Age, HLA, and Sex Define a Marked Risk of Organ-Specific Autoimmunity in First-Degree Relatives of Patients With Type 1 Diabetes

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OBJECTIVE

Autoimmune diseases can be diagnosed early through the detection of autoantibodies. The aim of this study was to determine the risk of organ-specific autoimmunity in individuals with a family history of type 1 diabetes.

RESEARCH DESIGN AND METHODS

The study cohort included 2,441 first-degree relatives of patients with type 1 diabetes who were prospectively followed from birth to a maximum of 29.4 years (median 13.2 years). All were tested regularly for the development of autoantibodies associated with type 1 diabetes (islet), celiac disease (transglutaminase), or thyroid autoimmunity (thyroid peroxidase). The outcome was defined as an autoantibody-positive status on two consecutive samples.

RESULTS

In total, 394 relatives developed one (n = 353) or more (n = 41) of the three disease-associated autoantibodies during follow-up. The risk by age 20 years was 8.0% (95% CI 6.8–9.2%) for islet autoantibodies, 6.3% (5.1–7.5%) for transglutaminase autoantibodies, 10.7% (8.9–12.5%) for thyroid peroxidase autoantibodies, and 21.5% (19.5–23.5%) for any of these autoantibodies. Each of the three disease-associated autoantibodies was defined by distinct HLA, sex, genetic, and age profiles. The risk of developing any of these autoantibodies was 56.5% (40.8–72.2%) in relatives with HLA DR3/DR3 and 44.4% (36.6–52.2%) in relatives with HLA DR3/DR4-DQ8.

CONCLUSIONS

Relatives of patients with type 1 diabetes have a very high risk of organ-specific autoimmunity. Appropriate counseling and genetic and autoantibody testing for multiple autoimmune diseases may be warranted for relatives of patients with type 1 diabetes.

Prospective studies that follow children from birth have established that autoimmunity can start very early in life and years before clinical symptoms appear (1–3). In type 1 diabetes, autoimmunity often appears between 6 months and 3 years of age (1–3), and the large majority of children who develop autoantibodies against multiple pancreatic islet antigens at this age progress to clinical disease (4). New disease paradigms have emerged from such studies, leading to extensive screening (5), the

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investigation of potential causes of the diseases (6), and consortia that attempt to prevent disease in asymptomatic individuals (7).

The BABYDIAB and subsequent BABYDIET studies were the first of these prospective from-birth studies of type 1 diabetes (8,9). These studies have been following newborn children who have a first-degree relative with type 1 diabetes on the basis that they have an elevated risk of developing the disease. Over the course of follow-up, the children were also observed to be at risk for developing other autoantibodies (10,11), similar to findings in patients with type 1 diabetes (12–15) and in their family members (16–18). The participants in these studies are now approaching 30 years of age and provide a unique opportunity to track the course of autoimmunity from birth to adulthood. The aim of the current study was to determine the risk of organ-specific autoimmunity in individuals with a family history of type 1 diabetes. Our findings demonstrate distinct age, sex, and genetic profiles for the autoantibodies associated with the three diseases examined, which occur with surprisingly high frequency in first-degree relatives of patients with type 1 diabetes. Our findings are relevant to the causes of autoimmunity and provide a strong rationale and practical guidelines for autoantibody testing in members of families affected by type 1 diabetes.

RESEARCH DESIGN AND METHODS

Study Population

Data from two German birth cohorts of individuals with a first-degree family history of type 1 diabetes born between 1989 and 2000 (BABYDIAB) or between 2000 and 2006 (BABYDIET) were combined and analyzed (8,9). Both studies prospectively examined the natural history of islet autoimmunity and type 1 diabetes, celiac disease–associated autoimmunity, and thyroid autoimmunity. The BABYDIAB study recruited 1,650 infants born to a mother or father with type 1 diabetes, and the BABYDIET study recruited 791 infants who had a mother, father, or sibling with type 1 diabetes. A subgroup of 150 children participated in the BABYDIET gluten intervention study (Clinical trial reg. no. NCT01115621, clinicaltrials.gov) to investigate whether delayed exposure to gluten reduces the risk of developing autoantibodies. That intervention failed to show any effect on autoantibody development, and all the participants continued with follow-up examinations according to the natural history protocol (8). The studies were approved by the ethics committees of Bavaria, Germany (Bayerische Landesärztekammer no. 95357 and Ludwig-Maximilians University no. 329/00, respectively) and were performed in accordance with the principles of the Declaration of Helsinki, including the provision of written informed consent from all participants or their parents.

Children were scheduled for follow-up and venous blood collection at ages 9 months, 2 years, and every 3 years thereafter, and for the 150 children who participated in the dietary intervention, every 3 months until 3 years of age and yearly thereafter. The median follow-up period from birth to the last sample was 13.2 years (interquartile range [IQR] 7.4–18.2 years) and from birth to the last contact was 16.1 years (11.2–20.0 years).

Autoantibody Measurements

The type 1 diabetes–associated islet autoantibodies (IAs) against insulin (IAA), GAD (GADA), IA2 (IA-2A), and zinc transporter 8 were measured at the Helmholtz Center, Munich, Germany, in venous blood samples obtained at all completed visits. The celiac disease–associated IgA autoantibodies against tissue transglutaminase 2 (TGA) and the thyroid autoimmune disease–associated autoantibodies against thyroid peroxidase (TPOA) were not measured in 46 and 30 children, respectively, because of insufficient sample volume. Measurements were made with radiobinding assays (10,11,19,20). The IA assays (laboratory 121) were evaluated in the Diabetes Autoantibody Standardization Program, which included other assays that gave similar performances (21–23). The genetic typing at the HLA-*DRB1*, HLA-*DQA1*, and HLA-*DQB1* loci and at single nucleotide polymorphisms (SNPs) rs689 (*INS* gene), rs2476601 (*PTPN22* gene), rs1990760 (*IFIH1* gene), and rs3184504 (*SH2B3* gene) was performed in 2,290 children (DNA sample was unavailable in the remainder) and has been described elsewhere (24,25).

Outcome Definition

IA positivity was defined as the development of persistent IAA, GADA, IA-2A, or zinc transporter 8, with sample values >99th percentile of control children. Persistence was defined as autoantibody positivity in at least two consecutive samples or in the last sample if the participant developed diabetes before providing a follow-up sample. The age at the first IA-positive sample was considered the age of seroconversion. The children were classified as multiple-IA positive if in addition to persistent IA positivity they tested positive for more than one of the IAs on at least one occasion. In children who were positive for GADA in the absence of other IAs, GADA was also assessed for its GAD-binding affinity (19) and positivity on an ELISA (Medizym anti-GAD ELISA Kit; MEDIPAN, Berlin, Germany). These children were classified as single-GADA positive if they had GADA with affinity $\geq 10^9$ L/mol or tested positive on the radiobinding assay and ELISA measurements (21). In children who were positive for IAA in the absence of other IAs, IAAs were assessed for their insulin-binding affinity and were classified as single-IAA positive if they had IAA with affinity $\geq 10^9$ L/mol (19). TGA positivity was defined as the development of persistent antibodies above a threshold representing the 99th percentile of control children (10,11). TPOA positivity was defined as the development of persistent antibodies above a threshold of 50 units/mL, as defined with a quantile-quantile plot analysis (11). For TPOA and TGA, persistence was defined as a positive result in two consecutive samples or autoantibody levels of >10 units/mL (TGA) or >100 units/mL (TPOA) in the last measured sample if a second sample was unavailable for testing.

Type 1 diabetes was diagnosed according to the criteria of the American Diabetes Association Expert Committee (26). Families were asked to report the occurrence of diabetes symptoms. In children with IAs, an annual oral glucose tolerance test was performed. Type 1 diabetes onset was defined as unequivocal hyperglycemia with acute metabolic decompensation, the observation on at least two occasions of a 2-h plasma glucose >200 mg/dL after an oral glucose test, or a random blood glucose concentration >200 mg/dL accompanied

by unequivocal symptoms. In 1997, fasting blood glucose >126 mg/dL on two occasions was added to the diabetes diagnosis criteria.

Families of children who were no longer in follow-up or refused to provide blood samples or perform oral glucose tolerance tests were regularly contacted by telephone to ask whether the child had developed diabetes. In cases of loss to follow-up, local diabetes registries or cohort studies were used as a second source of information on the development of type 1 diabetes in the study participants.

Table 1—Description of the study population

Characteristic	Frequency or duration
Girls, *n*/total (%)	1,188/2,441 (48.7)
Follow-up time, median (IQR)	13.2 (7.4–18.2)
Patients with type 1 diabetes, *n*/total (%)	115/2,441 (4.7)
Age at type 1 diabetes onset, median (IQR)	9.4 (5.0–12.7)
IA, TPOA, or TGA positive, *n*	394
IA positive, *n*/total (%)	165/2,441 (6.8)
TGA positive, *n*/total (%)	117/2,395 (4.9)
TPOA positive, *n*/total (%)	154/2,411 (6.4)
HLA DR3-DQ8/DR4-DQ8, *n*/total (%)	178/2,290 (7.8)
HLA DR4-DQ8/DR4-DQ8, *n*/total (%)	91/2,290 (4.0)
HLA DR3/DR3, *n*/total (%)	72/2,290 (3.1)

Statistical Analysis

Children in the BABYDIAB and BABYDIET cohorts were analyzed together. The cumulative dropout rate and the cumulative risks of developing autoantibodies (IA, TGA, or TPOA), multiple IAs, or type 1 diabetes were estimated with the Kaplan-Meier method. The log-rank test was used to compare categories in the Kaplan-Meier analysis. Follow-up was calculated from birth to the age of diagnosis of diabetes, to the age when autoimmunity first developed, or to the last contact or sample. Within IA-positive children, the Kaplan-Meier analysis was used to calculate the risk of progression to type 1 diabetes. Cox proportional hazard models were used to determine the multivariable hazard ratios (HRs) with 95% CIs for autoantibody risks and to determine the effect of the interaction between sex and HLA genotype on the risk of autoantibodies. Relatives with missing data were not included in the models.

In accordance with the timing of the visit schedule, the incidence of antibodies per 1,000 person-years was calculated from samples obtained within the following age intervals: 0–18 months, 18–42 months, 42–78 months, 78–114 months, 114–150 months, 150–186 months, and 186–204 months (1). The incidence of type 1 diabetes was calculated from the number of children who were newly diagnosed with type 1 diabetes and the number of children followed in each time interval as cases per 1,000 per year (per 1,000 person-years) for each time interval. SEs were calculated using the Cooper-Pearson method. Comparisons of IA incidence between age-groups were performed using Fisher exact test, and the age at autoantibody development was compared among IA, TGA, and TPOA using the Mann-Whitney *U* test. All statistical analyses were performed with SPSS version 25.0 (IBM Corporation, Armonk, NY) and SAS 9.4 (SAS Institute, Cary, NC) software.

RESULTS

In total, 2,441 children (1,188 girls) were enrolled in the prospective BABYDIAB and BABYDIET studies and tested for IA (*n* = 2,441), TGA (*n* = 2,395, 1,166 girls), and TPOA (*n* = 2,411, 1,172 girls) at scheduled visits. Of these children, 394 developed one or more autoantibodies (Table 1 and Supplementary Fig. 1), including 165 (6.8%) who seroconverted to IA positivity (76; 46.1% girls), 117 (4.9%) to TGA positivity (72; 61.5% girls), and 154 (6.4%) to TPOA positivity (107; 69.5% girls). This included 41 children (29 girls) who developed autoantibodies associated with multiple diseases (6 with IA and TGA, 23 with IA and TPOA; 11 with TGA and TPOA; and 1 with IA, TGA, and TPOA).

Of the 165 children with IA, 128 (77.6%) developed multiple IAs (23 developed two IAs, 47 developed three IAs, and 58 developed four IAs), and 37 children (22.4%) developed a single IA (Supplementary Fig. 1). Diabetes was diagnosed in 115 children (4.7%, 51 girls), including 10 who progressed to diabetes without an IA-positive sample before the onset of diabetes. Six children who developed diabetes had TGA (all were IA positive), and 13 had TPOA, including 2 of the 10 children without prior autoantibodies. The cumulative rate of withdrawal or loss to follow-up by age 20 years was 38.0% (95% CI, 35.8–40.2%).

Risk of Developing Islet, Celiac Disease, or Thyroid Autoimmunity

By 20 years of age, the IA risk was 8.0% (95% CI, 6.8–9.2%), the TGA risk was 6.3% (5.1–7.5%), and the TPOA risk was 10.7% (8.9–12.5%) (Fig. 1*A–C*). The risk of developing any of these autoantibodies was 21.5% by 20 years of age (19.5–23.5%) (Fig. 1*D*).

Age of Seroconversion, HLA, and Sex Distinctly Define the Risk of Each Autoimmunity

Age was the most disparate feature among the three disease-associated autoantibodies (Fig. 1*E*). The incidence of IA seroconversion was highest at the ages of 9 months (incidence 16.4 per 1,000 person-years, SE ± 2.8) and 2 years (18 per 1,000 person-years, SE ± 2.6) and decreased thereafter ($P < 0.0001$ incidence in first 2 years vs. later). The peak incidence of seroconversion to TGA occurred at an age of 2 years (13.9 per 1,000 person-years, SE ± 2.3), and the highest incidence of seroconversion to TPOA occurred broadly between the ages of 11 and 17 years (peak incidence at 14 years: 10.4 per 1,000 person-years, SE ± 1.8). The median age of seroconversion to IA was 3.8 years (IQR 1.8–7.6 years); to TGA, 5.1 years (3.0–8.0 years; $P \leq 0.0001$ vs. IA); and to TPOA, 11.3 years (8.3–14.5 years; $P \leq 0.0001$ vs. IA; $P \leq 0.0001$ vs. TGA).

IA, TGA, and TPOA were also defined by distinct HLA and sex profiles (Supplementary Figs. 2 and 3). The risk for IA was associated with HLA DR3/DR4-DQ8 (27.2% by 20 years of age [95% CI, 20.3–34.1%] in children with HLA DR3/DR4-DQ8; $P < 0.0001$ vs. the rest) and not with sex. TGA positivity was associated with HLA DR3/DR3 (35.9% by 20 years of

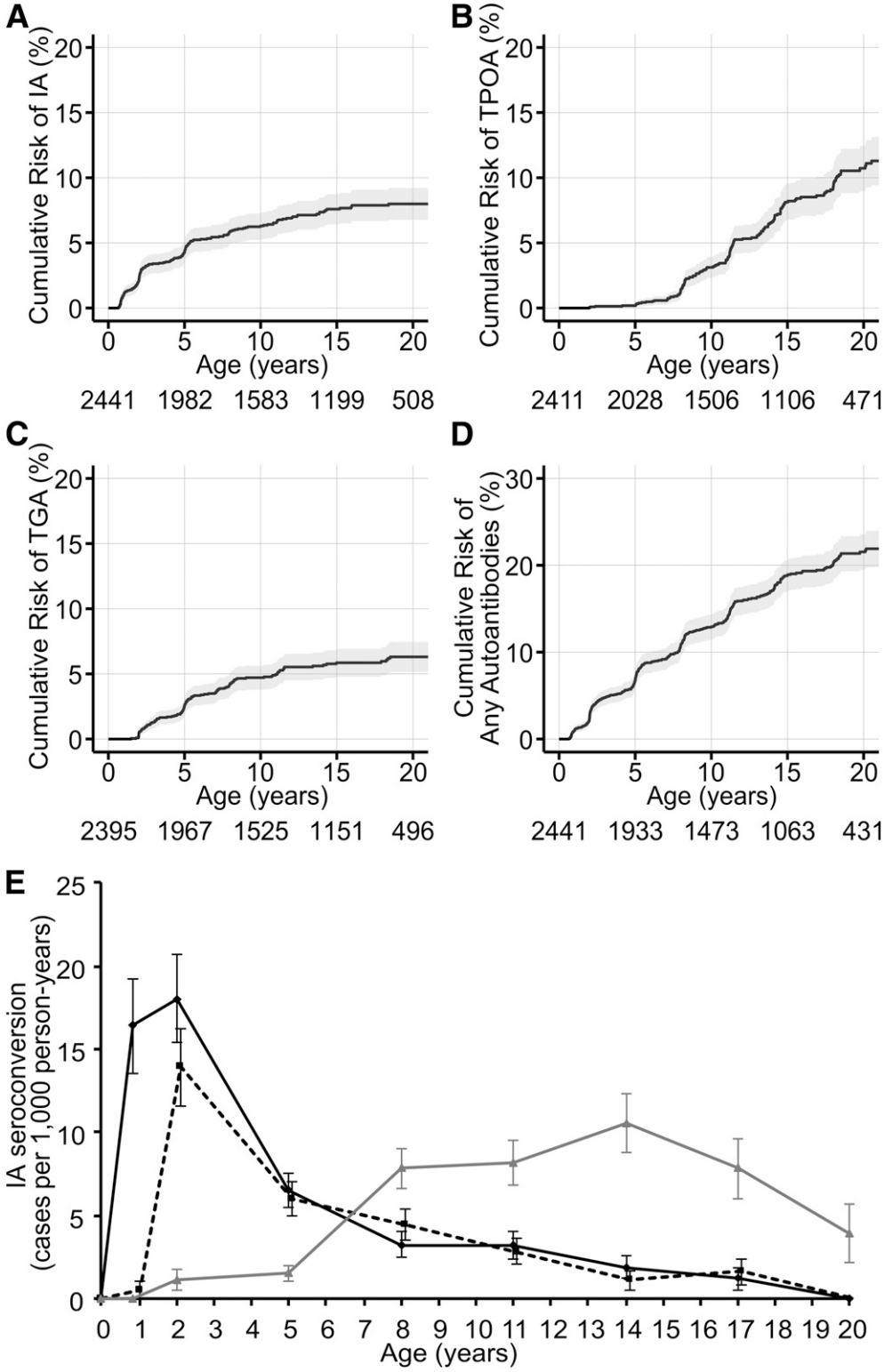

Figure 1—Cumulative risks and incidence curves of developing autoantibodies in the BABYDIAB and BABYDIET participants. Risk of IA (*A*), TPOA (*B*), TGA (*C*), and any of these autoantibodies (*D*). Incidence (cases per 1,000 person-years) of IA (solid line), TGA (dotted line), and (TPOA, gray line) (*E*). Errorbars represent SE.

age [22.4–49.4%] in children with HLA DR3/DR3; $P < 0.0001$ vs. the rest) and was higher in females (7.9% by 20 years of age [6.1–9.7%]) than in males (4.8% by 20 years of age [3.4–6.2%]; $P = 0.005$). TPOA positivity was also higher in females (15.2% by 20 years of age [12.3–18.1%]) than in males (6.3% by 20 years of age [4.3–8.3%]; $P < 0.0001$). Although no strong HLA association was observed, TPOA positivity was highest in children with the HLA DR3/DR3 genotype (26.1%

Table 2—Multivariable Cox proportional hazard models for the development of autoantibodies

	IA		TGA		TPOA		Any	
Variable	HR (95% CI)	*P* value*	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
HLA, reference x/x†								
DR3/DR4	9.7 (5.3–17.5)	<0.0001	15.9 (4.3–58.7)	<0.0001	2.2 (1.1–4.2)	0.026	5.3 (3.5–8.1)	<0.0001
DR4/DR4	5.8 (2.6–12.9)	<0.0001	7.1 (1.2–42.4)	0.033	2.3 (0.9–5.2)	0.055	3.6 (2.0–6.5)	0.0001
DR3/DR3	3.3 (1.1–9.8)	0.031	62.1 (17.2–223)	<0.0001	3.5 (1.6–7.4)	0.0012	6.4 (3.7–11.2)	<0.0001
DR3/x, DR4/x	1.8 (1.1–3.1)	0.024	8.4 (2.6–27.0)	<0.0001	1.3 (0.9–2.0)	0.22	2.0 (1.4–2.7)	0.0007
Sex (reference boys)	0.8 (0.6–1.2)	0.35	1.4 (0.8–2.2)	0.20	2.3 (1.6–3.3)	<0.001	1.3 (1.1–1.7)	0.016
INS (rs689)	1.6 (1.1–2.3)	0.026	1.1 (0.6–1.7)	0.837	1.1 (0.8–1.6)	0.52	1.3 (0.9–1.6)	0.078
PTPN22 (rs2476601)	1.5 (1.0–2.2)	0.046	1.6 (1.0–2.6)	0.047	1.0 (0.7–1.5)	0.92	1.3 (1.0–1.7)	0.022
IFIH1 (rs1990760)	2.2 (1.0–4.7)	0.043	0.9 (0.4–1.7)	0.69	1.4 (0.8–2.5)	0.26	1.5 (0.9–2.2)	0.061
SH2B3 (rs3184504)	0.8 (0.6–1.3)	0.44	0.9 (0.5–1.5)	0.60	1.5 (1.0–2.1)	0.032	1.1 (0.8–1.5)	0.33

**P* value is calculated from the Wald statistic. †x is any haplotype other than HLA DR3 or DR4-DQ8.

by 20 years of age [10.4–41.8%]) or the DR3/DR4-DQ8 genotype (16.7% by 20 years of age [8.9–24.5%]; $P = 0.0005$ for children with HLA DR3/DR3 or DR3/DR4-DQ8 vs. the rest). Remarkably, the risk of any of these antibodies by 20 years of age was 44.4% (36.6–52.2%) in the children with the HLA DR3/DR4-DQ8 genotype, 56.5% (40.8–72.2%) in those with the HLA DR3/DR3 genotype, and 37.4% (25.6–49.2%) in those with the HLA DR4-DQ8/DR4-DQ8 genotype. In contrast, the risk was 11.2% (8.2–14.2%) in children without an HLA DR3 or DR4-DQ8 haplotype.

Additional genes influenced the risk of developing each of the autoantibodies. SNPs in the *INS* gene, which has the strongest genetic association with type 1 diabetes after HLA (27), and in the *PTPN22*, *IFIH1*, and *SH2B3* genes, which are associated with multiple autoimmune diseases (27,28), were included in a Cox proportional hazards model with HLA and sex (Table 2). The risk of IA was influenced by *INS* (HR 1.6 [95% CI 1.1–2.3]; $P = 0.026$), *PTPN22* (1.5 [1.0–2.2]; $P = 0.046$), and *IFIH1* (2.2 [1.0–4.7]; $P = 0.043$) SNPs. *PTPN22* influenced the risk of TGA (1.6 [1.0–2.6]; $P = 0.047$), and *SH2B3* influenced the risk of TPOA (1.5 [1.0–2.1]; $P = 0.032$).

The earlier development of IA and TGA compared with TPOA provided the opportunity to determine whether the development of one autoantibody influences the risk of developing a second autoantibody (Supplementary Fig. 4). Children who were positive for IA or TGA by age 10 years had an increased risk of developing TPOA after age 10 years (18.2% by age 20 years [95% CI 7.3–27.9%]) than children who were IA and TGA negative (11.9% [4.7–18.5%]; $P = 0.0043$). The difference was also observed when the analysis was restricted to children with an HLA DR3/DR3, DR3/DR4-DQ8, or DR4-DQ8/DR4-DQ8 genotype (30.2% [3.4–49.6%] vs. 5.5% [0.2–10.5%]; $P = 0.0015$).

Interaction Between HLA and Sex on Autoantibody Risk

An interaction between HLA and sex on autoantibody risk was investigated using Cox proportional hazards models. The model incorporating the interaction was more effective in predicting the risk of any of the autoantibodies than the model that did not consider this interaction ($P = 0.0077$) (Supplementary Table 1). We therefore performed exploratory analyses after stratification by HLA genotype (Fig. 2). An interaction was most clearly observed when we compared the effect of sex on the risks in children with the DR4-DQ8/DR4-DQ8 or DR3/DR4-DQ8 genotype. Boys with the HLA DR4-DQ8/DR4-DQ8 genotype had a markedly reduced risk of TGA ($P = 0.014$ vs. girls) and TPOA ($P = 0.0084$ vs. girls), whereas no difference in the risk of TGA or TPOA was observed between boys and girls who had the DR3/DR4-DQ8 genotype. Strikingly and in contrast to the boys, in whom HLA affected the risk of IA ($P = 0.010$) and TGA ($P = 0.0006$), the risks in girls were not associated with HLA for IA ($P = 0.37$), TGA ($P = 0.07$), and TPOA ($P = 0.5$).

Diabetes Development and Prognosis After Autoantibody Seroconversion

The diabetes risk by 20 years of age was 6.2% (95% CI 5.0–7.4%) in all children. In total, 105 of 165 (64%) children with IA progressed to clinical or symptomatic diabetes (8 with a single IA and 97 with multiple IAs) at a median time after seroconversion of 4.7 years (IQR 1.5–8.2 years) (Supplementary Table 2). Only two IA-positive children have been followed for >20 years after seroconversion without developing clinical diabetes (Supplementary Table 3). The progression to diabetes after seroconversion was 87.7% (95% CI 79.9–95.5%) at 20-years follow-up in children with multiple IAs, and 29.2% (9.6–48.8%)

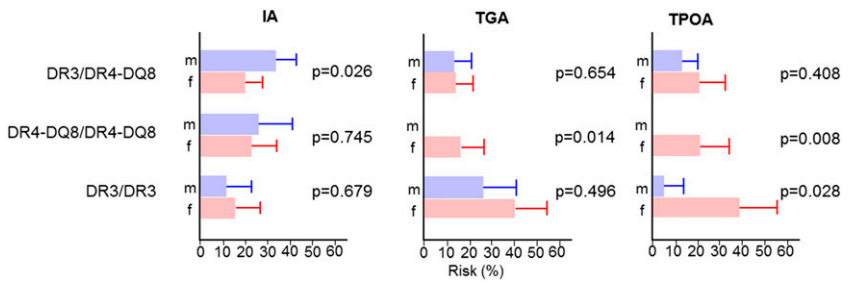

Figure 2—Cumulative risk of autoantibodies in relation to HLA and sex. Risks by age 20 years for IA, TGA, and TPOA in males (m) and females (f) were obtained from Kaplan-Meier analysis. The error bars represent the 95% CIs. *P* values compare females and males and were obtained from the log-rank test.

at 10 years follow-up in children with a single IA (Supplementary Fig. 5A). None of the 128 children with multiple IAs and 6 of the 37 children with a single IA ($P < 0.0001$) seroconverted to an autoantibody-negative status at 3.1–11.4 years of follow-up; none of these 6 children had an HLA DR3/DR4-DQ8, DR4-DQ8/DR4-DQ8, or DR3/DR3 genotype, and none developed clinical diabetes. The incidence of disease progression was relatively consistent over all age ranges, with an average of 79 per 1,000 person-years in IA-positive relatives (Supplementary Fig. 5B). The risk of clinical diabetes was not increased in children who developed TGA or TPOA without IA (Supplementary Fig. 5C).

CONCLUSIONS

We have traced the natural history of autoimmunity related to type 1 diabetes, celiac disease, and thyroid disease from birth to adulthood in first-degree relatives of patients with type 1 diabetes. We found that a surprisingly high frequency of relatives developed these autoantibodies, reaching ~50% by age 20 years in those with the HLA DR3/DR4-DQ8 or HLA DR3/DR3 genotype. The autoantibody-positive relatives usually developed antibodies associated with only one of the diseases, and the risk of any one of the autoantibodies was precisely defined by the HLA genotype, other risk genes, age, and/or sex. These findings suggest a strong familial predisposition for organ-specific autoimmunity, which manifests in a particular organ in an HLA-, an age-, and a sex-dependent manner.

The strength of this study is its long-term follow-up of a large number of participants from birth. All the participants were followed from the first year of life, and the oldest participant was 29.4 years of age at the time of analysis. The cohort had also been tested prospectively for a range of autoantibodies at regular intervals. Furthermore, it is the only birth cohort that was not selected for high-risk HLA genotypes; therefore, we were able to stratify risk across all HLA risk categories. A limitation of the study was that the 115 children who developed type 1 diabetes were not followed after disease onset, so the cumulative frequency of TGA and TPOA by the age of 20 years and the overlap among IA, TGA, and TPOA may have been underestimated. The family histories of celiac disease and thyroid autoimmune disease were also unavailable, so we could not assess the true extent of the familial clustering of autoimmunity associated with the three diseases. Autoantibodies associated with other autoimmune diseases that are associated with type 1 diabetes were not measured; therefore, it is likely that the risk of organ-specific autoimmunity in the relatives is underestimated. We did not analyze the clinical relevance of TGA and TPOA in the current study. Others have shown that these autoantibodies predict disease (29,30). More than one-third of the study population was lost to follow-up before the age of 20 years. However, these participants had similar characteristics to those who remained in the study. The study was performed in a population that is largely of European descent, and the findings may not be generalizable to other ethnic groups. Finally, although a number of susceptibility genes were associated with the risks of IA, TGA, and TPOA, we selected only four to include in the Cox proportional hazards model. These were selected either because they have been reported as the more strongly type 1 diabetes–associated genes (*INS*, *PTPN22*) or because they were reported to be associated with multiple autoimmune diseases (28). Nevertheless, the multivariable analyses included a large number of variables, and there should be caution in interpreting some of the nonsignificant findings, especially for variables with low category frequencies, which have wide CIs.

More than 20% of first-degree relatives of patients with type 1 diabetes and ~50% of the relatives with high-risk HLA genotypes developed persistent autoantibodies associated with one of the three autoimmune diseases. Both TGA and thyroid autoimmunity are more frequent in patients with type 1 diabetes, an association that is attributed, in part, to the sharing of susceptible HLA alleles and other genetic susceptibility regions (13–15). The frequencies of TGA and TPOA in relatives were higher than in previous studies that included predominantly parents of patients with type 1 diabetes (16,18) and were similar to those found in patients with type 1 diabetes (13–15). Sex modified the risks of TGA and TPOA as previously reported (31–33). Furthermore, sex interacted with HLA in modifying the risk of IA, TGA, and TPOA. Interaction between sex and IA on the risk for TPOA is also reported (34). The mechanism of these interactions is unknown. We also confirmed the influence of the *INS* gene on the risk of IA (35), but not on the risk of TGA or TPOA, and the influence of genes such as *PTPN22* on more than one disease-associated autoantibody (28).

A second important finding was that relatively few of the participants developed antibodies associated with different diseases. Age was a highly relevant factor defining which of the disease autoantibodies would develop as previously reported in patients with type 1 diabetes (36,37). For example, a 1-year-old child with HLA DR3/DR4-DQ8 was at highest risk of developing IA, but a teenager with HLA DR3/DR4-DQ8 was at highest risk of developing TPOA. Similarly, a preschool child with HLA DR3/DR3 was more likely to develop TGA, but a teenager with HLA DR3/DR3 was at highest risk of TPOA. Of interest, the risk for TPOA in adolescence was increased if children had developed IA or TGA and remained increased in children with the high-risk HLA genotypes, suggesting some, but incomplete, overlap in genetic and environmental determinants of the autoantibodies. This is consistent with previous twin studies that showed that heritability of thyroid autoimmunity is >50% (31,32) and that heritability includes additive genetic factors and environmental factors that are different for TPOA compared with IA (31).

The observed age-related differences have potential relevance to the causes of organ-specific autoimmunity. Thyroid activity changes during adolescence (38), whereas the islet β-cells undergo many changes in infancy, for example, in response to new nutrient sources of energy (39). Therefore, we predict that the targeted organ is directly involved in generating an autoimmune response and suggest that examining the changes and the environmental exposures that occur at these ages of peak incidence will help us to understand the causes of autoimmune diseases.

Our findings have practical value. A high risk of autoantibodies associated with these three autoimmune diseases was mainly restricted to first-degree relatives with either a DR3 or a DR4-DQ8 haplotype. Therefore, families in

which one member is affected by type 1 diabetes who are concerned about the disease risk can be advised to test for the HLA DR3, DR4, and DQ8 alleles, which can be done by typing three SNPs (40). Family members with HLA DR3 or DR4-DQ8 have an ~25% risk of autoimmunity associated with any of these three diseases. On the basis of the ages of peak incidence, we advise that testing for IA and TGA could be systematically undertaken at 2–5 years and 8–10 years of age to capture early and later autoantibody development and at 13–15 years of age and again in adulthood for TPOA. Family members found positive have opportunities to participate in clinical trials (7) or further testing for clinical disease stage (29,30).

In conclusion, we have shown that autoimmunity to one of three organs has a high rate of occurrence in the first-degree relatives of patients with type 1 diabetes. We suggest that regular measurement of a range of autoantibodies in relatives with susceptible HLA haplotypes is warranted.

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