

Autoantibody "Subspecificity" in Type 1 Diabetes

Risk for organ-specific autoimmunity clusters in distinct groups

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OBJECTIVE — Autoimmune thyroid disease (AIT), celiac disease, and Addison's disease are characterized by the presence of autoantibodies: thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TGAb) in AIT, tissue transglutaminase antibody (TTGAb) in celiac disease, and 21-hydroxylase antibody (21-OHAb) in Addison's disease. The objective of this study was to define the prevalence of these autoantibodies and clinical disease in a population with type 1 diabetes.

RESEARCH DESIGN AND METHODS — We screened 814 individuals with type 1 diabetes for TPOAb, TGAb, TTGAb, and 21-OHAb. Clinical disease was defined by chart review. Factors related to the presence of autoimmunity and clinical disease including age at onset of type 1 diabetes, duration of diabetes, age at screening, sex, and the presence of autoantibodies were reviewed.

RESULTS — The most common autoantibodies expressed were TPOAb and/or TGAb (29%), followed by TTGAb (10.1%) and 21-OHAb (1.6%). Specific HLA DR/DQ genotypes were associated with the highest risk for expression of 21-OHAb (DRB1*0404-DQ8, DR3-DQ2) and TTGAb (DR3-DQ2-DR3-DQ2). The expression of thyroid autoantibodies was related to 21-OHAb but not to TTGAb. The presence of autoantibodies was associated with and predictive of disease.

CONCLUSIONS — In this large cohort of individuals with type 1 diabetes, the expression of organ-specific autoantibodies was very high. The grouping of autoantibody expression suggests common factors contributing to the clustering.

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Abbreviations: 21-OHAb, 21-hydroxylase antibody; AIT, autoimmune thyroid disease; ICA, islet cell antigen; ICMA, immunochemiluminometric assay; mIAA, microinsulin autoantibody assay; TGAb, thyroglobulin antibody; TPOAb, thyroid peroxidase antibody; TSH, thyroid-stimulating hormone; TTGAb, tissue transglutaminase antibody.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Type 1 diabetes is a common autoimmune endocrine disorder occurring in ~1/300 individuals (1,2). Type 1 diabetes often occurs in the company of other autoimmune diseases, including autoimmune thyroid disease (AIT) (3), celiac disease (4,5), and Addison's disease (6). Type 1 diabetes, AIT, and Addison's disease form the well-recognized clinical triad associated with autoimmune polyendocrine syndrome type II (7).

AIT is common in the general population, particularly in older individuals (8). The association of type 1 diabetes and AIT has been well established and is highly dependent upon age, sex, and ethnicity (3,9–12). This association is linked to the expression of thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TGAb) (3,11). HLA alleles have been associated with the development of AIT (13–16) in the population with type 1 diabetes. Common HLA alleles have been identified as independent risk factors for AIT and type 1 diabetes (17), although the data for AIT are much less consistent than the data for type 1 diabetes.

The prevalence of celiac autoimmunity is high as 1 in 100 in certain populations (18,19). This risk is increased in individuals with type 1 diabetes (4,20) and by HLA DR3-DQ2 (5). Addison's disease is a much rarer autoimmune endocrine disease, occurring in 1 in 10,000 individuals (21,22). The population with type 1 diabetes is at an increased risk for Addison's disease, with ~2% expressing autoantibodies associated with Addison's disease (6,23). Risk for Addison's disease is related to HLA genotypes, specifically DRB1*0404-DQ8, DR3-DQ2 (6).

Celiac disease, Addison's disease, AIT, and type 1 diabetes are associated with the production of organ-specific autoantibodies. Highly sensitive radioimmunoassays have been developed for the detection of autoantibodies related to celiac disease (tissue transglutaminase antibody [TTGAb]), Addison's disease (21-hydroxylase antibody [21-OHAb]), and type 1 diabetes (insulin, GAD, and islet

cell antigen [ICA]512). AIT is associated with TPOAb and TGAb. Standard immunochimiluminometric assays (ICMAs) have been developed and are performed on a routine basis.

Our objective was to define the prevalence of autoantibodies to multiple organs in individuals with type 1 diabetes. We hypothesize that in patients with type 1 diabetes, autoimmune diseases cluster into “subspecific” groups, implying that the risk for additional autoimmunity is not equal for each disease. We hypothesize that these groups may result from shared genetic risk (both within and outside the major histocompatibility complex), from shared environmental factors, or by “collateral” priming to antigens shared by specific groups of organs (24).

RESEARCH DESIGN AND METHODS

We screened 814 individuals with type 1 diabetes, as diagnosed by the American Diabetes Association criteria (25), who were seen at the Barbara Davis Center from January 1993 through April 2004 for TPOAb, TGAb, TTGAb, and 21-OHAb. Autoantibodies were measured one time. Duration of diabetes was determined. Individuals were included in this analysis from onset of diabetes to duration >25 years. Charts were reviewed for evidence of clinical diagnosis of AIT, Addison's disease, and celiac disease diagnosed by 2004. Onset of disease, evaluation performed at onset, and treatment were recorded. Individuals provided informed consent. The University of Colorado Health Sciences Center's Combined Institutional Review Board approved the protocol.

21-OHAb was measured by the method previously described (26). Autoantibodies are reported as an index relative to a positive control sample. Positivity for 21-OHAb was defined as exceeding the highest index of 241 normal control subjects (an index >0.149) (6). The assays were performed in duplicate. Positive results were repeated.

TTGAb was measured by the previously described method (5). The antibody levels were expressed as an index relative to a positive control sample. The upper level of normal for TTGAb was established as three times the 100th percentile in 184 healthy control subjects (an index of 0.05) (5). The assays were performed in duplicate. Positive results were repeated.

GAD and ICA512 autoantibodies were measured simultaneously by combined GAD and ICA512 radioassay (full-length GAD65 and ICA512bdc cDNA clones). The cut points for positivity were set at indexes of 0.032 (mean \pm 2 SD, GAD) and 0.049 (mean \pm 6 SD, ICA512 autoantibodies), the 99th percentile of 198 normal control subjects. The microinsulin autoantibody assay (mIAA) in this study used 125 I insulin. The upper limit of normal (0.01) was chosen as the 99th percentile from receiver operating characteristic curves in 106 healthy control subjects and 105 patients with new-onset diabetes. A subject was classified as positive for islet autoantibodies when results for GAD and/or ICA512 autoantibodies were positive or if results for mIAA were positive before 1 week of insulin therapy.

TPOAb and TGAb were measured using an ICMA performed at Nichols Laboratory. A cutoff of >2 mIU/ml for both autoantibodies was considered positive. Measurements for the autoantibodies were performed in singlet with an automated system; the control measurements were performed at the beginning and end of the assay and at designated intervals.

HLA-DQB and DRB genotyping were performed using PCR probe-based genotyping kits (DynaL Biotech, Lafayette Hill, PA). DNA amplification is performed in a 9600 Thermal Cycler (Perkin Elmer, Norwalk, CT). The amplified DNA was added to strips containing immobilized sequence-specific oligonucleotides for DQB1 or DRB1.

Our current clinical practice is to obtain thyroid-stimulating hormone (TSH) and thyroid hormone levels (T_4 or free T_4) annually or with symptoms of AIT. For this study, AIT was defined by the presence of abnormalities of T_4 or free T_4 and/or TSH and treatment with thyroid hormone replacement for hypothyroidism or radioactive iodine, propylthiouracil, or methimazole for hyperthyroidism. Date of laboratory abnormalities was recorded as onset of AIT. Individuals with TTGAb were screened clinically for symptoms consistent with celiac disease. Patients with symptoms or persistently high positive TTGAb results were referred to a gastroenterologist for evaluation and small intestinal biopsy to confirm the diagnosis of celiac disease. Individuals with positive 21-OHAb results had ACTH stimulation testing.

Statistical analyses

Statistical analyses were performed using SAS 8.0. None of the variables analyzed were normally distributed. The Mann-Whitney test was used for nonnormally distributed continuous variables. Proportions were compared using the χ^2 test unless 20% of the cells had an expected value <5, in which case Fisher's exact test was used. Logistic regression was performed using potentially significant variables identified by univariate analysis ($P < 0.1$ in univariate analysis). Outcomes modeled included thyroid antibody positivity, AIT, and 21-OHAb positivity. Backwards stepwise elimination was used to obtain the best model. The relationship between the individual islet autoantibodies and the presence of thyroid, adrenal, and celiac autoimmunity was explored. Two-tailed P values <0.05 were considered statistically significant.

RESULTS— Of the 814 individuals, 384 (47%) were female. Median age of diabetes diagnosis was 9.5 years (interquartile range 5.83–13.08), median duration of diabetes was 3.4 years (0.08–10.33), and median age at sample was 14.8 years (10.75–21.17). All subjects had autoantibodies measured once.

Of the study group, 509 were positive for islet autoantibodies, 99 were negative for all three autoantibodies, and 206 were positive for only mIAA >1 week from the start of insulin therapy. The prevalence of positive autoantibodies is shown in Fig. 1. Of the study group, 236 (29%) were positive for either TPOAb and/or TGAb, 82 (10.1%) were positive for TTGAb, and 13 (1.6%) were positive for 21-OHAb. Thirty-six percent ($n = 295$) were positive for at least thyroid autoantibodies, TTGAb, or 21-OHAb; 34 subjects (4.1%) were positive for two of the autoantibodies, and 1 individual was positive for all three autoantibodies.

Fig. 2 shows relationships between the different autoantibodies. A positive association between the presence of thyroid autoantibodies and 21-OHAb was noted ($P = 0.003$).

The thyroid autoantibody-positive subjects were more likely to be female (58 vs. 43%, $P < 0.0001$), older (16.13 vs. 14.46 years, $P = 0.0013$), and 21-OHAb positive (3.81 vs. 0.69%, $P = 0.0029$) with a longer duration of diabetes (5.35 vs. 2.62 years, $P < 0.0001$). When these variables were combined in a model with

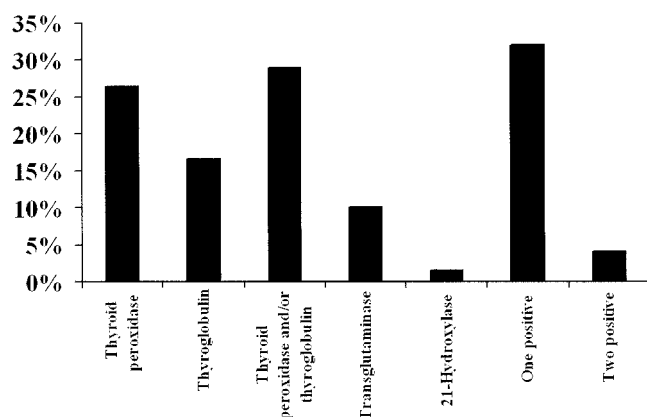


Figure 1—Prevalence of positive autoantibodies in 814 individuals with type 1 diabetes. The y-axis represents percentage.

thyroid autoantibody positivity as the outcome, female sex, duration of diabetes, and positivity for 21-OHAb were significantly associated with thyroid autoantibody positivity (Table 1). Analysis for associations between TTGAb positivity and sex, age at screening, duration of diabetes, and the presence of other autoimmunities showed no significant associations.

Individuals positive for 21-OHAb had a longer duration of diabetes (8.92 vs. 3.29 years, $P = 0.03$) and were more likely to be positive for thyroid autoantibodies (69 vs. 28%, $P = 0.003$) compared with those who were negative. Multiple logistic regression with these two variables showed only a significant association with thyroid autoantibodies and a trend for significance with duration of diabetes (Table 1).

Subjects positive for GAD autoantibodies (120 of 339) were more likely to be thyroid autoantibody positive compared with those who were GAD autoantibody negative (116 of 475) (35 vs. 24%, $P = 0.0008$). When those who had positive islet autoantibodies (159 of 309) were compared with those who had negative islet autoantibodies (11 of 99), there was an increase in thyroid autoimmunity in the positive group versus the negative group (31 vs. 11%, $P < 0.0001$).

Of the group, 804 individuals (99%) had DNA available for complete DQA and DQB typing. DR4 subtyping was performed on 97% (471 of 485) of those with DR4. Table 2 shows the proportion of subjects with each the major DR genotypes positive for thyroid, celiac, and adrenal autoimmunity. Our study confirms the DR3-DQ2 association with celiac au-

toimmunity: 33.3% of DR3-DQ2 homozygotes, 12.3% of DR3-DQ2 heterozygotes, and 4.4% of subjects without DR3-DQ2 were positive for TTGAb.

Analysis of HLA and association with thyroid autoimmunity in this cohort with type 1 diabetes has demonstrated an association with DR3-DQ2 homozygosity with 43.3% (26 of 60) of subjects homozygous for DR3-DQ2 positive for thyroid autoantibodies compared with 28.0% (208 of 744) DR3-DQ2/X or no DR3 alleles ($P < 0.02$).

The high diabetes risk DR3/4 genotype DQA1*0501-DQB1*0201, DQA1*0301-DQB1*0302 was also associated with 21-OHAb. Eight of 219 (3.7%) individuals with the DR3/4 genotype DQA1*0501-DQB1*0201, DQA1*0301-DQB1*0302 were positive for 21-OHAb compared with 5 of 585 (0.7%) without this genotype ($P = 0.009$). DR3/4 individuals with DRB1*0404 were more likely to be positive for 21-OHAb (5 of 32 [15.6%]) compared with those without DRB1*0404 (3 of 182 [1.6%], $P = 0.002$). There is a trend for an association between thyroid autoimmunity and adrenal autoimmunity in this high-risk group (DRB1*0401-DQ8, DR3-DQ2; $P = 0.053$).

Clinical data were available for 220 (93%) individuals with thyroid autoantibodies and 434 (75%) of those negative for thyroid autoantibodies. At the time of sampling, 42 of the positive group (19.1%) had a previous diagnosis or a new diagnosis of AIT. Over an average follow-up of 6.96 years, AIT was diagnosed in 35 subjects (31 hypothyroid and 4 hyperthyroid) in the positive group. The presence of TPOAb with or without TGAb conferred a high risk for thyroid disease compared with TGAb alone (75 of 201 vs. 2 of 20, $P < 0.02$). In the negative group, two

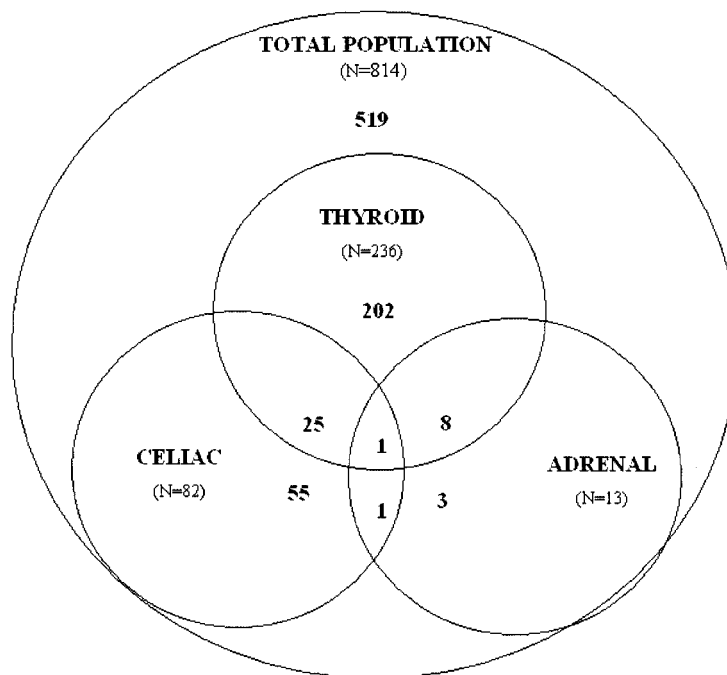


Figure 2—Venn diagram of positive antibodies. Circles represent thyroid, celiac, and adrenal autoimmunity. Overlapping areas represent individuals positive for more than one autoantibody. There is significant association between thyroid and 21-hydroxylase autoantibodies ($P < 0.0001$); no other significant association was noted.

Table 1—Multiple logistic regression for factors associated with thyroid antibody positivity, 21-hydroxylase positivity, and thyroid disease in the thyroid antibody–positive group*

Factors	Odds ratio (95% CI)	P value
Thyroid antibody positivity		
Sex (male is reference)	1.89 (1.38–2.59)	<0.0001
Duration of diabetes (for 5 years)	1.15 (1.04–1.28)	0.008
Positive 21-hydroxylase	5.74 (1.72–19.13)	<0.005
21-hydroxylase positivity		
Duration of diabetes (for 5 years)	1.31 (0.95–1.82)	0.10
Positive thyroid antibody	5.43 (1.65–17.86)	0.005
Thyroid disease		
Antithyroperoxidase level (50-unit increase)	1.08 (1.05–1.12)	<0.0001
Positive tissue transglutaminase	2.83 (1.18–6.79)	0.02
Positive 21-hydroxylase	4.58 (1.05–20.0)	0.043

*Only factors significant in univariate analysis were included in this model. The best model was attained by using backwards stepwise elimination.

individuals were hyperthyroid (one diagnosed before and one diagnosed after sampling). Seventeen were hypothyroid; for 15 of these, the date of diagnosis was known and occurred after the screening sample. Autoantibodies were obtained in four of the initially negative individuals; one was positive for TPOAb and TGAb. One individual was receiving lithium therapy.

In the thyroid autoantibody–positive group, those with disease had a higher level of TPOAb (393 vs. 39.0%, $P < 0.0001$) but no difference was seen in TGAb. These cases were more likely to be positive for TTGAb (18 vs. 8.4%, $P < 0.05$). Multiple logistic regression revealed that TTGAb and 21-OHAb positivity and TPOAb levels were significantly associated with AIT (Table 1).

Biopsies were performed in 19 (23%) individuals positive for TTGAb. Sixteen (84%) were positive (Marsh score II or greater), two were negative (Marsh score 0), and one was indeterminate (Marsh score I). Fifteen (17%) were consuming a gluten-free diet, 10 were positive for celiac disease on biopsy, and 5 had elevated TTGAb levels and no biopsy was performed. Of the individuals positive for 21-OHAb, five (38%) had Addison's disease.

CONCLUSIONS— We confirmed the high prevalence of a second organ-specific autoimmune manifestation in individuals with type 1 diabetes. One-third were positive for thyroid autoantibodies, TTGAb, and/or 21-OHAb. At least 25% of our group were positive for thyroid autoimmunity, 10% for TTGAb, and

1.5% for 21-OHAb. The high prevalence of autoimmune diseases has important implications for both the clinical care of individuals with type 1 diabetes and the pathogenesis of diabetes-associated autoimmunity.

The major limitation of this study was the cross-sectional nature of autoantibody ascertainment. Analysis regarding islet autoantibody association is limited by the known disappearance of islet autoantibodies with duration of diabetes. The effect of time is difficult to control in a cross-sectional study and should be addressed in future studies of patients prospectively followed from onset of diabetes. In addition, we classified subjects as positive or negative based on a cutoff value established in groups of normal control sub-

jects. Changing the cutoff value will change the prevalence of autoantibody-positive subjects. Follow-up for stability of positive versus negative classification and the development of disease is required to validate this classification.

Previously identified risk factors have been confirmed in this study. Sex, age at sample, and duration of diabetes were associated with the development of thyroid autoimmunity (3,9–11), but sex did not influence TTGAb and 21-OHAb. The lack of association of duration and sex with TTGAb is contrary to recently published data in a large group of Italian children with type 1 diabetes (27). The lack of relationship may be due to the smaller population screened. The association between DR3-DQ2 homozygosity and celiac disease among patients with type 1 diabetes (5) was observed in this group. Nearly 16% (5 of 32) of the individuals with DRB1*0404-DQ8, DR3-DQ2 expressed 21-OHAb, a remarkable prevalence for an autoantibody associated with a rare disease (6). In addition, a full 69% of individuals with 21-OHAb also expressed thyroid autoimmunity, thereby identifying a group at an even higher risk for the development of thyroid autoimmunity.

The risk for additional autoimmune diseases in the group with type 1 diabetes and thyroid autoimmunity was selective. The risk for 21-OHAb was increased in the group with thyroid autoantibodies, whereas the risk for TTGAb in the group with thyroid autoantibodies was similar

Table 2—HLA genotype and thyroid, celiac, and adrenal autoimmunity

HLA genotype	Thyroid autoimmunity	Celiac autoimmunity	Adrenal autoimmunity	Total study
n	234	81	13	804
DR3/4	58 (25)	26 (32)	8 (62)	223 (28)
DR3/3	26 (11)	20 (25)	1 (8)	60 (8)
DR3/1	8 (3)	3 (4)	1 (8)	35 (4)
DR3/2	4 (2)	4 (5)		15 (2)
DR3/7	3 (1)	5 (6)		21 (3)
DR3/X	13 (6)	7 (9)	1 (8)	62 (8)
DR4/4	26 (11)	7 (9)		62 (8)
DR4/1	13 (6)	3 (4)		60 (8)
DR4/2				2 (0.3)
DR4/7	10 (4)	1 (1)		33 (4)
DR4/X	23 (10)	4 (5)	1 (8)	143 (18)
DRX/X	50 (21)	1 (1)	1 (8)	88 (11)

Data are n (%). X does not equal DR4, DR3, DR2, DR7, or DR1. Thyroid and celiac autoimmunity were associated with DR3-DQ2 homozygosity ($P < 0.02$ and <0.0001 , respectively). Adrenal autoimmunity was associated with DR3-DQ2, DR4-DQ8 ($P = 0.009$).

to that of the entire population. Therefore, we hypothesize that autoimmunity to specific organs segregates together such that individuals with type 1 diabetes and thyroid autoimmunity have risk factors that are different (but possibly overlapping) from those with type 1 diabetes and celiac autoimmunity. This risk may be conferred by HLA. However, based on small numbers, our data argue against this hypothesis. The increased prevalence of thyroid autoimmunity in the 21-OHAb-positive group was seen even when controlling for DRB1*0404-DQ8, DR3-DQ2. Our data are not conclusive given the small numbers of subjects positive for 21-OHAb; we suggest confirming these observations in larger populations of people with type 1 diabetes.

These patterns of disease risk may also be conferred by shared environmental exposures, genes outside of the HLA, or collateral priming (24). Collateral priming is a process in which naïve T-cells can be primed to new antigens, bypassing signals usually required, by previously activated T-cells. In this manner, once an autoimmune process to a single organ starts (for example, the thyroid), T-cell response to antigens in the thyroid that are also in the adrenal gland may be initiated and risk for a second autoimmune reaction (for example, to the adrenal gland) increased. If this hypothesis were true, we would expect the increased risk for thyroid and adrenal autoimmunity to be present in the population without type 1 diabetes. The risk for thyroid autoimmunity in the population with Addison's disease has been well established with risk for hypothyroidism in the group with Addison's disease of ~15–20% (28–30). The converse risk for Addison's disease in the population with thyroid autoimmunity has been reported to be 2–3% (31). Somewhat contrary to this hypothesis is the observation that in the general population, the risk for AIT increases for those with celiac disease and vice versa (32–33). Further studies with larger populations of individuals with type 1 diabetes are needed to answer this basic question about initiation of subspecific groups of autoimmune disease.

Certain individuals demonstrated a high burden of disease: 35% (77 of 220) of those with thyroid autoimmunity and AIT, 38% (5 of 13) of those with 21-OHAb and Addison's disease, and 84% (16 of 19) of those with TTGAb and a

biopsy confirming celiac disease. Each disease has associated morbidity and, in the case of untreated Addison's disease, mortality. However, they may be detected in the preclinical phase by expression of autoantibodies. These organ-specific autoantibodies provide a simple way to screen for autoimmunity in a susceptible population and possibly prevent morbidity and mortality. However, the specific strategy for screening is an area of active debate and research (34–37). Long-term prospective studies are needed to identify the natural history of autoimmunity in patients with type 1 diabetes.

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