

Age at Development of Type 1 Diabetes- and Celiac Disease-Associated Antibodies and Clinical Disease in Genetically Susceptible Children Observed From Birth

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OBJECTIVE — To compare the ages and sequence in which antibodies associated with type 1 diabetes and celiac disease appear and overt diseases develop in children with an HLA-conferred susceptibility to both diseases.

RESEARCH DESIGN AND METHODS — We observed 2,052 children carrying genetic risks for both type 1 diabetes and celiac disease from birth until the median age of 5.7 years and analyzed diabetes- and celiac disease-associated antibodies in serum samples collected at 3- to 12-month intervals. Diabetes was confirmed by World Health Organization criteria and celiac disease by duodenal biopsies.

RESULTS — Altogether 342 children seroconverted to positivity for at least one diabetes-associated autoantibody and 88 to positivity for at least one celiac disease-associated antibody at the median ages of 3.0 and 1.5 years, respectively ($P < 0.001$). If only children with biochemically defined diabetes-associated autoantibodies against insulin, GAD, or IA-2A protein ($n = 146$) and children with tissue transglutaminase autoantibodies were compared ($n = 86$), the median seroconversion ages were 2.5 and 3.0 years ($P = 0.011$). Fifty-one children progressed to overt diabetes at 4.5 years and 44 children to celiac disease at 4.3 years ($P = 0.257$). Of the 19 children who developed both diabetes- and celiac disease-associated antibodies, 3 progressed to both diabetes and celiac disease.

CONCLUSIONS — Children with HLA-conferred susceptibility to type 1 diabetes and celiac disease develop celiac disease-associated antibodies mostly at a younger age or the same age at which they develop diabetes-associated autoantibodies. Clinical diabetes and celiac disease are commonly diagnosed at the same median age.

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The incidences of type 1 diabetes and celiac disease are increasing rapidly (1). These autoimmune diseases often occur together, as ~4.5% of subjects with recent-onset type 1 diabetes also have celiac disease, and the coexistence is even more common in subjects with long-standing type 1 diabetes (2,3). Shared

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susceptibility alleles in the HLA region probably contribute to this coexistence (4). Although appearance of diabetes- and celiac disease-specific antibodies strongly indicates commencement of autoimmunity (5), antibodies also predict progression to the respective clinical diseases. However, in the case of diabetes, in particular, the time from autoimmunity to overt disease may vary from months to years. Interestingly, clinical type 1 diabetes is usually diagnosed first and celiac disease within the following few years (6,7). The order is rarely reversed (8).

Although coexistence of type 1 diabetes and celiac disease has been studied mainly in clinical patients, Williams et al. (9) showed in a cross-sectional study that 5.4% of nondiabetic first-degree relatives of type 1 diabetic patients who were positive for diabetes-associated autoantibodies were positive also for tissue transglutaminase autoantibody (TGA). However, the findings of the Diabetes Autoimmunity Study in the Young (DAISY) indicated that the two types of antibodies rarely appeared simultaneously (10), whereas the German BabyDiab study suggested that celiac disease-associated antibodies invariably develop later than diabetes-associated autoantibodies (11,12).

Here we report the age and order in which the diabetes- and celiac disease-associated antibodies and the two clinical diseases developed in children who carried genetic type 1 diabetes and celiac disease susceptibility and participated in the type 1 Diabetes Prediction and Prevention (DIPP) study.

RESEARCH DESIGN AND METHODS

All study children were participants in the ongoing population-based DIPP study, which is a survey of the natural course of preclinical type 1 diabetes in genetically susceptible individuals born in the cities of Turku, Oulu, and Tampere in Finland (13). After parental consent, the newborns carrying HLA-DQB1 genotypes conferring susceptibility to type 1 diabetes (*02/*0302; *0302/x

[$x \neq *02, *0301, *0602, \text{ or } *0603$] and male infants in Turku with HLA-DQB1*02/x) (13) were observed from birth for the appearance of diabetes-associated antibodies. The at-risk children in Turku were examined at 3-month intervals until 2 years of age and then at 6-month intervals. In Oulu and Tampere, the children were examined at 3, 6, 12, 18, and 24 months and then at 12-month intervals. At every examination, a blood sample was drawn, processed, and stored as described (13).

For this study, we chose an 11-year cohort of DIPP study children carrying HLA alleles DQB1*02/DQB1*0302 or DQA1*05-DQB1*02 and born between November 1994 and December 2005. The HLA alleles were determined from cord blood spots dried on filter paper using a semiautomated technique (14). To document when diabetes autoimmunity began, islet cell autoantibody (ICA) was first measured from every blood sample drawn. If the sample was ICA-positive, autoantibodies against biochemically characterized autoantigens (insulin [IAA], GAD [GADA], and protein tyrosine phosphatase-related IA-2 protein [IA-2A]) were also analyzed in all samples drawn from that child since birth. To evaluate which proportion of children remaining ICA-negative but developing other diabetes-associated autoantibodies were missed when ICA alone was measured, we analyzed the four diabetes-associated autoantibodies in all samples drawn from a time-restricted cohort of 1,006 DIPP study children (15), in all samples drawn from children born to autoantibody-positive mothers, and in all samples drawn from children born on or after 1 January 2003. All children who developed diabetes-associated autoantibodies were permanently observed at 3-month intervals (13).

We used IgA-class TGA as the primary marker of celiac disease autoimmunity. We first measured TGA in the samples drawn during the year 2000 from all children with HLA-conferred diabetes and celiac disease susceptibility. The age of the oldest child in the year 2000 screening was 5.2 years. Later, TGA was first analyzed at the age of 1 year in the children with genetic celiac disease susceptibility. Taking into account all samples analyzed, the first assessment of TGA was made from samples collected at a median age (range) of 1.8 years (0.1–9.1). The children were retested annually until the end of March 2007, when the oldest

children were 12.3 years. When a child became TGA positive, antibodies against endomysium (EMA), reticulon (ARA), and gliadin (AGA-IgA and AGA-IgG) were also analyzed in all of the child's previous and forthcoming samples (5). TGA was also measured in the last available samples from the six children who withdrew from the follow-up before the year 2000. The age at seroconversion to antibody positivity was defined as the age when the first positive blood sample was drawn, irrespective of the slight variation in the intervals between the last autoantibody-negative and the first autoantibody-positive sample.

The diagnosis of type 1 diabetes was based on the World Health Organization criteria (16). Duodenal biopsies were recommended for all TGA-positive children. If biopsies showed villous atrophy, celiac disease was diagnosed and a gluten-free diet was recommended.

The ethics committees of the participating university hospitals approved the study. Written informed consent was obtained from the parents for autoantibody analysis and intestinal biopsies. The child's consent was also requested for children aged >7 years.

Type 1 diabetes-associated antibodies and celiac disease-associated antibodies

ICA was quantified using a standard indirect immunofluorescence method, and biochemical autoantibodies (IAA, GADA, and IA-2A) were quantified using radio-binding assays (17). TGA was measured with a recombinant human TGA kit (Celikey; Pharmacia Diagnostics, Freiburg, Germany) (18). EMA and ARA were determined by indirect immunofluorescence (19,20). Serum AGA-IgG and AGA-IgA were analyzed by an enzyme-linked immunosorbent assay (ELISA) (21).

Serum IgA was analyzed when a child's antibodies were measured for the first time. If the concentration was <0.05 g/l, IgG class TGA and AGA were analyzed by ELISA (21).

Positivity for diabetes-associated antibodies and celiac disease-associated antibodies

We measured ICA in all samples, and if the result was positive, we also analyzed IAA, GADA, and IA-2A in all previous and forthcoming samples for that child. We defined the age at seroconversion to positivity for diabetes-associated autoantibodies as the age when any of the

autoantibodies was positive for the first time and positivity for a biochemically defined diabetes-associated autoantibody as the age when the first of at least two consecutive samples positive for IAA, GADA, or IA-2A was drawn.

Because TGA was used for the primary screening of celiac disease autoimmunity, all children with celiac disease-associated autoantibodies were positive in at least one sample for TGA. The age at seroconversion to positivity for celiac disease-associated antibodies (TGA, AGA-IgA, AGA-IgG, EMA, or ARA) was defined as the age when the first one of these antibodies was positive, alone or in any combination. For IgA-deficient children, AGA-IgG and TGA-IgG were measured to determine the age at seroconversion.

Statistical analysis

We used Wilcoxon's test to compare differences between the median seroconversion ages and Cox's regression analysis to compare the development of diabetes-associated and celiac disease-associated antibodies. Tests were considered significant if two-sided $P < 0.05$. The statistical analyses were performed using SAS (version 9.2; SAS Institute, Cary, NC).

RESULTS

Children positive for diabetes-associated or celiac disease-associated antibodies

We first analyzed HLA-conferred type 1 diabetes susceptibility in 100,846 consecutive newborns (Fig. 1) and formed the cohort of this study by selecting from the diabetes-susceptible infants those 2,052 who also were at high genetic risk for celiac disease. The median age of the cohort children at the end of this study was 5.7 years (range 1.0–12.3 years). Boys made up 65% ($n = 1,332$) of the cohort because of the inclusion criteria of the DIPP study (13).

At least one sample in 342 children was positive for ICA (Fig. 1), whereas 146 children tested positive for at least one biochemical diabetes-associated autoantibody in at least two consecutive samples. These children included 19 who were continuously ICA-negative. Altogether 215 children were positive only for ICA, and 17 were positive in only one sample. At least one sample in 86 children was positive for TGA, and 2 children with IgA deficiency were positive for AGA-IgG. Consequently, 88 children were regarded

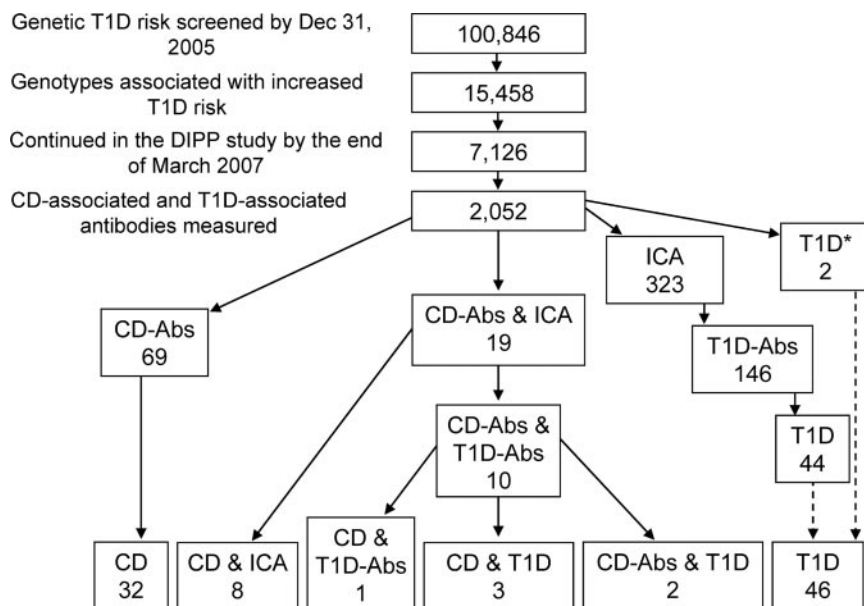


Figure 1—Flow chart of the children in the DIPP study. CD-Abs, at least one sample positive for TGA (IgA or IgG) and/or AGA-IgA, AGA-IgG, EMA, or ARA; ICA, at least one sample positive for ICA, or ICA and IAA, GADA, and/or IA-2A; T1D-Abs, two consecutive samples positive for IAA, GADA, and/or IA-2A. *Two children were negative for type 1 diabetes (T1D)-associated autoantibodies in the last follow-up sample drawn, but were positive in a sample drawn at the time of type 1 diabetes diagnosis.

as positive for celiac disease-associated antibodies.

Seroconversion to positivity for the first-appearing celiac disease-associated antibody occurred at the median age of 1.5 years (range 0.5–7.5), i.e., at a markedly younger age than the seroconversion to positivity for the first diabetes-associated autoantibody (3.0 years, range 0.4–11.1; $P < 0.001$) (Fig. 2). If only persisting biochemical diabetes-associated autoantibodies were accepted for analysis, the children seroconverted at a median age of 2.5 years (0.5–10.1), also clearly later than the children seroconverted to celiac disease-associated antibody positivity ($P = 0.007$). Restricting celiac disease-associated antibodies to TGA, the children seroconverted at a median age of 3.0 years (1.0–9.0), i.e., at the same age as the children seroconverted to positivity for diabetes-associated autoantibodies but later than they seroconverted to positivity for the first biochemically defined diabetes-associated autoantibody ($P = 0.011$) (Table 1). If we omit AGA-IgG and AGA-IgA from the analysis, the median age at seroconversion to positivity for TGA, EMA, or ARA was 2.5 years.

Of the 86 TGA-positive children, 66 children seroconverted first to AGA-IgG positivity. In 40 of them, the first sample positive for a celiac disease-associated autoantibody was for AGA-IgG alone. The

time from seroconversion to AGA-IgG positivity to positivity for another celiac disease-associated antibody (TGA, EMA, ARA, or AGA-IgA) was 0.8 years (range 0.2–6.1).

Interestingly, among the 86 children who seroconverted to TGA positivity, 39 (45%) reverted spontaneously to negativity, or the values fluctuated at least twice from negativity to positivity without dietary intervention. Although 23 children were TGA-positive in only one sample, all except one showed positivity in the same sample for AGA-IgG, AGA-IgA, EMA, and/or ARA. Antibody transience and fluctuation were common also among AGA-IgA (24 of 50 [48%]), AGA-IgG (22 of 78 [28%]), EMA (29 of 85 [34%]), and ARA (26 of 74 [35%]).

During the follow-up, 51 of the 342 children positive for diabetes-associated autoantibodies progressed to overt diabetes, and 43 of the 88 children positive for celiac disease-associated antibodies progressed to biopsy-proven celiac disease. One additional child developed skin biopsy-confirmed dermatitis herpetiformis at age 4.3 years. Although celiac disease-associated antibodies developed, on average, at a slightly younger age than the diabetes-associated autoantibodies, overt diabetes and celiac disease were diagnosed at the ages of 4.5 years (range 1.4–11.6) and 4.3 years (1.6–9.8),

respectively ($P = 0.257$). Twenty-five children lost TGA spontaneously before endoscopy, the parents of three children refused the procedure, and four children were on the endoscopy waiting list at the end of the follow-up.

Children with both diabetes-associated antibodies and celiac disease-associated antibodies

Nineteen (5.6%) children developed both diabetes-associated and celiac disease-associated antibodies. Eight children seroconverted first to positivity for diabetes-associated autoantibodies, eight first to positivity for celiac disease-associated antibodies, and three to positivity for both types of antibodies. The median ages of the 19 children who seroconverted to positivity for the first diabetes-associated autoantibody and the first celiac disease-associated antibody were 1.6 years (range 0.5–6.5) and 1.5 years (0.8–7.2), respectively. Accordingly, the children who seroconverted to positivity for both diabetes- and celiac disease-associated antibodies seroconverted at a younger age than those who were positive only for diabetes-associated antibodies (1.6 vs. 3.0 years; $P = 0.026$).

Of the 19 children with both diabetes-associated and celiac disease-associated antibodies, 10 were positive for the biochemical autoantibodies. Four of them developed first IAA, GADA, or IA-2A; four developed celiac disease-associated antibodies; and two developed both types of antibodies at the same age. The children seroconverted to positivity for biochemical autoantibodies and celiac disease-associated antibodies at the median ages of 1.4 years (range 0.5–8.9) and 1.4 years (0.8–2.6), respectively. The 3 children who progressed to clinical type 1 diabetes and celiac disease during the follow-up developed autoantibodies and overt diseases in random order (supplemental Fig. 3, available in an online appendix at <http://care.diabetesjournals.org/cgi/content/full/dc09-1217/DC1>).

CONCLUSIONS— Our findings suggest that celiac disease-associated antibodies often develop earlier than or at the same age as diabetes-associated autoantibodies in children with an HLA-conferred risk for both diseases, assuming that the children are followed up from birth at frequent intervals. The children who developed both diabetes- and celiac disease-associated antibodies generated

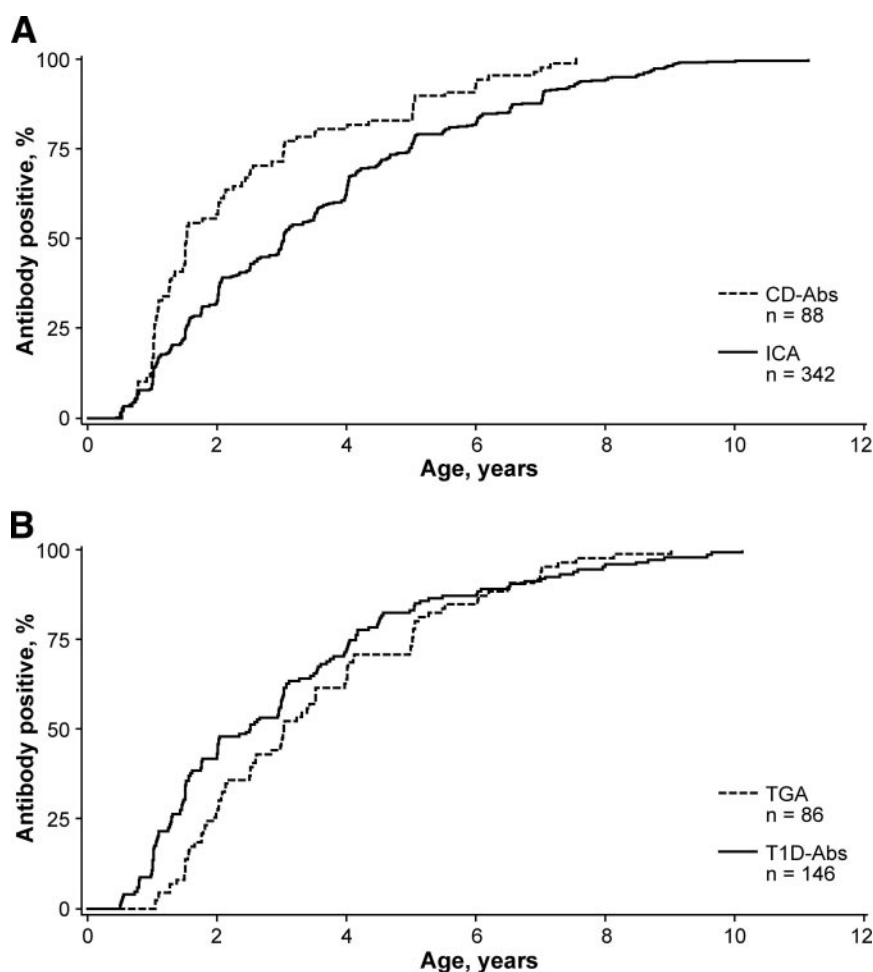


Figure 2—Cumulative seroconversion to positivity for type 1 diabetes (T1D)- and celiac disease (CD)-associated antibodies in the DIPP study children with HLA-conferred genetic type 1 diabetes and celiac disease susceptibility. A: Cumulative seroconversion to positivity for diabetes-associated and celiac disease-associated antibodies ($P < 0.001$). B: Cumulative seroconversion to positivity for one or more of the persisting biochemical diabetes-associated autoantibodies and to positivity for TGA ($P = 0.011$). TGA, at least one sample positive for TGA. For other abbreviations, see Fig. 1.

the two types of antibodies usually in a random order within a short time interval. Interestingly, the children with celiac disease-associated antibodies seroconverted to positivity for diabetes-associated autoantibodies earlier than those who did not have celiac disease-associated antibodies. However, overt diabetes and celiac disease were ultimately diagnosed at approximately the same age.

Our results differ clearly from those reported in the BabyDiab study, in which diabetes-associated autoantibodies developed earlier than celiac disease-associated antibodies (12). However, if the analysis was based only on IAA, GADA, or IA-2A and only on TGA, seroconversion to TGA positivity occurred in our DIPP study cohort at 3.0 years and seroconversion to positivity for the bio-

chemical diabetes-associated autoantibodies at 2.5 years. Meanwhile, in the BabyDiab study, the respective seroconversions were seen at 4.9 and 2.3 years. These differences between the two studies are probably explained mainly by the more frequent follow-up in the DIPP study, which permitted more accurate determination of the seroconversion time. Indeed, in the BabyDiab study the samples are collected from antibody-negative children at the ages of 9 months and 2, 5, 8, 11, and 14 years. None of our DIPP study children seroconverted to TGA positivity before age 1 year, although gluten-containing foods were almost without exception introduced between 4 and 6 months of age. However, 23 of the 86 TGA-positive children seroconverted to TGA positivity between 1 and 2 years of age, i.e., during the period when the sam-

ples in the DIPP study were still collected at 3- to 6-month intervals.

When we measured all diabetes- and celiac disease-associated antibodies to determine the median ages of commencement of the two autoimmune diseases in the DIPP study cohort, biochemical diabetes-associated autoantibodies developed slightly later than celiac disease-associated antibodies (2.5 vs. 1.5 years). In the BabyDiab study, celiac disease autoimmunity was defined as being positive for TGA in at least one sample, but the positivity had to be confirmed using both an ELISA and a radiobinding assay. In the DIPP study, we also defined celiac disease autoimmunity as being positive for TGA with an ELISA in at least one sample; this was obviously appropriate, because all children except one were simultaneously positive also for AGA-IgA, AGA-IgG, EMA, and/or ARA. In the BabyDiab study, the prevalence of celiac disease-associated antibody positivity among the children positive also for diabetes-associated autoantibodies was 3 of 107 (2.8%). All 3 of these children first developed diabetes-associated autoantibodies. In our DIPP study cohort, 10 of 146 (6.8%) of the children were positive for both types of antibodies, closely resembling British data (5.4%), but markedly exceeding the values obtained in the BabyDiab study (9,12). Differences in the study populations may have influenced these results, because in the BabyDiab study, all children had at least one parent with type 1 diabetes, whereas in the DIPP study, the children were selected from the general population based on HLA-conferred genetic risk. Unfortunately, comparison of our findings with those from DAISY, another long-term observational study, is hampered by the fact that the coexistence of diabetes-associated and celiac disease-associated antibodies has so far not been thoroughly presented.

Gluten has been proposed to be a trigger of not only celiac disease but also type 1 diabetes. If that is the case, avoidance of gluten ingestion might delay progression to type 1 diabetes (22,23). Our frequent follow-up of the children with a genetic susceptibility to celiac disease and diabetes indicates that celiac disease-associated autoimmunity often develops at a slightly younger age than diabetes-associated autoimmunity, supporting the hypothesis that a gluten-free diet might indeed prevent or delay diabetes autoimmunity. Interestingly, diabetes-associated autoantibodies developed at a younger

Table 1—Median age at seroconversion to positivity for type 1 diabetes–associated and celiac disease–associated antibodies

First seroconversion to positivity for any antibody of the group	n	Age (years)
Celiac disease–associated antibodies: TGA, AGA-IgA, AGA-IgG, EMA, or ARA	88	1.5 (0.5–7.5)
Celiac disease–associated antibodies without AGA: TGA, EMA, or ARA	86	2.5 (1.0–9.0)
Diabetes-associated autoantibodies: ICA, IAA, GADA, or IA-2A	342	3.0 (0.4–11.1)
Biochemical diabetes-associated autoantibodies: IAA, GADA, or IA-2A	146	2.5 (0.5–10.0)
First seroconversion to positivity for each antibody alone		
TGA	86	3.0 (1.0–9.0)
AGA-IgA	50	3.0 (0.6–10.0)
AGA-IgG	78	1.5 (0.5–11.5)
ARA	74	3.0 (1.0–10.5)
EMA	85	3.0 (1.0–9.0)
ICA	342	3.2 (0.4–11.1)
IAA	110	2.0 (0.5–10.3)
GADA	107	3.0 (0.7–10.1)
IA-2A	90	3.0 (0.5–9.8)

Data are n or median (range).

age in children who were positive for both diabetes- and celiac disease–associated antibodies than in children who were positive for diabetes-associated autoantibodies only.

We included in this study all children who developed diabetes-associated and celiac disease–associated antibodies during the follow-up using overt clinical type 1 diabetes as the end point of the study (24). Thus, we have data only until the diagnosis of diabetes from the children who progressed to diabetes, and some children may have developed celiac disease after the diagnosis of diabetes. However, the purpose of this study was to evaluate the early phases of diabetes and celiac disease autoimmunity, not to challenge previous studies analyzing the risk of celiac disease once type 1 diabetes has developed (2,3,6–8).

Our study has some obvious limitations. One is caused by the use of ICA as the primary autoantibody for diabetes autoimmunity screening in children born in 1994–2002, although we also analyzed the three biochemical autoantibodies in all samples drawn from the ICA-positive children. In children born between the beginning of 2003 and the end of the study, all four diabetes-associated autoantibodies were directly analyzed in all samples collected. Our recent study showed that >82% of the samples that

were positive for the first autoantibody were positive for ICA, whereas ICA or IAA accounted for 93% of the positive samples. The sensitivity of our current program when tested using ICA as the marker of commencement of diabetes-associated autoimmunity is 86% (24), and <10% of children with autoantibodies are missed if ICA only is analyzed. We have also shown that 95% of all IAA, GADA, and IA-2A seroconversions occur in young children in a narrow time window (–12 to 8 months) around the time of ICA seroconversion (17). Of the 2,052 children in this cohort, 19 were positive for biochemical diabetes-associated autoantibodies while being continuously ICA negative. Consequently, we probably missed a few children who were ICA-negative but had other diabetes-associated autoantibodies. Defining diabetes autoimmunity as positivity for the biochemical autoantibodies does not eliminate the problems associated with the use of ICA, but it excludes from the study population the children who are positive for ICA only. In any case, defining onset of diabetes-associated autoimmunity by seroconversion to persisting positivity for at least one of the biochemical autoantibodies allows better comparison of our data with those obtained in the BabyDiab study.

Another limitation in our DIPP cohort study is that we used AGA-IgG as one of

the celiac disease–associated antibodies. AGA is highly unspecific, and ~25% of the TGA-negative children with genetic celiac disease susceptibility were AGA-IgG–positive in the DIPP study (data not shown). However, we think that analyzing AGA-IgG together with other celiac disease–associated antibodies is justified, because seroconversion to AGA-IgG positivity may identify the time when the celiac disease process really begins in children who also develop other celiac disease–associated antibodies. AGA-IgG may also be important when celiac disease triggers other than gluten are searched for. We previously also measured antibodies to the deamidated gliadin peptide in part of the currently studied children (25); the findings showed high concordance with the AGA and TGA results.

The follow-up schedules of the children at the three study centers in Finland differed slightly, as the children were seen more frequently in Turku than in Oulu and Tampere (17). Analysis of the data at the three sites slightly postponed the seroconversion ages in Oulu and Tampere for celiac disease–associated antibodies but not for diabetes-associated autoantibodies for unknown reasons (data not shown). However, these changes in the seroconversion ages are minor and do not change the conclusions of our study. On the other hand, the frequent follow-up is also a major strength of our study, permitting rather accurate determination of events contributing to progression to overt clinical diabetes and celiac disease; even the less frequent follow-up schedule is three times more frequent than the follow-up in the older groups in the BabyDiab study.

In summary, this population-based, prospective follow-up study suggests that celiac disease–associated antibodies often develop earlier than or at the same age as type 1 diabetes–associated autoantibodies in children with a genetic susceptibility to both diseases.

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No other potential conflicts of interest relevant to this article were reported.

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