



CEEH Pilot Projects Program Abstracts from Year 6 (April 1, 2000 to March 31, 2001)

Role of Glutamate-L-cysteine Ligase in Cystic Fibrosis • Terrance J. Kavanagh, PhD,
Associate Professor,
Department of Environmental Health, UW

Cystic fibrosis is a debilitating systemic disorder that affects the lungs, the pancreas and the intestine. Most CF is thought to be due to a mutation in the cystic fibrosis transmembrane regulator protein (CFTR). However, other factors certainly contribute the severity and progression of this disease. Recently, it has been shown that CFTR may influence the ability of airway epithelial cells to transport the antioxidant glutathione. Glutathione is important in preserving lung function in the face of oxidative stress, which is a frequent occurrence in CF. Individuals with CF have been shown to have lower levels of GSH in their alveolar lining fluid. This suggests that CF may be characterized by an inability to supply the alveolus with sufficient GSH. We have recently shown that a polymorphism in the GSH biosynthetic enzyme glutamate-L-cysteine ligase (GLCL) is associated with risk for idiopathic pulmonary fibrosis, a condition that also is characterized by low GSH in the alveolar lining fluid. The aims of this proposal are to examine the prevalence of the GLCL polymorphism in CF patients, and to assess the association of various alleles with disease severity. This information will help to define the variability in disease severity seen in this condition and also lead to rational antioxidant therapy for these patients.

Candidate Genetic Polymorphisms for Stroke in Young Women • Stephen M. Schwartz, PhD, Associate Professor, Department of Epidemiology, UW

The role of genetics in stroke is not well understood. We propose a pilot study of three candidate genes with known functional mutations suspected of being involved in the pathogenesis of ischemic and hemorrhagic strokes. Specifically, using data from a study of early-onset stroke in women from the Puget Sound metropolitan region, the proposed pilot study will allow us to 1) determine the prevalence of susceptibility alleles for the atrial natriuretic peptide gene (ANP) and two matrix metalloproteinase genes (MMP), and 2) obtain preliminary estimates of the association of polymorphisms in these genes with ischemic and hemorrhagic stroke. Subjects were between the ages of 18 to 44 years with no previous history of clinical cerebrovascular or cardiovascular disease. A total of 41 ischemic stroke cases, 52 hemorrhagic stroke cases, and 250 controls have DNA available for genetic analysis. The results from this pilot study will help us

determine the feasibility of submitting a larger NIH grant that would allow us to build upon our existing study of population-based stroke cases and controls. This larger study will be a collaborative effort involving several investigators at the University of Washington and will enable us to collect additional stroke cases and their relatives, evaluate gene-environment interactions, and perform association and family-based analyses. Thus, the proposed study fits well with the goals of the UW Center for Ecogenetics and Environmental Health (CEEH).

Role of Platelet Thrombin Receptor (PAR1) Genetic Variants and Environmental Factors in the Susceptibility of Young Women to Acute MI • David S. Siscovick, MD, MPH, Professor, Departments of Epidemiology and Medicine, UW

There is increasing evidence that genetic prothrombotic factors are important in the occurrence of athero-thrombotic diseases such as acute myocardial infarction (MI). We have previously demonstrated the importance of several coagulation factor and platelet receptor genetic susceptibility markers in combination with cigarette smoking in the risk of MI using a case-control study of MI in young women. We are thus in a unique position to evaluate prothrombotic candidate genetic susceptibility markers and their interaction with environmental factors in the pathogenesis of early-onset athero-thrombotic disease. Thrombin is a multi-functional coagulation enzyme that is also important in platelet-dependent thrombosis following arterial wall injury. Very recently, we and others have identified several single nucleotide polymorphisms within the human platelet thrombin receptor gene (PAR1). We propose to perform genotype association studies for selected PAR1 polymorphisms in our case-control study of acute MI in young women and assess potential interactions with traditional cardiovascular risk factors such as cigarette smoking. The genetic influence on platelet thrombin receptor function will also be assessed through correlation of in vitro platelet thrombin aggregation response as well as platelet surface receptor levels with PAR1 genotypes. The results of these studies will provide important new information regarding the role of candidate prothrombotic genetic factors in the susceptibility to athero-thrombotic disease, and enhance our understanding of the interaction between genetic and environmental risk factors in the development of a common, but complex disorder.

The Role of CYP209 Genotype in Modulating the Risk from Exposure to Exogenous Substrates: Warfarin and the Risk of Adverse Bleeding Events • David Veenstra, PharmD, PhD, Assistant Professor, Department of Pharmacy, UW

CYP2C9 is a major liver enzyme (cytochrome P450) that is involved in the metabolism of substrates such as (S)-warfarin, tolbutamide, and benzo[a]pyrene. Two variant alleles of CYP2C9 have been identified, the *2 and *3 alleles. Both alleles are associated with diminished CYP2C9 activity. Warfarin is an oral blood-thinning agent used to prevent clotting events in patients with

thromboembolic diseases, and is metabolized primarily by CYP2C9. The use of warfarin is associated with significant risks of bleeding complications and requires frequent monitoring. The rate of major bleeding due to warfarin ranges from 0.9 to 2.7 percent per year.

It is our hypothesis that individuals with CYP2C9 *2 or 83 alleles are more likely to have a higher risk of bleeding events due to impaired metabolism, experience greater variability in anticoagulation levels, and require lower maintenance doses of warfarin. We propose to use a retrospective, case-control study design to examine the association between CYP2C9 genotype and three clinical parameters: bleeding episodes, dose requirements, and anticoagulation variability. Results will be stratified based on smoking exposure. Patients at the UWMC anticoagulation clinics will be identified according to their daily warfarin dose requirements. We will then collect clinical information and obtain blood samples from consenting study participants. Full sequencing of exons 3 and 7 of the CYP2C9 locus, which contain the *2 and *3 mutation sites, will be completed.

This proposed project is aligned with the CEEH's mission to understand molecular mechanisms underlying human variability in response to environmental exposures because CYP2C9 is a biotransformation enzyme of relevance to ecogenetics. The results of this study may directly influence the clinical management of warfarin patients, will be used in a cost-effectiveness study to evaluate the societal implications of genetic testing of warfarin patients, and may suggest the need for a larger, prospective study.