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Molecular diagnostics - Genetic testing

Cod: W132

ROLE OF GLUTATHIONE-S-TARNSFERASE P1 GENETIC VARIANTS IN ETIOPATHOGENESES OF TYPE2 DIABETES MELLITUS AND ITS EFFECT ON THE GLYCEMIC CONTROL PARAMETERS

A.H.M. Jarari [2], D.N. Shatiela [2], F. Elshaari [2], N.M. Jarari [3], J.R. Peela [1]

[1]*Department of Biochemistry and Medical Genetics,School of Medicine,St Matthews University,Grand Cayman,Cayman Islands*

[2]*Department of Biochemistry,Benghazi University,Benghazi,Libya*

[3]*Department of Pharmacology,Benghazi University,Benghazi,Libya*

(Libya)
abdallajarari@yahoo.com

Introduction: Glutathione-S-transferases (GSTs) are key enzymes of phase II related to the metabolism of numerous genotoxic compounds. Glutathione-S-transferases M1 (GSTM1) and T1 (GSTT1), the important subtypes of GSTs enzymes, are reportedly involved in detoxification of reactive oxygen species, which are considered to play a key role in the occurrence of various endocrine-related cancers. Another member of the glutathione-S-transferase (GST) family, GSTP1, which is located at 11q13, has a role in the detoxification of electrophilic compounds by glutathione conjugation . It has been found that two genetic polymorphisms in exon 5 and exon 6, lead to amino acid substitutions. However, only the transition in exon 5 was linked to activity of enzymes since this is located within the region coding for the enzyme's active site. The genetic change in exon 5 at the site -313, results in polymorphism at codon 105, where an adenosine-to-guanidine (A > G) transition causes an Ile-to-Val substitution.
Several investigators have determined the clinical or genetic factors associated with T2DM with interests to detoxification agents.
Patients and methods:Total of 67 type2 Libyan diabetes patients, 26 apparently healthy age and sex-matched individuals were selected from the high institute of medical professions' Benghazi to serve as controls. Clinical information and medical history were obtained through patients' interview.
Venous blood samples were drawn from all the participants after at least 10 hours fasting, and analyzed for blood glucose, HbA1c, total cholesterol, triacylglycerol, and HDL-c. LDL cholesterol was calculated according to Friedewald equation.
The data were analyzed, P values < 0.05 were considered to be statistically significant.
DNA is extracted from blood samples, To detect the GSTP1Ile105Val genotype loading digested products on 2.5%agarose gel electrophoresis.
Results: Diabetic patients had significantly higher fasting blood glucose, HbA1c, total cholesterol, triacylglycerol, and LDL-c, and lower HDL-c than control patients group.
Conclusion: In the present study, GSTP1Ile105Val may not play role in the etiopathogeneses of diabetes mellitus and on glycemic control parameters

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DISCOVERY OF NOVEL TRANSCRIPTS OF THE KALLIKREIN-RELATED PEPTIDASE (KLK) FAMILY WITH POTENTIAL UTILITY AS CANCER BIOMARKERS, USING NEXT-GENERATION SEQUENCING

P.G. Adamopoulos [1], C.K. Kontos [1], G. Ardavanis [2], A. Scorilas [2]

[1]*Department of Biochemistry and Molecular Biology, National and Kapodistrian University of Athens, Athens, Greece.*

[2]*First Department of Medical Oncology, "Saint Savvas", Anticancer Hospital, Athens*

(Greece)
ascorilas@biol.uoa.gr

BACKGROUND: Next-generation sequencing (NGS) technology has enabled genome-wide studies, providing massively parallel DNA sequencing. NGS applications constitute a revolution in genetics and have already paved new ways in clinical cancer research. The tissue kallikrein (KLK1) and kallikrein-related peptidases (KLKs) are secreted serine proteases with various expression patterns and physiological roles. KLK family members are aberrantly expressed in malignancies and involved in processes related to carcinogenesis, such as cell growth regulation, angiogenesis, invasion, and metastasis. The aim of this study was the discovery of novel alternatively spliced transcripts of the KLK5, KLK6, KLK7, KLK8, and KLK9 genes; such transcripts could represent novel, potential tumor biomarkers, exhibiting high sensitivity and specificity. METHODS: Total RNA extraction was performed from 56 human cancer cell lines, followed by first-strand cDNA synthesis using an oligo-dT-adaptor as primer. Next, 3#-RACE PCR and nested 3#-RACE PCR were carried out for the molecular cloning of novel transcripts of the targeted KLK genes. PCR products were then cleaned up and used for NGS library construction. The concentration of the created library was assessed using quantitative real-time PCR and a TaqMan MGB assay. Then, NGS was performed on an Ion PGM™ system. The obtained NGS data were analyzed with in-house–developed bioinformatic algorithms. RESULTS: NGS data analysis revealed novel splice junctions of the KLK5, KLK6, KLK7, KLK8, and KLK9 genes, supporting the existence of novel KLK transcripts, which were validated with PCR using variant-specific primers, followed by agarose gel electrophoresis and Sanger sequencing of the PCR products. Thus, 29 novel splice variants of these KLK genes were discovered and cloned. Most of these transcripts are predicted to encode novel protein isoforms with unknown enzymatic activity. CONCLUSIONS: 29 novel splice variants of the KLK5, KLK6, KLK7, KLK8, and KLK9 genes were discovered and cloned. Since most members of the KLK family represent important cancer biomarkers (e.g. PSA/KLK3 in prostate cancer), the quantification of the newly discovered KLK transcripts in human samples may have clinical applications in cancer and deserves further investigation.

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Molecular diagnostics - Genetic testing

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SYNDROMIC MENTAL RETARDATION CAUSED BY THE MICRODELETION, EXPERIENCE OF OUR LABORATORY IN ALGIERS

B. Ait Abdelkader [1], A.M. Ghouali [1], A. Chikouche [1]
[1]*CPMC ALGIERS*

(Algeria)
belgene.belaid@gmail.com

Isolated or syndromic mental retardation occurs in 2 to 3 % of the general population. It is mainly caused by chromosomal aberrations that are detected by karyotype. However this technique has a limited resolution.
The molecular cytogenetic techniques (FISH and CGH-array) have revolutionized the diagnosis of the syndromic mental retardations, in particular those resulting from a structural abnormality.
The microdeletion syndromes, object of our study, are displayed by FISH analysis within the "Centre Pierre et Marie Curie", Medical Biochemistry Laboratory, Cytogenetics Unit. There have been 77 suspected microdeletions, but only 24 confirmed ones by FISH analysis. This fact draws our attention to the FISH's efficiency threshold despite a resolving power less than 5 Mb.
In order to increase the diagnosed patients' rate, it would be preferable to resort to the CGH-array which provides a more accurate analysis. From this perspective, 10 samples have been sent to the Constitutional Cytogenetics Laboratory of the "Mère-enfan" Hospital in Lyon, France.
All of these techniques would allow us to diagnose a high number of patients with mental retardation. Nonetheless, a proportion of these retardations remain unexplained.

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Molecular diagnostics - Genetic testing

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TNFA GENETICS: A RISK FACTOR FOR SPONDYLOARTHRITIS

A. Aita [2], M.G. Lorenzin [1], R. Ramonda [1], D. Basso [1], P. Frallonardo [1], F. Navaglia [1], C. Zambon [1], S. Moz [1], L. Punzi [1], M. Plebani [1]
[1]*Department of Medicine DIMED, University of Padova (Italy)*
[2]*Department of Medicine-DIMED, University of Padova (Italy)*
(Italy)
ada.aita@tiscali.it

Background. Spondyloarthritis (SpA) are multifactorial diseases resulting of interplay among genetic background (mainly HLA-B27) and environmental factors. Autoinflammation and dysregulation of the immune-system also concur. TNF is primarily involved in these processes, supporting the beneficial effects of anti-TNF therapy. The aim of this work was to investigate whether single nucleotide polymorphisms (SNPs) of the autoinflammatory genes MEFV and TNFRSF1A, or SNPs in the promoter region of TNFA are associated with SpA and/or response to treatment.
Methods. 91 SpA (52.1±12.5 years; M/F:57/34; 55 with Psoriatic Arthritis-PA and 36 with Ankylosing Spondylitis- AS), and 223 controls (46±11 years; M/F:146/77) from the Veneto Region (Italy) were studied. Direct sequencing of MEFV (exons 2,3,5 and 10) and TNFRSF1A (exons 2,3,4 and 6) genes were performed. HLA-B27 and TNFA polymorphisms (-1031T>C;-857C>T;-376G>A;-308G>A;-238G>A) were assayed by RT-PCR.
Results. As expected, HLA-B27 was correlated with AS ($\chi 2$=120.1; p=0.000). 21 SNPs were identified in MEFV gene, 10 with a known potential functional significance. Variant alleles were extremely rare in our population (Minor allele frequencies-MAF<0.025) except for R202Q (MAF=0.27). None was associated with SpA diagnosis (p>0.05). Two SNPs were identified in TNFRS1A, the R92Q (MAF=0.034) and c.625+10A/G (MAF=0.479). None of them was associated with SpA (p>0.05). None of the studied TNFA SNPs was singly associated with SpA, while the haplotype C/G, resulting from -1031T>C/-308G>A combination, was significantly associated with a reduced risk of AS (p=0.015). The haplotypes resulting from the combination between HLA-B27 and -376G, -308G and -238G alleles in TNFA gene increased AS (p<0.005), but also PA (p<0.05) risk. The TNFRSF1A c.625+10A/G was associated with the response to anti-TNF therapy, assessed by BASDAI score lower/equal or higher than 4 at 10 month (p=0.031).
Conclusions. TNFRSF1A and MEFV gene SNPs are not associated with SpA in the north-East of Italy. AS risk appears to depend not only on HLA-B27, but also on the TNFA haplotype -1031C/-308G. The haplotypes resulting from HLA-B27 and TNFA -376G,-308G,-238G alleles, play also a role in enhancing PA risk. The TNFRSF1A c.625+10A/G impacts on the response to anti-TNFα therapy.

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Molecular diagnostics - Genetic testing

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THE INFLUENCE OF VDR GENE FOKI POLYMORPHISM ON LIPID PROFILE IN PATIENTS WITH JUVENILE IDIOPATHIC ARTHRITIS TREATED WITH ETANERCEPT

J. Basic [3], D. Pavlovic [3], T. Jevtovic-Stoimenov [3], J. Vojinovic [2], V. Milosevic [1], M. Despotovic [3], T. Cvetkovic [3], I. Stojanovic [3]

[1]*Clinic of Neurology, Clinical Centre Nis, Nis, Serbia*

[2]*Clinic of Pediatrics, Clinical Centre Nis, Nis,Serbia*

[3]*Institute of Biochemistry, Medical Faculty, University of Nis, Serbia*

(Serbia)
jelenabasic@yahoo.com

Introduction: Juvenile idiopathic arthritis (JIA) is an inflammatory arthritis of unknown etiology. Although it is speculated that there is lipid profile disbalance in JIA patients, genetic contribution of VDR gene FokI polymorphism on lipid profile and response to TNF blocking agents in JIA is not yet well established.
The Aim of this study was to investigate the distribution of VDR gene FokI C/T (F/f) polymorphism (rs10735810) in JIA patients compared to controls, as well as to evaluate whether this polymorphism can influence lipid profile and clinical response to etanercept (TNF receptor II.Fc fusion protein) in JIA patients after 1 year of treatment.
Methods: A total of 62 patients with JIA and 38 healthy children were screened for the FokI VDR gene polymorphism using the PCR-RFLP method. Lipid profile (cholesterol, triacylglycerol, HDL-C and LDL-C) was determined using standard biochemical analysis prior to and 12 months after etanercept therapy.
Results: The genotype frequency distributions of the VDR gene FokI polymorphism in the patients were significantly different from those of the controls ($p<0.001$). The frequency of the f allele was significantly higher in JIA patients compared to controls ($p<0.001$). There was statistically significant decrease of triacylglycerol ($p=0.008$) and increase of HDL-C levels ($p=0.011$) in patients after etanercept treatment in comparison to baseline values. According to the VDR gene FokI polymorphism, only the levels of HDL-C in patients with the genotype FF were significantly increased ($p=0.006$) after 1 year of treatment compared to the values from before. On the other hand, there was increase of HDL-C levels after treatment, but not statistically significant in patients with the genotypes Ff/ff.
Conclusion: The frequency of the f allele was significantly higher in JIA patients in comparison to controls. Our results showed that the F allele is associated with improvement of HDL-C levels in JIA patients treated with etanercept.
Key words: juvenile idiopathic arthritis, VDR gene FokI polymorphism, lipid profile, etanercept

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Molecular diagnostics - Genetic testing

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EVALUATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY WITHOUT HEMOLYSIS IN ICTERIC NEWBORNS

R. Dabboubi [2], S. Hamdi [1], A. Bouachba [2], A. Zoghlami [2], T. Ben Messaoud [2]

[1]*Childre's Hospital - Tunis*

[2]*Children's Hospital-Tunis*

(Tunisia)
taieb.messaoud@rns.tn

INTRODUCTION: Glucose-6- phosphate dehydrogenase (G6PD) deficiency is an inherited deficiency that may be the cause of neonatal jaundice. Our aim was to study the prevalence of G6PD deficiency without hemolysis in relation to neonatal jaundice.
Methods: This prospective descriptive study has been conducted on 152 icteric newborns recruited from the children's hospital "Bachir Hamza" in Tunis. The study was conducted over a two-month period (April-May) 2016. The dataset included: age, sex, total and direct bilirubin, hemoglobin, reticulocyte count, blood group and Rh of mother and newborn, direct Coombs, G6PD level and the type of treatment. All data was analyzed by using statistical method.
RESULTS: From 152 neonates, 31 neonates (20.3 %) were found to have G6PD deficiency. The male to female ratio was 1.39 (18 male and 13 female neonates). There was no difference in the mean bilirubin level, hemoglobin level between patients with G6PD deficiency and those without G6PD deficiency ($p>0.05$).
From 31 neonates with G6PD deficiency, hemolysis was seen in 12 neonates (38.7%) and the rate of G6PD deficiency without hemolysis was 12.5%. Out of 31 patients with G6PD deficiency, 2 patients (16.7%) had blood exchange transfusion. Rh and ABO incompatibility were seen patients. The etiologies are dominated by physiological jaundice in 25%, neonatal infection in 22% neonatal respiratory distress 16.44%, prematurity in 5.9% and maternal-fetal incompatibility in in 4.6%. Phototherapy associated etiological treatment are essential to the treatment
Conclusion: In this study the prevalence of G6PD deficiency in icteric newborns was considerably high and most of them were non hemolytic, so we recommend G6PD test as a screening program for every newborn at the time of delivery.

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Molecular diagnostics - Genetic testing

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COMPARISON AND COST EFECTIVENESS ANALYSIS BETWEEN MOLECULAR AND RAPID TECHNIQUE FOR THE DETECTION OF CLOSTRIDIUM DIFFICILE TEST IN A PUBLIC HOSPITAL

R. Benavente [1], F. Espinosa [1], L. Contreras [1], D. Baez [1]
[1]*HOSPITAL REGIONAL DE TALCA, CHILE*

(Chile)
rbenavente@hospitaldetalca.cl

Background: Clostridium difficile(CD) diagnosis is critical to public health and to prevent outbreaks in health centers. The absence of highly sensitive, specific, bacteria diagnostic methods, for economic reasons, contribute to make outbreaks extremely difficult to manage in short term. Faced with this situation, molecular tests have emerged as one of the main tools for disease diagnosis, with the consequent outbreak control. Objectives: To compare and establish differences between molecular methods and immunochromatographic CD detection diagnostic.
Materials and Methods: C. DIFF QUIK CHEK kit Complete® TECHLAB company, which detects both GDH as toxin A and B. The molecular analysis was through Amplivue enterprise system Quidel Molecular performed. Results: Of a total of 32 samples (100%) analyzed both by EIA and PCR, it was showed a 19% increase in positive detection for Clostridium difficile by PCR, which was expected by the greater molecular technique sensitivity (28% EIA v / s 47% PCR). In case of negative detection, or absence of pathogens in the sample, PCR showed less negative (EIA 59% v / s 53% PCR). In results by EIA Ag (+) / Tx profile there was 4 (-) samples, equivalent to 13% of the analysed universe, while no PCR sign was found. The bed day cost of medical service is USD 459. If we consider a dubious sample, resulting Ag (+) / Tx (-), the average time at which a positive result is obtained, if the patient is truly developing the disease, is 8 days, ie, the cost to our hospital is USD 3526, besides danger of an outbreak. After the 8th day, hospital personnel could take respective measures for this patient, such as isolation, proper antibiotic treatment, etc. The molecular technique has a cost of approximately USD 30.7, measures could be immediately started (isolation, treatment) and Hospital costs diminish the equivalent of 7 days day bed, which means USD 3400, with molecular determination cost included. Conclusions: The molecular analysis is a useful sensitive, specific, fast support for CD infection diagnosis, and helps medical personnel to make clinical decisions that allow them to develop all actions that could improve patient health and protect other users, as well as reduce overall infection management costs.

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Molecular diagnostics - Genetic testing

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HIGH RESOLUTION MELTING ANALYSIS IS VERY USEFUL TO IDENTIFY BRCA1 C.4964_4982DEL19 (RS80359876) FOUNDER CALABRIAN PATHOGENIC VARIANT ON PERIPHERAL BLOOD AND BUCCAL SWAB DNA

M. De Bonis [1], A. Minucci [1], E.D. Capoluongo [1]

[1]*Laboratory of Clinical Molecular and Personalized Diagnostics, Institute of Biochemistry and Clinical Biochemistry, Catholic University, Rome, Italy*

(Italy)
mari.debonis86@gmail.com

Introduction. Different BRCA1 founder mutations are confined to geographically isolated regions or specific populations. BRCA1 5083del19 mutation is recurrent and specific to individuals of Italian descent with a founder effect on the Calabrian population. The correct identification of this type of mutation is one of pitfalls of NGS mainly due to insufficient coverage, read lenght or alignment quality.
In view of this, we set up a rapid, low cost, high-throughput High Resolution Melting Analysis (HRMA) for genotyping the Italian BRCA1 5083del19founder mutation starting from peripheral blood and/or buccal swab DNA.
Methods. DNA samples were obtained from 30 subjects, 15 wild type (WT) and 15 mutated (M) for the BRCA1 5083del19, previously amplified by BRCA MASTR™ Dx (Multiplicom,Niel, Belgium), defined by NGS on the Illumina MiSeq® platform (Illumina Inc., San Diego, CA, USA) and confirmed by Sanger sequencing. To validate the assay, we performed HRMA analysis on a set of further 20 unknown samples, obtained from Italian HBOC patients, belonging to Calabria region. We also evaluated the sensitivity by mixing the heterozygote sample for BRCA1 c.4964_4982del19 variant and a reference WT DNA sample at ratios of 50%, 10%, 2.5% of the mutated sample.
PCR-HRMA were performed on the LightCycler® 480 Real-Time PCR System. Data, analyzed with LightCycler 480 Gene-Scanning Software version 1.2 (Roche Diagnostics), were normalized, temperature-shifted and converted to a derivative plot for analysis. Melting temperatures (Tms) were derived at the greatest dF/dT value of the derivative curve data.
Results. WT (95bp) and M amplicons (76bp) showed a clearly different melting profile; furthermore the WT and M samples presented evident differences in Tm (TmWT=79.3± 0.5; TmM =74.3±0.5). So, HRMA results were 100% concordant with direct sequencing. In addition, HRMA sensitivity appear superior to direct sequencing, allowing the detection of heterozygous sequence changes up to 2.5% of the mutated allele: this result is particularly promising above all when this assay could be applied to DNA from FFPE tumor samples.
Conclusion. We provide evidence about application of HRMA in unambiguously genotyping of the founder BRCA1 c.4964_4982del19 variant in individuals belonging to Calabria Italian region. In fact, HRMA was confirmed to be particularly suitable for the identification of BRCA1 c.4964_4982del19 variant, making this approach useful in clinical molecular diagnostics.

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Molecular diagnostics - Genetic testing

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COMPETITIVE PCR-HIGH RESOLUTION MELTING ANALYSIS (CPCR-HRMA) AS SCREENING METHOD FOR LARGE GENOMIC REARRANGEMENTS (LGRS) DETECTION: A NOVEL APPROACH TO IMPROVE MOLECULAR TESTING FOR BRCA GENES

E. De Paolis [1], G.L. Scaglione [1], A. Minucci [1], E.D. Capoluongo [1]

[1]*Laboratory of Clinical Molecular and Personalized Diagnostics, Institute of Biochemistry and Clinical Biochemistry, Catholic University, Rome, Italy*

(Italy)
elisadepaolis88@gmail.com

Background
To date, 1.700 BRCA1 variants and 1.900 BRCA2 variants have been reported. However, only 81 BRCA1 variants and 17 BRCA2 variants are Large Genomic Rearrangements (LGRs). The aim of the study was to investigate a Competitive PCR-High Resolution Melting Assay (cPCR-HRMA) as an innovative screening method for BRCA1 LGRs analysis. The principle of the method is to amplify simultaneously sequences of unknown germline BRCA1 copy number variation (CNV) and unchanged Albumin CNV.
Methods
We assessed the germline CNV status of exons 1, 2, 3 and 14 as training set of exons. cPCR-HRMA was performed in 30 wild type samples and in the following positive samples, previously analyzed by MLPA: 10 for exons 1 and 2 deletion, 5 for exon 3 deletion, 1 for exon 3 duplication and 4 for exon 14 deletion. HRMA was performed on target BRCA1 regions during exponential PCR phase in duplex reactions including Albumin as unchanged copy reference. Genotypes were assessed comparing the melting profiles and the fluorescence peaks height ratio (BRCA1/Albumin) in each reaction. Mean and SD of fluorescence peak height ratio of wild type samples was used to set an approximate scale of ratios for unchanged copy number (1x±SD), deleted (0.5x±SD) and duplicated (1.5x±SD) samples.
Results
Melting profiles showed a marked different behavior of both wild type and positive samples for all analyzed exons. Samples were correctly classified also by using fluorescence peaks height ratio scale. Furthermore, samples with point mutations and micro-rearrangements showed alternative typical melting profiles.
Conclusions
MLPA and MAQ techniques are routinely used for CNV analysis in BRCA1/2 genes. These approaches are expensive and time consuming, therefore the identification of screening methods is needed to optimize the diagnostic procedure. In addition, different computational methods based on massively parallel sequencing (MPS) have been devised to detect LGRs. Thus, HRMA has proven as innovative, efficient and fast screening method for CNV BRCA1 status, allowing an implementation of our diagnostic molecular BRCA1/2 pipeline. This novel approach enabled us to use c-PCR-HRMA as a fast screening method and also to use MLPA/MAQ as confirmatory tools for CNV status of positive c-PCR-HRMA results.

Reference:
Borun P, Kubaszewski L, Banasiewicz T, at al. Hum Genet. 2014 May; 133 (5):535-45.

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Molecular diagnostics - Genetic testing

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EFFECT OF ACRIFLAVINE ON THE 5-FLOUROURACIL SENSITIVITY IN COLORECTAL CANCER

E. Eftekhar [1], Z. Parisa [2]

[1]*Department of biochemistry,Hormozgan university of medical sciences, Bandar abbas,iran*

[2]*Department of physiology,Hormozgan university of medical sciences, Bandar abbas,iran*

(Iran (Islamic Republic of))
Eftekhar19@gmail. Com

Background:
5-fluorouracil (5-FU) -based chemotherapy improves the overall survival of advanced colorectal cancer (CRC) patients. However, only a small percent of patients respond to 5-FU, when used as a single agent. The aim of the present work was to study whether anticancer property of 5-FU can be potentiated by combination treatment with acriflavine (ACF), in CRC cells.
Method and Materials
The cytotoxic effect of ACF, 5-FU and irinotecan, on 3 human CRC cell lines (SW480, LS174T and HCT116) were studied using MTT assay. To determine the effect of ACF on the sensitivity of cells against 5-FU, cells were simultaneously cotreated with IC30 values of ACF and different concentrations of 5-FU or pretreated with IC30 doses of ACF and then different concentration of 5-FU.
Results
Pretreatment with ACF significantly sensitized CRC cells to the cytotoxic effects of 5-FU. Whereas simultaneous treatment with ACF and 5-FU could not change the resistance of CRC cells to 5-FU. In compare to irinotecan, ACF was a more potent agent for enhancing the antitumor activity of 5-FU.
Conclusion
Our findings, show for the first time that pretreatment with ACF markedly increases the cytotoxic effects of 5-FU on CRC cells. This effect is independent of the p53 status of cells.

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Molecular diagnostics - Genetic testing

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DESCRIPTIVE STUDY OF HFE GENE POLYMORPHISMS IN THE ALCORCON'S POPULATION.

F.J. García Íñigo [1], J.M. Acedo Sanz [1], M.L. Casas Losada [1]
[1]*Hospital Universitario Fundación de Alcorcón*

(Spain)
chesco.es@gmail.com

BACKGROUND

Hemochromatosis is a genetic disease associated with iron overload. Excess iron is deposited in various tissues (eg liver, heart muscle), resulting in fibrosis of these tissues. The main cause of the disease in Europe are autosomal recessive mutations in the HFE gene causing hereditary hemochromatosis type I.

METHODS

We analyzed the results of genetic studies of HFE in 638 patients who was suspected haemochromatosis, either ferric overload or by family history. HFE genetic study was performed by real-time PCR, containing the most frequent polymorphisms: C282Y, H63D and S65C.

RESULTS

Of the 638 patients studied, only 287 (45%) don't present any of the 3 common polymorphisms of the HFE gene. The mayor polymorphism, C282Y, appeared in 90 patients (14.1%), which can be further divided in 39 patients (6.1%) as C282Y heterozygotes, 15 patients (2.4%) as C282Y homozygotes, 31 patients (4.9%) as C282Y/H63D compound heterozygotes and 5 patients (0.8%) as C282Y/S65C compound heterozygotes. H63D polymorphism is common, occurring in 286 patients (44.8%), who are classified as follows: 203 patients (31.8%) as H63D heterozygotes, 48 patients (7,5%) as H63D homozygotes, 31 patients (4.9%) as C282Y/H63D compound heterozygotes and 4 patients (0.6%). S65C is the least common polymorphism, found 15 patients (2.3%) with this polymorphism: 6 patients (0.9%) as S65C heterozygotes, 5 patients (0.8%) as C282Y/S65C compound heterozygotes and 4 patients (0.6%) as H63D/S65C compound heterozygotes.

CONCLUSIONS

Unlike the literature indicates, in our population the most frequent polymorphism is the H63D, present in 44.8% of patients, but has not been reviewed if these patients are clinically affected (elevated ferritin and/or saturation transferrin). However, polymorphism S65C, as in the literature, is the least common, occurring in only 2.3% of patients, but also only 2 patients have elevated transferrin saturation with hyperferritinemia, and 1 patient present high ferritin levels (>1000 ng/mL) and hepatic iron overload. Most of the remaining 12 patients have elevated ferritin associated with metabolic syndrome. Therefore, the S65C polymorphism should only be study the in cases with a high suspicion of hemochromatosis and after having discarded the other polymorphisms.

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Molecular diagnostics - Genetic testing

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BRCA1 AND BRCA2 GERMLINE MUTATIONS IN ALGERIAN FAMILIAL BREAST AND / OR OVARIAN CANCER PATIENTS

N. Habak [1], A. Ladjroud [3], N. Benzidane [4], A. Chikouche [1], M. Ait Abdallah [1], K. Bouzid [2], L. Griene [1]

[1] *1) Department of hormonology and biochemistry, Unit of molecular genetics. Center Pierre and Marie Curie, Algiers, Algeria.*

[2] *2) Department of oncology. Center Pierre and Marie Curie, Algiers, Algeria*

[3] *2) Department of oncology. Center Pierre and Marie Curie, Algiers, Algeria.*

[4] *3) Department of senology. Center Pierre and Marie Curie, Algiers, Algeria*

(Algeria)
nawal.bioch@gmail.com

Introduction
Breast and ovarian cancers are the first and fourth leading causes of cancer death, respectively, among women in Algerian. Studies have shown lower age of onset and lower incidence of breast cancer in North African population. This association suggests that genetic factors such as mutation of BRCA1/ BRCA2 may contribute to high proportion of breast / ovarian cancer cases. Few reports have been published about the spectrum of BRCA1/2 mutations in Algerian women. The aim of our prospective study is to determine the prevalence and the nature of BRCA1/2 germline mutation in familial breast / ovarian cancer patients in Algeria.
Patients and methods
49 index cases from the 49 families included in this study has been selected for the DNA diagnosis of BRCA1/ 2 according to the selection criteria based on the number of first degree and / or second degree relatives with breast / ovarian cancer and the age of diagnosis.
Double stranded sequencing of all exons including flanking intronic regions of BRCA1 and BRCA2, respectively, was performed. The MLPA method was used to detect larger deletion and duplication.
Results: We identified 5 different deleterious mutations in BRCA1 and 3 different mutations in BRCA2.
•The total rate of deleterious BRCA1/2 mutation carriers was 44.8% in our cohort,
38.7% had mutation in BRCA1 and 6.1% in BRCA2,
•2 deleterious mutations in BRCA1 were previously described in other populations:
- the c.798_799 delTT, p.Ser267Lys fsx19 mutation identified in 8 Algerian families was also observed in families from Tunisia and Marocco.- microsatellite markers flanking and in the BRCA1 locus showed a common haplotype in c.798_799 delTT carriers.
- the c.211 A>G , p.Arg91Gly mutation identified in one Algerian family is a founder Spanish mutation.
•The c.83_84delTG, p.leu28Arg fsx 12 has been found in 7 independent families, all coming from the same region located in north Algeria.
• 6 Unclassified variants UVs were identified in12.2 % of all patients; a novel UV c.803A>G
p Asn 268 Ser, has been identified; which has not been described in public mutation databases
Conclusion: Our results suggest a relative spectrum of BRCA1/ BRCA2 mutation in Algerian breast / ovarian cancer, but 151 other index cases from 151 families are actually under investigation. The overall results may therefore, identified the real spectrum of BRCA1/2 mutation in familial breast/ovarian cancer in Algeria and, perhaps, some genetic particularity.

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Molecular diagnostics - Genetic testing

Cod: W145

MICRO RNAS hsa-mir-99B-5p AND hsa-mir-125A-5p IN MYELODYSPLASTIC SYNDROME

M.M. Kardum Paro [1], I. Mandac Rogulj [2], S. Ostojić Kolonić [2], Z. Flegar- Meštrić [1]

[1]*Department of Clinical Chemistry and Laboratory Medicine, Merkur University Hospital, Zagreb, Croatia*

[2]*Department of Internal Medicine, Merkur University Hospital, Zagreb, Croatia*

(Croatia)
mirjanamariana@gmail.com

Background. The pathogenesis of myelodisplastic syndrome (MDS) and the complex mechanism of disease transformation to more aggressive acute myeloid leukemia (AML) has been objective of many experimental studies. Due to molecular mechanisms investigated micro RNA (miRNA) profiling
of specific circulating microRNAs (miRNAs) could represent new noninvasive molecular target for detection and prognosis of MDS. The aim was to investigate the plasma expression levels of two miRNAs (hsa-miR-99b-5p and hsa-miR-125a-5p) specific for MDS.
Methods. The plasma expression levels of hsa-miR-99b-5p and hsa-miR-125a-5p were investigated in a molecular laboratory accredited according ISO 15189. Whole blood from 4 healthy volunteers and 33 MDS patients diagnosed in Reference center for MDS Croatian ministry of Health was drawn into EDTA containing tubes and processed to plasma separation, miRNAs extraction (miRNeasy Serum/Plasma Kit), reverse transcription (miScript II RT Kit) and qPCR measuring (miScript SYBR Green PCR Kit/Custom PCR Array). For the determination of the presence of RT and PCR inhibitors miRNA RT control assay (miRTC) and positive PCR control (PPC) were used. Data normalization (cell-miR-39-3p) and data analysis (web- based software) were done according to the manufacturer instructions (Qiagen). Results. With the recommended Ct cut-off set at 35 PCR array reproducibility and RT efficiency were satisfactory. No statistically significant differences of miRNAs expression levels between healthy volunteers and MDS patients were observed (hsa-miR-125a= 0,3049; hsa-miR-99b-5p =0,1336), but in MDS patients both miRNAs were up regulated - hsa-miR-125a even over 2-fold change of healthy volunteers (MDS/Health fold change 2,30 vs. 1,90).
Conclusions. Proper qPCR controls and data normalization eliminated technical differences that may be due to miRNA quality or expression levels qPCR measuring. Results have show significant up regulation of hsa-miR-125a expression level in MDS patients which could serve as an potential prognostic marker or a potential therapeutic target in MDS. In the future extended miRNA profiling with some additional miRNAs (let-7a, hsa-miR-16, hsa-miR-144, hsa-miR-651) should identify real small changes in miRNA expression levels that could be useful for diagnostic purposes in MDS.
Acknowledgments. This work was supported by Ana Rukavina Fondation, Zagreb, Croatia.

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Molecular diagnostics - Genetic testing

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INNATE IMMUNITY GENE POLYMORPHISMS IN WET AGE-RELATED MACULAR DEGENERATION (AMD)

A. Sarli [1], I. Skalidakis [2], A. Velissari [1], C. Koutsandrea [2], M.B. Petersen [3], G. Kitsos [4], M.M. Moschos [2], C. Kroupis [1]

[1]*Department of Clinical Biochemistry, Attikon University General Hospital, University of Athens Medical School, Athens, Greece*

[2]*Department of Ophthalmology, "G. Gennimatas" General Hospital, University of Athens Medical School, Athens, Greece*

[3]*Department of the Clinical Genetics and Department of Clinical Medicine, Aalborg University Hospital, Aalborg, Denmark*

[4]*Ophthalmology Department, University General Hospital of Ioannina, University of Ioannina, Ioannina, Greece*

(Greece)
ckroupis@med.uoa.gr

Background: Age-related Macular Degeneration (AMD) is a multifactorial degenerative ocular disease of the retina that leads to loss of central vision. Functional gene polymorphisms have already been associated with the disease (e.g. CFH in a previous work of our group). The goal of our study was to verify the correlation of another established polymorphism (ARMS2 A69S) with the disease and to investigate the role of innate immunity system and more specifically of the CD14-TLR4 complex. Thus, we examined for the first time the association of C-260T variation in the promoter of the CD14 gene and looked at two TLR4 polymorphisms (Asp299Gly, Thr399Ile) in Greek patients with the most severe form of AMD, the wet form.
Methods: Genomic DNAs were isolated from blood samples of 103 healthy controls and 120 Greek patients that were age- and sex-matched, and all of whom were clinically evaluated and have signed an informed consent form. For the genotyping of all the selected polymorphisms, PCR-RFLP analysis was performed with appropriate primers and restriction enzymes. Findings were confirmed with DNA Sequencing in an ABI 310 genetic analyzer. Statistical analysis was performed with SPSS vs. 21 and SNPStats software.
Results and conclusions: This study confirmed the association between the ARMS2 variation and AMD, detecting the T risk allele in a significantly higher frequency in the patient group compared with the control subjects (45% vs.29.13%, $p<0.001$, OR 1.99 CI 1.34-2.95). For the CD14 polymorphism, no statistically significant correlation was observed. As for the TLR4 polymorphisms, the percentage of heterozygotes was increased from 2.9% to 11.7% in the patient population for Asp299Gly and from 1.9% to 10% for the Thr399Ile polymorphism [ORs 4.40 ($p=0.01$) and 5.61 ($p=0.0088$), respectively].

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Molecular diagnostics - Genetic testing

Cod: W147

DEVELOPMENT OF HRMA METHOD FOR DETECTION OF GRN/MAPT MUTATIONS IN FRONTOTEMPORAL DEMENTIA

C. Florou-Chatziyiannidou [1], C. Koros [2], V. Papastefanopoulou [2], N. Andronas [2], J. Papatriantafyllou [2], L. Stefanis [2], S.G. Papageorgiou [2], C. Kroupis [1]

[1]*Clinical Biochemistry Department, Attikon University Hospital, Medical School, National and Kapodistrian University of Athens, Athens,GREECE*

[2]*Cognitive Disorders/Rare Dementias Clinic, 2nd Department of Neurology, Attikon University General Hospital, Medical School, National and Kapodistrian University of Athens, Athens, GREECE*

(Greece)
ckroupis@med.uoa.gr

BACKGROUND: Frontotemporal dementia (FTD) is as common as Alzheimer's disease in patients <65 years old. Two of the many genes that have been associated with the disease are the granulin gene (GRN) and the microtubule associated protein tau gene (MAPT), both mapped in 17q21 locus. GRN gene encodes for a secreted 593-amino acid protein that leads to brain neurodegeneration in cases of reduced concentration. MAPT gene transcribes six alternative splicing variants corresponding to isoforms varying from 352 to 441 amino acids in brain tissue, that mainly act as regulators of the stability of axonal microtubules. When MAPT is mutated, tau neuronal accumulation occurs. The pathogenic mutations of the GRN gene account for 3-12% of global FTD cases, whereas MAPT mutation frequency is generally 1-3%. So far, such mutations have been identified with the Gold standard DNA Sequencing method. The purpose of the study was the detection of pathogenic/potentially pathogenic GRN/MAPT mutations, for the first time in Greek FTD patients, through the development of a less laborious and cost-effecting screening method: HRMA (High Resolution-Melting curve Analysis).

PATIENTS AND METHODS: DNA was isolated with the High Pure PCR Template Kit (Roche) from peripheral EDTA blood of 18 Greek FTD patients, after they have signed an informed consent. Two GRN exons (3 and 12) and MAPT exon 13 were amplified with specially-designed primers in the Rotor-Gene Q 6000 (Qiagen) and subsequently were analyzed by HRMA in the presence of LCGreen Plus dye (Biofire Diagnostics). Primers for Cycle Sequencing with BigDye Terminator v1.1 and electrophoresis in ABI310 Genetic Analyzer were also designed for comparison of the results with the reference method of DNA Sequencing.

RESULTS AND CONCLUSIONS: Based on melting curve data analysis features, three DNA variants were detected: one in exon 3 of GRN gene, one in exon 12 of GRN gene and one in exon 13 of MAPT gene, all in three different patients. Sequencing method identified the GRN c.264delG mutation (p.E88fs), the GRN c.G1445A mutation (p.C482Y) and the MAPT c.2092G>A mutation (p.V698I) respectively. Method comparison indicates that the developing HRMA method is accurate, but further research for its validation is required. Future goals include the analysis of more samples of Greek FTD patients and the expansion of the method in the remaining GRN/MAPT exons, so that all such mutated patients are identified.

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Molecular diagnostics - Genetic testing

Cod: W148

AKT2 GENE POLYMORPHISMS IN POLYCYSTIC OVARY SYNDROME (PCOS)

A. Zerva [1], E. Trakakis [2], N. Poumpouridou [1], M. Tsagla [1], C. Kroupis [1], D. Kleanthi [1]

[1]*Department of Clinical Biochemistry, Attikon General University Hospital, National and Kapodistrian University of Athens, Athens, GREECE*

[2]*Department of Obstetrics and Gynecology, Attikon General University Hospital, National and Kapodistrian University of Athens, Athens, GREECE*

(Greece)
ckroupis@med.uoa.gr

Background: Polycystic Ovarian Syndrome (PCOS) is a common reproductive and endocrinologic disorder found in 6-10% of female population at reproductive age and is characterized by hyperandrogenism, oligo-ovulation, and polycystic ovarian morphology. AKT2 gene is widely expressed and its activation by insulin in adipocytes is reduced in type 2 diabetes, whereas experimental reduction of AKT2 leads to decreased insulin sensitivity and reduced glucose disposal, both of which are features of PCOS. The goal of this study was to investigate the association between AKT2 and Polycystic Ovary Syndrome. Four polymorphisms were studied; rs11671439, rs8100018, rs3730051 and rs2304188. The reason why AKT2 gene was chosen for this study is because its product affects metabolism of glucose, as well as mitogenic signaling and mediates cell survival in ovaries.

Methods: Genomic DNA was isolated from a total of 60 white patients with PCOS and 30 white control women that were age and sex- matched. Participation in research was offered to patients meeting the inclusion criteria, whereas control samples were healthy women with regular menstrual cycles. For genotyping of the four selected polymorphisms, novel real-time qPCR methods were developed using either the dual probe or the single probe format. All findings were confirmed using DNA Sequencing and statistical analysis was performed using SPSS and SNPstats.

Results and conclusions: This study confirmed that there was a statistically important difference ($p<0.042$) for SNP rs 2304188 between patients and controls regarding Minor Allele Frequency (MAF%). Also, a comparison of our results with those from the Genome Variation Server (GVS) for both patients and controls showed differences for both patients MAF % ($p<0.002$) and controls MAF% ($p<0.013$) for SNP rs3730051 and only controls MAF% ($p=0.014$) for SNP rs2304188. Furthermore, there was found an association between hirsutism and SNP rs2304188 ($p=0.044$) in PCOS patients. Finally, 8 cases of PCOS women (13.33% of patients) were found to have SNP rs8100018 in their DNA together with either rs11671439 or rs2304188, while no control women was bearing this combination.

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Molecular diagnostics - Genetic testing

Cod: W149

NEW MUTATION IN KIT EXON 11 IN A GASTROINTESTINAL STROMAL TUMOR

F. Sanchez-Jimenez [1], J.M. Maesa [1], E. Melguizo-Madrid [2]

[1]*Biología Molecular, Bioquímica Clinica, Hospital Universitario Virgen Macarena, Sevilla*

[2]*Bioquímica Clínica, Hospital Universitario Virgen Macarena, Sevilla*

(Spain)
concepcion.sanchez.r.sspa@juntadeandalucia.es

Background: KIT gene, in chromosome 4q12, encodes to a transmembrane glycoprotein belonging to type III receptor tyrosine kinase family that regulates cell functions as proliferation, apoptosis, chemotaxis and adhesion.
Some gain-of-function mutations in KIT are involved in pathogenesis of Gastrointestinal stromal tumors (GISTs) and also they are responsible for the development of resistance to imatinib, which is an inhibitor of specific protein tyrosine kinases used to treat unresectable and metastatic GISTs.
Mutations concern four exons (9, 11, 13 and 17), but mainly exon 11. Gastric GISTs with exon 11 deletions are more aggressive than those with substitutions. In addition, KIT exon 11 mutants respond well to imatinib. In-frame deletions in this exon are the most common mutations in GISTs. In this study we describe a new in-frame deletion in exon 11 of KIT that results in structural deleterious effects in the protein.
Methods: A sample of DNA extracted from biopsy of a GIST was amplified using specific primer pairs for exon 11 of KIT gene. First, the deletion was identified by running the PCR product on agarose gel electrophoresis. Then we proceed to sequence exon 11 PCR product as well as each allele independently after the bands were removed. They were purified separately from the gel by using ionic exchange columns. We performed Sanger dideoxy sequencing on ABI3130 equipment (Applied Biosystems). The results were compared with the reference sequence using BLAST. The most important database of somatic mutations in cancer were consulted (Cosmid, IntOGen). Ensembl GRCh38.p7 was used to get the cDNA and amino acids reference sequences.
Results: The electrophoretic profile identified the presence of a wild and a mutated allele of slightly lower molecular weight. We detected a six nucleotides in-frame deletion (ACCCAT) in heterozygosity in exon 11 of KIT gene: NM_000222: c.1650_1655delACCCAT. The subsequent analysis of the amino acid sequence showed a nonsense change with the generation of a novel stop codon: NP_000213. p.Pro551TyrfsX18. This mutation is supposed to disrupt the protein native structure and its function, probably resulting in pathogenic consequences that remains to be studied.
Conclusion:To our knowledge, the mutation described in this work is not included in Catalogue of Somatic Mutations in Cancer (COSMIC) nor in any other data base. Its nature as deletion with a nonsense result, would explain a pathogenic effect at the protein level.

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Molecular diagnostics - Genetic testing

Cod: W150

NON INVASIVE PRENATAL FETAL BLOOD GROUP GENOTYPING IN THE MONITORING OF ALLO-IMMUNISED ANTI-RH4 PREGNANT WOMEN: EXPERIENCE OF THE FRENCH NATIONAL CENTER FOR PERINATAL HEMOBIOLOGY (CNRHP).

N. Da-Silva Nelly [1], M. Vaubourdolle [4], S. Huguet-Jacquot [1], C. Toly-Ndour [1], E. Maisonneuve [3], S. Friszer [3], J. Jouannic [3], A. Cortey [2], A. Mailloux [1]

[1]*Centre National de Référence en Hémobiologie Périnatale (CNRHP), Hôpital Saint Antoine, AP-HP, Paris, France*

[2]*Centre National de Référence en Hémobiologie Périnatale (CNRHP), Hôpital Trousseau, AP-HP, Paris, France*

[3]*Départements de Gynecologie - Obstétrique et de Médecine fœtale , Hôpital Trousseau, APHP, Paris, France*

[4]*Pôle Biologie médicale et pathologie, Hôpitaux Universitaires Est Parisien, AP-HP, Paris, France*

(France)
agnes.mailloux@sat.aphp.fr

Background:
Maternal-feto blood group incompatibility is common and may result in hemolytic disease of the fetus and newborn (HDFN). This disease is characterised by anemia and hyperbilirubinemia which may lead to fetal hydrops, kernicterus or death. Three antibodies are associated with severe fetal disease: anti-RH1, anti-KEL1 and anti-RH4. CNRHP provide non invasive fetal genotyping as a routine service to help the practitioners to improve the accuracy follow-up in pregnant women anti-RH1and KEL1 allo-immunised but this assistance are not yet provided to monitoring pregnant women RH4 allo-immunised. Among the 300 patients/year followed by CNRHP, about 100 have severe immunisation (RH4 antibody dosage higher than 500UCHP/ml) leading to specific antenatal monitoring if partner are RH4.
Aim:
Set up and evaluation of non invasive prenatal fetal genotyping to guide the follow-up of allo-immunised anti-RH4 pregnant women.
Methods:
To set up non invasive fetal RHc genotyping, DNA from 19 plasmas of RH:-4 women between 12 and 38 weeks amenorrhea were isolated using manual methods. Then RHc allele was detected by PCR using an adapted published method (Finning et al. Transfusion, 2007, 47: 2126-33) and compared to RHc genotype determined from amniotic cell or to red blood cells phenotype of the babies at birth.
Results:
Non invasive fetal RHc genotyping set up result show a sensibility, specificity and a positive and negative predictive value of 100%.
Since April 2016, CNRHP does non invasive fetal RHc genotyping from peripheral maternal blood. 30 non invasive fetal RHc genotyping from allo-immunised anti-RH4 women with RH: 2.4 partners were done to identify foetuses at risk for HDFN. For 24% of the allo-immunized women, the pregnancy was found compatible.
Conclusion:
Non invasive RHc fetal genotyping is a powerful tool to diagnose a feto-maternel red blood cells incompatibility and allows to legitimize a costly and heavy specific antenatal monitoring only to pregnant women carrying incompatible fetus with every two weeks an anti-RH4 dosage and weekly a search for signs of fetal anemia (Velocimetry Doppler).

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Molecular diagnostics - Genetic testing

Cod: W151

DIAGNOSTICS OF INTRAUTERINE COMPLICATION LEADING TO PRETERM BIRTH BY USING MOLECULAR METHODS

M. Rabajdova [2], R. Dudic [1], V. Dudicova [1], P. Urdzik [1], M. Marekova [2]

[1]*Department of Gynecology and Obstetrics, Pavol Jozef Šafárik University in Košice, Faculty of Medicine, Slovakia*

[2]*Department of Medical and Clinical Biochemistry, Pavol Jozef Šafárik University in Košice, Faculty of Medicine, Slovakia*

(Slovakia (Slovak Republic))
maria.marekova@upjs.sk

Today, many pathological conditions complicate pregnancy. As well Pre-eclampsia (PE), Intrauterine Growth Restriction (IUGR), fetal growth retardation and preterm birth are involved in the increase of maternal and perinatal morbidity and mortality. The most common cause of maternal mortality is eclampsia, intracranial hemorrhage, renal failure, liver failure and HELLP (Hemolysis, Elevated Liver enzymes, Low Platelets) syndrome. To the studied serum markers of PE, IUGR and preterm birth include Vascular Endothelial Growth Factor (VEGF), Placental Growth Factor (PLGF) and antagonists of soluble Fms-like tyrosine kinase 1 (sFlt-1, known as sVEGFR1) and soluble Endoglin (ENG). The increased expression of hypoxia induced factors like (HIF-1), triggers overexpression of anti-angiogenic genes. We determined the transcriptional activity of individual pro and anti-angiogenic markers (VEGF, HIF-1, sEng, Flt-1, PlGF-1) in maternal blood samples from patients with spontaneous preterm birth, preterm birth in combination with IUGR + PE and term birth in combination with IUGR + PE comparing to physiologically terminated pregnancies.
Methods: We detected transcriptional activity of specific genes, from the peripherals blood of patients using chromatin immunoprecipitation capture and qReal-Time-PCR methods.
Results: The maximal differences in mRNA levels of PlGF-1, VEGF-A were detected in 2 groups; normal term birth group with complications and preterm labor with complications (both significantly lower than control, $p<0.001$). In contrast, the marked increase of mRNA levels was found in the same groups of patients for genes HIF-1, endoglin and Flt-1 ($p<0.001$).
Conclusion: Our results from molecular analysis, showed that transcriptional activity of endoglin gene, have been increasing depending of the degree of retardation of the patient population. As well as we saw that increased of oxidative stress, increasing the expression levels of anti-angiogenic genes, and reduction of the transcriptional activity of pro-angiogenic genes what can provide additional information during diagnostics of birth pathological complications.
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Molecular diagnostics - Genetic testing

Cod: W152

ADVANCES IN MOLECULAR DIAGNOSTICS WORKFLOW: COMPARISON OF THE DXN VERIS AND ROCHE COBAS® 6800 SYSTEMS

M. García-Álvarez [1], F. Gutiérrez [1], S. Zurita [1], R. Delgado [1]

[1]*Servicio de Microbiología. Hospital Universitario 12 de Octubre. Madrid. Spain.*

(United Kingdom)
bgmckeown@beckman.com

Background: The DxN VERIS Molecular Diagnostics System (DxN VERIS) from Beckman Coulter is a new addition to the molecular diagnostics market. The objective of this study was to compare workflow on the DxN VERIS and cobas® 6800 systems. This study included the four quantitative viral load assays for HIV-1, HCV, HBV and CMV. The workflow was carried out over a three day period and various parameters were investigated for both systems ranging from complexity of use, to Hands-On-Time (HOT) and Time-To-First/Last Results (TTFR/TTLR).

Methods: Duplicate samples for HIV-1, HCV, HBV and CMV were prepared to facilitate running on either the DxN VERIS or cobas® 6800 systems. Detailed timings were recorded for system maintenance, set-up/test preparation, sample preparation and post-run clean up for a variety of test assay/test combinations. Workflow logging and analysis was performed by NEXUS, Plano, TX.

Results: Overall the DxN VERIS system required fewer steps in terms of workflow and instrument set up with 10 steps compared to 26 steps for the cobas® 6800. The number of consumables required to run both systems was also different. The DxN VERIS required a total of five consumables compared to the cobas® 6800 which needed in excess of 15 consumables. The total HOT for the 100 samples analyzed on day 1 including instrument maintenance, system and/or test prep, sample prep and post run activities for the DxN VERIS was 19 minutes when compared with 43 minutes for the cobas® 6800.
The TTFR and TTLR for a "typical" day's testing (day two samples, total of 52 samples) was determined to be 79 minutes and 254 minutes respectively on the DxN VERIS. On the cobas® 6800 the TTFR and TTLR was 179 and 271 minutes respectively.
A final run time comparison was performed on 25 patient samples. Viral load assays for HIV-1, HCV and CMV were requested for each sample on both systems. The TTFR and TTLR on DxN VERIS were 80 and 357 minutes respectively. On the cobas® 6800 the TTFR and TTLR were 187 and 187 minutes respectively.

Conclusion: The DxN VERIS and cobas® 6800 both offer a more streamlined workflow compared to older molecular diagnostics platforms. In addition, the simplified workflow and instrument set-up lead to additional time savings on the DxN VERIS.
The DxN VERIS also offers single sample random access thereby speeding up the time to the first results becoming available.

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Molecular diagnostics - Genetic testing

Cod: W153

PERFORMANCE EVALUATION OF THE VERSANT ZIKA ASSAY

A. Miller [1], T. Battersby [1], A. Chmura [1], A. Lal [1], D. Monga [1], R. Malhotra [1], A. Patel [1], S. Hao [1], H. Huang [1], C. Wagner [1], G. Kritikos [1], J. Mosner [1]

[1]*Siemens Healthcare Diagnostics, Inc.*

(United States)
ashleigh.miller@siemens.com

Background: Zika (ZIKV) is a mosquito-borne virus of the family Flaviviridae related to Yellow Fever, Dengue, and West Nile. In February 2016, the World Health Organization (WHO) declared that the ongoing ZIKV outbreak in the Americas, associated microcephaly, and other neurological disorders, constitutes a Public Health Emergency of International Concern.1 Current Centers for Disease Control and Prevention (CDC) guidance calls for RT-PCR testing for Zika on plasma, serum, or urine collected from patients within 14 days post onset of symptoms.2

We evaluated the analytical sensitivity, specificity and clinical correlation of a qualitative diagnostic real-time PCR assay, the VERSANT® Zika RNA 1.0 Assay (kPCR).*

Methods: The VERSANT Zika RNA 1.0 Assay (kPCR) qualitatively detects ZIKV RNA in plasma, serum, and urine and targets two regions of ZIKV: NS2 and NS5. ZIKV RNA was extracted with Siemens' VERSANT Sample Preparation 1.0 Reagents and amplified on three real-time PCR systems. Analytical sensitivity was evaluated with a dilution series of NATtrol Zika Virus (ZeptoMetrix) (Source material from ZeptoMetrix and CDC). Cross-reactivity was evaluated with 61 closely related pathogens.

Clinical evaluation was performed with 347 serum, plasma, or urine samples collected from patients suspected or confirmed to contain ZIKV. The VERSANT Zika RNA 1.0 Assay (kPCR) results were compared to the CDC 1077 Assay (Trioplex RT-PCR).

Results: Evaluation of a dilution series of cultured ZIKV established an assay limit of detection in both plasma and serum of ≤ 0.05 U/mL (TCID50) and ≤ 0.075 U/mL in urine. No cross reactivity was observed with any tested pathogens, and no significant sequence homology was seen by in silico evaluation.

In clinical sample testing, the VERSANT Zika RNA 1.0 Assay (kPCR) detected ZIKV in 42 of 68 plasma samples with a percent positive agreement (PPA) of 100% with the CDC 1077 assay. In serum, the VERSANT Zika RNA 1.0 Assay (kPCR) detected ZIKV in 65 of 98 samples; PPA 90.6%. In urine, VERSANT Zika detected ZIKV in 52 of 97 samples; PPA 86.7%. In plasma/serum and urine matched samples, the VERSANT Zika RNA 1.0 Assay (kPCR) detected ZIKV in 68 of 78 samples; PPA 95.8%.

Conclusion: The VERSANT Zika RNA 1.0 Assay (kPCR) qualitatively detects ZIKV RNA in serum, plasma, and urine with a high degree of sensitivity, has high correlation between serum/plasma and urine samples, and has excellent performance with clinical specimens.

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Molecular diagnostics - Genetic testing

Cod: W154

MOLECULAR SCREENING FOR TSC1 AND TSC2 MUTATIONS IN GREEK PATIENTS WITH TUBEROUS SCLEROSIS SYNDROME USING NGS

A. Papadopoulou [4], G. Koutsodontis [7], A. Dinopoulos [3], R. Pons [2], V. Koute [5], P. Vorgia [6], A. Vratimos [7], D. Zafeiriou [1]

[1]*1st Department of Pediatrics, Aristotle University of Thessaloniki, Greece*

[2]*1st Department of Pediatrics, University of Athens, Agia Sophia Children's Hospital, Athens, Greece*

[3]*3rd Department of Pediatrics, Medical School, University General Hospital "ATTIKON", Athens, Greece*

[4]*3rd Department of Pediatrics, Medical School, University General Hospital "ATTIKON", Athens, Greece & DNAGENETIX, Medical Diagnostic Laboratory, Athens, Greece*

[5]*Department of Pediatrics, Faculty of Medicine, University of Thessaly, Greece*

[6]*Department of Pediatrics, University of Crete, Greece*

[7]*DNAGENETIX, Medical Diagnostic Laboratory, Athens, Greece*

(Greece)
a_papado@yahoo.com

Background
Tuberous Sclerosis (TSC) is a rare hereditary genetic disorder, inherited by an autosomal dominant manner. It is characterised by high clinical heterogeneity and the development of multiple hamartomas in various tissues. At the molecular level the syndrome involves the presence of pathogenic mutations in TSC1 (9q34) or TSC2 (16p13.3) genes. Next Generation Sequencing (NGS) is increasingly in use for the genetic diagnosis of TSC. Here, we report the first record of TSC1 and TSC2 mutations in TSC Greek patients.
Methods
Germline DNA was isolated from 17 pediatric patients recruited from 5 clinics. Mutational analysis was performed in the TSC1 and TSC2 genes using a custom solution-based sequence capture manufactured by Roche Nimblegen (Madison, WI), including exons 3-23 of TSC1 gene, 2-42 exons of TSC2 gene and 100 bp of the flanking intronic regions. The final estimated capture size was 21,361b (99.6% target bases covered). Sequence reads were mapped against the human chromosomes reference (hg38) using the GS Reference Mapper (Roche, version 2.6 and 2.7) and the JSI medical systems GmbH software. All pathogenic or likely pathogenic variants were confirmed by Sanger sequencing. MLPA was performed for the detection of larger deletions/insertions in both genes.
Results
The number of reads per run was estimated on average to be 129,783 of which 98,149 were mapped with a mean length of 431 bp. 14 different mutations were identified in the TSC1 and TSC2 genes in 14 patients whereas no mutations were found in 2 children. 7 of the mutations were novel and functional prediction models showed a pathogenic effect. Among the mutations identified, 7 were missense (6 in TSC2 37.3%, 1 in TSC1; 6.3%), 1 splicing (in TSC2; 6.3%), 6 small indels (4 in TSC2 25%, 2 in TSC1; 12.5%) and 2 large deletions (1 in TSC2 6.3% and 1 in TSC1; 6.3%). All but one cases were sporadic and their age of diagnosis ranged between birth day to 8 years. Most individuals with TSC2 mutations had mild or less severe neurological phenotype. Angiomyolipoma and renal cysts were not reported in patients with deletions.
Conclusions
Molecular screening for mutations in TSC1 and TSC2 genes using a SeqCap custom made NGS panel reached a mutation detection rate of 88% in the tested Greek patients which fulfilled the diagnostic criteria.

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Molecular diagnostics - Genetic testing

Cod: W155

SCREENING FOR MUTATIONS IN CLINICALLY CHARACTERIZED MODY2 TYPE DIABETES PATIENTS WITHOUT FAMILY HISTORY OF DIABETES.

M.S. Perez [2], A. De Dios [1], I.J. Chiesa [2], G.D. Frechtel [1], A.P. Lopez [1]
[1]*División genética. Hospital de Clínicas José de San Martín.UBA*
[2]*Medicina genómica. Laboratorio Manlab*

(Argentina)
maria.perez@manlab.com.ar

Background:
MODY2 is a form of Diabetes that is characterized by an early age at onset, a mild hyperglycemia and with a low risk of complications. Despite finding causal de novo mutations was uncommon in the related gene, in recent studies a higher frequency of this kind of mutations has been detected. The probable reason of this discrepancy is because typical recommendations regarding the diagnosis of Maturity-onset diabetes of the young 2 (MODY2) and screening for mutations include the existence of a family history as an exclusionary rule. Therefore, de novo mutations could be more frequent than was already described. In this work, we describe the study of mutations in four individuals that have clear clinical features of MODY2 but without family history of Diabetes and their parents that are clinically healthy, in search for de novo mutations in the GCK gene.
Methods:
Within a larger screening of GCK mutations in a cohort of patients clinically characterized as MODY2 with family history of Diabetes, we studied 4 patients with MODY2 clear characteristics but without family history of Diabetes and their both healthy parents.
Genetic studies were carried out as follows: DNA was extracted from blood samples by the MagNA Pure system (Roche) followed by PCR amplification employing specific primers for GCK gene, and finally, direct sequencing of PCR products. Paternity was analysed by AmpFLSTR Identifiler PCR Amplification Kit.
Bioinformatic tools used were ChromasLite and BLAST software for DNA sequences, and mutations found were analyzed using Mutation taster software.
Results:
The 4 patients studied have de novo mutations in the GCK gene, so being 4 out of 34, results in 11.76% of all the participants genotyped as MODY2.
Results obtained by the Identifyler method allowed us to discard a case of false paternity. All mutations were predicted to be deleterious using the software Mutation taster.
Conclusions:
The number of mutations in GCK/MODY2 is undoubtedly underestimated, as accepted criteria for performing genetic tests include family history of the pathology. These cases illustrate the importance of analyzing the GCK gene in patients with clinical features of MODY2, even in the absence of family history as it is essential for establishing a correct diagnosis, treatment and genetic counsel in the patients.

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Molecular diagnostics - Genetic testing

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AETIOPATHOGENESIS OF VITILIGO: BIOCHEMICAL AND MOLECULAR BASES

L. Postiglione [2], G. Varriale [1], G. Di Spigna [2], C. Cappelli [5], R. Di Francia [4], A. Sangermano [3]
[1]*AXIA Clinic, Naples, Italy*
[2]*Department of Translational Medical Sciences University "Federico II", Naples, Italy*
[3]*Dia-Chem Medical Biotechnology, Naples, Italy*
[4]*Oncologists Group Italian Researchers (GORI), Pordenone, Italy*
[5]*SENEBGENE, Naples, Italy*

(Italy)
loredana.postiglione@unina.it

Vitiligo is a common dermatosis etiopathogenesis not yet well defined. A genetic and biochemical study of autocytotoxic pathogenetic mechanisms in patients with vitiligo in association with family members has been carried out. 21 families have been enrolled: 43 members show vitiligo and 20 members do not establish this dermatological condition. 30 donors were used as control.
Either biochemical and molecular methods were used: analysis of serum haptoglobin and ceruloplamina with Nephelometry, analysis of homocysteine and other thiols (glutathione) in the blood by liquid chromatography using reverse phase HPLC (High-performance liquid chromatography) and fluorescence detection of the derivatised free thiols with fluorochrome, analysis of vitamin A and E in serum by liquid chromatography HPLC after extraction of the samples in the organic phase, identification, by molecular analysis with the PCR (Polymerase Chain Reaction) on genomic DNA from peripheral blood. Extraction of DNA is performed follwed by amplification and digestion with restriction enzymes and analysis of C677T polymorphism of the gene coding for enzyme MTHFR C677T (methylene-tetrahydrofolate reductase), A1298C polymorphism of the gene encoding the enzym MTHFR A1298C(methylene-tetrahydrofolate reductase), A66G polymorphism of the gene encoding the enzyme MTRR A66G (methionine synthase reductase), 844ins68 mutation of the gene encoding the CBS844 ins 68 (cystathionine beta-synthase enzyme), I278T mutation of the gene encoding the enzyme CBS I278T (cystathionine beta synthase).
Results compared to control values showed a decrease in haptoglobin values, an increase in plasma homocysteine values, a normal range of vitamin A and vitamin E and an increase in the prevalence of polymorphisms in homo- and heterozygous for the MTHFR C677T, MTRR A66G, and CBS I278T mutation. The first data would be indicative of a dysregulation, genetically based, of thiols production mechanisms

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Molecular diagnostics - Genetic testing

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PREVALENCE OF MUTATIONS IN A PANEL OF DIFFERENT GENES AMONG GEORGIANS

T. Ramishvili [2], T. Pabst [1], D. Steinberger [1], T. Phirtskhalaishvili [2], L. Zangurashvili [2], N. Kankia [2], N. Jashiashvili [2], M. Lomidze [2], M. Barnova [2], N. Abramishvili [2], S. Kavsadze [2], T. Dzagania [2], K. Kvatadze [2], I. Rtskhiladze [2]

[1]*bio.logis Zentrum für Humangenetik, Frankfurt am Main, Germany*

[2]*Laboratory, Medical Center Mrcheveli, Tbilisi, Georgia*

(Georgia)
tamarramishvili@yahoo.com

BACKGROUND
Personal Genomics Service(PGS) is the multi gene panel test which allows all interested persons to obtain relevant information on their genetics. PGS gives us information about the following 29 gene status: Alcohol metabolism(ADH1B,ALDH2), alpha-1 antitrypsin deficiency(AAT), Response to beta blockers(ADRB3), Antioxidants, detoxication, oxidative stress(SOD2), Blood pressure regulation(AGT,AGTR1), Iron storage(HFE),Family Mediterranean fever(MEFV), Favism(G6PD), Folic acid metabolism (SLC19A1), Fructose intolerance, hereditary(ALDOB), Hypercholesterolemia(APOB), Copper storage(ATP7B), Lactase persistence(LCT), Medium-chain acyl-CoA dehydrogenase(MCAD)-deficiency(ACADM), Crohn's disease(NOD2), Morbus Mülengracht (UGT1A1), Mucoviscidosis(CFTR), Osteoporosis risk(COL1A1), Sickle cell disease(HBB), Sports performance types(ACE,ACTN3) Thrombophilia disposition(F2,F5,MTHFR,PAI1), Atherosclerosis(PON1).Analysis of the results allows us to assess the distribution of gene frequency in Georgians.
METHODS
We retrospectively analyzed data of PGS from Medical Center Mrcheveli, in 32 patients from ethnical Georgians. From here 20 of them were clinically MEFV suspected and other 12 had no indication. The following data were collected: Gender, Age, Gene dispositions in each patient. Genetic study was performed in Bio.logis Zentrum für Humangenetik Frankfurt a. M. Germany,within the test of PGS, using the Competitive Allele Specific PCR (KASP)-Technology by Laboratory of the Government Chemist (LGC) Genomics, Fragment analysis on the AB Prism sequencer.
RESULTS
32 patients:17 male,15 female, mean Age 27 years(0-60 year),with the following gene prevalence:
LCT-32(100%)of patients(Pt),4Heterozygous(HT),28Homozygous(HM)
ADH1B-4(12.5%) Pt,1 HM,3HT,28wildtype(WT)
SOD2-22(68.8%) Pt,7HM,15HT,10WT
AGTR1-12(38%) Pt,0 HM,12HT,20WT
AGT-23(72%) Pt,6HM,17HT,9WT
HFE-11(34%) Pt,0 HM,11HT,21WT
SLC19A1-24(75%) Pt,8 HM,16HT,8WT
MTHFR-28(87.5%) Pt,10 HM,12HT,6compound-HT,4WT
MEFV-10(31%) Pt,4 HM,6HT,22WT
UGT1A1-22(69%) Pt,7 HM,15HT,10WT
NOD2-3(9%) Pt,0 HM,3 HT,29WT
COL1A1-8(25%) Pt,1HM,7HT,24WT
ACE-23(72%) Pt,12 HM,11HT,9WT
ACTN3-29(91%) Pt,11 HM,18HT,3WT
F2-2(6%) Pt,0 HM,2HT,30WT
PAI1-27(84%) Pt,12 HM,15 HT,5WT
PON1-27(84%) Pt,10 HM,13 HT,4compound-HT,5WT
ATP7B,APOB,ACADM,ALDH2,CFTR,HBB,G6PD-32(100%)WT
AAT,ADRB3,ALDOB,F5-1(3%) Pt,0 HM,1HT,31WT
CONCLUSIONS
We can conclude that the most frequent 5 genes are:ACTN3,SOD2,MTHFR,PAI1,PON1.

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Molecular diagnostics - Genetic testing

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IMPLICATION OF POLYMORPHIC MARKER IVS6A GATT IN THE CLINICAL EXPRESSION OF CYSTIC FIBROSIS

C. Sahli [1], S. Hadj Fredj [1], R. Dabboubi [1], K. Boussetta [2], A. Mehrezi [3], T. Messaoud [1]

[1]*Biochemistry Laboratory, Children's Hospital Bechir Hamza, Research Laboratory LR00SP03, Tunis, Tunisia*

[2]*Department of Pediatrics, Children's Hospital Bechir Hamza, Tunis, Tunisia*

[3]*Department of Pediatrics, Hospital Mongi Slim, Tunis, Tunisia*

(Tunisia)
chaymasahli@yahoo.fr

Background: Cystic fibrosis (CF) is the most common autosomal recessive disease in Caucasians, caused by mutation in Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). The analysis of some extra and intragenic markers within or closely linked to CFTR gene is useful as a molecular method in clinical linkage analysis. Indeed, knowing that the molecular basis of CF is highly heterogeneous in our population is explained in the present study. In this work, we are interested for the first time to study the polymorphic marker IVS6a GATT in a CF Tunisian population.
Methods: Our study involved 80 CF Tunisian patients with a positive sweat test. A cohort of 90 healthy controls was also enrolled. The analysis of the variant IVS6a GATT was conducted by analysis of the fragments on automatic sequencer (ABI Prism 310). A statistical analysis was performed on Statistical Package for the Social Sciences (SPSS) version 20 software.
Results: The analysis of genotypic distribution of IVS6aGATT showed a significant difference between the control and CF groups suggesting the involvement of this marker in cystic fibrosis. Furthermore, we noted that the 6 GATT repetition in the homozygous state is more common in CF patients than in the control group ($p < 0.05$). This while the 7GATT / 7GATT genotype is more common among controls compared to CF patients ($p = 0.002$). Regarding the interest of this polymorphism on the clinical expression of cystic fibrosis, we have noted no significant association between 6/6 genotype with different clinical conditions in CF patients outside the CFTR mutation. While a significant association was found between respiratory involvement and mixed (respiratory and digestive) and the 6/6 genotype in patients with the mutation F508del homozygous ($p < 0.05$). In addition, a significant association was also noted with gastrointestinal involvement for non F508del patients / F508del not ($p = 0.014$). Given that, phenotypic and genotypic heterogeneity of cystic fibrosis, several studies have sought to highlight the role of genetic markers linked to the CFTR gene in the expression and evolution of the disease.
Conclusion: Our study on the implication of polymorphic marker IVS6a GATT is one of the first works carried out in the Tunisian population and confirms the usefulness of this marker in the clinical expression of cystic fibrosis.

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ASSOCIATION ANALYSIS BETWEEN THE ANKYRIN REPEAT AND KINASE DOMAIN CONTAINING 1 (ANKK1) TAQIA POLYMORPHISM AND ALCOHOL DEPENDENCE

M. Santamaria Gonzalez [1], S. Menao [1], A. Ferrer [2], J.J. Puente [1]

[1]*Clinical biochemistry service. Lozano Blesa university hospital, Zaragoza*

[2]*Toxicology Unit, Lozano Blesa university hospital, Zaragoza*

(Spain)
airamsg@hotmail.com

BACKGROUND-AIM

The rewarding effects of alcohol are mediated through the mesolimbic dopamine system so the dopamine D2 receptor gene (DRD2) is considered a good candidate gene for alcohol dependence risk. The ankyrin repeat and kinase domain containing 1 (ANKK1) TaqIA polymorphism is located 10 kb downstream from DRD2 and it has been extensively studied as a marker of the gene for dopamine receptor D2 (DRD2) in drug addictions. The aim of the present study was to investigate the contribution of the TaqIA polymorphisms (rs1800497) in patients with alcoholism.

METHODS

Genetic association between TaqIA and alcohol dependence was investigated using a case-control approach. The case group is made up of 85 patients with alcoholism from detoxification centers. Alcohol dependence was determined by DSM-IV criteria. The control group is made up of 85 volunteers that do not have any kind of dependence. Then, genomic DNA was extracted from venous blood and DNA and amplified by PCR reaction. Taq1A was genotyped by PCR-RFLP and resolved by electrophoresis. All the data were entered into SPSS15.0 software as a database. Genotype and allele frequencies were compared between groups using chi-square test. A two-tailed type I error rate of 5% was chosen for the analysis was used for statistical analysis. If the P-value was less than 0.05, the difference was judged as significant.

RESULTS

Three genotypes were obtained from patients group: A2A2 (p2 = 0.36), A1A2 (2pq=0.42) y A1A1 (q2=0.22) and from control group: A2A2 (p2 = 0.59), A1A2 (2pq=0.36) y A1A1 (q2=0.05). None of the genotype distributions deviated significantly from those expected by Hardy–Weinberg equilibrium for cases or controls. We found evidence for association ($\chi 2$ = 13.74, P-value = 0.001) in TaqIA polymorphisms .

CONCLUSIONS

The findings from this study suggest an important association between TaqI A polymorphism and alcohol dependence so patients who carry TaqIA may have a higher risk for developing an alcohol use disorder.
ANKK1 variants may increase the likelihood that a person will become alcohol dependent. So additional studies, using larger sample sizes and new methods for simultaneous analysis of multiple polymorphisms.

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Molecular diagnostics - Genetic testing

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RET MUTATION STATUS IN MEDULLARY THYROID CANCER PATIENTS AND THE SIGNIFICANCE OF GENETIC SCREENING IN THEIR IMMEDIATE RELATIVES

K. Sifi [2], S. Hanachi [2], A.K. Lezzar [3], K. Boudaoud [3], N. Nouri [3], C. Benlatreche [1], N. Abadi [1]

[1]*Biochemistry Laboratory of the University Hospital of Constantine (2) Laboratory of Biology and Molecular Genetics University 3 of Constantine*

[2]*Biochemistry Laboratory of the University Hospital of Constantine , Laboratory of Biology and Molecular Genetics University 3 of Constantine*

[3]*Department of Endocrinology University Hospital of Constantine*

(Algeria)
sifikarima@yahoo.fr

Introduction
Medullary Thyroid Carcinoma (MTC) can occur in hereditary (25%) or sporadic form (75%).
In the hereditary forms, MTC is the major component of the Multiple Endocrine Neoplasia type 2 (MEN 2).
MEN 2 is caused by autosomal dominant RET proto-oncogene mutations. Early prophylactic total thyroidectomy before the development of MTC is the only curative treatment.
The aims of this study were to:
-Determine the frequency and the localization of the detected RET proto-oncogene changes in MTC case index and their relatives and to compare them with the data of the literature.
-Present the phenotype–genotype correlation in Algerian MEN2 families.
Patients and methods
DNA was extracted from the peripheral blood lymphocytes of a total of 40 persons, including 25 MTC probands and 15 of their unaffected kindred's. Exons 8,9,10, 11, 13, 14, 15 and 16 of the RET gene were amplified by PCR and sequenced. Informed consent was obtained from all subjects.
Results and Discussion
The C634Y RET exon11 germline mutation was detected in 8% of our MTC index cases and in 46.66% of their relatives. In relatives G691S and S904S polymorphisms identical to those of MEN2 index cases were found but in the homozygous state, suggesting that this haplotype has a modifying effect on the age of onset of MTC in MEN2A.
In sporadic MTC, the exon 11G691S SNP, was strongly present. Several studies have shown that this SNP is associated with sporadic MTC predisposition.
In our patients, the C634Y mutation was significantly associated with the presence of pheochromocytoma.
Conclusion
The identification of the C634Y mutation allowed us to offer to the mutated case index and their relatives prophylactic thyroidectomy.

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ASSOCIATION BETWEEN METHYLENETETRAHYDROFOLATE REDUCTASE GENE C677T POLYMORPHISM AND COLORECTAL CANCER SUSCEPTIBILITY

K. Sifi [2], S. Hanachi [2], K. Boudaoud [4], C.Y. Amrane [1], T. Filali [3], N. Abadi [2], C. Benlatreche [2]

[1]*Anatomy department of the University Hospital of Constantine*

[2]*Biochemistry Laboratory of the University Hospital of Constantine , Laboratory of Biology and Molecular Genetics University 3 of Constantine*

[3]*Oncology department of the University Hospital of Constantine ,Laboratory of Biology and Molecular Genetics University 3 of Constantine*

[4]*Radiotherapy department of the University Hospital of Constantine ,Laboratory of Biology and Molecular Genetics University 3 of Constantine*

(Algeria)
sifikarima@yahoo.fr

Introduction
The 5,10 methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism . The MTHFR C677T polymorphism creates a thermolabile enzyme which decreases enzyme activity, appears to interfere with the phenomena of carcinogenesis by reducing the DNA methylation levels and by monitoring the synthesis of DNA.
Numerous studies have highlighted the important role of MTHFR in carcinogenesis.
The aims of this study were to:
-Determine the allelic and genotype frequencies of MTHFR C677T polymorphism in patients with colorectal cancer (CRC) and healthy controls.
-Performe estimation of relative risk associeted with this polymorphism in CRC cancer compared to halthy controls.
Patients and methods
70 patients with colorectal cancer (CRC) and 101 healthy controls, were genotyped for the MTHFR C677T polymorphism by using the PCR / RFLP method.
Results
Allelic frequencies of MTHFR 677T and MTHFR 677C were 34,92 % and 65,07 % respectively in the control group, 43.75% and 56.25%, respectively, for patients with CRC.
The odds ratio 677 T/T vs 677 C/C and 677 C/T vs 677C/C were 9.82 (0.91-19.54) ($p<0.05$) and 1.4 (0.9-2.3) ($p<0.05$) respectively in CRC patients.
Conclusion
Our results have shown some evidence for possible genetic contribution of this polymorphism to the development of CRC, which is in agreement with the data of the littérarure.

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DEVELOPMENT OF A MULTIPLEX BIOCHIP ARRAY PLATFORM FOR THE RAPID, SIMULTANEOUS DETECTION OF NRAS MUTATIONS

R. Waugh [1], H. Murray [1], M. Crockard [1], J. Lamont [1], S. Fitzgerald [1]

[1]*Randox Biosciences, Crumlin, United Kingdom*

(United Kingdom)
scientific.publications@randox.com

Background. Tumour biomarker status is now routinely used to guide treatment decisions in patients with cancer. This is of particular benefit to patients with metastatic colorectal cancer (mCRC) due to heterogeneity in response and toxicities associated with available targeted therapies. KRAS exon 2 mutation status has been an established predictive marker to anti-EGFR therapies since 2008. However, recent evidence has demonstrated that NRAS mutational status is also predictive of response and as a consequence, it is now necessary to expand the mutational profiling of mCRC patients to include NRAS in order to effectively tailor treatment. This study reports the development of a biochip array to enable the rapid, simultaneous detection of 14 mutations within the NRAS gene (codons 12, 13, 59, 61, 117 and 146).
Methods. The assay exploits current technology enabling high multiplexing through use of a combination of multiplex PCR and biochip array hybridisation. Sample analysis can be completed, from template DNA, through PCR to data readout in less than 3 hours. Primers were designed and optimised for use in a single multiplex PCR reaction, with amplifiable target regions limited to ≤100bp. Assay specificity and limit of detection (LOD) of 14 mutations was determined using cell line blends and/or commercial reference standards (1% and 5% sensitivity levels). Cross-reactivity was analysed against KRAS cell line blends (exons 2, 3 and 4). The biochip assay was then challenged with a range of CRC samples (FFPE and fresh/frozen tissue) and mutational status confirmed using Sanger sequencing.
Results. 100% specificity was achieved for all 14 assay targets with assay LOD determined at 1%. No cross-reactivity was noted against KRAS. 100% agreement was also demonstrated between the biochip assay and Sanger sequencing for the range of samples analysed.
Conclusion. This biochip array platform is capable of detecting multiple NRAS mutations simultaneously with high discrimination and at least 1% sensitivity. Combining this array with the existing KRAS/BRAF/PIK3CA Array (EV3799A/B), would aid in the selection of candidate patients to receive anti-EGFR therapy. This extended mutational profiling of mCRC patients could thus improve drug efficacy and minimise adverse patient effects.