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**

2. **Reporting Period (start and end date of grant award period):** 1/1/2012-12/31/2012

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:** 4100057671

6. **Project Number and Title of Research Project:** Genetic Variants that Affect Susceptibility to Microvascular Complications of Diabetes

7. **Start and End Date of Research Project:** 1/1/2012-12/31/2012

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:

   $ 59,685

   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	Position Title	% of Effort on Project	Cost
Lonsdale	Principal Investigator	18	$31,282.38
Cohen	Coordinator	100	$18,313.62

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	Position Title	% of Effort on Project
None		

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 I Diabetic Complications	X NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify: _____)	Feb. 2013	$499,999	$

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

Yes X No_____

If yes, please describe your plans:
If the NIH grant mentioned above does not get funded, we will re-apply. We may also apply to the JDRF.

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

The current research project on linkage in T1D complications arises out of this work.

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_____ No X

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

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

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				

Non-Hispanic				
Unknown				
Total				

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

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_____ No X

If yes, please describe the collaborations:

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.

Aim 1) Determine whether specific HLA DR/DQ alleles or type 1 diabetes (T1D)-related Human Leukocyte Antigen (HLA) susceptibility haplotypes influence the development of microvascular complications of diabetes (MCD) among T1D patients.

In the work detailed below, we show that specific HLA alleles/haplotypes influence the risk for diabetic complications.

Type 1 diabetes represents a major health problem, and data show that its prevalence is rising [1]. By year 2030, over 3 million people are predicted to have type 1 diabetes in the United States alone [2]. As more people develop type 1 diabetes, the prevalence of the associated complications also increases. The major pathologies related to type 1 diabetes are the chronic microvascular complications: retinopathy, nephropathy, and neuropathy. These complications are responsible for much of the morbidity and mortality in patients, leading to blindness, end-stage renal disease, neuropathy and consequent amputation in many patients. Previous work has shown that while the type 1 diabetes-associated complications may be the result of persistent high blood sugar, they are also familial, suggesting the existence of a genetic contribution to these phenotypes [3-6]. However, the findings from studies of the genetics of microvascular complications are inconclusive and controversial [7-22].

Type 1 diabetes is a complex, autoimmune disease in which dendritic cells, macrophages, $CD4^+$ and $CD8^+$ T lymphocytes infiltrate the pancreas and destroy the insulin-producing β cells in the islets of Langerhans [23]. The Human Leukocyte Antigen (HLA) region on chromosome 6p21 is the major susceptibility locus for type 1 diabetes. The class II loci, *HLA–DRB1, -DQA1* and *-DQB1,* have the strongest effects on type 1 diabetes risk. Specifically, the haplotypes with the highest risk for type 1 diabetes among Europeans are DRB1*03:01-DQA1*05:01-DQB1*02:01 / DRB1*04:01-DQA1*03:01-DQB1*03:02 [24,25]. The class I HLA genes have also been implicated in type 1 diabetes risk, but these alleles have smaller effects on type 1 diabetes than do the class II HLA alleles [26]. While the influence of HLA on type 1 diabetes is well known [27], their role in the development of microvascular complications is less clearly understood. Some studies have reported significant associations of retinopathy or nephropathy with HLA class I or II alleles [7,8,14-22], while other studies have failed to report such associations [9-13].

In this study, we examined the association of HLA alleles with type 1 diabetes-related complications in a large Caucasian cohort. We report newly observed associations of complications with specifically chosen HLA alleles. These hypothesis-driven statistical associations may shed light on genetic influences that affect susceptibility to complications.

Materials and methods

Family identification and data collection

Families were ascertained through the presence of at least one family member with type 1 diabetes (the "proband"); most families were multiplex for type 1 diabetes, i.e., there were at least two affected offspring per family. Human Biological Data Interchange (HBDI) designated probands were used as the proband cases and controls. Families were invited to be part of the

Human Biological Data Interchange data collection through a series of advertisements sent to the entire mailing list of the Juvenile Diabetes Foundation International (JDFI) during the period 1988–1990 [28]. All member families were asked to complete a standardized confidential questionnaire sent by mail and the responses were added to the HBDI database. The questionnaire was administered to the proband (or parents, if the proband was a child) and also to additional family informants. Inquiries included demographic, medical, genealogical, and familial information about complications. Informed consent was obtained.

HBDI data

Our dataset included 425 families with cases diagnosed with type 1 diabetes before age 30. There were 2506 family members included from the HBDI database as of the end of 2004. Families were selected for inclusion in the HBDI sample based on the presence of at least one type 1 diabetes patient per family. Multiplex (> 1 case per family) families were preferentially sought. In this sample, all participants were diagnosed with type 1 diabetes. All patients in this study sample are Caucasian. A total of 49% of all subjects were female. We emphasize that, for this study, only 1 individual (the proband) per family was used in the analyses.

Assessment and definition of diabetes and diabetic complications

We included only patients with type 1 diabetes diagnosed before 30 years of age who required insulin treatment. The accuracy of the self-reported information with respect to presence/absence of complications (*e.g.* presence of retinopathy, yes or no) was evaluated by:

1) Including extra questions about related conditions in the questionnaire. The presence of macular edema or complete or partial blindness were considered indicators of retinopathy; the presence of end- stage renal failure, kidney failure, or repeated high urinary albumin levels were considered indicators of nephropathy. In cases of inconsistencies (*e.g.* presence of macular edema but not retinopathy), further investigations were carried out through phone interviews, around the time of data collection.

2) Data available from follow-up were used to confirm or update the presence/absence and progression of complications. Starting in 2004, follow-up questionnaires have been periodically sent to a subset of families to obtain updated information about development of complications, new cases of diabetes, and related medical history data, with 1000-2000 families targeted each year (for further description, please see (3)).

3) Collecting medical records. For the subset of patients [n=179] with medical records available, the presence of type 1 diabetes and complications was verified according to American Diabetes Association guidelines [29-32].

4) Information indicating *absence* of a complication in a subject was considered reliable only if the subject was without that complication for at least 15 years after type 1 diabetes onset.

Type 1 diabetes subjects and complications

Of the 425 probands in the sample, 128 had at least one complication, and 297 were free of complications. The majority of cases that had any complication had retinopathy (93.0%); fewer cases had nephropathy or neuropathy (Table 1).

Study design

We used a case-control study design nested on the cohort of the HBDI type 1 diabetes patients and, for some analyses, their affected siblings. The probands with at least one microvascular

complication were considered cases, and the probands without microvascular complications were considered controls. Every proband, whether case or control, has type 1 diabetes.
To identify genetic risk factors for microvascular complications, the presence of an allelic risk predictor was considered the "exposure." The outcome variable was defined as the presence of any microvascular complication(s). Analyses were also done treating retinopathy, nephropathy and neuropathy as separate outcomes. However, results for nephropathy and neuropathy are not reported due to small sample sizes. We included sex, age at type 1 diabetes diagnosis, and duration of type 1 diabetes as covariates to control for environmental factors that may influence the development of microvascular complications.

HLA genotyping
Genotyping of the HBDI cohort was performed by sequence-specific oligonucleotide probe (SSOP) technology and has been described previously [33-36]. Briefly, relevant polymorphic exons for each locus (exon 2 for class II alleles and exons 2 and 3 for class I alleles) were amplified by polymerase chain reaction with biotinylated primers, denatured, and hybridized to an array of unlabeled oligonucleotide probes (corresponding to known polymorphic sequence motifs) on a backed nylon membrane. Hybridization was visualized with a colorimetric detection system, and probe binding patterns were interpreted using Sequence COmpilation and REarrangement software (SCORE™) [37]. The HBDI collection was included as one of the extant cohorts in Type 1 Diabetes Genetics Consortium (T1DGC), and the HBDI samples were re-genotyped at higher resolution, with updated SSOP reagents, to ensure uniformity of resolution in the HLA genotyping data in the T1DGC [24,26,38,39].

Statistical analysis
We performed logistic regression among type 1 diabetes probands to determine associations with microvascular complications. We calculated odds ratios (ORs) and 95% confidence intervals (CIs), adjusting for sex, age of type 1 diabetes diagnosis (using 5-year intervals) and duration of type 1 diabetes. The duration variable was split into intervals of having type 1 diabetes for 0-29 yrs, 29-38 yrs, >38 yrs (approximately equal numbers of individuals in each category). As with an earlier study [3], we included sex as a covariate in the logistic regression. Age at type 1 diabetes diagnosis and duration were also included as covariates since these factors may be influential for the onset and development of complications. HLA alleles were included in the regression models as independent predictors for microvascular complications. Each HLA allele or (in the case of DQ-encoding loci) haplotype encoding the heterodimeric protein (e.g., DRB1*03:01, DRB1*04:01, DQA1*05:01-DQB1*02:01, DQA1*03:01-DQB1*03:02, B*39:06, B*44:02) was analyzed in separate regression models. A two tailed test was used and a $p < 0.05$ was considered statistically significant. All of the analyses were performed using the statistical package Stata 10.1 (Stata Corp., College Station, TX, 2003).

Multiple Testing
Correction for multiple tests was not required in our primary analysis of four HLA factors (2 *DRB1* alleles and 2 *DQA1-DQB1* haplotypes): DRB1*03:01 and DRB1*04:01 alleles and the DQA1*03:01-DQB1*03:02 and DQA1*05:01-DQB1*02:01 haplotypes. These four hypotheses were chosen *a priori* on the basis of prior knowledge that these four HLA factors are strongly associated with type 1 diabetes.

In an exploratory analysis of the HLA class I loci, multiple alleles were tested with the aim of generating hypotheses that could be tested in a larger follow-up study. These tests were based upon either the high prevalence of a particular allele in the population or prior association of type 1 diabetes and a particular allele. Six alleles were tested based on the high prevalence in the population (>30%) or because of prior knowledge: A*01:01, A*02:01, B*08:01, B*39:06, B*44:02, C*07:01.

Aim 2) Determine whether specific HLA DR/DQ alleles or susceptibility haplotypes influence the development of retinopathy, nephropathy, and neuropathy as separate phenotypes and analyze the distribution of HLA subtypes among affected-unaffected sib pairs to examine the familiality of our findings.

Patient characteristics

The clinical and familial characteristics of our study population are summarized in Table 1. We performed χ^2 test for gender and Student's t-test for duration of type 1 diabetes. For the 425 type 1 diabetes probands in the study, the mean durations of type 1 diabetes in complications cases (n=128) and controls (n=297) were 39.4 +/- 8.90 years and 31.2+/- 9.71 years, respectively. Duration of diabetes showed statistically significant differences between cases and controls. However, according to Student's t-test, age of type 1 diabetes diagnosis did not show a statistically significant difference, nor did gender, according to the Chi square test.

MHC Class II genes analyses

The distribution of HLA DRB1*03:01, DRB1*04:01, DQA1*05:01-DQB1*02:01, and DQA1*03:01-DQB1*03:02 alleles/haplotypes among the probands is shown in Table 2. Among the 425 probands, 19% of probands did not express either DRB1*03:01 or DRB1*04:01 alleles and 14% had neither the DQA1*05:01-DQB1*02:01 nor DQA1*03:01-DQB1*03:02 haplotype (data not shown). Sixty-two percent of probands had at least one DRB1*03:01 allele, and 49% of probands had at least one DRB1*04:01 allele. Sixty-five percent were positive for at least one DQA1*03:01-DQB1*03:02 and 58% for at least one DQA1*05:01-DQB1*02:01 haplotype.

MHC Class I genes analyses

We then sought to determine if there was an association of specific HLA class 1 genes with the presence of microvascular complications. We chose six independent class I risk alleles based on prior knowledge related to T1D or because of the high prevalence of these alleles in our study population; only the HLA-B*39:06 allele demonstrated a significant influence on susceptibility to complications after adjusting for covariates (Tables 2 & 3).

HLA-B*39:06

After adjusting for covariates in multivariable logistic regression models, the HLA-B*39:06 allele showed a notable increased risk for one or more complications (OR=3.27, 95% CI 1.36-7.89), and for retinopathy alone (OR=3.34, 95% CI 1.34-8.30). We also observed elevated risks for nephropathy alone and neuropathy alone, but these were not statistically significantly (data not shown).

To summarize: We found that the HLA DRB1*03:01 allele, and/or alleles at other (unknown) loci that are in linkage disequilibrium with that allele, protects against development of

complications. DRB1*04:01 appears to have no effect on complications risk. Also, although it was not part of our original aims, we took the opportunity to examine specific alleles in the HLA class I A and B loci.

Aim 3) Perform case-control association analysis as well as family-based association analysis to identify alleles that predispose T1D patients to develop MCD.

We performed a combined case-control and family data analysis, which increased the statistical significance of our finding for DRB1*03:01 showing the presence of this allele offers protection against microvascular complications.

Table 2 provides unadjusted and adjusted ORs for the presence of one or more microvascular complications (e.g., retinopathy, nephropathy, neuropathy) using specific HLA alleles as predictor variables. Table 3 shows the unadjusted and adjusted ORs for retinopathy alone. The adjusted estimates are controlled for sex, age at type 1 diabetes diagnosis, and duration of type 1 diabetes. Using a multivariable logistic regression model, adjusting for covariates, the presence of a DRB1*03:01 allele was protective both for one or more microvascular complications (OR=0.58, 95% CI 0.35-0.95) and for retinopathy alone (OR=0.58, 95% CI 0.35-0.96), which was the most frequent complication in the dataset. In the multivariable logistic regression model for DRB1*04:01, no relationship between DRB1*04:01 and microvascular complications was found for either retinopathy alone (OR=1.17, 95% CI 0.72-1.92) or for one or more microvascular complications (OR=1.13, 95% CI 0.70-1.81). (We also conducted a series of comparable nonparametric analyses, and the findings were qualitatively unchanged (data not shown).)

In investigating the family data, we applied our new genetic analysis software package which includes POPFAM—a generalized test of association that combines population-based and family-based information to increase power. We re-tested the association between the DRB1 alleles and microvascular complications, expanding the reported proband-based analysis to include 401 type 1 diabetic families. We used a nonparametric bootstrap procedure to estimate the distribution of our combined-test statistic (implemented in the program POPFAM (http://www.mathmed.org/wclstewart)). The combined-test compares 1) the transmission rates of DRB1*03:01 alleles between those offspring with and without microvascular complications, then 2) uses logistic regression to compute the slope coefficient for the alleles, DRB1*03:01 and not-DRB1*03:01, in cases and controls. Then, both lines of evidence are then combined through a weighted average that accounts for the correlation between the family-based and population-based components.

Thus, unlike the results for DRB1*03:01, the presence of DRB1*04:01 shows little evidence of influence on the risk for complications. We observed a similar trend (i.e., DRB1*03:01 appears mildly protective while DRB1*04:01 appears neutral) for nephropathy, but the OR did not reach statistical significance. Such a trend was not observed for neuropathy, but the sample size precluded detecting all but the strongest effects.

We examined the association of DQ haplotypes with the risk for one or more microvascular complications and the risk for retinopathy alone (Tables 2 & 3). DQA1*05:01-DQB1*02:01

(which is in linkage disequilibrium with DRB1*03:01) was significantly protective for the presence of one or more complications (OR=0.59, 95% CI 0.37-0.95) and for retinopathy (OR= 0.58, 95% CI 0.36-0.95). Because every individual with a DQA1*05:01-DQB1*02:01 haplotype also had a DRB1*03:01 allele (except for three individuals who were DRB1*03:01-positive and DQA1*05:01-DQB1*02:01-negative), the strong linkage disequilibrium between these two alleles means it is difficult to determine the origin of the protective effect. There was no influence of DQA1*03:01-DQB1*03:02 on either retinopathy or one or more complications. Analyses using randomly chosen type I diabetes siblings, instead of probands, gave similar results.

Our results suggest that, in type I diabetes, HLA- DRB1*03:01 or DQA1*05:01-DQB1*02:01 (or an allele in linkage disequilibrium with these alleles) *protects* against the presence of complications. The evidence is strongest for protection specifically against retinopathy.

MHC Class II genes and complications risk: Current study and past work
In our covariate-adjusted models, both DRB1*03:01 and DQA1*05:01-DQB1*02:01 were significant protective factors both for the presence of more than one microvascular complication (Table 2) and retinopathy alone (Table 3), although it is likely that the effect we see in our data arises mostly from the retinopathy phenotype. Analyses of the DRB1*04:01 allele, on the other hand, suggest its presence influences the risk for complications. Cruickshanks et al. [22] reported an association of retinopathy with HLA- DRB1*04:01, *among those negative for HLA-DRB1*03:01* (DRB1*04:01/X, X≠ DRB1*03:01), an observation similar to one seen in our analyses. Cruickshanks et al. found that type 1 diabetes patients with HLA- DRB1*04:01, who were negative for HLA- DRB1*03:01 were significantly more likely to have proliferative retinopathy (OR=5.43, 95% CI 1.04-28.30) than those negative for both alleles. However in a follow-up study, Wong et al. [13] investigated the effect of HLA- DRB1*03:01 and DRB1*04:01 on the development of diabetic retinopathy and they failed to observe a relationship between HLA- DRB1*03:01 or DRB1*04:01 and diabetic retinopathy. Dornan et al. [17] reported that DRB1*04:01 was a risk allele for retinopathy. The Cruickshanks et al. [22] study and our study also found DRB1*04:01 was a risk allele but only in subjects without DRB1*03:01. Jensen et al. [16] examined the effect of DRB1*03:01-DQA1*05:01-DQB1*02:01 and DRB1*04:01- DQA1*03:01-DQB1*03:02 haplotypes and the risk of retinopathy after 15 years of type 1 diabetes duration. Consistent with our findings, they observed that DRB1*03:01-DQA1*05:01-DQB1*02:01 is protective, but their findings were not statistically significant. They also reported, as we found here, that the DRB1*04:01-DQA1*03:01-DQB1*03:02 haplotype was neither a risk factor nor protective for developing retinopathy, although they did not examine the effect of the DRB1*04:01 allele without the presence of the DRB1*03:01 allele. Contrary to our findings and to those by Jensen et al., Agardh et al. [7] reported that the DRB1*03:01-DQA1*05:01-DQB1*02:01 haplotype was more frequent in patients with severe retinopathy. Concerning nephropathy, Svejgaard et al. [18] and the GoKinD study [8] reported that DRB1*04:01 was a protective allele for nephropathy. While we did not identify DRB1*04:01 as a protective allele for any of the complications, Svejgaard et al. and the GoKinD work do support the notion that HLA is involved in the development of microvascular complications. Other studies, however, failed to report any association of DRB1*03:01 or DRB1*04:01 with either retinopathy or nephropathy [9-15].

Thus, the earlier literature is somewhat contradictory, although most of the studies report an association of HLA class II alleles with some complications.

To discuss all the possible reasons for these contradictory results is beyond the scope of this work. However, differences in ascertainment, whether one examines "retinopathy" or "proliferative retinopathy", the definition of which diabetes patients are cases and which are controls, as well as analysis techniques used, all play a role. For example, in our sample, it may be that severe retinopathy was more likely to be noted in a self-report than mild retinopathy. The GoKinD study [8] and Rogus et al.'s [40] samples defined controls as not having nephropathy after at least 10 years diabetes duration. Our controls had type 1 diabetes for at least 15 years and 90% had diabetes for more than 20 years. Heitala et al. [41] included patients with type I diabetes onset age greater than 35 years. Our sample is one of the few that used probands from families multiplex for type I diabetes. The absence of retinopathy among the controls in these genetically loaded families suggests genetic factors played a greater role in protection from complications because of the strong evidence that inherited factors influence risk for complications [3-6]. Also, environmental factors such as smoking status that may influence the development of complications, smoking status is unlikely to be associated with any particular HLA allele or haplotype. Thus, while there can be several explanations for the contradictory results in the literature, the number of studies finding association with HLA class II alleles and complications strongly suggest that such an effect exists.

*Effect of DRB1*03:01 vs. DRB1*04:01*

Since DRB1*03:01 appears to have a protective effect on the risk of complications, we investigated the effect of DRB1*04:01 on its own, in the absence of DRB1*03:01 (i.e., excluding individuals who were heterozygous for DRB1*03:01 and DRB1*04:01). In an adjusted multivariable regression model, we observed a stronger positive association between DRB1*04:01 and the risk for retinopathy (closer to, but not reaching, statistical significance; OR=1.74, p-value=0.069; data not shown) compared with a model in which heterozygous DRB1*03:01/ DRB1*04:01 individuals were included. The failure to reach significance could be due to decreased sample size, because a quarter of the study population was heterozygous for the excluded DRB1*03:01/ DRB1*04:01 genotype. In a further examination, when stratifying on DQA1*03:01-DQB1*03:02, we observed that the DRB1*04:01 allele was associated with an even greater elevated risk of retinopathy. In the absence of DQA1*03:01-DQB1*03:02, DRB1*04:01 becomes a significant risk factor for the risk of retinopathy with borderline statistical significance (OR=2.67, 95% CI 0.94-7.60). In the presence of DQA1*03:01-DQB1*03:02, the DRB1*04:01 allele does not influence risk of complications (data not shown). While the sample size is too small to confidently assert this putative risk effect of DRB1*04:01 (17 DRB1*04:01 individuals in the absence of DQA1*03:01-DQB1*03:02), it is worthy of conducting further research to investigate whether an increased risk exists.

MHC Class I genes

After covariate adjustment, we found that the presence of the HLA-B*39:06 allele was associated with an elevated risk for both retinopathy alone and for the presence of one or more complications. HLA-B*39:06 has also been reported to be associated with the risk for type 1 diabetes risk, whether conditioned on the class II DR-DQ alleles or not [26,36,42]. However, no previous study has identified this allele as a risk factor for complications [14].

Other studies have reported associations between different class I alleles and microvascular complications in the Japanese population [14,20,21]. In an earlier study, Nakanishi et al. reported an association between HLA-A24 and retinopathy [20], and in a more recent study, Nakanishi et al. reported that the HLA-A24 allele was associated with early beta cell loss and with early development of diabetic retinopathy [21]. Mimura et al. investigated the relationship between HLA and proliferative diabetic retinopathy (PDR) and reported a higher frequency of the HLA-B62 and Cw4 alleles among type 1 diabetics with PDR compared with the non-PDR group [14]. These findings aside, the relationship between HLA class I alleles and complications has not been widely explored. Although among studies reporting associations, findings have been inconclusive [11,12,14,20,21].

Effect of age-of-onset of type I diabetes

Onset of complications is influenced by type 1 diabetes duration, and previous research suggests that age at onset and progression to type 1 diabetes are directly linked to the MHC class II genes [43]. Early age of type 1 diabetes onset is commonly associated with the high risk haplotypes HLA DRB1*03:01-DQA1*05:01-DQB1*02:01 and DRB1*04:01-DQA1*03:01-DQB1*03:02, especially the very high risk heterozygous genotype comprised of these two haplotypes [44,45]. This association with early onset suggests a stronger genetic predisposition to disease than other haplotypes [45]. The majority of patients who develop some degree of retinopathy do so by 15-20 years duration of type 1 diabetes [31,46]. Up to 40-50% of patients develop nephropathy or neuropathy within 15-20 years of the onset of type 1 diabetes [46]. Among probands in the current study, the average duration of type 1 diabetes was almost 34 years; the average age of diagnosis for type 1 diabetes was approximately 9 years of age. On average, the duration of type 1 diabetes in our sample exceeds the peak risk of 15-20 years for both case and control probands and thus variation in duration is unlikely to influence these analyses. In our data, DRB1*03:01-positive subjects do not have later onset of disease than DRB1*03:01-negative subjects; however, we retained the duration variable in the adjusted models because cases had a significantly longer duration of disease than controls.

Advantages and limitations

Our study examined *complications* as the phenotype of interest because complications are ultimately responsible for much of the morbidity and mortality seen with type 1 diabetes. Other HLA studies that have examined complications suffer from small sample sizes. Our work has the benefit of using one of the larger type 1 diabetes *multiplex family-based* datasets in the world (meaning that genetic factors may be more prominent among the subjects used in our study), a data set that also has information on all three microvascular complications. One limitation in our study is that we do not have HbA1c measurements or data on other environmental factors such as smoking status that may influence the development of complications, although smoking status is unlikely to be associated with any particular HLA allele or haplotype. Previous work has established that reducing blood glucose concentrations close to normal glycemic ranges also significantly reduces the incidence of diabetes-associated complications (although, in a recent study of T2D, tight control appears to increase mortality [47,48]). However, recent reports indicate that HbA1c may not adequately explain the risk for complications. Some patients with poor glycemic control do not develop complications, and some with good glycemic control develop complications [4,49,50]. The literature indicates that HbA1c may not be a necessary

predictor of complications and consequently it does not impede our ability to detect genetic risk factors for complications. Further, while our data include information on the presence/absence of complications, we lack information on the age of onset of complications. Information on age of onset would enable more accurate analyses. Survival analysis, for example, would be more powerful than a case-control design; however, we are precluded from doing survival analysis because we have no data on the timing of complications' onset. Nonetheless, our current data provide a solid indication that genes influence the expression of complications and suggest that HLA plays a role in risk.

In conclusion, these data indicate that, in addition to their strong association with disease susceptibility, HLA alleles and haplotypes are also associated with microvascular complications of type 1 diabetes. The formal possibility exists that the classical HLA loci themselves are not involved, but that alleles at other loci in linkage disequilibrium with the diabetes risk alleles are responsible for our findings. Lastly, further research needs to be conducted in separate study populations to validate these findings. Confirmation of these results could provide greater insights into the mechanisms leading to the development of microvascular complications. Ultimately, the findings of our study could lead to the ability to stratify risk of developing microvascular complications in type 1 diabetes patients.

Aim 4) Continue follow-up on identified T1D families. We will continue our annual follow-up program to track the development and progression of MCD in families over time.

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 871 questionnaires to participants in 2012 and have received 301 completed questionnaires/updates from individual participants, corresponding to a 35% response rate. These updates provide information on 4,298 individuals in the HBDI National Genetics Family Registry. Twenty-seven questionnaires were returned due to incorrect addresses. Of these, we were able to send questionnaires to different family members in 21 cases, thus only 6 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 490 individuals, with success on 88 cases (18%). 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.

Since 2008 we have updated a total of 22,469 individuals from the National Genetics Family Registry. In regards to the 2,700 participants who have been immortalized in the NDRI National Genetics Family Repository, we have updated information for 1,873 individuals (69%) since 2008. 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.

Table 1. Characteristics of type 1 diabetes by numbers of total proband cases and controls[a].

Characteristic	Cases	Controls
No. of subjects (in 425 families)	128	297
Females (n, %)	60 (46.9)	132 (44.4)
Age of type 1 diabetes diagnosis (yr±SD)	8.7 ± 5.3	9.1 ± 7.1
Duration of type 1 diabetes [yr±SD][b]	39.4 ± 8.9	31.2 ± 9.7
Retinopathy (n, %)	119 (93.0)	N/A
Nephropathy (n, %)	46 (35.9)	N/A
Neuropathy (n, %)	35 (27.3)	N/A
>1 complication (n, %)	54 (42.2)	N/A

[a]'Case' refers to a proband with type 1 diabetes and at least 1 microvascular complication. 'Control' refers to a proband with type 1 diabetes only and no history of microvascular complications.
[b]Statistically significant difference between cases and controls according to Student's t-test.

Table 2. Results of logistic regression models for one or more microvascular complications among probands with T1D.

Allele/haplotype	Total probands N (%)	Cases N (%)	Controls N (%)	Unadjusted OR (95% CI), p-value	Adjusted OR* (95% CI), p-value
No. of DR-typed subjects	**400**	**120**	**280**		
DRB1*03:01-negative	151 (37.7)	54 (45.0)	97 (34.6)	Referent	Referent
DRB1*03:01-positive	249 (62.3)	66 (55.0)	183 (65.4)	0.65 (0.42, 0.051	0.58 (0.35, 0.030
DRB1*04:01 -negative	205 (51.3)	57 (47.5)	148 (52.9)	Referent	Referent
DRB1*04:01 -positive	195 (48.8)	63 (52.5)	132 (47.1)	1.24 (0.81, 0.326	1.13 (0.70, 0.624
No. of DQ-typed subjects	**425**	**128**	**297**		
DQA1*03:01-DQB1*03:02-negative	150 (35.3)	47 (36.7)	103 (34.7)	Referent	Referent
DQA1*03:01-DQB1*03:02-positive	275 (64.7)	81 (63.3)	194 (65.3)	0.92 (0.59, 0.687	0.81 (0.49, 0.390
DQA1*05:01-DQB1*02:01-negative	179 (42.1)	63 (49.2)	116 (39.1)	Referent	Referent
DQA1*05:01-DQB1*02:01-positive	246 (57.9)	65 (50.8)	181 (60.9)	0.66 (0.44, 1.00), 0.052	0.59 (0.37, 0.031
No. of HLA-B-typed subjects	**425**	**128**	**297**		
HLA-B*39:06-negative	393 (92.5)	115 (89.8)	278 (93.6)	Referent	Referent
HLA-B*39:06-positve	32 (7.5)	13 (10.2)	19 (6.4)	1.65 (0.79, 0.182	3.27 (1.36, 0.008

* Adjusted for sex, age at type 1 diabetes diagnosis, duration of type 1 diabetes

Table 3. Results of logistic regression models for retinopathy among probands with T1D.

Allele/haplotype	Total probands N (%)	Cases N (%)	Controls N (%)	Unadjusted OR (95% CI), p-value	Adjusted OR* (95% CI), p-value
No. of DR-typed subjects	**400**	**120**	**280**		
DRB1*03:01-negative	151 (37.7)	50 (45.1)	101 (35.0)	Referent	Referent
DRB1*03:01-positive	249 (62.3)	61 (55.0)	188 (65.1)	0.66 (0.42, 1.02), 0.063	0.58 (0.35, 0.96), 0.031
DRB1*04:01 -negative	205 (51.3)	52 (46.9)	153 (52.9)	Referent	Referent
DRB1*04:01 -positive	195 (48.8)	59 (53.2)	136 (47.1)	1.28 (0.82, 1.98) 0.275	1.17 (0.72, 1.92), 0.520
No. of DQ-typed subjects	**425**	**128**	**297**		
DQA1*03:01-DQB1*03:02-negative	150 (35.3)	43 (36.1)	107 (35.0)	Referent	Referent
DQA1*03:01-DQB1*03:02-positive	275 (64.7)	76 (63.9)	199 (65.0)	0.95 (0.61, 1.48) 0.821	0.85 (0.51, 1.41) 0.531
DQA1*05:01-DQB1*02:01-negative	179 (42.1)	59 (49.6)	120 (39.2)	Referent	Referent
DQA1*05:01-DQB1*02:01-positive	246 (57.9)	60 (50.4)	186 (60.8)	0.66 (0.43, 1.00), 0.053	0.58 (0.36, 0.029
No. of HLA-B-typed subjects	**425**	**128**	**297**		
HLA-B*39:06-negative	384 (92.5)	107 (89.8)	278 (93.6)	Referent	Referent
HLA-B*39:06-positve	31 (7.5)	13 (10.1)	19 (6.4)	1.60 (0.76, 0.217	3.34 (1.34, 0.01

*Adjusted for sex, age at type 1 diabetes diagnosis, duration of type 1 diabetes

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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, the number of the publication and

an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

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 1diabetes	EM Lipner, Y Tomer, JA Noble, MC Monti, JT Lonsdale, B Corso, WCL Stewart & DA Greenberg	Human Immunology	July 2012	☐Submitted ☐Accepted XPublished
2.				☐Submitted ☐Accepted ☐Published
3.				☐Submitted ☐Accepted ☐Published

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

Yes_____ No X

If yes, please describe your plans:

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

Please refer to the application instructions in order to complete sections A, B, C, and D of the Biographical Sketch.

A. Personal Statement

Dr. Lonsdale has extensive experience and expertise in all aspects of the provision of human tissues for research. Dr. Lonsdale has extensive experience as PI/Project Leader on numerous research projects and in providing scientific and administrative oversight, including as PI on the Genome Tissue Expression (GTEx) project, the Biospecimen Methodological Study (BMS) and PA State Formula Research Grants since 2003.

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