# Association Between Type 1 Diabetes Age of Onset and HLA Among Sibling Pairs

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**In this study, we report type 1 diabetes age-of-onset association with HLA class II (DRB1, DQB1, and DPB1) and class I (A) genes in 222 multiplex families from the Human Biological Data Interchange. Linear regression showed a small ($R^2$ = 0.26) but significant correlation in the ages of onset among sib pairs. A strong association in age of onset between members of sib pairs was observed when the analysis was performed using contingency tables that split sibs into three age-at-onset ranges (0–10, 11–20, and 21–36 years). The association is strongest for sib pairs that share both haplotypes and is nonsignificant for sib pairs that do not share any DR-DQ haplotypes. A goodness-of-fit test revealed that DR-DQ haplotype sharing cannot account for all the association in age of onset among sib pairs. The age-of-onset distribution of DR-DQ haplotypes is affected by the DPB1 and A alleles present. The strongest associations were found with the A locus: DR3/DR4 genotype frequency decreases with age of onset in this data set only among A*0101$^-$ individuals, and A*2402 is strongly associated with younger age of onset in many DR-DQ haplotypes. *Diabetes* 48:1658–1661, 1999**

Type 1 diabetes is an autoimmune disease characterized by the destruction of insulin-producing β-cells in the pancreas (1). Although multiple genes have been implicated, HLA-linked genes are the major type 1 diabetes susceptibility markers known to date, and, under a multiplicative model, constitute ~53% (2) of the type 1 diabetes genetic component (3,4). It is widely recognized that the major component of HLA type 1 diabetes susceptibility involves DRB1, DQA1, and DQB1 genes (5–9).

Clinical onset of type 1 diabetes is not confined to childhood. Age-dependent HLA heterogeneity has been observed in Caucasian type 1 diabetic patients, indicating that high-risk HLA genotypes occur at a higher frequency among the younger-age-of-onset groups (10), whereas older age at diagnosis is associated with an increase in heterogeneity of DRB1 and a decrease in heterogeneity of DPB1 (11). The heritability for age of onset has been estimated to be 74% in a British twin study (12).

In this study, we explore the HLA contribution to the correlation in age of onset between affected siblings. We also test whether DRB1 and DQB1 can account for all the HLA association with type 1 diabetes age of onset in this data set.

## RESEARCH DESIGN AND METHODS

The Human Biological Data Interchange (HBDI) is a repository for cell lines derived from type 1 diabetic families. The HBDI collection was established in part for the purpose of mapping type 1 diabetes–associated genes by linkage analysis. Most of the HBDI families are nuclear families with unaffected parents and at least two affected siblings (multiplex). In this study, we report age-of-onset association with class II (DRB1, DQB1, and DPB1) and class I (A) alleles. The typing results for 222 families here analyzed are reported elsewhere (2 and A.M.V., G.T., H.A.E., J.A.N., unpublished observations). All of the families analyzed were Caucasian. Only two affected sibs from each family have been included in this analysis. Of the 222 families, 213 are not recombinant for DR-DQ-DP-A. Results reported for DPB1 or A loci refer to a sample size of 213 sib pairs.

## RESULTS

**Age-of-onset distribution.** The mean age of onset in the 444 affected individuals was 12.06 years, with a standard deviation of 8.53, a maximum of 36 years, and a minimum of 6 months. On average, the first (oldest) sib was 2.45 ± 8.82 years older at diagnosis than the second sib (paired $t$ test, $P < 5 \times 10^{-5}$).

**Association between ages of first and second sibs at clinical onset.** The first question addressed in this study is the amount of correlation in age at onset within sib pairs. Linear regressions were performed first on all pairs, then on pairs subdivided by their identity-by-descent (IBD) status, and then according to HLA-DR-DQ status in the share 2 category (Table 1; Fig. 1). The correlation among sibs who share both haplotypes is highly significant, although $R^2$ is small (0.3). The value of $R^2$ does not increase substantially even when looking only at the share 2 category within the genotype DR3-DR4 (Table 1). The correlation levels observed in this study are lower than those reported for 33 British sib pairs by Fava et al. (12) for sib pairs, although this discrepancy could be due to the differences in ascertainment between the studies (Fava et al. excluded sib pairs if one sib was born after the other had developed type 1 diabetes).

To leave out some of the variation contained in plots such as those from Fig. 1, we built contingency tables dividing the data into age-of-clinical-onset intervals: 0–10, 11–20, and 21–36 years. A strong association in age of onset between sibs in a pair was observed. The association is strongest for pairs of sibs that share both haplotypes; it is nonsignificant for those that do not share any DR-DQ haplotypes (Table 2). Given that the $\chi^2$ statistic used depends on sample size, the lack of significance observed among sib pairs whose IBD is 0

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HBDI, Human Biological Data Interchange; IBD, identity by descent.

TABLE 1
Linear regression applied to age of onset in sib pairs affected by type 1 diabetes

| | $R^2$ | Significance of $F$ | Number of pairs |
|---|---|---|---|
| All pairs | 0.134 | $1.9 \times 10^{-8}$ | 222 |
| Share 0 | 0.022 | 0.62 | 14 |
| Share 1 | 0.043 | 0.06 | 81 |
| Share 2 | | | |
| All | 0.260 | $1.04 \times 10^{-9}$ | 127 |
| DR3-DR4 | 0.319 | $2.3 \times 10^{-5}$ | 50 |
| DR4-DR4 | 0.202 | 0.08 | 16 |
| DR3-DR3 | 0.155 | 0.23 | 11 |
| DR3-DRX | 0.001 | 0.89 | 16 |
| DR4-DRX | 0.356 | 0.001 | 27 |
| DRX-DRX | 0.469 | 0.09 | 7 |

DRX refers to DR-DQ haplotypes except those containing DRB1*0301 or any DR4 subtype.

could be due to the small number of pairs analyzed, which is not an issue with IBD 2 and IBD 1 sib pairs. For this reason, we report the average observed-to-expected ratio among sib pairs falling in the same age category (Table 2). Regardless of the significance value, which is sample size–dependent, such a ratio reflects the strength of the actual association in age of onset between sibs in a pair. The results shown clearly indicate that the association in age of onset grows stronger with higher DR-DQ haplotype sharing.

We then examined if the association in age of onset between sib pairs is stronger than what can be explained by the DR-DQ genotype shared. To do so, we computed the expected probabilities under the assumption that DR-DQ genotype sharing accounts for age-at-clinical-onset association, which was our null hypothesis, and then tested for goodness of fit. Let $P_{(0–10)}$ be the frequency among patients with age of onset 0–10 years of genotype G, $P_{(11–20)}$ the frequency among patients with age of onset 10–20 years of genotype G, and $P_{(21–36)}$ the frequency among patients with age of onset 21–36 years of genotype G. Assume further that there are $N$ pedigrees in the data set for which both affected

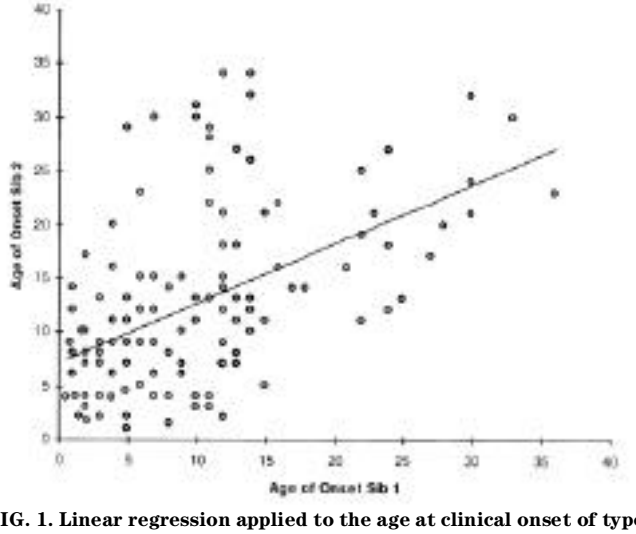


**FIG. 1. Linear regression applied to the age at clinical onset of type 1 diabetes in 127 sib pairs sharing both DR-DQ haplotypes.**

TABLE 2
Association in age of onset between sib pairs as revealed by contingency table analysis

| | $\chi^2$ (4 degrees of freedom) | $P$ value | Number of sib pairs | Observed-to-expected ratio |
|---|---|---|---|---|
| Share 0 | 3.36 | 0.5 | 14 | 0.99 |
| Share 1 | 12.02 | 0.017 | 81 | 1.21 |
| Share 2 | | | | |
| All | 32.65 | $1.4 \times 10^{-6}$ | 127 | 1.68 |
| DR3-DR4† | 16.85 | 0.002 | 50 | 2.01 |
| Non–DR3-DR4† | 18.80 | 0.001 | 77 | 1.58 |

Observed-to-expected ratio is the average ratio of observed to expected number of sib pairs where both sibs fall in the same age range. †Sibs share both haplotypes.

sibs share genotype G (identical by descent). Then, under the null hypothesis, the expected number of sib pairs sharing genotype G where the age of onset of the first sib falls in age range $x$ and that of the second sib in age range $y$ will be $2Np_xp_y$ if $x \neq y$ and $Np_x^2$ if $x = y$, and the $\chi^2$ test for goodness of fit on each 3×3 table, one for each genotype, will have 4 degrees of freedom.

Our results (Table 3) indicate that DR-DQ genotype sharing cannot account for all the age association among sib pairs. Clearly, DR3-DR4 and DR4-DR4 identical sib pairs do not fit under the null hypothesis ($P < 0.026$ and $< 0.047$, respectively), so there must be other genetic and/or environmental factors that are responsible for age-of-onset association between affected sibs. Because we also typed the DPB1 and class I A loci for these sib pairs, we looked at the effect of these loci on age of onset, conditional on the DR-DQ haplotypes present.

**Inclusion of HLA class II DPB1 into the analysis.** Tait et al. (11) reported an increase in DP heterogeneity with increasing age of onset. In principle, we could repeat the analysis performed on DR-DQ genotype–matched sib pairs, matching also for DP. Unfortunately, with the inclusion of DPB1 data, the sample sizes of each genotype are extremely small; consequently such a comparison has little statistical power. We have instead looked at the age distributions of two common DR-DQ haplotypes in patients and stratified them by the associated DPB1 alleles. The results shown in Table 4 illus-

TABLE 3
Goodness-of-fit results to the hypothesis that DRB1-DQB1 genotype sharing accounts for all of the age-of-onset association among sib pairs

| Genotype | Significance level ($P$) |
|---|---|
| DR3/DRB1*0404 DQB1*0302 | <0.018 (4 df) |
| Combined goodness of fit for DR3/DRB1*0401 DQB1*0302 DR3/DRB1*0402 DQB1*0302 DR3/DRB1*0404 DQB1*0302 | <0.026 (12 df) |
| DR4/DR4 | <0.047 (4 df) |
| Non–DR3/DR4 | <0.0032 (4 df) |

df, degrees of freedom.

TABLE 4
Observed haplotype frequencies in three age-of-onset groups stratified by the associated DPB1 allele

| | Age of onset (years) | | |
|---|---|---|---|
| DPB1 | 0–10 | 11–20 | 21–36 |
| DRB1*0101 DQB1*0501† | | | |
| 0401 | 3.9 | 1.0 | 2.8 |
| Other | 1.6 | 2.7 | 3.5 |
| DRB1*0301 DQB1*02‡ | | | |
| 0101 | 8.1 | 7.6 | 4.2 |
| 0201 | 3.5 | 4.5 | 6.3 |
| 0401 | 8.1 | 11.5 | 7.1 |
| 0402 | 0 | 0.7 | 1.4 |
| 1501 | 1.5 | 0.3 | 0 |
| Other | 6.5 | 10.4 | 4.9 |

Data are %. †$P < 0.05$ ($\chi^2$; 2 degrees of freedom); ‡$P < 0.019$ ($\chi^2$; 10 degrees of freedom).

trate how the age-of-onset frequency distribution of the same DR-DQ haplotype can vary depending on the DPB1 allele present. Individual alleles show some interesting trends, but these trends are not necessarily consistent when several DR-DQ haplotypes are considered (data not shown). Although these results do not prove conclusively a role of DPB1 in type 1 diabetes age of onset, they do suggest that alleles at this locus may modulate the DR-DQ effect on the clinical onset of the disease.

**Inclusion of HLA class I A into the analysis.** We looked at DRB1-DQB1 haplotypes in association with alleles at the A locus. The results, summarized in Table 5, indicate that A*2402 has a significant effect on the age-of-onset distribution of DR-DQ haplotypes occurring at a higher frequency among young-age-of-onset patients. Nakanishi et al. (13) have demonstrated the presence of subtle but definite residual β-cell function in Japanese patients with type 1 diabetes of long duration. In their study, 95% of patients without residual β-cell function had this allele. In a recent study of the HBDI type 1 diabetic families, an association of A*2402 was found with type 1 diabetes that could not be attributed to linkage disequilibrium of high-risk DR-DQ haplotypes (J.A.N., A.M.V., G.T., H.A.E. unpublished observations). The involvement of A*2402 with complete β-cell destruction and type 1 diabetes may explain the association of this allele with younger age at onset. Further, A*0101 appears to be associated, although not in a strictly linear way, with older age of onset among DR3 haplotypes.

Several studies have reported a decrease of DR3/DR4 genotypes among older-age-of-onset individuals compared with early-onset patients (10,11,14–16). One interpretation of this finding is that a restricted number of DR and DQ haplotypes are associated with early age at diagnosis, possibly by influencing the nature of β-cell autoimmunity and the rate of progression of β-cell destruction (11). In this data set, however, no trend relating to age of onset and the frequency of DR3/DR4 genotypes was found (Fig. 2*A*). One possibility is that other loci might be involved in increased frequency of DR3/DR4 at a younger age of onset. Tait et al. (11) reported a significantly higher frequency of A1-DR3 haplotypes in the group whose age at onset was ≥31 years, which is consistent with the results reported in Table 5. We thus investigated if the presence of the A*0101 allele affected the age-of-onset distribution of DR3/DR4 individuals. When the group of DR3/DR4 affected individuals (excluding DR4 haplotypes containing DQB1*0301) was subdivided into those with one or two copies of A*0101 (A*0101$^+$) and those with no copies of A*0101 (A*0101$^-$), the difference between groups was significant ($P < 0.025$) (Fig. 2*B*). Moreover, a Cochran-

TABLE 5
DRB1-DQB1 haplotype frequencies in three age-of-onset groups stratified by the associated A allele

| | Age of onset (years) | | |
|---|---|---|---|
| A | 0–10 | 11–20 | 21–36 |
| DRB1*0404 DQB1*0302† | | | |
| 2402 | 4.1 | 1.8 | 0 |
| Other | 6.8 | 7.2 | 11.9 |
| DRB1*0301 DQB1*02‡ | | | |
| 0101 | 11.9 | 18.1 | 15.6 |
| 0201 | 6.8 | 5.8 | 2.2 |
| 2402 | 3.4 | 1.8 | 0.7 |
| Other | 8.6 | 9.4 | 6.7 |
| DRB1*0401 DQB1*0302‡ | | | |
| 0201 | 12.9 | 8.3 | 17.2 |
| 0301 | 1.6 | 1.1 | 2.2 |
| 2402 | 2.3 | 0.4 | 0.0 |
| 2601 | 0.5 | 0.7 | 3.0 |
| Other | 8.6 | 8.3 | 6.7 |

Data are %. †$P < 0.009$; ‡$P < 0.05$.

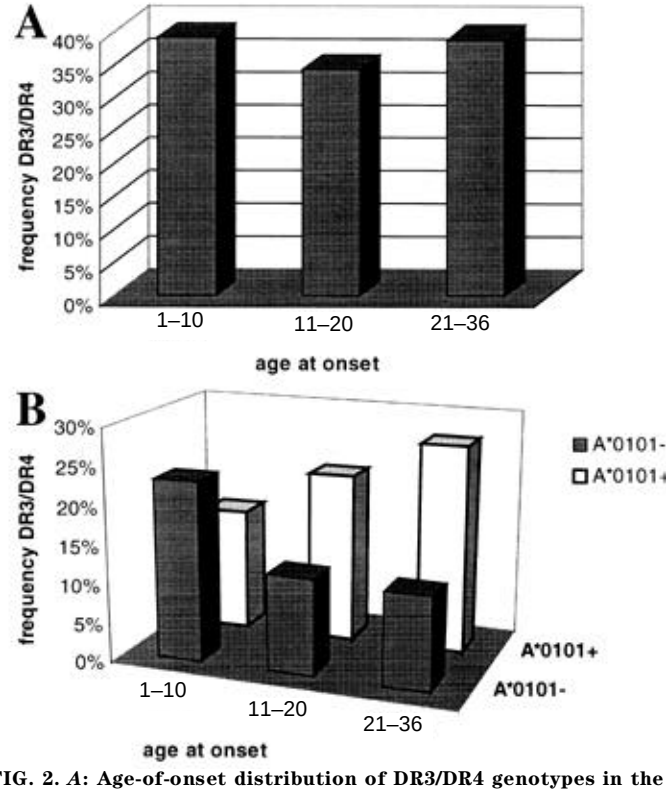


**FIG. 2. *A*: Age-of-onset distribution of DR3/DR4 genotypes in the 222 sib pairs studied (excluding DR4 subtypes containing DQB1*0301). *B*: DR3/DR4 genotypes subdivided by whether they are associated with at least one copy of A*0101 (A*0101$^+$) or not (A*0101$^-$).**

Armitage trend test (17) revealed the increasing linear trend found among A*0101$^+$ DR3/DR4 genotypes to be significant at $P < 0.041$ and the decreasing linear trend among A*0101$^-$ DR3/DR4 genotypes to be significant at $P < 0.009$ (Fig. 2*B*). The reverse direction of these trends could explain why no trend was found when both A*0101$^+$ and A*0101$^-$ DR3/DR4 genotypes were pooled together (Fig. 2*A*).

## DISCUSSION

We found a strong HLA association with type 1 diabetes age of onset by looking at pairs of siblings. The age of onset of one of the two sibs in a type 1 diabetes multiplex pedigree, however, is a poor predictor of the age of onset of the other sib, given the large variance of the age-of-onset distribution. The two ages are not independent but are strongly associated in sib pairs that share at least one HLA DR-DQ haplotype, indicating that genes in the HLA region are responsible in part for this effect. Indeed, the age association between sibs in a pair is stronger than can be explained by DR-DQ sharing alone. We have shown that alleles at both DPB1 and A appear to modulate the DR-DQ effect on age of onset. Obviously, these results must be interpreted with caution. From a statistical point of view, given that several tests have been performed, it is possible that some of the significant results observed are due to type I error. From a biological point of view, although it is clear that other genes outside DR-DQ influence type 1 diabetes age at clinical onset, these loci need not necessarily be HLA-A or DPB1. In light of the tight linkage disequilibrium present in this genetic region, it is possible that the patterns reported here are due to other major histocompatibility complex genes whose effect is detected through HLA-A or DPB1.

It must be noted, however, that class I involvement in age at onset of type 1 diabetes has been previously reported (11,18–19). The hypothesis has been put forth that since class II molecules are involved in triggering the autoimmune response, they affect the initial events in the development of type 1 diabetes. On the other hand, HLA class I molecules play a role in the final step of the immune response (destruction of target cells) and therefore may affect the rate of pancreatic β-cell destruction. Based on these considerations, we might expect class I associations with the age of onset (19). The strong effect of A*2402 gives further support to this hypothesis, given that this allele has been reported to be associated with total β-cell destruction. The effect of A*0101 alleles on DR3/DR4 haplotypes is more difficult to explain but may reflect the heterogeneity of DR3 haplotypes with respect to type 1 diabetes susceptibility.

Our results stress the importance of looking at other HLA loci in addition to DR and DQ and their association with the clinical features of type 1 diabetes.

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