Genetics of Autoimmune Thyroiditis in Type 1 Diabetes Reveals a Novel Association With *DPB1**0201: Data From the Type 1 Diabetes Genetics Consortium

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BACKGROUND

Autoimmune thyroiditis occurs in 10–25% of patients with type 1 diabetes (T1D). Most of these patients are also positive for thyroid peroxidase (TPO) antibodies. Thyroid dysfunction complicates T1D metabolic control and is a component of the autoimmune polyglandular syndrome (APS, type 2 or 3). Previous studies of isolated T1D and of T1D combined with other autoimmune disorders showed genetic susceptibility for alleles in HLA-*DQB1* and -*DRB1* and also *CTLA4* and *PTPN22*.

RESEARCH DESIGN AND METHODS

We analyzed the Type 1 Diabetes Genetics Consortium Autoantibody Workshop data by differentiating those T1D probands with and without TPO antibodies or thyroid disease with respect to polymorphisms in HLA, *CTLA4*, *INS*, *PTPN22*, and *VDR*, taking into account the ethnic origin. Genotype and clinical/immunogenic phenotype data were analyzed by gene counting methods and logistic regression analysis.

RESULTS

The presence of TPO antibodies (25.2%) and thyroid disease (8.4%) was associated with older age, female sex, and presence of other autoantibodies (GAD65, ATPase, 21-OH) (all $P < 0.001$). The highest prevalence was in patients of Hispanic ancestry (31%) and the lowest in those of African ancestry (8%). In T1D non-Hispanic whites, HLA-*DRB10101 is significantly ($P < 0.0001$) less frequent in TPO-positive than in TPO-negative individuals, whereas HLA-*DRB1**0404, -*DQB1**0301, and -*DPB1**0201 are significantly ($P < 0.0001$) more frequent. Subjects with a high titer of TPO autoantibodies and with thyroid disease were associated with female sex and older age and negatively associated with *DRB1**0401-*DQB1**0302 ($P < 0.0001$). No significant differences were observed for an association of TPO positivity or thyroid disease with single nucleotide polymorphisms in the *INS*, *CTLA4*, or *VDR* loci, with nominal significance ($P = 0.01$) for *PTPN22* R620W variant.**

CONCLUSIONS

Thyroid autoimmunity is highly prevalent in T1D patients of non-Hispanic white, Asian, or Hispanic origin. The strongest disease risk is conferred by female sex and older age. This risk is modulated by HLA-*DRB1* and HLA-*DPB1* loci. The immunogenetic profile for T1D with thyroid autoimmunity may identify distinct pathways regulating polyglandular autoimmunity and disease.

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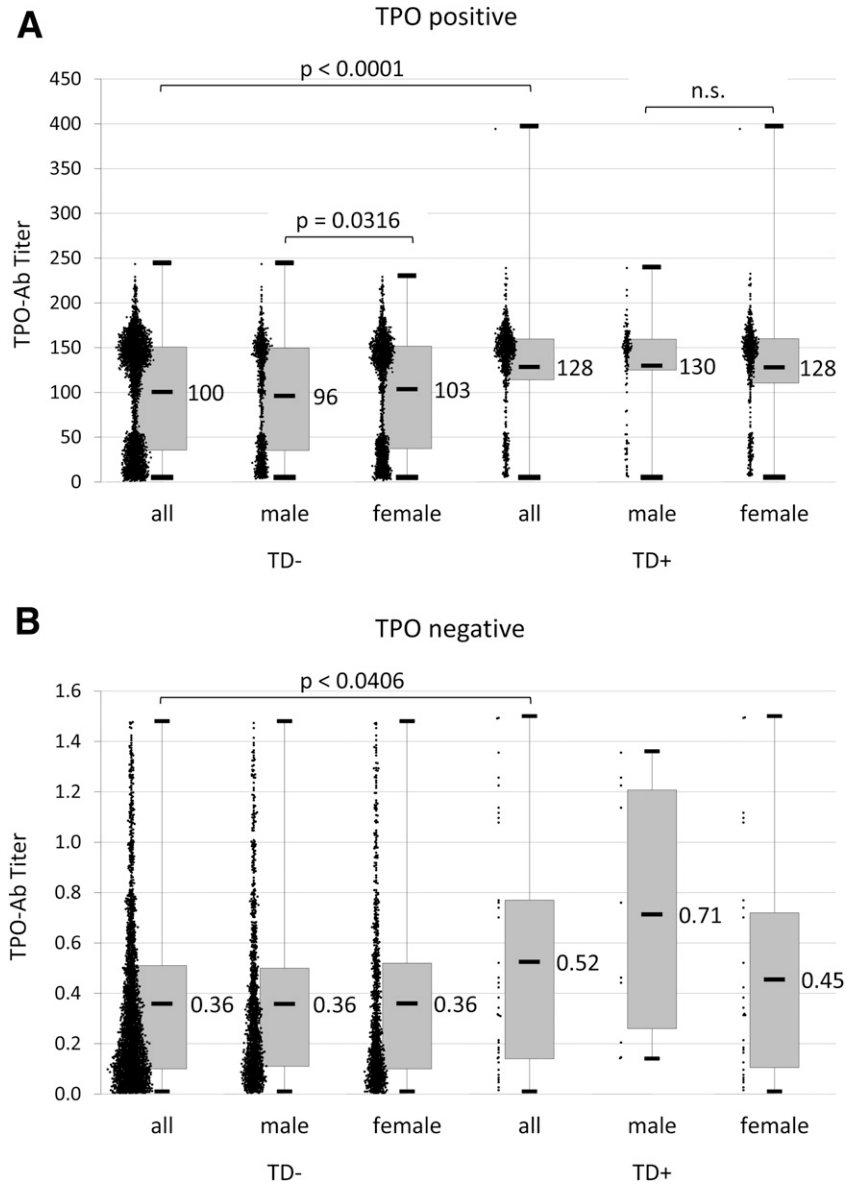

Figure 1—*A*: TPO-Ab titers in TPO-Ab+ T1D patients. *B*: TPO-Ab titers in TPO-Ab− T1D patients.

Type 1 diabetes (T1D) results from the β-cell–specific destruction mediated by T cells in genetically susceptible individuals after triggering events. This organ-specific destruction is not just confined to the pancreatic islets of Langerhans but may also affect the thyroid and other tissue targets leading to the autoimmune polyglandular syndrome type 2 (APS 2) (1). These immune-mediated disorders may occur combined in the same individual but also may be isolated diseases clustered in families (2). A shared genetic predisposition has been shown to be common in all autoimmune endocrinopathies, specifically for T1D and thyroid autoimmunity (3).

Autoimmune thyroid diseases (AITDs) are among the most common immune-mediated disorders and they enhance the risk to develop β-cell autoimmunity or overt T1D (4). Thyroid dysfunction worsens diabetes as thyroid hormones play a crucial role in energy and metabolic regulation, with recent data pointing to both central as well as peripheral mechanisms involving AMPK (5,6). Furthermore, the pathogenesis of thyroid and β-cell autoimmunity displays similar T-cell infiltration and presumed activation signals (7,8). Signatures of T-cell imbalance can also be observed in the peripheral blood as indirect markers of the immune response (9).

The Type 1 Diabetes Genetics Consortium (T1DGC) generated a large biobank of renewable (e.g., Epstein Barr virus–transformed B-cell lines) and nonrenewable (e.g., serum and DNA) resources to clarify the genetic basis for T1D. The T1DGC has identified more than 40 susceptibility loci by genome-wide association and linkage studies (www.t1dbase.org). It also analyzed humoral immunity in affected sibling pairs by measuring thyroid peroxidase (TPO) autoantibodies (TPO-Ab) as indicators of AITD. These data have permitted, for the first time, an estimation of the prevalence of AITD in a large sample of familial T1D. Furthermore, the unique collection of ethnic subpopulations permitted an ethnic-specific characterization of T1D with associated diseases as well as TPO antibody status.

RESEARCH DESIGN AND METHODS

In total, 7,083 subjects with T1D were screened for TPO-Ab; 7,055 were genotyped for *HLA* class I *(HLA-A*, *-B*, and *-C)* and class II *(HLA-DRB1*, *-DQA1*, *-DQB1*, *-DPA1*, and *-DPB1)* alleles. Genotypes were assigned by high-resolution analysis using a PCR-based sequence-specific oligonucleotide probe system (10) for the eight distinct HLA genes. A total of 2,182 subjects were genotyped for T1D-associated single nucleotide polymorphisms (SNPs), including those in *CTLA4, INS, PTPN22*, and *VDR*. Targeted SNP typing used an Illumina custom array. The largest ethnic group comprised

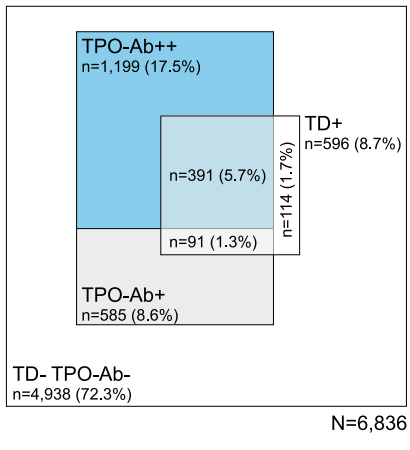

Figure 2—The relative proportions of T1D patients either with thyroid autoimmunity and TPO-Ab+ or TPO-Ab++ and/or thyroid disease are represented by the areas within the quadrangle.

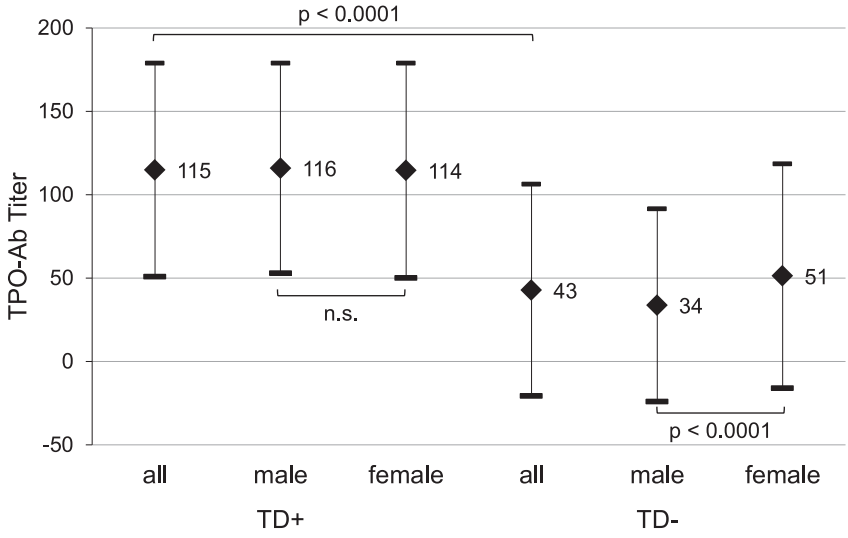

Figure 3—T1D patients with thyroid disease have significantly higher TPO-Ab titers than those without a thyroid disorder.

5,219 non-Hispanic whites (NHWs) with the HLA genotype.

TPO-Ab were measured by radioimmunoassay (KRONUS, Star, ID), and thresholds for positivity were defined according to the manufacturer's instructions. TPO antibody titers were documented from 6,836 T1D subjects, while positivity or negativity for TPO was recorded from only 247 participants. Presence or absence of thyroid disease was defined in a previously collected detailed clinical questionnaire for T1DGC study participants.

Statistical analysis was performed using BiAS for Windows, epsilon 2007, for calculating allele frequencies. For each of the 47 most common (minor allele frequency >3%) alleles, allelic association was tested for seven related phenotypes (TPO, TD [thyroid disease], TPO+TD+, TPO+TD−, TPO−TD+, TPO-Ab++, and TPO-Ab++TD++) by logistic regression, clustered by family, with age and sex as covariates. Significance levels were determined using robust variance estimators. To correct for multiple testing, a $P <$ 0.001 threshold was used for statistical significance. Screening for HLA allele associations was restricted to the 5,266 NHW subjects with HLA genotypes. Allelic associations identified in NHWs were tested in other ethnic groups and combined using the DerSimonian and Laird random-effects method to assess heterogeneity. Analyses were carried out using Stata (version 11.2; StataCorp, College Station, TX). Phased DR-DQ haplotypes were obtained from family data, in most cases including both parents as well as unaffected siblings.

Table 1—Association of thyroid autoimmunity with age and sex

		Age (years)			Sex	
Phenotype	*n*	Mean	SD	*P*	% Female	*P*
Total	7,083	20.8	12.5		50.0	
TPO+	1,785	23.6	13.2	<0.001	63.9	<0.001
TD+	596	28.8	14.4	<0.001	74.7	<0.001
TPO+TD+	482	27.7	14.1	<0.001	74.5	<0.001
TPO+TD−	1,303	22.0	12.4	<0.001	59.9	<0.001
TPO−TD+	93	32.4	13.9	<0.001	75.3	<0.001
TPO−TD−	4,943	19.5	11.9		44.5	

Table 2—Ethnic differences in the prevalence of thyroid autoimmunity

			Age (years)		Sex	
Ethnicity	*n*	Frequency (%)	Mean	SD	Male	Female
Other	20	0.2	10.7	4.7	16 (80)	4 (20)
Asian	380	5.4	17.4	7.6	183 (48.2)	197 (51.8)
Black	710	10.0	14.8	8.2	311 (43.8)	399 (56.2)
Hispanic	495	7.0	14.0	7.9	245 (49.5)	250 (50.5)
NHW	5,478	77.3	22.5	13.1	2,788 (50.9)	2,690 (49.1)
Total	7,083		20.8	12.5		

RESULTS

Phenotypes of TPO Positivity and Thyroid Disease in T1D

Six clinical/immunogenic phenotype groups were distinguished by antibody profile and diagnosis of thyroid disease: *1*) patients with or without TPO antibodies (TPO+ vs. TPO−), *2*) those with or without thyroid disease (TD+ vs. TD−), *3*) those with both TPO–Ab and thyroid disease (TPO+TD+), *4*) those with TPO-Ab but without thyroid disease (TPO+TD−), *5*) those with thyroid disease without TPO-Ab (TPO−TD+), and, as the reference group for the latter three phenotypes, *6*) those T1D patients without thyroid disease or TPO autoantibody positivity (TPO−TD−). It should be noted that TPO positivity was found in both low titer (TPO-Ab+ <75 units/L) and high titer (TPO-Ab++ >75 units/L) subject samples. Out of 7,083 T1D cases, there were 1,785 (25.2%) who tested positive for TPO antibodies, and 596 T1D cases (8.4%) reported a concomitant thyroid disorder.

The bimodal distribution of TPO antibody titers (TPO-Ab++/TPO-Ab+) was observed in both sexes and was not different when stratified by age of T1D onset (Fig. 1). The majority of TPO-positive T1D patients were TPO-Ab++ (17.5%), only 8.6% were TPO-Ab+. As shown in Fig. 2, only small groups had TPO-Ab+ and thyroid disease (1.3%) or were TPO−TD+ patients (1.7%). T1D patients with thyroid disease had a significantly ($P < 0.0001$) higher mean titer of TPO-Ab (115 units/L) compared with those without thyroid disease (43 units/L) (Fig. 3).

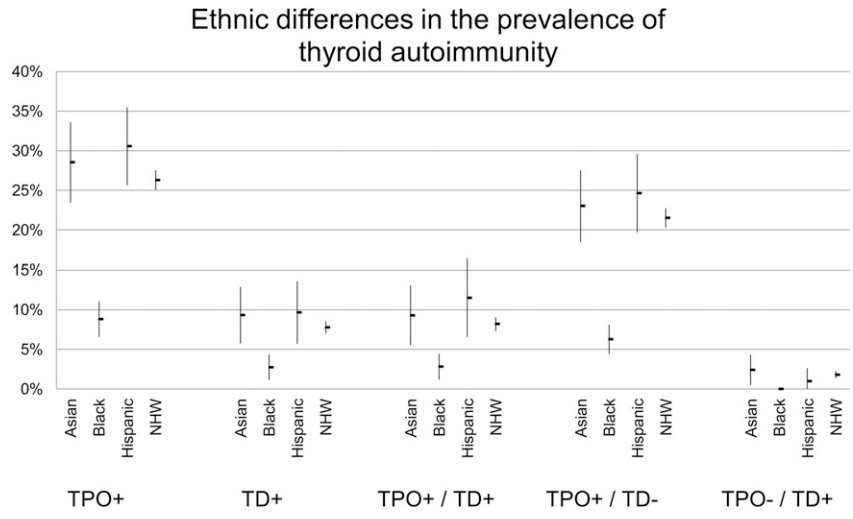

Figure 4—Ethnic differences in the prevalence of thyroid autoimmunity.

TPO positivity was associated with both age at sampling time and with female sex. Compared with the total study population, T1D patients who were TPO+ are the youngest (19.5 years), followed by TPO+TD− (22.0 years), TPO+TD+ (27.7 years), and TPO−TD+ (32.4 years) ($P < 0.001$). Female sex also predisposes to TPO+TD− patients (59.9% female, $P < 0.001$). Moreover, there were differences within the ethnic groups (Table 2 and Fig. 4), with the highest prevalence of TPO autoantibody positivity in Hispanics (29.7%), which is consistent with the highest prevalence in TPO+TD+ patients (12%) despite their younger mean age (14 years in Hispanics vs. 22.5 years in NHWs).

Table 3—Association between TPO and GAD65 autoantibody positivity

		GAD65, *n* (%)		
	TPO	Negative	Positive	*P*
NHW	Negative	2,288 (60)	1,525 (40)	<0.001
	Positive	688 (47.2)	769 (52.8)	
Hispanic	Negative	194 (57.7)	142 (42.3)	0.003
	Positive	60 (42.3)	82 (57.7)	
Black	Negative	290 (46.8)	330 (53.2)	0.001
	Positive	20 (27)	54 (73)	
Asian	Negative	148 (59.2)	102 (40.8)	0.004
	Positive	46 (42.2)	63 (57.8)	

were significantly older (23.6 vs. 20.8 years) and more often female (63.9 vs. 50%), as were T1D TD+ patients (28.8 years and 74.7% female) (Table 1). For the combined phenotypes, TPO−TD− thyroid disease (OR 2.27, $P < 0.001$), regardless of TPO antibodies (TPO+TD+, 74.5% female; TPO−TD+, 75.3% female; $P < 0.001$). The sex bias is less apparent, although still significant, for

Table 4—Association between TPO and ATPase autoantibody positivity

		ATPase, *n* (%)		
	TPO	Negative	Positive	*P*
NHW	Negative	3,152 (86.3)	501 (13.7)	<0.001
	Positive	835 (60.5)	545 (39.5)	
Hispanic	Negative	257 (79.8)	65 (20.2)	<0.001
	Positive	78 (57.8)	57 (42.2)	
Black	Negative	487 (82.8)	101 (17.2)	<0.001
	Positive	38 (55.1)	31 (44.9)	
Asian	Negative	218 (90.1)	24 (9.9)	<0.001
	Positive	76 (73.8)	27 (26.2)	

TPO positivity is associated with GAD65 positivity and ATPase positivity. In those TPO-positive T1D subjects, more than one-half in each ethnic group is also positive for GAD65 (52.8% NHWs, 57.7% Hispanics, 73.0% blacks, and 57.8% Asians, all $P < 0.004$) (Table 3). TPO positivity is less associated with ATPase positivity (39.5% NHWs, 42.2% Hispanics, 44.9% blacks, and 26.2% Asians, all $P < 0.001$) (Table 4). TPO positivity is also associated with 21-hydroxylase (21-OH) in NHWs ($P < 0.001$) and Hispanics ($P = 0.008$) but not in blacks ($P = 0.287$) (Table 5). None of the 359 Asians tested were positive for 21-OH. There is no evidence for association between TPO positivity and IA-2 (Table 6) or TG (Table 7) positivity in any ethnic group.

Allele Frequencies of the MHC

In the series of HLA class I and class II genes in the MHC, 10 alleles showed significant allele frequency differences in one or more phenotypic subgroups of thyroid autoimmunity (Table 8) as identified by logistic regression analysis: class I HLA-*A**2402 and class II HLA-*DQA1**0101 and -*DQA1**0301; HLA-*DQB1**0301, -*DQB1**0302, and -*DQB1**0501; HLA-*DRB1**0101, -*DRB1**0401, and -*DRB1**0404; and HLA-*DPB1**0201. However, *DRB1**0404 is in complete linkage disequilibrium (LD) with *DQA1**0301-*DQB1**0302, and also *DRB1**0101 is in complete LD with *DQA1**0101-*DQB1**0501. In both cases, the HLA-DQ associations were secondary to LD with the HLA-*DRB1* allele. Of the remaining alleles, the effects of *A**2402, *DQB1**0301, *DRB1**0404, *DPB1**0201 are independent, whereas the effects of *DQA1**0301 and *DRB1**0401 are secondary to LD with *DQB1**0301.

For most of the alleles associated with thyroid autoimmunity, the pattern of association is similar for different phenotypic subgroups (Table 9). There is consistent association of *DPB1**0201 both with patients with TPO positivity

Table 5—Association between TPO and 21-OH autoantibody positivity

		21-OH, *n* (%)		
	TPO	Negative	Positive	*P*
NHW	Negative	3,771 (98.9)	42 (1.1)	<0.001
	Positive	1,403 (96.3)	54 (3.7)	
Hispanic	Negative	336 (100)	0 (0)	0.008
	Positive	138 (97.2)	4 (2.8)	
Black	Negative	618 (99.7)	2 (0.3)	0.287
	Positive	73 (98.6)	1 (1.4)	
Asian	Negative	250 (100)	0 (0)	—
	Positive	109 (100)	0 (0)	

and manifest thyroid disease (22.7% TPO−TD+) and with cases with thyroid disease but without TPO antibodies (10.6% TPO+TD−) versus those without TPO antibodies and thyroid disease (14% TPO−TD−) (odds ratio [OR] 1.95, $P = 4 \times 10^{-4}$ and OR 1.30, $P = 3 \times 10^{-4}$, respectively). The protective effect of *A**2402 has the strongest effect in individuals with manifest thyroid disease who are also TPO positive (7.3% TPO+TD+ vs. 12.0% TPO−TD−; OR 0.59, $P < 0.0001$). There was no evidence supporting an effect of A*2402 in manifest thyroid disease without TPO antibodies (11.6% TPO−TD+ vs. 12.0% TPO−TD−; OR 1.02, $P > 0.5$). The *DRB1**0401 and *DQB1**0302 alleles were significantly less frequent in subjects with TPO-Ab++ than with TPO-Ab+. In addition, they were also significantly less frequent in TPO+ subjects with high titers and thyroid disease (TPO-Ab++TD+) than in TPO+ subjects with low titers and no thyroid disease (TPO-Ab+TD−). This was seen for the *DRB1**0401-*DQB1**0302 haplotype but was not observed when *DRB1**0401 was present on the same haplotype with *DQB1**0301 or when *DQB1**0302 was present on the same haplotype as *DRB1**0404 (data not shown).

Association analyses in T1D patients of Asian, African, and Hispanic ancestry exhibited similar trends in the pattern of allele frequencies for *A**2402, *DRB1**0101, *DRB1**0404, *DPB1**0201, *DRB1**0401, and *DQB1**0302 alleles. The pooled estimate of effect sizes remained statistically significant for each of these alleles, even when combined with results for NHWs. However, the association of *DQB1**0301 with TPO phenotypes showed significant heterogeneity across ethnicities ($P < 0.015$), and the pooled estimate of the effect of *DQB1**0301 was no longer significant ($P > 0.4$ for TPO+; $P > 0.9$ for TPO+TD−). There were weaker indications of population differences in the association of the *DRB1**0401-*DQB1**0302 haplotype with TPO antibody titers ($P > 0.1$). Although the association of both *DRB1**0401 and *DQB1**0302 alleles was consistent across populations, it remained significant ($P < 0.001$) in the pooled analyses.

Other Gene T1D Risk Loci

No significant association could be detected for SNPs in the *CTLA4*, *INS*, or *VDR* genes with association to TPO positivity in T1D subjects. Although there were only slight significant differences in minor allele frequency for some SNPs, this could not be confirmed by regression analysis at a significance criterion of $P < 0.001$ to correct for multiple testing. The *PTPN22* SNP coding for the R620W variant (rs2476601) reached nominal significance for association with TPO positivity (TPO+; $P < 0.01$) in comparison with TPO-negative participants.

Table 6—Association between TPO and IA-2 autoantibody positivity

		IA-2, *n* (%)		
	TPO	Negative	Positive	*P*
NHW	Negative	2,036 (53.4)	1,777 (46.6)	0.734
	Positive	786 (53.9)	671 (46.1)	
Hispanic	Negative	127 (37.8)	209 (62.2)	0.681
	Positive	57 (40.1)	85 (59.9)	
Black	Negative	286 (46.1)	334 (53.9)	0.902
	Positive	33 (44.6)	41 (55.4)	
Asian	Negative	189 (75.6)	61 (24.4)	0.588
	Positive	86 (78.9)	23 (21.1)	

CONCLUSIONS

We found a high prevalence of thyroid autoimmunity as well as thyroid disorders in the population of T1D subjects participating in the T1DGC Autoantibody Workshop. Participants of African ancestry had a lower prevalence of thyroid autoimmunity compared with those of Asian ancestry who had the highest prevalence; NHWs were classified as intermediate for thyroid autoimmunity. Ethnic differences in polyendocrine autoimmunity of T1D have not yet been described and warrant further investigation. Due to the smaller numbers in the Hispanic and African ancestry groups, the specific genetic contributions are underpowered in our study to address genetic contribution. These findings, however, provide new insights into the polyendocrine autoimmunity of T1D.

The bimodal distribution of TPO positivity in low-titer and high-titer groups is certainly of interest. Latent thyroid autoimmunity can be distinguished from advanced forms that are more frequently associated with manifest thyroid disease. Follow-up testing of both TPO-Ab and thyroid function should, therefore, be advised for all T1D subjects who are found to be antibody positive but unaffected by thyroid disease. Whether thyroid autoimmunity will progress to overt disease cannot be predicted at present. Yet as the autoimmune pathophysiology runs a slow course with advancing age, more T1D patients will develop thyroid disease in the long run.

Recently, four gene loci were associated with autoantibody positivity at genome-wide significance in a large cross-sectional cohort of 6,160 T1D affected siblings (11). Brorsson et al. (11) tested for association with seven disease-specific autoantibodies and provided evidence that genetic control of antibody formation is distinct from T1D risk.

Conversely, testing for multiple islet autoantibodies was shown to be a useful predictive marker for risk of progression to T1D onset in patients with thyroid disease (12). Hereby, the immunogenetic profile for T1D with thyroid autoimmunity

Table 7—Association between TPO and TG autoantibody positivity

		TG, *n* (%)		
	TPO	Negative	Positive	*P*
NHW	Negative	3,537 (92.8)	276 (7.2)	0.292
	Positive	1,339 (91.9)	118 (8.1)	
Hispanic	Negative	318 (94.6)	18 (5.4)	0.304
	Positive	131 (92.3)	11 (7.7)	
Black	Negative	600 (96.8)	20 (3.2)	0.153
	Positive	74 (100)	0 (0)	
Asian	Negative	234 (93.6)	16 (6.4)	0.276
	Positive	98 (89.9)	11 (10.1)	

may identify distinct pathways regulating polyglandular autoimmunity and disease.

In the ethnic group of NHWs, the strongest genetic association for thyroid autoimmunity with T1D is observed with *DPB1**0201, found in nearly a fifth of all T1D patients with a thyroid disorder and in 18.1% of those with TPO positivity. Only the risk for having the *DRB1**0404 allele was higher in patients with TPO positivity (OR 1.84) and thus greater than in T1D cases with thyroid disorders (OR 1.45). In contrast, significant protection was found with *DRB1**0101 (OR 0.55 for TPO) and *A**2402 present (OR 0.80 for TPO, OR = 0.72 for TD) (Table 9).

These findings are in agreement with earlier reports of genetic susceptibility in thyroid autoimmune disease that is shared across the spectrum of several autoimmune disorders (2,3,13,14). Those results demonstrate that HLA genes in the MHC are linked with Graves disease, not only in Caucasians but also in Han Chinese from Taiwan (15,16). The largest component of genetic susceptibility for several autoimmune diseases resides in the MHC (13,17). The specific role of HLA-DP contributing to the risk in T1D has only recently been shown (10). Here, alleles of HLA-DP confer both significant susceptibility as well as protection. *DPB1**0201 confers the highest risk in those T1D cases with thyroid disease but without antibodies and only slightly less risk in those with antibodies. In both groups, *DPB1*0201* is the most prevalent risk allele (22.7% of TD patients without autoantibodies and 19.6% in TD cases with autoantibodies) that is otherwise observed only in 14% of T1D patients without thyroid autoimmunity.

The relatively high frequency of *DPB1**0201 in Caucasian populations varies between 10–20% (www.allelefrequencies.net) (18). Some populations have higher frequencies, such as Italians and Sardinians (18–20%) in comparison with central Europeans (Germans and Austrians, 13–15%), Scandinavians (Norwegians and Swedes, 10–14%) or Bretons and British (10–15%). Whether these differences contribute to the regional variations in prevalence of thyroid autoimmunity should be investigated more thoroughly. HLA-*DPB1* now comprises 155 alleles and its variability is increasingly recognized for its importance in bone marrow transplantation (19).

HLA-*DPB1* alleles have been shown to be associated with Graves disease in a study of multiplex families from the U.K. (20,21) differentiating the risk of HLA-DR17 haplotypes. Graves disease also had a significant association with *DPB1**0501, appearing as the major susceptibility gene with a population-attributable risk of 48.4% in a combined case-control and family study of Han Chinese (22). As Graves disease shows a slightly higher prevalence in Asians, the different frequencies of HLA risk and protection alleles may contribute to this geoepidemiological variation of autoimmune disease as well as to antibody formation (23–25). The varying frequencies of HLA-DR, -DQ, and -DP alleles across populations are paralleled by autoimmune disease incidence data with other risk factors such as higher birth weight (26) or viral infections (27). The high prevalence of TPO in the Asian T1D patients from the T1DGC cohort mirrors this enhanced ethnic susceptibility. Our analysis indicated the HLA class I *Cw**1203 allele conferring an enhanced risk (data not shown) to develop thyroid autoimmunity in T1D patients. However, due to the comparatively low number of Asian individuals in this study, there was insufficient power to prove this.

T1D varies across populations with a somewhat slower course of onset in Japanese accompanied by more APS 3 variants (APS3v) and a female predominance. These APS3v patients show an association with *DRB1*0405-DQB1**0401 (28). In the T1DGC Autoantibody Workshop data, we could not identify whether a later onset of T1D is associated with a higher frequency of thyroid autoimmunity. The major antigen in thyroid autoimmunity is TPO (29). Whether TPO epitopes engage in a specific interaction with the HLA-DR and/or HLA-DP peptide binding pocket in a similar manner as it has been presumed for GAD65, the major T1D antigen, is unknown (8,30,31). As the T1DGC recruited affected sibling

Table 8—Association of thyroid autoimmunity and thyroid disease with HLA alleles

	TPO−TD− (7,450)*	TPO+TD+ (822)*			TPO+TD− (2,088)*			TPO-TD+ (172)*		
HLA allele	% (*n*)	% (*n*)	*P*	OR (95% CI)	% (*n*)	*P*	OR (95% CI)	% (*n*)	*P*	OR (95% CI)
*A**2402	12.0 (893)	7.3 (60)	<0.0001	0.59 (0.44–0.80)	10.6 (222)	0.139	0.88 (0.74–1.04)	11.6 (20)	>0.5	1.02 (0.64–1.62)
*DRB1**0101†	7.3 (545)	3.9 (32)	<0.001	0.51 (0.34–0.77)	4.4 (92)	1E-05	0.58 (0.45–0.74)	11.0 (19)	>0.1	1.49 (0.91–2.44)
*DRB1**0404‡	4.9 (362)	7.8 (64)	<0.0001	1.78 (1.31–2.43)	8.6 (179)	5E-09	1.87 (1.51–2.30)	5.8 (10)	>0.3	1.48 (0.70–2.66)
*DQB1**0301	4.1 (302)	5.7 (47)	<0.05	1.43 (1.03–2.00)	6.5 (136)	7E-06	1.87 (1.30–2.00)	6.4 (11)	>0.2	1.43 (0.81–2.53)
*DPB1**0201	14.0 (1,045)	19.6 (161)	<0.0001	1.50 (1.22–1.84)	17.6 (367)	3E-04	1.30 (1.23–1.49)	22.7 (39)	<0.0001	1.95 (1.35–2.81)

*Data in parentheses are number of alleles in each group. †Haplotype *DRB1**0101-*DQA1**0101-*DQB1**0501. ‡Haplotype *DRB1**0404-*DQA1**0301-*DQB1**0302.

Table 9—Association of thyroid autoimmunity with HLA alleles in NHWs

	TPO− (7,622)*	TPO+ (2,910)*			TD− (9,918)*	TD+ (1,030)*		
HLA allele	% (*n*)	% (*n*)	*P*	OR (95% CI)	% (*n*)	% (*n*)	*P*	OR (95% CI)
*A**2402	12.0 (913)	6.3 (183)	0.006	0.80 (0.68–0.94)	11.7 (1,159)	8.4 (87)	0.006	0.72 (0.56–0.92)
*DRB1**0101†	7.4 (564)	4.3 (124)	<0.001	0.55 (0.44–0.68)	6.7 (661)	5.2 (54)	>0.1	0.78 (0.57–1.06)
*DRB1**0404‡	4.9 (372)	8.3 (243)	<0.0001	1.84 (1.52–2.23)	5.7 (565)	7.8 (80)	<0.005	1.45 (1.12–1.88)
*DQB1**0301	4.1 (313)	6.3 (183)	<0.0001	1.52 (1.26–1.84)	4.6 (459)	5.9 (61)	>0.1	1.23 (0.94–1.61)
*DPB1**0201	14.2 (1,084)	18.1 (528)	<0.0001	1.31 (1.16–1.49)	14.9 (1,480)	19.8 (204)	<0.0001	1.37 (1.14–1.63)

*Data in parentheses are number of alleles in each group. †Haplotype *DRB1**0101-*DQA1**0101-*DQB1**0501. ‡Haplotype *DRB1**0404-*DQA1**0301-*DQB1**0302.

pairs and only few simplex cases (as trios), we cannot assess whether the prevalence of AITD in multiplex T1D sibling relationships is higher than in simplex (i.e., sporadic) cases. However, the prevalence of TPO positivity is similar to data reported in earlier studies (14).

These findings have implications for the care of T1D patients, particularly those with Asian ancestry, as these individuals should be HLA genotyped and monitored for thyroid autoimmunity more carefully than the other groups investigated here. However, these results stress that all patients with T1D, particularly those with advancing age and women, need to have regular thyroid monitoring during follow-up investigations. Currently, genetic markers do not permit stratification of those with a higher risk with sufficient specificity. Nonetheless, the identification of risk or protective alleles represents the first step in the process of personalized medicine. Ultimately, unraveling the functional impact of these risk alleles will identify the mechanisms underlying multiple endocrine organ destruction.

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References

1. Eisenbarth GS, Gottlieb PA. Autoimmune polyendocrine syndromes. N Engl J Med 2004; 350:2068–2079
2. Barker JM, Yu J, Yu L, et al. Autoantibody "subspecificity" in type 1 diabetes: risk for organ-specific autoimmunity clusters in distinct groups. Diabetes Care 2005;28:850–855
3. Badenhoop K, Walfish PG, Rau H, et al. Susceptibility and resistance alleles of human leukocyte antigen (HLA) DQA1 and HLA DQB1 are shared in endocrine autoimmune disease. J Clin Endocrinol Metab 1995;80:2112–2117
4. Pilia S, Casini MR, Cambuli VM, et al. Prevalence of type 1 diabetes autoantibodies (GAD and IA2) in Sardinian children and adolescents with autoimmune thyroiditis. Diabet Med 2011; 28:896–899
5. López M, Varela L, Vázquez MJ, et al. Hypothalamic AMPK and fatty acid metabolism mediate thyroid regulation of energy balance. Nat Med 2010;16:1001–1008
6. Duntas LH, Orgiazzi J, Brabant G. The interface between thyroid and diabetes mellitus. Clin Endocrinol (Oxf) 2011;75:1–9
7. McLachlan SM, Nagayama Y, Pichurin PN, et al. The link between Graves' disease and Hashimoto's thyroiditis: a role for regulatory T cells. Endocrinology 2007;148:5724–5733
8. Ellis RJ, Varela-Calvino R, Tree TI, Peakman M. HLA Class II molecules on haplotypes associated with type 1 diabetes exhibit similar patterns of binding affinities for coxsackievirus P2C peptides. Immunology 2005;116:337–346
9. Fountoulakis S, Vartholomatos G, Kolaitis N, Frillingos S, Philippou G, Tsatsoulis A. HLA-DR expressing peripheral T regulatory cells in newly diagnosed patients with different forms of autoimmune thyroid disease. Thyroid 2008;18:1195–1200
10. 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
11. Brorsson CA, Onengut S, Chen W-M, et al.; Type 1 Diabetes Genetics Consortium. Novel association between immune-mediated susceptibility loci and persistent autoantibody positivity in type 1 diabetes. Diabetes 2015;64:3017–3027
12. Pietropaolo M, Peakman M, Pietropaolo SL, et al. Combined analysis of GAD65 and ICA512(IA-2) autoantibodies in organ and non-organ-specific autoimmune diseases confers high specificity for insulin-dependent diabetes mellitus. J Autoimmun 1998;11:1–10
13. Criswell LA, Pfeiffer KA, Lum RF, et al. Analysis of families in the Multiple Autoimmune Disease Genetics Consortium (MADGC) collection: the PTPN22 620W allele associates with multiple autoimmune phenotypes. Am J Hum Genet 2005;76:561–571
14. Triolo TM, Armstrong TK, McFann K, et al. Additional autoimmune disease found in 33% of patients at type 1 diabetes onset. Diabetes Care 2011;34:1211–1213
15. Heward JM, Allahabadia A, Daykin J, et al. Linkage disequilibrium between the human leukocyte antigen class II region of the major histocompatibility complex and Graves' disease: replication using a population case control and family-based study. J Clin Endocrinol Metab 1998;83:3394–3397
16. Chen PL, Fann CS, Chang CC, et al. Linkage of Graves' disease to the human leucocyte antigen region in the Chinese-Han population in Taiwan. Clin Endocrinol (Oxf) 2007;66:646–651
17. de Bakker PI, McVean G, Sabeti PC, et al. A high-resolution HLA and SNP haplotype map for disease association studies in the extended human MHC. Nat Genet 2006;38:1166–1172
18. Gonzalez-Galarza FF, Christmas S, Middleton D, Jones AR. Allele frequency net: a database and online repository for immune gene frequencies in worldwide populations. Nucleic Acids Res 2011; 39:D913–D919
19. Robinson J, Mistry K, McWilliam H, Lopez R, Parham P, Marsh SG. The IMGT/HLA database. Nucleic Acids Res 2011;39:D1171–D1176
20. Ratanachaiyavong S, Fleming D, Janer M, et al. HLA-DPB1 polymorphisms in patients with hyperthyroid Graves' disease and early onset myasthenia gravis. Autoimmunity 1994;17: 99–104
21. Ratanachaiyavong S, McGregor AM. HLA-DPB1 polymorphisms on the MHC-extended

haplotypes of families of patients with Graves' disease: two distinct HLA-DR17 haplotypes. Eur J Clin Invest 1994;24:309–315

22. Chen PL, Fann CS, Chu CC, et al. Comprehensive genotyping in two homogeneous Graves' disease samples reveals major and novel HLA association alleles. PLoS One 2011;6:e16635

23. Shapira Y, Poratkatz BS, Gilburd B, et al. Geographical differences in autoantibodies and anti-infectious agents antibodies among healthy adults. Clin Rev Allergy Immunol 2012; 42:154–163

24. Shapira Y, Agmon-Levin N, Shoenfeld Y. Defining and analyzing geoepidemiology and human autoimmunity. J Autoimmun 2010;34: J168–J177

25. Shapira Y, Agmon-Levin N, Shoenfeld Y. Geoepidemiology of autoimmune rheumatic diseases. Nat Rev Rheumatol 2010;6:468–476

26. Järvinen TM, Harjutsalo V, Kinnunen L, Miettinen ME, Tuomilehto-Wolf E, Tuomilehto J. A population-specific diabetogenic haplotype HLA-A2,Cw1,B56,DR4,DQ8 is associated with high birthweight in Finnish diabetic families. Genes Immun 2008;9:207–213

27. Kubinak JL, Ruff JS, Hyzer CW, Slev PR, Potts WK. Experimental viral evolution to specific host MHC genotypes reveals fitness and virulence trade-offs in alternative MHC types. Proc Natl Acad Sci U S A 2012;109:3422–3427

28. Horie I, Kawasaki E, Ando T, et al. Clinical and genetic characteristics of autoimmune polyglandular syndrome type 3 variant in the Japanese population. J Clin Endocrinol Metab 2012;97:E1043–E1050

29. McLachlan SM, Rapoport B. Thyroid peroxidase as an autoantigen. Thyroid 2007;17:939–948

30. Menconi F, Osman R, Monti MC, Greenberg DA, Concepcion ES, Tomer Y. Shared molecular amino acid signature in the HLA-DR peptide binding pocket predisposes to both autoimmune diabetes and thyroiditis. Proc Natl Acad Sci U S A 2010;107: 16899–16903

31. Ge X, James EA, Reijonen H, Kwok WW. Differences in self-peptide binding between T1D-related susceptible and protective DR4 subtypes. J Autoimmun 2011;36:155–160