Age, HLA and sex define a marked risk of organ-specific autoimmunity in first degree relative of patients with type 1 diabetes

Christiane Winkler PhD\*1,2, Manja Jolink\*1, Annette Knopff1, Nana-Adjoa Kwarteng1, Peter Achenbach MD1,2,3, Ezio Bonifacio PhD4,5, Anette-G. Ziegler MD1,2,3

\*Shared first authorship

1 Institute of Diabetes Research, Helmholtz Zentrum München, German Research Center for Environmental Health, Munich-Neuherberg, Germany

2 Forschergruppe Diabetes e.V. at Helmholtz Zentrum München, German Research Center for Environmental Health, Munich-Neuherberg, Germany

3 Forschergruppe Diabetes, Technical University Munich, at Klinikum rechts der Isar, Munich, Germany

4 DFG-Center for Regenerative Therapies Dresden, Faculty of Medicine, TU Dresden, Dresden, Germany

5 Paul Langerhans Institute Dresden of the Helmholtz Center Munich at University Hospital Carl Gustav Carus and Faculty of Medicine, TU Dresden, Germany

Short title: Familial risks of autoantibodies

The authors dedicate this work to the memory of Gian Franco Bottazzo who discovered islet cell autoantibodies in patients with polyendocrine autoimmunity.

Address for correspondence:

Anette-G. Ziegler, MD

Institute of Diabetes Research, Helmholtz Zentrum München,

Ingolstaedter Landstr. 1, 85764 Neuherberg, Germany

E-mail: anette-g.ziegler@helmholtz-muenchen.de

Phone: 0049 89 3137 2896, Fax: 0049 89 3187 3144

Word count: 3874

Tables/Figures: 4

Abstract

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 for up to a maximum of 28.6 years (median, 13.2 years). All were tested regularly for the development of autoantibodies associated with type 1 diabetes (islet), coeliac 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 (353) or more (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% (95% CI, 5.1-7.5%) for transglutaminase autoantibodies, 10.7% (95% CI, 8.9-12.5%) for thyroid peroxidase autoantibodies, and 21.5% (95% CI, 19.5-23.5%) for any of these autoantibodies. Each of the three disease-associated autoantibodies was defined by distinct human leukocyte antigen (HLA), sex, genetic, and age profiles. The risk of developing any of these autoantibodies was 56.5% (95% CI, 40.8%-72.2%) in HLA DR3/DR3 relatives and 44.4% (95% CI, 36.6%-52.2%) in HLA DR3/DR4-DQ8 relatives.

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 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). They follow 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 subjects were also observed to be at risk of 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 this 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 (ClinicalTrials.gov NCT01115621) 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 (IQR, 11.2­-20.0 years).

*Autoantibody measurements*

The type 1 diabetes-associated islet autoantibodies (IA) against insulin (IAA), glutamic acid decarboxylase (GADA), insulinoma antigen 2 (IA-2A), and zinc transporter 8 (ZnT8A) were measured at the Helmholtz Center, Munich 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 rs689 (*INS* gene), rs2476601 (*PTPN22* gene), rs1990760 (*IFIH1* gene), and rs3184504 (*SH2B3* gene) was performed in 2290 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, IA2A, or ZnT8A, with sample values above the 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 IA on at least one occasion. In children who were positive for GADA in the absence of other islet autoantibodies, GADA was also assessed for its GAD-binding affinity (19), and positivity on an enzyme-linked immunosorbent assay (ELISA; Medizym anti-GAD ELISA Kit, Medipan, Berlin, Germany). These children were classified as single-GADA positive if they had GADA with affinity ≥109 l/mol or tested positive on the RBA and ELISA measurements (21). In children who were positive for IAA in the absence of other IA, IAA were assessed for their insulin-binding affinity, and were classified as single-IAA positive if they had IAA with affinity ≥ 109 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 U/ml, as defined with a quantile-quantile (QQ) plot analysis (11). For TPOA and TGA, persistence was defined as a positive result in two consecutive samples or autoantibody levels of > 10 U/ml (TGA) or > 100 U/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 islet autoantibodies, 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 2 occasions of a 2-hour plasma glucose greater than 200 mg/dL after an oral glucose test; or a random blood glucose concentration greater than 200 mg/dL accompanied by unequivocal symptoms. In 1997, fasting blood glucose greater than 126 mg/dL on 2 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 if 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.

*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 IA, 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% confidence intervals (CI) 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. Standard errors (SE) were calculated based on the Cooper-Pearson method. Comparisons of islet autoantibody incidence between age groups were performed using Fisher’s exact test and the age of autoantibody development was compared between IA, TGA, and TPOA using the Mann-Whitney U test.

All statistical analyses were performed with IBM SPSS version 25.0 (IBM Corp., Armonk, NY), and SAS 9.4 (SAS Institute, Cary, NC).

Results

In total, 2,441 children (1,188 girls) were enrolled in the prospective BABYDIAB and BABYDIET studies and tested for IA (n = 2441), TGA (n = 2395, 1166 girls), and TPOA (n = 2411, 1172 girls) at scheduled visits. Of these children, 394 developed one or more autoantibodies (Table 1, Figure S1), 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 (six with IA and TGA, 23 with IA and TPOA, 11 with TGA and TPOA, and one with IA, TGA, and TPOA).

Of the 165 children with IA, 128 (77.6%) developed multiple IA (23 developed two IA, 47 developed three IA, 58 developed four IA), and 37 children (22.4%) developed single IA (Figure S1). 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 two 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% (95% CI, 5.1-7.5%), and the TPOA risk was 10.7% (95% CI, 8.9-12.5%; Figure 1A-C). The risk of developing any of these autoantibodies was 21.5% by 20 years of age (95% CI, 19.5-23.5%) (Figure 1D).

*Age of seroconversion, HLA, and sex distinctly define the risk of each autoimmunity*

Age was the most disparate feature between the three disease-associated autoantibodies (Figure 1E). The incidence of IA seroconversion was highest at ages of 9 months (incidence 16.4 per 1,000 person-years; SE, ± 2.8) and 2 years (incidence, 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 to 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 was 5.1 years (IQR, 3.0-8.0 years; p=<0.0001 vs IA), and to TPOA was 11.3 years (IQR, 8.3-14.5 years; p=<0.0001 vs IA; p=<0.0001 vs TGA).

IA, TGA, and TPOA were also defined by distinct HLA and sex profiles (Figures S2, S3). 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 rest), and not with sex. TGA positivity was associated with HLA DR3/3 (35.9% by 20 years of age; 95% CI, 22.4%-49.4% in HLA DR3/3 children; p<0.0001 vs rest) and was higher in females (7.9% by 20 years of age; 95% CI, 6.1%-9.7%) than males (4.8% by 20 years of age; 95% CI, 3.4%-6.2%; p = 0.005). TPOA positivity was also higher in females (15.2% by 20 years of age; 95% CI, 12.3%-18.1%) than in males (6.3% by 20 years of age; 95% CI, 4.3%-8.3%; p < 0.0001). Although no strong HLA association was observed, TPOA positivity was highest in children with the HLA DR3/3 genotype (26.1% by 20 years of age; 95% CI, 10.4%-41.8%) or DR3/4-DQ8 genotype (16.7% by 20 years of age; 95% CI, 8.9%-24.5%; p=0.0005, HLA DR3/3 or DR3/DR4-DQ8 children vs rest). Remarkably, the risk of any of these antibodies by 20 years of age was 44.4% (95% CI, 36.6%-52.2%) in the HLA DR3/4-DQ8 children, 56.5% (95% CI, 40.8%-72.2%) in the HLA DR3/3 children, and 37.4% (95% CI, 25.6%-49.2%) in the children with the HLA DR4-DQ8/DR4-DQ8 genotype. In contrast, the risk was 11.2% (95% CI, 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 *PTPN22*, *IFIH1*, and *SH2B3*, which are genes 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* (HR, 1.5; 95% CI, 1.0-2.2; p = 0.046), and *IFIH1* (HR, 2.2; 95% CI, 1.0-4.7; p = 0.043) SNPs. *PTPN22* influenced the risk of TGA (HR, 1.6; 95% CI, 1.0-2.6; p = 0.047), and *SH2B3* influenced the risk of TPOA (HR, 1.5; 95% CI, 1.0-2.1; p = 0.032).

The earlier development of IA and TGA as compared to TPOA provided the opportunity to determine whether the development on one autoantibody influences the risk of developing a second autoantibody (Figure S4). Children who were positive for IA or TGA by age 10 years had an increased risk to develop 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%; 95% CI; 4.7-18.5; p=0.0043). The difference was also observed when the analysis was restricted to children with a HLA DR3/3, DR3/DR4-DQ8 or DR4-DQ8/DR4-DQ8 genotype (30.2%; 95% CI, 3.4-49.6 vs 5.5%; 95% CI, 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; Table S1). We, therefore performed exploratory analyses after stratification by HLA genotype (Figure 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 (eight with single IA and 97 with multiple IA) at a median time after seroconversion of 4.7 years (IQR, 1.5-8.2 years; Table S2). Only two IA-positive children have been followed for more than 20 years after seroconversion without developing clinical diabetes (Table S3). The progression to diabetes after seroconversion was 87.7% (95% CI, 79.9%-95.5%) at 20-years follow-up in children with multiple IA, and 29.2% (95% CI, 9.6%-48.8%) at 10-years follow-up in children with a single IA (Figure S5a). None of the 128 children with multiple IA and six of the 37 children with single autoantibodies (p < 0.0001) seroconverted to an autoantibody-negative status at 3.1-11.4 years of follow-up; none of these six children had a HLA DR3/DR4-DQ8, DR4-DQ8/DR4-DQ8, or DR3/3 genotype and none developed clinical diabetes. The incidence of disease progression was relatively consistent over all age ranges, with an average of 79 per 1000 person years in IA positive relatives (Figure S5b). The risk of clinical diabetes was not increased in children who developed TGA or TPOA without IA (Figure S5c).

Conclusions

We have traced the natural history of type 1 diabetes-, celiac disease-, and thyroid disease-related autoimmunity 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 relatives 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-, age-, and 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, and we were, therefore, 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 between 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 and it is, therefore, 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). Over 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 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 4 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 include a large number of variables and there should be caution in interpreting some of the non-significant findings, especially for variables with low category frequencies, which have wide confidence intervals.

More than 20% of first-degree relatives of patients with type 1 diabetes and around 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 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, an HLA DR3/DR4-DQ8 1-year-old child was at highest risk of developing IA, but an HLA DR3/DR4-DQ8 teenager was at highest risk of developing TPOA. Similarly, a preschool HLA DR3/DR3 child was more likely to develop TGA, but an HLA DR3/DR3 teenager 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 show that heritability of thyroid autoimmunity is over 50% (31,32), and that heritability includes additive genetic factors and environmental factors that are different for TPOA as compared to 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 beta 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 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 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 a ~25% risk of autoimmunity associated with any of these three diseases. Based on the ages of peak incidence, we advise that testing for IA and TGA could be systematically undertaken at 2 to 5 years and 8 to 10 years to capture early and later autoantibody development, and at 13 to 15 years and again in adulthood for TPOA. 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, and we suggest that the regular measurement of a range of autoantibodies in relatives with susceptible HLA haplotypes is warranted.

Acknowledgments

This work was funded by the German Federal Ministry of Education and Research (BMBF) to the German Center for Diabetes Research (DZD e.V.).

We thank Sandra Hummel for overseeing the BABYDIET intervention. We thank all the study nurses and technicians for their assistance in the studies over the past 30 years of the study. We also thank all the study participants and the pediatricians and family doctors in Germany for participating in the BABYDIAB and BABYDIET studies.

Declaration of interests: All the authors have completed and submitted the ICMJE form for the disclosure of potential conflicts of interest and none were reported.

Author Contributions: AGZ is the principal investigator of the BABYDIAB and BABYDIET studies and designed the studies and concept. AGZ, EB, CW, MJ, AK, NAK, and PA contributed to the collection of the data. EB, AGZ, CW, MJ, and NAK performed the statistical analysis. CW, MJ, AK, EB, AGZ, and PA contributed to the interpretation of the data. CW, MJ EB, and AGZ drafted the manuscript. CW, MJ, AK, EB, AGZ, NAK, and PA critically reviewed the manuscript for important intellectual content.

Dr. Ziegler 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.

References

1. Ziegler AG, Bonifacio E; BABYDIAB-BABYDIET Study Group. Age-related islet autoantibody incidence in offspring of patients with type 1 diabetes. *Diabetologia* 2012;55:1937-43.

2. Parikka V, Näntö-Salonen K, Saarinen M, Simell T, Ilonen J, Hyöty H, Veijola R, Knip M, Simell O. Early seroconversion and rapidly increasing autoantibody concentrations predict prepubertal manifestation of type 1 diabetes in children at genetic risk. *Diabetologia* 2012;55:1926-36.

3. Krischer JP, Lynch KF, Schatz DA, Ilonen J, Lernmark Å, Hagopian WA, Rewers MJ, She JX, Simell OG, Toppari J, Ziegler AG, Akolkar B, Bonifacio E; TEDDY Study Group. The 6 year incidence of diabetes-associated autoantibodies in genetically at-risk children: the TEDDY study. *Diabetologia* 2015;58:980-7.

4. Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, Winkler C, Ilonen J, Veijola R, Knip M, Bonifacio E, Eisenbarth GS. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. *JAMA* 2013;309:2473-9.

5. Raab J, Haupt F, Scholz M, Matzke C, Warncke K, Lange K, Assfalg R, Weininger K, Wittich S, Löbner S, Beyerlein A, Nennstiel-Ratzel U, Lang M, Laub O, Dunstheimer D, Bonifacio E, Achenbach P, Winkler C, Ziegler AG; Fr1da Study Group. Capillary blood islet autoantibody screening for identifying pre-type 1 diabetes in the general population: design and initial results of the Fr1da study. *BMJ Open* 2016;6:e011144

6. TEDDY Study Group. The Environmental Determinants of Diabetes in the Young (TEDDY) study: study design. *Pediatr Diabetes* 2007;8:286-98.

7. Bingley PJ, Wherrett DK, Shultz A, Rafkin LE, Atkinson MA, Greenbaum CJ. Type 1 Diabetes TrialNet: A Multifaceted Approach to Bringing Disease-Modifying Therapy to Clinical Use in Type 1 Diabetes. *Diabetes Care* 2018;41:653-61

8. Ziegler AG, Hummel M, Schenker M, Bonifacio E. Autoantibody appearance and risk for development of childhood diabetes in offspring of parents with type 1 diabetes: the 2-year analysis of the German BABYDIAB Study. *Diabetes* 1999;48:460-8.

9. Hummel S, Pflüger M, Hummel M, Bonifacio E, Ziegler AG. Primary dietary intervention study to reduce the risk of islet autoimmunity in children at increased risk for type 1 diabetes: the BABYDIET study. *Diabetes Care* 2011;34:1301-5.

10. Hummel S, Hummel M, Banholzer J, Hanak D, Mollenhauer U, Bonifacio E, Ziegler AG.. Development of autoimmunity to transglutaminase C in children of patients with type 1 diabetes: relationship to islet autoantibodies and infant feeding. Diabetologia 2007; 50:390-394.

11. Bonifacio E, Mayr A, Knopff A, Ziegler AG. Endocrine autoimmunity in families with type 1 diabetes: frequent appearance of thyroid autoimmunity during late childhood and adolescence. Diabetologia. 2009;52(2):185-92.

12. Riley WJ, Maclaren NK, Lezotte DC, Spillar RP, Rosenbloom AL. Thyroid autoimmunity in insulin-dependent diabetes mellitus: the case for routine screening. *J Pediatr.* 1981;99(3):350-4.

13. Barker JM. Clinical review: Type 1 diabetes-associated autoimmunity: natural history, genetic associations, and screening. *J Clin Endocrinol Metab* 2006;91:1210-7.

14. Triolo TM, Armstrong TK, McFann K, Yu L, Rewers MJ, Klingensmith GJ, Eisenbarth GS, Barker JM. [Additional autoimmune disease found in 33% of patients at type 1 diabetes onset.](https://www.ncbi.nlm.nih.gov/pubmed/21430083) *Diabetes Care* 2011;34:1211-3

15. Kozhakhmetova A, Wyatt RC, Caygill C, Williams C, Long AE, Chandler K, Aitken RJ1, Wenzlau JM, Davidson HW, Gillespie KM, Williams AJK. A quarter of patients with type 1 diabetes have co-existing non-islet autoimmunity: the findings of a UK population-based family study. *Clin Exp Immunol* 2018;192:251-8.

16. Parkkola A, Härkönen T, Ryhänen SJ, Uibo R, Ilonen J, Knip M; Finnish Pediatric Diabetes Register. Transglutaminase antibodies and celiac disease in children with type 1 diabetes and in their family members. *Pediatr Diabetes.* 2018;19(2):305-313.

17.Lendrum R, Nelson PG, Pyke DA, Walker G, Gamble DR. Islet-cell, thyroid, and gastric autoantibodies in diabetic identical twins. *Br Med J.* 1976;1(6009):553-5.

18. Jaeger C, Hatziagelaki E, Petzoldt R, Bretzel RG. Comparative analysis of organ-specific autoantibodies and celiac disease--associated antibodies in type 1 diabetic patients, their first-degree relatives, and healthy control subjects. *Diabetes Care* 2001;24(1):27-32.

19. Giannopoulou EZ, Winkler C, Chmiel R, Matzke C, Scholz M, Beyerlein A, Achenbach P, Bonifacio E, Ziegler AG. Islet autoantibody phenotypes and incidence in children at increased risk for type 1 diabetes. *Diabetologia* 2015;58:2317-23.

20. Achenbach P, Lampasona V, Landherr U, Koczwara K, Krause S, Grallert H, Winkler C, Pflüger M, Illig T, Bonifacio E, Ziegler AG. Autoantibodies to zinc transporter 8 and SLC30A8 genotype stratify type 1 diabetes risk. *Diabetologia* 2009;52:1881-8.

21. Lampasona V, Schlosser M, Mueller PW, Williams AJ, Wenzlau JM, Hutton JC, Achenbach P. Diabetes antibody standardization program: first proficiency evaluation of assays for autoantibodies to zinc transporter 8. *Clin Chem* 2011;57:1693-1702.

22. Schlosser M, Mueller PW, Törn C, Bonifacio E, Bingley PJ; Participating Laboratories Diabetes Antibody Standardization Program: evaluation of assays for insulin autoantibodies. *Diabetologia* 2010;53:2611-20.

23. Törn C, Mueller PW, Schlosser M, Bonifacio E, Bingley PJ; Participating Laboratories Diabetes Antibody Standardization Program: evaluation of assays for autoantibodies to glutamic acid decarboxylase and islet antigen-2. *Diabetologia* 2008;51:846-52.

24. Schenker M, Hummel M, Ferber K, Walter M, Keller E, Albert ED, Janka HU, Kastendiek C, Sorger M, Louwen F, Ziegler AG. Early expression and high prevalence of islet autoantibodies for DR3/4 heterozygous and DR4/4 homozygous offspring of parents with Type I diabetes: the German BABYDIAB study. *Diabetologia* 1999;42: 671-7.

25. Winkler C, Krumsiek J, Buettner F, Angermüller C, Giannopoulou EZ, Theis FJ, Ziegler AG, Bonifacio E. Feature ranking of type 1 diabetes susceptibility genes improves prediction of type 1 diabetes. *Diabetologia* 2014;57:2521-9.

26. Puavilai G, Chanprasertyotin S, Sriphrapradaeng A. Diagnostic criteria for diabetes mellitus and other categories of glucose intolerance: 1997 criteria by the expert committee on the diagnosis and classification of diabetes mellitus (ADA), 1998 Acta Diabetol 123 WHO consultation criteria, and 1985 WHO criteria. World Health Organization. *Diabetes Res Clin Pract* 1999;44:21-6.

27. Pociot F, Akolkar B, Concannon P, Erlich HA, Julier C, Morahan G, Nierras CR, Todd JA, Rich SS, Nerup J. Genetics of type 1 diabetes: what's next? *Diabetes.* 2010;59(7):1561-71.

28. Brorsson CA, Pociot F, and the Type 1 Diabetes Genetics Consortium. Shared genetic basis for type 1 diabetes, islet autoantibodies, and autoantibodies associated with other immune-mediated diseases in families with type 1 diabetes. *Diabetes Care* 2015;38:S8-S13.

29. Jonsdottir B, Larsson C, Carlsson A, Forsander G, Ivarsson SA, Lernmark Å, Ludvigsson J, Marcus C, Samuelsson U, Örtqvist E, Larsson HE; Better Diabetes Diagnosis Study Group. Thyroid and Islet Autoantibodies Predict Autoimmune Thyroid Disease at Type 1 Diabetes Diagnosis. *J Clin Endocrinol Metab.* 2017;102(4):1277-1285

30. Gidrewicz D, Potter K, Trevenen CL, Lyon M, Butzner JD. Evaluation of the ESPGHAN Celiac Guidelines in a North American Pediatric Population. *Am J Gastroenterol.* 2015;110(5):760-7.

31. Hansen PS, Brix TH, Iachine I, Kyvik KO, Hegedüs L. The relative importance of genetic and environmental effects for the early stages of thyroid autoimmunity. A study of healthy Danish twins. *Eur J Endocrinol* 2006;154:29–38

32. Wang B, Hawa MI, Rijsdijk FV, Fain PR, Paschou SA, Boehm BO, Steck AK, Snieder H, Leslie RD. Heritability of thyroid peroxidase autoantibody levels in type 1 diabetes: evidence from discordant twin pairs. *Diabetologia* 2015;58(9):2079-86

33. Liu E, Lee HS, Aronsson CA, Hagopian WA, Koletzko S, Rewers MJ, Eisenbarth GS, Bingley PJ, Bonifacio E, Simell V, Agardh D; TEDDY Study Group. Risk of pediatric celiac disease according to HLA haplotype and country. *N Engl J Med. 2014*;371(1):42-9

34. Jonsdottir B, Larsson C, Lundgren M, Ramelius A, Jönsson I, Larsson HE; DiPiS study Group3. Childhood thyroid autoimmunity and relation to islet autoantibodies in children at risk for type 1 diabetes in the diabetes prediction in skåne (DiPiS) study. *Autoimmunity.* 2018;51(5):228-237.

35. Hermann R, Laine AP, Veijola R, Vahlberg T, Simell S, Lähde J, Simell O, Knip M, Ilonen J. The effect of HLA class II, insulin and CTLA4 gene regions on the development of humoral beta cell autoimmunity. *Diabetologia* 2005;48(9):1766-75.

36. Holl RW, Bohm B, Loos U, Grabert M, Heinze E, Homoki J. Thyroid autoimmunity in children and adolescents with type 1 diabetes mellitus. Effect of age, gender and HLA type. *Horm Res.* 1999;52: 113-8

37. De Block CE, De Leeuw IH, Vertommen JJ, Rooman RP, Du Caju MV, Van Campenhout CM, Weyler JJ, Winnock F, Van Autreve J, Gorus FK; Belgian Diabetes Registry. Beta-cell, thyroid, gastric, adrenal and coeliac autoimmunity and HLA-DQ types in type 1 diabetes. Clin *Exp Immunol* 2001;126:236-241.

38. Weber G, Vigone MC, Stroppa L, Chiumello G. Thyroid function and puberty. *J Pediatr Endocrinol Metab* 2003;16 Suppl 2:253-7.

39. Stolovich-Rain M, Enk J, Vikesa J, Nielsen FC, Saada A, Glaser B, Dor Y. Weaning triggers a maturation step of pancreatic β cells. *Dev Cell* 2015;32(5):535-45.

40. Nguyen C, Varney MD, Harrison LC, Morahan G. Definition of high-risk type 1 diabetes HLA-DR and HLA-DQ types using only three single nucleotide polymorphisms. *Diabetes* 2013;62:2135-2140.

Table 1. Description of the study population

|  |  |
| --- | --- |
| Characteristic | Frequency or duration |
| Girls, n/total (%) | 1188/2441 (48.7%) |
| Follow-up time, median (IQR) | 13.2 (7.4-18.2) |
| T1D cases, n/total (%) | 115/2441 (4.7%) |
|  age at T1D onset, median (IQR) | 9.4 (5.0-12.7) |
| IA, TPOA, or TGA positive (n) | 394 |
|  IA positive, n/total (%) | 165/2441 (6.8%) |
|  TGA positive, n/total (%)  | 117/2395 (4.9%) |
|  TPOA positive, n/total (%) | 154/2411 (6.4%)  |
| HLA *DR3-DQ8/DR4-DQ8,* n/total (%) | 178/2290 (7.8%) |
| HLA *DR4-DQ8/DR4-DQ8,* n/total (%) | 91/2290 (4.0%) |
| HLA *DR3/3,* n/total (%) | 72/2290 (3.1%) |

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

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Variable | IA HR (95% CI) | p-value\* | TGA HR (95% CI) | p-value | TPOA HR (95% CI) | p-value | Any 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 |

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

Figure Legends

Figure 1: Cumulative risks and incidence curves of developing autoantibodies in the BABYDIAB and BABYDIET participants. Risk of (A) islet autoantibodies, IA; (B) thyroid peroxidase autoantibodies, TPOA; (C) transglutaminase autoantibodies, TGA; and (D) any of these autoantibodies. (E) Incidence (cases per 1,000 person-years) of IA (solid line), TGA (dotted line), and (TPOA, gray line). Error bars represent SE.

Figure 2: Cumulative risk of autoantibodies in relation to HLA and sex. Risks by age 20 years for IA (islet autoantibodies), TGA (transglutaminase autoantibodies) and TPOA (thyroid peroxidase autoantibodies) in males (m, blue) and females (f, red) were obtained from the Kaplan-Meier analysis. The error bars represent the 95 confidence intervals. P values compare females and males and were obtained from the log rank test.