Predictors of the Initiation of Islet Autoimmunity, Progression to Multiple Autoantibodies and Clinical Diabetes: The TEDDY Study

Running Title: Seroconversion, Multiple Autoantibodies and Type 1 Diabetes

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Abbreviations

CI	confidence intervals
FDR	first degree relative
GADA	glutamic acid decarboxylase autoantibodies

GP	general population
HLA	human leukocyte antigen
HR	hazard ratio
IA	islet autoimmunity
IAA	insulin autoantibodies
IA-2A	insulinoma antigen-2 autoantibodies
IQR	interquartile range
PH	proportional hazard

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Abstract

Objective: To distinguish among predictors of seroconversion, progression to multiple autoantibodies and from multiple autoantibodies to type 1 diabetes in young children.

Research Design and Methods: Genetically high-risk newborns (n=8502) were followed for a median of 11.2 y (IQR 9.3-12.6 y); 835 (9.8%) developed islet autoantibodies and 283 (3.3%) were diagnosed with type 1 diabetes. Predictors were examined using Cox proportional hazard models.

Results: Predictors of seroconversion and progression differed, depending on the type of first appearing autoantibody. Male sex, Finnish residence, having a sibling with type 1 diabetes, the HLA DR4 allele, probiotic use before age 28 days, and SNP rs689_A (INS) predicted seroconversion to IAA-first. Increased weight at 12 months and SNPs rs12708716_G (CLEC16A) and rs2292239_T (ERBB3) predicted GADA-first. Having a father with type 1 diabetes, the SNPs rs2476601_A (PTPN22) and rs3184504_T (SH2B3) predicted both. Younger age at seroconversion predicted progression from single to multiple autoantibodies as well as progression to diabetes, except for those presenting with GADA-first. Family history of type 1 diabetes and the HLA DR4 allele predicted progression to multiple autoantibodies, but not diabetes. Sex did not predict progression to multiple autoantibodies, but males progressed more slowly than females from multiple autoantibodies to diabetes. SKAP2 and MIR3681HG SNPs are newly reported to be significantly associated with progression from multiple autoantibodies to type 1 diabetes.

Conclusions: Predictors of IAA-first vs GADA-first autoimmunity differ from each other and from the predictors of progression to diabetes.

Introduction

The detection of multiple islet autoantibodies, recognized as the initial stage of type 1 diabetes (1), is usually preceded by presence of a single autoantibody. Some individuals are diagnosed with diabetes with a single autoantibody; others may progress to multiple autoantibodies, but not to clinical diabetes, over an extended period of time. Progression depends on the age at seroconversion and the number of autoantibodies present (2-11). A family history of type 1 diabetes and genetic factors are less predictive (3, 9,11) of progression than they are of seroconversion..

Multiple reports describe different patterns of islet autoantibody initial presentation, subsequent spreading and their relationship to diabetes onset (12,13). There seems to be a number of factors that contribute to the initiation of islet autoimmunity (IA) and affect the risk of progression through to type 1 diabetes. One of the factors is which islet autoantibody that appears first (4, 13). This paper focuses on the identification of predictors for the detection of a first autoantibody (seroconversion from autoantibody negative to autoantibody positive), the progression from single to multiple autoantibodies and from multiple autoantibodies to type 1 diabetes in The Environmental Determinants of Diabetes in the Young (TEDDY) study. The hypothesis is that there are different predictors at each step, that class II HLA genes are more related to initiation of seroconversion than in progression to diabetes once islet autoantibodies are manifest. Differences in the significance of other predictors may shed light on environmental exposures or gene-environment interactions which are not uniform across disease stages.

Methods

Participants. TEDDY is a prospective cohort study funded by the National Institutes of Health with the primary goal to identify environmental causes of type 1 diabetes. It includes six clinical research centers - three in the U.S.: Colorado, Georgia/Florida, and Washington State and three in Europe: Finland, Germany, and Sweden. Detailed study design and methods have been previously published (14-16). Written informed consents were obtained for all study participants from a parent or primary caretaker, separately, for genetic screening and participation in the prospective follow-up.

The high-risk HLA genotypes for participants screened from the general population were as follows: DRB1*04-DQA1*03-DQB1*03:02/DRB1*03-DQA1*05-DQB1*02:01 (DR3/4), DRB1*04-DQA1*03-DQB1*03:02/DRB1*04-DQA1*03-DQB1*03:02 (DR4/4), DRB1*04-DQA1*03-DQB1*03:02/DRB1*08-DQA1*04-DQB1*04:02 (DR4/8) and DRB1*03-DQA1*05-DQB1*02:01/DRB1*03-DQA1*05-DQB1*02:01 (DR3/3). Additional genotypes were included for first degree relatives (FDRs) of a subject with type 1 diabetes: DRB1*04-DQA1*03-DQB1*03:02/DRB1*04- DQA1*03-DQB1*02:02 (DR4/4b), DRB1*04-DQA1*03-DQB1*03:02/DRB1*01- DQA1*01-DQB1*05:01 (DR4/1), DRB1*04-DQA1*03-DQB1*03:02/DRB1*13-DQA1*01-DQB1*06:04 (DR4/13), DRB1*04-DQA1*03-DQB1*03:02/DRB1*09- DQA1*03-DQB1*03:03 (DR4/9), and DRB1*03-DQA1*05-DQB1*02:01/DRB1*09- DQA1*03-DQB1*03:03 (DR3/9). The HLA-DR-DQ genotype abbreviations shown in parentheses will be used throughout this paper. Genotyping was confirmed by reverse blot hybridization at the central HLA Reference Laboratory at Roche Molecular Systems, Oakland, CA (16), along with the *INS*-23Hph1 (rs689), *CTLA4* T17A (rs231775) and *PTPN22* R620W (rs2476601) SNP primer pairs. The study was approved by local Institutional Review or Ethics Boards and is monitored by an External Evaluation Committee formed by the National Institutes of Health.

SNP genotyping was performed by the Center for Public Health Genomics at the University of Virginia, using the Illumina Immunochip which is a custom array for genotyping of SNPs selected from regions of the human genome firmly associated with autoimmune diseases (17). The final selection of SNPs containing approximately 186,000 SNPs in 186 regions, for 12 autoimmune diseases was decided by the Immunochip Consortium. TEDDY previously examined whether any of 41 non-HLA SNPs previously shown to be associated with type 1 diabetes conferred risk for islet autoimmunity (IA) (18).

Islet Autoantibodies. Islet autoantibodies to insulin (IAA), glutamic acid decarboxylase (GADA) or insulinoma antigen-2 (IA-2A) were measured in two laboratories by radio binding assays. In the U.S., all sera were assayed at the Barbara Davis Center for Childhood Diabetes at the University of Colorado Denver; in Europe, all sera were assayed at the University of Bristol, U.K. Both laboratories demonstrated high sensitivity and specificity as well as concordance (19). All positive islet autoantibody samples and 5% of the negative samples were re-tested in the other reference laboratory and deemed confirmed if concordant. Persistent islet autoimmunity was defined as confirmed positive IAA, GADA or IA-2A in at least two consecutive samples.

Statistical Methods. Time-to-event analyses using multivariable Cox proportional hazards (PH) models were applied to examine factors related to the risk of each disease stage leading to clinical diabetes: development of islet autoantibody (IA) positivity, progression from a single to multiple autoantibodies, and progression from multiple autoantibodies to type 1 diabetes. Separate cause-specific PH models for competing risks were used to study the risk (cause-specific hazard ratios) of

GADA-first and IAA-first, respectively by censoring IA events from other causes at the event time [20]. The magnitude of the association was described by hazard ratios (HR) with 95% confidence intervals (CI).

IA positivity was defined as confirmed positive autoantibodies to GADA, IAA, or IA-2A in at least two consecutive samples by both TEDDY laboratories. The time to the development of IA was the age at the initial of two or more consecutive positive tests. Children negative for IA were right censored at the date of the last negative sample drawn for autoantibodies.

Progression from a single to multiple autoantibodies was defined as the development of a second IA in single IA positive children. The time-to-event variable was the duration calculated from the onset date of the first IA to the onset date of a second IA or right censored for children without multiple autoantibodies at the date of the last negative sample drawn for the other autoantibodies.

Similarly, progression from multiple autoantibodies to type 1 diabetes was defined as the development of type 1 diabetes in multiple IA positive children. The time-to-event variable was the duration calculated from the date at onset of multiple IA to the diagnosis date of type 1 diabetes or right censored for those who did not develop type 1 diabetes by the date of the last TEDDY visit.

Covariates

The factors under examination included those previously published by TEDDY (21, 22). In addition, nineteen SNPs were included in the multivariable Cox models. Accounting for population stratification (ancestral heterogeneity) were made by including the top two principal components calculated from the ImmunoChip data as covariates in the Cox models. (23) The nineteen SNPs were identified from forty-five SNPs in type 1 diabetes risk loci (rs689 in INS, forty-one SNPs exmined by TEDDY, and three SNPs recently available in TEDDY (rs11755527 in BACH2, rs12444268, rs917997)) by forward selection procedures with Cox regression of them on each of the

risks of IA, IAA-first, GADA-first, progression from a single to multiple autoantibodies, and progression from multiple autoantibodies to type 1 diabetes, with inclusion criteria of p-value < 0.05. (11,18)

Age at seroconversion was normalized using a log-transformation and then included as a covariate in the Cox analyses of progression from a single to multiple autoantibodies, and from multiple autoantibodies to type 1 diabetes.

Constant risks were assumed in order to compare the average annual hazard rate of development of IA, IAA-first IA, GAD-first IA, progression from single autoantibody to multiple autoantibodies and progression from multiple autoantibodies to type 1 diabetes stratified by follow-up time period (≤ 2 years and > 2 years). (24)

Data were analyzed using the Statistical Analysis System software (version 9.4; SAS Institute, Cary, NC). Two-tailed p-values less than 0.05 were considered to be statistically significant. No adjustment in type 1 error was made for multiple comparisons except in the context of the multivariable Cox regression model.

Results

The TEDDY study has enrolled and followed, from three months of age, a cohort of 8676 infants at elevated genetic risk for autoimmune type 1 diabetes; 174 children were excluded due to HLA ineligibility or indeterminate autoantibody status, leaving 8502 in the analysis. Children were followed quarterly for a first-appearing islet autoantibody and progression to diagnosis of diabetes. Follow up of children with one or more islet autoantibodies continued on this schedule, whereas children who were autoantibody negative were followed semiannually after 4 years of age. The

median (interquartile range, IQR) age at the last follow up was 11.2 (9.3-12.6) years (range 2 months to 15.3 years).

As of February 29, 2020, 835 children (9.8%) have developed islet autoantibodies; 701 with a single autoantibody (308 IAA, 370 GADA, 23 IA-2) and 134 had multiple autoantibodies at the first detection. Of the 701, who developed a single autoantibody first, 346 (49.4%) progressed to multiple autoantibodies and 189 (54.6%) of these progressed to diagnosis of diabetes. Of those with multiple autoantibodies at seroconversion, 94 (70.1%) progressed to diabetes. (Figure 1). Omitted are 40 children who were diagnosed with diabetes, but had no autoantibodies detected; most had limited follow-up prior to dropping out of the study. An additional 42 children were excluded with a single autoantibody (24 IAA, 13 GADA, and 5 IA-2) who were diagnosed with diabetes without having multiple autoantibodies observed.

The proportion of individuals seroconverting to IAA-first (31.2%) as compared to GADA-first (18.6%) was significantly higher among those from Finland than in other sites (p=0.0001), among siblings of children with type 1 diabetes (7.5% vs. 2.7%, p=0.004), among those with DR4/8 (20.5% vs. 12.2%, p=0.003) or FDR specific genotypes (4.5% vs. 1.4%, p=0.012) as compared with other groups. Only children with a DR3/3 genotype (22.2% vs. 9.7%, p=0.00001) and those from Sweden had a significantly higher proportion of children seroconverting to GADA-first (38.1%) as compared to IAA-first (30.5%, p=0.039) (Table 1).

First appearance of IA (Table 2)

Predictors for the appearance of IA differed depending on the type of first appearing autoantibody. Males, as compared to females were at higher risk for IA (HR=1.30, 95%CI=1.13, 1.49, p<0.001) and this was among those presenting with IAA-first (HR=1.35, 95%CI=1.07,1.70, p=0.013). The IAA-first-related association was also found with respect to increased IA risk when there was a diabetes affected sibling (HR=5.06, 95%CI=3.22,7.96, p<0.001), DR4 allele as compared to DR3/3 (HRs ranging from 2.10 to 2.68, all p<=0.001), individuals from Finland as compared to the US (HR=2.19, 95%CI=1.47,3.28, p<0.001), the protective effects of the introduction of probiotics within the first 28 days of life (HR=0.48, 95%CI=0.28,0.84, p=0.010), and the SNPs rs689_A (INS (HR=0.58, 95%CI=0.45,0.73, p<0.001) and rs2327832_G (TNFAIP3) (HR=0.76, 95%CI=0.61,0.95, p=0.015). The SNP rs7202877_C (CTRB2) (HR=1.28, 95%CI=1.01,1.61, p=0.039) was associated with increased IAA risk.

Increased weight z-score at 12 months was a predictor (HR=1.17, 95%CI=1.05,1.30, p=0.003), as were the SNPs rs12708716_G (CLEC16A) (HR=0.81, 95%CI=0.69,0.95, p=0.010), and rs11258747_A (PRKCQ) (HR=1.19, 95%CI=1.00,1.41, p=0.045) for GADA-first. The SNPs rs2292239_T (ERBB3) (HR=1.17, 95%CI=1.00,1.36, p=0.054), rs11755527_C (BACH2) (HR=1.15, 95% CI=1.00,1.33, p=0.056) and rs7804356_G (SKAP2) (HR=0.83, 95%CI=0.69,1.00, p=0.054),were only marginally significant for GADA-first. The FDR-specific genotypes included in the cohort were at significantly lower risk as compared to those with the DR3/3 genotype (HR=0.016, 95%CI=0.06,0.46, p=0.001).

Having a father with type 1 diabetes (as compared to the general population) was a predictor (HR=2.41, 95%CI=1.87,3.11, p<0.001) as were the SNPs rs2476601_A (PTPN22) (HR=1.44,

95%CI=1.15,1.80, p=0.002, HR=1.42, 95%CI=1.15,1.76, p=0.001),) and rs3184504_T (SH2B3) (HR=1.27, 95%CI=1.08,1.50, p=0.005, HR=1.34, 95%CI=1.15,1.56, p<0.001), for IA for both IAA-first and GADA-first appearing autoantibodies, respectfully. Four SNPs, rs763361_A(CD226) (HR=1.13, 95%CI=1.02,1.25, p=0.016), rs1990760_G(IFIH1) (HR=0.88, 95%CI=0.79,0.97, p=0.014), rs11203203_A(UBASH3A) (HR=1.12, 95%CI=1.01,1.24, p=0.039) and rs4948088_A(COBL) (HR=0.74, 95%CI=0.57,0.97, p=0.031), were significant for IA but not for IAA-first nor GADA-first.

Progression from Single to Multiple Autoantibodies and Multiple Autoantibodies to type 1 diabetes (Table 3)

Among those who seroconverted to a single autoantibody, there was no discernable difference in a multivariate proportional hazards model of the rate of progression to multiple autoantibodies between those who seroconverted to IAA-first vs. GADA-first (HR=1.00, 95%CI= 0.77,1.29, p=0.99). Those who initially presented with multiple autoantibodies were at higher risk (HR=1.91, 95%CI=1.42,2.56, p<0.001) of progressing to diagnosis of diabetes than those with only a single autoantibody. Increasing age at either initial seroconversion or progression from single to multiple autoantibodies was significantly associated with a lower rate of progression to multiple autoantibodies (HR=0.65, 95%CI=0.57,0.75, p<0.001) or type 1 diabetes (HR=0.62, 95%CI=0.51,0.75, p<0.001), respectively. A father with type 1 diabetes (HR=1.42, 95%CI=1.00,2.01, p=0.049) or a sibling (HR=1.73, 95%CI=1.05,2.85, p=0.031) elevated risk (compared to those without first-degree relative with type 1 diabetes) for progressing from single to multiple autoantibodies, but not diabetes. All the DR 4 allele genotypes were significant predictors as compared to the DR3/3 genotype for progressing from single to multiple autoantibodies (HR

ranging from 1.80 to 2.63, 0.001<p<0.021), but none were significantly related to progression from multiple autoantibodies to type 1 diabetes. The HLA predictors for progression to multiple autoantibodies were significant only in those who first presented with IAA, but not GADA, with the exception of the DR3/4 and DR4/4 vs. DR3/3, which were significant for both, and those with a father with type 1 diabetes. Notably, male sex imparted a lower risk (HR=0.63, 95%CI=0.49,0.83, P=0.021) for progressing from multiple autoantibodies to type 1 diabetes, even though sex was not significantly associated with progression from single to multiple autoantibodies. Five SNPs, 2 with reduced risk and 3 with increased risk, were significant diabetes risk factors; although none were significant for the development of autoantibodies or progression from single to multiple autoantibodies: rs1004446_A (INS) HR=0.81 (95% CI=0.65,1.00, p=0.053), rs1534422_G (MIR3681HG) HR=1.32 (95%CI=1.09,1.60, p=0.005), rs2327832_G (TNFAIP3) HR=1.40 (95% CI=1.12,1.74, p=0.003), rs3825932_A (CTSH) HR=0.76 (95% CI=0.62, 0.93, p=0.007), and rs7804356_G (SKAP2) HR=1.26 (95% CI=1.01,1.58, p=0.043).

The Effect of Age

The effect of increasing age at seroconversion was highly statistically significantly associated with a decrease in the risk of seroconverting (and the type of first appearing autoantibody), progression from single to multiple autoantibodies and progression from multiple autoantibodies to type 1 diabetes. Yet, progression from multiple autoantibodies to type 1 diabetes was not related to the age of seroconversion when the first autoantibody at seroconversion was GADA (HR=0.99, 95%CI=0.62,1.57, p=0.97), but it was when the first appearing autoantibody was IAA (HR=0.45, 95%CI=0.31,0.67, p<0.001) or when the initial seroconversion was to multiple autoantibodies (HR=0.44, 95%CI=0.28,0.68, p<0.001). Additionally, a closer inspection (Supplemental Figures

S1,S2) reveals that among those children who have not progressed within 2 years, the age effect was substantially reduced. That is, the hazard rate in the first 2 years of age or progression from single to multiple autoantibodies was significantly higher in the first 2 years of follow up, than it was during the remainder follow up period (Table 4), with the exception of the risk of developing GADA as the first appearing autoantibody and the risk of progressing from multiple autoantibodies to type 1 diabetes.

Discussion

Type 1 diabetes is a chronic disease characterized by the loss of functional pancreatic islet beta-cells. Appearance of islet autoantibodies precede the clinical disease for a highly variable time from months to more than 15 years . In 2015, it was proposed that the disease is a continuum of identifiable stages prior to the clinical diagnosis of diabetes (1). Stage 1 was defined as the presence of two or more islet autoantibodies with normoglycemia, stage 2 as two or more islet autoantibodies with dysglycemia and stage 3 as onset of clinical disease. Clinical intervention would be needed to prevent subsequent morbidity and mortality. It has also been noted that at-risk individuals progress through these stages at different rates, determined in part by the age at which seroconversion to autoantibody positivity occurred and the number of autoantibodies present (25-29). TEDDY (20) and TrialNet (10) have shown that some individuals who seroconvert to a single autoantibody will progress to having multiple autoantibodies and have subsequent increased type 1 diabetes risk. The type 1 diabetes risk in those who do not progress to multiple autoantibodies remains elevated as compared those who have not seroconverted, as reported by TEDDY and elsewhere (30). Predictors for the initial occurrence of autoantibodies and for the progression to type 1 diabetes differ.

First, although family history of type 1 diabetes and HLA (at least among the high-risk genotypes included in TEDDY) are highly related to the initial seroconversion and progression to multiple autoantibodies, a major finding is that this association is lost with progression from multiple autoantibodies (Stage 1 disease) to type 1 diabetes (Stage 3 disease). It is notable that the family history of type 1 diabetes as a predictor for progression from single to multiple autoantibodies is related to whether the affected family member is a sibling (for IAA appearing first) or a father (for either IAA or GADA appearing first). It has long been known that the risk for type 1 diabetes is eight times higher if a sibling has the disease compared to a fivefold increase when the father is affected. The present observation may suggest that this epidemiological observation may be explained by two different etiologies, one related to IAA-first and the other to GADA-first.

Second, the age at seroconversion is a consistent predictor throughout, although for those whose first autoantibody was GADA, age is not significantly associated with progression to type 1 diabetes. An important finding is that the risk of type 1 diabetes among those with multiple autoantibodies decreases with increasing age of initial seroconversion. These results confirm earlier findings in the German BABYDIAB study (2), and the Finnish DIPP study (3,4). These studies were not limited to the TEDDY HLA-defined high-risk population and the results combined therefore underline the importance of considering the type of initially appearing autoantibody and the age at development of multiple autoantibodies when evaluating predictors for progression to diagnosis of diabetes. One possible explanation may be related to the very infrequent appearance of IAA as the first appearing autoantibody after age 2 and the relatively constant seroconversion to GADA after 5 years of age (11,13). It remains to be seen whether this trend remains for those developing multiple autoantibodies at older ages, since there are other reports (7) that describe the decreasing type 1

diabetes risk with increasing age. With the caveats that TEDDY is a young population and follow-up is limited, it is clear, however, that the type 1 diabetes risk through the first two decades of life declines overall with increasing age at seroconversion and increasing time from seroconversion.

This study is not without its limitations. The relationship between predictors, age and the progression through different type 1 diabetes stages might not be generalizable to other HLA-defined populations, even though we did not observe that HLA was associated with progression to diabetes diagnosis in multiple autoantibody positive children. Despite this study's size of 8502 children, the age range reported herein is still limited and there are still relatively few type 1 diabetes cases observed in the TEDDY cohort.

Yet, the TEDDY cohort represents, depending on country, about 40-50% of children expected to develop diabetes before 18 years of age (16). The age at screening for the presence of islet autoantibodies in both the general population or among FDR and its associated heterogeneity with respect to diabetes predictors (5,6) should be considered when subjects are to be enrolled in secondary prevention studies. It is of importance to know that about 10% of the TEDDY children had an FDR, a proportion consistent with the proportion of FDR among population-based children and adolescents newly diagnosed with type 1 diabetes (31,32). The association between non -HLA genetic factors and the risk for a first appearing autoantibody, first observed after 6 (20,13) as well as 9 (11) years of follow up, underscore the need for gene-environment interaction analyses as INS polymorphism remained associated (protective) with IAA-first, but ERBB3_T does not, with an additional five years of follow up. Conversely, CLEC16A was previously reported to be associated with IA and is now shown to related to a protective effect on GADA-first. PTPN22 and SH2B3

remained associated with increased risk for either one. The INS gene polymorphism may be related to preproinsulin expression in the thymus and thereby affect central tolerance (33). CLEC16A regulates mitophagy and controls beta-cell function (34). PTPN22 and SH2B3 polymorphisms are both associated with a number of autoimmune disorders including type 1 diabetes (35,36). A number of significant associations, not previously reported in TEDDY, have been found for IA (CD226, IFIH1, UBASH3A), IAA-first (CTRB2), GADA-first (PRKCQ), single to multiple autoantibodies (SKAP2), sometimes specific to whether there was IAA-first (MIR3681HG and rs12444268). SKAP2 and MIR3681HG were newly reported to be significantly associated with progression from multiple autoantibodies to type 1 diabetes. CTSH and TNFAIR3 had previously reported to be significiantly associated with progression to type 1 diabetes and remained significantly associated in this analysis.

Finally, caution should be exercised in interpreting statistically significant findings due to the number of comparisons that have been made (inflating the Type 1 error). Additionally, the HRs reported herein with respect to IAA or GADA-first appearance are all derived separately from cause-specific models and assume independence of the event type. While some findings reported are novel, perhaps reflecting the increasing age of the cohort, others are confirmed in independently reported studies with different population, lending strength to their credence. The continuous follow up of the TEDDY children expanding the longitudinal observations with more observed cases of seroconversion and type 1 diabetes suggest confirmation of those relationships that have remained consistent after 6 (21,13), 9 (11) and now 12 years of follow up. Adjusting the significance level for multiple comparisons when conducting epidemiological research, especially in the context of a multivariate analysis has both supporters (37) and detractors (38,39). No matter what side of the

argument the reader falls on, the associations reported herein should be viewed in the larger context of the results of other studies and other populations to be properly interpreted.

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Data availability

The datasets generated and analyzed during the current study will be made available in the NIDDK Central Repository at https://repository.niddk.nih.gov/studies/teddy.

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Electronic supplementary material: A complete list of the members of the TEDDY Study Group can be found in the online version of this article.

References

1. Richard A. Insel, Jessica L. Dunne, Mark A. Atkinson, Jane L. Chiang, Dana Dabelea, Peter A. Gottlieb, Carla J. Greenbaum, Kevan C. Herold, Jeffrey P. Krischer, Ake Lernmark, Robert E. Ratner, Marian J. Rewers, Desmond A. Schatz, Jay S. Skyler, Jay M. Sosenko, and Anette-G. Ziegler: Staging Presymptomatic Type 1 Diabetes: A Scientific Statement of JDRF, the Endocrine Society, and the American Diabetes Association. *Diabetes Care* 2015; 38:1964-1974.

2. Hummel M, Bonifacio E, Schmid S, et al. Brief communication: early appearance of islet autoantibodies predicts childhood type 1 diabetes in offspring of diabetic parents. Ann Intern Med 2004;140:882-886.

3. Bauer W, Veijola R, Lempainen J, et al. Age at Seroconversion, HLA Genotype and Specificity of Autoantibodies in Progression of Islet Autoimmunity in Childhood. J Clin Endocrinol Metab 2019;104:4521-4530

4. Ilonen J, Hammais A, Laine AP, et al. Patterns of β-cell autoantibody appearance and genetic associations during the first years of life. Diabetes 2013;62:3636-40

5. Frederiksen BN, Kroehl M, Barón A, et al. Assessing age-related etiologic heterogeneity in the onset of islet autoimmunity. Biomed Res Int 2015;2015:708289

6. Xu P, Krischer JP; Type 1 Diabetes TrialNet Study Group. Prognostic Classification Factors Associated With Development of Multiple Autoantibodies, Dysglycemia, and Type 1 Diabetes-A Recursive Partitioning Analysis. Diabetes Care 2016;39:1036-44

7. Cantor AB; Krischer JP; Cuthbertson DD; Schatz DA; Riley WJ; Malone J; Schwartz S; Quattrin T; Maclaren NK: Age and Family Relationship Accentuate the Risk of Insulin Dependent Diabetes Mellitus (IDDM) in Relatives of Patients with IDDM. *J Clin Endocrinol Metab* 80(12):3739-3743, 1995.

8. 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: 309:2473-79.

9. Krischer JP, Liu X, Vehik K, Akolkar B, Hagopian WA, Rewers MJ, She JX, Toppari J, Ziegler AG, Lernmark Å; TEDDY Study Group. Predicting Islet Cell Autoimmunity and Type 1 Diabetes: An 8-Year TEDDY Study Progress Report. *Diabetes Care*. 2019 Jun;42(6):1051-1060.

10. Bingley P, Boulware D, Krischer J, and the Type 1 Diabetes TrialNet Study Group. The implications of autoantibodies to a single islet antigen in relatives with normal glucose tolerance: development of other autoantibodies and progression to type 1 diabetes. *Diabetologia*. 2016. 59: 542-549. doi:10.1007/s00125-015-3830-2.

11. Krischer JP, Liu X, Lernmark Å, Hagopian WA, Rewers MJ, She JX, Toppari J, Ziegler AG, Akolkar B, and the TEDDY Study Group. The Influence of Type 1 Diabetes Genetic Susceptibility Regions, Age, Sex, and Family History to the Progression from Multiple Autoantibodies to Type 1 Diabetes: A TEDDY Study Report. Diabetes. 2017;66:3122-3129

12. Vehik K, Bonifacio E, Lernmark Å, Yu L, Williams A, Schatz D, Rewers M, She JX, Toppari J, Hagopian W, Akolkar B, Ziegler AG, Krischer JP; TEDDY Study Group. Hierarchical Order of Distinct Autoantibody Spreading and Progression to Type 1 Diabetes in the TEDDY Study. Diabetes Care. 2020 Sep;43(9):2066-2073.

13. Krischer JP, Lynch KF, Lernmark Å, Hagopian WA, Rewers MJ, She JX, Toppari J, Ziegler AG, Akolkar B; TEDDY Study Group. Genetic and Environmental Interactions Modify the Risk of Diabetes-Related Autoimmunity by 6 Years of Age: The TEDDY Study. Diabetes Care 2017; 40:1194-1202

14. TEDDY Study Group. The Environmental Determinants of Diabetes in the Young (TEDDY) study: study design. Pediatr Diabetes 2007; 8:286-298

15. TEDDY Study Group. The Environmental Determinants of Diabetes in the Young (TEDDY) study. Ann N Y Acad Sci 2008; 1150:1-13

16. Hagopian WA, Erlich H, Lernmark Å, et al.; TEDDY Study Group. The Environmental Determinants of Diabetes in the Young (TEDDY): genetic criteria and international diabetes risk screening of 421 000 infants. Pediatr Diabetes 2011; 12:733-743

17. Parkes M, Cortes A, van Heel DA, Brown MA. Genetic insights into common pathways and complex relationships among immune-mediated diseases. Nat Rev Genet 2013;14:661-673

18. Törn C, Hadley D, Lee HS, Hagopian W, Lernmark Å, Simell O, Rewers M, Ziegler A, Schatz D, Akolkar B, Onengut-Gumuscu S, Chen WM, Toppari J, Mykkänen J, Ilonen J, Rich SS, She JX, Steck AK, Krischer J; TEDDY Study GroupTEDDY Study Group. Role of type 1 diabetes associated SNPs on risk of autoantibody positivity in the TEDDY study. Diabetes 2015;64:1818-1829

19. Bonifacio E, Yu L, Williams AK, et al. Harmonization of glutamic acid decarboxylase and islet antigen-2 autoantibody assays for national institute of diabetes and digestive and kidney diseases consortia. J Clin Endocrinol Metab 2010; 95:3360-3367

20. Klein, J.P., van Houwelingen, H.C., Ibrahim, J.G., & Scheike, T.H. (Eds.). (2013). Competing Risks. In Handbook of Survival Analysis (1st ed.). Chapman and Hall/CRC.

21. 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 May;58(5):980-7.

22. Krischer JP, Lynch KF, Lernmark Å, Hagopian WA, Rewers MJ, She JX, Toppari J, Ziegler AG, Akolkar B; TEDDY Study Group. Genetic and Environmental Interactions Modify the Risk of Diabetes-Related Autoimmunity by 6 Years of Age: The TEDDY Study. Diabetes Care 2017; 40:1194-1202

23. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D: Principal components analysis corrects for stratification in genome-wide association studies. Nature Genet 2006;38:904-909

24. Kalbfleisch, J.D. and Prentice, R.L. (2002). Chapter 2 Failure Time Models. In The Statistical Analysis of Failure Time Data (eds J.D. Kalbfleisch and R.L. Prentice), John Wiley and Sons, New York

25. Bauer, Witold , Veijola Riitta , Lempainen Johanna , Kiviniemi Minna , Härkönen Taina , Toppari Jorma , Knip Mikael , Gyenesei Attila , Ilonen Jorma . Age at Seroconversion, HLA Genotype, and Specificity of Autoantibodies in Progression of Islet Autoimmunity in Childhood. J Clin Endocrinol Metab 2019 Oct 1;104(10):4521-4530. doi: 10.1210/jc.2019-00421.

26. Pöllänen Petra M , Ryhänen Samppa J , Toppari Jorma , Ilonen Jorma , Vähäsalo Paula , Veijola Riitta , Siljander Heli , Knip Mikael Dynamics of Islet Autoantibodies During Prospective

Follow-Up From Birth to Age 15 Years J Clin Endocrinol Metab 2020 Dec 1;105(12):e4638-e4651. doi: 10.1210/clinem/dgaa624.

27. Bonifacio E, Weiß A, Winkler C, Hippich M, Rewers MJ, Toppari J, Lernmark Å, She JX, Hagopian WA, Krischer JP, Vehik K, Schatz DA, Akolkar B, Ziegler AG; TEDDY Study Group. An Age-Related Exponential Decline in the Risk of Multiple Islet Autoantibody Seroconversion During Childhood. Diabetes Care. 2021 Feb 24:dc202122. doi: 10.2337/dc20-2122. Online ahead of print.PMID: 33627366

28. Hoffmann VS, Weiß A, Winkler C, Knopff A, Jolink M, Bonifacio E, Ziegler AG. Landmark models to define the age-adjusted risk of developing stage 1 type 1 diabetes across childhood and adolescence. BMC Med. 2019 Jul 9;17(1):125. doi: 10.1186/s12916-019-1360-3.PMID: 31286933

29. Emanuele Bosi, Susan Geyer, Jay Sosenko, Dorothy J. Becker, Manuela Battaglia, Heba M. Ismail, Mark A. Atkinson, Louis H. Philipson, Jerry P. Palmer And Carmella Evans-Molina Metabolic Markers Associated with Increased Risk for Progression from Single to Multiple Autoantibodies in Type 1 Diabetes Relatives
Diabetes 2018 Jul; 67

30. Anand Vibha , Li Ying , Liu Bin , Ghalwash Mohamed , Koski Eileen , Ng Kenney , Dunne Jessica L. , Jönsson Josefine , Winkler Christiane , Knip Mikael , Toppari Jorma , Ilonen

Jorma , Killian Michael B. , Frohnert Brigitte I. , Lundgren Markus , Ziegler Anette-Gabriele , Hagopian William , Veijola Riitta , Rewers Marian for the T1DI Study Group. Islet Autoimmunity and HLA Markers of Presymptomatic and Clinical Type 1 Diabetes: Joint Analyses of Prospective Cohort Studies in Finland, Germany, Sweden, and the U.S. Diabetes Care 2021 Jun; dc201836.

31. Cowie CC, Casagrande SS, Menke A, Cissell MA, Eberhardt MS, Meigs JB, Gregg EW, Knowler WC, Barrett-Connor E, Becker DJ, Brancati FL, Boyko EJ, Herman WH, Howard BV, Narayan KMV, Rewers M, Fradkin JE, editors. Diabetes in America. 3rd ed. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases (US); 2018 Aug. PMID: 33651524.

32. Patterson CC, Karuranga S, Salpea P, Saeedi P, Dahlquist G, Soltesz G, Ogle GD. Worldwide estimates of incidence, prevalence and mortality of type 1 diabetes in children and adolescents: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. 2019 Nov;157:107842.

33. Anjos S, Polychronakos C. Mechanisms of genetic susceptibility to type I diabetes: beyond HLA. Mol Genet Metab. 2004 Mar;81(3):187-95. doi: 10.1016/j.ymgme.2003.11.010. PMID: 14972324.

34. Schuster Cornelia , Gerold Kay D , Schober Kilian , Probst Lilli , Boerner Kevin , Kim Mi-Jeong , Ruckdeschel Anna , Serwold Thomas , Kissler Stephan. The Autoimmunity-Associated Gene

CLEC16A Modulates Thymic Epithelial Cell Autophagy and Alters T Cell Selection Immunity 2015 May 19;42(5):942-52. doi: 10.1016

35. Chaouali Marwa,Fernandes Veronica,Ghazouani Ezzedine Pereira Luisa Kochkar Radhia Association of *STAT4*, *TGFβ1*, *SH2B3* and *PTPN22* polymorphisms with autoimmune hepatitis Experimental and Molecular Pathology 2018, 105:279-284

36. Lee YH, Rho YH, Choi SJ, Ji JD, Song GG, Nath SK, Harley JB. The PTPN22 C1858T functional polymorphism and autoimmune diseases-a meta-analysis. *Rheumatology* 2007; 46: 49–56.

37. Thompson JR. Invited commentary: Re: "Multiple comparisons and related issues in the interpretation of epidemiologic data." Am J Epidemiol 1998;147:801-806

38. Savitz DA and Olshan AF, Multiple Comparisons and Related Issues in the Interpretation of Epidemiologic Data. Am J Epidemiol 1995;142:904-908

39. Savitz DA and Olshan AF, Describing data requires no adjustment for multiple comparisons: a reply from Savitz and Olshan. Am J Epidemiol 1998;147:813-814

Table 1. Characteristics of TEDDY children

Characteristic		No IA (n=7667)	IA overall (n=835)	IAA-first (n=308)	GADA-first (n=370)	Multiple ab+ (n=480)	Type 1 diabetes (n=283)
Age at onset (yrs.) mean (SD)		NA	4.45 (3.39)	3.38 (3.20)	5.40 (3.39)	4.35 (3.13)	6.52 (3.46)
Age at onset (yrs.) median (IQR)		NA	3.32 (1.57, 7.15)	2.03 (1.03, 4.57)	4.79 (2.35, 8.29)	3.31 (1.82, 6.47)	6.43 (3.30, 9.26)
Country	US	3339 (43.6)	287 (34.4)	98 (31.8)	141 (38.1)	162 (33.8)	94 (33.2)
	Finland	1603 (20.9)	201 (24.1)	96 (31.2)	69 (18.6)	128 (26.7)	82 (29.0)
	Germany	514 (6.7)	60 (7.2)	20 (6.5)	19 (5.1)	39 (8.1)	27 (9.5)
	Sweden	2211 (28.8)	287 (34.4)	94 (30.5)	141 (38.1)	151 (31.5)	80 (28.3)
Family History	FDR: Mother	298 (3.9)	41 (4.9)	12 (3.9)	17 (4.6)	27 (5.6)	18 (6.4)
	FDR: Father	368 (4.8)	86 (10.3)	32 (10.4)	40 (10.8)	60 (12.5)	35 (12.4)
	FDR: Sibling	111 (1.4)	40 (4.8)	23 (7.5)	10 (2.7)	26 (5.4)	19 (6.7)
	General Population	6890 (89.9)	668 (80.0)	241 (78.2)	303 (81.9)	367 (76.5)	211 (74.6)
Sex	Female	3813 (49.7)	378 (45.3)	137 (44.5)	172 (46.5)	214 (44.6)	136 (48.1)
	Male	3854 (50.3)	457 (54.7)	171 (55.5)	198 (53.5)	266 (55.4)	147 (51.9)
HLA Genotype	DR3/4	2915 (38.0)	403 (48.3)	144 (46.8)	181 (48.9)	261 (54.4)	162 (57.2)
	DR4/4	1506 (19.6)	155 (18.6)	57 (18.5)	57 (15.4)	98 (20.4)	52 (18.4)
	DR4/8	1340 (17.5)	128 (15.3)	63 (20.5)	45 (12.2)	64 (13.3)	35 (12.4)
	DR3/3	1662 (21.7)	120 (14.4)	30 (9.7)	82 (22.2)	37 (7.7)	20 (7.1)
	FDR Specific*	244 (3.2)	29 (3.5)	14 (4.5)	5 (1.4)	20 (4.2)	14 (4.9)

*FDR-specific HLA-DR-DQ genotypes are DR4/4b, DR4/1, DR4/13, DR4/9, and DR3/9.
Data are presented as number (percentage) unless otherwise indicated.

Table 2. Association between factors and risk of developing islet autoimmunity (IA), IAA-first and GADA-first. Adjusted (cause-specific) hazard ratios (HR) were estimated from multivariable proportional hazards models.

		IA (n=701)		IAA-first IA (n=308)		GADA-first IA (n=370)	
Factor	**Comparison**	**HR (95% CI)**	**p**	**HR (95% CI)**	**p**	**HR (95% CI)**	**p**
Sex	**Male vs. Female**	1.30 (1.13,1.49)	<.001	1.35 (1.07,1.70)	0.013	1.22 (0.99,1.50)	0.066
Family history	**FDR father vs. GP**	2.41 (1.87,3.11)	<.001	2.23 (1.45,3.42)	<.001	2.95 (2.06,4.22)	<.001
	FDR sibling vs. GP	2.96 (2.11,4.14)	<.001	5.06 (3.22,7.96)	<.001	1.60 (0.82,3.12)	0.172
	FDR mother vs. GP	1.30 (0.92,1.84)	0.141	1.01 (0.53,1.90)	0.985	1.40 (0.82,2.39)	0.218
HLA genotype	**DR3/4 vs. DR3/3**	1.86 (1.51,2.29)	<.001	2.61 (1.73,3.93)	<.001	1.26 (0.96,1.64)	0.091
	DR4/4 vs. DR3/3	1.40 (1.10,1.79)	0.007	2.10 (1.33,3.32)	0.001	0.77 (0.54,1.09)	0.134
	DR4/8 vs. DR3/3	1.41 (1.09,1.83)	0.010	2.68 (1.69,4.24)	<.001	0.79 (0.54,1.16)	0.225
	FDR Specific vs. DR3/3	0.73 (0.46,1.15)	0.178	1.62 (0.80,3.27)	0.178	0.16 (0.06,0.46)	0.001
Country	**Finland vs. US**	1.53 (1.20,1.96)	0.001	2.19 (1.47,3.28)	<.001	1.04 (0.72,1.51)	0.829
	Germany vs. US	1.01 (0.74,1.40)	0.935	1.07 (0.63,1.81)	0.813	0.63 (0.36,1.11)	0.110
	Sweden vs. US	1.19 (0.99,1.43)	0.060	1.21 (0.88,1.65)	0.239	1.16 (0.90,1.50)	0.257
Probiotics introduction age	**< 28 days vs. ≥ 28 days**	0.66 (0.48,0.91)	0.010	0.48 (0.28,0.84)	0.010	0.73 (0.45,1.20)	0.215
Weight z-score at 12 months		1.14 (1.06,1.23)	<.001	1.12 (0.99,1.25)	0.062	1.17 (1.05,1.30)	0.003
rs689_A (INS)		0.73 (0.64,0.84)	<.001	0.58 (0.45,0.73)	<.001	1.00 (0.83,1.21)	0.997
rs2476601_A (PTPN22)		1.42 (1.23,1.63)	<.001	1.44 (1.15,1.80)	0.002	1.42 (1.15,1.76)	0.001

rs3184504_T (SH2B3)		1.26 (1.14,1.40)	<.001	1.27 (1.08,1.50)	0.005	1.34 (1.15,1.56)	<.001
rs12708716_G (CLEC16A)		0.85 (0.76,0.94)	0.002	0.88 (0.74,1.05)	0.165	0.81 (0.69,0.95)	0.010
rs2292239_T (ERBB3)		1.17 (1.05,1.30)	0.004	1.14 (0.96,1.35)	0.140	1.17 (1.00,1.36)	0.054
rs763361_A (CD226)		1.13 (1.02,1.25)	0.016	1.10 (0.93,1.29)	0.264	1.09 (0.94,1.26)	0.262
rs1990760_G (IFIH1)		0.88 (0.79,0.97)	0.014	0.90 (0.76,1.07)	0.236	0.86 (0.74,1.01)	0.063
rs11203203_A (UBASH3A)		1.12 (1.01,1.24)	0.039	1.11 (0.93,1.32)	0.245	1.09 (0.93,1.27)	0.305
rs4948088_A (COBL)		0.74 (0.57,0.97)	0.031	0.70 (0.44,1.11)	0.126	0.86 (0.59,1.25)	0.425
rs2327832_G (TNFAIP3)		0.92 (0.81,1.04)	0.169	0.76 (0.61,0.95)	0.015	0.91 (0.76,1.10)	0.330
rs7202877_C (CTRB2)		1.14 (0.98,1.32)	0.082	1.28 (1.01,1.61)	0.039	1.12 (0.89,1.41)	0.324
rs11258747_A (PRKCQ)		1.09 (0.97,1.23)	0.127	1.16 (0.96,1.39)	0.127	1.19 (1.00,1.41)	0.045
rs12251307_A (RBM17, IL2RA)		0.85 (0.73,1.00)	0.049	0.91 (0.71,1.18)	0.481	0.77 (0.60,0.98)	0.036
rs11755527_C (BACH2)		1.03 (0.93,1.14)	0.551	0.88 (0.75,1.04)	0.146	1.15 (1.00,1.33)	0.056
rs7804356_G (SKAP2)		0.95 (0.84,1.07)	0.402	0.99 (0.81,1.20)	0.903	0.83 (0.69,1.00)	0.054
rs12444268_A		0.97 (0.87,1.08)	0.551	0.96 (0.80,1.14)	0.620	0.94 (0.80,1.11)	0.489
rs1534422_G (MIR3681HG)		0.99 (0.90,1.10)	0.911	1.03 (0.87,1.21)	0.752	0.98 (0.85,1.14)	0.821
rs3825932_A (CTSH)		0.93 (0.84,1.03)	0.179	0.90 (0.76,1.07)	0.218	1.03 (0.88,1.21)	0.688
rs1004446_A (INS)		0.93 (0.83,1.05)	0.231	0.95 (0.79,1.15)	0.616	0.91 (0.77,1.08)	0.296

PC1*		0.97 (0.87,1.07)	0.499	1.03 (0.86,1.24)	0.760	0.94 (0.82,1.09)	0.423
PC2*		1.16 (1.05,1.29)	0.003	1.22 (1.03,1.45)	0.023	1.11 (0.97,1.28)	0.120

***PC1 and PC2 were the top two principal components estimated from the ImmunoChip data.**

Table 3. Association between factors and risk of progression from single autoantibody to multiple autoantibodies and progression from multiple autoantibodies to type 1 diabetes. Adjusted hazard ratios (HR) were estimated from multivariable proportional hazards models.

		Single to multiple autoantibodies						Multiple ab+ to type 1 diabetes	
		Overall		Subgroup by autoantibody at seroconversion				Overall	
		n/N=346/701		IAA-first IA (n/N=167/308)		GADA-first IA (n/N=168/370)		n/N=283/480	
Factor	**Comparison**	**HR (95% CI)**	**p**	**HR (95% CI)**	**p**	**HR (95% CI)**	**p**	**HR (95% CI)**	**p**
Autoantibody at seroconversion	**IA2A vs. GADA**	1.12 (0.57,2.22)	0.736						
	IAA vs. GADA	1.00 (0.77,1.29)	0.992						
Initial ab	**Multiple vs. Single ab+**							1.91 (1.42,2.56)	<.001
Age (yr) at seroconversion*		0.65 (0.57,0.75)	<.001	0.63 (0.51,0.77)	<.001	0.64 (0.52,0.80)	<.001	0.62 (0.51,0.75)	<.001
Sex	**Male vs. Female**	1.04 (0.82,1.31)	0.752	1.04 (0.72,1.51)	0.823	0.89 (0.63,1.26)	0.505	0.63 (0.49,0.83)	0.001
Family history	**FDR father vs. GP**	1.42 (1.00,2.01)	0.049	1.52 (0.91,2.54)	0.111	1.35 (0.80,2.27)	0.260	0.99 (0.65,1.51)	0.970
	FDR sibling vs. GP	1.73 (1.05,2.85)	0.031	1.97 (1.01,3.86)	0.047	0.97 (0.32,2.97)	0.958	1.36 (0.77,2.39)	0.290
	FDR mother vs. GP	1.50 (0.83,2.71)	0.182	1.81 (0.70,4.65)	0.219	1.37 (0.60,3.12)	0.460	1.55 (0.88,2.74)	0.127
HLA genotype	**DR3/4 vs. DR3/3**	2.63 (1.75,3.94)	<.001	5.42 (1.66,17.67)	0.005	2.44 (1.52,3.92)	<.001	1.12 (0.66,1.89)	0.680
	DR4/4 vs. DR3/3	2.28 (1.44,3.60)	<.001	5.82 (1.74,19.48)	0.004	1.84 (1.03,3.30)	0.041	1.00 (0.56,1.79)	0.987
	DR4/8 vs. DR3/3	1.80 (1.09,2.96)	0.021	3.93 (1.17,13.22)	0.027	1.93 (0.95,3.93)	0.070	0.89 (0.48,1.67)	0.724
	FDR Specific vs. DR3/3	2.09 (0.98,4.47)	0.057	4.74 (1.16,19.40)	0.030	3.82 (0.80,18.18)	0.092	1.37 (0.59,3.16)	0.458
Country	**Finland vs. US**	1.29 (0.84,1.97)	0.246	1.62 (0.89,2.94)	0.113	1.22 (0.61,2.40)	0.575	1.02 (0.63,1.67)	0.931

	Germany vs. US	0.69 (0.40,1.18)	0.177	0.75 (0.36,1.56)	0.447	0.71 (0.27,1.90)	0.496	1.24 (0.72,2.14)	0.432
	Sweden vs. US	0.69 (0.52,0.93)	0.014	0.74 (0.46,1.18)	0.202	0.69 (0.46,1.05)	0.081	0.82 (0.59,1.16)	0.266
rs689_A (INS)		0.70 (0.56,0.88)	0.002	0.83 (0.58,1.18)	0.292	0.62 (0.44,0.86)	0.005	1.05 (0.81,1.36)	0.704
rs2476601_A (PTPN22)		1.31 (1.05,1.63)	0.017	1.08 (0.79,1.49)	0.631	1.79 (1.30,2.47)	<.001	1.15 (0.88,1.51)	0.307
rs3184504_T (SH2B3)		0.98 (0.84,1.15)	0.809	0.91 (0.71,1.17)	0.467	1.08 (0.85,1.38)	0.531	1.02 (0.85,1.22)	0.834
rs12708716_G (CLEC16A)		0.88 (0.73,1.05)	0.157	0.80 (0.59,1.07)	0.126	0.98 (0.74,1.29)	0.864	1.19 (0.97,1.45)	0.095
rs2292239_T (ERBB3)		1.12 (0.94,1.34)	0.212	1.08 (0.80,1.46)	0.633	1.12 (0.87,1.44)	0.397	1.08 (0.89,1.31)	0.444
rs763361_A (CD226)		0.86 (0.73,1.01)	0.070	0.84 (0.67,1.07)	0.158	1.00 (0.78,1.29)	1.000	1.12 (0.92,1.35)	0.265
rs1990760_G (IFIH1)		0.99 (0.83,1.18)	0.928	1.22 (0.93,1.59)	0.145	0.87 (0.67,1.12)	0.282	1.12 (0.92,1.37)	0.255
rs11203203_A (UBASH3A)		1.19 (1.01,1.40)	0.041	1.01 (0.77,1.33)	0.930	1.35 (1.06,1.72)	0.014	1.05 (0.87,1.27)	0.613
rs4948088_A (COBL)		0.83 (0.52,1.33)	0.440	1.01 (0.47,2.20)	0.977	0.77 (0.41,1.45)	0.418	1.27 (0.77,2.09)	0.343
rs2327832_G (TNFAIP3)		0.91 (0.74,1.13)	0.419	0.81 (0.58,1.12)	0.199	0.98 (0.70,1.37)	0.904	1.40 (1.12,1.74)	0.003
rs7202877_C (CTRB2)		1.00 (0.79,1.27)	0.999	0.96 (0.68,1.34)	0.805	1.13 (0.78,1.64)	0.512	1.13 (0.86,1.47)	0.382
rs11258747_A (PRKCQ)		1.10 (0.92,1.33)	0.298	1.14 (0.86,1.51)	0.365	1.04 (0.80,1.36)	0.749	0.96 (0.78,1.18)	0.713

rs12251307_A (RBM17, IL2RA)		0.68 (0.51,0.90)	0.007	0.81 (0.54,1.22)	0.314	0.61 (0.39,0.94)	0.026	1.03 (0.77,1.39)	0.839
rs11755527_C (BACH2)		1.11 (0.94,1.30)	0.224	1.44 (1.12,1.84)	0.004	0.92 (0.72,1.19)	0.546	0.85 (0.70,1.03)	0.101
rs7804356_G (SKAP2)		0.87 (0.71,1.07)	0.189	0.92 (0.68,1.23)	0.574	0.72 (0.52,0.98)	0.038	1.26 (1.01,1.58)	0.043
rs12444268_A		1.25 (1.05,1.50)	0.014	1.45 (1.09,1.92)	0.010	1.08 (0.83,1.40)	0.557	0.88 (0.71,1.08)	0.219
rs1534422_G (MIR3681HG)		0.90 (0.76,1.06)	0.192	0.73 (0.56,0.94)	0.016	0.91 (0.71,1.16)	0.428	1.32 (1.09,1.60)	0.005
rs3825932_A (CTSH)		0.92 (0.78,1.08)	0.306	0.83 (0.65,1.06)	0.139	1.01 (0.80,1.29)	0.907	0.76 (0.62,0.93)	0.007
rs1004446_A (INS)		0.91 (0.75,1.09)	0.304	0.85 (0.64,1.13)	0.263	1.09 (0.82,1.46)	0.554	0.81 (0.65,1.00)	0.053
PC1†		1.17 (0.95,1.45)	0.137	1.03 (0.78,1.36)	0.838	1.42 (0.99,2.03)	0.059	0.77 (0.60,0.99)	0.044
PC2†		1.19 (0.98,1.45)	0.075	1.30 (1.04,1.63)	0.024	1.10 (0.78,1.54)	0.597	0.94 (0.75,1.19)	0.604

*** Age at seroconversion was log-transformed.**

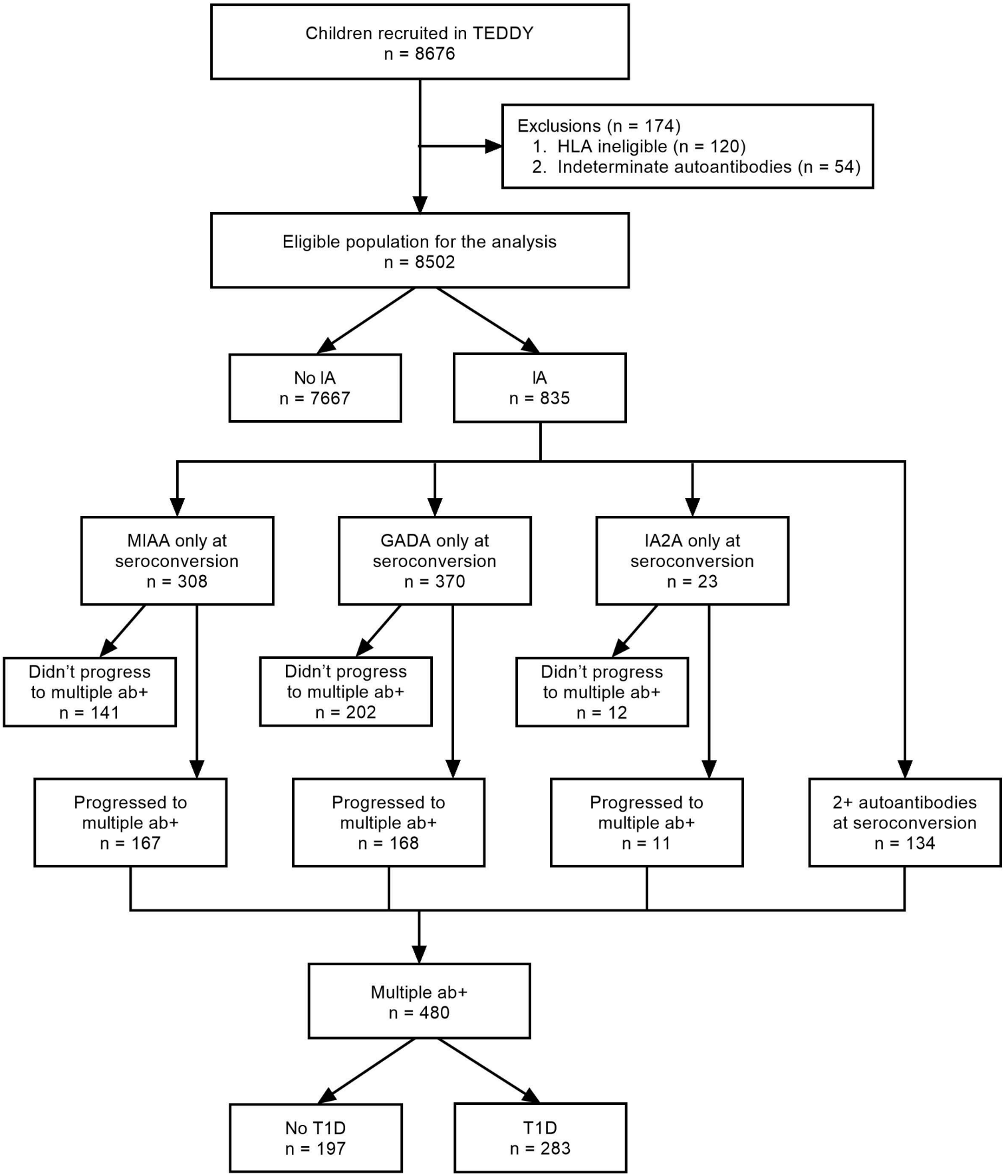
† PC1 and PC2 were the top two principal components estimated from the ImmunoChip data.

Table 4. Annual hazard rate of development of IA, IAA-first IA, GAD-first IA, progression from single autoantibody to multiple autoantibodies and progression from multiple autoantibodies to type 1 diabetes stratified by follow-up time period (≤ 2 years and > 2 years) assuming exponential survival distribution.

	Annual hazard rate (95% CI) stratified by follow-up time period		P-value
	0 - 2 years	**> 2 years**	
Development of IA	0.018 (0.016, 0.020)	0.011 (0.010,0.012)	<0.001
Development of IAA-first IA	0.010 (0.008,0.012)	0.0030 (0.0026, 0.0036)	<0.001
Development of GADA-first IA	0.0046 (0.0036, 0.0058)	0.0058 (0.0052, 0.0066)	0.07
Progression from single to multiple autoantibodies	0.30 (0.27, 0.34)	0.05 (0.04, 0.07)	<0.001
Progression from multiple autoantibodies to type 1 diabetes	0.12 (0.10, 0.15)	0.14 (0.12, 0.16)	0.32

Figure legends:

Figure 1. TEDDY study population.

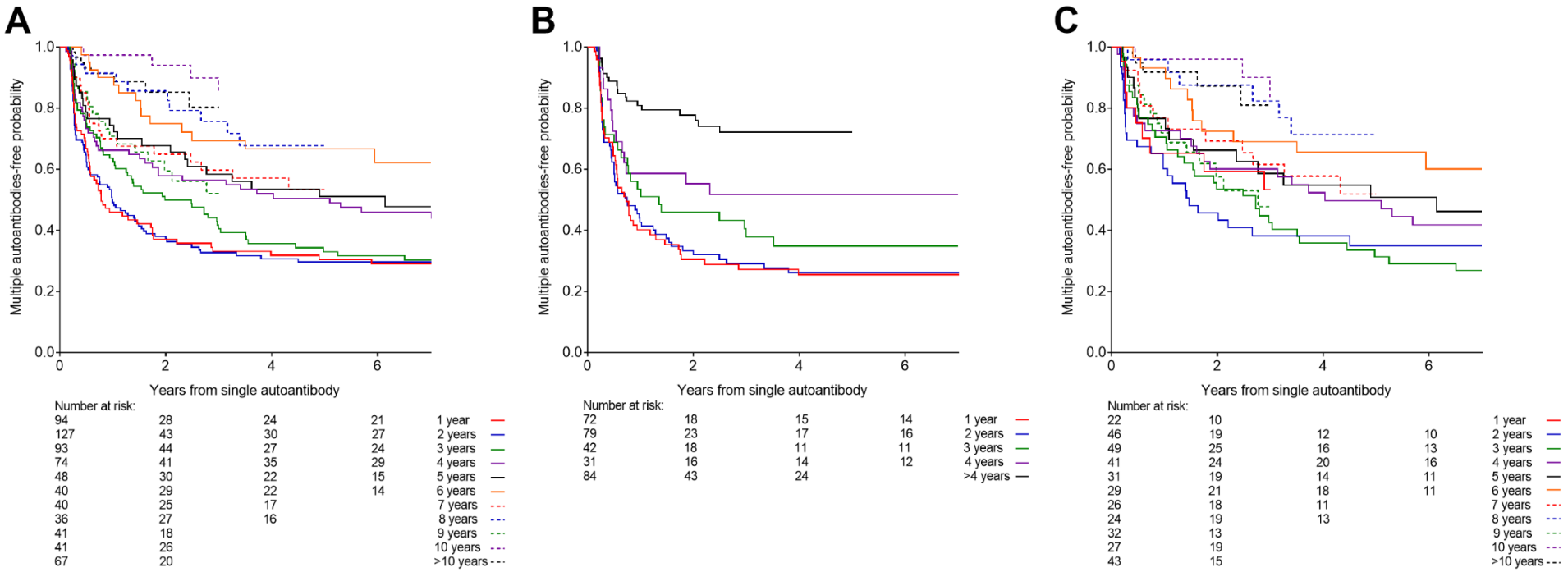

Children recruited in TEDDY
n = 8676
Exclusions (n = 174)
1. HLA ineligible (n = 120)
2. Indeterminate autoantibodies (n = 54)
Eligible population for the analysis
n = 8502
No IA
n = 7667
IA
n = 835
MIAA only at seroconversion
n = 308
GADA only at seroconversion
n = 370
IA2A only at seroconversion
n = 23
Didn't progress to multiple ab+
n = 141
Didn't progress to multiple ab+
n = 202
Didn't progress to multiple ab+
n = 12
Progressed to multiple ab+
n = 167
Progressed to multiple ab+
n = 168
Progressed to multiple ab+
n = 11
2+ autoantibodies at seroconversion
n = 134
Multiple ab+
n = 480
No T1D
n = 197
T1D
n = 283

Online-Only Supplemental Material

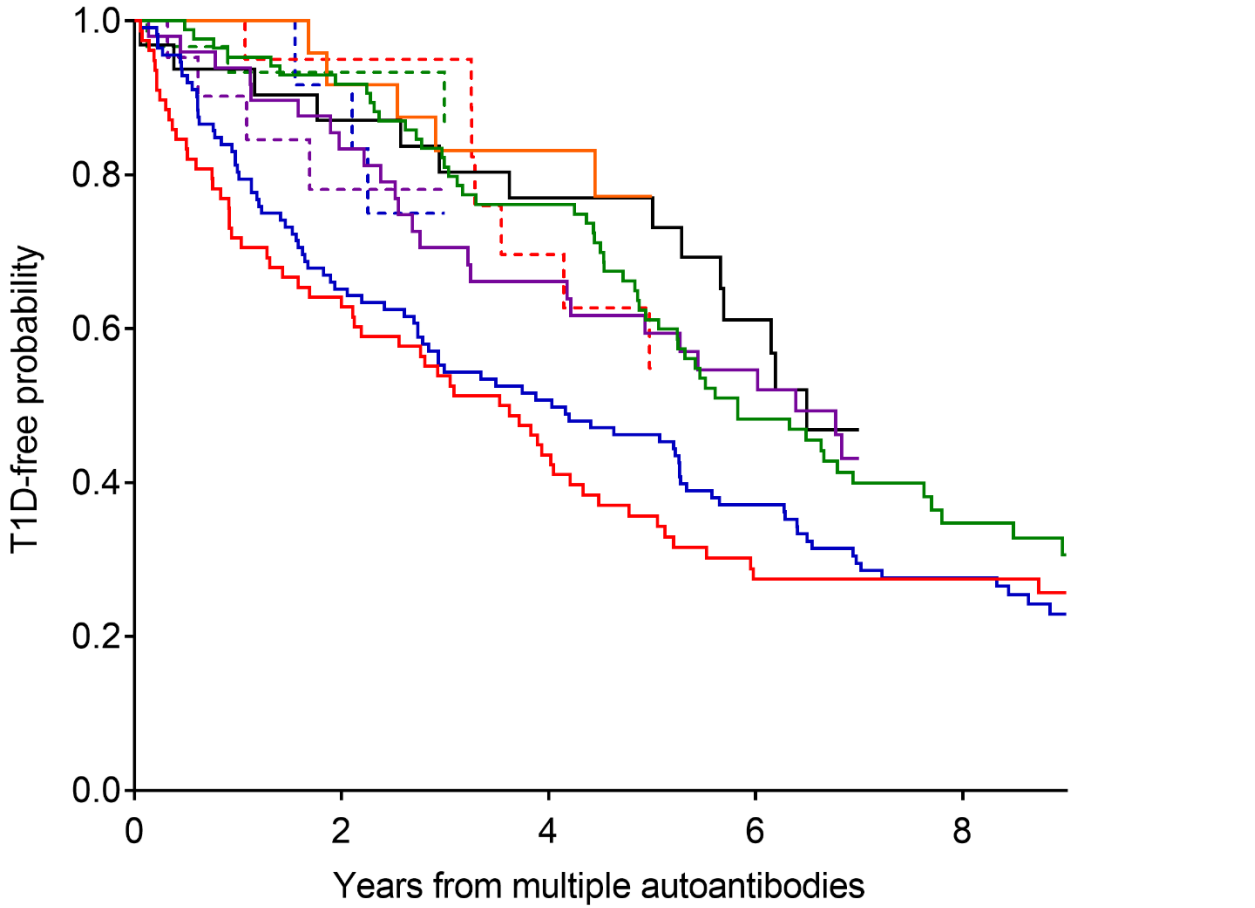
Predictors of the Initiation of Islet Autoimmunity, Progression to Multiple Autoantibodies and Clinical Diabetes: The TEDDY Study

Jeffrey P. Krischer, Xiang Liu, Åke Lernmark, William A. Hagopian, Marian J. Rewers, Jin-Xiong She, Jorma Toppari, Anette-G. Ziegler, Beena Akolkar, on behalf of the TEDDY Study Group

Supplemental Figure S1. Progression from single ab+ to multiple ab+ by age at seroconversion among subjects (a) with MIAA or GADA (n=678, log-rank test, p<0.0001), (b) with IAA only at seroconversion (n=308, log-rank test, p<0.0001), and (c) with GADA only at seroconversion (n=370, log-rank test, p=0.0002).



Supplemental Figure S2. Progression from multiple ab+ to type 1 diabetes by age at seroconversion.



Number at risk:

78	49	34	20	17	1 year
115	73	56	41	28	2 years
86	77	61	36	20	3 years
50	39	30	21		4 years
33	26	22	14		5 years
25	22	16			6 years
20	16	11			7 years
18	11				8 years
31	22				9 years
24	10				>9 years

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