



JOINT EVENT

10<sup>th</sup> International Conference on

**Genomics and Molecular Biology**

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May 21-23, 2018 Barcelona, Spain

# Posters

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**Regulation of miRNAs expression by mutant p53 gain of function in cancer****Tzitzijani Madrigal Domínguez**  
UAM Iztapalapa, Mexico

p53 is a tumor suppressor protein encoded by the TP53 gene which is located in chromosome 17p13.1. In response to environmental and cellular stress p53 activates the expression of genes and microRNAs (miRNAs) involved in cell cycle arrest, senescence and apoptosis. The TP53 is the most frequently mutated gene in human cancers. It has also been demonstrated that some mutant p53 proteins not only lose tumor suppressor activity, but also acquire novel oncogenic functions also known as “gain of function” (GOF) that are independent of wild-type (WT) p53. Recent studies have shown that mutant p53 can regulate gene expression and exert oncogenic effects through specific miRNAs. We transfected p53 mutants (p53R273, p53R175H, p53R248Q) into p53-null Saos2 cells, profiled the miRNA expression by miRNA PCR array, we selected and validated the expression of miR-182, miR-200b, miR-3151 and miR-509-5p by real-time quantitative PCR and observed that mutants of p53 have a global negative effect for human miRNome expression, however some miRNAs were upregulated. Here we found tumor suppressors miRNAs downregulated like miR-200b, miR-3151 and miR-509-5p or oncomiRNAs like miR-182 upregulated. Many studies have reported that patients with tumors carrying p53 mutations have worse prognosis and poorer response to conventional anticancer treatments than those bearing p53 WT protein, therefore, our study contributes to the understanding of regulation of miRNAs by mutants of p53 that could explain in part the role of mutant p53 proteins in the development of cancer and may help propose new target therapies.

**Biography**

Tzitzijani Madrigal Domínguez completed her Graduation in Biology from Universidad Autónoma Metropolitana (UAM) in 2010; studied at Molecular Oncology Laboratory and received her MA in Experimental Biology from UAM in 2013. She is currently a PhD student in Experimental Biology Program-UAM. She is a Member of Mexican Association for Cancer Research which is a civil association, made up of scientists recognized both nationally and internationally as leaders in the development of basic, clinical, pharmacological and social research projects associated with the study of cancer.

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**Genome-wide evaluation of loci and candidate genes underlying important traits in soybean (*Glycine max* (L.) Merr.)****Wenbin Li, Xue Zhao, Yingpeng Han, Weili Teng, Jian Luo, Lei Feng and Chanjuan Zhang**  
Northeast Agricultural University, China

Disease resistance and seed quality are important traits for soybean breeding. Better understanding of the genetic architecture and genomic landscape of soybean germplasm with targeted traits is the precondition of molecular design breeding of soybean. Construction of a favorable data platform including phenotyping and genotyping pools and efficient analytical approaches were the fundamental tasks for molecular breeding work. Therefore, more than 500 diverse soybean accessions were sequenced using specific-locus amplified fragment sequencing (SLAF-seq) to establish a genotype database. In total, 64 141 single nucleotide polymorphisms (SNPs) with minor allele frequencies (MAFs) > 0.05 were found among the 512 tested accessions. The genotyped soybean germplasm has been phenotyped for some important soybean quality traits including soybean fatty acid components and seed vitamin E content under multi-environmental conditions. Resistance to different pathogens including resistance to soybean cyst nematode (SCN), soybean white mold (SWM), soybean root rot (SRR) and soybean mosaic virus (SMV) has also been phenotyped. A set of loci were found to be associated with the above traits by GWAS and some of them were confirmed by bi-parental mapping which has been used for molecular assisted selection breeding. A set of candidate genes for disease resistance that have been evaluated via sequence polymorphism and differential expression in special donors were cloned and were staged in functional genomics research.

**Biography**

Wenbin Li completed his PhD in Plant Genetics and Breeding in 1988 at Northeast Agricultural University of China. He is working at Soybean Research Institute of Northeast Agricultural University for more than 15 years as a Director and Professor. His major research areas are covered by soybean functional genomics, gene characteristics for agronomic important traits, and molecular breeding. He has published over 80 peer reviewed papers in numbers of international journals, such as *New Phytologist*, *The Plant Journal*, *TAG*, *BMC Genomics* and *Heredity*. Recently, he became a member of Executive Committee for World Soybean Association, and an Associate Editor of BMC Genomic.

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**Comparison of the effect of caloric restriction types on the expression of some cancer-associated genes via RNA-seq**Nehir Ozdemir Ozgenturk<sup>1</sup>, Ali Yasir Koç<sup>1</sup>, Zehra Omeroglu Ulu<sup>1</sup>, Soner Dogan<sup>2</sup>, Bilge Guvenc Tuna<sup>2</sup> and Salih Ulu<sup>1</sup><sup>1</sup>Yildiz Technical University, Davutpasa Campus, Turkey<sup>2</sup>Yeditepe University, Turkey

Pathways and Co-expression Network Analysis of Immune-Related Genes in Short-term Calorie Restricted Mice RNA-seq technology was performed a comparative transcriptome analysis of the MMTV-TGF- $\alpha$  female mice thymus tissues that were fed *ad libitum* (AL), cronic calorie restriction (CCR) (85% of AL fed mice) and intermittent calorie restriction (ICR) (3 weeks AL fed, 1 week 40% of AL fed mice) from 10 weeks of age to 17 weeks of age or 18 weeks of age. The results of RNA-seq analysis, a total of 6091 significantly differentially expressed genes (DEGs) were identified. 2821, 2825 and 445 significantly DEGs were detected between AL-CCR, CCR-ICR and AL-ICR fed groups, respectively. These DEGs were classified according to cellular components, biological processes and molecular functions Gene Ontology (GO) main categories. 188 of 2821, 36 of 445, 176 of 2825 genes were identified to be involved in immune system process (GO:0002376) biological processes GO categories. KEGG pathway and the gene co-expression network analysis between AL-CCR, CCR-ICR and AL-ICR fed groups immune-related DEGs were done using String database. For network analysis, nodes and edges were presented the interaction between immune-related DEGs. Calorie restriction is to reduce the amount of calorie received without malnutrition. This manipulation method has positive effects on life span, cancer formation and immune response in the long run. However, there are also some systems such as lymph organs that respond quickly to lack of calories. Thymus is one of these lymph organs. In this study, two type calorie restriction practices (CCR/ICR) were used on MMTV-TGF- $\alpha$  transgenic mice and a group of mice were fed *ad libitum* (AL) as a control group. It is aimed to determine the changes that the nutrition type will bring about in the expression of genes. The manipulation started at the 10th week of the life of the mice and ended at the 17th and 18th week. RNA-Seq-based transcriptome were performed to the RNAs obtained from the thymus of the sacrificed mice. Through RNA-Seq results "Differential Expressed Genes" (DEGs) were determined. 6091 of them have been identified as statistically significant ( $p < 0.05$ ). The AKT1, CTCF, PTEN genes known to cause breast tumor development were selected by data banks. The selected genes were searched in three different RNA-Seq data and expression levels were determined for three genes in three different medium. Changes in expression level are displayed via graphics.

**Biography**

Nehir Ozdemir Ozgenturk completed her Graduation at Ege University; Master's Degree in Plant Breeding Department and PhD in the Department of Justus Liebig University. In 2003, she worked at the Cereal Research Center in Canada as a Post-doc. Also she worked at Georgia Medical School for four months with Nato fellowship. She has scientific paper in various scientific journals, publications and presentations at international conferences.

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**Mussel adhesive protein-conjugated vitronectin (MAP151-V) induces anti-inflammatory activity on LPS-stimulated macrophages and UVB-irradiated keratinocyte**Kyung Bae Pi<sup>1</sup>, Seul Gee Um<sup>1</sup>, Jung Mo Ahn<sup>1</sup>, Beom Seop Rho<sup>1</sup>, Ki Beom Lee<sup>1</sup>, Sung Gil Park<sup>2</sup>, Ho Jin Kim<sup>1</sup> and Yoonjin Lee<sup>3</sup><sup>1</sup>Biotechnology & Business Center, Incheon business information Technopark, Incheon, Korea<sup>2</sup>R&D center, Advanced BioTech Co., Ltd, Pilot Plant 12 Gaetbeol-ro, Yeonsu-gu, Incheon, Korea<sup>3</sup>Cosmocos Corporation, Incheon, Korea

Skin inflammation and dermal injuries is a major clinical problem due to the current therapies limited to established scars with poor understanding of healing mechanisms. Unique adhesive and biocompatibility properties of mussel adhesive proteins (MAPs) are known for their great potential in many tissue engineering and biomedical applications. Previously it was successfully demonstrated that redesigned hybrid type MAP, fb-151, mass produced in gram-negative bacterium *Escherichia coli*, could be utilized as a promising adhesive biomaterial. However, the biological activity of vitronectin-bound recombinant fb-151 has not been established. The aim of this study was to develop a novel recombinant protein using MAP and vitronectin and to elucidate the anti-inflammatory effects of these on macrophages and keratinocytes. We investigated the anti-inflammatory activities of recombinant fb-151 conjugated vitronectin (MAP151-V). LPS (Lipopolysaccharide) was used as a stimulant for macrophages and UVB was used as a stimulant for keratinocytes. Macrophages stimulated by LPS increased the expression of iNOS and COX-2, which are inflammatory factors, while the MAP151-V-treated groups suppressed the expression of iNOS and COX-2 in a dose-dependent manner. In addition, keratinocyte stimulated with UVB showed reduced expression of iNOS and COX-2 MAP151-V treatment. Interestingly, in UVB-irradiated keratinocytes, inflammatory cytokines such as IL-1 $\beta$ , IL-6 and TNF- $\alpha$  were significantly reduced by MAP151-V treatment. These results suggest that MAP151-V has a more effective anti-inflammatory activity on keratinocyte, suggesting its use as a skin inflammation and therapeutic agent of skin..

**Biography**

Kyung Bae Pi completed his MS from Kyung Hee University, South Korea. He is a Senior Researcher and Project Leader of Incheon Business Information Technopark, South Korea. He has published more than 14 papers in reputed journals and has been serving as an Editorial Board Member of *repute*.

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**Comparative genomics analysis of 29 *Lactococcus lactis* strains****Schermann Sabine**

DuPont Nutrition and Health, France

**L**actococcus *lactis* is a lactic acid bacterium widely used in the dairy industry to produce diverse cheeses. Several decades of meticulous microbial selection have provided large collections of strains with appropriate technological attributes such as fast milk acidification, improved bacteriophage resistance and desired aroma production. The objective of this study is to link specific phenotypes to the genetic content of select strains using a pan-genome approach following whole genome sequencing. Whole genome sequences were generated for 29 *L. lactis* subsp. *cremoris* or subsp. *lactis* proprietary strains using Illumina sequencing. The 29 draft genomes ranged in size between 2.40 and 2.90 Mb (mean: 2.57 Mb) and were organized into 94 to 332 contigs, reflecting a varying content of repeated sequences, notably insertion sequences. The number of predicted CDS varied between 2,644 and 3,521 per genome (mean: 2,813). In most genomes, putative plasmid-based contigs could be detected, although this prediction of plasmid nature is not trivial. Overall, 81,578 CDS were classified into 10,604 gene families (pan-genome), including 1,142 core genes and 4,769 unique genes. In this study, many novel genes and functions could be identified easily within a set of 29 *L. lactis* strains having a potential for industrialization. Although this species is well-known for its small genome size, our data indicate a significant strain-to-strain genetic diversity in agreement with already observed physiological distinctive features thus paving the way for further genomic analyses.

**Biography**

Schermann Sabine obtained her Master's Degree in Bioinformatics at Université Paul Sabatier (Toulouse, France) in 2011. Just after her studies, she joined the DuPont Company as a Bioinformatician. She works in the Research and Development Department-Nutrition and Health division of the same company. Her team works on the selection and study of lactic acid bacteria aimed to be used in the dairy industry. Her main missions are tool development, Linux server maintenance and the conduction of genomics analyses.

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**A PtDRG1, desiccation response gene from *Pyropia tenera* (Rhodophyta) exhibits chaperone function and enhance abiotic stress tolerance****Dong Woog Choi**

Chonnam National University, South Korea

*Pyropia* are commercially valuable marine red algae that grow in the intertidal zone and extremely tolerant to desiccation stress. We identified and reported the desiccation response genes (DRGs) based on comparison of the transcriptomes of *P. tenera*. Among them, *PtDRG1* encodes a polypeptide of 22.6 kDa that located in chloroplast. *PtDRG1* does not share sequence homology with known genes in public database except for several red algae species. Transcription of the *PtDRG1* gene was upregulated by osmotic stress induced by mannitol or H<sub>2</sub>O<sub>2</sub> as well as desiccation stress but did not respond to heat. When *PtDRG1* was over-expressed in *Escherichia coli* and *Chlamydomonas*, the transformed cells grew much better than control cells under high temperature as well as osmotic stress induced by mannitol and NaCl. In addition, *PtDRG1* significantly reduced the thermal aggregation of substrate protein at heat stress condition. These results demonstrate that *PtDRG1* have a chaperone function and plays a role in tolerance mechanism for abiotic stress in *Pyropia*. This study shows that red algae have unknown stress proteins such as PtDRG1, and that these proteins have chaperon function and play a role in stress tolerance in red algae as stress proteins such as dehydrin work in green plants.

**Biography**

Dong Woog Choi has completed his PhD from Seoul National University, South Korea and Postdoctoral studies from University of California at Riverside, USA. He is the Professor in the Department of Biology Education, Chonnam National University, South Korea.

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**Onco-Plus: an integrated database and computational protocol for discovery of lead molecules targeting unique DNA****Akhilesh Mishra, Pradeep Pant and B Jayaram**  
IIT Delhi, India

The specific binding of transcription factors (TFs) to specific DNA sequences make the DNA motifs promising drug targets for the coordinated regulation of gene expression. Here, we present Onco-Plus, an integrated database of regulatory motifs of cancer genes clubbed with Unique Sequence Predictor (USP) (<http://www.scfbio-iitd.res.in/software/onco/NavSite/index.htm>) and a software suite for targeting DNA for drug discovery. USP identifies unique sequences (i.e. these sequences occur only once in the entire genome and if targeted would presumably show no off-target binding /side effects) for each of the identified regulatory DNA motifs at the specified position in the genome by extending a given DNA motif, in 5'→3' or 3'→5' or in both directions. For each identified motif, three possible unique sequences could be generated. Taking off from the identified unique sequences as drug targets, a rapid virtual screening against a million-compound library could be performed (<http://www.scfbio-iitd.res.in/PSDDF/tool4.php>) to generate a list of hit molecules (potential candidate drugs) with their predicted binding free energies. This methodology is demonstrated on E2F transcription factor binding site for the WNT10B gene, implicated in breast and endometrial cancers and a few small molecules are proposed as potential drug candidates. Being fast and cost effective, this protocol could be of considerable value in generating new potential drug candidates to inhibit desired sequences for further experimental studies.

**Biography**

Akhilesh Mishra has done his Master's in Bioinformatics from Jamia Millia Islamia, New Delhi, India. He is pursuing his PhD from Indian Institute of Technology, New Delhi. He is the Recipient of CSIR-UGC-SRF and BINC award from the Department of Biotechnology, India. He has published many research articles in reputed peer reviewed journals.

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**Population genomics reveals adaptive divergence in global populations of common carp (*Cyprinus carpio*)**Jian Xu<sup>1</sup>, Zixia Zhao<sup>1</sup>, Yanliang Jiang<sup>1</sup>, Hanyuan Zhang<sup>1</sup>, Yan Zhang<sup>1</sup>, Chuanju Dong<sup>2</sup>, Ruyu Tai<sup>1</sup> and Peng Xu<sup>3</sup><sup>1</sup>Chinese Academy of Fishery Sciences, Beijing, China<sup>2</sup>Henan Normal University, Xinxiang, China<sup>3</sup>Xiamen University, Xiamen, China

The common carp, *Cyprinus carpio*, is one of the most important cyprinid species cultured in Europe and Asia and globally accounts for over 70% of freshwater aquaculture production worldwide. Various populations of *C. carpio* distribute all over the world and mainly in the Eurasian continent, showing distinct biological characteristics including scale, color, body shape, etc. However, the genetic mechanism underlying the traits were not very clear yet. Here we present a population genomic analysis on 14 populations of *C. carpio*. High throughput SNP genotyping of 2,587 representative individuals from worldwide 14 populations demonstrates different genetic component for *C. carpio* in two subspecies (*C. carpio* subsp. *haematopterus* and *C. carpio* subsp. *carpio*). Quality control of SNPs were conducted with following parameters (call rate>95%, genotype rate>95%, MAF>5%). A maximum-likelihood tree was constructed with RAxML and displayed with iTOL software (<http://itol.embl.de/upload.cgi>), and all SNPs were used to investigate the PCA with SMARTPCA. Population structure were finished using STRUCTURE with 2,000 iterations. The resulting structure matrix was plotted using STRUCTURE PLOT v2.0. LD (linkage disequilibrium) block average lengths of 14 populations range from 3.94kb to 36.67kb. We calculated the  $\pi$  distribution for each linkage group using a sliding window method in VCFTOOLS. The window width was set to 10 kb, and the stepwise distance was 10 kb. The  $\pi$  values from the main populations were compared, and the ratios were sorted. Fst and Tajima's D values were also calculated using VCFTOOLS with the parameters “-weir-fst-pop” and “-TajimaD”, respectively. We identified the regions with the 5% highest p ratios and the regions with the 5% highest Fst values. Genes within selective sweep regions were identified by genome scanning among different populations, including *gdf6a*, *grb7*, *mtnr1ba*, *tgfb2*, etc. Gene ontology and KEGG enrichment analyses by DAVID software unveiled potential trait-related GO terms and pathways which were associated with body shape, scaling patterns and skin color, such as TGF-beta signaling pathway, ECM-receptor interaction and so on. The population genomics analysis paves the way for better evolutionary studies and improved genome-assisted breeding of *C. carpio*.

**Biography**

Jian Xu has completed his PhD from Peking Union Medical School, China. He is an Associate Professor and Vice Director of Center of Aquatic Genomics of Chinese Academy of Fishery Sciences, Beijing, China. He has published 33 papers in reputed journals and has been serving as an Editorial Board Member of *Frontiers in Genetics*.

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**Expression of alpha 1 intensities in haptoglobin 2-1 and its association with clinical course in aneurysmal subarachnoid hemorrhage****Bong Jun Kim<sup>1</sup>, Young Mi Kim<sup>1</sup> and Jin Pyeong Jeon<sup>1,2</sup>**<sup>1</sup>Institute of New Frontier Research - Hallym University College of Medicine, Republic of South Korea<sup>2</sup>Hallym University College of Medicine, Republic of South Korea

**Introduction & Aim:** Delayed cerebral ischemia (DCI) contribute to poor clinical outcome following subarachnoid hemorrhage (SAH). Haptoglobin (Hp) comprised of two light ( $\alpha$ ) and two heavy ( $\beta$ ) chains has anti-oxidant effect by free hemoglobin (Hb) binding. Among three phenotypes, Hp1-1 (two  $\alpha$ 1), Hp2-1 ( $\alpha$ 1 and  $\alpha$ 2), and Hp2-2 (two  $\alpha$ 2), higher protective effect for toxic free Hb is reported in Hp2-2 than Hp1-1. However, few studies have focused on Hp2-1 in determining outcome. This study aims to examine the  $\alpha$ 1 and  $\alpha$ 2 expression and to evaluate the association with outcomes in Hp2-1.

**Methodology:** Eighty-seven patients were prospectively enrolled: Hp1-1 (12, 13.8%); Hp2-1 (36, 41.4%); and Hp2-2 (n=39, 44.8%). Phenotypes was confirmed by western blotting. The relative intensities were measured as  $\alpha$  intensities divided by the albumin intensities and expressed as the median (25<sup>th</sup>-75<sup>th</sup> percentile). The difference in  $\alpha$  intensities according to DCI, angiographic vasospasm (AV) and outcome (mRS 0-2) in 6 months were analyzed.

**Results:** DCI (n=21, 53.8%) and AV (n=22, 56.4%) were more frequently observed in Hp2-2 than Hp1-1 (DCI, n=3 (25.0%) and AV, n=3 (25.0%)). The  $\alpha$ 1 intensities in Hp2-1 without DCI (0.70 (0.54-0.89)) and AV (0.65 (0.32-0.88)) were significantly higher than that with DCI (0.24 (0.14-0.32),  $p<0.001$ ) and AV (0.32 (0.17-0.67),  $p=0.046$ ). For  $\alpha$ 2 intensities, no significant difference was noted according to DCI ( $p=0.377$ ) and AV ( $p=0.459$ ). The  $\alpha$ 1 ( $p=0.359$ ) and  $\alpha$ 2 ( $p=0.233$ ) intensities did not differ significantly according to outcome.

**Conclusions:** Higher  $\alpha$ 1 intensities in Hp2-1 can be associated with lower DCI and AV. The  $\alpha$ 1 intensity degree may provide additional information on individual risk of secondary injury following SAH in Hp2-1.

**Biography**

Bong Jun Kim completed his Graduation from Hallym University, Department of Biomedical Sciences and studied experimentation in the virology laboratory for about a year during his undergraduate degree. In the same year, he acquired a certificate for handling experimental animals. He majored in Medical Genetics with a Master's degree. He studied COPD-associated gene mutations in a Korean cohort through next-generation genome analysis and statistical analysis. Currently, he collaborates with Neurosurgeons as a member of industry-academia cooperation group and studying neurosurgical diseases.

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**Exome analyses in subfamily trios from large family tree in the south-eastern Moravia (Czech Republic) population with high incidence of parkinsonism****Radek Vodicka**

University Hospital Olomouc, Czech Republic

There has been previously described higher prevalence of Parkinsonism in small isolated region from the South-Eastern Moravia. We used NGS Ion AmpliSeq Exome method (IonTorrent) for two (A and B) subfamily trios. Each trio comprised of two affected and one healthy person. DNA exome libraries were sequenced on IonPI chips. Variants were predicted using Torrent Suite and Ion Reporter softwares. Aligned reads (BAM files) were then analyzed using Ion Reporter Whole Exome Trio workflow. Final filtering was done with respect to population frequency, variant effects and with respect to the presence of variants in Parkinsonism disease responsible genes. Last filter was done with respect to the segregation of the disease. Almost whole exome was sequenced with coverage 1-20 and 90% of exome was covered more than 20x in all the samples. Together more than 70,000 variants with average base coverage depth 75 were analyzable in both trios before filtering. After filtering there were found 99 and 96 variants in trio A and B respectively. The most potentially associating variants with parkinsonism are as given in tabulated form as follows:

Trio A:	Trio B:
SLC18A2 p.Gly195Ser:c.583G>A	TENM4 p.Asn965Ser:c.2894A>G
DRD1 p.Ala353Val:c.1058C>T	MON2 p.Gln531Arg:c.1592A>G
AP2A2 p.Asn401Ser:c.1202A>G	MTCL1 p.Ala482Val:c.1445C>T
CCDC88C p.Leu1696Pro:c.5087T>C	NEPRO p.Val297Ile:c.889G>A
ZFHX3 p.Met2102Thr:c.6305T>C	FAM131A p.Leu280Val:c.838C>G
ARAP2 p.Pro159Ser:c.475C>T	ADH1C p.Arg48His:c.143G>A
CYP4F11 p.Trp29Ser:c.86G>C	SYNE1 p.Lys3729Asn:c.11187G>T
MRPS15 p.Thr252Ile:c.755C>T	RXFP2 p.Thr222Pro:c.664A>C
MRPS28 p.Arg48Pro:c.143G>C	AKAP11 p.Ile183Met:c.549A>G
PRELID2 p.Val62Met:c.184G>A	ZNF19 p.Pro216Ser:c.646C>T
FAM171A1 p.Ser844Leu:c.2531C>T	LRRK2 p.Arg1514Gln:c.4541G>A
CAPRIN2 p.Arg373His:c.1118G>A	OSBPL1A c.115_116insAATT
FAM186B p.Ala727Val:c.2180C>T	SACS p.Met1359Thr:c.4076T>C
CROT p.Glu118Asp:c.354A>C	ZFHX3 p.Met2102Thr:c.6305T>C
MPDZ p.Cys119Ser:c.356G>C	COL18A1 p.Ala1381Thr:c.4141G>A

Detailed whole exome analyses in genetic isolated parkinsonism patients could contribute to further understanding of molecular-genetic mechanism and background of the disease.

**Biography**

Radek Vodicka has completed his PhD study of Medical Genetics at the Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic in 2003. In 2015 he was appointed as an Associate Professor in the same field. Since 2001, he has been working in the DNA Diagnostics Laboratory at the Institute of Medical Genetics, University Hospital Olomouc, Czech Republic. He is also working as an Associate Professor at the Faculty of Medicine and Dentistry, Palacky University Olomouc. He has published more than 35 papers.

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**NGS analysis of miRNA expression patterns concerning therapeutic protein production and protein glycosylation in recombinant CHO DG44 cells**Ann Cathrin Leroux<sup>1,2</sup><sup>1</sup>University of Ulm, Germany<sup>2</sup>Sartorius Stedim Cellca GmbH, Germany

Mature miRNAs are 19-25 bp long RNA duplexes, which are associated with RISC (RNA induced silencing complex) and bind to mRNAs leading to translational expression. It has been shown, that miRNAs also play a role in regulation of productivity and cell growth in CHO cells. This work investigates the role of miRNA in recombinant protein production in CHO DG44 cells including a variety of different products with production-relevant final product concentrations (up to 8 g/l in fed-batch mode). Additionally, the influence of miRNA on the glycosylation pattern of the recombinant protein is investigated. In detail, 24 clonal cell lines expressing four different therapeutic proteins are selected based on final product concentrations and glycosylation patterns. These clones were cultivated in an ambr<sup>®</sup> 15 system in fed-batch mode collecting process and glycosylation data. Cell samples are used for NGS of small RNA on the Illumina NextSeq System. Between 150 and 200 predicted small RNAs were found in each cell line. Of these, 10 to 20% are putative novel and could not be found in miRBase 2, Rfam 13.0 or RNAdB 2.0. Overall, 394 distinct miRNAs could be identified. Differential analysis revealed that there is great variety between therapeutic protein groups, as differentially expressed miRNAs was only recurred in maximum two of these groups. In a follow-up study, 5 to 10 differentially expressed miRNAs of each product group will be tested for their effects on therapeutic protein production and glycosylation in CHO DG44 cells.

**Biography**

Ann Cathrin Leroux studied BSc in Medical Biotechnology at University of Rostock, Germany and Pharmaceutical Biotechnology at Ulm University, Germany. She is currently working on her PhD at Sartorius Stedim Cellca GmbH and Ulm University. She has completed Master studies at Ulm University and University of Applied Sciences Biberach, Germany. Her master thesis research field includes: Influence of promoters on the productivity of CHO DG44 cells, performed at Sartorius Stedim Cellca GmbH. She has done her Bachelor studies at University of Rostock, Germany.

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**SEQprocess: A pipeline tool for processing of next generation sequencing data with modularized customization**Taewoon Joo<sup>1</sup>, Ji Hye Choi<sup>1</sup>, Ji Hye Lee<sup>1</sup>, So Eun Park<sup>1</sup>, Youngsic Jeon<sup>2</sup>, Sae Hoon Jung<sup>1</sup> and Hyun Goo Woo<sup>1</sup><sup>1</sup>Ajou University School of Medicine, South Korea<sup>2</sup>Yonsei University College of Medicine, South Korea

Next-Generation Sequencing (NGS) technology is now widely used in biomedical research field. The application of NGS technologies includes the identification sequence variants of DNA or RNA and the quantitation of RNA abundances or DNA copy numbers. Previously several softwares for NGS data processing pipelines have been released. However, the softwares do not cover the recently updated GDC pipelines which is a standardized pipeline used in the processing of the TCGA data. Moreover, there is no comprehensive tools that handle the recent clinical applications of NGS technology such as cell-free DNA, small RNA, and exosomal RNA sequencing data. Here, we developed a SEQprocess that can provide NGS processing pipelines covering the GDC pipeline as well as the new data for clinical applications. SEQprocess is implemented in an R program to provide an automated and user friendly interfaces. SEQprocess also provide a flexible customization framework by modularizing the multiple NGS processing steps that can be easily included or excluded in the process. In addition, SEQprocess automatically generate a report that summarize the processing steps, which will ensure reproducibility of the NGS data analysis.

**Biography**

Ji Hye Choi has completed her Doctor of Science Degree majoring in Biomedical Informatics and Convergence Medicine from Ajou University of Medicine, Suwon, Republic of South Korea in August 2017 and currently is a Research Fellow at the same university. Her research interests are Genomics, Bioinformatics and System Biology. She has authored more than five papers in reputed journals including *Cancer Research*, *Nature communication*, *Experimental & Molecular Medicine*, *Oncotarget* and *BMC Bioinformatics*.

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**Danshensu rescues ischemia/reperfusion caused hepatocyte damage**Chun Ya Liang<sup>1</sup>, Maw Sheng Sun<sup>1</sup> and Chan Yen Kuo<sup>2,3</sup><sup>1</sup>Show Chwan Memorial Hospital, Taiwan<sup>2</sup>National Central University, Taiwan<sup>3</sup>Hsin Sheng Junior College of Medical Care and Management, Taiwan

**Introduction:** Apoptosis of hepatocyte, under ischemia/reperfusion (IR) conditions, has been identified as an essential process in the progression of liver transplantation. Under these conditions, mitochondria can become a threat to the cell because of their capacity to generate reactive oxygen species (ROS). Additionally, ROS overproduction may induce inflammation. As ROS accumulation appear to cause hepatocyte damage or death, there has been considerable interest in identifying the candidate natural products involved and in developing strategies to reduce oxidative stress.

**Materials & Methods:** In this study, we use Danshensu as an candidate product to speculate whether has the protective effect on apoptotic hepatocyte upon IR. To speculate the apoptotic phenomena was reversed by Danshensu, we detected the p53, cleaved-caspase 3 expression by western blotting, as well as caspase-3 activity. Additionally, we analyzed the ROS levels by 2',7'-dichlorofluorescein diacetate (DCF-DA) staining. We also detected the cell viability by WST-1.

**Results & Discussion:** Results showed that Danshensu alleviated hypoxia-caused cell apoptosis via ROS overproduction. However, the precise roles of ROS in liver as a regulatory, protective, or deleterious mediator are still unresolved questions and need to be further investigated.

**Conclusion:** We suggested that Danshensu is a good strategy for treating hepatocyte damage upon IR.

**Biography**

Yi-Ru Ho completed her Graduation from Department of Molecular Biology and Human Genetics, Tzu-Chi University. Currently, she is a Research Assistant in Department of Medical Research Chang Bing Show Chwan Memorial Hospital. She is interested in "Primary cell culture of ADMSC, cell culture of neuron cell, MTT assay, DNA extraction, qPCR, Western blot, flow cytometry of cell cycle and cell marker, Luciferase assay, siRNA transfection, Enzyme-linked immunosorbent assay, intraperitoneal injection of mice".

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**The protective effect of simvastatin against UVB-induced corneal endothelial cell death**Yi Ru Ho<sup>1</sup> and Chan Yen Kuo<sup>2,3</sup><sup>1</sup>Chan Bing Show Chwan Memorial Hospital, Taiwan<sup>2</sup>Hsin Sheng Junior College of Medical Care and Management, Taiwan<sup>3</sup>National Central University, Chungli, Taiwan

Ultraviolet B (UVB) radiation is a risk factor for uveitis, and excessive UVB exposure causing corneal endothelium injury, including apoptosis, is a serious condition. Therefore, drugs that can inhibit apoptosis in corneal endothelial cells represent an effective strategy for treating uveitis. Simvastatin is widely used as a specific inhibitor of 3-hydroxy-3-methyl-glutaryl-CoA reductase, can reduce levels of low density lipoprotein (LDL) cholesterol, and exerts anti-inflammatory effects. However, the effect of simvastatin on uveitis remains unclear. Therefore, the aim of this study was to elucidate whether UVB promotes the initiation of apoptosis in corneal endothelial cells and subsequently contributes to uveitic injury reversible by simvastatin treatment. Our findings indicated that simvastatin alleviated UVB-induced corneal endothelial cell apoptosis via caspase-3 activity.

**Biography**

Yi-Ru Ho completed her Graduation from Department of Molecular Biology and Human Genetics, Tzu Chi University. Currently, she is a Research Assistant in Department of Medical Research Chang Bing Show Chwan Memorial Hospital. She is interested in "Primary cell culture of ADMSC, cell culture of neuron cell, MTT assay, DNA extraction, qPCR, Western blot, flow cytometry of cell cycle and cell marker, Luciferase assay, siRNA transfection, Enzyme-linked immunosorbent assay, intraperitoneal injection of mice".

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## SPARC enhance acute corneal repair after chemical injury in a rat dry eye model

Yun-Ching Cheng<sup>1</sup>, Wen-Yang Lai<sup>1</sup>, Wan-Yu Hsieh<sup>2</sup> and Shu-Ching Hsu<sup>3</sup>

<sup>1</sup>Chang Bing Show Chwan Memorial Hospital, Taiwan

<sup>2</sup>National Cheng Kung University, Taiwan

<sup>3</sup>National Institute of Infectious Diseases and Vaccinology, Taiwan

**Objective:** The objective of this study was to report the evaluation of efficacy of mesenchymal stem cells (MSC) conditional medium for the treatment of severe keratitis in dry eye disease in early-access program. Unique abilities of MSC could be used to develop new treatment approaches for dry eye disease.

**Materials & Methodology:** An SD rat dry eye model was used in which chemical damage corneal break decreased the Schirmer test score by at least 40%. The eye symptom score, breakup time of tear film (BUT) and Schirmer test score were compared before and after treatment in the two groups (MSC conditional medium in normoxia and hypoxia). The repair ability of conditional medium of MSC by wound weal assay using HUVEC and cancer cell line. We identified the specific component of hypoxia conditional medium of WJ-MSC (Wharton's jelly) by mass spectrometry (MS-MS). Classification of specific high expression protein was determined by western blot analysis.

**Results & Discussions:** Hypoxia conditional medium of WJ-MSC showed significant high repair ability than normoxia conditional medium of WJ-MSC in SD rat dry eye model. SPARC was identified as major protein in hypoxia conditional medium. According to the different days of conditional medium by western blot analysis, SPACR (secreted protein, acidic and rich in cysteine) was increased as time-dependent manner. Cell mobile ability was being increase by SPACR in serum-free culture condition of HUVEC. In SD rat dry eye model, rat continuing treatment with PBS, Artificial tears and SPACR for 2 weeks and determine the recovery by BUT and Schirmer test score. SPACR show the best recovery ability than others.

**Conclusions:** In a rat dry eye model, SPARC, the special expression in hypoxia conditional medium, show great recovery ability. Cell based assay, SPARC enhance the cell wound weal. According these results, we consider that SPARC is a potential therapeutic agent for use in the treatment of dry eye syndrome.

## Biography

Yun-Ching Cheng has completed her PhD from National Sun Yat-sen University and Post-doctoral studies from Cancer Research of NHRI in Taiwan. Her studies focus on the role of hypoxia condition-expression of proteins/genes in cancer metastasis and the repair ability of hypoxia-induced proteins in MSC. She has published more than five papers in reputed journals.

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# Accepted Abstracts

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**Integration of pharmacogenomics in patient-centered healthcare**

**Adrijana Kekic**

Mayo Clinic, Arizona, USA

**Intended Audience:** This workshop is intended for clinicians, researchers, and payers interested in results of implementation of pharmacogenomics in patient-centered model of care.

**Scope of the Workshop:** The workshop is focused on sharing examples of lessons learned from the clinic. Areas of practice covered will include cardiology, psychology, transplant, anesthesia and palliative care. The intent of this workshop is to provide an interactive forum for discussion on the role and integration of PGx at bedside, with specific emphasis on chronic disease management, polypharmacy, therapy selection and pre-emptive prescribing.

**Goals of the Workshop:** The goal of this workshop is to discuss the impact of PGx in patient centered model of care; increase enthusiasm for implanting PGx testing in the clinic; encourage collaborations in PGx discoveries and clinical implementations.

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**Bridging pheno-plasticity with genetic profile of the hydrophyte *Ludwigia stolonifera* (Guill. & Perr.) P.H. Raven: With reference to its expansion to new habitats****Azza Badr Hamed and Wafaa M Amer**  
Cairo University, Egypt

*Ludwigia* L is a pantropic genus of aquatic and subaquatic herbs. Recently, *L. stolonifera* (Guill. & Perr.) P.H. Raven became a dominant aquatic macrophyte in Egypt and it expanded from fresh water into salty habitats. Interestingly, the plant showed morphological plasticity in several characters such as leaf shapes, vesicles, flower sex, fertilization efficiency, fruit parameters and number of produced seeds/fruit. Accordingly, in Egypt this species morphologically grouped into seven morphotypes (1–7). The lack of information about the plant genome further complicates the identification of these morphotypes. Thus, it was crucial to investigate the morphotypes in terms of karyotyping and genetic profile to understand if this morphological plasticity is genetically based or it is an impression of habitat diversity. In this study, seven morphotypes located in different Egyptian habitats were compared using karyotyping and random amplified polymorphic DNA (RAPD-PCR) technique. Karyotyping indicated that some morphotypes were tetraploid ( $2n=32$ ), while others morphotypes were triploid ( $2n=24$ ). The measured similarity percentage based on RAPD data revealed a highest value (98.6%) between the triploid M4 and M5 morphotypes; similarly between the tetraploid M1 and M2 morphotypes showed 95.4%. Meanwhile, the lowest similarity (73.4%) was between the aquatic morphotype (M2) and the salt affected wetland morphotype (M3). The possible link between the genetic composition and ecological variation of this phenoplastic species will be discussed further.

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**Visualization and data mining of tremendous cancer transcriptome data****Zefang Tang**

Peking University, China

The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) projects produced RNA-Seq data for tens of thousands of cancer and non-cancer samples, providing an unprecedented opportunity for data mining, cancer drug target discovery and data visualization. In recent years, promising cancer drugs including panitumumab and bevacizumab have been developed that inhibit cancer cells by selectively targeting over-expressed EGFR or VEGF genes in cancer cells, while leaving normal cells unharmed. Genetic alterations will influence gene expression directly or indirectly. It is a frequently used strategy to discover candidate cancer drug targets through the finding of cancer specific expressed genes. This study aims to investigate normalization methods for integrating different expression datasets, explore effective approaches to obtain differentially expressed genes, profile the prognostic genes and transcripts in survival analyses, characterize the distribution of cancer specific genes or transcripts, and analyze their biological functions. Meanwhile, we will develop tools for visualizing integrated expression data, with the aim to disseminate such data to the wide research community. We also plan to find useful biomarkers for early diagnosis. Finally, by investigating the association between genetic alterations and over-expression, we aim to elucidate the underlying genetic mechanisms of differentially expressed genes.

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**Shifts in core bacterial microbiome of gorgonian sea fan related to necrotic-patch disease: Local confinement of pathobiome may facilitate recovery****Elena Quintanilla Alcaide<sup>1</sup> and Justus Liebig<sup>2</sup>**<sup>1</sup>BIOMMAR - Universidad de los Andes, Colombia<sup>2</sup>University Giessen, Germany

Microbiome disruptions triggering disease outbreaks are increasingly threatening corals worldwide. In the Tropical Eastern Pacific, a necrotic-patch disease affecting gorgonian corals (sea fans, *Pacifigorgia* spp.) has been observed in recent years. Massive mortalities of *Pacifigorgia* spp. have also been registered as a consequence of the incidence of this disease. However, the composition of the microbiome and its disease-related disruptions remain unknown in these gorgonian corals. Here, we analysed 16S rRNA gene amplicons from tissues of healthy colonies (n=20) and from symptomatic-asymptomatic tissues of diseased colonies (n=19) of *Pacifigorgia cairnsi* to test for disease-related changes in the bacterial microbiome. We found that potential endosymbionts dominate the core microbiome in healthy colonies. Moreover, healthy tissues differed in community composition and functional profile from those of the symptomatic tissues but did not show differences to asymptomatic tissues of the diseased colonies. Thus, potential endosymbionts in the core microbiome seem to be replaced by a set of more diverse bacteria in the symptomatic tissues. Furthermore, according to a comparative taxonomy-based functional profiling, the taxa that replaces the core microbiome in symptomatic tissues is characterized by heterotrophic, ammonia oxidizer and de-halogenating bacteria, while is depleted in nitrite and sulfate reducers. In conjunction, our results suggest that the bacterial consortium associated with the disease behaves opportunistically. We also conclude that the confinement of the pathobiome to symptomatic tissues may facilitate colony recovery by the potential breakage of affected-necrotic areas, hence contributing to colony resistance to disease and ultimately to the population resilience.

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**Identification of overlooked genes in DEG analysis by integrating metabolic network topology analysis****Emine Ravza Öztürk and Alper Yılmaz**  
Yıldız Technical University, Turkey

Most of the gene expression studies reveal differentially expressed genes (DEG) followed with gene set enrichment analysis (GSEA). Although this approach is practical for reducing the number of targets to engage, it is very much prone to overlook important targets. This is because the enrichment analysis ignores the metabolic pathway topology. A single gene in DEG list that is involved in very crucial reaction will not be identified as “enriched” if there are more than few genes are found in same pathway with this candidate gene. Thus, metabolic network topology should be strongly integrated with DEG analysis to uncover genes from a given DEG list which affect critical points in metabolic network. In our study, we parsed and merged available pathway and reaction data to construct whole human metabolic network. Then, by graph theory algorithms, identified critical nodes in whole network, perturbation of which would impact the whole network. To pinpoint overlooked targets in already published or calculated DEG lists, we gathered available DEG lists and expression data and mapped resulting DEG to metabolic network. Our approach could recover previously undetected important genes. As a result, the veil called “enriched gene” is lifted so that not enriched but critically important genes are exposed.

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**Regulation of aberrant inflammatory responses and development of vascular disease through the IL-1RI co-receptor TILRR****Eva E Qvarnstrom**

University of Sheffield, UK

Members of the toll-like and IL-1 receptor family (TIR) are central regulators of immune and inflammatory responses. Signal activation is induced through ligand binding and controlled by system-specific co-receptors. We have identified a novel component of the IL-1 receptor complex, the co-receptor TILRR (FREM-1 isoform 2). TILRR associates with the signalling receptor and magnifies IL-1 induced activation of the transcription factor NF- $\kappa$ B by enhancing signal amplification at the level of the receptor complex and potentiate recruitment of the MyD88 adapter. TILRR-controlled MyD88 dependent activation is regulated in a Ras-dependent manner, reflected in alterations in cytoskeletal structure and cell adhesion, and in release of cytoskeletal bound I $\kappa$ B $\alpha$ . In *silico* simulations using agent based modeling of the NF- $\kappa$ B network predicts the cytoskeletal control of inhibitor levels provides a mechanism for rapid signal calibration, and enables activation-sensitive regulation of NF- $\kappa$ B induced inflammatory responses. Recent studies have used *in vivo* models to assess the role of TILRR in host defense, vascular disease and lung fibrosis. Results show that TILRR expression is increased in inflammatory cells during development of myocardial infarction and in areas of inflammation, such as the atherosclerotic plaque and lymphoid tissue in the lung, but present at low levels in healthy tissue. Further, they demonstrate that genetic deletion or antibody blocking of TILRR function reduces development of disease progression, and suggest that TILRR provides a novel rational target for site- and signal specific inhibition of inflammatory responses in disease.

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**Prediction analysis of transient receptor potential ion channel and acetylcholine receptor genes in b lymphocytes from chronic fatigue syndrome/myalgic encephalomyelitis patients****Helene Cabanas, Anne Klein, Samantha Johnston, Donald Staines, Anu Chacko, Thao Nguyen, Emily Knauth and Sonya Marshall Gradisnik**  
Griffith University, Gold Coast, Australia

Calcium (Ca<sup>2+</sup>) and acetylcholine (ACh) signaling are important in B cell activation and potential antibody development. The aim of the study was to examine the effects of key genes responsible for these mechanisms from transient receptor potential (TRP) ion channels and acetylcholine receptors (AChRs) in isolated B cells from chronic fatigue syndrome/myalgic encephalomyelitis patients (CFS/ME). Flow cytometric protocols were used to determine B cell purity, followed by single-nucleotide polymorphisms (SNP) and genotype analysis from 21 TRP ion channel genes and 9 AChR genes examined by iPLEX Gold assay. Exome analysis was conducted using Illumina HiSeq platform and SNP association and genotype was determined using ANOVA (Analysis of Variance) and PLINK analysis. The SNP predictions on splicing events were realized using Automated Splice Site and Exon Definition Analysis server. Eleven CFS/ME patients (mean age 31.8±SD 5.5 years) defined according to the Fukuda criteria and 11 non-fatigued controls (mean age 33.9±5.1 years) were included. Seventy-eight SNPs were associated with CFS/ME: 35 were mAChR M3, the remaining were nAChR delta, nAChR alpha 9, TRPV2, TRPM3, TRPM4, mAChRM2 and mAChRM5. Mutations in the above genes can create or abolish splicing cryptic sites, which could induce important consequences on protein expression. The mutations can also strengthen or weaken binding sites by affecting the affinity with spliceosome elements, consequently inducing an increase or decrease in protein expression. These findings warrant further examination of the above genes in a larger cohort to investigate their potential role in CFS/ME.

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**MicroRNA expression profiling in placenta and maternal plasma in early pregnancy loss**Mohammad Kazem Hosseini<sup>1</sup>, Tuba Gunel<sup>1</sup>, Ece Gumusoglu<sup>1</sup>, Ali Benian<sup>1</sup> and Kilic Aydinli<sup>2</sup><sup>1</sup>Istanbul University, Turkey<sup>2</sup>Medicus Health Center, Turkey

Early pregnancy loss (EPL), is determined as the unintentional expulsion of an embryo or fetus prior to the 12th week of gestation. EPL frequency is ~15% in pregnancies. Fetal development and growth is associated with placental function and vessel development; therefore, the placental genome would represent a useful EPL model for epigenetic and genomic studies. An important factor of placental development and function is epigenetic regulation of gene expression. MicroRNAs (miRNAs) are the primary epigenetic regulators which have an important role in placental development and function. In the present study, maternal plasma and villous tissue were collected from 16 EPL cases during 6<sup>th</sup>-8<sup>th</sup> gestational weeks (GWs) and 8 abortions (control group) in 6<sup>th</sup>- 8<sup>th</sup> GWs. Detection of the differences in miRNA expression was performed using microarrays and dysregulated miRNAs were validated by RT-qPCR. miRNA microarray findings revealed that four miRNAs, including hsa-miRNA (miR-125a-3p, hsa- miR-3663-3p, hsa-miR-423-5p and hsa-miR-575) were upregulated in tissue samples. In maternal plasma, two miRNAs (hsa-let-7c, hsa-miR-122) were upregulated and one miRNA (hsa-miR-135a) was downregulated. A total of 6 out of 7 dysregulated miRNAs were validated using RT-qPCR. The aim this study was to detect dysregulated miRNAs in maternal plasma and villous cells and identify the target genes of dysregulated miRNAs and their associated pathways. The target gene analyses have revealed that the affected genes are primarily associated with cell migration, proliferation, implantation, adhesion, angiogenesis and differentiation and all are involved with EPL pathogenesis. Therefore, the present study may contribute to the understanding of the molecular mechanisms which lead to EPL.

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**A new case of de novo chromosome 19p13.12 deletion in an Omani girl with global developmental delay and multiple congenital anomalies****Musallam Al Araiimi, Salma Al Harasi, Nishath Hamza, Manal Al Kharusi, Hibah Al Hasni, Ali Al Yahmadi and Waad Allah Mula Abed**  
National Genetic Centre, Royal Hospital, Oman

**1** 9p13.12 deletion syndrome is a rare genetic disorder in which a small section of the short arm of chromosome 19 is deleted. It is a newly identified syndrome which is characterized by developmental delay, learning impairment and facial dysmorphism. We report a 4-year-old Omani girl with 19p13.12 micro-deletion syndrome. She was born as full-term to a non-related parent with global developmental delay, hypotonia and dysmorphism. She presented with multiple phenotypic skeletal abnormalities, hypotonia, and facial dysmorphism including frontal bossing, down slanting palpebral fissures, maxillary hypoplasia, bi-temporal narrowing, arachnodactyly and strabismus. Skeletal survey radiographs revealed thin long bones and square shaped of some vertebral bodies. Computed tomography (CT) and Magnetic Resonance Imaging (MRI) of the brain were unremarkable. Parents and the older sibling daughter were asymptomatic. Using array comparative genomic hybridization (CGH) analysis, a novel 1,594 kbp deletion at 19p13.12 was identified with 53 genes on which 35 are OMIM genes. These include *NFIX* (OMIM #164005), *MAN2B1* (OMIM #609458), *NFIX* (OMIM # 615094), *CACNA1A* (OMIM # 601011) and *GCDH* (OMIM # 608801) that could be responsible for the presented phenotypes (global developmental delay and various skeletal anomalies). This was found to be a de novo mutation by investigating the parents. We present this patient as the first case reported in Oman and the Gulf region.

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**Biological activities of *Ipomoea carnea* (Jacq.) extracts and chlorpyrifos on the cotton worm *Spodoptera littoralis* (Boisd.)**Mamdouh I Nassar<sup>1</sup>, Mohamed T Taha<sup>2</sup>, Hala M I Mead<sup>3</sup> and Mohamed G M Salama<sup>3</sup><sup>1</sup>Cairo University, Egypt<sup>2</sup>Al-Azhar University, Egypt<sup>3</sup>Plant Protection Research Institute, Egypt

Biological activities of different safety botanical extracts were very potent against many insect species. The cotton leaf worm, *Spodoptera littoralis* (Boisd.) is a highly polyphagous insect that causes damages to more than 112 plant species around the world. The toxic effect of *Ipomoea carnea* (*I. carnea*) extracts against 4<sup>th</sup> instar larvae revealed that LC<sub>50</sub> and LC<sub>90</sub> values of *I. carnea* extracts were 24.622 and 164.947 ppm, respectively while, LC<sub>50</sub> and LC<sub>90</sub> of 4<sup>th</sup> instar larva treated with chlorpyrifos insecticide was 9.497 and 91.126 ppm, respectively. On the other hand all the tested treatments were significantly affected during the larvae, pupae and adult stage. LC<sub>50</sub> of *I. carnea* extracts produced the highest significant increase of total immature duration which was 28.66 days, followed by chlorpyrifos, 28.40 days compared to controls (22.57 days). Adult longevity was slightly affected while the female fertility was decreased by 1277.65 and 1300.79 due to LC<sub>50</sub> of *I. carnea* and chlorpyrifos, respectively compared to controls (1969.23). Moreover, larvae, pupae and adult deformation of *S. littoralis* were obtained after treatment of 4<sup>th</sup> instar treated with *I. carnea* extracts (16%, 6%, and 10%) and chlorpyrifos compound (0%, 6%, and 6%) of larvae, pupa and adult stages, respectively. This study suggested that *I. carnea* was very essential to attract alternatives to synthetic chemical pesticides for pest management as they reportedly pose threat to the environment and human health.

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**Human artificial chromosomes and TAR cloning technology for genomes studies and biomedicine****Natalya Kouprina**

National Cancer Institute-NIH, USA

Transformation-associated recombination (TAR) cloning allows selective isolation of full-length genes and genomic loci as large circular Yeast Artificial Chromosomes (YACs) in yeast. The method has a broad application for structural and functional genomics, long-range haplotyping, characterization of chromosomal rearrangements and evolutionary studies. Also, the benefit of combining the TAR gene cloning technology with the HAC gene delivery system for gene expression studies will be discussed. Human artificial chromosome HAC-based vectors offer a promising system for delivery and expression of full-length human genes. HACs avoid the limited cloning capacity, lack of copy number control and insertional mutagenesis due to integration into host chromosomes that plague viral vectors. Recently we engineered the HAC with a single *LoxP* gene adopter site and a defined structure and demonstrated its utility for delivery of several full-length genes and correction of genetic deficiencies in human cells. We also showed that phenotypes arising from stable gene expression can be reversed when cells are “cured” of this HAC by inactivating its kinetochore in proliferating cell populations, a feature that provides a control for phenotypic changes attributed to expression of HAC-encoded genes, thereby aiding in proper interpretation of gene function studies. Also, we demonstrated that HAC-bearing ES cells were indistinguishable from their wild-type counterparts: they retained self-renewal potential and full capacity for multi-lineage differentiation during mouse development, whereas the HAC itself was mitotically and transcriptionally stable during this process. The HAC vectors have a great potential for genes function studies, gene therapy, regenerative medicine, screening of anticancer drugs and biotechnology.

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**Association between plasminogen activator inhibitor-1-675 4g/5g insertion/deletion polymorphism and chronic obstructive pulmonary disease****Rabab El Wahsh**

Menoufia University, Egypt

Molecular pathology of chronic obstructive pulmonary disease (COPD) is still being investigated to discover relationships with disease pathogenesis. Evidence of plasminogen activator inhibitor-1 (PAI-1) overexpression in the sputum and the blood of COPD patients is growing. We aimed to investigate the potential relation between PAI-1 promoter 4G/5G insertion/deletion polymorphism and COPD development. In a case-control study, we genotyped 117 COPD patients and 160 control subjects for PAI-1 promoter 4G/5G polymorphism by an allele-specific polymerase chain reaction analysis. All subjects were male smokers. In the co-dominant model, there was a significant difference in the distribution of 5G/5G, 4G/5G and 4G/4G genotypes between COPD patients and controls ( $p=0.002$ ). In the recessive model, carriers of 4G/4G genotype were significantly higher in COPD patients than controls ( $p=0.01$ ). Carriers of 4G/4G genotype were at higher risk to develop COPD than those carrying 5G/5G or 4G/5G genotypes (crude odds ratio (OR)=2.10, 95% confidence interval (CI)=1.19-3.73, adjusted OR=2.5, 95% CI=1.22-3.99). PAI-1 4G/5G genetic variations are associated with COPD development in males.

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**Biophysical and biochemical insights into the mechanisms of action by Red $\beta$  during homologous recombination****Sivaraman Subramaniam**

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Repair of DNA breaks by single-strand annealing (SSA) is a major mechanism for the maintenance of genomic integrity. SSA is promoted by proteins (single-strand-annealing proteins [SSAPs]) such as eukaryotic RAD52 and  $\lambda$  phage Red $\beta$ . These proteins use a short single-stranded region to find sequence identity and initiate homologous recombination. Using biophysical single molecule techniques, we have shown that homology is recognized by Red $\beta$  monomers that weakly hold single DNA strands together. Upon annealing, homodimerization of Red $\beta$  clamps the double-stranded region and nucleates nucleoprotein filament growth. In this manner, DNA clamping ensures and secures a successful detection for DNA sequence homology. Red $\beta$  clamp is characterized by a structural change and a remarkable stability against force up to 200 pN. Our findings not only present a detailed explanation for SSAP action but also identify the DNA clamp as a very stable, non-covalent, DNA-protein interaction. Using protein biochemistry and recombination assays, we have shown that C-terminally truncated Red  $\beta$ , whilst still able to promote annealing and nucleoprotein filament formation, is unable to mediate homologous recombination. As evaluated by co-immunoprecipitation experiments, the dsDNA recombination function relates to the Red $\alpha$ -Red $\beta$  interaction, which requires not only contacts in the C-terminal domain but also at the N-terminus. Mutations of critical amino acids affected either dsDNA recombination or both ssDNA and dsDNA recombination, indicating two separable functions: one critical for dsDNA recombination and the other for recombination per se. These data further advance Red recombination model and show that Red $\beta$  and RAD52 SSAPs share ancestral and mechanistic roots.

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**Regenerative competence in root explants of *Rhynchosstylis gigantea*, an endangered species: *In vitro* study****Vishal Sharma**

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Orchids constitute one of the largest and diverse family of angiosperms families with 30,000-35,000 species in 600-800 genera, still in an evolutionary flux. They have out-smarted and out-numbered their counterparts due to their long-lasting flowers of myriad shapes, sizes and colors. Their latter utility accounts for a highly lucrative trade in floriculture. Tissue culture technique has been exploited as means of *ex situ* conservation, particularly in outbreeders like orchids which generate a great deal of heterozygosity in the progenies Beechey (1970) suggested possibility of using aerial roots in micro-propagating orchids. The utility of roots as explant source is being increasingly realized due to their easy availability, low oxidation rate & ease with which they can be planted. Keeping this in view, presently we report the pioneer attempt to use root explants from *in vivo* grown fox tail orchid, *R. gigantea* (Lindl.) Ridl, a native of Thailand. *R. gigantea* exhibit free fertility within and beyond the taxonomic limits and has been used as breeding material for raising floriculturally significant hybrids. Besides being victim of its own beauty & utility *R. gigantea* is progressively losing its natural habitat and is getting rarer with every passage of time and figures prominently in Appendix II of the Convention on International Trade in Endangered species of Wild Fauna and Flora (CITES, 2012, 2017). The neo-formations in the *in vivo* root explants of *Rhynchosstylis gigantea* depend upon their location, maturity level and chemical regime. The regeneration is affected by polarity showing basipetal gradient. The distal ones with intact tips with well-developed root caps showed an extended growth with sub-apical formation of globular structure whereas, the proximal explants responded to the presence of cytokinin (Kn) medium according to Mitra et al. (1976) The effect of cytokinins was accentuated in the additional presence of NAA and the higher organogenetic responses are observed in explants when BAP, Kn was used in dose double than that of NAA. The regenerated plantlets were acclimatized & transferred to pots filled with moss, pine bark, brick & charcoal pieces (2:4:1:1) with 90% survival. In conclusion, the results clearly indicate that the root segments as a reliable method of clonal propagation bereft of somaclonal as the root explants is an effective alternative to shoot meristem for micro-propagation due to their easy availability and does not require the sacrifice of mother plant and provide exciting opportunities to raise large numbers of true-to-type plantlets.

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