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TYPE 1 DIABETES: PATHOPHYSIOLOGY AND PREVENTION

Autoantibodies against ATP4A are a feature of the abundant autoimmunity that develops in first-degree relatives of patients with type 1 diabetes

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Abstract

Objective: Type 1 diabetes is associated with autoantibodies to different organs that include the gut. The objective of the study was to determine the risk of developing gastric parietal cell autoimmunity in relation to other autoimmunity in individuals with a family history of type 1 diabetes.

Methods: Autoantibodies to the parietal cell autoantigen, H^+/K^+ ATPase subunit A (ATP4A) was measured in 2218 first-degree relatives of patients with type 1 diabetes, who were prospectively followed from birth for a median of 14.5 years. All were also tested regularly for the development of islet autoantibodies, transglutaminase autoantibodies, and thyroid peroxidase autoantibodies.

Results: The cumulative risk to develop ATP4A autoantibodies was 8.1% (95% CI, 6.6–9.6) by age 20 years with a maximum incidence observed at age 2 years. Risk was increased in females (HR, 1.9; 95% CI, 1.3–2.8; $p = 0.0004$), relatives with the HLA DR4-DQ8/DR4-DQ8 genotype (HR, 3.4; 95% CI, 1.9–5.9; $p < 0.0001$) and in participants who also had thyroid peroxidase autoantibodies (HR, 3.7; 95% CI, 2.5–5.5; $p < 0.0001$). Risk for at least one of ATP4A-, islet-, transglutaminase-, or thyroid peroxidase-autoantibodies was 24.7% (95% CI, 22.6–26.7) by age 20 years and was 47.3% (95% CI, 41.3–53.3) in relatives who had an HLA DR3/DR4-DQ8, DR4-DQ8/DR4-DQ8, or DR3/DR3 genotype ($p < 0.0001$ vs. other genotypes).

Conclusions: Relatives of patients with type 1 diabetes who have risk genotypes are at very high risk for the development of autoimmunity against gastric and other organs.

KEYWORDS
autoimmunity, H^+/K^+ ATPase, parietal cell autoantibodies, type 1 diabetes, islet autoantibodies

1 | INTRODUCTION

Type 1 diabetes is characterized by the presence of circulating autoantibodies to islet antigens and an increased prevalence of autoantibodies targeting other organs such as the thyroid, gut and adrenal gland.[1 4] Many of these autoantibodies also appear in first-degree relatives of patients with or without concomitant islet autoantibodies.[5] The increased prevalence of autoimmunity other than islet autoantibodies in patients with type 1 diabetes and their relatives is likely to be in part due to shared genetic susceptibility at HLA and other gene regions.[6] Despite the shared susceptibility, there are differences in the age at which thyroid, transglutaminase, and islet autoantibodies develop, suggesting different etiological factors.[5] Gastric parietal cell antibodies are also more prevalent in patients with type 1 diabetes.[3] Here, we have measured autoantibodies against the parietal cell autoantigen H^+/K^+ ATPase subunit A (ATP4A) in a cohort of over 2000 first-degree relatives of patients with type 1 diabetes prospectively followed from birth to identify genetic and temporal similarities and differences between the manifestation of autoantibodies to different organs and antigens.

2 | METHODS

2.1 | Study population

Children with a first-degree family history of type 1 diabetes recruited to the BABYDIAB or BABYDIET prospective studies between 1989 and 2006[7,8] were included. Children were followed for the development of autoimmunity associated with type 1 diabetes, celiac disease, and thyroid autoimmune disease.[5] Blood samples were obtained at age 9 months, 2 years, 5 years and every 3 years thereafter or, for 150 children in the BABYDIET study, every 3 months until age 3 years and subsequently at age 5 years and every 3 years thereafter. Follow-up was increased to six-monthly intervals in children who developed islet autoantibodies. The median follow-up period from birth was 14.5 years (interquartile range [IQR], 7.9–19.9 years). Children were HLA typed at the HLA-DRB1, HLA-DQA1, and HLA-DQB1 loci as previously described.[9,10] The studies were approved by the Bavarian ethics committees (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 the participants or their parents.

2.2 | ATP4A autoantibody measurements

The coding sequence was obtained as a synthetic gene (BaseClear B.V., The Netherlands) and was cloned into the pCMV6-AC-IRES-GFP-Puro (Origene, Rockville, MD, USA) vector containing a NanoLuc, thus creating an NH_2-terminal fusion. Recombinant NanoLuc-fused antigen was expressed in HEK 293 T cells by transfecting 2 μg expression vector DNA using polyethylenimine (Polysciences, Warrington, PA, USA). Recombinant protein was harvested in the supernatant after 48 h. The recombinant antigens were then aliquoted and stored frozen at −80°C. Antibodies were measured by luciferase immunoprecipitation assays[11,12] similar to those previously described for antibodies to Coxsackie B virus[13] and tetraspanin 7.[14] Briefly, serum (2 μl) was added to buffer (25 μl) containing 5 million light units (counts per second [cps]) of luciferase-tagged protein in deep 96-well plates, incubated at room temperature for 2 h, followed by the addition of 50 μl buffer containing glycine-treated Protein A Sepharose CL-4B (GE Healthcare, Chicago, IL). Plates were incubated for 1 h at 4°C, the Protein A Sepharose was transferred to a microplate (OptiPlate-96, Perkin Elmer) and washed 10 times with 200 μl of wash buffer before adding substrate (25 μl). The captured light units were measured on a multimode microplate reader (GloMax Navigator, Promega). The values were converted to arbitrary units using a calibration curve of an anti-NanoLuc antibody (kind gift from Promega) diluted in buffer (20 mM KH_2PO_4, 150 mM NaCl, pH 7.2, 50% Glycerol, 0.01% wt/vol NaN_3) over a range of 1–100 arbitrary units and included in every assay. Positivity was defined as greater than the 99th centile of values in a control population of islet autoantibody negative children and adolescents and corresponded to 10 arbitrary units. ATP4A autoantibodies were measured in the last available sample from the 2218 relatives, and then in all previously available samples for those who had values above 10 arbitrary units.

2.3 | Other autoantibody measurements

Radiobinding assays[15,16] were used to measure islet autoantibodies against insulin, insulinoma antigen 2 (IA-2), glutamic acid decarboxylase (GAD), and zinc transporter 8 (ZnT8) in serum at all completed visits. IgA autoantibodies against tissue transglutaminase 2 (TG), and the thyroid autoimmune disease-associated autoantibodies against thyroid peroxidase (TPO) were measured by radiobinding assays.[1,17]

2.4 | Statistical analysis

Participants were considered positive for an autoantibody if the autoantibody was above the positive threshold in two or more consecutive samples or in the last available sample.[1,17] The Kaplan–Meier method was used to determine the cumulative risks of developing autoantibodies and the log rank test was used to compare categories. Follow-up was calculated from birth to the age when autoimmunity first developed or to the last sample in participants who were negative. Univariable and multivariable hazard ratios (HRs) and their 95% confidence intervals (CI) for autoantibody development were determined using Cox proportional hazard models. The incidence of antibodies was expressed as per 1000 person-years and was calculated for the age intervals 0–18 months, 18–42 months, 42–78 months, 78–114 months, 114–150 months, 150–186 months, and 186–204 months. Standard errors were calculated based on the Cooper-Pearson method. All statistical analyses were performed with SAS 9.4 (SAS Institute, Cary, NC) and IBM SPSS version 25.0 (IBM Corp., Armonk, NY).

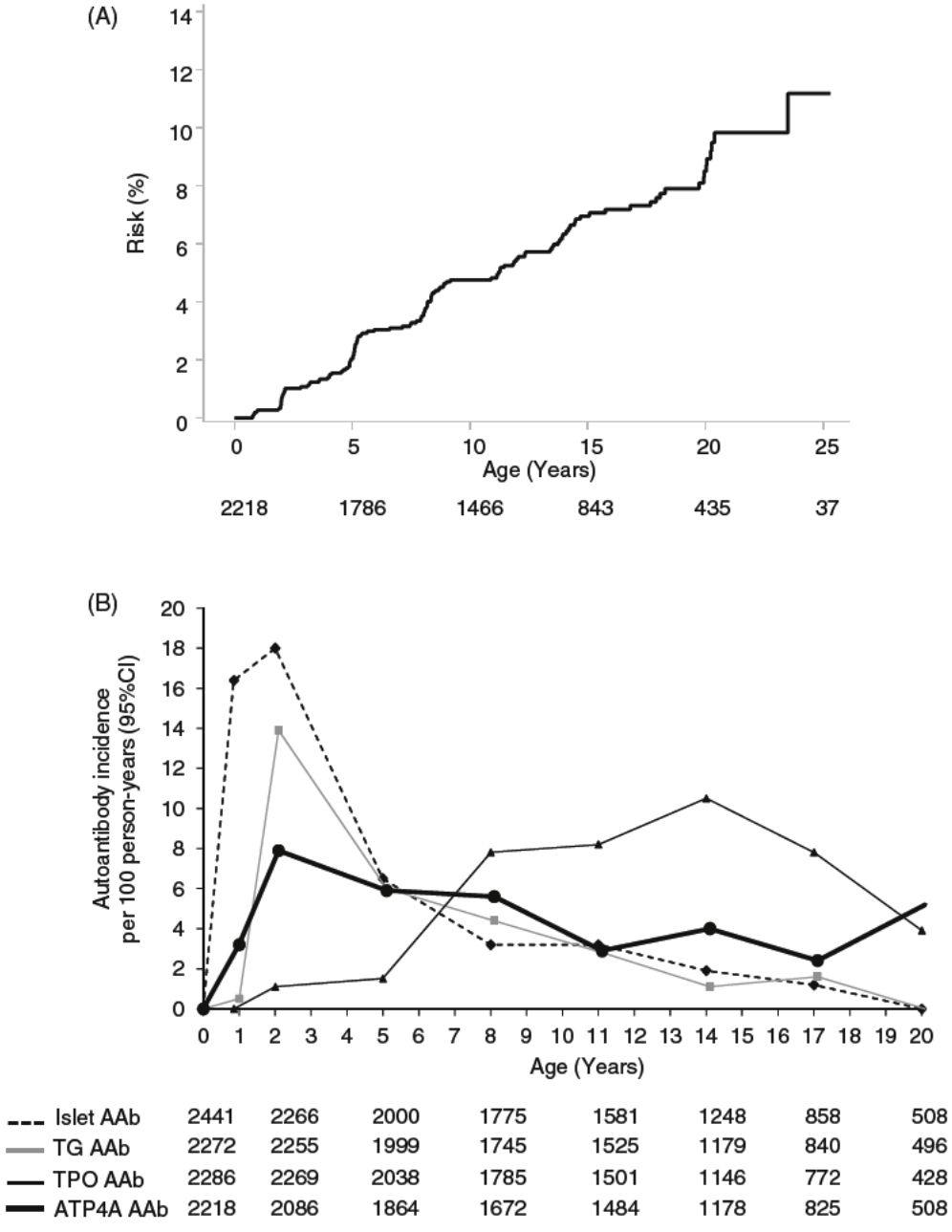

FIGURE 1 Cumulative risk and age-related incidence curve of ATP4A autoantibodies in first-degree relatives of patients with type 1 diabetes. (A). Cumulative risk of ATP4A autoantibodies (*y* axis) with increasing age (*x* axis) in 2218 participants followed from birth. The numbers of children remaining in follow-up are indicated below each time-point and the shaded area indicates the 95% confidence interval. (B). Incidence (cases per 1000 person-years) of ATP4A autoantibodies (thick solid line). As comparison, the incidence of islet autoantibodies (dashed line), transglutaminase autoantibodies (thin gray line), and thyroid peroxidase autoantibodies (thin black line) in the participants are shown. Numbers under the *x* axis indicate participants remaining in follow-up.

3 | RESULTS

3.1 | ATP4A autoantibody prevalence and age of appearance

ATP4A autoantibodies were measured in 2218 first-degree relatives of patients with type 1 diabetes. ATP4A autoantibodies were observed in 128 participants ranging in first appearance from age 9 months to 23 years. The cumulative prevalence of ATP4A autoantibodies was 4.7% (95% CI, 3.7–5.7) by age 10 years and 8.1% (95% CI, 6.6–9.6) by age 20 years (Figure 1A).

ATP4A autoantibodies incidence rose to a peak of 7.9/100,000 per year at around 2 years of age and remained above 5/100,000 per year until 8 years of age (Figure 1B). In comparison, the peak incidence for islet autoantibodies was observed at age 9 months to 2 years, TG autoantibodies at age 2 years, and TPO autoantibodies at 14 years of age.

3.2 | Factors associated with ATP4A autoantibodies

Sex and HLA class II genotypes were associated with ATP4A autoantibody risk (Table 1). The risk of developing ATP4A autoantibodies was higher in females as compared to males (HR, 1.9; 95% CO, 1.3–2.8; $p = 0.0004$). Risk was elevated in participants with the HLA DR4-DQ8/DR4-DQ8 genotype (risk by age 20 years, 19.7%; HR, 3.4; 95% CI, 1.9–5.9; $p < 0.0001$) but not in

participants with HLA DR3-DQ8/DR4-DQ8 (risk, 7.0%; HR, 1.3; 95% CI, 0.7–2.4; $p = 0.49$) or HLA DR3/3 genotypes (risk, 6.8%; HR, 1.0; 95% CI, 0.4–2.8; $p = 0.98$) as compared to participants without these genotypes (risk, 7.6%). The index case of type 1 diabetes (maternal, paternal, sibling) was not associated with ATP4A autoantibody risk.

TABLE 1 Cox proportional hazards (univariable) for the development of ATP4A autoantibodies

Variable	Category (*n*)	Hazard ratio	95% CI	*p* Value
Sex (reference male)	Female (1188)	1.93	1.34–2.77	0.0004
FDR (reference mother)	Father (924)	0.91	0.63–1.32	0.62
	Sibling (58)	0.89	0.22–3.65	0.88
HLA (reference other)	DR3-DQ8/DR4-DQ8 (178)	1.26	0.66–2.41	0.49
	DR4-DQ8/DR4-DQ8 (91)	3.39	1.93–5.93	<0.0001
	DR3/3 (72)	1.01	0.37–2.75	0.98
Islet AAb	Positive (169)	1.99	1.12–3.53	0.019
Transglutaminase AAb	Positive (118)	0.80	0.35–1.82	0.60
Thyroid Peroxidase AAb	Positive (158)	3.67	2.45–5.50	<0.0001

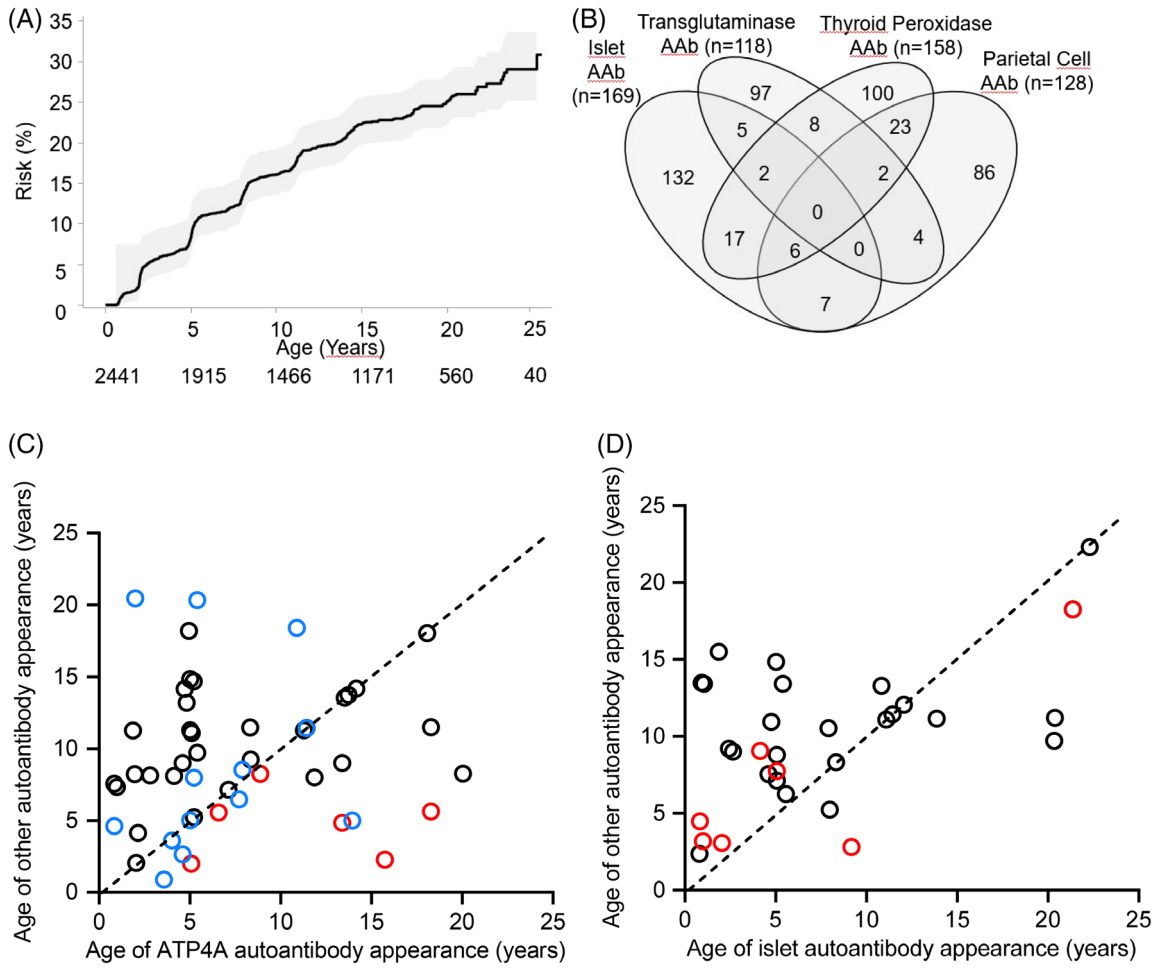

FIGURE 2 Risk and overlap of autoantibodies to ATP4A, islet autoantigens, transglutaminase or thyroid peroxidase in first-degree relatives of patients with type 1 diabetes. (A). Cumulative risk to develop ATP4A-, islet-, transglutaminase- or thyroid peroxidase autoantibodies (*y* axis) with increasing age (*x* axis) in participants followed from birth. The numbers of children remaining in follow-up are indicated below each time-point and the shaded area indicates the 95% confidence interval. (B). Venn diagram of the autoantibody combinations in 489 participants with at least one of the autoantibodies. (C). The age of appearance of ATP4A autoantibodies (*x* axis) versus the age of appearance of islet (blue), transglutaminase (red), and thyroid peroxidase autoantibodies (black) in children with ATP4A autoantibodies. (D). The age of appearance of islet autoantibodies (*x* axis) versus the age of appearance of transglutaminase (red), and thyroid peroxidase autoantibodies (black) in children with islet autoantibodies.

3.3 | ATP4A autoantibody associations with other autoimmunity

The development of ATP4A autoantibodies was more frequent in participants who developed TPO or islet autoantibodies. ATP4A autoantibody risk in participants who developed TPO autoantibodies was 21.8% by age 20 years (HR, 3.7; 95% CI, 2.5–5.5; $p < 0.0001$). Participants who developed islet autoantibodies had a marginally increased risk (risk, 13.8%; HR, 1.9; 95% CI, 1.1–3.5; $p = 0.02$) and risk was not elevated in participants who developed TG autoantibodies (risk, 9.5%; HR, 0.8; 95% CI, 0.4–1.8; $p = 0.60$).

The risk for any of the islet, TPO, TG, or ATP4A autoantibodies was 24.7% (95% CI, 22.6–26.7) by age 20 years (Figure 2A). Any of these autoantibodies was observed in 489 participants (Figure 2B). Among these, 74 (15.1%) had autoantibodies against multiple organs, including 42 who had ATP4A autoantibodies as well as islet ($n = 13$), TPO ($n = 31$) and/or TG ($n = 6$) autoantibodies. For those with ATP4A and other autoantibodies, no difference in the relative age of appearance was observed between ATP4A autoantibodies and islet

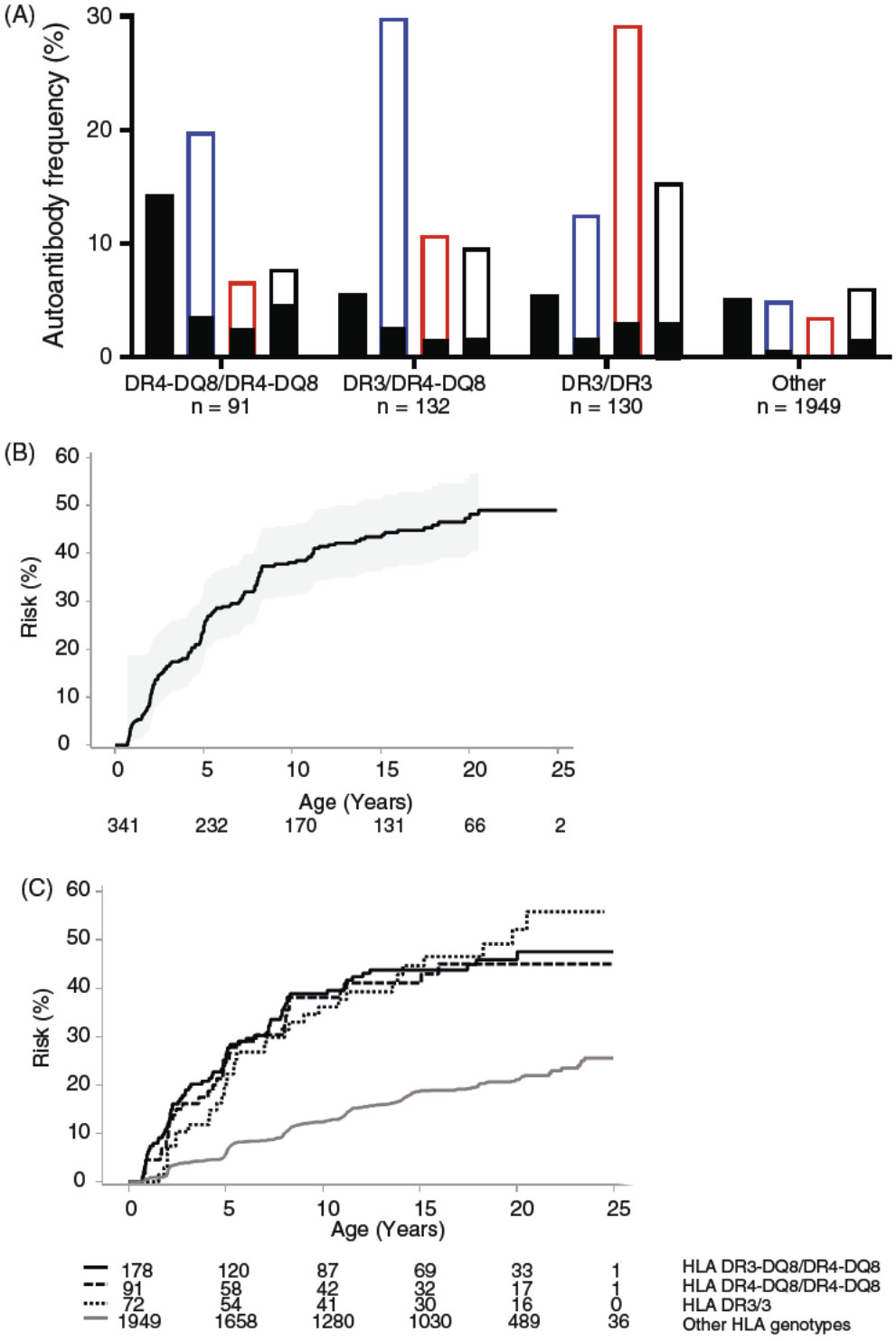

FIGURE 3 Autoantibodies and HLA DR-DQ genotypes. (A). Frequency (%, *y* axis) of participants with ATP4A autoantibodies (filled black bars), islet autoantibodies (open blue bars), transglutaminase autoantibodies (open red bars), and thyroid peroxidase autoantibodies (open black bars) stratified for HLA genotype. The filled black portions within the islet-, transglutaminase- and thyroid peroxidase-autoantibody bars indicate those who also had ATP4A autoantibodies. (B). Cumulative risk to develop any of ATP4A-, islet-, transglutaminase- or thyroid peroxidase autoantibodies (*y* axis) with increasing age (*x* axis) in participants followed from birth who have an HLA DR3/DR4-DQ8, DR4-DQ8/DR4-DQ8 or DR3/DR3 genotype. The numbers of children remaining in follow-up are indicated below each time-point and the shaded area indicates the 95% confidence interval. (C). Cumulative risk to develop any of ATP4A-, islet-, transglutaminase- or thyroid peroxidase autoantibodies (*y* axis) with increasing age (*x* axis) in participants followed from birth stratified by HLA genotype as HLA DR3/DR4-DQ8 (solid black line), DR4-DQ8/DR4-DQ8 (dashed line), DR3/DR3 (dotted line) or other genotypes (solid gray line). The numbers of children remaining in follow-up are indicated below each time-point.

autoantibodies (6 prior, 2 at the same age, and 5 after islet autoantibodies). ATP4A autoantibodies appeared after TG autoantibodies in all 6 with both autoantibodies, and most often before ($n = 18$) or at the same age ($n = 9$) as TPO autoantibodies in the 31 children with both autoantibodies (Figure 2C). Islet autoantibodies were more frequently observed before TG autoantibodies (5 prior, 0 at the same age, and 2 after TG autoantibodies), and before TPO autoantibodies (16 prior, 5 at the same age, and 4 after TPO autoantibodies; Figure 2D).

3.4 | HLA class II genotype and ATP4A, islet, transglutaminase and TPO autoantibodies

HLA class II genotype strongly influenced which autoantibody developed in the relatives (Figure 3A). Islet autoantibodies (51.4%) and ATP4A autoantibodies (37.1%) were the most frequently observed among autoantibody positive participants with the HLA DR4-DQ8/DR4-DQ8 genotype. Despite the increased susceptibility for both ATP4A and islet autoantibodies in relatives with the HLA DR4-DQ8/DR4-DQ8 genotype, there was no excess in the overlap between these two autoantibodies in the 91 children with the genotype (13 with ATP4A autoantibodies, 18 islet autoantibodies, and 3 of these had both; $p = 0.72$). Islet autoantibodies (71.6%) were the most frequently observed among autoantibody positive participants with the HLA DR3/DR4-DQ8 genotype; TG autoantibodies (63.6%) the most frequent among autoantibody positive participants with the HLA DR3/DR3 genotype; and TPO autoantibodies (42.1%) the most frequent among autoantibody positive participants with other HLA genotypes. Remarkably, the risk for any of the autoantibodies was 47.3% by age 20 years (95% CI, 41.3–53.3) in participants with HLA DR3/3, DR3/DR4-DQ8, or DR4-DQ8/DR4-DQ8 genotypes (Figure 3B). Although the risk for specific organ autoantibodies varied by HLA genotype, the risk for any one of the four antibodies was very similar for each of these three genotypes (HLA DR3-DQ8/DR4-DQ8: 45.9%, 95%CI 37.9–53.9; HLA DR4-DQ8/DR4-DQ8: 44.9%, 95%CI 33.5–56.4; HLA DR3/3: 52.1%, 95%CI 38.8–65.4; Figure 3C).

4 | DISCUSSION

Autoantibodies to the gastric parietal cell autoantigen ATP4A were found in 8.1% of relatives of patients with type 1 diabetes by age 20 years. ATP4A autoantibodies developed throughout childhood and adolescence, as early as age 9 months, and with a peak incidence around age 2 years. The risk for developing ATP4A autoantibodies was increased in females, in individuals with the HLA DR4-DQ8/DR4-DQ8 genotype, and those who developed TPO autoantibodies. Relatives with HLA DR4-DQ8/DR4-DQ8, DR3/DR4-DQ8, or HLA DR3/3 genotypes had a marked risk of autoimmunity with around 50% risk for developing autoantibodies to ATP4A, pancreatic islet antigens, TPO, or TG by age 20 years.

The findings are from a large cohort of first-degree relatives of patients with type 1 diabetes followed from birth for up to 32 years. The cohort participants are, therefore, genetically at risk for autoimmunity that is associated with type 1 diabetes, which includes ATP4A autoantibodies.[3] A potential limitation of the study is that screening for ATP4A autoantibodies was performed on the last sample available from participants. It is possible, therefore, that participants with transient ATP4A autoantibodies were missed and that the risk for the autoantibodies is slightly underestimated. ATP4A autoantibodies are often detected in the absence of or only mild clinical complications[18,19] Since our study did not have clinical or biochemical data related to pernicious anemia, we cannot determine the clinical significance of ATP4A autoantibodies in the relatives.

The association of ATP4A autoantibodies with female sex, thyroid autoimmunity and HLA DR4 are consistent with previous reports in patients with type 1 diabetes[20] The marked risk in the relatives with the HLA DR4-DQ8/DR4-DQ8 genotype is novel. This genotype is strongly associated with the risk for developing type 1 diabetes and autoantibodies against IA-2 and insulin.[21 23] Of interest, there was relatively little overlap between islet and ATP4A autoantibodies in relatives with this genotype, suggesting that the two autoimmune entities are likely to have distinct etiologies. There was also no association between ATP4A and TG autoantibodies, although both target gut autoantigens and both had a peak age of incidence around age 2 years. Unlike ATP4A and islet autoantibodies, the ATP4A and TG autoantibodies had distinct HLA genotype associations suggesting that HLA may determine the likely specificity of gut autoimmunity. A similar scenario is observed for pancreatic islet autoimmunity where HLA DR4-DQ8 is linked to the development of autoantibodies to insulin and HLA DR3 is linked to the development of autoantibodies to GAD.[24,25]

A remarkable observation is that around 50% of the relatives of patients with type 1 diabetes and with two HLA DR3 or DR4-DQ8 haplotypes or heterozygous for these haplotypes developed islet, gut or thyroid autoimmunity by age 20 years. Although the three possible genotypes had different autoantibody associations, any autoantibody risk was around 50% for each of the genotypes. Familial autoimmunity is, therefore, strongly determined by genetics and its target specificity determined by HLA, age, and sex. The contribution of common and/or specific environmental factors to the development of each autoimmunity remains to be determined.

AUTHOR CONTRIBUTIONS

Marie-Luise Zielmann, Manja Jolink, Christiane Winkler, Anne Eugster, Denise Müller, Marlon Scholz, Anette-G. Ziegler, and Ezio Bonifacio contributed to the production and/or collection of the data and critically reviewed the manuscript for important intellectual content. Christiane Winkler, Manja Jolink, Anette-G. Ziegler, and Ezio Bonifacio contributed to the interpretation of the data. Christiane Winkler, Manja Jolink, and Ezio Bonifacio performed the statistical analysis. Ezio Bonifacio and Anette-G. Ziegler drafted the manuscript. Anette-G. Ziegler is the principal investigator of the BABYDIAB and BABYDIET studies and designed the studies and concept. Ezio Bonifacio is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The BABYDIAB and BABYDIET studies were approved by the ethics committees of Bavaria, Germany (Bayerische Landesärztekammer no. 95357 and Ludwig-Maximilians University no. 329/00, respectively).

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