The High Prevalence of Autoantibodies to Tissue Transglutaminase in First-Degree Relatives of Patients With Type 1 Diabetes Is Not Associated With Islet Autoimmunity

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OBJECTIVE — To determine the extent of celiac autoimmunity in type 1 diabetic patients and the overlap between islet and celiac autoimmunity in their nondiabetic relatives.

RESEARCH DESIGN AND METHODS — IgA antibodies to tissue transglutaminase were determined in serum taken from 433 type 1 diabetic patients and 1,442 nondiabetic first-degree relatives. Samples were taken from 347 schoolchildren, who were also assayed for IgA anti-endomyosial antibodies (EMAs). Markers of islet autoimmunity (islet cell antibodies and autoantibodies to insulin, glutamate decarboxylase, and protein tyrosine phosphatase IA-2) had previously been measured in all relatives.

RESULTS — In the absence of known celiac disease, the prevalence of transglutaminase antibody levels above the 97.5th percentile of the schoolchildren was 13.4% in diabetic patients and 7.0% in nondiabetic relatives. EMAs were found in addition to transglutaminase antibodies in 2.6% of probands and in 1.9% of first-degree relatives, but none of the schoolchildren. Transglutaminase antibodies were found to persist in 10 of 30 patients and in 30 of 59 relatives with follow-up samples taken at least 2 years after the initial sample. Of 186 nondiabetic relatives with islet autoantibodies, only 10 also had transglutaminase antibodies.

CONCLUSIONS — We found a high prevalence of celiac autoimmunity in patients and first-degree relatives of children with type 1 diabetes, but we found limited overlap between islet and celiac autoimmunity in nondiabetic relatives. The high prevalence of celiac autoimmunity may be explained by shared genetic susceptibility and identifies a population within which screening for the disease may be justified.

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Abbreviations: EMA, anti-endomyosial antibody; ICA, islet cell antibody; IDS, Immunology in Diabetes Society; JDF, Juvenile Diabetes Foundation; tTG, tissue transglutaminase.

A table elsewhere in this issue shows conventional and Systeme International (SI) units and conversion factors for many substances.
degree of overlap between islet and celiac autoimmunity, thus providing an indication of the extent to which these diseases share a common etiology.

**RESEARCH DESIGN AND METHODS** — IgA antibodies to tTG were measured on the first available serum sample from the first 500 families recruited to the Bart’s Oxford family study, a prospective population-based study of probands and relatives of children diagnosed with type 1 diabetes before 21 years of age in the Oxford region of the U.K. (27). Samples were available from 433 probands diagnosed between 1985 and 1992 (median age 12.2 years, range 0.8–22.4, 248 male) and 1,442 nondiabetic relatives, including 410 fathers (median age 41 years, range 21.8–70.2), 461 mothers (median age 38.5 years, range 23.1–55.5), 273 brothers (median age 13.2 years, range 0–30.5), and 298 sisters (median age 13.1 years, range 0–26.2). A clinical history of autoimmunity was collected from each family on entry to the study and updated by fieldworker visits or postal questionnaire.

Sera collected from unselected schoolchildren (median age 11.3 years, range 9.0–13.8) also living in the Oxford region of the U.K. were used to define thresholds for each antibody.

**Assays**

**Strategy.** IgA antibodies to tTG were measured on all samples from probands and nondiabetic relatives. Those samples found to have tTG antibodies above the 97.5th percentile of 347 schoolchildren (median age 10.6 years, range 9.0–13.3) were assayed for IgA EMAs. ICA and antibodies to GAD, IA-2, and insulin had previously been measured on all samples (28). To investigate temporal changes in antibody levels, tTG antibodies were measured in the most recent sample, taken at least 2 years after initial testing from those relatives and probands with tTG antibodies above the 97.5th percentile of the schoolchildren. Those follow-up samples with persistently raised tTG antibodies were then tested for EMAs.

**Assay for transglutaminase antibodies.** IgA antibodies to tissue transglutaminase were measured using a radiobinding assay that has previously been described (15). Serum was incubated overnight with in vitro transcribed and translated human $^{35}$S-labeled human tissue transglutaminase C (received from Dr. E. Bonifacio), and IgA immune complexes were then precipitated with sheep anti-human IgA (Therapeutic Antibodies, Llandysul, Ceredigion, U.K.). After washing and adding scintillant (Microscint 40; Packard Instrument, Pangbourne, Berks, U.K.), the precipitates were counted in a scintillation counter (Topcount; Packard Instrument). Results were expressed in arbitrary units derived from a standard curve. The standards consisted of dilutions of serum taken from a patient with celiac disease with normal human serum to span the range from 0.4 units (1/512) to 100 units (1/2). The inter-assay coefficient of variation of the IgA-tTG antibody assay was 19% at 8 units and 28% at 51 units.

The threshold level for tTG antibodies (0.84 units) was defined by the 97.5th percentile of 347 schoolchild samples. In a previous study, 24 of 25 samples from adults and 11 of 11 children with untreated celiac disease were found to have antibody levels above this threshold (15). IgA EMAs. All samples with tTG antibodies above the 97.5th percentile of 347 schoolchildren were assayed for IgA EMAs using an indirect immunofluorescence technique on sections of monkey esophagus (The Binding Site, Birmingham, U.K.) or human umbilical cord, as previously described (7). Samples from 47 nondiabetic relatives and 26 probands with tTG antibody levels below the 97.5th percentile of the schoolchildren were assayed in parallel to act as negative controls. Our laboratory participates in the U.K. National External Quality Assurance Scheme quality assurance program.

**GAD and IA-2$_{ic}$ autoantibody assays.** All samples were assayed for autoantibodies to GAD and IA-2$_{ic}$ with radiobinding assays, as previously described (28), using in vitro transcribed and translated human $^{35}$S-GAD$_{65}$ or $^{35}$S-IA-2$_{ic}$ as label. Immune complexes were isolated with protein A sepharose (Amersham Pharma- cia Biotech, Little Chalfont, Bucks, U.K.). Samples assayed for GAD and IA-2$_{ic}$ antibodies were considered positive if they had levels above the 97.5th percentile of 2,860 schoolchildren (8). The ICA assay achieved 78% sensitivity with 98% specificity on the samples included in the First IDS Combined Antibody Workshop (29).

**Islet cell antibody assay.** Islet cell autoantibodies were measured by indirect immunofluorescence using sections of human pancreas, as previously described (9). End point titers of test samples were converted to Juvenile Diabetes Foundation (JDF) units by comparison with a standard curve in which log$_{2}$ JDF units were plotted versus log$_{2}$ of end point titer of the standard sera. Samples assayed for ICAs were considered positive if they had levels >4 JDF units, the 97.5th percentile of 2,860 schoolchildren (8). The ICA assay achieved 78% sensitivity with 98% specificity in the First IDS Combined Antibody Workshop (29).

**Statistical analyses**

Proportions were compared using the $\chi^2$ test. Tests were considered significant if the two-tailed $P$ value was <0.05.

**RESULTS**

**Clinical celiac disease**

Three mothers and one father gave a clinical history of celiac disease on entry to the study. None of these relatives had islet autoantibodies, and they were excluded from further analysis. Two probands and one mother, each of whom had transglutaminase antibodies greater than the 97.5th percentile of the schoolchildren before diagnosis (two with EMAs), gave a clinical history of celiac disease after entry into the study.
Subclinical autoimmunity

The distribution of antibodies to tTG is shown in Fig. 1.

**Proband**

Of 433 probands, 58 (13.4%) had tTG antibody levels above the 97.5th percentile of the schoolchildren. Of these, 29 (11.7%) were male (7 with EMAs) and 29 (15.7%) were female (4 with EMAs). Of 106 samples collected within 7 days of diagnosis of diabetes, 26 (25%) had raised antibodies to tTG (two with EMAs) compared with only 32 of 327 (9.8%) with diabetes of longer duration (median 230 days, range 44–1,541) ($P=0.0004$). Antibodies to tTG above the 97.5th percentile of the schoolchildren were found in 25 of 144 (17.4%) probands, 10 years of age compared with 33 of 289 (11.4%) older probands ($P=0.08$).

**Relatives**

In the absence of known celiac disease, 100 nondiabetic relatives (7.0%) had tTG antibody levels above the 97.5th percentile of the schoolchildren (28 fathers [6.8%], 41 mothers [9.0%], 17 brothers [6.2%], and 14 sisters [4.7%]). Of these relatives, 27 also had EMAs (6 fathers, 16 mothers, 1 brother, and 4 sisters), giving an overall prevalence of 1.9% for those with both tTG antibodies and EMAs.

None of the 26 samples from probands or 47 samples from nondiabetic relatives with tTG antibody levels below the 97.5th percentile of the schoolchildren were found to have EMAs.

**Follow-up samples**

Follow-up samples were available from 30 of the 58 probands (19 male) and 59 of the 100 relatives (19 male) with IgA tTG antibodies above the 97.5th percentile, in the absence of known disease. The median period of follow-up was 4.3 years (range 2.1–7.5) for the probands and 3.8 years (range 2.1–13.3) for the relatives. The changes in IgA tTG antibody levels during follow-up are shown in Fig. 2. Ten of 30 probands (5 male) and 30 of 59 relatives (9 male) had IgA tTG antibodies above the 97.5th percentile in the follow-up sample (Fig. 2), including 1 of the 2 probands and 16 of the 17 relatives who originally had EMAs. In the follow-up samples from relatives and probands with persistently raised IgA tTG antibodies, 13 relatives (2 male) and 1 male proband were found to have EMAs, including 1
mother who had been EMA-negative in the initial sample. The four relatives who lost EMA included a mother who had developed clinically overt celiac disease.

**Islet autoimmunity**

There was no association between gut and islet autoimmunity in relatives with no known disease. The distribution of islet and celiac autoimmunity in the nondiabetic relatives is shown in Table 1. A total of 100 nondiabetic relatives were positive for tTG antibodies. These included 10 of 186 (5.4%) relatives with islet autoantibodies and 90 of 1,252 (7.2%) relatives negative for islet antibodies ($P > 0.25$). Celiac autoimmunity did not segregate with multiple–islet antibody positivity, because coexisting antibodies to tTG were found in 8 of 160 relatives with one islet antibody, 1 of 12 relatives with two islet antibodies, 1 of 9 relatives with three islet antibodies, and 0 of 5 relatives with four antibodies. Similarly, islet autoantibody positivity was associated with neither EMAs nor persistent tTG antibodies. Of 30 relatives with persistent tTG antibodies, 4 (3 with EMAs) had islet autoantibodies, whereas 26 (13 with EMAs) did not ($P > 0.25$). Only 1 of 15 relatives with samples taken before the onset of diabetes (median 4.2 years, range 0–9.15) had both tTG antibodies and EMAs.

**CONCLUSIONS** — We found a high prevalence of IgA tTG antibodies in type 1 diabetic patients (13.5%) and their non-diabetic first-degree relatives (7.0%) in the absence of known clinical celiac disease, compared with 2.5% in schoolchildren from the same region and 2% in adult blood donors (15). IgA EMAs were found in addition to IgA tTG antibodies in 2.6% of the probands and 1.9% of the first-degree relatives compared with none of the schoolchildren and 1% of the blood donors. We did not perform biopsies to confirm the presence of abnormal gut mucosa, but others have found subclinical celiac disease in the majority of patients (16) and relatives (25–26) with EMAs. Our previous studies suggest that the sensitivity of IgA tTG antibodies is at least equivalent to that of IgA EMAs in biopsy-confirmed celiac disease and that IgA tTG antibodies measured by radioimmunoassay may persist longer or may be detectable earlier than the antibodies assayed by immunofluorescence (15). Persistent IgA tTG antibodies in the absence of EMAs may also indicate latent disease, although transient IgA tTG antibodies could reflect assay imprecision close to the threshold. In this study, 40 of 89 individuals tested on two occasions had persistent antibodies, and in one of these individuals, an increase in tTG antibody level in a second sample collected after 3 years was accompanied by the development of EMAs. These findings suggest that not only patients with type 1 diabetes but also their relatives could be considered suitable for active screening for celiac disease (24).

Our findings are consistent with those of other studies in which the prevalence of IgA tTG antibodies in different populations of patients with type 1 diabetes has been found to be between 3.6 and 11.6% (21–23). However, comparisons between studies are difficult because of differences in methodology and in the percentile used in the various control groups to set the thresholds that define antibody positivity. The high prevalence of tTG antibodies found at diagnosis of diabetes in our study may reflect the transient rise in serum IgA at clinical onset (31).

In this study, the prevalence of EMAs in children with type 1 diabetes is at the lower end of the 3–12% range previously reported (16,17,19–21,25). However, the overall prevalence of latent celiac disease in our study may be underestimated, because individuals with IgA deficiency would not be identified by our strategy. Individuals with EMAs but without IgA tTG antibodies would also have been missed by our two-stage screening strategy, although in a previous study, tTG antibodies were detected in all but 3 of 75 samples with EMAs (15). Two previous studies have investigated the prevalence of EMAs in first-degree relatives of children with type 1 diabetes. An Algerian study in a population with a high background prevalence of celiac disease reported EMAs in 5% of relatives of diabetic children (25), and a German prospective study in the offspring of type 1 diabetic parents found a 1.6% cumulative incidence of EMAs by 8 years of age following screening with IgG tTG antibodies (26).

A causal association between celiac disease and diabetes has been suggested (32–33), because diagnosis of diabetes precedes celiac disease in the majority of patients with both conditions (34–35). Consistent with this hypothesis, celiac disease was diagnosed after diabetes in two probands, whereas no nondiabetic siblings were known to have celiac disease. The association of celiac autoimmunity with diabetes is consistent with a shared genetic susceptibility and possibly with a breakdown in immune regulation.

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**Table 1** — The distribution of islet and celiac autoimmunity in the nondiabetic relatives without known celiac disease

<table>
<thead>
<tr>
<th>Islet antibody</th>
<th>$t\text{TG} &lt; 97.5$th percentile</th>
<th>$t\text{TG} \geq 97.5$th percentile</th>
<th>$t\text{TG} + \text{EMA}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1,162</td>
<td>90</td>
<td>24</td>
</tr>
<tr>
<td>Single marker</td>
<td>152</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>ICA</td>
<td>58</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>IA-2</td>
<td>14</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>GADA</td>
<td>54</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>IAA</td>
<td>26</td>
<td>3</td>
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</tr>
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<td>Two markers</td>
<td>11</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>ICA, IA-2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ICA, GADA</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ICA, IAA</td>
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<td>0</td>
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<tr>
<td>IA-2, GADA</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IA-2, IAA</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GADA, IAA</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Three markers</td>
<td>8</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ICA, IA-2, GADA</td>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ICA, IA-2, IAA</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ICA, GADA, IAA</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IA-2, GADA, IAA</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Four markers</td>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>1,338</td>
<td>100</td>
<td>27</td>
</tr>
</tbody>
</table>

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However, we did not find an association between TG antibodies and islet antibodies in the nondiabetic relatives, even in those relatives with multiple islet autoantibodies, who are at a particularly high risk of progression to diabetes (8). This suggests that celiac autoimmunity is not associated with an additional risk of progression to type 1 diabetes in this population and therefore does not support the concept of gluten as a common environmental trigger (32).

Using the two-step screening strategy described, we have found evidence of a high prevalence of subclinical celiac disease in patients and in first-degree relatives of type 1 diabetic children in the U.K. and a limited overlap between islet and celiac autoimmunity in nondiabetic relatives. Therefore, we conclude that the high prevalence of celiac autoimmunity in families of children with type 1 diabetes may be explained by shared genetic susceptibility, thus identifying a population in which screening for the disease may be justified if early intervention is proven beneficial.

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References
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