of 6 plenary sessions in which 27 papers were presented and one session, where 11 posters were presented, since not all participants have submitted abstracts. Sessions focused on: warfarin pharmacogenomics, cancer pharmacogenomics, pharmacogenomics of psychiatric diseases, pharmacogenomics of other diseases, such as cardiovascular disease, hemoglobinopathies, transplantation, and adverse drug reactions.

The program as well as the abstracts of the plenary and poster sessions that are submitted to this meeting are provided below, while a comprehensive meeting report of this symposium is also available [1].

## Plenary Session 1: INTRODUCTION

*Patrinos GP (University of Patras, Greece):* Translation of genetic knowledge into clinical practice: The expectations and realities of personalized medicines

*Innocenti F (University of Chicago, USA):*

Pharmacogenomics: an Odyssey

*Hodges L (Stanford University, USA):* PharmGKB: The Pharmacogenomics Knowledge base

*van Shaik R (Erasmus University Medical Center, the Netherlands)* Gene variants in tamoxifen metabolizing enzymes CYP2D6 and CYP2C19 predict breast cancer endocrine therapy outcome

*Marsh S (Pharmacogenomics Centre, Canada)* Irinotecan pharmacogenomics

*Joseph D (University of Guelph, Canada)* Polymorphisms of glutathione transferase genes and enzymes: implications for cancer risk and cancer treatment

*Innocenti F (University of Chicago, United States)* Early experience with genome-wide association studies in cancer chemotherapy

## Plenary Session 2: TRANSLATION OF PHARMACOGENOMICS INTO CLINICAL PRACTICE: THE WARFARIN PARADIGM

*Allan Rettie A (University of Washington, United States):* Pharmacogenetics of vitamin K antagonists and resistance to anticoagulants

*Deloukas P (Wellcome Trust Sanger Institute, United Kingdom):* A whole genome scan for polymorphisms influencing warfarin dosing

*Kamali F (Newcastle University, United Kingdom):* The International Warfarin Pharmacogenetics consortium

*Rane A (Karolinska University, Sweden)* Warfarin pharmacogenomics - Translation into clinical practice

*Michel Eichelbaum (Institute of Clinical Pharmacology Stuttgart, Germany)* Pharmacogenomic-guided drug therapy: Where do we stand in 2009

## Plenary Session 4: PHARMACOGENOMICS IN NEUROPSYCHIATRIC DISEASES

*Arranz MJ (Kings College Institute of Psychiatry, United Kingdom)* Pharmacogenetics and critical candidate genes involved in drug response for schizophrenia

*Kronenberg S (Schneider Childrens Medical Center of Israel, Israel)* Pharmacogenomics of anti-depressants

*Delanty N (Beaumont Hospital, Ireland)* Pharmacogenomics for epilepsy treatment

*Squassina A (University of Cagliari, Italy)* Pharmacogenomics of the response to mood stabilizers in bipolar disease

*Lesko L (Food and Drug Administration, United States)* Level of evidence to validate pharmacogenetic biomarkers

## Plenary Session 3: CANCER PHARMACOGENOMICS

*Dzadzadzuszko R (Medical University of Gdansk, Poland)* Response to EGFR inhibitors: A paradigm for integrating germ-line and somatic molecular determinants

## Plenary Session 5: PHARMACOGENOMICS OF OTHER DISEASES

- Patrinos GP (University of Patras, Greece)*  
Pharmacogenomics for hemoglobinopathies
- Thervet E (Hopital Necker, France)* Impact of heritable genetic variability on renal transplantation
- Niemi M (University of Helsinki, Finland)* Statin pharmacogenomics for cardiovascular patients
- Vasiliou V (University of Colorado, United States)*  
Pharmacogenomics: Aldehyde Dehydrogenases in human disease

## Plenary Session 6: ADVERSE DRUG REACTIONS

- Becker M (Children's Mercy Hospital, United States)*  
Identifying genomic causes of Adverse Drug Reactions in children

## Poster Session

- *Sims AM (Astra Zeneca, Macclesfield, United Kingdom), Abstract No 1.*
- *Jung'a JO (Institute of Primate Research, Nairobi, Kenya), Abstract No 2*
- *Topolcan O (Faculty of Medicine, Plzen, Czech Republic), Abstract No 3*
- *Dolžan V (University of Ljubljana, Ljubljana, Slovenia), Abstract No 4*
- *Balboa E (Universidad De Santiago de Compostela, la Coruna, Spain), Abstract No 5*
- *Chaido D (University of Athens, Athens, Greece), Abstract No 6*
- *Paré L (Hospital Sant Pau, Barcelona, Spain), Abstract No 7*
- *Ragia G (Democritus Univeristy of Thrace, Alexandroupolis, Greece), Abstract No 8*
- *Lezhava A (RIKEN, Omics Science Center, Yokohama, Japan), Abstract No 9*
- *Zukic B (Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia), Abstract No 10*

## PLENARY SESSIONS

### PharmGKB: The Pharmacogenomics Knowledge Base

#### Hodges L

The Pharmacogenomics Knowledge Base, or PharmGKB ([www.pharmgkb.org](http://www.pharmgkb.org)), is a free, public resource that provides online information about gene-drug-disease relationships to inform the contribution of genetic variation on variation in drug response. The knowledge base is built upon information from PubMed and other sources, as well as primary genotypic and phenotypic data submitted by affiliated partners, such as the International Warfarin Pharmacogenetics Consortium. Highlights of PharmGKB include highly curated drug-gene-disease relationships and genotype-phenotype associations, which are used to generate key drug-

centered biomolecular pathways (60 to date) and summaries of Very Important Pharmacogenes (42 VIPs to date). A new feature at PharmGKB is an updated user interface design to enable pinpointed searches of specific drug-gene-disease relationships. Recent additions to the website include information on Clinical Pharmacogenomics (PGx) and Well Known Pairs of Gene-Drug PGx Relationships (26 to date), which highlight clinically relevant drug-gene relationships and current government guidance for patient genotyping to inform personalized medicine. Lastly, PharmGKB, in its effort to provide current and relevant pharmacogenomic information, encourages users to register and offer comments at [feedback@pharmgkb.org](mailto:feedback@pharmgkb.org). PharmGKB is supported by NIH/NIGMS GM61374.

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### Pharmacogenomics guided therapy: Where do we stand in 2009

#### Eichelbaum M

Genetic factors have been suggested depending on the drug, to account for 20 to 95 % of the variability in drug disposition and effects. The best recognized examples of genetic polymorphisms that influence drug response in humans are highly penetrant monogenic traits of drug metabolizing enzymes (DME). Inherited difference in a single gene of DME has such a profound effect on the pharmacokinetics of a drug resulting in more than a 100 fold difference in systemic drug exposure with clinically important effect on drug response. Loss of function or gene duplication of DME genes have been identified as mechanisms of severe and life-threatening toxicity and poor treatment response, respectively. There is a growing list of genetic polymorphisms in drug transporters and targets that have been shown to influence drug response.

A major limitation in implementing pharmacogenetic testing in the clinical setting is the lack of clinical trials demonstrating that such testing can improve drug therapy by reducing toxicity and increasing efficacy. Randomized prospective studies are considered to be the gold standard. But we do not have the resources to carry these trials especially for the many old drugs on the market. Moreover due to inclusion and exclusion criteria they represent a highly selective patient population and do not represent the patient population seen in clinical practice. Thus there is a need to carry out both retrospective and prospective studies in a naturalistic clinical setting. The major limitations of the studies carried out so far is the poor definition of the phenotype, too small sample size, replication of the data in an independent cohort and incomplete genotyping of functional relevant variants. In addition non-genetic factors such as phenocopying and drug adherence which can profoundly modify drug response are often not taken into account. Furthermore drug response involves many genes and

therefore new strategies are needed to identify, for a given drug, the relevant genes and genetic polymorphisms and the pathways and processes in their interaction. These new strategies include genome-wide haplotype mapping, gene expression analyses and proteomic methods.

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### **Response to EGFR inhibitors: a paradigm for integrating germline and somatic molecular determinants**

*Dziadziuszko R*

Two classes of epidermal growth factor receptor (EGFR) inhibitors are currently used in clinical practice and evaluated in trials involving non-small cell lung cancer (NSCLC) patients: small molecule, orally available tyrosine kinase inhibitors (TKIs) and monoclonal antibodies. Erlotinib and gefitinib (EGFR TKIs) are used as monotherapy whereas cetuximab (monoclonal antibody) showed minor improvement in overall survival when added to front-line chemotherapy in a large phase III clinical study in NSCLC. EGFR TKIs have marginal efficacy when used in unselected NSCLC populations, but cause prolonged regressions and disease stabilizations in some patients, pointing towards specific genomic characteristics that is associated with their efficacy. To date, several somatic and germline molecular determinants of sensitivity to EGFR TKIs have been found. The somatic characteristics of increased efficacy include activating mutations in tyrosine kinase domain of EGFR gene, high EGFR gene copy number, high EGFR protein level, and absence of K-ras mutations in the tumor. The germline features include exon 1 polymorphism. The predictive value for the benefit from EGFR targeted therapies depends on the line of treatment used (i.e. first vs. second/third line treatment of advanced disease) and comparator treatment (chemotherapy vs. best supportive care). Currently, EGFR mutations appear to be the best predictors of survival benefit with EGFR TKIs in the first line setting as compared to chemotherapy, whereas high EGFR gene copy number appears to characterize those patients who achieve some benefit in pretreated patients who are not candidates for further cytotoxic treatment. The value of these markers in the adjuvant setting is not yet established. Ongoing clinical trials with EGFR inhibitors and biomarker correlative programs will be presented.

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### **Glutathione transferase polymorphisms: implications for cancer risk and cancer treatment**

*Joseph PD*

Glutathione transferases (GSTs; EC 2.5.1.18) catalyze the conjugation of glutathione (GSH) with electrophiles, such as quinones, epoxides, alkylating agents, and lipid peroxidation products. Since many of these substrates are cytotoxic and genotoxic, their conjugation to GSH is a cellular defense mechanism. GSH conjugation is expected to be an important aspect of the detoxication of several classes of cancer chemotherapeutic agents, such as

nitrosoureas, nitrogen mustards, and platinum compounds.

Large interindividual variations of GST enzyme expression have been observed in the human population. These variations result from a combination of genetic polymorphisms, differences in diet, and other poorly understood factors, such as gender, age, and drug or carcinogen exposure.

The human genome encodes at least 16 cytosolic GSTs, grouped into seven classes: Alpha, Zeta, Theta, Mu, Pi, Sigma, and Omega. Some GST genes are highly polymorphic. The frequencies of the null alleles of GSTM1 and GSTT1 are as high as 50% in some populations. Each of the 12 cytosolic GSTs resequenced by the Environmental Genome Project displays at least one non-synonymous coding region single nucleotide polymorphism (SNP), including the well-known I105V variant of GST P1-1.

Thiotepa, irinotecan, melphalan, busulfan, and cisplatin are among the cancer drugs that may be subject to polymorphic GSH conjugation. However, in contrast to enzymes such as P450 2D6, arylamine N-acetyltransferase, or thiopurine S-methyltransferase, GST polymorphisms are not usually associated with striking pharmacokinetic effects; this is probably due to the multiplicity of GST enzymes with overlapping substrate specificities. Many molecular-epidemiological studies have tested possible associations between GST polymorphisms and disease risk or response to therapy, but few consistent correlations have been found. A clearer understanding of GST pharmacogenomics requires a more complete examination of the effects of GST polymorphisms on enzyme expression, induction, and catalytic activity.

GST T1-1 catalyzes the detoxication of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and has glutathione peroxidase activity, but it also bioactivates haloalkanes, such as ethylene dibromide. We have expressed two of the eight reported non-synonymous SNPs of the GSTT1 gene as recombinant proteins in *E. coli*. GST T1-1 variant E173K showed reduced catalytic activity, altered substrate specificity, and improper protein folding. Such studies, which provide insight into the structure and function of GST enzymes, can identify SNP variants of possible pharmacogenomic importance. (supported by NSERC Canada)

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### **Early experience with genome-wide association studies in cancer chemotherapy**

*Innocenti F*

In clinical pharmacogenomics, genome-wide association studies (GWAS) have been so far usually applied to disease risk assessment. More recently, studies have been also conducted to discover novel gene variation that is associated with inter-patient differences in outcome of cancer chemotherapy. GWAS can be used for the discovery of novel heritable markers of patient survival, as well as the

side effects of chemotherapy, which can be severe in cancer patients. GWAS uses hundreds of thousands of heritable markers to scan the entire genome of patients. At the time of this meeting, one GWAS has been published in childhood lymphoblastic leukemia patients treated with chemotherapy (Yang et al., JAMA 2009). In study, minimal residual disease (MRD) at the end of the induction chemotherapy was used as the phenotypic endpoint. A SNP in the IL15 showed an association with MRD, and the authors also provide convincing mechanistic data (from ex vivo cytotoxicity and expression studies) to corroborate these findings. IL15 is a proliferation stimulatory cytokine that has shown protection from glucocorticoid-induced apoptosis, and its high expression has been associated with CNS relapse. This study had a validation cohort, and enrolled about 500 patients. Several other SNP candidates were located in intergenic regions and in genes that were not yet annotated.

Our group has also performed a GWAS in about 300 advanced cancer patients treated with gemcitabine (cytotoxic compound) with and without bevacizumab, and preliminary data have been recently presented (Innocenti et al., ASCO 2009). This study has identified a SNP in the IL17F gene that confers reduced overall survival. The association was of genome-wide significance, and our most significant SNP is H161R. Wild-type 161H interleukin-17F has demonstrated a strong anti-angiogenesis effect in vitro. The variant 161R form of interleukin-17F is a natural antagonist of the anti-angiogenic effects of wild-type interleukin-17F. As angiogenesis has been thought to play an important role in the growth and metastatic spread of pancreatic cancer, we hypothesize that the angiogenesis potential of tumors of patients with the variant 161R interleukin-17F is higher than tumors with wild-type 161H interleukin-17F, conferring worse prognosis. However, other possible and more complex mechanisms related to the pro-inflammatory effects of interleukin-17F cannot be excluded.

GWAS studies show the promise of moving from candidate genes approaches and providing an unbiased assessment of the role of heritable variation for cancer therapeutics.

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### **Irinotecan Pharmacogenomics**

#### Marsh S

Irinotecan (Camptosar®), a topoisomerase I inhibitor developed in the mid-1990s, is an anticancer drug approved in combination therapy for the treatment of advanced colorectal cancer. Response rates of combination therapy including irinotecan are significantly higher than those with the individual drugs alone. However, adverse drug reactions (ADRs) occurring from irinotecan treatment are prevalent, with up to 36% of patients experiencing severe, life-threatening toxicities including diarrhea and neutropenia. Multiple genes play a role in irinotecan pharmacokinetics and pharmacodynamics. Primarily the UGT1A1 enzyme has been strongly associated with these ADRs. A frequently

studied dinucleotide repeat polymorphism within the UGT1A1 gene promoter region ([TA]<sub>7</sub>TAA; UGT1A1\*28) is strongly correlated with neutropenia and diarrhea in cancer patients receiving irinotecan. This UGT1A1\*28 variant, leading to a detrimental effect on the clinical outcome, is common and occurs at a frequency of 30-45% in Caucasian and African populations, and approximately 15% in Asian populations. Recently, the US FDA has approved a genetic test for UGT1A1\*28, and has altered the Camptosar® package insert to take into account this genotype-toxicity relationship. However, the administered dose of irinotecan is directly related to the clinical utility of screening for UGT1A1\*28. In addition UGT1A1\*28 does not explain the whole story of irinotecan toxicity. Other variants within UGT1A1 and UGT1A gene family members, ABC transporters, and pharmacodynamic genes have been identified, which may, in part, explain the remaining ADRs.

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### **Pharmacogenetics and critical candidate genes involved in drug response for schizophrenia**

#### Arranz MJ

Using a candidate-gene approach, pharmacogenetic research has identified a number of genes related to variability in response to antipsychotic treatment. The most significant findings relate polymorphic variants in genes coding for cytochrome P450 group (CYP) metabolising enzymes with propensity to develop side-effects and level of response. Individuals possessing CYP poor metabolising variants are more likely to develop side-effects due to toxic accumulation of drug metabolites. On the other hand, individuals with rapid metabolising variants may fail to respond when given normal doses of drugs and may require higher doses to obtain clinical improvement. This knowledge can be used for the adjustment of clinical doses accordingly, resulting in higher treatment efficacy with lower adverse reactions. Additionally, pharmacogenetic research has confirmed the importance of dopaminergic and serotonergic in antipsychotic treatment. Findings of association between dopaminergic and serotonergic genetic variants with response variability have validated these systems as therapeutic targets. Less research has been conducted on other drug-targeted neurotransmitter systems. However, significant genetic findings indicate that adrenergic receptors may participate in the development of adverse reactions after treatment with antipsychotic medications. Other genes related to the aetiology of the disease, but not directly targeted by psychotropic medications (BDNF, COMT, NRG1) may contribute to treatment variability, indicating an indirect mechanism of action.

However, most reported associations have moderate genetic effects and therefore are of limited clinical value. Several algorithms combining information

from several genes have been developed, with an improved prediction level and therefore, increased clinical value. Nevertheless, currently available pharmacogenetic tests are very rarely used as prescription aid in psychiatry. Improved predictive levels, including information from clinical, environmental and genetic factors determining treatment response may increase the role of pharmacogenetic testing in everyday's clinical practice.

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### Pharmacogenetics of antidepressants

#### Kronenberg S

In 1998, Smeraldi et al.<sup>1</sup> published the first report on pharmacogenetics of antidepressants. They have reported that carriers of the 'L' allele of the 5-HTT gene, the serotonin transporter, were more likely to respond to the SSRI fluvoxamine. Many studies were published since, but two merit further discussion. The first is a meta-analysis conducted by Serretti et al.<sup>2</sup> In this meta-analysis, studies with a combined n of over 1,400 patients were analyzed. The results were impressive; the 'LL' and 'LS' genotypes conferred both favorable response and remission rates. The second is the largest pharmacogenetic study to date. The sample was collected in the course of the sequenced treatment alternatives to relieve depression (STAR\*D), a prospective, multicentre, randomized clinical trial involving over 4000 out-patients. The study, conducted by Kraft et al.<sup>3</sup> concluded by stating: "SSRI response in major depression is not determined by DNA variation at this [5-HTT] locus". It is difficult to reconcile between the results obtained by a well designed meta-analysis and the largest pharmacogenetic study as of yet. It is possible that if 5-HTT 'L' allele has a small effect size on the response to SSRIs, it may be completely hidden by cofounders. In addition, only lately it was discovered that the 'L' allele has a SNP that alters its activity to be similar to that of the 'S' allele<sup>4</sup>. Thus, the 'LA' allele functions similarly to the 'S' allele, while the 'LG' allele confers higher 5-HTT expression. Since only some of the recent studies further divide the 'L' allele into 'LA' and 'LG', it has become even more difficult to draw conclusions. At this time, the verdict on the pharmacogenetics importance of 5-HTT is still pending.

Other genes of interest are: TPH1; TPH2; 5HTR2A; ABCB1; GRIK2; GRIK4; CREB1; GRIA3 and FKBP5<sup>5</sup>. While TPH1, TPH2 and 5HTR2A are serotonergic genes, the other genes in this list go beyond this system. As our understanding of the genetic underpinnings of depression is growing, HPA-axis and signal transduction genes become increasingly more relevant.

One way of dealing with the problem of small effect sizes, is to consider the additive effect of two genes and more.

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<sup>1</sup> Smeraldi, *Molecular Psychiatry* (1998) 3:508–511,

<sup>2</sup> Serretti, *Molecular Psychiatry* (2007) 12:247–257

<sup>3</sup> Kraft, *Biological Psychiatry* (2007) 61:734–742

<sup>4</sup> Hu, *Am J Hum Genet* (2006) 78:815-826

<sup>5</sup> Kronenberg, *Pharmacogenomics* (2008) 9:1725-1736

Recently, Rotberg et al.<sup>6</sup> reported that carriers of both the 5-HTT 'S' allele and the TPH2 'T' allele in hetero- or homozygosity are expected to respond even more poorly than those patients who were genotyped only in the 5-HTT gene.

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### Pharmacogenomics for Epilepsy Treatment

#### Delanty N

Epilepsy is a heterogeneous group of brain disorders characterised clinically by the occurrence of recurrent unprovoked seizures. The epilepsies may be divided into generalised and partial (localisation-related) types, and have a variety of aetiologies, including both genetic predisposition and acquired insults such as CNS infection, head trauma, and stroke. Approximately one third of people with epilepsy have refractory drug-resistant epilepsy which may have a profound impact on quality of life of patients and their families. In addition treatment may be limited by the occurrence of dose-related and idiosyncratic adverse effects. Thus, the pharmacogenomic exploration of both epilepsy predisposition and treatment response may be a particularly fruitful avenue of study. This presentation will outline updated knowledge of current pharmacogenomic investigation in epilepsy. It will summarise prior single nucleotide polymorphism association studies, and outline ongoing whole genome association work. It will also focus on a number of areas of study exploring the pharmacogenomics of important treatment-limiting idiosyncratic adverse effects. The presentation will close with a description of some ongoing multi-centre collaborative studies in a number of important common epilepsies.

#### **Pharmacogenomics of the response to mood stabilizers in bipolar disease**

#### Squassina A

Bipolar Disorder (BD) is a chronic and often severe psychiatric illness characterized by manic and depressive episodes affecting 1-5% of the general population. BD is a leading cause of premature mortality due to a high rate of suicide and associated medical conditions. Among the most effective treatments, mood stabilizers represent the keystone in acute-mania, depression and maintenance treatment of BD. To date, Lithium (Li) is the most effective and first choice prophylactic treatment of BD. Chronic Li treatment has also been associated with a significantly reduced risk of suicide in patients with mood disorders.

Besides the high rate of BD patients showing an excellent response (~30-40%), a significant fraction

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<sup>6</sup> Rotberg, *Eur Neuropsychopharmacol* (2008) 18:S224

of patients do not respond or respond partially to the prophylactic treatment with Li.

It has been shown that inter-individual differences in drug response are modulated by a complex pattern of interactions between environmental and biological factors with a great genetic contribute. The role of genetics in Li response has been widely supported by family studies that investigated the heritability of the response across generations. Relatives of patients who respond to Li have an increased likelihood of responding to the treatment, thus suggesting the presence of a genetic mediation.

To date, pharmacogenetic strategies have been employed with the aim of dissecting the complexity of the genetic interactions involved in modulating response to mood stabilizers treatments particularly focusing on Li. Linkage studies performed on BD patients responders to Li, have identified some chromosomal regions that may predict the response to Li treatment (7q11.2, 18q23, 12q23-q24). However, no specific genes within these regions have been showed to be clearly involved in treatment response in further studies.

Candidate genes approaches have so far focused on genes codifying for elements of biological pathways shown to be target of Li, such as proteins of the intracellular second messenger cascade mediated by Inositol, the AMPc mediated pathway and the GSK3 $\beta$  protein.

Together with findings from gene expression studies, these data have provided intriguing insights into the understanding of the genetic burden of lithium response. To date, only one genome wide association study has been carried out employing a time to recurrence approach for defining the response to Li (Perlis et al., 2009).

This study pointed to polymorphisms in several chromosomal regions though none of them reached a genome wide significance threshold.

Taken together, these results strongly support the necessity of further efforts that will pave the path to personalized therapy for BD treatment and will provide the essential tools for the discovering of new therapies.

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#### **Level of Evidence to Validate Pharmacogenetic Biomarkers**

##### Lesko LJ

There is no consensus definition of what "clinical utility" is or is not; as a result the evidence needed to support "clinical utility" depends on the clinical context for using a biomarker and the individual stakeholder in biomarker decisions.

This presentation will discuss framework for defining "clinical utility" and will rely on real-life examples to demonstrate how evidence is context-specific.

There are several strategic pathways to validating biomarkers that differ in efficiency and levels of evidence.

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#### **Identifying Genomic Causes of Adverse Drug Reactions in Children**

##### Becker ML

Pediatric medicine has evolved over the last century to recognize that the pediatric patient is not just a "miniature adult", and both manifestation of disease and drug response often differ greatly in the pediatric population. Examples of differences in disease expression and drug toxicity will be discussed briefly. A brief review of ontogeny will follow. To consider what makes children different from adults when evaluating drug response and toxicity, one must take into account the concept of ontogeny. Pathways used for drug detoxification and elimination often overlap with endogenous compounds instrumental for normal growth and development. The role of development in drug response affects aspects of drug absorption, distribution, metabolism and excretion. For example, drug metabolizing enzymes change throughout fetal and postnatal life; the developmental expression of different pathways at different times may lead to variability in drug disposition and response. Developmental trajectories will be discussed and specific examples of the role of genotype and the differential expression of drug metabolizing enzymes will be reviewed.

The last portion of this talk will focus on an example of our approach to a clinical ADR question with pharmacogenomics. Methotrexate (MTX) is the most common second-line therapeutic agent used to treat Juvenile Idiopathic Arthritis (JIA) worldwide. There is significant variability in response and toxicity to this medication in both adults and children. Common side effects of this drug include gastrointestinal toxicity and hepatotoxicity, both of which can result in drug dose adjustment or discontinuation. Preliminary adult data suggest that MTX polyglutamation and folate gene polymorphisms may be useful for guiding therapy. I will report the population variability in total intracellular MTX (MTXglu<sub>tot</sub>) concentrations and patterns of intracellular MTX polyglutamate subtypes (MTXglu<sub>1-7</sub>) in JIA patients receiving a stable dose of MTX. We have identified clinical contributors to this observed variability, and further associations with genotype, specifically of genes within the folate pathway are also being investigated. Future directions of our investigations with MTX will be discussed.

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#### **Pharmacogenomics for hemoglobinopathies**

##### Patrinos GP

The switch from embryonic  $\gamma$ -globin to adult  $\beta$ -globin production is a complex process, involving many factors and regulatory elements, which has not been fully elucidated yet. The application of pharmacogenomics in hemoglobinopathies therapeutics is particularly attractive because of the limited therapeutic possibilities presently available

and narrow therapeutic fetal hemoglobin (Hb F)-inducing agent index. The current state-of-the-art in the field of pharmacogenomics for  $\beta$ -type hemoglobinopathies therapeutics will be presented. In particular, an update on the correlation between the inter-individual variations and the therapeutic outcome relevant to Hb F levels in hemoglobinopathies patients will be provided, along with an attempt to delineate the increased Hb F levels with novel genetic defects in the  $\gamma$ -globin gene regulatory regions. Also, results from genome-wide transcription profiling in patients with  $\beta$ -type hemoglobinopathies who do or do not respond to hydroxyurea treatment and downstream functional assays for data verification will be presented in relation to gene discovery for reactivation of  $\gamma$ -globin gene transcription. Also, in an attempt to shed light on the events and genes involved in this maturation process, we established conditions for the generation of primary erythroid precursor cultures from human fetal liver, umbilical cord blood (high HbF levels), and adult peripheral blood (low HbF levels) and assessed using whole-genome transcription profiling differences in expression levels. Detailed analysis of the genes that exhibit differential patterns of expression will provide more clues as to the regulation of the human globin switching process.

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#### **Pharmacogenomics: Aldehyde Dehydrogenases in Human Disease**

*Vasiliou V*

The aldehyde dehydrogenase superfamily contains NAD(P)<sup>+</sup>-dependent enzymes that catalyze the oxidation of aldehydes to their corresponding carboxylic acids. Nineteen aldehyde dehydrogenase genes have been identified in the human genome. Mutations in these genes and subsequent inborn errors in aldehyde metabolism are the molecular basis of several diseases, including Sjögren-Larsson syndrome, type II hyperprolinemia,  $\gamma$ -hydroxybutyric aciduria and pyridoxine-dependent seizures. Aldehyde dehydrogenase enzymes also play important roles in embryogenesis and development, neurotransmission, oxidative stress and cancer.

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#### **Poster Session**

##### **Abstract No 1.**

##### **Genotyping and Phenotyping of Butyrylcholinesterase in Drug Development**

*Sims AM, White R, Jolly N, Surry D, Pinel T, Brealey C, Goodall R*

Butyrylcholinesterase (BChE) is an important drug metabolising enzyme in plasma known to be responsible for the metabolism of several xenobiotics including cocaine, heroin and organophosphate. Pharmacogenetic and phenotypic variation of the enzyme, have been studied in detail because individuals with low BChE activity can display prolonged apnoea and paralysis

following administration of the muscle relaxants suxamethonium and mivacurium. In addition, BChE has been used in drug design strategies for pro-drugs and ante-drugs given its very high turnover numbers with these drug substrates and ability to contribute to very rapid metabolic conversion to the active drug or inactive metabolite. Clearly, there is a need to investigate the extent to which pharmacogenetic differences in the gene encoding BChE (BCHE) contribute to variation in exposure to these drugs. In this study we have assembled data from a panel of healthy volunteers and from individuals with known phenotypic variation in BChE. BChE activities and genotypes were determined and these observations correlated with the metabolic turnover of a novel ante-drug development candidate. There was a wide range of BChE activity and this correlated well with the metabolic turnover of the ante-drug. Genotyping of individuals allowed separation of the range of BChE activity with genotype and indicated that, for the low to intermediate range, the ante-drug substrate was less affected by decrease in enzyme activity than the model substrate butyrylthiocholine.

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##### **Abstract No 2.**

##### **Exogenous stress affects hepatocyte proliferation in-vitro**

*Jung'a JO, Mitema SO, Gutzeit HO*

Aquatic animals inhabit very unstable natural environments, and are also always in danger of exposures to intermittent variable toxic man made substances, which often impair their normal tissue and cellular homeostasis. Cellular protein chaperones otherwise known as Heat Shock Proteins (HSPs) have been described to play very important roles in maintenance of cellular homeostasis. The aim of this study was to establish and validate an in-vitro cellular bioassay test system for analysing HSP70 expressions in Nile tilapia (*Oreochromis niloticus*) from Lake Victoria, a tropical freshwater fish of great ecological and economical importance. The suitability of the established in-vitro primary hepatocyte cultures from *Oreochromis niloticus* for HSP70 protein analyses was evaluated by microscopy, flow cytometry and western immunoblots followed by enhanced chemiluminescence, (ECL) method. The effects of temperature and toxic concentrations of cadmium, copper and zinc on in-vitro expressions of HSP70 were studied using one-dimensional SDS-PAGE followed by western immunoblots and the proteins detected using ECL method. Inductions of HSP70 in *O. niloticus* using sub-lethal concentrations of cadmium and estimated 96-h LC50 values for p-DDE and  $\beta$ -HCH in tilapia were studied using Quantitative reverse transcriptase coupled with polymerase chain reaction (RT-PCR). The results obtained using light and electron microscopy followed with single-cell flow cytometric DNA counts showed that nearly 90% of the

hepatocytes seeded at a density of 2.5X10<sup>5</sup> cells per well did not significantly proliferate ( $p < 0.05$ ) but remained viable for at least three weeks with fetal calf serum (FCS) supplement, which also resulted into increased culture longevity.

Mixed primary hepatocyte cultures expressed consistent levels of albumin, a liver-specific protein and basal HSP70. Western immunoblot analyses of the effects of heat shock on the in-vitro cultures showed a transient but increased accumulation of HSP70 to 200% compared to controls set at 100% eight hours post treatment ( $p < 0.05$ ). Cadmium ions (Cd<sup>2+</sup>) induced increased expression of HSP70 to 170% at concentrations of between 20-30  $\mu$ M ( $p < 0.05$ ). However, copper and zinc ions only showed an increased induction of HSP70 at a higher concentration (100  $\mu$ M), where Cd<sup>2+</sup> exhibited toxic effects. Quantitative RT-PCR analyses of liver HSP70 mRNA expressions from cadmium, p-DDE and  $\beta$ -HCH exposed fish confirmed the results of the in-vitro western immunoblots. However, only inductions by cadmium resulted into statistically increased expressions of HSP70 mRNA ( $p < 0.05$ ).

The results of these studies revealed that mixed in-vitro liver primary cultures are a good bioassay model system for assessing cellular toxicity but the use of HSP70 alone as a marker of toxicity in wild tilapia populations may be misleading due to the presence of high basal levels of HSP70. Furthermore, HSP70 induction appeared to be dependent upon the toxic substance used. The degree of HSP70 expressions appeared to be stressor-specific as observed in the enhancement of the heat shock-induced synthesis of HSP70.

The different HSP70 species that showed enhanced induction upon stress exposure resembled the HSP pattern that is characteristic for secondary exposure effect and not for the observed initial heat shock expression.

Such results support the notion that low doses of toxic chemical compounds that induce increased expressions of molecular chaperones may, under certain conditions, have beneficial effects related to a stimulation of endogenous cytoprotective mechanisms.

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#### **Abstract No 3.**

##### **Significance of Methylation Status and Expression of mRNA of RECK in Lung Tissue of Patients with NSCLC**

*Topolcan O, Pesta M, Kulda V, Safranek J, Vrzalová J, Treska V, Holubec L*

Reversion-inducing cysteine-rich protein with Kazal motifs (RECK) is a glycoprotein which negatively regulates the activity of matrix metalloproteinases (MMPs). One mechanism of RECK expression regulation is promoter methylation. In our study, we analyzed relation among promoter methylation status, level of RECK mRNA expression and disease free interval (DFI) and overall survival of patients with non-small cell lung cancer (NSCLC).

Methylation status of RECK promoter and expression of RECK mRNA were analyzed in paired tissue samples (tumor and control) of 50 patients with NSCLC who had

undergone surgery in the years 2005-2007. Methylation status of RECK promoter was assessed using methylation specific PCR. Level of RECK mRNA expression was measured using the RT real-time PCR method.

We observed significantly lower expression of RECK mRNA in NSCLC tissue in samples with positive methylation promoter RECK status in comparison with samples with negative methylation promoter status ( $p=0.0400$ ). We recorded lower expressions of RECK mRNA in NSCLC tissue vs. normal tissue ( $p=0.0032$ ). There was significantly lower expressions of RECK in epidermal carcinoma tissue in comparison with adenocarcinoma tissue ( $p=0.0051$ ). We found significant differences in expressions of RECK in stage IB-IIIa in comparison IA ( $p=0.0455$ ). There were no statistical significance in relation of RECK expression and DFI or OS and also no significance in relation of methylation status of RECK and DFI or OS.

Our results show that the RECK mRNA expression is down regulated by RECK promoter methylation in NSCLC tissue. RECK could be classified as tumor suppressor gene and is an interesting target of further research of MMPs inhibitors.

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#### **Abstract No 4.**

##### **Pharmacogenetics – a step further towards personalized DMARD treatment of rheumatoid arthritis**

*Dolžan V, Bohanec Grabar P, Logar D, Rozman B*

Rheumatoid arthritis (RA), a chronic autoimmune disease, leads to progressive joint deformation, dysfunction and disability. Treatment with disease modifying antirheumatic drugs (DMARDs) such as low dose methotrexate (LD-MTX) and leflunomide reduces pain, improves function and prevents or retards disease progression. Good response is achieved in only half of patients on either agent and a range of toxicities is reported. The risk for failure on a particular drug may in part be due to inter-individual variability due to genetic polymorphisms in metabolic pathways associated with specific drugs. The aim of our study was to identify pharmacogenetic predictors of response to DMARDs. Among RA patients studied 211 were treated with LD-MTX and 105 with leflunomide. Disease activity score of 28 joints count (DAS28) was assessed and toxicity recorded from patients' files. TaqMan SNP genotyping assays were used to genotype selected polymorphisms in folate and adenosine pathway and in genes linked to leflunomide action.

We confirmed the association of SNPs in folate transporter genes SLC19A1 A80G and ABCB1 C3435T with overall LD-MTX toxicity ( $p=0.039$  and  $p=0.032$ , respectively). MTHFD1 1958G allele conferred to low disease activity ( $p=0.021$ ).

Similar to previous reports on adenosine pathway AMPD1 34T conferred to low disease activity ( $p=0.012$ ) while ATIC 347G allele increased the risk

for LD-MTX toxicity ( $p=0.024$ ). Our study also identified the first pharmacogenetic predictors of leflunomide toxicity. CYP1A2 -163CC genotype associated with low inducibility increased leflunomide toxicity ( $p=0.002$ ). Also a SNP causing Lys7Gln substitution in leflunomide target enzyme DHODH increased leflunomide toxicity ( $p=0.005$ ). In conclusion, our results suggest that pharmacogenetic markers could be used in directing treatment choices in RA in clinical practice.

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**Abstract No 5.**

**High incidence of a TYMS exonic polymorphism in stage II/III rectal tumor tissue**

*Balboa E, Duran G, Lamas MJ, Gomez-Caamaño A, Celeiro-Muñoz C, Carracedo A, Barros F.*

Thymidylate synthase (TYMS) is the major target enzyme for the fluoropyrimidines metabolism. Tandemly repeated (TSER) sequences on TYMS gene enhancer region are polymorphic in humans and different among ethnic groups. That polymorphism has been associated to enzyme expression and related to response to 5FU and capecitabine treatment, but current literature about this link show contradictory results. We have detected a polymorphism at codon 15 in the TYMS gene. A T base in TYMS exon 1 position 43 instead of the normal C base leads to an aminoacid exchange (pro/ser).

The heterozygosity of that polymorphism in spanish population is low (0.05), but rises to near 0.60 in rectal tumoral tissues.

We found no relationship between this polymorphism and the response to 5-FU and capecitabine based neoadjuvant treatment in locally advanced rectal cancer, however the aminoacid exchange might be affecting the expression of TYMS in tissue and modulating the influence of the other polymorphisms, thus impacting on the conflicting results reported in the literature.

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**Abstract No 6.**

**Relationship Between a Polymorphism of the e-NOS Gene and Myocardial Infarction in a Subgroup of Greek MI Patients**

*Chaido D, Drakoulis N, Cokkinos DV*

The Glu298Asp polymorphic variant of endothelial nitric oxide synthase (e-NOS) has been associated with myocardial infarction (MI), but data relating to this variant are lacking, especially in Greece. Accordingly, we examined a possible association between the Glu298Asp polymorphism of the e-NOS gene and MI in a subgroup of the Greek population.

The study population consisted of 204 patients with a history of MI (Group A) and 197 control subjects without a history of MI and/or angina, clinical evidence of coronary heart disease, and a normal electrocardiogram (Group B).

All subjects were selected from the general population of the greater Athens area. Age and BMI were not different between groups, while males were 89% in Group A versus

74% in B ( $p<0.0028$ ). From each participant genomic DNA was extracted from peripheral blood leukocytes. The e-NOS Glu298Asp polymorphism was determined by Real-Time Polymerase Chain Reaction (RT-PCR) with melting curve analysis of PCR products from acceptor (5'-end-labeled) and donor probes (3'-end-labeled with fluorescein) specific for the polymorphism.

The frequencies of the Glu298Asp (GG, GT and TT) genotypes were present in 83 (40.7%), 94 (46.1%), 27 (13.2%) of the 204 patients with MI; and 94 (47.7%), 90 (45.7%), 13 (6.6%) of the 197 healthy control subjects, respectively.

The risk for MI in Glu298Asp TT was 2.159 (95% confidence interval [CI], 1.079 to 4.319),  $p=0.0403$  versus GG+GT and 2.352 (95% CI, 1.140 to 4.855),  $p=0.0293$  versus GG.

The risk for the T allele was 1.36 (95%CI, 1.01 to 1.83),  $p=0.0473$  versus G allele.

This risk was higher when the genetic factor was combined with other risk factors, such as age < 60 years and cigarette smoking.

Smoking increased the odds ratio (OR) by 3.962 (95% CI: 1.412-11.11,  $p = 0.0058$ ) for TT versus GG and by 3.222 (95% CI: 1.188-8.741,  $p = 0.0276$ ) for TT versus GG and GT combined, suggesting a significant interaction between smoking and e-NOS gene polymorphism for MI. However, no association between the variant 298Asp allele and risk of MI was observed in non-smokers (OR = 1.030, 95% CI: 0.2579-4.116,  $p = 1.000$ ).

This study indicates that Glu298Asp polymorphism of the e-NOS gene probably increases the risk for MI in the Greek population, especially when combined with smoking, suggesting a gene-environment interaction.

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**Abstract No 7.**

**VEGF pathway genes: novel functional variations**

*Paré L, Evans P, Duan S, Kashyap S, Zhang W, Dolan ME, Innocenti F.*

Vascular endothelial growth factor (VEGF), its receptors and signaling effectors play a central role in tumor-induced angiogenesis. Targeting tumor angiogenesis is an established antitumor therapy. Studies on germline variation of VEGF have proposed that heritable variation might predict efficacy of angiogenesis inhibitors as well as predisposing factors for cancer. However, very little information is available on variation of genes in the VEGF pathway and their molecular effects on gene expression. Our objective was to identify novel genetic information in the VEGF pathway and functional single nucleotide polymorphisms (SNPs) that are associated with changes in gene expression.

Twenty-three genes were prioritized based upon their biological significance in endothelial function and VEGF signaling. These genes were resequenced, with full coverage for ATK1, FRS2, KRAS, MAPK11, MAPK3, NRAS, PGF, PIK3R5, VEGFB and standard

coverage for CRK, FLT1, GRB2, ITGAV, ITGB5, MAP2K6, MAPK1, MAPK14, NRP1, PIK3C2A, PIK3C2B, PRKCA, PRKCE, RAF1. Unrelated HapMap DNA samples from the African Yoruba (YRI) people (n=24) and from European CEPH families (CEU) (n=23) were used for resequencing conducted through a grant obtained by the NHLBI Genotyping and Resequencing Service.

In the same HapMap samples, mRNA expression analysis was performed using the Affymetrix GeneChip Array in lymphoblastoid cell lines (LCLs). This exploratory analysis used a linear regression in PLINK to select common SNPs (minor allele frequency > 5%) significantly associated with mRNA expression in each population.

LD analysis was conducted on all the SNPs identified for each gene by using the Haploview. Genomic positions 10 million SNPs from NCBI dbSNP130 were retrieved and tested to search for SNPs within the TFBS conserved regions (TFBS Conserved track) and miRNA binding sites (TS miRNA sites track) in the UCSC genome browser.

We identified a total of 2,691 variants, of which 2,062 were not previously available in dbSNP build 128. Overall, 119 SNPs showed significant associations with mRNA expression in the CEU or YRI cell lines. Only PIK3C2B, PIK3R5, and PRKCE had SNPs associated with expression in both CEU and YRI.

28 SNPs were found in putative TFBS conserved regions and 5 SNPs were located in the miRNA regulatory sites predicted by TargetScan software.

We discovered novel functional SNPs in the VEGF-pathway genes that are associated with the variation of gene expression in HapMap LCLs.

This work provides fundamental information to select SNPs for further testing in molecular and clinical genetic studies of angiogenesis inhibitors and cancer risk in patients of different ethnicities.

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#### **Abstract No 8.**

#### **CYP2C9 and Kir6.2 gene polymorphisms in association with sulphonylurea-induced hypoglycaemia in type 2 diabetic patients**

*Ragia G, Petridis I, Christakidis D, Tavridou A, Manolopoulos VG.*

Hypoglycaemia is a common adverse effect of sulphonylurea oral hypoglycaemic agents. CYP2C9 mediates the metabolism of all sulphonylureas, while Kir6.2 is the internal subunit of the receptor through which sulphonylureas promote insulin secretion. Impaired metabolism of sulphonylureas due to gene polymorphisms of metabolic enzyme CYP2C9 or altered sulphonylureas-receptor properties due to Kir6.2 gene polymorphisms might lead to hypoglycaemia. In the present study we explored the association of CYP2C9 variant alleles CYP2C9\*2 and CYP2C9\*3 and Kir6.2 E23K amino-acid substitution with the incidence of hypoglycaemic events in patients with Type 2 Diabetes Mellitus (T2DM) receiving the sulphonylureas glimepiride and gliclazide. 92 T2DM patients receiving sulphonylurea and reporting drug-associated hypoglycaemia and 84 T2DM patients receiving sulphonylurea and having never experienced hypoglycaemia were included in the study.

CYP2C9\*2, \*3 alleles and Kir6.2 E23K substitution were detected by use of PCR-RFLP analysis.

Eleven out of 92 subjects (12%) experiencing hypoglycaemia carried the CYP2C9\*3 allele, as opposed to only 1 out of 84 subjects (1.2%) free of sulphonylurea-induced hypoglycaemia. In a model adjusted for age, BMI, mean daily dose of sulphonylurea, duration of T2DM and renal function, CYP2C9\*1/\*3 genotype increased the hypoglycaemia risk in response to sulphonylurea (odds ratio: 1.687; p=0.011). However, no differences in CYP2C9\*2 allele frequency between the two groups were found. Kir6.2 E23K polymorphism was equally distributed between two groups and did not affect the sulphonylurea-induced hypoglycaemia risk.

The presence of CYP2C9\*3 allele increases the risk of hypoglycaemia in sulphonylurea treated T2DM patients, possibly due to impaired metabolism of these drugs. CYP2C9 genotyping might thus be a useful tool for predicting adverse effects caused by sulphonylureas and help clinicians in safer prescribing of oral hypoglycaemic agents.

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#### **Abstract No 9.**

#### **Rapid screening of clinical samples for codon-specific mutations by the Smart Amplification Process**

*Lezhava A*

We developed modified SmartAmp2 assays that enabled detection of any change in a single codon using a single assay. Rapid SMAP-2 screening assays are suitable for routine clinical identification of critical amino acid substitutions such as codon 12 mutations in KRAS. Primers bracketing the first two nucleotides of KRAS codon 12 were designed so that all possible alleles would be amplified by the SmartAmp2 assay. In combination with the peptide nucleic acid (PNA) with exact homology to the wild-type allele, our assay amplified all mutant alleles except for the wild-type sequence. With this new assay design (termed PNA-clamp SmartAmp2), we could detect KRAS mutations within 60 minutes, including sample preparation. We compared results from PNA-clamp SmartAmp2 assay, polymerase chain reaction-restriction fragment length polymorphism, and direct sequencing of clinical samples from pancreatic cancer patients and demonstrated perfect concordance. The PNA-clamp SmartAmp2 method is a rapid, simple, and highly sensitive detection assay for cancer mutations.

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#### **Abstract No 10.**

#### **Fine tuning of human TPMT gene transcription: role of promoter VNTR polymorphism**

*Zukic B, Pavlovic S.*

Thiopurine S-methyltransferase (TPMT; EC 2.1.1.67) represents one of the examples of pharmacogenetics

applied for individualizing drug therapy. TPMT enzyme catalyzes inactivation of thiopurine drugs, such as 6-mercaptopurine, widely used in treatment of various diseases.

TPMT activity is polymorphic and trimodal distribution has been demonstrated in Caucasians. Low, intermediate and high methylator groups has been defined, but there is no clear separation between groups. Patients with inherited TPMT deficiency, expressing low or intermediate TPMT activity, do not tolerate standard doses of thiopurines and they need a dose reduction to avoid toxicity. Level of TPMT enzyme activity is essential for balance of therapeutic and toxic effects of thiopurines. The majority of TPMT polymorphisms, correlated to decreased TPMT activity, represent SNPs. There are polymorphisms that influence different TPMT gene expression. The major candidate for potential additional influence to TPMT activity is promoter of this gene.

Promoter of TPMT gene is a TATA-less one, polymorphic, containing a variable number of tandem repeats (VNTR), from 3 to 9. There are three types of repeats: A, B and C, all GC-rich. The architecture of repeats, AnBmC, is always maintained.

We investigated the influence of number and type of promoter tandem repeats on transcription of human TPMT gene. We performed functional analysis of tandem repeats by introducing various VNTR CAT constructs into K562 cells, and analyzed interaction of VNTR and transcription factors by EMSA and supershift assays.

We have confirmed that transcription factors Sp3 and EKLF directly bind to VNTR region. Functional assays revealed that TPMT promoter with the highest activity was the one with VNTR\*4b type (AB2C).

Promoters with 5, 6 and 7 VNTR alleles all had successively lower activities. VNTR\*8 activity was two times higher than activity of VNTR\*7 types. We found differences in activity between the constructs containing the same number, but different type of tandem repeats. The most prominent difference was observed between VNTR\*4 variants (A2BC and AB2C). We have shown that number and type of VNTRs in the TPMT gene promoter determine level of TPMT transcription. VNTR architecture (distance and configuration from A to C repeats) probably spatially and in sequence specific manner, modulate TPMT transcription. VNTR region may be responsible for diversity in TPMT activity among defined groups of TPMT methylators.

Further investigations will reveal if VNTR region of *TPMT* gene could be considered as a pharmacogenetic marker and confirm if it could be of clinical importance for guiding thiopurine therapy.

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