Wednesday, August 3, 2016

Poster Session: 9:30 AM - 5:00 PM

Molecular Pathology/Probes

B-183

Association between Plasminogen Activator Inhibitor-1 4G/5G Promotor Genotype and its Circulating Levels in Middle Eastern Population

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Background

Plasminogen activator inhibitor-1 (PAI-1) is the main inhibitor of tissue-type plasminogen activator (tPA) and urokinase plasminogen activator (uPA) and, as such, plays an important role in the regulation of fibrinolysis. Elevated (PAI-1) levels are associated with increased venous thromboembolism (VT) risk in the general population. The relation of PAI-1 4G/5G promotor genotype [NM_000602.4] (c.-817dupG) to venous thrombosis has been investigated primarily in case-control studies, which have produced inconsistent findings. Most studies, however, have reported higher PAI-1 plasma levels in individuals with 4G/4G. the evidence regarding the relationship between an elevated PAI-1 plasma level or PAI-1 genetic polymorphism and the risk of VT is conflicting. There is insufficient information to recommend use of either PAI-1 levels or genotype in evaluating risk of thrombophilia. The aim of the present study was to investigate the frequency of the 4G/5G PAI-1 promoter genotype in Middle Eastern population and the relation of the genotype to circulating PAI-1 levels.

Method

A total of 1930 individual (837 men and 1093 women; 18 to 76 years old) with no personal history of VT from 4 different middle eastern countries (Egypt (833), Saudi Arabia (512), Qatar (373) and United Arab Emirates(212)) included in the study. PAI-1 4G/5G promotor genotype was determined using validated reverse hybridization polymerase chain reaction (PCR) derived from ViennaLab Diagnostics GmbH Vienna, Austria. PAI-1 level was measured quantitatively using a validated solid phase sandwich enzyme linked-immuno-sorbent assay (ELISA) derived from Invitrogen Corporation, CA, USA.

Results

There were 285 subjects with the 4G/4G genotype (14.7%), 885 with 4G/5G genotype (54.85%), and 760 with 5G/5G genotype (39.38%). PAI-1 levels were significantly higher homozygotes for the PAI-1 gene deletion allele (4G/4G) (P<.001) and heterozygous PAI-1 gene deletion allele (4G/5G) (P<.001), while the 5G homozygotes allele had the lowest levels of PAI-1.

Conclusion:

There is a significant correlation between gene variants of PAI-1 and circulating PAI-1 antigen levels in Middle Eastern individuals without clinical evidence or history of venous thromboembolism and thus the PAI-1 genotype and levels could be used along with clinical data and other laboratory findings in evaluation risk of thrombophilia.

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Genotype distribution of high risk human papillomavirus in women from the state of Jalisco, Mexico

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Background: The majority of cervical cancer (CC) diagnoses are attributed to persistent infection of high risk HPV (Human Papillomavirus). Particularly, HPV 16 followed by HPV 18, are responsible for approximately 70% of cervical cancers. In 2015, the Public Health Laboratory *(Laboratorio Estatal de Salud Pública-LESP)* implemented quantitative real-time polymerase chain reaction (PCR) molecular technology in order to increase accuracy of HPV testing. Our aim was to identify high risk genotypes: HPV 16, HPV 18 and a 12 VPH genotype pool: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 in women from the 13 sanitary regions of Jalisco, Mexico. **Methods:** In this cross-sectional study from April 14 to October 15, 2015, a total of 44,349 cervical cell samples of women ages 35 to 64 years attending health centers, mobile health caravans, regional hospitals and dysplasia clinics of the 13 sanitary regions of the state of Jalisco, were assessed for HPV genotype distribution by real-time PCR (Cobas ®4800 HPV; clinical sensitivity: 92.9%, specificity: 71.0%). Cervical cells were obtained from ectocervix and endocervix of the uterus of every woman and maintained in PreservCyt® solution.

Results: From the 44,349 cervical cell samples, 3,791 were positive to some type of HPV. From these, we found a total of 457 positive samples (12.05%) for HPV 16 and a total of 179 (4.72%) positive samples for HPV 18. In addition, 10 samples were positive to HPV 16+18 (0.26%) and 3,145 (82.97%) samples to the HPV 12 pool. Table 1 shows the genotype distributions of each region in Jalisco.

Table 1. HPV genotype distribution in women from the 13 sanitary regions of Jalisco

SANITARY REGIONS IN THE STATE OF JALISCO	HPV-POSITIVE CERVICAL CELL SAMPLES				
	TOTAL (n)	HPV 16	HPV 18	HPV (16+18)	HPV 12 POOL
COLOTLAN	77	12 (15.6 %)	1 (1.3 %)	1 (1.30%)	63 (81.80%)
LAGOS DE MORENO	162	18 (11.1 %)	7 (4.3 %)	-	137 (84.60%)
TEPATITLAN DE MORELOS	163	22 (13.5 %)	5 (3.1 %)	-	136 (83.40%)
LA BARCA	422	46 (10.9 %)	27 (6.4 %)	1 (0.24%)	348 (82.46%)
TAMAZULA DE GORDIANO	60	3 (5.0 %)	4 (6.7 %)	-	53 (88.30%)
CIUDAD GUZMAN	213	36 (16.9 %)	4 (1.9 %)	1 (0.47%)	172 (80.73%)
AUTLAN DE NAVARRO	252	21 (8.3 %)	15 (6.0 %)	1 (0.40%)	215 (85.30%)
PUERTO VALLARTA	179	23 (12.9 %)	7 (3.9 %)	-	149 (83.20%)
AMECA	315	36 (11.4 %)	17 (5.4 %)	-	262 (83.20%)
ZAPOPAN	376	57 (15.2 %)	14 (3.7 %)	1 (0.27%)	304 (80.83%)
TONALA	313	31 (9.9 %)	14 (4.5 %)	-	268 (85.60%)
TLAQUEPAQUE	342	37 (10.8 %)	18 (5.3 %)	1 (0.29%)	286 (83.61%)
GUADALAJARA	917	115 (12.5 %)	46 (5.0 %)	4 (0.44%)	752 (82.06%)

Data are expressed as the total number of HPV-positive cervical cell samples (n) and HPV genotypes as the total number of cases (n) and percentage (%); HPV: Human papillomavirus.

Conclusion:: : Quantitative real-time PCR technology for HPV testing has allowed rapid, accurate and reproducible results for CC early detection. In Jalisco, the primary high risk VPH genotypes were VPH 16 followed by VPH 18, both of which have an important clinical significance in CC.

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Comparison of Genefinder HPV Liquid Beads Microarray PCR Kit and Hybrid Capture 2 Assay for Detection of HPV Infection

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Background: Along with advances in methodological technologies, various assays for detecting high-risk human papillomavirus (HR HPV) have been introduced. The Genefinder HPV liquid beads microarray PCR kit is one of the recently developed. Our aim was to compare the performance of Genefinder to Hybrid Capture 2 for detection of HR HPV.

Methods: A total of 900 cervical swab specimens were obtained. We submitted all specimens for HR HPV detection with HC2 and Genefinder, and then additionally analyzed the discordant or both positive results using restriction fragment mass polymorphism (RFMP) genotyping analysis.

Results: HC2 detected 12.8% cases and Genefinder detected 15.8% cases with 13 HR HPV types. Also, Genefinder detected 27.4% cases for the 32 detectable HPV types. The overall agreement rate was 93.2% with 0.724 kappa coefficient. Discordant results between these two assays were observed in 56 cases. HC2 showed sensitivity of 83.5% and specificity of 95.9%, while Genefinder showed sensitivity of 85.4% and specificity of 91.9%. For HPV 16 or HPV 18 detection, Genefinder showed 95.0% or 66.7% of sensitivity and 99.2% or 100%, respectively. Overall coinfection rate was 15.4% (38/247) in Genefinder analysis.

Conclusion: Considering the high agreement rate with HC2, high sensitivity and the ability to differentiate 32 HPV genotypes including HPV 16/18, Genefinder could be used as a laboratory testing method for the screening of HPV infections. The use of Genefinder may also contribute to future research associated with the significance of various HPV types and multiple coinfections.

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Copy number variation results of intellectually disabled patients using agilent and affymetrix chromosomal microarrays

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Background: Chromosome microarray analysis can detect causative genetic abnormalities in up to 15% of patients with intellectual disabilities who had a normal G-banded karyotype.The ability of these assays to detect chromosome imbalances is influenced by the microarray design, coverage and probe density. AgilentTechnologies designs chromosomal arrays with longer oligonucleotides and uses hybridization of patient and reference DNAs while Affymetrix designs arrays with shorter probes and compare patient with an ideal sample in virtual database. Aims: The aim of this study was to carry out the verification of two microarray techniques in our laboratory using Agilent and Affymetrix platforms, and compare the concordance across platforms concerning copy number variation (CNV) results. Patients and Methods: A blind genomic analysis was performed retrospectively in 12 samples from patients with intellectual disabilities who had been previously tested by a reference laboratory. DNA was extracted with DNeasy Blood and Tissue kit (QIAGEN) and quality was assed using Nanodrop2000 (Thermo Scientific). Two comparable arrays with resolution of approximately 100 kb and higher probe densities in ClinGen regions were chosen: Cytoscan 750k (Affymetrix) and SurePrint G3 CGH ISCA v2 180k (Agilent) and experiments were performed according to the manufactures´s instructions. Affymetrix data were analyzed with Chromosome Analysis Suitv3.1software; 25 probes, 50Kb and -0,45log2 ratiowere defined for losses callings and 50 probes, 100Kband 0,30log2 ratiofor gains. Agilent data were analyzed with Cytogenomics v2.9.2.4 software with a three-probe minimum aberration call, log2ratio ≥0,25 for gains and ≤ 0,25 for losses. CNVs were classified in accordance with the guidelines from the American College of Medical Genetics. Results: Taking together, the reference reports(based on an Agilent 180k array) showed three aberrations classified as pathogenic, five variants of uncertain significance (VOUS) and fourteen benign CNVs longer than 200kb. In our analyses, all the pathogenic and VOUS callings were detected in both Agilent and Affymetrix assays. CNVs considered benign, on the other hand, were more frequently reported when using Agilent. Five of the benign CNVs were not detected by Cytoscan 750k either because there were no probes in those specific chromosome regions, or because few probes (<20) were altered. Among these CNVs, four were located in pericentromeric regions and one was non-coding located in the long arm of chr22. Conclusion: Using Cytoscan 750k and SurePrint G3 ISCA 180k arrays we were able to define correctly all the pathogenic and VOUS previously reported in 12 patients.One third of the CNVs classified as benign, though, were detected using SurePrint ISCA 180k only, mainly due to differences in the array designs. Nevertheless, the regions that are not covered by Cytoscanbear CNVs common in the population according to the Database of Genomic Variants (DGV) and do not affect the calling of causative aberrations. Therefore, we concluded that the results were highly concordant. There is evidence that the methodologies are equally efficient for the detection of CNVs with clinical importance, although because of the small number of cases studied, further investigation will be needed.

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Utilization and reliability of genetic testing by analysis of repeat measurements from a large data repository

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Background: Repeat testing for the same genetic allele or mutation is typically unnecessary. However, this may occur from lack of awareness about prior testing, unavailability of previous results or desire to retest for confirmation due to concerns about reliability of results or clinical consequences to patients and their family members. This study examined the frequency of repeat genetic testing to assess utilization and reproducibility of results. **Methods:** Hemochromatosis (HFE), factor V Leiden (FVL), and *HLA-B*57:01* (B5701) test information was collected from the Veteran Affairs (VA) Healthcare Corporate Data Warehouse for results reported for up to 15 years since 2014. The frequency of cases undergoing repeat testing was evaluated. Cases with incomplete or missing information were excluded from analysis involving specific genotypes. Statistical analysis involving differences between proportions of specific genotypes were performed by the chi-square method.

Results: A total of 46,929 HFE, 47,050 FVL, and 9,358 B5701 cases were validated for evaluation from 118, 120, and 94 VA healthcare facilities respectively. One or more repeat tests were observed from 3,530 (7.5%) HFE, 3,762 (8.0%) FVL, and 704 (7.5%) B5701 cases of which 712 (20.2%) HFE, 753 (20.0%) FVL and 282 (40.1%) B5701 were retested at another facility. Frequency of discrepant results after retesting were 27/2,827 (0.96%) for HFE, 24/3,786 (0.63%) for FVL, and 0/675 for B5701. Retesting was more frequent for homozygous C282Y/C282Y 283/2,608 (10.9%) and H63D/H63D 93/1,099 (8.5%) genotypes compared to compound (C282Y/H63D) 135/1,758 (7.7%), C282Y 301/4,477 (6.7%), and H63D 467/7,506 (6.2%) heterozygotes and wild genotypes, 1,505/22,626 (6.7%), *P*<.001. Among FVL tests, homozygous, heterozygous and wild type retesting rates were 22/197 (11.2%), 488/5,596 (8.7%) and 3,205/40,456 (7.9%), respectively, *P*=.032. There was no difference in retesting frequency between negative 680/8,767 (7.8%) and positive 23/410 (5.6%) B5701 genotypes, *P*=.16. **Conclusion:** These results show that HFE, FVL and H5701 measurements are reliable based on low rates of discrepant results when retested at different times and locations on the same patient . These findings provide supporting evidence that retesting is generally unnecessary to confirm results. Causes for the few discrepancies observed were not evaluated but may have been due to problems associated with mislabeling, analytical errors or clerical mistakes. Since many repeat tests were performed at another facility, it is likely that retesting was, in part, caused by lack of awareness of previous results. Retesting was more common for abnormal HFE and FVL genotypes, possibly due to higher likelihood of clinical findings that prompted testing as well as possible intent to confirm abnormal results due to impact on clinical management and patients' family members. In summary, the three genetic tests evaluated were shown, in practice, to be reliable among a large and diverse group of laboratories but sometimes inappropriately repeated.

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Compound heterozygous mutations identified in a Brazilian infant with glycogen storage disease type Ia

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Background: The liver is the largest internal organ in the body and it is associated with more than 100 different forms of diseases that affectpeople of any age. The diagnostic of liver diseases can be difficult since its symptoms can be unclear, non-specific and easily confused with other health problems. Liver diseases can be caused by a variety of factors, such as defective genes,viruses, medications and drugs. **Objective:** To identify, by whole exome sequencing (WES), the genetic cause of liver disease in an infant who had typical clinical features of lysosomal acid lipase deficiency (LAL-D) but who was LAL-D negative based on the measurement of LAL enzyme level and activity. **Case report:** A 6-month-old male infant born from healthy and non-consanguineous parents presented a poor weight gain, watery stools, steatorrhea, hepatomegaly with hepatic steatosis, hypertriglyceridemia and hypoglycemia. Family history was negative. LAL-D was proposed and discarded based on the measurement of LAL enzyme level and activity in the blood. The patient presents a good response oflow-fat diet. **Methods:** Genomic DNA was extracted from blood sample, following standard protocols. WES was performed using the Illumina Nextera technology according to the manufacturer's instructions and sequenced on the Illumina HiSeq 2500 platform. About 200 thousands exons of 20,500 genes were captured. A total of 158,193,788 sequences were obtained. The average coverage was 98-fold, with 96.0% of the target bases being covered at least 10X. **Results:** Two heterozygous mutations were identified on *G6PC* gene: a known pathogenic mutation in exon four (rs80356482; ENST00000253801:c.562G>C; p.Gly188Arg) and a previously undescribed mutation in exon three (ENST00000253801:c.510delA; p.Ile171Serfs*62).This mutation results in a frameshift and stop signal 62 codons downstream, causing a premature truncation of the G6PC protein at amino acid position 233. Online prediction program Mutation Taster suggested that this variant is a disease causing mutation with a probability value of 1.0 due to the truncated protein. The two mutations were identified in trans configuration, defining this individual as a compound heterozygote. **Discussion:** Glycogen storage diseases (GSD) type Ia is associated with abnormalities in the *G6PC* gene. Mutation in this gene result in a deficiency in the glucose-6-phosphatase (G6Pase) enzyme. GSD are a group of disorders in which stored glycogen cannot be metabolized into glucose to supply energy for the body. GSD type I (GSDI) or von Gierke's disease is the most common of the GSD and it is inherited as an autosomal recessive genetic disorder. Although its recessive nature, compound heterozygous mutations can cause genetic disease in the heterozygous state, since both alleles are defective. Others compound heterozygous mutations have already been described in patients with GSD Ia.**Conclusios:** WES revealed an important, sensitive and efficient tool for the molecular diagnosis in genetic and phenotypic complex diseases, where the phenotype is not suggestive of a particular candidate gene or set of genes, such as liver diseases. Furthermore, it has important implications for prognosis, carrier testing, genetic counselling and prenatal diagnosis.

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Molecular cytogenetic characterization of a add(13)(q32) detected in a boy with multiple abnormalities

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Array comparative genomic hybridization (array-CGH) is a molecular analysis designed to detect gains and losses of genetic material in clinically significant regions of the human genome. Array-CGH detects the presence of microdeletions and microduplications that would be undetectable by conventional cytogenetic technique.We report here the cytogenetic investigation of one-year-old male with cardiovascular disease, optic atrophy, microcephaly, hypotonia, low weight, short stature, dimorphisms and global developmental delay.The initial standard chromosome study revealed 46,XY, add(13)(q32) karyotype. Array-CGH was performed to delineate the origin of additional genomic gain in chromosome 13. Array-CGH analysis additionally revealed a 29.1 Mb pathogenic duplication in the 2p25.3-p23.2 region and confirmed a 7.3 Mb deletion in the 13q33.3q34 region (arr [hg18] 2p25.3p23.2 (20,341-29,172,594) ×3, 13q33.3q34 (106,815,039-114,114,568) ×1). This suggests the additional chromosomal segment attached on chromosome 13q has originated from chromosome 2p with breakpoints in 2p25.3p23.2.The duplication in 2p25.3p23.2 region encompasses more than 100 genes and the deletion in chromosome 13q33.3q34 includes more than 40 genes.There are few reports of patients with pure duplication in 2p. The clinical findings are associated to variable phenotypes as cardiac anomalies, facial dimorphism and psychomotor delay. The 13q33.3q34 deletion has been associated with intellectual deficit, growth impairment and microcephaly. There is a report that described a patient with similar chromosomal rearrangement, but not characterized at the molecular level, that presented a clinical phenotype oflow birth weight , convulsions, neuroblastoma, low-set malformed ears, hypertelorism, micrognathia, micropenis and polydactyly with conventional karyotype 46, XY , der (13) t (2; 13) (p23 ; q34) dn. Moreover, we found in Decipher database a case of patient with coloboma, global development delay, mild intellectual deficit, intrauterine growth retardation, microcephaly, micrognathia, valgus foot deformity and ventricular septal defect, carrying a 13.55Mb terminal duplication in the short arm of the chromosome 2, as well a 5.87Mb terminal deletion in the long arm the chromosome 13. Parental karyotype analysis could help to characterize this chromosomal rearrangement and evaluate the recurrent risk. However, Array-CGH is a useful method for identifying unknown additional and unbalanced rearranged chromosomes that are not detected by conventional cytogenetic analysis.

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Validation of fluorescence in situ hybridization assay for detection of the BCR/ABL rearrangement

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The BCR/ABL1 gene rearrangement is generated by a t(9;22)(q34;q11) reciprocal translocation. In most cases, it is cytogenetically visualized by the Philadelphia (Ph) chromosome. The Ph chromosome is the typical hallmark in chronic myeloid leukemia (CML), but can also be present in acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). Ph chromosome detection by conventional cytogenetic can be hampered by low quantity and quality of metaphases. Furthermore, BCR/ABL1 rearrangements may be hidden due to cryptic rearrangements or complex aberrations. About 5-10% of CML patients lack cytogenetic evidence of the Ph translocation but show BCR/ABL fusion by fluorescence in situ hybridization (FISH). FISH can be performed in dividing and non-dividing cells, which is important when dealing with leukemia cells with low proliferation. Although manufacturers evaluate the performance of most FISH probes prior to commercialization, it is necessary to revalidate all probes before the implementation of assay for clinical diagnostic. Therefore, clinical laboratories must adopt protocols for verify the performance of the assay. In this context, this study aimed to validate FISH assay for detection of BCR/ABL1 translocation following recommendations from the American College of Medical Genetics (ACMG). It was used the BCR/ABL1 translocation, dual fusion probe manufactured by Cytocell®. Metaphase cells obtained from five karyotypically normal male blood samples were used to localize the probe and determine its analytical sensitivity and specificity. To establish a reference range (normal cutoff), it was estimated the false positive rate from 10 uncultured normal bone marrow samples and 10 uncultured normal blood samples. Two analysts scored 500 interphase cells (250 per analyst). All probe signal patterns were recorded. The cutoffs for each signal pattern were calculated using the beta inverse (BETAINV) function available in Microsoft Excel software. The BCR/ABL1 probe presents the ABL1 (9q34) and BCR (22q11.2) probes labeled with red and green fluorophores, respectively. A normal result using this probe should show two green and two red signals (2G2R). Two fusion signals in addition to one green and one red signals (2F1G1R) indicate the presence of classical translocation. The probe demonstrated 100% specificity and analytical sensitivity. After analysis, three and seven atypical signal patterns were respectively identified in bone marrow and blood samples. . It was not observed change in the cutoffs with increasing cell count. The signal patterns and its cutoffs for bone marrow samples were 1F1G1R (3.5%), 2G1R (6.0%) and 1G2R (2.5%). The signal patterns and its cutoffs for blood samples were 1F1G1R (1.5%), 1F2G1R (1.5%), 1F2G2R (2.5%), 2G1R (2.5%), 1G2R (3.5%), 2G3R (1.5%) and 1G1R (1.5%). The cutoffs obtained with BETAINV function were validated using a sample of 200 cells. The analyses of normal and abnormal samples by FISH were in agreement with the karyotype analysis. It showed high reproducibility and quality in different hybridizations. The probe specificity and sensibility was higher than recommended by the ACMG.

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Developing and evaluating a new *in-house* genotyping test for *CYP2D6* gene

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Background: The ability to metabolize drug is influenced by genetic variability. Interindividual differences result in a wide variation in drug response, leading to adverse drug reactions, therapeutic failure, and affects patient well-being and survival. Most of the commercialized drugs are metabolized by polymorphic enzymes and mainly by one or more cytochrome P450 (CYP) enzymes. *CYP2D6* gene (MIM #124030) is one of the most polymorphic pharmacogenes. Variations include point mutations, copy number variations (CNV), indels, conversions and gene rearrangements. These variants increase or decrease CYP2D6 enzyme activity resulting in four phenotypes with significant clinical implication: poor (PM), intermediate (IM), extensive (EM) and ultrarapid (UM) metabolizers. Genotyping and phenotyping test are used in clinical practice to identify variations in *CYP* allelic variants. For *CYP2D6,*more than 109 allelic variants were reported by the Human Cytochrome P450 (*CYP*) Allele Nomenclature Database. **Objective:** Since most commercial genotyping kits are available for just few alleles, this study aimed to develop, validate and evaluate a new in-house *CYP2D6* genotyping test using Sanger sequencing and TaqMan® Copy Number Assay. **Methods:** Genomic DNA samplesfrom 10 individuals previously genotyped by Luminex xTAG® CYP2D6 Kit were used for validation. Coding sequences and flanking regions of *CYP2D6* gene were amplified by PCR. Bidirectional Sanger sequencing was performed with BigDye® Terminator v3.1 Cycle Sequencing Kit and sequenced on ABI 3730 DNA Analyzer. Sequences were analyzed using SeqScape Software v3.0 and compared with the *CYP2D6**1reference sequence (Accession Number: AY545216.1) to identify polymorphisms. CNV was determined by TaqMan Copy Number Assay (Assay ID: Hs04083572_cn) and RNaseP assay (Assay ID: 4403326) served as the internal control. Relative quantification was performed with CopyCaller® Software v2.1 using the comparative ΔΔCT method. For each individual, the genotype that best represents the set of polymorphisms and CNV datawere defined according to The Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee, PharmGKB, and LOVD databases. The phenotype was predicted as PM, IM, EM or UM, based on CYP2D6 diplotypes and the activity score system recommend by the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for codeine therapy. The results from the method developed by this study and the commercial kit were compared. **Results:** The results were 70% concordant between the two methodologies. The differences were due to the coverage of the present in-house assay, once it can detect more variants than the commercial kit, classifying haplotypes more accurately. In addition, one difference was due to a *CYP2D6* deletion that was not identified by the commercial kit. Although these differences, phenotype did not change for the patients. **Conclusions:** Strategy of CNV determination in combination with polymorphism analysis is of utmost importance to correct phenotype prediction. The new method developed combines these two assays creating a more accurate, sensitive and reliable genotyping test than the offered by most commercial genotyping kits. Although this method does not detect all polymorphism of *CYP2D6* since it does not sequence the entire gene, it can detect more than 140 variants reported in the databases and can identify undescribed mutations, contributing to new haplotypes identification.

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Validation of Multiplex Ligation-Dependent Probe Amplification (MLPA) kit P081 and P082 for use in Neurofibromatosis Type 1 clinical routine diagnosis

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Neurofibromatosis Type 1 (Nf1) is one of the most common inherited neurological disorders, affecting about 1: 3.000 individuals. Mutations in the *NF1* gene [(350 kb, 60 exons located on the long arm of chromosome 17 (17q11.2)] are the cause of Nf1 disease, but there are others conditions that may confound the diagnosis. Nf1 is characterized by multiple *cafe-au-lait* spots, freckling, Lisch nodules and neurofibromas and clinical diagnosis is achieved with criteria established by Neurofibromatosis Conference at National Institute of Health, USA (NIH - 1988). Our study aimed to validate the SALSA MLPA PROBE MIX P081 and P082 kits for use in clinical practice to detect deletions of *NF1* gene in patients with clinical diagnosis of Nf1. For this purpose, 32 individuals were selected, of which 25 patients with clinical diagnosis of Nf1 and 7 non-Nf1 affected individuals (supposed healthy). All Nf1 patients were invited from the Neurofibromatosis Outpatient Reference Center at Hospital das Clínicas - Belo Horizonte / MG / Brazil. The MLPA analysis showed that 100% (7/7) of non-Nf1 individuals showed no deletion or duplication in the *NF1* gene. For the 25 patients with Nf1 clinical diagnosis, 76% (19/25) were negative for deletions / duplications in the neurofibromin gene. Microdeletions (former whole-gene deletion) were found in four (20% - 5/25) Nf1 patients with heterozygosity and one patient had only exon 47 deletion in heterozygosity. To further confirm the one exon deletion of the last patient, *NF1* gene was sequenced by massively parallel sequencing that showed, in fact, to be a point mutation in the annealing site of one of the probes from MLPA P081 kit targeting exon 47 region. In addition, one patient (4%) with positive clinical diagnosis for Nf1 remained inconclusive in MLPA analysis. Two samples were sent for analysis in another reference laboratory and the results for molecular alterations were confirmed. Importantly, the negative result does not exclude the diagnosis of Nf1. The present results suggest MLPA analysis may benefit Nf1 suspected individuals that present only one criterion or uncertain diagnosis criteria.

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Improving NPM1 exon 12 sequencing using PCR amplification with a high-fidelity enzyme

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Background: Nucleophosmin (NPM1) exon 12 mutations consist in the most prevalent genetic lesion in adult acute myeloid leukemia. These mutations are small insertion/deletions in the beginning of exon 12, which code for the nucleolar localization signal of the protein. NPM1 exon 12 mutations and are associated with favorable prognosis, therefore the mutational status is crucial for treatment decision making. **Aims:** The aim of this study was to evaluate different approaches for NPM1 exon 12 sequencing and define the methodology that is most cost-effective and yields good quality sequences. **Patients and Methods:** 155 Brazilian patients diagnosed with de novo myeloid acute leukemia from February 2011 to September 2014 were included in this study. All patients were screened for NPM1 exon 12 mutations through cDNA sequencing. Twenty patients with known genotypes were chosen for DNA sequencing. Bone marrow samples were collected in PAXgene tubes (QIAGEN/BD); RNA was extracted using PAXgene Bone Marrow RNA Kit; DNA was extracted from 1ml of PAXgene solution plus blood using QIAamp DNA Blood Mini (QIAGEN). RNA was reverse transcribed into cDNA with Improm II Reverse Transcription System (Promega). Exon 12 and 3´part of exon 11 were PCR amplified from both DNA and cDNA with primers containing mutations to avoid pseudogene amplification. Amplification was carried out with Platinum Taq DNA Polymerase (Life Technologies) and with PfuUltra High-fidelity (Agilent Technologies). PCR products were column purified with the illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare Life Science). Sequencing reactions of both strands were performed using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Thermo Scientific). **Results:** Sequencing results from DNA and cDNA were completely concordant. Nevertheless, forward DNA sequencing after PCR with Taq Polymerase was hampered by the presence of extra peaks following capillary electrophoresis, probably because of the presence of a poly-T region in the 3´extremity of exon 11 that may cause DNA polymerase slippage. These peaks were not seen when DNA was amplified with the high-fidelity enzyme. **Conclusion:** In technical terms, the best method for NPM1 mutation evaluation was cDNA sequencing, although for a clinical laboratory this may not be a cost effective test due to the need of rapid sample transportation, RNA extraction and cDNA synthesis. Alternatively, DNA sequencing using PfuUltrahigh-fidelity yields good results, although in a few cases (when patients carry a rare T deletion polymorphism in intron 11) the forward DNA sequence may be misinterpreted.

B-196

Multiplex Ligation-Dependent Probe Amplification (MLPA) as a diagnostic tool for detection of copy number variations in Charcot-Marie-Tooth and HNPP diseases

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Background: Charcot-Marie-Tooth (CMT) and Hereditary Neuropathy with Liability to Pressure Palsies (HNPP) are common inherited disorders of the sensory and peripheral nervous system that present distinct clinical manifestations and histology. Both diseases are demyelinating neuropathiesthat are mainly related to copy number variations (CNVs) of peripheral myelin protein 22 gene (*PMP22,* MIM# 601097). CMT type 1A (CMT1A) is the most commom form of CMT and is characterized by severely reduced motor nerve conduction velocities.The majority of CMT1A cases (>98%) results from duplication of region encompassing the *PMP22* gene*,* located on chromosome region 17p11.2-p12. In addition, deletions of the *PMP22* gene are the usual cause of HNPP (85%).The incidence of CMT1A is about one in 2.500 individuals and for HNPP is somewhat lower, aroundone in 6.250. Several methods for the identification of CMT1A and HNPP CNVs are reported. Multiplex Ligation-Dependent Probe Amplification (MLPA) has been introduced into DNA diagnostic laboratories for the detection of molecular genetic alterations that are of diagnostic and prognostic significance, such as gene duplications and deletions. **Aims:** Validate the MLPA kit P405 in CMT and HPNN patients previously evaluated by Short Tandem Repeat technique (STR).The MLPA kit is capable of discriminate CMT1A, CMT1B, X-linked CMT and HNPP diseases. **Methods:** Seven patients with CMT or HNPP were tested using the commercial MLPA kit P405 versionA1 (MRC-Holland), following manufacturer's instructions.The P405-A1 CMT probemix contains probes for copy number detection of *PMP22*, *COX10* and *TEKT3* genes in the CMT/HNPP region of chromosome 17p12 and also probes for *GJB1* (X-linked CMT gene) and *MPZ* (CMT1B gene).The analysis was performed using the Coffalyser v.140721.1958 software. Furthermore, all patients were also evaluated by STR using eight markers for CMT (D17S9A, D17S9B, D17S4A, D17S2220, D17S2224, D17S2227, D17S2228 and D17S2230) and six markers for HNPP (D17S9B, D17S9A, D17S2220, D17S4A, D17S2227 and D17S2230). **Results:**The MLPA results were concordant with STR results in all patients. Three patients did not present any variation in *PMP22*, two patients presented *PMP22* duplications and two presented *PMP22* deletions. In addition, X chromosomal variations were detected in two patients by MLPA that cannot be identified by STR markers. Although both patients presented duplications of X-linked genes, one patient presented a *PMP22* deletion. **Conclusions:** The MLPA technique appears to be more informative and high-throughput than STR test. Moreover, the former technique is robust, sensitive and sequence-specific test for detection of *PMP22* gene duplications and deletions in molecular diagnosis of CMT1A and HNPP. Therefore, MLPA could be recommended as a diagnostic tool for CMT disease.

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Array-based comparative genomic hybridization identified genomic imbalance in a patient with apparently *de novo* balanced translocation

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Balanced translocations are relatively common, requiring a two-break event and involving the exchange of chromosome segments between two, usually non-homologous chromosomes. Although the great majority of cases with apparently balanced translocations are not associated with abnormal phenotypes, they can reduce the fertility and increase the risk of unbalanced gametes and abnormal progeny. In approximately 6% of balanced chromosomal rearrangements carriers, intellectual

disability, dysmorphic features and congenital anomalies can be found. The abnormal phenotype might be the result of genomic imbalance or aberrant expression caused by direct breakage of a dosage sensitive gene. Here, we report a seven-years-oldboy with intellectual disability and speech delay.Chromosomal analyses of this patient showed an apparently balanced translocation between chromosomes 2 and 9. The karyotype was designated as 46,XY,t(2;9)(q33;p22)dn. Array-based comparative genomic hybridization (array-CGH) though revealed a ~7.6 Mb interstitial deletion on chromosome 9 at 9p23-p22.2 (arr [hg18] 9p23p22.2 (10,166, 368-17,766, 067) ×1). We performed chromosomal analyses of samples obtained from his parents and the karyotypes were normal. The deletion in 9p23p22.2 region encompasses more than 15 genes, including TYRP1 (tyrosinase-related protein 1) and FREM1 (FRAS1 related extracellular matrix 1) genes. Deletions of the short arm of chromosome 9 are associated with distinct clinical phenotypes. There are reports in the literature of terminal and interstitial deletions with distinct sizes in chromosome 9p (OMIM #158170) associated with the 9p deletion syndrome. This syndrome is characterized by a variable phenotype that includes facial dysmorphism, hypotonia, intellectual disability and others. Additionally, we identified ~195 Kb duplication in 10p11.21 that could be classified as VOUS (variant of unknown significance).Studies suggested that the link between an apparently balanced translocation and the appearance of abnormal phenotype may be partly explained by the presence of cryptic and complex chromosomal rearrangements and that more breaks may lead to imbalances. Some studies were based only on FISH approaches and, therefore, small interstitial imbalances may have been missed. The development of the array-CGH overcame the limitations of cytogenetic and FISH approaches and provided the opportunity to screen the entire genome for cryptic genomic gains and losses.This study underlines the importance of a genome-wide approach in patients with an apparently balanced chromosome rearrangement and abnormal phenotype. It is crucial to examine an apparently balanced rearrangement after detection because it can be more complex than suggests.

B-198

Leukemic survival factor SALL4 contributes to defective DNA damage repair

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Background: SALL4 is aberrantly expressed in human myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). We have generated a SALL4 transgenic (SALL4B Tg) mouse model with pre-leukemic MDS-like symptoms that transform to AML over time. This makes our mouse model applicable for studying human MDS/AML diseases. In this study, by searching the common gene/pathway in leukemia initiating cells/populations (LICs), we have identified a novel mechanism that enables SALL4 contributes to leukemic development.

Methods: The molecular mechanism of SALL4 contributing to leukemogenesis was explored through gene expression profiling studies on leukemic initiating populations. Data were analyzed by dChip (http://biosun1.harvard.edu/complab/dchip/) and GSEA 2.0 algorithm (http://www.broad.mit.edu/gsea/). The regulations of a number of potentially relevant genes were further verified by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Mitomycin C (MMC) clonogenic assay, chromosomal spread assay and Homologoous recombination (HR) and nonhomologous DNA end joining (NHEJ) reporter assay were performed to study the role of SALL4 in DNA damage repair and response. The gene expression data on a cohort of 542 AML patients were downloaded from publicly available Gene Expression Omnibus (GEO) dataset under series accession number GSE13159. Another cohort of 75 primary human MDS/AML samples was collected in Beijing from December 2009 to December 2015. This study was approved by the institutional review board of Peking Union Medical College Hospital and informed consent was obtained from all subjects.

Results: Characterization of the leukemic initiation population in this model leads to the discovery that Fancl (Fanconi anemia, complementation group L) is down-regulated in SALL4B Tg leukemic and pre-leukemic cells. Similar to the reported Fanconi anemia (FA) mouse model, chromosomal instability with radial changes that can be detected in pre-leukemic SALL4B Tg bone marrow (BM) cells after DNA damage challenge. Results from additional studies using DNA damage repair reporter assays support a role of SALL4 in inhibiting the homologous recombination pathway. Intriguingly, unlike the FA mouse model, after DNA damage challenge, SALL4B Tg BM cells can survive and generate hematopoietic colonies. We further elucidated that the mechanism by which SALL4 promotes cell survival is through Bcl2 activation. Parallel human study also showed that SALL4 expression of MDS/AML patients with complex aberrant karyotype was significantly higher than patients with normal or other abnormalities.

Conclusion: In summary, our studies demonstrate for the first time that SALL4 has a negative impact in DNA damage repair, and support the model of dual functional properties of SALL4 in leukemogenesis through inhibiting DNA damage repair and promoting cell survival.

B-199

Verification of the Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay

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Background: Cystic Fibrosis (CF) is the most common life-threatening autosomal recessive disorder among Caucasian populations with an incidence of 1 in 3000 and an estimated 1 in 28 carrier frequency. Caused by inheriting two defective copies of the cystic fibrosis transmembrane conductance regulator gene (CFTR), CF can affect multiple organ systems. CF typically presents with persistent pulmonary infections, sinus infections and pancreatic insufficiency. CF has a highly variable presentation, with milder forms of the disease often presenting later in life. Diagnosis is established through newborn screening programs followed by confirmation with sweat chloride testing and CFTR gene variant identification. Early identification of disease causing CF mutations is important in order to minimize the decline of lung and pancreatic function. The most common CFTR mutation (ΔF508) is responsible for ~66% of CF disease within the US population. Greater than 2000 variants have been identified and both the carrier rate and variant frequency vary between different ethnic populations. The American College of Medical Genetics (ACMG) recommends screening for a minimum of 23 common CF variants that identify 88% of Caucasian carriers but only 72% of Hispanic and 65% of African American carriers. Expanding the number of variants screened will increase the likelihood of identifying carriers in Caucasians and other ethnic groups. The objective of our study was to perform an in-house verification of the recently FDA approved Illumina MiSeqDx® Cystic Fibrosis 139 Variant IVD assay with split sample comparisons to the Luminex xTAG® 60 Variant Kit v2. This assay contains all ACMG and American College of Obstetricians and Gynecologists recommended variants.

Methods: DNA from 62 peripheral blood specimens previously submitted for CFTR testing by xTAG® Cystic Fibrosis 60 V2 Assay were de-identified and genotypes blinded. The Illumina MiSeqDx® Cystic Fibrosis 139 Variant assay was performed according to the manufacturer's protocol. The INTROL™ CF Panel III Control (MMQCI) was used to verify additional mutations not present in patient samples. Barcoded libraries were created using the CF 139-variant oligonucleotide probe pool. Amplified libraries were purified, normalized, pooled, sequenced, and analyzed using the MiSeqDx® and MiSeq Reporter 2.2.31.

Results: Prior to library preparation 14.5% (9/62) samples failed to meet established DNA quality measures and were removed from testing. Of the remaining 53 samples there was 100% intra-assay concordance. 64% (32) of samples were positive for at least one CF variant and 36% (19) contained no identified CF variants. A total of 43 variants were identified across 36 alleles and 4 manufactured controls. 99% (81/82) of CFTR amplicons had a read depth of >30X with 1 amplicon resulting in <30X in 5% of samples and an average read depth of > 1000X. A sample call rate of 99.9% was observed across all samples and variants. Cluster densities on the MiSeqDx ranged from 422-987K/mm^2 and resulted in an average of 8 million reads/run. We did not observe any additional variant pickups with the expanded panel within our highly Caucasian population.

Conclusion: Our verification of the Illumina MiSeqDX demonstrated 100% concordance with the Luminex xTAG® 60 Variant Kit v2.

B-200

Identification of three novel pathogenic mutations in NF1 gene

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Neurofibromatosis type 1 (Nf1) is a genetic autosomal dominant disease characterized by café au lait spots, Lisch nodules, neurofibromas, osseous dysplasia and optical gliomas. Nf1 is the most common genetic disease caused by a single gene [1]. The gene *NF1* is located at chromosome 17 (17q11.2) near the centromere, whose product is a protein (Neurofibromin - 2,485 amino acids) predominantly expressed in neurons, Schwann cells, oligodendrocytes, and leukocytes. The Neurofibromin is a tumor suppressor protein, that functioning as negative regulator of cellular RAS-MAPK signaling pathway. Any mutation that disrupts the function of protein could lead to uncontrolled cell growth and potentially tumorigenesis. More than 90% of these mutations are small deletions and insertions, splicing mutations, and nonsense or missense mutations. A minority of patients (4-5%) has exonic or whole-gene deletions/duplications. The *NF1* gene has the highest rate of mutation in a human gene and the causes remain unclear.In this work, we described three novel mutations in the *NF1* gene, related to typical Nf1 clinical features. The mutations were identified by Massive Parallel Sequencing complete of *NF1* gene of individuals previously diagnosed for Nf1 and with negative result for exonic or whole-gene deletions/duplications. The clinical diagnosis of Nf1 was based on the clinical diagnostic criteria outlined in the National Institutes of Health (NIH) consensus development conference in 1988. The products of PCR were loaded on Ion 316 chip and sequenced with an Ion Personal Genome Machine (PGM) System (Thermo Fisher). Entire *NF1* coding exons and their intron boundaries (25 bp) were coverage (99.44%). Screening for *NF1* deletions was performed using the SALSA P081/082 (MRC-Holland) NF1 MLPA assay following instruction's manufacturer.All the three mutations (g.248760_248763del, g.250559dup and g.69115delG) resulted in a frame shift predicted to generate a premature stop codon at amino acid positions 2268, 2305 and 102, respectively. All of them were classified as pathogenic following the Standards and Guidelines for interpretation of sequence variants by the American College of Medical Genetics and Genomics (ACMG - 2015).The knowledge of new pathogenic mutations is helpful to best clinical decisions for Nf1 treatment and diagnosis.

B-201

New Molecular Assays for the Simultaneous Detection of Gastric Pathogens Using the VERSANT MiPLX Solution

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Background: Gastroenteritis accounts for more than 1.7 billion cases globally, leading to 2 million deaths annually. Children under 5 years are especially at risk, with over 700,000 deaths every year.[1] These infections can be caused by a diversity of pathogens including viruses, bacteria, and parasites. Conventional methods in the clinical laboratory can take days to identify the cause. Here we describe several new molecular assays for the simultaneous detection of gastric pathogens including Norovirus, *Clostridium difficile*, *Salmonella*, *Campylobacter,* and Giardia in stool samples using the VERSANT® kPCR Molecular System with MiPLX software.

Methods: Raw stool samples were collected from patients with Norovirus, *C. difficile*, *Salmonella*, *Campylobacter,* and Giardia infections. Ten samples of each pathogen were processed. Either 60 mg of stool was weighed, or a 10 µL loop of stool was added to pretreatment buffer. Following pretreatment, samples were loaded onto the VERSANT kPCR Molecular System for automated nucleic acid extraction and plate setup. A single extracted sample was able to be split into PCR wells for up to six different assays. Amplification and detection was performed for all pathogens using the same thermal cycling method on the QuantStudio 5 (ThermoFisher Scientific).

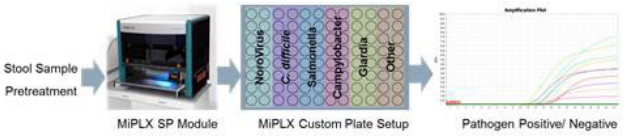

Figure 1: The VERSANT® kPCR Molecular System with MiPLX software for automated nucleic acid extraction, plate setup, and amplification/detection.

Results: The new "Company Name" gastrointestinal assays, as automated on the VERSANT kPCR Molecular System with MiPLX software, detect multiple pathogens including Norovirus, *C. difficile*, *Salmonella*, *Campylobacter,* and Giardia. Norovirus, *Salmonella*, *Campylobacter,* and Giardia were detected in both 60 mg and 10 µL loops of stool samples.

Conclusion: The VERSANT MiPLX Solution, consisting of the VERSANT kPCR Molecular System with MiPLX software, provides an automated and simplified workflow for detection of up to six gastrointestinal pathogens from one sample in a single run. The VERSANT MiPLX Solution provides a customized and flexible approach for performing high-throughput testing with a short turnaround time for the detection of desired pathogens. 1) World Health Organization (WHO). 2013.

B-202

Implementation of a molecular diagnostic method for the quantitative detection of Zika virus

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The Zika virus is a single-stranded positive sense RNA molecule belonging to the Flaviviridae family of Flaviviruses. The Flavivirus genus also includes the better known Dengue, Chikungunya, and West Nile Viruses. The World Health Organization (WHO) recently declared that the current (2016) outbreak of Zika virus in Brazil and other parts of South America poses a significant global health risk that is likely to reach pandemic status. Additionally, the United Stated Centers for Disease Control (CDC) has issued an alert level 2 (practice enhanced precautions) travel advisory for affected regions of South and Central America. Patients with an active Zika virus infection, known as Zika fever, do not present with particularly aggressive symptoms. The major cause for concern with Zika viral infection has, instead, been the possibility that Zika virus may cause microcephaly in the fetuses of infected pregnant women. Early detection of active viral infection is thus of paramount importance as it can be used to trigger more aggressive ultrasound monitoring in pregnant women with the goal of identifying the early signs of microcephaly.

Zika, and other members of the flaviviridae family, share high amounts of structural homology that render standard immunoassay-based testing methodologies of limited utility when used in viral typing. Molecular based methods, however, are well suited to identifying minor differences in the envelope and glycoprotein regions of the viral genome, and make both detection and quantification of active viral infection possible. We therefore sought to implement and validate a one-step real-time PCR-based quantitative assay (qRT-PCR) for the detection of active virus in patient plasma.

Primer sequences for real-time detection of Zika virus were provided upon request from the CDCs Arbovirus Disease Branch in Fort Collins Colorado. Primer sequences were specific for two regions of the viral genome include a viral envelope glycoprotein and one of the nonstructural protein coding regions. We performed the validation studies for our assay using a combination of commercially available viral cultures (Zeptometrix), Zika patient proficiency samples (CDC), and synthetic double-stranded DNA oligonucleotide fragments (gBlock, Integrated DNA Technologies). We determined the lower limit of quantification of our qRT-PCR assay to be $1x10^3$ copies using the GeneBlock fragments, which were also used to determine the linearity of our assay over an analytical measurement range of of $1x10^3$-$1x10^{12}$ copies. Cycle thresholds were set empirically on a per run basis and verification of the amplified PCR products was carried out by visualization of the

amplicons (75-90bp) on a QIAxcel capillary electrophoresis system. Precision studies were carried out for inter and intra-assay variability and yielded a coefficient of variation of <15% at the low end of the quantification limit. Using a combination of patient samples and synthetically designed gBlock fragments we have implemented and validated a qRT-PCR test for Zika virus, which is currently available in our institution. Having an "in-house" assay allows for faster turnaround times for our patients and clinical colleagues and enables us to provide a higher level of clinical care.

B-203

A Laboratory Developed, 106 Mutation Cystic Fibrosis Carrier Screening Test Using the Agena MassARRAY®: A Review of the First 123,000 Tests

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Background: In 2001 and 2004, ACOG and ACMG recommended population-based carrier screening for cystic fibrosis (CF) using a panel of 23 mutations. Since then, some have argued that expanded panels with additional mutations would be useful in some populations. We co-developed a 106 mutation CF screening test with Agena Biosciences (formerly Sequenom, Inc.). Analytical verification of the assay has been presented (Farkas et al. JMD 2010;12:611-9). Here we report the data obtained from the first 123,172 test preformed at Mayo Clinic, and ask whether extended carrier screening panels can increase the sensitivity of CF carrier detection. **Methods**: The MassARRAY® CF screening test was validated according to CLIA guidelines. The workflow consists of 8 multiplex PCR reactions, removal of dNTPs and primers with Agencourt® AMPure® XP resin (Beckman-Coulter), 8 multiplex single base extension (SBE) reactions, desalting with an ion exchange resin, spotting the extended SBE products onto a MassARRAY chip, and analysis by MALDI-TOF mass spectrometry. 48 samples and controls are run per batch in 384 well plates. All steps are automated, and the test requires 9 hours to complete. The method requires only general purpose laboratory reagents, thus the test is inexpensive at under $30 per test. Since validation, the MGL has tested **123,172** clinical samples. **Results**: Validation of accuracy included analysis of a set of 43 samples that contained 60 different mutations and potential interferences (such as E75Q and 711+3A>G), 28 of these samples were compound heterozygotes. In addition, 594 samples were tested in parallel (15 runs) with the current Luminex 70+5 commercial assay, 25 of these samples had one mutation detected by the Luminex assay. For both sets of samples, there were no discordant results. Of the clinical samples tested, **3,153** were for a possible or known diagnosis of CF, pancreatitis, or male infertility; the remainder had indications for testing of routine carrier screening, a family history of CF, a partner that was a known CF carrier, or no indication given. Of the samples tested, **472** had two mutations (**226** homozygotes and **246** compound heterozygotes). Of these, there were **203** deltaF508 homozygotes, and **184** cases with one deltaF508 with another mutation. There were **4,647** cases with one CF mutation identified. Of the 123,172 total cases tested, this represents a **3.8%** carrier frequency, which is concordant with literature estimates. These numbers do not include 498 carriers of one copy of the R117H variant with a 7T allele. These results are reported, but not as CF carriers - rather carriers of a mild variant not typically associated with classical CF. Of the cases with one mutation identified, **4169 (89.7%)** were ACMG panel mutations; **3,293** of which were **dF508**. We have detected **66** different mutations, including all of those on the 23 mutation ACMG panel. **Conclusions**: Of all detected mutations, **10.3%**, were non-ACMG panel mutations, indicating that a significant fraction of the population can benefit from screening with an expanded panel. The MassARRAY instrument has proven to be a robust, cost effective platform for a laboratory developed CF carrier screening test.

B-204

A rare cytogenetic finding: Ring chromosome 22

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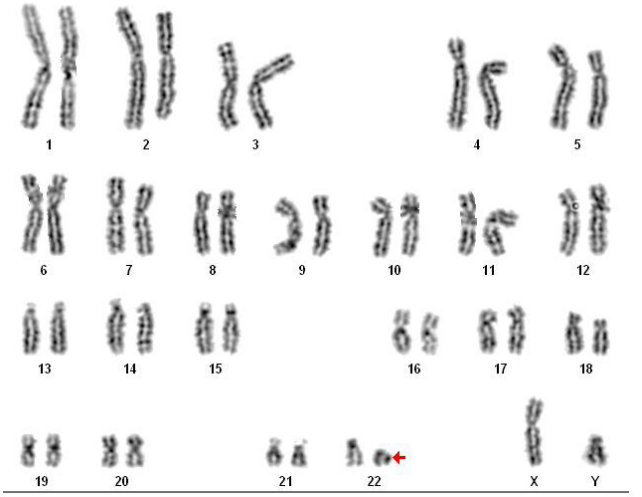
Background: Ring chromosome 22 is a rare cytogenetic finding and was first described in 1968. Since then about 60 patients have been reported. The major features of this syndrome includes intelectual disability, hypotonia and developmental delay. Here we describe a 4 years old male with intelectual disability, developmental delay and a Karyotype with ring chromosome 22.

Methods: A 400-bands Karyotype were performed on methaphase chromosomes obtained from peripheral blood lymphocyte.

Results: The patient´s karyotype showed the presence of one chromosome 22 shaped-like ring. The karyotype: 46,XY,r(22)(p13q13) (Figura1), was observed in 20 metaphases.

Conclusion: The majority of r(22) are formed de novo but there are reports of familial trasnmission, therefore the karyotype of the father and mother of the patient should be performed. The phenotypic differences of this syndrome could be the result of diferent size deletions in chromosome 22. The most frequently observed features of individuals with ring chromosome 22 overlap with the features of 22q13 deletion Syndrome, in which haploinsufficiency of SHANK3/PROSAP2 is suggested to be responsible. A few cases of ring chromosome 22 were characterized by molecular studies, and in most of them the segment containing the locus ARSA was deleted. This report underscores the variability in ring structure and clinical presentation of the patients and adds information to the limited literature on this rare disorder.



B-205

A fertile patient with an unusual karyotype finding for a mosaic of Turner Syndrome and a 4p16 translocation

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Background: Turner Syndrome is the most common sex chromosome abnormality in females, occurring in approximately one in 2500 live births. Women with Turner Syndrome are at extremely high risk for primary ovarian insufficiency (POI) and infertility. Although about 70-80% have no spontaneous pubertal development and 90% experience primary amenorrhea, the remainder may possess a small residual of ovarian follicles at birth or early childhood. The t(4;8)(p16;p23) is the second most common constitutional chromosomal translocation and is caused by an ectopic meiotic recombination between the olfactory receptor gene clusters (ORGC), located on chromosome 4p and 8p. Given that ORGCs are scattered across the genome and make-up about 0.1% of the human genome , translocations between 4p16 and other regions might be mediated by ectopic recombination between different ORGC. Purpose: To describe a fertile patient with an unusual karyotype finding for a mosaic of Turner Syndrome and a 4p16 translocation and her son with the inherited translocation and intellectual disability.

Method: A 400-band Karyotype was performed in peripheral blood lymphocyte of the patient and her son. The patient had 30 metaphases analyzed and her son 20 metaphases.

Results: The patient´s karyotype revealed 90% of metaphases with a monosomy of the sex chromosome X and the translocation between short arm of chromosome 4 and the long arm of chromosome 8: mos45,X,t(4;8)(p?16;q22)[27]/46,XX,t(4;8)(p?16;q22)[3](Figure1). The patient´s son karyotype revealed in the twenty metaphases a translocation between short arm of chromosome 4 and the long arm of chromosome 8: 46,XY,t(4;8)(p?16;q22) (Figure2).

Discussion: Primary ovarian insufficiency is a classic feature of Turner Syndrome. The pregnancy outcomes in spontaneous pregnancy are more favorable than those after oocyte donation in Turner patients but the risk of fetal chromosomal abnormalities remains high. In this case a molecular study would be important to define the breakpoints of the translocation and correlate with patient´s son phenotype.

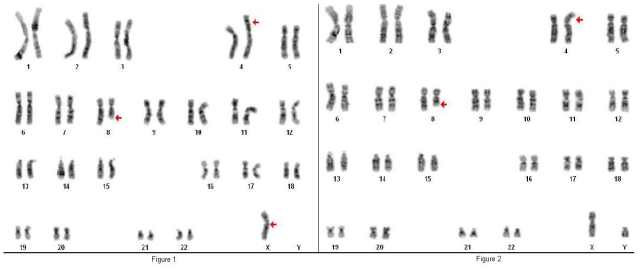

Figure 1 Figure 2

B-206

Multiplex Real-time PCR assays for Rapid Detection of group A, B streptococcus and Streptococcus pneumoniae

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Background : Streptococcus are subdivided into five main pathogenic groups commonly found in human (A, B, C, D and G). Especially group A, B streptococcus and *streptococcus pneumoniae* are risk factors cause of human disease. Streptococcus is a major infectious agent with significant morbidity and mortality, rapid and sensitive diagnosis is difficult. In this regard, real-time polymerase chain reaction (PCR) based on TaqMan technology enables the accurate detection of bacterial genome over a broad range without the necessity for post-PCR handling. In this study, we developed a multiplex real-time PCR assay (mrt-PCR) capable of simultaneously detecting group A , B streptococcus and *Streptococcus pneumoniae.*

Method: The specific primers and probes were designed based on highly conserved the capsular polysaccharide synthesis regulatory protein (cpsB) gene region of group A streptococcus, the cAMP factor (cfb) gene region of group B streptococcus and the pneumolysin (ply) gene region of *streptococcus pneumoniae*. We evaluated the analytical sensitivity, specificity, reproducibility, and reportable range of bacterial DNA load using the mrt-PCR assay. Also we established streptococcus national standards through collaborative studies with Korea Food & Drug Administation (KDFA). We have performed stability testing of certified national standards that consist of working standard (WS) and positive control (PC), since 2012.

Result: This assay showed a greatest linearity within a range from 5 x 10^2 copies/mL to 1 x 10^{10} copies/mL. The limit of detection (LOD) was 250, 350, 600 copies/mL and the percent coefficient of variation (%CV) value in the intra- and inter analysis was 0.29/0.88, 0.35/1.51, and 0.46/2.2 or group A, B streptococcus and *streptococcus pneumoniae* respectively. None of various bacteria showed a cross reactivity with three types of streptococcus. We confirmed the stability of streptococcus WS and PC, respectively.

Conclusions: The multiplex real-time PCR assay showed good analytical sensitivity, specificity and high reliability with a broad range. This assay is a powerful tool for the rapid and cost-effective diagnosis of Streptococcus.

B-207

The Shortening of Leukocyte Telomere Length in Type 2 Diabetes Mellitus with LINE1 Gene DNA Hypermethylation

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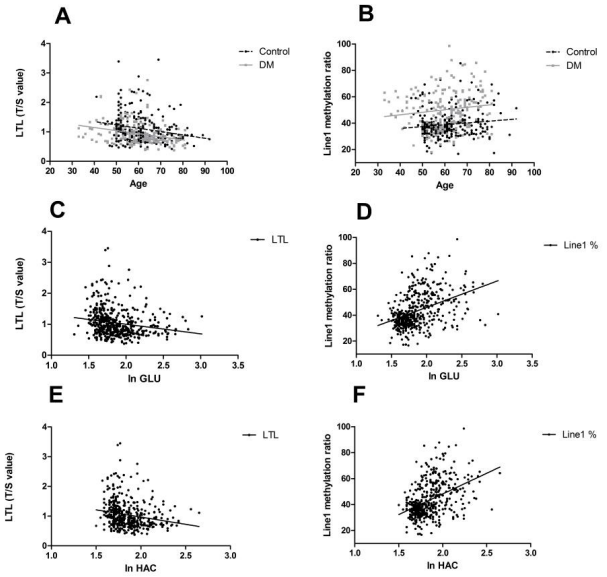
Background: Short telomeres have been linked to age-related diseases, including diabetes, but their results are inconsistent, DNA methylation has been reported with the development of type 2 diabetes mellitus (T2DM) and the shortening of telomere length, respectively. This study aims to investigate the cross-talking of leukocyte telomere length (LTL) and DNA methylation in T2DM.

Methods: We performed a hospital-based case-control study of 278 cases of T2DM patients defined as glycated hemoglobin (HbA1c) ≥6.5 %, or fasting glucose ≥126 mg/dl (7 mmol/l) and 184 of healthy normal controls visiting the hospital for a health examination, with sex and age matched, from the Peking Union Medical College Hospital, China. Circulating leukocytes were collected for LTL determination and DNA methylation of LINE1 measured by a quantitative PCR method. Biochemical variables, including serum glucose (Glu), HbA1c, homocysteine, hs-CRP, lipids-related indexs, systolic and diastolic blood pressure and body mass index (BMI) were collected. All analyses involved the use of SPSS 16.0. A two-sided P<0.05 indicated statistical significance. **Results:** LTL was significantly shortened in T2DM comparing with controls (0.93±0.38 vs. 1.11±0.48, P<0.001) and decreased steadily with age in both controls and T2DM. Reversely, we found significant increase of LINE1 DNA methylation in T2DM compared with controls (50.21±14.50 vs. 38.81±9.95, P<0.001). Spearman correlation showed LTL negatively correlated with LINE1 methylation (r=-0.280, P<0.001), ln Glu (r=-0.191, P<0.001), ln HbA1c (r=-0.196, P<0.001) adjusted for sex and age. For the multivariate linear regression analysis, we applied a principal component assay to avoiding multicollinearity of Glu and HbA1c, and found Glu, HbA1c and LINE1 methylation were stably negative related with LTL.**Conclusion:** Shortening of LTL is independently associated with the risk of incident T2DM, and closely related with the DNA methylation of LINE1. These findings provide novel insights into an epigenetic mechanism for shortened LTL in DM.



B-208

Genetic diagnosis of Familial Hypercholesterolaemia using a rapid biochip array assay for 40 common LDLR, ApoB and PCSK9 mutations.

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Background Familial Hypercholesterolaemia leads to a lifelong increase in plasma LDL levels with subsequent increase in premature vascular disease. Early diagnosis and treatment is the key to effective management of this condition. This research aims to produce a simple and cost effective genetic test which could identify the majority (71%) of mutations causing FH in the UK and Ireland.

Methods Biochip array technology was used to detect 40 point mutations in LDLR, ApoB and PCSK9 genes, over two 5X5 arrays. This technology uses multiplex allele specific PCR and biochip array hybridisation, followed by a chemiluminescence detection system and software for automated mutation calling.

Results The FH biochip array assay was validated in the Belfast Genetics Laboratory using 199 cascade screening samples previously sequenced for known FH causing family mutations, the overall sensitivity was 99%. The assay was then used for routine testing of 663 patients with possible FH, from clinics across the UK and Ireland. A total of 49(7.4%) mutation positive individuals were identified, however for the clinics in England the detection rate was 12.9%. Further analysis of 120 biochip negative patients, using DNA sequencing, did not identify any false negatives.

Conclusions The FH biochip array provides a rapid and reliable genetic test for the majority of FH causing point mutations in the UK and Ireland. A total of 32 samples can be run in 3 hours. This allows clinics to evaluate additional patients for a possible diagnosis of FH such as patients with high LDL, patients with early onset coronary disease and patients with relatives known to have FH.

B-210

Validation of a new panel for sexually transmitted infections detection at richet laboratory - Rio de Janeiro, Brazil

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Background: Sexually transmitted infections (STIs) are a public health problem, and their prevalence is rising even in developed nations. There are over 30 pathogens considered responsible for STIs; however, only eight of them are clearly related to the prevalence in the majority of clinically relevant cases. We can mention *Trichomonas vaginalis, Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma urealyticum, Chlamydia trachomatis, Neisseria gonorrhoeae*, *Herpes simplex* and *Treponema pallidum*. Technological development in laboratory diagnosis has been relevant, allowing the direct detection of pathogens in clinical samples by molecular techniques [1]. Within the diagnostic technologies by amplification of genetic material, the CLART® STI's A&B test panel offers the solution to unify the analysis techniques in addition being very useful in the diagnosis of those microorganisms that are difficult to cultivate. The aim of this study was to validate to implement the STIs panel evaluating other sample types not yet validated by the manufacturer. Also to demonstrate the relevance in detecting unexpected laboratory findings.

Methods:149 samples received at the laboratory were used for this study.

41 of these samples were from urine, 25 general swabs, 5 semen, and 78 of liquid cytology in Surepath® BD (medium not recommended by the manufacturer).

The CLART® STIs A&B (CLART® Technology from Genomica S.A.U) was used for the detection and typing of bacteria, fungi and parasites causing STIs and the results were compared with those obtained previously using the techniques ABBOTT® CT/NG (Chlamydia trachomatis, Neisseria gonorrhoeae) and TIB MOLBIOL® - distributed by ROCHE® - (Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma urealyticum).

Results: On liquid cytology samples, the concordance of results was 100%, 83% in urine, and 55% in swab. Despite of the swab case (55%), it is important to consider the collection area, as the recommended by the manufacturer is the urethral swab. Eleven tested samples from the liquid cytology were ordered to HPV test; therefore, they had no previous result for any of the microorganisms involved in STD's. However, after being tested, the positivity of these samples 27% for others microorganisms associated with STIs (*Neisseria gonorrhoeae*, *Mycpoplasma hominis* and *Candida albicans* were the most frequently found). In the case of semen, the results were not conclusive. It is necessary to increase the number of samples for better evaluation.

Conclusion: The panel showed to be faster than conventional methods and can get a reduction in overall time of diagnosis, more than 24 hours in many cases, allowing the physician to make adjustments in medication and / or therapies administered to each patient. The CLART® STIs kit has presented good results in liquid cytology and urine samples even not being the liquid cytology the specimen indicated by the manufacturer. For extraction

methodologies, all automated platforms from Qiagen, Abbott and Biomerieux showed satisfactory results.

B-211

Evaluation of the Invader Genotyping and TaqMan Copy Number Variation Assays for *CYP2D6*

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Background: Cytochrome P450 2D6 is involved in phase I metabolism of many different classes of commonly used drugs with four broad clinical CYP2D6 phenotypes that are inferred from genotypes: poor metabolizer (PM), intermediate metabolizer (IM), extensive metabolizer (EM), and ultrarapid metabolizer (UM). Accurate CYP2D6 genotyping is challenging due to the highly polymorphic nature of the gene and the presence of the pseudogenes. The aim of this study was to validate the Invader® (Hologic, Inc., Bedford, MA, USA) CYP2D6 genotyping assay and the TaqMan™ (Life Technologies, Carlsbad, CA) copy number variation (CNV) assay.

Methods: Fifty de-identified patient samples and twenty-nine Coriell DNA were assessed. After multiplexed PCR the Invader® genotyping assay detects twenty-seven variants on three non-overlapping fragments. Genotypes were determined based on the fluorescence signal ratios of the wildtype and mutant alleles. For CYP2D6 copy number analysis, three distinct TaqMan™ real-time PCR assays targeted different regions of the gene. The copy number was determined using the CopyCaller™ Software by comparing the Crossing point (Ct) of the calibrator gene RNase P (two copies). Discrimination of the duplicate alleles was done by long-range PCR followed by the Invader® genotyping or single nucleotide extension (SNE). Invader® results were compared to INFINITI™ system (Autogenomics, Inc., Carlsbad, CA) and Sanger sequencing.

Results: Genotypes and copy number determined by the Invader® and TaqMan™ assays for the twenty-nine Coriell DNA samples were 100% concordant with previously published results except for one sample for copy number analysis. This sample was omitted from the copy number validation because of a low CopyCaller™ confidence score. The Invader® genotyping for fifty patient DNA were 100% concordant with the INFINITI™ system except for two samples, which was due to the lack of the *45 allele in the INFINITI™ panel. The presence of the allele *45 was confirmed by Sanger sequencing. Copy number analysis on the patient DNA were 100% concordant with the results obtained using the long-range PCR. Invader® inter-run and intra-run and TaqMan™ inter-run precision studies showed 100% reproducibility. Analytical sensitivity was determined to be 10 ng of DNA for the Invader® assay and 2 ng of DNA for the TaqMan™ assay, respectively. For determining the duplicate allele, seventeen DNA samples with three copies of CYP2D6 were compared to results with long-range PCR with SNE analysis. Sixteen samples showed the same results with the exception of one sample, determined to be *29/*43X2 by the Invader® test and *1/*1X2 by the comparative method, which did not test for *43. The inter-run precision of the duplicate allele discrimination assay showed 100% reproducibility.

Conclusion: Evaluation of CYP2D6 genotyping using the Invader® and TaqMan™ CNV assays shows that the results are valid, robust and highly reliable, which makes these assays particularly relevant for the implementation in the clinical practice.

B-213

Dengue Outbreak in São Paulo-Brazil (2015): Use Polymerase Chain Reaction for Detection and Typing of the Ethiological Agent

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Background: Dengue viruses (DENV) are widespread in Brazil and have been implicated with several outbreaks around the country. An important increase in the number of cases have been reported last year, particularly during summer and early autumn at São Paulo city, Brazil and an emergency unit for initial care and diagnosis was set up in the North region of São Paulo City. The discovery of Chikungunya (CHIKV) and Zika (ZIKV) viruses in Brazil reinforced the need for rapid and reliable assay to detect the presence of these infections among the patients. The objective of this study is detect and characterize DENV using real-time 3M Integrated Cycler PCR instrument in samples collected during an epidemic in São Paulo, Brazil. **Methods:** We analyzed 1,461 samples from suspected cases of DENV or other arboviruses infections attended at the the Basic Health Unit (BHU) Jardim Vista Alegre, North region of São Paulo City, Brazil during the epidemic period of 2015 were assayed at the Special Techniques Laboratory, Hospital Israelita Albert Einstein (HIAE), São Paulo, Brazil. For the detection of anti-dengue antibodies of types 1-4, immunoassay Dengue Virus IgM Capture Dx Select ™ (Focus Diagnostics Cypress, California USA) was utilized. Simplexa Dengue test using the 3M Integrated Cycler real-time PCR instrument (Focus Diagnostics Cypress, California USA) was applied for the qualitative detection and discrimination of DENV1-4 serotypes. An internal control RNA was used to monitor the efficiency of the extraction process and to detect the inhibition of RT-PCR. In addition, some of the samples with negative results for DENV were also analyzed using the Light Mix Chikungunya Kit - Virus (TIB MOLBIOL- Berlin, Germany) in Cobas z480 equipment (Roche Diagnostics, USA). **Results:** Of the 1,461 samples studied, 1.048 (71.73%) were positive and 413 (28.27%) negative for the 3M Integrated Cycler, whereas the ELISA test detected 312 (21.35%) and 207 (14.16%) samples for IgM and IgG, respectively. Indeterminate serology results appeared in 129 (8.82%) and 46 (3.15%) samples for IgM and IgG, respectively. DENV1 was the predominant strain, found in 99.7% (1.059 / 1.062) DENV positive samples, followed by 0.2% (2 / 1,062) DENV-4 and 0.1% (1 / 1,062) DENV-1/2 coinfection. DENV-3 was not detected in the study population, as well as CHIKV, that was not detected in 409 analyzed samples for this virus. **Conclusion:** As expected, the search for DENV using PCR allowed earlier detection of this infection when compared to IgG/IgM antibodies assays. The use of PCR is an alternative to NS1 test to detect the viral infection in the first days of infection. Furthermore, PCR

is the sole technique that allows distinguishing the different types of DENV. CHIKV was not found in the samples analyzed.

B-214

Prevalence of CARD15 among Crohn's disease patients and its relation to disease severity

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Crohn's disease is a chronic, episodic, inflammatory condition of the gastrointestinal tract. It causes complications outside the gastrointestinal tract. The exact cause of Crohn's disease is unknown; with genetic and environmental implications.The aim of this work was to study the prevalence of CARD15/NOD2 gene variants among Crohn's disease patients and correlate the genotypes with the extent and severity of the disease. **Subjects and Methods**: This study included100 patients and 100 age matched healthy controls. All subjects undergone immunological investigations, ASCA IgA and IgG and p ANCA by ELISA immunoassay, **The Crohn's disease endoscopy index of severity (CDEIS**) was calculated as a subscore for each segment explored, including the presence of deep ulcers, superficial ulcers, the estimated surface area of affected mucosa and ulcerated mucosa. **CDAIndex:** where Inactive (< 150), Mild activity (150-245), Moderate activity (>245) Severe (>450 Detection of CARD15/NOD2 polymorphisms was done after DNA extraction followed by PCR amplification of the targeted area then digestion of the amplified sequence (PCR-RFLP) by the appropriate restriction enzyme (**R702W, G908R and 1007fs). Results**: The CARD15 was positive among 17% of Crohn's disease patients while it was positive among 20% in control group. The distribution of CARD15variants in Crohn's disease patient group was as follows: **1007 FS** variant was positive in 6% of patients, **R702W** was positive in 7% of patients and **G908 R** was positive in 4% of patients. While in the control group; **1007 FS** variant was positive in10% of patients, **R702W** variant was positive in 5% of patients **G908R** variant was positive in 5% of patients. It was found that 19.0% of patients was inactive (<150) ,64.0% showed mild activity (150-245) 17.0% of cases showed moderate activity (>245) . The percentage of CARD15 gene was higher in ileal location of Crohn's disease and higher in inflammatory behavior, but didn't reach statistical significance. There was positive correlation between CARD15 gene, CDAI and ASCA, IgA, IgG and ANCA respectively.CARD15 status was higher with mild activity of CDAI, but didn't reach statistical significance. The present study showed the percentage of positive CARD15 gene in ASCA positive (17.6%) while in ANCA positive (0.0%). Seromarkers showed positive ASCA in 18% while negative ASCA was in 82%. Positive ANCA was found in 2% while Negative ANCA was found in 98.0%. Positive CARD15 was 17.6% with ASCA Positive, while Positive CARD15 was 82.4% with ASCA Negative. There were no statistical significant differences between CARD15 gene and ASCA and ANCA respectively **Conclusions.** The presence of CARD15 (R702W, G908R and L1007fs) mutations in unaffected persons indicates additional genes or environmental factors are necessary for CD development Thus CARD15 mutations were not sole factor implicated in susceptibility to Crohn's disease. However it could help in prioritization of genetic mechanisms that contribute to the expression of IBD. The identification of key genes serves to prioritize development of new therapeutic approaches in IBD as well as help in the development of predictive risk models

Key words: CARD15, CD, CDAI, PCR-RFLP.

B-215

Validation of a qualitative PCR for Zika virus in a private hospital in Sao Paulo, Brazil

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Background: Zika virus (ZKV) is an RNA virus from the genus *Flavivirus* which causes a tropical disease that is transmitted to humans by infected *Aedes* mosquitoes, the same vector of dengue (DENV) and Chikungunya (CHIKV) viruses. In 2015, the first autochthonous ZKV infection was confirmed in Brazil and there was a dramatic increase in reports of ZIKV infection. Although the disease is self-limiting, the Ministry of Health of Brazil recent reports an unusual increase of cases of microcephaly among newborns in the Northeast region, which indicates a possible association between ZIKV infection in pregnancy and fetal malformations. Guillain Barré syndrome cases have also been related to ZIKV infection. Clinical diagnosis is difficult due to the similar symptoms with DENV and CHIKV, so accurate diagnosis is crucial for patient management and public health interventions. Laboratory diagnosis can be accomplished by testing samples to detect viral RNA and anti- Zika IgM/IgG antibodies. However, only molecular test can identify ZKV in the first days after infection. In order to offer a high quality molecular ZKV diagnostic test in our service, we validated a laboratory developed test for this purpose.

Methods: Nucleic acids from plasma and urine samples were extracted using QIAamp Viral RNA Mini Kit (Qiagen) followed by a real time PCR assay using SuperScript® III Platinum® One-Step qRT-PCR Kit (Invitrogen™). According to CAP guidelines, we evaluated accuracy, reproducibility, analytical sensitivity and analytical specificity of the test.

Results: For accuracy, we compared the results of 30 samples and obtained 100% of correlation. Analytical sensitivity of the method was evaluated by testing dilutions of a sample with known copy number and was established in 800 copies/mL. Reproducibility was check using two negatives and two positive´s samples, tested in three different days by different persons. The results were concordant. For the specificity, we tested 10 samples positives for DENV type 1 and one sample positive for CHIKV, and all of them were negative for ZKV.

Conclusion: The results of validation demonstrated that the test is reliable and useful for detection of ZKV in plasma samples, and combined with its fast turnaround time and decreased hands-on time, make this assay highly suitable for the rapid diagnostics of ZKV infections in the clinical laboratory. Molecular methods are sensitive tools for the diagnosis of this viral infection in the early viremic stages of illness. Since ZKV, CHIKV and DENV infections produce similar clinical signs, it is important for physicians to differentiate them rapidly, particularly in pregnant women's.

B-216

Validation of *CFTR* complete gene sequencing, a suitable test for highly heterogeneous Brazilian population

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Background: Cystic fibrosis (CF) is a genetic disorder caused by deleterious variants in *CFTR* gene. Although p.F508del is the most common CF-causing variant, over 2000 variants have been described. The frequencies of specific variants oscillate widely across populations, and are sometimes shaped by founder effects. Generally, the first screening in *CFTR* is for p.F508del. Beyond this variant, a number of screening panels have been proposed, such as the American College of Medical Genetics (ACMG) panel composed of 23 variants. Due to the heterogeneous composition of most populations, screening panels often bypass low frequency pathogenic variants segregating in specific populations. That implies that some locally relevant deleterious variants might be missed, causing misdiagnosis, inefficient carrier screening and equivocal genetic counseling. For this reason, complete gene sequencing is the most efficient methodology for *CFTR* genetic testing, especially useful for highly heterogeneous and understudied populations. Our aim in this study was to validate the complete *CFTR* gene sequencing in a population from São Paulo, Brazil, by Next Generation Sequencing (NGS), a high-throughput, reliable and progressively accessible technology.

Methodology: DNA extracted from whole-blood samples of twenty-one patients with previously known variants for *CFTR* gene was used. Sixteen samples were positive for pathogenic variants. Of those, fourteen samples had a pathogenic or probably pathogenic variant in both alleles. Five samples were negative either for pathogenic, probably pathogenic or variants of uncertain significance (VUS). *CFTR* gene library was built using the Ion AmpliSeq *CFTR* panel, which includes exons, intron-exon boundaries and UTRs. Amplicons were fully sequenced in Ion Torrent PGM Sequencer. Pathogenic variants and regions with coverage below 50X were verified using Sanger sequencing. Validation followed CAP guidelines, evaluating accuracy, reproducibility, specificity and sensitivity of the test. To test for reproducibility, replicates were done: 2 positive samples were sequenced in triplicate, and 3 samples (2 positive and 1 negative) were sequenced in duplicate. The reproducibility tests were performed in different routines by different analysts. Read mapping and variant calling were performed in two softwares each, with the following combination: Torrent Mapping Alignment Program (TMAP) + Torrent Variant Caller (TVC) vs. 4.2 and Burrows-Wheeler Aligner (BWA) + Atlas2 vs. 1.4.3. Final variants were classified following the ACMG guidelines for interpretation of sequence variation.

Results: All samples were successfully sequenced for the entire gene. Variants were entirely concordant with previous results, resulting in 100% accuracy. In total, seventeen different pathogenic or probably pathogenic variants were detected: 7 indels (5 frameshift, 2 inframe), 10 substitutions (3 missense, 5 nonsense, and 2 splice-site), indicating 100% sensitivity and specificity. Variant p.F508del was detected in 8

patients (2 homozygotes). One VUS missense substitution was also detected. Results of replicates showed successful reproducibility.

Conclusion: We validated the complete sequencing of *CFTR* gene in a NGS platform, which resulted in a reliable genetic test for CF molecular screening. Based on our results we are confident that this genetic test can be securely offered to the highly admixtured Brazilian population.

B-218

Molecular tension probes to investigate the mechanopharmacology of smooth muscle cells

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Background: The contractile phenotype of airway smooth muscle cells is an established characteristic of asthma, a chronic lung disease characterized by airflow obstruction. Airway smooth muscle (ASM) cells generate cellular contractile forces that lead to bronchoconstriction and airflow obstruction. Bronchodilators are used to relax the airway smooth muscle cells to relieve the obstruction and increase airflow. Therefore, an assay measuring cellular forces can potentially screen bronchodilators for relative efficacy. To examine this, we used fluorescent molecular tension sensors to determine the EC50 values of albuterol in normal and asthmatic ASM cells and demonstrate that molecular tension probes are a potential diagnostic tool for personalized medicine. In this study, we measured integrin-mediated forces in ASM cells using molecular tension sensors to study the effects of chronic nicotine exposure in asthma by determining the EC50 value of albuterol in normal and asthmatic ASMs exposed to nicotine. **Methods:** Diseased and healthy human airway smooth muscle cells isolated from asthmatic or healthy donors were used in this study. Titin-based gold nanoparticle sensor surfaces were fabricated as following: I27 constructs were recombinantly expressed where the I27 domain of titin, an immunoglobulin domain found in the sarcomere, was flanked by a linear RGD peptide, a canonic motif found in fibronectin, at the N-terminal for cellular adhesion and two cysteines at the C-terminal for the attachment onto 9 nm gold nanoparticles. The gold nanoparticles were immobilized onto a glass surface through silane - NHS lipoic acid chemistry. In addition, the N-terminal of the I27 domain was decorated with a fluorescent dye that is quenched when in close proximity to the gold nanoparticle. Smooth muscle cells were added to the sensor surface where the integrin cellular receptors recognize the adhesion peptide and stretch the sensor, thus generating fluorescence signal at adhesion sites. Upon addition of the bronchodilator albuterol, we obtained a fluorescent dose response curve from which the EC50 values were determined. **Results:** The EC50 value for normal ASM cells incubated on the titin-based sensor and treated with the albuterol was 9 +/- 2 nM. The addition of nerve growth factor (NGF), a neurotrophin implicated in asthma pathobiology, shifted the EC50 to 45 +/- 11 nM. In comparison, EC50 of asthmatic smooth muscle cells without NGF was 21 +/- 4 nM and was shifted in the presence of NGF to 140 +/- 41 nM. Chronic nicotine treatment of asthmatic smooth muscle cells resulted in the EC50 value of 198 +/- 38 nM. **Conclusion:** We obtained EC50 values of albuterol for cells plated on molecular tension sensors as a readout of the contractile phenotype of asthmatic and normal airway smooth muscle cells. When comparing normal and asthmatic smooth muscle cells, the latter showed higher EC50 values both in the absence and in the presence of NGF. However, chronic nicotine treatment of asthmatic cells resulted in the highest EC50 value, which showed that nicotine changes the contractile phenotype of smooth muscle cells *in vitro*.

B-219

Expression of Matrix Metalloproteinases (MMPs) MMP-3 and MMP-8 Before and After Treatment with Etoricoxib in Patients with Chronic Periodontal Disease

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Introduction: In inflammatory periodontal pathology, presence of bacteria in subgingival level causes the host cells with the inflammatory response, resulting in the synthesis of MMPs, degradation of extracellular matrix and destruction of periodontal tissue and bone.

Aim: This study sought to highlight the expression of matrix metalloproteinases MMP - 8 and MMP - 9 in patients with chronic periodontal disease before and after treatment with etoricoxib.

Material and methods: we included in our study 23 patients with chronic periodontal disease and 11 healthy patients (control group), aged between 35 and 65 years, 16 female and 18 male, followed-up as outpatients at dental office *Dentissimo Dental Care* Timisoara, Romania.

The study was conducted during September 2014 - January 2015. Patients were administered Etoricoxib in the pharmacological dose of 90 mg, for 6 months, 7 days/month.

Biological material was collected by gingival curettage. RNA was extracted from biological material that has been amplified by RT-PCR. Gene expression of interest was highlighted by agarose gel migration.

Results: The analysis of gene expression before treatment revealed overexpression of MMP-3 in 19 (82.6%) patients and overexpression of MMP- 8 in 21 (91.3 %) of patients included in our study.

Following treatment with etoricoxib, we noted decrease of studied gene expression in two patients from the experimental group.

Conclusions: The results obtained showed the biological effects of this drug in the pathology of periodontal disease; two matrix metalloproteinases can be used in the therapeutic management of this condition.