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CELL MODELS IN PHARMACOGENOMIC RESEARCH

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BACKGROUND-AIM

The potential of pharmacogenomic (PGx) research is to improve general health care by on one side reducing adverse drug reactions (ADRs) and on the other side by increasing the treatment efficacy. However, a lot of factors are affecting progress in PGx field for example: the need for large clinical populations of treated patients and control/placebo-treated cohorts; the difficulty in evaluating drug response; the interactions of underlying biochemical pathways (in either adverse or therapeutic drug effects) are often not fully understood etc. Therefore, the use of cell models would enormously decrease the time and costs of PGx research. Three steps where cell models could improve PGx research are: i) identification of PGx markers before clinical studies; ii) explanation of biochemical pathways of drug distribution, metabolism, elimination as well as therapeutic and adverse effects and iii) the pharmacokinetic evaluation of drug distribution, metabolism, elimination needed for development of dosage algorithms including PGx data.

METHODS

Methods such as genome wide association studies (GWAS) or sequencing have greatly facilitated the identification of gene loci and variations and have contributed to selection and rational introduction of genetic variation into clinical studies. In addition, the experiments on the cells or animals remain necessary in order to explain the function of such genes and variations. In cell models, usually plasmid like methods are used to investigate gene regulatory variations, while gene knock out, silencing or overexpression methods are used to investigate gene function and involvement in drug metabolism.

RESULTS

We have seen an example of OCT1, which was shown to be responsible for the cellular uptake of imatinib and therefore relevant for the success of the CML therapy, but imatinib was also shown not to be a substrate of OCT1 at all. Recently novel technology CRISPR/Cas9 allows for a relatively easy and quick disruption of genes and we are pursuing the implementation of this technology in elucidation of imatinib active transport mechanism which is responsible for the uptake of this drug in to the target cells and thus for its therapeutic efficiency.

CONCLUSION

The new and emerging methodology will provide ever more reliable and perhaps even quantitative information on the clinical relevance of particular genetic variants.

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THE EFFECT OF CEA OVEREXPRESSION ON 5-FLUOROURACIL-INDUCED APOPTOSIS AND AUTOPHAGY IN COLORECTAL CANCER CELLS

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BACKGROUND-AIM

Carcinoembryonic antigen (CEA) is the most frequently used tumor marker in colorectal cancer (CRC). An increase in serum CEA concentration after surgery in CRC patients has been considered as signal of tumor recurrence. Previously, it has been shown that CEA overexpression increase resistance to anticancer drug 5-fluorouracil (5-FU) in CRC cells. In the present study the effect of CEA overexpression on 5-FU-induced apoptosis and autophagy in CRC cells was investigated.

METHODS

The Chinese hamster ovary (CHO) cell line and human colorectal cancer cell line SW742 were stably transfected with pcDNA3.1 (+) containing full length human CEA cDNA using calcium-phosphate co-precipitation and electroporation methods, respectively. CEA-expressing clones were obtained after G418 selection. The CEA content of transfected cell lines was determined by ELISA kit and the corresponding band of CEA (180KD) was shown using western blot. The transfected cells were treated with 5-FU (250 μM) for 72 h. Apoptosis was detected using DNA fragmentation assay and was quantified through DNA content assay by flowcytometry. For the analysis of autophagy induction, the development of acidic vesicular organelles (AVO) was quantified using an inverted fluorescent microscope.

RESULTS

Transfected cells significantly express higher level of CEA than control parental cells. The results of DNA content assay showed that CEA transfected CHO and SW742 have a significantly lower apoptotic rate (71% and 79%, respectively) compared with the control untransfected cells. In both cell lines DNA fragmentation (hallmark of apoptosis) was more pronounced in control groups than CEA transfectants. The presence of ladder DNA in gel correlated well with the presence of cells with fractional DNA content detected by flowcytometry. Analysis of autophagy induction shows that there are no differences between 5-FU treated CEA-transfected and untransfected cells regarding to AVO formation.

CONCLUSION

Our findings demonstrate that CEA overexpression increased resistance to 5-FU treatment probably through inhibition of apoptosis. Additionally, CEA expression has no effect on 5-FU induced autophay. Inhibition of CEA expression may augment therapeutic effect of 5-FU based chemotherapy.

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GENOTYPIC AND FENOTYPIC CHARACTERIZATION OF CYP2C19 FROM A MESTIZA POPULATION IN COLOMBIA

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BACKGROUND-AIM

CYP450 system represents a family of enzymes that catalyze the metabolism of a wide variety of drugs, more than any other enzyme family consists of several isozymes and within which includes CYP2C19. This catalyzes the metabolism of several drugs commonly prescribed as diazepam, some barbiturates, tricyclic antidepressants, omeprazole and its structural analogues. The polymorphism of the gene transcribes populations divided into three phenotypic subgroups: extensive metabolizers (EM), intermediate metabolizers (IM) and poor metabolizers (PM). The vast majority of individuals EM require a dose four times greater than the PM, to achieve similar effects and serum concentrations of the drug. It is necessary to know the CYP2C19 gene polymorphism in a population because you can not generalize a treatment for a disease globally. This highlights the importance of personalized drug therapy, in order to provide treatment and optimal doses, improving drug response and reducing the adverse effects that might arise. Objective: To establish the frequencies of alleles *1, *2 and *3 of CYP2C19 gene and phenotypes according sorting genetic profile in a Colombian mestizo population.

METHODS

A descriptive cross-sectional study with a sample of 100 adults mestizos of Valledupar, Colombia. Genotyping was performed by PCR-CTPP, according to the technique of Yoshiko Ishida.

RESULTS

In the studied individuals dominated the native allele with 47% followed by 35% and 18% for mutations *2 and *3 respectively. At least 74% of the participants carried a copy of allele *1 in their genotype and not found homozygous subjects of *2 and *3. The frequency of phenotypes according inferred genetic profile were 70%, 16% and 14% for MI, EM and PM respectively. These results agree with those reported in mestizos from another city of the Colombian Caribbean (Barranquilla), but differs from that observed in other regions of country, which can be explained by the predominance of defective *2 allele in Africans (20%), whence derives mainly from the mestiza race of the Caribbean region of Colombia.

CONCLUSION

These findings constitute valuable information for doses of drug personalized adjusted to genetic profile of mestizos of the Colombian Caribbean.

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ESSENTIAL OIL OF TWO AROMATIC PLANTS AGAINST HOSPITAL STRAINS

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BACKGROUND-AIM

Plants are very used by the pharmaceutical industry because side effects of drugs so concerned users turn to natural treatments.
Ammoides verticillata is an aromatic plant belonging to the family of Apiaceae, commonly called Nounkha or Noukha, name comes from the Persian Nankhah (Nan=bread and khah=flavour) reffering to its use to flavor bread. Largely spread in North Africa, Ethiopia and Turkey.
The plant show several therapeutic effects and it's used as: diuretic, analgesic, carminative, anti-diarrheal, anti-histamine, febrifuge, anthelmintic and anti-asthmatic.
In Algeria, decoction of aerial parts used against flu and fever, and as fresh infusion with lemon slices -in hot season- to avoid infections.
Mentha pulegium (pulegium means repulse fleas) is also an aromatic plant belonging to the family Lamiaceae and widespread in northern Europe, the Mediterranean region and Asia, called by locals Fliou, it is a great emmenagogue, digestive, tonic and sudorific, to repulse insects, against colds and flu, reduce asthma attacks and regulate menstruation, used also in some culinary preparations to flavor sauces, desserts and drinks.

METHODS

Our work focuses on the activity of the essential oil of these two plants on hospital strains, for that the extraction was done by hydrodistillation, and the plants revealed an important yield (2.58% and 1.36% w/w respectively for Ammoides verticillata and Mentha pulegium).
The antimicrobial activity was revealed by aromatogramme against a sample of 130 strains.

RESULTS

The essential oil of Ammoides verticillata is active against 90.76% of the strains with +++ (3 crosses) index:
- 92.15% among Enterobacteriaceae are sensitive, given that 7.85% that are resistant represented by Pseudomonas aeruginosa;
- 81.25% of Staphylococcus aureus are sensitive;
- 91.66% of Streptococci are sensitive.

Mentha pulegium essential oil is generally moderately active against 83.84% of the strains with ++ (2 cross) index:
- For Enterobacteriaceae: 84.31% are sensitive;
- Staphylococcus aureus: 81.25% are sensitive;
- 66.67% of Streptococci are sensitive.

CONCLUSION

- The essential oils from Ammoides verticillata and Mentha pulegium have good activity against bacteria, with the use of contact method;
- The yields of essential oil from our plants are important;
- The chemical composition published by previous researches of these two plants open more possibilities to search for new biological activities.

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BUTYRYLCHOLINESTERASE (BCHE) GENOTYPING FOR POST-SUXAMETHONIUM OR MIVACURIUM APNOEA: EXPERIENCE OF A FRENCH CLINICAL LABORATORY.

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BACKGROUND-AIM

Butyrylcholinesterase (BChE) deficiency (OMIM 177400) is characterized by prolonged apnea after the use of muscle relaxants (suxamethonium – SC - or mivacurium – MI -) in patients who have mutations in the BCHE gene. It is an uncommon but serious adverse event, with an incidence estimated at 1 per 1,800 anaesthetic cases. Currently close to 70 natural mutations have been documented in human BCHE. Most of them have an adverse effect on BChE activity. This may occur either by deleterious effects of point mutations on catalytic functioning, or by point mutations that affect protein expression, which may result in an absence of BChE altogether. Here we report our experience in BChE deficiency exploration.

METHODS

Between January 2012 and December 2014, we genotyped 59 patients referred after prolonged post-succinylcholine or mivacurium apnea. Total serum BChE activity was measured with butyrylthiocholine iodide (BTC) as substrate on a Cobas® 6000 system (Roche Diagnostics, GmbH, Mannheim, Germany). High resolution melting-curve analysis were applied for genotyping Atypical-variant (c.293A>G, p.Asp70Gly, rs1799808) and Kalow-variant (c.293A>G, p.Asp70Gly, rs1803274). Additional DNA sequencing of BCHE coding regions was provided when the two-mutation screen was negative or inconsistent with enzyme activity or clinical history.

RESULTS

Genotyping identified 56 patients with BChE deficiency attributable to BCHE mutations. All except one presented a deficiency in BChE activity. The genotype of 48 patients was established by the two-mutation screen (detection rate: 86 %). Additional sequencing studies revealed five other mutations. Among them, four were not previously described nor were included in any mutation databases (7% of the patients). The most common genotypes abnormality were compound homozygous atypical-variant and homozygous Kalow-variant (n: 26, 46%) and compound homozygous atypical-variant and heterozygous Kalow-variant (n: 13, 23%). No difference of BChE activity was observed between the different genogroups. On the remaining three patients, two had normal BChE activity and gene, and one was diagnosed with BChE deficiency related to a liver transplantation.

CONCLUSION

A two-mutation screen approach can identify the BCHE mutations for nearly 90% of patients with post-SC or MI apnea. This approach which is cost-effective produced results in less than one week. Due to BCHE mutation heterogeneity, subsequent analysis must be realized when the two-mutation screen is inconclusive.

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CYTOCHROME P450 (CYP) GENE POLYMORPHISMS AND RESPONSE TO ESCITALOPRAM TREATMENT IN ELDERLY PATIENTS WITH LATE-ONSET DEPRESSION.

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BACKGROUND-AIM

Escitalopram is the selective serotonin reuptake inhibitor most commonly used for the symptomatic treatment of major depressive disorders. Escitalopram is inactivated by the polymorphic cytochrome P450 (CYP) 3A4 and 2D6 enzymes. Aim of this study is to investigate the relationships between CYP2D6 and CYP3A4 gene polymorphisms and the responder/non-responder phenotype to escitalopram treatment in patients with late-onset depression (LOD) attending a geriatric ward.

METHODS

85 patients with a clinical diagnosis of late-onset MDD according to DMS-IV-TR criteria were consecutively recruited at the geriatric unit of the IRCCS "Casa Sollievo della Sofferenza". The responder phenotype was defined as an observed reduction ≥50% on the HAM-D 21 score at six-months follow-up. The high-throughput analysis of five variants in the CYP3A4 gene and fifteen variants in the CYP2D6 gene was made by means of the Infinity analyzer (Autogenomics, Inc. Vista, CA, USA) using CYP3A4 and CYP2D6I assays according to manufacturer instructions. Genetic analyses were made in blinded fashion.

RESULTS

At follow-up 24 patients showed a responder phenotype whereas 61 patients showed a non-responder phenotype. No variants in the CYP3A4 genes were observed in both responder and non-responder patients. Conversely, several CYP2D6 variants were identified. No differences were observed in the distribution of CYP2D6 variants associated with a reduced enzyme activity (45.83% vs 52.46%; p=0.328) as well as those associated with a increased enzyme activity (11.48% vs 0%; p=0.079). These variants, however, were present only in NR patients.

CONCLUSION

If confirmed, our preliminary results suggested that the analysis of CYP2D6 gene may be useful in identify patients with LOD with different responses to escitalopram treatment.

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READY TO USE CE-IVD SMART ELISA KITS FROM SANQUIN REAGENTS FOR INFLIXIMAB AND ADALIMUMAB LEVELS CORRELATE WITH THE GOLDEN STANDARD AND CAN BE USED FOR TREATMENT OPTIMISATION IN PATIENTS.

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BACKGROUND-AIM

An increasing number of studies indicate that the success of treatment with anti-TNF therapy in patients depends on circulating levels of these drugs. For (cost-)effective and safe treatment, assays to reliably measure drug levels in patients therefore seem indispensable. The objective was to test the performance of the newly developed, ready to use (RtU) CE-IVD SMART ELISA kits from Sanquin Reagents for measuring circulating levels of adalimumab (ADL) and infliximab (IFX), and to verify its role in optimising the treatment of patients.

METHODS

The RtU complete SMART ELISA kits for ADL(Humira®) and IFX(Remicade®) were developed to measure levels in serum, Li-heparin and EDTA plasma. The drugs bind to recombinant TNF coated on the plate through anti-TNF antibodies. Subsequently, the drug is detected with drug specific HRP-conjugated monoclonal antibody. Controls are developed for the clinical low and normal concentration range. The performance of the new SMART kits was tested and we did a method comparison with the in-house test of Sanquin Diagnostic Services, the current golden standard.

RESULTS

Both kits cover the clinically relevant drug concentration range. For samples with concentrations within the clinically relevant area, all recommended dilutions (1:200, 1:1500, 1:2000) give similar results (%CV<10%). In addition, both intra- and inter-assay reliability are good (%CV<10%) as well as the precision of the SMART ELISA kits, including for the low (±1 μg/mL) and high control (±5 μg/mL) (%CV<10%). The new SMART kits highly correlated with the well-established tests of Sanquin Diagnostic Services (slope 0.9-1.1, correlation ~0.95). Concordance tables for drug concentrations in the low, adequate and high drug concentration range show that more than 95% of the samples were categorized identical.

CONCLUSION

This study shows that RtU CE-IVD SMART ELISA kits from Sanquin Reagents for ADL and IFX levels are highly reliable with results that are identical to the results from Sanquin Diagnostic Services. This makes the new SMART RtU CE-IVD ELISA kits from Sanquin Reagents an excellent tool for diagnostic laboratories to monitor drug levels in individual patients in order to achieve (cost-) effective, safe and personalised treatment .

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NEXT GENERATION SEQUENCING FOR TESTING PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA: A PRELIMINARY REPORT

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BACKGROUND-AIM

Familial hypercholesterolemia (FH) is a common Mendelian disorder associated with early coronary heart disease that can be treated by cholesterol-lowering drugs. Individuals with FH are at high risk of premature coronary artery disease, due to lifetime exposure to high levels of circulating Low Density Lipoprotein Cholesterol (LDLC). The presence of mutations in the LDL receptor (LDLR) gene, which is responsible for the cellular uptake of LDLC, is the most common cause of FH. Mutations in the apolipoprotein B and E (APOB-E) and proprotein convertase subtilisin/kexin type 9 (PCSK9) genes have also been described. In this study, we combined systematic clinical selection of hypercholesterolemic patients and next-generation sequencing (NGS) in order setup a NGS-based pipeline for the screening of HF affected individuals.

METHODS

DNA was obtained from 10 individuals with total cholesterol >230 mg/dl. NGS was performed on 454 GS Junior (Roche) using ADH MASTR kit (Multiplicom) capable to detect mutations in the coding and promoter regions of LDLR, PCSK9, APOE and part of the exon 26 of APOB genes. Data analysis was performed by an “ad hoc” bioinformatic tool developed in our lab.

RESULTS

Pathogenic mutations were found in 3 patients (30%). Mutations identified were: p.D221G in exon 4 of LDLR, p.K3449E in exon 26 APOB (that is a novel mutation, because not previously reported in literature) and, finally, p.L21_L22ins2L in exon 1 of PCSK9. One patient was found carrier of p.A391T, p.R3638Q, p.C130E and p.L21_L22ins2L variants in LDLR, APOB, APOE and PCSK9 genes, respectively.

CONCLUSION

In this preliminary study, with a single 454 GS Junior run, ADH MASTR kit allowed a definitive FH molecular diagnostic screening in 4 hypercholesterolemic patients. We underline that the lower cost and workload associated with NGS-based testing may increase access to this type of test above all in the context of population screenings. Finally, in presence of a precisely identified gene defect, targeted pharmacologic therapies, as PCSK-9-inhibitors (MTP-inhibitor lomitapide) and the ApoB synthesis inhibitor (mipomersen) can be used.

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MOLECULAR ANALYSIS AND IN SILICO CHARACTERIZATION OF TWO NOVEL BUTYRYLCHOLINESTERASE (BCHE) GENE MUTATIONS: P.LEU88HIS AND P.ILE140DEL.

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BACKGROUND-AIM

Butyrylcholinesterase (BChE) deficiency (OMIM 177400) is characterized by prolonged apnea after the use of muscle relaxants (suxamethonium or mivacurium) in patients who have mutations in the BCHE gene. Currently, close to 70 natural mutations have been documented in human BCHE. Here, we report the in silico characterization of two novel BCHE gene mutations: c.347T>A (p.Leu88His) and c.502_504delATT (p.Ile140del).

METHODS

The proband is a 32-year-old woman who presented a marked BChE deficiency activity (|BChE|: 1,344 U/L, reference interval: 4,260 – 11,250 U/L). Sequencing of the whole coding region of BCHE revealed that she harboured four different mutations in a compound heterozygous state. Two were the well-known atypical variant (c.293A>G, p.Asp70Gly, rs1799808) and the Kalow-variant (c.185C>T, p.Ala34Val, rs1803274). The others were not previously described: c.347T>A (p.Leu88His) and c.502_504delATT (p.Ile140del). The functional effects of these novel mutations were predicted using various programs: Provean, Mutation t@sting, Meta-SNP and PredicSNP. Structural theoretical models (one for each mutation alone and one with combined mutations) were created for variants through comparative modelling using the RaptorX server. The root mean square deviations (RMSDs) of the mutant structures with respect to the wild-type structure were calculated using Chimera 1.9 sofware.

RESULTS

All in silico prediction programs identified these mutations as potentially deleterious. According to Provean results, p.Leu88His mutation (score: -5.072, cut-off score for deleterious effect: -2.5) was as deleterious as the atypical variant (score: -5.559) and p.Ile140del was more deleterious (score: -12.256). The RMSD values of the modelled mutants indicated likely pathogenicity for all mutations (RMSD > 0.15). Disruption of the catalytic triad of the enzyme is observed in structural theoretical models variants with p.Ile140del mutation.

CONCLUSION

Overall, our observations prove that p.Leu88His and p.ILe140del variants are deleterious.

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COMPARISON OF TWO DIFFERENT IMMUNOASSAYS TO MEASURE LEVELS OF INFLIXIMAB AND AUTOANTIBODIES

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BACKGROUND-AIM

Tumor necrosis factor α (TNF-α) is a proinflammatory cytokine that is involved in many inflammatory processes, such as rheumatoid arthritis, Crohn's disease, ulcerative colitis or psoriasis. It has been found that the treatment with neutralizing antibodies for the cytokine improves patient´s quality of life with pain remission and clinical recovery.
Infliximab (IFX) is a monoclonal antibody anti-TNF-α indicated in some of these diseases, due to its ability to block the proinflammatory action of TNF-α. However, treatment is expensive, and it has been found that some patients have a poor response (among 20-40%), due to production of antibodies to the same drug, thereby decreasing viability and considerably reducing the effectiveness of treatments.
Aim: Evaluate the transferibility of results between two methodologies, both with technology enzime linked immunosorbent assay (ELISA), for measurement of Infliximab and antibodies to Infliximab (ATI)

METHODS

Methods: Sera concentrations of infliximab and ATI were measured by two different sandwich-ELISA assays, following the instructions of the manufacturers:
• Promonitor® IFX Determination of drug and anti-drug antibodies concentration (Menarini, Italy).
• NF Blocker monitoring and antibodies against TNF blocker (ImmuDiagnostik, Germany).
Patients: Serum samples were collected from 40 patients (24 male, mean age 45,3 years (SD:15,7) treated with Infliximab, and frozen at -80° C until measurement.
Statistical analysis: Passing-Bablok regression and Kappa statistic was performed using the MedCalc software.

RESULTS

Passing-Bablok regression showed differences between the two methods for Infliximab concentrations. These differences are directly proportional to drug concentrations. The regression equation was:
Immunodiagnostk= -8,4(IC95%:-583,5-45,2)+ 1,4(IC95%: 1,3-1,5)xPromonitor
To compare ATI, we used the Kappa statistic to categorical variables. We categorized ATI as positive or negative using the cut-offs predefined by manufacturers. We obtained a poor correlation between methods (κ = 0,45 (IC95: 0,1473-0,7551)).

CONCLUSION

Based on these results, the methods are not interchangeable. It would be necessary enlarge the sample size, and try to compare the results obtained with other methods commercially available, before making decisions

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TRAMADOL-BASED POST-OPERATIVE ANALGESIA IS BIASED BY CYP2D6 GENOTYPES

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BACKGROUND-AIM

Tramadol is an opioid analgesics commonly used to relieve pain after surgery. Tramadol is administered as inactive molecule, and is metabolized in the active form by the hepatic enzyme cytochrome P450(CYP)2D6. Aim of this study is to evaluate the influence of the functional polymorphisms in the CYP2D6 gene on the efficacy of tramadol-based protocols in post-surgical pain treatment.

METHODS

This was a prospective cohort study of 40 consecutive patients underwent thoracic/abdominal surgical operation and treated with tramadol-based protocols for post-surgical pain treatment. In prevision of post-surgical pain of mild (M1), moderate (M2) or severe (M3) pain, M1 patients underwent tramadol 200 mg, ketoprofen 320 mg, ranitidine 100 mg, metoclopramide 20mg in 48hrs, M2 patients underwent tramadol 400mg, ketoprofen 640mg, ranitidine 200mg, metoclopramide 40mg in 48hrs and M3 patients underwent the same protocol of M2 patients plus morphine 20mg in 48hrs. Levels of analgesia has been evaluated by means of the Verbal Numerical Rate (VNR) scale. At 24 hrs a blood sample was obtained from all patients. Genetic analyses of the 16 polymorphisms in the CYP2D6 was made using the INFINITITM Analyzer with the CYP4502D6-I Assay. Hierarchical longitudinal linear model statistic analysis was used to evaluate differences in the estimated means (±SE) of VNR scores as compared with CYP2D6-associated metabolizer phenotypes.

RESULTS

The analysis revealed that 18 subjects harbor CYP2D6 mutations possibly leading to an extensive metabolizer phenotype (EM), 17 subjects have mutations possibly leading to an intermediate metabolizer phenotype (IM), whereas 5 subjects show mutations possibly leading to a poor metabolizer phenotype (PM). The analysis reveal a significant difference in the response to post-surgical analgesia. The VNR estimated mean was significantly higher in IM than in EM subjects (3.332±0.191vs2.657±0.189; p=0.015),and in EM subjects as compared with PM subjects (2.657±0.189vs1.741±0.343; p=0.024). Accordingly, the VNR estimated mean was significantly higher in IM subjects than in PM subjects (p=0.002).

CONCLUSION

The analysis of the CYP2D6 gene may be useful to identify groups of patients with a different response to post-surgical analgesia.

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NOVEL THERAPIES FOR CANCER TREATMENT: DESIGNING HIGH AFFINITY AND SELECTIVITY LIGANDS AGAINST SIRT1

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BACKGROUND-AIM

The word 'sirtuin' (SIR) stands for Silent Information Regulator. SIRT1 is the most studied mammalian sirtuin and predominantly localises to the nucleus. Many sirtuins targets are involved in cancer and in many types of cancers, SIRT1 is found to be overexpressed. Recent observations support SIRT1 being both an oncogene and a tumour suppressor, depending on the cancer etiology and type of tissue. To answer the question "How can sirtuins function as both oncogenes and tumour suppressors?" we propose to develop highly selective ligands and study in a range of cancer cell lines the modulated activity of SIRT1. Aptamers are a novel and particularly interesting targeting modality, with a unique ability to bind to a variety of targets including proteins, peptides, enzymes, antibodies and various cell surface receptors. Aptamers are single stranded oligonucleotides that vary in size between 25 and 50 bases long and are derived from combinatorial libraries through selective targeting. They offer unique benefits compared to other targeting agents, in that they bind with high affinity and selectivity, are not immunogenic or toxic and have good clearance from the system, are easily and quickly synthesised using in vitro techniques, and are stable and consistent.

METHODS

The SELEX methodology is based on the idea of following an evolutionary process of selection, partition and amplification rounds to generate nucleic acids as therapeutic reagents. Since DNA molecules adopt stable and intricately folded three dimensional shapes, they are capable of providing a scaffold for the interaction with functional side groups of a ligand.

RESULTS

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CONCLUSION

To test the above hypothesis we plan to follow the specific methodological approaches:
• Identification of aptamers against SIRT1.
• Characterisation of the interactions between selected aptamers and SIRT1 in vitro.
• Characterisation of the interactions between selected aptamers and SIRT1 in a range of cancer cell lines.
• Compare the results that will be obtained by using siRNA.

Pharmacogenetics, pharmacogenomics, personalized medicine

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COMBINED EVALUATION OF GENOTYPE AND PHENOTYPE OF THIOPURINE S-METHYL TRANSFERASES (TPMT) AS A PROFIT TOOL IN THE CLINIC MANAGEMENT OF PATIENTS IN CHRONIC THERAPY WITH AZATHIOPRINE

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BACKGROUND-AIM

Occurrence of adverse events (ADR) often occur during treatment with azathioprine (AZA) in patients with chronic autoimmune diseases. The response to AZA is influenced by the activity of thiopurine s-methyl transferases enzyme (TPMT): a low activity leads to accumulation of toxic metabolites, a high activity results in a higher production of methylated metabolite and therefore a lower therapeutic efficacy. To date 3 TPMT gene polymorphisms are associated with reduced enzyme function: 238G/C, 460G/A, 719A/G. Response to AZA can be predicted genetically with the study of polymorphysms and biochemically with the study of the enzyme activity. Integrated evaluation of TPMT genotype/phenotype is a useful tool in the clinical management of patients receiving AZA preventing ADR and/or side effects.

METHODS

223 patients afferent to Medical Genetics of Niguarda Ca' Granda Hospital (Milan), were genetically analyzed for the 3 TPMT gene polymorphisms. TPMT genotypes were analyzed by PCR, direct sequencing and enzymatic digestion. The enzymatic TPMT activity was evaluated with HPLC assay.

RESULTS

199 patients resulted wild type (wt) and have tolerated therapy, 12 were found to be mutated and do not use AZA therapy, 12 patients resulted wt, but have developed ADR. For this last group of patients TPMT enzymatic activity was evaluated by HPLC. Referring to the literature, was used as cut-off for TPMT enzymatic activity 58.8/ng/ml/h: 8 patients resulted below the cut-off while 4 patients diplayed normal enzymatic activity.

CONCLUSION

Genetic analysis of TPMT gene can predict the occurrence of ADR related to treatment with AZA predetermining TPMT activity levels; this text is not influenced by pharmacological and intra-individual variables. Conversely, genetic analysis focus only on three variables explaining about 80% of the altered TPMT activity. The biochemical test predicts dose-dependent ADR but the enzymatic assay suffers from pharmacological and/or individual variables. An integrated genotype/phenotype assessment of TPMT is a useful tool in the clinical management of patients receiving AZA for preventing ADR.

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IMPACT OF GENETIC POLYMORPHISMS ON 6-THIOGUANINE NUCLEOTIDE LEVELS AND TOXICITY IN PEDIATRIC IBD PATIENTS TREATED WITH AZATHIOPRINE

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BACKGROUND-AIM

Thiopurine-related toxicity results in discontinuation of therapy in up to 30% of patients with inflammatory bowel disease (IBD). Although thiopurine S-methyltransferase (TPMT) is implicated in toxicity, not all toxicity can be attributed to TPMT polymorphisms. We investigated effects of polymorphisms of genes involved in thiopurine and folate metabolism pathways on 6-thioguanine nucleotide (6-TGN) levels and toxicity.

METHODS

Retrospective clinical data and blood samples were collected from 132 pediatric IBD patients treated with azathioprine (AZA). Eighty-seven genetic polymorphisms of 30 genes were screened using a MassARRAY® system and 70 polymorphisms of 28 genes were selected for further analysis.

RESULTS

TPMT genotype ($P < 0.001$), concurrent use of mesalazine ($P = 0.006$), ABCC5 (rs2293001) ($P < 0.001$), ITPA (rs2236206 and rs8362) ($P = 0.010$ and $P = 0.003$), and ABCB1 (rs2032582) ($P = 0.028$) were all associated with ratio of 6-thioguanine nucleotides to AZA dose. ADK (rs10824095) ($P = 0.004$, odds ratio [OR] = 6.220), SLC29A1 (rs747199) ($P = 0.016$, OR = 5.681), and TYMS (rs34743033) ($P = 0.045$, OR = 3.846) were associated with neutropenia. ABCC1 (rs2074087) ($P = 0.022$, OR = 3.406), IMPDH1 (rs2278294) ($P = 0.027$, OR = 0.276), and IMPDH2 (rs11706052) ($P = 0.034$, OR = 3.639) had a significant impact on lymphopenia.

CONCLUSION

The present integrative study describes most of the suggested candidate genes related to the thiopurine metabolism pathway and toxicity. This is the first study to extensively analyze SNPs associated with thiopurine therapy in pediatric IBD patients among the Asian population. This study describes candidate genetic polymorphisms in genes whose products may affect pharmacokinetics (including drug absorption, metabolism, and elimination), and which may predict the relative likelihood of benefit or risk from thiopurine treatment. These findings may serve as a basis for personalized thiopurine therapy in pediatric IBD patients, although our data need to be validated in further studies.