

# Geisinger Clinic

## Annual Progress Report: 2007 Formula Grant

### Reporting Period

July 1, 2009 – December 31, 2009

### Formula Grant Overview

The Geisinger Clinic - Weis Center for Research received \$92,771 in formula funds for the grant award period January 1, 2008 through December 31, 2009. Accomplishments for the reporting period are described below.

### Research Project 1: Project Title and Purpose

*Genetics of Morbid Obesity* - The high prevalence of obesity in the US is well known. Obesity can lead to a number of related medical conditions, including hypertension and diabetes. An important but often overlooked condition associated with obesity is non-alcoholic steatohepatitis (NASH). This disease is marked by accumulation of fat in the liver, liver inflammation, and fibrosis. NASH can lead to liver failure, and is thought to be a precursor to liver cancer. NASH is often under diagnosed because of the lack of reliable non-invasive tests. There is evidence for a genetic pre-disposition to the development of NASH. The purpose of this study is to identify specific genetic variants that are associated with NASH. This information will allow individuals at high risk for the disease to be identified and monitored, and should provide new information on the underlying causes of this disease.

### Duration of Project

1/1/2008 - 12/31/2009

### Project Overview

The long term goal of this project is to develop better ways to diagnose and treat patients with non-alcoholic steatohepatitis (NASH), an often undiagnosed but severe liver disease. The specific objective of this study is to identify genetic variants that are associated with the disease in a cohort of morbidly obese patients (patients with a body mass index greater than 40). NASH is highly prevalent in morbidly obese patients. Subjects for the research will come from the comprehensive bariatric surgery program at Geisinger Medical Center. All patients that enter the program are invited to participate in a research study of the molecular and genetic factors associated with obesity and related diseases. To date, more than 1,000 individuals have enrolled as research subjects. DNA samples from a subset of obese patients with NASH and a subset of obese patients with normal livers (controls) will be analyzed in a genome wide association study to identify specific genetic variants that are different between the two groups. A DNA pooling strategy will be employed in combination with high density single nucleotide polymorphism

(SNP) allotyping, using Affymetrix 500K SNP microarrays. The initial genome wide scan will be used to identify candidate genomic regions that contain variants that appear to be associated with NASH. In the second phase of the study, more detailed genetic analysis of these candidate regions will be carried out to validate the initial findings, and to map the specific NASH-associated variants. This will be accomplished by individual genotype analysis of SNPs in candidate regions.

### **Principal Investigator**

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### **Other Participating Researchers**

Glenn Gerhard, MD, Christopher Still, DO - employed by Geisinger Clinic

### **Expected Research Outcomes and Benefits**

The outcome of this research will be the identification of specific genetic variants that are associated with NASH, an often undiagnosed but severe liver disease. This would have several benefits. Simple genetic tests could be developed using this information that would identify individuals at high risk for the disease. These high risk individuals could then be monitored more closely to improve the likelihood of early stage detection, before liver damage is irreversible. For example, patients with a known genetic risk would be candidates for liver biopsy, which is currently the only reliable way to diagnose NASH. Importantly, patients at low risk might be spared liver biopsy, which is an invasive, sometimes painful, and occasionally risky procedure. A second benefit of identifying the genetic variants that are associated with NASH is that knowledge of the NASH-associated genes would provide new insight into the underlying mechanism that lead to this disease. Currently, knowledge of the biochemical mechanisms that cause NASH is limited. Understanding the causal mechanisms provides targets for therapeutic intervention. Thus, a possible long term benefit of this research is the development of new treatments for NASH.

### **Summary of Research Completed**

Aim 2 of this project was to genotype candidate genes to validate their association with obesity-associated liver disease in our cohort of morbidly obese patients consented through the Geisinger Clinic Department of Nutrition and Weight Management. During this portion of the project period we analyzed a genetic variant that was previously reported to be associated with hepatic fat deposition. This is a non-synonymous SNP (rs738409) in the *PNPLA3* gene (patatin-like phospholipase domain-containing protein 3, also called adiponutrin). This is a liver-expressed triacylglycerol lipase that is thought to be involved in mobilizing stored fat.

DNA samples from 1,008 patients from the Geisinger obesity cohort were genotyped for SNP rs738409 using the TaqMan assay on an Applied Biosystems 96 well instrument. Genotype calls were >99%. The data were analyzed using the Golden Helix genetic statistical analysis software package. The results showed a highly significant association of the SNP genotype with the extent of fat deposition in the liver. The latter was determined by histological analysis of liver biopsies obtained from the research subjects at the time of their undergoing gastric bypass surgery. Only 1% of patients with no fat deposits in the liver (grade 0 steatosis) were homozygous for the minor allele (genotype GG) while 29% were heterozygous for SNP rs738409. In contrast, 10% of patients with grade 3 steatosis (fat deposits throughout two-thirds or more of the liver tissue) were homozygous for the minor allele (CC) and 54% were heterozygous. The P value for the association of SNP rs738409 genotype with steatosis grade was < 0.005. In contrast, the SNP genotype was not independently associated with liver fibrosis. Furthermore, there was no association of the SNP genotype with serum lipid levels, including triglycerides.

These results further confirm the association of this gene and genetic variant with fat deposition in the liver. Because this is a non-synonymous variant it is highly probable that the polymorphism alters the enzyme's function or activity in a way that predisposes individuals to liver steatosis. Conversely, the major allele variant could protect individuals from some harmful effects of obesity on the liver (especially considering that the mean body mass index of the genotyped patients is approximately 50, yet individuals homozygous for the major allele or heterozygous were significantly more likely to have fatty-deposit-free livers). Our results also suggest that the genetic variant affects liver steatosis by a mechanism that does not simply relate to circulating lipid levels.