Molecular diagnostics

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MICRORNAS EXPRESSIONS PROMOTE TO THE EXPANSION OF CANCER STEM CELL GENERATION IN PROSTATE CANCER

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BACKGROUND-AIM

Prostate cancer (PCa) is the second most common cause of cancer and the sixth leading cause of cancer death worldwide. However, reliable prognostic and diagnostic biomarkers are lacking to distinguish indolent from localised and metastatic PCa. Therefore, in this study, we focus on the roles of miR141 and 145 and their association with stem cells in different stages of prostate cancer

METHODS

In this study, we used quantitative real-time PCR to identify the miRNA expression of miR-141 and miR-145, and prostate cancer stem cell (PCSCs), apoptosis, and cellular ROS levels were analyzed by flow cytometry in benign prostatic hyperplasia (BPH), locliased PCa, and Metastatic PCa.

RESULTS

The results showed that the fold change mean expression of miR-141 was upregulated in localised PCa and metastatic PCa compared to a BPH control. In contrast, miR-145 was downregulated in localised PCa and metastatic PCa as compared to BPH. However, the mean expressions of CD44+/CD24− and CD133 showed higher expression in localized PCa and metastatic PCa than in BPH. The levels of apoptosis, reactive oxygen species (ROS), prostate-specific antigen (PSA), and testosterone also showed a significant increase ($p<0.001$) in both localized PCa and metastatic PCa as compared with BPH. Moreover, all studied markers showed significant ($p<0.001$) diagnostic potential in estimating cases of metastatic PCa and those of localized PCa except PSA. The inter-correlation of different studied variables in localised PCa and metastatic PCa, respectively. In localised PCa, significant ($P<0.05$) positive correlation was found between MiR-141 ($r=0.30$, $P = 0.049$), MiR-145 ($r=0.32$, $P = 0.034$), whereas in metastatic PCa were significant ($P<0.05$) negative correlation between Gleason grade ($r=-0.30$, $P = 0.045$).

CONCLUSIONS

Taken together, our findings define distinct miRNA expression patterns that coordinately regulate the tumorigenicity of PCa.

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HEREDITARY HAEMOCHROMATOSIS (HH) TYPE 1, HFE GENE MUTATIONS FREQUENCY IN A REGION OF SOUTHERN EUROPE

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BACKGROUND-AIM

HH type I is one of the most common autosomal recessive diseases in the caucasian population. It is due to mutations in the HFE gene of the short arm of chromosome 6. This disease is characterized by a disorder in iron metabolism, producing an increase in intestinal absorption of iron and the consequent storage in multiple tissues causing damage. The aim of this study is to establish the frequency (%) of mutations for HH type I in our population in order to establish the genetic algorithm that fits this region of Baix Empordà, Catalunya.

METHODS

Eighty-two patients were screened for HFE gene mutations C282Y, H63D and S65C using molecular genetics assays (real time PCR) during 2021 and 2022. The inclusion criteria consisted of patients with situations of Ferritin> 300 ng/mL and Transferrin Saturation Index > 45%. The data in this prospective study was analyzed by SPSS from IBM.

RESULTS

The frequency (%) obtained for mutations in HFE consists of 2 heterozygotes for C282Y (2.43%), 3 homozygous for C282Y (3.65%), 19 heterozygotes for H63D (23.17%) and 8 homozygotes for H63D (9.75%). We also found a compound heterozygous genotype (C282Y/H63D) in 4 individuals (4.87%). We found no patients for S65C mutations (0.0%).

CONCLUSIONS

The distribution of HFE gene mutations found in our group matches the trends observed in other European countries: high frequency for the H63D mutation, followed by C282Y mutations and low or no frequency for the S65C. Despite the migratory exchange in our area of the Baix Empordà our distribution of mutations is in line with the total of the Spanish State. These results allow us to maintain our molecular diagnosis algorithm giving priority to the study of mutations in H63D followed by C282Y.

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ANALYSIS OF MUTATIONS IN THE DPYD GENE AND ITS INFLUENCE ON TREATMENT WITH FLUOROPYRIMIDINES

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BACKGROUND-AIM

Fluorouracil is an antineoplastic indicated in the treatment of various types of cancer. Dihydropyrimidine dehydrogenase (DPD) is the central enzyme in the metabolism of this drug, and its activity is subject to interindividual variability and genetic polymorphisms. The DPD enzyme is encoded by the DPYD gene, in which there are several variants that cause reduced or absent enzymatic activity. Both the European Medicines Agency and the Spanish Agency for Medicines and Health Products have issued recommendations on the analysis of the most frequent mutations in the DPYD gene before the start of treatment with fluoropyrimidines.

METHODS

A descriptive study carried out over nine months in 439 cancer patients with a polymorphisms in the DPYD gene study. Prior to choosing chemotherapy treatment with fluoropyrimidines, a peripheral blood sample was received in an EDTA tube to extract the DNA from lymphocytes and study the mutations with the Elucigene DPYD kit. Polymerase chain reaction (PCR) amplified sequences (amplicons) that are separated by capillary electrophoresis. GeneMapper 4.0 analysis software allows amplicons to be identified and labeled according to their size and dye color.
The mutations detected by this technique and with the highest frequency of appearance in patients are the following: c.1905+1G>A, c.1679T>G, c.2846A>T, c.1129-5923C>G, c.1236G >A, c.483+18G>A; the presence of these last three mutations in the same patient corresponds to haplotype B3.

RESULTS

Some of the most frequent mutations in the DPYD gene were found in 14 patients, all being heterozygous, giving rise to a frequency of 3.2% in our population. The most frequently identified mutations correspond to the B3 haplotype in 7 patients (50.0%), followed by the 2846A>T mutation in 3 patients (21.4%) and the 1905+1G>A, 1236G>A and 483 mutations. +18G>A in 1 patient each (7.1%).

CONCLUSIONS

Mutations in the DPYD gene, in heterozygosity, decrease DPD activity by 30-70%, so there is an increased risk of severe or even fatal drug toxicity when fluoropyrimidines are administered to these patients.
All of the above phenotypes for the DPYD gene correspond to intermediate metabolizers, so the recommendation in these patients is to reduce the initial dose of fluoropyrimidine by 50%, followed by a gradual increase if there is no toxicity.

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ENERGETIC AND GENETIC MECHANISMS OF TRANSFORMATION OF NORMAL CELLS INTO MALIGNANT CELLS

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BACKGROUND-AIM

Each cell needs energy to maintain its vital structure, for the synthesis of reserve substances and for the exercise of various specific cellular functions and various movement processes; plasma movement, nuclear division and cell synthesis.
Aim and Method
The main stimulus for malignant tumor is hypoxia from predominant anaerobe metabolism. Following this information aim of this work was to emphasis levels of energy in cells, in types of malignant disease, due to the dys-regulation of genes, using the bio-luminescence method, running an Analyzer of bio-luminescence LKB.

METHODS

The main stimulus for malignant tumor is hypoxia from predominant anaerobe metabolism. Following this information aim of this work was to emphasis levels of energy in cells, in types of malignant disease, due to the dysregulation of genes, using the bioluminescence method, running an Analyzer of bioluminescence LKB.
The principle of the reaction: ATP + luciferin + O_2----> oxyluciferin + AMP + PPi + CO_2 + Light, reaction catalyzed by the enzyme luciferase from the ATP research kit.

RESULTS

Concentrations of micro-moles, μM ATP, into malignant diseases were determined with Bio-luminescence
Concentration analysis of ATP in mean values, ($\bar{x}$) with Standard Deviation, (SD) :
-ATP in normal T Cells, $\bar{x}$ = 1.39 μM ATP, (SD = 0.41)
-ATP in normal B cells, $\bar{x}$ =0.35 μM ATP, (SD = 0.42)
-ATP in T cells of malignant diseases, $\bar{x}$= 0.17 μM ATP, (SD = 0.46)
-ATP in B cells of malignant disease=, $\bar{x}$ =3.06 μM ATP, (SD = 0.45)
-ATP in B cells of Leukemia, $\bar{x}$ = 4.33 μM ATP, (SD =1.5)
- ATP in T cells of Leukemia, $\bar{x}$ = 0.09 μM ATP, (SD=1.7)
Regarding the study of malignant diseases, was noted the increased measured values: 4.30 - 4.55 μM ATP for chronic lymphatic leukemia and respectively 3.20 - 3.65 micromoles ATP for neoplasms or with bone metastases.

CONCLUSIONS

Following this information, we can say that a certain type of chronic disease is due to the dys-regulation of genes in a certain time and way of life. The structural and biochemical changes in malignant cells will influence the development of characteristic bio-energetic processes that at their level will condition the expression of cellular oncogenes to the detriment of anti-oncogenes that are expressed up to now, normally in an aerobic metabolism.

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DIAGNOSTIC REORIENTATION IN A PATIENT WITH A MARFANOID PHENOTYPE

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BACKGROUND-AIM

Marfan syndrome is a systemic connective tissue disorder of variable phenotypic expressivity involving the cardiovascular, skeletal and ocular systems (tall stature, joint laxity, myopia, cardiac problems). Autosomal dominantly inherited, it occurs as a result of a pathogenic variant in the FBN1 gene.
17-year-old patient who comes to Genetic Counseling consultation to perform study for marfanoid phenotype: tall stature, pectus excavatum, mild scoliosis, kyphotic attitude and myopia magna. Personal history: mother with similar phenotype, brother with pectus excavatum, maternal grandfather with retinitis pigmentosa.

METHODS

NGS (Next Generation Sequencing) panel was performed for genes associated with Marfan syndrome: ACTA2, COL3A1, COL5A1, FBN1, FBN2, MYH11, SMAD3, TGFB2, TGFBR1, TGFBR2, TGFB3, ADAMTSL4, in Illumina NextSeq mass sequencer, detecting the pathogenic variant c.2008C>T; p.Arg670* of the ADAMTSL4 gene in heterozygosis, associated with ectopia lentis, of autosomal recessive inheritance. Since the findings don´t justify the marfanoid phenotype of the patient, it was decided to perform MLPA (Multiplex Ligation dependent Probe Amplification) analysis of the FBN1 and TGFBR2 genes to study deletions and duplications in chromosomes 15q21.1 and 3p24.1 respectively. The result obtained was negative.

RESULTS

Subsequently, the detection of the SRY gene of the Y chromosome and a double dose of the X chromosome, compatible with Klinefelter syndrome, found during the review of data from the NGS panel previously performed, is reported by telephone from the external laboratory. The chromosomal formula 47,XXY was confirmed by karyotyping.

CONCLUSIONS

Klinefelter syndrome is characterized by having more than one X chromosome and is the most frequent sex chromosome disorder in males (1 in 600 male newborns). It shares certain phenotypic features with Marfan syndrome, including tall stature and to a lesser extent elongated limbs or joint laxity, which in this case has made diagnosis by NGS difficult. Most cases of Klinefelter's syndrome, because of their low phenotypic expression, are not diagnosed or are diagnosed late. The patient's phenotype is consistent with the family phenotype, who despite appearing to be Marfanoid, was actually Klinefelter.

Molecular diagnostics

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A CASE ANALYSIS OF CHANGES IN TEST INDEX FOR CHOLELITHIASIS COMPLICATED WITH CHOLECYSTITIS

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BACKGROUND-AIM

Calculous cholecystitis accounts for more than 90% of chronic cholecystitis, and acute exacerbation of chronic cholecystitis can cause serious complications that require surgical treatment if necessary.

METHODS

A retrospective analysis of the chief complaint, diagnosis, and treatment process of cholelithiasis complicated with cholecystitis admitted to Jiangsu Provincial Hospital of Chinese Medicine, combined with domestic and foreign literature, the clinical manifestations of the disease, and the diagnostic value provided by the examination report were described.

RESULTS

The patient was diagnosed with cholelithiasis complicated with cholecystitis by Computed Tomography (CT). During the course of the disease, the white blood cell count decreased for the first time, the C-reactive protein (CRP) level was normal at this time, and the procalcitonin (PCT) level was increased. After a few hours, the white blood cell count was elevated, and both CRP and PCT levels were elevated.

CONCLUSIONS

The PCT is more valuable than CRP in the diagnosis of acute exacerbation of chronic cholecystitis. Cholelithiasis Complicated with Cholecystitis is more common in women. Acute calculous cholecystitis is an acute inflammation caused by the obstruction of the cystic duct by stones, resulting in the retention of bile in the gallbladder and secondary bacterial infection. Patients with mild clinical symptoms can first control inflammation through non-surgical methods. However, patients with severe clinical symptoms should undergo surgical treatment as soon as possible, especially the elderly, whose condition changes rapidly and should pay more attention to. Patients usually need to pay attention to developing good living habits and seek medical treatment in time after illness.

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PATIENT WITH CIRRHOSIS DUE TO A PATHOGENIC VARIANT IN THE TERT GENE. A CASE REPORT.

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BACKGROUND-AIM

Human telomeres consist of thousands of hexameric TTAGGG nucleotide repeats and protein components that bind to them. Defects in genes involved in telomere maintenance result in short telomeres that lead to a spectrum of rare disorders known as telomere biology disorders (TBD).

METHODS

We present the case of a 44-year-old woman with cryptogenic cirrhosis, portal hypertension and esophageal varices that eventually required a liver transplant. She was referred to our Genetics Department to study the clinical exome genes from whole exome sequencing, filtering the analysis for genes related to hemochromatosis and Wilson disease due to high clinical suspicion. The exonic regions of the genome were captured using the xGen Exome Panel v2.0 kit (Integrated DNA Technogies) and next generation sequencing (NGS) was performed in the NextSeq™ 550 system (Illumina).

RESULTS

NGS results showed our patient was a carrier of the pathogenic variant c.187C>G (p.His63Asp) in HFE, a gene associated with hemochromatosis. No variants were found in ATP7B, the gene associated with Wilson disease. These findings did not explain our patient's phenotype and it was decided to reanalyse the case by filtering for the HPO term "Cirrhosis" (HP:0001394). This reanalysis detected the pathogenic variant c.2147C>T (p.Ala716Val) in heterozygosity in TERT, the gene that encodes the telomerase reverse transcriptase. Further studies were conducted and telomere length was measured by quantitative polymerase chain reaction. This analysis showed a very short telomere length (6.1 ± 0.2 kbp) given the age of our patient.

CONCLUSIONS

Taken together, these results suggest that our patient suffers from an autosomal dominant TBD caused by the pathogenic variant found in the TERT gene. Pathogenic variants in TERT are associated with different phenotypes with incomplete penetrance and variable expressivity. Individuals with adult onset of the disease usually present pulmonary fibrosis (most common manifestation), bone marrow failure, liver cirrhosis and a higher risk for haematological malignancies. Given the increased risk of developing pulmonary fibrosis and haematological complications, our patient should be referred to a lung specialist and haematologist for careful follow-up.

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THE DEVELOPMENT OF MONOCLONAL ANTIBODIES AGAINST OPISTHORCHIS VIVERRINI CYSTEINE PROTEASE (OVCP) AS AN EFFECTIVE DIAGNOSTIC TOOL

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BACKGROUND-AIM

Opisthorchis viverrini (O. viverrini), the human liver fluke, is the major health problem in several counties, especially in Southeast Asia. The infection not only causes gastro-hepatic symptoms but long-term infection also leads to aggressive bile duct cancer, cholangiocarcinoma (CCA), which poor prognosis and high mortality. Hence, the early diagnosis of O. viverrini infection could be interrupted that situation. The current diagnostic procedure is stool examination by microscopic-based methods which restricted by low parasite egg numbers and low parasitemia. The molecular diagnosis prompts the chance to evaluate the light infection, but currently, inconvenient for routine use due to special equipment requirements and unstable sensitivities. This study aims to develop the monoclonal antibody against parasite-secreted protein, O. viverrini cysteine protease (OvCP), using phage display technologies for using in detection of OvCP in other specimens.

METHODS

The OvCP deduced amino acid sequence was analyzed and predicted of B-cell epitopes used for short peptide synthesis. The synthetic peptides were used to screen the phage library simultaneously with OvCP recombinant protein (rOvCP). The potentiated phages were collected, rescued, and reassembled in XL1-blue Escherichia coli . The positive clones of phagemids were isolated and the single chain variable fragment (scFv) were computationally predicted. The scFv fragments were digested from the phagemid and subcloned into pOPE101 expression vector and expressed in XL1-blue E. coli. The recombinant scFv against OvCP epitopes were purified using Ni-NTA agarose affinity chromatography and dropwise dialyzed against PBS, pH 7.4 for refolding.

RESULTS

The scFv monoclonal antibodies were evaluated for specific recognition and detection limit of rOvCP using indirect ELISA and western blot analysis. The result indicated that recombinant scFv monoclonal antibodies against OvCP were successfully produced, and it could bind specifically to rOvCP at a concentration of lower than one ng.

CONCLUSIONS

In conclusion, this result suggested that our produced scFv monoclonal antibodies will be the potential candidate for developing an effective diagnostic procedure of O. viverrini infection in human secretion other than stool specimens in the future.

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ULTRA-SENSITIVE MINIMAL RESIDUAL DISEASE (MRD) MONITORING FOR CANCER PATIENTS USING SUPERRCA MUTATION ASSAYS WITH FLOW CYTOMETER READOUT

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BACKGROUND-AIM

Rare tumor-specific mutations in patient samples serve as excellent markers to monitor the course of malignant disease and responses to therapy in clinical routine, and improved assay techniques are needed for broad adoption. We describe herein - superRCA assays - which provides for rapid and highly specific detection of DNA sequence variants present at very low frequencies in DNA samples. Using a standard flow cytometer we demonstrate precise, ultra-sensitive detection of single-nucleotide mutant sequences from malignant cells against a 100,000-fold excess of DNA.

METHODS

Sequence of interest are first enriched by targeted PCR amplification from a patient sample and converted to DNA circles that are subjected to rolling-circle amplification (RCA). Padlock probes specific for mutant or wild-type sequences are then used to probe the repeated sequences of the RCA products with exquisite specificity, followed by RCA of the circularized probes. The large DNA clusters that result from each starting DNA circle are referred to as superRCA products.

RESULTS

The low detection limit and high precision of superRCA are consequences of the highly selective genotyping of the repeated target sequences in combination with the large numbers of products that may be conveniently analyzed by flow cytometry. NGS-analysis failed to detect the remaining mutation after initial treatment which was therefore paused, subsequently leading to a relapse for this patient. Even low levels of remaining leukemic markers in the post SCT-setting would prompt clinical action, mainly by reducing immunosuppressants to boost the immunological effect of the SCT in order to eradicate remaining malignant clones that risk giving rise to leukemic relapse. For the Lung cancer patients superRCA can precisely tracking the patients' response to the immune therapy by tracking recurring driver mutations presented in the patients' primary tumor.

CONCLUSIONS

The superRCA assay procedure is suitable for routine use by virtue of its high sensitivity and simplicity. With ultra-high sensitivity, tt's even possible to monitoring the status of Leukaemia patient with samples with equal utility comparing to the bone marrow samples as well as in the plasma ctDNA samples from the Lung cancer patients.

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PREVALENCE OF MYCOPLASMA AND UREAPLASMA SPP. IN ROUTINE GYNECOLOGICAL CARE IN BAIX EMPORDà, CATALUNYA

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BACKGROUND-AIM

Ureaplasmas (Ureaplasma urealyticum (UU) and Ureaplasma parvum(UP)) and mycoplasmas (Mycoplasma genitalium (MG) and Mycoplasma hominis(MH)) are frequently isolated from the genital tract. They are potentially pathogenic species that play a controversial etiological role. Some publications relate these colonizations with abortions in the first trimester, non-gonococcal urethritis and/or success in fertility treatments.
The aim of this study consists of evaluating the prevalence of mycoplasmas and ureaplasmas in the routine asymptomatic gynecological population.

METHODS

It consists of a 24-month retrospective descriptive study, based on the analysis of molecular diagnostic results from our laboratory. The sampling was random and included women of childbearing age (age range: 16-45 years) who attended the gynecology service. The detection of UU, UP, MG and MH was carried out from genital swabs by multiplex PCR.

RESULTS

A total of 2429 women met the inclusion criteria, 49% of the patients did not report any type of abdominal and/or genital symptoms in the last two weeks. Among the 1190 asymptomatic women, the prevalence of pathogens was 81.57%, with the following distribution: U. parvum 43.1%, U. urealyticum 24%, M. hominis 13.1% and M. genitalium 1, 37% In 29 (1.20%) positive patients, Ureaplasma spp and Mycoplasma hominis co-infection was recorded, all of them were between 18 and 29 years of age.

CONCLUSIONS

Data show that a high rate of women of childbearing age harbor these microorganisms in their genital microbiota. We believe that our research can be used to consider whether asymptomatic samples should be screened for M. genitalium, M. hominis, U. ueralyticum, and U. parvum. mainly in pregnant women and in asymptomatic women under study for fertility. In subsequent studies we suggest establishing possible pathogenic links and/or success in assisted reproduction treatments.

Molecular diagnostics

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HIGH PROPORTION OF RR-TB AND MUTATIONS CONFERRING RR OUTSIDE OF THE RRDR OF THE RPOB GENE DETECTED IN GENEXPERT MTB/RIF ASSAY POSITIVE PULMONARY TUBERCULOSIS CASES, IN ADDIS ABABA, ETHIOPIA

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BACKGROUND-AIM

Globally, access to affordable and rapid TB diagnostics remained a major challenge for many developing countries which bear the greatest burden of TB delaying the initiation time to treatment. This study aimed to assess the GeneXpert MTB RIF assay probe utility for the detection of pulmonary TB and RR TB cases in Addis Ababa, Ethiopia.

METHODS

A cross-sectional study was performed from October 2019 to July 2020 in Saint Peter TB Specialized Hospital in Addis Ababa metropolitan area, Ethiopia. This study enrolled 216 clinically suspected new presumptive pulmonary TB cases confirmed by GeneXpert MTB/RIF Assay. Data were entered in Microsoft Excel 2019 and exported to IBM SPSS Statistics for Windows, Version 26.0. Descriptive analysis and binary and multivariate logistics regression were performed and all statistical significance was determined at a 95% confidence level.

RESULTS

The majority of the study participants, 55.1% [119/216] were males aged 6 – 80 years. The prevalence of RR MTB was 11.11% [24/216]. A higher proportion of RR TB was found in female patients [54.2%, 13/24], in persons whose residence is urban [79.2%, 19/24], in persons who had a history of contact with active and LTBI [33.3%, 8/24], and in persons who had a history of HIV and IDUs [41.7%, 10/24]. Occupation (AOR 22.868, 95% CI 1.655-316.022, p=0.019), history of previous PTB+ (AOR 4.222, 95% CI 1.020-17.47, p=0.047), and history of HIV and IDUs (AOR 4.733, 95% CI 1.416-15.819, p=0.012) were independent predictors associated with RR-TB emergence. The commonest mutation 62.5% [15/24] was found in the probe E region. There was no mutation associated with probe A, probe B, and probe C regions, as well as no combination of missed probes, was revealed. However, 12.5% [3/24] of RR TB patients were found without unidentified missed probe types detected outside of the RRDR. The highest proportion of 35.6% [77/216] RR TB was detected in samples of medium DNA load.

CONCLUSIONS

The proportion of RR-TB we observed in this study was high. Similarly, a higher proportion of RR TB was detected outside of the RRDR. Moreover, a significant number of the GeneXpert MTB/RIF Assay probes were identified as unhybridized and this critical observation would mean that most of the probes had no or minimal utility in this geographical region.

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EVALUATION OF TWO DIFFERENT RNA EXTRACTION INSTRUMENTS FOR MOLECULAR DIAGNOSIS OF SARS-COV-2 AT A REFERENCE LABORATORY

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BACKGROUND-AIM

Molecular detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) plays a crucial role in the early diagnosis of coronavirus disease (COVID-19). The sensitivity is related to the efficiency of RNA extraction and amplification. Therefore, evaluating the extraction instruments and kits is essential for the quality control of tests for SARS-CoV-2. We conducted a comparison of two automated RNA extraction instruments for the detection of SARS-CoV-2 in upper respiratory specimens.

METHODS

We collected 58 SARS-CoV-2 positive nasopharyngeal swab (NPS) samples in viral transport medium. RNAs were extracted from the NPS samples using the Maelstrom 9600 (Taiwan Advanced Nanotech) and the KingFisher Flex (Thermo Fisher) in parallel. The extracts were tested for three different target genes of SARS-CoV-2 (E, RdRP/S, and N genes) by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) using AllplexTM SARS-CoV-2 assay (Seegene, inc.) and the CFX96TM system (Bio-rad). Cycle threshold (Ct) values were evaluated for the comparison of the two extraction instruments.

RESULTS

The sensitivity of the two extraction instruments was both 100%. The median Ct values for Maelstrom 9600 were 18.57 (interquartile range, IQR: 16.32 – 28.25) for E gene, 21.07 (IQR: 18.57 – 30.57) for RdRP/S gene, and 17.24 (IQR: 14.75 – 27.05) for N gene. The median Ct values for KingFisher Flex were 18.63 (IQR: 16.46 – 28.63) for E gene, 19.89 (IQR: 17.50 – 30.46) for RdRP/S gene, and 17.52 (IQR: 15.12 – 27.04) for N gene. Statistical analysis by the Wilcoxon matched-paired signed rank test revealed a significant difference in RdRP/S gene ($P < 0.0001$) and N gene ($P = 0.0061$). The correlation between the two instruments was very high for all three genes (E gene, rs = 0.9871; RdRP/S gene, rs = 0.9790; N gene, rs = 0.9825).

CONCLUSIONS

Both extraction instruments showed similar efficiency and high correlation in SARS-CoV-2 detection, except slight difference according to the target gene.

Molecular diagnostics

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GENETIC POLYMORPHISM OF ALCOHOL DEHYDROGENASE 2 (ADH1B) IN ASSOCIATION WITH ALCOHOL CONSUMPTION IN NEPALESE POPULATION.

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BACKGROUND-AIM

Alcohol is the world's third largest risk factor for disease burden. Liver is the main site of alcohol metabolism. The alcohol metabolizing gene, ADH1B is mostly ethnic and race dependent. Functional polymorphisms found within the genes encoding ADH1B, might lead to either increased or decreased rate of enzymatic metabolism of ingested ethanol. Thus, it's necessary to assess SNP of ADH1B gene in subjects consuming alcohol to explore their genotypic influence on alcohol consumption. This study aims to observe genotypic and allele frequency in alcoholic and non- alcoholic groups and to explore genotypic influence of ADH1B gene polymorphism on alcohol consumption

METHODS

A total of 82 EDTA blood samples were taken from alcoholic and non-alcoholic subjects. The samples were subjected to molecular analysis for detection of ADH1B polymorphism by PCR-CTPP method. The ultimate products were visualized by 1.5% agarose gel electrophoresis. . The resulting data was then entered into excel sheet and analyzed by SPSS v 21.0.

RESULTS

The homozygous form ADH1B*1/*1 genotype was found to be prevalent in higher frequencies in all study participants. The frequency of ADH1B*1 allele was 97.55% and ADH1B*2 allele was 2.45%. Similarly, ADH1B*1/*1 genotype was found to be 98.1%, 76.2% and 100% in aadibasi/janajati, bhraman/chheteri and madhesi ethnicities respectively. Likewise, individuals consuming alcohol for a longer duration have ADH1B*1/*1 genotype causing them to tolerate the effects of metabolic products arising from alcohol metabolism for such a chronic time.

CONCLUSIONS

It can be concluded that the presence of ADH1B*1/*1, ADH1B*1/*2 and ADH1B*2/*2 genotype alters alcohol metabolism, its consumption and tolerance. The presence of ADH1B*1/*1 genotype increases the tolerance towards alcohol and makes individual more liable to alcoholism preceding the onset of alcohol use disorders. Thus, result might indicate the presence of ADH1B*2 allele to be protective against alcoholism and its subsequent consequence.

Molecular diagnostics

P1676

GENETIC CONFIRMATION OF TUBEROUS SCLEROSIS. APROPOS OF A CASE.

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BACKGROUND-AIM

Tuberous sclerosis (TS) is an autosomal dominant neurocutaneous genetic disorder. Clinical manifestations include pigmentary skin changes, brain malformations, cardiac rhabdiomyomas, renal angiomyolipomas and pulmonary lymphangioleiomyomatosis. Early-onset epilepsy is present in 85% of patients.

METHODS

A 54 year old woman who presented with headache crisis and blood pressure of 240/120 mmHg was referred to Internal Medicine for ambulatory monitoring. On examination, an abdominal mass was palpated in the left hypochondrium, an ultrasound was performed showing a large mass and two smaller ones compatible with angiomyolipomas. After an exhaustive examination, palpebral angiofibroma was observed in the right eye, periungual angiofibromas in the hallux, second and fourth toes and chagrim plaque in the lumbar dorsum. A cranial CT scan showed a subependymal nodule in the frontal horn of the left ventricle. Ophthalmology was consulted due to a hypopigmented lesion in both eyes, ruling out astrocytoma.
The patient met 4 major criteria for the diagnosis of ET, so it was decided to extract blood for genetic study by massive sequencing of TSC1 and TSC2 genes.

RESULTS

A heterozygous change was detected in the TSC2 gene consisting of a transversion of a cytosine by an adenine that produces the change of serine from position 1114 to a tyrosine. The transition of a cytosine by a thymine producing the change of the glutamine at position 1115 by a premature stop codon was also observed.

CONCLUSIONS

ET is caused by a mutation in one of the TSC1 or TSC2 genes. It is characterized mainly by a disorder of brain development and the appearance of benign tumors.
The patient is a heterozygous carrier of the pathogenic change c.3343C>T (p.Gln1115*) in the TSC2 gene, being compatible with her diagnosis. She is also a carrier in cis of the c.3341C>A (p.Ser1115Tyr) change in the same gene, with uncertain clinical significance.
Analysis of the c.3343C>T change in relatives at risk of being carriers is recommended. Early diagnosis and identification of the mode of transmission is important to provide good genetic counseling.
Periodic evaluation of patients is important for early identification of tumors or other complications, with the aim of early treatment to improve prognosis.

Molecular diagnostics

P1677

A CASE REPORT OF L2-HYDROXYGLUTARIC ACIDURIA PRESENTING WITH LEARNING DISABILITY AND CEREBELLAR SIGNS

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BACKGROUND-AIM

L2-hydroxyglutaric aciduria (L2HGA) is a slowly progressive, autosomal recessive rare neurometabolic disorder due to mutations in L2HGDH gene, coding for the mitochondrial enzyme L2-hydroxyglutarate dehydrogenase (L2HGDH). Deficiency of L2HGDH enzyme leads to accumulation of L2-hydroxyglutaric acid in cells causing a leukoencephalopathy predominantly affecting the cerebellum. L2HGA presents with psychomotor retardation, macrocephaly, bilateral cerebellar involvement, behavioral disorders, seizures, pyramidal and extra pyramidal signs. The disease is first described by Duran et al. Up to now only 300 cases have been reported worldwide.

METHODS

We present a case of a Sri Lankan boy born to a third degree consanguineous parentage, with two healthy siblings, who had a normal birth, post natal period and a normal development up to 4 years. He presented at 12 years of age with bilateral cerebellar signs and tonic extensor spasms in all four limbs. His past history revealed inattention, behavioral problems and very poor school performance from 4 years of age.
Urine organic acid analysis by gas chromatography/mass spectrometry (GCMS) revealed a massive peak of 2-hydroxyglutaric acid (2HGA). Mutation analysis revealed two heterozygous pathogenic variants in L2HGDH gene in exon 3, c.293A>G (p.His98arg) and exon 7,c. 829c>T (p.Arg277#) confirming the genetic diagnosis of autosomal recessive L2HGA due to compound heterozygosity.
Parental genetic testing confirmed the carrier status of L2HGA.
Child was started on therapy with riboflavin, levocarnitine and co-enzyme Q. However, he did not respond to treatment well and the condition remains static over the past one year.

RESULTS

To our knowledge, this is the first case of L2HGA presented in Sri Lanka. Riboflavin, a flavin adenine dinucleotide (FAD) precursor activates the L2HGDH enzyme reducing the neurotoxicity of L2HGA. A good response have been observed in some cases when used before established neuronal damage.

CONCLUSIONS

In a child presenting with learning disability with neurological involvement, performing a urine organic acid profile by gas chromatography and mass spectrometry (GCMS) is of paramount importance in ruling out rare metabolic causes such as L2HGA.

Molecular diagnostics

P1678

PREVALENCE OF MTFHR GENOTYPING AT THE UNIVERSITY HOSPITAL SVETI DUH IN CROATIA

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BACKGROUND-AIM

Despite decades of studies showing a lack of association between methylenetetrahydrofolate reductase (MTHFR) polymorphisms and the onset of various thromboembolic conditions, MTHFR genotyping is routinely requested which leads to increased lab costs and patient confusion. In order to investigate the current state of MTHFR genotyping requests, we analyzed 170 patient results in 2 years and compared various patient diagnoses in which MTHFR genotyping is more likely to be requested at our hospital.

METHODS

DNA was manually isolated from patient blood samples using the GeneProof PathogenFree DNA Isolation Kit (GeneProof, Czech Republic) in accordance with the manufacturer’s protocol. In order to determine the presence of the common MTHFR variant (C677T), real-time PCR was performed using the GeneProof MTHFR C677T PCR Kit (GeneProof, Czech Republic). Resulting patient genotypes were sorted by type (wild type C/C, heterozygote C/T, mutant T/T) and prevalence and compared to published data. Patient genotypes were matched with their diagnoses and grouped into medical conditions to determine the rationale of hospital departments requesting MTFHR genotyping.

RESULTS

In 2 years, the majority of conducted tests (total: 170) included MTHFR genotyping (86%). Patient genotypes were mostly wild type C/C (47%) or heterozygote C/T (42%) while a smaller subset carried the mutant T/T variant (11%)-a pattern consistent with published data. Despite the scientific consensus, the majority of patients with diagnosed or suspected thromboembolisms (90%) and gynaecological conditions (64%) were tested for the MTHFR variant. Patients with neurological, psychiatric and/or ophthalmic conditions were appointed for testing in all cases (100%) likely due to MTHFR involvement in neurotransmitter metabolism.

CONCLUSIONS

Our hospital data shows high prevalence of MTHFR genotyping for diagnoses with low or inconsequential involvement of the MTHFR variant C677T. This redundancy not only provides additional lab costs, but also leads to patient concern over their lab results. This analysis provides an insight into the diagnostic context likely associated with MTHFR requests and provides an opportunity for further educational activities in our hospital.

Molecular diagnostics

P1679

ANALYTIC AND CLINICAL PERFORMANCE OF A FULLY-AUTOMATED EBV DNA QUANTITATIVE TEST

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BACKGROUND-AIM

Regular screening for Epstein-Barr virus (EBV) DNA using quantitative RT-PCR is recommended for early intervention in at-risk patients. Harmonization of quantitative RT-PCR assays is critical to avoid misinterpretation of results. The aim of this dual center study was to evaluate the analytical and clinical performance of the cobas EBV (Roche Molecular Diagnostics) test for use on the cobas 6800/8800 Systems (Roche), and to compare it with different commercially available EBV-DNA assays.

METHODS

In this study, the cobas EBV (Roche), EBV R-Gene (bioMerieux), artus EBV RG PCR (Qiagen), RealStar EBV PCR kit 2.0 (altona) and Abbott EBV RealTime (Abbott) assays were compared. For analytic performance, a 10-fold dilution series of EBV reference material, normalized to the WHO standard, for clinical performance, anonymized leftover EBV-DNA-positive EDTA plasma samples were used.

RESULTS

When analytic performance was estimated, the cobas EBV deviated -0.0097 log10 from target values. The other tests showed deviations between 0.0037 and -0.12 log10. For clinical performance, Bland-Altman bias and Deming regression analyses showed statistical correlation for cobas EBV to both R-Gene and Abbott RealTime assays with 95% CI for slope and intercept: Bland-Altman (-0.14, -0.12) and (0.09, 0.10), Deming (0.86, 1.08) and (-0.19, 0.53), respectively. In contrast, Bland-Altman analysis detected an offset of cobas EBV to artus 95% CI for slope (0.04, 0.58) and Deming analysis detected proportional bias with the RealStar assay 95% CI for slope (0.50, 0.93). Linearity and accuracy of cobas EBV data from both study sites were excellent [Bland-Altman 95% CI for slope: (-0.02, 0.12); Deming 95% CI for slope and intercept: (0.96, 1.08) and (-0.35, 0.10)]. The overall time required for the cobas EBV assay was 190 min. For the comparator assays, this time ranged from 180 to 267 min. The cobas EBV assay required the shortest hands-on time.

CONCLUSIONS

The fully automated cobas EBV assay proved to be a good tool for accurate quantitation of plasma EBV-DNA in the routine diagnostic laboratory. It showed the closest¬ correlation to the reference material, followed closely by R-Gene, Abbott and RealStar assays. The cobas EBV assay may improve guideline adoption for EBV diagnosis, monitoring and treatment.

Molecular diagnostics

P1680

EXPRESSION OF GENES RESISTANCE TO ISONICIDE/RIFAMPICIN IN PATIENTS WITH TB

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BACKGROUND-AIM

TB is a human disease caused by the bacteria Mycobacterium tuberculosis that mainly affects the lungs. Likewise worldwide, the burden of multidrug resistant tuberculosis (MDR-TB) persists for more than 10 years and causes a high mortality. To describe the genetic profile of Mycobacterium tuberculosis and the pattern of resistance to rifampicin and isoniazid in patients from East Lima.

METHODS

Descriptive, retrospective study. The study populations were: patients with suspicion and diagnosis of pulmonary tuberculosis who reside in Lima this year and who attended the HNHU during 2019. Inclusion criteria: BK (+) of sputum and genotyping results using the GenoType MTBDRplus method of rifampicin (RIF) and isoniacid (INH). A validated collection instrument was used, information on cases registered at HNHU during 2019 was obtained. A descriptive analysis, mean, mode, standard deviation was performed. Statistical software STATA V. 16 was used.

RESULTS

Of 2700 patients with sputum BK study, M. tuberculosis was identified in 2314 of whom genotyping was performed using the GenoType MTBDRplus method of rifampicin (RIF) and isoniacide (INH). The average age of patients was 34.38 (+16.09 years), with a minimum age of 10 years and a maximum of 95 years. The male gender represents 71.5% of the patients. 78.1% of patients did not receive treatment, 14.8 had received previous treatment. 77% of patients were pansensitive and MDR was 13.6%. The presence of the mutation of the rpo B gene was 12.6%, of the kat G gene was 10.8% and the Inh A gene in the present mutation. Rifampicin had a resistance of 15.4% and the isonicide had 20.9%. Male patients present higher proportions of MDR cases (14.4%) compared to female patients (11.7%). Patients with a history of anti-TBC treatment had higher cases of MDR TB (32%) compared to never treated (10.2%). The largest cases of MDR-TB were hospitalized patients (38%) and emergency patients (25%) compared to SJL criminal patients (12.9%).

CONCLUSIONS

Of the patients studied, 86% were pansensitive, MDR-TB represented 13.6%. The male gender presented more cases of MDR-TB. Hospitalized patients presented higher cases of MDR-TB. The mutation of the rpo B gene was 12.6%, of the kat G gene it was 10.8% and the Inh A gene did not present mutation.

Molecular diagnostics

P1681

EVALUATION OF GENETIC MUTATION PROFILE OF MDS/MPN PATIENTS BY A CUSTOMIZED NGS PANEL

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BACKGROUND-AIM

Myelodysplastic/myeloproliferative neoplasms (MDS/MPN) include chronic myelomonocytic leukemia (CMML), MDS/MPN with neutrophilia (MDS/MPN-N), MDS/MPN with SF3B1 mutation and thrombocytosis (MDS/MPN-SF3B1-T), and MDS/MPN, not otherwise specified (MDS/MPN, NOS) as its subtype according to the 5th edition of the World Health Organization classification of haematolymphoid tumours. Herein, we evaluated genetic mutation profiles of each subtype of MDS/MPN patients by a custom NGS panel.

METHODS

Data of 36 patients diagnosed with MDS/MPN who underwent targeted NGS analysis in Severance hospital from January 2017 to August 2022 were collected retrospectively. Subtypes of these patients consisted of 27(75%) CMML, 3(8.3%) MDS/MPN-N, 2(5.6%) MDS/MPN-SF3B1-T, 4(11.1%) MDS/MPN, NOS. Genomic DNA was extracted from diagnostic bone marrow aspirate or peripheral blood samples. After library preparation and target enrichment using a customized capture probe targeting 531 genes associated with hematologic malignancies, sequencing was done with Illumina platform. NGS data analysis was done by using an in-house bioinformatics pipeline. Identified somatic variants were classified into four tiers based on the guidelines established by Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists guidelines.

RESULTS

Of the 36 patients, 94 tier 1/2 somatic mutations involving 29 genes were detected. The overall average of tier 1/2 variants per patient was 2.61. As for the average of mutations per patient for each subtype, CMML had the highest value of 2.81 and the patient with the most mutations, which was six, also had the diagnosis of CMML. Variant allele frequency of the mutations ranged from 6.2 to 98.4%. Commonly mutated genes in overall MDS/MPN patients were TET2(18%), ASXL1(17%), NRAS(6%), CBL(5%), SRSF2(5%). Each subtype differed in its commonly mutated genes: TET2, ASXL1, CBL, NRAS, SRSF2 in CMML; ASXL1, JAK2, KIT, NRAS, RUNX1, TET2, ZRSR2 in MDS/MPN-N; SF3B1, ASXL1, ATRX, JAK2 in MDS/MPN-SF3B1-T; ASXL1, DNMT3A, IDH2, JAK2, SF3B1, U2AF1 in MDS/MPN, NOS.

CONCLUSIONS

This study showed genetic mutation profiles of each subtype of MDS/MPN patients. With more extensive study, these genetic mutation profiles can be useful in the differential diagnosis of MDS/MPN.

Molecular diagnostics

P1682

EXPLORING THE ASSOCIATION BETWEEN ND1 GENE POLYMORPHISM AND SERUM LEPTIN LEVELS IN NORTH INDIAN PREECLAMPSIA PATIENTS

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BACKGROUND-AIM

Pre-eclampsia(PE), affecting 5-7% pregnant women worldwide, is a major cause of maternal and perinatal morbidity. PE is defined as new onset of hypertension (blood pressure >140/90 mmHg) and proteinuria (24h urinary protein >300mg/24h or protein-creatinine ratio ≥0.3) after 20weeks of gestation. Despite advances in our understanding of pathophysiologic causes involving genetic and environmental factors, the genetic basis is still more to be explored. In this context, an important emphasis has been given to mitochondrial ND1 gene, encoding for NADH dehydrogenase and serum leptin levels. ND1 gene polymorphism will affect energy balance which may influence serum leptin levels. Leptin plays an important role in regulation of conceptus development and fetal growth apart from modulating satiety and energy homeostasis.
In our study the role of ND1 gene polymorphism (G331A) and serum leptin in PE has been explored.

METHODS

Hospital-based case-control study, with Institutional Ethical Clearance was conducted over a period of 18months at the Department of Biochemistry in collaboration with Department of Obstetrics and Gynaecology, Maulana Azad Medical College and associated hospitals, New Delhi, India, and comprised of 70 patients diagnosed with preeclampsia and 70 healthy controls. DNA from peripheral whole blood samples was extracted by column binding method and analysed for ND1 polymorphism by PCR-RFLP. Serum leptin level was estimated by sandwich-ELISA technique. Statistical analysis was done with SPSS v25, IBM, USA. p-value < 0.05 was considered significant.

RESULTS

ND1 polymorphism was present in 4.3% of PE cases and 2.9% of controls however it was not statistically significant. When compared to control group, serum leptin was significantly higher(p<0.01) in PE cases. Although, the difference in serum leptin levels was not significant between GG and AA genotypes in PE cases, slightly higher risk of PE was associated with AA genotype with Odds ratio of 0.66 (95% CI=0.106-4.057).

CONCLUSIONS

We found that ND1 polymorphism and serum leptin levels act independent of each other in PE. Since ours was a hospital-based study, it may not truly reflect real association of ND1 polymorphism in PE. So, larger multicentric studies should be conducted.

Molecular diagnostics

P1683

PREVALENCE OF HUMAN PAPILLOMAVIRUS IN LATVIAN POPULATION IN YEAR 2022

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BACKGROUND-AIM

According to statistics, cervical cancer is one of the most commonly diagnosed types of cancer in the world and in Latvia as well. Human papillomavirus (HPV), which is the causative agent of cervical cancer, is one of the most frequent viral infections of the reproductive system and can be easily diagnosed. To reduce the incidence of cervical cancer, a nationwide screening program is of a great importance. According to the Global Strategy and general recommendations of the World Health Organization, high-risk HPV type DNA testing by highly specific and sensitive molecular diagnostics methods is recommended.

METHODS

Data on the women who have responded to national screening programm in Latvia were gathered in SIA "Central Laboratory" from the 1st of July, 2022 until the 1st of December, 2022. High-risk HPV type DNA testing was done by Cobas 6800, Roche. The test system detects and differentiates HPV type 16, 18 and makes a pull of other high-risk HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 or 68).

RESULTS

Overall, from July 1st until December 1st 2022 in SIA "Central laboratory" 22 207 high-risk HPV DNA tests were performed. It was calculated that 11,1 % of the received samples were positive to at least one high risk-HPV type. According to statistics data, single-type HPV infections are more common than multiple-type HPV infections. Overall calculated co-infection rate was 0,9 % in the analyzed samples. Simultaneous infection with HPV type 16 and other high-risk HPV types was detected in 145 samples, HPV type 18 and other high-risk HPV types in 34 samples, HPV type 16 and HPV type 18 in 11 samples and simultaneous infection with HPV type 16, 18 and other high-risk HPV types in 5 samples.

CONCLUSIONS

Testing data indicates that the prevalence of high-risk HPV types in Latvia is comparable with the global average. Detected HPV infections can be single-type and multiple-type, the most widespread one is single-type with any of high-risk HPV types. It is crucial to maintain and promote the national cervical cancer screening program that includes broad testing. Testing with molecular diagnostics methods provides valuable statistical data that helps evaluate the prevalence of high-risk HPV in Latvian population.

Molecular diagnostics

P1684

THE RELATIONSHIP BETWEEN THE C677T POLYMORPHISM OF THE MTHFR GENE AND BREAST CANCER

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BACKGROUND-AIM

Breast cancer is the most common cancer in women. It is currently the leading cause of female cancer deaths between the ages of 35 to 64 years.
A family history of breast cancer increases the risk by a factor of 2 or 3. Some mutations result in a very high risk of breast cancer.
The objective of our study is the evaluation of the influence of the various risk factors, the identification of the MTHFR C677T polymorphism in the controls and in the patients and finally the establishment of a possible relationship between this polymorphism and breast cancer in patients.

METHODS

Our study conducted on 52 subjects, 26 breast cancer patients and 26 controls.
Molecular analysis using the PCR / restriction enzyme digestion technique Hinf#.

RESULTS

Our results show a high frequency of breast cancer between the age of 45-54 years with a mean age of 49.61 ± 9 35 years, the majority of breast cancers are infiltrating ductal carcinomas (92%) with predominance grade ### (54.91%). 11.54% of our controls are heterozygous C / T, 7.69% are mutated homozygotes and 80.77% are normal homozygotes. The frequencies of the C and T alleles are respectively 85.19% and 14.81%, 73.91% of our patients presented the C / C genotype, 13.4% the C / T genotype and 13.4% the T / T genotype. The frequency of their C and T alleles is 80.83% and 19.57% respectively.
The calculation of the Odds ratios, indicates the absence of association between the MTHFR C677T polymorphism and the breast cancer and this that it is for the model CC vs TT (OR = 1.85 and p-value = 0.52) or model TT + CT vs CC (OR = 1.24 and p-value = 0.75). This framework has allowed us to identify certain risk factors for breast cancer.

CONCLUSIONS

In conclusion, our study found no evidence of a significant association between the MTHFR C677T polymorphism and mammary carcinogenesis.

Molecular diagnostics

P1685

DEVELOPMENT OF ASSAYS TO DETECT CLINICALLY RELEVANT ß-LACTAMASE GENES USING MULTIPLEX REAL-TIME PCR

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BACKGROUND-AIM

Resistance to ß-lactam antibiotics in Gram-negative bacilli is an increasing problem worldwide. Early detection of these strains is crucial for effective patient management, as well as for infection control and surveillance purposes. Therefore, we developed assays consisting of six real-time PCR reactions to detect frequently encountered ß-lactamases genes.

METHODS

We targeted genes encoding six families of ESBLs (TEM, SHV, CTX-M, VEB, PER, and OXA-10); seven families of AmpC ß-lactamases (CMY, DHA, FOX, ACC, ACT, MIR, and MOX); LAT-1 and CFE-1 AmpC ß-lactamases; 11 families of carbapenemases (IMI, SME, KPC, GES, IMP, VIM, NDM, OXA-23, OXA-24, OXA-48, and OXA-58); and SPM-1, SIM-1, GIM-1, and NMC-A carbapenemases. 16S rRNA genes from Gram-negative bacteria were chosen as a target gene for an internal PCR control. PCR conditions were optimized with 24 genomic DNAs and 17 synthetic DNAs as positive controls. Thirteen negative control strains were used to evaluate them for the false-positive reaction. A total of 248 clinical isolates were then screened for 30 ß-lactamase genes.

RESULTS

The assays were initially optimized with twenty-two genomic DNAs extracted from positive control strains, one bla_{GES-5} and one $bla_{OXA-232}$ genomic DNAs, and 17 synthetic bla DNAs. Only the expected PCR products were amplified. Then, thirteen negative control strains were used to evaluate the assays for the false-positive reaction. No amplification was observed except for three strains. One M. morganii was positive for bla_{DHA} and two K. pneumoniae were positive for bla_{SHV}. After the initial testing, 248 clinical isolates were analyzed using the real-time PCR protocol. Out of 248 clinical isolates selected, 184 strains produced more than one ß-lactamase. These results were validated with other published assays.

CONCLUSIONS

Developed assays will be useful to rapidly screen frequently encountered ß-lactamase genes, and they will provide an additional tool for optimum antibiotic therapy and infection control and surveillance.

Molecular diagnostics

P1686

GENOMIC PROFILING BASED ON CERVICAL SMEAR AND BLOOD IN PATIENTS WITH ENDOMETRIAL CANCER

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BACKGROUND-AIM

When diagnosing and prognostically grading endometrial cancer patients, cervical swab samples can be a novel, non-invasive alternative to surgical staging. We investigated the value of blood-based circulating tumor DNA (ctDNA) and genomic DNA (gDNA) extracted from cervical swabs in patients with endometrial cancer.

METHODS

Since January 2021, prospectively enrolled patients were those having surgery for endometrial cancer, pre-invasive endometrial disease, or benign endometrial illness. Blind vaginal sampling was used to gather cervical swab samples before to surgery. Blood samples were taken at the time of operation and then every three months following. A panel spanning 100 endometrial cancer-related genes was used to extract and analyze DNA. The Illumina NovaSeq 6000 System was used to sequence the prepared libraries, and PiSeq (Dxome) was used to analyze the data.

RESULTS

A total of 175 patients enrolled with 131 cervical swabs and 204 blood samples, including 103 endometrial cancer patients. When compared to matched blood-based ctDNA, cervical swab-based gDNA performed much better, detecting cancer with a sensitivity of 67% and specificity of 93%. Regardless of the use of adjuvant medication and despite the low sensitivity, baseline blood tests successfully identified patients with poor progression-free survival, including those with uterine restricted illness. Serially collected blood showed negative conversion in 64% of patients. Cervical swab-based gDNA had a greater percentage of pathogenic mutation discovery as compared to whole blood, with at least one harmful mutation being detected in 69 out of 108 patients. Additionally, gDNA from cervical swabs might predict MSI score, with an AUC of 0.95. The gDNA-based ProMisE classification indicated predictive value based on MSI score and pathogenic somatic mutation.

CONCLUSIONS

Our research shows that the two non-invasive approaches for genetic profiling may be complimentary in treating endometrial cancer at all stages. The serial collection of whole blood-based ctDNA may be helpful for therapeutic monitoring in advanced stage disease. Cervical swab-based gDNA may be used as a one-stop prognostic solution because it may be self-collected and evaluated prior to hysterectomy.

Molecular diagnostics

P1687

CONCORDANCE OF CK AND ALDOLASE IN THE DIAGNOSTIC APPROACH OF MUSCLE DAMAGE

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BACKGROUND-AIM

Aldolase is an enzyme involved in glucose metabolism and it is mainly found in muscle tissue. It is used in diagnosis and follow-up of musculoskeletal diseases, in which a more specific enzyme, creatine kinase (CK), a muscle enzyme involved in the storage and transference of energy, is also elevated. The determination of CK has been shown to be the most sensitive marker in cases of suspected muscle damage, except in cases of polymyositis (PM) or dermatomyositis (DM), in which aldolase is the only elevated enzyme. PM and DM incidences are 2-10/10#/year and 4- 6/10#/year, respectively. A systematic determination of both, CK and aldolase, is often made for the study of muscle damage regardless of diagnosis. Thus, studying the concordance between CK and aldolase may allow reducing the number of determinations, saving aldolase measurement only to those specific cases, which would reduce health care costs.

METHODS

Retrospective study conducted between 2019 and 2020 analyzing the concentrations of CK (30-200 U/L - men, 29-168 U/L - women) and aldolase (0-7 U/L) (Alinity c, Abbott) from routine extraction in a tertiary hospital. A correlation study was performed by analyzing the Pearson correlation coefficient and a concordance study by analyzing Cohen's kappa index stratified by sex using reference values.

RESULTS

The study included 1488 patients (874 women and 614 men) with a mean age of 35 years. A correlation coefficient between both analytes of 0.84 was obtained. In the concordance study, Cohen's kappa index for women was 0.46 and 0.29 for men.

CONCLUSIONS

The study of both analytes has shown the existence of a high correlation. However, the concordance study has presented a low concordance index for both sexes. Since this study was carried out without considering the final diagnosis of the patients and using the reference values provided by the trader, it would be necessary to establish our own reference values and a cut-off point considering the final diagnosis to verify its concordance. This may lead to a better use of these analytes which may also help reducing health care costs. This study also highlights the possible need for concordance studies of these analytes and its role in the diagnostic approach of muscular damage by other laboratories.

Molecular diagnostics

P1688

THE I / D POLYMORPHISM OF THE ACE GENE AND C677T OF THE MTHFR GENE AND THE RISK OF SCHIZOPHRENIA IN A POPULATION OF EASTERN ALGERIA

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BACKGROUND-AIM

Schizophrenia is a complex psychotic disorder that affects approximately 1% of the general population. Interactions between environmental factors and genetic vulnerability have been suggested as etiological factors of this disease. Several studies have shown the association of the 677T allele of the MTHFR gene with high levels of homocysteine and low levels of folate and vitamin B12. Likewise, the Insertion (I) / Deletion (D) polymorphism of the ACE gene seems involved since the activity of the angiotensin converting enzyme in different brain regions of schizophrenics is increased.
the purpose of the study is to establish a possible relationship between the C677T polymorphism of the MTHFR gene and the plasma level of homocysteine on the one hand and the Insertion (I) / Deletion (D) polymorphism of the gene encoding the ACE and the activity of the angiotensin converting enzyme on the other hand in east Algerian schizophrenics.

METHODS

Our study involved 114 controls and 42 schizophrenics. The search for I / D polymorphism of the ACE gene was carried out by a simple PCR followed by separation of the PCR products by agarose gel electrophoresis and The C677T mutation of the MTHFR gene was detected by PCR / RFLP using the restriction enzyme Hinf I.

RESULTS

Our results showed a strong association between the homozygous mutated TT genotype of the MTHFR gene (57.14% vs 5.36%) and schizophrenia with odds ratios of TT vs CC genotypes = 30.87 CI (7.21-153.23) $p < 0.00001$ and TT + CT vs CC = 9.16IC (3.47-24.78) $p < 0.00001$.
The genotypic frequencies of the I / D polymorphism of the ACE gene were : 13.33% vs 40.35%, are heterozygous ID, 66.66% vs 51.75% are homozygous DD and 13.33% vs 5.26% are homozygous II respectively in patients and witnesses. The odds ratios I /I vs DD is not significant 1.97 (0.50-7.69) with a $p = 0.21$.

CONCLUSIONS

Our results showed that the TT genotype of the MTHFR gene was a risk factor for the development of the disease, however no association between I / D polymorphism of ACE and susceptibility to schizophrenia was revealed.

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Molecular diagnostics

P1689

CUSTOMIZED MOLECULAR TESTS FOR MINIMAL RESIDUAL DISEASE: A TOOL FOR THERAPEUTIC DECISIONS IN FOLLICULAR LYMPHOMA PATIENTS

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BACKGROUND-AIM

Monitoring of minimal residual disease (MRD) is based on accurate laboratory tests which can recognize a small fraction of malignant cells that survived the course of treatment. Several studies of the B-non-Hodgkin lymphoma group have been presented that MRD positivity at the end of treatment is a negative prognostic index. Follicular lymphoma (FL) is a chronic indolent malignancy, which response highly to immuno-chemotherapy followed by prolonged remissions but with inevitable relapse in most patients. The GALLIUM study showed that immuno-chemotherapy combinations, 6 cycles of bendamustine-obinutuzumab (BO), is the most efficacious treatment, but with severe side effects .In addition, 90% of patients achieved negative MRD from peripheral blood (PB) or bone marrow (BM) after only 3 BO cycles. These findings raise the possibility for MRD based treatment approach, where the duration of chemotherapy could be decided according to MRD status at mid-induction (MI).
AIM: Identification of a marker in the PB and/or BM of each FL patient which will be use to the design of a sensitive RQ-PCR test for MRD detection for follow-up.

METHODS

Qualitative PCR for translocation 14:18 and for the VDJ rearrangement sequence of the immunoglobulin heavy chain was used for marker identification. MRD monitoring was done by RQ-PCR. MRD monitoring for VDJ was designed specifically to each patients based on his VDJ sequence. MRD was assessed on PB and BM at MI.

RESULTS

Marker for monitoring was identified at 11/21 patients in this study. After 3 combined therapy cycles, 7 patients were found to have MRD negativity by RQ-PCR therefore continue with immuno-therapy only. MRD positivity was assessed in BM of 3 patients (0.11%, 0.022%, and 0.133%). These 3 patients continued with the combined therapy. RQ-PCR sensitivity was 10-4-10-5.

CONCLUSIONS

This study will reveal the ability to use sensitive and specific MRD that has to be designed for each patient according to his marker and enable the establishment of a treatment with a high safety profile for FL patients. Correlation between MRD status and clinical and safety parameters will be assessed at the end of the study, 30 months after the first treatment, taking into account MRD status from different time point of the follow-up period.

Molecular diagnostics

P1690

PATHOGENIC VARIANT IN NF1 GENE: DOES IT ALWAYS CONSTITUTE A CASE OF TYPE 1 NEUROFIBROMATOSIS?

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BACKGROUND-AIM

NF1 is a multisystem disease caused by alterations in the NF1 gene. It shows an autosomal dominant inheritance pattern, with almost complete penetrance, and variable expressivity, both high inter- and intra-family. The most common symptoms are café-au-lait spots, freckles in the armpits and groin area, Lisch nodules, and neurofibromas.

METHODS

A 28-year-old woman attended the clinic for a pigmented skin lesion in the left preauricular region of 3-4 years of evolution, which on physical examination showed morphological changes. Family history of melanoma (father).

RESULTS

Biopsy of the skin lesion with atypia: melanoma in situ. Having a family history of melanoma, a genetic panel was indicated which incidentally results in a NF1 gene pathogenic variant (NM-000267.3: c.2033dup p. (Ile679Aspfs*21)), with an allelic fraction of 0.28. A Sanger sequencing method showed disturbances in two independent genomic DNA (gDNA) samples. The presence of the variant in any of the pseudogenes described for NF1, by long-range amplification followed by specific amplification of exon 18; performed for both gDNA and complementary DNA (cDNA), was ruled out. To exclude the possibility of a preferential wild type allele amplification, the patient´s parents genetic testing was suggested.

CONCLUSIONS

The genetic results show the mosaicism of a pathogenic variant in the NF1 gene, associated to neurofibromatosis but not to melanoma. Specific follow up and counseling for NF1 was recommended. During post test evaluation, some lesions were observed on the left arm that could be compatible with neurofibromas, therefore she was referred to the dermatology clinic for evaluation.

This case reflects two relatively frequent phenomena in cancer predisposition syndromes, phenotypic overlap between syndromes and genetic mosaicism which are difficult to detect by conventional molecular diagnosis approaches until now. The implementation of multigene analysis by Next Generation Sequencing (NGS) reveals a higher incidence than the one previously described.

Molecular diagnostics

P1691

CAPTURING TETRASPANIN POSITIVE-EVS WITH NP-TRFIA FACILITATES THE DETECTION OF RENAL CELL CARCINOMA

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BACKGROUND-AIM

Renal cell carcinoma (RCC) is a major lethal urological cancer which is often discovered incidentally. New and non-invasive biomarkers are needed for the early diagnosis of RCC. Extracellular vesicles (EVs) are considered a promising new biomarker target for diagnosis of various malignancies. However, investigation of EVs typically demands their isolation from body fluids, which is a difficult and time-consuming process. The aim of our study was to develop EV-based assay for the early and non-invasive detection of RCC using a highly sensitive nanoparticle-aided time-resolved fluorescence immunoassay (NP-TRFIA).

METHODS

EVs were isolated from control HEK293 and RCC4 and 786-O RCC cell lines by SEC column (qEV-Izon). EVs- and PE (protein enriched)-fractions were characterized using NP-TRFIA assay constructed with tetraspanins (CD9, CD63, CD81, and CD151). Tetraspanin biomarkers were further characterized using RCC cell culture medium as well as serum samples from RCC (n=14), benign (n=17), and healthy (n=9) individuals. This study was conducted following the guidelines of Helsinki Declaration.

RESULTS

Our NP-TRFIA showed 2-20-fold higher signal intensity on EVs compared to PE-fractions. Among tetraspanins, CD63 showed 3-5-fold higher expression on EVs-derived from RCC4 and 786-O cell lines compared to HEK293 control. Assay consisting of double monoclonal CD63 antibody, CD63-CD63 assay, demonstrated significant discrimination of RCC patients from benign ($p=0.001$), and healthy ($p= 0.002$) individuals, respectively. Similarly, the CD81-CD81 assay also enabled significant separation of RCC patients compared to benign ($p=0.016$), and healthy ($p= 0.003$) controls, respectively.

CONCLUSIONS

This result suggests that RCC cell lines derived EVs and serum of RCC patients showed higher amounts of CD63-positive EVs compared to control sources. Detection of these tetraspanin-positive EVs using our NP-TRFIA approach may play a vital role in the early detection of RCC. This study demands further validation with larger cohorts of samples.

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Molecular diagnostics

P1692

PILOT ASSESSMENT OF THE DIAGNOSTIC ACCURACY OF CEPHEID GENEXPERT HIV-1 QUAL FOR EARLY INFANT DIAGNOSIS

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BACKGROUND-AIM

The World Health Organization (WHO) recommends testing HIV-exposed infants 4 to 6 weeks after birth using an HIV polymerase chain reaction (PCR) test for early infant diagnosis (EID). In Malawi, access to EID remains low, with only 30% of exposed infants less than 2 months old receiving HIV PCR results. Cepheid recently released GeneXpert (Xpert) HIV-1 Qual, a simplified HIV PCR test that can be performed near the point of care. The purpose of this pilot study was to assess of the diagnostic accuracy of Xpert HIV-1 Qual for the diagnosis of HIV infection among infants and children. In Malawi, PCR testing is only available in central laboratories, leading to long turn-around times (TATs) to as long at 29 days from sample collection to result printing.

METHODS

The pilot was conducted at Queen Elizabeth Hospital laboratory in Blantyre, Malawi between December 2015 and January 2016. Consecutive dried blood spot (DBS) samples from HIV-exposed infants and children aged 6 weeks to 18 months, were tested with Xpert HIV-1 Qual in parallel with the Abbott RealTime HIV-1 Qualitative assay, the reference test, within 2 months of sample collection. The sample size was determined by the number of Xpert HIV-1 Qual cartridges available.

RESULTS

Paired testing was done on 378 samples, of which 17 (4.5%) were positive on the Abbott assay. Xpert HIV-1 Qual detected all 17 HIV positive on Abbott: sensitivity 100% (95% CI; 80.5% to 100%). Xpert HIV-1 Qual detected 358/361 HIV-negative cases on Abbott: specificity 99.2% (95% CI; 97.6% to 99.8%). Assuming the sensitivity and specificity values found in our study, the negative predictive value (NPV) would be 100% assuming an HIV prevalence between 1% and 10%, and the positive predictive value (PPV) be 55.8% at an HIV prevalence of 1%, increasing to 93.3% at an HIV prevalence of 10%.

CONCLUSIONS

Assuming the sensitivity and specificity values found in our study, the negative predictive value (NPV) would be 100% assuming an HIV prevalence between 1% and 10%, and the positive predictive value (PPV) be 55.8% at an HIV prevalence of 1%, increasing to 93.3% at an HIV prevalence of 10%.

Molecular diagnostics

P1693

DIAGNOSTIC UTILITY OF ARRAY-CGH IN THE DETECTION OF 5P15.2-P15.1 DELETION SYNDROME

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BACKGROUND-AIM

17-month-old male patient referred to the Neuropediatrics clinic due to paroxysmal episodes, who suffered intense skin pallor, with a preserved level of consciousness although hyporeactive, accompanied by global progressive hypotonia. The pregnancy and delivery were normal, with a low birth weight of 2350 grams. His parents were not related by blood.In the current examination, the gait is still unstable, little babbling, and he communicates mainly through gestures. It also highlights a peculiar phenotype, nonspecific dysmorphic features: epicanthus, short and flat nasal root, micrognathia, and facial asymmetry.He comes for a review at 2 years, he walks and runs clumsily in a peculiar way, with his head turned to the side. Fine motor skills are good, he speaks very little. Toys do not attract his attention, he does not interact much with other children. His parents say that when he gets angry or is very happy, he bites compulsively.

METHODS

Considerating the psychomotor retardation,the behavioral alterations and the peculiar phenotype, a molecular cytogenetic study of Comparative Genomic Hybridization Array was requested.The methodology is based on the extraction of genomic DNA from a blood sample and the application of the CGH array technique using a CGX array that includes 180,000 probes, using the PerkinElmer platform.

RESULTS

A 2.37 Megabase deletion is detected in the chromosomal region 5p15.2-p15.1, which can be classified as a possible cause of the patient's pathology.Therefore, the patient has a male genetic pattern, compatible with the chromosome formula (according to ICSN 2009 nomenclature): arr 5p15.2-p15.1 (14, 796,253-17, 170,028) x1

CONCLUSIONS

The deletion identified in the patient, which shares many characteristics with cri du chat syndrome, has been described in some cases associated with mental retardation, developmental delay and dysmorphia. In addition, the deletion of the ANKH gene (5p 15.1), included in this deletion, is the cause of craniometaphyseal dysplasia, which is a generalized skeletal disorder characterized by progressive hyperostosis and sclerosis of the craniofacial bones.
The genetic study of both parents was carried out, the results concluded that the deletion found was not present in the parents, therefore it must be classified as a "de novo" appearance.

Molecular diagnostics

P1694

DIAGNOSTIC ACCURACY VALIDATION OF ABBOTT M2000 FOR HIV VIRAL LOAD TESTING ON DBS SAMPLES (VERSION1.0); MALAWI PILOT STUDY.

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BACKGROUND-AIM

The strict requirements for storage and transport of plasma samples from clinics to laboratories for HIV viral load (VL) testing, limits access to HIV VL monitoring among patients on antiretroviral therapy (ART) in resource-limited settings. Dried blood spots (DBS) provide an alternative to plasma because there are no cold chain requirements and DBS can be stored at room temperature for up to 3 months. The Malawi Ministry of Health has adopted the Abbott m2000 system (Abbott) as the national standard for VL testing. As part of the switch from bioMérieux NucliSENS EasyQ/Easy Mag (NucliSENS) to Abbott, we did a study in Thyolo District Laboratory in Malawi to assess the diagnostic accuracy of the Abbott m2000 system for HIV VL testing on BDS samples.

METHODS

EDTA venous blood was collected from 412 patients on ART in August and September 2015, and processed into DBS and plasma samples. Plasma samples were tested on NucliSENS, and DBS samples were tested on Abbott and NucliSENS. The diagnostic accuracy of DBS VL at a threshold of 1,000 cells/ml was assessed using the plasma VL result as the reference.

RESULTS

Of the 412 study participants, 257 (62.4%) were females. DBS VL measured on Abbott had a sensitivity of 88.2% (95% CI: 72.5 – 96.7%) and specificity of 91.1% (95% CI: 87.8 – 93.7%) compared to plasma NucliSENS. DBS VL measured on NucliSENS had a sensitivity of 91.4% (95% CI: 76.9-98.2%) and specificity of 92.0% (95% CI: 88.8 – 94.6%) compared to plasma NucliSENS. Assuming a prevalence of VL >=1,000 copies/ml of 10%, DBS had a positive predictive value (PPV) of 52.4% and negative predictive value (NPV) of 98.6% on Abbott, and a PPV of 55.9% and NPV of 99.0% on NucliSENS.

CONCLUSIONS

DBS had satisfactory diagnostic accuracy, making DBS samples suitable for VL testing on Abbott.

Molecular diagnostics

P1695

ROLE OF SERUM TRANSFORMING GROWTH FACTOR – β1 (TGF-β1) IN ATOPIC DISEASES

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BACKGROUND-AIM

Transforming growth factor β (TGFβ) is a cytokine consisting of TGFβ1, 2, and 3 isoforms. TGFβ1 is the most abundant isoform in most tissues, including skin and lung. TGFβ1 has immunosuppressive functions, promoting the differentiation of anti-inflammatory T regulatory cells, suppressing Th1 and Th2 responses and may participate in the immune response associated with atopic diseases. The aim of this study was to investigate TGF-β1 levels in patients with atopic diseases (atopic dermatitis and allergic asthma) in comparison to healthy individuals and to evaluate relationship between markers of atopy (total IgE, IL-5, eosinophils).

METHODS

In total, 42 subjects with atopy (23 with mild to moderate atopic dermatitis – AD, 19 with mild to moderate allergic asthma – AA) and 15 age-matched healthy subjects were involved in the study. Blood eosinophil count was determined by standard methods. Measurements of total IgE, IL-5, TGF-β1 levels in serum were evaluated by ELISA.

RESULTS

Subjects with AD or AA had significantly higher IL-5 levels compared to the controls (121.65 ± 13.31 and 101.12 ± 12.96 vs 59.55 ± 7.05 pg/ml), higher blood eosinophil count (0.36 ± 0.12 and 0.23 ± 0.05 vs 0.06 ± 0.01 x10/9/l) and lower TGF-β1 levels (9.87 ± 0.67 and 9.27 ± 0.73 vs 11.91 ± 0.65 ng/ml, respectively, $p<0.05$). A tendency was observed that total IgE is higher in patients with AD and AA compared to the control group (643.42 ± 307.32 and 896.51 ± 337.78 vs 11.46 ± 6.08 kU/l, $p=0.08$). In addition, a negative significant correlation was found between TGF-β1, blood eosinophil count ($r=-0.28$, $p<0.05$) and total IgE ($r=-0.27$, $p<0.05$) in the atopic subjects. There was no significant correlation between IL-5 and TGF-β1 in all subjects groups.

CONCLUSIONS

The study findings let us suggest that TGF-β1 may play an important role in the pathogenesis of atopic dermatitis and allergic asthma and may be helpful marker in monitoring the course of atopic disease.

Molecular diagnostics

P1696

DELETION SYNDROME DETECTED BY CGH ARRAY

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BACKGROUND-AIM

2 year old famale patient was admitted from the operating room after exploratory laparotomy on suspicion of intestinal perforation after endoscopic placement of a gastrostomy button, performed due to failure to thrive.
Personal history: Her father has two healthy male children, her mother, with uterine fibroids, dichorionic diamniotic twin pregnancy , urgent cesarean delivery at 33 weeks of gestation. Her brother is healthy.Birth weight: 1,300 grams.
Clinical judgement: small for her gestational age and cutis-scalp aplasia.
She is being studied due to her short stature, poor psychomotor development (she still cannot walk on her own), dysmorphic appearance, microcephaly, aplasia of the left parietal cutis, as well as swallowing difficulties.

METHODS

The first analysis demostrated:C Reactive Protein: 7.4 mg/mL [0.02-5],hemoglobin 10.1 g/dL [11-17].
A molecular cytogenetic study of Array of Comparative Genomic Hybridization (Array-CGH) is requested after evaluation by Endocrinology and Neurology.The methodology of this technique is based on the extraction of genomic DNA from a blood sample and the application of the CGH array technique using a CGX array that includes 180,000 probes, using the PerkinElmer platform.

RESULTS

A deletion of 1.90 Megabases was detected in the chromosomal region 19q13.11 which, according to the bibliography described, must be classified as causing pathology.
The patient has a genetic pattern compatible with the chromosome formula (according to ICSN 2009 nomenclature): arr 19q13.11 (33,201,844-35,102,421)x1.

CONCLUSIONS

19q13.11 microdeletion syndrome is characterized by prenatal and postnatal growth retardation, eating disorders, microcephaly, intellectual deficit with speech disturbances, hypospadias, and ectodermal dysplasia with aplasia of the scalp; sparse hair, eyebrows and eyelashes, thin dry skin and dysplastic nails.This genetic syndrome has a very low prevalence.
To complete this case, we proceeded to the genetic study of both parents. The results concluded that the deletion found was not present in its parents, therefore it must be classified as a "de novo" appearance.
It is very important to carry out correct genetic counseling, since it is a "de novo" mutation, there is no high risk that other siblings may develop the same pathology.

Molecular diagnostics

P1697

MOLECULAR DIAGNOSIS OF INFREQUENT MULTISYSTEM SYNDROME

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BACKGROUND-AIM

Pediatric male patient for evaluation and genetic study presenting symptoms of hexadactyly and obesity. The patient also presents a significant developmental delay, mainly in the language area.
Previously performed genetic tests Array CGH, Prader Willi Syndrome and Fragile X Syndrome were negative.

METHODS

Finally, the study of the genes related to Bardel Bield Syndrome is valued. This syndrome is characterized by obesity, psychomotor retardation, hypotonia, polydactyly, hearing loss and retinitis pigmentosa.
The genetic study is carried out using Next Generation Sequencing of the genes related to this syndrome: ARL6, BBIP1, BBS1, BBS10, BBS12, BBS2, BBS4, BBS5, BBS7, BBS9, CCDC28B, CEP290, IFT27, LZTFL1, MKKS, MKS1, SDCCAG8, TMEM67, TRIM32, TTC8, WDPCP.

RESULTS

The result obtained in the genetic study was the following:
The homozygous presence of a pathogenic variant in the TTC8 gene has been identified, which could confirm the hypothesis of Bardet-Biedl syndrome:
--Gene TTC8 NM_144596.4 c.677G>A p.(Trp226Ter) rs948160026.
It presents an Autosomal Recessive inheritance. It is not described in the clinical databases or in the scientific bibliography consulted at the date of issuance of the report. It has a low population frequency and the predictors estimate that the variant has a pathogenic effect.
The carrier study of the variant found in both parents of the patient is carried out, confirming that they are heterozygous carriers, thus confirming the homozygous state of the patient and therefore compatible with Bardet-Bield Syndrome.

CONCLUSIONS

Bardet Bield syndrome is a ciliopathy of genetic origin, characterized by retinal dystrophy, obesity, polydactyly, genitourinary and renal anomalies, learning disabilities and hypogonadism, among other less frequent manifestations. The approach to patients affected by this syndrome requires multidisciplinary management. There is no specific treatment, but rather it is based on addressing its clinical manifestations (obesity, learning disabilities, renal anomalies).

Molecular diagnostics

P1698

ABDOMINAL PAIN AND SYNDROME OF INAPPROPRIATE ANTIDIURETIC HORMONE SECRETION (SIADH): AN UNUSUAL FORM OF PRESENTATION OF VARIEGATE PORPHYRIA

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BACKGROUND-AIM

Porphyria is a group of liver disorders in which porphyrins build up in the body due to a deficiency in the haem biosynthesis pathway. Symptoms are non-specific and include abdominal pain, vomiting and confusion, so the diagnosis is often delayed.

A 51-year-old woman presented at the hospital reporting of acute abdominal pain with poor control despite morphics and tachycardia. Moreover, presented severe hyponatremia and orange urine. Examination of the cardiovascular, respiratory and nervous system detected no abnormalities. Blood tests showed: Na+ 119 mmol/L, K+ 3.8 mmol/L, Cl- 85 mmol/L, urea 30 mg/dL, creatinine 0.69 mg/dL, CRP 2.0 mg/L, Leukocytes 6.93×109/µL. CT of the abdomen, chest and head were unremarkable.

METHODS

To rule out porphyria, it is recommended to carry out biochemical analysis of blood and urine. Firstly, screening Hoesch test was performed by mixing 2.5mL of photoprotected urine and 2.5mL Ehrlich reagent (2% p-dimethylaminobenzaldehyde HCl 6N). A sample of 24-hour urine was analysed by High Resolution Liquid Chromatography to identify elevated metabolites. Eventually, since porphyria is generally considered genetic in nature, we searched for possible mutations. A blood sample with EDTA-K3 was analysed by NGS (Next-Generation Sequencing).

RESULTS

During acute crisis, the urinary elimination of PBG (Porphobilinogen) and ALA (Acid-Delta-Aminolaevulinic) precursors was abnormally high, easily proved by Hoesch test. Once we found the screening test was positive, we proceeded to the quantification of different metabolites of the biosynthetic pathway of the haem group: Acid-Delta-Aminolaevulinic, Porphobilinogen, Coproporphyrins, Uroporphyrins , Pentacarboxilporphyrins, Hexacarboxilporphyrins, Heptacarboxilporphyrins. All of them were well above the upper reference limits.

Eventually, genetic analysis revealed a heterozygous mutation in the PPOX gene, associated with variegate porphyria, of autosomal dominant inheritance.

CONCLUSIONS

Porphyria is a rare disease with a non-specific onset of symptoms which could lead to misdiagnosis. Therefore, it is important to carry out a comprehensive analysis that goes beyond conventional colorimetric tests to prevent worsening of the pathology.

Molecular diagnostics

P1699

PREVALENCE OF CARBAPENEM-RESISTANT ENTEROBACTERALES (CRE) GENOTYPE IN THE REPUBLIC OF KOREA 2018-2022

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BACKGROUND-AIM

Carbapenem-resistant Enterobacterales (CRE) can be categorized into carbapenemase-producing Enterobacteriaceae (CPE) and carbapenem-resistant non-CPE that are due to efflux pump, changes in outer membrane protein permeability, etc. CRE genes mainly exist in plasmids and can easily spread in gram-negative bacteria due to active horizontal gene transfer. So far, more than 2,100 CRE genotypes have been reported at varying degrees across countries. Based on the reporting by Seegene Medical Foundation, this study looks into the CRE genotypes in South Korea.

METHODS

From January 2018 to November 2022, 10,762 cases of CRE media were analyzed by Seegene Medical Foundation. Genetic testing for 5 CRE genotypes (IMP, OXA, VIM, NDM, KPC) was conducted by a laboratory-developed test (LDT) using PCR with primers listed on Table 1.

RESULTS

The number of CRE genotype testing requests to Seegene Medical Foundation continuously increased from 247 cases in 2018 to 3,672 cases in 2022 while the positivity rate for CRE genotype has also increased from 58.7% in 2018 to 79.5% in 2019, 90.2% in 2020, 90.5% in 2021, and 92.8% in 2022 (Figure 1). At each genotype level, KPC consisted the most of CRE genotype findings and continued to increase except for 2022. The second most numerous genotype was NDM, followed by OXA-48. Characteristically, there were 865 cases identified as OXA-48 in 2022, constituting 23.56% (Figure 2).

CONCLUSIONS

According to Korea Disease Control and Prevention Agency (KDCA), the number of CRE patients have continued to increase every year, which is broadly consistent with our findings regarding increased testing requests and positivity rates.As the positivity rate for CRE increases every year, infection control in hospitals, such as monitoring patients according to CRE genotypes, seems to become more and more important.

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Molecular diagnostics

P1700

3 ASSESSMENT OF THE DIAGNOSTIC ABILITY OF METAGENOMIC NEXT-GENERATION SEQUENCING AND DDPCR IN THE SUSPECTED MYCOBACTERIUM TUBERCULOSIS INFECTION

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BACKGROUND-AIM

The Metagenomics next-generation sequencing (mNGS) and GeneXpert MTB/RIF assay (Xpert) exhibited a sensitivity for tuberculosis (TB) diagnostic performance. Some suspected tuberculosis diagnoses were still lack of etiological evidence. Droplet digital polymerase chain reaction (ddPCR) has higher sensitivity than real-time PCR for the identification of trace DNA. However, research directly evaluating the ability of clinical samples mNGS, ddPCR and Xpert in mycobacterium tuberculosis comple (MTB) infection is still scarce.

METHODS

Samples from 217 patients presenting with suspected active MTB infection were collected between March 1, 2021, and November 21, 2021. All samples underwent synchronous tuberculous testing with Xpert, and mNGS, ddPCR. We conducted a study to evaluate diagnostic performance of mNGS, Xpert, ddPCR analysis and the clinical final diagnosis for detection of Mycobacterium tuberculosis complex in multiple types of direct clinical sample. Clinical final diagnosis was used as the reference standard.

RESULTS

100 of 217 participants had a clinical final diagnosis of active MTB infection, including cases of 86 pulmonary TB and cases of 14 extrapulmonary MTB. Compared to clinical final diagnosis, mNGS produced a sensitivity of 86% for all active MTB cases, which was lower than ddPCR (99%) but much higher than Xpert (64%). When the results of Xpert and mNGS were stratified according to the mycobacterial load detected by IS6110 and IS1081 ddPCR, the MTB load was lower in Xpert negative with respect to the mNGS positive samples, confirming the high sensitivity of mNGS and ddPCR in the diagnosis of MTB.

CONCLUSIONS

ddPCR provides a sensitive test of MTB examination compared with Xpert and mNGS for MTB, as defined by the high concordance between ddPCR assay and clinical final diagnosis. It significantly improves the etiology diagnosis of MTB.

Molecular diagnostics

P1701

NEW VARIANT FOUND IN HUWE1 GENE IN AN INDIVIDUAL WITH INTELLECTUAL DISABILITY: A CASE REPORT

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BACKGROUND-AIM

The HUWE1 gene encodes for a protein of the E3 ubiquitin ligase family located on the X chromosome (Xp11.22). It it known to be involved in the early development and function of the nervous system; and has been related with cases of X-linked intellectual disability and other neurodevelopmental disorders. We present the finding of a new variant in HUWE1 and the associated phenotype.

METHODS

Case description.

RESULTS

A 13-year-old male patient, first-born of an unrelated couple, was referred to the neuropediatrician for evaluation due to learning disorders and concentration problems. The patient also presented with writing and comprehension difficulties, global psycholinguistic developmental delay, communication problems (speech and hearing), autism spectrum disorder (ASD), strabismus (Duane syndrome), color blindness and frontal headache. His mother had no relevant phenotypic features, except for frontal headache. A genetic study was requested.

Human whole exome sequencing was performed (NextSeq/MiSeq, Illumina). Variant Interpreter, ClinVar, Varsome and Franklin databases were consulted, obtaining the following result: the test revealed a variant in HUWE1 gene (NM_031407.5) c.12074A>G p.(His4025Arg) in exon 78/84. The variant was considered of unknown significance. The inheritance of HUWE1 is X-linked and the carrier status was in hemizygosis.

Familial segregation analysis showed that the variant was present in the patient's younger brother (with a learning disorder phenotype), mother, uncle and maternal grandmother. This variant is not found in the general population and is not currently described in the literature or databases. However, its location in the gene together with other described pathogenic mutations allows us to suspect its relationship with the patient's phenotype.

CONCLUSIONS

The new variant found in the patient and his family is located in the HECT domain of HUWE1 where other pathogenic variants have been found, highlighting the existence of a wide phenotypic variability for XLID and different related genes (including HUWE1).

Molecular diagnostics

P1702

EVALUATION OF THE ANALYTICAL PERFORMANCES OF TWO DIAGNOSTIC KITS BASED ON MULTIPLEX RT-PCR FOR THE DETECTION AND SIMULTANOUS DISCRIMINATION OF HSV-1 AND HSV-2 AND FOR THE DETECTION OF VZV IN HUMAN SAMPLES USING THE SENTINAT® 200 AUTOMATED INSTRUMENT

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BACKGROUND-AIM

The infections caused by herpes viruses represent an issue for the healthcare systems worldwide due to their widespread. Furthermore, it is often difficult to differentiate the type of infection based on the symptoms alone, because of their similarity, thus Real Time PCR assays are used to identify the specific virus infection. The aim of this study is to evaluate the analytical performances of STAT-NAT® SN200 HSV-1&2 and STAT-NAT® SN200 VZV kits (Sentinel Diagnostics) to respectively detect and quantify Human alphaherpesvirus 1 and 2 (HSV-1&2) and Human alphaherpesvirus 3 (VZV-Virus Varicella Zoster) on different human matrices, using the fully automated system SENTiNAT® 200 (Sentinel Diagnostics).

METHODS

Human plasma, blood and swabs were analyzed to evaluate the performances of STAT-NAT® SN200 HSV-1&2 and STAT-NAT® SN200 VZV kits to determine the analytical sensitivity (LoD, LoQ), linearity, precision and specificity on 22 pathogens, following the CLSI (Clinical and Laboratory Standards Institute) guidelines. DNA from samples was extracted using SENTiNAT® X48 Pathomag Extraction kit (Sentinel Diagnostics). The extraction, PCR set up and amplification reaction were performed by SENTiNAT® 200, a fully automated, samples-to-result platform.

RESULTS

For STAT-NAT® SN200 HSV-1&2 kit analytical sensitivity in blood for HSV-1&2 is 750 cps/mL (LoD/LoQ); in plasma is 400 cps/mL (LoD) and 450 cps/mL (LoQ) for HSV-1, 250 cps/mL (LoD,LoQ) for HSV-2; in swab is 300 cps/mL (LoD/LoQ) for HSV-1 and 250 cps/mL (LoD/LoQ) for HSV-2. Linearity range is: $2.5x10^2$ - $1x10^7$ cps/mL for HSV-1 and $2x10^2$ - $1x10^8$ cps/mL for HSV-2.
For STAT-NAT® SN200 VZV analytical sensitivity (LoD) is 500 cps/mL in blood, 250 cps/mL in plasma and swab, LoQ is 600 cps/mL in blood, 350 cps/mL in plasma and swab. Linearity range is: $2.0x10^2$ - $1x10^7$ cps/mL.
For both kits, reproducibility and repeatability %CV is <10%; analytical specificity is 100%.

CONCLUSIONS

The evaluated STAT-NAT® kits, used in combination with the SENTiNAT®200 instrument, offer a complete, automated, sample-to-result solution for the simultaneous detection and quantification of HSV-1&2 and VZV in different human matrices, providing sensitive, precise and reproducible results.

Molecular diagnostics

P1703

EVALUATION OF THE ANALYTICAL PERFORMANCES OF TWO DIAGNOSTIC KITS BASED ON MULTIPLEX RT-PCR FOR THE DETECTION AND QUANTIFICATION OF BKV AND JCV IN HUMAN SAMPLES USING THE SENTINAT® 200 AUTOMATED INSTRUMENT

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BACKGROUND-AIM

Polyomaviruses are ubiquitous, species-specific, members of the Papovaviridae family. Human Polyomavirus 1 (BKV) and Human Polyomavirus 2 (JCV) are the most commonly known viruses that can cause mild pathologies in childhood, and more serious disease states if reactivated o acquired by immunocompromised patients. While BKV is mainly associated with nephropathies, JCV can cause progressive multifocal leukoencephalopathy. Both viruses are diagnosed by multiplex Real-Time PCR assays. The aim of this work was to evaluate the analytical performances of STAT-NAT® SN200 BKV and STAT-NAT® SN200 JCV (Sentinel Diagnostics) kits for the detection and quantification, respectively, of BKV and JCV on different human matrices, using the fully automated system SENTiNAT® 200 (Sentinel Diagnostics).

METHODS

Human plasma and urine samples were analyzed to evaluate the performances of STAT-NAT® SN200 BKV kit and human plasma and blood samples were analyzed to evaluate the performances of STAT-NAT® SN200 JVC kit to determine the analytical sensitivity (LoD, LoQ), linearity, precision and specificity on 22 pathogens, following the CLSI (Clinical and Laboratory Standards Institute) guidelines. DNA from samples was extracted using SENTiNAT® X48 Pathomag Extraction kit (Sentinel Diagnostics). The extraction, PCR set up and amplification reaction were performed by SENTiNAT® 200, a fully automated, sample-to-result platform.

RESULTS

Data obtained on STAT-NAT® SN200 BKV kit are the following: LoD in plasma 190 IU/mL, in urine 200 IU/mL; LoQ in plasma 200 IU/mL, in urine 250 IU/mL. Linearity range is $1.0x10^2$ – $1.0x10^8$ IU/mL; reproducibility and repeatability %CV is <10%.
Data obtained on STAT-NAT® SN200 JCV kit are the following: LoD in plasma and blood 500 IU/mL; LoQ in plasma 600 IU/mL, in blood 750 IU/mL. Linearity range is $5.0x10^2$ – $1.0x10^7$ IU/mL; reproducibility and repeatability %CV is <10%. Analytical specificity is 100% for both kits.

CONCLUSIONS

The evaluated STAT-NAT® kits, used in combination with the SENTiNAT®200 instrument, offer a complete, automated, sample-to-result solution for the simultaneous detection and quantification of BKV and JCV in different human matrices, providing sensitive, precise and reproducible results.

Molecular diagnostics

P1704

EVALUATION OF THE ANALYTICAL PERFORMANCES OF THREE DIAGNOSTIC KITS BASED ON MULTIPLEX RT-PCR FOR THE DETECTION AND QUANTIFICATION OF HHV-6, HHV-7 E HHV-8 IN HUMAN SAMPLES USING THE SENTINAT® 200 AUTOMATED INSTRUMENT

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BACKGROUND-AIM

There are 8 Herpes viruses able to infect humans: they remain latent in the host even after the primary infection has resolved and can reactivate in physical conditions like immunodepression and transplantations. Related infections can be diagnosed by multiplex real-time PCR assays. Aim of this study is to evaluate the analytical performances of STAT-NAT® SN200 HHV-6, STAT-NAT® SN200 HHV-7 and STAT-NAT® SN200 HHV-8 (Sentinel Diagnostics) for the detection and quantification respectively of Human betaherpesvirus 6, Human betaherpesvirus 7 e Human herpesvirus 8 on different human matrices, using the fully automated system SENTiNAT® 200 (Sentinel Diagnostics).

METHODS

Human plasma and blood samples were analyzed to evaluate the performances of STAT-NAT® SN200 HHV-6, STAT-NAT® SN200 HHV-7 and STAT-NAT® SN200 HHV-8 kits relatively to the analytical sensitivity (LoD, LoQ), linearity, precision and specificity on 22 pathogens, following the CLSI (Clinical and Laboratory Standards Institute) guidelines. DNA from samples was extracted using SENTiNAT® X48 Pathomag Extraction kit (Sentinel Diagnostics). The extraction, PCR set up and amplification reaction were performed by SENTiNAT® 200, a fully automated, sample-to-result platform.

RESULTS

With STAT-NAT® SN200 HHV-6 kit the following data were obtained: sensitivity in blood 490 IU/mL (LoD) and 560 IU/mL (LoQ), in plasma 380 IU/mL (LoD and LoQ). Linearity range is $5x10^1$ - $1.0x10^8$ IU/mL.
With STAT-NAT® SN200 HHV-7 kit the following data were obtained: sensitivity in blood 250 cps/mL (LoD), 350 cps/mL (LoQ), in plasma 202 cps/mL (LoD) and 300 cps/mL (LoQ). Linearity range is $1.0x10^2$ - $1x10^7$ cps/mL.
With STAT-NAT® SN200 HHV-8 the following data were obtained: sensitivity in blood 500 cps/mL (LoD and LoQ), in plasma 200 cps/mL (LoD) and 300 cps/mL (LoQ). Linearity range is $2.0x10^2$ - $1x10^7$ cps/mL.
For all the tested kits, the analytical specificity is 100% and reproducibility and repeatability %CV is <10%.

CONCLUSIONS

The evaluated STAT-NAT® kits, combined with the SENTiNAT®200 instrument, offer a complete, automated, sample-to-result solution for the simultaneous detection and quantification of HHV-6, HHV-7 and HHV-8 in different human matrices, providing sensitive, precise and reproducible results.

Molecular diagnostics

P1705

MENéTRIER'S DISEASE IN CHILDREN. A CASE REPORT

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BACKGROUND-AIM

Ménétrier's disease consist of thickening of the gastric folds secondary to hyperplasia of the mucosa cells, and is frequently associated with loss of enteric proteins and hypoalbuminaemia. It is a rare pathology in children, and his etiology is unknown, although it is related to infectious, allergic, and immunological causes.

METHODS

The patient was a 3 year old boy who presented oliguria and lower extremities and bilateral periorbital edema. The boy presented 4days vomiting whitout fever or abdominal pain. He had recurrent diarrhea for the last twelve months. On physical examination, the patient presented a good general condition, his abdomen was soft and depressible with no signs of ascites.

RESULTS

Laboratory tests showed normal leve of hemoglobin, peripheral leukocyte 21.000/ul with a 68% linfocytes and 2% eosinóphils. Serum total protein 3.5g/dL, reference value (RV): 6-8g/dL, albumin (2g/dL, (RV: 3.8-5.4g/dL), calcium, 7.3 mg/dL;; and C-reactive protein, <0.03 mg/dL
Renal and liver function tests were normal. Hypoalbuminemia has been attributed to gastrointestinal loss. The differential diagnosis includes: celiac disease (Ac. Anti-transglutaminase (IgA): neg and Ac. Anti-deaminated gliadin (IgG): neg), eosinophilic gastroenteritis: <10% eosinophils), Crohn's: ruled out.
The serology for Cytomegalovirus (CMV) was positive to IgG and negative to IgM. Abdominal ultrasonography suggested diffuse thickening of the gastric wall. Gastrointestinal endoscopy revealed enlarged gastric folds, erythema, and hemorrhagic erosions covered with whitish mucus throughout the gastric body. A gastric biopsy showed foveolar hyperplasia and dilated gastric glands; CMV-DNA was detected in the tissue sample by polymerase chain reaction (PCR) assay.

CONCLUSIONS

Although the allergic and immunological cause can be postulated, the strongest association in the enf. Pediatric de menetrier is CMV infection (up to 70% of case). Many detected cases have shown this association through the detection of serum antibodies but new diagnostic methods such as PCR can facilitate the diagnosis of this disease.

Molecular diagnostics

P1706

SCREENING OF THROMBOPHILIA ASSOCIATED GENES VARIANTS DURING THE COVID-19 PANDEMIC: A RETROSPECTIVE STUDY IN CAMPANIA POPULATION

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BACKGROUND-AIM

During Spring 2021 the widespread use of COVID-19 adenovirus-based vaccines has provided evidence for the occurrence of rare venous thrombotic events (VTE) in association with platelet aggregation, thrombocytopenia with severe clinical course in recipients of Vaxzevria (ChAdOx1, AstraZeneca) and Janssen (Ad.26.COV2.S, Johnson & Johnson).
These observations have led to an increase in requests for testing of hereditary thrombophilia risk factors. The aim of this study was to evaluate the frequencies of some VTE associated genetic variants in Campania population and compare them with the general population.

METHODS

The study cohort was comprised of 2492 consecutive patients (1520 females/972 males; age range 12-89) referred to the Synlab SDN IRCCS (Pagani, Salerno) from April 2021 to December 2022.
Genomic DNA was isolated from whole blood by Qiasymphony DSP DNA Mini Kit (QIAGEN). Genotypes were identified by the Genequality AB-Thrombo Type Plus (AB ANALITICA).
Gene variants provided were: Prothrombin G20210A (FII), Factor V Leiden G1691A (FVL), Factor V H1299R (FV), Methylenetetrahydrofolate reductase C677T and A1298C (MTHFR) and Plasminogen activator inhibitor-1 4G/5G (PAI-1).

RESULTS

In examined population, the frequencies of homozygous mutated and heterozygous polymorphic variants were respectively: 0.16% and 6.10% for FVL; 0.52% and 12.36% for FV; 0.12% and 7.74% for FII; 26.36% and 47.99% for MTHFR C677T; 7.58% and 42.38% for MTHFR A1298C; 26.24% and 51.12% for PAI-1.

CONCLUSIONS

Our study showed that homozygous pathogenic variants of FVL, FV and FII were observed less frequently in Campanian subjects (respectively 0.16%, 0.52% and 0.12%) than in general population (respectively 2-3%, 1-3% and 0.7-4%).
Moreover the Campania cohort has higher frequency of MTHFR homozygous mutated genotypes at the 677 locus (26.36%), and a lower frequency at the 1298 locus (7.58%) respectively reported in literature at 10-15% and 14%.
Finally, in our group frequency for PAI-1 polymorphism was comparable to general population.
The COVID-19 pandemic has been an opportunity to study thrombophilia risk factors on a large scale. In our study population, differences in frequencies were observed between our results and the general population, suggesting an uneven distribution of genetic risk factors.

Molecular diagnostics

P1707

IN SILICO INCLUSIVITY ASSESSMENT OF THE FTD SARS-COV-2 AND FTD SARS-COV-2/FLUA/FLUB/HRSV ASSAYS

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BACKGROUND-AIM

The genetic drifts observed in the matrix (M1) gene of a recent influenza A H3N2 outbreak in March 2022 in Denmark confirmed the vital role of surveillance programs. Such programs ensure a correct diagnosis of currently circulating strains and allow a quick public health response. Siemens Healthineers has established a systematic surveillance program for its FTD SARS-CoV-2* and FTD SARS-CoV-2 /FluA/FluB/HRSV* assays.

METHODS

To assess the assays' inclusivity, sequences for each pathogen were downloaded from the GISAID database. Incomplete sequences, low-coverage sequences, and animal sequences were excluded from the analysis. Primers and probes were aligned (up to four mismatches per oligonucleotides) to check for potential matches producing the amplicons.

RESULTS

The FTD SARS-CoV-2/FluA/FluB/HRSV assay targeting SARS-CoV-2, influenza A, influenza B, and human respiratory syncytial viruses A and B scored an overall ≥99.94% in silico inclusivity. In addition, recent published data showed genetic drifts in the matrix gene (influenza A assay target gene) of influenza A H3N2. The analysis of the described strains (seven analyzed sequences) showed the genetic drifts happened outside the targeted region. An oligonucleotide alignment revealed that all seven sequences were detected with no mismatch.
The FTD SARS-CoV-2 assay has a dual-target design, allowing it to maintain 100% inclusivity for all analyzed sequences since its development: from 901 sequences (March 2020) to >11 million sequences (July 2022). With the Omicron variant's emergence, the mutation rate seemed to slightly increase, as the number of sequences without mismatches in the N gene decreased from 97.71% to 96.14%. This increase was not observed for the ORF1ab region. Only 0.04% of sequences showed mismatches in both regions.

CONCLUSIONS

The FTD SARS-CoV-2 and FTD SARS-CoV-2/FluA/FluB/HRSV assays showed excellent in silico inclusivity performance, demonstrating their suitability to detect currently circulating respiratory viruses, including a new influenza A H3N2 strain. Inclusivity of 100% observed over the past 3 years for SARS-CoV-2 demonstrates that the N gene- and ORF1ab-targeted regions remained conserved throughout the virus's evolution.
*CE-IVD labeled for diagnostic use in the EU.

Molecular diagnostics

P1708

URINARY MICROBIOME AND BLADDER CANCER: COULD FIRST-MORNING URINE BE A POTENTIAL BIOMARKER?

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BACKGROUND-AIM

Nowadays, several evidence support the involvement of dysbiosis in urological diseases, confirming that the bladder niche is no longer considered sterile. In fact, several studies focused on the relationship between bladder microbial composition and urologic disorders, particularly in bladder cancer. Specifically, microbial composition of urine in the midstream and catheter samples results differently in bladder cancer patients.

We decided to investigate the microbiome of first-morning urine samples from patients undergoing transurethral resection of bladder tumor (TURBT), in order to characterize the microbial profile potentially associated with bladder cancer.

METHODS

The urine free-floating bacterial community was analyzed in samples collected by patients at University Hospital "Federico II" in Naples. A first-morning urine sample (FM-U; n=28) and a catheter sample (CAT-U; n=25) were harvested during the TURBT procedure. A first-morning urine sample from healthy subjects was used as control (HC-U; n=28). The urine microbiome was studied by sequencing the bacterial hypervariable V3-V4-V5 regions of the bacterial 16S rRNA with Illumina Next Generation Sequencing (NGS) platform. Sequencing data were evaluated to identify operational taxonomic units (OTU) and statistical analysis was performed using dedicated pipelines.

RESULTS

Firstly, we compared the microbiome of FM-U and CAT-U samples, and no differentially abundant taxa were detected, suggesting that these two specimens are comparable. Then, we compared the first-morning urine samples of patients with those of healthy subjects, and differences in the bacterial community profile were detected. For a subset of TURBT patients, the diagnosis of bladder cancer was histologically confirmed. The latter showed a specific microbial signature, associated with the presence of the tumor.

CONCLUSIONS

On the basis of our findings, we propose to use the first-morning urine as an advantageous alternative and less-expensive sample to profile the TURBT patients-associated microbiome. Further investigations are needed to better clarify the role of the microbiome in the development and progression of bladder cancer.

Molecular diagnostics

P1709

EVALUATION OF CLINICAL IMPLEMENTATION OF DPYD GENOTYPING PRIOR TO TREATMENT WITH FLUOROPYRIMIDINES

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BACKGROUND-AIM

Fluoropyrimidine treatment can result in severe toxicity in up to 30% of patients and is often the result of reduced activity of the key metabolic enzyme dihydropyrimidine dehydrogenase (DPD), mostly caused by genetic variants in the gene encoding DPD (DPYD). Since May 2020, the Spanish Agency of Medicines and Health Products (AEMPS) recommends the determination of at least 4 polymorphisms of the DPYD gene: (c.1905+1G>A and c.1679T>G, non-functional; and c.2846A>T and c.1129-5923C>G, c.1236G>A (HapB23), with reduced functionality) before initiating treatment with fluoropyrimidines.
In this study we determined the frequency of these polymorphisms among oncology patients in our center and estimate the percentage of intermediate or poor metabolizers in our population.

METHODS

We carried out a descriptive, retrospective, 17-month study (July-2021-December 2022), with a total of 281 oncology patients who were candidates for treatment with fluoropyrimidines. Four DPYD gene polymorphisms (c.1905+1G>A, c.1679T>G, c.2846A>T, c.1129-5923C>G) were analyzed with the LightMix® in-vitro diagnostics kit Multi-SNiP DPYD, using the allelic discrimination technology with real-time PCR (RT-PCR) using FRET-Taqman probes, in the Cobas 480z sequencer.

RESULTS

Of the total number of patients, 4.27% (12/281) presented in heterozygosis any of the polymorphisms studied, being phenotypically classified as intermediate metabolizers with reductions of 25% or 50% of the enzymatic activity of DPYD.
The 2.84% (8/281) were heterozygous for c.1129-5923C>G and 1.07% (3/281) were heterozygous for c.1679T>A and only the 0.35% (1/281) presented heterozygosity at c.1905 1G>A. No patient was identified with a reduction of the enzymatic activity more than 50%. In all cases, the DPYD genotype study was performed before starting treatment, decreasing the dose of fluoropyrimidines in patients with polymorphisms, as indicated in the literature (1,2). None of the patients showed severe toxicity reactions.

CONCLUSIONS

The implementation of DPYD deficiency screening through genotyping of DPYD gene polymorphisms allows a more accurate prediction of the toxicity of treatment with fluoropyrimidines, managing to adjust the dose in each patient and thus reduce the adverse effects caused by treatment.

Molecular diagnostics

P1710

IGE SENSITIZATION TO MAIZE ASSESSED BY MOLECULAR ALLERGY EXPLORER MULTIPLEX IMMUNOASSAY

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BACKGROUND-AIM

Allergy to maize was reported in many countries in which the consumption of corn is widespread.

METHODS

We report the case of a 47-year-old female patient with history of recurrent anaphylaxis after the consumption of polenta and other maize-derived products and rhinoconjunctivitis after eating popcorn with positive skin prick-prick test to canned corn. Molecular diagnosis was performed by an in vitro allergy explorer based on IgE multiplex platform, with allergen extracts and molecules coupled to nanoparticles in solid phase as macroarray and lab protocol integrating powerful cross-reactive carbohydrate determinants inhibitor.

RESULTS

Serum specific IgE to nsLTP biomarker rZea m 14 (1.51 kUA/L) confirmed IgE sensitization to maize. Due to their high structural stability, nsLTPs resist to both heat and pepsin digestion and allergy to nsLTP usually triggers severe reactions like anaphylaxis. Maize has been found to cross-react with other cereals, such as rice, wheat or barley through lipid transfer protein rZea m 14, a major allergen, which has shown sequence identity with the other LTPs. Besides this, maize also cross-react with fruits, such as peach (rPru p3), cherry, cowpea, apricot, grapes (rVit v1) which can also elicit systemic reactions, but in this case such sensitizations were not detected (≤ 0.1 kUA/L).

CONCLUSIONS

Precision allergy molecular diagnosis using a multiplex allergy explorer test is useful in clinical practice to assess the detailed IgE sensitization profile to maize, which in individual cases is associated with severe reactions such as anaphylaxis.

Molecular diagnostics

P1711

ANALYSIS OF MUTATIONS IN THE EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) FROM LIQUID BIOPSY (CIRCULATING TUMOUR DNA, CTDNA) IN PATIENTS WITH ADVANCED NON-SMALL CELL LUNG CANCER

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BACKGROUND-AIM

Analysis of mutations in exons 18-21 of the epidermal growth factor receptor(EGFR) is recommended in patients with advanced-stage non-small cell lung cancer,as they allow tumor cells to acquire treatment resistance. Tyrosine kinase inhibitor drugs(TKIs) block the signaling of this receptor, contributing to the blocking of tumor growth. Resistance mutations against TKIs associated with EGFR mutations have been described. Identification of mutations by liquid biopsy or analysis of circulating tumor DNA(ctDNA) may allow the selection of those patients in whom replacing conventional chemotherapy(first line treatment)with a targeted therapy and the use of TKIs against which the tumor does not show resistance may contribute to a significant clinical improvement
The aim was determination of EGFR mutation frequency in liquid biopsy specimens in investigated population and evaluation of correlations between EGFR mutation,clinical characteristics of the patients, treatment changes, clinical evolution and adverse effects

METHODS

We studied EGFR mutations in exons 18,19,20 and 21 in double-centrifuged plasma obtained from 22 patients(7 men,15 women,ages between 44-87 years old)diagnosed with advanced non-small cell lung cancer and therapeutic failure with IdyllaTMctEGFR Mutation Assay(Biocartis).Statistical analysis was performed with spss v27.0

RESULTS

In our study group, lung adenocarcinoma is more common in women(68%), non-smokers(82%), and in patients between 70-79 years old
64% of patients present EGFR mutation. The most frequent was L858R mutation(33%), followed by exon 19 mutations(21%) and T790M mutation(13%). In case of detection of EGFR mutations, patients received targeted therapy drugs, mainly erlotinib(second-line treatment). In patients with EGFR mutations who required third-line treatment, the treatment of choice was Osimertinib
The group of patients with aged between 50 and 59 years, those with L858R mutation and those who are treated with Osimertinib showed the best clinical evolution. Only 2 patients has skin lesions as adverse effect of TKIs

CONCLUSIONS

Liquid biopsy in the follow-up of advanced lung adenocarcinoma could be very useful for choosing the most appropriate treatment for each patient

Molecular diagnostics

P1712

MONITORING CIRCULATING TUMOR DNA IN NON-SMALL CELL LUNG CANCER PATIENTS BY TARGETED NGS: PRELIMINARY DATA ON THE CORELAB PROJECT

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BACKGROUND-AIM

Liquid biopsy is a non-invasive promising strategy to detect and monitor circulating cancer-derived material obtained from several body fluids instead of tumor tissue; it helps dissecting cancer heterogeneity and avoiding stressful and painful procedures. The analysis of circulating cell-free DNA (cfDNA) from plasma has given promising results in lung cancer. However, despite the increasing implementation of immunotherapy-based treatment, the prognosis of advanced Non-Small Cell Lung Cancer (NSCLC) patients remains miserable, and therefore research of specific predictive biomarkers is actively pursued.
CORELAB is an ongoing multi-centric project sponsored by Regione Toscana (Italy), with the aim to discover new predictive biomarkers of activity and efficacy of immune check point inhibitors in NSCLC. Our group contributed to the project analyzing the mutational status of plasma derived circulating tumor DNA (ctDNA).

METHODS

Blood samples were collected for each patient before immunotherapy (T0) and after 2 months of treatment (T1). cfDNA was isolated from plasma, then quantified in terms of ng/µl using a QubitTM dsDNA HS Assay Kit and in the end the mutational status was evaluated by NGS sequencing using the OncomineTM lung cfDNA Assay, a panel of 11 lung cancer related genes.

RESULTS

50 baseline samples and 39 matching T1 samples were successfully processed and analyzed. At T0 19/50 patients were wild type for the examined genes and 31/50 had at least one variant. The most frequently mutated genes were TP53 and KRAS. Less frequent mutations were found in: BRAF, EGFR, MAP2K1, MET, NRAS and PIK3CA. For 39 patients a follow-up after two months of treatment was feasible. 16/39 patients that were wild type at T0 remained wild type at T1, 17/39 had a reduction in ctDNA level, whereas 6/39 had an increase.

CONCLUSIONS

The adopted targeted-NGS method fulfilled the purpose in detecting low allelic fraction mutations in cfDNA, ensuring high levels of sensitivity and specificity. Mutation frequencies in KRAS and TP53 were in line with the literature for immunotherapy NSCLC candidates. Mutational analysis of ctDNA as liquid biopsy allowed a dynamic monitoring of the disease through the assessment of the presence of specific tumor-related mutations and the evaluation of their rate over time.

Molecular diagnostics

P1713

SPECTRAL DATA PROCESSING IN 3D FLUORESCENCE METABOLOMICS OF ENDOMETRIAL CANCER

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BACKGROUND-AIM

Endometrial cancer (EC) is the most common cancer of the female reproductive tract in developed countries. Five-year survival rates correlate to the stage at diagnosis, but currently, no validated screening test is available. Native fluorescence is a phenomenon that characterizes the physiological state, but can also reflect the pathological state of the biological system. The aim of this study was to identify specific biomarkers by mathematical processing of synchronous excitation spectra (SES) of blood serum.

METHODS

Fluorescence spectral analysis was performed on the blood serum samples of gynecological patients with EC (n=68) which were compared with samples of patients with endometriosis (n=13) as well as with a control group of healthy volunteers (n=47). SES were measured with a Perkin Elmer LS 55 luminescence spectrophotometer and processed in the WinLab software. Receiver operating characteristic curves (ROC) and the one-way ANOVA test were utilized to achieve the goal of discriminating the spectral characteristics of serum between the observed groups.

RESULTS

To assess the differences in the composition of serum, the selected peaks of SES were analyzed and mathematically processed. The intensity ratios R300/330 and R330/280/225/300 provided a clear differentiation between EC and healthy subjects. The ratios represent 4 specific peaks: tyrosine at 225 nm, tryptophan at 280 nm, indole metabolites of tryptophan at 300 nm and NADH at 330 nm. The mean ratios of R300/330 and R330/280/225/300 were: 1.50 ± 0.44 and 0.33 ± 0.09 in controls, 1.25 ± 0.34 and 0.52 ± 0.19 in endometrial samples, whereas 0.94 ± 0.25 and 0.62 ± 0.18 in EC samples. The difference between the groups was statistically significant ($p < 0.0001$).

CONCLUSIONS

The current findings of this research specify that concentration of NADH and metabolism of tryptophan are misregulated in EC patients as compared with that in healthy individuals. This provides an excellent discrimination between the spectral features of analyzed samples, with a specificity and sensitivity of 93%. Substantial variation between the observed groups supports the premise for integrating this fluorescent non-invasive diagnostic monitoring into clinical practice.
The project was supported by the grant 1/0435/23

Molecular diagnostics

P1714

THE ADVANTAGES OF NEXT GENERATION SEQUENCING FOR CORRECTLY DIAGNOSING RARE RHEUMATIC AUTOINFLAMMATORY DISEASES: A SINGLE-CENTER STUDY

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BACKGROUND-AIM

Autoinflammatory diseases (AIDs) are a heterogeneous group of rare inherited rheumatic disorders with overlapping clinical features. Over 30 genes have been confirmed as causing AIDs. Sanger sequencing is the gold-standard approach for genetic diagnosis, but cannot be exhaustive, especially if limited to a few genes as routine performed in our laboratory. To improve it, our aim was to analyse the benefit of using a next generation sequencing (NGS) platform in the diagnostic process.

METHODS

Thirty patients with autoinflammatory-like features were enrolled by the Rheumatology Unit and sequenced. Sanger sequencing of selected exons in three AIDs genes (MEFV, MVK, TNFRSF1A) was initially performed using standard protocol in an ABI 3500 Genetic Analyzer. Then, NGS analysis run in MiSeq sequencer (Illumina) using a custom Sophia Genetics kit (CS-SOPHiA Customer Fever & Autoinflammatory Disease) and a standard V2 (2x250bp) kit (Illumina). The panel includes 17 AIDs-associated genes (ADA2, CARD14, ELANE, IL10RA, IL10RB, IL1RN, LPIN2, MEFV, MVK, NLRP12, NLRP3, NLRP7, NOD2, PSMB8, PSTPIP1, TNFRSF11A, TNFRSF1A). Reads were aligned to GRCh37 human genome. Variant calling and evaluation of filtered variants were performed using SOPHiA DDM software (v.5.10.15).

RESULTS

Sanger sequencing revealed 5 silent point polymorphisms in exons 2 and 3 of MEFV and 2 likely benign intronic variants (SNPs) in intron 9 of MVK and intron 6 of TNFRSF1A, which had no or negligible significance. The NGS analysis showed at least 500X coverage of the target areas. A total of 98 different missense variants with unknown significance (VUS) (98.9% of cases) or potentially deleterious were identified in heterozygosity in 13 genes. Of note, 29 patients presented at least one of the VUS variant, thus helping the clinician to correctly diagnose AIDs. L37K in IL10RB and G39V in NLRP12 were the two more frequently detected SNPs (62% and 44.8%, respectively). Interestingly, 18.4% of the identified SNPs were somatic variants, uncovered in 6 genes.

CONCLUSIONS

Custom NGS panel for AIDs is a very useful tool for detecting a wide range of variants, even somatic, otherwise not identified with routine Sanger. The NGS approach allowed a diagnostic improvement for almost all patients, thus limiting the delay in diagnosis and therapy.

Molecular diagnostics

P1715

MOLECULAR DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLEMIA: COMPARISON OF GUIDELINES FOR PATHOGENICITY EVALUATION OF GENETIC VARIANTS

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BACKGROUND-AIM

Familial Hypercholesterolemia (FH) is the most frequent genetic disease, with a prevalence of 1:250 for the heterozygous form, characterized by high level of LDL-cholesterol and higher cardiovascular risk. It is caused by pathogenic variants in three main causative genes (LDLR, APOB and PCSK9). There are two forms of this disease: the heterozygous (HeFH) and the homozygous one (HoFH), characterized by the presence of one and two pathogenic variants, respectively. We aim to compare the pathogenicity evaluation of a large number of variants identified in 717 index patients analyzed by our laboratory, according to different guidelines.

METHODS

The pathogenicity evaluation of 193 variants, identified in the laboratory from 2008 to 2022, was made comparing the ACMG's general guidelines (Richards et al. 2015) with the most recent FH-specific suggestions (Chora et al. 2018 for APOB and PCSK9 and ClinGen – Chora et al. 2022 - for LDLR).

RESULTS

The most recent guidelines led to an increased number of LDLR variants classified as uncertain significance (VUS) respect to the general ones (38 vs 18 variants) and, conversely, a decreased number of pathogenic/likely pathogenic ones (92 vs 113 variants). The criteria most impacting the difference in classification are related with the functional characterization, the number of unrelated patients with the variant and the consideration of missense variants in LDLR. Four HoFH patients resulted reclassified as HeFH+USV, despite a clear variant/phenotype segregation among relatives. No differences were observed between the different guidelines in the evaluation of the 45 variants identified in APOB and the 11 ones identified in PCSK9.

CONCLUSIONS

Pathogenicity assessment is a crucial point in molecular diagnostics of FH allowing to correctly identify HoFH. Despite new guidelines suggested criteria specific for the FH genetic features that are very useful for a standardization of pathogenicity evaluation, their application resulted in many variants classified as USV. For rarest variants, guidelines could be improved giving more strength to the few available evidence, when no benignity criteria are present.

Molecular diagnostics

P1716

FREQUENCY OF GENETIC VARIANTS ASSOCIATED WITH FAMILIAL PSEUDOHYPERKALEMIA. IS IT A RARE CONDITION?

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BACKGROUND-AIM

Familial pseudohyperkalemia (FP) is an inherited, mild, non-hemolytic subtype of hereditary stomatocytosis that is associated with a temperature-dependent anomaly in red cell membrane permeability to potassium that leads to high in vitro potassium levels in samples stored below 25°C. All families identified so far have mutations in the ABCB6 gene (2q35) but the prevalence is unknown. However, some studies point to an european prevalence from 1/370 to 1/500.
The aim of this work is to study the prevalence of the ABCB6 gene variants associated with FP and the positivity of the FP screening protocols in our hospital.

METHODS

Ninety nine protocols for the screening of FP were carried out in the Medicine laboratory department. The screening protocol is based on taking 7 aliquots from a lithium heparin tube for incubation at 4ºC, 25ºC and 37ºC for 4 and 6 hours for subsequent potassium analysis.
Six thousand six hundred and eighty eight patients studied previously by clinical exome in the Clinical Genetics department were selected. The variants collected for study are:
V454A/V454A. Bolivian variant. FP in homozygosity.
R276W. Cardiff-2 variant. FP in heterozygosity.
R723Q. Cardiff-2 variant. FP in heterozygosity.
R375W. Falkirk variant. FP in heterozygosity.
R375Q. Lille variant. FP in heterozygosity.

RESULTS

Seventy nine protocols have been reported as compatible with FP (79.8%) and 20 negative (20.2%).
Sixty four patients were found with the V454A variant, 4 of them were homozygous, with an allele frequency (AF) of 0.00508. Ninety six patients with the R276W variant were found, 0 of them were homozygous, with an AF of 0.00718. No patients with R723Q, R375W and R375Q variants were found. In total, 100 cases with variants associated with PF were found (4 V454A/V454A and 96 R276W) out of the 6688 patients analyzed, which implies a prevalence of 1/67 patients.

CONCLUSIONS

Our prevalence is higher than that estimated for the European population. Due to our high positivity rate in PF screening tests with a high prevalence, which correlates with the study of ABCB6 gene frequencies (1 out of 67 patients), PF should be considered in the differential diagnosis in those patients with elevated potassium without a justifiable cause.

Molecular diagnostics

P1717

THE INVOLVEMENT OF MOLECULAR AND EPIGENETIC MECHANISMS IN TMAO REGULATION LEVELS

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BACKGROUND-AIM

Trimethylamine-N-oxide (TMAO) is a microbial byproduct of choline metabolism. It is processed in the liver and excreted into the blood circulation. Its high levels are associated with increased atherosclerotic lesion formation and risk of cardiovascular disease. miRNAs are small noncoding RNA implicated in different cellular processes. Their levels are altered in several human pathological conditions, such as cardiovascular diseases. They are involved in lipoproteins metabolism and cholesterol synthesis. In particular, miR-146a-5p hepatic expression has been correlated with TMAO circulated levels.
The aim of this study is to investigate a correlation between TMAO levels and people' lifestyle, diet, drugs and pathologies, such as cardiovascular diseases, and to identify the molecular and epigenetic entailment in TMAO regulation levels

METHODS

To investigate the possible correlation between TMAO and miRNA expression level, we have enrolled 20 subjects and for each one the plasma levels of TMAO, specific miRNAs, such as miR-146a-5p, and stem cell biomarkers, like CD34, will be examined.
The TMAO quantitative method was developed and validate on two different analytical platforms in LC/MS-MS, using the isotopic dilution method

RESULTS

In a preliminary phase the TMAO levels have been compared with subjects' lifestyle, diet, drugs, pathologies, hepatic biomarkers, total and serum proteins. Our preliminary results showed physiological TMAO values (2.25-5.79 µmol/L) in 64.3% of patients. Whereas the 35.1% of subjects exhibited high TMAO levels. In particular, the 7.1% of patients showed TMAO levels between 5.8 and 6.2 µmol/L, associated to a moderate atherosclerotic risk, in 28.6% of subjects TMAO levels were >10 µmol/L, related to high atherosclerotic risk. Moreover, our data highlighted that high TMAO levels were significantly correlated with daily alcohol consumption (Fisher Test, $p<0.05$).
The preliminary analysis of miR-146a-5p expression levels in plasma suggests an inverse correlation between TMAO and miR-146a-5p levels

CONCLUSIONS

Our study is important for elucidating how the epigenetic molecular mechanisms interact with TMAO levels regulation, in different human pathologies, such as atherosclerosis and cardiovascular diseases

Molecular diagnostics

P1718

GENETIC DIAGNOSIS OF CITRULLINEMIA TYPE 1. A CASE REPORT

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BACKGROUND-AIM

Citrullinemia type I (CT1) is an autosomal recessive disorder caused by deficiency of the cytosolic enzyme argininosuccinate synthetase 1, the third step in the urea cycle, in which citrulline is condensed with aspartate to form argininosuccinic acid. This deficiency is caused by pathogenic variants in the ASS1 gene. CT1 has a heterogeneous clinical presentation, including severe hyperammonemic events within the first 28 days of life, a more variable phenotype presenting after the neonatal period, and individuals with mild or no symptoms. The diagnosis of CT1 is established in patients with elevated plasma ammonia (>250 µg/dL) and citrulline concentration (>500 µmol/L), and absent argininosuccinate and/or by the identification of biallelic pathogenic (or likely pathogenic) variants in ASS1 on molecular genetic testing.

METHODS

We present the case of a full-term newborn male being studied due to two older siblings dying days after their birth after several vomiting episodes. At 48 hours of life, after taking a bottle of formula, he began vomiting and showed an elevation of plasma ammonia concentration (298 µg/dL) and lactic acid (65 mg/dL), with low serum urea concentration (3 mg/dL) and a tendency towards metabolic alkalosis. The newborn screening results showed a high concentration of plasma citrulline (422 µmol/L). A urea cycle disorder was suspected as the first possible diagnosis, so a genetic study was requested.

RESULTS

An analysis of the ASS1 gene was carried out by next-generation sequencing of the exonic and adjacent intronic regions for the detection of punctual variants. This analysis was able to identify the homozygous variant c.836G>A (p.Arg279Gln) in ASS1, classified as pathogenic and related to CT1, which confirmed the diagnosis of CT1. Currently, the patient is stable, on treatment, and is being followed by gastroenterology pediatricians.

CONCLUSIONS

We can conclude that CT1 is a rare disorder that can be potentially lethal. Therefore, an early and accurate diagnosis is essential in order to improve the patients' quality of life and to provide adequate genetic counseling to them and their families. This is why genetic testing is of utmost importance, as it's necessary for both a confirmatory diagnosis and the identification of new variants with possible clinical meaning.

Molecular diagnostics

P1719

SUSCEPTIBLE HLA ALLELES PREDISPOSE TO CYCLOSPORINE INDUCED NEUROTOXICITY

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BACKGROUND-AIM

Neurotoxicity is a well-established complication of cyclosporine A (CSA) therapy after hematopoietic stem cell transplantation. There is potent genetic association in contribution of HLA genes in development of drug hypersensitivity. Delayed-type or nonimmediate drug hypersensitivity reactions often involve the activation of drug-specific T cells. HLA-binding peptides with drugs leads to the stimulation of T cells. The aim was to identify the human leukocyte antigen (HLA) susceptible alleles which predispose to cyclosporine induced neurotoxicity (CIN) in HSCT patients.

METHODS

Two parallel samples in EDTA vacutainers drawn from 102 patients who underwent allogeneic HSCT. DNA extracted by commercial kit method (mini QIAGEN). HLA typing performed by sequence specific primer methodology according to protocol provided by Olerup commercial kits. Results interpreted on software (SCORE) provided by Olerup company. Cyclosporine trough levels obtained during neurological event. CIN were labeled, if central nervous system (CNS) symptoms present along with posterior reversible encephalopathy syndrome (PRES) on CT/MRI scan.

RESULTS

Mean age of patients were 11.4+ 11.1 (range 1 to 47) years with 65 (64%) were males. Majority of patients were of beta-thalaessemia major and aplastic anemia 51 (50%) and 32 (31%) respectively. Overall HLA-A*02 (33.3%), HLA-DRB1*0(46.1%) and HLA-DRB1*1(35.3%) respectively were the most frequent alleles. In 102 CSA users, 31(30.2%) developed neurotoxicity related symptoms with HLA-A*24(20.9%), HLA-A*11(19.3%), DRB1*0(27.4%), and DRB1*15(27.4%) most frequent alleles. While 13(12.7%) were labeled as CIN by CT-scan with HLA-A*24 (22.2%) and HLA-DRB1 *15 (27.7%) were most frequent allele followed by DRB1*11 and DRB1*03 (16%). The mean CsA trough level in CIN vs no-CIN was 691+ 370.3 mg/dl, and 410+253.3 mg/dl.

CONCLUSIONS

HLA-DRB1*15 was most common allele in conf-CIN patients. Screening of HLA-DRB1*15 prior to the use of CSA therapy might be required to predict probability of CIN.

Molecular diagnostics

P1720

HETEROPLASMY AND VARIABLE EXPRESSIVITY IN MITOCHONDRIAL DISEASE

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BACKGROUND-AIM

Within each mitochondria are thousands of mitochondrial DNA (mtDNA) molecules that encode critical genes for oxidative phosphorylation and are maternally inherited. The coexistence of wild-type and mutated mtDNA molecules is called heteroplasmy, but a minimum percentage of mutated mtDNA is necessary for symptoms to appear (threshold effect). Among the best-studied pathogenic mutations is m.3245A>G in the MT-TL1 gene, which can cause mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes syndrome (MELAS) when heteroplasmy is 50-70%, and maternally inherited diabetes mellitus and deafness (MIDD) at levels below 40%.

METHODS

25-year-old patient with a pervasive developmental disorder diagnosed at 2 years of age with no determined cause and hypertrophic cardiomyopathy.
One year later, the patient debuted with diabetes, presenting a maternal history of type 1 and 2 diabetes. Additionally, he presented deafness and proteinuria.

RESULTS

The patient was referred to the Genetics unit where a clinically targeted exome was performed for the cardiomyopathy, in which only a heterozygous Variant of Uncertain Significance was found in the SCN5A gene, which is associated with Brugada syndrome.
Because of diabetes debut, he was referred back to the Genetics unit, where a genetic study of mtDNA and Mody type diabetes by massive sequencing (NGS) was carried out. The presence in heteroplasmy (43.8%) of the pathogenic variant m.3243A>G was identified. This mutation was also found in the mother, with a heteroplasmy of 13%.
Finally, with these results, the patient was diagnosed with mitochondrial diabetes and MELAS syndrome.

CONCLUSIONS

The mitotic distribution of mitochondria is random, so the daughter cells do not necessarily receive the same load of mutated mtDNA. In addition, the number of mitochondria with the presence of the variant in blood decreases over time. This would explain the difference in the percentage of heteroplasmy between mother and son.
MtDNA continues to replicate independently of cell division, so different tissues may vary in their heteroplasmy load. For this reason, despite the fact that the percentage of heteroplasmy does not reach the threshold for MELAS, the patient was eventually diagnosed based on the clinical presentation.

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Molecular diagnostics

P1721

CONTRIBUTION OF GENETICS TO THE DIAGNOSIS AND RECLASSIFICATION OF ALPORT SYNDROME

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BACKGROUND-AIM

Hereditary renal diseases (HKD) constitute a broad group of disorders of genetic cause. Autosomal dominant polycystic kidney disease (ADPKD) stands out for its frequency, with Alport syndrome (AS) in second place and is considered a rare disease. It is an underdiagnosed disease partly due to its clinical heterogeneity and variable expressivity. It can be transmitted X-linked (80%), autosomal recessive (15%) or dominant (5%).

METHODS

Descriptive observational study of 386 individuals with a diagnosis of chronic kidney disease and suspected genetic cause, who underwent a genetic study using a panel of 44 genes related to HRD (SOPHiA Genetics). We analyzed those probands with any pathogenic or probably pathogenic variant.
Sequencing of the libraries was performed on a MiSeq (Illumina Inc), bioinformatics analysis of the data and variant annotation was performed using SOPHiA DDM 5.8.0.3 software, and variant review by querying major databases (ClinVar, Exac, HGMD, NCBI, PKD Foundation, LOVD).

RESULTS

Of the 386 patients studied, 132 informative results were obtained, 20% corresponding to Alport syndrome, of which: 35% developed deafness, whereas only 7% had vision loss. One-third required renal replacement therapy, of which 70% had variants in COL4A3 or COL4A4.
The correlation between clinical and genetic diagnosis was only 11%, the reasons for the study being mainly unaffiliated CKD, segmental and focal glomerulosclerosis, and even ADPKD.
65% of the variants found were in heterozygosis and dominant inheritance, with COL4A3 being the most frequently implicated gene.

CONCLUSIONS

We observed that in AS there is a low degree of concordance between clinical and genetic diagnosis, making the genetic study a basic tool for its correct diagnosis. This lack of correlation is mainly due to its clinical heterogeneity, since the clinical spectrum is wide, even in individuals who share the same variant within the same family, and no genotype-phenotype correlation has been found.
In our healthcare setting, in contrast to the scientific evidence published to date, autosomal dominant inheritance is the majority.
We found few cases of recessive transmission, which could be associated with the variable expressivity of the syndrome rather than with this type of inheritance.

Molecular diagnostics

P1722

HPV INFECTION IN UNVACCINATED ADULTS IN AZERBAIJAN.

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BACKGROUND-AIM

Human Papilloma Virus (HPV) is primarily considered a sexually transmitted disease with over 100 genotypes. Some HPV genotypes are a source of risk for malignant transformation. Two HPV serotypes (16 and 18) are highly associated with precancerous cervical lesions and cervical cancer. This study aimed to investigate the prevalence of HPV infection among men and women in Azerbaijan. Moreover, we will investigate the HPV 16 and HPV 18 genotype prevalence.

METHODS

In total, 403 unvaccinated men and women aged 18-60 years vaginal and urethral smear materials from “INCI LABORATORIES” were collected from 01 January 2022 to 14 January 2023 in Azerbaijan. Genotyping of HPV was accomplished in Real-Time PCR technique. 21 HPV genotypes (6, 11, 16, 18, 26, 31, 33, 35, 39, 44, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) were analysed.

RESULTS

110 HPV-positive results were observed from 403 patients’ genital and urethral smear materials. Between HPV-positive results patients 104 were women and 6 were men. HPV 16 and HPV 18 genotype-positive cases were 32 (7.9 %) and 17 (4.2%) respectively.

CONCLUSIONS

The results of this series of cases suggest that a higher prevalence of genital HPV was found in women. Additionally HPV 16 genotype was more prevalent, while HPV 18 was among investigated cervical lesions patients in Azerbaijan. However, further studies are needed to clarify HPV infection’s genotype prevalence in investigated patients in Azerbaijan.

Molecular diagnostics

P1723

ASSOCIATION OF MUC5B PROMOTOR GENETIC VARIANT WITH THE DEVELOPMENT OF IDIOPATHIC PULMONARY FIBROSIS

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BACKGROUND-AIM

Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive interstitial pneumonia limited to the lung, with a poor prognosis. Currently the prevalence is 16/100.000 habitants.
There is a well-recognized genetic component to IPF susceptibility. The genetic variant in the promoter region of MUC5B gene (NG_031880.1:g.1927G>T,rs35705950) has been associated with an increased risk of developing IPF.
Although the precise mechanisms through which MUC5B dysregulation contributes to IPF development are currently unknown, MUC5B overexpression may cause mucociliary dysfunction and disruption of the normal reparative mechanisms in the lung.
MUC5B rs35705950 not only predisposes to IPF but has also been associated with improved survival.
To assess the influence of the rs35705950 genotype on the risk of developing IPF and to evaluate the influence on disease progression.

METHODS

The total study population of 197 patients was divided into a cohort of 58 symptomatic patients diagnosed with IPF by chest CT scan or lung biopsy (March 2021 to September 2022) and family history (cases), and another cohort of 139 asymptomatic patients (controls).
The influence of MUC5B on disease progression was evaluated by an observational and retrospective study, where disease progression was defined as a forced vital capacity (FVC) loss≥5% per year. Patients were classified as progressing (≥5%pred.) or stable (<5%pred.).
To analyse the genotypic distribution’s relationship (noncarrier GG, heterozygous GT and homozygous TT) between cases and controls and disease progression a chi-square test ($\chi 2$) was carried out.

RESULTS

There was statistically significant differences in the prevalence of the genetic variant: in the control cohort (n=139) prevalence was 28% (99GG, 39GT, 1TT), whereas in the IFP cohort (n=58) the prevalence rose to 71% (17GG, 11GT and 6TT).($\chi 2$ test p-value=3.678x10-8).
Of the 58 patients with family history of IPF, 36.84% progressed functionally. However, there was no statistically significant relationship with MUC5B genotype.

CONCLUSIONS

In this population, the presence of the genetic variant rs35705950 is a risk factor for developing IPF. However, no relationship with disease progression could be identified. Further research is needed to better understand the genetic implications of this variant.

Molecular diagnostics

P1724

COMPARISON OF ELECTROPHORETIC AND SPECTROPHOTOMETRIC DNA CONCENTRATION MEASURING METHOD

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BACKGROUND-AIM

High DNA quality and quantity are crucial for further downstream applications. Therefore, DNA isolation is usually followed by DNA purity, integrity and concentration measurement. Based on different principles, there are several DNA concentration measuring methods. We hypothesis that these methods differ in determined DNA concentration. The aim of our study was to compare two DNA concentration measuring methods: electrophoretic with fluorescence detection and high-sensitivity UV-spectrophotometric.

METHODS

DNA was isolated from 40 remaining whole blood samples, collected by venipuncture in 3 mL Vacuette® tube (K3EDTA, Greiner Bio-One, Kremsmunster, Austria), using column-based extraction with Roche® High Pure PCR Template Isolation Kit (Roche, Mannheim, Germany). In each isolate, DNA concentration was measured with two methods. First, by microfluidic, automated DNA electrophoresis with fluorescence detection on Agilent 4200 Tapestation System using Genomic DNA ScreenTape (both Agilent, Waldbronn, Germany), and second, by absorbance measuring at 230, 260 and 280 nm on DeNovix® DS-11 FX+ spectrophotometer (DeNovix, Wilmington, USA). For spectrophotometric method, 1 μL of each DNA isolate was analyzed in triplicate and average value was taken for statistical analysis. Obtained DNA concentrations were compared using Bland-Altman analysis and Passing-Bablok regression analysis on MedCalc v.20.013 (MedCalc, Ostend, Belgium).

RESULTS

Bland-Altman analysis (DeNovix vs. Tapestation) showed statistically significant systematic and proportional bias (mean 5.52 ng/μL, 95%CI:2.70-8.35 ng/μL and 15.81%, 95%CI:7.34-24.28%, respectively) indicating higher DNA concentration values measured on DeNovix spectrophotometer. Passing-Bablok regression analysis showed only statistically significant proportional deviation (y = 2.84 (-2.41 to 7.69) + 0.79 (0.63 to 0.97) x).

CONCLUSIONS

Methods are not comparable indicating slightly higher DNA concentrations obtained by the spectrophotometric method. This may be due to the different method principles: electrophoretic protocol includes SYBRTM Green I fluorescent dye, which makes it more dsDNA specific when compared to the spectrophotometric method. However, for routine laboratory work these differences are almost negligible and both methods can be considered acceptable.

Molecular diagnostics

P1725

GENETIC DIAGNOSIS OF CAPILLARY MALFORMATION-ARTERIOVENOUS MALFORMATION SYNDROME: A CASE REPORT

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BACKGROUND-AIM

Capillary malformation-arteriovenous malformation syndrome (MC-AVM) is an autosomal hereditary rasopathy whose prevalence is approximately 1:100,000 with a penetrance between 89-98%. It is characterized by the presence of multifocal cutaneous capillary and vascular malformations associated with arteriovenous fistulas and can cause life-threatening complications such as bleeding, congestive heart failure or neurological involvement.

METHODS

A 16-day-old infant who, from birth, presented a 9x4cm salmon-colored macule in the lower back-left flank area with no evolution. At 6 months, new smaller macules appeared in different locations. Magnetic resonance angioma of the entire neuraxis revealed the existence of multiple galeal and subgaleal capillary angiomas, with no signs of vascular malformation.

RESULTS

Due to these results, MC-MAV syndrome was established as clinical suspicion. The first genetic study using clinical exome directed at rasopathies was not informative and therefore no alteration in said genes that would justify the phenotype of the patient. In a subsequent genetic study by massive sequencing, the heterozygous presence of an undescribed pathogenic variant in the RASA1 gene was identified (NM_002890.3(RASA1):c.1995_1999delCAAAG (p.Ser665Argfs*3)), being a 5-nucleotide deletion that causes a frameshift change at position 665 of the protein. Consequently, this would cause the substitution of a serine amino acid for arginine and, due to the change in the reading frame, a premature stop codon would be generated 3 amino acids later. Pathogenic variants in this gene are associated with capillary and arteriovenous malformations type 1 (OMIM: 608354). After this, a segregation study was carried out in both parents, revealing with high probability that the previously described variant identified in the daughter had not been inherited. inherited, thus being a de novo variant.

CONCLUSIONS

Clinical suspicion of MC-AVM syndrome usually begins with pink spots on the skin and the diagnosis is confirmed by detection of pathogenic variants in the RASA1 gene. All this will allow, by performing cerebral and spinal angioresonance, to prevent possible complications.

Molecular diagnostics

P1726

DETECTION OF ACQUIRED EGFR T790M RESISTANCE MUTATION IN LUNG CANCER: A COMPARISON OF LIQUID BIOPSY AND TISSUE BIOPSY MOLECULAR ANALYSIS

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BACKGROUND-AIM

Activating mutations of the epidermal growth factor receptor gene (EGFR) in lung cancer are predictive biomarkers of response to first- and second-generation tyrosine kinase inhibitors (TKI). Patients that initially respond to these drugs develop cancer progression due to the acquired T790M resistance mutation in EGFR exon 20. Liquid biopsy is a method of molecular testing preferentially performed on circulating tumor DNA (ctDNA) extracted from plasma samples. The aim of this study is to present the case report and clarify the “plasma first” versus “tissue first” approach in EGFR retesting.

METHODS

A patient data and informed consent were extracted from the database of the Institute for Pulmonary Diseases of Vojvodina. DNA was isolated from the transbronchial needle aspiration (TBNA) specimen and from the K2 EDTA blood plasma using the cobas® DNA and cfDNA Sample Preparation Kits, respectively. Real-time PCR analysis was based on the cobas® EGFR Mutation Test V2.

RESULTS

In a 70-year-old woman malignant infiltration in the right upper lobe of the lung and multiple bone metastases were observed. Bronchoscopic samples were positive for lung adenocarcinoma. EGFR testing detected the presence of deletion in exon 19. Afatinib (second-generation TKI) was chosen as a therapy in May 2019. During the treatment, the patient showed a partial radiological response until May 2022, when the follow-up CT scan indicated progression of the disease. Liquid biopsy was performed and confirmed deletion in exon 19. It was repeated in June and July 2022 and the samples showed only deletion in exon 19. After 39 months (August 2022) afatinib therapy was stopped. Rebronchoscopy was performed and in a new sample of TBNA the presence of deletion in exon 19 and T790M mutation was detected. The patient was administered osimertinib (third-generation TKI) which binds irreversibly to EGFR with T790M mutation. Our patient is still on osimertinib therapy, with stable disease and good quality of life.

CONCLUSIONS

Liquid biopsy is a minimally invasive technique that should be preferred as the first approach for EGFR acquired resistance setting. Due to the lower sensitivity of the liquid biopsy, tissue tumor genotyping is still the gold standard. Liquid and tissue biopsy should be complementary approaches in EGFR retesting.

Molecular diagnostics

P1727

COMPONENT-RESOLVED ALLERGY DIAGNOSIS IN IDENTIFYING STINGING WASP SPECIES

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BACKGROUND-AIM

Proper recognition of the insect responsible for a sting reaction in a patient allergic to Hymenoptera venom is critical for appropriate management. The wasp stinger usually has no barbs, multiple stings being possible. Because vespids have similar coloring and appearance, the majority of stung allergic individuals are not able to differentiate species from the genera Vespula, Dolichovespula, Vespa and Polistes.

METHODS

We present the case of an adult female patient living in a region in Southern Romania where most stings of insects from the Vespidae family are inflicted usually by wasps from genus Vespula and, rarely from genus Polistes. She was stung by unidentified wasps: once in the foot, with generalized urticaria, and afterwards in the upper lip, with angioedema and dyspnea, both times with care by an emergency physician. A kit with adrenaline autoinjector was prescribed. Component-resolved allergy diagnosis was performed by a multiplex allergy explorer immunoassay used for the recognition of IgE sensitizations against specific Hymenoptera insect venom components.

RESULTS

The detection of specific IgE antibodies against rPol d 5 (CCD-free recombinant Antigen 5) in serum (0.47 kUA/L) reveals primary sensitization to the European paper wasp (Polistes dominulus) venom. Serum specific IgE antibodies against common wasp (Vespula vulgaris) venom and its specific allergen component rVes v 1 (phospholipase A1), against long-headed wasps (Dolichovespula spp) venom, as well as specific IgE antibodies against honey bee (Apis mellifera) venom and its components nApi m 1 (phospholipase A2) and rApi m 10 (icarapin variant 2), were not detected ($\leq$ 0.1 kUA/L). These differentiating biomarker allergens were used to allow the discrimination between primary IgE sensitisation to vespid and honeybee venoms, while rPol d 5 was used for the precise molecular diagnosis of paper wasp venom allergy.

CONCLUSIONS

Because in any region of the continental Europe, wasps may coexist, at least species from the genera Vespula and Polistes, component-resolved diagnosis is highly valuable for precision allergy diagnosis, the majority of Hymenoptera sting victims being not able to identify a vespid sting culprit.

Molecular diagnostics

P1728

RISKS OF FOOD ALLERGIC REACTIONS DUE TO IGE SENSITIZATION AGAINST HOUSE DUST MITE MOLECULAR ALLERGENS OTHER THAN TROMPOMYOSIN AND PARAMYOSIN

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BACKGROUND-AIM

IgE sensitization to tropomyosin from house dust mites (HDMs) locomotory muscles is usually considered a risk for HDMs-crustaceans-mollusks syndrome and allergy to edible insects, Der p 10 being considered an invertebrate panallergen. Der p 11 may also be involved due to cross-reactivity with molluskan paramyosins.

METHODS

We report the case of an adult male patient with HDMs-allergic rhinitis in which we assessed the risk of allergic reactions to foods due to IgE sensitization to allergenic molecules other than trompomyosin and paramyosin. IgE HDMs molecular profiling was performed by a new-generation macroarray immunoassay based on a proprietary nano-bead technology.

RESULTS

Serum specific IgE antibodies against HDMs major recombinant allergens from the NPC2 family, rDer p 2 (2.18 kUA/L) and rDer f 2 (5.33 kUA/L), along with peritrophin-like protein rDer p 23 (0.54 kUA/L) were detected, supporting the diagnosis of HDMs-induced respiratory allergy. The multiplex immunoassay revealed no IgE sensitization to cysteine protease group 1 major allergens (Der p 1, Der f 1), to tropomyosins from mites (rDer p 10, rBlo t 10), fish nematode Anisakis simplex (rAni s 3), cockroach Periplaneta americana (rPer a 7), shrimp Penaeus monodon (rPen m 1), or to HDMs paramyosin (rDer p 11). Sensitization to the group 2 allergens (Der f 2, Der p 2), thermostable and resistant to digestion, may be considered a risk factor for oral mite anaphylaxis or pancake syndrome. Detected specific IgE antibodies against HDM rDer p 20 (4.26 kUA/L), cockroach Blattella germanica rBla g 9 (2.56 kUA/L) and black tiger shrimp Penaeus monodon rPen m 2 (0.18 kUA/L) support the IgE sensitization to invertebrate panallergen arginine kinase, which, being volatile, more unstable and less resistant than tropomyosin, may be considered a risk factor for respiratory symptoms induced by crustacean steam inhalation and for anaphylaxis to edible invertebrates such as silkworm Bombyx mori pupa, an Asian delicacy.

CONCLUSIONS

Molecular allergy diagnosis using a multiplex macroarray immunoassay allows to assess the risks of food allergy to oral mites and edible invertebrates in HDMs-allergic patients.

Molecular diagnostics

P1729

MOLECULAR IGE SENSITIZATION PROFILING IN OCCUPATIONAL LATEX ALLERGY WITHOUT LATEX-FRUIT/VEGETABLE SYNDROME

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BACKGROUND-AIM

Several molecular Hevea brasiliensis latex allergens are more important for health care workers (HCWs) than in patients with spina bifida (SB), while cross-reactive ones may be involved in latex-fruit/vegetable syndrome. HCWs represent a significant risk group for latex allergy due to the usual regular wearing of natural rubber gloves. Molecular allergens Hev b 5 and Hev b 6.02 are found in higher amounts on the internal surfaces of such gloves.

METHODS

We present the case of a female adult health care worker, an intensive care unit nurse, with clinically relevant latex allergy to natural rubber gloves and no history of allergic reactions to cross-reactive foods such as banana, kiwifruit, avocado, papaya, mango, fig, chestnut, paprika or potato. She never ate maracuja and cassava. Molecular IgE sensitization profiling was performed by a state-of-the-art macroarray ELISA-based multiplex immunoassay, with recombinant allergens, used as a molecular allergy explorer

RESULTS

Serum specific IgE antibodies against the major allergens involved in HCWs natural rubber latex allergy, acidic structural protein rHev b 5 and pro-hevein rHev b 6.02, were detected (47.44 kUA/L and 8.29 kUA/L, respectively). Specific IgE antibodies against the minor allergen rHev b 11, a class I chitinase, were also found (0.93 kUA/L), but sensitization to the rHev b 8 profilin, a plant panallergen, was not revealed. Serum specific IgE antibodies against the small rubber particle protein rHev b 3, a minor allergen in latex-allergic patients related to occupational exposure, were detected (0.54 kUA/L), while those against the rubber elongation factor rHev b 1, a major allergen for latex allergy in SB patients, not involved in cross-reactivity with edible fruits, were not discovered (≤ 0.1 kUA/L).

CONCLUSIONS

IgE sensitisation profiling using recombinant allergens is useful in the precision allergy diagnosis of natural rubber latex allergy in HCWs.

Molecular diagnostics

P1730

IDENTIFICATION OF A DELETION IN DMD GENE IN A PATIENT WITH DILATED CARDIOMYOPATHY.

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BACKGROUND-AIM

Dystrophinopathies, including Duchenne (DMD, MIM:310200) and Becker (BMD, MIM:300376) Muscular Dystrophies, represent varying clinical presentations of an X-linked, progressive neuromuscular diseases caused by mutations in the DMD gene (Xp21.2), encoding the dystrophin protein. These two conditions, severe-DMD or milder-DMB, differ in their age of onset, rate of progression and severity of neuromuscular and cardiac involvement.

METHODS

39 year-old male patient was referred due to palpitations and ECG changes (T-wave inversion). Echocardiography: dilated cardiomyopathy with moderate systolic dysfunction (LVEF 38%) and apical hypertrabeculation. No previous muscle exploration. No family history of heart disease. Personal history: dyslipidemia, and Hodgkin's lymphoma.
Genetic study: Molecular analysis with a panel of 251 genes related to Cardiomyopathies, Cardiac Arrhythmias and Sudden Death, using Next Generation Sequencing (NGS) (Agilent enrichment kits and Illumina HiSeq1500 platform).

RESULTS

A deletion (735 pb), in hemizygosis, in exon 48 of the DMD gene (NM_004006.3) was found in the patient and it is associated with Dilated Cardiomyopathy and DMD/B. This variant has been classified as pathogenic (ACMG). It was confirmed by MLPA (P034-DMD-1, MRC Holland®).
Additionally, 2 Variants of Uncertain Significance (VUS) were detected in BAG3 and DTNA genes, respectively.

CONCLUSIONS

Exon 48 deletion of the DMD gene results in a truncated but functional protein. These patients present a very mild or asymptomatic dystrophinopathy and it is possible that many cases of BMD are undiagnosed. Likewise, cardiac disease is not necessarily related to the degree of muscular involvement or may even be the predominant manifestation.
NGS panels allow demonstrate infrequent hereditary syndromes, as occurs in this case of DMB, whose prevalence could be higher than described previously. Thanks to these molecular biology tools, it has been possible to perform a better diagnostic characterization of this patient, which will permit adequate treatment as well as genetic counselling.

Molecular diagnostics

P1731

GENETIC DIAGNOSIS OF SEVERE JUNCTIONAL EPIDERMOLYSIS BULLOSA 1B

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BACKGROUND-AIM

A 15-day-old infant is taken to the hospital emergency room due to bullous lesions that do not subside after antibiotic treatment. As the clinician suspect bullous impetigo and due to wounds infection by Kblebsiella pneumoniae, she is admitted for intravenous antibiotic treatment. Instead of subsiding, the lesions spread and blisters with serous content appear all over the body. Consultation with dermatology service is performed, emphasizing the presence of friction blisters.
Mutations in the LAMB3 gene are related to Intermediate Generalized Junctional Epidermolysis Bullosa 1A and Severe Generalized Junctional Epidermolysis Bullosa 1B, characterized by the formation of blisters at the level of the lamina lucida in the basement membrane of the skin. 1A is an adult, non-lethal form, characterized by lifelong skin blistering. On the contrary, 1B is a severe form with bullous lesions that appear at birth and extensive denudation of the skin and mucous membranes that can be hemorrhagic. Death usually occurs within the first six months of life.

METHODS

A genetic study of epidermolysis bullosa was carried out. After DNA extraction, quantification and amplification, analysis by ultrasequencing (NGS) of the adjacent exonic and intronic regions of the genes of interest was performed for the detection of specific variants and possible copy number variations (CNV).

RESULTS

The variant of clinical pathogenic significance c349C>T, (p.Gln117Ter) was detected in homozygosis in the LAMB3 gene. This sequence change creates a premature translational stop signal and is therefore expected to result in an absent or disrupted protein product. Given this result, it was recommended to carry out the study in the parents, which showed up the same mutation in heterocygosis.

CONCLUSIONS

The importance of the genetic study lies, on the one hand, in confirming or ruling out clinical suspicion and, on the other, in offering genetic counselling. Results are consistent with the autosomal recessive inheritance pattern presented by pathologies related to the LAMB3 gene. Therefore, the probability that these people have another affected child is 25%. Parents were referred to the Assisted Reproduction Consultation for considering in vitro fertilization and preimplantational genetic diagnosis, in case of wanting more children.

Molecular diagnostics

P1732

CASK-RELATED INTELLECTUAL DISABILITY: A CASE REPORT

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BACKGROUND-AIM

Mutations in the CASK gene have been associated with X-linked intellectual disability or microcephaly with pontocerebellar hypoplasia. More than 35 CASK gene mutations have been identified in people with CASK-related intellectual disabilities. This gene is located on the short arm of the X chromosome (Xp11.4) and encodes a calcium-calmodulin-dependent serine protein kinase. This protein is found mainly in neurons, where it helps control the expression of other genes involved in brain development. Males tend to have more severe signs and symptoms than females, although the most severe forms of microcephaly with cerebellar hypoplasia mainly affect females. This may be due to the fact that only a small number of males survive to birth.

METHODS

1-year-old girl of consanguineous parents with microcephaly and pontocerebellar hypoplasia, detected by ultrasound in the 32nd week of gestation and confirmed after birth. QF-PCR was performed on amniotic fluid and was normal for the chromosomes studied. CHG-array was performed. Due to the results, it was decided to extend the study and perform whole exome sequencing.

RESULTS

CHG-array shown two regions of homozygosity of more than 5 Mb were found on chromosomes 7 and 16. The targeted exome for genes contained in the homozygosity regions was negative. The whole exome performed identified the presence in heterozygosis of the pathogenic variant c.2041C>T; p. (Arg681*) in the CASK gene. This result confirms a genetic diagnosis of intellectual disability, microcephaly and pontocerebellar hypoplasia in the patient, with an X-linked dominant inheritance pattern. Study of the variant in the mother shows that she is not a carrier of the altered gene.

CONCLUSIONS

After confirmation of the de novo origin of the pathogenic variant in CASK and taking into account that the tests performed did not rule out germline mosaicism, the patient's mother was referred to assisted reproduction for further study.

Molecular diagnostics

P1733

PATIENT DIAGNOSED WITH COWDEN SYNDROME. A CASE REPORT

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BACKGROUND-AIM

Cowden syndrome (CS) or hamartoma tumor syndrome is an autosomal dominant genetic disorder associated with a germline mutation in the tumor suppressor gene PTEN (Protein thyrosine phosphatase with homology to tensin) located on chromosome 10q22-23. It is characterized by the presence of multiple hamartomas and nodules in the skin and oral mucosa, together with abnormalities in the breast, thyroid and polyps in the gastrointestinal tract, with a tendency to malignancy, especially in the breast and thyroid.

METHODS

Case information
A 32-year-old patient presented with multiple lesions on the tongue and alveolar ridge that made chewing difficult. Physical examination showed a right cervical mass with no adenopathies and an abdominal mass in the hypogastrium. Biochemistry showed elevated tumor markers (Cyfra21.1: 35 ng/mL VN<2.1, CEA (carcinoembryonic antigen): 47ng/mL VN<5), with thyroid, renal and hepatic function within the normal range. Thyroid ultrasound showed an asymmetric multinodular goiter of right predominance with compression and tracheal displacement to the left. Abdominal CT showed a large abdominal-pelvic mass with neoplastic aspect. She was evaluated by a multidisciplinary team and total thyroidectomy and right salpingo-oophorectomy were performed, without finding neoplastic cells.
Among the family antecedents: father died due to intestinal tumor, brother operated for intestinal polyps and sister recently hysterectomized with bilateral oophorectomy.

RESULTS

Given the family history and the suspicion of CS, a genetic study was performed and a mutation in the PTEN + gene was found.

CONCLUSIONS

The diagnosis of this syndrome is confirmed by the demonstration of a mutation in PTEN. This gene inhibits tumor growth by acting as a regulator of cell growth enhanced by tyrosine kinase.
Early diagnosis of CS is important to ensure adequate follow-up given the risk of developing malignant tumors such as breast cancer (65%), thyroid cancer (3-10%) and endometrial cancer (5-10%). In addition, a family genetic study should be performed due to the high penetrance of the disease.
After diagnosis, the patient is monitored by a multidisciplinary team of endocrinology, oncology, genetics and dermatology.

Molecular diagnostics

P1734

FACIOSCAPULOHUMERAL DYSTROPHY TYPE 2 AND DIGENIC INHERITANCE

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BACKGROUND-AIM

We present a 61-year-old female patient with a history of parental consanguinity who presented progressive weakness. She reports loss of strength in her extremities. Furthermore, the weakness made it difficult for her to walk properly. An elevated creatine kinase (CK) level of >10.000 U/L (39-308) was note on serology. She maintains elevated CK levels around 2.500 U/L.

Facioscapulohumeral dystrophy (FSHD) (OMIM #158901) is an inherited progressive muscle pathology characterized by weakness and asymmetric muscle atrophy of face, shoulders, and lower extremities. It presents a prevalence of 1/100,000 inhabitants, high penetrance and variable expressivity. There are two types, FSHD1 and FSHD2, with same signs and symptoms, but differ in their genetic cause and frequency, 95% and 4%, respectively. In FSHD2, the alterations are due to changes in a region D4Z4 from long arm of chromosome 4. Usually, this region is hypermethylated, but the hypomethylation is associated with mutations in the SMCHD1 gene. D4Z4 hypomethylation favors DUX4 expression, which associated to the development of muscle diseases.

FSHD2 has an autosomal dominant digenic inheritance pattern, so it is necessary to present simultaneously hypomethylation of D4Z4 and the pathogenic variant of SMCHD1.

METHODS

We performed NGS based exome myopathies panel to identify pathological variants. These variants were confirmed by Sanger sequencing. An MS-MLPA study was performed to determine the methylation status of D4Z4.

RESULTS

An uncertain significance heterozygous variant was found in the SMCHD1 gene (c.5213C>T; p.Ala1738Val). The methylation status of D4Z4 is analysed, observing 22.8% methylation, FSHD2 (<25% methylation levels). Both findings are compatible with FSHD2.

CONCLUSIONS

Pathologies based on digenic inheritance are not very common and have a complex diagnosis, so a high index of diagnostic suspicion is essential.

CK levels tend to be elevated in patients with FSHD, but only molecular analysis will confirm the diagnosis. One of the most striking features in this case was the persistently elevated CK. In these diseases, CK levels do not usually exceed 500U/L in repeated measurements, so these results, together with the predominant weakness in lower extremity muscles, led to the suspicion of muscular dystrophy.

Molecular diagnostics

P1736

BOSMA ARHINIA-MICROPHTALMIA SYNDROME ASSOCIATED WITH SMCHD1 MUTATION

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BACKGROUND-AIM

Bosma arhinia-microphthalmia syndrome (BAMS,MIM#603457) is an extremely rare and striking condition, (prevalence: <1/1.000.000), with fewer than 50 cases reported thus far. Arhinia is variably associated with absent sinuses, hypertelorism, microphthalmia and nasolacrimal duct abnormalities. In its most severe presentation, it is associated with reproductive defects.

We report on a novel case of BAMS, the proband was a 33-years-old male patient, son of a non-consanguineous couple, from a normal pregnancy who was born with arhinia, left microphthalmia, and choanal agenesis. In addition, he lacks secondary sexual characteristics, suffers delayed puberty and impotence. He is referred to a molecular study. Furthermore, a hormonal study is requested.

METHODS

Clinical exome sequencing was used (Sophia-Genetics, 4572-genes) to search for BAMS causing variants. Sanger sequencing was performed for variant validation.

RESULTS

Molecular analysis revealed a likely pathogenic heterozygous missense variant in the SMCHD1-(NM_015295) gene:c.400G>T;p.Ala134Ser, associated with BAMS.

Regarding the hormonal study, total-testosterone was at 0.2µg/L(2.4-8.7), with free-testosterone at 1.1ng/L (7.0-40.0). LH of <0.1UI/L (0.6-12.0), FSH of <0.1UI/L (1.0-12.0), PSA of <0.07µg/L (0.1-3.5) a pattern consistent with hypogonadotrophic hypogonadism. GH, IGF1, Prolactin, TSH, and cortisol were normal. One of the consequences of hypogonadotrophic hypogonadism is reduced bone density with the result of fractures. Bone densiometry scan showed a low Z-score in lumbar region of -1.8.

After the diagnosis, he was prescribed Testogel. Subsequent analyzes showed an improvement in free-testosterone (22.9ng/L) and total-testosterone (6.1ng/L) levels, with the consequent improvement in bone density.

CONCLUSIONS

Given the clinical suspicion of a rare disease it is important to carry out a correct molecular diagnosis to identify the specific disease, to describe new variants or to support the relevance of already described variants. This information is necessary to give a correct genetic advice, diagnosis as well as to design the best treatment to improve his quality of life. Ultimately, this will help patients diagnosed with BAMS to have an understanding of their diagnosis as well as expected treatments and future complications.

Molecular diagnostics

P1737

VDR GENE POLYMORPHISMS IN CHILDREN WITH GROWTH HORMONE DEFICIENCY

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BACKGROUND-AIM

Growth Hormone Deficiency (GHD) is a rare disease defined by a total or partial impairment of growth hormone's production. However, vitamin D deficiency is frequent and may be associated with numerous pathologies. Few studies have investigated the association between GHD and vitamin D deficiency. The aim of this study is to analyse VDR gene polymorphisms related to vitamin D status in order to assure a better care for GHD patients.

METHODS

A case control study was conducted at Children's hospital of Tunis in collaboration of Farhat Hached's hospital of Sousse, included 39 GHD patients and 48 healthy subjects. Genetic analysis of VDR gene polymorphisms (BsmI, ApaI, TaqI, FokI and Tru9I) was performed using PCR-RFLP technique. Haplotype was analysed using Haploview software and statistical analysis was accomplished using SPSS.

RESULTS

Our study showed significant difference between patients and healthy subjects in vitamin D concentration (p=0,049) and in calcium concentration which was lower in the GHD group (p=0,018). The comparison of the allelic and genotypic frequencies of the five polymorphisms revealed one association between FokI polymorphism and GHD. A significant difference was established in patients between ApaI genotypes and PTH (p=0,019) and ALP (p=0,035). FokI genotypes were associated with phosphore (p=0,021). One haplotype CTAGT was significally different between patients and healthy subjects (p=0,002).

CONCLUSIONS

Our study showed that hypovitaminosis D is frequent in GHD patients even when treated with rhGH, which highlight the importance of vitamin D supplementation during treatment. More studies need to be conducted to evaluate the implication of the haplotype CTAGT in both vitamin D status and pathology.

Molecular diagnostics

P1738

MILD PHENOTYPE IN CYSTIC FIBROSIS PATIENT BEARING CFTR COMPLEX ALLELES

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BACKGROUND-AIM

Cystic fibrosis (CF) is a rare genetic multisystemic disease. To date, more than 2000 mutations have been reported in Cystic Fibrosis Transmembrane Conductance Regulator gene (CFTR), determining different degrees of CFTR dysfunction and different cystic fibrosis phenotypes. In the present study, we report the extremely rare complex genotype F508del-TG12T5/S977F-TG12T5 in cystic fibrosis Tunisian patient with a mild phenotype.

METHODS

Our study focused on an 8-month-old infant with cystic fibrosis from a non consanguineous marriage. The sweat test was carried out by pilocarpine iontophoresis (Exsudose technique). Molecular study of the CFTR gene was determined by direct sequencing of all 27 coding exons and their intron-exon junctions.

RESULTS

Our patient is the first male infant of a Tunisian couple. Mild respiratory manifestations were observed since the age of 3 months without digestive involvement. CF was suspected and a sweat test was recommended. An intermediate chlorides levels were obtained (48 and 62 mmol/L) .
Molecular study allowed us to identify for the first time in Tunisia the rare complex allele S977F-TG12T5 in association with F508del-TG12T5. A previous study also showed the association of S977F-TG12T5 in trans with F508del responsible for a less severe phenotype of cystic fibrosis.

CONCLUSIONS

The characterization of these complex alleles shows their impact on the cystic fibrosis phenotype. These findings will be important in the development of new therapeutic approaches in patients with cystic fibrosis. Functional studies and bioinformatics analysis of these complex allelic help to better understand genotype/phenotype correlation.

Molecular diagnostics

P1739

CIRCULATING TUMOR DNA AS A PREDICTIVE AND PROGNOSTIC BIOMARKER FOR BRCA NON-MUTATED PATIENTS WITH PLATINUM-SENSITIVE RECURRENT OVARIAN CANCER RECEIVING TRIPLET MAINTENANCE THERAPY (OPEB-01)

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BACKGROUND-AIM

OPEB-01 study aims to assess the efficacy and safety of triplet maintenance therapy (olaparib, pembrolizumab, and bevacizumab) in BRCA non-mutated patients with platinum-sensitive recurrent ovarian cancer (NCT04361370). Our objective was to assess the feasibility of serially collected circulating tumor DNA (ctDNA) as a predictive and prognostic biomarker in this patient cohort.

METHODS

Peripheral blood samples of patients were collected every 3 months during the triplet maintenance. Cell-free DNA extracted from patients' plasma was target-enriched with a panel with 112 cancer-related genes, and was sequenced using the NovaSeq6000 System (Illumina, CA, USA). The data was analyzed using our in-house Next-Generation Sequencing (NGS) algorithm.

RESULTS

Among 44 patients enrolled, serially collected ctDNA samples were available from 34 patients, amounting to a total of 288 samples. At data cutoff, nine patients have progressed (PD). Analysis of pre-treatment ctDNA showed that the detection rate of tier 1 or 2 somatic variants was 88.9% (8/9) in patients who have progressed and 52.0% (13/25) in patients who are still on the maintenance therapy. The detection rate of significant variants that were matched with tumor mutations identified in tissue NGS results, was 77.8% (7/9) in PD patients. In five patients out of the seven patients with PD, tissue-matched variants were detected several months earlier than the PD date based on imaging. The median lead time was 6.4 months, ranging from 3.6 to 10.8 months. TP53 was the most frequently modified gene among those patients, followed by KRAS, CDK12, RB1, RAD52, NOTCH4.

CONCLUSIONS

Analysis of ctDNA in recurrent OC patients can be useful in monitoring therapeutic response and early detection of recurrence. Updated data from additional analysis of ctDNA profile will be shared at the meeting.

Molecular diagnostics

P1740

EVALUATION OF GENE EXPRESSIONS OF ER STRESS MARKERS ATF6, IRE1 AND PERK IN NEUTROPHILS AS A BIOMARKER IN THE TREATMENT OF RELAPSING-REMITTING MULTIPLE SCLEROSIS ATTACKS

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BACKGROUND-AIM

Multiple sclerosis (MS) is a chronic neurodegenerative disease of central nervous system (CNS) associated with uncontrolled inflammation and autoimmunity, caused by the attack of autoreactive T cells to myelin. Most common type is relapsing remitting MS (RRMS) with periods of recovery and recurrence. Corticosteroids are administered to relapsed patients. Currently used biomarkers don't correlate strongly with treatment efficacy. Therefore, it is necessary to find molecular biomarkers that reflect therapeutic efficacy and that can be measured in blood. ER stress occurs when misfolded proteins accumulate in the ER lumen. Biopsy samples and post-mortem brain tissues of MS patients showed increased ER stress. According to recent research, neutrophils have an important role in MS and may be more pathogenic under ER stress. The unfolded protein response (UPR) is initiated by three ER proteins: IRE1, PERK, and ATF6. When ER stress can be relieved by the UPR, the cell survives. If ER stress cannot be alleviated, the UPR triggers the apoptosis process. ER stress in neutrophils has never been studied before in MS. The aim of this study was to investigate the IRE1, PERK and ATF6 gene expressions in neutrophils as biomarkers that can be used to examine the therapeutic efficacy of corticosteroids during and after relapse.

METHODS

For this purpose, whole blood samples were collected from 52 healthy controls, and RRMS patients during an MS attack (relapse) before corticosteroid treatment (BT) (n=10), after treatment (AT) (n=9), and 1 month after the treatment (1M) (n=10), by Neurology Clinics, Ankara City Hospital, Ankara, Turkey. Neutrophils were isolated from these samples using the gradient centrifugation separation, and RNAs were isolated from them. cDNAs were synthesized from these RNAs and used in qRTPCR studies.

RESULTS

According to the fold change analyses, significant differences were observed on BT group vs. controls for PERK (P=0.042) and for IRE1 (P=0.017). PERK and IRE1 expression were significantly lower in the BT group than in controls, but this difference disappeared after treatment. There was no significant difference for ATF6.

CONCLUSIONS

These are the preliminary results of an ongoing study.
Acknowledgment: This study was supported by TUBITAK (218S578)

Molecular diagnostics

P1741

USING BIOFIRE FILMARRAY MENINGITIS/ENCEPHALITIS (ME) PANEL TO SURVEY CENTRAL NERVOUS SYSTEM INFECTIONS IN TAIWAN MEDICAL CENTER

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BACKGROUND-AIM

Patients with suspected meningitis are treated empirically by pending diagnostic results, which means lengthy hospitalization and unnecessary antimicrobial dosage might increase the overall medical cost. CSF culture, as the organism identification standard procedure, may take >48 hours to reveal the conclusion report. Because of the long turn-around time, 70-85% of patients suffered from bacterial meningitis have not received promptly antimicrobial therapy in advance. Therefore, rapid diagnostic tests, to determine the bacterial etiology of meningitis, should be seriously taken into consideration.
To instantly identify the pathogens from central nervous system infections by Biofire ME Panel test, we collected and analyzed the pathogen distribution on meningitis/Encephalitis surveillance to know better about the efficacy of the panel test.

METHODS

Meningitis/Encephalitis (ME) system is a multiplex polymerase chain reaction (PCR) technology which simultaneously amplifies nucleic acids from multiple targets in single biochemical reaction including six bacteria, seven viruses and one yeast. BioFire ME Panel, in May 2020, was introduced in China Medical University Hospital to provide short TAT laboratory diagnostic service. Afterwards, from May 2021 to October 2022, we retrospectively study multiple respiratory pathogens in lumber-punctured CSF specimens of CNS infection.

RESULTS

From May 2021 to October 2022, we retrospectively collected 778 lumbar puncture cerebrospinal Fluid (CSF) samples of central nervous system infection for pathogen detection by BioFire ME Panel. According to the results, 59 (7.58%) were tested any of the panel target pathogens positive, among which 2 specimens were found two pathogens co-infection , HSV-2+E. coli and HSV-1+CMV.

CONCLUSIONS

Laboratory turn-around time to clinical diagnosis is critical against bacterial meningitis, which could be fatal to patients in 24 to 48 hours. Punctual in-time treatments based on rapid identification of the pathogen as bacterial, viral, or yeast could be crucial to healthcare. BioFire ME Panel is a rapid method to detect the pathogens of central nervous system infections , to improve diagnosis rate of viral infection, and to shorten hospitalization and further decrease antimicrobial therapy expense.

Molecular diagnostics

P1742

APO E GENETYPE DISTRIBUTION IN HEALTHY ADULT WORKING AT THE UNIVERSITY COLLEGE HOSPITAL, IBADAN.

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BACKGROUND-AIM

Apo E genotype is a widely studied single nucleotide polymorphism and it plays a central role in lipid metabolism, however few studies have been done on its genotypic polymorphism influence in African population. This study investigated the distribution of Apo E genotype among healthy adults in University College Hospital, Ibadan. Nigeria.

METHODS

This cross-sectional study was conducted amongst 128 randomly selected apparently healthy members of staff between ages 19 to 62 years at the University College Hospital, Ibadan, Nigeria, amongst 128 apparently healthy members of staff between ages 19 to 62 years. DNA was extracted from leucocytes using EDTA blood. Apo E genotypes were determined using the Seeplex Apo E ACE genotyping kit.

RESULTS

Majority (53.13%).of the study participants had Apo E3/E3 followed by Apo E3/E4 (21.09%), E2/3(14.84%), and Apo E4/E4 (4.69%) while Apo E2/E2 and E2/E4 had equal distribution of 3.13%.

CONCLUSIONS

The Apo E genotype E3/E3 was observed to be the most common genotype.

Molecular diagnostics

P1743

CLINICAL AND GENETIC CHARACTERIZATION OF FAMILIAL MEDITERRANEAN FEVER AMONG OF GEORGIAN PATIENTS

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BACKGROUND-AIM

Familial Mediterranean Fever (FMF) is an autosomal recessive hereditary disease, characterized by recurrent attacks of fever, serositis and arthritis, which primarily affects non-Ashkenasi Jews, Armenians, Arabs and Turks. A small number of FMF cases are also described in other ethnic groups. Very few data are available on the presence and genetic spectrum of FMF in Georgian patients. The purpose of our study was to find FMF cases in ethnically Georgian patients through genetic testing; to investigate distribution of FMF gene mutation in this ethnic group and to compare mutation distribution in Georgians with population at risk (Jews, Armenians, Arabs and Turks).

METHODS

220 patients from ethnical Georgians, with clinically suspected diagnosis of FMF, mean age 23.19 year (0-73 year), 121 male, 99 female, underwent molecular genetic studies using polymerase chain reaction. Genetic study was performed in Bernhard-Nocht-Institut for Tropical Medicine and in diagnosticum Zentrum für Humangenetik. We also registered clinical manifestations, severity of disease, treatment and its efficacy (using standardized questionnaire) and correlated them with mutation.

RESULTS

MEFV gene mutations were found in 156 patients. The M694V Mutation was predominant. Distribution of mutations are: M694V – 104 (66.7%), M68OJ/M694V - 9 (5,7%), M694V/R761H - 5 (3,2%), M694V/E148Q - 4 (2,6%) , V726A/ E167D-2 (1,3%) ,other mutations - 32 (20,5%). 75 patients (48,1%) were Homozygous M694V/ M694V, 56 (35,9%) - were Heterozygous, 25 (16.0%) were compound heterozygous for M694V and other mutation. Family history of FMF was positive only in 14(12,8%) cases. Frequency of clinical symptoms: fever in 99 patients (90,7%), abdominal pain in 91 (83,6%), abdominal operation in 39 (35,8%), arthralgia in 63 (58,3%). Renal function was deteriorated in 11 (10,1%) cases, 1 patient (0,9%) was on hemodialysis, renal biopsy (RB) was done in 3 (2,8%) cases. Treatment with colchicine was performed in 42 cases (38,5%).

CONCLUSIONS

FMF is present in ethnical Georgians. Most frequent mutation is M694V. Distribution of mutations is more similar to north African Jews and differs from Armenians in Armenia and Turks.

Molecular diagnostics

P1744

ASSOCIATION OF INTERLEUKIN-22 TRANSMEMBRANE RECEPTOR AND BINDING PROTEIN WITH ITS LEVELS IN TUBERCULOSIS

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BACKGROUND-AIM

Around 40% of Indians harbor Tuberculosis (TB) infection in their body and around 10% develop an active infection. T helper cells provide the primary host response which decides whether the infection remains latent or becomes active. However, the entire cluster of host protective factors are not yet fully understood. Many recent studies have suggested the protective role of interleukin-22 (IL-22) in TB patients. It helps in cellular regeneration and secretes antimicrobial substances. IL-22 action is modulated at cellular level with the help of its transmembrane receptor and blocked by soluble binding protein (IL-22R1 and IL-22R2 respectively). The present study aims to determine their gene expression levels in the peripheral blood of TB patients and compare to healthy individuals.

METHODS

170 subjects were recruited in the study after taking due informed consent - 85 sputum positive TB patients as cases and 85 asymptomatic healthy individuals as controls. Venipuncture was done by trained phlebotomists to collect 5mL whole blood in plain and EDTA vacutainers. Serum IL-22 levels were estimated using enzyme linked immunosorbent assay. Total RNA was isolated from whole blood and converted to cDNA. Gene expression analysis of IL-22R1 and IL-22R2 was done using real time PCR. Statistical analysis was performed using SPSS.

RESULTS

Serum IL-22 was significantly lower in TB patients with a median (IQR) of 18.55 (5.08) pg/mL, compared to healthy controls with 49.38 (162.88) pg/mL ($p<0.0001$). IL-22R1 was significantly upregulated and IL-22R2 downregulated with 2.01- and 0.79-fold change ($p<0.0001$ and 0.04) respectively. IL-22R1 showed a significant positive correlation and IL-22R2 showed a negative correlation with IL-22 levels. On ROC analysis, IL-22 discriminated TB patients from healthy controls with an AUC of 0.9.

CONCLUSIONS

IL-22 levels were found to be significantly decreased in TB. Upregulation of IL-22R1 and downregulation of IL-22R2 may be a host mechanism to combat the infection, as there is growing evidence that IL-22 can modulate mycobacterial growth. This is also the first study to check for diagnostic efficiency of IL-22 and was found to be promising with a high sensitivity and specificity in discriminating TB individuals from healthy people.

Molecular diagnostics

P1745

MUTATIONAL SPECTRUM OF THE CFTR GENE IN A GREEK (CRETAN) SUBPOPULATION

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BACKGROUND-AIM

Cystic fibrosis (CF), a common monogenic disease, is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The distribution and frequency of CFTR variants fluctuate in different countries and ethnic groups. The spectrum of pathogenic variants of the CFTR gene has not been extensively studied in Greek subpopulations, previously. Therefore, in the present study, we aim to assess the population frequency of CFTR gene mutations in the samples of healthy unrelated individuals from the populations of Crete.

METHODS

For this purpose, 3504 individuals lived in Crete, were studied for common mutations using an assay which identifies 50 mutations in total and also analyses the intron 8 polyT tract with accurate measurement of the adjacent TG repeat.

RESULTS

A total of 14 different CFTR mutations, already reported as cystic fibrosis (CF) or CFTR related disorders variants, were detected. High frequencies of ΔF508 (49.4%), R334W (13.8%) and 2789+5G>A (10.3%) were found. D1152H and W1282X were also frequent mutations (6.9 and 4.6%). Three mutations were expressed in a percentage of 3.4% and the rest 6 mutations identified were expressed in a rate of 1.1%

CONCLUSIONS

A wide spectrum of CFTR variants was identified, confirming the highest CFTR allelic heterogeneity previously reported in Mediterranean area. Additionally, better knowledge about the CFTR sequence variation spectrum may contribute to more efficient genetic testing in the Cretan subpopulation.

Molecular diagnostics

P1746

EVALUATION OF OPTIMIZED SAMPLE PREPARATION FOR SIMPLEXATM C. DIFFICILE DIRECT PCR ASSAY

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BACKGROUND-AIM

Fast and reliable detection of infections with Clostridioides difficile (C. diff) is crucial to prevent outbreaks in hospitals and to allow adequate and fast patient treatment. According to the European Guideline immunoassays can be used for the detection of C. diff. associated diarrhea. Those assays detect Glutamatdehydrogenase (GDH) resp. Toxin A and B and are used in daily lab Routine. In case of discrepant immunoassay results confirmatory PCR testing is recommended. The aim of this study was to establish a workflow to perform the full C. diff diagnostic pathway including immuoassays and PCR by using only one stool sample extraction.

METHODS

Retrospectively, 25 left over samples with positive C. diff result and 5 samples with negative C. diff result were collected. All samples were pre-tested with immunoassays (LIAISON® C. difficile GDH and LIAISON® C. difficile Toxin A/B) as well as the sample-to-answer PCR device LIAISON® MDx using the SimplexaTM C. difficile Direct assay. During this study two PCR measurements were conducted in parallel. The first one was carried out according to the instruction of use, using the Sample Prep Kit for the stool sample extraction. For the second one 200 µl of extraction dilution from the immunoassays was pipetted into the Sample Prep Kit buffer to avoid the handling with the native stool sample. The qualitative results of both runs were compared. Percentage of positive and negative agreement (PPA and NPA) as well as correlation between CT values was obtained.

RESULTS

The results achieved with the workflow stated in the IFU and the optimized one showed qualitatively a 100% agreement. The fluctuations of the CT-values were neglectable.

CONCLUSIONS

This study showed that for the combined use of the LIAISON® C. difficile GDH, LIAISON® C. difficile Toxin A/B and the SimplexaTM C. difficile Direct assays one stool sample extraction is sufficient. 200 µl of the stool sample extraction prepared for the immunoassays can be transferred to the PCR buffer. With mixture can then be used for the PCR diagnostics using the LIAISON® MDx.

Molecular diagnostics

P1747

NEWLY IDENTIFIED ACVRL1 PATHOGENIC GENE VARIANT CAUSING HEREDITARY HEMORRHAGIC TELANGIECTASIA (HHT). A SIX-GENERATION FAMILY STUDY.

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BACKGROUND-AIM

Hereditary Hemorrhagic Telangiectasia (HHT), also known as Rendu-Osler-Weber syndrome, is a rare autosomal dominant disorder characterized by arteriovenous malformations due to defects in angiogenesis. Clinical manifestations such as epistaxis, skin/mucose telangiectases and a positive family history are enough for diagnosis, which can be genetically confirmed. In the Mediterranean area, HHT2 is the most common subtype, mainly affecting the ACVRL1 gene. We present a clinical and genetic family study covering six generations affected by HHT2.

METHODS

A 55 year-old male was clinically identified as index case. A family tree was completed includying six generations with clinically affected members. The three youngest generations were selected for a comprehensive disease assessment and genetic testing.
13 whole blood samples were obtained. A targeting exome analysis was performed covering five genes associated with HHT (ACVRL1, BMPR2, ENG, GDF2, SMAD4). Only changes with a number of readings >20x and a frequency >30% were considered variants. Detected variants were confirmed by Sanger sequencing, described according to the reference sequence (HGMD database), and contrasted with different databases and in silico prediction tools.

RESULTS

The proband was found to be heterozygous for the variant c.752_772+2dup in the ACVRL1 gene, consisting of a 23 nucleotide duplication in intron 6 splice donor site.
Four of his relatives – one uncle and his daughter, one sister and one of his own daughters – were positive for the same variant, coming from his paternal line.
Amongst the positives, 3/5 have presented with recurrent epistaxis between the 2nd and the 5th decade of life, whereas 2/5 keep asymptomatic to date (being now 48 and 29 years old). None of the negatives or their descendants have shown any bleeding symptoms.

CONCLUSIONS

This ACVRL1 variant has not been previously described, however the intronic variant c.772+3_772+4dupAA has been reported as pathogenic. Furthermore, its location in the splice donor site could have an effect on ARNm processing. Altoghether, and considering that the clinical manifestations of the carriers are consistent with an HHT phenotype, we conclude that the new variant could be classified as pathogenic.

Molecular diagnostics

P1748

MITOCHONDRIAL DNA DISORDERS: A CASE REPORT

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BACKGROUND-AIM

Mitochondrial diseases do not follow the rules of mendelian inheritance, but a maternal inheritance pattern since only females can transmit them to their offspring. Moreover, their clinical manifestations are conditioned by the phenomena of heteroplasmy, threshold effect and mitotic segregation. When the sequence of all these mitochondrial DNA molecules is identical, it is known as homoplasmy.

METHODS

7-year-old boy with epilepsy, obesity, hypotonia and language delay, who was admitted to the emergency department for confusion, fatigue, hypertension, urinary retention, tendency to sleep and respiratory distress. A blood test was performed showing significant hypomagnesemia, lactic acidosis and elevated myocardial damage enzymes, so an electrocardiogram was performed, showing left ventricular hypertrophy. Lesions suggestive of thiamine deficiency were observed in a brain magnetic resonance imaging. Due to this, exome sequencing targeted to mitochondrial disease and thiamine transporter defects was requested. Three weeks after admission, the patient died of sudden cardiac arrest that did not respond to resuscitation.

RESULTS

The presence of mutations in nuclear DNA related to mitochondrial diseases is ruled out, however, it is identified the presence in homoplasmia of the variant of uncertain significance m.4290T>C in the MT-TI tRNA has been identified. If the pathogenicity of the variant is confirmed, this result would be compatible with a genetic diagnosis in the patient of Encephalopathy, with a mitochondrial inheritance pattern. This variant is associated with cardiomyopathy, encephalopathy, mitochondrial complex I and IV deficiency, hypomagnesemia, hypertension and hypercholesterolemia.

CONCLUSIONS

A study of the mother of the variant found is carried out and, depending on the results, the study will be extended to the maternal family. It is important to emphasize the importance of knowing when a mutation occurs in nuclear DNA or mitochondrial DNA, as this will determine the type of inheritance. Knowing the type of inheritance will allow an adequate genetic counseling.

Molecular diagnostics

P1749

EXOMA AND CGH ARRAY ARE COMPLEMENTARY AND HELP TO UNDERSTAND COMPLEX NEUROLOGICAL DISEASES CASE REPORT

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BACKGROUND-AIM

CGH array is a method used to analyze the genome, able to detect gains or losses of genetic material in the DNA sequence called CNVs (Copy Number Variants).
We present the case of a 2-year-old girl with a normal prenatal history and healthy parents with a clinical picture of chronic cerebellar ataxia, mild global hypotonia, psychomotor retardation predominantly motor and language, repetitive stereotyped movements, slow eye movements, episodes of self-absorption and gait instability.
The variants detected in array CGH were not enough to explain her clinical picture. The study was extended to the whole exome.

METHODS

Exome: DNA was extracted automatically and amplified using Twist technology, including the 21,285-gene exome for sequencing on the Illumina NextSeq 1000TM Sequencing System.
Genomic array: SNPS/CNVS Cytoscan® HD Affymetrix commercial standard genomic array was performed. Analysis software used: Affymetrix Chromosome Analysis Suite (ChAS) v.4.2.1. Reference assembly: Hg_38.

RESULTS

1) Variants of pathological significance detected by CGH array:
- Gain arr[GRCh38]16p13.11(15,353,154_16,219,095)x3, 866 kbp
- Gain Mosaic GRCh38]16p13.13p12.3(12,305,467_21,167,052)x2~3
8,862 kpb.
2) Variants detected in Complete Exome (which would justify cerebellar ataxia):
- POLR3A (NM_007055), c.896C>A; p. (Ser299*). VUS, AR (Likely pathogenic) and POLR3A (NM_007055), c.1431+42T>C. rs182783920. VUS, AR.
- CACNA1A (NM_000068), c.2005G>A; p. (Asp669Asn), VUS, AD.
- EIF2AK2 (NM_001135651), c.688-3T>C. rs374454967, VUS, AD
- SPEN (NM_015001) c.194C>T; p. (Ser65Leu). rs749477752. VUS, AD.
VUS: Variant of uncertain significance. AD: Autosomal dominant. AR: Autosomal recessive.

CONCLUSIONS

1. The results obtained with CGH array partially explained the clinical signs. The exome gives us another partial view of the possible cause of the disease, with the sum of the considered causes being the best option.
2. Our results suggest the clinical usefulness of the joint use of exome and CGH array in complex cases, being complementary the use of both techniques to elucidate the multifactorial causes of complex disease.

Molecular diagnostics

P1750

PREVALENCE OF DISTRIBUTION OF 21 HUMAN PAPILLOMAVIRUS GENOTYPES IN A GROUP PATIENTS IN ALBANIAN POPULATION

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BACKGROUND-AIM

Human Papillomavirus is one of the most common sexually transmitted infections (STIs). It belongs to the Papillomaviridae family and among 200 types are discovered. Based on the high oncological potential due to association with cervical cancer or precancerous lesions, 30-40 genotypes are divided into HR-high risk genotypes that cause cervical cancer neoplasia and LR-low risk genotypes which cause mild dysplasia. Our aim was to determine the most frequent genotypes of HPV and the investigation for the multinfection or co–infection in order to help the limited database available of HPV prevalence in Albanian population.

METHODS

Manual Genomic Column DNA Express extraction REF K-1-1/E Sacace Biotechnologies was used to determine genotypes, while HPV Genotype 21 Real – TM Quant REF V21-100FRT detection was used for amplification. Data analysis were performed with IBM SPSS Statistics 26 package.

RESULTS

In this study 365 patients coming from 6 regions of Albania were screened for 21 genotypes of HPV in Genius LAB. The region of Tirana represents 65.9% of all patients screened. The prevalence of positive cases was 55.3%, whereas high-risk (HR) HPV infection rate was 84.61%, Low-risk (LR) HPV infection rate was 26.92% and mix HR and LR-HPV infection rate were 15.38%. The overall prevalence of HPV is high in the age group between 16 – 30 years old, which presents 63.5% of positive cases. The most common genotype was 53 followed by 31 and 44 genotypes, the infection ratio was 13.3%, 11.1% and 11.1% respectively. The prevalence of multi-infection cases represents 38.46% of all positive cases, where HR co-infection was 34.6%. The dual infection was the most common infection among multinfection, the 53- 66 genotype co-infection was the most common infection with a rate 15.3% of all positive cases.

CONCLUSIONS

Infection at a young age and the appearance of carcinoma at a late age shows the importance of early diagnosis with molecular diagnosis to prevent the development of cervical cancer.

Molecular diagnostics

P1751

ASSOCIATION OF SAMAD7,BMP, POU5F1P1 GENE POLYMORPHISMS WITH COLORECTAL CANCER IN TUNISIAN POPULATION

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BACKGROUND-AIM

Colorectal cancer (CCR) is a disease with high incidence and is characterized by a long preclinical stage, with a progression from early adenoma to invasive cancer taking years. These characteristics of CCR make it more suitable for genetics population screening than any other malignancy. That's why we target polymorphisms of genes coding for principal actors implicated in its carcinogenesis ;SAMAD7, BMP and POU5F1P1. So Taking into account our Mediterranean dietary intake and other potential confounding non genetics risk factors, our aim wasto study the association of five polymorphismsof these three genes with CCR.

METHODS

This case-control study involved 64 patients and 59 controls recruited from the CHU Sahloul Sousse gastroenterology service.The genotyping was carried out by PCR-RFLP for RS6983267 (POU5F1P1), rs961253 (BMP), rs1957636 (BMP), rs10795668 (ATP5C1), and PCR-t-ARMS for RS4939827 (SMAD7). Statistical analysis was performed using SPSS 20.

RESULTS

Among non genetics factors,age, sex and the consummation of dark chocolate, vegetables, blue fish and fig were used to adjust the association between our five polymorphisms and the CRC according to the dominant or recessive model. Using the dominant model, we found that carrying the variant allele of rs4939827, rs6983267, rs961253 multiply the risk of developing CRC by 3.8 (p=0,01), 2.8 (p=0,03), 2.6 (p=0,03)respectively. For the rs1957636 when applying the recessive model, we noted that carrying the homozygous variant allele multiply the risk of developing CRC by 2.8 (p=0,02). These associations may be explained by the role of SAMAD7, BMP and POU5F1P1.In fact SAMAD7 is an antagonist of the transforming growth factor beta, which controls proliferation, differentiation, and other functions in many cell types, BMP is a Wnt pathway inhibitor that regulates crucial aspects of cell fate determination, cell migration, cell polarity, neural patterning and organogenesis during embryonic development.

CONCLUSIONS

Among the five studied polymorphisms rs961253, rs1957636,rs4939827 and rs6983267 seem to be associated with CRC. So these polymorphisms can be suggested to be introduced in genetics biomarkers panel for early diagnostic.

Molecular diagnostics

P1752

ANOMALY OF SEXUAL DIFFERENTIATION, A CASE REPORT.

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BACKGROUND-AIM

BACKGROUND: The patient is a 29-year-old man who consults for infertility after several attempts to have a child and is unable to do so. Hormonal analysis shows serum levels of FSH 46.6 mIU/mL; LH 30.2 mIU/mL; Testosterone 180ng/dL and SHBG 13 nmol/L, suggesting infertility of primary origin and testicular dysfunction. Seminogram: complete azoospermia. Normal seminal volume.
Examination: Patient with younger appearance with respect to his age, absence of facial hair. Broad hip constitution. Thin hands with hardly any musculature. Penis of normal appearance. Hypoplastic scrotum. Atrophic left testicle of small size, the right testicle is not located in the inguinal canal or abdominal level.
The patient underwent gynecomastia surgery in adolescence in both breasts due to a possible genicomastia. Suspected diagnosis: possible incomplete true hermaphroditism.

METHODS

A peripheral blood karyotype and a study of Y chromosome microdeletions (AZFa, AZFb, AZFc gene) were performed.

RESULTS

DIAGNOSIS: Karyotype according to ISCN 2020 with chromosomal resolution of 500 bands: chromosomal formula: 46.XX. In the cytogenetic study performed a discordance between the reported gender and the chromosomal sex was detected.
According to the clinical information reported, the patient presents an alteration in the development of the external genitalia (left testicle and absence of right testicle) which is compatible with an anomaly in sexual differentiation (ADS) which is further supported by the karyotype study (male 46,XX) and by the analysis of chromosome microdeletions which confirms the absence of the Y chromosome (AZFa, AZFb, AZFc gene) and of the SRY gene

CONCLUSIONS

ASD is considered a rare disease (approximately 1/20,000), and according to the literature, 10% of XX males are SRY negative, like the present case, present hypospadias, ambiguous or undescended genitalia, infertility, gynecomastia and different degrees of inadequate virilization of the external genitalia.
The patient is currently on testosterone treatment, was counseled about his desire to have children, and is in treatment with several specialists for his disease.

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Molecular diagnostics

P1753

MOLECULAR STUDIES OF TCF4 GENE AND CORRELATION WITH LATE-ONSET FUCHS ENDOTHELIAL CORNEAL DYSTROPHY IN THE GREEK POPULATION

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BACKGROUND-AIM

Late-onset Fuchs' endothelial corneal dystrophy (FECD) is a hereditary, progressive, bilateral and irreversible disorder that is characterized by thickening of Descemet's membrane, microscopic collagenous protuberances known as guttae and accelerated loss of corneal endothelial cells. Patients initially complain of blurred vision and as the disease progresses, painful epithelial edema develops. Untreated cases of FECD often result in blindness and the only treatment is corneal transplantation.

DNA polymorphisms in many genes have been implicated, among them TCF4 on chromosome 18q encoding a transcription factor protein E2-2, which is involved in regulating cellular growth and differentiation in the cornea. In our previous published study, we confirmed the association of an intronic TCF4 SNP (rs613872) with the disease in our population. The purpose of this present study is to investigate further into another intronic point of interest in the same gene, the CTG18.1 trinucleotide repeat expansion.

METHODS

DNA was isolated from EDTA blood from a well-ascertained group of 36 Greek patients with FECD (Krachmer scale ≥2) and 58 healthy individuals, age- and sex-matched after obtaining their informed consent. Triplet-repeat primed PCR (TP-PCR) and fragment analysis in an ABI SeqStudio genetic analyzer was performed along with our real-time qPCR genotyping for the SNP in the LightCycler (Roche). Statistical analysis of both genetic results was performed with SNPStats.

RESULTS

The TCF4 expansion method was validated adequately. An expanded allele was defined as having over 40 trinucleotide repeats. 20 out of 36 patients (56%) possessed at least one expanded allele compared to only 3 out of 58 healthy controls (5%, [odds ratio=17.62 (95% C.I.=1.68-7.59)]. The frequency of TCF4 risk G allele was increased to 40% in patients with FECD compared to 17% in healthy subjects [odds ratio=3.59 (95% C.I.=3.93-78.97)].

CONCLUSIONS

Since 58% of the FECD patients possessed a range of 2-4 risk alleles in both TCF4 loci, we conclude that TCF4 is strongly and statistically associated with late-onset FECD in a Greek population. The missing heritability could be attributed to other genes such as ZEB1, AGBL1, SLC4A11, LOXHD1 that deserve further attention in the future.

Molecular diagnostics

P1754

PREDICTIVE GENOMIC MEDICINE FOR HEAD & NECK CANCERS

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BACKGROUND-AIM

In the last year, the Aiom-Airtum data (www.aiom.it) estimate 9,300 new cases of head and neck cancers in Italy, of which 7,000 among men and 2,300 among women; the data identify the neoplasms of the upper aero-digestive regions, which do not include tumors of the salivary glands, those of the nasal and paranasal sinuses and, obviously, the thyroid for which, in consideration of the high incidence, a separate classification is used, even though it is part of the head and neck district. The tumors are confirmed to be more frequent in males between the ages of 50 and 69, with a highly declining mortality rate. In the majority of cases (over 90%) these are squamous cell carcinomas that develop from the epithelia that cover the mucous membranes of the implicated district. These types of tumors are not frequently hereditary, even if familial epidemiology has been found.

METHODS

A total of n=47 samples (15 females and 32 males) were selected from Head & Neck Surgery Unit, Istituto tumori Fondazione Pascale, Naples, Italy, and enrolled in the last couple of years. The samples were all subjected to molecular testing using a multigene panel of 58 genes related to general cancer predisposition, customized in our laboratory, including also BRCA1 and BRCA2. The strategy used involves an enrichment with specific probes (made for us by Agilent Technologies) and subsequent sequencing with the Illumina MiSeq platform. Data analysis was performed with the companion Alissa software.

RESULTS

We identified a total of five pathogenic mutations, annotated in clinical databases as ClinVar, in BRCA1 (two different variants), BRCA2, MUTYH and BRIP1 (corresponding to about 10% of our patients). The results obtained show that there is also a frequent predisposition for head and neck tumors similar to other types of neoplasia that are more studied (for example breast and ovarian cancers and colon cancer).

CONCLUSIONS

Therefore, predictive genomic medicine aimed at the early identification of germline mutations in head and neck neoplasia is of great importance for early tumor detection in risk cancer assets within affected families.

Molecular diagnostics

P1755

THE ROLE OF THE P-53 GENE AND THE P-53 PROTEIN IN NON-HODGKIN MALIGNANT LYMPHOMAS

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BACKGROUND-AIM

P-53 gene mutations are the most common genetic abnormalities of cancer. They have been extensively studied in various mature B-cell malignancies, including chronic lymphocytic leukemia (CLL). Identifying different p53 gene mutations is very important because these mutations have an impact on the patient's clinical course in CLL.

METHODS

A sandwich ELISA colorimetric quantitative method was used for direct detection of the p53 isoform protein, the product of gene p53: Specificity: human p53 protein (aa20-25); Format: Purified product: Monoclonal antibody clone: Isotype DO-1: IgG2a. The antibody is suitable for the techniques: ICC / IF and ELISA. The research antibody PAb 1620 has been reported to be specific for the conformation of the normal p-53 protein, and PAb 240 antibodies bind specifically to denatured p-53 protein. Compatible sample types: cell culture supernatants, plasma, serum; solid support: 96-well microplate; firm: Ray Biotech Life, Inc.

RESULTS

Laboratory examinations: Hemoleukogram with 5 Diff and cytological examination of the blood smear on the peripheral blood in the May Grunwald-Geimsa staining, and bone marrow puncture, BM with medullary forcing. The cases were classified as CLL with >5000 lymphocytes in absolute value, present at the cytological examination of the blood smear, from the peripheral blood or LLC with less than 10% prolymphocytes based on the peripheral blood smears May-Grunwald Giemsa, stained.
Immunophenotyping: The diagnosis of CLL was confirmed by immune phenotyping. All samples that entered the study were lymphocytes with positive CD19#, CD20##, CD5# and CD23# cell receptors. The CD38+ receptor was considered positive if the distinct lymphocytes of the population showed a higher intensity of staining than the granulocytes in the sample and was associated with the presence of protein ZAP-70.

CONCLUSIONS

In recent years, more attention has been paid to the importance of the p53-expressed protein in CLL, and a combination with low survival and non-response to classical conventional chemotherapy, due to mutations in the p53 gene, with progression to Richter Syndrome. Identifying different p53 gene mutations is very important because these mutations have an impact on patients' clinical course in CLL with the p53 protein mutant isoform.

Molecular diagnostics

P1756

EXPLORING THE POTENTIAL OF SERUM PERIOSTIN AS A PREDICTIVE BIOMARKER FOR EARLY-ONSET IDIOPATHIC PULMONARY FIBROSIS: A FOLLOW-UP STUDY

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BACKGROUND-AIM

The diagnostic value of periostin and Krebs von den Lungen-6 (KL-6) in idiopathic pulmonary fibrosis (IPF) has been reported, but the superiority of serum periostin or KL-6 as a biomarker in early-onset IPF is yet to be determined.

METHODS

A total of 51 IPF patients with anti-fibrotic therapy who underwent twice high-resolution computed tomography (HRCT) fibrosis scoring evaluation and 27 healthy controls were retrospectively enrolled from the First Affiliated Hospital of Guangzhou Medical University between January 2020 and May 2022. Serum levels of periostin and KL-6 by enzyme-linked immunosorbent assay (ELISA) and clinical diagnosis test were evaluated in both cohorts.

RESULTS

The levels of serum KL-6 levels in patients with initial diagnosis and with anti-fibrotic therapy decreased from 1680.71 ± 1842.60U/mL to 1263.25 ± 1488.19 U/mL (P<0.05), while serum periostin levels decreased from 73.92 ± 13.48pg/mL to 43.78 ± 15.52 pg/mL (P<0.001). In survival probability analysis, the combined performance of periostin-KL-6 was noteworthy compared with periostin and KL-6 alone (AUC: 0.894, 0.875, 0.639, respectively). Significant performances were observed between periostin levels and total fibrosis score < 100 than KL-6 in the stage of early-onset IPF (r: 0.2266 > 0.1118), while KL-6 showed a better when total fibrosis score > 100, namely in the later stage of IPF (r: 0.2197 > 0.1050).

CONCLUSIONS

Our findings indicate that serum periostin expression was more remarkable than KL-6 in the early-onset IPF diagnosis (Total fibrosis score < 100), and innovative HRCT fibrosis score stratification was a significant supplementation in UIP-IPF patients.

Molecular diagnostics

P1757

LOW-FREQUENCY ALLELE VARIANTS IN NGS MULTI-GENE PANELS FOR HEREDITARY CANCER TESTING: ARTIFACTS, CHIP OR MOSAICS? MANAGING THE RESULTS IN THE LABORATORY ROUTINE.

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BACKGROUND-AIM

Next-Generation Sequencing (NGS) gained greater importance and interest in hereditary tumours, and in particular breast, ovarian, and colon cancer syndromes. This technology increased the chance of detecting germline DNA mutations in cancer-related genes, guiding patients' management, and surveillance strategies in both patients and families. NGS-based multi-gene panels (MGP) are the methods of choice for the diagnosis of such syndromes, because they allow target sequencing of selected relevant genes, reaching high sequencing coverage and sensitivity. That means increasing the number of genetic variants with a variant allele frequency (VAF) lower than the predicted heterozygous threshold and therefore more unexpected genomic results with higher complexity in their interpretation.

METHODS

We investigated this phenomenon in patients who underwent a hereditary-cancer test with a MGP for hereditary cancer syndromes in our laboratory. The study examined the prevalence and characteristics of low-frequency genomic variants in 1896 patients who were tested for 22 cancer-related genes. We filtered the variants excluding those representing potential sequencing errors. Then we considered those mapping on target regions and not repeated among samples, so unique. Finally, we selected those clinically relevant.

RESULTS

A percentage equal to 0.47% of tested patients turned out to have a clinically relevant low VAF mutation. Additional tests on secondary tissues established that the most part of the patients had a blood-confined low VAF variant, representing an event of Clonal Haematopoiesis of Indeterminate Potential (CHIP). Fewer patients, instead, were identified with a pathogenic constitutional mosaicism. Moreover, cascade screening in their families, identified positive heterozygous offspring.

CONCLUSIONS

As mosaic patients and positive offspring can have access to proper clinical surveillance and management, the results obtained raised a flag on the importance of low VAF mutations analysis in NGS testing for hereditary cancer syndromes. Because there are no standard guidelines for the analysis of such variants in NGS diagnostic pipelines, we designed a new algorithm to investigate and confirm low VAF genetic variants that we put it into the diagnostic routine.

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Molecular diagnostics

P1758

INFREQUENT IGE SENSITIZATION PROFILE TO HOUSE DUST MITE MOLECULAR ALLERGENS

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BACKGROUND-AIM

Recent data from Eastern Europe revealed that in patients with allergic diseases the most prevalent IgE sensitization profiles to house dust mite (HDM) molecular allergens are sensitizations against group 2 allergens (Der p 2, Der f 2), group 1 and 2 allergens (Der p 1, Der f 1, Der p 2, Der f 2), group 1 and 2 allergens and Der p 23, group 1 and 2 allergens along with Der p 5, Der p 7 and Der p 23, and monosensitization to Der p 23

METHODS

Molecular IgE sensitization profiling was performed by a new generation macroarray ELISA-based multiplex immunoassay, used as a molecular allergy explorer to detect serum specific IgE against HDM allergenic recombinant molecules rDer p 1, rDer f 1, rDer p 2, rDer f 2, rDer p 5, rDer p 7, rDer p 10, rDer p 11, rDer p 20, rDer p 21, and rDer p 21. We searched in our database of IgE sensitization profiles to HDM molecular allergens the ones with a low frequency (less than 5%).

RESULTS

We report the case of a HDM IgE sensitization profile with concomitant serum specific IgE antibodies against cysteine proteases rDer p 1 (4.53 kUA/L) and rDer f 1 (0.4 kUA/L), MD-2-like proteins belonging to the NPC2 family rDer p 2 (10.08 kUA/L) and rDer f 2 (9.15 kUA/L), lipid binding protein rDer p 21 (5.6 kUA/L) and peritrophin-like protein rDer p 23 (3.52 kUA/L), but with no serum specific IgE antibodies detected against lipid binding proteins rDer p 5 and rDer p 7, tropomyosin rDer p 10, paramyosin rDer p 11 and arginine kinase rDer p 20 ($\leq$ 0.1 kUA/L).

CONCLUSIONS

Besides less frequent IgE sensitization profiles reported in Eastern Europe such as monosensitizations to Der p 5, Der p 7 and Der p 20, concominant sensitization to group 1 and 2 allergens together with Der p 7 and Der p 23 or with Der p 5, Der p 21 and Der p 23, we found a particular molecular HDM IgE sensitization profile that may influence the prescription and efficacy of allergen immunotherapy.

Molecular diagnostics

P1759

SEXUAL AMBIGUITY: A SINGLE CASE STUDY.

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BACKGROUND-AIM

Sexual ambiguity or disorder of sexual development is a discrepancy between the external and internal genitalia and the secondary sexual characteristics on the one hand, and on the other hand, a discrepancy between the genetic sex and the gonadal sex and this is a consequence of the SRY(Sex-determining Region on Y) gene carried on the Y chromosome.
The interest of our research is to describe the anatomical-clinical, paraclinical and therapeutic aspects of these sexual ambiguities and to search for the SRY gene in children and adults with this anomaly; using PCR(Polymerase Chain Reaction).

METHODS

This is a prospective and descriptive study with the main aim of searching for the SRY gene. Our study focused on a 3-year-old Fulla patient with sexual ambiguity referred by the endocrinology and paediatrics departments of the University Hospital of Constantine. We collected clinical data through questioning and blood sampling for the determination of biological parameters and the search for the SRY gene.

RESULTS

This is a male pseudohermaphroditism in a three-year-old child whose genetic and gonadal sex is male, and whose phenotypic sex is female, which led to an error in her civil status and the legal sex was declared female. Fulla was therefore raised as a girl.
The clinical examination showed a little girl with an altered general condition presenting a skin fold of dehydration following her untreated diarrhoea which lasted several days.
Abdominal-pelvic ultrasound which showed absence of uterus and ovaries and presence of testicles in intra-abdominal position, testosterone and LH at the upper limit.
The karyotype showed a chromosome formula of type 46, XY. The SRY gene test came back positive showing a conspicuous band of the SRY gene.

CONCLUSIONS

Sexual ambiguity is a set of pathological conditions of varying severity, which requires specialised multidisciplinary management involving endocrinologists, paediatricians, geneticists, surgeons and psychologists.

Molecular diagnostics

P1760

TRISOMY 13 WITH MILD PHENOTYPIC EXPRESSION

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BACKGROUND-AIM

Patau syndrome is a chromosomal disorder that occurs in 1/20000 live births with a survival that rarely exceeds one year of life. It is associated with a severe polymalformative congenital síndrome, frequently with abnormalities of the middle structure.
This case describes a newborn with a polymalformative syndrome: anorectal malformation, postaxial polydactyly, syndactyly and micrognathia.

METHODS

Cytogenetic study was performed by G-banding karyotype test in peripheral blood, in vitro fluorescent hybridization (FISH), Array-CGH-60K, and study of the pattern of inactivation of the X chromosome.

RESULTS

The FISH test result was: nuc ish Xcen(DXZ1)x2,21q22(D21S259,D21S341,D21S342)x2,18p11(D18Z1)x2,13q14(RB1)x3. Karotype test of patient showed: 46,X+der(13;X)(p11;q12.1). These alterations were confirmed by chromosome painting FISH using a specific probe of chromosome 13. Array-CGH detected a partial trisomy of chromosome 13 and a partial monosomy of X: arr Xp22.33p11.23(2687082-48756025)x1–13q12.3q34(30805425-115083342)x3. The pattern of X chromosome inactivation showed a preferential inactivation of the derived X chromosome. Karyotype and Array-CGH were performed on parents, which were normal.

CONCLUSIONS

Cytogenetic testing revealed that the patient presented with a derivative chromosome which includes the translocation between the long arm of chromosome 13 and the long arm of chromosome X. This implies partial trisomy 13q (84Mb) and partial monosomy Xp (46Mb) that does not include the SHOX gene. These alterations are related to Patau Syndrome and Turner Syndrome, respectively.
Partial monosomy X without involvement of the SHOX gene could explain Turner Syndrome with mild phenotypic expression. In the same way, the pattern of preferential inactivation of the derivative chromosome could explain the mild phenotypic associated with of Patau syndrome. Paternal genetic testing revealed de novo acquisition of this derivative chromosome.
Detection by molecular cytogenetics testing (FISH or Array-CGH) of any autosomal aneuploidy in females, requires confirmation by karyotype test due to the possible intervention of the X chromosome in the chromosomal rearrangement. This is important because X chromosome inactivation can alter the phenotypic expression of a recognized clinical entity.

Molecular diagnostics

P1761

GENETIC POLYMORPHISMS IN GEORGIAN WOMEN WITH UNEXPLAINED RECURENT PREGNANCY LOSS (RPL)

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BACKGROUND-AIM

Inherited thrombophilia is associated with an increased risk of venous thrombosis and has been considered a risk factor for RPL. However, there is limited evidence of importance to guide screening for and management of these conditions before and during pregnancy. Little is known about the prevalence of each hereditary thrombophilia among different ethnic groups.
Detection of frequency and types of thrombophilia gene mutations (GM) in Georgian women with RPL with and without personal and/or family history of thrombosis, as well as healthy women.
436 Georgian women with two or more incidents of pregnancy loss and 41 healthy women were investigated.
Women were divided into Group I (280) with and Group II (156) without personal and/or family history of thrombosis.
Control group (CG) - 41 women with 2 live births, without any pregnancy complications, miscarriages and personal and family history of thrombosis.

METHODS

All cases were detected by PCR method:
Factor V Leiden - G1691A, Factor VHR2 Haplotype - H1299R
Prothrombin Factor II - G20210A ,Plasminogen activator inhibitor – PAI -1
MTHFR C677T and MTHFR A1298C

RESULTS

Prevalence of FVL GM was significantly higher in Group I (10 ,8 %) compared to Group II (5.9%) and control (CG – 2.4%).
Prevalence of FII GM in Group I– 9,6 % and 9,7 % in Group II, (CG - 4,8%).
Prevalence of MTHFR homozygote in patients with RPL was significantly higher than in controls (I group-12.9 %; II group- 12,8% and CG – 3,7%).
Prevalence of combined mutations didn’t differ significantly between groups with RPL (9,28% in I group and 8,97% in II group, but it was significantly higher than in control – 2,4%.

CONCLUSIONS

Received results to indicate that it is reasonable to conduct investigation on all Georgian women with RPL, despite personal and/or family history of thrombosis. Especially taking into account that when timely started, adequate treatment is very successful.

Molecular diagnostics

P1762

HIGH-RISK HUMAN PAPILLOMAVIRUS GENOTYPE DISTRIBUTION AMONG WOMEN WITH GYNECOLOGY COMPLAINTS IN NORTHWEST ETHIOPIA

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BACKGROUND-AIM

Human papillomavirus (HPV) genotypes differ by geographic location. With the advent of HPV vaccination and HPV-based cervical screening tests in Ethiopia, nationwide data set on the genotype distribution of HPV among women has paramount importance in the fight against cervical cancer. However, there is limited data in this regard in the northwest part of the country.

METHODS

A health facility-based cross-sectional study was conducted at Felege Hiwot Comprehensive Specialized Hospital (FHCSH), Bahir Dar - Ethiopia. Women aged >30 years who visited the hospital gynecology unit from 01 March 2019 to 30 October 2021 were included. Following general and pelvic examinations, a senior gynecologist collected cervical swabs for HR-HPV detection using the Abbott Alinity m system (Abbott Molecular, Des Plaines, IL, USA), and extended genotyping was carried out with the INNO-LiPA HPV Genotyping Extra II assay (INNO-LiPA; Fujirebio Europe, Ghent, Belgium) as per the manufacturer protocols at the Institute of Virology, Leipzig University Hospital, Germany.

RESULTS

We included 355 women with a mean age of 46.4+11.4 years. The proportion of HR-HPV was 53.0% (n=188; 95%CI: 47.8-58.1%). From these samples, 13 different HR-HPV types with a total of 258 sequences were identified. The detection of HR-HPV increased significantly with an increase in the age of the participants. The predominant identified HR-HPV was HPV16, 50.4% (130/258: 95%CI: 29.4-39.2%) followed by HPV31 (9.7%), HPV33 (8.5%), HPV39 and HPV68 each (5.8%) and HPV18 (4.7%). Of the total HR-HPV-positive women, 23.9% (45/188) were infected with two and more (up to five) multiple HR-HPV types. All HPV16, HPV18, HPV35, and HPV45 genotypes (as a single or in coinfections) were found to be associated with either high-grade lesions or cervical cancer.

CONCLUSIONS

HR-HPV infection was reportedly higher among women in the present study area. Based on our findings, we strongly recommend the nonavalent HPV vaccine for immunization and any HPV-based screening method to take into consideration the predominant genotypes circulating in the country. The role of multiple HPV infections in high-grade cervical lesions entails further study in Ethiopia.

Molecular diagnostics

P1763

PROTEIN KINASE A (PKA) IN HUMAN URINE SAMPLES: A RELEVANT OR IRRELEVANT NOVEL ANALYTE?

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BACKGROUND-AIM

Fully involved in cellular maintenance, cAMP-dependent Protein Kinase A (PKA) has been proposed as putative biomarker in different pathological conditions, including cancer. Recently, an active PKA form has been identified in human sera and PKA autoantibodies have been detected in cancer patients. However, their serum functions, as well as diagnostic significance, remain largely unknown. Different methods have been developed for PKA detection over the years, even though no one refers to a laboratory diagnostic procedure so far. Among these, ELISA assay constitutes the most widespread technique for PKA recognition. To the best of our knowledge, there is no evidence showing PKA presence in human urine samples, therefore we explored the chance of detecting this enzyme even in this biological specimen.

METHODS

Using ELISA assays, we tested thirty urine samples from randomly recruited volunteers, whose health conditions were kept hidden. Four different sera, unrelated to the urine donors, were also included in our analysis as positive control for PKA recognition. Finally, Western blotting (WB) was employed as an additional method for PKA detection in urines.

RESULTS

Among the thirty-screened urines by quantitative sandwich ELISA, we recognized detectable PKA levels in five different samples, two of which exhibited a considerable high concentration. Interestingly, PKA concentration ranged between 88.9 and 336.8 U/L within the positive urines, while a similar but slightly higher extent was detected in sera samples (from 143.2 to 398.2 U/L). To corroborate these results, we also evaluated the PKA presence in both positive and negative ELISA urines by WB. Remarkably, immunoblotting analysis confirmed the PKA positivity.

CONCLUSIONS

Despite quite preliminary, these findings first identify PKA in human urine specimens. Assuming a possible correlation with specific health conditions, the non-ubiquitous presence of PKA enforces its potential clinic usage as a diagnostic analyte in laboratory medicine. Collaterally, we further recognized WB as alternative tool for detecting PKA in different body fluids.

Molecular diagnostics

P1764

DETECTION OF P.C282Y AND P.H63D MUTATIONS IN HEREDITARY HEMOCHROMATOSIS (HH) PATIENTS

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BACKGROUND-AIM

Hereditary hemochromatosis (HH) is an autosomal recessive disorder of iron metabolism that can result in multi-organ dysfunction caused by increased iron deposition, primarily in the liver. In this study we conducted molecular analysis of the two most common mutations (p.C282Y and p.H63D) in the hemochromatosis gene (HFE) for patients with suspected HH. Serum ferritin levels and iron concentrations in patients with p.C282Y or p.H63D mutation in one or both alleles of the HFE gene are also considered.

METHODS

From 2021-2022, DNA extraction (Qiagen, GmbH, Germany) and molecular analysis with allele-specific polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) (Applied Biosystems, USA) were done in 197 patients. After restriction, PCR products were analyzed electrophoretic on polyacrylamide gels (Invitrogen, USA). Serum ferritin levels and iron concentrations were also measured (Beckman Coulter AU 680, USA). All methods used are accredited according to the ISO 15189 standard.

RESULTS

Out of 197 patients, 8 were homozygous p.C282Y (4.1%), 15 heterozygous p.C282Y (7.6%), 6 homozygous p.H63D (3.0%) and 67 heterozygous p.H63D (34.0%). The other 101 tested patients had no mutations. The value range for proven homozygous p.C282Y patients were serum ferritin value 112.1-3336.2 μmol/l, heterozygous p.C282Y 61.1-779.0 μmol/l, homozygous p.H63D 168.1-1256.0 μmol/l and heterozygous p.H63D 52.1-2046.0 μmol/l. The iron concentrations range were 17-47 μmol/l for homozygous p.C282Y patients, 4-65 μmol/l heterozygous p.C282Y 13-52 μmol/l, homozygous p.H63D had 13-51 μmol/l and heterozygous p.H63D 6-77 μmol/l.

CONCLUSIONS

In the observed patients, serum ferritin and/or iron concentrations above the limit of reference values were present, so they were sent for molecular testing on HH. Homozygosity for p.C282Y mutation is associated with type 1 HH manifest clinical symptoms of the disease but homozygous for H63D as well as heterozygosity for p.C282Y and p.H63D are not associated with an increased risk of iron overload unless other risk factors are present. Therefore, molecular analysis should always be considered in case of elevated serum ferritin and iron values or if clinical symptoms are present to prevent increased iron deposition and organ damage.

Molecular diagnostics

P1765

MOLECULAR MARKERS PREDICTING THE PROGRESSION AND PROGNOSIS OF HUMAN PAPILLOMAVIRUS INDUCED CERVICAL LESIONS TO CERVICAL CANCER.

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BACKGROUND-AIM

Persistent Human Papillomavirus (HPV) infection is linked with 99% of cervical cancer (CC) cases. HPV types 16 and 18 alone are responsible for 75% of the total number of CC cases and thus are considered to be high risk HPV types (HR HPV) CC is the third most common cancer among women globally. Approximately, 7000 patients die from it yearly. It is worthy to note that not every patient with HPV related precancerous lesions will progress to CC. Using molecular and viral biomarkers can be of great help for early detection and prediction of HPV induced cervical lesions that are likely to progress to CC.

METHODS

Three databases (PubMed, Google Scholar, EBSCO) were searched for articles with keywords CC screening, HPV, and recent molecular biomarkers. The Search time frame was limited to the last 7 years to get the most updated scientific evidence. Studies on HPV induced cancers other than CC were excluded independently; a total of 200 eligible articles were retrieved by authors

RESULTS

The interaction between viral oncoproteins and the cellular genetic apparatus alters the expression of many genes at different phases of the disease. There was an association between cervical lesions induced by HR-HPV and the overexpression of markers of oxidative DNA damage and several cellular proteins. Proposed markers showing form of dysregulation and associated with high risk lesions are p16INK4a, programmed cell death-1 (PD-1)/programmed cell death ligand 1, mismatch repair enzymes(MMR), miRNA-377, claudin family (CLDN).Furthermore, advanced older cervical lesions were associated with high methylation levels and higher risk to progress to cervical carcinoma.

CONCLUSIONS

The study of altered genes in CC can suggest molecular markers that will improve the screening programs capacity to to identify high risk cervical lesions and even categorize the patients according to their prognosis and treatment potential so that adequate and aggressive treatment options are directed towards patients with poorer survival. It will also show the possibility of using specific immunotherapy with those patients as a step towards precision medicine. Making such tests valuable for decreasing CC cases in low- and middle-income countries will help reduce the global CC burden and mortality.

Molecular diagnostics

P1766

GLUCOSE 6 PHOSPHATE DEHYDROGENASE DEFICIENCY

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BACKGROUND-AIM

Glucose 6 phosphate dehydrogenase (G6PD) deficiency is the most common enzyme disorder of red blood cells, with a prevalence of 20% in tropical and subtropical areas. Its inheritance is X-linked dominant: males inherit the mutation in hemizygosis and females in heterozygosis. Numerous variants associated with different enzymatic activity and severity of hemolysis have been described.

METHODS

A 4-day-old male patient of Indian descent presented to the emergency department for skin and conjunctival jaundice. First Rh+ pregnancy of an Rh- woman.

RESULTS

Physical examination: the newborn presents signs of bilirrubin encephalopathy: high-pitched cry, scanty movements and generalized tremor.
Complementary tests: laboratory investigations revealed highlights a total bilirubin of 50.7 mg/dL (0.2-1.2) predominantly unconjugated, normal blood count and negative direct Coombs. Given the severity of the values, he was diagnosed with extreme hyperbilirubinemia and was admitted to the Neonatal Intensive Care Unit. The patient underwent exchange transfusion and intensive phototherapy, measures that allowed bilirubin levels to decrease to 19.27mg/dL.
Etiological study: a metabolic study was conducted: organic acids in urine were normal, pyruvate kinase in erythrocytes: 137.3mU/1000m (60-220) and G6PD: 49.3 mU/1000m (221-557). Considering these findings, a genetic study for G6PD deficiency was requested, finding the c.563C>T variant in hemizygosis in the G6P gene, corresponding to the type 2 or Mediterranean variant, predominant in some regions of Asia such as India.

CONCLUSIONS

Most cases of neonatal hyperbilirubinemia are due to physiological causes, but there are specific cases where etiological diagnosis is essential, especially in extreme cases such as the one presented. Given the high prevalence of G6PD deficiency, it should be taken into account as a possible cause. The clinical manifestations depend on the degree of enzyme deficiency and can range from jaundice to chronic hemolytic anemias. Early diagnosis is essential and will allow the patient to be educated to avoid triggering factors that cause hemolytic crises and their consequences

Molecular diagnostics

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ARBOLEDA-THAM SYNDROME. A CASE REPORT

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BACKGROUND-AIM

Arboleda-Tham syndrome (ARTHS, OMIM#616268) is a newly rare genetic disorder characterized by global developmental delay with intellectual disability, speech delay, facial dysmorphism, congenital heart anomalies, gastrointestinal problems, visual defects, microcephaly, neonatal hypotonia and seizures, among others, with variable clinical expression. It is an autosomal dominant hereditary disease caused by heterozygous pathogenic variants in the KAT6A gene.

METHODS

We describe the case of a 15-year-old boy who was referred to the Clinical Genetics Unit due to intellectual disability, learning difficulties, speech delay, abnormal phenotype and two episodes of febrile seizures. At age 5, he presented a convulsive crisis, acute encephalitis and double pneumonia. He has functional chronic abdominal pain and myopia.

RESULTS

A karyotype, 60k array-CGH and molecular analysis for fragile X syndrome were requested, finding normal results. A blood sample was sent to an external laboratory to perform whole exome sequencing (WES), but no pathogenic/likely pathogenic variants were found. One year later, the patient was included in a research project and WES data was reanalysed in another laboratory. The exome reanalysis detected the heterozygous variant c.2944_2945delAG (HGVS: p.[(Arg982GlyfsTer4)];[Arg982=]) in KAT6A, associated to ARTHS. The family segregation analysis was performed in our laboratory by Sanger sequencing. The variant was not found in any of our patient's parents, demonstrating that the variant has occurred de novo in our patient.

CONCLUSIONS

The variant detected in our patient is a frameshift variant that has not been previously reported in the literature. Applying the American College of Medical Genetics and Genomics (ACMG) standards, the variant c.2944_2945delAG in KAT6A is classified as pathogenic and it is responsible for our patient's phenotype. Our patient presents the most common manifestations of ARTHS: intellectual disability, speech delay, gastrointestinal problems, visual alterations and epilepsy. WES studies are a useful diagnostic tool in neurodevelopmental disorders. However, the analysis of WES data is complex and sometimes reanalyses are necessary to identify the pathogenic variant responsible for the patient's phenotype.