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# HLA Class I and II Alleles are Associated with Microvascular Complications of Type 1 Diabetes

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## Abstract

Although HLA alleles are associated with type 1 diabetes, association with microvascular complications remains controversial. We tested HLA association with complications in multiplex type 1 diabetes families.

Probands from 425 type 1 diabetes families from the Human Biological Data Interchange (HBDI) collection were analyzed. The frequencies of specific HLA alleles in patients with complications were compared with the frequencies in complications-free patients. The complications we examined were: retinopathy, neuropathy, and nephropathy. We used logistic regression models with covariates to estimate odds ratios.

We found that the DRB1*03:01 allele is a protective factor for complications (OR=0.58; p = 0.03), as is the DQA1*05:01-DQB1*02:01 haplotype found in linkage disequilibrium with DRB1*03:01 (OR= 0.59; p = 0.031). The DRB1*04:01 allele showed no evidence of association (OR=1.13; p = 0.624), although DRB1*04:01 showed suggestive evidence when the carriers of the



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protective DRB1*03:01 were removed from the analysis. The class II DQA1*03:01-DQB1*03:02 haplotype was not associated with complications, but the class I allele B*39:06 (OR=3.27; P = 0.008) suggested a strong positive association with complications.

Our results show that in type 1 diabetes patients, specific HLA alleles may be involved in susceptibility to, or protection from, microvascular complications.

### Keywords

Retinopathy; nephropathy; neuropathy; microvascular complications; genetics; type 1 diabetes; logistic regression; association; HLA

## 1. Introduction

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 US alone [2], (American Diabetes Association). 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 (see Discussion) [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.

## 2. Materials and methods

### 2.1 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. HBDI designated probands were used as the proband cases and controls. Families were invited to be part of the Human Biological Data

Interchange (HBDI) 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.

### 2.2 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.

### 2.3 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.

### 2.4 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).

### 2.5 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.

### 2.6 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 COmpliation 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].

### 2.7 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).

### 2.8 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.

## 3. Results

### 3.1 Patient characteristics

The clinical and familial characteristics of our study population are summarized in Table 1. We performed $\chi^2$ 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. The difference in mean duration of diabetes between cases and controls was statistically significant ($p < 0.0001$), as was the difference in medians (data not shown). 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 $\chi^2$ test.

### 3.2 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.

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).)

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.

### 3.3 MHC Class I genes analyses

To better guide future studies involving the genetics of microvascular complications, we sought to identify specific HLA class 1 genes that might warrant further consideration. 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).

## 4. Discussion

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.

### 4.1 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 for 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 is 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]. 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.

### 4.2 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.

### 4.3 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].

### 4.4 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.

### 4.5 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.

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## 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[c] (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, 1.00), 0.051 | 0.58 (0.35, 0.95), 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, 1.90), 0.326 | 1.13 (0.70, 1.81), 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, 1.41), 0.687 | 0.81 (0.49, 1.32), 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, 0.95) 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, 3.46) 0.182 | 3.27 (1.36, 7.89) 0.008 |

[c]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[a] (95% CI), *p*-value |
|---|---|---|---|---|---|
| No. of DR-typed subjects | 400 | 111 | 289 | | |
| DRBl *03:01-negative | 151 (37.7) | 50 (45.1) | 101 (34.9) | Referent | Referent |
| DRBl *03:01-positive | 249 (62.3) | 61 (54.9) | 188 (65.1) | 0.66 (0.42, 1.02), 0.063 | 0.58 (0.35, 0.96), 0.031 |
| DRBl *04:01 -negative | 205 (51.3) | 52 (46.8) | 153 (52.9) | Referent | Referent |
| DRBl *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 | 119 | 306 | | |
| DQAl *03:01-DQBl *03: 02-negative | 150 (35.3) | 43 (36.1) | 107 (35.0) | Referent | Referent |
| DQAl *03:01-DQBl *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 |
| DQAl *05:01-DQBl *02: 01-negative | 179 (42.1) | 59 (49.6) | 120 (39.2) | Referent | Referent |
| DQAl *05:01-DQBl *02: 01-positive | 246 (57.9) | 60 (50.4) | 186 (60.8) | 0.66 (0.43, 1.00), 0.053 | 0.58 (0.36, 0.95), 0.029 |
| No. of HLA-B-typed subjects | 416 | 119 | 297 | | |
| HLA-B*39:06-negative | 385 (92.5) | 107 (89.9) | 278 (93.6) | Referent | Referent |
| HLA-B*39:06-positive | 31 (7.5) | 12 (10.1) | 19 (6.4) | 1.64 (0.77, 3.50), 0.199 | 3.34 (1.34, 8.30), 0.01 |

[a]Adjusted for sex, age at type 1 diabetes diagnosis, duration of type 1 diabetes.