HEALTH SCIENCES

HS - 01

A SYSTEMS VIEW OF THE HOX-OME

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In mouse and humans, the correct location of body parts along the A-P axis is specified by the expression boundaries of 39 Hox genes. Regulated in a spatial and temporal fashion, these genes, dubbed as 'master genetic switches', instruct embryonic cells on a particular route of morphogenesis. Focusing on Hoxc8, we have established its first network map from the results from our previous genome-wide screening for Hoxc8-associated molecules using a combination of CHIP-based screening and cloning and 2-DGE proteomics screening. Together with available literature data on the transcriptome and proteome of Hoxc8, the predominant signaling pathways were identified. The molecular networks and interaction of diverse signaling pathways revealed the important and overlapping roles played by Hoxc8 during both embryonic development and carcinogenesis.

Keywords: Hoxc8, cancer, embryogenesis, systems biology, molecular networks

HS - 02

ALLELIC DISCRIMINATION FOR SINGLE NUCLEOTIDE POLYMORPHISM IN INTERLEUKIN 28B GENE USING 5' NUCLEASE ASSAY

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With the development of single nucleotide polymorphism (SNP) genotyping assays and the availability of fluorogenic probes that anneal specifically to its complementary sequence, it is now possible to use real-time PCR to detect SNP alleles in purified genomic DNA samples. DNA was extracted from peripheral blood of patients clinically diagnosed with chronic hepatitis B or C using the Taqman sample-to-SNP kit. Real-time PCR was performed on the Rotor-Gene 3000 instrument (Corbett Research) with a SNP genotyping assay for rs8099917. The rs8099917 G to T polymorphism on chromosome 19, located near IL28B gene which encodes interferon-λ3 has been shown to be associated with sustained virologic response in patients with chronic hepatitis C treated with pegylated interferon-α plus ribavirin. Recently, it has also been shown that the G allele of rs8099917 was associated with higher rate of response in chronic hepatitis B patients treated with interferon-á. Thus, a reliable method for the accurate identification of IL28B SNP is important for the management of chronic hepatitis. Allelic discrimination was performed using the Rotor-Gene 6.1 software. The allele frequencies in Hardy-Weinberg equilibrium among cases were 0.75 for the T allele and 0.25 for the G allele. Fifty six per cent was identified as homozygous for the wild-type T/T genotype, 38% was identified as heterozygous for the G/T genotype, and 6% was identified as homozygous for the G/G variant genotype. Overall, the 5' nuclease assay provides a rapid and automated method for detecting SNP in the IL28B gene.

Keywords: 5' nuclease assay, chronic hepatitis, interleukin 28B gene, single nucleotide polymorphism

HS - 03

ANTIPROLIFERATIVE ASSESSMENT AND MECHANISTIC PROBING OF THE PROMISING ANTI-CANCER BISINDOLE ALKALOID GLOBOSPIRAMINE FROM *Voacanga globosa*

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Globospiramine, a new bisindole alkaloid from the Philippine endemic Apocynaceae plant *Voacanga globosa* was observed to possess potent antituberculosis and anti-Alzheimer's activity. As part of a continuing study to elaborate the biological potency of globospiramine, investigations directed towards its anti-cancer activity were undertaken. Cell viability assays (MTT and SRB) showed globospiramine to be cytotoxic to various cancer cell-lines at very low microgram/mL or micromolar concentration ranges (HUVEC GI_{50} = 5.4 μg/mL; K-562 GI_{50}=1.4 μg/mL; HeLa CC_{50} =6.1 μg/mL; T47D IC_{50}=1.11 μM; MDA-MB-231 IC_{50}=1.17 μM; PC-3 IC_{50}=1.77 μM). Reporter assay on T47D cells lines transfected with pHRE-luc plasmid showed no effect on HIF activity at low concentrations but had a significant inhibitory effect at concentrations greater than 5 mM under both chemical and physical hypoxia. In the mitochondrial respiration assay, globospiramine showed increased mitochondrial respiration rate slightly at 0.1 mM indicating little mitochondrial uncoupling activity. In the anti-tubulin polymerization assay, globospiramine appeared to have a different mechanism of action revealing total non-specific cell death with crenated shrunken cells and at some point, may have led to loss of microtubules during apoptosis or via calcium entry – a behavior not seen in related anticancer alkaloids, vincristine and vinblastine.

Keywords: globospiramine, *Voacanga globosa*, bisindole alkaloids, anti-cancer, mitochondrial cytotoxicity, anti-tubulin polymerization.

HS - 04

APOLIPOPROTEIN E ε4 AS A FACTOR IN THE EVALUATION OF THE GENETIC RISK OF ALZHEIMER'S DEMENTIA IN FILIPINOS

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In the Philippines, there are 3.8 million people above 65 years old who are at risk of developing Alzheimer's dementia. This report presents the results of a cross-sectional study to determine the genotypic distribution of *ApoE* in affected and unaffected groups of Filipinos, who either consulted in the hospital for Alzheimer's dementia or were screened in a community setting. Peripheral blood was collected from a total of 600 subjects, composed of 124 patients who consulted at the Memory Center of St. Luke's Medical Center, Quezon City, and 476 individuals who participated in a community-based cohort. DNA from the buffy coat was analyzed for *ApoE* genotype by PCR-RFLP (Hha I) method. The most frequent genotype for both groups was ε3/ε3: 51.6% for the hospital group and 66.6% for the community group. The clinical profile of all the subjects is: 96 with Alzheimer's dementia, 110 with mild cognitive impairment, 369 with no dementia, and 25 with diagnoses other than dementia. In this group of Filipinos, the *ApoE* ε4 allele was significantly associated with risk for Alzheimer's dementia, compared with the no dementia group, with a p-value of 0.0481 and an odds ratio of 1.611. The frequencies of the risk ε4 allele were as follows: 14.1% for those diagnosed with Alzheimer's dementia, 9.1% for those with mild cognitive impairment, 9.2% for those with no dementia, and 6.0% for those with diagnoses other than dementia. These results suggest that the detection of the presence of the *ApoE ε4* allele could be a useful tool for the assessment of genetic risk for Alzheimer's dementia in Filipinos when integrated with the patient's clinical data and family history.

Keywords: Alzheimer's disease, dementia, apolipoprotein E, PCR-RFLP, genetics

HS - 05

ASSOCIATION OF ANGIOTENSIN CONVERTING ENZYME DD GENOTYPE WITH INCREASED RISK OF SEVERE CORONARY STENOSIS IN THE FILIPINO POPULATION

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The evidence supporting the association of the angiotensin converting enzyme (*ACE*) and the susceptibility to coronary artery disease (CAD) across other populations remains debatable. In this study, the association between *ACE* I/D polymorphism and the development of severe stenosis was determined among Filipinos who underwent coronary angiography in St. Luke's Medical Center, Philippines. A total of 215 patients aged 18-85 years of age were enrolled for the study with 166 patients with e"70% angiographically established stenosis (severe stenosis) and 49 with <70% stenosis as controls. The *ACE* I/D polymorphism was assessed by polymerase chain reaction wherein primer binding sites flanked the 287 bp *alu*-sequence deletion, hence discriminating the I and D alleles with difference in PCR amplicon sizes in an agarose gel electrophoresis. The *ACE* I/D polymorphism showed concordance with Hardy-Weinberg Law with allele frequencies of 0.61 and 0.39 for the I and D alleles, respectively. Multiple logistic regression analysis after adjustment with several potential confounders showed that DD genotype poses a higher risk of developing severe stenosis when compared with to the II genotype with an OR of 4.37 (p value 0.033, CI 1.13-16.9). A univariate analysis with a larger sample size (n=500, e"70% stenosis = 382, <70% stenosis = 118) was also performed for several other genes related to CAD. The homozygosity to angiotensinogen Thr235 in reference to having at least one Met235 allele (MetMet + MetThr) also showed significant association to e"70% stenosis with an OR of 3.38 (p value 0.033, CI 1.16-9.84). In conclusion, this study supports previous findings indicating that the RAS pathway may play a role in the pathogenesis of coronary artery disease.

Keywords*:* Coronary artery disease; Stenosis; Angiotensin converting enzyme; Angiotensinogen; Cardiovascular genomics

HS - 06

ASSOCIATION OF LIPOPROTEIN LIPASE HINDIII POLYMORPHISM WITH CORONARY ARTERY DISEASE IN FILIPINO PATIENTS

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The Genomics and Cardiovascular Medicine Initiative is a research program aimed at elucidating the genetic basis of cardiovascular disease in Filipino patients seen at the Heart Institute of St. Luke's Medical Center. One of the gene polymorphisms studied is the Lipoprotein lipase (LPL) HindIII T>G and has been associated with coronary artery disease (CAD) in a number of ethnic groups including Asians and Caucasians. To determine genotype and allele frequencies and establish disease association of the LPL HindIII polymorphism in Filipino CAD patients, we performed PCR-RFLP genotyping of DNA samples from patients who underwent coronary angiography. Out of 1,271 patients enrolled (mean age=58.50), 880 (69.2%) were males, and severe stenosis (e"70%) was seen in 848 (66.7%) patients (from St. Luke's Cardiovascular Disease Information System). Genotype frequencies were as follows: TT=830 (62.9%), TG=237 (18.6%), and GG=234 (18.4%). The T allele frequency was 0.72. Chi-square test revealed a significant association between LPL HindIII and obesity (p=0.009) but not with other risk factors. Adapting a case-control association study using % stenosis as parameter, genotype frequencies of normal patients, deviated from Hardy-Weinberg equilibrium but several significant associations were established. In normal patients, LPL HindIII variant was associated with obesity (p=0.031) and myocardial infarction (p=0.044). The T allele in normal patients had a high OR (2.638, CI: 1.098-6.348) for obesity and low OR (0.425, CI: 0.209-0.866) for myocardial infarction, indicating that in normal individuals the presence of the allele could mean increased susceptibility to obesity and/or protection from myocardial infarction.

Keywords: lipoprotein lipase, CAD, Filipino, allele frequency, PCR-RFLP

HS - 07

ASSOCIATION OF URINARY IODINE EXCRETION LEVEL TO PHYSIOLOGICAL STATUS OF FILIPINO WOMEN

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Urinary iodine excretion (UIE) is currently the most practical biochemical marker for iodine nutrition. For this biomarker, international groups have recommended school-aged children as useful target group for surveillance because of their combined high vulnerability, easy access, and applicability to a variety of survey activities and as an acceptable proxy for the iodine status of the general population. But the relevance of this group to others, especially among pregnant and lactating women, is not well established. In the present study, UIEs of 442 pregnant and 830 lactating women were compared to UIEs of 1272 age- and BMI-matched non-pregnant, non-lactating women covered in the Biochemical Phase of the 7th National Nutrition Survey conducted by the Food and Nutrition Research Institute, Department of Science and Technology. Among pregnant women, median UIE was 100 μg/L (adequate e"150 μg/L) with 26.4% having values <50 μg/L. Median UIE among non-pregnant women, on the other hand, was 142 μg/L with only 17.5% having values <50 μg/L. Likewise, median UIEs among lactating and non-lactating women were 78 μg/L and 141 μg/L, respectively. Median UIEs for both pregnant and lactating women indicate insufficient iodine status. Further, median UIE levels for the 1st, 2nd and 3rd trimesters of pregnancy were 113, 107 and 89 μg/L, respectively. Iodine nutrition among non-pregnant, non-lactating women was optimal based on median UIE (e"100 μg/L) and the percentage (<20%) of women having UIE<50 μg/L. In conclusion, physiological status such as pregnancy and lactation possibly increases the demand and utilization of iodine. In pregnancy, this may also be true as gestation progresses. To achieve optimal nutrition in these vulnerable groups, an increase in dietary intake is recommended. In addition, monitoring of their iodine status, as well as supplementation during pregnancy and lactation should be considered.

Keywords: iodine, urinary excretion, biomarker, pregnancy, lactation

HS - 08

DEVELOPMENT OF A PANEL OF PROTEIN DIAGNOSTIC BIOMARKERS USING NANO-LC-ORBITRAP MS/MS IN URINARY PROTEOME BEFORE RADICAL PROSTATECTOMY OF PATIENTS WITH PROSTATE CANCER

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Global analysis of protein structures *via* nano-LC/MS/MS provides a wealth of information. Bioinformatics allows simultaneous identification of all the contributing proteins in a disease. Given the limitations of PSA test for prostate cancer, these developments in instrumentation, the facile and non-invasive acquisition of urine as a source of proteins similar to blood can provide an alternative for the discovery of novel protein biomarkers. This research aims to identify the protein biomarkers for the early detection of prostate cancer using gradient SDS-PAGE followed by LC-MS/MS analysis aided by Protein Prospector, SwissProt and XCalibur. Transthyretin, hemoglobin alpha and beta were the three protein biomarkers identified in patients with prostate cancer. They are linked to high TNM stage and Gleason scores. Uromodulin and mannan binding lectin protease 2 can distinguish BPH from prostate cancer. These proteins belong to acute phase response proteins like C-reactive proteins which are associated with inflammation and oxidative stress. Hemoglobin alpha and beta as carriers of oxygen justify them as excellent biomarkers. Novel mutations due to oxidation and their reaction mechanism are also shown and discussed. Using a high-throughput method, a panel of biomarkers was identified simultaneously making it possible to differentiate urine of the normal controls from the patients with prostate cancer and another panel of protein biomarkers to distinguish benign prostate hyperplasia from prostate cancer.

Keywords: panel of biomarkers, transthyretin, hemoglobin subunit alpha and beta, uromodulin, mannan binding lectin protease 2, TNM stage, Gleason score, BPH, prostate cancer

HS – 09

ASSESSMENT OF HEAVY METAL (ARSENIC, CADMIUM, LEAD AND MERCURY) CONTENTS OF COMMONLY-CONSUMED SEAFOOD AND PRODUCTS BY FILIPINO ADULTS IN METRO MANILA

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Fish consumption is the main route of heavy metal exposure to humans which poses health risks if taken in large amounts. This study aims to validate methods of analysis for heavy metals, to prepare in-house food reference materials, and determine exposure assessment of commonly consumed seafood and products in Metro Manila. Validation data showed that the methods for analysis of heavy metals (arsenic, cadmium, mercury and lead) are fit for intended use. Mean correlation coefficients for heavy metals detection were between 0.99943 – 0.99977. Mean limits of detection were 0.3470 ppb, 0.1043 ppb, 1.8099 ppb and 0.2970 ppb for As, Cd, Hg and Pb, respectively. The Horwitz ratio was used for the test of method precision. The data showed that the method precision was less than the recommended Horwitz ratio (d”2). Highest levels of heavy metals were found in dried Indian sardine (9.282 ug/g As) and dried anchovy (0.273 ug/g Cd, 0.154 ug/g Hg, and 0.208 ug/g Pb). On the other hand, lowest levels were found in tilapia (0.044 ug/g As), shrimp (0.0003 ug/g Cd), canned sardines 1 (0.007 ug/g Hg), and mussel (0.028 ug/g Pb). Heavy metal contents of seafood and products were lower than the provisional tolerable weekly intake. The target hazard quotients for all the seafood and products also showed values less than 1, which suggests that health risks were insignificant. In conclusion, analysis of the heavy metal contents of seafood and products revealed that the values were below the provisional tolerable weekly intake by these metals indicating that no risk is posed by the consumption of fish most commonly eaten by Filipino adults.

Keywords: heavy metals, validation, target hazard quotient, provisional tolerable weekly intake, arsenic, cadmium, lead, mercury

HS – 10

LIPIDEMIC EFFECTS OF KAMIAS (*Averrhoa bilimbi* L.) FRUIT EXTRACT ON MALE WISTAR RATS

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Kamias (*Averrhoa bilimbi* L.) is a tropical fruit known for its many medicinal properties. The present study investigated the effects of fruit extracts on the lipidemic profile of male Wistar rats. Different concentrations of aqueous and ethanolic fruit extracts were fed and the rats were tested for blood cholesterol, high-density and low-density lipoproteins, and triglycerides. Rats were given high-fat diet for fourteen days using rat pellets amended with egg yolk. A control group with normal diet was also maintained. Extracts were administered through oral gavage once a day for fourteen days at different treatment concentrations: 25%, 50%, 75% and pure concentrations. Simvastatin and water were used as control treatments. Blood samples were taken through the tail vein during the initial and final phases. Results processed using Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) show significant reduction in cholesterol, low-density lipoprotein and triglycerides among rats treated with pure extracts. This reduction is seen to be directly associated with concentration of treatments. As treatment concentration increases, effects on lipidemic parameters were greater. Further, the results are comparable to Simvastatin, a commercially – available drug used to lower blood cholesterol. Cholesterol level of rats given pure and 75% fruit extracts were at 85.22 and 88.46 mg/dL of blood respectively, significantly better than Simvastatin's 98.91 mg/dL. The study concludes that Kamias is a potential natural cholesterol control and can significantly replace statin drugs. It is recommended that a thorough chemical analysis be done on the fruit for better drug applications.

Keywords: Kamias, *Averrhoa bilimbi* L, cholesterol, lipidemic, Simvastatin

HS – 11

ROLE OF SEX AND *SORTILIN-RELATED RECEPTOR 1* VARIANTS IN FILIPINOS WITH COGNITIVE IMPAIRMENT

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This study investigated the association of 6 *sortilin-related receptor 1* (*SORL1)* variants with cognitive impairment. *SORL1* SNPs 8, 9, 10, 13, 19 and 23 were genotyped using TaqMan® SNP Genotyping Assays in 484 Filipinos: 335 females and 149 males). One hundred had Alzheimer's disease (AD), 109 who had mild cognitive impairment (MCI), 18 who had other types of cognitive impairment, and 257 who had normal cognitive functions. Cognitive impairment (CI), which includes AD, MCI and other cognitive impairment cases, was associated with SNP 23 (p=0.041). None of the *SORL1* variants was associated with AD. SNPs 8, 9, 10 and 23 were associated with MCI, (p=0.028, p=0.034, p=0.034 and p=0.025, respectively). Based on these results, *SORL1* may be used as a biomarker in the early detection or diagnosis of cognitive impairment and other dementias. The role of sex in the association of *SORL1* variants and cognitive impairment was also evaluated. The results suggest that the SNP 23 may have a significant association with CI in females. The results showed association between SNP 23 (p=0.033) and the female sex in the MCI group. For males, all six *SORL1* variants did not show any association with the CI groups. This suggests that *SORL1* may affect cognitive impairment and the interplay of sex-specific risk factors, sex-specific disease course, and sex-specific survival of a disease, through a female-dependent mechanism. SORL1 plays a crucial role in the formation of amyloid plaques – the primary cause of AD. Although no association was observed between the variants and AD, the findings provide evidence that *SORL1* may predispose individuals to CI. Further evaluation of the usefulness of *SORL1* variants as predictors of progression of CI to AD needs to be done.

Keywords: Cognitive impairment, *sortilin-related receptor 1,* SNPs