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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

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The HLA-DRB1*15:01-DQA1*01:02-DQB1*06:02 haplotype is linked to protection from the development of type 1 diabetes (T1D). However, it is not known at which stages in the natural history of T1D development this haplotype affords protection. We examined a cohort of 3,358 autoantibody-positive relatives of T1D patients in the Pathway to Prevention (PTP) Study of the Type 1 Diabetes TrialNet. The PTP study examines risk factors for T1D and disease progression in relatives. HLA typing revealed that 155 relatives carried this protective haplotype. A comparison with 60 autoantibody-negative relatives suggested protection from autoantibody development. Moreover, the relatives with DRB1*15:01-DQA1*01:02-DQB1*06:02 less frequently expressed autoantibodies associated with higher T1D risk, were less likely to have multiple autoantibodies at baseline, and rarely converted from single to multiple autoantibody positivity on follow-up. These relatives also had lower frequencies of metabolic abnormalities at baseline and exhibited no overall metabolic worsening on follow-up. Ultimately, they had a very low 5-year cumulative incidence of T1D. In conclusion, the protective influence of DRB1*15:01-DQA1*01:02-DQB1*06:02 spans from autoantibody development through all stages of progression, and relatives with this allele only rarely develop T1D.

HLAs are antigen-presenting molecules that play a key role in mediating adaptive immune responses. HLA class I and class II molecules are restricting elements for CD8 and CD4 T-cell responses, respectively. They are encoded by polymorphic genes in the HLA complex, and a number of HLA gene variants have been linked to immune-mediated and autoimmune diseases (1,2). In type 1 diabetes (T1D), certain HLA class I and class II variants increase disease risk; these include the class I molecules encoded by A*02:01, A*24, and B39 and the class II molecules encoded by the

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DRB1*04 (DR4)-DQA1*03:01-DQB1*03:02 and DRB1*03:01 (DR3)-DQA1*05:01-DQB1:02:01 haplotypes. In T1D, HLA-encoded susceptibility confers up to 50–60% of the overall genetic risk from inherited alleles (3).

Other HLA variants are linked to genetic protection from T1D. Among four HLA-DR2 (DRB1*15) haplotypes observed in Caucasians, the DRB1*15:01-DQA1*01:02-DQB1*06:02 haplotype is commonly found in Caucasians and is negatively associated with T1D (4–8). Individuals with this haplotype are extremely rare (<1%) in most T1D populations studied (9,10). The disease association of various HLA-DR2 haplotypes expressing diverse linkage patterns of DRB1 and DQA1/DQB1 alleles (5,10–13) or unusual DQA1/DQB1 alleles in *cis* with the usual DRB1*15:01 allele (14,15) suggests that protection from T1D maps largely to the DQA1*01:02 and DQB1*06:02 alleles, which together encode for the HLA-DQ6 heterodimer. DRB1*15:01-DQA1*01:02-DQB1*06:02 has been previously linked to protection from T1D development among first-degree relatives with autoantibodies to islet cell autoantigens (7,16–19). This suggests that genetic protection may not always prevent the triggering of islet autoimmunity in relatives; yet, they rarely develop diabetes.

Nevertheless, it is not known at which stages during the natural history of T1D development that this haplotype is protective, including the initial triggering of autoantibodies, the development of multiple autoantibody responses to islet antigens, and the occurrence of metabolic changes indicating β-cell function deterioration eventually leading to clinically manifest diabetes. Such knowledge is of potential importance, as it could yield insights for devising preventive strategies against the disease. To this end, we examined the Pathway to Prevention (PTP) cohort of the Type 1 Diabetes TrialNet (TrialNet), a consortium of investigators studying the natural history, risk factors, and prevention of T1D (20). The cohort includes over 3,000 relatives of T1D patients who were found to express T1D-associated autoantibodies and are followed longitudinally with repeat oral glucose tolerance and autoantibody testing until the development of T1D. This is the first study to examine the protective influence of DRB1*15:01-DQA1*01:02-DQB1*06:02 throughout the progression to T1D.

RESEARCH DESIGN AND METHODS

Subjects

These analyses include 3,358 relatives (first to third degree) of patients with T1D who expressed T1D-associated autoantibodies and therefore are at increased risk for disease development. The relatives were identified by TrialNet, a National Institutes of Health–sponsored consortium that conducts clinical research studies in T1D patients and their relatives, including prevention trials and intervention trials following T1D diagnosis. As a control group for these PTP relatives, 60 autoantibody-negative relatives were studied; the TrialNet Coordinating Center generated randomized lists of autoantibody-negative PTP participants, which were then provided to TrialNet Clinical Centers for recruitment. All subjects signed informed consent and the study was approved by the ethics boards of all participating institutions. Demographic characteristics are shown in Table 1. Participants self-reported race and ethnicity in the screening form.

Table 1—Characteristics of PTP participants by autoantibody status

	AAb+ (*n* = 3,358)	AAb− (*n* = 60)	*P*
Age (years)	18.2 ± 13.4 (*n* = 3,325)	16.6 ± 10.1 (*n* = 60)	0.23
BMI (kg/m^2)	21.7 ± 6.7 (*n* = 3,222)	20.7 ± 5.3 (*n* = 57)	0.15
Sex (% female)	53.2	52.5	0.91
Relation to the proband			
Offspring	18.5 (*n* = 613)	18.6 (*n* = 11)	0.60*
Parent	21.2 (*n* = 705)	15.2 (*n* = 9)	
Sibling	52.0 (*n* = 1,726)	52.5 (*n* = 31)	
Other	8.3 (*n* = 276)	13.5 (*n* = 8)	
Unknown	(*n* = 38)	(*n* = 1)	
Duration of follow-up (years)	3.0 ± 2.3	4.5 ± 1.7	<0.0001
Race			
White	87.1 (*n* = 2,872)	96.4 (*n* = 53)	0.04**
Other	12.9 (*n* = 424)	3.6 (*n* = 2)	
Unknown	(*n* = 62)	(*n* = 5)	
Ethnicity			
Hispanic or Latino	12.3 (*n* = 394)	3.6 (*n* = 2)	0.05*
Not Hispanic or Latino	87.7 (*n* = 2,806)	96.4 (*n* = 54)	
Other	(*n* = 107)	(*n* = 1)	
Unknown	(*n* = 51)	(*n* = 3)	

Data are mean ± SD or %. AAb, autoantibody. *The comparison does not include "other" and "unknown." **The comparison does not include "unknown."

Study Design

The TrialNet PTP Study, formerly known as the Natural History Study, screens relatives of T1D patients for the presence of islet autoantibodies (20). Between 2000 and 30 September 2014, TrialNet screened approximately 140,000 relatives of patients with T1D for T1D-associated autoantibodies to glutamic acid decarboxylase 65 (GAD65A), tyrosine phosphatase-like insulinoma-associated protein (IA-2A), and insulin (mIAA). Relatives positive for any of these autoantibodies were subsequently tested for islet cell antibodies, and a subset were also tested for autoantibodies to the zinc transporter 8 (ZnT8A), as described below. Initially, all those with at least one autoantibody were prospectively followed with testing every 6 months for autoantibodies and metabolic evaluation by oral glucose tolerance test (OGTT). After 2012, some participants with single autoantibodies, who are considered at lower risk, have been followed at yearly intervals. The autoantibody-negative relatives had the same baseline testing as those who were autoantibody positive; they have been followed with autoantibody and OGTT measurements at yearly intervals. For the OGTTs, samples for plasma glucose and C-peptide measurement were obtained in the fasting state and at 30, 60, 90, and 120 min after ingestion of a 1.75 g per kilogram glucose dose (maximum: 75 g of carbohydrate). Dysglycemia was defined by a fasting glucose value ≥110 mg/dL, a 2-h value of 140–199 mg/dL, and/or a 30-, 60-, 90-min glucose value ≥200 mg/dL. The diagnosis of T1D was made according to American Diabetes Association guidelines. This required either two consecutive OGTTs in which fasting glucose values were ≥126 mg/dL and/or 2-h glucose values ≥200 mg/dL and/or HbA_{1c} ≥6.5% and/or clinical symptoms associated with random glucose ≥200 mg/dL (21). An OGTT was repeated for diagnostic confirmation if the fasting glucose value was ≥126 mg/dL and/or the 2-h glucose value was ≥200 mg/dL. If both thresholds were not exceeded on the confirmatory OGTT, participants were followed as before. We calculated the progression scale at 6 months (PS6M), which is a measure of change in the glucose sum (from the 30-, 60-, 90-, and 120-min values of OGTTs) over a 6-month period (22). Autoantibody-positive individuals who do not progress to T1D with at least 2 years of follow-up (nonprogressors) are expected to have an average PS6M value of zero. We also evaluated early C-peptide secretion during OGTTs as defined by the 30 − 0 min difference in C-peptide levels. This measure correlates highly with the first-phase insulin response of an intravenous glucose tolerance test (23).

Laboratory Procedures

All autoantibodies were measured using standardized radioimmunoassays (24,25). Screening identified 3,358 relatives expressing at least one autoantibody. Following discovery of the ZnT8 autoantigen in 2007 (26) and the development of a specific radioimmunoassay, a subset of 1,940 autoantibody-positive relatives was tested for ZnT8A. Samples that were positive for mIAA and/or GADA were also tested with the new electrochemiluminescence (ECL) assays (ECL-IAA and ECL-GADA, respectively) at the Barbara Davis Center Autoantibody/HLA Core Laboratory, as previously described (27–29). Both the ECL-IAA and ECL-GADA identify autoantibody responses that are more disease specific and are stronger predictors of T1D risk than the standard radioimmunoassays. ECL results were available from 931 autoantibody-positive relatives; of whom, 346 had multiple autoantibodies and 243 had T1D. Relatives were typed for HLA class II DRB1, DQA1, and DQB1 alleles using DNA-based typing with oligonucleotide probes, as previously reported (30). C-peptide levels were measured by an immunoenzymometric assay using the Tosoh AIA-600II autoanalyzer (Tosoh Bioscience, South San Francisco, CA) (31). The glucose oxidase method was used to measure the plasma glucose.

Data Analysis

The χ^2 tests and t tests were used to compare groups. Paired t tests were used to assess changes over time (at the beginning and end of follow-up). Cumulative incidence was determined by Kaplan-Meier analysis. Log-rank testing was used to compare cumulative incidence. Proportional hazards regression was used to assess time-dependent associations. 95% CIs are included with estimates of risk. Adjustments for multiple comparisons were not performed.

RESULTS

Characteristics at Baseline

In the PTP study, 3,358 PTP study participants were autoantibody positive and HLA typed for class II DRB1, DQA1, and DQB1 alleles. A group of 60 autoantibody-negative relatives were also typed. Key baseline characteristics are shown in Table 1 for both autoantibody-positive and autoantibody-negative relatives. Baseline characteristics for the autoantibody-positive relatives, according to the absence or presence of DRB1*15:01-DQA1*01:02-DQB1*06:02, are shown in Table 2; from here onward, we will refer to the two categories of autoantibody-positive relatives as 0602+ and 0602−, respectively. The 0602+ group had a higher mean age ($P < 0.0001$) with higher BMI values ($P < 0.0001$); sex was not significantly different. The 0602+ relatives more frequently included parents and less frequently included siblings of T1D probands ($P = 0.01$) (Table 2). The 60 autoantibody-negative relatives were similar in age, BMI, and sex to the autoantibody-positive relatives (Table 1); >85% of the relatives were white and not Hispanic in all the groups compared.

Frequency of DRB1*15:01-DQA1*01:02-DQB1*06:02

The frequency of DRB1*15:01-DQA1*01:02-DQB1*06:02 was higher among autoantibody-negative than that among autoantibody-positive relatives [10/60 (17%) vs. 155/3,358 (4.6%)]; the odds ratio (OR) was highly significant, consistent with protection from autoantibody development by DRB1*15:01-DQA1*01:02-DQB1*06:02 positivity (OR 0.242 [95% CI 0.120, 0.486], $P < 0.001$).

Table 2—Characteristics of PTP participants by DRB1*15:01-DQA1*01:02-DQB1*06:02 status

	0602+ (*n* = 155)	0602− (*n* = 3,203)	*P*
Age (years)	23.7 ± 14.5 (*n* = 154)	17.9 ± 13.3 (*n* = 3,171)	<0.0001
BMI (kg/m^2)*	23.9 ± 7.1	21.6 ± 6.6	<0.0001
Sex (% female)	59.1	53.0	0.14
Relation to the proband			
Offspring	16.9 (*n* = 26)	18.5 (*n* = 587)	0.01*
Parent	31.1 (*n* = 48)	20.8 (*n* = 657)	
Sibling	42.9 (*n* = 66)	52.4 (*n* = 1,660)	
Other	9.1 (*n* = 14)	8.3 (*n* = 262)	
Unknown	(*n* = 1)	(*n* = 37)	
Duration of follow-up (years)	3.5 ± 2.2	3.0 ± 2.3	0.01
Race			
White	89.6 (*n* = 138)	87.0 (*n* = 2,734)	0.35**
Other	10.4 (*n* = 16)	13.0 (*n* = 408)	
Unknown	(*n* = 1)	(*n* = 61)	
Ethnicity			
Hispanic or Latino	8.7 (*n* = 13)	12.5 (*n* = 381)	0.17*
Not Hispanic or Latino	91.3 (*n* = 136)	87.5 (*n* = 2,670)	
Other	(*n* = 4)	(*n* = 103)	
Unknown	(*n* = 2)	(*n* = 49)	

Data are mean ± SD or %. *The comparison does not include "other" and "unknown." **The comparison does not include "unknown."

Frequencies of Autoantibody Positivity at Baseline

Table 3 compares baseline GADA, IA-2A, and mIAA autoantibody patterns between 0602+ and 0602− participants. The 0602+ relatives had lower proportions of autoantibody positivity for all autoantibodies: GADA (72.9% vs. 80.4%, $P = 0.02$), IA-2A (17.4% vs. 35.5%, $P < 0.0001$), and mIAA (29.7% vs. 38.2%, $P = 0.03$). The 0602+ relatives had a higher proportion of single autoantibodies (84.5% vs. 59.0%, $P < 0.0001$), most commonly GADA, and lower proportions of multiple autoantibodies (two autoantibodies, 11.0% vs. 27.9%, $P < 0.0001$; three autoantibodies, 4.5% vs. 13.0%, $P < 0.0001$). These findings were mirrored in the smaller subset with available ZnT8A measurements (n = 1,940); 0602+ relatives less frequently expressed ZnT8A (12.9% vs. 36.1%, $P < 0.0001$) and four autoantibodies (5.9% vs. 10.5%, $P < 0.0001$).

Table 3—Frequency of autoantibody positivity in 0602+ and 0602− relatives at screening

	0602+ (*n* = 155)	0602− (*n* = 3,203)	*P*
Entire data set (*n* = 3,203)			
GAD65A	72.9 (113/155)	80.4 (2,574/3,203)	0.02
IA-2A	17.4 (27/155)	35.5 (1,136/3,203)	<0.0001
mIAA	29.7 (46/155)	38.2 (1,124/3,203)	0.03
1 AAb	84.5 (131/155)	59.0 (1,890/3,203)	<0.0001
2 AAb	11.0 (17/155)	27.9 (895/3,203)	<0.0001
3 AAb	4.5 (7/155)	13.0 (418/3,203)	<0.0001
	0602+ (*n* = 85)	0602− (*n* = 1,855)	*P*
Subset tested for ZnT8A (*n* = 1,940)			
GAD65A	78.2 (67/85)	80.2 (1,488/1,855)	0.75
IA-2A	18.8 (16/85)	36.1 (670/1,855)	0.001
mIAA	24.7 (21/85)	39.0 (723/1,855)	0.008
ZnT8A	12.9 (11/85)	36.1 (669/1,855)	<0.0001
1 AAb	81.2 (69/85)	48.3 (896/1,855)	<0.0001
2 AAb	8.2 (7/85)	22.5 (418/1,855)	<0.0001
3 AAb	4.7 (4/85)	18.6 (346/1,855)	<0.0001
4 AAb	5.9 (5/85)	10.5 (195/1,855)	<0.0001

Data are % (*n*/*N*), unless stated otherwise. AAb, autoantibody.

When GADA and mIAA were measured by ECL assays, ECL positivity was associated with increased risk (27,28); the proportions with ECL positivity were lower in 0602+ relatives than in 0602− relatives for GADA (21/47 [44.7%] vs. 550/883 [62.3%], $P = 0.02$] and mIAA (5/47 [11.6%] vs. 305/884 [34.8%], $P < 0.001$).

Progression to Multiple Autoantibodies

Figure 1*A* compares cumulative incidence curves between the 0602+ and 0602− relatives for progression from one to two or more autoantibodies in the entire data set (not including ZnT8A). The progression to multiple autoantibodies was lower (log-rank test, $P = 0.001$) in 0602+ relatives. The 5-year conversion estimates (95% CI) for 0602+ and 0602− relatives were 19.0% (11, 31.5) and 33% (30.4, 36.7), respectively. The hazard ratio (HR) was 0.42 (0.25, 0.72). Overall, 14/131 (10.7%) 0602+ relatives and 413/1,890 (21.8%) 0602− relatives converted from single to multiple autoantibody positivity on follow-up ($P = 0.003$, OR 0.43 [95% CI 0.24, 0.75]). Data are also shown for siblings only (Fig. 1*B*) (log-rank test, $P = 0.0135$): the 5-year conversion estimates (95% CI) for 0602+ and 0602− relatives were 20.0% (9.8, 38.4) and 44% (38.7, 49.2), respectively. The HR was 0.40 (0.19, 0.85). Overall, 7/48 (14.6%) 0602+ relatives and 239/831 (28.8%) 0602− relatives converted from single to multiple autoantibody

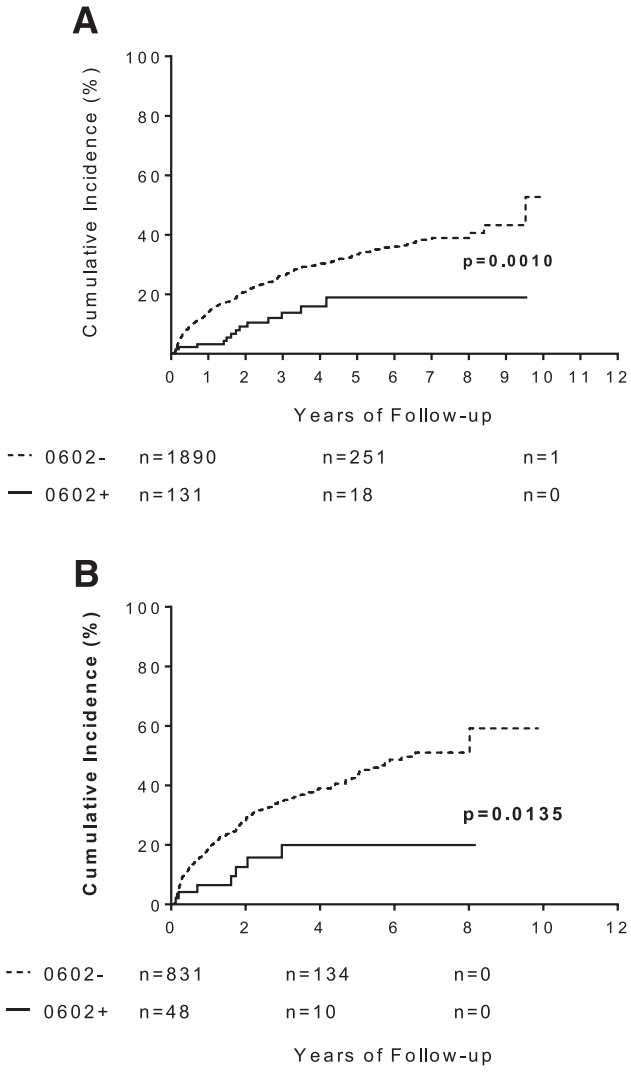

Figure 1—Cumulative incidence of conversion from single to multiple autoantibodies in 0602+ and 0602− relatives. *A*: Entire data set. *B*: Siblings only.

positivity on follow-up (P = 0.03, OR 0.42 [95% CI 0.19, 0.96]). Both HRs and ORs indicate strong protection by DRB1*15:01-DQA1*01:02-DQB1*06:02 positivity from the development of multiple autoantibodies.

Metabolic Measures at Baseline

Table 4 compares frequencies of abnormal glucose tolerance at baseline between 0602+ and 0602− relatives with autoantibodies for those relatives who had data for all OGTT time points. The 0602+ relatives had a lower frequency of diabetic range values (fasting glucose values ≥126 mg/dL and/or 2-h glucose values ≥200 mg/dL) in their baseline OGTTs (P = 0.004). Among autoantibody-positive relatives whose glucose values were in the nondiabetic range, 0602+ relatives also had a lower frequency of dysglycemic OGTTs (P = 0.013). The sum of glucose (glucose sum) values from 30 to 120 min was also lower in those individuals (509 ± 90 mg/dL vs. 530 ± 109 mg/dL, P = 0.008; data not shown).

In 0602+ relatives, the proportion with an impaired early C-peptide response (as defined by 30 − 0 min C-peptide difference values <2.0 ng/mL), which correlates with the first-phase insulin response (23), was significantly lower than in 0602− relatives [10/150 (6.6%) vs. 723/3,071 (23.5%), $P < 0.001$; data not shown). The Diabetes Prevention Trial-Type 1 (DPT-1) Risk Score (DPTRS) (32), which is based on glucose and C-peptide measures along with age and BMI, was also lower in 0602+ relatives (5.5 ± 1.1 vs. 6.1 ± 1.3, $P < 0.001$; data not shown).

Changes in Glycemia From Baseline

The change in glucose concentrations from baseline to the 6-month visit, as indicated by the PS6M, was lower in 0602+ relatives (2.3 ± 77.2 ng/mL [n = 75] vs. 25.9 ± 101.9 ng/mL [n = 1,341], P = 0.01]. Table 5 shows OGTT glucose values at baseline and at the 24-month (± 3 months) visit in 0602+ and 0602− relatives. The 0602− relatives showed a statistically significant increase at all OGTT time points and in the glucose sum over the 24 months; in contrast, there were no significant changes for 0602+ relatives. Moreover, 0602+ relatives showed a slight decrease in the glucose sum values from baseline, whereas there was an appreciable increase in the 0602− relatives ($P < 0.001$).

Diagnosis of T1D

The cumulative incidence for T1D (Fig. 2*A*) was much lower for 0602+ relatives ($P < 0.0001$). The 5-year T1D incidence estimates for 0602+ and 0602− relatives were 3.8% (95% CI 1.3, 10.5) and 28.5% (26.4, 30.8), respectively. The HR (95% CI) for the presence of 0602 was 0.11 (0.04, 0.29), indicating a strong protective effect of DRB1*15:01-DQA1*01:02-DQB1*06:02 positivity. Among those with dysglycemia at baseline (Fig. 2*B*), progression to T1D occurred less frequently in the 0602+ relatives (2/21 [9.5%] vs. 204/664 [30.7%]) (HR 0.24 [0.05, 1.03], P = 0.05). When we analyzed relatives with higher risk because of the presence of HLA-DR3 or -DR4 and multiple autoantibodies, protection was still evident: only 17.6% (3/17) of the 0602+ relatives have progressed to T1D

Table 4—Proportions of 0602+ and 0602− relatives with abnormal glucose tolerance at baseline OGTT

	0602+	0602−	OR (95% CI)	*P*
Diabetes	2/155 (1.3)	219/3,203 (6.8)	0.13 (0.03, 0.79)	0.004
Dysglycemic	21/143 (14.7)	664/2,781 (23.8)	0.55 (0.34, 0.88)	0.013

Data are *n/N* (%), unless stated otherwise.

Table 5—Glucose values (mg/dL) at baseline and 24 months in 0602+ and 0602− relatives

	0602+ (n = 50)			0602− (n = 671)		
	Baseline	24 months	P	Baseline	24 months	P
0 min	88 ± 6	89 ± 8	n.s.	89 ± 9	91 ± 11	<0.01
30 min	135 ± 21	138 ± 23	n.s.	144 ± 28	146 ± 30	<0.05
60 min	134 ± 31	135 ± 33	n.s.	140 ± 36	148 ± 44	<0.001
90 min	121 ± 27	117 ± 31	n.s.	125 ± 34	133 ± 47	<0.001
120 min	111 ± 27	105 ± 25	n.s.	117 ± 27	124 ± 45	<0.001
Glucose sum	500 ± 83	495 ± 90	n.s.	526 ± 104	551 ± 146	<0.001

Data are mean ± SD, unless stated otherwise. n.s., not significant.

compared with 34.1% (548/832, $P < 0.0001$) of 0602− relatives. However, only 1/131 (0.8%) 0602+ relatives with a single autoantibody developed T1D compared with the 3/17 (17.6%) 0602+ relatives with multiple autoantibodies ($P < 0.0001$). Overall, only 4 of the 155 0602+ relatives developed diabetes on follow-up. The characteristics of the 0602+ individuals diagnosed with diabetes are shown in Table 6. One of the four relatives was 44.7 years of age at diagnosis and positive only for GADA. The others were all less than 16 years of age at diagnosis and had at least two autoantibodies; two subjects had high BMI values.

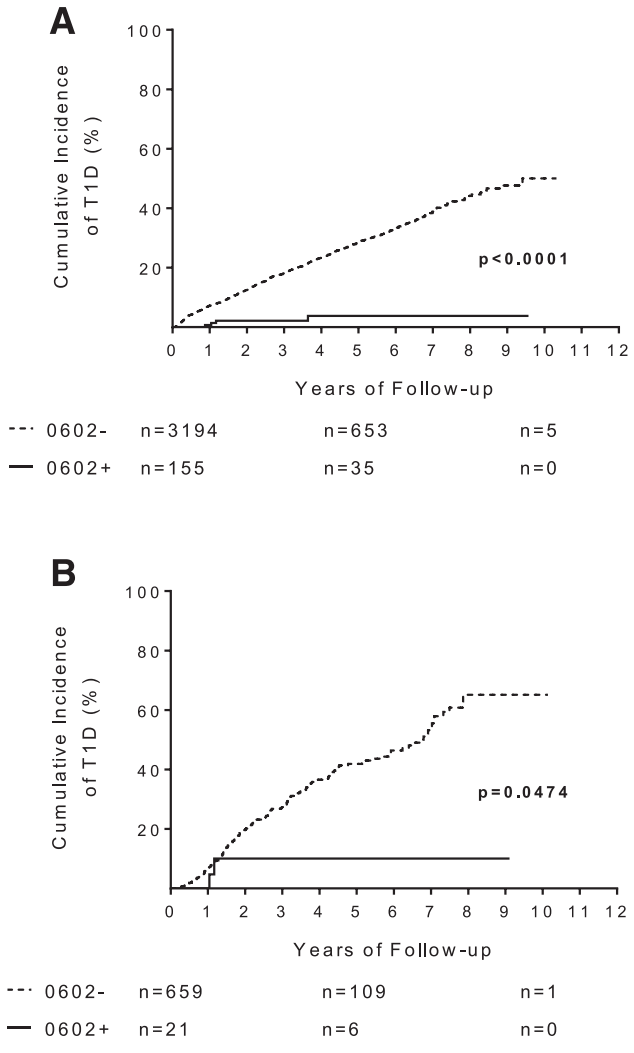

Figure 2—Cumulative incidence of T1D in 0602+ and 0602− relatives. *A*: Entire data set. *B*: Subset of relatives with dysglycemia at baseline.

Frequency of Predisposing HLA Haplotypes

We investigated whether the presence of autoantibodies and the reduced incidence of T1D in 0602+ relatives were related to a potential lack of HLA predisposition on the other chromosome. We present data for all such relatives, and then for those with a single autoantibody at screening and on follow-up, those who acquired multiple autoantibodies on follow-up, and those who already had multiple autoantibodies at screening (Table 7). We determined the frequencies of HLA-DR3 and -DR4 haplotypes, also accounting for the contribution of the DQ locus in 0602+ and 0602− relatives. Considering that in the 0602+ group one chromosome is fixed, we compared the frequency of DR3 or DR4 haplotypes alone, so that the frequency is independent of the other chromosome. Specifically, we compared the frequency of

1. DR3, meaning any DR3 haplotype (DRB1*03) without DR4 (any DR4, DRB1*04), and more specifically the T1D-associated DR3 haplotype (DRB1*03:01-DQA1*05:01-DQB1*02:01) without any DR4; overall, 8 (0.3%) DR3-positive subjects did not have the T1D-associated DR3.
2. DR4, meaning any DR4 haplotype (DRB1*04) without DR3 (any DR3, DRB1*03), and more specifically the T1D-associated DR4 haplotypes bearing HLA-DQ8 (DRB1*04-DQA1*03:01-DQB1*03:02) without DR3; overall, 212 (8.1%) of the DR4-positive subjects did not have T1D-associated DR4-DQ8 haplotypes. Although not directly compared in the Table 7, this frequency did not differ among 0602+ and 0602− relatives (for all the groups compared P = 0.5–0.9).
3. DR3/DR4 heterozygous genotypes, any DR3 haplotype (DRB1*03) together with any DR4 haplotype (DRB1*04), and then T1D-associated genotypes encoded by DRB1*04-DQA1*03:01-DQB1*03:02 together with DRB1*03:01-DQA1*05:01-DQB1*02:01.

The comparisons predictably show that there were differences in the frequency of DR3/DR4 genotypes among 0602+ and 0602− relatives in all subsets compared, which are explained by the fact that 0602+ relatives cannot have this genotype. There were no statistically significant differences for DR3 haplotypes in any comparison. However, the frequency of DRB1*04 haplotypes, with and without DQA1*03:01-DQB1*03:02, was lower among 0602+ relatives in comparisons of all autoantibody-positive relatives and of relatives with a single autoantibody at screening and on follow-up. Such differences in DR4 haplotypes were not observed in comparisons of relatives who multiple autoantibodies acquired on follow-up and of relatives who had multiple autoantibodies at screening.

We then compared the frequency of DRB1*04-DQA1*03:01-DQB1*03:02 (alone) in 0602+ and 0602− relatives with multiple autoantibodies (either at screening or on follow-up) and found that only 1/16 (6.2%) of the 0602+ relatives had T1D compared with 220/691 (31.8%) of 0602− relatives (P = 0.02, OR 0.14, Fisher exact test). None of the 0602+ relatives with a single autoantibody and DRB1*04-DQA1*03:01-DQB1*03:02 had developed T1D.

Finally, we compared the occurrence of T1D, multiple autoantibodies, positivity for each autoantibody, and dysglycemia at baseline and at the end of the follow-up for 0602+ relatives according to the presence of DR3 or DR4-DQ8 (DR4B1*04-DQA1*03:01-DQB1*03:02) on the other chromosome. The above parameters did not differ in this comparison, except that relatives that were 0602+ and DR4-DQ8+ were more frequently positive for multiple autoantibodies (16/45, 35.6%) than those carrying DR3 (9/54, 16.7%; P = 0.0382) (Supplementary Table 1).

DISCUSSION

Prior studies of islet autoantibody-positive relatives showed that those carrying the protective DRB1*15:01-DQA1*01:02-DQB1*06:02 haplotype have a very low risk of developing T1D; such relatives are unlikely to express multiple autoantibodies (7,16–19). In addition, in most 0602+ relatives, T1D did not develop even in the presence of HLA alleles that confer susceptibility on the other chromosome. However, prior studies have not examined the effect of DRB1*15:01-DQA1*01:02-DQB1*06:02 throughout the natural history of islet autoimmunity.

Previous studies have not compared the frequency of DRB1*15:01-DQA1*01:02-DQB1*06:02 between autoantibody-positive and autoantibody-negative relatives. Our cross-sectional analysis indicates that autoantibody-negative relatives carry DRB1*15:01-DQA1*01:02-DQB1*06:02 at similar frequency to that of Caucasian populations (www.allelefrequencies.net) but higher compared with autoantibody-positive relatives; moreover, we show that autoantibody development is less likely in 0602+ relatives, a finding that will require confirmation in prospective studies of autoantibody-negative relatives.

Among autoantibody-positive relatives, we show that those with DRB1*15:01-DQA1*01:02-DQB1*06:02 are less

Table 6—Baseline characteristics of 0602+ relatives who developed T1D

T1D case	Age of onset (years)	Relation to the proband	Sex	Ethnicity	BMI (kg/m^2)	OGTT status	GADA	IA-2A	mIAA	ZnT8A	Other HLA-DRB1-DQA1-DQB1
1	44.7	Offspring	F	Caucasian	26.0	Normal	Pos	Neg	Neg	Neg	0301–0501–0201
2	15.0	Other	F	Hispanic	20.6	Dysglycemia	Pos	Pos	Pos	Pos	0301–0501–0201
3	15.6	Offspring	M	Caucasian	28.8	Diabetic range	Neg	Pos	Neg	Pos	0301–0501–0201
4	12.0	Sibling	M	Caucasian	31.9	Dysglycemia	Pos	Pos	Pos	Neg	0405–0301–0302

F, female; M, male; Neg, negative; Pos, positive.

Table 7—Frequencies of HLA haplotypes in 0602+ and 0602− relatives

	0602+		0602−			0602+		0602−		
	All AAb+					Single AAb at screening and on follow-up				
Haplotypes or genotypes	*n/N*	%	*n/N*	%	*P*	*n/N*	%	*n/N*	%	*P*
DRB1*03+, (DRB1*04−)	57/155	36.8	725/2,447	29.6	0.07	48/117	41.0	440/1,261	34.9	0.19
DRB1*03:01-DQA1*05:01-DQB1*02:01+, (DRB1*04−)	54/155	34.8	720/2,447	29.4	0.18	45/117	38.5	437/1,261	34.7	0.41
DRB1*04+, (DRB1*03−)	54/155	34.8	1,339/2,447	54.7	<0.0001	35/117	29.9	528/1,261	41.9	0.01
DRB1*04-DQA1*03:01-DQB1*03:02+, (DRB1*03−)	45/155	29.0	1,136/2,447	46.4	<0.0001	29/117	24.8	445/1,261	35.3	0.02
DRB1*04+ and DRB1*03+	0/155	0.0	755/3,203	23.6	<0.0001	0/117	0.0	268/1,477	18.1	<0.0001
DRB1*04-DQA1*03:01-DQB1*03:02+ and DRB1*03:01-DQA1*05:01-DQB1*02:01+	0/155	0.0	659/3,203	20.6	<0.0001	0/117	0.0	216/1,477	14.6	<0.0001
	Single AAb at screening → multiple AAb					Multiple AAb at screening				
	n/N	%	*n/N*	%	*P*	*n/N*	%	*n/N*	%	*P*
DRB1*03+, (DRB1*04−)	4/14	28.6	79/309	25.6	0.76	5/24	20.8	206/973	21.2	1.0
DRB1*03:01-DQA1*05:01-DQB1*02:01+, (DRB1*04−)	4/14	28.6	78/309	25.2	0.75	5/24	20.8	205/973	21.1	1.0
DRB1*04+, (DRB1*03−)	7/14	50.0	188/309	60.8	0.41	12/24	50.0	623/973	64.0	0.19
DRB1*04-DQA1*03:01-DQB1*03:02+, (DRB1*03−)	6/14	42.9	165/309	53.4	0.58	10/24	41.7	526/973	54.1	0.3
DRB1*04+ and DRB1*03+	0/14	0.0	115/413	27.8	0.01	0/24	0.0	373/1,313	28.4	0.0007
DRB1*04-DQA1*03:01-DQB1*03:02+ and DRB1*03:01-DQA1*05:01-DQB1*02:01+	0/14	0.0	104/413	25.2	0.02	0/24	0.0	339/1,313	25.8	0.001

frequently positive for each autoantibody studied, including ZnT8A, which was not included in earlier reports. The most prevalent autoantibody among 0602+ relatives was GADA, most often as a single autoantibody, which implies a lower risk of future T1D among autoantibody-positive relatives (33–36). The 0602+ relatives much less frequently expressed IA-2A, ZnT8A, and multiple autoantibodies, markers of more advanced islet autoimmunity and more rapid disease progression that typically appear closer to diagnosis.

In addition, our study is the first to show that 0602+ relatives were less often positive for GADA and IAA measured by ECL assays, which identify autoantibody responses more strongly associated with progression (27,28). Although others have observed lower proportions of multiple autoantibodies in 0602+ individuals, we provide the first prospective data showing decreased progression from single to multiple autoantibodies in 0602+ relatives.

The metabolic data also showed evidence of protection by the DRB1*15:01-DQA1*01:02-DQB1*06:02 allele. Both diabetic range and dysglycemic OGTTs were less common at baseline in the 0602+ relatives. In addition, the frequency of an impaired early C-peptide response was also lower at baseline in 0602+ relatives, indicating better β-cell function. The longitudinal assessments of changes in glycemia were consistent with a protective effect of DRB1*15:01-DQA1*01:02-DQB1*06:02. Average PS6M values approached zero, the value expected for nonprogressors, over a 6-month period. In contrast to 0602− relatives, 0602+ relatives showed essentially no change in glycemia over a 24-month period. Even among those with dysglycemia, fewer progressed to T1D.

Our study also shows that 0602+ relatives less frequently bear DR4 haplotypes, including those carrying the high-risk DQA1*03:01-DQB1*03:02 allele. Further analysis by autoantibody status reveals that this difference is true for relatives with a single autoantibody, whereas the frequency of DR4 haplotypes among relatives with multiple autoantibodies is similar among 0602+ and 0602− relatives. This suggests that the presence of DR4 among 0602+ relatives is associated with multiple autoantibodies; consistent with this, 0602+ relatives with DR4-DQ8 are more likely to have multiple autoantibodies than 0602+ relatives with DR3. However, the risk of T1D was much lower in 0602+ relatives with multiple autoantibodies and DRB1*04-DQA1*03:01-DQB1*03:02 compared with 0602− relatives with the same features. Moreover, the frequency of dysglycemia was not influenced by DR4-DQ8 or DR3 in 0602+ relatives. These observations suggest that protection that is mediated by DRB1*15:01-DQA1*01:02-DQB1*06:02 most often overcomes HLA-encoded susceptibility on the other chromosome.

However, protection is not absolute. A small minority of the 0602+ relatives had multiple autoantibodies or hyperglycemic OGTTs at baseline or during follow-up, and four relatives progressed to diabetes. Of these relatives, three had at least two autoantibodies and all carried predisposing HLA haplotypes on the other chromosome. Although limited by the small numbers of subjects available for analysis, 0602+ relatives with multiple autoantibodies have higher T1D risk compared with those with a single autoantibody, but this is estimated to be about 50% lower than the risk in 0602− relatives with multiple autoantibodies. Future studies could investigate whether these relatives have DRB1*15:01-DQA1*01:02-DQB1*06:02 with mutations or alleles that weaken the protective effect. For example, we previously reported that DRB1*15:01-DQA1*01:02-DQB1*06:02 haplotypes carrying allele 15 at the microsatellite D6S265 (109 kb centromeric of HLA-A) confer reduced protection from T1D; this effect was observed in patients from Sweden who tended to develop disease at an older age (37). It should also be noted that among the 0602+ relatives who developed T1D in our study, the one with single GADA positivity was much older than most PTP participants.

In general, the 0602+ relatives were older and had greater BMI values than the 0602− relatives and included higher proportions of parents and lower proportions of siblings. However, as an older age and a higher BMI are likely outcomes of the protective effect of DRB1*15:01-DQA1*01:02-DQB1*06:02 from disease progression, we chose not to perform adjustments for them in our analyses. These are not true confounders but rather direct outcomes of the protective effect from T1D development. By being protected from T1D, 0602+ relatives maintaining autoantibody positivity over time are likely to be identified at an older age, with associated higher BMI values. Consistent with this, 0602+ relatives with autoantibodies were more often parents rather than siblings of T1D probands. Schisterman et al. (38) elegantly described how unnecessary adjustments could bias results; in this case, adjusting for age and BMI would be an unnecessary adjustment that would misleadingly diminish the impact of DRB1*15:01-DQA1*01:02-DQB1*06:02 positivity. Nonetheless, we analyzed siblings separately and showed that even among them, DRB1*15:01-DQA1*01:02-DQB1*06:02 protects from the acquisition of multiple autoantibodies.

The protective effect of DRB1*15:01-DQA1*01:02-DQB1*06:02 may result from interference with the immunologically mediated pathogenesis of T1D. We show that DRB1*15:01-DQA1*01:02-DQB1*06:02 markedly reduces the likelihood of autoantibody development and the spreading of autoimmune responses as determined by the number of autoantibodies. In addition, it diminishes the likelihood and the progression of glucose abnormalities, such as dysglycemia, and ultimately inhibits progression to T1D. Given the function of antigen-presenting HLA molecules, the DR or DQ molecules encoded by the protective haplotype may influence the presentation of islet cell autoantigen epitopes, a mechanism that could be operative in both the thymus and peripheral lymphoid tissues, where tolerogenic presentation of self-molecules, including T1D autoantigens, has been shown (39). Thus, potential mechanisms of protection include thymic deletion of autoreactive T cells (40), the induction of regulatory T cells, or the induction of less inflammatory responses. Models of affinity, competition, and determinant capture are consistent with these mechanisms and have been proposed to explain the genetic protection from HLA molecules (41,42). For example, the heterodimer encoded by DQA1*01:02-DQB1*06:02 displays high stability and increased ability to bind diabetogenic self-epitopes compared with predisposing molecules (43–46). These mechanisms, and ultimately whether certain HLA molecules protect from T1D or increase risk, may also be linked to variation in the density of expression of HLA-DQ molecules on the cell surface. Reports indicate that T1D-protective HLA-DQ molecules (including the DQ molecule encoded by DQA1*01:02-DQB1*06:02) are more stable than predisposing ones, and this may lead to more sustained tolerogenic presentation of islet autoantigen peptides (47).

In closing, DRB1*15:01-DQA1*01:02-DQB1*06:02 reduces the risk of developing autoantibodies, progressing from single to multiple autoantibodies, and developing dysglycemia and ultimately protects from overt diabetes. This recognition has implications for the design of recruitment strategies for prevention trials relevant to each of the stages of disease progression recently proposed (48), from genetic predisposition to the triggering of autoimmunity, the spreading of the autoimmune response, the development of dysglycemia, and later clinical disease. A treatment that mimics or replicates the protection afforded by DRB1*15:01-DQA1*01:02-DQB1*06:02 might delay or prevent T1D at any of these stages.

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