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Pharmacogenetics - Personalized Medicine

Cod: M309

GOLD NANOPARTICLES: A NOVEL NANOPARTICLE FOR RADIOSENSITIZATION IN ORAL CAVITY CANCER

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The advances in nanotechnology provide promising results to develop an innovative and novel technique to detect and cure cancer simultaneously. Several studies have demonstrated that metallic nanoparticles with high atomic numbers may enhance the cytotoxic effect of radiotherapy (RT); thereby, have a potential to be used as a radiosensitizer. Therefore, gold nanoparticles (GNPs) are ideal candidates due to their unique surface, chemistry, electronic, and optical properties. In this respect, the purpose of this study was to develop a metallic based nanoparticle including gold (Au) functionalized with polyethylene glycol (PEG) and determine its cytotoxic effect on RT resistant oral cavity cancer cell line (UPCI-SCC-131). GNPs were synthesized by using sodium citrate (Na3C6H5O7) solution as a reducing agent and characterised with SEM. UPCI-SCC-131 cell line was cultured in MEM at 37°C with 5% CO2. Cells were seeded at 1x104 cells/well into 96-E plates and exposed to GNPs at various concentrations (100, 50, 25, 20, 10 and 5 μg/mL). Cell viability was monitored by xCELLigence RTCA DP system for 48 h. The percentage of cell viability was calculated by the ratio of cell index of control cells to cisplatin and nano-drug applied cells. Different sizes (15 and 20 nm) of GNPs applied to colony formation assay combined with RT.

In vitro studies demonstrated that GNPs had no cytotoxic effect at all concentrations. According to these results, 25 μg/ml of GNPs were selected. Colony formation assay showed that control cells did not grow into a colony only after 10 Gy radiation. On the other hand,

15 nm GNPs combined with RT inhibited colony formation at 0.5 Gy. However, 20 nm GNPs were not effective up to 5 Gy. In conclusion, GNPs have a potential to enhance the cytotoxic effect of RT and 15 nm GNPs is a better radiosensitizer for oral cavity cancer cells. However, in vivo studies are required.

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EFFECT OF SINGLE NUCLEOTIDE POLYMORPHISMS IN ADH1B, ADH4, ADH1C, OPRM1, DRD2, BDNF AND ALDH2 GENES ON ALCOHOL DEPENDENCE AND INCIDENCE IN A CAUCASIAN POPULATION

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Alcohol is probably the most frequently used addictive substance worldwide. Aim of this study is to determine the frequency distribution of SNPs within ADH1B, ADH4, ADH1C, ALDH2 genes encoding enzymes involved in alcohol metabolism and BDNF, OPRM1, DRD2 genes associated with other CNS processes in a southeastern European Caucasian population. An alcohol-addiction specific algorithm was generated (TGS) that may predict alcohol dependence and/or alcohol addiction prevalence in a population. In total, samples of 1276 volunteers were analyzed after de-identification and anonymization. Allele distribution of the examined polymorphisms in the present Greek population cohort were: rs1229984 (ADH1B): GG(wt)=64.14%, GA=29.86%, AA=4.00%, rs1693482 (ADH1C): CC(wt)=57.45%, CT=36.76%, TT=5.80%, rs1799971 (OPRM1): AA(wt)=72.43%, AG=28.72%, GG=1.89%, rs1800497 (DRD2): TT(wt)=1.98%, CT=27.18%, CC=70.84%, rs1800759 (ADH4): CC(wt)=34.25%, CA=48.12%, AA=17.63%, rs6265 (BDNF): GG(wt)=65.99%, GA=31.02%, AA=2.99%, rs671 (ALDH2): GG(wt)=99.84% GA=0.16%, AA=0.00%. Mutant rs1229984 allele A was ~6.5x more frequent in the Greek than in the European population (18.93% v/s 2.88%). Mutant rs1693482 allele T was ~1.7x more frequent in the European than in the Greek population (24.18% v/s 40.46%]. Mutant alleles for polymorphisms rs1800759 and rs1799971 show similar frequencies in both northern and southern Europeans. One rs671 mutant A allele was detected in the Greek population (0.08%). The mutant rs1800497 allele T was ~1.2x more frequent in the European than in the Greek population (15.57% v/s 18.79%) and the mutant rs6265 allele A was ~1.1x more frequent in the European than in the Greek population (18.50% v/s 19.68%]. In conclusion, the analyzed Southeastern population may differ genetically from north Europeans, due to influences from neighboring Asian and African populations.

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FREQUENCY DISTRIBUTION OF ARG389GLY AND SER49GLY POLYMORPHISMS IN A CAUCASIAN SOUTH-EASTERN POPULATION FROM GREECE.

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BACKGROUND

Functionally important β1 Adrenergic Receptor gene polymorphisms, Arg389Gly and Ser49Gly, have been associated with cardiac function in numerous studies but no causative cardiovascular diseases association could be documented. Moreover, both polymorphisms seem to have an impact on β blockers response. Homozygous Arg389 individuals are considered good responders and homozygous Gly389 as poor or non responders. Purpose of our study is the frequency distribution of these two polymorphisms in a Caucasian South- Eastern population from Greece and the comparison with existing data from other populations.

METHODS

A sample of 585 male and 616 female Caucasians from Greek general population volunteered to participate in this genotyping analysis after anonymization and de-identification. There were no specific inclusion or exclusion criteria. rtPCR (Melting Curve Analysis) followed DNA extraction from buccal swabs, results were analysed by SPSS.

RESULTS

Of all subjects, 46% were homozygous for the wild type Arg389 variant, 45% were heterozygous and 9% were homozygous for the polymorphic Gly389 variant. No statistically important difference in genotype distribution between the two genders was documented. Respectively, for Ser49Gly polymorphism, 81% of the subjects was homozygous for Ser49 variant, 18% was heterozygous and only 1% was homozygous for the polymorphic Gly49 variant. No gender association of either polymorphism was observed. A strong linkage disequilibrium that characterizes these two polymorphisms was confirmed. Haplotype Arg389Gly/Ser49Ser appears at the highest frequency (39%).

CONCLUSIONS

The present study data comparison for Ser49Gly polymorphism distribution of the Caucasian South-Eastern population from Greece differs significantly from both European and global population ($p<0,0001$). Furthermore, there is a statistically significant distribution difference between Greek and global population ($p<0,0001$) for Arg389Gly polymorphism while genotype distribution seems to be similar to European.

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DRUG-METABOLIZING ENZYME CYP2C19 POLYMORPHISMS IN GREEK BREAST CANCER PATIENTS.

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Background: Tamoxifen is a potent antiestrogen metabolized by the CYP450 system, predominantly by the CYP2D6 enzyme, to its active hydroxylated metabolites. CYP2C19 maps to chromosome 10 (10q24.1-10q24.3) and is also involved in the tamoxifen metabolism. The CYP2C19*2 loss of function polymorphism, known for null enzymatic activity, is associated with the clinical efficacy of tamoxifen in Estrogen Receptor positive (ER+) breast cancer patients.
Methods: CYP genotype specified in 31 non-related Greek breast-cancer patients. The CYP2C19 gene is highly polymorphic. For the analysis of the 3 most common CYP2C19 allelic variants: the wild-type CYP2C19*1, the CYP2C19*2 (aberrant splice site, c.681G>A; rs4244285) and the CYP2C19*3 (premature stop codon, p.W212X; rs4986893), we isolated whole blood genomic DNA (Qiagen method, manual preparation), used PCR (abi geneamp 9700 thermal cycler, PE Applied Biosystems) and microarray technology (AmpliChip CYP450 Test), classifying patients into two phenotypes.
Results: 26 patients were wild-type homozygous and 5 patients heterozygous (CYP2C19*1/CYP2C19*2). The frequencies of CYP2C19*1 and CYP2C19*2 variants were 0.897 and 0.103 respectively.
Conclusions: The prevalence of CYP2C19 allelic variants is in accordance with other Caucasian populations. The defective CYP2C19*2 polymorphism, associated with the most benefit from adjuvant tamoxifen treatment, is found in 10.3% of breast cancer patients. No patient carried the CYP2C19*3 allelic variant.

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DIGITAL PCR AS A CANDIDATE REFERENCE METHOD FOR PRECISION MEDICINE

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For the promise of precision medicine to be maximized there is a need for high-accuracy, reproducible and traceable diagnostics to identify markers that tell a physician the stage, therapy options and likely prognosis of a given condition. Diagnostic research is advancing such analyses in the context of a number of cancers where the presence of key mutations (such as variants in genes including KRAS, EGFR and BRAF) are guiding treatment and impacting on patients. However, for such approaches to become more widely established, reference methods and materials to facilitate traceability and enable compliance with Clinical Standards (eg ISO 17511 and 15189) will be needed. Molecular counting using techniques such as digital PCR offer the potential to achieve SI traceability through the concept of enumeration. The aim of this study was to investigate the accuracy and reproducibility of digital PCR (dPCR) when measuring key genetic targets used in guiding treatment decision in precision medicine and investigate the potential role this high accuracy method could have in supporting traceability of such diagnostic approaches. Accuracy and reproducibility of dPCR was evaluated using DNA reference panels containing clinically tested mutations used to direct therapy. Absolute and relative (with mixtures of wild type sequence) quantification was assessed at a range of different concentrations using different assays, detection chemistries and instruments. The methodology was further tested by performing inter laboratory comparisons. Using reference panels dPCR was demonstrated to be highly reproducible with quantitative discrepancies instruments and chemistries being less than 20%. Inter laboratory reproducibility was also high with 18 out of 21 laboratories. For all of these comparisons no calibrator was needed demonstrating that dPCR is a highly reproducible technique that has the potential to be a reference method for improving inter laboratory reproducibility.

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FREQUENCY OF HLA-B*57:01 ALLELE IN HIV INFECTED PATIENTS ADMITTED TO PREVENTIVE SCREENING FOR ABACAVIR® THERAPY: EXPERIENCE FROM THE IMMUNOGENETICS LABORATORY OF PARMA

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BACKGROUND
Abacavir® (ABC) is an antiretroviral drug recommended for Human Immunodeficiency Virus (HIV) infected individuals. The most important adverse event associated with ABC use is a severe hypersensitivity reaction (HSR) that affects 5-8% of the patients. HSR to ABC has been reportedly associated with the presence of HLA-B*57:01 allele. Allele frequency is highly variable among ethnic groups: the frequency is about 5% in Caucasian populations, while other populations, expecially Africans, have lower allele frequencies. This study was designed to establish HLA-B*57:01 frequency in our population of HIV infected patients to validate the effectiveness of prospective HLA-B*57:01 screening to prevent the HSR to ABC®.
METHODS
Our study involved 212 HIV-infected patients, from January 2013 to July 2016, admitted to the prospective HLA-B*57:01 screening for the prevention of ABC- correlated HSR, and 210 healthy blood donors. Patients mean age was 40 years, range 14 -74; 67 females (32%) and 145 males (68%), 53% (113 patients) was of Italian origin. Controls mean age was 48, range 21-73, 111 females (53%) and 99 males (47%). HLA-B low resolution genotyping has been performed using SSO-PCR (HistoSpot, BAG) and/or SSP-PCR (One Lambda). In HLA-B*57 positive samples we searched for the presence of HLA-B*57:01 allele using high resolution technique, as SSO-PCR (HistoSpot 4D, BAG) and/or SSP-PCR (Olerup).
RESULTS
Within the 212 HIV-infected patients, 15 (7%) were carriers of HLA-B*57 allele; 5 of Italian origin and 10 of African origin. High resolution typing allowed to establish HLA-B*57:01 allele among 5 Italian patients, but not in any African patients; a percentage (6.2%) of controls (13 patients) were carriers of HLA-B*57 allele. HLA-B*57:01 allele frequency was 4.42% in Italian HIV infected patients and 5.24% in control population.
CONCLUSIONS
Our results showed HLA-B*57:01 allele frequency in HIV-infected local patients is similar in controls and in Italian population (about 5%). Finally, considering the clinical importance of HSR to ABC® treatment in HIV infected patients and the frequency of HLA-B*57:01 carriers reported in this study we suggest the preventive use of genetic screening in Italian population before ABC® treatment.

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PHARMACOGENETICS OF STATINS RESPONSE: PRELIMINARY RESULTS OF A MULTICENTRIC STUDY.

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BACKGROUND
The response to statin therapy has a large interindividual variability (20-60%) in terms of reducing LDL-Cholesterol (LDL-C). For this reason, the study of the effect in the statin therapy response of genetic variants involved in lipid and statin metabolism can provide relevant insights to understand and explain this interindividual variability.

Our aim is to study the effect of 8 polymorphisms (TaqIB and I405V polymorphisms of the CETP gene, R219K of ABCA1 gene, CYP2D6 * 3, CYP2D6 * 4 and CYP2D6 * 6 of the gene CYP2D6, and CYP2C9 * 2 and CYP2C9 * 3 of the gene CYP2C9) in the reduction of LDL-C after treatment with simvastatin, atorvastatin or rosuvastatin in a Spanish population.

METHODS
171 patients were included in a prospectively way during two years. Clinical data and LDL-C were collected in the first and second visit (before and after treatment). The patients were genotyped by real time PCR and the method was validated by Sanger sequencing.
Multivariable linear regression models were used to explain the LDL-C variation between the first and the second visit, in each treatment group.

RESULTS
The most relevant results of the linear models explaining LDL-C variation with and without including genetic polymorphisms were the following: the inclusion of TaqIB genotypes significantly improves the variance explained by the model in rosuvastatin patients, from R2= 0,420 to R2= 0,720, (p value = 0,04 Regression coefficient (B) + confidence interval (IC) (95%)=29,05 (1,14-56,96)).
The inclusion of R219K polymorphism in the models explaining LDL-C variation within simvastatin (from R2= 0,066 to R2 0,097, p value= 0,11; B + IC (95%) =4,98 (-13,26-4,99) and atorvastatin patients (from R2= 0,162 to R2 0,223, p value= 0,06; B + IC (95%) =15,09 (-1,18-31,33)) is approaching statistical significance.

CONCLUSIONS

TaqIB variant (CETP gene) influences in rosuvastatin response in a statistically significant way. Furthermore, the presence of R219K variant (ABCA1) seems to influence in simvastatin and atorvastatin response, but no statistical significance was achieved. These are preliminary results and a larger number of patients are necessary to complete the study.

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CLOPIDOGREL-PATHWAY GENE POLYMORPHISMS AND CLINICAL RISK-STRATIFICATION OF PATIENTS WITH STEMI UNDERGOING PRIMARY PCI

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BACKGROUND: Accurate risk stratification has an important role in the management of patients with ST-elevation acute myocardial infarction (STEMI) treated by primary percutaneous coronary intervention (PCI). The incidence of major adverse cardiovascular events (MACE) in this population is about 10%. The MACE occurence can be partially explained by variability of clopidogrel response attributed to pharmacogenetics of clopidogrel metabolism or residual platelet reactivity in peripheral whole blood. The aim of the study was to investigate the association between clopidogrel-pathway gene polymorphisms and the Controlled Abciximab and Device Investigation to Lower Late Angioplasty Complications (CADILLAC) risk score on risk prediction of 30 day MACE in patients with STEMI treated by primary PCI.

METHODS: This prospective study included 140 consecutive patients referred to primary PCI for STEMI. The clopidogrel-metabolizing pathway SNPs used were: ABCB1 (rs1045642), CYP2C19*2 (rs4244285), CYP2C19*17 (rs12248560), P2RY12 (rs2046934), and PON1 (rs854560, rs662). The primary end point MACE was defined as death, nonfatal infarction or immediate target vessel revascularization. Patients were followed-up at 30 days after primary PCI.

RESULTS: Thirty-day MACE was 4.3%. All SNPs tested were in Hardy-Weinberg equilibrium ($p > 0.05$). Among the SNPs tested, only CYP2C19*17 was significantly associated with MACE. Addition of CYP2C19*17 T allele to CADILLAC score increased the area under the ROC (0.700 vs. 0.832). The addition of CYP2C19*17 T allele to CADILLAC score enhanced net reclassification improvement and integrated discrimination improvement, suggesting effective discrimination and reclassification.

CONCLUSIONS: These data revealed the combination of the established CADILLAC score and CYP2C19*17 could derive a more accurate prediction for clinical outcomes in STEMI patients.

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TRANSMEMBRANE BAX INHIBITOR MOTIF-6 (TMBIM6/ BI-1) PROTECTS AGAINST CISPLATIN-INDUCED TESTIS DAMAGE

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Cisplatin (cis-diamminedichloroplatinum) is an anti tumor drug. Despite of its curative activities, it has side affect on kidney, ear and bone marrow. The side effect has also been observed in testis; the testosterone production capacity is highly suppressed after cisplatin treatment. To examine the effect of cisplatin on Transmembrane BAX Inhibitor motif-6 (TMBIM6/ BI-1) in testis, wild type (BI-1WT) and knockout mice (BI-1-KO) mice and BI-1 overexpressing leydig cell (TM3 cell) were applied in this study. When exposed to cisplatin, the heme oxygenase activity is highly increased and the StAR level, a rate limiting enzyme for testosterone, and 3-β-HSD are less decreased and relatively maintaining testosterone in the BI-1 WT, compared with the knock-out condition. Excessive post-translational oxidation of protein disulfide isomerase (PDI), intra-ER ROS accumulation and folding capacitance alteration were also observed in cisplatin-BI-1 KO mice. Higher levels of endoplasmic reticulum (ER) stress were consistently observed in KO mice compared with the WT mice, indicating that the testosterone production capacity is higher in BI-1WT in comparison to BI-1-KO. This study indicates that BI-1 protects against cisplatin-induced toxicity both in vitro and in vivo by inducing HO-1 and through enhancing ER folding capacitance.