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Newborn HLA-DR,DQ genotype screening: age- and ethnicity-specific type 1 diabetes risk estimates

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Abstract

Objective—Certain human leukocyte antigen (HLA)-DR,DQ genotypes have been associated with type 1 diabetes mellitus (T1DM) risk, although it is unknown whether the association is due to alleles, haplotypes, genotypes, the formation of heterodimers, or all of the above. To characterize the role of the HLA-DR,DQ genotype and ethnicity on the onset age of T1DM, we analyzed these factors in patients with T1DM and the general population.

Methods—One thousand three hundred twenty-two well-characterized patients with T1DM were compared with 3339 children from the general population of Denver, Colorado, USA. Because of the extensive available data across age and ethnic groups, this study population is unique.

Results—The HLA-DR3/4,DQB1*0302, DRX/4,DQB1*0302 (where X = 1, 4, 8, and 9), and HLA-DR3/3 genotypes were associated with T1DM, supporting previous research. Additionally, the DR3/9 genotype showed a positive association with T1DM, which has not previously been described in Caucasian populations. The HLA-DR3/4*0302 genotype was most strongly associated with T1DM in diabetic individuals with the youngest onset age. Genotype frequencies were similar between Hispanics and non-Hispanic whites, except for the DR3/3 genotype, which was more likely to be found in non-Hispanic whites.

Conclusions—These results indicate that there are multiple alleles and genotypes associated with T1DM and that the risk associated with different genetic markers depends on the age of disease onset, suggesting that some markers may be involved in more rapid disease progression.

Keywords

age of onset; ethnic groups; gene frequency; genetic predisposition to disease; genetic screening

Type 1 diabetes mellitus (T1DM) is an autoimmune disease resulting from destruction of insulin-producing beta cells of the pancreas. Genetic studies of T1DM have uncovered a major role in disease susceptibility for genes in the class II subregion of the human leukocyte antigen (HLA) region on the short arm of chromosome 6 (1–9). Specific HLA genotype/haplotype combinations appear to determine the extent of disease risk, as the HLA-DR4,DQB1*0302, HLA-DR3,DQB1*0201 heterozygote has the highest risk ratio for T1DM (2). This synergistic effect can be explained by the formation of certain DQ *trans*-dimers, which may be responsible

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for the increased risk (2,10–16). It is likely that the DQA1*0301/DQB1*0201 *trans*-dimer contributes to the high risk, as this haplotype confers an increased risk in an infrequent *cis*-encoded haplotype, although DR4/DR7 genotypes have DQA1*0301/DQB1*0201 in *trans* without an increased risk of T1DM (16). In addition, the DQA1*0501/DQB1*0302 *trans*-dimer is likely to contribute to an increased disease risk in the DR3/4 heterozygote (10,17).

Racial and ethnic differences exist in the incidence of T1DM, even within the same geographic area (18,19), with highest incidence rates in non-Hispanic whites as compared with other races and ethnic groups (15,18–20). Frequencies of specific alleles and haplotypes have been found to be similar between Hispanics and non-Hispanic whites (21–23), although haplotype relative risks between the two ethnic groups differed (24,25). This could indicate the presence of ethnically different HLA-linked genetic modifiers acting upon susceptibility genotypes or an effect of genetic admixture on predisposing factors to T1DM (24–26).

The incidence of T1DM peaks at 8–12 yr of age; however, the disease can be diagnosed at any age. It has been hypothesized that the rate of progression to T1DM and age at onset are directly related to the HLA-DR,DQ genotypes (27–30). Children who were diagnosed with T1DM at a younger age were more likely to have a relative with T1DM, suggesting that genetics contribute more significantly to T1DM when it is diagnosed at a younger age (31,32). As is the case for the genetic association with many complex diseases, the contribution of HLA genes appears to be strongest when T1DM is diagnosed in childhood, especially for HLA-DR3/4 heterozygotes, which demonstrates the overall heterogeneity of T1DM (28).

Most knowledge of diabetes risk contributed by HLA-DR,DQ genes comes from studies of first-degree relatives of T1DM patients and may not be applicable to the general population. Additionally, T1DM risk estimates for the state of Colorado have not been described. We initiated this study in order to characterize differences in HLA-DR,DQ genotype frequencies, age at onset, and ethnicity in patients with T1DM and the general population of Colorado.

Methods

Subjects

Subjects with T1DM were ascertained from the patient database of the Barbara Davis Center for Childhood Diabetes (BDC). All patients who had complete HLA-DR,DQ genotyping and known age of onset, less than 30 yr, were included in the analysis. HLA typing was considered complete if DRB1 and DQB1 were known, or if DQB1 and DQA1 were known. The DRB1 allele can be inferred, based on linkage disequilibrium patterns, from the DQA1 and DQB1 alleles. Date of diagnosis ranged from 1981 to 2001. To limit subjects to those with T1DM that was autoimmune in origin, we excluded patients not on insulin 1 yr after diagnosis, patients with type 2 diabetes or diabetes secondary to other causes, or patients negative for T1DM antibodies, if tested, which was confirmed by chart review. T1DM antibody test data were available for 96.5% of the cases. Race and ethnicity information was obtained through patient self-report and is available for the majority (89.4%) of cases. Age of onset and gender were available for 100% of cases. Approximately 53.3% of patients with T1DM were male, 75.6% were non-Hispanic white, 9.4% were Hispanic, and 15.0% were of other or unknown race/ethnicity.

General population subjects were ascertained from the newborn screening population of the Diabetes Autoimmunity Study in the Young (DAISY), which offers HLA-DR,DQ genotype testing to parents of newborns at a Denver hospital. Because the screening is offered to all English-speaking parents, with a >85% rate of participation, this group is considered to be a representative sample of the general population in Denver. DAISY has been screening newborns at this hospital since 1994 (33); however, newer, higher-resolution genotype testing

was initiated in 2000, as described below. The general population subjects were limited to children genotyped with the higher-resolution testing, and therefore are limited to children less than 5 yr of age. Information about gender and race/ethnicity is self-reported at the time of screening. Approximately 51.7% of general population subjects were male, 49.7% were non-Hispanic white, 30.9% were Hispanic, and 19.4% were of other or unknown race/ethnicity. For all subjects, Hispanic ethnicity is defined as Mexican, Puerto Rican, Cuban, Spanish, or other Hispanic descent. All Hispanic ethnic groups were combined into one group in the analysis.

Population comparability

The BDC is a referral center where the majority of children diagnosed before 5 yr of age in Colorado are seen. Recent comparison with the population-based, Centers for Disease Control and Prevention (CDC)-funded Diabetes Registry, SEARCH for Diabetes in Youth has demonstrated that 60% of all Colorado children with T1DM are seen at the BDC at time of diagnosis, and over 80% within 5 yr of diagnosis (D. Dabelea, personal communication). BDC patients with T1DM with complete HLA typing were included, who are more likely to be older, which could introduce a bias by age or ethnicity. Because of these limitations, the T1DM subjects selected for this analysis may not be an accurate representation of the general population of Colorado. To assess the comparability, gender, race/ethnicity, and onset age of the selected subjects with T1DM diagnosed in 1986–2001 were compared to those of all BDC patients with T1DM diagnosed in 1986–2001 and those of the Colorado Insulin-Dependent Diabetes Mellitus (IDDM) Registry participants diagnosed in 1978–1988. This analysis was limited to non-Hispanic white, Hispanic, and African American participants, as other ethnic groups represented <3% of the study population. The subjects with T1DM chosen for analysis were not significantly different from the overall BDC patient population with T1DM for the variables of gender, race, or onset age (data not shown). However, the subjects with T1DM were more likely to be Hispanic than the Colorado IDDM Registry participants (11.8 vs. 8.2%, $p = 0.014$). The subjects with T1DM and the overall BDC population had somewhat younger age at onset compared to the Colorado IDDM Registry patients ($p < 0.0001$). These differences could be due to possible changes in the demographics of T1DM between 1978–1988 and 1986–2001, with younger age at onset of T1DM, as described by several registries (34–37), and an increased proportion of Hispanic individuals in the Colorado population (17.1 in 2000 vs. 11.8% in 1980), as evidenced by census data (38,39). The subjects with T1DM selected for this analysis can be considered an accurate representation of patients with T1DM in Colorado.

While the general population children who serve as controls in these analyses have been followed for up to 4.4 yr, it is possible that some of them may develop diabetes later on. However, with the general population risk of T1DM of 1:300 by age 20, fewer than 10 of the 3339 controls would be expected to develop T1DM between ages 4 and 20. Our inability to exclude these future cases is unlikely to substantially influence the results of this study.

HLA typing

For DAISY general population subjects, whole cord blood is sent to the HLA typing laboratory at Roche Molecular Systems, Inc., Alameda, CA, USA, where it is used in polymerase chain reaction (PCR) amplification. The polymorphic exons 2 of DRB1 and DQB1 loci were specifically amplified using a pool of 11 biotinylated primers (8, 5′ and 1, 3′ primers for DRB1 and one primer pair for DQB1) in a single PCR reaction. DNA typing is performed by hybridizing the amplified DNA to a nylon membrane (strip) containing 18 immobilized sequence-specific oligonucleotide probes (15 DRB1 and three DQB1 probes) in linear arrays and detecting the bound biotinylated PCR product with streptavidin–horseradish peroxidase and a chromogenic substrate, details of which have been published (33,40). HLA typing for subjects with T1DM was completed at the BDC by using the Dynal RELI™ SSO HLA-DQB1

and DRB1 DNA typing kit (Bromborough, UK). In order to compare subjects with T1DM, which were typed for DQA and DQB, with the general population, typed for DRB and DQB, it was necessary to convert the DQ genotypes into DR genotypes, using well-defined linkage disequilibrium patterns and HLA haplotypes for the DQ/DR loci. However, it was not always possible to distinguish between certain DR types, and for this reason, DR10, 11, 12, 13, and 14 are all combined into one group, designated as DR-Y.

Statistical analysis

Using Statistical Analysis Software (SAS, Cary, NC, USA), we used χ^2-tests for statistical comparisons among the studied groups. Odds ratios were calculated using Woolf's method for haplotypes and genotypes. The Bonferroni correction for multiple comparisons was used when appropriate. Multiple linear regression using backward elimination was used to examine the effect of gender, race/ethnicity, and HLA genotype, as well as interactions between these variables, on age of onset. Due to the age limitation of the general population subjects, it was not possible to examine age as a continuous independent variable. To examine the effect of HLA-DR,DQ genotype on onset age, subjects with T1DM were categorized into age groupings and compared with all general population subjects. The age categories were chosen to match the categories of the Colorado IDDM Registry (41).

Results

HLA-DR,DQ genotype associations with T1DM

Table 1 displays the frequencies of selected HLA-DR,DQ genotypes in subjects with T1DM and in general population subjects, and the associated odds ratio for each genotype, as compared with all other genotypes. The highest odds ratio was found for the HLA-DR3/4,DQB1*0302 genotype, indicating that subjects with T1DM were much more likely to have this genotype than the general population. The DR3/4,DQB1*0301 genotype also occurred more frequently in subjects with T1DM, although to a much lesser extent. Other genotypes exhibiting elevated odds ratios include HLA-DR3/3 and DR4/4,DQB1*0302 homozygotes. Subjects with T1DM were more likely than the general population to have DR4, DQB1*0302 combined with DR1, 8, 9, or Y, as these genotype combinations resulted in slightly increased odds ratios (Table 1). The combination of the predisposing DR3 allele with DR-Y or with the protective DR2,DQB1*0602 was found more often in the general population than in subjects with T1DM. However, the DR3/2 genotype, in the absence of DQB1*0602, was more frequent in subjects with T1DM. Other alleles in combination with DR3 were neutral, with the notable exception of the DR3/9 genotype, which was more likely to be found in subjects within in T1DM than in the general population.

Ethnic differences

When genotypes were stratified by ethnicity, the proportions by subject group for each genotype were similar to the overall distributions for non-Hispanic whites and Hispanics, and each genotype yielded a similar degree of risk within these two ethnic groups (Table 2). The odds ratios for the DR3/3 genotype are not significantly different between Hispanics and non-Hispanic whites; however, the frequency of the DR3/3 genotype was significantly different in Hispanic compared to that in non-Hispanic white subjects with T1DM, after adjusting for gender and onset age ($p = 0.048$). The frequency differences for other genotypes were not statistically significant.

HLA-DR,DQ genotype effect on onset age

Table 3 lists the frequencies and odds ratios for selected HLA-DR,DQ genotypes, stratified by onset age of T1DM. Greater deviation of genotype frequency from the general population was

found in subjects with T1DM with the youngest age at onset possessing the HLA-DR3/4,DQB1*0302 genotype, as indicated by the odds ratio of 37.01, compared to an odds ratio of 14.15 for those diagnosed between 5 and 9 yr of age, 19.38 for 10–14 yr old, and 8.65 for ages 15–29. Nearly 50% of subjects with T1DM diagnosed before the age of 5 possess this high-risk genotype, and as a result, the magnitude of the odds ratios associated with other moderate risk genotypes is diminished or non-significant, with the exception of DR3/9, with an odds ratio of 12.21. The DR3/9 genotype did not demonstrate a significantly increased odds ratio in any other age group. The odds ratios for DR4/4,DQ*0302 and DR3/3 homozygotes did not differ by age.

The results of the multiple linear regression indicated that HLA genotype was significantly ($p < 0.0001$) associated with age at onset of T1DM, after controlling for the effects of gender and race/ethnicity, although genotype only accounts for a fraction of the variability in onset age ($R^2 = 0.044$).

Discussion

The results of this study provide additional evidence for the association between T1DM and certain high-risk HLA-DR,DQ genotypes, such as HLA-DR3/4, DQB*0302 and DR3 and DR4 homozygotes. The genotype consisting of DR9 and DR4,DQB1*0302 was associated with an increased risk in the non-Hispanic white population. The only genotype with a higher degree of risk in non-Hispanic whites was DR3/4,DQB1*0302.

Additionally, the HLA-DR3/9 genotype was positively associated with T1DM. This particular genotype is relatively infrequent in the general population, found in only 0.24% of the general population subjects. The odds ratio associated with this genotype was 4.66 for all subjects with T1DM and was strongest for youngest age at diagnosis (12.21 in the age group of 0–4 yr). Although previous studies have documented an increased risk of T1DM associated with the DR3/9 genotype, the associations have only been within Chinese, Japanese, Korean, Filipino, and African American populations (16,17,42–47). To our knowledge, an association between this genotype and T1DM has not previously been reported in a Caucasian population. Because this genotype has a very low frequency within the Caucasian population, it is possible that previous associations were undetected. A possible explanation for this effect of this genotype is the formation of the heterodimer DQA1*0301/DQB1*0201. This heterodimer is formed in *trans* in the DR3/9 genotype, as well as the high-risk DR3/4 genotype. However, DR4/DR7 genotypes also have the DQA1*0301/DQB1*0201 heterodimer in *trans*, but without an increased risk of T1DM, so this heterodimer cannot be the sole explanation for the high risk associated with DR3/9 and DR3/4. The functional properties of HLA molecules, as well as genetic evidence, imply that the effect of the same HLA molecule on T1DM may not vary across ethnic groups or geographic populations (16). This study provides evidence that the positive association between T1DM and the DR3/9 genotype is consistent across several racial/ethnic groups.

The DR3/9 genotype also contains the DQB1*0303 allele, which could account, in part, for the effect of this genotype. Support for DR9-DQB1*0303 as a susceptibility haplotype is also evidenced by an increased odds ratio associated with the DR4/9 genotype. The DQB1*0303 allele, previously thought to be neutral in determining susceptibility to T1DM (11), is identical to the high-risk DQB1*0302 allele, except that it contains aspartic acid at residue 57. Aspartic acid at this position has previously been associated with protection against T1DM (10–14) although the association of DR4-DQB1*0401 and *0402, both Asp-57, has been well established among Asian populations (2,15,16). Asp-57 protection was not demonstrated in Mexican Americans (23). The hypothesis of an Asp-57 protective effect is not consistent with the association between DR3/9 and T1DM, confirming studies of other alleles, and suggesting

that DQB residues other than position 57, as well as heterodimers, impact susceptibility to T1DM (10,48). Additionally, it has been demonstrated that absence of Asp at position 57 may be more important in determining T1DM susceptibility in non-DR3/non-DR4 genotypes (13). It is likely that there are other factors involved in the increased association with DR3/9 established in this analysis.

Ethnic differences

The results of this study suggest a weaker association between HLA and T1DM within the Hispanic population than within the non-Hispanic white population, consistent with previous studies (21,49,50). The magnitude of the risk in cases possessing DR4,DQB1*0302 is similar between Hispanics and non-Hispanic whites. The odds ratio for the DR3/3 genotype is higher for Hispanics than that for non-Hispanic whites, although not statistically different. The proportion of the general population with the DR3/3 genotype is lower in Hispanics than in non-Hispanic whites (0.29 vs. 1.87%); thus, with greater numbers of Hispanic subjects, it is likely that the DR3/3 genotype in Hispanics would result in a significantly increased odds ratio, consistent with the evidence of risk heterogeneity associated with different DR3 haplotypes (19,25). These results support previous data (50) and could indicate that the DR3 haplotype, when present, yields a greater risk of T1DM within the Hispanic population. These observations may also reflect differences with respect to linkage disequilibrium on DR3 haplotypes between Hispanics and non-Hispanic whites. Hispanics with DR3 are more likely to also carry B18 than non-Hispanic whites with DR3, suggesting that loci other than DRB1 and DQB1 may be involved in determining disease risk.

Genetic contribution to age at onset

Previous studies have attempted to determine whether the variability in age at onset and disease progression is related to HLA-DR,DQ genotypes (27–32,51). Additionally, it has been hypothesized that rate of progression to disease and age at onset are directly correlated and that HLA-DR,DQ genotypes may also influence disease progression (27–30,51). Higher proportions of HLA-DR3/4 heterozygotes and HLA-DR3+patients have been found in children diagnosed at younger ages (52). However, not all studies have supported a genetic link (53).

The results of this study do provide evidence that age of onset of T1DM is genetically determined. Nearly half of patients diagnosed before the age of 5 possessed the high-risk genotype DR3/4,DQB1*0302. This genotype increased the risk of T1DM 40-fold in the youngest age group, higher than that at any other age group. However, past the onset age of 5 yr, other genotypes become more important in their contribution to risk of T1DM. These results provide additional support for the overall heterogeneity of T1DM and suggest that the disease and risk factors for disease change with increasing age of diagnosis. Analyzing all age groups of patients with T1DM together could potentially obscure the role of specific HLA alleles at different ages.

This study was not without limitations, including the young age of the general population and the differences in HLA typing methods used, which necessitated the grouping of several genotypes into one category (DR-Y), preventing us from examining these individual DR alleles. Additionally, screening enrollment of general population DAISY subjects is limited to English speakers, which may not be representative of all Colorado Hispanics. However, this eliminated less than 9% of newborns eligible for screening. The BDC is a referral center where the majority of children diagnosed before 5 yr of age in Colorado and the Rocky Mountain region are seen, while older children are less likely to be referred. This referral pattern may differ by ethnicity. The study was performed using residents of Colorado, which has a relatively high incidence of diabetes, as compared to other US populations (41,54–56), and a sizeable

Hispanic population (38,39). This population may not be precisely representative of the US population.

However, the advantages of a study such as this are numerous. Examining genotypes as opposed to alleles allowed us to examine the effects of combinations of alleles. Due to the relatively large number of subjects with T1DM and general population subjects used in the analysis, it is possible to divide the subjects with T1DM into age and ethnic groupings to determine whether the influence of specific HLA-DR,DQ genotypes is different for different ages at onset and ethnic groups.

In conclusion, the results of this study suggest that T1DM is a disease of allelic heterogeneity and that the disease and risk factors for disease change with increasing age of diagnosis. Additionally, the degree of risk conferred by certain genotypes is different in subjects with T1DM of dissimilar racial/ethnic background. A novel finding was the positive association of T1DM with the HLA-DR3/9 genotype, not previously reported in Caucasian populations. These findings have important implications for risk assessment of T1DM in the general population, and within different racial/ethnic groups. HLA-DR,DQ genotype may be useful in predicting time to disease onset in prediabetic individuals, helping to guide strategies to test possible interventions for the prevention of T1DM.

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Table 1

HLA-DR,DQ genotype frequencies and odds ratios in patients with type 1 diabetes mellitus (T1DM) and general population

HLA-DR,DQB1	Type 1 diabetic individuals (n = 1322) n (%)	Population frequency (n = 3339) n (%)	Odds ratio (95% CI)	p-value α = 0.0025
3/4,*0302	400 (30.26)	78 (2.34)	18.14 (14.08–23.37)	< 0.0001
4/4,*0302	146 (11.04)	100 (2.99)	4.02 (3.09–5.23)	< 0.0001
1/4,*0302	96 (7.26)	75 (2.25)	3.41 (2.50–4.64)	< 0.0001
8/4,*0302	36 (2.72)	50 (1.50)	1.84 (1.19–2.84)	0.0051
9/4,*0302	13 (0.98)	11 (0.33)	3.01 (1.34–6.72)	0.0049
7/4*0302	39 (2.95)	82 (2.46)	1.21 (0.82–1.78)	0.34
Y/4,*0302[†]	116 (8.78)	242 (7.25)	1.29 (1.03–1.63)	0.0281
2,*0602/4,*0302	6 (0.45)	79 (2.37)	0.19 (0.08–0.43)	< 0.0001
2/4,*0302	9 (0.68)	33 (0.99)	0.69 (0.33–1.42)	0.3166
3/3	103 (7.79)	44 (1.32)	6.33 (4.42–9.06)	< 0.0001
3/1	45 (3.40)	73 (2.19)	1.58 (1.08–2.30)	0.0171
3/8	15 (1.13)	30 (0.90)	1.27 (0.68–2.36)	0.4573
3/9	11 (0.83)	6 (0.18)	4.66 (1.72–12.63)	0.0009
3/7	33 (2.50)	86 (2.58)	0.97 (0.65–1.45)	0.8769
3/Y[†]	45 (3.40)	182 (5.45)	0.61 (0.44–0.85)	0.0034
3/2,*0602	7 (0.53)	81 (2.43)	0.21 (0.099–0.46)	< 0.0001
3/2	22 (1.66)	27 (0.81)	2.08 (1.18–3.66)	0.0098
3/4,*0301	30 (2.27)	33 (0.99)	2.33 (1.41–3.83)	0.0006
4/X,4/4,*0301[†]	58 (4.39)	277 (8.30)	0.51 (0.38–0.68)	< 0.0001
All others (X/X)[‡]	92 (6.96)	1750 (52.41)	0.068 (0.054–0.085)	< 0.0001

HLA, human leukocyte antigen.

[†] Y = DR 10, 11, 12, 13, or 14.

[‡] X = not DR3, not DR4.

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Table 2

HLA-DR,DQ genotype frequencies in non-Hispanic whites and Hispanics

HLA-DR,DQB1	Non-Hispanic white			Hispanic		
	Cases (n = 999) n (%)	Controls (n = 1661) n (%)	OR (95% CI)	Cases (n = 124) n (%)	Controls (n = 1032) n (%)	OR (95% CI)
3/4,*0302	317 (31.73)	42 (2.53)	17.92 (12.83–25.02)	39 (31.45)	29 (2.81)	15.87 (9.35–26.94)
4/4,*0302	102 (10.21)	50 (3.01)	3.66 (2.59–5.19)	19 (15.32)	42 (4.07)	4.27 (2.39–7.60)
1/4,*0302	72 (7.21)	37 (2.23)	3.41 (2.27–5.11)	8 (6.45)	32 (3.10)	2.16 (0.97–4.79)
8/4,*0302	19 (1.90)	15 (0.90)	2.13 (1.08–4.21)	6 (4.84)	30 (2.91)	1.70 (0.69–4.16)
9/4,*0302	10 (1.00)	2 (0.12)	8.39 (1.83–38.36)	1 (0.81)	6 (0.58)	1.39 (0.17–11.64)
7/4*0302	31 (3.10)	45 (2.71)	1.15 (0.72–1.83)	3 (2.42)	31 (3.00)	0.80 (0.24–2.66)
Y/4,*0302[†]	79 (7.91)	86 (5.18)	1.57 (1.15–2.16)	11 (8.87)	119 (11.53)	0.75 (0.39–1.43)
2,*0602/4,*0302	4 (0.40)	35 (2.11)	0.19 (0.066–0.53)	0	34 (3.29)	—[§]
2/4,*0302	6 (0.60)	9 (0.54)	1.11 (0.39–3.13)	0	20 (1.94)	—[§]
3/3	84 (8.41)	31 (1.87)	4.83 (3.17–7.34)	4 (3.23)	3 (0.29)	11.43 (2.53–51.70)
3/1	36 (3.60)	59 (3.55)	1.02 (0.67–1.55)	3 (2.42)	8 (0.78)	3.17 (0.83–12.12)
3/8	10 (1.00)	14 (0.84)	1.19 (0.53–2.69)	4 (3.23)	11 (1.07)	3.09 (0.97–9.87)
3/9	8 (0.80)	4 (0.24)	3.34 (1.01–11.13)	1 (0.81)	0	—[§]
3/7	28 (2.80)	52 (3.13)	0.89 (0.56–1.42)	2 (1.61)	20 (1.9)	0.83 (0.19–3.59)
3/Y[†]	34 (3.40)	99 (5.96)	0.56 (0.37–0.83)	3 (2.42)	51 (4.94)	0.48 (0.15–1.55)
3/2,*0602	4 (0.40)	55 (3.31)	0.12 (0.04–0.32)	2 (1.61)	14 (1.36)	1.19 (0.27–5.31)
3/2	15 (1.50)	9 (0.54)	2.80 (1.22–6.42)	2 (1.61)	8 (0.78)	2.10 (0.44–9.99)
3/4,*0301	24 (2.40)	23 (1.38)	1.75 (0.98–3.12)	1 (0.81)	5 (0.48)	1.67 (0.19–14.41)
4/X,4/4,*0301	43 (4.30)	167 (10.05)	0.40 (0.29–0.57)	4 (3.23)	66 (6.40)	0.49 (0.17–1.36)
All others (X/X)[‡]	73 (7.31)	827 (49.79)	0.80 (0.062–0.103)	11 (8.87)	503 (48.74)	0.10 (0.05–0.19)

HLA, human leukocyte antigen.

[†]Y = DR 10, 11, 12, 13, or 14.

[‡]X = not DR3, not DR4.

[§]Unable to calculate due to zero cell.

Table 3

HLA-DR,DQ genotype frequencies and odds ratios, by age at onset of type 1 diabetes mellitus (T1DM)

	Onset age 0–4 (n = 279)		Onset age 5–9 (n = 443)		Onset age 10–14 (n = 390)		Onset age 15–29 (n = 210)		Controls (n = 3339)
HLA-DR,DQB1	**n (%)**	**OR (95% CI)**	**n (%)**	**OR (95% CI)**	**n (%)**	**OR (95% CI)**	**n (%)**	**OR (95% CI)**	**n (%)**
3/4,*0302	131 (47.0)	37.01 (26.73–51.22)	112 (25.3)	14.15 (10.37–19.29)	121 (31.0)	19.38 (14.20–26.46)	36 (17.1)	8.65 (5.66–13.21)	78 (2.34)
4/4,*0302	30 (10.75)	3.90 (2.54–5.99)	52 (11.74)	4.31 (3.03–6.12)	34 (8.72)	3.09 (2.06–4.63)	30 (14.29)	5.40 (3.50–8.34)	100 (2.99)
1/4,*0302	12 (4.30)	1.96 (1.05–3.64)	41 (9.26)	4.44 (2.99–6.59)	25 (6.41)	2.98 (1.87–4.74)	18 (8.57)	4.08 (2.39–6.96)	75 (2.25)
8/4,*0302	4 (1.43)	0.96 (0.34–2.67)	13 (2.93)	1.99 (1.07–3.69)	11 (2.82)	1.91 (0.99–3.70)	8 (3.81)	2.61 (1.22–5.57)	50 (1.50)
9/4,*0302	1 (0.36)	1.09 (0.14–8.46)	8 (1.81)	5.56 (2.23–13.91)	3 (0.77)	2.35 (0.65–8.44)	1 (0.48)	1.45 (0.17–11.27)	11 (0.33)
7/4*0302	6 (2.15)	0.87 (0.38–2.02)	21 (4.74)	1.97 (1.21–3.23)	10 (2.56)	1.05 (0.54–2.03)	2 (0.95)	0.38 (0.093–1.56)	82 (2.46)
Y/4,*0302[†]	15 (5.38)	0.73 (0.43–1.24)	31 (7.0)	0.96 (0.65–1.42)	43 (11.03)	1.59 (1.13–2.23)	27 (12.86)	1.89 (1.24–2.89)	242 (7.25)
2,*0602/4,*0302	2 (0.72)	0.30 (0.073–1.22)	3 (0.68)	0.28 (0.089–0.90)	1 (0.26)	0.11 (0.015–0.76)	0	—[§]	79 (2.37)
2/4,*0302	1 (0.36)	0.36 (0.049–2.65)	3 (0.68)	0.68 (0.021–2.24)	4 (1.03)	1.04 (0.37–2.95)	1 (0.48)	0.48 (0.065–3.52)	33 (0.99)
3/3	19 (6.81)	5.47 (3.15–9.51)	29 (6.55)	5.25 (3.25–8.48)	35 (8.97)	7.38 (4.67–11.66	20 (9.52)	7.88 (4.56–13.64)	44 (1.32)
3/1	8 (2.87)	1.32 (0.63–2.77)	16 (3.61)	1.68 (0.97–2.91)	16 (4.10)	1.91 (1.1–3.32)	5 (2.38)	1.09 (0.44–2.73)	73 (2.19)
3/8	2 (0.72)	0.78 (0.19–3.35)	4 (0.90)	1.01 (0.35–2.87)	6 (1.54)	1.72 (0.71–4.17)	3 (1.43)	1.60 (0.48–5.28)	30 (0.90)
3/9	6 (2.15)	12.21 (3.91–38.11)	3 (0.68)	3.79 (0.94–15.20)	1 (0.26)	1.43 (0.17–11.89)	1 (0.48)	2.66 (0.32–22.18)	6 (0.18)
3/7	4 (1.43)	0.55 (0.20–1.51)	13 (2.93)	1.14 (0.63–2.07)	11 (2.82)	1.10 (0.58–2.07)	5 (2.38)	0.92 (03.7–2.30)	86 (2.58)
3/Y[†]	7 (2.51)	0.45 (0.21–0.96)	18 (4.06)	0.73 (0.45–1.21)	12 (3.08)	0.55 (0.30–0.99)	8 (3.81)	0.69 (0.33–1.41)	182 (5.45)
3/2,*0602	1 (0.36)	0.15 (0.020–1.04)	2 (0.45)	0.18 (0.45–0.75)	1 (0.26)	0.10 (0.014–0.75)	3 (1.43)	0.58 (0.18–1.86)	81 (2.43)
3/2	3 (1.08)	1.33 (0.40–4.42)	8 (1.81)	2.26 (1.02–5.00)	4 (1.03)	1.27 (0.44–3.65)	7 (3.33)	4.23 (1.82–9.83)	27 (0.81)
3/4,*0301	6 (2.15)	2.20 (0.91–5.30)	10 (2.26)	2.31 (1.13–4.73)	9 (2.31)	2.37 (1.12–4.98)	5 (2.38)	2.44 (0.94–6.33)	33 (0.99)
4/X,4/4,*0301[‡]	6 (2.15)	0.24 (0.11–0.55)	20 (4.51)	0.52 (0.33–0.83)	20 (5.13)	0.60 (0.38–0.95)	12 (5.71)	0.67 (0.37–1.25)	277 (8.30)
All others (X/X)[‡]	15 (5.38)	0.052 (0.031–0.087)	36 (8.13)	0.080 (0.057–0.11)	23 (5.91)	0.057 (0.037–0.087)	18 (8.57)	0.085 (0.052–0.14)	1750 (52.4)

HLA, human leukocyte antigen.

[†] Y = DR 10, 11, 12, 13, or 14.

[‡] X = not DR3, not DR4.

[§] Unable to calculate due to zero cell.