

Comparative Analysis of Organ-Specific Autoantibodies and Celiac Disease-Associated Antibodies in Type 1 Diabetic Patients, Their First-Degree Relatives, and Healthy Control Subjects

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OBJECTIVE — In type 1 diabetes the coexistence with other endocrine diseases and organ-specific autoantibodies has been frequently reported leading to the concept of autoimmune polyendocrine syndrome (APS). In addition, an association of type 1 diabetes with celiac disease has been described. These disorders share a similar genetic background, and first-degree relatives of type 1 diabetic patients may also be affected significantly. Screening for specific antibodies allows early diagnosis of these disorders.

RESEARCH DESIGN AND METHODS — In the present cross-sectional study, we analyzed sera from 197 recent-onset type 1 diabetic patients at the time of diagnosis, 882 first-degree relatives, and sera of 150 healthy control subjects for prevalence and co-occurrence of the following antibodies (method): insulin autoantibodies (radioimmunoassay); GAD and IA-2 antibodies (radioligand assay); islet cell antibody, anti-adrenal cortex antibodies, and anti-gastric parietal cell antibodies (indirect immunofluorescence); anti-thyroglobulin and anti-thyroid peroxidase antibodies; and gliadin IgG/A and tissue-transglutaminase IgA (enzyme-linked immunosorbent assay).

RESULTS — The overall frequency of gastric parietal cell antibodies and adrenal antibodies did not differ significantly among groups. In contrast, type 1 diabetes-associated antibodies and thyroid antibodies were significantly more frequent both in recent-onset type 1 diabetic patients and in the group of first-degree relatives ($P < 0.05$). The prevalence of gliadin IgG/IgA and transglutaminase IgA was significantly higher in the group of recent-onset type 1 diabetic patients ($P < 0.05$), but the difference between first-degree relatives and control subjects did not reach statistical significance. Focusing on the coexistence of antibodies, the group of recent-onset type 1 diabetic patients presented with 27.4% of the subjects testing antibody-positive-specific for two or more of the envisaged disorders (i.e., type 1 diabetes, autoimmune thyroiditis, and celiac disease) compared with 3.1% in the group of first-degree relatives and 0 of 150 in the control population ($P < 0.05$).

CONCLUSIONS — We conclude that, in an active case-finding strategy, recent-onset type 1 diabetic patients should be routinely screened at least for concomitant autoimmune thyroid disease and additionally for celiac disease. Screening in their first-degree relatives should include at a minimum the search for thyroid autoimmunity in addition to screening for pre-type 1 diabetes.

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Abbreviations: APS, autoimmune polyendocrine syndrome; ELISA, enzyme-linked immunosorbent assay; GADA, GAD65 antibodies; GPC, gastric parietal cell; IAA, insulin autoantibodies; ICA, islet cell antibodies; TG, thyroglobulin; TPO, thyroid peroxidase; tTGC, tissue transglutaminase C; WHO, World Health Organization.

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

The first evidence that type 1 diabetes might be a disease of autoimmune origin came from the observation that type 1 diabetes is often associated with other endocrine autoimmune disorders. Type 1 diabetes was later added as a third component to the description of Schmidt's syndrome (1), consisting originally of autoimmune thyroiditis and adrenalitis. This overlap of different autoimmune disorders has led to the concept of autoimmune polyendocrine syndrome (APS) with the clinical or subclinical involvement of several organs in the same subject or family (2–4). In addition, celiac disease is significantly associated with type 1 diabetes (5–8), which is supposed to be the result of an interplay among genetic, hormonal, and immunological factors (9–11). Based on the hypothesis that the frequent coexistence of these diseases can be explained at least in part by their similar genetic background, we addressed the question whether first-degree relatives of type 1 diabetic patients might be significantly affected by these disorders.

Circulating autoantibodies are a hallmark of clinical or subclinical autoimmune polyendocrine disease, particularly in APS II (i.e., Carpenter's syndrome, type 1 diabetes, Hashimoto thyroiditis, adrenalitis) and APS III (type 1 diabetes, autoimmune thyroid disease, pernicious anemia). The measurement of specific antibodies allows early diagnosis of these disorders including pre-clinical stages of celiac disease. Focusing on type 1 diabetes as the index diagnosis to consider screening for potential APS and celiac disease, we aimed to determine the significance of the different antibody prevalences and levels of coexistence in 882 first-degree relatives of type 1 diabetic patients. For conclusive interpretation of the data, we determined additionally autoantibody prevalences in 197 recent-onset type 1 diabetic patients and in 150 healthy individuals, serving as positive versus negative control subjects, respectively. We screened our study population for islet cell antibod-

Table 1—Prevalence of type 1 diabetes- and thyroid disease-associated autoantibodies in patients with type 1 diabetes at recent onset, first-degree relatives of type 1 diabetic patients, and healthy control subjects

	Recent-onset type 1 diabetic patients	First-degree relatives	Healthy control subjects
<i>n</i>	197	882	150
Type 1 diabetes-associated antibodies			
ICA	82.1*	4.9*	1.3
Anti-GADA	76.0*	7.6*	2.6
Anti-IA-2 antibodies	44.4*	4.0*	0.6
IAA	37.8*	3.4	0.6
≥1 antibody ⁺	93.4*	11.6*	4.0
Thyroid disease-associated antibodies			
Anti-TPO ± TG antibodies	18.4*	7.8*	3.2

*Significantly different ($P < 0.05$).

ies (ICA), insulin autoantibodies (IAA), GAD65 antibodies (GADA), anti-IA-2 antibodies, anti-adrenal cortex antibodies, anti-gastric parietal cell (GPC) antibodies, anti-thyroglobulin (TG) antibodies, and anti-thyroid peroxidase (TPO) antibodies. In addition, screening for celiac disease was performed by measuring antibodies to gliadin (IgG/IgA) and IgA autoantibodies to tissue transglutaminase C (tTGC), the recently identified target autoantigen of the endomysial antibodies (12). The data gained in this analysis are supposed to provide a rationale for screening programs for active case-finding strategies in specialized centers (e.g., diabetes outpatient clinics).

RESEARCH DESIGN AND METHODS

Subjects and human sera

The study population consisted of 197 patients at onset of type 1 diabetes, diagnosed according to World Health Organization (WHO) criteria. The median age of the recent-onset type 1 diabetic patients was 16 years (range 5–27), and 112 were men. The study population also included 882 first-degree relatives who were recruited from the Giessen-Bad Oeyenhausen prospective family study (13), of which 485 were parents (median age 43 years, range 22–59), 382 were siblings, and 15 were offspring of type 1 diabetic patients (median age 16 years, range 2–41). As a control group, we additionally analyzed sera from 150 healthy individuals without a family history of diabetes who were age- and sex-matched to the group of first-degree relatives. The serum samples were drawn before insulin treat-

ment was instituted in the recent-onset type 1 diabetic patients, and in the first-degree relatives, blood was drawn close to the time of diagnosis of the index patient. Sera were immediately stored in aliquots at -20°C in our serum bank before testing. Informed consent was obtained before blood sampling.

Antibody assays

The type 1 diabetes-associated autoantibodies (i.e., ICA, IAA, and GADA) and the intracytoplasmic domain of the tyrosine phosphatase-like protein IA-2 (anti-IA-2) were measured by standard methods, established in our laboratory and evaluated in Immunology of Diabetes Society proficiency tests as described previously (13). Briefly, ICA were detected by the indirect immunofluorescence technique on cryostat sections of human pancreas, blood group O. GADA and anti-IA-2 antibodies were measured in a fluid phase radioligand binding assay with human GAD65 or the tyrosine phosphatase-like protein IA-2 as substrate. IAA were determined in a competitive radioimmunoassay. Adrenal cortex autoantibodies and GPC antibodies were detected by indirect immunofluorescence on 4- μm cryostat sections of fresh frozen guinea pig adrenal gland tissue or stomach. The celiac disease-associated IgG/IgA directed to gliadin and IgA targeting tTGC were evaluated by means of a newly developed enzyme-linked immunosorbent assay (ELISA) utilizing highly purified tissue transglutaminase from guinea pig as substrate (Medipan Diagnostica, Selchow, Germany). Thyroid autoantibodies directed to TG and microsomal antigens (TPO) were determined in an ELISA based on recom-

binant human TG or TPO. The standardization of the TPO antibodies assay was carried out against the WHO standard serum NIBSC 66/387 (Medizintechnik; Elias, Freiburg, Germany).

Statistics

For comparison between groups, χ^2 statistics, Fisher's exact test, and Mann-Whitney U test were applied where appropriate. A P value of <0.05 was considered significant. The data analysis was carried out using the SPSS 6.1.3 software.

RESULTS

Prevalence of autoantibodies

The overall frequencies of type 1 diabetes- and thyroid disease-associated antibodies in the study population and in the healthy control subjects are shown in Table 1 and Fig. 1. Because type 1 diabetes was chosen as the index diagnosis in this study, we naturally observed a significantly higher frequency of humoral markers of type 1 diabetes-associated autoimmunity in the group of recent-onset type 1 diabetic patients and in the group of first-degree relatives compared with healthy control subjects ($P < 0.05$). In addition, there was a general increase of thyroid autoimmunity in the recent-onset type 1 diabetic patients and in the first-degree relatives compared with healthy control subjects. The prevalence of anti-TPO ± TG antibodies was significantly higher both in the recent-onset type 1 diabetic patients and in their first-degree relatives compared with the control group ($P < 0.05$). Because it has long been realized that thyroid autoimmunity increases with age, we analyzed the frequencies in the subgroup of parents compared with siblings and offspring. We confirmed significantly higher prevalences against thyroid antigens in the older age group (parents) ($P < 0.05$) (data not shown). Nevertheless, looking at the overall frequencies of anti-TPO ± TG antibodies in the group of first-degree relatives compared with the age- and sex-matched control group, we still observed a significant increase in thyroid autoimmunity in the first-degree relatives compared with the control subjects ($P < 0.05$). This finding suggests an increased level of thyroid autoimmunity independent of age in first-degree relatives of patients with type 1 diabetes.

Table 2 and Fig. 1 show the overall frequency of celiac disease-associated anti-

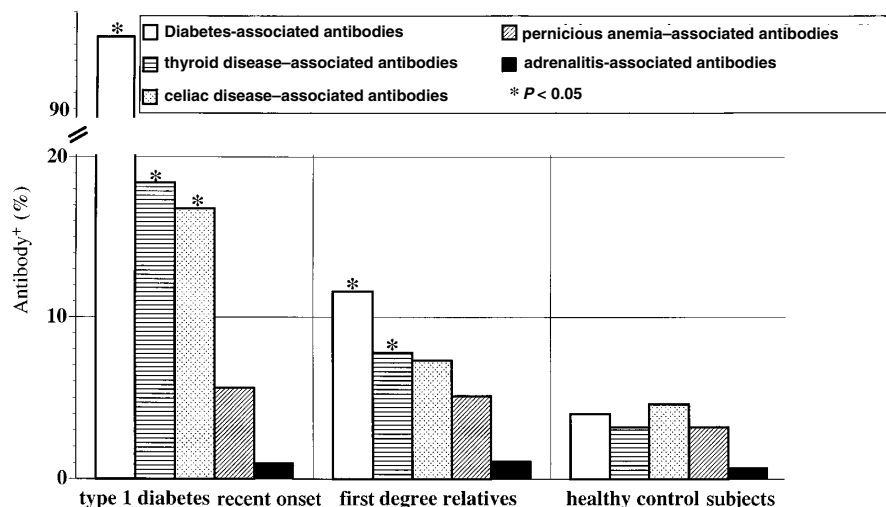


Figure 1—Antibody prevalences in type 1 diabetic patients, first-degree relatives, and healthy control subjects. Data are stratified for disease-specific antibodies (recent-onset type 1 diabetes, autoimmune thyroid disease, celiac disease, pernicious anemia, and Addison's disease). In cases in which more than one antibody specificity is pertinent for the respective disease, the data are shown as positivity for ≥ 1 antibody. Significant differences ($P < 0.05$) are encoded with an asterisk (*).

bodies, GPC antibodies, and adrenal cortex antibodies in the study population and in the control group. We observed a significant increase in the frequency of anti-gliadin IgG, anti-gliadin IgA, and anti-transglutaminase IgA as potential markers for celiac disease in the group of recent-onset type 1 diabetic patients ($P < 0.05$). In addition, there was a trend for higher prevalences of celiac disease-associated antibodies in the group of first-degree relatives compared with the control group, but this difference did not reach statistical significance. The prevalence for GPC antibodies did not differ significantly between groups; however, there was a significant increase in GPC antibodies positivity depending on older age in the group of first-degree relatives ($P < 0.05$) (data not shown). Adrenal cortex antibodies were only detected at very low prevalence levels and were not significantly different between groups.

Co-occurrence of autoantibodies

As shown before, the antibody prevalence study has revealed significant differences for three of the five disorders under consideration (i.e., recent-onset type 1 diabetes, autoimmune thyroid disease, and celiac disease). Figure 2 shows the data analysis with regard to co-occurrence of disease-specific antibodies by focusing on antibody positivity for two or more of the envisaged disorders (recent-onset type 1 diabetes, autoimmune thyroid disease, and

celiac disease). In the healthy control subjects, the overall level of antibody positivity and titers were low, and we did not observe any subject with positivity for more than one disease-specific antibody (0/150). By contrast, in the group of patients with recent-onset type 1 diabetes, a significant ($P < 0.05$) proportion of patients was positive for two or more disease-specific antibodies (54/197, 27.4%). The level of coexistence for thyroid antibodies- and/or celiac disease-associated antibodies was 11.2 and 9.6%, respectively, and 6.6% were triple positive. Interestingly, when looking at the group of first-degree relatives, we found 27/882

(3.1%) double or triple positive, also demonstrating a significant ($P < 0.05$) overlap of disease-specific antibodies in first-degree relatives of type 1 diabetic patients compared with the control subjects ($P < 0.05$). Thus, we observed a significant clustering of disease-specific antibodies both in recent-onset type diabetic patients (thyroid antibodies and celiac disease-associated antibodies) and in their first-degree relatives (thyroid antibodies in addition to type 1 diabetes-associated antibodies).

CONCLUSIONS — The clinically established entity of APS II and III reflect a significant overlap of different endocrine disorders of autoimmune origin in one subject, leading to differential therapeutic concepts (4). Recently, several reports have described a frequent association of celiac disease and type 1 diabetes (6,7,14,15), which has obvious therapeutic implications. A gluten-free diet may improve the diarrhea in some patients with type 1 diabetes where the reason for diarrhea is underlying celiac disease, and the diet may improve control of diabetes by normalizing the nutritional-hormonal balance (5,16,17). These considerations provide a strong rationale for screening strategies for the early diagnosis of suspected APS and/or celiac disease in populations at risk. In the present study, we have chosen type 1 diabetes as the index diagnosis to consider screening and have included first-degree relatives because of the suspected genetic predisposition of these disorders.

For screening purposes, detection of autoantibodies is most efficient to diagnose preclinical endocrine dysfunction. This is well established for pre-type 1 diabetes by

Table 2—Prevalence of organ-specific autoantibodies in patients with type 1 diabetes at recent-onset, first-degree relatives of type 1 diabetic patients, and healthy control subjects

	Recent-onset type 1 diabetic patients	First-degree relatives	Healthy control subjects
n	197	882	150
Celiac disease-associated antibodies			
Anti-gliadin IgG	10.2*	5.6	3.2
Anti-gliadin IgA	7.6*	2.6	2.0
Anti-transglutaminase IgA	9.7*	3.2	2.6
≥ 1 antibody ⁺	16.8*	7.3	4.6
Pernicious anemia-associated antibodies			
Anti-GPC antibodies	5.6	6.0	3.2
Adrenalitis-associated antibodies			
Anti-adrenal antibodies	1.0	1.1	0.7

*Significantly different ($P < 0.05$).

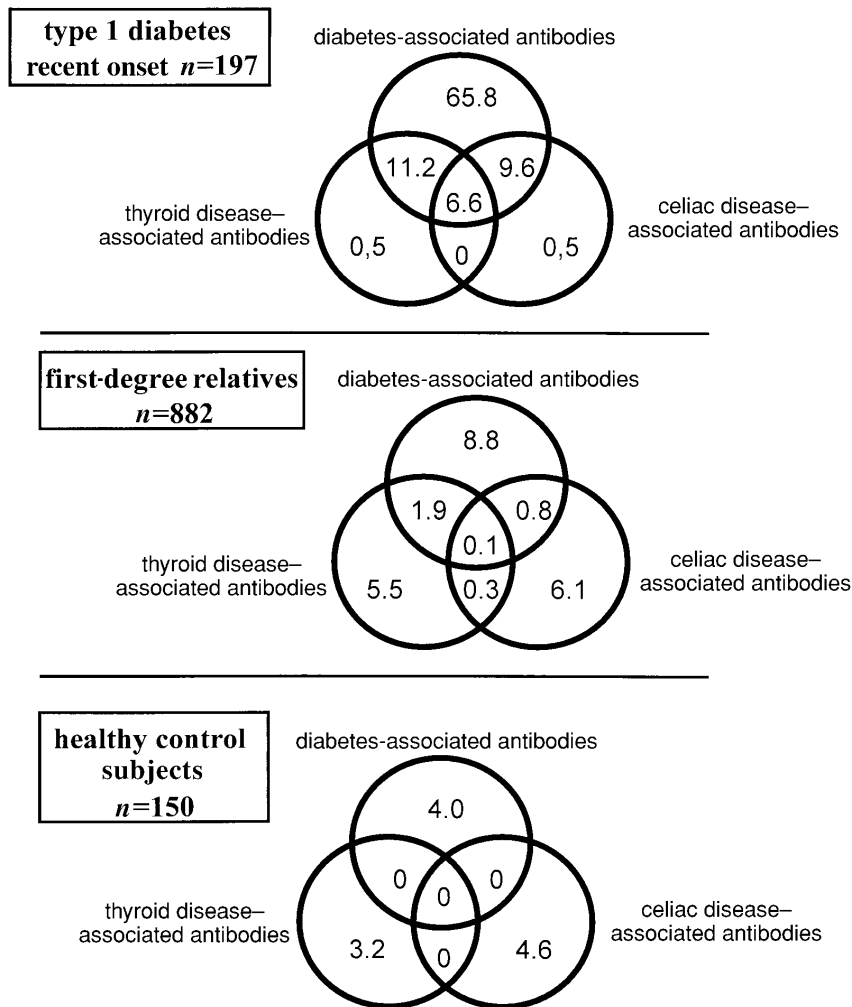


Figure 2—Coexistence of disease-specific antibodies relevant for type 1 diabetes, autoimmune thyroid disease, and celiac disease in healthy control subjects, type 1 diabetic patients at onset of the disease, and their first-degree relatives. Significant differences for the proportions of individuals being double or triple positive both for the group of recent-onset type 1 diabetic patients and for the group of first-degree relatives compared with control subjects ($P < 0.05$).

assessment for ICA, IAA, GADA, and anti-IA-2 antibodies (13,18,19). Moreover, autoimmune thyroid disease may present with anti-TPO antibodies and/or anti-TG antibodies (20,21), and anti-GPC antibodies or anti-adrenal cortex antibodies are significantly associated with pernicious anemia or Addison's disease-associated adrenalitis, respectively (22–26). In celiac disease, a major breakthrough was the identification of tTGC as the target antigen of the endomysial antibodies (12,15,27,28). The panel of antibodies, selected for screening in our present study, therefore included ICA, IAA, GADA, anti-IA-2, anti-TPO antibodies, anti-TG antibodies, anti-GPC antibodies, and anti-adrenal cortex antibodies and was

supplemented with anti-gliadin IgG/IgA and anti-tTGC antibodies. Because in clinical practice, even in specialized centers, it is not suitable to screen all individuals for the whole panel of antibodies, the data arising from this analysis should provide a rationale for an active case finding strategy that is efficient and based on epidemiological data.

Focusing on the group of recent-onset type 1 diabetic patients, we observed autoantibody frequencies very similar to previously published data, confirming a reasonable sensitivity and specificity of the assay systems used. Signs of autoimmune thyroid disease were found in one of five recent-onset type 1 diabetic patients, and one of six was positive for one or more of

the celiac disease-associated antibodies, which is well in accordance with recent observations from other investigators (8,29,30). Interestingly, 6.6% (1 of 15) were triple positive for thyroid antibodies and celiac disease-associated antibodies in addition to type 1 diabetes-related autoimmune markers. We conclude from these high frequencies and levels of coexistence that recent-onset type 1 diabetic patients should be screened at a minimum for presence of thyroid antibodies and celiac disease-associated antibodies to facilitate differential and optimal therapy. As described by others, the prevalences for anti-GPC antibodies and anti-adrenal cortex antibodies were low (26,31) and did not differ among groups. A screening for these antibodies in recent-onset type 1 diabetic patients without a certain level of suspicion seems not to be justified.

In the group of first-degree relatives of type 1 diabetic patients, 11.6% were positive for at least one of the diabetes-associated autoantibodies. Based on these antibodies, refined prediction models for future development of type 1 diabetes are clinically well established, and several intervention studies are underway aiming to prevent type 1 diabetes in these individuals at risk. In contrast, only few data exist in first-degree relatives of type 1 diabetic patients comprising the whole panel of antibodies relevant for APS II/III and celiac disease, which is in the focus of our present study. In 7.8% of the cases (1 of 13), we observed signs of anti-thyroid autoimmunity and in 2% (1 of 50) a coexistence of type 1 diabetes-associated antibodies with anti-thyroid antibodies. We suggest from these data that screening in first-degree relatives of patients with type 1 diabetes should include the search for thyroid autoimmunity in addition to screening for pre-type 1 diabetes. Celiac disease-associated antibodies were not significantly higher compared with the control group. Although there was a positive trend with 7.6% positivity for celiac disease-associated antibodies in the group of first-degree relatives compared with only 4.6% in the control group, this difference was not statistically significant. The rate at which celiac disease is diagnosed depends on the level of suspicion for the disease. From our data, we cannot recommend a general screening of first-degree relatives for celiac disease-associated antibodies. Nevertheless, a potentially increased risk for celiac disease at least in the subgroup of children

from diabetic parents should be considered because of a recent report that offspring of type 1 diabetic parents have a seven times higher risk to develop tTGC than healthy control subjects (32). In our group of first-degree relatives, offspring were the minority, representing only 1.7% of the population, while the majority were siblings and parents of type 1 diabetic patients. As in the group of recent-onset type 1 diabetic patients, the overall frequencies for anti-GPC antibodies and anti-adrenal cortex antibodies were low and not significantly different from the control group. Thus, a general screening for anti-GPC antibodies and anti-adrenal cortex antibodies in first-degree relatives of type 1 diabetic patients cannot be recommended.

Because of the cross-sectional design of the present study, we cannot answer the question related to an optimal time point for screening or repeated blood sampling. However, at least for celiac disease, a follow-up study in type 1 diabetic patients has shown that celiac disease tends to develop soon after diabetes has occurred. Routine screening for celiac disease repeatedly during the first years after the diagnosis of type 1 diabetes is suggested by the authors (33).

In summary, our data provide evidence that in an active case finding strategy, recent-onset type 1 diabetic patients should be screened routinely for concomitant autoimmune thyroid disease and additionally for celiac disease. Screening in their first-degree relatives should include at a minimum the search for thyroid autoimmunity in addition to screening for pre-type 1 diabetes and can be extended for anti-tTGC antibodies and anti-gliadin antibodies depending on the level of suspicion for celiac disease (e.g., in offspring of diabetic parents). A general screening for anti-GPC and anti-adrenal cortex antibodies without clinical suspicion for the disease appears not to be justified. In case of autoantibody positivity, further diagnostic tools should be considered including metabolic testing or jejunal biopsy to verify the diagnosis. The screening procedure suggested in our present study is noninvasive by using standardized antibody assays performed on small amounts of serum and is suitable for large-scale screening. It has the potential to identify undiagnosed cases of APS and/or celiac disease, which is essential to prevent complications such as hypothyroidism, nutritional deficiencies, and other severe complications associated with these disorders.

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