2ND ANNUAL AUB BASIC BIOMEDICAL RESEARCH DAY

THEME: MULTIDISCIPLINARY APPROACHES TO SCIENTIFIC DISCOVERY

Charles Hostler Auditorium

Saturday, February 25, 2012

8:30 am - 2:00 pm

Table of Content

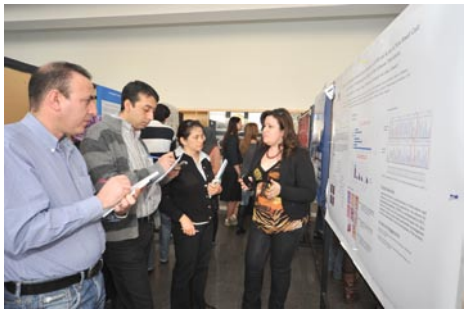
Awardees of the 2011 AUB Basic Biomedical Research Day

Oral presentation

- **Akram Ghantous**, FAS: *Epigenetic Inhibition of Epidermal Carcinogenesis by Parthenolide: Role of NF-κB Signaling*

Poster presentation

- **Dania Al-Labban**, FAS: *Over-Expression of Connexin-43 Attenuates Breast Adenocarcinoma Cell Lines growth and invasion in a β-Catenin Signaling Dependent Pathway*
- **Jamal El-Saghir**, FM: *Heterocellular Interaction between Cancer Cells and Stem Cells in Cancer Metastasis*
- **Ramy Mourad**, FEA: *Designing Pooling Systems for Protein-Protein Interaction Inference Using LDPC Codes*



Schedule of events

8:30 am - 8:45 am	Welcome note: **Dr. Ayad Jaffa**, Assistant Dean for Interdisciplinary Programs **Dr. Mohamed H. Sayegh**, Raja N. Khuri Dean, Faculty of Medicine Vice President of Medical Affairs **Dr. Ahmad Dallal**, University Provost
8:45 am - 9:00 am	2012 Farouk Jabre Award Presentation *President Dorman, Provost Dallal, Dean Sayegh, Trustee Jabre*
9:00 am - 9:45 am	Key note speaker **Dr. Aida Al Aqeel** ***Title**: Personalized Translation Genomics in the Middle East; Challenges and Opportunities*
10:00 am - 10:15 am	Coffee break
	<u>Presentations by the 2011 Farouk Jabre Award recipients</u>
10:15 am – 10:35 am	**Dr. Robert Habib**: *Characterization of Bubble Oscillations in the Infant Lung during Bubble Nasal Continuous Positive Airway Pressure (NCPAP) Support: Effects on Gas Transport and Exchange*
10:35 am - 10:55 am	**Dr. Marwan Sabban:** *TiO2-Paramagnetic coated particles for organ-specific homing of stem cells and tumorcidal therapy*
10:55 am - 11:15 am	**Dr. Raya Saab:** *Identification of repressed genes involved in heterochromatin foci in Cyclin D1-driven senescence*
11:15 am - 2:00 pm	Poster viewing followed by lunch, award presentation for the top 4 posters and closing

Objectives

- serve as a platform to bring together the research community of the different AUB faculties, showcase the basic biomedical research that is performed at AUB

- provide an intellectual environment for scientific exchange among the various researchers at AUB

- provide a platform for students, postdoctoral fellows and junior investigators to present their scientific findings and to foster collaboration within the AUB family of investigators

Eligibility

working in biomedical research in all academic units at AUB

- Students
- Trainees
- Residents
- Fellows
- Post docs

Organizing Committee

Chairperson

- Ayad Jaffa, Assistant Dean of Interdisciplinary Programs, FM, Department of Biochemistry and Molecular Genetics

Members

- Hala Muhtasib, FAS, Department of Biology
- Kamal Bouhadir, FAS, Department of Chemistry
- Marwan Sabban, FM, Department of Anatomy, Cell Biology and Physiological Sciences
- Nahla Hwalla, FAFS, Dean
- Fadi Karameh, FEA, Department of Electrical and Computer Engineering
- Ghassan Dbaibo, FM, Department of Pediatrics and Adolescent Medicine and Department of Biochemistry and Molecular Genetics
- Nayef Saadeh, FM, Department of Human Morphology
- Zaher Dawy, FEA, Department of Electrical and Computer Engineering
- Nadine Darwiche, FM, Department of Biochemistry and Molecular Genetics
- Assaad Eid, FM, Department of Anatomy, Cell Biology and Physiological Sciences
- Soha Yazbek, FHS, Department of Medical Laboratory Sciences
- Nada Melhem, FHS, Department of Infectious Diseases and Microbiology Medical Laboratory Sciences
- Yumna Maalouf, FM, Medical Dean's Office

Keynote Speaker

Aida Ibrahim M. Al Aqeel, MD, DCH, FRCP (Edin/Lond), FACMG

Senior Consultant and Head Pediatrics Medical Geneticist, Metabolist

Consultant Endocrinologist

Riyadh Military Hospital

Adjunct Principal Scientist, King Faisal Specialist Hospital & Research Centre

Email: aalaqeel@rmh.med.sa

Dr. Al-Aqeel graduated from Kuwait University, with honors; had three fellowships in Genetic and Metabolic Disorders including molecular genetics fellowship; obtained the American Board of Medical Genetics in 1999. She has been awarded several awards including, among many others, the Outstanding Investigator Award from the American Federation of Medical Research, February 2002, Science Award from European Society of Human Genetics, May 2005, and the Outstanding Physician Award from the 10th International Congress of Inborn Errors of Metabolism, Chiba, Japan, Sept 2006.

Dr. Al Aqeel is the first Saudi Geneticist and Metabolist. She been involved in many administrative and teaching activities, including CAGS (Centre of Arab Genomic Studies), Dubai, UAE; EMAME (Medical Ethics and Scientific Misconduct) in the WHO – EMRO, Cairo, Egypt, writing policies for implementing genetics services as primary health care services for WHO-EMRO. Human Variome Project consortium and working group member.

She has been on the editorial board of several national and international journals, including the editorial board of American Journal of Bioethics, Cardio genetics journal

She has many novel discoveries including:

1. She has discovered a new Genetic Syndrome, which carries her name. She also found the causative gene, the protein, and the mouse model. This discovery has been published and reviewed in Nature Genetics as “the first genetic evidence for the understanding of human growth and development”. She was awarded the Outstanding Investigator Award by the American Federation of Medical Research for this discovery.
2. She has described a genetic disorder (BDPKU), never described in Saudi Arabia which affects the new born and leads to handicap if not treated, and found the causative enzyme and the molecular defect, has treated patients with an ideal doses of the cofactors without the need for diet.
3. She has described a genetic disorder leading to early rickets in children (VDDRII), found the causative gene and innovated anew therapy.
4. She is the leading Professional in the Arab and Muslim World to establish ethical Frame work for research and patient care in Genetics and Genomics. Therefore I was invited to

write a commentary to Nature Genetics on "Islamic Ethical Framework for Research into and Prevention of Genetic Diseases", among other publications

5. She is the first Saudi geneticist to establish Embryonic Stem Cell Therapy for genetics metabolic disorders by a research grant from King AbulAziz City for Science and Technology- Guaranteed around $10,000,000.

She has been actively involved in research for implementing improved health services, including:

1. Pre implantation Genetic diagnosis for the prevention of genetic disorders
   Co-Principal Investigator – involved in 50% of the research activities.

2. Molecular Genetics of Deafness in Saudi Arabia- Co Principle investigator, under submission.
3. Treatment of Genetics disorders at the molecular level- Principal Investigator, to be submitted to King Abdulaziz City for Science and Technology.

She has been invited to teach and lecture to several Universities and conferences including: Harvard Medical School; North Western University; Mount Sinai School of Medicine; Yale University; University of California, Irvine, USA; World Health Organization Geneva; Centre of Disease Control, USA; University of Oslo and Medical Advisory Board of Biotechnology, Oslo, Norway; National Institute of Health (NICHD), USA; Wellcome Trust Foundation ,Cambridge, UK; Human Variome and Human Genome meetings Panel of experts, NIH, WHO, Brocher Foundation Newborn Screening Panels, Human Genome Meeting 2011 among others.

She has published over 70 manuscripts on neurometabolic genetic disorders in peer reviewed, editorialized journals, three of these publications are in nature Genetics, presented over 100 abstracts and gave over 100 invited speeches, nationally and internationally. She is the co-author for two books including Atlas of metabolic diseases, Arnold Health Sciences; and Human Genomics and Hereditary Disorders in the developing world, Oxford University Press.

2011 Farouk Jabre Award Recipients

- **Dr. Robert Habib**, FM and **Dr. Alan Shihadeh**, FEA: *Characterization of Bubble Oscillations in the Infant Lung during Bubble Nasal Continuous Positive Airway Pressure (NCPAP) Support: Effects on Gas Transport and Exchange*

- **Dr. Kamal Bouhadir**, FAS, **Dr. Tarek Ghaddar**, FAS and **Dr. Marwan Sabban**, FM**:** *TiO2-Paramagnetic coated particles for organ-specific homing of stem cells and tumorcidal therapy*

- **Dr. Raya Saab**, FM and **Dr. Noel Ghanem**, FAS**:** *Identification of repressed genes involved in heterochromatin foci in Cyclin D1-driven senescence*

2012 Farouk Jabre Award Recipients

- **Dr. Georges Nemer**, FM and **Dr. Zakaria Kambris**, FAS: *The nephrin gene: A renal gene implicated in cardiovascular diseases*

- **Dr. Marwan Sabban**, FM and **Dr. Hala Muhtasib**, FAS: *Targeted therapy of breast cancer stem cells and metastasis by the hypoxiaactivated quinoxaline 1,4-dioxide DCQ*

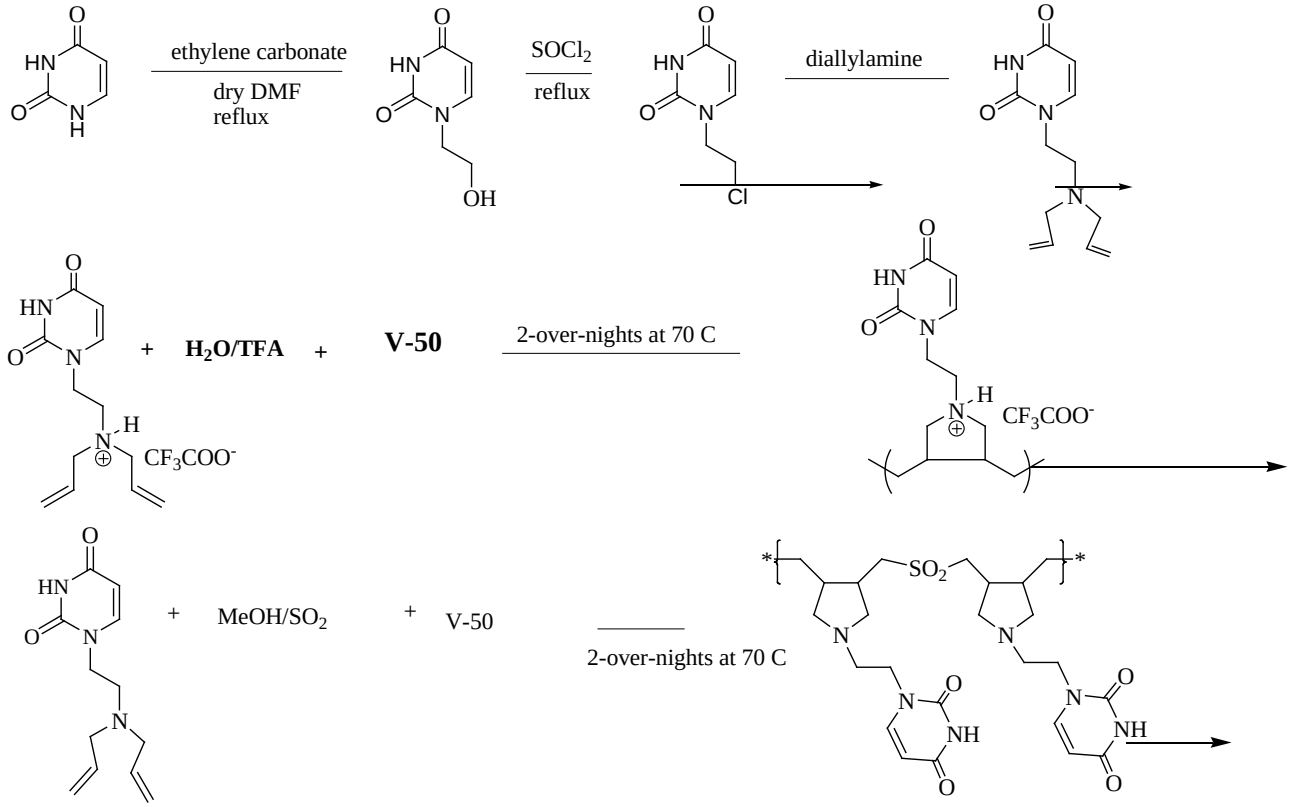
ABSTRACT # 1

SYNTHESIS AND CYCLOPOLYMERIZATION OF 1-(2-DIALLYLAMINOETHYL)URCAIL

A. Ezzeddine and K. Bouhadir*

Department of Chemistry, American University of Beirut, Beirut, Lebanon
*Email: kb05@aub.edu.lb

Gene therapy has been investigated over the years resulting in different strategies to combat inherited diseases. An efficient and yet attractive approach is to correct the mutated gene in the affected individual[1]. Synthetic oligonucleotides are currently used to repair such genes.[1] Moreover, polydiallyldimethylamonuim chloride (PDADMAC) and its copolymers have been utilized in a wide range of applications such as wastewater treatment and purification, paper and textile industries, cosmetic and personal care industries, biological, medical, and food applications.[2] As a result Poly (diallylalkyl ammonium salts) could be used as a potential synthetic backbone for such materials. We report herein the synthesis and cyclopolymerization of 9-(2-diallylaminoethyl)uracil while evaluating the effect of monomer concentration on the isolated polymer yield. We have characterized the resulting polymers with FTIR, UV-VIS and NMR.



Key words: Uracil, diallylamine, polymers, cyclopolymerization

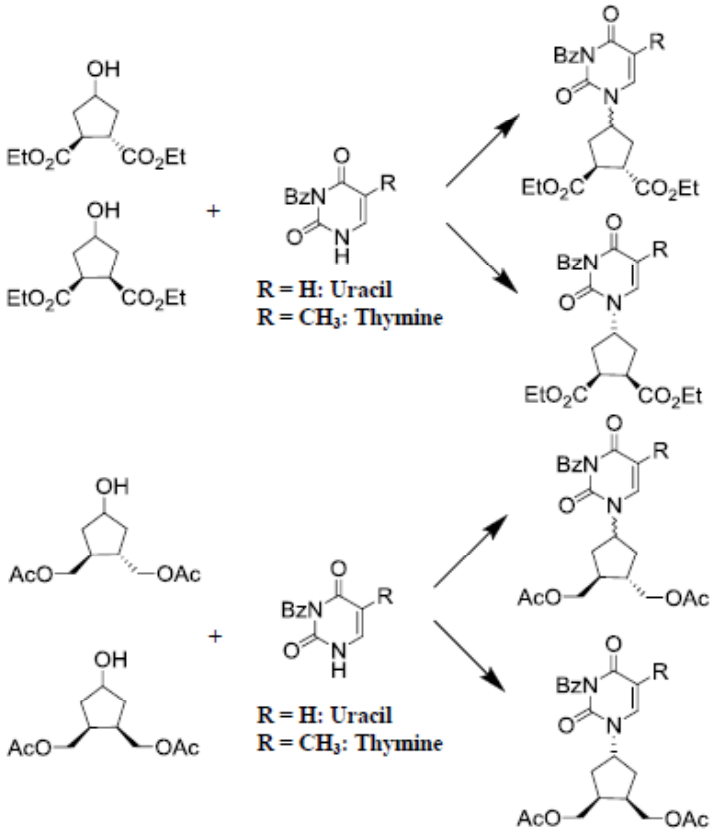
ABSTRACT # 2

SYNTHETIC PYRIMIDINE-BASED NUCLEOSIDES

A. Koubeissi, M. Farah, N. Alkhalil and K. Bouhadir*

Laboratory of Organic Chemistry, Department of Chemistry, American University of Beirut, Beirut, Lebanon
*Email: kb05@aub.edu.lb

Synthetic oligodeoxynucleotides (ODNs) have received increasing interest over the years due to their potential use in therapeutic and diagnostic applications.
We are interested in the design and synthesis of modified nucleosides that could be utilized to form synthetic polynucleotide analogs thereafter. These include a variety of 3,4-substituted carbocyclic nucleoside analogs with two functional groups attached to a cyclopentane ring and hence, could be connected to each other to form synthetic polynucleotide analogs such as a new form of peptide nucleic acids (PNAs). We have utilized the Mitsunobu reaction to couple the protected pyrimidine bases to alcohols in most of these adducts. We will report the synthetic protocols followed to synthesize these molecules as well as some of the characterization of the final products.



Key words: Modified nucleosides, nucleic bases, Mitsunobu reaction

ABSTRACT # 3

CORTICAL LAYERS ESTIMATION FOR MODELED EEG POTENTIAL BASED ON REALISTIC CORTICAL MICRO-ARCHITECTURE

H. Farhat and F. Karame*

Department of Electrical and Computer Engineering, Faculty of Engineering and Architecture, American University of Beirut, Beirut, Lebanon
*Email: fk14@aub.edu.lb

Background

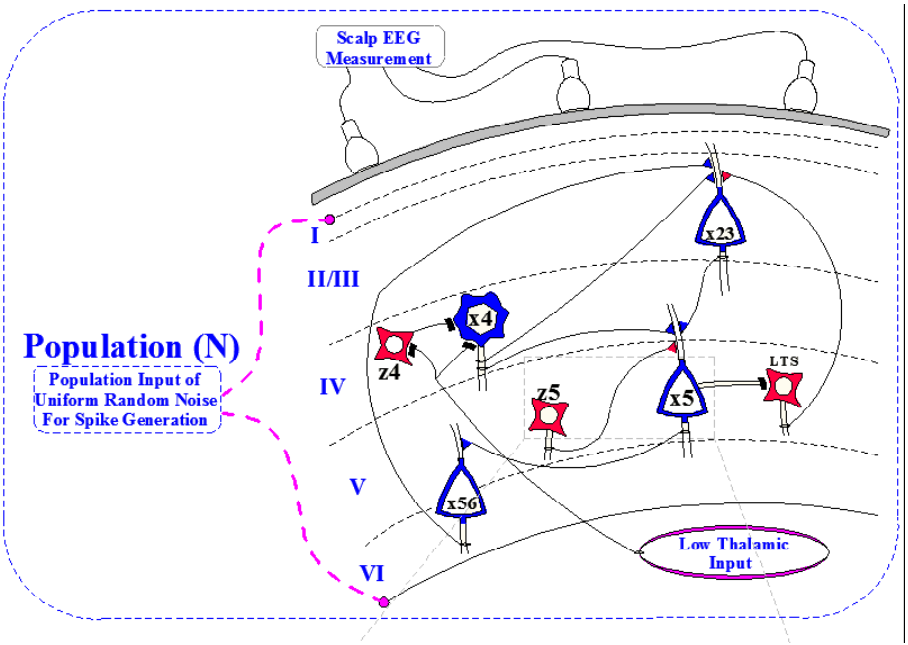
EEG modeling is a promising tool for understanding global brain dynamics. While many models assume EEG signals as the output of homogeneous neuronal populations, other efforts delve into the detailed dynamics at the single neuronal level. In this work, we will try to develop models that build on a compromise of these two approaches. In general, we aim to account on the interaction taking place between different brain regions to study the contribution of different structures in the generation of rhythmic EEG. Our work will focus on an intermediate population level that accounts for inter-neuronal connectivity known to exist within and among sensory systems, thus linking between structures that produce such a rhythmic activity and others along which the activity propagates. The simulated activity is then hypothesized to account for the EEG signals under sensory tasks.

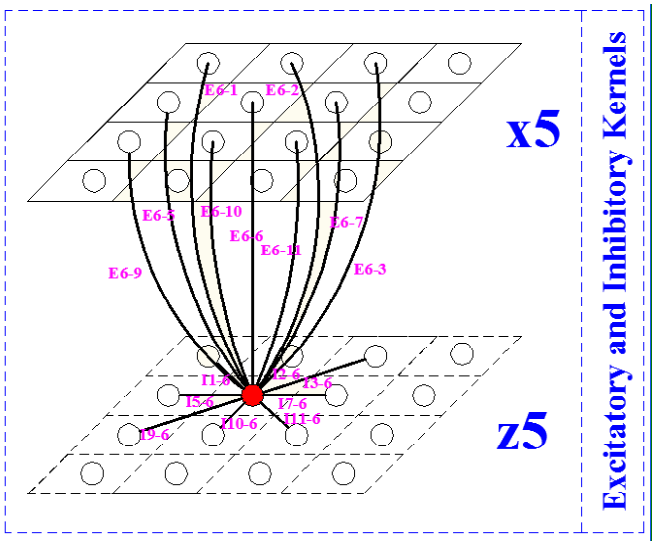
Aim

In short, in our simulation to produce the EEG, 10 to 15 populations (representing the six well known cortical layers) hosting a specific number of neurons for each layer (mainly 16) will be used (see figure below). A unique uniform random noise to produce the firing spikes will drive each population where uniqueness helps attain the randomness in the process of EEG generation and which will be vital at the estimation level. The estimation is the core of the model where we can discover, using only the observed EEG, the underlying states of the cortical layers in addition to the parameters (weights) connecting these states.

Methods

To estimate the parameters of the model, we use a nonlinear time-varying estimation tool known as the Unscented Kalman Filter (UKF) and/or Square-Root Kalman which also shows significance potential to correctly identify hidden states (neuronal activity) and the parameters (connectivity) based on simulated EEG signals.





Key words: EEG, cortical layers, Unscented Kalman Filter

ABSTRACT # 4

IDENTIFICATION OF PROTEIN-PROTEIN INTERACTIONS USING COMPRESSED SENSING

R. Mourad and Z. Dawy*

Department of Electrical and Computer Engineering, Faculty of Engineering and Architecture, American University of Beirut, Beirut, Lebanon
*Email: zd03@aub.edu.lb

The availability of whole-genome sequences has made it possible for researchers to study the large-scale biochemical and biological function of proteins to elucidate protein structure, protein-protein interactions, and drug interactions, among many other studies in systems biology. Large quantities of data coming from high throughput experiments are being used to produce large networks of interactions and study their structure. This higher-level view of networks provides global properties of the biological system and helps understand patterns of behavior that might predict gaps in the incomplete interaction map of an organism. In this work, we use Compressed Sensing (CS), a recently developed sampling theory for sparse signals, to address the problem of efficient and reliable detection of protein-protein interactions in yeast two-hybrid (Y2H) high throughput experiments [1], [2]. The proposed method is applied to a simulation study in which 940 prey proteins were screened against up to 5 active baits in low, mild, and high experimental false positive and false negative error rates. The results were benchmarked against the current state-of-the-art in systems biology, the Shifted Transversal Design (STD) and Interpool decoder [3], [4]. The obtained results indicate improved error-correction and computational efficiency for the majority of the cases considered. The performance of the proposed method was also tested against a real experimental data set, where 12,675 prey proteins were screened against 12 bait proteins, with comparable performance. The results of the simulation study and application to experimental data provide validity for the method described in this work. Additionally, the error-correction properties of several commonly used pooling designs were investigated, with the conclusion that the STD pooling design is the most suitable for error-correction and identifiability in biological applications of CS, demonstrating the lowest row and column coherence among other matrix designs considered.

References:

1. R. Baraniuk, “Compressive sensing,” *IEEE Signal Processing Magazine*, vol. 24, no. 4, pp. 118–121, July, 2007.

2. E. Candes and M. Wakin, “An introduction to compressive sampling,” *IEEE Signal Processing Magazine*, vol. 25, no. 2, pp. 21–30, March, 2008.

3. N. Thierry-Mieg, “A new pooling strategy for high-throughput screening: the shifted transversal design," *BMC Bioinformatics*, vol. 7, January, 2006. doi: 10.1186/1471-2105-7-28.

4. N. Thierry-Mieg and G. Bailly, “Interpool,” *Bioinformatics*, vol. 24, no. 5, pp. 696–703, January, 2008.

ABSTRACT # 5

COMPARATIVE ANALYSIS OF BODY COMPOSITION ASSESSMENT METHODS IN 8-10 YEAR OLD LEBANESE CHILDREN

S. Kassem-Youssef, J. Chahine, L. Nasreddine*, F. Naja and N. Hwalla

Department of Nutrition and Food Sciences, American University of Beirut, Beirut, Lebanon
*Email: ln10@aub.edu.lb

Introduction
Recent studies suggest that estimation of body fat from anthropometric indicators and bioelectric impedance (BIA) may produce systematic errors across different ethnic groups. This study aims at evaluating the predictive validity of five previously published anthropometry-based equations and BIA in estimating body fat among 8 -10 year old Lebanese children.

Methods
A total of 158 children (77 boys and 81 girls) were recruited. Anthropometric measurements (weight, height, skin-fold thickness at 5 different sites) were obtained. Total body water was used as the reference method and was assessed using deuterium dilution, with the use of age and gender specific constants for the determination of Fat Free Mass and Fat Mass. Body fat was estimated using 5 published anthropometry-based equations as well as by BIA. Correlation and agreement between methods were assessed using Pearson Correlation analysis and Bland-Altman plots. Estimates of percent body fat (%BF) from various methods were compared using repeated measures ANOVA with Bonferroni corrections.

Results
Correlations coefficients between deuterium dilution and other methods ranged between 0.77 and 0.86 in boys and between 0.62 and 0.71 in girls ($p<0.05$), the highest being for BIA in boys and Bray's equation in girls. Significant differences were found between %BF obtained using deuterium dilution and all methods under study ($p<0.034$). Bland Altman plots showed systematic bias towards a larger difference between techniques at higher percent fat mass.

Conclusion
These findings call for the development of valid body composition assessment equations in Lebanese children and emphasize the need for further research, especially in Middle-Eastern countries, to help clarify ethnic differences in body composition.

Key words: Body composition; prepubertal children; Deuterium Dilution technique

ABSTRACT # 6

ASSOCIATION BETWEEN OBESITY AND THE METABOLIC SYNDROME INDICATORS AMONG ADOLESCENTS IN LEBANON

A. El-Aily[1†], C. Haikal[1†], M.Tabet[1†], MZ. Habbal[2], N. Adra[1], L. Nasreddine[1*] and N. Hwalla[1*]

[1]Department of Nutrition and Food Science, Faculty of Agricultural and Food Sciences,
[2]Pathology and Laboratory Medicine, Faculty of Medicine, American University of Beirut, Beirut, Lebanon
[†]Contributed equally to this work
*Email: nahla@aub.edu.lb, ln10@aub.edu.lb

Background
Prevalence of metabolic syndrome (MS) in obese adolescents was reported to range between 18% and 44%, depending on country of origin, thus suggesting an ethnic based association between obesity and MS.

Aim
This study aims to investigate the magnitude of the association between obesity, insulin resistance and components of MS among adolescents in Lebanon.

Subjects and methods
The sample included 263 adolescents at 4th and 5th tanner stages of puberty (104 obese; 78 overweight; 81 normal weight). Anthropometric, biochemical and blood pressure measurements were performed. Body Fat was assessed using dual-energy X-ray absorptiometry.

Results
According to International Diabetes Federation criteria, MS was identified in 21.2% of obese, 3.8% of overweight and 1.2% of normal weight subjects. The most common metabolic abnormalities among subjects having MS were elevated waist circumference (96.2%), low HDL (96.2%), and hypertriglyceridemia (73.1%). Insulin resistance was identified in all subjects having MS. Regression analyses showed that percent body fat, waist circumference and BMI were similar in their ability to predict the MS in this age group.

Conclusions
MS was identified in a substantial proportion of Lebanese obese adolescents, thus highlighting the importance of early screening for obesity-associated metabolic abnormalities and of developing successful multi-component interventions addressing adolescent obesity.

Key words: Metabolic syndrome, obesity, body fat, BMI, waist circumference, insulin resistance, adolescents, Lebanon

ABSTRACT # 7

H. PYLORI INFECTION IN LEBANON: PREVALENCE, RISK FACTORS AND ASSOCIATION WITH METABOLIC SYNDROME

P. Moghames[1], H. Gali-Muhtasib[2], N. Hwalla[1, 4], A. Sibai[3, 4], L. Nasreddine[1, 4], H. Shouaib[1], M.Fatfat[2] and F. Naja[1, 4*]

[1]Department of Nutrition and Food Sciences, [2]Department of Biology, [3]Department of Epidemiology and Population Health, [4]Members of the Public Health and Nutrition (PHAN) Research Group at the American University of Beirut, Beirut, Lebanon
[*]Research Advisor

Introduction
H. pylori is classified by WHO as type I carcinogen for its association with gastric cancer. This bacterium has also been associated with diverse extra digestive diseases, namely metabolic syndrome (MetS). The aim of this study was to investigate the prevalence and correlates of *H.pylori* infection among Lebanese adults and to evaluate its association with MetS.

Methods
The data for this study were drawn from the national Nutrition and Non-Communicable Diseases Risk factors survey conducted in Lebanon between years 2008 and 2009. Stored blood samples from survey participants older than 18 years of age and with no chronic diseases were tested for *H.pylori* infection by ELISA. Data available included sociodemographic and lifestyle characteristics, blood pressure, biochemical indices (serum HDL, LDL, TAG, glucose) and anthropometric measurements such as weight, height, and waist circumference. The International Diabetes Federation criteria were used to classify study participants with metabolic syndrome.

Results
A total of 309 blood samples were analyzed. The prevalence of *H. pylori* infection in the study sample was 52% (95% CI: 46.43%-57.57). No difference in prevalence estimates was observed among males and females. In addition, age was not associated with the presence of the infection. Among all the demographic and socioeconomic factors studied, only crowding index was associated with *H. pylori* infection; a higher crowding index was associated with a higher prevalence of the infection (OR: 1.93; 95% CI: 1.04-3.58). The serum levels of glucose HDL, LDL, TG, and the incidence of MetS were all comparable among infected and non infected individuals.

Conclusion
The prevalence of *H pylori* infection in Lebanon is comparable with that of other developing countries. Crowding index is a significant predictor of the infection. *H.pylori* infection was not associated with MetS or any of its components in this Lebanese adult population.

Key words: *H. pylori*, metabolic syndrome, Lebanon

ABSTRACT # 8

DCQ REDUCES HYPOXIA INDUCED AGGRESSIVE PHENOTYPE IN BREAST CANCER

K. Ghattass*[1], M. Haddadin[2], ME. Sabban[3] and H. Gali-Muhtasib[1]

[1]Department of Biology, [2] Department of Chemistry[3], Department of Human Morphology, American University of Beirut, Beirut, Lebanon
*Email: kig01@aub.edu.lb

Introduction

It is believed that eliminating the highly resistant hypoxic cells within the tumor is an exceptionally promising approach for treatment of aggressive tumors. Among viable approaches is the use of hypoxia activated drugs. 2-benzoyl-6,7-dichloro-3-phenylquinoxaline 1,4-dioxide(dcq) is a synthetic quinoxaline dioxide first synthesized at aub. For a decade, our lab has shown that dcq preferably targets tumor cells within their hypoxic microenvironment. Here we present new data on dcq's antineoplastic and antimetastatic effects in breast cancer cell lines with different metastatic potentials.

Methods

To test for the hypoxic selectivity of dcq, cells were treated with different concentrations of the drug for different time intervals either under hypoxia or normoxia. The effect of dcq was investigated using mtt proliferation assay, colony formation assay (clonogenic survival), western blot (protein expression), invasion assay, flow cytometry of pi stained dna (cell cycle analysis), elisa of secreted vegf, and dcfh assay (ros production).

Results

Ddcq effectively induces apoptosis and decreases the colony forming ability in mcf-7 and mda-mb-231 cells more so under hypoxia than oxia. The targeted signaling molecules appear to be distinct in the two cell lines. In mcf-7, dcq induces dna damage induces phosphorylation of p53, targets hif-1α to proteasomal degradation, decreases p21 levels, and reverses the hypoxia-induced increase in vegf. In mda-mb-231, dcq reduces hif-1 α, however this was accompanied by dramatic increase in p21 and cleavage of caspases 3 and 9, effects not seen in mcf-7.

Conclusion

Dcq is a hypoxia activated cytotoxin that has not yet failed to present extremely promising results against all tested cancer cell lines. Currently, several studies are being conducted to decipher its mechanism of action. However, further preclinical investigations are required for DCQ to advance from laboratory benches to clinical trials.

ABSTRACT # 9

PARTHENOLIDE INHIBITS TUMOR PROMOTION: EPIGENETIC REGULATION OF P21

A. Ghantous[1], M. Saikali[2], R. Schneider-Stock[3] and N. Darwiche[2*]

[1] Cell and Molecular Biology PhD Student, Department of Biology, [2] Department of Biochemistry and Molecular Genetics, American University of Beirut, Beirut, Lebanon, [3] Pathology Institute, Friedrich-Alexander University of Erlangen-Nuremberg, Germany
*Email: nd03@aub.du.lb

Introduction
The promotion stage in the multistep process of tumorigenesis is mostly epigenetic and reversible, thus, a suitable target for chemoprevention. The transcription factor NF-kB is crucial for tumor promotion; therefore, we investigated whether the NF-kB inhibitor, parthenolide, currently in cancer clinical trials, shows chemopreventive properties and regulates NF-kB target genes by epigenetic mechanisms.

Methods
Cell growth assays, soft agar colony formation, cell cycle analyses by flow cytometry, NF-κB gel shift and luciferase reporter assays, westerns, real time PCR, chromatin immuno-precipitation (ChIP) assays, siRNA, tumor mouse models, tissue microarrays

Results
Parthenolide, at specific concentrations, selectively inhibited the growth of neoplastic keratinocytes while sparing normal ones in well-characterized *in vitro* models of human and murine epidermal carcinogenesis. In JB6P+ cells, a model of tumor promotion, parthenolide abrogated promoter-induced cell proliferation and anchorage-independent growth, blocking promoted cells in S-G_2/M phases. Furthermore, parthenolide decreased basal and promoter-induced NF-kB activity and modulated the expression of the NF-kB target genes, *p21* and *cyclin D1*, which play crucial roles in tumor promotion and are epigenetically regulated in epidermal carcinogenesis. In parthenolide-treated cells, *p21* transcription correlated with relaxed chromatin, but not with p65/NF-kB binding, at the *p21* promoter. However, *cyclin D1* transcription correlated with p65/NF-kB binding, rather than with chromatin structure, at the *cyclin D1* promoter. Using p21-siRNA and human colon carcinoma HCT116 wild type and p21-/- clonal cell variants, we showed that p21 is implicated in increased cell sensitivity to parthenolide. Interestingly, parthenolide, at low concentrations (0.25 mg/Kg), inhibited tumor growth of promoted JB6P+ cells in xenograft NMRI mouse chemoprevention protocols, in which the drug was administered intraperitoneally every other day over a ten-day period and stopped upon injection of tumor cells or stopped upon tumor appearance. Similarly to its effects *in vitro*, parthenolide decreased *in vivo* tumor cell proliferation, by Ki76 immunostaining, while decreasing p65/NF-kB and increasing p21 immunostains.

Conclusion
These results demonstrate the chemopreventive properties of parthenolide at low concentrations and suggest its potential use in epigenetic cancer therapy

Key words: Parthenolide, tumor promotion, epigenetics, NF-κB, p21

ABSTRACT # 10

CLN3 GENE, A NOVEL BIOMARKER FOR BREAST CANCER

M. Raad [1,2†], KA. Sarhane [2,3†], B. Noutsi [2,3], E. Saad Aldine [4], F. Boulos [5], A. Tfayli [4] and R-M. Boustany *[2,3]

[1]Department of Biology, AUB, [2]Department of Pediatrics and Adolescent Medicine, Program in Neurogenetics, [3]Department of Biochemistry and Molecular Genetics, [4]Breast Center of Excellence, NK Basile Cancer Institute,[5]Department of Pathology and Laboratory Medicine, American University of Beirut, Beirut, Lebanon
[†]Equal contributors [*]Email: rb50@aub.edu.lb

Background
CLN3p is an integral membrane protein encoded by the CLN3 gene. It positively regulates cell growth and is anti-apoptotic. CLN3 decreases ceramide generation upstream. Defects in this gene have been linked to a neurodegenerative disease (Batten disease). It is overexpressed at both the mRNA and the protein level in a number of cancer cell lines, including numerous human breast (BT-20, BT-549, and BT-474), and murine mammary cancer lines.

Aims

1) To assess the expression of CLN3 using qRT-PCR in normal breast tissue specimens, in ductal breast carcinoma in situ (pre-malignant, DCIS) and in invasive ductal carcinoma, grades I-III (malignant, IDC).
2) CLN3 overexpression profiles will be compared in the three patient cohorts.
3) Clinical significance of this CLN3 overexpression in discrimination of breast tumors (benign vs. DCIS vs. IDC grade I, II and III) will be analyzed.
4) Last but not least, CLN3 overexpression will be correlated with various clinicopathological factors and patient outcomes, including disease free survival, recurrences and metastasis.

Materials and Methods
RNA is extracted from formalin-fixed paraffin embedded breast tissue blocks from AUBMC pathology, and analyzed by qRT-PCR in accordance with an IRB-approved protocol.

Results
250 out of 1300 potential samples have been analyzed so far. Greater than 1-fold CLN3 overexpression was found in 33% of cases of pre-malignant DCIS, 61% of grade I invasive ductal carcinoma (malignant, IDC), 81% of grade II IDC and in 59% of grade III IDC.

Discussion and Conclusions
The abrupt increase in CLN3 overexpression from DCIS (pre-malignant) to IDC (malignant) shows that CLN3 might be the trigger or switch needed for cancerous transformation of pre-malignant tissue to invasive ductal carcinoma. The drop in expression from grade II to grade III initially appeared counterintuitive. It, however, is best explained by loss of some CLN3 expression due to abundant hypoxic foci and necrosis within grade III tumors.Quantification of CLN3 expression in breast tissue biopsies may be considered as a novel and independent biomarker for differentiating between benign, pre-malignant and malignant tumors of the mammary gland. It also may have predictive value vis-à-vis clinical outcomes.

Key words: CLN3, breast cancer, cancer biomarker, tumor marker, intraductal breast carcinoma, invasive ductal breast carcinoma, ceramide, apoptosis

ABSTRACT # 11

CLN3, A NOVEL MOLECULAR TARGET FOR BREAST CANCER

J. Makoukji[1*] and R-M. Boustany[1]
[1]Neurogenetics Program and Division of Pediatric Neurology, Departments of Pediatrics & Adolescent Medicine and Biochemistry and Molecular Biology, American University of Beirut Medical Center, Beirut, Lebanon
*E-mail: jm53@aub.edu.lb

Introduction

Breast cancer (BC) is the most common cancer in women, accounting for 23% of all female malignancies. In addition, BC incidence has shown an alarming rate increase worldwide. Elucidation of the underlying biology and molecular pathways for this disease is necessary with the goal of personalizing therapeutic options for individual patients, hence, improving clinical outcomes. The CLN3 protein is anti-apoptotic, and defects in the CLN3 gene cause accelerated apoptotic death of neurons in juvenile Batten disease. Previous work from this laboratory has shown upregulation of CLN3 protein in a number of cancer cell lines, including human and murine breast cancer cell lines, as well as solid colon and breast cancer. Dysregulated apoptotic pathways are often implicated in development of the oncogenic phenotype. The aim of this study is to establish CLN3 expression in a breast cancer cell line (MCF7), and to determine the effect of CLN3 levels on cell growth and apoptosis.

Methods

CLN3 expression will be determined by real-time PCR in MCF7 cells compared to normal breast epithelial cells (MCF10A). Blocking CLN3 protein expression will be achieved by transfecting MCF7 cells with scrambled siRNA or with siRNA directed against CLN3. Impact of CLN3 expression on cancer cell growth, apoptosis and ceramide production will be determined using trypan blue dye exclusion, propidium iodide staining, and the DGK assay, respectively. The effect of different chemotherapeutic agents on cancer cell growth and apoptosis will be determined under different CLN3 expression states. Efficacy of Fenretinide, an activator of ceramide generation, sodium butyrate a modifier of histone acetylation, and 5-Aza-2'-deoxycytidine which alters methylation status will be established for different CLN3 expression states.

Preliminary results

CLN3 messenger RNA is overexpressed in MCF7 cells compared to MCF10A cells, making MCF7 cells an excellent *in vitro* model to study the impact of CLN3 expression. We also established the efficacy of CLN3 siRNA showing the blockage of CLN3 protein expression in MCF7 cells. Blocking CLN3 expression inhibited growth and viability of MCF7 cancer cells, and increased apoptosis as shown by propidium iodide staining.

Conclusion: Targeting CLN3 overexpression may be an option for therapy in breast cancer. In other words, CLN3 may be a novel molecular target for cancer drug discovery, possibly acting via modulation of ceramide pathways.

Key words: CLN3, breast cancer, MCF7 cells, cell growth, apoptosis, ceramide

ABSTRACT # 12

REACTIVE OXYGEN SPECIES MEDIATE THYMOQUINONE INDUCED CELL DEATH IN HTLV-1 POSITIVE AND NEGATIVE CELL LINES OF T-CELL LEUKEMIA

E. Dergarabedian* and H. Gali-Muhtasib

Department of Biology, American University of Beirut, Beirut, Lebanon
*Email: emd00@aub.edu.lb

Background
Thymoquinone (TQ), the main constituent of the volatile oil of Nigella sativa seed has been used in folk medicine for over 2000 years. Tested both in vitro and in vivo, TQ has shown anti-neoplastic potential against a wide range of tumors.

Aims
In this study, we aimed at assessing TQ's effects against HTLV-1 positive cells (HuT-102 and MT-2) and HTLV-1 negative peripheral T-cell lymphomas (CEM and Jurkat) and determining the so far poorly understood anticancer mechanism of action of TQ.

Methods
We evaluated the effects of TQ on cell death and cell cycle by MTT, Trypan blue, propidium iodide, TUNEL and annexin. The levels of reactive oxygen species (ROS) were determined using DCFH assay. The intermediary roles of H_2O_2 and O_2^- were also studied using catalase (CAT) and superoxide dismutase (SOD), respectively. Finally the intracellular levels of glutathione (GSH); the major antioxidant in the cell were determined using a colorimetric detection kit.

Results
Treatment with TQ decreased viability in all cancerous cell lines. Interestingly, HTLV-1 negative cell lines were more sensitive to TQ than the HTLV-1 positive counterparts. The mode of cell death was mainly through apoptosis and involved the generation of ROS in both Jurkat and Hut-102 cells. The two main oxidants, superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) were found to be responsible for cell death since CAT and SOD both partially abolished the oxidant shift. CAT provided significant protection against TQ-induced cell death in both HTLV-1 positive and negative cells while SOD only provided a significant protection in HTLV-1 negative Jurkat cells. Finally we showed that TQ depleted GSH levels.

Conclusions
TQ causes cell death through both ROS generation and inhibition of ROS elimination. TQ selectivity to cancer cells while sparing the normal lymphocytes makes it a potentially interesting anticancer drug for the treatment of leukemia.

Key words: Thymoquinone, cell death, reactive oxygen species, T-cell leukemia, HTLV-1

ABSTRACT # 13

CHEMOPREVENTIVE ACTIVITIES OF SESQUITERPENE LACTONES FROM INDIGENOUS LEBANESE PLANTS IN SKIN CANCER: MODULATION OF AP-1 AND NF-KB SIGNALING

M. Saikali,[1] A. Ghantous,[2] N. Saliba[3,4] and N. Darwiche[1,4*]

[1]Departments of Biochemistry &Molecular Genetics, [2]Biology, [3]Chemistry, and [4]IBSAR, American University of Beirut, Beirut, Lebanon
*Email: nd03@aub.edu.lb

Introduction

Most cancers are of epithelial origin of which skin cancer is the most frequent. Numerous naturally occurring compounds have been identified as skin chemopreventive agents, and many of the best-selling anticancer drugs are plant-derived. We screened for anti-cancer activities of Middle Eastern plant extracts used in folk medicine and we identified the Lebanese indigenous plants, *Centaurea ainetensis* and *Achillea falcata*, to possess potent and selective activities against skin cancer cells. Bioassay-guided fractionation of these plants' crude extracts led to the isolation of the sesquiterpene lactones, Salogravolide A (Sal A) from *Centaurea ainetensis* and 3-β-methoxy-iso-seco-tanapartholide (β-tan) from *Achillea falcata*. Our aims were to investigate the effects of Sal A and β-tan on i) the growth of skin tumor cells, ii) promoter-induced cell proliferation and transformation, iii) the regulation of key AP-1 members and target genes and iv) the modulation of AP-1 and NF-κB transcriptional activities.

Methodology & Results

Using well established *in vitro* models of human and mouse epidermal carcinogenesis, and MTT cell viability assays, we have shown that these purified sesquiterpene lactones preferentially inhibited the proliferation of papilloma and malignant skin cells at concentrations that do not affect the growth of normal cells. Furthermore, both Sal A and β-tan, at low concentrations show promising anti-promoting activities by inhibiting promoter-induced cell proliferation and anchorage-independent growth in soft agar. Elevated levels of the transcription factors, activator protein 1 (AP-1) and nuclear factor κB (NF-κB) are hallmarks of tumor promotion and are upregulated in skin cancer. Western blot analysis showed that both sesquiterpene lactones downregulate key members of the AP-1 complex, in addition to modulating several of AP-1 and NF-κB key downstream target genes. Finally, both compounds differentially regulate promoter-induced AP-1 and NF-κB transcriptional activities.

Conclusion

These results highlight the potential chemopreventive properties of the sesquiterpene lactones, Sal A and β-tan, isolated from indigenous Lebanese plants, in skin cancer through; inhibiting promoter-induced cell proliferation, cell transformation and AP-1 and NF-κB transcriptional activities as well as modulating members and targets of the AP-1 transcription factor. We are currently testing their chemopreventive and therapeutic properties using an established ultraviolet irradiation mouse epidermal carcinogenesis model.

Key words: Chemoprevention, skin cancer, sesquiterpene lactone, AP-1, NF-κB

ABSTRACT # 14

THE ZINC CHELATOR TPEN IS A PROMISING THERAPEUTIC ANTI-CANCER AGENT THAT INDUCES APOPTOSIS IN HUMAN COLON CANCER CELLS.

M. Fatfat[1], A. Zaki[1], K. Machaca[2] and H. Gali-Muhtasib[1]

[1]Department of Biology, American University of Beirut, Beirut, Lebanon, [2]Department of Physiology and Biophysics, Weill Cornell Medical College, Doha, Qatar

Background and Aims
Zinc is an essential trace element needed by all cells for their proper growth and development. In addition, Zinc is a cofactor for a large number of proteins including transcription factors. Here we aimed to define the role of zinc in regulating cell cycle progression, cell growth and cell death. The results of this study could form the basis for identifying the efficacy of zinc chelation in cancer therapy. Our results indicate that the zinc chelator, TPEN, induces apoptosis in HCT116 p53+/+ human colon cancer cell lines through the intrinsic mitochondrial pathway in a caspase-dependent manner.

Methods
We evaluated the effects of TPEN on cell death and cell cycle by MTT, and propidium iodide staining of DNA content. The levels of reactive species (ROS) were determined using DCFH assay. Intracellular zinc was measured by zinquin assay. The effect of treatment on the mitochondrial membrane potential was studied using rhodamine 123. Percentages of apoptotic cells were measured by TUNEL and Annexin assays. Western blotting was done to determine the effect of TPEN on the protein expression levels.

Results
Treatment with TPEN decreased the viability of HCT116 cancer cell lines in a concentration- and time-dependent manner. PI staining with flow cyotmetry, TUNEL and Annexin assays showed that the addition of TPEN at 3 µM and 5µM for 24h induced pre G_1 increase and caused apoptosis. In addition, TPEN disrupted the mitochondrial membrane potential as evidenced by the reduction of the levels of rhodamine 123 fluorescence, suggesting that apoptosis by TPEN is through the intrinsic mitochondrial pathway. The exogenous addition of the strong antioxidant NAC and of $ZnSO_4$ inhibited apoptosis by TPEN. At the molecular level, TPEN was found to degrade the X-linked inhibitor of apoptosis (XIAP) protein and this degradation was blocked by the addition of the pancaspase inhibitor, suggesting that caspases are involved in TPEN-induced apoptosis.

Conclusions
Our results suggest that Zn^{2+} chelation by TPEN may be a promising therapeutic approach against colon cancer.

Key words: Zinc chelator, TPEN, colon cancer, apoptosis, caspases

ABSTRACT # 15

AMPK, MTOR AND TUBERIN: NOVEL BIOLOGICAL PATHWAYS LINKING DIABETES TO COLORECTAL CANCER

S. Abou Merhi[1], A. Jaffa[2], FN. Ziyadeh[3] and A. Eid[1*]

Department of Anatomy, Cell Biology and Physiological Sciences[1], Department of Biochemistry and Molecular Genetics[2] and the Department of Internal Medicine[3], Faculty of Medicine, American University of Beirut, Beirut, Lebanon
*Email: ae49@aub.edu.lb

Introduction
Diabetes and cancer are prevalent diseases whose incidence is increasing worldwide. Recent studies found that people with diabetes were 38% more likely to be diagnosed with colorectal cancer. While diabetes and especially type 2 diabetes and cancer share many risk factors, the biological links between the two diseases are poorly characterized.

Results
We have evidence that in human epithelial colorectal cancerous cells, either high glucose or insulin inactivates adenine monophosphate kinase (AMPK), induces the loss of function of the tumor suppressor gene, tuberous sclerosis complex 2, encoding tuberin, activates the mTOR/S6Kinase pathway and enhances the generation of mutagnic DNA, 8-oxodG. In rodent models of diabetes, we find that loss of function of tuberin is associated with loss of function and mutations of OGG1 gene and accumulation of significant amounts of 8-oxodG and activation of the mTOR/S6Kinase pathway. These observations indicate a critical role for AMPK, tuberin and mTOR in cancer development/progression in diabetes.

Conclusion
We hypothesize that inactivation of AMPK and tuberin and activation of mTOR pathways through an oxidative stress-dependant mechanism in diabetes results in downregulation of the DNA repair enzyme 8-oxoG-DNA glycosylase (OGG1) and accumulation of 8-oxodG, which in turn accelerates colorectal tumor development and increases tumor burden. Our work may identify new therapeutic approaches to treat cancer development/progression in diabetes.

Key words: Diabetes, colorectal cancer, AMPK/tuberin/mTOR, oxidative stress, OGG1, 8o-xodG

ABSTRACT # 16

BOTH PML NUCLEAR BODIES AND RNF4 ARE REQUIRED FOR ARSENIC/INTERFERON-INDUCED DEGRADATION OF THE HTLV-I ONCOPROTEIN TAX

Z. Dassouki[1*], U. Shahin[3*],Y. Kfoury[1], H. El Hajj[1], R. Nasser[1], K. Zibara, M. El-Sabban[2], R. Nasr[1], O. Hermine[4], H. de Thé[3] and A. Bazarbachi[1]

[1]Department of Internal Medicine, and [2]Department of Human Morphology, American University of Beirut; [3]UMR 7212 CNRS, U944 Inserm, Laboratoire Associé au Comité de Paris de la Ligue contre le Cancer, Paris, France; [4]CNRS UMR 8603 and Department of Hematology, Necker Hospital, Paris, France;

*Equal contribution

Adult T cell leukemia (ATL) is one of the rare human cancers initiated by a transforming retrovirus, HTLV-I. After many years of controversy, it is accepted that the viral transactivator protein Tax plays a critical role in initiating the leukemic process. We previously showed that the combination of arsenic trioxide (As) and interferon-alpha (IFN) triggers Tax proteolysis resulting in apoptosis of HTLV-I transformed cells and cure murine ATL derived from Tax transgenics. However, the biochemical pathways involved in Tax degradation remain unclear. Here we show that As/IFN induces the polysumoylation of Tax leading to its subsequent ubiquitylation and proteasomal degradation in both Tax transfected and HTLV-1 transformed cells. Furthermore, this combination fails to degrade Tax when the lysine sites involved in Sumoylation and Ubiquitynation are mutated. Fusing ubiquitin but not SUMO1 to lysine mutant restores Tax degradation. Strikingly, IFN treatment results in complete colocalization between Tax nuclear bodies and Promyelocytic Leukaemia Protein nuclear bodies (PML-NBs). Furthermore, As/IFN induced-Tax SUMOylation is impaired in PML silenced cells, strongly suggesting that Tax SUMOylation occurs in PML nuclear bodies. We also demonstrate that the RING-domain-containing ubiquitin E3 ligase, RNF4 (also known as SNURF), targets poly-SUMO-modified Tax for degradation. This degradation is mediated by Tax ubiquitylation and subsequent recruitment of the 20S proteasome into Tax nuclear bodies. RNF4 depletion leads to the accumulation of poly-SUMO chains and impaired As/IFN induced Tax ubiquitylation. All together, our results elucidate for the first time the molecular mechanism of Tax degradation upon As/IFN treatment.

Key words: HTLV-I, tax, PML, RNF4

ABSTRACT # 17

TP53-DEPENDENT REPRESSION OF CDK2 CONTRIBUTES TO INDUCTION OF CYCLIN D1-INDUCED SENESCENCE AND TUMOR SUPPRESSION

H. Zalzali*, M. Harajly and R. Saab

Department of Pediatric and Adolescent Medicine, American University of Beirut, Beirut, Lebanon
*Email: hz13@aub.edu.lb

Background
Oncogene induced senescence is a tumor suppressor mechanism that is activated in response to tumor-promoting stimuli, and is thought to contribute to the phenotype of premalignant human lesions in various tissues. Much is understood about the process of senescence *in vitro*, but the factors affecting senescence *in vivo* are still being elucidated.

Methods
We used a transgenic mouse model of Cyclin D1-driven senescence in pineal cells (causing a premalignant lesion to the brain tumor pineoblastoma) to further understand molecular mechanisms underlying the progression of senescence in vivo. These mice develop premalignant pineal hyperplasia with features of senescence, and tumors progress only when either the p53 or the RB tumor suppressor pathways are disrupted.

Results
Cyclin D1 – driven senescence evolved in pineal cells over a period of weeks, with initial cell cycle arrest correlating with p53 pathway activation, followed days later by RB protein hypophosphorylation and activation. Interestingly, cell cycle exit that preceded senescence was associated with p53-dependent repression of the Cyclin-dependent kinase Cdk2. Pineal tumors arising in mice with a compromised p53 (p53 knockout background), or mice with compromised RB pathways (p18Ink4c knockout background), both showed increased Cdk2 expression. Inhibition of Cdk2 in cultured tumor cells of both backgrounds resulted in evidence of senescence and decreased cell accumulation.

Conclusion
Our findings indicate that the p53 and the RB pathways play temporally distinct roles in senescence induction in Cyclin D1-expressing cells and that p53-dependent Cdk2 repression plays a role in tumor suppression. Cdk2 may be a useful therapeutic target in tumors that have evaded senescence, regardless of whether this occurred by eluding the RB or the p53 pathway.

Key words: Senescence, tumor suppression, cyclin-dependent kinase

ABSTRACT # 18

BHLH PROTEINS AND TRANSCRIPTIONAL REGULATION OF CARDIAC GENES

A. Osman, R. Tanos, M. Fardon, K. Kassab and G. Nemer

Department of Biochemistry and Molecular Genetics, Faculty of Medicine, American University of Beirut, Beirut, Lebanon

Introduction

The HAND2 and Hey2 genes encode bHLH (basic Helix-Loop-Helix) proteins that were shown to be important players in cardiac morphogenesis by forming heterodimers with other transcriptional factors such as PAN. A G758T mutation within the coding regions of the HAND2 gene has been identified in a family with familial CHD within the Lebanese population. A somatic mutation in Hey2 leading to an Ala229Thr substitution was also recently documented in patients having VSD (ventricular septal defect) at our center. ,by utilizing Gelshift assays ,and co-transfection assays using a reporter gene coupled to luciferase we were able to demonstrate that these mutations alter protein function and are responsible of the underlying phenotype. in terms of regulation of GATA4 promoter, the dose dependent activation of HAND2 was lost in the mutants as well as loss of synergy obtained between HAND2 and PAN, similarly for HAND2 HEY2 interaction However for the Hey2 mutants, the interaction with GATA4 has remained unchanged The differential effects these mutations have on the physical interaction between the proteins will help unravel common and/or divergent downstream targets that will explain the differential clinical phenotypes obtained when one or the other gene is mutated.

Aim

To demonstrate that these mutations alter protein function and are responsible of the underlying phenotype.

Methods

Gel Shift assays as well as co-transfection assays using a reporter gene coupled to luciferase were used to assess the binding activity of the mutated protein to the DNA as well as their functional interactions with their partners.

Results

Our results has demonstrated a dose dependent activation of the GATA4 promoter by HAND2 and PAN, we have also shown a loss of activation by the HAND2 mutants over the GATA4 promoter when compared to the wild type as well as loss of synergy between HAND2 and PAN from 6x to 2x in activating the same promoter. Similar results were demonstrated for the interaction between HAND2 mutants with Hey2 wild type. However for the Hey2 mutants, the interaction with GATA4 has remained unchanged suggesting differential pathways during development.

Conclusion

We have characterized the effects two mutations in two genes encoding bHLH proteins on DNA binding and protein interaction. The differential effects these mutations have on the physical interaction between the proteins will help unravel common and/or divergent downstream targets that will explain the differential clinical phenotypes obtained when one or the other gene is mutated.

ABSTRACT # 19

PLAYERS OF EMBRYONIC ATRIAL PACEMAKER CELLS

Z. Samadi [1], L. Mansour [1], K. Kassab [1], M. Fardon [1], F. Bitar [2] and G. Nemer[1]

Departments of Biochemistry[1] and Genetics and Pediatrics[2], Faculty of Medicine, American University of Beirut, Beirut, Lebanon

Introduction

Following gastrulation, the first definitive organ to form and function in the embryo is the heart. Tbx3, Tbx5 & Hey2 are transcriptional factors that play an important role in cardiac development abnormalities that lead to congenital heart defects (CHDs) which are the leading non-infectious cause of mortality in newborns. Tbx3 a member of T-box transcription factors controls the sinoatrial node gene program and imposes pacemaker function on the atria. Tbx3+2a is a splice variant of Tbx3 which was shown to be functionally as efficient as Tbx3. Tbx5 is one of the earliest marker of heart development and atrial identity and was shown to be mutated in patients with Holt-Oram syndrome. Hey2 belongs to the HEY family of basic helix-loop-helix (bHLH) transcription factors, and is critical for normal ventricular septation during embryonic development as well as normal myocardial function.

Objective

Our study aims at assessing the DNA binding and the protein-protein interactions between the mentioned families & isoforms in order to better understand their role during heart development and CHDs.

Methods: - Gel Shift to assess the binding activity of each transcription factor.

- Luciferase assay was performed to assess the effect of Tbx3, Tbx3+2a, Tbx5, Hey2 on GATA4, eNOS and ANF promoters as well as the functional interaction between Tbx3, Tbx3+2a, Tbx5, with Hey2 on the GATA4 promoter.
- Co-Immunoprecipitation to assess the physical interaction between Tbx3, Tbx3+2a, Tbx5, with Hey2.

Results

Tbx3 and its isoform Tbx3+2a are functionally distinctive. ANF, GATA4 and eNOS promoters were activated by Tbx3 while no effect was seen using Tbx3+2a. Furthermore, Tbx3 along with Hey2 synergistically suppress GATA4 promoter, while Tbx3+2a in combination with Hey2 has even a more dramatic effect. The GATA4 promoter was activated by Tbx5 while suppressed by Hey2. Co-immunoprecipitation shows physical interaction between Tbx3+2a and Hey2 while no interaction between Hey2 with neither Tbx3 nor Tbx5.

Conclusion

We have shown for the first time a differential interaction between members of the bHLH and T-box families of transcription factors. Our results will help dissect the pathways by which these interactions affect the development of the sinoartrial node gene program.

ABSTRACT # 20

ROLE OF MTOR IN PODOCYTE INJURY AND ALBUMINURIA IN TYPE 1 DIABETES

K. Braysh[1], H. Abboud[2] and A. Eid[1*]

Department of Anatomy, Cell Biology and Physiological Sciences[1], Faculty of Medicine, American University of Beirut, Beirut, Beirut, Lebanon, and the Department of Medicine.[2]. University of Texas Health Science center at San Antonio, San Antonio, Texas, USA
*Email: ae49@aub.edu.lb

Introduction
Glomerular podocyte apoptosis represents a critical mechanism for excessive loss of urinary albumin that eventuates in kidney fibrosis. Pharmacological doses of the mTOR inhibitor rapamycin reduce albuminura in diabetes by unknown mechanism. We explored the hypothesis that mTOR mediates podocyte injury in diabetes.

Results
High glucose (HG) induces apoptosis of cultured podocytes and increases the levels of Nox4 and NADPH oxidase activity. HG also inhibits the phosphorylation of AMPK on the activating site Thr^{172}, increases the phosphorylation of tuberin on its inactivating sites Thr^{1462} and decreases it on the activation site. HG also activates mTOR and enhances the phosphorylation of its substrate S6 kinase. Inhibition of mTOR by low doses of rapamycin prevents HG–induced expression of Nox4, NADPH oxidase activity and podocyte apoptosis. Inhibition of mTOR had no effect on AMPK or tuberin phosphorylation indicating that mTOR is downstream of these two signaling molecules. In isolated glomeruli of OVE26 type 1 diabetic mice, there is similar decrease in the phosphorylation/activation of AMPK, enhanced phosphorylation of tuberin on the inactivating site Thr^{1462} and activation of mTOR and S6 kinase together with increase in Nox4 and NADPH oxidase activity.

Conclusion
Our data provide evidence for understanding a novel function of mTOR in Nox4-derived ROS generation and podocyte apoptosis that contribute to urinary albumin excretion in type 1 diabetes. Thus mTOR inhibition may represent a therapeutic modality of diabetic kidney disease.

Key words: Diabetic nephropathy, AMPK/tuberin/mTOR, oxidative stress, NADPH oxidases, podocyte depletion

ABSTRACT # 21

INVOLVEMENT OF RENAL CYTOCHROME P450 AND ARACHIDONIC ACID METABOLITES IN DIABETIC NEPHROPATHY

S. Eid[1], F. Ziyadeh[2], A. Jaffa[3] and A. Eid[1*]

Department of Anatomy, Cell Biology and Physiological Sciences[1], the Department of Internal Medicine[2] and the Department of Biochemistry and Molecular Genetics[3], Faculty of Medicine, American University of Beirut, Beirut, Lebanon
[$] Email: ae49@aub.edu.lb

Introduction
Diabetic nephropathy (DN) a serious complications of diabetes is characterized by hypertrophy, matrix accumulation, and proteinuria leading to end stage renal disease. Proximal tubular cells contribute to the hypertrophic response of the kidney. Early stages of DN are also associated with alterations in renal sodium handling as well as hypertension; processes linked by involvement of the arachidonic acid (AA) metabolites 20-HETE and EETs. Indeed, metabolism of AA to various intermediates is increased in a rat model of diabetes. It is known that AA is metabolized by several cytochrome 450 (CYP) isoforms to produce 20-HETE and EETs.

Results
The levels of CYPs 2B, 2C, and 4F were assessed in a rat model of type 1 diabetes. Our data show an induction of expression and activity of these CYPs in the diabetic rats, this induction was reduced in diabetic rats treated with insulin. Immunoblot analysis of microsomes derived from cultures of rat proximal tubular cells, incubated in normal (NG) or high glucose (HG), confirmed the induction of CYPs 2B and 4F by glucose. As a functional measure of cellular hypertrophy, the incorporation of S^{35}-methionine into a primary culture of rat renal cortical cells was monitored. Incubation of cells with HG results in an approximately 30% increase in methionine incorporation. When the incubation in HG is performed in the presence of 0016HETE, a specific inhibitor of 20-HETE production, methionine incorporation is decreased to control levels. Thus, inhibition of CYPs attenuates the cellular hypertrophy caused by increased levels of glucose.

Conclusion
Our results indicate that hyperglycemia in diabetes has a significant effect on the expression of AA-metabolizing CYPs, manifested by increased AA metabolism, and might thus alter kidney function through alteration of type and amount of AA metabolites. Inhibition of this CYP-mediated response alleviates the hypertrophic response of these cells to high glucose.

Key words: Diabetic nephropathy, Cytochromes P450, oxidative stress, 20HETE, EETs

ABSTRACT # 22

MOLECULAR MECHANISMS OF MECHANICAL STRETCH-INDUCED LEPTIN SECRETION AND VASCULAR SMOOTH MUSCLE HYPERTROPHY

C. Ghantous[1], L. Dib[1], M. Al-Hariri[2], A. Jaffa[2] and A. Zeidan*[1]

[1] Department of Anatomy, Cell Biology and Physiological Sciences, and [2]Department of Biochemistry and Molecular Genetics, Faculty of medicine, American University of Beirut, Beirut, Lebanon
*E-mail: az29@aub.edu.lb

Obesity is associated with increased leptin production that may contribute to cardiovascular pathology through a multiplicity of effects. Leptin has been shown to contribute to vascular remodeling through various mechanisms, including production of vascular smooth muscle (VSMC) hypertrophy; however, the mechanisms underlying the vascular hypertrophic effect of leptin remain unknown. We used rat portal vein (RPV) organ culture to investigate the effect of mechanical stretch (mimicking hypertension) on autocrine secretion of leptin and the effect of exogenous leptin (3.1 nM) on VSMC. Stretching the RPV for 6h significantly up-regulated leptin and leptin receptor (leptin obese receptor) expression. The dry weight to wet weight ratio and [3H]leucine incorporation rates were significantly increased by leptin in unstretched blood vessels. Leptin-induced VSMC hypertrophy was associated with significant ERK1/2 and cofilin phosphorylation and ROS production. Moreover, leptin significantly increased polymerization of actin in unstretched blood vessels, as reflected by an increase in the F-/G-actin ratio, effects that were significantly attenuated by a leptin receptor antibody, the ROCK inhibitor Y-27632 as well as the ERK1/2 inhibitor PD98059. Leptin-induced VSMC hypertrophy in unstretched tissues significantly attenuated by an anti-leptin antibody (166 ng/ml), and the NADPH oxidase inhibitor apocynin (1 mM) indicating the involvements of ROS production in leptin-induced VSMC hypertrophy. Our results indicate that the activation of ERK1/2, RhoA/ROCK pathway and ROS production plays a pivotal role in leptin signaling, leading to the development of VSMC hypertrophy through a mechanism involving altered actin dynamics.

Key words: Vascular smooth muscle cells, hypertrophy, leptin

ABSTRACT # 23

KININ SIGNALING IN RENAL GLOMERULAR CELLS

H. Chalhoub*[1], M. Al Hariri[1], F. Ziyadeh[1,2], A. Eid[3] and A. Jaffa[1]

[1]Department of Biochemistry and Molecular Genetics. [2]Department of Internal Medicine, [3]Department of Anatomy, Cell Biology and Physiological Sciences, Faculty of Medicine. American University of Beirut, Beirut, Lebanon
*Email: hc29@aub.edu.lb

Diabetic nephropathy (DN) is a major health epidemic and is the main cause of morbidity and mortality in diabetes. It is the most common cause of end-stage renal failure. A pivotal event initiated by DN is glomerular injury, characterized by mesangial matrix deposition and podocyte loss. Microalbuminuria, an early marker of DN, signifies high risk for progressive renal failure and is strongly correlated with glomerular injury. The risk factors and mechanisms involved in the pathogenesis of DN are still not completely defined. Previous data generated from our laboratory demonstrated novel mechanisms and functions B2 kinin receptors in DN. Diabetic B2R-/- null mice display reduced albumin excretion rate (AER), and reduced glomerular and tubular injury compared to diabetic B2R+/+ mice. In the current study we aimed to understand the cellular mechanisms through which activation of B2 kinin receptors contribute to the initiation and progression of DN. Stimulation of rat podocytes and mesangial cells with bradykinin (BK) resulted in increased expression of connective tissue growth factor (CTGF) and this effect is associated with increased expression of oxidative stress enzymes Nox 1 and Nox 4 and activation of sphingosine kinase and transactivation of sphingosine 1 phosphate receptors. In addition BK resulted in the phosphorylation of p42/p44 MAPK and AKT. These findings provide insights into novel aspects of B2 receptor signal transduction pathways and their functional significance in pathogenesis of DN and identify novel targets for interventional strategies.

Key words: Diabetic nephropathy, podocytes, mesangial cells, oxidative stress, signal transduction

ABSTRACT # 24

CROSS-TALK BETWEEN BRADYKININ AND LEPTIN AND VASCULAR REMODELING

M. Al Hariri[1]*, H. Chalhoub[1], L. Dib[2], A. Habib[1], F. Ziyadeh[1,3], A. Zeidan[2] and A. Jaffa[1]
Departments of Biochemistry and Molecular Genetics[1] ,Department of Anatomy, Cell Biology and Physiological Sciences[2] and Department of Internal Medicine[3], Faculty of Medicine, American University of Beirut, Beirut, Lebanon
*Email:ma147@aub.edu.lb

Atherosclerosis is the leading cause of death in diabetes, and is also a major source of morbidity and mortality. Early atherosclerotic lesions are characterized by endothelial dysfunction, accumulation of inflammatory cells, VSMC proliferation and migration, and extracellular matrix deposition in the vessel wall. Although the association of chronic hyperglycemia and dyslipidemia with diabetic micro-and macrovascular disease is well recognized, the factors and cellular signaling mechanisms that link hyperglycemia and dyslipidemia with atherosclerotic vascular disease are not fully defined. Both bradykinin and leptin have been shown to promote vascular remodeling, but the cross-talk between the two systems has not been explored. Treatment of vascular smooth muscle cells (VSMC) with BK stimulated the mRNA levels of leptin, ObRa (functional leptin receptor isoform) and NOX4. On the other hand treatment of VSMC with leptin induced the mRNA levels of B1-kinin receptors and downregulated the expression of connective tissue growth factor (CTGF). Both BK and leptin stimulation resulted in the activation of p42/p44 MAPK and AKT. These findings are the first to demonstrate that BK can stimulate leptin expression and its receptor in VSMC. Insights into the cellular mechanisms and interrelationships between BK and leptin may provide a novel mechanistic pathway through which both factors interact to promote vascular remodeling.

Key words: Vascular remodeling, leptin, oxidative stress, cell signaling, bradykinin

ABSTRACT # 25

NFATC1 AND ITS PARTNER ARE ESSENTIAL FOR VALVE FORMATION IN THE HEART

T. Farhat[1]*, Z. Abdul Sater[1], F. Bitar[2] and G. Nemer[1]
Departments of Biochemistry and Molecular Genetics[1] and Pediatrics[2], Faculty of Medicine, American University of Beirut, Beirut, Lebanon
*Email: tif01@aub.edu.lb

Background
The vertebrate heart is a structurally and functionally complicated organ. Valve morphogenesis is a multistep process regulated by complex signaling pathways including calcineurin/nuclear factor of activated T cells (NFATc) signaling and vascular endothelial growth factor (VEGF). Mutations in the genes encoding these proteins might thus be associated with cardiac valvular and septal malformations, which account for the majority of congenital heart defects.
We have previously shown that mutations in NFATc1 gene were detected in two patients one with tricuspid atresia and transposition of the great arteries and the other with aortic stenosis.
The aim of this study is to investigate the effect of NFATc1 mutations on the interaction with other transcription factors as well as the transcriptional regulation of the VEGF promoter in order to understand the mechanisms underlying the cardiac defects.

Methods
Co-transfection assays using the VEGF promoter fused to Luciferase were performed to assess the activity of each mutant alone and in combination with other transcription factors (Tbx5, Tbx20, and GATA5) and compared it to the wild type.

Results
The preliminary results showed a pronounced inhibition of activation of the VEGF promoter by the NFATc1 mutants. In addition, differential interactions between NFATc1/Tbx5, NFATc1/GATA5, NFATc1/Tbx20, GATA5/Tbx20, and GATA5/Tbx5 were observed suggesting multiple pathways regulating VEGF expression.

Conclusion
On one hand, disruption of the interactions between NFATc1 mutants and the other transcription factors affects the transcriptional regulation of VEGF. The change in the level of VEGF expression during valve formation possibly inhibits cardiac cushion formation leading to defective valves and causing congenital heart defects. On the other hand, the characterization of transcriptional regulators of the VEGF gene will give insight on the molecular mechanisms behind proper valve development.

Key words: Congenital heart disease, valvulogenesis, nuclear factor of activated T cells, vascular endothelial growth factor

ABSTRACT # 26

POMEGRANATE (*PUNICA GRANATUM*) ATTENUATES ACUTE KIDNEY INJURY BY DOWNREGULATING NOX1 AND NOX4 NADPH OXIDASE IN MICE EXPOSED TO CIGARETTES SMOKING

S. Boutary[1], A. Al Turk[1], M. Abed El Aziz[1], B. Abiad[1], A. Eid*[1*] and A. Husary[2]
Department of Anatomy, Cell Biology and Physiological Sciences[1] and the Department of Internal Medicine[2], Faculty of Medicine, American University of Beirut, Beirut, Lebanon.
*Email:ae49@aub.edu.lb

Introduction
Pomegranate, through its antioxidant properties, may be useful to treat or prevent human diseases. We have evidence suggesting that oxidative stress contributes to the pathogenesis of cigarettes smoking induced-acute kidney injury. We tested the hypothesis that pomegranate prevents cigarettes smoking-induced renal oxidative stress, attenuating renal injury.

Methods
Adult male C57BL/6J mice weighing between 22-25g were divided into four groups: Control (C) received placebo by oral gavage once daily, control mice that received pomegranate juice (80µmole/Kg) twice daily by oral gavage. Mice in group 3 (S) were exposed to cigarette smoke and group 4 (SP) mice were exposed to cigarette smoke and received pomegranate (80µmole/Kg) twice daily. At day 4 the animals were sacrificed and the kidney removed for biochemical and histological studies.

Results
Early indices of renal injury assessed by hypertrophy, fibrosis and renal expression of collagen IV were significantly greater in cigarettes smoking mice when compared to their control littermates. These indices of renal injury were significantly reduced in mice that received pomegranate. Cigarettes smoking induced-renal injury were paralleled by an increase in ROS production. More interestingly, our data show a significant increase in NAD(P)H oxidase-dependent ROS production accompanied by an increase in Nox1 and Nox4 protein expression. These observations were reversed by pomegranate treatment, suggesting that increased generation of NADPH oxidase derived reactive oxygen species mediates cigarettes smoking-induced kidney injury.

Conclusion
We conclude that cigarettes smoking induced kidney injury results from the generation of ROS by NAD(P)H oxidase Nox 1 and Nox 4 and this was reversed by pomegranate treatment. These findings suggest that the consumption of pomegranate may ameliorate kidney injury induced by cigarettes smoking and may introduce a new paradigm in the treatment of cigarettes smoking-induced complications.

Key words: Kidney injury, oxidative stress, NADPH oxidases, cigarettes smoking, pomegranate

ABSTRACT # 27

AUTISM SUSCEPTIBILITY GENES IN THE LEBANESE POPULATION

J. Soueid *, S. Kourtian, S. Ghanem and R-M. Boustany

Neurogenetics Program and Division of Pediatric Neurology, Departments of Pediatrics and Biochemistry and Molecular Genetics, American University of Beirut, Beirut, Lebanon
*E-mail: js61@aub.edu.lb

Introduction
Autism is a neurodevelopmental disorder characterized by three core symptom domains: ritualistic-repetitive behaviors, impaired non-verbal communication and language development. Although autism is considered to be genetic in 20-25% of cases, its extreme heterogeneity has defied genetic classification. Over the last years, the identification of a large number of autism susceptibility genes has led to an increased appreciation of the contribution of de novo and inherited copy number variation (CNV). Our aims are to confirm previously identified autism susceptibility genes in cases of autism in Lebanon and/or uncover novel autism susceptibility genes specific to the Lebanese population.

Methods
We are using Cytogenetics 2.7M Microarray technology to detect CNVs in a subset of Lebanese autistic children, and/or to map homozygous regions in each family. The Lebanese population is ideal for homozygosity mapping because of a high degree of shared ancestry and the likelihood of additional healthy siblings per family.

Results
Homozygous regions specific to autistic children compared to parents and siblings includes previously described autism, schizophrenia and mental disorder susceptibility genes such as CNTNAP2, GLO1, NRXN1 and GRIK2. More interestingly, we uncovered several novel groups of candidate genes. The first subset is involved in glutamatergic transmission already known to be important in autism. These genes are GABRA1, GRIA1 and ALDH9A1. Another subset implicates neuronal cell adhesion molecules including NLGN3, NRXN1 and CNTN3. Other subsets consist of genes like PTPRT and GOLSYN known to play a role in synapse formation, and others related to the mitochondrion and DNA remodeling. Moreover, comparing genomes of 12 autistic children narrowed the analysis to 6 homozygous regions found in 6 or more children. The VPS13B gene associated with Cohen syndrome, a disorder with autistic features was common to 10/12 children. The GPHN gene found in 7/12 patients encodes a protein that plays a role in GABA receptor clustering.

Conclusion
Uncovering a set of genes responsible for autism in the Lebanese population will facilitate diagnostic work-up and genetic counseling for autistic spectrum disorders in Lebanon. Also, discovery of novel autism susceptibility genes may potentially reveal untapped and novel therapeutic targets.

Key words: Autism, microarray technology, homozygosity mapping, copy number variations

ABSTRACT # 28

A NOVEL ROLE FOR TBX5 IN AORTIC COARCTATION

N. Daou, A. Fahed, R. Safa, F. Bitar and G. Nemer

Department of Biochemistry and Molecular Genetics and Department of Pediatrics, American University of Beirut, Beirut, Lebanon

Background
Heart formation is a complex process initiated early in mammalian embryogenesis with the formation of a cardiogenic field and ending with the formation of distinct left and right chambers separated by atrial and ventricular septa. Cell specification, differentiation, growth and migration underpin normal cardiac development, processes that are thought to occur through specific programs of gene expression. Multiple transcription factors play important roles in cardiac development such as Tbx5, a member of the T-box family implicated in vertebrate tissue patterning and differentiation. Mutations in Tbx5 have been reported in patients with Holt-Oram syndrome, and recent studies do suggest its involvement also in isolated cases of CHDs.

Objective
Our objective is to use whole exome screening to detect mutation in genes encoding cardiac enriched proteins that might be implicated in familial and sporadic cases of CHD.

Methods
Whole exome sequencing done at Harvard Medical School was followed up by functional characterization of the mutation. Site directed mutagenesis was used to generate the cDNA encoding the mutated protein. Gel shift assays, cellular localization, and co-transfection assays were used to assess the function of the mutated protein and compare it to the wild type.

Results
We detected a Tbx5 missense mutation (V263M) in a family with two patients with coarctation of the aorta. Paradoxically, only one of the patients has the mutation in addition to his father who is phenotypically normal. The mutation was reconstructed in an expression vector and functional studies do point to a slight reduced transcriptional activity of the mutated protein. In addition, the interaction was conserved with GATA4 but was dramatically affected with GATA5.

Conclusion
This is the first report describing the involvement of Tbx5 in aortic coarctation. Further studies are under way to understand the frequency of this mutation and the mechanisms underlying its expression and function in the aorta.

ABSTRACT # 29

RB CONTROLS THE NUMBER OF NEW BORN NEURONS IN THE OLFACTORY BULB BY REGULATING NEUROGENESIS IN THE ADULT SUBVENTRICULAR ZONE

N. Rayan[1], V. Renaud[2], B. Maarouf[1], J. Carine[1], S. Al Lafi[1], SS. Ruth[2] and G. Noël[1]*

[1] Department of Biology, American University of Beirut, Beirut, Lebanon and [2] Department of Cellular and Molecular Medicine, University of Ottawa, Ontario, Canada
*Email: ng13@aub.edu.lb

Background
The retinoblastoma protein, pRb, is a tumour suppressor gene that controls the G1/S phase checkpoint and the coordination between cell proliferation and differentiation during brain development. Although expressed at high level, Rb's function in the adult brain remains unknown and Rb conditional knockout mice in the telencephalon die at birth.

Methods
To investigate Rb's function in nerve regeneration in the brain, we generated an inducible Rb deletion specifically in stem cells and progenitor cells by crossing Nestin-CreERT2; YFP/YFP reporter mice with Rb floxed/floxed mice. Upon tamoxifen administration, Cre translocates into the nucleus, deletes Rb and activates the expression of the yellow fluorescent protein, YFP in Nestin-positive cells.

Results
5-10 week old mice were injected with tamoxifen and sacrificed 4 and 8 weeks later. Adult neurogenesis was assessed in the subventricular zone (SVZ) and the olfactory bulb (OB) in Rb mutant versus control animals. We found that loss of Rb causes a two fold increase in progenitor cell proliferation in the SVZ and a 1.5 fold increase in the number of neuroblasts migrating along the rostral migratory stream (RMS) to the OB in Rb mutant compared with control mice. These neuroblasts exit properly the cell cycle and initiate their differentiation programs as indicated by the expression of *Dlx2*, *Dlx1, Sp8* and *Pax6* early differentiation markers of distinct subtypes of GABAegic neurons as well as GAD67 and NeuN, two late differentiation markers. We then examined whether these neuroblasts give rise to distinct subtypes of GABAergic neurons including calretinin- (CR), calbindin-(CB), and tyrosine hydroxylase-expressing neurons (TH). We found that loss of Rb does not alter the ratios of CR, CB or TH produced in 10 weeks old mice but leads to a proportional increase in their respective numbers as a result of the increase in proliferation detected earlier.

Conclusion
This study is the first to identify a requirement for Rb in neuronal regeneration in the brain through the control of the rate of progenitor proliferation without affecting cell differentiation and with respect to the spatio-temporal specification of distinct subtypes of GABAergic neurons. These results have long-term implications on regenerative medicine after neuronal injury or loss.

Key words: Adult neurogenesis, subventricular zone, olfactory bulb, GABAergic neurons

ABSTRACT # 30

GALACTOSYLCERAMIDE (GALCER) AS POTENTIAL TREATMENT FOR JUVENILE NEURONAL CEROID LIPOFUSCINOSIS (JNCL)

H. Harati[1,2]*, L. Haliw[2], S. Cotman[2] and R-M. Boustany[1]

[1]Neurogenetics Program and Division of Pediatric Neurology, Department of Pediatrics and Adolescent Medicine and Department of Biochemistry and Molecular Genetics, American University of Beirut, Beirut, Lebanon; [2]Center for Human Genetic Research, Massachusetts General Hospital, Harvard Medical School, Boston MA, USA
*E-mail: hh80@aub.edu.lb

Introduction
JNCL is a neurodegenerative disorder caused by *CLN3* gene defects that negatively modulate cell growth/apoptosis. CLN3 protein harbors antiapoptotic motifs and a galactosylceramide (GalCer) lipid raft-binding domain and transports GalCer to cell surface. There is a GalCer deficit in lipid rafts (LR) of CLN3-deficient cells. CLN3-defective brain/cells have elevated ceramide (Cer). Previous hypotheses suggest that low GalCer in LR leads to increased Cer generation to overcome GalCer deficit, which leads to neuronal cell death. *Cln3*$^{\Delta ex7/8}$ knock-in mouse mimics human disorder exhibiting early onset and accumulating JNCL storage material, gliosis, motor disturbances and a shortened lifespan.

Methods
Daily injections of 20 mg/kg GalCer, or vehicle only, were administered to homozygous *Cln3*$^{\Delta ex7/8}$ mice from 5 to 17 weeks old. Mice were tested in rotarod and pole climbing locomotor tests. DGK assay was used to quantify ceramide levels, subcellular fractionation to quantify GalCer in cell fractions, and fluorescent immunohistochemistry to quatify GalCer, Subunit C (storage marker), and S-100 and GFAP (inflammation and gliosis markers) in tissues.

Results
Cer levels in *Cln3*$^{\Delta ex7/8}$ mouse brain were significantly elevated compared to wild-type control. Adding GalCer significantly normalized brain ceramide in *Cln3*$^{\Delta ex7/8}$ mice. Exogenous GalCer corrected the reduced LR/Golgi GalCer ratios in *Cln3*$^{\Delta ex7/8}$ mouse brain. GalCer levels, were increased in many brain regions in GalCer-treated versus vehicle-treated *Cln3*$^{\Delta ex7/8}$ mice. Exogenous GalCer also decreased subunit c in selected brain regions, liver and kidneys of GalCer-treated *Cln3*$^{\Delta ex7/8}$ mice versus their vehicle-treated counterparts. Brains from GalCer-treated *Cln3*$^{\Delta ex7/8}$ mice also tended to display reduced gliosis, as measured by GFAP ans S100 immunostaining. In locomotor activity tests, an enhanced benefit was observed.

Conclusion
Exogenous GalCer partially corrected GalCer deficit in LR, diminished subunit C, lowered brain ceramide and improved behavior in homozygous *Cln3*$^{\Delta ex7/8}$ mice. Thus, CLN3 defects impairs GalCer trafficking and increased brain ceramide, which may accelerate neurodegeneration in JNCL. GalCer supplementation will be further explored as a treatment option for JNCL.

Key words: JNCL, *Cln3*$^{\Delta ex7/8}$ mice, galactosylceramide (GalCer), ceramide (Cer)

ABSTRACT # 31

NOVEL MUTATIONS IN THE GATA4 GENE IN PATIENTS WITH TETRALOGY OF FALLOT

L. Gharibeh* and G. Nemer

Department of Biochemistry and Molecular Genetics, Faculty of Medicine, American University of Beirut, Beirut, Lebanon
*Email : Gharibeh.lara@gmail.com

Introduction
Heart morphogenesis is a very complex process. Many studies in vertebrates have shown a revolutionary conserved pathway from crescent formation till the adult heart. This process is governed by the action of transcription factors including GATA4 and Tbx5. Mutations in these genes encoding these proteins might be associated with Tetralogy of Fallot (TOF), one of the prominent congenital heart defects.

Objectives
The aim of this study is to characterize three novel GATA4 mutations associated with sporadic cases of TOF and to prove that they are disease-causing.

Methods
-Site Directed mutagenesis to introduce the mutations.
-Immunofluorescence to screen the subcellular localization of GATA4 mutants.
-EMSA to assess binding affinity of the mutated GATA4 protein to DNA.
-Western Blot to quantify and ensure the presence of the proteins.
-Luciferase assay to assess the transcriptional regulation of GATA4 mutants on the ANF promoter.
-Co-immunoprecipitation to study the interaction between GATA4 and Tbx5.

Results
We are suspecting that the 3 mutations in the GATA4 will decrease the transcriptional activation of the ANF promoter without affecting GATA4 ability to translocate into the nucleus. The ability to bind DNA and the interaction with Tbx5 should be affected only for the 3rd mutant since the mutation is at the level of the 2nd zinc finger domain.

Conclusion
The role of GATA4 in congenital heart diseases specifically in Tetralofy of Fallot will be assessed and that will make GATA4 a biomarker for Tetralogy of Fallot.

Key words: GATA4, congenital heart disease, Tetralogy of Fallot,TBX5

ABSTRACT # 32

CERAMIDE REGULATES THE TRANSLOCATION AND PHOSPHORYLATION OF PKCΘ

R. Hage-Sleiman*, A. Hamze, R. Attieh and G. Dbaibo

Department of Biochemistry and Molecular Genetics, American University of Beirut, Beirut, Lebanon
*Email: rh113@aub.edu.lb

Background and aims
Protein kinase C θ (PKCθ) plays a central role in the activation of T cells during immune response to infections. This enzyme is regulated by phosphorylation and translocation to immunological synapses where lipid rafts accumulate along with aggregates of TCR/CD3 and other signaling receptors. The sphingolipid ceramide is a key lipid involved in many signaling pathways of cell growth, survival and death. It regulates many PKC isoforms; more specifically it inhibits PKCθ kinase activity. In this study, we intend to elucidate the mechanism of inhibition of PKCθ by ceramide.

Methods
This study is performed on Jurkat T cells activated either by PMA (phorbol ester) or anti-CD3/anti-CD28 treatments. T cells are either treated by exogenous ceramide or stimulated to accumulate endogenous ceramide by bacterial sphingomyelinases or anti-Fas treatment. The effects of ceramide on the translocation and expression level of total and phosphorylated forms of PKCθ are studied in both the lipid raft and non-raft fractions of the plasma membrane. The kinase activity of PKCθ is monitored in response to both exogenous and endogenous accumulation of ceramide.

Results
Upon T cells activation using PMA, PKCθ translocates to the membrane and more specifically to the lipid raft. However, ceramide inhibits translocation from membrane or cytosol to the lipid raft, specific phosphorylation and membrane activity of PKCθ. Flotillin-1, a lipid raft marker, normally expressed in the lipid raft fractions of control cells is no more visible after treatment with ceramide.

Conclusion
After treatment with ceramide, PKCθ is no more able to translocate to lipid raft or get phosphorylated and activated. Based on the results obtained with respect to flotillin-1, we can conclude that ceramide might be either partially disturbing the lipid raft or completely disrupting it. Consequently, the survival signaling pathway triggered by PKCθ is dysregulated. Our hypothesis is supported in literature where disruption of the integrity of lipid rafts by ceramide was described to inhibit lymphocyte responses through changes in the distribution of several critical proteins such as kinases. These results are very important to define the relationship between the sphingolipid ceramide and the immune response in order to develop specific modulators of the immune response in future studies.

Key words: Ceramide, lipid raft, protein kinase C θ

ABSTRACT # 33

REGULATION OF CERAMIDE GENERATION DURING ADENOVIRAL INFECTION

S. Ghayad*, M. Esmerian, R. Attieh, M. Yehia and G. Dbaibo

Department of Biochemistry and Molecular Genetics, American University of Beirut, Beirut, Lebanon
*Email: sg35@aub.edu.lb

Viruses use various strategies to manipulate host cell signaling pathways in order to promote viral replication and avoid elimination by host immune responses. The long-term goals of this study are to define the relationship between adenoviral infection and the cellular lipid signaling pathway driven by ceramide. Ceramide is a putative regulator of the stress response in the cell. It plays an important role in the regulation of cell differentiation, cell cycle regulation, and apoptosis in response to various stresses to the cell including cytokine action and genotoxic damage. Study of adenoviral infection of cells in culture showed that infection leads to the gradual accumulation of ceramide. Several of our observations indicate that, during adenoviral infection, ceramide accumulation plays an important role in the virus-host cell interaction: 1) most of the accumulated ceramide is synthesized de novo during the mid to late stages of viral infection; i.e. an infected and dying cell is driven to expend valuable energy to synthesize ceramide; 2) inhibition of the de novo synthesis of ceramide during adenoviral infection results in significant delay of cell lysis indicating that ceramide plays a necessary role in the virus-host cell interplay that results in cell lysis; and 3) the accumulated ceramide appears to be responsible for inducing significant dephosphorylation of SR proteins that are necessary for effective splicing of viral and cellular mRNA. These observations raise the possibility that adenovirus increases the levels of cellular ceramide either directly, or indirectly, by the action of one or more of its gene products.

Keywords: Ceramide, adenovirus, SR proteins

ABSTRACT # 34

REGULATION OF THE CERAMIDE PATHWAY BY THE ADENOVIRAL E4ORF4 GENE

M. Esmerian*, S. Ghayad, R. Attieh, M. Yehia and G. Dbaibo
Department of Biochemistry and Molecular Genetics, American University of Beirut, Beirut, Lebanon
*Email: sg35@aub.edu.lb

Adenoviral infections are among the most common human viral infections. Adenovirus encodes for several viral proteins that target key components of cellular regulatory pathways in order to insure propagation of the virus and to manipulate the host machinery in the interest of viral replication. At least 7 proteins are encoded by the E4 region from the different open reading frames (orf). Of these proteins, E4orf4 was shown to induce p53-independent apoptosis. This function appears to be dependent on its ability to bind the Bα subunit of the protein phosphatase 2A (PP2A). Moreover, E4orf4 was shown to regulate alternative splicing of late adenoviral genes by inducing PP2A-dependent dephosphorylation of SR splicing factors. We had shown that adenoviral infection results in the accumulation of the sphingolipid ceramide, a known regulator of cell cycle arrest and apoptosis. The adenoviral gene(s) responsible for the increase in ceramide levels have not been mapped. In preliminary studies, we showed that E1A was unlikely to be the primary gene responsible for ceramide accumulation. However, we have shown that a viral mutant that lacks all E4 genes except E4orf4 induces a more rapid and potent accumulation of ceramide when compared with wild type virus whereas a mutant virus that lacks all E4 genes results in only a modest accumulation of ceramide. This suggested that E4orf4 plays an important role in regulating ceramide accumulation.

Key words: Ceramide, adenovirus, E4orf4

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