National Disease Research Interchange

Annual Progress Report: 2010 Formula Grant

Reporting Period

January 1, 2010 – June 30, 2011

Formula Grant Overview

The National Disease Research Interchange received $62,393 in formula funds for the grant award period January 1, 2011 through December 31, 2011. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

The Development of Diabetic Retinopathy: Going from Genetic Susceptibility to Functional Analysis - The Human Biological Data Interchange (HBDI) database is a vast repository of family and medical information focused on the study of type 1 diabetes (T1D) and its complications. Previously, our work has confirmed that genetic factors influence susceptibility to microvascular complications of diabetes. We will now test for functional gene expression differences in retinopathy-affected vs. non-retinopathy-affected diabetic retina and correlate the expression data with a) the results of case-control association studies in HBDI samples previously typed and b) the results of family-based association and linkage analysis on a candidate locus for susceptibility to retinopathy integrating newly typed HBDI family members. We will also continue our program of administering follow-up questionnaires to T1D families, allowing us to track clinical changes in T1D and complications.

Anticipated Duration of Project

1/1/2011 – 12/31/2011

Project Overview

The overall goal of this project is to identify genetic variants that lead to susceptibility or protection from the microvascular complications of diabetes (MCD). We are specifically searching for genetic variants that predispose to development of, or protection from, type 1 diabetes (T1D) complications but that are *not necessarily* related to T1D susceptibility. Identifying such genetic variants will allow us to predict which patients are at greatest risk for the blindness, kidney failure and nervous system disease caused by MCD, as well as determine the mechanisms by which high blood glucose leads to MCD. Our specific aims are to: 1) Plan and execute gene expression profiling comparing retinopathy-affected and non-retinopathy-affected diabetic retinas and normal retinas 2) Perform family-based linkage and association analysis to identify loci and alleles involved in MCD expression 3) Continue follow-up on identified T1D families.

Specific Aims:

Aim 1) While SNP analysis led to the discovery of genes of potential interest, this is still only a statistical finding. Functional analysis is needed to prove the biology underlying the statistical finding and indicate the biologic pathway by which known genetic variants lead to protection from/susceptibility to retinopathy. We plan microarray gene expression profiling to identify individual genes whose expression is correlated with the retinopathy phenotype (confirming that the candidate gene found by SNP analysis plays a role in the pathway) and to identify multiple genes that are interactively involved in mediating retinopathy pathogenesis.

Aim 2) Perform family-based association and linkage analysis, stratifying the data on observations such as HLA type, presence/absence of other MCDs, and specific genotypes at other loci associated with T1D, as well as identify new loci linked to MCDs. This will allow us to test for possible interaction of loci in the development of MCD. Family-based association analysis will also eliminate the possibility of biased results due to population stratification.

Aim 3) Continue our annual follow-up program to track the development and progression, or lack thereof, of MCD in families over time.

Principal Investigator

John T. Lonsdale, PhD
Research Director
National Disease Research Interchange
1628 John F. Kennedy Boulevard
8 Penn Center, Suite 1500
Philadelphia, PA 19103

Other Participating Researchers

Cathie Miller, PhD, Davlyn Tang, BS – employed by National Disease Research Interchange
Maria Cristina Monti, PhD – employed by Columbia University, New York

Expected Research Outcomes and Benefits

The clinical and familial data contained in the HBDI database is an immense and invaluable resource for the development and testing of hypotheses regarding the genetic and environmental factors leading to susceptibility to diabetic complications. It is the complications of diabetes that eventually take the biggest toll on the lives of T1D patients. The most common complications include: 1) retinopathy of the eye – which distorts vision and leads to blindness, 2) neuropathy – a degenerative nerve condition that can cause excruciating pain in the extremities, decreased blood flow and even require amputation, and 3) nephropathy – a serious kidney disorder that prevents proper filtering of the blood which may require dialysis or transplantation.

Better ways of preventing, treating and curing diabetes will inevitably stem from a better understanding of those factors that contribute to complications. We have already demonstrated that SNPs within specific genes are associated with susceptibility to development/protection of diabetic complications. The next step is to explore the functional differences in expression patterns to determine if there is a correlation with the specific genes identified through SNP analysis. The information contained in the HBDI database, coupled with newly available genetic data, provides us a unique opportunity to screen these culprit genes for specific linkage markers to complication occurrence. These studies hope to provide a mechanism for better screening, prevention and treatment of complications with the ultimate aim of reducing the heavy burden on patients and society.

Summary of Research Completed

Aim 1.
The goal of aim 1 is to generate and analyze independent evidence of the importance of the promising genetic region found to be significant for association with complications of type 1 diabetes (T1D). We will perform microarray analysis comparing expression patterns from deceased donor diabetic eyes not affected by retinopathy, diabetic eyes affected by retinopathy and non-diabetic normal eyes to determine differentially expressed genes downstream of our affected genetic region of interest and also to determine potential additional pathways which may be altered.

To date, eyes have been procured and retinas dissected and preserved from 6 donors. Two normal eyes have been recovered from 2 non-diabetic donors, ages 70 and 73, and 4 eyes have been recovered from 4 donors with diabetes, with ages of 66, 69, 72 and 73.

Aim 2.
During this reporting period we established the data base for the linkage study, downloaded the 6000, genome wide, publically available CIDR-typed SNPs into the data base and checked the data, family connectivities, etc. We also prepared the software to be used for analysis of the data. The software is designed to choose SNPs, set up the analysis runs, and run the analyses without user intervention after the user chooses the chromosomal region and analysis parameters and phenotypes to be analyzed. Linkage analyses have begun.

We began with linkage and association analyses on chromosome 12 because our previous case-control association analysis suggested association of retinopathy with the vitamin D receptor (VDR). Showing evidence of linkage to VDR would confirm the genetic influence of this locus on MCDs.

Large linkage and association studies have failed to detect a signal for VDR among type 1 diabetics. However, other studies did find evidence of association of VDR gene polymorphisms with severe diabetic retinopathy. The finding of linkage strongly suggests that VDR is directly related to the development of microvascular complications alone, i.e., it does not increase susceptibility to type 1 diabetes. The Type 1 Diabetes Genetics Consortium genotyped 40 SNPs in the VDR gene. We later typed 29 additional SNPs around the VDR to provide maximum information. In the VDR SNP saturation study we identified 2 SNPs as being associated with

our retinopathy cases: rs3890733 (p-value < 0.03; OR = 0.6) and rs4760655 (p-value < 0.01; OR =1.7).

Upon closer investigation of potential risk haplotypes, we found that the haplotypes defined by these two SNPs appear to have protective (TA: 0.30 case, 0.41 control), neutral (CA: 0.26 case, 0.27 control), and risk (CG: 0.44 case, 0.32 control) effects on diabetic retinopathy. The TG haplotype (i.e. both minor alleles in *cis*) was not observed in our sample.

SNP rs4760655 was also associated with neuropathy cases (*p-value* 0.01521, OR 2.051). No significant associations were found with nephropathy cases. We did not see any evidence of population stratification in these data.

For linkage analysis, chromosome 12 was genotyped at 200 SNPs. We selected families with at least one member suffering from severe diabetic retinopathy. Family members with at least 20 years T1D duration but without retinopathy were classified as "unaffected". Parents were classified as "unknown". Only parents and T1D-affected siblings were included in the linkage analysis. For the case-control study, we selected 95 unrelated cases with retinopathy, 38 cases with nephropathy, 31 cases with neuropathy and 167 controls for a multivariate case-control association analysis. The controls were defined as T1D patients without MCD or family history of MCD and with diabetes' duration of at least 20 years.

We found evidence for linkage to a VDR locus (44.97 – 92.0 cM) using the Kong & Cox linkage statistic (*p-value* < 0.02). Furthermore, in the case-control analysis using the same markers, we found an association at rs855272 (*p-value* < 0.04; OR = 1.4), and at rs1978161 (*p-value* < 0.01; OR =1.5), located in the VDR gene.

Our preliminary linkage results provide evidence in support of the hypothesis that VDR is involved in the pathogenesis of diabetic retinopathy which is substantiated by the positive association results.

VDR is extensively expressed in the retina, but to date, no one has characterized VDR expression patterns as a function of the known VDR mutations in Caucasians. The identification of causal variants of the VDR gene may lead to more effective treatment, especially for diabetics suffering from severe proliferative retinopathy, and could provide valuable insight into the molecular mechanisms underlying diabetic retinopathy.

Aim 3.
The goal of specific aim 3 is to perform our annual follow-up on a subset of 1000 registry families we had not contacted in recent years. To date this year, a total of 938 questionnaires have been sent out and 170 have been received, and updated information entered into the database. This corresponds to a response rate of 18.1%.