Failed Genetic Protection: Type 1 Diabetes in the Presence of HLA-DQB1*06:02

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Certain HLA class II genes increase the risk for type 1 diabetes (T1D) development while others provide protection from disease development. HLA class II alleles encode MHC proteins on antigen-presenting cells, which function to present peptides and activate CD4 T cells. The DRB1*15:01 (DR15)-DQA1*01:02-DQB1*06:02 (DQ6) haplotype provides dominant protection across all stages of T1D and is a common haplotype found in Caucasians. However, it is present in <1% of people with T1D. Knowing which metabolic, immunologic, and genetic features are unique to individuals who fail genetic protection and develop T1D is important for defining the underlying mechanisms of DQB1*06:02-mediated protection. We describe a T1D cohort with DQB1*06:02 (n = 50) and compare them to individuals with T1D and without DQB1*06:02 (n = 2,759) who were identified over the last 26 years at the Barbara Davis Center for Diabetes. The age at diagnosis was similar between the cohorts and normally distributed throughout childhood and early adulthood. The average hemoglobin A1c was 10.8 ± 2.8% (95 ± 7 mmol/mol) at diagnosis in those DQB1*06:02 positive. The majority of T1D DQB1*06:02+ individuals were positive for one or more islet autoantibodies; however, there was a greater proportion who were islet autoantibody negative compared with those T1D DQB1*06:02− individuals. Interestingly, DQB1*03:02, which confers significant T1D risk, was present in only those DQB1*06:02+ individuals with islet autoantibodies. This is one of the largest studies examining patients presenting with clinical T1D in the presence of DQB1*06:02, which provides a population to study the mechanisms of failed genetic protection against T1D.

Genome-wide association studies have identified over 50 genetic polymorphisms that increase the odds of developing type 1 diabetes (T1D), the immune-mediated form of diabetes (1–4). Over half of the genetic risk for T1D is conferred by HLA genes, especially the class II DR and DQ genes that are in linkage disequilibrium on chromosome 6 (5,6). HLA class II genes encode MHC proteins that are present on antigen-presenting cells, such as B cells, dendritic cells, and macrophages, which function to present processed proteins and activate CD4 T cells (7). Specific HLA alleles are known to increase risk for T1D development, including DRB1*04 (DR4)-DQA1*03:01-DQB1*03:02 (DQ8) and DRB1*03 (DR3)-DQA1*05:01-DQB1*02:01 (DQ2) haplotypes (8,9). Other HLA genes are strongly associated with protection from T1D development, namely, DRB1*15:01 (DR15)-DQA1*01:02-DQB1*06:02 (DQ6), which is a common haplotype found in European Americans with a frequency of about 14% but present in <1% of people with T1D (10–15). Further, genetic studies have mapped the protection to the DQ6 allele, as opposed to the DR15 allele that is in linkage disequilibrium with DQ6 (16,17).

The natural history of T1D occurs in stages that begin with the presence of islet autoantibodies, including those directed against insulin and β-cell proteins (GAD, IA-2, and zinc transporter 8 [ZnT8]), then impaired glucose tolerance, and finally clinical T1D onset marked by hyperglycemia and the need for insulin treatment (18). The Pathway to Prevention Cohort of Type 1 Diabetes TrialNet, an international consortium of investigators studying the natural history of T1D and conducting T1D prevention trials (19), recently identified a cohort of relatives with islet autoantibodies and the DQB1*06:02 allele. From 3,358 autoantibody-positive relatives of people with T1D, 155 (5%) were positive for the DRB1*15:01 (DR15)-DQA1*01:02-DQB1*06:02 (DQ6) haplotype (20). These relatives were less likely to develop additional autoantibodies,
Institutional Review Board approved the study.

written informed consent, and the Colorado Multiple

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database, and participants self-reported race and ethnic-

06:02

These results con

IAA, 20 DK units for GADA, 5 DK units for IA-2A, and

were applied to all participants with an index of 0.010 for

converted to DK units. The same positive cutoff values

2010, and index values obtained before this time were

1999 or later and 2010 or later, respectively. Harmonized

using radio-binding assays as previously described (23,24).

(ZnT8A), and insulin (IAA) were measured from serum

2,809 T1D patients, we identi

patients presenting with clinical T1D at our center. From

2,809 T1D patients, we identified 50 patients who had the

DQB1*06:02 allele. This is one of the largest studies

examining the metabolic and immunologic features of

patients presenting with clinical T1D in the presence of

DQB1*06:02.

RESEARCH DESIGN AND METHODS

Subjects and Study Design

Subjects were recruited from the Barbara Davis Center for

Diabetes Clinics, a major referral center for children and

adults with new-onset T1D, between 1993 and 2019.

These analyses include 2,809 patients who met the Ameri-

can Diabetes Association diagnostic criteria for diagnosis

with diabetes and were diagnosed with T1D. Of the 2,809

patients, we identified 50 patients who required insulin

treatment and had at least one DQB1*06:02 allele. Demo-

graphic characteristics were collected in a research

database, and participants self-reported race and ethnic-

ity. Peripheral blood was obtained for islet autoantibody

measurements and HLA typing. All participants provided

written informed consent, and the Colorado Multiple

Institutional Review Board approved the study.

Laboratory Procedures

Islet autoantibodies to GAD (GADA), IA-2 (IA-2A), ZnT8

(ZnT8A), and insulin (IAA) were measured from serum

using radio-binding assays as previously described (23,24).

IAA and ZnT8A were measured in patients diagnosed in

1999 or later and 2010 or later, respectively. Harmonized

assays for GADA and IA-2A were available starting in

2010, and index values obtained before this time were

converted to DK units. The same positive cutoff values

were applied to all participants with an index of 0.010 for

IAA, 20 DK units for GADA, 5 DK units for IA-2A, and

index 0.020 for ZnT8A. HLA typing was performed using

sequence-specific oligonucleotide probes for DRB1, DQA1,

DQB1, A, and B, as described previously (25).

Statistical Analysis

Statistical analyses were performed using GraphPad

Prism 8.0 software (GraphPad Software). Fisher exact

tests were used to compare categorical variables between

DQB1*06:02+ participants and DQB1*06:02− participants

and between DQB1*06:02+ participants who were islet

autoantibody positive and negative. The categorical vari-

ables analyzed include race/ethnicity, islet autoantibody

positivity, number of islet autoantibodies, presence of di-

abetic ketoacidosis (DKA), and HLA type. As our sample

sizes are unequal between the DQB1*06:02+ (n = 50) and

DQB1*06:02− (n = 2,759) groups, our power calculations

reveal 80% power to detect a medium effect size (based

upon a Cohen’s h value of 0.4), which indicates the ability

to detect a clinically significant difference in these vari-

ables between the groups.

Unpaired t tests were used to compare continuous vari-

ables (HbA1c, islet autoantibody levels, and age) between

DQB1*06:02+ participants and DQB1*06:02− participants

and between DQB1*06:02+ participants who were islet

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with our sample sizes indicate that we have 80% power to
detect a difference of 0.4 SDs in these continuous variables. 
P values <0.05 were considered significant.

Data Resource and Availability

All data generated and analyzed during this study are

included in the published article and the Supplementary

Material.

RESULTS

Characteristics of T1D Participants by

HLA-DQB1*06:02 Status

Over the course of 26 years, we identified 50/2,809 (1.8%) indi-

viduals with T1D and an HLA-DQB1*06:02 allele (Table

1). The majority of these individuals (62.4%) were seen at

initial diagnosis or within 1 year of diagnosis (Supplemen-

tary Material). It has been hypothesized that

abetic ketoacidosis (DKA), and HLA type. As our sample

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P values <0.05 were considered significant.
Islet Autoantibody Positivity

The majority of 0602\textsuperscript{+} participants (70\%) were positive for one or more islet autoantibodies, yet more individuals in the 0602\textsuperscript{+} group were antibody negative in comparison with the 0602\textsuperscript{−} group (30\% vs. 14\%, \(P < 0.004\)) (Table 1). This resulted in the overall number of islet autoantibodies being lower in the 0602\textsuperscript{+} group compared with the 0602\textsuperscript{−} group (\(P < 0.009\)); however, there was not a difference in the percentage of individuals positive for one or multiple autoantibodies (Table 1). Positivity for GADA, IAA, and ZnT8A was similar between the 0602\textsuperscript{+} and 0602\textsuperscript{−} participants; IAA was only considered positive if measured within 21 days of T1D diagnosis. However, IA-2A was less frequently positive in 0602\textsuperscript{+} compared with 0602\textsuperscript{−} individuals (39\% vs. 60\%, \(P < 0.005\)). When examining islet autoantibody levels (Fig. 2A–D), IA-2A levels were lower in the 0602\textsuperscript{+} group compared with the 0602\textsuperscript{−} with a mean of 70 vs. 161 DK units (\(P < 0.001\)). IAA levels were also lower in the 0602\textsuperscript{+} group compared with 0602\textsuperscript{−} with a mean index of 0.022 vs. 0.050 (\(P = 0.006\)).

Characteristics of DQB1\textsuperscript{06:02+} Patients by Autoantibody Status

The cohort of 0602\textsuperscript{+} individuals with T1D were diagnosed at ages between 3 months and 28.9 years with no difference between those with and without autoantibodies (\(P = 0.069\)) (Fig. 1B). As expected, these individuals were hyperglycemic at the time of T1D diagnosis as measured by hemoglobin A1c levels averaging 10.8 ± 2.8\% (95 ± 7 mmol/mol) (Table 2). The rates of DKA were similar between those with and without autoantibodies (Table 2). As there were several individuals with a very early age of diabetes onset (<1 year of age), monogenic forms of diabetes were investigated. Two 0602\textsuperscript{+} individuals were

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**Table 1.—Characteristics of T1D participants by HLA-DQB1\textsuperscript{06:02} status**

|                        | DQB1\textsuperscript{06:02+} \((n = 50)\) | DQB1\textsuperscript{06:02−} \((n = 2,759)\) | \(P\) value |
|------------------------|-------------------------------------------|---------------------------------------------|-------------|
| Age at diagnosis, years (mean ± SD) | 10.8 ± 6.0 | 12.5 ± 8.7 | 0.069 |
| Sex (% female)          | 52 (n = 26) | 46 (n = 1,266) | 0.395 |
| Race/ethnicity (%)      | Space for data | Space for data | 0.540 |
| White                  | 86 (n = 43) | 80 (n = 2,213) | \space |
| Hispanic               | 6 (n = 3) | 5 (n = 142) | \space |
| Other                  | 6 (n = 3) | 8 (n = 218) | \space |
| Unknown                | 2 (n = 1) | 7 (n = 186) | \space |
| Islet autoantibody status (%) | Space for data | Space for data | \space |
| GADA                   | 55 (27/49) | 52 (1,399/2,676) | 0.773 |
| IA-2A                  | 39 (19/49) | 60 (1,587/2,663) | 0.005 |
| IAA\textsuperscript{*} | 35 (13/37) | 34 (479/1,398) | 1.00 |
| ZnT8A                  | 51 (18/35) | 62 (553/898) | 0.289 |
| Number of islet autoantibodies (%)** | Space for data | Space for data | 0.009 |
| 0 Ab                    | 30 (14/47) | 14 (247/1,827) | 0.004 |
| 1 Ab                    | 19 (9/47) | 26 (474/1,827) | 0.398 |
| 2 Ab                    | 21 (10/47) | 33 (607/1,827) | 0.115 |
| ≥3 Ab                   | 30 (14/47) | 27 (499/1,827) | 0.741 |

Ab, autoantibodies. \textsuperscript{*}IAA positive values were excluded from analysis if measured >21 days after T1D diagnosis. **Includes samples for which at least three islet autoantibodies were measured; overall \(P\) value is reported, followed by those for each category.

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**Figure 1—**A: Distribution of age at diagnosis with T1D in DQB1\textsuperscript{06:02+} participants \((n = 50)\). B: Age at diagnosis of T1D DQB1\textsuperscript{06:02+} participants that had islet autoantibodies measured \((n = 49)\). Mean age of autoantibody-negative \((Ab^{−})\) participants was 8.5 ± 5.0 years \((n = 16)\), and mean age of autoantibody-positive \((Ab^{+})\) participants was 11.8 ± 6.2 years \((n = 33)\); \(P = 0.069\).
discovered to have mutations in the gene encoding the
ATP-sensitive potassium-channel subunit Kir6.2 (KCNJ11),
which are known to cause neonatal diabetes (26) (Supple-
mental Table). One participant was autoantibody nega-
tive, while the other had an islet autoantibody. A separate
autoantibody-negative participant presented with diabetes
and later had clinical symptoms suggestive of Wolfram
syndrome (27). The participant was found to have a mu-
tation in the WFS1 gene (Supplementary Material).

**Frequency of T1D Predisposing HLA Alleles**

First, the type and frequency of DQB1 alleles were de-
termined in both the 0602− and 0602+ cohorts. As
depicted in Fig. 3, the 0602− individuals with T1D have
percentages of T1D risk alleles as defined by DQB1*03:02
(DQ8), DQB1*02:01 (DQ2), or both DQ8 and DQ2 that are
similar to those reported by other large T1D cohorts (3,5).
The percentages of these alleles are independent of auto-
antibody positivity. However, there are stark differences in
the 0602+ cohort based upon the presence of islet auto-
antibodies. Those 0602 positive with autoantibodies are
more likely to have DQB1*03:02 (42%) as the other DQ
allele, whereas in the 0602− group without autoantibodies,
the DQB1*03:02 allele was not present ($P = 0.002$) (Table
2). This is in contrast to DQB1*02:01, which is present in
similar frequencies in the 0602− group independent of
autoantibodies (Table 2). When analyzing the 0602− group
with autoantibodies for known T1D class II risk alleles
(e.g., DR3, DR4, DQ8, or DQ2), 82% (27/33) had at least
one on the other chromosome (Supplementary Material).

**Figure 2**—A–D: Islet autoantibody levels in DQB1*06:02+ and DQB1*06:02− T1D individuals for IAA (A), IA-2A (B), GADA (C), and ZnT8A (D). $P = 0.006$ for IAA, $P < 0.001$ for IA-2A, $P = 0.20$ for GADA, and $P = 0.76$ for ZnT8A. IAA values were included if measured within 21 days of T1D diagnosis. The dotted line is at the cutoff for each autoantibody positivity.

**Table 2—Characteristics of T1D participants with DQB1*06:02* by autoantibody status**

| HbA1c at diagnosis, |
|---------------------|
| % [mmol/mol] (mean ± SD) |
| (n = 50)* |
| Ab+ (n = 33) |
| Ab− (n = 16) |
| P value |

| DKA at diagnosis (%) |
|----------------------|
| Yes |
| No |
| Unknown |

| HLA-DQ status (%) |
|-------------------|
| DQB1*03:02 |
| DQB1*02:01 |
| Other |

*Islet autoantibodies were measured in 49/50 0602− individuals with T1D.
Next, to gain insight into further potential genetic risk for T1D with \( \text{DQB1}^\text{*06:02} \), we conducted HLA class I typing for HLA-A and HLA-B alleles. We also conducted class I typing on a subset of 0602− T1D participants (Supplementary Material). Several A and B alleles have been shown to confer T1D risk (28,29), and the frequencies of these alleles in 0602+ and 0602− participants with autoantibodies are depicted in Table 3. Remarkably, 94% (31/33) of 0602+ participants with autoantibodies had at least one A risk allele defined as A*02, A*03, or A*24 compared with 80% (221/276) of those 0602− T1D participants with a trend toward statistical significance \((P = 0.058)\) (Table 3). Known T1D risk B alleles (B*15, 18, 39, 41, 49, and 50) were less frequent in both 0602+ and 0602− groups compared with the A alleles. B risk alleles are more frequent in 0602− T1D individuals compared with 0602+ individuals (51% vs. 24%, \( P = 0.007 \)) (Table 3).

Taken together, HLA typing indicates that those T1D individuals with \( \text{DQB1}^\text{*06:02} \) have high frequencies of both known T1D risk class II and class I alleles.

**DISCUSSION**

The HLA-DQB1*06:02 allele is known to provide dominant protection across the stages of T1D and from the initial development of clinical diabetes. As such, only a limited number of 0602+ T1D cases have been described in the literature. Here, we present one of the largest studies to characterize \( \text{DQB1}^\text{*06:02} \) individuals with clinical T1D to provide insights into the potential mechanisms of failed genetic protection in the presence of a strongly protective HLA allele. These 50 patients were identified over 26 years of performing HLA-DQ typing and measurement of islet autoantibodies in patients presenting to a large tertiary referral center. Less than 2% of our cohort with clinical T1D had the \( \text{DQB1}^\text{*06:02} \) allele, which is evidence of the profound protection afforded by the DQ6 allele against T1D development. Characterizing the rare 0602+ individuals who have developed clinical T1D provides a framework for understanding the underlying mechanisms behind failed genetic protection, which can offer important insights into T1D pathogenesis.

In general, T1D can be diagnosed at any age but typically peaks between the ages of 10 and 14 years, which coincides with the start of puberty (30). The 0602+ T1D patients have no exception with an average age at diagnosis of 11 years, which was similar to the 0602− patients in our study. An older age was observed in autoantibody-positive relatives with \( \text{DQB1}^\text{*06:02} \) in the TrialNet cohort; however, only a very small percentage of those individuals progressed to clinical T1D (20). Our data do not suggest that age of diagnosis is delayed by the presence of the DQ6 allele, but our presented results and those from the TrialNet cohort indicate that \( \text{DQB1}^\text{*06:02} \) affords protection in the presence of islet autoimmunity. However, as we report here, rare instances where the \( \text{DQB1}^\text{*06:02} \) allele fails to provide genetic protection from T1D do occur. Therefore, protection via the presence of the DQ6 allele is not absolute.

In fact, approximately two-thirds of our 0602+ T1D patients had islet autoantibodies. It is also appreciated that autoantibody-negative T1D can encompass different types of diabetes including monogenic forms of diabetes (31), and we did identify monogenic forms of diabetes with mutations in the \( \text{KCNJ11} \) and \( \text{WFS1} \) genes in three 0602+ individuals. Maturity-onset diabetes of the young (MODY) is another monogenic form of diabetes, which is more common in autoantibody-negative patients (32). Comprehensive testing for monogenic forms of diabetes including MODY needs to be completed in the 0602+ patients without antibodies, as this subset of individuals does not have genetic risk for T1D, to discern whether these individuals truly have autoantibody-negative T1D. Finally, type 2 diabetes can be difficult to distinguish from autoantibody-negative T1D. Although we cannot completely exclude this possibility, the majority of our autoantibody-negative T1D participants were prepubertal, and all participants required insulin treatment at diagnosis, thus making a diagnosis of type 2 diabetes less likely.

### Table 3—HLA class I typing in autoantibody-positive participants by \( \text{DQB1}^\text{*06:02} \) status

| HLA-A allele (%) | \( \text{DQB1}^\text{*06:02} \) | \( \text{DQB1}^\text{*06:02} \) | \( P \) value |
|-----------------|----------------------|----------------------|--------------|
| 02  | 36 (24/66) | 33 (184/552) | 0.680 |
| 03  | 20 (13/66) | 11 (61/552) | 0.068 |
| 24  | 8 (5/66) | 10 (56/552) | 0.663 |
| Other | 36 (24/66) | 46 (251/552) | 0.190 |
| Individuals with a risk allele (02, 03, and 24) | 94 (31/33) | 80 (221/276) | 0.058 |

| HLA-B allele (%) | \( \text{DQB1}^\text{*06:02} \) | \( \text{DQB1}^\text{*06:02} \) | \( P \) value |
|-----------------|----------------------|----------------------|--------------|
| 15  | 9 (6/66) | 15 (42/276) | 0.240 |
| 18  | 3 (2/66) | 7 (20/276) | 0.273 |
| 39  | 0 (0/66) | 3 (8/276) | 0.362 |
| 41  | 0 (0/66) | 1 (2/276) | 1.00 |
| 49  | 2 (1/66) | 1 (3/276) | 0.578 |
| 50  | 0 (0/66) | 1 (2/276) | 1.00 |
| Other | 86 (57/66) | 72 (199/276) | 0.018 |
| Individuals with a risk allele (15, 18, 39, 41, 49, and 50) | 24 (8/33) | 51 (70/138) | 0.007 |
The autoantibody profiles of those with 0602+ and 0602− T1D showed equivalent percentages of patients positive for autoantibodies directed against GAD65 and ZnT8 with similar levels between the groups. However, IAA and IA-2A levels were lower in the T1D 0602+ group compared with the 0602− group. Interestingly, a much lower percentage of autoantibodies directed against IA-2 was observed in the 0602− T1D patients. This finding could indicate less autoimmunity directed against IA-2, or the potential exists that there are autoantibodies directed against the extracellular domain of IA-2 (33), as the measured antibodies in our assay bind to intracellular IA-2 epitopes. A recent report describes antibodies to a novel variant of IA-2 with three amino acid substitutions, which has been shown to increase the prediction of T1D in autoantibody-positive relatives (34). It will be important to measure these alternate IA-2 autoantibodies in our 0602+ T1D patients.

The potential mechanisms of failed genetic protection in the presence of DQB1*06:02 are important to define. Our HLA typing data indicate that both known class I and II T1D risk alleles are frequently present in the 0602+ T1D group. This implicates islet-reactive CD8 and CD4 T cells in disease development as class I and II molecules function to present peptides to these respective T-cell subsets. Remarkably, 94% of the autoantibody-positive 0602+ group had an A risk allele with much lower frequencies of B risk alleles, implicating the role of HLA-A molecules in 0602+ T1D patients. CD8 T cells reactive to islet peptides presented by HLA-A2 are known to exist in the peripheral blood of patients with T1D (35,36) and within the residual islets of T1D organ donors (37,38). Thus, the presence, frequency, and function of islet-reactive CD8 T cells will be important to measure in 0602+ T1D patients.

When examining the other DQ allele in addition to 0602, we observed a high proportion of T1D risk-conferring alleles, mainly DQ8 (DQB1*03:02) and DQ2 (DQB1*02:01). These class II risk alleles did segregate by autoantibody status in that those with islet autoantibodies were more likely to have DQ8 on the other chromosome. In fact, DQ8 was only observed in those T1D 0602+ individuals with autoantibodies. Preproinsulin peptides have been eluted from antigen-presenting cells expressing DQ6 and DQ8, indicating that both heterodimers are capable of binding and presenting insulin peptides (39,40). Interestingly, an epitope within the signal peptide was presented by both DQ8 and DQ6, albeit in different binding registers (40). It will be important to study the function and activation status of CD4 T cells that respond to similar insulin peptides presented by DQ6 and DQ8 in individuals with and without T1D.

An alternate mechanism includes epitope stealing in which DQ6 preferentially binds T1D autoantigens such that DQ8 cannot present these antigens thus leading to less effector T-cell responses. Binding and peptide elution data from DQ6 and DQ8 expressing antigen-presenting cells are consistent with this mechanism (40,41). Epitope stealing may be lacking in the ~50% of our 0602+ T1D cases with DQ8; however, additional mechanisms may still be involved for those with a strongly protective HLA allele to develop T1D.

In conclusion, we describe a very rare cohort of individuals that developed clinical T1D in the presence of HLA-DQB1*06:02, which is ordinarily known to confer strong protection against disease development. Studying the clinical, immunologic, and genetic features of individuals that failed genetic protection holds promise for understanding mechanisms that ultimately lead to β-cell destruction in T1D.

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**Duality of Interest.** No potential conflicts of interest relevant to this article were reported.

**Author Contributions.** K.M.S., L.Y. and A.W.M. designed the studies and wrote the manuscript. A.M.M., A.A.A., K.A.M., E.E.B., and T.A. performed the experiments.

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**Figure 3**—HLA alleles in DQB1*06:02+ and DQB1*06:02− T1D individuals compared by islet autoantibody positive (Ab+) versus autoantibody negative (Ab−) status. n = 33 for 0602+ Ab+, n = 16 for 0602− Ab−, n = 2,077 for 0602+ Ab+, and n = 670 for 0602− Ab−.
experiments and reviewed the manuscript. L.P. conducted independent statistical analysis on the study data. A.W.M. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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**References**

1. Concannon P, Erlich HA, Julier C, et al.; Type 1 Diabetes Genetics Consortium. Type 1 diabetes: evidence for susceptibility loci from four genome-wide linkage scans in 1,435 multiplex families. Diabetes 2005;54:2995–3001
2. Todd JA, Walker NM, Cooper JD, et al.; Genetics of Type 1 Diabetes in Finland; Wellcome Trust Case Control Consortium. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. Nat Genet 2007;39:857–864
3. Barrett JC, Clayton DG, Concannon P, et al.; Type 1 Diabetes Genetics Consortium. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. Nat Genet 2009;41:703–707
4. Bradfield JP, Gu HO, Wang K, et al. A genome-wide meta-analysis of six type 1 diabetes cohorts identifies multiple associated loci. PLoS Genet 2011;7:e1002293
5. Erlich H, Valdes AM, Noble J, et al.; Type 1 Diabetes Genetics Consortium. HLA DR-DQ haplotypes and genotypes and type 1 diabetes risk: analysis of the type 1 diabetes genetics consortium families. Diabetes 2008;57:1084–1092
6. Hu X, Deutsch AJ, Lenz TL, et al. Additive and interaction effects at three amino acid positions in HLA-DQ and HLA-DR molecules drive type 1 diabetes risk. Nat Genet 2015;47:899–905
7. Nakayama M, Simmons KM, Michelis AW. Molecular interactions governing autoantigen presentation in type 1 diabetes. Curr Diab Rep 2015;15:113
8. Concannon P, Rich SS, Nepom GT. Genetics of type 1A diabetes. N Engl J Med 2009;360:1646–1654
9. Noble JA, Erlich HA. Genetics of type 1 diabetes. Cold Spring Harb Perspect Med 2012;2:a007732
10. Todd JA, Bell JJ, McDevitt HO. HLA-DQ beta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. Nature 1987;329:599–604
11. Morel PA, Dorman JS, Todd JA, McDevitt HO, Trucco M. Aspartic acid at position 57 of the HLA-DQ beta chain protects against type 1 diabetes: a family study. Proc Natl Acad Sci U S A 1988;85:8111–8115
12. Baich JM, Weeks T, Giles R, Hoover M, Stastny P, Capra JD. Analysis of HLA-DQ genotypes and susceptibility in insulin-dependent diabetes mellitus. N Engl J Med 1990;322:1836–1841
13. Pugliese A, Kawasaki E, Zeller M, et al. Sequence analysis of the diabetes-protective human leukocyte antigen-DQB1*0602 allele in unaffected, islet cell antibody-positive first degree relatives and in rare patients with type 1 diabetes. J Clin Endocrinol Metab 1999;84:1722–1728
14. Redondo MJ, Kawasaki E, Mulgrew CL, et al. DR- and DQ-associated protection from type 1A diabetes: comparison of DRB1*1401 and DQA1*0102–DQB1*0602. J Clin Endocrinol Metab 2000:85:3793–3797
15. Klitz W, Maisers M, Spellman S, et al. New HLA haplotype frequency reference standards: high-resolution and large sample typing of HLA DR-DQ haplotypes in a sample of European Americans. Tissue Antigens 2003;62:296–307
16. Erlich HA, Griffith RL, Bugawan TL, Ziegler R, Alper C, Eisenbarth G. Implication of specific DQB1 alleles in genetic susceptibility and resistance by identification of IIDD siblings with novel HLA-DQB1 allele and unusual DR2 and DR1 haplotypes. Diabetes 1991;40:478–481
17. Hooper ML, Marta RT. Molecular modeling of HLA-DQ suggests a mechanism of resistance in type 1 diabetes. Scand J Immunol 1997;45:193–202
18. Insel RA, Dunne JL, Atkinson MA, et al. Staging presymptomatic type 1 diabetes: a scientific statement of the JDFR, the Endocrine Society, and the American Diabetes Association. Diabetes Care 2015;38:1964–1974
19. Siims EE, Geyer S, Johnson SB, et al.; Type 1 Diabetes TrialNet Study Group. Who is enrolling? The path to monitoring in type 1 diabetes TrialNet’s Pathway to Prevention. Diabetes Care 2019;42:2228–2236
20. Pugliese A, Bouhle D, Yu L, et al.; Type 1 Diabetes TrialNet Study Group. HLA-DRB1*15:01–DQA1*01:02–DQB1*06:02 haplotype protects autoantibody-positive relatives from type 1 diabetes throughout the stages of disease progression. Diabetes 2016;65:1109–1119
21. Zelisiewski D, Tiercy JM, Boulard C, et al. Extensive study of DRB, DQA, and DQB gene polymorphism in 23 DR2-positive, insulin-dependent diabetes mellitus patients. Hum Immunol 1992;33:140–147
22. Noble JA, Valdes AM, Cook M, Kitz W, Thomson G, Erlich HA. The role of HLA class II genes in insulin-dependent diabetes mellitus: molecular analysis of 180 Caucasian, multiplex families. Am J Hum Genet 1996;59:1134–1148
23. Yu L, Rewers M, Giann R, et al. Antigenic determinants in the amino terminus of IA-2 (ICA512) in childhood and adult autoimmune diabetes: new biomarker development. Diabetes Care 2017;40:561–566
24. James EA, Abreu JRF, McGinty JW, et al.; Immunology of Diabetes Society T Cell Workshop. Diabetologia 2018;61:658–670