Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** National Disease research Interchange (NDRI)

2. **Reporting Period (start and end date of grant award period):** 1/1/2011 to 6/30/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John T Lonsdale, PhD

4. **Grant Contact Person's Telephone Number:** 800 222 6374 x 271

5. **Grant SAP Number:** 4100054863

6. **Project Number and Title of Research Project:** 01- The Development of Diabetic Retinopathy: Going from Genetic Susceptibility to Functional Analysis

7. **Start and End Date of Research Project:** 1/1/2011 to 6/30/2013

8. **Name of Principal Investigator for the Research Project:** John T Lonsdale, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 62,393.00

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Lonsdale, John	PI	5.6	9,997.15
Miller, Cathie	HBDI Director	25	7,047.31
Tang, Davlyn	Project Manager	50	9,713.82

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
Greenberg, David	Statistical Consultant	2
Monti, Maria Cristina	Statistical Consultant	10

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No ____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes X____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Genetic Mechanisms of Type 1 Diabetes Complications	☒NIH ☐ Other federal (specify:___) ☐Nonfederal source (specify:_)	June, 2013	$3,687,707	$
Genes for Type 1 Diabetes Complications	☐ NIH ☐ Other federal (specify:___) ☒Nonfederal source (specify: American Diabetes Association)	August, 2013	$1,200,000	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes_____ No ____X_____

If yes, please describe your plans:

12. Future of Research Project. What are the future plans for this research project?

Analysis of the RNA-seq expression data will continue in collaboration with David Greenberg PhD, Director of Neurogenetics, Battelle Center for Mathematical Medicine and Department of Neurology, Nationwide Children's Hospital, Columbus, Ohio and Yaron Tomer MD, Professor Medicine, Endocrinology, Diabetes and Bone Disease, Mt Sinai Hospital, NY. Results will be published in appropriate journals. We plan to identify the gene(s) that increase complications' susceptibility and determine the mechanisms by which that susceptibility can be interrupted, thus devising preventative or curative treatments.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes X____ No

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female			1	1
Unknown				
Total			**1**	**1**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			1	1
Unknown				
Total			**1**	**1**

	Undergraduate	Masters	Pre-doc	Post-doc
White			1	1
Black				
Asian				
Other				
Unknown				
Total			**1**	**1**

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No ____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes_____ No ____X_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of

your institution (e.g., entire university, entire hospital system)?

Yes ____X_____ No_____

If yes, please describe the collaborations:

Collaborations developed with David Greenberg PhD at Columbia University, New York, NY and later at Nationwide Children's Hospital, Columbus, Ohio and with Yaron Tomer MD, Professor of Medicine, Endocrinology, Diabetes and Bone Disease, Mt Sinai Hospital, NY, to enhance analysis of the RNA-seq expression data.

16(B) Did the research project result in commercial development of any research products?

Yes_____ No ____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No ____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

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.

Aim 1. To perform microarray studies to determine functional differences in genes transcriptionally downstream from our significant genetic locus of interest and to identify multiple genes that are interactively involved in mediating retinopathy pathogenesis. The objective is to compare global gene expression in retinopathy-affected diabetic retina vs. non-retinopathy-affected diabetic retina vs. non-diabetic normal eyes. This comparison will provide deeper understanding into the genetic basis of the development of retinopathy in the diabetic eye.

In previous work, we found evidence showing that the vitamin D receptor gene is both linked and associated with complications. In order to pursue this finding and find other genes that may affect the development of retinopathy, NDRI collected retinal tissue samples from T1D patients with retinopathy, T1D patients *without* retinopathy, and retinal tissue from subjects without T1D. Our goal was to compare the expression of RNA from these three groups. By comparing the RNA, we can discover how gene expression differs, especially between patients with and without retinopathy. This information will then give us clues as to what genes different between the two groups, how alternative splicing, for example, differs and may affect retinopathy expression, and, especially, whether the vitamin D receptor is involved in susceptibility and, if so, which alleles may be leading to susceptibility. These data have taken a very long time to generate and, given the volume of information, will take some time to analyze and understand. However, we anticipate results before the end of 2013.

Rationale

To stay at the forefront of transcriptional profiling analysis, we modified the technological approach for Aim 1. At the time of our grant submission, microarray analysis was the "gold standard" for transcriptional analysis. However, recent developments in RNA-seq technology have quickly shifted the transcriptional profiling field away from microarray experiments. Three key factors are attracting scientists to the RNA-seq approach: 1) the technology overcomes the gene and probe limitations that are associated with microarray analysis, 2) the RNA-seq read lengths have increased and 3) the costs for RNA-seq experiments have dropped dramatically. Collectively, the rapid changes in technology and lower costs prompted us to replace the proposed Affymetrix GeneChip Human Gene 1.0 ST Array approach with RNA-seq analysis.

Sample collection

It was originally proposed that whole globes would be procured from 3 different deceased donor pools: 1) at least 15 diabetic non-retinopathy-affected individuals, 2) at least 15 diabetic retinopathy-affected individuals, and 3) at least 15 non-diabetic normal eyes. However, collection of the requisite number of eyes within the original proposed project timeframe proved problematic, resulting in a request for an 18 month no-cost project extension. The quality of the eyes is paramount to ensure success, and it is important we carry out a single expression analysis experiment with the maximum number of donors to generate statistically significant results. The key parameter in determining retinal quality is the *post mortem* interval (PMI) to preservation by snap-freezing, and despite NDRI's national leadership position in recovering eyes for research, it proved difficult to recover eyes with a PMI below 8 hours at the required frequency. The situation is complicated because the retina is extremely sensitive to anoxia and hypoxia, meaning that donors who were ventilator-dependent at the time of death were ineligible for study. Further compounding the collection issue is the fact that medical-social history data of sufficient quality to confirm the disease status of the eyes is frequently not available at the time of donor referral from eye banks to NDRI.

The final distribution of donor types from which eyes were recovered was as follows: 8 donors without any history of diabetes or physical evidence of retinopathy; 10 donors with diabetes but without any history or physical evidence of retinopathy and 12 donors with diabetes and with physical evidence of retinopathy. Whole eyes were procured from consented human donors, stored within 8 hours of death at 4°C in RNAlater, and shipped to the tissue processor. Within 48 hours of procurement, the retina was microdissected from the eye and snap-frozen in RNAlater (Invitrogen) and stored at -80°C. Dissection of the globes and processing of the retinas was carried out in the laboratory of Marco Zarbin MD, PhD, FACS, Professor and Chair, Institute of Ophthalmology and Visual Science and Professor of Neurosciences, UMDNJ-New Jersey Medical School, and Chief, Department of Ophthalmology, University Hospital, Newark, New Jersey.

Retinal RNA isolation

RNA isolation and RNA-seq reactions were performed by the Penn Genomics Frontiers Institute (PGFI) at the University of Pennsylvania, Philadelphia, PA. Total RNA was isolated from 30 retinas (~100 mg tissue per retina) via the Trizol (Invitrogen) extraction protocol. A small portion of each retina was retained for future experiments, particularly genotyping. The total RNA samples were subsequently processed with the RNAeasy (Qiagen) protocol to further

purify the RNA population and stabilize the transcriptional integrity of each sample. The RNA concentrations and RIN values were determined by NanoDrop (Agilent) measurements and Bioanalyzer (Agilent) analyzes on total RNA nanochips. The average RNA concentrations and RIN values were 383.7 ng/µl and 7.33, respectively.

mRNA isolation and cDNA library generation

The total RNA from 30 retinas was used as template to construct sequencing libraries for RNA-seq analysis via the Illumina platform. The TruSeq RNA Sample Preparation Kit v2 (Illumina) was used to convert 1 µg of total RNA from each sample into sequencing templates via Low Sample Protocol. This protocol is optimized for 0.1 to 4 µg of the total RNA. Two rounds of purification with oligo-dT beads were used to purify mRNA from total RNA. During the final elution step, the mRNA was fragmented and primed with random primers for first strand cDNA synthesis with Superscript III (Invitrogen). Next, the samples were subjected to second strand synthesis; the double stranded (ds) cDNA products were purified from the reaction mix with AMPure XP beads. To optimize addition of the library adapters, the ends of the samples were converted to blunt ends and subsequently the 3' ends were adenylated to create complementary overhangs for the library adapters. The adapters were ligated onto the ds cDNA products and polymerase chain reaction (PCR) was used to selectively enrich the library with ds cDNA products that have adapters on both ends.

cDNA library verification

ds cDNA products were purified from the PCR reaction mix with AMPure XP beads. The libraries were validated by Bioanalyzer (Agilent) analysis on DNA 1000 (quantitative range 0.5-50 ng/µl) chips. The DNA 1000 chips results were used standardize the concentrations of each library to 10 nM. The libraries were further quantified by Bioanalyzer (Agilent) analysis on High Sensitivity DNA chips (quantitative range 5-50 pg/µl).

cDNA pooling for RNA-seq analysis

Based on the High Sensitivity DNA chips results, groups of six samples were pooled together at equimolar concentrations; the sample pools (n=5) were then quantified by Bioanalyzer (Agilent) analysis on High Sensitivity DNA chips. Once verified, 5 pM of each library pool was then clustered onto the Illumina flow cells and amplified using via cBOT (Illumina). All the data was collected and aligned by PGFI.

RNA-seq data analysis

Analysis of the RNA-seq expression data is being carried out in collaboration with David Greenberg PhD, Director of Neurogenetics, Battelle Center for Mathematical Medicine and Department of Neurology, Nationwide Children's Hospital, Columbus, Ohio and Yaron Tomer MD, Professor Medicine, Endocrinology, Diabetes and Bone Disease, Mt Sinai Hospital, NY.

RNA-seq analysis allows the examination of all of the RNA that is produced in a tissue. The expectation is that the specific RNAs (and, by implication, which proteins) which are more or less frequent in diseased tissue compared to healthy tissue will inform us which genes are turned on or turned off in the diseased vs. normal tissue.

We are particularly interested in the vitamin D receptor because that has been implicated in susceptibility to retinopathy in our earlier work. However, we will have information on all changes in gene expression and can use that to give us clues to which genes participate in causing blindness.

Aim 2. Analyze the SNP data generated with support of the PA CURE program 2009 Formula grant. New members of families were assayed for SNPs representative of the region of interest in the genetic locus previously determined to be significant. Because we have family data, we can additionally test any statistically significant results obtained from the case-control study previously done using family-based association analysis. Multipoint linkage analysis is able to determine if a locus found for the complication is actually related to the complication alone or to T1D. We will also analyze separate groups of patients/families (data stratification) by HLA type, presence/absence of other MCDs and look for interaction among other T1D-related loci (e.g., the insulin VNTR). This approach was helpful in identifying other loci in a genetic study of Familial Primary Arterial Hypertension.

During this grant period, in addition to establishing the data base infrastructure and assembling the data for analysis, we:

1) Looked for linkage and association with the vitamin D receptor because our preliminary results suggested association of retinopathy with this gene.

2) Performed the association analysis to determine if the HLA-DRB1 alleles that predispose to T1D (DRB1*03:01 and DRB1*04:01) influence the expression of T1D microvascular complications.

3) Performed a linkage analysis looking for loci on chromosome 6 that are linked to T1D complications. We also examined gene-gene interaction of the loci we discovered with the T1D-associated DRB1 alleles.

<u>Analysis of markers around the Vitamin D receptor gene</u>
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 only, 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.

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.

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. The RNA-seq experiment (described later) is an attempt to confirm the finding about VDR.

<u>HLA DRB1 allele association analysis</u>
We tested whether the DRB1 alleles DRB1*03:01 and DRB1*04:01 are associated with T1D complications. These two alleles represent the strongest genetic effect on susceptibility to T1D. We found that the presence of 03:01 actually *protects* against complications while 04:01 is either neutral or predisposed to complications. We also tested alleles in the HLA class I genes and found that the allele B*39:06 may strongly predispose to complications, but our sample was not large enough to be definitive. This work was recently published[1].

Linkage analyses on chromosome 6
Partly in an attempt to confirm the protective effect of DRB1*03:01 on complications and to determine if DRB1*04:01 had an effect or not, we undertook a linkage analysis on chromosome 6 to see if linkage data could help resolve the issue. In the event, we discovered two loci related

to the expression of complications and then tested the interaction of these loci with the DRB1 alleles using a technique we developed that stratifies the linkage data on the presence of the allele being tested[2].

We used the following phenotype definitions: An individual was designated as affected if he/she had T1D with onset <30 years of age and the presence of retinopathy, nephropathy and/or neuropathy. An individual was designated unaffected if he/she had T1D with onset <30 years of age and was free of complications for at least 20 years. Phenotypes definitions included "any complication", meaning T1D individuals with the presence of at least one complication. We also analyzed families in which the proband had only a given complication, i.e., retinopathy alone, nephropathy alone, and neuropathy alone. For each phenotype definition, we stratified families based on the DRB1*03:01 or DRB1*04:01 status of the family's proband.

Linkage results on chromosome 6 and looking for evidence of gene-gene interaction

As noted above, in our association analysis of DRB1 alleles, we showed that the HLA alleles that exert the strongest effect on susceptibility to T1D, the DRB1*03:01 and DRB1*04:01 alleles, also affect susceptibility to microvascular diabetic complications, but in unanticipated ways. Recall that the DRB1*03:01 allele *protects* against diabetic complications while the DRB1*04:01 allele either has no effect or somewhat increases risk for complications.

Linkage was performed using logarithm of odds (LOD) score analysis. Multi-point heterogeneity LOD scores (HLOD scores) were calculated using the Genehunter program and also using the program MERLIN. We assumed a penetrance of 25% and tested both a dominant and recessive mode of inheritance for the analyses reported here, choosing the mode of inheritance that led to the higher LOD score[3].

In our linkage analysis, we analyzed the data using three different phenotype definitions: the presence of "any complication", the presence of retinopathy, or the presence of nephropathy. We found statistically significant evidence for the existence of two loci that influence the expression of complications, one at 42 cM (from the telomere of 6p) and one at 65 cM. These two loci are on either side of the HLA locus (52 cM), which unsurprisingly showed strong evidence of linkage. (This was unsurprising because in order to have complications, subjects must first have T1D and the HLA locus is the strongest genetic influence on T1D susceptibility.) When we confined ourselves to the phenotype of "retinopathy", we observe a picture similar to that for the "any complication" phenotype. This occurred because the majority of patients with complications had retinopathy or nephropathy and few had neuropathy. Thus we confined our further analyses to retinopathy and nephropathy only.

The figures below show the results of the linkage analyses and also the results of linkage under stratification, in which only families of probands with specific HLA DRB1 alleles are included.

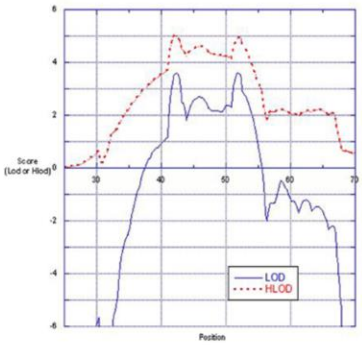
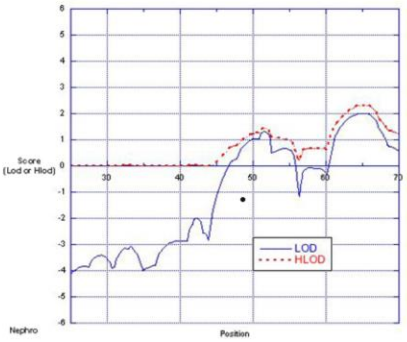


Fig 1. Analysis of Retinopathy Phenotype



Fig, 2. Analysis of Nephropathy phenotype.

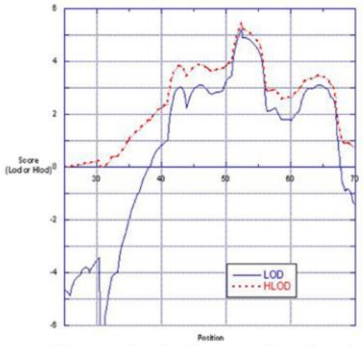


Figure 3. Retinopathy Analysis including only Families with DRB1*03:01 HLA Allele

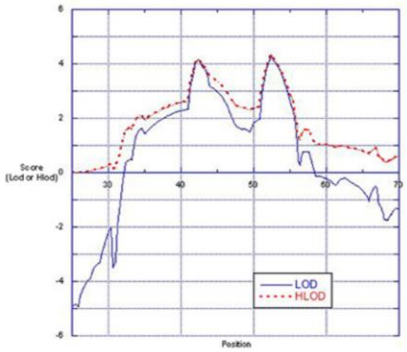


Figure 4. Retinopathy Analysis including only Families with DRB1*04:01 HLA Allele

The y axis in the figures shows the evidence in favor of linkage (LODs and HLODs (heterogeneity LOD score, which takes into account genetic heterogeneity)). Figure 1 shows an analysis in which the phenotype includes only retinopathy families. A maximum LOD score (indicating the presence of a gene that co-segregates with the phenotype, in this case, retinopathy) occurs at the HLA region (~52 centimorgans) but there is a peak occurring before the HLA region (~42 cM) that is statistically significant (LOD=3.5) even before taking heterogeneity into account (HLOD=4.5). There is also a broad area of elevated HLOD score (but low LOD score) at around 65 cM, but not a peak, *per se*. This suggests there is linkage to this region but that another genetic influence is also at work. Since we only examined chromosome 6, a genome-wide statistical correction is not necessary. Figure 2 shows an analysis in which the affected phenotype is nephropathy. That there is no peak corresponding to the peak seen telomeric to HLA in the retinopathy analysis, but there is a notable peak occurring centromeric to the HLA region at 65 cM, with a very similar HLOD score. This suggests differing genetic influences on different complications. Not only does the locus at 65 cM exert a strong effect on susceptibility to nephropathy (figure 2) but, under the right circumstances, also affects r (see

stratification results below). Figure 2 also shows that the 42 cM locus has no effect on nephropathy susceptibility, as shown by the fact that lod scores are negative for analysis of the nephropathy phenotype at 42 cM.

If these two novel loci affect complications' susceptibility, our finding of associations between HLA-DRB1 alleles and complications suggests we might expect to see evidence of interaction between these two loci and the associated HLA alleles. Our lab has developed a method to detect gene-gene interaction using linkage data that has a high reliability[4]. Details of these findings are below.

Figures 3 and 4 show further analysis of retinopathy that includes only those families in which the proband carries a specific HLA-DRB1 allele. In figure 3, only families with the DRB1*03:01 allele are included. The telomeric peak (42 cM) is much diminished but the evidence for linkage at the 65 cM peak is now statistically significant and the LOD and HLOD score almost the same, indicating that we have eliminated the "other" genetic influence that was causing the LOD score to be low (see figure 1). It appears that the heterogeneous influence is related to the DRB1*04:01 allele, since it has been diminished when the frequency of that allele is reduced in the stratification. This suggests that the 65 cM locus interacts with the 03:01 allele. Similarly, in figure 4, only probands with the DRB1*04:01 allele are included. The LOD score for the telomeric peak is strengthened, but the 65 cM peak is gone, suggesting that 04:01 interacts with the telomeric peak but not the locus at 65 cM.

We emphasize these results prove the *existence* of complications-related genes in these regions, and suggest they interact with HLA alleles, but the areas are too broad and encompass too many genes to determine the origin of the signals.

To our knowledge, this is the first evidence for a) major genetic influences on diabetic complications and b) the first firm evidence for gene-gene interaction in complications susceptibility. Many diabetics develop complications and, while research has shown that maintaining stable blood glucose levels can delay complications onset, it is also true that some patients are more susceptible to complications and some, a notable proportion of our data set, do not develop complications even 20-30 years after diabetes onset (and before maintaining highly stable glucose levels was feasible). I believe it is not too much to say that when we identify the genes that predispose to about half of the retinopathy families (estimates of the amount of heterogeneity being about 50%) and as much as 75% of the nephropathy families, being able to interrupt the processes leading to complications at the most fundamental level will be feasible.

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, 1,971

questionnaires were sent out and 440 were returned, corresponding to a response rate of 22%. The information in these updates provides information on 10, 482 individuals in the HBDI National Genetics Family Registry.

In regards to the NDRI National Genetics Family Repository participants for whom we have banked cell lines and DNA, we have updated medical-social information on 672 of these individuals. 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 the registry and repository.

References

1. Lipner E.M., T.Y., Noble J.A., Monti M.C., Lonsdale J.T., Corso B., Stewart W.C.L., Greenberg D.A. (2013). HLA Class I and II Alleles are Associated with Microvascular Complications of Type 1 Diabetes. Human Immunology (in press).

2. Rodriguez-Murillo, L., Subaran, R., Stewart, W.C., Pramanik, S., Marathe, S., Barst, R.J., Chung, W.K., and Greenberg, D.A. (2010). Novel loci interacting epistatically with bone morphogenetic protein receptor 2 cause familial pulmonary arterial hypertension. The Journal of heart and lung transplantation : the official publication of the International Society for Heart Transplantation 29, 174-180.

3. Greenberg, D.A., Abreu, P., and Hodge, S.E. (1998). The power to detect linkage in complex disease by means of simple LOD-score analyses. American Journal of Human Genetics 63, 870-879.

4. Hodge, S.E., Rodriguez-Murillo, L., Strug, L.J., and Greenberg, D.A. (2008). Multipoint LODs provide reliable linkage evidence despite unknown limiting distribution: type I error probabilities decrease with sample size for multipoint LODs and mods. Genet Epidemiol 32, 800-815.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
X_____No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
X_____No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
X_____No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1.HLA class I and II alleles are associated with microvascular complications of type 1 diabetes	Lipner EM, Tomer Y, Novle JA, Monti MC, Lonsdale JT, Corso B, Stewart WCL, Greenberg DA	Human Immunology	07/2012	☐Submitted ☐Accepted X Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes X____ No_____

If yes, please describe your plans:

The research included performing linkage analysis on existing marker data on chromosome 6 to search for loci influencing diabetic complications. The results of those investigations are currently being prepared for publication. Analysis of the RNA-seq expression data will be published in appropriate journal(s).

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No X

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No___X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Lonsdale, John T.	POSITION TITLE Vice President, Research
eRA COMMONS USER NAME (credential, e.g., agency login) JOHNLONSDALE	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Newcastle Upon Tyne, England, UK	B.Sc (Double First Class Honors)	1981	Biochemistry & Microbiology
University of Newcastle Upon Tyne, England, UK	PhD	1985	Microbial Biochemistry

A. Personal Statement

Dr. Lonsdale has extensive experience as PI/Project Leader on numerous research projects and in providing scientific and administrative oversight, including as NDRI PI on the NIH-funded Human Tissues and Organs Research resource (HTOR), Genome Tissue Expression (GTEx) and Biospecimen Methodological Study (BMS) projects. He has extensive experience and expertise in all aspects of the provision of human tissues for research, with particular knowledge of those pertaining to researcher recruitment, application processing, protocol development and service. He is responsible for liaison with NIH program officers and IC representatives.

B. Positions and Honors

1985-1995	Biochemistry Department, SmithKline Beecham Pharmaceuticals, Brockham Park, England, UK
1995-2001	Assistant Director, Anti-Infectives Research, SmithKline Beecham Pharmaceuticals, Collegeville, PA USA
2001-2002	Director, Microbial Biochemistry - Antimicrobials and Host Defense CEDD, GlaxoSmithKline, Collegeville, PA, USA
2002	Director, Biochemistry - Microbial, Musculoskeletal and Proliferative Diseases CEDD, GlaxoSmithKline, Collegeville, PA, USA
2003-2012	Research Director, NDRI, Philadelphia, PA, USA
2012-present	VP, Research, NDRI, Philadelphia, PA, USA

C. Selected Peer–reviewed Publications

MC Monti, JT Lonsdale, C. Montomoli, R. Montross, E. Schlag and DA Greenberg. Familial risk factors for microvascular complications and differential male-female risk in a large cohort of American families with type 1 diabetes. *J Clin Endocrinol Metab 92:4650-4655. 2007.*

Lipner EM, Tomer Y, Noble JA, Monti MC, Lonsdale JT, Corso B, Stewart WC, Greenberg DA. HLA class I and II alleles are associated with microvascular complications of type 1 diabetes. *Hum Immunol May;74(5):538-44. 2013*

Lonsdale J, Thomas J, Salvatore M, Phillips R, Lo E, Shad S, Hasz R, Walters G, Garcia F, Young N, Foster B, Moser M, Karasik E, Gillard B, Ramsey K, Sullivan S, Bridge J, Magazine H, Syron J, Fleming J, Siminoff L, Traino H, Mosavel M, Barker L, Jewell S, Rohrer D, Maxim D, Filkins D, Harbach P, Cortadillo E, Berghuis B, Turner L, Hudson E, Feenstra K, Sobin L, Robb J, Branton P, Korzeniewski G, Shive C, Tabor D, Qi L, Groch K, Nampally S, Buia S, Zimmerman A, Smith A, Burges R, Robinson K, Valentino K, Bradbury D, Cosentino M, Diaz-Mayoral N, Kennedy M, Engel T, Williams P, Erickson K, Ardlie K, Winckler W, Getz G, Deluca D, Macarthur D, Kellis M, Thomson A, Young T, Gelfand E, Donovan M, Meng Y, Grant G, Mash D, Marcus Y, Basile M, Liu J, Zhu J, Tu Z, Cox NJ, Nicolae DL, Gamazon ER, Im HK, Konkashbaev A, Pritchard J, Stevens M, Flutre T, Wen X, Dermitzakis ET, Lappalainen T, Guigo R, Monlong J, Sammeth M, Koller D, Battle A, Mostafavi S, McCarthy M, Rivas M, Maller J, Rusyn I, Nobel A, Wright F, Shabalin A, Feolo M, Sharopova N, Sturcke A, Paschal J, Anderson JM, Wilder EL, Derr LK, Green ED, Struewing JP, Temple G, Volpi S, Boyer JT, Thomson EJ, Guyer MS, Ng C, Abdallah A, Colantuoni D, Insel TR, Koester SE, Little AR, Bender PK, Lehner T, Yao Y, Compton CC, Vaught JB, Sawyer S, Lockhart NC, Demchok J, Moore HF. The Genotype-Tissue Expression (GTEx) project. *Nat Genet*. May 29;45(6):580-5. *2013*

D. Research Support

ACTIVE

2U42 OD11148-23 (PI: Lonsdale) 7/1/2013 – 6/30/2018
NIH/OD

Human Tissue and Organ Research Resource
The primary mission of the Human Tissue and Organ Research Resource (HTOR) program is to procure and distribute human biomaterials to the biomedical research community.

GTEx RFP 210-120 (PI: Lonsdale) 03/29/13 – 05/31/14
NIH/NCI

Genotype-Tissue Expression Project
The primary mission of the GTEx project is to collect and analyze up to 50 tissues from each of approximately 250 deceased donors using methods specially developed to preserve tissues of the highest quality with the lowest possible *post mortem* interval.

Health Research Formula Fund Award (PI: Lonsdale) 1/1/2011 – 06/30/2013
Health Research Formula Fund Award (PI: Lonsdale) 1/1/2013 – 12/31/2013
Pennsylvania State

T1D Microvascular Complications: Defining Phenotypes to Identify Susceptibility Loci in Candidate Genes
This project will test for associations between specific genes and the presence or absence of complications in individuals with type 1 diabetes with a major goal of better defining the genetic contribution to complications.