



CEEH Pilot Projects Program Abstracts from Year 11 (April 1, 2005 to March 31, 2006)

Population Genetics and Molecular Evolution of SPRR1B • Joshua Akey, PhD,
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The goal of ecogenetics is to understand how genetic variation and environmental exposures interact to mediate susceptibility to complex disease. In order to understand how genetic risk factors vary between individuals within and between populations, population genetic and evolutionary studies are also an important component to ecogenetics research. To this end, we have performed an initial evolutionary analysis of 399 environmental response genes that were sequenced as part of the National Institutes of Environmental Health Sciences (NIEHS) SNPs Program at the University of Washington. This preliminary analysis suggests that the small proline-rich protein 1B (SPRR1B) gene possesses one of the strongest signatures of balancing selection to be described to date. SPRR1B plays an important role in terminal differentiation of the human epidermis, is upregulated in response to environmental insults, and is an early biomarker for bronchial carcinogenesis. However, little is known about how the amount, structure, and patterns of genetic variation in SPRR1B are distributed in geographically diverse populations. The goal of this proposal is to bridge this lack of knowledge by studying the population genetics and molecular evolution of SPRR1B. In specific aim 1, we will re-sequence SPRR1B in 80 individuals from ten human populations and 15 non-human primates. These data will provide the first large-scale assessment on patterns of SPRR1B genetic variation with and between populations and allow detailed evolutionary hypotheses to be tested. In particular, patterns of linkage disequilibrium, haplotype structure, confirmation of the signature of balancing selection, and comparative genomics analyses will be performed. In specific aim 2, we will perform phylogenetic footprinting to identify putative cis-regulatory motifs in SPRR1B. The data generated in this project will furnish detailed information on the population genetics and molecular evolution of SPRR1B, which will provide the necessary foundation for subsequent disease based association and gene-x-environment studies.

CYP1B1, COMT, and GST Polymorphisms as Modifiers of the Effect of HRT on the Risk of Breast Cancer • Kathleen Malone, PhD, Associate Member, Fred Hutchinson Cancer Research Center

Breast cancer is the most common cancer in women and predominantly affects older women. Hormone replacement therapy (HRT) is reemerging as a risk factor for breast cancer and is the dominant source of exogenous estrogen for post-

menopausal women. Estrogen has been shown to have direct genotoxic effects via the formation of catechol estrogens (CE). Genetic polymorphisms along the estrogen metabolism pathway involved in the formation and inactivation of CEs need to be investigated within the CYP1B1, COMT, and GST genes as potential modifiers of the effect of HRT. CYP1B1 is responsible for converting estradiol into CE, COMT is responsible for inactivating CEs through methylation, and the GST genes are involved in CE inactivation through conjugation of the CE. We hypothesize that a profile of susceptibility alleles within these genes increases the risk of breast cancer overall and among the subset of women who have used HRT.

To test these hypotheses, we propose to genotype functional single nucleotide polymorphisms (SNPs) in CYP1B1, COMT, and the GST genes to investigate whether alleles leading to higher enzyme activity in CYP1B1 and alleles leading to lower protein activity in COMT, GSTM1, GSTP1, and GSTT1 alter the risk of breast cancer overall and among HRT users. No prior study has targeted these pathway components in combination. An emerging paradigm suggests that hormone-mediated factors like HRT exhibit differential effects according to histologic type and hormone receptor status. Thus, a subset analysis will be performed in ER+/PR+ tumors and within lobular tumors. This study will be conducted on the foundation of a population-based case-control study of breast cancer in women ages 65-79 (891 cases and 878 controls). This study offers the best study to-date to test this hypothesis due to the large sample of post-menopausal women with blood samples available to analyze and the pathway based approach.

Genetic Variations in Calcium Sensing Receptor (CASR) and Colon Cancer • Ulrike Peters, PhD, Assistant Member, Research Assistant Professor, Department of Epidemiology, UW and FHCRC

Colorectal cancer is the third leading cause of cancer death in the US. Diet is considered a major environmental cause of colorectal cancer. Several studies, including randomized clinical trials, have provided strong evidence for a beneficial effect of calcium on colorectal cancer. The overall goal of our research is to better understand the molecular mechanisms underlying the preventive effects of calcium and to study interactions between calcium intake and key genes of the calcium pathway. This research is in line with the goal of the CEEH to study “Mechanisms Underlying Human Variability in Response to Environmental Exposures” – in our case calcium. The aim of this proposal is to identify the genetic variation in the calcium sensing receptor (CASR) a key gene of the calcium pathway that has not been sufficiently studied. Therefore, we propose to: 1) sequence the CASR gene in 32 unrelated individuals; and 2) use re-sequencing information to identify tagging single nucleotide polymorphisms (tagSNPs) in the CASR gene based on functionality and an algorithm to capture the common genetic variation. Results from this study will strongly support the application for funding to evaluate associations of genetic polymorphisms in CASR and gene-

environment interactions between CASR and calcium intake and colon cancer risk, using samples from a previously completed population-based case-control study (1676 cases, 2004 controls). This research will shed light on the mechanisms underlying the variability in response to the protective effect of calcium against colon cancer. Genetic variation may be found to alter the risk of colon cancer or modify the chemo-preventive response to calcium supplements in the US. This study will build a multidisciplinary collaboration between Peters, a new research assistant professor and Farin of the CEEH Functional Genomics Facility Core.

Functional Protein Analysis in Asbestosis • Tim K. Takaro, MD, PhD, Assistant Professor, Departments of Environmental and Occupational Health Sciences and Epidemiology, UW; FHCRC

Asbestosis is a major cause of pulmonary fibrosis. Epidemiologic evidence strongly suggests that genetic factors play an important role in disease susceptibility in populations exposed to occupational or environmental asbestos. The Beta-Carotene and Retinol Efficacy Trial (CARET) coordinated by the Fred Hutchinson Cancer Research Center (FHCRC) has stored serum and whole blood samples on over 4,000 asbestos exposed workers and 12,000 non-asbestos exposed smokers. This cohort offers unprecedented opportunities to study factors that influence asbestosis. We propose to show whether the serum stored from this cohort can be used to determine levels of proteins relevant in asbestosis. Using the CEEH proteomics Core, we will a) measure differences in levels of cytokines and other proteins relevant to the disease process by standard ELISA methods and b) assess differential patterns of protein expression with matrix assisted laser desorption/ionization time-of-flight spectroscopy (MALDI-TOF). This effort will provide a rich database for future studies to elucidate potential novel proteins in this human model of pulmonary fibrosis. We will validate the MALDI-TOF scan results with an ELISA analysis of nitrotyrosine modified proteins. As such a protein pattern analysis has not been previously reported in asbestos disease, a manuscript is expected from this work. Additionally, the improved phenotypic characterization of asbestosis through this proteomics approach will help direct proposed future genomic studies of this cohort. That will lead to a better understanding of the biological mechanisms in asbestos related pulmonary fibrosis will help to identify potential therapeutic targets in this untreatable progressive respiratory condition. The proposal utilized the outstanding collaborative opportunities fostered by the CEEH and, in addition to involving the FHCRC, also involves the Cardiovascular and Respiratory Toxicology Research Cores, and the Functional Genomics and Proteomics Facility Cores.