

G_xE News

A Newsletter of the

Center for Environmental Genomics and Integrative Biology

*"An NIH-NIEHS Center of Excellence
at the University Of Louisville"*

Kenneth S. Ramos, Ph. D., Director

J. Christopher States, Ph. D., Deputy Director

Jeannie Bowman, Center Administrator

The Center for Environmental Genomics and Integrative Biology focuses on the elucidation of gene, protein and metabolic networks involved in cardiovascular disease, cancer and developmental origins of health and disease, and the role of environmental factors in these disorders.

CONTENTS

[Director's corner](#)

Emphasis on biomarkers, upcoming grant application deadlines

[Member accolades](#)

Recognition and accomplishments of our members

[Member highlight](#)

Get to know our members

[Career development for new environmental health investigators](#)

Young investigators....

[Pilot projects program](#)

Newly funded pilot grants

[Bioinformatics, biostatistics and computational biology core](#)

Recent activities and projects

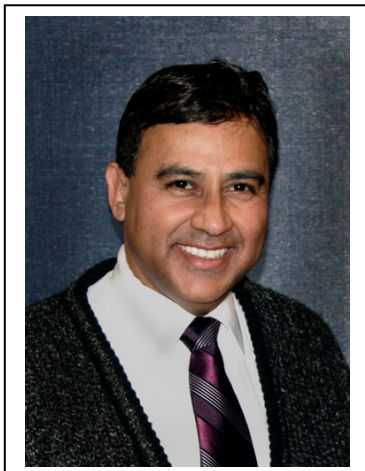
[Community outreach and education core](#)

Recent activities

[New Grants](#)

Newly funded awards to CEGIB members

[Recent CEGIB Member Publications](#)



DIRECTOR'S CORNER

The time is ripe for the Center for Environmental Genomics and Integrative Biology to bring its resources and manpower to bear upon the development of biomarkers with clinical applications. As we begin our second year of operations, my goal is to focus Center efforts on:

- Biomarkers of cardiovascular disease related to disturbances in serum protein profiles.
- Biomarkers of cancer progression based on high throughput genome sequencing and haplotype analysis.
- Identification of master genes for developmental control that can be targeted for therapeutic intervention to mitigate adult onset chronic diseases.

Vital to our efforts is an initiative formulated at the NIH through the pairing of two existing programs. One is the NIEHS's four year Exposure Biology Program (EBP) with goals to improve exposure assessment technology and identify biomarkers for common pathogenic mechanisms that reflect the human response to environmental agents. The other is the National Human Genome Research Institute (NHGRI) program aimed at finding genetic risk factors for environmental diseases. These two programs were combined to become the Genes and Environment Initiative (GEI). The ultimate goal is to help develop technologies that can precisely measure environmental exposures and that ultimately lead to better biomarkers of physiological response. In addition, new biomarkers will be used in genome association studies for specific illnesses to unravel the molecular underpinnings of disease progression.

I want to remind CEGIB members of several important NIEHS grant application due dates in October and November:

- Implementation Planning Grants for Educational, Behavioral, or Social Studies for Translation of Genetic Factors in Common Diseases (U34) ([RFA-DK-08-003](#))
Letters of Intent Receipt Date: October 24, 2008
Application Due Date: November 25, 2008
<http://grants.nih.gov/grants/guide/rfa-files/RFA-DK-08-003.html>
- Translation of Common Disease Genetics into Clinical Applications (R21) ([RFA-DK-08-004](#))
Letters of Intent Receipt Date: October 24, 2008
Application Due Date: November 25, 2008
<http://grants.nih.gov/grants/guide/rfa-files/RFA-DK-08-004.html>
- Epigenomics of Human Health and Disease (R01) ([RFA-RM-08-017](#))
NIH Roadmap Initiatives
Application Receipt Date(s): October 28, 2009
<http://grants.nih.gov/grants/guide/rfa-files/RFA-RM-08-017.html>

Links to these RFA's and other announcements of grant opportunities can be found at <http://www.niehs.nih.gov/> and <http://www.niehs.nih.gov/funding/grants/announcements/index.cfm>.

I encourage you to make use of CEGIB facility cores in your grant applications and to start formalizing collaborations to compete in the next round of pilot project grant applications in Spring 2009. Please contact me directly to discuss your research plans and needs. I look forward to a productive quarter ahead.

– Ken Ramos



MEMBER ACCOLADES

CEGIB was featured in the Winter 2008 edition of MEDICINE Magazine, a publication of the University of Louisville School of Medicine which highlights important developments in the School. The article may be viewed at: <http://louisville.edu/hsc/medmag/w08/gene.html>.

Several of our members and their students received recognition this fall for their research.

Students

Mohammad Al-Ghoul, a Postdoctoral Fellow in Roland Valdes' Clinical Chemistry Program and Laboratory presented a poster titled "A novel prognostic test: Digoxin and dihydrodigoxin in breast cancer therapy at the 6th Annual Ohio Valley Affiliates for life Sciences (OVALS) Conference, Louisville, KY April 2008 and a related work on "Digoxin and its metabolite dihydrodigoxin in breast cancer therapy at the 34th CLAS International Meeting, Coral Springs, FL in June of 2008. These data are setting the stage for new uses of plant-derived molecules in treatment of cancer with potentially strong environmental component through dietary influences.

Immaculate Amunom, completed her Ph.D. under the direction of Russ Prough and was hooded at the Spring 2008 Graduate Convocation. She received a Graduate Dean's Citation at the award ceremony. The title for her dissertation was "Lipid Aldehydes Are Substrates And Transcriptional Regulators Of Cytochromes P450". She joined the Department of Drug Metabolism at XenoTech LLC in Lenexa KS as a Scientific Staff member. This is a successful biotech company started by Andrew Parkinson.

Marjories Bon Homme, an advanced Postdoctoral Fellow in the Clinical Chemistry Program and in Mark Linder and Valdes' laboratories presented an abstract at the 34th CLAS International Meeting in Coral Springs, FL 2008 on "Repeated exposure to acetaminophen protects against a subsequent lethal dose through selective replenishment of mitochondrial glutathione.

L. Jay Stallons, graduate student in Glenn McGregor's laboratory received a travel award and was invited to give a platform presentation at the annual meeting of the Environmental Mutagenesis Society held in Atlanta in October 2007. The title of his talk was, "Evidence from mutation spectra that DNA polymerase eta is the preferred translesion DNA polymerase and may be error-free or error-prone". A poster describing this work was awarded a first-place award in the basic science graduate student division at Research! Louisville, November 2007.

Nick Watson, graduate student in Glenn McGregor's laboratory was invited to speak at the 2nd annual Laboratory for Fluorescence Dynamics Advanced Imaging Workshop held in October 2007. The title of his talk was "Intranuclear dynamics of proteins required for resolution of DNA replication forks blocked by genotoxic carcinogens". He completed his Ph.D. research this year and was hooded in the Spring 2008 commencement.

Pete Womack, as graduate student in Roland Valdes' lab published two abstracts in the Clinical Chemistry Journal on the cytotoxic effects of digoxin on lung cancer cells involving cell-dependent apoptotic mechanisms (Clin Chem, 2008;54(suppl):A136 (Abstract C118)) in addition to one dealing with Uzara promoting cell death in lung and breast cancer cells (Clin Chem, 2008;54(suppl):A134 (Abstract C110)

Center Members

Craig McClain is serving on the 2008 Nominating Committee for the [American Association for the Study of Liver Diseases](#) (AASLD), and was elected a member of the AASLD-NIH Liaison Committee (2008-2010). He also gave an invited presentation on "Drug Induced Liver Toxicity" at the American Academy of Dermatology Annual Meeting.

Russ Prough serves as the President of the International Society for the Study of Xenobiotics for 2008-2009. He opened the 10th European Regional Meeting in Vienna, Austria on May 19 and the 15th North American Regional Meeting in San Diego, CA on October 12, 2008.

Ken Ramos is serving as President of the Society of Toxicology for the 2008-2009 year. He chaired the Scientific Program Committee at the 2008 national meeting in Seattle, WA March 16 – 20, 2008. He also serves as a member of the NIH search committee for the next director of the National Institute of Environmental Health Sciences. Earlier this year he organized and chair a symposium at the annual meeting of the American Association for the Advancement of Science entitled “Understanding the linkages between toxicity and disease”.

Mark Rothstein delivered the Siegel Memorial Lecture at Duke Law School on environmental epigenetics. His presentation, "Exposed Today, Grandchildren Pay?", was the topic of an article [Looking Ahead to Legal and Ethical Implications of Epigenetics](#) that appeared in the April issue of the NIEHS newsletter, *Environmental Factor*.

Chris States chaired a symposium on Arsenic and Cardiovascular Disease at the national Society of Toxicology meeting held in Seattle March 16-20, 2008. He presented recent studies from his laboratory investigating in utero arsenic exposure induced changes in liver microRNAs and mRNAs associated with accelerated atherosclerosis in a talk entitled “Prenatal arsenic exposure alters hepatic developmental programming predisposing to atherosclerosis”. **Sanjay Srivastava** also presented in the symposium. His talk entitled “Arsenic-Induced Atherogenesis: Contribution Of Oxidative Stress, Inflammation And Unfolded Protein Response “ in which he showed remarkable time- and dose-dependent increases in atherosclerosis in arsenic exposed ApoE-knockout mice.

Roland Valdes, Jr. Organized and Co-Chaired a 2-day national meeting titled “Translating Proteomic Discoveries into Clinical Diagnostics”, an AACC sponsored meeting in Seattle, WA held in May 2008. This meeting was focused on breaking new ground in the development of biological markers based on proteomic discoveries and novel techniques and importantly how to transition those discoveries to research and clinical applications. He also presented several invited lectures: one titled “Pharmacogenetics in Laboratory Medicine: Optimizing Therapeutics” during the Norton Hospital Pediatric Pharmacology Symposium in Louisville, KY held on January 11, 2008; one on “Overview of Pharmacogenetics in Medical Practice: Applications in Warfarin Therapy” presented at the University of Florida Molecular Medicine Symposium, Lake Buena Vista, FL March 2008; one titled “Understanding Pharmacogenetics and Its Role in Laboratory Medicine” presented at the University of Arizona as the Paul Finley Lectureship in Tucson on April 24, 2008; and one presented at a Luminex Corporation Scientific Advisory Board Meeting and titled “Personalized Medicine Interface Tool (PerMIT)” in Austin TX in May 2008. Valdes was also a key co-investigator on two new grants: an NIH-NHLBI SBIR Phase I Award to PGXL Laboratories, LLC titled “Development of a Personalized Medicine Interface for the Safe and Effective Treatment of Patients Undergoing Warfarin Therapy” with a former clinical chemistry postdoctoral student, Dr Kristen Reynolds, as PI which was funded to PGXL Laboratories.



MEMBER HIGHLIGHT

The ever-expanding role of estrogen in health and disease

Carolyn Klinge has been a faculty member in the Department of Biochemistry and Molecular Biology since 1996. Her research focuses on the molecular mechanisms by which estrogens regulate physiological functions in a cell-type specific manner. Trained in genetics and pharmacology with a focus on regulation of DNA replication in breast tumors, Carrie started working on estrogen receptor (ER) as a post-doc at the University of Rochester School of Medicine with Russell Hilf and Robert Bambara. This was in 1985 when there was just one ER which had not yet been cloned and Carrie went to the local slaughterhouse at 6:00 AM two mornings a week to collect fresh calf uteri for ER purification. Transitioning from a cold room biochemist to a molecular biologist during her time at the UofR, she became intrigued by the diverse effects of estrogens and the critical role of estrogen in breast cancer as well as gender-selective diseases. Now there are two ERs, ER α and ER β which have functions opposite from one another in breast, prostate and colon cancer cells. Carrie's work on ER-DNA interaction and the role of the estrogen response element sequence on ER conformation demonstrated that DNA is an allosteric modulator of ER interaction with coregulators. Her research commitment to women's health has evolved into four main projects plus several exploratory projects and collaborations.



Carolyn Klinge, Ph. D.

The primary focus of Carrie's laboratory is to determine how estradiol (E₂) and other ER ligands regulate the expression of specific genes, including microRNAs, in a cell-type and promoter context-specific manner. The mechanisms by which estrogens regulate nuclear-mitochondrial signaling is the focus of an NIH R01 grant now in its 9th year. In an NIH/NCI R21 funded project, Research Associate Nalinie Wickramasinghe and graduate student Tissa Manavalan are examining the role of miRNAs in mediating estrogenic and anti-estrogenic signaling in breast cancer cells. To date, miR-21 has been identified as an estrogen-regulated microRNA and this work, a collaboration with CEGIB member Yong Li, has been submitted for publication (*Endocrinology*). The longer term goal to determine the role of microRNA expression in endocrine-resistant breast cancer is the focus of a new NIH R01 application currently under review.

The third project is funded by the Susan G. Komen For the Cure Foundation and addresses the role of an orphan nuclear receptor called COUP-TFII in anti-estrogen-sensitivity and the mechanism for the decrease in COUP-TFII expression in endocrine-resistant breast cancer. Doctoral candidate Krista Riggs will soon defend her Ph.D. dissertation on this research.

A fourth project is supported by grants from Joan's Legacy and the Lungevity Foundations. In that work, Research Associate Margarita Ivanova and technician Susan Dougherty are examining the mechanisms accounting for the gender disparity in lung adenocarcinoma. Women are at higher risk of lung adenocarcinoma than men and the roles of ER β -interacting proteins and MUC-1 in this disparity are being investigated. ER-MUC-1 signaling is the focus of doctoral candidate Yoannis Imbert's research on dry eye disease, another female-selective disorder.

In work supported by the American Heart Association, Carrie recently reported that the dietary phytochemical resveratrol, which is enriched in red wine and was recently featured in the health column of *Courier-Journal* for its anti-aging activity, has cardioprotective effects in the vascular endothelium. The data show that nanomolar concentrations of resveratrol rapidly activate ER α 's membrane-initiated (non-genomic) signaling pathway in HUVECs and this research was published in the July 2008 issue of *FASEB J*. Related work, a collaboration with Ryoichi Kizu of Doshisha Women's College of Liberal Arts in Kodo, Japan, and Steven Meyers, Dept. Pharmacology & Toxicology, UofL, described the rapid activation of

MAPK, AKT, and eNOS by diesel exhaust particulate extracts (DEPEs) in HUVEC and was recently published in *Toxicol. Lett.*

Carrie has several other active collaborations in addition to those already discussed. With David J. Schultz (Biology), Carrie's lab is examining the anti-cancer activity of anacardic acid. This research is supported by a Research on Women grant from the UofL Office of the Vice President for Research. The role of ER signaling in thyroid cancer is the focus of a collaboration with Richard E. Goldstein (Surgery) and post-doc Akhilesh Kumar. Albert R. Cunningham of the Brown Cancer Center has identified chemicals by SAR that have specifically increased rat mammary tumors and Carrie's lab is testing the estrogenic/antiestrogenic activity of these chemicals in cell-based assays. In collaboration with Mary Huff at Bellarmine University, the nongenomic ER-mediated effects of cadmium and arsenite in lung adenocarcinoma cells are being examined.

In addition to carrying on her own research, Carrie provides research opportunities is PI of an NIH T35 (NIDDK) training grant supporting the summer research stipends and support costs for 6 second year medical student scholars in endocrine research. Steven Winters and Richard Goldstein are co-PIs on this training grant.

Since coming to the UofL, Carrie's research grants have totaled over \$3.1 million, she has trained 2 Ph.D. and 2 M.S. students, and published 48 peer-reviewed papers in journals including *Cancer Research*, *Journal of Biological Chemistry* and *Molecular Endocrinology*. One might question how Carrie manages



Members of the Carolyn Klinge lab:

Left-right: Farzan Pouranfar, Yoannis Imbert, Piyumika Sooriyampola, Dr. Margarita Ivanova, Dr. Carolyn Klinge, Susan Dougherty, visiting sabbatical Professor Joan Magnusen, Krista Robinson, Tissa Manavalan, and Dr. Nalinie Wickramasinghe.

her many estrogen-o-centric projects. She says having great people in her lab is key and that she considers her research a stewardship of taxpayer and Komen or Joan's Legacy donor investments. That is a sacred trust and so she tries to do her best to advance our understanding of estrogen action. She also said that lap swimming at Lakeside and running around her Highlands neighborhood give her thinking time.

CAREER DEVELOPMENT

-Russ Prough

Our past Career Development Awardees have achieved several hallmarks of their support from CEGIB. **Qunwei Zhang** received an American Heart Association Great Rivers Affiliate grant entitled "Mechanism underlying the susceptibility of diabetics to ultrafine particles" for \$121,000 from July 2008-June 2010 and a Health Effects Institute Walter A. Rosenblith New Investigator Award entitled "Activation of Endothelial cell and gene expression in lungs following exposure to ultrafine particles" for \$300,000 from March 1, 2007-Feb 28, 2010. He has just heard that he received a KSEF grant entitled "Oxidative stress and endothelial cell dysfunction in animal exposure to ultrafine particles and cigarette smoke" for \$100,000 from July 2009 through June 2010. He published four papers in 2007-2008 and attended 7 conferences on environmental health related topics. He has submitted a number of new grant applications to continue building his career support.

Yong Li received a Scientist Development Grant from the American Heart Association entitled "MicroRNAs in Atherosclerosis" from January 2008 through December 2011 and has submitted an NSF Career Award Project entitled "Exploring novel functions of mammalian microRNAs" to run from April 2009 through March 2014. He also published 2 articles in peer-reviewed journals and attended the 2007 Keystone Symposium on MicroRNA functions in NF- κ B signaling pathways, the 2007 MDM2 International Workshop on Lentiviral-virus based genetic library for microRNA at Woods Hole and its applications in p53 signaling pathway, the 2008 NF- κ B symposium, Banff, Alberta, Canada in 2008 and the 2008 Society of Toxicology meeting in Seattle, Washington.

The Career Development Awardees for the second year of CEGIB have been selected and their development plans initiated. The first is **Ming Ouyang**, Ph.D., who is a new faculty member from the Department of Computer Engineering & Computer Science at the J. B. Speed School of Engineering. He obtained his Ph.D. in the Department of Computer Science, Rutgers University and then was an instructor in that department. He subsequently joined the faculty at the Department of Computer and Information Sciences, University of Delaware and participated in teaching a course entitled "Introduction to Computer Science: He joined the faculty at UofL in 2007. He has a grant from the Federal Aviation Administration and is a Co-investigator on a grant from the EPA Environmental Bioinformatics and Computational Toxicology Center.



Ming Ouyang



David J. Samuelson

The second is **David J. Samuelson**, Ph.D., an Assistant Professor in Biochemistry & Molecular Biology who joined UofL August 1, 2007 after completion of postdoctoral work at the McArdle Laboratory for Cancer Research, University of Wisconsin Madison. His work on the identification of breast cancer susceptibility genes leads directly to the studies he proposes to pursue in his new position and as part of his CEGIB career development award. He received a M.S. in Quantitative Genetics, at Virginia Tech, Blacksburg, VA, his Ph.D. in Cancer Biology, University of Arizona, Tucson and pursued successful postdoctoral training with Michael Gould at the McArdle Laboratory. He has just completed submission of grants to a number of foundations and the National Institutes of Health.



PILOT PROJECTS PROGRAM

-Doug Darling

The CEGIB Pilot Project Program continues to provide support for developing new research directions in the field of environmental genomics and integrative biology. This grant program emphasizes projects which will lead directly to new NIH R01 applications. Successful projects often develop collaborations between young faculty members with expertise in a novel technology and senior investigators with experience in environmental health science. .

The 4 projects funded during the 2007 cycle have been highly successful, and Lu Cai, Yong Li, David Powell, and David Scott, and their collaborators, are to be congratulated. The \$105,000 invested in these projects has supported successful applications for external grant awards totaling over \$830,000 in funding. In addition, 11 publications have been produced by these investigators.

Applications for the current year were submitted in mid-February and reviewed both externally and by an internal council. Many applications were judged to have a strong focus on a novel environmental question, as well as strong preliminary studies showing an excellent chance of leading to future external funding. The following applications were funded:

William L Dean, Effect of Butadiene Metabolite on Angiogenesis

Irene Litvan, Polymorphic Genes of Detoxification Enzymes as Risk Factors for PSP

M. Michele Pisano, Developmental Neurotoxicity of Prenatal Cigarette Smoke Exposure

Qunwei Zhang, Carcinogenesis of Nickel Nanoparticles

We congratulate these individuals and their collaborators on their excellent applications, and look forward to continued success with these projects.

The next round of Pilot Project funding will be announced in the fall of 2008, so start developing your innovative ideas of importance to biomarkers of environmental health and disease.

[To ↑
TOP](#)



William Dean



Irene Litvan



Michelle Pisano



Qunwei Zhang

FACILITY CORE ACTIVIES

BIOINFORMATICS, BIostatISTICS AND COMPUTATIONAL BIOLOGY CORE (BBCB)

The BBCB has been active in educational activities and has organized or supported two well attended workshops.

NIEHS dbSNP Workshop:

This workshop was held January 10-11, 2008 at Louisville's Brown Hotel and was attended by 79 regional researchers. Debbie Nickerson from the University of Washington conducted the workshop with assistance from several faculty and staff. The workshop provided an overview of the latest approaches for identifying and genotyping single nucleotide polymorphisms (SNPs) and for evaluating genetic associations across the human genome. Topics included the extraction of SNP data from public resources, approaches for SNP discovery by re-sequencing, software tools for haplotype inference and optimal SNP selection for genotyping, platform approaches for SNP genotyping, and the analyses of these datasets for genotype-phenotype studies. The workshop also included hands-on tutorials on a variety of software tools used for variation analysis. The workshop agenda is available online at <http://egp.gs.washington.edu/agenda.html>.

Annual Bioinformatics Summit:

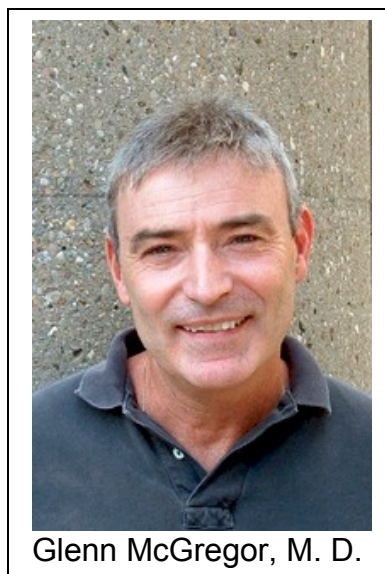
A Kentucky/Tennessee Bioinformatics Summit was held March 28-30, 2008 at Lake Barkley State Park in Cadiz, Kentucky. The conference was attended by nearly two hundred regional researchers. Plenary sessions on pathways to prediction, biomedical informatics and regulatory analysis were among the highlights of the summit. The proceedings, including a meeting summary and selected meeting abstracts have been published in BMC Bioinformatics, and may be found at <http://www.biomedcentral.com/1471-2105/9/S7/I1>.

Large datasets, Microarrays, MicroRNAs (miRNAs) and mRNAs:

The BBCB has been active providing CEGIB researchers with expertise in statistical analysis of high throughput data sets, and in the use of online tools and databases. Analysis of high throughput datasets, acquisition of complementary information from online databases, and use of computational tools is daunting for many researchers. The BBCB provides the skills necessary to integrate a researcher's data with complementary public domain datasets, and to perform the custom analysis necessary to drive the next generation of discovery in environment health research. Three on-going projects focused on data obtained by microarray of microRNAs and mRNAs have made great progress with assistance from the BBCB. The following summaries of these projects demonstrate the benefits of BBCB expertise.

Global gene expression in response to UV

Glenn McGregor's laboratory studies the accessory DNA polymerases of the Y family that catalyze direct DNA synthesis past bulky lesions that persist into S phase. These enzymes are able to accommodate helical distortions induced by such lesions because the "fingers" of the traditional palm-thumb-fingers configuration of these DNA polymerases have an open configuration. These enzymes bypass replication-blocking lesions at the cost of fidelity and processivity. Although mutagenic bypass avoids lethal double strand



Glenn McGregor, M. D.

breaks, these enzymes are responsible for virtually all mutations induced by genotoxic carcinogens. Higher eukaryotic cells, including human, have four members of this family classified based on structural homology to the budding yeast Rad30A protein. These are POL η , POL ι , POL κ , and REV1. POL η has the unique ability to faithfully bypass UV-induced TT dimers and is the molecular defect in xerodermapigmentosum variant (XP variant) syndrome. In the absence of POL η , another more error prone Y family polymerase (likely POL ι) bypasses these most frequent UV lesions in an error-prone fashion. This leads to increased UV mutagenesis and the rapid onset of aggressive skin cancer in XP variant patients.

It is widely accepted that point mutations in critical growth-control genes are the basis for malignant transformation, and it follows that reducing the frequency of such mutations will reduce the incidence of carcinogen-induced cancer. Accordingly, McGregor's laboratory is actively pursuing antimutator strategies based on the inhibition of specific members of the Y family. They developed combinatorial *pol η* and *pol ι* knockout mice to examine UV-induced tumorigenesis as modulated by these enzymes. As expected, *pol η ^{-/-}* mice showed high UV-induced mutation frequencies and developed tumors rapidly, validating this mouse model as the first for XP variant syndrome. They hypothesized that pol ι is the error-prone polymerase responsible for UV-induced mutations, and would be a likely target for antimutator strategies. To test this, they generated *pol η ^{-/-}pol ι ^{-/-}* double knockout mice, and consistent with their hypothesis, the cells from these mice have greatly reduced UV-induced mutation frequencies. Unexpectedly, these mice developed tumors *faster* than mice deficient in pol η single knockout mice, and the tumors were more aggressive with greater metastatic potential. The increased carcinogenic potential in light of decreased mutation frequency implies that pol ι acts as a tumor suppressor, separate from its known functions as an error-prone polymerase. Clearly, there are critical gaps in our knowledge of the cell biology of these enzymes that must be examined before modulation of this pathway can become a practical strategy for cancer prevention. In order to fill this gap, McGregor's group initiated, with the collaboration of BBCB core members, a systems biology approach to understanding the modulation of UV-induced changes in microRNA and mRNA, as influenced by the Y family polymerases. Together with McGregor's graduate student Lindsay J. Stallons, the CEGIB BBCB is providing the expertise supporting microarray experiments analyzing the role of pol η and pol ι in mRNA and miRNA alterations in response to UV. The Biostatistics group is working to provide support for microarray analysis of mRNA and miRNA experiments, and the Informatics group is working to gather data from public domain resources to integrate miRNA target data, and transcription factor information to identify possible functional cellular interactions that would explain the expression data. The ultimate goal of this project is to identify mechanisms of carcinogenesis involving Y-family polymerases in order to determine methods of intervention and cancer prevention.

Environmental Pollutants and Non-alcoholic Fatty Liver Disease:

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the United States, and its incidence is rising. By histology, NAFLD resembles alcoholic liver disease, although affected individuals, by definition, do not drink. NAFLD encompasses a spectrum of liver pathology beginning with fat accumulation (steatosis), which may trigger an inflammatory and fibrotic response (steatohepatitis or NASH) and progress to cirrhosis and hepatocellular carcinoma. NAFLD is most commonly initially detected as an asymptomatic ALT elevation on routine blood tests.



Matthew Cave, M. D.

NAFLD is generally believed to be the hepatic manifestation of obesity and the metabolic syndrome. However, recent reports document NASH in lean petrochemical workers as well as cancer patients treated with chemotherapy. Some, but not all, patients with NAFLD develop progressive disease. A “two hit” model has been proposed to explain this phenomenon. Multiple “second hits” including insulin resistance, pro-inflammatory cytokine production, and oxidative stress have been described. It is unknown why these “second hits” do not affect all NAFLD patients, but differential exposure to environmental toxicants could play a role.

Matthew Cave is interested in the role of environmental toxicants and nutrient-toxicant interactions in NAFLD. He and his co-workers have identified an extremely high incidence of NASH (78%) in non-obese polyvinyl chloride workers from Louisville, Kentucky which was associated with insulin resistance, oxidative stress and increased pro-inflammatory cytokine production (Figure 1). He has coined the term toxicant associated steatohepatitis (TASH) to describe this condition. Furthermore, they have performed animal studies demonstrating down-regulation of glutathione-S-transferases, key enzymes of xenobiotic metabolism, in several murine models of diet-induced obesity. Combined, these data suggest that members of the general population, particularly those who are obese, may be sensitized to NAFLD/TASH mediated by common environmental pollutants (Figure 2). However, the role of environmental pollutants in NAFLD affecting the general population has never been studied, and is the subject of this project.

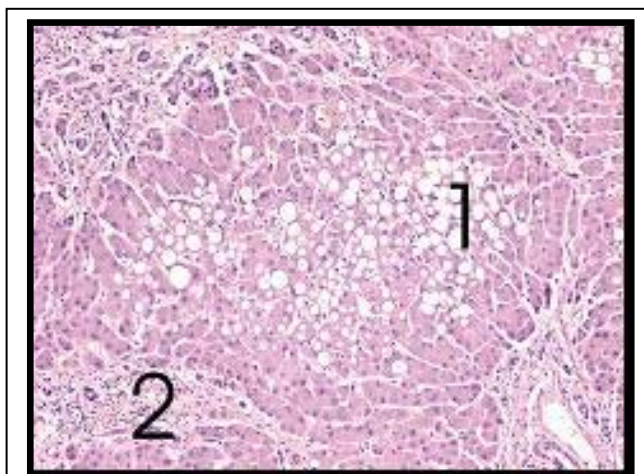


Figure 1: H&E stain of a liver biopsy from a polyvinyl chloride worker showing NASH cirrhosis with steatosis (1), inflammatory infiltrate and fibrosis (2).

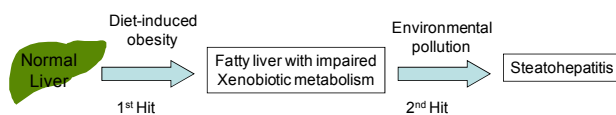


Figure 2: Environmental pollution is an underappreciated second hit in the genesis and progression of NAFLD/NASH.

The National Health and Nutrition Examination Survey (NHANES) is a large, population-based study of thousands of Americans conducted by the Centers for Disease Control (CDC) and the National Center for Health Statistics (NCHS). The primary purpose of NHANES is to collect information on the health and nutrition of the U.S. household population. In addition to a detailed interview and physical exam, NHANES participants undergo thorough laboratory testing. From 1999-2004, laboratory testing included routine liver function tests as well as serum, urine, and air levels of 200 potentially relevant toxicants. These pollutants belong to the following categories: polyfluorinated compounds (PFCs), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), volatile organic compounds, pesticides, arsenicals, heavy metals, phenols, polybrominated diphenyl ethers (PBDEs), phthalates, non-ortho-substituted or coplanar polychlorinated biphenyls (cPCBs), and polychlorinated dibenzo-*p*-dioxins (PCDDs).

Liver disease, and specifically NAFLD, is common in the adult NHANES population. Elevated serum alanine amino transferase (ALT) concentration has been used to estimate the prevalence of liver disease using pooled data from NHANES 1999-2004, which included 14,855 adult

participants. Over 40% of adult NHANES participants were found to have elevated ALT in prior studies. Furthermore, 69% of all ALT elevations were *not* explained by viral hepatitis, alcohol consumption, or hereditary hemochromatosis, suggesting that they were due to NAFLD. With the assistance of the BBCB, Cave and his collaborators, including Cam Falkner, Mirhir Patel and CEGIB member Craig McClain, are mining NHANES to explore for an association between pollutant exposure and unexplained ALT elevation. The Biostatistics group is providing statistical support for this data mining. For each pollutant in NHANES, subjects are stratified by quartile of exposure. Then, the data are analyzed for increasing adjusted odds ratios for unexplained ALT elevation across quartiles of increasing exposure. Significant increase in the adjusted odds ratio for unexplained ALT elevation with increasing level of exposure will document an association between chemical exposure and NAFLD.

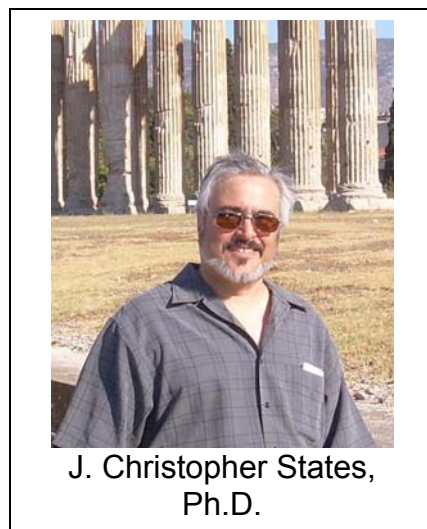
The study is expected to identify several pollutants associated with NAFLD in the general U.S. population, thus providing a plausible explanation for the increasing incidence of NAFLD. Targeting these toxicants for confirmatory and mechanistic studies in animal models will lead to an increased understanding of the role of environmental pollution in NAFLD, a liver disease affecting approximately 25% of Americans. Ultimately, these studies could lead to recommendations for patients either with or at risk for NAFLD to avoid environments and occupations which could place them at increased risk. Additional benefits may be gained by improved regulations by OSHA and the EPA which could potentially reduce NAFLD and its devastating complications, cirrhosis and hepatocellular carcinoma.

Arsenic and vascular disease

The major cause of death in the U.S. is cardiovascular disease. Epidemiological studies indicate that chronic arsenic ingestion causes cardiovascular disease in humans. However, the mechanism of arsenic-induced arterial disease is unknown. Chronic arsenic exposure is associated with increased mortality from myocardial infarction and stroke. In addition, gangrene of the digits is a frequent manifestation of arsenic-induced arteriosclerosis.

Widespread exposure to arsenic in drinking water in the U.S. likely contributes to atherogenesis and death from cardiovascular disease. The role that early life arsenic exposure may play in development of atherosclerosis in adult life is of particular interest. Deaths from myocardial infarction caused by advanced arteriosclerosis of infants whose mothers consumed water with high levels of arsenic suggest that transplacental arsenic exposure is a risk factor for arterial disease in humans.

Arsenic exposure via consumption of contaminated drinking water is a worldwide problem affecting the health of millions of people not only in third world countries like Bangladesh but also in the U.S. Chronic arsenic exposure is well established to cause cancer of the skin, lung and bladder. Less well appreciated is the high level of cardiovascular and peripheral vascular disease resulting from chronic arsenic exposure. In addition, until quite recently animal models to study arsenic induced vascular disease were not available until CEGIB members Chris States and Sanjay Srivastava demonstrated that arsenic exposure via drinking accelerated atherosclerosis in the ApoE-knockout mouse. They have shown that both post-natal and pre-natal exposures are



J. Christopher States,
Ph.D.

capable of inducing disease, suggesting that epigenetic mechanisms may play a role in arsenic induced vascular disease.

Arsenic interferes with DNA methylation. Because DNA methylation is known to play a major role in epigenetic control of gene expression, arsenic has the potential to dysregulate gene expression. In particular, DNA methylation plays a major role in maintenance of genetic imprinting which is the programmed suppression of either the paternal or maternal allele resulting in a functionally hemizygous phenotype. The effect may be on developmentally specific genes or may persist into adulthood. Dysregulation of genetic imprinting of several oncogenes plays a role in carcinogenesis. Transplacental arsenic exposure causes demethylation and aberrant increased expression of estrogen receptor α (ER α) and cyclin D1 genes in livers of exposed mice.

It is now well understood that atherosclerosis is a multifactorial disease process. More recent work also indicates that atherosclerosis may also be a multi-organ disease, with disease-modifying risks being derived from distal sites. Indeed, underlying liver disease has been shown to be a risk factor for developing atherosclerosis in humans, and is independent of other risks associated with chronic liver diseases (e.g., metabolic syndrome). Whereas the mechanisms by which liver disease modulates atherosclerosis are currently poorly understood, reasonable working hypotheses exist. First, it is proposed that systemic oxidative stress and inflammation that occurs during liver disease contributes to atherogenesis; this hypothesis is supported by the observation that the risk of atherogenesis increases with severity of hepatic inflammation. Second, abnormal lipid metabolism caused by underlying liver disease could contribute to atherogenesis; indeed, hepatic steatosis is associated with overproduction of VLDL in humans. Furthermore, atherosclerosis shows a clear correlation with NAFLD and circulating markers of liver damage/disease associated with metabolic syndrome. Whereas most of the studies in humans have been performed with nonalcoholic fatty liver disease (NAFLD), the States group has proposed that there are parallels between this disease and arsenic toxicity. For example, arsenic is known to cause liver damage in humans and in animal models with histologic lesions, in the latter analogous to those observed in advanced NAFLD. Thus, inflammation can be cause or consequence of liver disease.

In exciting preliminary studies aided by resources from the BBCB, States and colleagues used microarrays to analyze mRNA and miRNA abundances in livers of mice exposed to arsenic in utero. The BBCB analyzed the microarray data to derive altered pathways from the intersection of miRNA targets with mRNAs that changed expression. The data demonstrate that *in utero* exposure to arsenic increased expression of pro-inflammatory pathways in the liver which could lead to a heightened inflammatory responses thus leading to liver disease while providing the inflammatory stimulus for atherosclerosis. Importantly, this effect of *in utero* arsenic exposure occurred in absence of feeding the mice a high-fat diet. They are now confirming these microarray results with protein analyses and will build on these exciting results in future studies.

INTEGRATED HEALTH SCIENCES FACILITY CORE (IHSFC)

David Tollerud & Roland Valdes

The IHSFC has been focusing on ways to assist CEGIB members in biomarker development. Announcements of upcoming workshops and translational opportunities will be forthcoming.



COMMUNITY OUTREACH AND EDUCATION CORE (COEC)

-Irma Ramos

After months working in Shelbyville to build trust and strengthen collaborations with several community-based organizations, four lay health workers have been hired. These individuals are deeply committed to move forward the goals and missions of our community outreach core. After meaningful deliberations among them, they decided to self-identify as “Promotoras de la Salud Ambiental” (Environmental Health Promoters).

A successful community-based outreach program must be based on two essential elements: 1) the need for a solid infrastructure that persists within the community; and 2) the need to build a self-sustainable program. Working toward these goals, considerable efforts were spent this past year to identify

individuals with leadership potential who have resided within the community for some time years. In this newsletter I am pleased to introduce Ms. Guadalupe Vega as the leader of the Promotoras de la Salud Ambiental in Shelbyville. She is the mother of two grown children and has been living in Shelbyville for over 16 years, and working with one of the oldest community-based organizations in that area, El Centro Latino.

The basic training of the lay health workers was formally launched on Saturday, May 24, 2008. Three training sessions have been completed at El Centro Latino. Training is taking place during the weekends in order to accommodate the needs of lay health workers who often hold part-time employment elsewhere. In addition to the 4 lay health workers we also have two volunteers working with the group. These volunteers have proven to be a great asset to our program and we are fortunate to have them collaborating with us. The basic training of the lay health will continue for several months and focus on providing workers with the tools and knowledge needed for them to be able to connect community Shelbyville residents with the systems of education, government, social and medical services in the local community. Of particular note is the training being provided for our workers to perform one-on-one interviews of community residents that can yield a positive response from residents and that insures that principles of safety and community engagement are preserved.

While continuing their basic training, lay health workers will obtain their Human Subject Training (CITI) certification in preparation for their community interviews. Plans are now in place to complete 800 hundred surveys among Hispanics residing in the Shelbyville Area. The data obtained will be used to develop and refine our educational and interventional programs.



Promotoras de la Salud Ambiental: Digna, Rita, Isabel and Guadalupe (promotoras coordinator, far right)

NEW GRANTS RECENTLY AWARDED TO CEGIB MEMBERS

Nigel G. Cooper, Ph. D.

1R01EY017594-01A2 (Cooper)

06/01/2008- 05/31/2012

NIH/NEI

\$1,480,000 (Total costs)

The Role of CamKII in Cell Death and Survival Responses of Retinal Ganglion Cells

The project will explore the signal transduction pathways linked to gene regulatory networks which control cell death and survival processes in rat retinal ganglion cells, whose death is a major contributing factor for visual impairment in retinal and choroidal vessel occlusion, anterior ischemic optic neuropathy, glaucoma, and diabetic retinopathy.



Nigel Cooper

William M. Pierce, Jr., Ph. D.

W81XWH-08-1-0047 (Pierce)

3/1/2008 – 9/30/2009

US Department of Defense - USAMRMC

\$944,000 (Total costs)

High Technology Mass Spectrometry: Toxic Industrial Chemical Biomarkers

This project will define the chemical signatures of the toxic industrial chemicals (TICs) acrolein and acrylonitrile in human blood, and devise rapid, high throughput screening technology to determine exposure. Studies will first establish patterns of chemical reactivity and adduct formation. After establishing this knowledge, work will be focused on dose- and time-dependent formation of TIC-protein and TIC-DNA adducts, proper sample preparation techniques and ranges of sensitivity, specificity and precision of analysis. Finally, with this knowledge established, a technology platform will be developed for rapid, high throughput screening.



Bill Pierce

J. Christopher States, Ph. D.

1R21ES015812-01A1 (States)

04/01/2008- 03/31/2010

NIH/NIEHS

\$404,000 (Total costs)

Transplacental Arsenic Induced Hepatic Dysfunction and Vascular Disease

The project will establish the lower limit of in utero arsenic exposure that will accelerate

atherogenesis in ApoE-knockout mice and will characterize by histochemical methods the arsenic exposure induced changes in liver indicating a hyper-inflammatory response.



Chris States