National Disease Research Interchange

Annual Progress Report: 2009 Formula Grant

Reporting Period

July 1, 2010 – December 31, 2010

Formula Grant Overview

The National Disease Research Interchange received $73,679 in formula funds for the grant award period January 1, 2010 through December 31, 2010. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Fine Mapping of Genetic Susceptibility for Microvascular Complications in Patients with Type 1 Diabetes - The Human Biological Data Interchange (HBDI) database is a vast repository of family and medical information focused on the study of type 1 diabetes and its complications. A previous project confirmed that genetic factors likely influence susceptibility to microvascular complications of diabetes, including retinopathy, kidney disease and neuropathy. In this project we will test for associations between specific genes and the presence or absence of complications by using HBDI samples newly typed by the T1DGC (Type 1 Diabetes Genetic Consortium). To enhance our analyses and maintain the scientific value of our dataset, we will continue our ongoing program of administering follow-up questionnaires. Finally, we will begin a SNP saturation study of statistically important genes. The additional information we collect will better define the genetic contribution to diabetes complications.

Duration of Project

1/1/2010 – 12/31/2010

Project Overview

The overall goal of this project is to identify the mutations within genes associated with susceptibility to the microvascular complications of diabetes (MCD). We are specifically searching for mutations that predispose to T1D complications but that are not necessarily related to T1D susceptibility. Identifying such polymorphisms will allow us to predict which patients are at greatest risk for the blindness, kidney failure and nerve disease caused by MCD. Our specific aims are to: 1) Analyze the SNP data for confirmation of specific genetic susceptibility to retinopathy among T1D patients using case-control association analysis 2) Genotype "representative" SNPs on new members of the HBDI families to be able to perform family-based association and linkage analyses 3) Continue our annual follow-up program to track the development and progression, or lack thereof, of MCD. 4) Plan and execute analysis of other

genomic region of interest found to be important in the development of retinopathy in patients with T1D.

While previous SNP analysis led to the discovery of genes of potential interest, further analysis to discover whether causative mutations within these genes of interest are present is necessary to more fully understand the contributions of these genes to the development of MCD. We plan to extend these analyses and use new genetic data to discover mutations within genes correlated with the development of MCD.

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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 amputation, and 3) nephropathy – a serious kidney disorder that prevents proper filtering of the blood and 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 shown that some families with T1D are more susceptible to developing complications than others. We now know of some of the genes that potentially play a role in the susceptibility. The next step is to explore more fully the correlation of which SNPs within these genes may be responsible for these observed differences in susceptibility. 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 SNPs to complication occurrence. The long-range goal is 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. Analyze the SNP data generated with support of the 2008 PA Formula grant. The goal is to confirm the role of specific markers of genetic susceptibility to retinopathy (within a specific gene on chromosome 12) among T1D patients using a case-control association analysis. The SNP data were obtained on "Cases", defined as T1D patients with retinopathy and "controls", defined as patients with at least a 20-year history of T1D but without complications and without 1st degree relatives with complications.

Our goal in specific aim 1 was to analyze the SNP data using a multivariate case-control association analysis. The gene selected for the SNP saturation study already had 40 SNPs genotyped in the HBDI sample by T1D consortium and 30 extra SNPs had been genotyped giving an excellent coverage for a case-control analysis.

Using the cohort of HBDI type 1 diabetes patients, "cases" were defined as patients with retinopathy. "Controls" were T1D patients without any complications after 20 years with T1D and without family history of complications in T1D siblings. The first phenotype of interest is "presence of T1D for at least 20 years and no retinopathy". Thus, people who meet this criteria are coded as having the trait, while siblings who have T1D plus the complication are coded as lacking the trait. Thus, we are looking for loci that "protect" against the development of the complication.

Multivariate models that incorporated a cluster function to account for potential cofounders including age, duration of diabetes, and sex were carried out. Groups were stratified to determine whether some markers were specifically associated with subgroups defined by presence/absence of other complications (retinopathy, nephropathy, neuropathy, hypertension, and cardiac conditions).

A set of 95 cases with retinopathy, 38 cases with nephropathy, 31 cases with neuropathy and 167 controls without microvascular diabetic complications were selected from the HBDI cohort genotyped by T1DGC. The SNP saturation study demonstrated a significant region within the gene selected for study that was associated with the presence of retinopathy (p-values ranging from 0.0002391 – 0.009694, OR 1.69-1.864).

Aim 2. Perform SNP analysis of additional family members to allow for family-based association and linkage analyses. New members of the families analyzed for aim 1 (families of cases and families of controls) will be selected and SNPs representative of the region of interest of the gene determined in Aim 1 will be genotyped.

Because we have family data, we can additionally test any statistically significant results from the case-control study using family-based association. Multipoint linkage analysis, besides, is able to determine if a locus found for the complication is actually related to the complication alone or to T1D.

Analysis of each family genotyped by T1DGC was done to select informative families to allow for family-based association and linkage analysis. 392 individuals from 88 families were selected

for additional SNP analysis. The selection of informative families included families with affected children who were discordant for development of complications. Due to updated family information from returned completed questionnaires, an additional 6 families were eligible for inclusion (Table 1). Individuals noted as absent of complication were included only upon either medical record review or other confirmation from the individual's physician.

Validation analysis of markers that meet the stringent criteria for association using the case-control design with retinopathy is being carried out using family based genetic association analysis (TDT – transmission disequilibrium test and the PDT –pedigree disequilibrium test) with the individuals shown in Table 1.

We are looking for loci that "protect" against the development of the complication. In order to use linkage analysis, families must have at least one sib with the trait (lack of complication) and one sib without the trait (presence of complication). Because all sibs in the analysis have T1D, the only way to differentiate linkage to the target phenotype from T1D is to have at least one T1D sib without the trait and one with the trait. There are 88 families that fit the classification of at least one sib with the trait (without complication) and one sib without the trait (with complication).

In order to distinguish loci that may predispose to complications from loci that predispose to diabetes, we performed additional analysis on 200 families which do not overlap the 88 families who have members who lack complications. These families were used to examine linkage at loci with complications protection determined from the first analysis of the 88 families discordant for complications. If the T1D+ complication analysis indicates linkage at the same loci that the protection analysis indicates, then the locus is not specific for complications. If no linkage is indicated in the T1D+ complications group, then the locus may be specific for complications.

We are currently finalizing the analysis. Preliminary results demonstrate a confirmation of the previously identified region as specific for retinopathy.

Aim 3. Continue our annual program of participant follow-up using the updated family questionnaire to track development or progression of microvascular complications among patients with both T1D and T2D. Data gathered will be an essential component of our primary aim.

Our goal was to continue the annual program of participant follow-up using an updated family questionnaire to track development/progression or lack of development/progression of microvascular complications among patients with both T1D and T2D. To this end, we sent 1000 questionnaires to participants in 2010. To date, we have received 228 completed questionnaires/updates from individual participants. The information in these updates provides information on 4,298 individuals in the HBDI National Genetics Family Registry. Seventy questionnaires were returned due to incorrect addresses. Of these, we were able to send questionnaires to different family members in 47 cases, thus only 23 families have been confirmed lost to follow-up. Furthermore, we instituted a program of follow-up contact through email and phone calls. To date, we have attempted to contact 1,253 individuals, with success on 778 cases (62.1%). We continue to follow-up with these families in order to maintain the ability

to track development/progression or lack of development/progression of microvascular complications. Of note, in three years (2008-2010) we have updated 11,891 individuals from the National Genetics Family Registry. In regards to the participants who have been immortalized in the NDRI National Genetics Family Repository, we have updated 1,323 (49%) of these individuals in the last 3 years. Thus, our continued effort to perform annual updates has led to a significant rate of follow-up with participants and has resulted in continued interest and enhanced the usefulness of our database and repository.

The NDRI National Genetics Family Registry now includes epidemiologic and diagnostic data on 6,664 extended families (92,831 individuals). 8,948 subjects had type 1 diabetes (48% were females). 6,250 individuals reported type 2 diabetes, 3,429 had diabetes type unknown or unclassified (e.g., type 1 diabetes diagnosed after age 30), 44 patients had MODY (Maturity-Onset Diabetes of the Young), 126 patients had gestational diabetes and 74,034 subjects were not affected by diabetes. 90% of type 1 diabetes patients in the database were Caucasian, 1.8% were Hispanic, 0.6% American Indian, 1.8% Asian, 1.2% African-American, 0.2% bi-racial and 1.2% Pacific Islander.

A subset of 550 families were included in the HBDI genetic repository at the Coriell Institute in Camden, NJ. Blood was drawn from members of these families, cell lines were immortalized and DNA was extracted and stored. Among these are 424 nuclear families genotyped by the T1D Genetic Consortium: 2,532 Caucasian patients that were used as the study population for this study. The genetic data are provided to HBDI, which links the genotypes with the subjects' epidemiological and clinical information present in the HBDI database. Within this population, there are 895 T1D, including 424 probands and 471 affected siblings (Table 2) . Of these 424 families, 408 are multiplex, with 2 or more affected siblings and unaffected parents. 861 of the T1D are living with a mean age of 42.3 +/- 12 years. The average age of onset for diabetes is 12.2 +/- 9.4 for all T1D affected individuals, with probands having an earlier age of onset (9.1 +/- 6.5 years) than affected siblings (13.9 +/- 8.35 years) as expected. The average duration of diabetes is 30.3 +/-10 years for all T1D. Currently 221 individuals confirm presence of retinopathy, 94 confirm presence of neuropathy and 83 confirm presence of nephropathy.

<u>Aim 4.</u> To perform a SNP saturation study on additional candidate genes associated with retinopathy. Beside the genomic region already explored through the support of the 2008 PA Formula grant, there are additional genes shown to be associated with retinopathy in our preliminary analyses. In depth gene SNP analysis studies could identify specific polymorphisms responsible for the observed genetic signal.

To comprehensively evaluate the most promising SNPs in a genetic region found to be significant for association or linkage with complications, an additional 15 SNPs were performed on 392 individuals from 88 families. Individual SNPs were selected based on previous analysis demonstrating significance to the development of, or protection from, the development of retinopathy. The individuals selected for further analysis were selected from the families genotyped by T1DGC. Informative families were selected to allow additional family-based association and linkage analysis. The selection of informative families included families with affected children who were discordant for complications.

Validation analysis of markers that meet the stringent criteria for association using the case-control design with retinopathy is being carried out using family-based genetic association analysis (TDT – transmission disequilibrium test and the PDT –pedigree disequilibrium test) with the above individuals.

We are looking for loci that "protect" against the development of the complication. In order to use linkage analysis, families must have at least one sib with the trait (lack of complication) and one sib without the trait (presence of complication). Because all sibs in the analysis have T1D, the only way to differentiate linkage to the target phenotype from T1D is to have at least one T1D sib without the trait and one with the trait. There are 88 families that fit the classification of at least one sib with the trait (without complication) and one sib without the trait (with complication).

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N. of HBDI Families	*retinopathy*	*nephropathy*	*neuropathy*	*any complication*
At least 1 sibling with and at least 1 sibling without:	88	77	42	88
At least 2 siblings with:	49	17	11	65
At least 2 siblings without:	318	356	357	222
Trios: two parents and 1 affected sibling with:	144	79	63	249

Table 1. Description of type 1 diabetes HBDI families with at least 1 sibling with and at least 1 sibling without retinopathy, nephropathy or neuropathy.

Type 1 Individuals	*N*	*Probands*	*Siblings*
N of Subjects	895	424	471
No of Multiplex	408	na	na
Females	415	188	218
Living	861	381	455
Age (yr)	42.3 +/-12	42.2 +/- 11	41.3 +/- 11.7
Age of Onset (yr)	12.2 +/- 9.4	9.11 +/- 6.5	13.9 +/- 8.5
Duration of diabetes	30.3 +/- 10	32.9 +/- 9.7	27.6 +/- 8.9
Presence of Retinopathy	221	109	104
Presence of Neuropathy	94	48	42
Presence of Nephropathy	83	40	42

Table 2. Characteristics of type 1 diabetes patients and number of probands, siblings and parents only (description of third or fourth degree relatives of probands are not included, as reliable information about presence of complications is not available). Data are n (%) or means±SD.