Geisinger Clinic

Annual Progress Report: 2009 Formula Grant

Reporting Period

January 1, 2010 – June 30, 2010

Formula Grant Overview

The Geisinger Clinic - Weis Center for Research received $104,117 in formula funds for the grant award period January 1, 2010 through December 31, 2011. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Genetic Factors Associated with Aneurysms - The goal of this project is to identify genetic variants associated with abdominal aortic aneurysms (AAA). This will provide new insights into the disease mechanism, leading to new non-surgical therapies. Knowledge of AAA-associated genetic variants could also be used to identify individuals with increased genetic risk so that they could be diagnosed and treated before their aneurysm ruptures, which would reduce their chance of dying from a ruptured AAA.

Anticipated Duration of Project

1/1/2010 - 12/31/2011

Project Overview

AAA rupture is the thirteenth leading cause of death in the US and a major cause of death in the elderly. At the present time there are no simple laboratory tests to diagnose AAAs, and no non-surgical treatments.

A substantial genetic risk for AAA is well established. AAA is a complex disease, with risk influenced by an unknown number of genes that act in combination with environmental factors such as smoking. Knowledge of the genetic factors that influence AAA disease would shed new light on the underlying pathophysiology and provide new ways to identify patients at risk for the disease.

Several genetic loci have already been linked to AAA disease, but these account for only a fraction of the genetic risk. In a previous study we carried out a case-control genome wide association study (GWAS) using pooled DNA samples from AAA and matched controls and high density single nucleotide polymorphism (SNP) arrays. These GWAS data identify additional candidate AAA-associated regions. The primary aim of this project is to validate the association of candidate SNPs with AAA in an existing collection of DNA samples from patients

with AAA and matched controls. This will be done by individual genotype analysis of candidate SNPs. The second aim is to investigate the utility of a panel consisting of multiple AAA-associated SNPs to quantify AAA risk.

Principal Investigator

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Other Participating Researchers

None

Expected Research Outcomes and Benefits

The primary goal of this project is to identify genomic variants that are associated with AAA. Identifying the functional genetic variants that are associated with AAA will provide new insights into the disease pathophysiology and identify novel targets for non-surgical therapeutic interventions. At the present time, there are no medical therapies for AAA; surgery or endovascular repair is the only available treatment modality. A secondary goal is to obtain preliminary data to support use of a panel of AAA-associated variants to quantify AAA risk. Patients with elevated genetic risk could be targets of increased surveillance and subsequent diagnosis, leading to appropriate treatment prior to AAA rupture. Because most AAAs are asymptomatic many patients with AAA are undiagnosed. Since most AAA-related mortalities result from rupture of undiagnosed aneurysm, tools that increase diagnosis in a population will reduce mortality. Also, because increased genetic risk could be identified early in life, high risk individuals could be targeted for other interventions, such as smoking cessation or serum lipid management, which would reduce or eliminate non-genetic risk factors.

Summary of Research Completed

Study population and samples:
Patients for this project are enrolled through the Geisinger Clinic Department of Vascular Surgery. During the reporting period 168 new patients were enrolled into the study, bringing the total to 1,504 consented subjects. These include 923 patients with a confirmed diagnosis of abdominal aortic aneurysm (AAA) and 581 patients without AAA but matched to the AAA cases for age, gender, and smoking history, the main non-genetic risk factors for AAA. These research subjects provide blood and serum samples for research on the genetic and molecular basis of AAA. Blood samples are used to extract genomic DNA.

The other source of research subjects for this study is the Geisinger MyCode project. This project enrolls adult patients who make outpatient visits to Geisinger primary care clinics.

Enrolled subjects provide blood and serum samples for research. Collection of research blood samples is linked to a clinically ordered blood draw. This is done by placing an electronic order entry for the research blood draw into the patient's electronic medical record. Research blood samples are transported to the Geisinger Clinic Genomics Core in the Weis Center for Research on the Geisinger Medical Center campus where they are banked for future research use.

Patients that are enrolled in the MyCode project also provide authorization for Geisinger investigators to access data in their medical records for research. This is accomplished using an IRB-approved "data broker" process, in which a member of the Geisinger Biostatistics and Research Data Core accesses the patient's medical record to obtain the approved research data and provides that data to investigators in a manner that protects the confidentiality of the patient's information.

More than 16,000 Geisinger patients have consented to participate in the MyCode project. These provide a representative sample of the Geisinger outpatient cohort.

Access to MyCode samples is regulated by the MyCode Governing Board, which reviews and approves use of the samples and data for specific research uses. For this study, we obtained 482 MyCode DNA samples with associated clinical and demographic data (de-identified) to use as controls. These samples were matched for age, gender, and smoking history to the AAA cases.

Validation of AAA-associated genetic variants in the Geisinger population:
Aim 1 of the study is to use DNA samples from these AAA cases and controls to validate the association of genetic variants reported to be associated with AAA. Such replication of candidate disease-associated variants is a necessary step in establishing the true association. At this time the vast majority of candidate disease-associated genetic variants are single nucleotide polymorphisms (SNPs) that were identified in genome wide association studies. SNPs are the most common genomic sequence variants, and occur when a single base in the genome sequence is substituted with another base.

Of several SNPs that were genotyped in the Geisinger AAA and control samples one was SNP rs10752728 that was identified in a genome wide association study carried out by DeCode Genetics, a genetic research company located in Iceland. This SNP has also been linked to coronary artery disease and intracranial aneurysm, and therefore may be a marker for one or more genomic variants that affect biological mechanisms common to these disorders. SNP rs10752728 lies within a region of linkage disequilibrium that spans ~58,000 base pairs, but is >100,000 base pairs from the nearest known protein coding genes, *CDKN2B* and *CDKN2A*, which encode cyclin-dependent kinase inhibitors 2B and 2A, proteins involved in control of cell division. The AAA-associated genomic region does produce transcribed non-coding RNAs of unknown function. Deletion of the corresponding genomic region in mice has been reported to alter *CDKN2B* and *CDKN2A* expression, suggesting a distant-acting gene regulatory function in the AAA-associated genomic region. Deletion of this region also alters proliferation of aortic smooth muscle cells, which could be relevant to the biology of AAA.

We determined the SNP rs10757278 genotype using a TaqMan assay carried out in an Applied Biosystems 7500 Fast Real-Time PCR instrument in AAA cases and controls from the Geisinger

MyCode Project. The SNP genotype was significantly associated with AAA by chi-squared analysis ($P = 0.007$). The calculated odds ratio for AAA was 1.2.

Investigate the utility of a panel of AAA-associated SNPs to quantify AAA risk:
Individual disease-associated SNPs nearly all have small to moderate effects on disease risk. Of the several AAA-associated SNPs that have been validated in independent populations the odds ratios for AAA are in the range of 1.2 to 1.3. Aim 2 of this study is to determine the value of a panel of AAA-associated SNPs in quantifying the genetic risk of AAA.

To determine the effect of cumulative allele burden on AAA disease risk AAA-associated SNPs were genotyped in more than 2,000 Geisinger AAA cases and matched controls. A simple genetic model that assumed additive effects of risk alleles and no genetic interactions was used.

The distribution of total risk allele burden for the 3 genetic variants (from 0 to 6 risk alleles) was different in the AAA case and control groups, with the case group skewed toward more total risk alleles ($P < 0.0001$). Consistent with this finding, the relative odds ratio for AAA disease increased as a function of the number of risk alleles. For individuals with 3 or more risk alleles the odds ratio for AAA disease was increased 2.4-fold compared to individuals with 0-2 alleles ($P < 0.0001$). For individuals with 6 risk alleles the odds ratio was increased 3.5-fold ($P < 0.01$). The association of allele burden with AAA was also examined by linear regression analysis. The linear regression line had a positive non-zero slope ($P < 0.01$, $R^2 = 0.8$). The AAA disease odds varied by 3.7-fold from 0 to 6 risk alleles.

These results suggest that a panel of a sufficient number of appropriate SNP markers will have adequate information content to identify patients with a high genetic risk of AAA.