AUTHOR

Vibha Anand, Ying Li, Bin Liu, Mohamed Ghalwash, Eileen Koski, Kenney Ng, Jessica L. Dunne, Josefine Jönsson, Christiane Winkler, Mikael Knip, Jorma Toppari, Jorma Ilonen, Michael B. Killian, Brigitte I. Frohnert, Markus Lundgren, Anette-Gabriele Ziegler, William Hagopian, Riitta Veijola, Marian Rewers; for the T1DI Study Group.

TITLE & JOURNAL

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 1 October 2021; 44 (10): 2269–2276.

YEAR

2021 (October), Vol 44(10)

DOI

https://doi.org/10.2337/dc20-1836

VERSION

Final Draft (AAM)

Islet Autoimmunity and HLA Markers of Presymptomatic and Clinical Type 1 Diabetes: Joint Analyses of Prospective Cohort Studies in Finland, Germany, Sweden, and The United States

Vibha Anand, PhD[1], Ying Li, PhD[1], Bin Liu, PhD[1], Mohamed Ghalwash, PhD[1,2], Eileen Koski, MPhil[1], Kenney Ng, PhD[1], Jessica L. Dunne, PhD[3], Josefine Jönsson, MSc[5], Christiane Winkler, PhD[13,14,15], Mikael Knip, MD, PhD[6,7,8,9] Jorma Toppari, MD, PhD[10] , Jorma Ilonen, MD, PhD[11], Michael B. Killian, BS[4], Brigitte I. Frohnert, MD, PhD[12], Markus Lundgren, MD, PhD[5] , Anette-Gabriele Ziegler, MD, PhD[13,14,15], William Hagopian, MD, PhD[4], Riitta Veijola, MD, PhD[16] and Marian Rewers, MD, PhD[12] for the T1DI Study Group[17] (see Supplemental information for the full list of study group members)

[1]IBM T.J. Watson Research Center, Cambridge and Yorktown Heights, MA and NY, USA
[2]Ain Shams University, Cairo, Egypt
[3]JDRF, New York, NY, USA
[4]Pacific Northwest Research Institute, Seattle, WA, USA
[5]Department of Clinical Sciences Malmö, Lund University/CRC, Skåne University Hospital, Malmö, Sweden
[6]Tampere Center for Child Health Research, Tampere University Hospital, Tampere, Finland
[7]Pediatric Research Center, Children's Hospital, University of Helsinki and Helsinki University Hospital, Helsinki, Finland
[8]Research Program for Clinical and Molecular Metabolism, Faculty of Medicine, University of Helsinki
[9]Folkhälsan Research Center, Helsinki, Finland
[10]Institute of Biomedicine and Population Research Centre, University of Turku and Department of Pediatrics, Turku University Hospital, Turku, Finland
[11]Immunogenetics Laboratory, Institute of Biomedicine, University of Turku and Clinical Microbiology, Turku University Hospital, Turku, Finland
[12]Barbara Davis Center for Diabetes, University of Colorado, Denver, CO, USA
[13]Institute of Diabetes Research, Helmholtz Zentrum München, German Research Center for Environmental Health, Munich-Neuherberg, Munich, Germany
[14]Forschergruppe Diabetes e.V. at Helmholtz Zentrum, Munich, Germany
[15]Forschergruppe Diabetes, Technical University Munich, Germany
[16]Department of Pediatrics, PEDEGO Research Unit, University of Oulu and Oulu University Hospital, Oulu, Finland
[17]Center for Computational Health at IBM Research, JDRF and DAISY, DiPiS, DIPP, DEW-IT, BABYDIAB study sites

Corresponding Author:

Vibha Anand, PhD
Research Staff Member, Center for Computational Health
IBM T.J. Watson Research Center
75, Binney Street, Cambridge, MA 02142
Tel. 1-617-693-3026
email: anand@us.ibm.com

Short Title: Presymptomatic and Clinical Type1 Diabetes

Word count main text: 3988 [4000] **abstract:** 250 [250] **Tables**: 2 **Figures**: 2

ABSTRACT

OBJECTIVE: To combine prospective cohort studies, by including HLA harmonization, and to estimate risk of islet autoimmunity and progression to clinical diabetes.

RESEARCH DESIGN AND METHODS: Prospective cohorts in Finland, Germany, Sweden and the US have followed 24,662 children at increased genetic risk for development of islet autoantibodies and type 1 diabetes. Following harmonization, the outcomes were analyzed in 16,709 infants-toddlers enrolled by age 2.5 years.

RESULTS: In the infant-toddler cohort, 1413 (8.5%) developed at least one autoantibody confirmed at two or more consecutive visits (seroconversion), 865 (5%) developed multiple autoantibodies, and 655 (4%) progressed to diabetes. The 15-year cumulative incidence of diabetes varied in children with one, two or three autoantibodies at seroconversion: 45% (95% CI 40-52%), 85% (78-90%), and 92% (85-97%), respectively. Among those with single autoantibody, their status two years after seroconversion predicted diabetes risk: 12% (10-25%) if reverting to autoantibody negative, 30% (20-40%) if retaining single autoantibody, and 82% (80-95%) if developing multiple autoantibodies. HLA-DR-DQ affected the risk of confirmed seroconversion and progression to diabetes in children with stable single autoantibody. Their 15-year diabetes incidence for higher vs. lower risk genotypes was 40% (28-50%) vs. 12% (5-38%). The rate of progression to diabetes was inversely related to age at development of multiple autoantibodies ranging from 20%/year to 6%/year in children developing multi-positivity ≤2 years or >7.4 years, respectively.

CONCLUSIONS: The number of islet autoantibodies at seroconversion reliably predicts 15-year type 1 diabetes risk. In children retaining single autoantibody, HLA-DR-DQ genotypes can further refine risk of progression.

Keywords: Type 1 Diabetes, Autoantibodies, Prospective-cohort, Child

Abbreviations: HLA: human leukocyte antigen; IA: islet autoimmunity; GADA: glutamic acid decarboxylase autoantibody; IA-2A: insulinoma antigen-2 autoantibody; IAA: insulin autoantibody; ZnT8A: zinc transporter 8 autoantibody, T1DI Study Group: Type 1 Diabetes Intelligence Study Group.

Type 1 diabetes is a chronic autoimmune endocrine disease that affects an estimated 1 in 300 children and up to 1 in 120 adults in the U.S. (1) and more in high-risk Nordic countries (2,3). The causes of the underlying islet autoimmunity (IA) are poorly understood, and no durable prevention or cure is available. The genetic and environmental determinants of type 1 diabetes have been extensively investigated in cohort studies that followed children at increased genetic risk for development of IA and progression to diabetes (4–8). Observations from these cohorts (9) led to the staging of the natural history of type 1 diabetes into: stage 1 - normoglycemia with presence of multiple islet autoantibodies, stage 2 – dysglycemia, and stage 3 - clinical (symptomatic) diabetes (10).

While the average annual rate of progression from stage 1 to stage 3 is ~11% (11), the individual risk is difficult to predict due to large variability in the progression rate (5,8,12–14). To overcome this limitation, the five active cohort studies in Finland (DIPP) (6), Germany (BABYDIAB and BABYDIET) (5), Sweden (DiPiS) (8), and the U.S. (DAISY (4) and DEWIT (7)) harmonized and combined their data for joint analyses in collaboration with IBM Research and JDRF, known as the T1DI study group. We are reporting the risk estimates for development of IA and progression to clinical diabetes stratified by the number of autoantibodies and HLA-DR-DQ genotype in children followed from infancy.

Additionally, we are reporting the subsequent risk of progression to clinical diabetes stratified by age at seroconversion. In contrast to an earlier report (9) that stratified the risk by the maximum number of antibodies ever expressed over an extended period of time, here the risk is stratified by the number of autoantibodies observed at the time of

initial seroconversion. This approach better reflects information available to a screening program.

RESEARCH DESIGN AND METHODS

Study Populations in the Combined Dataset

The prospective cohort studies included in this report enrolled: 1) infants from the general population identified through newborn screening as carrying increased risk HLA-DR-DQ genotypes (4,6–8) and first-degree relatives of people with type 1 diabetes (regardless of their HLA genotype (4,5)). All study participants underwent HLA screening, however, the eligibility criteria varied (see the Supplementary Appendix - Study Sites, Newborn Screening and Recruitment). Age at the initial follow-up visit ranged from 2 months to 21.6 years; participants were followed at 3 to 36 months intervals for development of islet autoantibodies, according to the study specific protocols, for up to 26 years. Type 1 diabetes was diagnosed according to the ADA criteria (15).

All individual study protocols were approved by local Institutional Review Boards and the sites submitted de-identified data to IBM in accordance with HIPAA and GDPR regulations. IBM Research aggregated and harmonized the data and performed the analyses.

T1DI study cohorts

Overall harmonized cohort: The visits with positive autoantibody measurements deemed to be maternally transferred were excluded. Children lacking evidence of positivity for any of the islet autoantibodies prior to diagnosis of diabetes were excluded (n=54); most were lost to follow-up early, only to be found diagnosed with type 1 diabetes years after their last study visit. The overall harmonized cohort for analyses included 24,662 subjects with

285,217 study visits, median 10 visits and 8.7 years of follow up per subject. Of those, 4165 (17%) subjects reported family history of type 1 diabetes. Further characteristics of this cohort are shown in Table 1 and Supplemental Tables S1 and S2.

Infant-toddler cohort: For key analyses presented in this report, we selected a more homogeneous sub-cohort from the overall cohort, referred to hereafter as the infant-toddler cohort (see supplemental Figure S1 for summary of cohort selection). The infant-toddler cohort only includes subjects who were initially tested for islet autoantibodies to insulin, insulinoma antigen-2, and GAD at or before 2.5 years of age. DIPP participants who were born prior to 2003 were excluded (n=4,297) as they were only screened using islet cell antibodies (ICA) assay. The infant-toddler cohort includes 16,709 subjects with 215,757 study visits, median 12 visits per subject, and 10.4 years of follow up (Table 1).

Data variables and HLA harmonization

A minimal set of common features were extracted and standardized from the submitted datasets (Supplemental Table S1). The HLA genotypes were harmonized across these studies (see HLA Risk Groups below). Subject-level (static) features included: date of birth month and year, sex, family history and relationship to type 1 diabetes proband, HLA-DR-DQ genotype, breast feeding (ever), and age at diagnosis of type 1 diabetes. The visit-level (dynamic) variables included age at each visit, autoantibodies (titer level and positive/negative outcome) to insulin (IAA), glutamic acid decarboxylase (GADA), insulinoma antigen-2 (IA-2A), and zinc transporter 8 (ZnT8A) as well as height, weight, plasma glucose levels and HbA1c. The individual titer levels for antibody assays were not harmonized for this study and will be presented in our future work. Instead, the binary outcomes, i.e. (positive/negative) of autoantibody measurement, submitted by each study

site were used for this study. Similarly, data for standardized height (to centimeters), weight (to kilograms), HbA1c (to NGSP, US standard), and glucose levels (to mg/dL) will be presented in our future work. Socio-demographics, breastfeeding and family history (and relationship) where known are described in Supplemental Table S2 by the individual study site.

HLA Risk Groups. Genotypes from individual studies were harmonized into four risk groups – A, B, C, D, ordered by decreasing risk (A = DR4-DQ8/DR3-DQ2.5 represents the highest risk). This harmonization was performed based on prior risk information (16) of HLA-DRB1, DQA1 and DQB1 alleles (17–19). Five broad "haplotype groups" were defined as follows - DR3-DQ2 included DQB1*02 positivity together with DQA1*05. All DQB1*03:02 positive subjects were defined to be positive for DR4-DQ8, and all other haplotypes were grouped as either neutral (X), protective (Y) or highly protective (Z) (16,20,21). Please see Supplemental Table S3 for haplotype groups. For each subject, two haplotypes were individually assigned to one of the five haplotype groups, and then together mapped to one of the four HLA Risk Groups as described in Supplemental Table S4. In the infant-toddler cohort, 2212 (14%) subjects were assigned to HLA risk group A, 6632 (40%) to group B, 2508 (15%) to group C, and 5179 (31%) to group D. A total of 178 subjects (<1%) could not be assigned to any HLA risk group because of missing genotype information (missing at least one haplotype or both and/or a missing allele) and were excluded for analyses. In the infant-toddler cohort, the proportion of subjects with the highest risk genotypes (HLA Group A) were higher in the U.S cohorts: 22% in DAISY and 28% in DEWIT than in the European cohorts: 13% in DIPP, 12% in DiPiS, and 6% in BABYDIAB. BABYDIAB enrolled only first-degree relatives and did not use HLA

genotypes as eligibility criteria, albeit HLA typing was performed. Please see Supplemental Table S5 for details of HLA risk group assignment. Furthermore, please note, of the 3525 (21%) subjects with family history of type 1 diabetes in the infant-toddler cohort, 254 (7%) were assigned to HLA Group A, 801 (23%) to Group B, 739 (21%) to Group C, 1577 (45%) to Group D and 154 (4%) subjects remained unassigned.

Islet autoantibodies: Methods used by each study to measure islet autoantibodies to insulin, IA-2, and GAD are summarized in the Supplementary Appendix in measurement of islet autoantibodies section. These assays have evolved greatly over the past 26 years. Each of the studies employed rigorous quality control procedures to control for a drift in the assays and their laboratories have participated with satisfactory results in all concurrent Diabetes Autoantibody Standardization program (DASP) (22) and its successor, the Islet Autoantibody Standardization program (IASP) (23) proficiency workshops. For this study, a binary result (positive/negative) was produced for each islet autoantibody measurement.

Study endpoints and Islet Autoimmunity (IA) definitions

The primary study endpoints in the infant-toddler cohort were:

- confirmed seroconversion, defined as positivity for the same islet autoantibody at two or more consecutive visits regardless of the interval between the visits. Confirmed seroconversion age was defined as the age at the first of the consecutive positive visits;
- positivity for multiple islet autoantibodies at confirmed seroconversion or subsequently; the age at multiple was defined as the age when the second autoantibody was first detected; and

- clinical diabetes.

Islet autoimmunity (IA) was primarily defined by the number of positive autoantibodies (i.e. 1, 2, or 3) at confirmed seroconversion. Separately, for children with a single autoantibody at seroconversion, we examined their autoantibody status two years post seroconversion based on a recent report (24) of persistence or reversion. Therefore, we report on development of IA at analytic timepoint of 2 years past confirmed seroconversion as follows:

S-0 - single autoantibody at confirmed seroconversion with reversion to no antibodies 2 years later

S-0 - single autoantibody at confirmed seroconversion with no subsequent development of any additional antibody, referred to as *stable single;*

S-M - single autoantibody at confirmed seroconversion with development of multiple antibodies within 2 years

While all five cohort studies measured zinc transporter 8 autoantibodies (ZnT8A), these were not included in the analyses, as they were generally measured only if the subject tested positive for one or more of the other three autoantibodies or had developed diabetes. Inclusion of ZnT8A did not change the overall results (data not shown).

Statistical Analyses:

Survival analyses were performed to generate cumulative risk estimates by number of autoantibodies at seroconversion (and 2 years later if single autoantibody positive) and compare those by age and HLA risk groups. Age at development of multiple islet autoantibodies was stratified into quartiles for comparisons of the annual incidence rates of progression to diabetes (11). For all analyses of progression to diabetes, event time was defined as the age at diagnosis of clinical diabetes or the age at last follow-up visit

for those who did not progress. Kaplan-Meier curves were plotted with 95% confidence intervals and the log-rank test was used to test for statistical differences. When stratifying by HLA risk groups, or by number of islet autoantibodies, pairwise statistical comparisons were made. We calculated positive predictive value (PPV) and sensitivity (SENS) of number of islet autoantibodies to development of type 1 diabetes by using inverse probability of censoring weighting (25) to handle censored observations. Significance was tested at $P < 0.05$ and analyses were conducted using python v3.6 and statistical package R.

RESULTS

In the infant-toddler cohort of 16,709 subjects, 1413 (8.4%) had confirmed seroconversion and 865 (5.2%) developed multiple autoantibodies (stage 1 type 1 diabetes) (Table 1). The median age at confirmed seroconversion was 4.0 years and it was 3.8 years for development of multiple autoantibodies in children who developed multiple autoantibodies at seroconversion or thereafter. Overall, 655 (3.9%) children were diagnosed with clinical stage 3 type 1 diabetes.

Incidence of islet autoimmunity and clinical diabetes by the HLA risk group

In the infant-toddler cohort, the risk of confirmed seroconversion to a single autoantibody, multiple autoantibodies and clinical diabetes differed by the HLA risk groups ($P < 0.0001$ for all three comparisons). By the age of 15 years, confirmed seroconversion to a single autoantibody occurred in 14% (95% CI 12.5-17.5%) of children in HLA Group A (DR3-DQ2.5/DR4-DQ8.1), 9% (8.0-10.0%) in Group B, 8% (7.0-9.0%) in Group C, and 7.5% (7.5-11.0%) in Group D (Figure 1A). A large proportion (45%) of children in Group D, were first-degree relatives. Confirmed seroconversion to multiple autoantibodies by age 15

occurred in 7% (6.0-9.0%) of children in the HLA risk Group A, 2.5% (2.0-3.0%) in Group B, 1% (0.5-1.5%) in Group C, and 1% (0.5-2.0%) in Group D (Figure 1B).

Clinical diabetes developed by age 15 in 16% (95% CI 14.0-18.0%) of children in the HLA risk Group A, 6% (5.0-7.0%) with Group B, 4% (3.0-5.0%) with Group C, and in 2.5% (2.0-3.0%) in Group D (Figure 1C).

Type 1 diabetes incidence by the number of autoantibodies

Among children with confirmed seroconversion in the infant-toddler cohort (N=1413), majority (N=1047, 75%) were single autoantibody positive at seroconversion. The 15-year cumulative incidence of diabetes from seroconversion varied ($P < 0.0001$) in those with one (N=1047), two (N=281), or three autoantibodies (N=85) at confirmed seroconversion: 45% (95% CI 40-52%), 85% (78-90%), and 92% (85-97%), respectively (Figure 2A).

The PPV of one, two or three autoantibodies to develop type 1 diabetes in 15 years since seroconversion were 0.53 (95% CI: 0.47-0.59), 0.67 (0.60-0.73), 0.71 (0.63-0.79) respectively. Similarly, the SENS of one, two or three autoantibodies to develop type 1 diabetes in 15 years since seroconversion were 0.35 (0.33-0.38), 0.40 (0.38-0.42), and 0.42 (0.39-0.44) respectively.

The 15-year cumulative incidence of diabetes in children seroconverting with single autoantibody varied significantly ($P < 0.0001$) when 2 years follow-up post seroconversion was considered (Figure 2B). The 15-year cumulative incidence among those reverting to autoantibody negativity (S-0), retaining *stable* single autoantibody (S-S) and developing

multiple autoantibodies (S-M) by 2 years past seroconversion were: 12% (10-25%), 30% (20-40%), and 82% (80-95%) respectively.

The PPV of single autoantibody status at 2 years post seroconversion, i.e. (S-0), (S-S), and (S-M), to develop type 1 diabetes in 15 years were 0.21 (95% CI: 0.11-0.31), 0.31 (0.21-0.41), and 0.67 (0.55-0.79) respectively. Similarly, the SENS of (S-0), (S-S), and (S-M) to develop type 1 diabetes in 15 years were 0.18 (0.11-0.25), 0.25 (0.19-0.30), and 0.40 (0.36-0.44) respectively.

Furthermore, among children positive with stable single autoantibody (S-S) in the infant-toddler cohort, the 15-year cumulative incidence of diabetes varied significantly ($P < 0.0001$) by the higher vs. lower risk HLA genotypes: 40% (28.0-50.0%) for HLA Group A or B vs. 12% (5.0-38.0%) for HLA Group C or D genotypes (Figure 2C). Please also see Supplemental Figure S4(b) for stratification by individual HLA risk groups A to D in the infant-toddler cohort.

A summary of the cumulative incidence of diabetes in the overall cohort stratified by HLA risk groups and the number of islet autoantibodies at seroconversion (and 2 years post seroconversion) is given in Supplemental Table S6.

Type 1 diabetes incidence by the age at development of multiple islet autoantibodies

Of the 865 subjects who developed multiple autoantibodies in the infant-toddler cohort, 812 had confirmed seroconversion (at least 2 consecutive positive visits). To analyze the effect of age on the rate of progression to clinical diabetes, the age at development of multiple autoantibodies was divided in quartiles: 1Q: $\leq$2.0 years (n=202, of which 177

have developed diabetes), 2Q: 2.0-3.8 years (n=205, 151 with diabetes), 3Q: 3.8-7.4 years (n=202, 126 with diabetes), and 4Q: 7.4-18 years (n=203, 59 with diabetes). During the initial 10 years of follow-up since development of multiple autoantibodies, the overall incidence of diabetes was 12 per 100-person-years. This rate decreased ($P < 0.0001$) with increasing age quartile to 20, 12, 11 and 6 per 100 person-years, 1Q through 4Q, respectively (Table 2, and Supplemental Figure S5). However, the annual incidence rate was stable within each age quartile over time. In the infant-toddler cohort, the 10-year cumulative incidence of diabetes in children with multiple autoantibodies was double in ≤2 years age group when compared to >7.4 years age group (87% vs. 44%, Table 2).

CONCLUSIONS

We combined and harmonized data from five prospective cohorts of type 1 diabetes in the US and Europe in a single dataset. From these we generate robust risk estimates for development of islet autoimmunity and progression to clinical diabetes by number of islet autoantibodies and HLA-DR-DQ genotypes for up to 15 years of follow-up. In contrast to the earlier report (9), that retrospectively defined IA based on the maximum number of antibodies ever expressed between birth and age 15 years, our analysis is based on the number of autoantibodies observed at the time of initial seroconversion and 2 years later. The baseline for the estimated risk of progression to diabetes in the previous report (9) was the age when study subjects achieved maximum autoantibodies, while in the current report it is the age at initial seroconversion. The latter more closely represents the risk from the perspective of a screening program, where risk prediction cannot include information that is not yet available. The two reports defined risk by number of islet autoantibodies and the

baseline for follow up in a different way and yielded different risk estimates, an important consideration in the context of diabetes risk counseling.

While this previous work also analyzed a combined dataset from three of the same studies (DIPP, BABYDIAB and DAISY) as in our combined cohort, the analyses presented here go beyond in several important ways. We include two additional cohorts (DiPiS and DEW-IT), and up to 6 more years of follow-up of the study participants from the previous studies. To our knowledge, the TIDI study cohort is the largest dataset of prospectively collected information concerning predictors of childhood type 1 diabetes, with 24,662 children followed for up to 26 years, and a sub-cohort of 16,709 children followed since infancy.

Three major findings are shown herein. First, children who initially seroconvert to multiple autoantibodies had greater cumulative risk of type 1 diabetes than those who initially develop a single autoantibody, despite that many in the latter group have subsequently developed multiple autoantibodies. While this is consistent with previous observations that stratified the risk by the maximum number of autoantibodies ever expressed (9,11,26), our findings highlight the importance of the earliest biomarkers in the evolution of islet autoimmunity. We have estimated positive predictive value and sensitivity of number of autoantibodies at seroconversion for developing type 1 diabetes in 15 years while accounting for censoring in the past observational studies. These highlight the importance of risk evaluation based on single vs. multiple autoantibody development at the earliest time point in course of islet autoimmunity development.

Second, the younger the age of multiple autoantibody appearance, the greater the rate of progression to clinical type 1 diabetes consistent with previous studies (9,27).

However, this was found both in the infant-toddler cohort as well as in the overall cohort (please see Table S7 for overall cohort).

Third, the HLA-DR-DQ genotype significantly influences progression to diabetes among children seroconverting and remaining stable positive for single autoantibody at least 2 years past seroconversion. Previous reports (28,29) have missed this effect, by not sub-dividing initially single autoantibody positive children according to their status 2 years later. However, we confirmed previous reports that the HLA effect was negligible in those with multiple autoantibodies at seroconversion.

A concern when screening for childhood risk of type 1 diabetes is the uncertainty of single autoantibody at seroconversion. Their overall rate of developing type 1 diabetes during childhood is substantial (30%) but much less than those with multiple autoantibodies (>80%). Our findings suggest that addition of genetic markers and a repeat islet autoantibody test 2 years later may improve individual risk assessment in the single autoantibody group. Here we showed that HLA-DR-DQ genotypes may be useful in this regard. Interestingly, our analyses also suggest that single autoantibody positive children in HLA risk Groups C or D develop diabetes at a later age than Groups A or B. Thus, our findings emphasize that at confirmed seroconversion, the single autoantibody positive subjects (75% in this large cohort) have a substantially lower rate of progression to diabetes compared to multiple autoantibodies subjects. Among those remaining positive for single autoantibody, the risk can be stratified based on HLA-DR-DQ genotypes. Among those with multiple autoantibodies, the risk can be stratified by age at development of islet autoimmunity. We believe these

findings can positively inform recruitment in prevention trials and pave way for screening protocols.

Advantages of this report include the large dataset representing populations at moderate (Germany and the US) to high risk of type 1 diabetes (Finland and Sweden) and children followed from birth for up to 26 years. Substantial input from multiple investigators representing these studies made it possible to harmonize and jointly analyze the data. Harmonization of the HLA-DRB1-DQA1-DQB1 genotypes across the five cohorts was an unprecedented challenge. In the populations studied, a limited number of stable haplotypes was expected (30), so that even when not all three loci were typed, it was usually possible to infer the specific haplotypes. Then, using disease odds ratios from large collections of cases like the Type 1 Diabetes genetics consortium (16), we have shown it is readily possible to assign HLA genotype risk groups. For islet autoantibody tests, laboratories serving each study have long participated in the Diabetes Autoantibody Standardization program (DASP) (22) and its successor, the Islet Autoantibody Standardization program (IASP) (23). Consistent participation in the proficiency workshops at 18-month intervals has allowed the laboratories to adhere to standardized quality control procedures and monitor the accuracy of the assays, leading to broadly comparable islet autoantibody data.

Our study has some limitations. The study population is predominantly Caucasian, the harmonized dataset does not contain information on non-HLA genotypes though some of the participating studies submitted these data and this may expand. Subjects eligible for prospective follow-up had increased HLA-conferred genetic susceptibility but subjects were also included on the basis of positive family history of type 1

diabetes. The latter may carry non-HLA susceptibility genes more often than observed in the population. The majority of our HLA risk Group D subjects were positive for family history and that is probably reflected in their diabetes risk that was clearly higher than in the general population. Since the HLA genotyping in the original studies was crude (circa 2000), there is also a far broader representation of HLA genotypes in our cohort than can be found in a typical pre-selected type 1 diabetes study (31), and in that regard our cohort is a bit more like general background population. We did not evaluate the type or order of appearance of autoantibodies or specific autoantibody combinations in this study. Understanding relation of various IA profiles to genetic background will be a focus for our future work. The order of appearance of autoantibodies has been shown to be related to HLA-DR-DQ genotype, at least in one study (32). The T1DI study group agreed to use binary outcomes of autoantibody titers for the current analyses and the titer values are being harmonized for forthcoming manuscripts. The original study protocols included somewhat different eligibility criteria and follow-up visits frequency. Longer intervals between study visits hamper identification of the true seroconversion time. The TEDDY study (31), will overcome this limitation by following 8,676 high-risk children recruited at 3-4 months of age with islet autoantibody assessment every 3 months in the initial 4 years of life and every 6 months thereafter, until age 15. When the entire TEDDY cohort passes the 15-year mark, in late 2024, its dataset will provide higher resolution answers regarding seroconversion and risk of clinical diabetes. The T1DI study illustrates the variability in approaches to screening and follow-up for childhood diabetes in diverse settings in the U.S. and Europe. However, it also provides a proof of principle that such diverse

dataset can be harmonized and jointly analyzed to generate robust risk estimates for children and adolescents. In contrast, very few data on islet autoimmunity and genetic markers are currently available in the adult population.

Our results are generally consistent with the published literature. In the past, it has been difficult to generalize results from specific birth-cohort studies due to marked differences in study populations, eligibility criteria, and follow-up protocols. Our large and HLA harmonized dataset is already being used to explore more granular patterns of the development of islet autoantibodies (type, timing, and titer) and dysglycemia in relation to HLA and family history background, sex, growth, geography, and diet. Future application of novel analytical methods (33) such as machine learning and data-driven approaches, should increase our understanding of type 1 diabetes pathogenesis and prediction. This may include the application of tools already developed in other settings to visualize data-driven clusters (34) and disease progression models (35,36). These approaches require large and diverse datasets such as the T1DI cohort that we hope will pave the way to a more precise approach to prediction and prevention of type 1 diabetes.

Acknowledgements: The authors thank the participants of the DAISY, DiPiS, DIPP, DEW-IT and BABYDIAB studies.

William Hagopian, Markus Lundgren, Marian Rewers, Riitta Veijola and Anette Ziegler, as representatives of the data originating sites, are the overall guarantor of this project with responsibility for the integrity of the data. Vibha Anand, as a representative of IBM, is the technical research lead for this project with responsibility for the accuracy of the data analysis. Frank Martin is a representative of JDRF, the convenor and funder of the overall initiative.

Reference to prior publication in abstract form: https://doi.org/10.2337/db19-1345-P

Funding: This work was supported by funding from JDRF (IBM: 1-RSC-2017-368-I-X, 1-IND-2019-717-I-X), (DAISY: 1-SRA-2019-722-I-X, 1-RSC-2017-517-I-X, 5-ECR-2017-388-A-N), (DiPiS: 1-SRA-2019-720-I-X, 1-RSC-2017-526-I-X), (DIPP: 1-RSC-2018-555-I-X), (DEW-IT: 1-SRA-2019-719-I-X, 1-RSC-2017-516-I-X) as well as NIH (DAISY: DK032493, DK032083, DK104351; and DK116073; DiPiS: DK26190 and the CDC (DEW-IT: UR6/CCU017247).

The DIPP study was funded by JDRF (grants 1-SRA-2016-342-M-R, 1-SRA-2019-732-M-B); European Union (grant BMH4-CT98-3314); Novo Nordisk Foundation; Academy of Finland (Decision No 292538 and Centre of Excellence in Molecular Systems Immunology and Physiology Research 2012-2017, Decision No. 250114); Special Research Funds for University Hospitals in Finland; Diabetes Research Foundation, Finland; and Sigrid Juselius Foundation, Finland.

The BABYDIAB study was funded by the German Federal Ministry of Education and Research to the German Center for Diabetes Research.

The DiPiS study was funded by Swedish Research Council (grant no. 14064), Swedish Childhood Diabetes Foundation, Swedish Diabetes Association, Nordisk Insulin Fund, SUS funds, Lion Club International, district 101-S, The royal Physiographic society, Skåne County Council Foundation for Research and Development as well as LUDC-IRC/EXODIAB funding from the Swedish foundation for strategic research (Dnr IRC15-0067) and Swedish research council (Dnr 2009-1039).

Additional funding for DEW-IT was provided by the Hussman Foundation and by the Washington State Life Science Discovery Fund.

Duality of interest statement: The authors report no duality of interest relevant to the current study. VA, YL, MG, EK, KN are current employees of IBM. BL, YL are former employees of IBM and performed this work while at IBM. JD performed this work as an employee of JDRF and is now an employee of Jansen Inc.

Author contributions: VA is the PI for the IBM Research study and wrote the first and final draft of this manuscript and takes responsibility for all analyses presented. YL, BL helped with sub-analyses, MG helped with feature engineering (seroconversion and multiple positivity), KN, EK, JD provided logistical support for the project and draft revisions of the manuscript and were responsible for the initial conceptualization of the T1DI cohort study. WH and JI helped with HLA harmonization scheme. WH, ML, AL, MK, JT, BF, AZ, RV, MR are site primary and co-investigators and were responsible for collecting study data, JJ, CW and MKillian helped with site specific data collection and

support processes. All authors made substantial contributions to conception and design of the manuscript, participated in drafting the manuscript or revising it critically for important intellectual content, and gave final approval of the version to be submitted.

References:

1. Maahs DM, West NA, Lawrence JM, Mayer-Davis EJ. Chapter 1: Epidemiology of Type 1 Diabetes. Endocrinol Metab Clin North Am. 2010 Sep;39(3):481–97.

2. Berhan Y, Waernbaum I, Lind T, Mollsten A, Dahlquist G, for the Swedish Childhood Diabetes Study Group*. Thirty Years of Prospective Nationwide Incidence of Childhood Type 1 Diabetes: The Accelerating Increase by Time Tends to Level Off in Sweden. Diabetes. 2011 Feb 1;60(2):577–81.

3. Harjutsalo V, Sund R, Knip M, Groop P-H. Incidence of Type 1 Diabetes in Finland. JAMA. 2013 Jul 24;310(4):427.

4. Rewers M, Bugawan TL, Norris JM, Blair A, Beaty B, Hoffman M, et al. Newborn screening for HLA markers associated with IDDM: diabetes autoimmunity study in the young (DAISY). Diabetologia. 1996 Jul;39(7):807–12.

5. 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 Mar;48(3):460–8.

6. Kupila A, Muona P, Simell T, Arvilommi P, Savolainen H, Hämäläinen AM, et al. Feasibility of genetic and immunological prediction of type I diabetes in a population-based birth cohort. Diabetologia. 2001 Mar;44(3):290–7.

7. Wion E, Brantley M, Stevens J, Gallinger S, Peng H, Glass M, et al. Population-wide infant screening for HLA-based type 1 diabetes risk via dried blood spots from the public health infrastructure. Ann N Y Acad Sci. 2003 Nov;1005:400–3.

8. Larsson HE. A Swedish approach to the prevention of type 1 diabetes. Pediatric Diabetes. 2016 Jul 1;17(S22):73–7.

9. Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, et al. Seroconversion to Multiple Islet Autoantibodies and Risk of Progression to Diabetes in Children. JAMA. 2013 Jun 19;309(23):2473–9.

10. Insel RA, Dunne JL, Atkinson MA, Chiang JL, Dabelea D, Gottlieb PA, et al. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. Diabetes Care. 2015 Oct;38(10):1964–74.

11. Bonifacio E. Predicting type 1 diabetes using biomarkers. Diabetes Care. 2015 Jun;38(6):989–96.

12. Skyler JS. Characterizing Subgroups of Type 1 Diabetes. Diabetes. 2014 Nov 1;63(11):3578–80.

13. Arif S, Leete P, Nguyen V, Marks K, Nor NM, Estorninho M, et al. Blood and Islet Phenotypes Indicate Immunological Heterogeneity in Type 1 Diabetes. Diabetes. 2014 Nov;63(11):3835–45.

14. Veijola R, Koskinen M, Helminen O, Hekkala A. Dysregulation of glucose metabolism in preclinical type 1 diabetes. Pediatr Diabetes. 2016;17 Suppl 22:25–30.

15. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 2014 Jan 1;37(Supplement 1):S81–90.

16. Erlich H, Valdes AM, Noble J, Carlson JA, Varney M, Concannon P, et al. HLA DR-DQ Haplotypes and Genotypes and Type 1 Diabetes Risk: Analysis of the Type 1 Diabetes Genetics Consortium Families. Diabetes. 2008 Apr 1;57(4):1084–92.

17. Siljander HTA, Simell S, Hekkala A, Lähde J, Simell T, Vähäsalo P, et al. Predictive characteristics of diabetes-associated autoantibodies among children with HLA-conferred disease susceptibility in the general population. Diabetes. 2009 Dec;58(12):2835–42.

18. Wenzlau JM, Liu Y, Yu L, Moua O, Fowler KT, Rangasamy S, et al. A common nonsynonymous single nucleotide polymorphism in the SLC30A8 gene determines ZnT8 autoantibody specificity in type 1 diabetes. Diabetes. 2008 Oct;57(10):2693–7.

19. Salonen KM, Ryhänen S, Härkönen T, Ilonen J, Knip M, Finnish Pediatric Diabetes Register. Autoantibodies against zinc transporter 8 are related to age, metabolic state and HLA DR genotype in children with newly diagnosed type 1 diabetes. Diabetes Metab Res Rev. 2013 Nov;29(8):646–54.

20. Emery LM, Babu S, Bugawan TL, Norris JM, Erlich HA, Eisenbarth GS, et al. Newborn HLA-DR,DQ genotype screening: age- and ethnicity-specific type 1 diabetes risk estimates. Pediatr Diabetes. 2005 Sep;6(3):136–44.

21. Ilonen J, Reijonen H, Herva E, Sjöroos M, Iitiä A, Lövgren T, et al. Rapid HLA-DQB1 genotyping for four alleles in the assessment of risk for IDDM in the Finnish population. The Childhood Diabetes in Finland (DiMe) Study Group. Diabetes Care. 1996 Aug;19(8):795–800.

22. Bingley PJ, Bonifacio E, Mueller PW. Diabetes Antibody Standardization Program: first assay proficiency evaluation. Diabetes. 2003 May;52(5):1128–36.

23. Lampasona V, Pittman DL, Williams AJ, Achenbach P, Schlosser M, Akolkar B, et al. Islet Autoantibody Standardization Program 2018 Workshop: Interlaboratory Comparison of Glutamic Acid Decarboxylase Autoantibody Assay Performance. Clinical Chemistry. 2019 Sep 1;65(9):1141–52.

24. Vehik K, Lynch KF, Schatz DA, Akolkar B, Hagopian W, Rewers M, et al. Reversion of β-Cell Autoimmunity Changes Risk of Type 1 Diabetes: TEDDY Study. Diabetes Care. 2016 Sep;39(9):1535–42.

25. Vock DM, Wolfson J, Bandyopadhyay S, Adomavicius G, Johnson PE, Vazquez-Benitez G, et al. Adapting machine learning techniques to censored time-to-event health record data: A general-purpose approach using inverse probability of censoring weighting. Journal of Biomedical Informatics. 2016 Jun;61:119–31.

26. Steck AK, Vehik K, Bonifacio E, Lernmark A, Ziegler A-G, Hagopian WA, et al. Predictors of Progression From the Appearance of Islet Autoantibodies to Early Childhood Diabetes: The Environmental Determinants of Diabetes in the Young (TEDDY). Diabetes Care. 2015 May;38(5):808–13.

27. Krischer JP, Liu X, Lernmark Å, Hagopian WA, Rewers MJ, She J-X, et al. The Influence of Type 1 Diabetes Genetic Susceptibility Regions, Age, Sex, and Family History on the Progression From Multiple Autoantibodies to Type 1 Diabetes: A TEDDY Study Report. Diabetes. 2017;66(12):3122–9.

28. Bingley PJ, Gale E a. M, European Nicotinamide Diabetes Intervention Trial (ENDIT) Group. Progression to type 1 diabetes in islet cell antibody-positive relatives in the European Nicotinamide Diabetes Intervention Trial: the role of additional immune, genetic and metabolic markers of risk. Diabetologia. 2006 May;49(5):881–90.

29. Koskinen MK, Lempainen J, Löyttyniemi E, Helminen O, Hekkala A, Härkönen T, et al. Class II HLA Genotype Association With First-Phase Insulin Response Is Explained by Islet Autoantibodies. J Clin Endocrinol Metab. 2018 01;103(8):2870–8.

30. Klitz W, Maiers M, Spellman S, Baxter-Lowe LA, Schmeckpeper B, Williams TM, et al. New HLA haplotype frequency reference standards: high-resolution and large sample typing of HLA DR-DQ haplotypes in a sample of European Americans. Tissue Antigens. 2003 Oct;62(4):296–307.

31. Hagopian WA, Erlich H, Lernmark A, Rewers M, Ziegler AG, Simell O, et al. The Environmental Determinants of Diabetes in the Young (TEDDY): genetic criteria and international diabetes risk screening of 421 000 infants. Pediatr Diabetes. 2011 Dec;12(8):733–43.

32. Krischer JP, Lynch KF, Schatz DA, Ilonen J, Lernmark Å, Hagopian WA, et al. The 6 year incidence of diabetes-associated autoantibodies in genetically at-risk children: the TEDDY study. Diabetologia. 2015 May 1;58(5):980–7.

33. Dugas M. Clinical Research Informatics: Recent Advances and Future Directions. Yearb Med Inform. 2015 Aug 13;10(1):174–7.

34. Kwon BC, Eysenbach B, Verma J, Ng K, deFilippi C, Stewart WF, et al. Clustervision: Visual Supervision of Unsupervised Clustering. IEEE Transactions on Visualization and Computer Graphics. 2018;PP(1):1–1.

35. Kwon BC, Achenbach P, Dunne JL, Hagopian W, Lundgren M, Ng K, et al. Modeling Disease Progression Trajectories from Longitudinal Observational Data. arXiv:201205324 [cs] [Internet]. 2020 Dec 9 [cited 2020 Dec 24]; Available from: http://arxiv.org/abs/2012.05324

36. Kwon BC, Anand V, Severson KA, Ghosh S, Sun Z, Frohnert BI, et al. DPVis: Visual Analytics with Hidden Markov Models for Disease Progression Pathways. IEEE Trans Vis Comput Graph. 2020 Apr 7;

Table1−T1DI study cohorts

Study, Site, Enrollment period, age at enrollment	Age range, [Median age] in cohort at follow-up visits	Cohort Type	Cohort Subjects N (# visits)	Confirmed sero-conversion† N (% of cohort)	Multiple auto antibodies ever || N (% of cohort)	T1D cases N (% of cohort)	T1D cases excluded‡ N
BABYDIAB, Germany, 1989-2000, 9 m	0.0-28.5 y, [2.1 y]	Overall	**2364** (27,179)	220 (9.0%)	123 (5.0%)	95 (4.0%)	12
BABYDIAB, Germany, 1989-2000, 9 m	0.0-28.5 y, [2.1 y]	Infant-toddler	**2346 (**27,130)	220 (9.0%)	123 (5.0%)	95 (4.0%)	0
DAISY, Colorado, 1993-2006, 9 m*	0.6-29.8 y, [7.4 y]	Overall	**2539** (26,803)	199 (8.0%)	129 (5.0%)	101 (4.0%)	5
DAISY, Colorado, 1993-2006, 9 m*	0.6-25.1 y, [7.0 y]	Infant-toddler	**2170** (23,402)	165 (8.0%)	105 (5.0%)	81 (3.7%)	0
DEWIT, Washington, 1995-2001, 2008-2012	0.4-45.2 y, [8.1 y]	Overall	**3748** (9,196)	173 (5.0%)	170 (5.0%)	56 (1.5%)	8
DEWIT, Washington, 1995-2001, 2008-2012	0.4-16.5 y, [2.2 y]	Infant-toddler	**559 (**1,490)	17 (3.0%)	18 (3.0%)	8 (1.4%)	0
DiPiS, Sweden, 2000-2004, 24 m	0.0-13.0 y, [4.0 y]	Overall	**4359** (34,298)	184 (4.0%)	100 (2.0%)	75 (1.7%)	1
DiPiS, Sweden, 2000-2004, 24 m	0.0-13.0 y, [4.0 y]	Infant-toddler	**4353** (34,280)	184 (4.0%)	100 (2.0%)	75 (1.7%)	0

DIPP§, Finland, 1994-2009, 2-6 m	0.0-22.9 y, [4.9 y]	Overall	**11,652** (187,741)	837 (7.0%)	526 (5.0%)	399 (3.4%)	28
DIPP§, Finland, 1994-2009, 2-6 m	0.0-22.9 y, [5.0 y]	Infant-toddler	**7281** (129,455	827 (11.0%)	519 (7.0%)	396 (5.4%)	0
All	0.0-45.2 y, [4.9 y]	Overall	**24,662** (285,217)	1613 (7.0%)	1048 (4.2%)	726 (2.9%)	54
All	0.0-28.5 y, [4.6 y]	Infant-toddler	**16,709** (215,757)	1413 (8.4%)	865 (5.2%)	655 (3.9%)	0

* FDRs were eligible to enroll if younger then 8y (1993-1995) or younger than 4 y (1996-2004)
† Seroconversion per T1DI study cohort definition in Methods section, without using ZnT8 positivity
‡ T1D cases without autoantibody measurement or seropositivity in the follow up period
§ DIPP subjects born before 2003 were screened with only ICA (N=4,297)
|| Without ZnT8 in the autoantibody count

Table2−Risk of progression to type 1 diabetes among children positive for multiple islet autoantibodies in infant-toddler cohort (N=812)

Quartiles of age distribution at development of multiple islet autoantibodies	N	Cumulative 5-year incidence of diabetes	Cumulative 10-year incidence of diabetes	Cumulative 15-year incidence of diabetes	Average annual incidence of diabetes over 10 years (per 100 per year)
≤ 2.0 years	202	64% [57-70%]	87% [80-91%]	95% [89-98%]	19.9
>2.0 and ≤ 3.8 years	205	42% [35-49%]	72% [64-77%]	82% [74-88%]	12.4
>3.8 and ≤ 7.4 years	202	35% [28-42%]	67% [58-74%]	83% [72-90%]	10.8
>7.4 and ≤ 18.1 years	203	28% [20-35%]	44% [32-54%]	54% [36-67%]	5.6
Total	812				12.2

Figure captions

Figure 1A – Cumulative incidence of confirmed seroconversion to single islet autoantibody from birth by HLA risk group from birth in the infant-toddler cohort.

Figure 1B – Cumulative incidence of confirmed seroconversion to multiple islet autoantibodies from birth by HLA risk group from birth in the infant-toddler cohort.

Figure 1C – Cumulative incidence of type 1 diabetes from birth by HLA risk group in the infant-toddler cohort.

Figure 2A – Cumulative incidence of type 1 diabetes by the number of positive islet autoantibodies (IA=1, IA=2 or IA=3) at confirmed seroconversion in the infant-toddler cohort.

Figure 2B – Cumulative incidence of type 1 diabetes among subjects with single autoantibody at confirmed seroconversion in the infant-toddler cohort.

S-0: single autoantibody at seroconversion with reversion to autoantibody negativity 2 years post seroconversion

S-S: single autoantibody at seroconversion and at 2 years post seroconversion

S-M: single autoantibody at seroconversion progressed to multiple autoantibodies 2 years post seroconversion

Figure 2C − Cumulative incidence of type 1 diabetes by HLA risk group among subjects with stable single autoantibody (S-S) 2 years post seroconversion in the infant-toddler cohort.

(A) (B) (C)

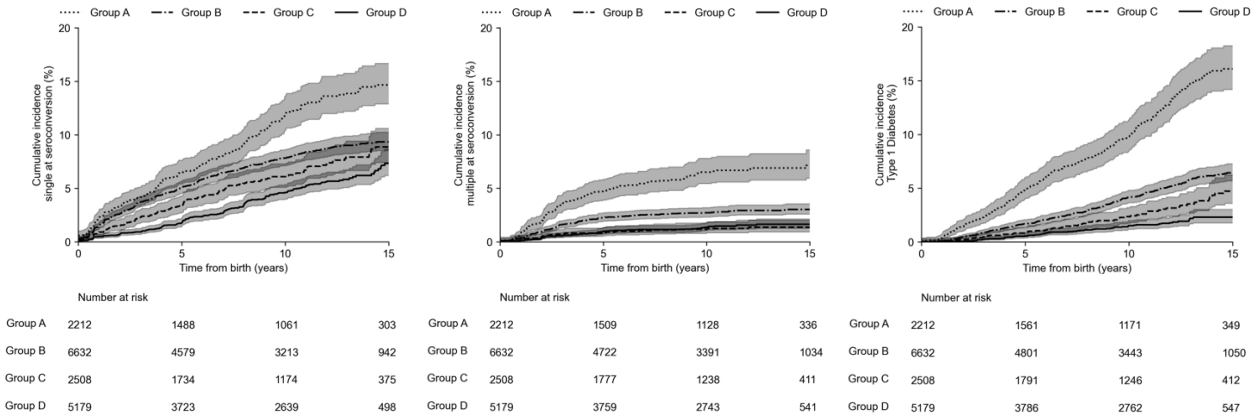

Figure 1: Cumulative incidence of confirmed seroconversion from birth by HLA risk group in the infant-toddler cohort (A) to single islet autoantibody, (B) to multiple islet autoantibodies; (C) Cumulative incidence of type 1 diabetes from birth by HLA risk group in the infant-toddler cohort.

(A) (B) (C)

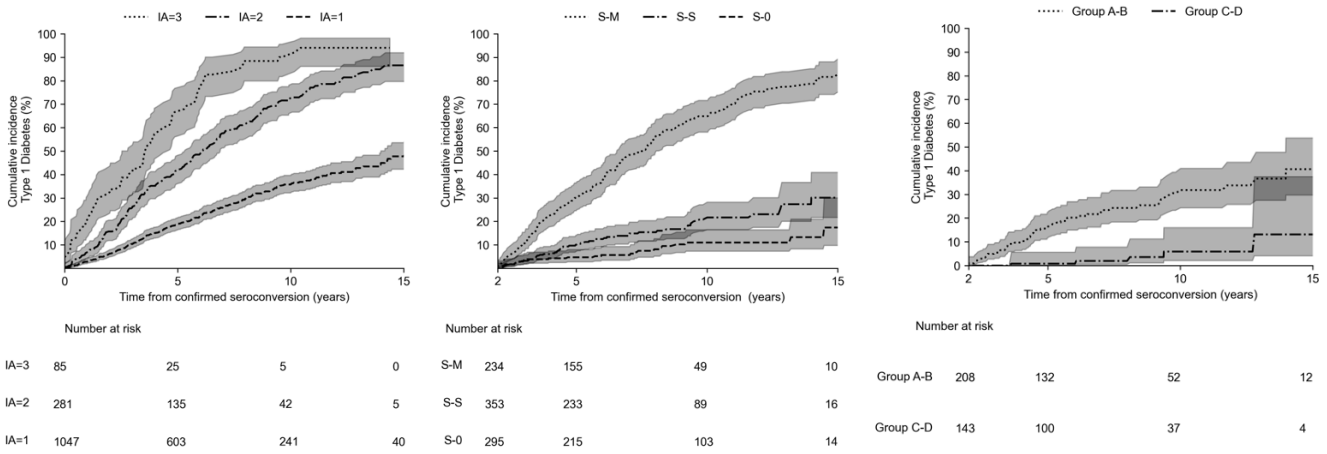

Figure 2: Cumulative incidence of type 1 diabetes in the infant-toddler cohort (A) by the number of positive autoantibodies (IA=1, IA=2 or IA=3) at confirmed seroconversion, (B) among subjects with single autoantibody at confirmed seroconversion (IA=1) by sub-types (S-0, S-S, S-M 2 years post seroconversion), (C) among stable single (S-S) autoantibody positive subjects by HLA risk group (A or B) vs. (C or D).

IA = Islet autoantibodies

S-0: single autoantibody with subsequent reversion to autoantibody negativity 2 years post seroconversion

S-S: single autoantibody at seroconversion and 2 years post seroconversion

S-M: single autoantibody at seroconversion subsequently progressed to multiple autoantibodies 2 years post seroconversion

AUTHOR	Vibha Anand, Ying Li, Bin Liu, Mohamed Ghalwash, Eileen Koski, Kenney Ng, Jessica L. Dunne, Josefine Jönsson, Christiane Winkler, Mikael Knip, Jorma Toppari, Jorma Ilonen, Michael B. Killian, Brigitte I. Frohnert, Markus Lundgren, Anette-Gabriele Ziegler, William Hagopian, Riitta Veijola, Marian Rewers; for the T1DI Study Group,
TITLE & JOURNAL	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 1 October 2021; 44 (10): 2269–2276.
YEAR	2021 (October), Vol 44(10):
DOI	https://doi.org/10.2337/dc20-1836
VERSION	Final Draft (AAM)