

Center of Excellence in Environmental Toxicology

Second Annual Symposium

Genes and Environmental Health

November 19, 2007
University of Pennsylvania



GENES AND ENVIRONMENTAL HEALTH

SECOND ANNUAL SYMPOSIUM

Presented by

CENTER OF EXCELLENCE IN ENVIRONMENTAL TOXICOLOGY
UNIVERSITY OF PENNSYLVANIA SCHOOL OF MEDICINE

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CEET

NIEHS



UNIVERSITY OF
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Abramson Cancer Center

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Contents

Keynote Speakers	1
Symposium Agenda	2
Mission of CEET	4
Members of CEET	5
Directors of CEET	6
Poster Abstracts	7
New Members to CEET	24
Grant Abstracts: Major Initiatives in Gene-Environment and Exposure Biology Research in CEET	29
Acknowledgements	38

Keynote Speakers



Margaret R. Spitz

Margaret R. Spitz is Professor of Epidemiology and Chair of the Department of Epidemiology at The University of Texas M. D. Anderson Cancer Center. She holds the Olga Keith Wiess Distinguished University Chair for Cancer Research. Dr. Spitz received her M.D. from the University of Witwatersrand Medical School in Johannesburg, South Africa, and earned an M.P.H. degree from The University of Texas School of Public Health, where she currently holds an academic appointment.

Dr. Spitz has earned several honors in the field of cancer epidemiologic research including the 2002 Award for Research Excellence in Epidemiology or Prevention from the American Association of Cancer Research and the American Cancer Society, the Rosalind Franklin Science Award for Women in Science from the National Cancer Institute in 2003, the Dr. William Cahan Distinguished Professor Award from the Flight Attendants Medical Research Institute in 2004, and in 2006, the 13th annual Hadassah Horn Memorial Lectureship at the Weizmann Institute, Israel. She serves on several external scientific advisory boards of major cancer centers. Dr. Spitz was co-chair of NCI's Lung Cancer Progress Review Group, and she serves on NCI's Board of Scientific Advisors.

Her research interest is in studying genetic markers of susceptibility to tobacco-related cancers with the long-term goals of identifying high-risk subgroups who can benefit from intensive cancer screening and chemopreventive interventions. Her group is well positioned to develop risk-assessment models for lung cancer. Active collaborations have been established with other Lung SPORE programs and the International Lung Cancer Consortium. She is also constructing genetic profiles for use in individualizing therapy and understanding the functional consequences of chemoprevention, chemotherapy or radiotherapy response. Dr Spitz also serves as PI of projects in the Head and Neck SPORE and on the internal advisory committees of the Prostate, Bladder, and Ovarian SPOREs at MDACC.



Thomas Kensler

Dr. Thomas Kensler received his A.B. from Hamilton College and his Ph.D. in toxicology from M.I.T.. Following postdoctoral fellowships at the McArdle Laboratory for Cancer Research, University of Wisconsin and at the National Cancer Institute in Bethesda, MD, he joined the faculty of the Johns Hopkins Bloomberg School of Public Health in 1980. From 2000 to 2006 he served as director of the Division of Toxicology. He holds joint appointments in the Departments of Biochemistry and Molecular Biology, Pharmacology and Molecular Sciences, and Oncology. He also holds several Visiting Professorships in China. His research interests are in environmental carcinogenesis and cancer prevention. He is currently a member of the NIH Chemo/Dietary Study Section and serves as the Cancer Prevention editor for the journal *Carcinogenesis*. He has received several honors including the 2007 AACR-American Cancer Society Award for Research Excellence in Cancer Epidemiology and Prevention.

Symposium Agenda

- 8:15 A.M. – 8:45 A.M. **Registration**
Poster Set-Up
Continental Breakfast
- 8:45 A.M. – 9:00 A.M. **Welcome**
Dr. Arthur H. Rubenstein, Dean School of Medicine
- 9:00 A.M. – 9:15 A.M. **Gene-Environment Initiatives of CEET**
Dr. Trevor M. Penning, Director CEET
- 9:15 A.M. – 10:30 A.M. **Gene-Environment Interactions in Lung Cancer**
Center for Gene-Environment Interaction in Lung Cancer
Dr. Alexander S. Whitehead
Co-Director, Genes & Environment Core CEET
Professor of Pharmacology
- Candidate Genes in Lung Cancer Susceptibility*
Dr. Trevor M. Penning
Director, CEET
Professor of Pharmacology
- Biomarkers of Exposure and Response to Cigarette Smoke*
Dr. Ian A. Blair
Co-Director, Oxidative Stress Core CEET
Director, Center for Cancer Pharmacology
A.N. Richards Professor of Pharmacology
- 10:30 A.M. – 11:00 A.M. **Break**
- 11:00 A.M. – 12:00 P.M. **Keynote Lecture**
Genetic Susceptibility to Lung Cancer: an Epidemiologic Approach
Dr. Margaret Spitz
Professor and Chair, Department of Epidemiology
University of Texas M.D. Anderson Cancer Center
- 12:00 P.M. – 1:30 P.M. **Networking Lunch**
Poster Viewing

-
- 1:30 P.M. – 2:00 P.M. ***High-Throughput Genotyping in Complex Disorders***
Dr. Hakon Hakonarson
Director, Center for Applied Genomics
The Children's Hospital of Philadelphia
- 2:00 P.M. – 3:00 P.M. **Epigenetics**
Epigenetic Consequences of Preimplantation Embryo Manipulation
Dr. Marisa Bartolomei
Professor of Cell & Developmental Biology
- Long-term Effects of Embryo Culture on Gene Expression and Behavior in Offspring***
Dr. Richard Schultz
Professor of Biology
- 3:00 P.M. – 4:00 P.M. **Master Regulators of Gene Expression**
Regulation of the SMN Complex, Orchestrator of Cellular RNAs, by Cell Stress
Dr. Gideon Dreyfuss
Isaac Norris Professor
Investigator, Howard Hughes Medical Institute
- Clock-Genes and Circadian-Rhythm***
Dr. John Hogenesch
Associate Professor of Pharmacology
Associate Director PENN Genomics Institute
- 4:00 P.M. – 4:15 P.M. **Break**
- 4:15 P.M. – 5:15 P.M. **Keynote Lecture**
Cell Survival Responses to Environmental Stresses via the Keap1-Nrf2-ARE Pathway
Dr. Thomas J. Kensler
Professor of Environmental Health Sciences
The Johns Hopkins School of Public Health
- 5:15 P.M. – 6:00 P.M. **Reception with Program Officials from NIEHS**

Mission of the Center of Excellence in Environmental Toxicology

The Center of Excellence in Environmental Toxicology (CEET) was launched in 2005 and receives grant support from the National Institutes of Environmental Health Sciences. It is one of only twenty-two designated Environmental Health Science Centers in the nation.

The CEET mission is to understand the mechanistic link between environmental exposures and diseases of environmental etiology. Understanding these processes can lead to early diagnosis, intervention and prevention strategies. The end result will be to improve environmental health and medicine in our region.

The CEET is a flexible entity that marshals excellence in basic, translational, patient oriented and population-based research in the School of Medicine and Children's Hospital of Philadelphia to facilitate an integrative approach to environmental health/medicine. Although primarily housed in the School of Medicine, the fifty CEET Investigators belong to sixteen departments and five schools at the University of Pennsylvania.

The CEET marries its relevant research excellence to diseases of environmental etiology that affect our urban region. The CEET includes a research core in Lung and Airway Disease (asthma, lung cancer, mesothelioma, and chronic obstructive pulmonary disease) because of the poor air-quality and air-pollution in our region (ozone, fine particulate matter, allergens, SO₂, NO₂ and CO emissions). The CEET also has a research core in Endocrine and Reproduction Disruption because of the high incidence of adverse pregnancy outcomes that lead to low-weight birth and birth and developmental defects in our region. These organ-based cores are linked to our cores in disease mechanism, which include Oxidative Stress and Oxidative Stress Injury and Genes and the Environment.

The CEET enables its investigators to conduct predictive toxicology by employing Toxicogenomic and Toxicoproteomic approaches to identify the genomic and proteomic fingerprints that can be assigned to toxicant class, and to different stages of diseases of the environment. It is engaged in identifying and validating Biomarkers for these diseases.

The CEET conducts research relevant to the forty-five Superfund Sites that permeate the region. Studies elucidate mechanisms of chemical toxicity; exposure levels, risk assessment and health hazard; bioremediation approaches; and effects on ecosystems and biodiversity.

The CEET works with and disseminates research findings to select local communities to empower them with new knowledge so that they are better informed to tackle issues of health disparities and environmental justice. To improve the environmental health of these and similar affected communities, the CEET is actively involved in the education of health care professionals (Residency Program in Occupational and Environmental Health, Nursing concentration in Occupational and Environmental Health, and Masters of Public Health Programs).

The CEET will also disseminate its mission and its research findings to all stakeholders including community organizations, local, state and federal officials and agencies (Pennsylvania Department of Health, Pennsylvania Department of Environmental Protection, Environmental Protection Agency) to affect change in environmental health and public health policies.

CENTER OF EXCELLENCE IN ENVIRONMENTAL TOXICOLOGY

University of Pennsylvania School of Medicine

ADMINISTRATIVE CORE:

Director: Trevor Penning, Ph.D.

Deputy Director: Ted (Edward) Emmett, M.D, M.S.

RESEARCH CORE I

Oxidative Stress and Oxidative Stress Injury

Co-Director: Ian Blair, Ph.D.

Co-Director: Harry Ischiropoulos, Ph.D.

Benoit Giasson, Ph.D.

Jeffrey Field, Ph.D.

Aron Fisher, M.D.

Garret FitzGerald, M.D.

Toshinori Hoshi, Ph.D.

Virginia Lee, Ph.D.

Linda McCauley, M.N., Ph.D.

Vladimir Muzykantov, M.D., Ph.D.

Trevor Penning, Ph.D.

Richard Schultz, Ph.D.

Rebecca Simmons, M.D.

Stephen Thom, M.D., Ph.D.

RESEARCH CORE II

Endocrine/Reproduction Disruption

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Co-Director: Samuel Parry, M.D.

Kurt Barnhart, M.D., M.S.C.E.

Marisa Bartolomei, Ph.D.

Phyllis Dennery, M.D.

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Jianghong Liu, Ph.D., RN

Mary Mullins, Ph.D.

Katherine Nathanson, M.D.

Trevor Penning, Ph.D.

Tim Rebbeck, Ph.D.

Richard Schultz, Ph.D.

Rebecca Simmons, M.D.

Wenchao Song, Ph.D.

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Lung and Airway Disease

Co-Director: Rey Panettieri, M.D.

Co-Director: Steve Albelda, M.D.

Yassine Amrani, Ph.D.

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Richard Doty, Ph.D.

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James Kreindler, M.D.

Vera Krymskaya, Ph.D.

Milton Rossman, M.D.

Anil Vachini, M.D.

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Genes and the Environment

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Jason Christie, M.D., M.S.C.E.

Hakon Hakonarson, M.D., Ph.D.

John Hogenesch, Ph.D.

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Caryn Lerman, Ph.D.

Linda McCauley, M.N., Ph.D.

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Jennifer Pinto-Martin, Ph.D, M.P.H.

Trevor Penning, Ph.D.

Dan Rader, M.D.

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Toxicogenomics

Co-Director: Don Baldwin, Ph.D.

Co-Director: John Tobias, Ph.D.

FACILITY CORE II

Toxicoproteomics

Director: Chao-Xing Yuan, Ph.D.

FACILITY CORE III

Biomarker

Director: Ian Blair, Ph.D.

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Charles Branas, Ph.D.

Pamela Dalton, Ph.D.

Ira Harkavy, Ph.D.

Mary Hufford, Ph.D.

Howard Kunreuther, Ph.D.

Jianghong Liu, Ph.D., RN

Judith McKenzie, M.D., M.P.H.

Kevin Osterhoudt, M.D., M.S.C.E.

Directors of CEET



Trevor M. Penning
Director, CEET

Trevor M. Penning, Ph.D.

Trevor M. Penning, Ph.D., Professor of Pharmacology, Biochemistry and Biophysics, and OB/GYN is the Director of the Center of Excellence in Environmental Toxicology (CEET) at the University of Pennsylvania School of Medicine. Dr. Penning was interim-chair of Pharmacology from 1994-1996, and was Director of the Office of Postdoctoral Programs, and Associate Dean for Postdoctoral Research Training, School of Medicine from 1997-2001, and was Director of Biomedical Postdoctoral Programs (BPP) from 2001-2005. As Director of BPP, he oversaw the appointments, training and education of 850 postdoctoral fellows across the Schools of Medicine, Veterinary Medicine, and Dental Medicine. He is internationally recognized for his research on steroid hormone enzymology and mechanisms by which polycyclic aromatic hydrocarbons cause cancer. His research is now focused on the emerging role of Aldo-Keto Reductases (AKRs) in hormonal and chemical carcinogenesis. (www.med.upenn.edu/ak) He currently is the recipient of four R01 grants from the NIH. He has published over 150 peer-review articles and is the recipient of five U.S. patent applications. He has served on the Editorial Boards of the Journal of Biological Chemistry, Chemical Research in Toxicology, and Steroids. He is a prominent member of the Cancer Etiology Study Section at NIH. He was elected to the Johns Hopkins Society of Scholars in 1998. He is a consultant to the WHO International Agency for Research on Cancer. He was Program Chair for the Division of Chemical Toxicology of the American Chemical Society 2005-2006. In 2005, he assumed the Directorship of the CEET. The CEET is the first Environmental Health Sciences Center in the Commonwealth of Pennsylvania and is supported by NIEHS.



Edward A. Emmett
Deputy Director, CEET

Edward A. Emmett, M.D., M.S.

Dr. Emmett, Professor and Director of Academic Programs in Occupational Medicine, is active in clinical practice, research and education in Occupational and Environmental Medicine. His past experience includes Founding Director of the Divisions of Occupational Medicine in the Schools of Public Health and Medicine and of the Center for Occupational and Environmental Health at Johns Hopkins University from 1978 to 1988, and Chief Executive of the National Occupational Health and Safety Commission for Australia from 1988 to 1996. Dr. Emmett's research contributions include studies of occupational and environmental skin diseases, ultraviolet radiation effects on skin and eyes, the toxicity of polycyclic aromatic hydrocarbons, PCBs, organometals, monomers used in plastics and resins, and other substances. He is currently studying the effects of community pollution by perfluorooctanoates.

Dr. Emmett is a recipient of the Fight for Sight Citation for Clinical Research, and of the Kehoe Award of Merit from the American College of Occupational and Environmental Medicine. He is on several editorial boards and has been a member of many national and international committees. He is certified by the American Board of Toxicology and the American Board of Preventive Medicine in Occupational Medicine. Current activities include Chair of the Human Health and Environment program of the Pennsylvania Consortium for Interdisciplinary Environmental Policy and working with the United Auto Workers Union (UAW) and General Motors Corporation as their "Risk Communicator" to better translate research results into preventive actions at the workplace.

Oxidative Stress and Oxidative Stress Injury

O1 Benzo[*a*]pyrene-7,8-dihydrodiol produces reactive oxygen species via the Aldo-Keto Reductase (AKR) pathway in A549 cells: Involvement of BP-mediated redox cycling and alteration in redox status

Authors: Jong-Heum, Park (Center of Excellence in Environmental Toxicology, Department of Pharmacology, University of Pennsylvania)

Tacka, Kirk A (Center of Excellence in Environmental Toxicology, Department of Pharmacology, University of Pennsylvania), *Dipti, Mangal* (Center for Cancer Pharmacology, Department of Pharmacology, University of Pennsylvania)

Blair, Ian A (Centers for Cancer Pharmacology and Excellence in Environmental Toxicology, Department of Pharmacology, University of Pennsylvania)

Penning, Trevor M (Center of Excellence in Environmental Toxicology, Department of Pharmacology, University of Pennsylvania)

PAHs require metabolic activation to exert their deleterious effects. Among these pathways, Aldo-Keto Reductases activate PAH *trans*-dihydrodiols to the corresponding ortho-quinones which cause oxidative DNA damage. We used the fluorescent probe 2'7'-dichlorofluorescein diacetate to detect *in situ* ROS formation in A549 cells (which highly express AKR 1A1 and 1C isoforms) when exposed to PAH metabolites. BP-7,8-dione and BP-7,8-diol produced ROS which was suppressed by ROS attenuators. However, *anti*-BPDE and BP-4,5-diol did not produce any detectable ROS. Flow cytometric assays showed that 2-fluoro-3,4-dihydroxy-5-nitrobenzophenone, a catechol *ortho*-methyl transferase inhibitor enhanced both BP-7,8-dione and BP-7,8-diol-mediated ROS formation, suggesting the involvement of AKR-mediated conversion of BP-7,8-diol to BP-7,8-dione. ROS formation was correlated with changes in redox potential (GSSG / GSH and NADP⁺ / NADPH) in A549 cells. These data show that AKR-mediated PAH activation results in ROS formation, change in cellular redox state and may cause oxidative DNA damage in human lung cells. (Supported by NIH, R01-CA39504, R01-ES015857)

O2 Analysis of 8-Oxo-2'-deoxyguanosine in human bronchoalveolar cells by immunoaffinity liquid chromatography/mass spectrometry

Authors: Mangal, Dipti (Centers for Cancer Pharmacology and Excellence in Environmental Toxicology, University of Pennsylvania)

Park, Jong-Heum (Centers for Cancer Pharmacology and Excellence in Environmental Toxicology, University of Pennsylvania)

Lee, Seon Hwa (Centers for Cancer Pharmacology and Excellence in Environmental Toxicology, University of Pennsylvania)

Penning, Trevor M (Centers for Cancer Pharmacology and Excellence in Environmental Toxicology, University of Pennsylvania)

Blair, Ian A (Centers for Cancer Pharmacology and Excellence in Environmental Toxicology, University of Pennsylvania)

8-Oxo-2'-deoxyguanosine(8-oxo-dGuo) is an important pro-mutagenic DNA lesion resulting from oxidative damage. However, accurate quantification of 8-oxo-dGuo is difficult because artifactual formation occurs during the isolation and hydrolysis of DNA from cells and tissue samples. To avoid this problem, a cold guanidine thiocyanate non-phenolic method was used to extract DNA from cells. In addition, Desferal was used in Chelex-treated buffers to remove transition metal ions and prevent artifactual 8-oxo-dGuo formation through Fenton Chemistry. Immunoaffinity purification removed interfering substances that co-eluted with 8-oxo-dGuo during subsequent chromatography and stable isotope dilution liquid chromatography multiple reaction monitoring/mass spectrometry (LC-MRM/MS) provided maximal specificity and sensitivity. Human bronchoalveolar cells were treated with potassium bromate, methyl methane sulfonate and polycyclic aromatic hydrocarbon metabolites in order to induce 8-oxo-dGuo formation. A linear dose response curve to potassium bromate was observed by LC-MRM/MS and there was an excellent correlation with the Comet assay when coupled to hOGG1. (Supported by NIH P01CA

Oxidative Stress and Oxidative Stress Injury

O3 Human AKRs display quinone reductase activity with PAH *o*-quinones

Authors: Shultz, Carol A (Department of Biochemistry & Biophysics, University of Pennsylvania)

Quinn, Amy M (Centers of Excellence in Environmental Toxicology and Cancer Pharmacology, University of Pennsylvania)

Penning, Trevor M (Centers of Excellence in Environmental Toxicology and Cancer Pharmacology, University of Pennsylvania)

Human aldo-keto reductases (AKRs) metabolically activate pro-carcinogenic polycyclic aromatic hydrocarbon *trans*-dihydrodiols to highly reactive *o*-quinones. However, the quinone reductase (QR) activity of human AKRs, where *o*-quinones are reduced back to the catechol, has not been previously studied. QR activity could either detoxify *o*-quinones or increase futile redox cycling, amplifying reactive oxygen species (ROS). In this study, we now show that recombinant human AKR1C1, 1C2, 1C3, 1C4, 1B1, and 1B10 have substantial QR activity. The enzymatic reduction of *o*-quinones completely oxidized NADPH, implying that all the catechol formed auto-oxidized back to the *o*-quinone. In each case, enzymatic *o*-quinone redox cycling greatly exceeded the rate of PAH *trans*-dihydrodiol oxidation catalyzed by the respective enzyme. The exception was AKR7A2, which had the highest QR activity, but did not oxidize *trans*-dihydrodiols. The QR activity of AKRs may increase PAH-mediated oxidative stress. (Supported by PO1 CA92354, RO1 CA39504, P30-ES013508 awarded to T. M. P.)

O4 Methylated-bay region and fjord-region PAH *o*-quinones form mono- and *bis*- conjugates with N-acetyl-L-cysteine and glutathione

Authors: Shultz, Carol A (Department of Biochemistry & Biophysics, University of Pennsylvania)

Mangal, Dipti (Centers of Excellence in Environmental Toxicology and Cancer Pharmacology, University of Pennsylvania)

Gopishetty, Sridhar (Centers of Excellence in Environmental Toxicology and Cancer Pharmacology, University of Pennsylvania)

Harvey, Ronald G (The Ben May Institute for Cancer Research, The University of Chicago, Chicago, Illinois)

Blair, Ian A (Centers of Excellence in Environmental Toxicology and Cancer Pharmacology, University of Pennsylvania)

Penning, Trevor M (Centers of Excellence in Environmental Toxicology and Cancer Pharmacology, University of Pennsylvania)

Aldo-keto reductases metabolically activate carcinogenic polycyclic aromatic hydrocarbon *trans*-dihydrodiols to highly reactive *o*-quinones. Previously, methylated-bay region 7,12-dimethylbenz[a]anthracene-3,4-dione and fjord-region benzo[g]chrysene-11,12-dione and benzo[c]phenanthrene-3,4-dione reaction products were trapped with 2-mercaptoethanol. Mono- and *bis*-thioether conjugates were formed indicating that both 1,6- and 1,4- Michael additions occurred to the *o*-quinone. We examined the formation of the hindered-bay region *o*-quinone conjugates with N-acetyl-L-cysteine and glutathione. Each *o*-quinone:scavenger pair yielded two predominant products, identified as mono- and *bis*-conjugates. In other systems, *o*-quinone glutathionyl conjugates were observed to either protect proteins thiols from electrophilic attack or become more redox active than the parent *o*-quinone, thereby amplifying ROS. (Supported by PO1 CA92354, RO1 CA39504, P30-ES013508 awarded to T. M. P.)

Oxidative Stress and Oxidative Stress Injury

O5 The pattern of p53 mutations caused by PAH *o*-quinones is driven by 8-oxo-dGuo formation while the spectrum of mutations is determined by biological selection for dominance

Authors: Field, Jeffrey (Center of Excellence in Environmental Toxicology, Department of Pharmacology, University of Pennsylvania)

Park, Jong-Heum (Center of Excellence in Environmental Toxicology, Department of Pharmacology, University of Pennsylvania)

Vendantam, Srilakshmi (Center of Excellence in Environmental Toxicology, Department of Pharmacology, University of Pennsylvania)

Oliva, Andrea (Center of Excellence in Environmental Toxicology, Department of Pharmacology, University of Pennsylvania)

Batra, Abhita (Center of Excellence in Environmental Toxicology, Department of Pharmacology, University of Pennsylvania)

Blair, Ian (Centers of Excellence in Environmental Toxicology and Cancer Pharmacology, Department of Pharmacology, University of Pennsylvania)

Penning, Trevor M (Centers of Excellence in Environmental Toxicology and Cancer Pharmacology, Department of Pharmacology, University of Pennsylvania)

PAHs (polycyclic aromatic hydrocarbons) are suspect lung cancer carcinogens that must be metabolically converted into DNA-reactive metabolites. P4501A1/P4501B1 plus epoxide hydrolase activate PAH to (+) anti-benzo[*a*]pyrene (-) anti-BPDE which causes bulky DNA adducts. Alternatively, \pm diol epoxide ((Aldo-Keto Reductases (AKRs) convert intermediate PAH *trans*-dihydrodiols to *o*-quinones, which cause DNA damage by generating reactive oxygen species (ROS). In lung cancer, the types or pattern of mutations in p53 are predominantly G to T transversions. The locations of these mutations form a distinct spectrum characterized by single point mutations in a number of hotspots located in the DNA binding domain. One route to the G to T transversions is via oxidative DNA damage. A HPLC-ECD assay was used to detect the formation of 8-oxo-dGuo in p53 cDNA exposed to representative quinones, BP-7,8-dione, BA-3,4-dione and DMBA-3,4-dione under redox cycling conditions. Concurrently, a yeast reporter system was used to detect mutations in the same cDNA samples. Nanomolar concentrations of PAH *o*-quinones generated 8-oxo-dGuo (detected by HPLC-ECD) in a concentration dependent manner that correlated in a linear fashion with (-) anti-BPDE \pm mutagenic frequency. By contrast, micromolar concentrations of (generated (+) *trans*-anti-BPDE-N2-dGuo adducts (detected by stable-isotope dilution LC/MS methodology) in p53 cDNA that correlated in a linear fashion with mutagenic frequency, but no 8-oxo-dGuo was detected. Previous studies found that mutations observed with PAH *o*-quinones were predominately G to T transversions and those observed with (\pm) anti-BPDE are predominately G to C transversions. However, mutations observed with either PAH-treatment occurred randomly through the DNA-binding domain of p53. Here we find that when the mutants were screened for dominance, the dominant mutations clustered at or near hotspots primarily at the protein-DNA interface, while the recessive mutations are scattered throughout the DNA binding domain without resembling the spectra observed in cancer. There were little differences in the spectrums generated by the different mutagens. We conclude that mutagenesis can drive the pattern of mutations, but that biological selection for dominant mutations drives the spectrum of mutations observed in p53 in lung cancer. (Supported by P30 ES013508.)

Oxidative Stress and Oxidative Stress Injury

O6 Quantitative analysis of dihydroxyeicosatrienoic acids by stable isotope dilution chiral LC-electron capture APCI/MS

Authors: Mesaros, Clementina (Centers of Excellence in Environmental Toxicology and Cancer Pharmacology, University of Pennsylvania)

Lee, Seon Hwa (Centers of Excellence in Environmental Toxicology and Cancer Pharmacology, University of Pennsylvania)

Blair, Ian A (Centers of Excellence in Environmental Toxicology and Cancer Pharmacology, University of Pennsylvania)

Cytochrome P450 (CYP) derived epoxyeicosatrienoic acids (EETs) are formed by CYP-mediated metabolism of arachidonic acid. EETs have vasodilator and anti-inflammatory properties. They are rapidly converted to dihydroxyeicosatrienoic acids (DHETs), which are also vasoactive, by cellular epoxide hydrolases. Determination of chirality of the DHETs is important in order to distinguish enzymatic from non-enzymatic pathways of formation. Previous studies have described the analysis of DHETs by LC-MS but none of them have been used in combination with chiral separation. Derivatization of DHETs to their pentafluorobenzyl bromide-ester (PFB) derivatives facilitates the electron capture APCI/MS detection and allowed for normal phase chromatography to be used for separation. Specific precursor and product ions were selected for each DHET-PFB derivative for LC-MRM/MS analysis. ¹³C isomer DHETs (universally labeled) were also derivatized to their corresponding PFB-esters. This method also allows for the DHET enantiomers to be directly analyzed with high sensitivity and specificity.

(Supported by CEET grant P30ES013508)

O7 Bioactive lipids derived from human monocytes and mouse macrophages

Authors: Wei, Cong (Center of Cancer Pharmacology, Department of Pharmacology, University of Pennsylvania School of Medicine)

Rangiah, Kannan (Center of Cancer Pharmacology, Department of Pharmacology, University of Pennsylvania School of Medicine)

Shuvaev, Vladimir V (Institute for Environmental Medicine, University of Pennsylvania School of Medicine)

Blair, Ian A (Centers of Excellence in Environmental Toxicology and Cancer Pharmacology, Department of Pharmacology, University of Pennsylvania School of Medicine)

In atherosclerotic lesions, 15-LOX-1 is up regulated and localized at the sites of macrophage accumulation. Numerous studies have focused on the potential biological roles of the 15-LOX-derived lipid mediators such as 15(S)-hydroperoxyeicosatetraenoic acid [15(S)-HPETE] and 15(S)-hydroxyeicosatetraenoic acid [15(S)-HETE]. However, it is not clear that these are the biologically important 15-LOX-1 metabolites involved in modulating the macrophage infiltration. The Blair laboratory has recently identified 15-oxo-eicosatetraenoic acid (15-oxo-ETE) as a metabolite of cyclooxygenase-2-derived 15(S)-HETE. To determine if 15-oxo-ETE can be derived from the 15-LOX-1 pathway in macrophages and determine the biological activity of 15-oxo-ETE, a macrophage cell line (R15L) that stably expresses human 15-LOX-1 was utilized. We use stable isotope dilution LC-electron capture atmospheric pressure chemical ionization (APCI)/MS/MS to identify the 15-LOX-1 metabolites generated from R15L cells and primary human monocytes. R15L cells are treated with 10 μ M arachidonic acid (AA) and 5 μ M calcium ionophore (CI) A-23187. Metabolites [e.g. 15(S)-HPETE, 15(S)-HETE and 15-oxo-ETE] are identified in R15L cells as well as in primary human monocytes after stimulation of both AA and CI. 15-oxo-ETE identified as a novel metabolite in this manner are synthesized, purified and added to human umbilical vein endothelial cells (HUVEC). 15-oxo-ETE causes HUVEC cells to undergo apoptosis in a dose-dependent manner within 24 hours. These results will also prompt further investigation into the formation of 15-oxo-ETE-Glutathione (15-oxo-ETE-GSH) adducts in macrophage cells.

Endocrine and Reproduction Disruption

R1 Follicular fluid F2-isoprostanes: A novel assessment of oxidative stress in IVF patients

Authors: Butts, Samantha (Department of Obstetrics and Gynecology, Penn (HUP))

Lin, Kat (Department of Obstetrics and Gynecology, Penn (HUP))

Shaunik, Alka (Department of Obstetrics and Gynecology, Penn (HUP))

Barnhart, Kurt (Department of Obstetrics and Gynecology, Penn (HUP))

FitzGerald, Garret (Penn Institute for Translational Medicine & Therapeutics)

Coutifaris, Christos (Department of Obstetrics and Gynecology, University of Pennsylvania)

Objective: A pilot study to demonstrate the isoprostane 8,12-iso-iPF₂ α as a marker of oxidative stress in the follicular fluid of IVF patients, and to examine the relationship between isoprostane levels and patient's age, infertility diagnosis, and pregnancy outcome.

Design: Cross-sectional

Materials and Methods: Patients underwent standard ovarian stimulation protocols for IVF. At time of oocyte retrieval, follicular fluid from the first puncture of each ovary was collected, processed to isolate the follicular fluid from the granulosa cells, and stored at -80°C. Analysis of 8,12-iso-iPF₂ α levels was accomplished using reverse phase solid phase extraction and liquid chromatography/electrospray ionization/mass spectrometry. Clinical history and pregnancy outcomes were assessed through chart review and embryology records. Statistical analysis was performed using the Wilcoxon Rank Sum and Kruskal-Wallis tests, and Spearman rank correlation coefficient.

Results: Follicular fluid samples from 29 IVF patients with varying infertility diagnoses were obtained. Ages ranged 27 to 45 years, with a median of 33 years. 62% achieved pregnancy, of which two-thirds resulted in delivery and one-third in spontaneous abortion.

Isoprostane levels are measurable in follicular fluid, on the order of nanogram per milliliter (ng/ml), which is comparable to that demonstrated in plasma. The 8,12-iso-iPF₂ α levels in the first-puncture follicular fluid ranged from 0.176 to 3.097 ng/ml. Similar levels were seen for both the right and left follicles first retrieved on each side ($p=0.04$), with an average of 0.897 ng/ml for the right and 0.907 ng/ml for the left.

Average 8,12-iso-iPF₂ α for those who achieved pregnancy was 0.867 ng/ml, but higher (0.990 ng/ml) in those who did not get pregnant. While not reaching significance, the average 8,12-iso-iPF₂ α for patients with diminished ovarian reserve was 2.050 ng/ml, which was higher than levels for any other diagnosis. No correlation between isoprostane levels and age was found.

Conclusions: Isoprostanes have been a powerful research tool in the study of neurodegenerative diseases, such as Alzheimer's Disease and Down's Syndrome. For the first time, we have demonstrated that isoprostane levels are quantifiable in follicular fluid, in similar concentrations to that found in plasma. That isoprostane levels are similar between the right and left follicles suggests that oxidative stress affects both ovaries equally, despite seemingly disparate responses in follicular development during ovarian stimulation. The lack of correlation between isoprostane levels and age was not unexpected, since the older women in our study did not exhibit decreased ovarian reserve with elevated day 3 FSH levels.

As shown in this pilot study, 8,12-iso-iPF₂ α serves as a feasible, objective marker for oxidative stress in studying infertility. Future directions include specific investigations regarding different infertility diagnoses, including diminished ovarian reserve, and further correlation with plasma or urine isoprostane levels. (Supported by P30 ES013508)

Endocrine and Reproduction Disruption

R2 The identification of mouse sperm proteins that change their thiol status during epididymal maturation

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Sperm undergo biochemical and functional changes in the epididymis. During epididymal transit, there is an increase in the oxidation of sulfhydryl groups of sperm proteins. Sperm proteins from the caput epididymis contain more free sulfhydryl groups than disulfide bonds. The oxidation of sulfhydryl groups during epididymal transit is correlated with the stabilization of sperm tail structures. We are using a large-scale proteomic approach to identify proteins containing sulfhydryl groups that become oxidized during epididymal maturation. A CyDye DIGE Fluor saturation dye labeling protocol was used to label all reduced cysteine thiols of proteins. By comparing caput and cauda epididymal sperm populations whose proteins were either reduced or not reduced prior to labeling with the fluorescent dyes, proteins that show differences in disulfide bonding were observed. Each protein lysate (unreduced caput sperm, reduced caput sperm, unreduced caudal sperm, reduced caudal sperm) was labeled separately with Cy5. As an internal control for protein normalization, equal amounts of protein lysates from the four samples were combined and labeled with Cy3. Each individual Cy5-labeled sample were mixed together with the internal control sample and separated by two-dimensional PAGE in a single gel. Statistical analysis (One-Way ANOVA) between four protein populations selected 23 candidate spots that were less abundant only in unreduced caudal sperm. The protein sizes of 19 candidates were approximately 10 to 30 kDa. Individual spots are being picked and identified by mass spectrometry analysis. (Supported in part by NIH grants P01 HD-06274 and P30 ES013508-02)

R3 Proteomic technology detects cervicovaginal calgranulin B in women who deliver prematurely

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Objective: Using proteomic technology, identify candidate biomarkers from cervicovaginal fluid of pregnant women that may be linked to preterm labor.

Methods: Cervicovaginal fluid were collected from pregnant women, stored at -80C and delivery dates were subsequently determined, term (n=46) and preterm (n=19). Samples were spotted onto WCX2 chips and analyzed using SELDI-TOF-MS. Spectra were obtained for each sample and peak differences were determined. Four protein peaks with significantly greater intensity in preterm patients were identified.

Results: Proteomic analysis revealed 9 peaks that were significantly different between preterm and term women. Two of these peaks were determined to be fragments of Calgranulin B: candidate markers at 2178Da and 2509Da.

We conclude that: there is a distinctive cervicovaginal protein signature differentiating preterm and term samples and that a specific Calgranulin B fragment found in higher intensity in preterm subjects, may play a role in the inflammation-induced preterm labor pathway. Calgranulin A and B have previously been detected in amniotic fluid and serum of pregnant women with PTL and inflammation. (Supported by S001 MP050088-02 and P30 ES013508)

Endocrine and Reproduction Disruption

R4 Proteomics-based approach for identifying novel biomarkers of preterm birth

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Spontaneous preterm birth is a major contributor of perinatal morbidity and mortality. However, the diagnosis of preterm labor that leads to preterm birth is difficult, and hence the need for novel biomarkers. Cervical-vaginal fluid (CVF) is a complex mixture of secretions from the vagina, endocervix, endometrium and oviduct; and can potentially serve as an important diagnostic site to monitor maternal and fetal health in pregnant women because of its minimally invasive collection method. The present study utilized SILAC methodology to generate labeled ($^{13}\text{C}_6^{15}\text{N}_2$ -Lys, $^{13}\text{C}_6^{15}\text{N}_1$ -Leu) proteins secreted by immortalized human endocervical (End1) and vaginal (VK2) cells in culture. Equal amounts of protein from SILAC-labeled End1 and VK2 supernatants were combined at 1:1 ratio, depleted of six major serum proteins and digested with trypsin. The tryptic peptides were separated by strong cation exchange chromatography and analyzed by LTQ-MS. Proteins were identified by Trans-Proteomic Pipeline software using the Sequest database. These SILAC labeled proteomes model proteins unique to cervical-vaginal secretions, and will be used to investigate CVF samples from pregnant women for novel biomarker discovery. (Supported by NIH Grant 5P30ES013508)

Lung and Airway Disease

L1 Ozone modulates murine small airway responsiveness to agonists

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Ozone (O₃), a pollutant known to induce airway hyper-responsiveness (AHR), poses an increased risk to human health in patients with obstructive airway diseases & asthma. A site at which significant changes occur is at the small airways. This study aims to show the effect of *in vivo* ozone exposure at low & high concentrations on murine small airways.

Male Balb/c mice were exposed to either a low (0.6 ppm) or high (6 ppm) concentration of O₃ or forced air (controls) for 2 hours. Precision cut lung slices (PCLS; 250 μm thickness) containing a small airway (~ 0.01 mm² lumen area) were prepared immediately after exposure. Small airways were contracted to carbachol the following day. EC₅₀ and Emax values were calculated by measuring the airway lumen area with respect to baseline status. High concentrations of O₃ caused significant AHR of small airways to CCh (Control: 1.14 ± 0.38 μM; Ozone: 0.31 ± 0.10 μM; p=0.02), with no effect seen at low concentrations. No change was seen in maximum contraction at either high or low doses compared to controls.

These effects may be due to altered myocyte function. New therapeutic approaches to altered ozone-induced myocyte contraction may decrease ozone-induced morbidity associated with asthma.

L2 The beta 2-agonist, arformoterol, enhances production of the lung-protective surfactant protein D (SP-D) in alveolar epithelial cells.

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SP-D plays multiple roles in clearance of inhaled particles while minimizing both the adaptive and innate inflammatory responses that can potentially damage pulmonary epithelium. Deficiency in SP-D in mice leads to emphysema, an important pathological characteristic of chronic obstructive pulmonary disease (COPD). The novel beta 2 adrenergic agonist, arformoterol has been shown to be beneficial in COPD but its mechanism of action is incompletely understood. Beta 2 receptors are widely distributed in the respiratory tract including alveolar type II cells, the principle sites of production for the surfactant proteins including SP-D. We hypothesized that arformoterol may function to resolve inflammation in part by an upregulation of SP-D production. The results indicate that type II alveolar epithelial cells responded to arformoterol in a dose-dependent manner between 1-100 nM, leading to increases in both intracellular and secreted SP-D. We conclude that the long-acting beta 2-agonist, arformoterol, increases the ability of type II alveolar epithelial cells to produce SP-D which in turn may modulate immune responses in the lung environment and is responsible for a subset of the beta 2-agonists' therapeutic effects. (Supported by P30 ES 013508.)

Lung and Airway Disease

L3 Combination of stress and asthma inhibits the glucocorticoid receptor (GR) and CAAT enhancer binding protein (C/EBP)-mediated production of the immunoprotective surfactant protein D (SP-D) in the lung

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SP-D is an important immuno protective molecule produced in the lung epithelial cells in response to corticosteroids. Psychosocial stress may exacerbate asthma by decreasing corticosteroid sensitivity of epithelial cells and affecting release of SP-D. We studied the effects of stress and asthma on lung expression of GR, and C/EBP in allergen sensitized mice. Direct treatment of epithelial cells with corticosteroid was studied in isolated epithelial cells *in vitro*. Combination of stress and asthma significantly inhibited SP-D in the lung and this inhibition was associated with reduced gene expression for GR and C/EBP, transcription regulators responsible for SP-D production. Combined *in vitro* treatment of epithelial cells with IL-4 (a cytokine mediating the allergic airway response) and dexamethasone also inhibited both GR and C/EBP. Our data indicate that impaired production of the immunoprotective SP-D is associated with stress-induced exacerbation of allergic inflammation. This effect maybe mediated through a combination of increased levels of circulating glucocorticoids due to stress and the inflammatory cytokine IL-4 which is released during allergic inflammation. Supported by R01 AI055593, P30 ES013508.

L4 Shaedler's *Escherichia coli* induces surfactant protein D (SP-D) expression and prevents lactobacillus salivarius translocation into the lung in IgA knockout mice

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SP-D may play a protective role during dissemination of bacteria to the lung. Germ-free (GF) IgA^{-/-} and wild type (WT) adult mice were colonized orally with the commensal Shaedler's *Escherichia coli* or the *Lactobacillus salivarius*. Translocation of these bacteria into the lung was assessed by organ homogenate plating. SP-D protein levels were assessed by Western Blott. Expression of SP-D gene was studied by real-time PCR. Levels of SP-D were higher in WT GF mice than in IgA^{-/-} mice suggesting impairment of the pulmonary innate immune system of these animals. On day 3-7 after inoculation, translocation of *L.salivarius* to the lung was observed in the lactobacilli colonized IgA^{-/-} mice, but not in their WT counterparts. *E. coli* showed similar colonization levels to lactobacilli, but no translocation in either IgA^{-/-} or WT mice. Colonization of mice with *E. coli* prior to *L.salivarius* resulted in diminished pulmonary translocation of the latter in IgA^{-/-} mice. *E. coli* pretreated IgA^{-/-} mice had higher levels of SP-D in the BAL and lung, than animals monoassociated with *L. salivarius*. *E. coli* pretreatment stimulated increase in SP-D, implicate an important role for this pulmonary innate immune molecule in protection from translocation. (Supported by P30 ES-013508)

Lung and Airway Disease

L5 Metabolism of Benzo[a]pyrene in human bronchoalveolar H358 cells by LC-MS

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Benzo[a]pyrene (B[a]P), is a representative polycyclic aromatic hydrocarbon (PAH), which can be metabolically activated by three enzyme pathways, P450-peroxidase, P4501A1/1B1, and aldo-keto reductases (AKRs). The profile of B[a]P metabolites in human lung epithelial cells using modern methods is not well studied even though lung is a major PAH exposure site due to the inhalation of first and second hand tobacco smoke. In this study, B[a]P-metabolites generated by P450 peroxidase (B[a]P-1,6- and 3,6-dione), by P4501A1/1B1 (3-hydroxy-B[a]P, B[a]P-7,8- and 9,10-dihydrodiols, and B[a]P-r-7,t-8,t-9,c-10-tetrahydrotetrol), and by AKRs (B[a]P-7,8-dione) in human bronchoalveolar H358 cells were quantified by RP-HPLC with radiometric detection and identified by LC-MS. LC/MS data were acquired using a Thermo Finnigan TSQ Quantum Ultra spectrometer with an atmospheric pressure chemical ionization source operated in the positive-ion mode and on-line chromatography was performed with a Waters Alliance 2690 HPLC system. The eluent on-line was monitored in the selected reaction monitoring mode and full-scan modes and ion-transitions compared to those obtained with authentic synthetic standards. Progress curves showed a lag-phase in the formation of B[a]P metabolites which were formed in the following amounts: 3-hydroxy-B[a]P > B[a]P-9,10-dihydrodiol > B[a]P-7,8-dihydrodiol > B[a]P-tetrol > B[a]P-1,6-dione and B[a]P-7,8-dione over 24 h. Northern blot analysis showed induction of P4501B1 and AKR1C1 by B[a]P in a time-dependent manner, suggesting that B[a]P stimulates its own metabolism by induction of these key enzymes. By contrast B[a]P-3,6-dione was formed without a lag-phase. Pretreatment with 10 nM TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) eliminated the lag-phase but the levels of the individual metabolites were unaltered suggesting that TCDD induction alters the kinetics of metabolite formation but not metabolite levels. This study provides evidence for the formation of reactive B[a]P metabolites from each of the pathways of PAH-activation in human lung bronchoalveolar cells (Supported by P01-CA092537 and R01-CA39505)

L6 Analysis of CYP1A1/1B1-independent benzo[a]pyrene DNA-adduct formation in human lung cancer cells by LC-MS

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Activation of benzo[a]pyrene (B[a]P) by CYP1A1/1B1, to 7,8-dihydroxy-9, 10-epoxy, 7,8,9,10-tetrahydrobenzo[a]pyrene (B[a]PDE), is a widely accepted metabolic pathway. Induction of the CYP1 family in human bronchoalveolar H358 cells requires pre-treatment with 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). An unexpected increase in B[a]PDE-DNA-adduct formation was observed in cells not subjected to TCDD. A stable isotope dilution, liquid chromatography-mass spectrometry assay was used to analyze the B[a]PDE-derived deoxyguanosine adducts. H358 and TCDD-induced H358 cells were treated with increasing concentrations of TCDD before treatment with 2 μ M (-)-B[a]P-7,8-dihydrodiol. Increasing the TCDD concentration caused a decrease in DNA-adduct formation. These results are contrary of what is typically observed in liver cells. Inhibition of CYP1 with tetramethylstilbene (TMS) and CYP3A with itraconazole was also tested. DNA-adduct levels remained the same for TMS treatment; however, itraconazole treatment resulted in decreased DNA-adduct formation. It is likely that another P450, such as CYP3A5, is able to metabolize B[a]P to B[a]PDE. (Supported by NIH grants 1R01CA130038, 5P30ES013508, 1F32ES016683, and 5R25CA101871)

Lung and Airway Disease

L7 Expression profiling of pathways of polycyclic aromatic hydrocarbon activation in normal human bronchial epithelial cells

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Polycyclic aromatic hydrocarbons (PAH) are environmental pollutants and human carcinogens that are metabolically activated to the proximate carcinogenic *trans*-dihydrodiols. These can be further activated by cytochrome P450 (P450) 1A1 and 1B1 to yield mutagenic diol-epoxides or by aldo-keto reductases (AKR) 1A1 and 1C1-1C4 to yield reactive and redox-active ortho-quinones. Immortalized human bronchial epithelial cells (HBECs) were evaluated for AKR and P450 expression to gain an understanding of which PAH activation pathways are present in normal human lung tissue. Western blot and functional enzyme assays revealed that HBECs express AKR1A1, AKR1B10, and AKR1C1/2 protein, but do not express AKR1B1 or AKR1C3. AKR1C activity was induced 10-fold over 24 h by treatment with 10 μ M R-sulforaphane, an inducer of genes containing an antioxidant response element (ARE). Expression of Nrf2, a key regulator of ARE-mediated transcription, was confirmed by Western blot analysis. P450 expression was not detected by Western blot; however, functional assay showed minimal induction of P450s 1A1 and 1B1 after 10 nM TCDD treatment. On balance, basal and inducible AKR expression exceeds that seen for P450 isoforms.

Lung and Airway Disease

L8 Dietary flaxseed abrogates lung tumor growth in a mouse model of benzo-[a]-pyrene-induced lung carcinogenesis while improving overall animal health

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Introduction: We evaluated the chemopreventive properties of the dietary supplement, flaxseed (FS), in a model of tobacco-induced lung carcinogenesis. **Rationale:** Although FS has chemopreventive properties in breast, colon and prostate cancers, it has never been tested in lung cancer. **Methods:** Mice were injected i.p. with Benzo[a]pyrene (B[a]P) (1 mcg) once weekly for 3 weeks and given a 10% FS or an isocaloric 0% FS control diet. Lungs were harvested at 5 months and evaluated for tumor formation. **Results:** Lung tumor nodules were smaller in the 10% FS fed mice compared to 0% FS fed mice as confirmed by image analysis ($p < 0.03$) and there was a trend towards decreased nodule size ($p = 0.09$). The % of lung area infiltrated by tumor was $4.6\% \pm 1\%$ for 0% FS and $2.4\% \pm 0.8\%$ for 10% FS, a difference that may be enhanced as tumors grow. Mouse weight, a sign of animal health, was higher in 10% FS fed mice than in controls that received B[a]P ($23 \pm 0.8\text{g}$ vs. $20 \pm 0.7\text{g}$, respectively, $p < 0.01$). **Conclusions:** The selected dose and mode of administration of B[a]P generated a reproducible lung carcinogenesis model. Dietary FS retards lung tumor growth and incidence while improving animal health. (Supported by 1 P30 ES013508-02 from the NIEHS.)

Genes and Environment

G1 Histone deacetylase 1 regulates gene expression during mouse preimplantation embryogenesis

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Superimposed on the activation of embryonic genome in the preimplantation mouse embryo is the formation of a transcriptionally repressive state during the late two-cell stage. Several lines of evidence suggest that histone deacetylases (HDAC) may underlie the development of the transcriptionally repressive state during zygotic gene activation (ZGA) by mediating changes in the chromatin structure. In this study, we investigated expression and possible functions of Class I HDACs HDAC1, HDAC2, and HDAC3 during mouse preimplantation embryogenesis. We show that HDAC1 is likely a major deacetylase and its expression inversely correlates with changes of histone H4K5 acetylation state. RNAi-mediated reduction of HDAC1 leads to hyperacetylation of histone H4 and a developmental delay even though expression of HDAC2 and HDAC3 is significantly induced in Hdac1-suppressed embryos. RNAi-mediated reduction of HDAC2 has no noticeable effect on preimplantation development, suggesting that individual HDACs have distinct functions during preimplantation development. We also demonstrate that HDAC1 knockdown results in elevated levels of expression of a subset of genes that are normally repressed following genome activation and that this expression correlates with hyperacetylation of histone H4. Results of these experiments suggest that HDAC1 is involved in the development of a transcriptionally repressive state that initiates in 2-cell embryos. This research was supported by grant HD 22681 from the NIH to R.M.S and P30 ES013508.

G2 Differences in smoking topography associated with CYP2A6 genotype

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Approximately 80% of nicotine is inactivated when metabolized to cotinine. Variations in CYP2A6 genotype alter the rate of nicotine metabolism. Previous research has demonstrated that smokers with variant alleles associated with slower metabolism smoked fewer cigarettes compared to those smokers who were homozygous normal. Smokers can also control their nicotine administration on a per cigarette basis by adjusting smoking topography measures, such as number of puffs, puff volume, puff velocity, and puff duration. Participants smoked one of their preferred brand cigarettes through a smoking topography device and provided a blood sample for genotyping as part of the baseline session of a large nicotine replacement therapy study. Smokers with genotypes associated with slow or poor metabolism took significantly smaller puff volumes than those with genotypes associated with intermediate/normal nicotine metabolism. Analyses indicate no significant association with CYP2A6 genotype and other measures. These results suggest that in addition to smoking fewer cigarettes, those who metabolize nicotine slowly may also be taking smaller puff volumes on the cigarettes they smoke relative to those who metabolize nicotine normally. (Supported by P50 CA084718 and P30 ES013508.)

Genes and Environment

G3 Sex differences in the associations between polymorphism in the 5,10-methylenetetrahydrofolate reductase gene, smoking and homocysteine concentrations

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A low folate, high homocysteine phenotype is associated with several pathologies including cardiovascular disease and birth defects. Such a phenotype may be the result of genetic and environmental factors. We identified sex-specific interactions between C677T polymorphism in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene and smoking that affect homocysteine concentrations in a cohort of young, reproductive age (20 to 26 years old) men and women from Northern Ireland. Among men the associations of both red blood cell folate and smoking status with homocysteine levels were influenced by MTHFR C677T genotype. Thus, male MTHFR 677TT homozygotes are at the highest risk of having elevated homocysteine via presumptive interactions with smoking and low folate status. In contrast the associations of both red blood cell folate and smoking status with homocysteine in women appeared to be independent of MTHFR C677T genotype. Regardless of MTHFR genotype, women who smoke are at higher risk of elevated homocysteine than women who do not smoke, an observation that may be attributable to the suppression of red blood cell folate levels in the smokers. (Supported by AR47663, ES013508, HD039195, The Wellcome Trust, and the British Heart Foundation)

Genes and Environment

G4 The Cystathionine Beta Synthase 844ins68 allele abolishes methylenetetrahydrofolate reductase 677C>T genotype specific differences in folate/homocysteine phenotype

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A low folate, high homocysteine phenotype is a feature of many diseases. The effect of the Cystathionine Beta Synthase (CBS) 844ins68 polymorphism on folate and homocysteine concentrations was examined alone and in the context of the Methylenetetrahydrofolate Reductase (MTHFR) 677C>T polymorphism in a Northwestern European male population. The MTHFR 677TT genotype is known to be associated with decreased folate and increased homocysteine relative to CT heterozygotes and CC homozygotes in this and other populations. MTHFR 677TT homozygotes who were also CBS 844ins68 carriers had folate and homocysteine concentrations similar to those of individuals with the MTHFR 677CT and CC genotypes. Serum folate levels in MTHFR 677TT subjects carrying the CBS 844ins68 allele were 27.7% higher than in non-carriers (11.16 vs 8.74 nmol/L $p=0.0338$), and homocysteine levels were 24.1% lower (6.66 vs 8.77 $\mu\text{mol/L}$ $p=0.0449$). These findings suggest that the CBS 844ins68 allele may normalize folate and homocysteine levels in MTHFR 677TT individuals. (Supported by P30 ES013508)

Community Outreach and Education Core

COEC1 **Community outreach: The Teen Research and Education in Environmental Science (TREES) summer program for high school students**

Author: Field, Jeffrey (Center of Excellence in Environmental Toxicology, Department of Pharmacology, University of Pennsylvania)

Beginning in 2007, the Center for Excellence in Environmental Toxicology launched a community outreach education program for High School students called the Teen Research and Education in Environmental Science (TREES) summer program. The TREES program is a unique hands-on research experience for high school students to introduce them to laboratory science. Seven students were recruited from local high schools for the five week program. They were taught by eight graduate student mentors, three high school student mentors and three tenured faculty members, all of whom volunteered their time to guide the students one-on-one or in the small group. There was a daily lecture on an environmental issue and a mini-course in toxicology. TREES students also watched and discussed movies about environmental issues (e.g. An Inconvenient Truth, Thank You for Smoking). Other classes focused on “survival” skills such as laboratory safety, library and internet research, ethics, scientific writing and presentation skills. There was also a college admissions workshop, library tours and a formal campus tour. Students also prepared reports on a natural product that originated from an environmentally sensitive region of the world. The program also included two weeks of structured laboratory exercises to teach basic lab techniques such as pipeting, weighing, microbial techniques, and several spectrophotometer level assays. This set the basic training for what is the most unique aspect of the program: an individually guided research project on a topic chosen by the student. The projects were developed in consultation with the mentors and faculty and then executed by the student. They presented their results in a report, a poster and a PowerPoint presentation. The PowerPoint presentation was presented to the group and invited guests in a student-run symposium. Students were encouraged to develop projects in their community based on their issues and present their work back at school and in local science fairs.

COEC2 **Undergraduate-led classroom education as a tool to reduce lead poisoning**

*Authors: Pepino, Rich (Center of Excellence in Environmental Toxicology, University of Pennsylvania)
Kramek, Niva (University of Pennsylvania)*

Individuals can take action to mitigate the risks posed by exposure to urban environmental hazards such as heavy metals and poor indoor air quality. To discover the extent to which classroom education can improve awareness both of environmental health risks and methods of individual action, a series of lessons on two topics-lead poisoning prevention and tobacco exposure prevention-was delivered to West Philadelphia public school students in three grades (4, 7, and 9) by undergraduate students at the University of Pennsylvania. Using innovative teaching methods and with an interdisciplinary focus, these lessons focused on hazard identification, impacts to human health, risk mitigation techniques, and environmental advocacy as a method of empowerment. At the end of each of the two semesters, the school children at all levels presented examples of their new knowledge in all target areas and pledged to serve as environmental health ambassadors, demonstrating potential for improvement in their community's environmental health.

Community Outreach and Education Core

COEC3 Community exposure to perfluorooctanoate: A successful model for risk communication in CBPR studies

Authors: Emmett, Edward A (Center of Excellence in Environmental Toxicology, Department of Emergency Medicine, University of Pennsylvania)

Hufford, Mary (Center of Excellence in Environmental Toxicology, University of Pennsylvania)

Freeman, David (Decatur Community Association)

Zhang, Hong (Grand Central Family Medicine)

Rodway, Nancy (Adena Health System)

We conducted an interdisciplinary health research, education, and intervention project in an economically disadvantaged region. The project addressed residents of the area served by the Little Hocking Water Association (LHWA). Some of the residents within this district have water service with the LHWA and others have private water supplies. The area encompassed by the LHWA will hereinafter be referred to as the Little Hocking Water Association District (LHWAD). LHWAD residents, located in the Appalachian region of Southeastern Ohio, are disproportionately exposed to a persistent environmental pollutant, perfluorooctanoate/ perfluorooctanoic acid (known as C8).

This health research, education, and intervention project was conducted through an Environmental Justice Partnership between the community association in the affected community, a local health care provider, and environmental health researchers. An effective partnership linking these groups and the use of the stakeholder model proposed by the Commission on Risk Assessment and Risk Management ensured that: the community was aware of basic environmental/occupational health concepts, issues, and resources; the community had a role in identifying and defining problems and risks related to environmental and occupational exposures and stressors; the community was included in the dialogue, was integral in shaping research and policy approaches to the problem, and actively participated with researchers and health care providers in developing responses and setting priorities for education and intervention strategies.

There has been substantial community distrust engendered by the events surrounding exposure to C8 in the Little Hocking and neighboring Marietta-Parkersburg areas. A major hypothesis of this research was that the Environmental Justice Partnership will provide effective outcomes for the community in terms of understanding, ownership and implementation of preventive practices to reduce exposure, and will thereby lead to an increase in community trust.

The sample size was chosen to have the statistical power necessary to determine the important routes of exposure to C8 within the studied population. (Supported by P30 ES013508)

New Members of CEET



Marisa Bartolomei

Marisa Bartolomei, Ph.D. is Professor in the Department of Cell and Developmental Biology and Assistant Investigator, Howard Hughes Medical Institute. She received her undergraduate degree from the University of Maryland in biochemistry and then obtained a PhD from Johns Hopkins University School of Medicine. She trained as a postdoctoral fellow with Shirley Tilghman at Princeton University. She joined the faculty of the University of Pennsylvania School of Medicine in 1993.



Charles Branäs

Charles Branäs, Ph.D. works to improve health and healthcare and is recognized for his innovative studies to reduce gun violence and enhance emergency medical care. As Co-Director of the Penn Cartographic Modeling Laboratory, much of his work incorporates human geography and spatial interactions. He is regularly invited to speak before local, national and international audiences and sits on various boards and scientific panels at the Centers for Disease Control, the National Institutes of Health, the American Trauma Society, the American Public Health Association, and elsewhere.

As principal investigator of several federally-funded studies, Dr. Branäs often leverages science to dispel popular myths and inform public policy. His research has been covered by numerous news outlets including the New York Times, USA Today, National Public Radio, and the UK Guardian. Most recently, his work on the risk of being shot in big cities and small towns, as well as the Philadelphia Gun and Alcohol Study, have made news and informed the debate over “gun control”. Other prominent research that Dr. Branäs directs includes a study of emergency medical care in the US and its implications for homeland security.



Michael E. Burczynski

Michael E. “Ted” Burczynski, Ph.D. is currently an Associate Director in the Biomarker Laboratory in the Department of Clinical Translational Medicine at Wyeth Research. Dr. Burczynski received his Ph.D. in Pharmacology from the University of Pennsylvania School of Medicine in 1999. He completed a post-doctoral fellowship at Johnson and Johnson in 2001 in the field of toxicogenomics and joined Wyeth in 2001.

Dr. Burczynski currently serves as an Adjunct Assistant Professor in the Department of Pharmacology at the University of Pennsylvania School of Medicine. In addition to research and review articles in the field of pharmacogenomics, he has published two textbooks: *An Introduction to Toxicogenomics* was published in 2003 by CRC Press and *Surrogate Tissue Analysis: Genomic, Proteomic and Metabolomic Approaches* was published by Taylor and Francis in 2005. His research efforts now focus on the validation and use of pharmacogenomic and protein-based biomarker assays to achieve translational objectives in clinical studies.

New Members of CEET



Hakon Hakonarson

Hakon Hakonarson, M.D., Ph.D. is the Director of the Center for Applied Genomics (CAG) at the Children's Hospital of Philadelphia (CHOP), a high-throughput highly automated genotyping facility founded to identify the genetic causes of complex medical disorders in children with the objective of developing new therapies. The Center is a \$40 million commitment from CHOP to collect and genotype approximately 100,000 children over a 4 year period. Dr. Hakonarson has an extensive track record in human genetics and has developed an international reputation amongst his peers. As such, he has served in several senior posts in the past, including as the Head of Inflammatory and Pharmacogenomics Research and the Vice President of Clinical Sciences and Development for biopharmaceutical company, deCODE genetics, and as the Chief Scientific Officer for the CRO, Encode Ltd. Dr. Hakonarson has also been the principal or co-principal investigator on several NIH sponsored grants, and he has published numerous high-impact papers in some of the most prestigious scientific medical journals, including *Nature Genetics*, *The Journal of the American Medical Association*, *The Journal of Clinical Investigation* and *The Proceedings of the National Academic Sciences*. With ten years of experience in pioneering genomic research and genome-wide mapping and association studies, Dr. Hakonarson had the intimate knowledge of the complexities of large-scale genomics projects and he has put together the necessary infrastructure and workflow processes to unravel these complexities in his role as the Director of the Center for Applied Genomics.

To this date, the Center for Applied Genomics has genotyped DNA samples from over 35,000 children and their parents, generating over 20 billion genotypes, made several novel discoveries and established numerous enabling collaborations with key scientists at the entire CHOP/PENN campus. Among the major discoveries to this date is the discovery of a novel gene for Type 1 diabetes that was recently published in *Nature*, with Dr. Hakonarson as the lead scientific author. (Hakonarson et al, *Nature*, 2007). Other research projects include autism, ADHD, asthma, obesity, many of which have distinct environmental factors. The Center for Applied Genomics and its research have also gained nationwide attention and were featured in the *Wall Street Journal*, *Science*, and *JAMA*, among other publications.

New Members of CEET



John Hogenesch

John Hogenesch, Ph.D. did his graduate work in the laboratory of Dr. Chris Bradfield at Northwestern University working on signal transduction pathways mediated by bHLH-PAS transcription factors. In his thesis work, he discovered five novel mammalian members of the bHLH-PAS family, termed MOP1-5 for members of PAS superfamily (Hogenesch et al., *JBC*, 1997). Characterization revealed one of the orphans, MOP3, was the partner of a related bHLH-PAS member, Clock, a master regulator of circadian (Hogenesch, et. al, *PNAS*, 1998). This circuitry comprises the molecular basis of circadian rhythmicity in mammals. To build on these interests, he joined the laboratory of Steve Kay at the Scripps Research Institute and the Novartis Research Foundation as a postdoctoral fellow in 1999 working on circadian rhythmicity in mammals. In 2000, he joined as a staff scientist at the Institute and later as the head of genomics there. At GNF, he worked on the assembly of the complete mammalian transcriptomes (Hogenesch et al., *Cell*, 2001), as well as on the mRNA characterization of their expression (Su et al, *PNAS*, 2002). Current interests include the development of genome wide methodologies for the study of cellular pathways using cDNAs and siRNAs (Conkright et al., *Molecular Cell*, 2003; Sato et al, *Neuron*, 2004 ; Willingham et al., *Science*, 2005; Sato et al., *Nature Genetics*, 2006). In 2006, Dr. Hogenesch joined the department of Pharmacology at the University of Pennsylvania School of Medicine as an Associate Professor.



James Kreindler

James Kreindler, M.D. is an Assistant Professor of Pediatrics in the Division of Pulmonary Medicine. Dr. Kreindler received his B.A. in Biology from the Johns Hopkins University in 1994 and his M.D. from the Mount Sinai School of Medicine in 1998. He completed a residency in Pediatrics at the Children's Hospital of New York of Columbia University and a fellowship in Pediatric Pulmonary Medicine at the Children's Hospital of Pittsburgh. During his fellowship, Dr. Kreindler became interested in the effects of cigarette smoke on chloride secretion in human airway cells. He currently holds a K08 award from the National Heart, Lung, and Blood institute to study the mechanism by which cigarette smoke inhibits chloride secretion in human bronchial epithelial cells.



Jianghong Liu

Jianghong Liu, Ph.D., RN is a new faculty member in the School of Nursing and Masters of Public Health Program in the School of Medicine. She obtained her Masters degree in Maternal-Child Health Nursing and an interdisciplinary Ph.D. from UCLA where she integrated knowledge across Nursing, Psychology, Psychiatry, and Public Health. She received postdoctoral training on developmental psychopathology at the University of Southern California.

Dr. Liu's research focuses on the early health risk factors in relation to childhood behavioral problems. Her research has three main features: an interdisciplinary collaborative approach; work in international settings; and, the use of longitudinal methodology. Her specific research areas include prenatal/postnatal risk factors, environmental toxicity and early nutritional deficits in the development of childhood cognitive impairments and antisocial behavior. Her work has been published in journals including the *American Journal of Psychiatry*, *Archives of Pediatrics & Adolescent Medicine*, and *Current Opinion in Pediatrics*. Dr. Liu currently holds a Research Scientist Development Award (K01) from NIH/NIEHS and is the Principal Investigator of the Jintan Child Health Project in China. This longitudinal study was begun in 2004 and follows children into adolescence to investigate the influence of lead exposure and micronutrient deficiency on children's behavior. Dr. Liu is a member of Society of Toxicology.

New Members of CEET



Jennifer Pinto-Martin

Jennifer Pinto-Martin, Ph.D., M.P.H. received her M.P.H. in 1982 and her Ph.D. in Epidemiology from the University of California, Berkeley, in 1984, and began her academic career at Columbia University College of Physicians and Surgeons. She was appointed to the faculty at the University of Pennsylvania in 1990, where she now holds the Viola MacInnes/Independence Chair in the School of Nursing. Dr. Pinto-Martin has a secondary appointment in the Department of Biostatistics and Epidemiology in the School of Medicine, and is a Senior Scholar in the Center for Clinical Epidemiology and Biostatistics (CCEB). She has been a member of both the Society for Epidemiology Research and the Society for Pediatric Epidemiology Research for over a decade and served as the President of the latter in 1993-94. She has also been on the editorial board for the Society's journal (*Paediatric and Perinatal Epidemiology*) for over a decade.

Dr. Pinto-Martin has been involved in longitudinal epidemiologic research on neonatal brain injury and has had continuous NIH support for this research since 1984. A major focus of her publications has been on the etiology and long-term consequences of neonatal brain injury. She is currently the Director and Principal Investigator of the Pennsylvania Center for Autism and Developmental Disabilities Research and Epidemiology (PA-CADDRE), one of six such centers funded by the Centers for Disease Control and Prevention for five years, to study the prevalence and etiology of autism spectrum disorders (ASD). The Study to Evaluate Early Development (SEED), a large, multi-site, case-cohort study of ASD will begin in March 2007, and will ultimately include 2,700 children, 900 on the autism spectrum and two comparison groups. Dr. Pinto-Martin is also the Principal Investigator on an R01 from NIH to assess the prevalence of ASD in the NBH cohort, thereby bringing her two major research interests together in one project.

Other areas of research include early identification of ASD. PA-CADDRE has launched a statewide training program for providers and early childhood educators on the appropriate screening for ASD. In addition, Dr. Pinto-Martin is working with the International Clinical Epidemiology Network on a study on the prevalence of ASD and other childhood disabilities in India.

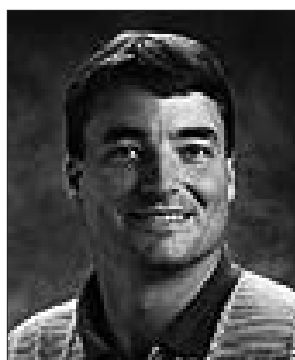
New Members of CEET



Judith Green-McKenzie

Judith Green-McKenzie, M.D., M.P.H. is Assistant Professor, Director of Clinical Practice and Associate Residency Director in Occupational Medicine at the University of Pennsylvania School of Medicine in Philadelphia. She is active in clinical practice, research, education and administration in Occupational and Environmental Medicine. She is the course director for the Epidemiology and Biostatistics series and for the Population Occupational Medicine Module as well as co-facilitator for the monthly Journal Club and sits on the Residency Advisory committee for the University of Pennsylvania Occupational Medicine Residency Program. She is also an Associate Professor in the Graduate Program in Public Health Studies, University of Pennsylvania School of Medicine.

Dr. McKenzie graduated from Princeton University where she received the Frederick Douglass Prize for leadership and scholarship. She went on to study medicine at Yale University School of Medicine where she was a Commonwealth Fellow. She completed her training in Internal Medicine at New York University (NYU)/Bellevue Hospital where she subsequently served as an attending physician in the Internal Medicine and the Virology Clinics from 1990-1995. She was a Clinical Assistant Professor, Module Leader and Associate Director of the Internal Medicine Ambulatory Care Clerkship at NYU / Bellevue where she developed curricula, precepted residents and interns, and taught the medical interview. She went on to complete a Masters of Public Health and an Occupational Medicine fellowship in Occupational Medicine at the Johns Hopkins School of Hygiene and Public Health. She was one of the recipients of the first resident research awards given at the American College of Occupational and Environmental Medicine national meeting.



Kevin C. Osterhoudt

Kevin C. Osterhoudt, M.D., M.S.C.E., is an Associate Professor of Pediatrics at the University of Pennsylvania. He is board-certified in Pediatrics, Pediatric Emergency Medicine and Medical Toxicology and is a trained clinical epidemiologist. Dr. Osterhoudt's clinical activities include service as an attending physician in the Emergency Department of The Children's Hospital of Philadelphia and inpatient and outpatient toxicology consultation. He also serves as the Medical Director of The Poison Control Center at CHOP. He was appointed by the Pennsylvania Chapter of the American Academy of Pediatrics as its Point Person for Environmental Toxicology, and represented the Academy at recent EPA hearings regarding regulations for airborne ozone.

Major initiatives in gene-environment and exposure biology research in CEET

GENE-ENVIRONMENT INTERACTIONS

PA-DOH-center of gene-environmental interactions in lung cancer

(P.I. Whitehead)

CEET Investigators: Albelda, Baldwin, Blair, Lerman, Penning, Vachani

Lung cancer is the leading cause of cancer death in the U.S., and incidence in Pennsylvania ranks second in the nation. In Philadelphia County the yearly rates for men and women are respectively 115.7 and 70.0 per 100,000, whereas in Dauphin County the corresponding rates are much lower at 83.9 and 47.6 per 100,000. In the former region, air pollution as well as health disparities typical of large urban settings likely contribute to the 50% higher burden of lung cancer. Tobacco smoke contains two major types of chemical carcinogens: nitrosaminoketones (NNK) and polycyclic aromatic hydrocarbons (PAH); the latter are also produced by fossil fuel combustion. Smokers with lung cancer, control disease-free smokers, and non-smokers with lung cancer, will be recruited through the University of Pennsylvania and Penn State Schools of Medicine, located respectively in Philadelphia and Dauphin Counties. Biomarkers for NNK and PAH will be measured to estimate exposures to carcinogens derived from tobacco smoke and environmental pollution. Variants of more than 50 genes involved in the metabolic activation and subsequent detoxification of NNK and PAH, as well as DNA adduct repair genes, will be genotyped to determine associations with susceptibility to lung cancer. Biomarkers of folate status and functional polymorphisms in folate-metabolizing enzymes will also be evaluated as potential direct or indirect modifiers of disease risk. Risk estimates for genetic and phenotypic variables will be calculated for the following categories of subjects with lung cancer: residents of geographic locations with high or low levels of air pollution, regardless of race; African Americans in locations with high levels of air pollution; Caucasians in locations with high or low levels of air pollution; smokers and non-smokers in each of the above categories. This program has the potential to identify genetic and/or phenotypic risk factors for lung cancer applicable to the general population, those living in areas with high levels of environmental pollution, and minorities. It is likely that the data generated in the course of this program will be invaluable for effectively addressing issues of health disparities and "environmental justice" and may thereby facilitate significant improvements in the health of Pennsylvanians.

GENE-ENVIRONMENT INTERACTIONS

2P50CA093372-03 Gene discovery in melanoma etiology

(P.I. Rebbeck)

CEET Investigator: Kanetsky

Melanoma has a complex, multifactorial etiology that includes genetic predisposition, UV exposure and somatic genetic changes. This project is focused on the discovery of genetic alterations that play a role in the development of melanoma and the use of those genetic biomarkers in the development of two models. These two models will provide information about: 1) risk factors for the probability of having melanoma, and 2) risk of metastatic failure in those individuals who do develop melanoma. Genetic biomarkers will be developed for these models by: 1) analyzing the association between germline variants in DNA damage response genes and risk for melanoma; 2) evaluating the mutation spectrum of a panel of receptor tyrosine kinase genes that may play a role in malignant transformation and genome instability in melanomas; and 3) identifying patterns of genomic instability in melanoma using a whole genome approach. We hypothesize that by combining established risk factors with novel genetic biomarkers from these three sources, accurate models for both risk of developing disease and risk of metastatic failure in patients who do develop melanoma can be defined for every individual. These risk profiles also will contain important biological information on melanoma initiation and progression that is needed to develop effective prevention, staging and treatment strategies. The specific aims of this project are: Specific Aim 1: To identify low penetrance melanoma susceptibility alleles in DNA damage response genes, evaluate their interaction with other genetic variants and risk factors in a case-control study and develop a multivariate melanoma etiology model. Specific Aim 2: To analyze a panel of 50 primary melanoma cell lines for mutations in candidate receptor tyrosine kinase genes and validate frequent mutations in 50 patient lesions. Specific Aim 3: To develop a novel molecular profile of melanoma using array-based comparative genomic hybridization (aCGH) to characterize amplifications and deletions across the entire genome at 1Mb resolution. Specific Aim 4: To validate somatic genetic alterations as risk factors for use in prognostic models that will distinguish melanoma patients at minimal and high risk of metastasis, thus requiring different management strategies.

GENE-ENVIRONMENT INTERACTIONS

CDC Grant# U10DD00182-01 Center for autism and developmental disabilities research and epidemiology (CADDRE)

(P.I. Pinto-Martin)

The most significant advance related to the etiology of autism spectrum disorders (ASD) has been recognition of the strong genetic influence on ASD occurrence, although no specific genes have been identified. The cause of ASD is probably multifactorial and one likely scenario is a multiple gene interaction (it is believed that at least 15 loci could contribute to etiology) possibly in response to certain environmental stimuli. It is also generally thought that the causal events leading to the disorder arise prenatally, although recent concerns about the effects of postnatal vaccine exposure or antibiotic treatment raise the issue of a possible postnatal insult that could contribute to development of the disorder in some susceptible children. In the face of these considerable gaps in our understanding of the causes of ASD, large population-based epidemiologic studies of ASD etiology are lacking. The Study to Explore Early Development (SEED) is designed to address this critical need. The study strengths are: 1) Case-cohort design with multiple-source, population-based ascertainment of case and comparison groups; 2) Confirmation of developmental status of all subjects based on standardized clinical evaluation procedures; 3) Uniform subject inclusion criteria and data collection protocols applied across all study sites; 4) Large sample size and study power; 5) Ability to stratify by phenotypic subgroups to investigate etiologic heterogeneity; 6) In-depth exploration of multiple research domains; and 7) Joint collection of genetic and environmental data. Biologic samples are collected from both children and biologic parents. These samples will provide DNA needed for genetic analyses as well as biomarkers of exposures and, potentially, disease. Blood specimens from parents are valuable not only as a source of parental DNA, but to allow exploration of correlation of biomarkers within and across families. Case children will include children with ASD, as defined below. A sample of children who have developmental problems excluding ASD will be called the neurodevelopmentally impaired comparison group (NIC). Both case and NIC groups will be drawn from children in the cohort who are evaluated or receive services for developmental problems. A second comparison group of children (subcohort) will be drawn randomly from the entire cohort. Comparisons between cases and the subcohort will identify risk factors for ASD relative to children from the general population, most of which are developing normally. Comparisons between cases and the NIC may provide the opportunity to distinguish risk factors for ASD independent of factors shared with other developmental problems.

GENE-ENVIRONMENT INTERACTIONS

R01-CA114478 Inherited genetic variation and predisposition to testicular germ cell tumors

(P.I. Nathanson)

CEET Investigators: Baldwin, Kanetsky

Testicular germ cell tumors (TGCT) are the most common cancer in men ages 20-40. The incidence of TGCT has more than doubled over the past forty years, without clear etiology. Both genetic effects and environmental exposures, specifically during the pre-natal period, are likely to play an important role in determining TGCT susceptibility. TGCT is known to develop from primordial germ cells (PGCs). We hypothesize that variation in genes that impact upon the differentiation and maturation of PGCs will be important determinants of TGCT susceptibility and based on this hypothesis have selected three important pathways for study: 1) male germ cell development, 2) androgen and estrogen biosynthesis and metabolism, and 3) IGF signaling. The proteins involved in early male germ cell development, normally only expressed in PGCs, are markers of, and are overexpressed in, TGCT. Markers of increased exposure to estrogen (or relatively decreased exposure to androgen) in utero and exogenous estrogen exposures, such as endocrine disruptors, have been associated with TGCT case status in multiple studies. IGF signaling is necessary for testis differentiation and maturation in mice and interacts synergistically with the estrogen-signaling pathway. We will analyze the contribution of genetic variants in these pathways to TGCT risk using a population-based case-control study in the Philadelphia metropolitan area. Our goal is the collection of 550 TGCT cases and 1100 age matched controls without a history of TGCT, which will yield 500 and 1000 white cases and controls, respectively, available for final analyses. All cases will be enumerated through the New Jersey and Pennsylvania state cancer registries. We will use a two-tiered approach for case recruitment: hospital clinic-based followed by registry-based. Controls will be identified through random digit dialing. Both cases and controls will complete a questionnaire addressing known, presumed, and hypothesized risk factors for TGCT and provide a blood sample or buccal swab. Pathological slides will be reviewed to confirm the diagnostic sub-type of TGCT. Haplotypes and functional SNPs will be typed in the genes of interest. Analyses will be conducted for specific variants, common haplotypes, alone and in conjunction with each other and exposure data after appropriate adjustment for potential confounders. The findings from this study will greatly contribute to our understanding of determinants of TGCT susceptibility.

EXPOSURE BIOLOGY

2P50CA084718-07 Transdisciplinary Tobacco Use Research Center (TTURC)

(P.I. Lerman)

Despite two decades of research, treatments for smoking cessation remain limited. Over the past 4 years, our TTURC has begun to address this gap in knowledge and practice. In our pharmacogenetic trials of nicotine dependence treatment, we have provided the first evidence for effects of specific genetic variants on smoking cessation and response to pharmacotherapy, generated new data on bi-behavioral mechanisms of response to treatment, developed new tools and applied new methods to analyze smoking cessation clinical trial data, and identified pre-treatment measures that can be used in clinical practice to tailor choice of treatment for individual smokers. In addition, we have completed a national survey of over 1000 physicians in the AMA, and have begun to identify emerging health policy and ethical issues in the translation of our research to clinical practice. A milestone event in the evolution of our TTURC was the move to the Penn in 2001. This move provided new opportunities for transdisciplinary collaboration, an enhanced institutional commitment, and access to state-of-the-art facilities for biomedical informatics, genotyping, and animal models research. The mission of the Penn TTURC is to translate new discoveries in basic neuroscience, pharmacology, genetics, and behavioral science to improve treatment for nicotine dependence. The specific aims are to: 1) Conduct 4 inter-related projects which test new treatments for nicotine dependence and test novel approaches to optimize the use of existing treatments; 2) Support and integrate TTURC research through a state-of-the-art Biomedical Informatics Core and Genetics Core; 3) Facilitate the diffusion and clinical integration of TTURC research through a Research to Practice Core which addresses key policy and ethical issues; 4) Train tobacco control scientists through a Training Core; and 5) Coordinate and facilitate transdisciplinary collaboration through an Administrative Core. Spanning from basic animal research to clinical trials ("bench to trench"), the ultimate objective of the TTURC is to develop new treatments that can be readily translated to the clinical setting in order to maximize the efficacy of pharmacotherapy for individual smokers.

EXPOSURE BIOLOGY

5U01HD050088-02 Network for premature birth research analytical core

(P.I. Parry)

CEET Investigators: Baldwin, Blair

The ultimate goal of the parent grant is to study the genetic and environmental etiologies and mechanisms of spontaneous preterm birth. The underlying hypothesis is that mothers who deliver spontaneous pre-term births have a unique maternal genetic polymorphism profile including gene-gene and gene-environment interactions distinct from mothers who deliver at term, and that maternal serum proteomic and metabolomic profiles can identify preterm labor patients who deliver preterm births spontaneously. The award mandates the following functions of the Genomic and Proteomic Network for Preterm Birth Research and serves as an organizational and technical model for the Training Program and the CEET:

1. To establish an Analytical Core that will: prepare and store samples for genotyping, proteomic and lipidomic and oxidative stress analysis; perform whole genome amplification of genomic DNA; perform high-throughput genotyping on a genome-wide basis and on selected markers for candidate genes; perform DNA microarray analyses to profile transcriptome changes in tissues of interest; analyze tissues and biological fluids for markers of preterm birth using proteomics; analyze tissues and biological fluids for lipid and oxidative stress-related biomarkers of preterm birth; and interpret and integrate genetic, microarray, proteomic, lipidomic and oxidative stress analyses.
2. To consult on the design of genetic studies of preterm birth by: assisting the Steering Committee in overall study design, sample size calculations, and statistical analysis; advising the Steering Committee on candidate genes and ancestry informative markers to be evaluated; advising on optimal sample collection protocols

EXPOSURE BIOLOGY

1UO1-ES016004-01 Biomarkers of exposure and response to cigarette smoke

*(P.I. Blair)**CEET Investigators: Baldwin, Penning, Troxel*

Exposure to tobacco smoke (mainstream and environmental) is a leading cause of death in the US. Cigarette smoke is an extremely complex mixture, including numerous polycyclic aromatic hydrocarbons (PAHs), in both the mainstream and sidestream (environmental) smoke fractions. Cigarette smokers provide an extreme model of PAH exposure that will permit both exposure and biological response biomarkers to be developed. Evidence exists that PAHs are causative agents in lung, skin, and bladder cancer. Furthermore, tobacco smoke is associated with oxidative stress, pancreatic cancer, cardiovascular disease, and chronic obstructive pulmonary disease (COPD), although the specific role of PAHs is not clear. Interestingly, the cardiovascular effects of sidestream smoke are almost as great as mainstream smoke. This proposal stems from significant advances we have made in the quantification of protein, lipid, and DNA biomarkers using stable isotope methodology and our basic research into enzyme regulation during oxidative stress. Previous methods for the analysis of oxidative DNA damage have been fraught with numerous methodological problems so that the current state-of-the-art involves the use of a COMET assay to measure 8-oxo-2'-deoxyguanosine (dGuo) lesions. We have devised a more quantitative method based on immunoaffinity stable isotope dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) that can be readily elaborated to studies of tobacco smokers. We also showed that oxidative stress could induce the formation of aldo-keto reductases (AKRs) of the 1C family. AKR1C3 is the enzyme, which we recently showed is responsible for the conversion of prostaglandin (PG) D₂ to the potent bronchoconstrictor 11 β -PGF₂. This provides an additional potential link between oxidative stress and COPD as well as the potential for a new therapeutic strategy, which involves AKR1C3 inhibition. Finally, preliminary studies have revealed that a DNA-adduct that can only arise from lipid peroxidation is present in the urine of cigarette smokers but is completely absent in urine from non-smokers. We will build on these new findings by developing panels of *in vivo* biomarkers of exposure and biological response, which will make it possible to distinguish a cohort of non-smokers from a cohort of disease-free tobacco smokers. The hypothesis will be tested by conducting research under the following three specific aims: Aim 1. To discover whether B[a]P and B[a]P-7,8-dione induce AKR1C/2 in NHBE cells and increase oxidative stress to form 8-oxo-dGuo and H μ dGuo in DNA, induce AKR1C3 in HASM cells and increase the biosynthesis of the potent bronchoconstrictor 11 β -PGF₂, as potential urine and EBC biological response biomarkers of PAH exposure. Aim 2: To discover secreted proteins following treatment of NHBE and HASM cells with B[a]P and its oxidative metabolites as potential serum biological response biomarkers of PAH exposure. Aim 3: To conduct predictive and refinement analyses of *in vivo* exposure and response biomarkers in urine together with biological response biomarkers in EBC and serum in order to distinguish non-smokers from disease-free tobacco smokers. Completion of the research will provide a panel of biomarkers of exposure and biological response to tobacco smoke and will have utility in future studies to elucidate the relationship between gene environment interactions and diseases such as cancer, cardiovascular disease, and COPD.

EXPOSURE BIOLOGY

P50 Discover-RFA-ES-06-001 Center for ozone, airway biology, and translational studies in asthma

(P.I. Panettieri)-Pending

CEET Investigators: Beers, Blair, Haczku, Krymskaya, Penning

This proposal is an interdisciplinary effort that focuses on defining the cellular and molecular mechanisms by which ozone enhances airway hyperresponsiveness (AHR) and exacerbates asthma. The central hypothesis states that ozone directly modulates airway epithelium and smooth muscle cell function to diminish innate immune responses and to amplify AHR in asthma. Despite considerable research effort, the precise mechanisms regulating AHR to ozone and the contribution of ozone-induced airway inflammation to the pathogenesis of airways disease remain unclear. To address our central hypothesis, four projects and three core units are proposed. In Project 1, the mechanisms by which ozone enhances allergen-induced AHR by promoting neutrophil-mediated airway inflammation and by directly increasing agonist-induced force generation of airway smooth muscle will be defined. Project 2 will characterize the mechanisms by which ozone alters surfactant protein D (SP-D) structure and/or expression, converting SP-D from an immunoprotective to a pro-inflammatory molecule in asthma. Project 3 will identify unique lipid and DNA adduct biomarkers that phenotypes subjects with asthma according to disease severity and ozone responsiveness. Project 4 will identify alterations in candidate genes and pathways related to oxidative stress, redox balance and innate immunity that predict ozone responders in both healthy susceptible individuals and in subjects with asthma. The major strength of our program is the productive interaction of an established team of investigators from clinical, environmental, immunological, cell biological and molecular pharmacological sciences. Three core units support four projects. Core A (Human Studies) will study ozone-challenged healthy and asthmatic subjects and obtain sputum, bronchoalveolar lavage and serum in well-characterized asthma cohorts. Core B (Murine) will perform murine physiology, histology and cytology in allergen- and ozone-induced animals. Core C (Administrative) will provide administrative and fiscal support. Using hypothesis-driven molecular studies of genes, proteins, cells, tissues, animal models and asthmatic patients, this interdisciplinary approach will provide new insight into the mechanisms by which ozone exacerbates asthma and identify new biomarkers and candidate gene alterations that predict asthma severity and ozone responsiveness.

EXPOSURE BIOLOGY

T32 Training grant-RFA-ES-07-002: A combined training program in genes and environmental health

(P.I. Whitehead)-Pending

CEET Investigators: Albelda, Barhart, Bartolomei, Blair, Bladwin, Emmett, Fisher, FitzGerald, Gerton, Harkonarson, Hogenesch, Ischiropoulos, Kanetsky, Lamitina, Lerman, McCauley, Nathanson, Panettieri, Parry, Pinto-Martin, Rebbeck, Rossman, Schultz

This application seeks to establish an institutional training program entitled "Combined Program in Genes and Environmental Health". Support is requested for a steady state pool of 7 pre-doctoral and 3 post-doctoral trainees (i.e. 31 and 12 trainee years respectively) over 5 years. The program aims to produce uniquely qualified biomedical researchers whose skill-set spans the disciplines of Genetics/Genomics and Environmental Health/Exposure Biology. It will draw on the particular faculty, programmatic and teaching strengths in these disciplines that are represented at the University of Pennsylvania School of Medicine. The Department of Pharmacology will take the lead in providing the administrative and formal teaching aspects of the program. Research opportunities will be provided by faculty working in a wide range of departments and affiliated with several relevant Centers and Multi-Investigator initiatives. The program will be a component of the recently established NIEHS-supported Center of Excellence in Environmental Toxicology which will provide the intellectual and technical underpinning of the training. Pre-doctoral trainees will be recruited to the program from their home Graduate Groups at the end of the first year and will be expected to undertake rotations approved by the executive committee overseeing the program. Such trainees will take mandatory didactic courses that will be developed for the program *de novo*, and will also be required to select additional didactic courses from an existing list of courses that are relevant to the program. A new practical summer course at the end of the second year will be an important technical training component. Progression to thesis work with one of the 39 program faculty will be subject to the same qualifying criteria as for all PhD candidates in this University. The program will employ a "dual mentorship" model for both thesis research and postdoctoral trainee research: projects will be jointly supervised by faculty with expertise in genetics and exposure biology. Faculty trainers include 19 in the former and 20 in the latter categories; trainers include basic science and clinical researchers as well as junior and senior faculty who will be paired, where possible, to provide "mentorship training". The program will be subject to the same institutional governance as other training programs run under the auspices of Biological Graduate Studies office, and will be managed by an Executive Committee with equal representation of Genetics and Exposure Biology faculty trainers

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www.med.upenn.edu/ceet/

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