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Accelerated progression from islet autoimmunity to diabetes is causing the escalating incidence of type 1 diabetes in young children

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ABSTRACT

The incidence of type 1 diabetes is rising worldwide, particularly in young children. Since type 1 diabetes is preceded by autoimmunity to islet antigens, there must be a consequent increase in the incidence of islet autoimmunity in young children or a more rapid rate of progression to diabetes once islet autoimmunity initiates. This study was to determine whether the incidence of islet autoimmunity or the rate of progression from autoimmunity to diabetes onset has changed over a 20-year period in children genetically predisposed to type 1 diabetes. Between 1989 and 2010, children who were first-degree relatives of patients with type 1 diabetes and who were born in Germany were prospectively followed from birth without intervention. A total of 324 children (BABYDIAB study) born between 1989 and 2000 and 216 children (TEDDY study) born between 2004 and 2010 with matched HLA genotypes were recruited before age 3 months and included for analysis. Children were followed for the development of autoantibodies to insulin, GAD, and IA-2, and for progression to diabetes. The cumulative frequency of diabetes by age 4 years was 2.5% (95% CI 0.8–4.2%) in BABYDIAB children and 6.2% (95% CI 2.3–10.1%) in TEDDY children ($p = 0.03$). The cumulative frequency of islet autoantibodies by age 4 years was similar in the children from both studies (11.3% vs 13.9%). Progression to diabetes from the development of islet autoantibodies was markedly increased in autoantibody-positive children from the more recently recruited TEDDY cohort (50% progression within 85.2 months for BABYDIAB children vs 9.6 months for TEDDY children; $p = 0.009$), also if children were further selected on the basis of high-risk HLA genotypes or the development of autoantibodies to multiple islet antigens ($p = 0.01$). The findings suggest that recent increasing incidence of type 1 diabetes in young children could be due to weakening of mechanisms that normally regulate autoimmune destruction of islet beta cells.

1. Introduction

The incidence of type 1 diabetes (T1D) is increasing worldwide. The relative annual increase varies between countries and has recently been reported to be on average 3.9% in Europe [1]. The increase is highest in children aged 1–4 years and data from Finland suggest that the increase is exponential rather than linear [2,3]. Because of the high escalating increase in young children the formerly described peak T1D incidence around puberty [4] is no longer pronounced and we are starting to observe a constant incidence from age 1 year to 15 years of age [3]. T1D is an autoimmune disorder where disease onset is preceded by a pre-clinical period of islet autoimmunity [5]. Check points in the pre-clinical pathogenesis include the initiation of islet autoimmunity measured by islet autoantibodies directed against insulin, glutamic acid decarboxylase (GAD), insulinoma-associated protein 2 (IA-2), and zinc transporter 8 (ZnT8), the spreading of the first islet autoantibody reactivity to multiple autoantigens, and eventual decline in glucose homeostasis [6]. The rate of this process is variable, with more than 50% of multiple islet autoantibody-positive

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individuals developing diabetes within 5–10 years [7,8]. With respect to the increasing T1D incidence in young age groups, it is unknown which aspects of the pre-clinical phases are affected. Increased incidence could be caused by elevation in the incidence of early autoimmunity or by faster progression to diabetes after the development of islet autoimmunity. Answers to this question will help us focus on which pre-clinical check points should be investigated in the search for causes and mechanisms of immune mediated beta cell destruction, and the rising incidence of T1D in early childhood. Here we have taken advantage of 20 years of prospective follow-up for the development of islet autoimmunity and diabetes in children in Germany to address this question. The findings suggest that increasing early diabetes incidence is mediated by mechanisms affecting progression from autoimmunity to disease.

2. Materials and methods

2.1. Participants

Children included in the analysis are first-degree relatives of T1D patients and have participated in the prospective BABYDIAB study (recruitment at birth from 1989 to 2000; [9]) and The Environmental Determinants of Diabetes in the Young (TEDDY) study (recruitment at birth from 2004 to 2010; [10]). The BABYDIAB study was conducted exclusively in Germany, whereas the TEDDY study is conducted in multiple countries, including Germany [9,10]. The recruitment sources within Germany for first-degree relatives who participate in BABYDIAB and TEDDY were similar and cover the whole of Germany. The BABYDIAB study followed children of parents with T1D regardless of HLA genotype, whereas TEDDY had eligibility criteria based on HLA DRB1 and DQB1 genotype [10]. Thus, for analysis, children were matched for HLA genotype by selecting the BABYDIAB children with the 10 TEDDY-eligible HLA genotypes (Fig. 1). Overall, 216 first-degree relatives with eligible HLA genotypes were enrolled into the TEDDY study between 2004 and 2010, including 193 offspring (89.4%) and 23 siblings (10.6%) of T1D patients. Of the 1650 enrolled BABYDIAB children, 324 had TEDDY-eligible HLA genotypes (Fig. 1). The distribution of these 10 genotypes was similar between the TEDDY children and the 324 BABYDIAB children (Electronic supplementary material [ESM] Table 1). TEDDY children were followed intensively with 3-monthly blood samples until age 4 years and 6-monthly samples thereafter. BABYDIAB children had blood samples taken at age 9 months, 2, 5, 8, 11, 14, 17 and 20 years, and once children developed islet autoantibodies, at 6-monthly intervals. Coordination of the BABYDIAB and TEDDY studies in Germany was performed in the Diabetes Research Institute in Munich through direct contact with the families and the family pediatrician. The study was carried out in accordance with the Helsinki Declaration. All families gave written informed consent to participate in the BABYDIAB study and the TEDDY study. Both studies were approved by the ethical committee of Bavaria, Germany (Bayerische Landesärztekammer Nr. 95357 and 04089).

2.2. Outcome measures

Autoantibodies to insulin (IAA), GAD (GADA), and IA-2 (IA-2A) were measured in all samples. Autoantibodies in the BABYDIAB samples were measured in the Munich central laboratory. For TEDDY samples, measurement was performed in the central European laboratory in Bristol and when positive also in the US central laboratory in Denver. The three laboratories use similar IAA, GADA and IA-2A assays and have been shown to have similar sensitivity and specificity in the Diabetes Autoantibody Standardization Program (DASP) workshops [11,12], and for GADA and IA-2A also in NIDDK harmonization activities [13]. Autoantibody outcome in BABYDIAB children was persistent autoantibodies (positive in at least 2 consecutive samples). Outcome in TEDDY

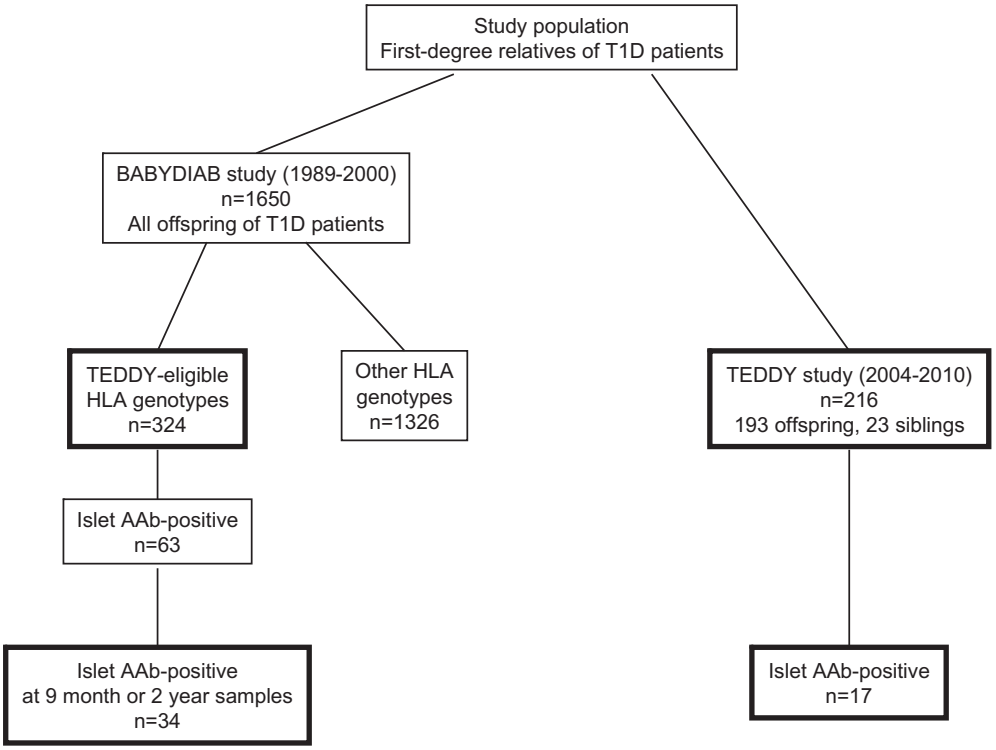

Fig. 1. Flow chart of study populations. Heavy boxed categories were analyzed for islet autoantibody (AAb) development or diabetes development.

children was persistent autoantibodies (positive in the European central laboratory in at least 2 consecutive samples). Since February 2010, TEDDY central laboratories used the harmonized assay for the measurement of GADA and IA-2A [13]. All islet autoantibody-positive TEDDY children also met criteria for positivity in the harmonized assay. For both studies, islet autoantibody outcome was also considered positive if the child developed diabetes prior to obtaining a follow-up sample. The age of the first positive sample was taken as the age of islet autoantibody development.

In both BABYDIAB and TEDDY studies, families with islet autoantibody-positive children were asked to report occurrence of symptoms of diabetes; regular testing for diabetes (every 6–12 months) was performed from the age of 3 years in islet autoantibody-positive children in the form of fasting blood glucose and oral glucose tolerance testing. Diabetes onset was defined according to ADA criteria which include unequivocal hyperglycemia with acute metabolic decompensation, or the observation on at least two occasions of (1) a 2-h plasma glucose >200 mg/dl after an oral glucose challenge, or (2) a random blood glucose >200 mg/dl if accompanied by unequivocal symptoms [14]. Since 1997, a fasting blood glucose >126 mg/dl on two occasions was also included as a diabetes diagnosis criterion in both studies.

2.3. Data analysis

Statistical analyses of diabetes development, islet autoantibody development, and progression to diabetes from first autoantibody-positive sample were performed using life-table method and compared by log rank test. The Mann–Whitney *U* test was used to compare continuous variables between groups. For all analyses, a two-tailed *p*-value of 0.05 was considered significant. Statistical analyses were performed using the GraphPad Prism 3 program (GraphPad Software, Inc., San Diego, CA, USA) and the Statistical Package for Social Science (SPSS 18.0; SPSS, Chicago, IL, USA).

3. Results and discussion

Seventeen of the TEDDY children and 63 of the BABYDIAB children developed islet autoantibodies, and 9 and 34 developed typical T1D that was acute onset and has required insulin treatment since diagnosis. A higher cumulative prevalence of diabetes up to age 4 years was observed in the TEDDY children (6.2%; 95% CI 2.3–10.1%) as compared to the BABYDIAB children (2.5%; 95% CI 0.8–4.2%; $p = 0.03$; Fig. 2a), consistent with observed increasing trends in this age group in Europe. This increase was not accompanied by an increase in the cumulative prevalence of islet autoantibodies, which was overlapping in children from both studies (Fig. 2b). The cumulative autoantibody risk was 11.3% (95% CI 7.8–14.8%) in BABYDIAB and 13.9% (95% CI 7.3–20.5%) in TEDDY at 4 years of age.

Concordant with these data, the autoantibody-positive TEDDY children showed a rapid progression to diabetes from when they were first islet autoantibody-positive (Fig. 3a). A 50% cumulative progression to diabetes was observed within 9.6 months. This is markedly faster than observations in other studies of islet autoantibody-positive first-degree relatives [7,15–18]. In order to compare the progression rate to diabetes between the autoantibody-positive TEDDY and BABYDIAB children, we selected all the 17 autoantibody-positive TEDDY children (median antibody-positive age 16.2 months, range 10.1–26.2 months) and the 34 BABYDIAB children who developed their islet autoantibodies at their 9 month or 2 year sample (median antibody-positive age 21.6 months, range 10.5–25.7 months; $p = 0.7$ vs TEDDY). This selection was added to avoid age bias in the two groups since we have previously found that age at first autoantibody positivity was associated with the rate of progression to diabetes [19]. In contrast to the TEDDY children, but similar to progression rates in previous studies [7,15,17,18], 50% progression to diabetes in the antibody-positive BABYDIAB children was 85 months ($p = 0.009$ vs TEDDY children, Fig. 3a). This difference was also observed when the children were stratified into those with the very high-risk HLA DR3/4 and DR4/4 genotypes (Fig. 3b) and those with other risk genotypes (Fig. 3c), and if only children who developed multiple islet autoantibodies were included in the analysis ($p = 0.01$). Median time from initial autoantibody-appearance to diabetes onset in the TEDDY children was 0.5 years (interquartile range 0.2–0.8 years) as compared to 4.7 (2.0–7.5) years in the BABYDIAB children ($p = 0.0004$, ESM Fig. 1). These data suggest, therefore, that within similarly selected cohorts of at risk neonates in a relatively homogeneous Caucasian population, there has been

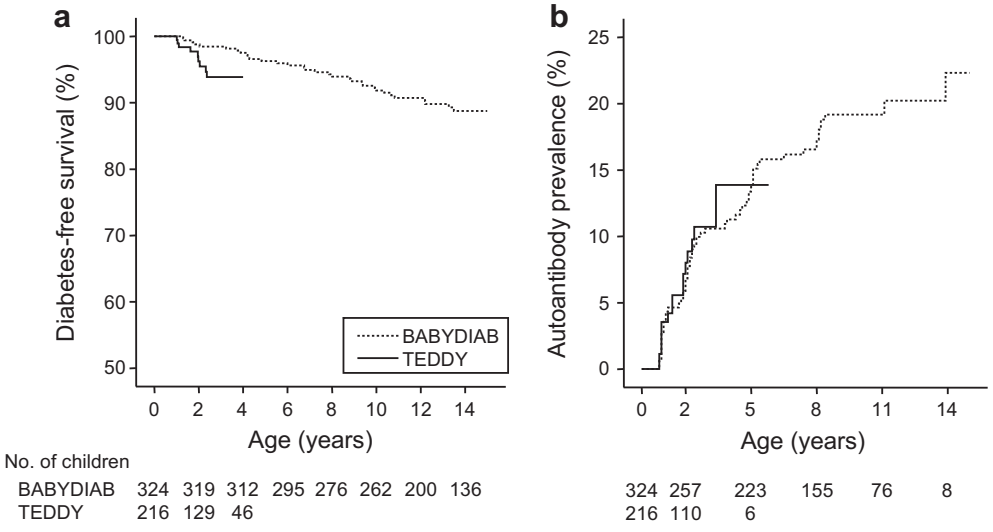

Fig. 2. Progression to diabetes and islet autoantibody development in children at T1D risk. (a) Observed cumulative progression to diabetes among children from the TEDDY study was significantly faster than the progression among children up to age 4 years from the BABYDIAB study ($p = 0.03$). (b) In contrast, cumulative risk for the development of autoantibodies to insulin, GAD or IA-2 within the first years of life was identical between the children from both studies. Numbers below the *x*-axis indicate the number of diabetes-free children (a) or autoantibody-negative children (b) remaining on follow-up with respect to age.

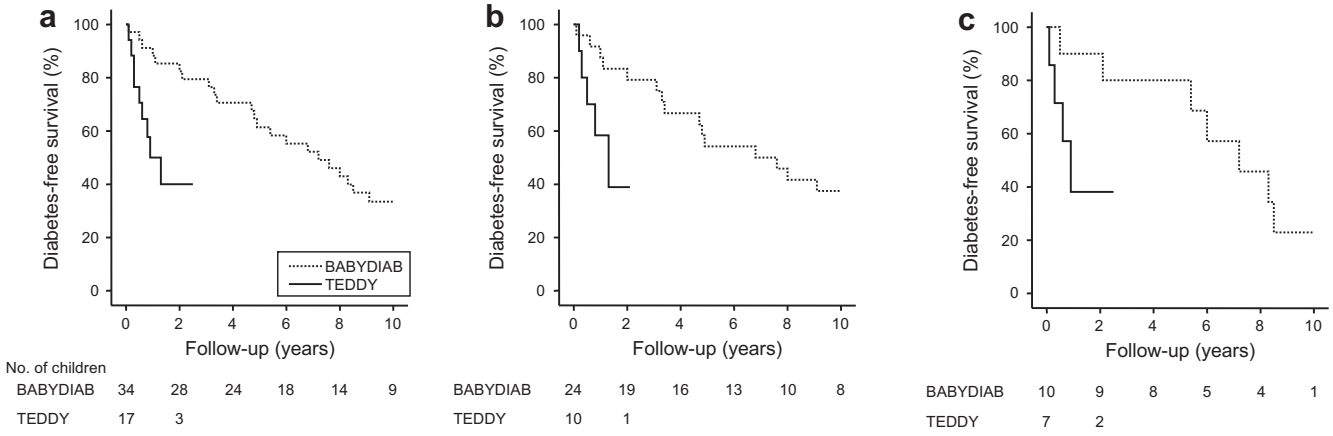

Fig. 3. Progression to diabetes in islet autoantibody-positive children. (a) Among children who developed autoantibodies to insulin, GAD or IA-2, cumulative progression to diabetes was significantly faster in the TEDDY study compared to the BABYDIAB study ($p = 0.009$). The different rates of progression were independent of whether children carried the high-risk HLA DR3/4 or 4/4 DQ8 genotypes (b) or other T1D-associated HLA genotypes (c). Children were followed from the first autoantibody-positive sample. Numbers below the *x*-axis indicate the number of diabetes-free children remaining on follow-up.

a marked increase in the progression rate from autoimmunity to diabetes in recent years.

Further data from the TEDDY and BABYDIAB study support this. Examining the cumulative frequency of the IAA, GADA and IA-2A showed that the development of IAA was similar between the TEDDY and BABYDIAB children (ESM Fig. 2a) but GADA and IA-2A, which have been considered later pre-clinical markers in children, developed 6–12 months earlier in the TEDDY children (ESM Fig. 2b and c).

In trying to understand the meaning of the data, we first wanted to be convinced that the observations were not simply artifacts due to differences between the two studies. We expect that there is unlikely to be a selection bias in the children included for analysis since they were all first-degree relatives of T1D patients and the HLA genotype distribution in both groups was similar. All children were born in Germany and there were no regional or ethnical differences between the two groups of children. Monitoring for the development of islet autoantibodies is more frequent in children in the TEDDY study compared to children in the BABYDIAB study. However, this should bias results toward a later detection of islet autoantibodies in the BABYDIAB study (i.e. closer to diabetes onset) and therefore cannot account for the observation of faster progression in the TEDDY autoantibody-positive children. Islet autoantibodies were measured in different laboratories in the two studies. Thus, a bias toward more sensitive and/or less specific detection of autoantibodies could be present due to assay differences. Both laboratories use very similar methods and have similar sensitivities and specificities in international workshops [11–13]. Islet autoantibody positivity was confirmed in the US central laboratory in 16 of the 17 TEDDY autoantibody-positive children (one child had insufficient sample for confirmation). Moreover, almost all children who had a positive islet autoantibody outcome within the age range of 0–4 years in the two studies eventually developed multiple islet autoantibodies and/or diabetes (15 of 17 TEDDY children, and all 34 BABYDIAB children) indicating that the autoantibody-positive outcomes in both studies were unlikely to contain an influential number of false-positive results. Diabetes onset was acute in the TEDDY children and all children have required insulin treatment since diagnosis suggesting that it is unlikely that the markedly faster progression in TEDDY children is due to improved early diabetes detection. Finally, the findings in the autoantibody-positive children in the TEDDY study are so striking with respect to their rapid progression to diabetes that bias would need to markedly select for rapid progression in the TEDDY group and we expect this to be improbable. Thus, we are convinced that the observations are real and represent a change in the natural history of T1D at young age in Germany over the last 2 decades.

The findings are limited to cases with a family history of T1D and may not be representative of all childhood T1D. Nevertheless, the results suggest that one reason for the increasing incidence of T1D in children is a faster progression from the initial insult that causes islet autoimmunity to near complete beta cell destruction at diabetes onset in at risk children. This could be due to increased magnitude or duration of the insult that causes islet autoimmunity, heightened immune response, or weaker mechanisms of regulation of the immune response. It is possible that the factors that cause these effects are encountered prior to immunization as for example what has been suggested for Bacillus Calmette–Guerin vaccination [20,21], at, or after immunization. An interplay between genetics and environment is likely as recently discussed [22] and suggested by gene-associated effects of cow's milk [23]. Although we do not have concrete insights into what these factors are, the findings highlight the need to examine the etiology of T1D as the etiology of islet autoimmunity and the etiology of progression to disease onset since the factors that influence one are not necessarily the same as those that will influence the other.

In conclusion, the data suggest that mechanisms regulating progression from autoimmunity to diabetes appear more likely to be responsible for the rising early T1D incidence than those initiating autoimmunity. Furthermore, they point to the substantial differences in diabetes progression rate between individuals as an opportunity to discover causes of disease and opportunities for intervention to delay or prevent disease.

Role of the funding source

None of the funding sources have been involved in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

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Appendix. Supplementary material

Supplementary material related to this article can be found online at doi:10.1016/j.jaut.2011.02.004.

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