Supplementary Appendix

Supplement to: **Vibha Anand et al., 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**

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The T1DI Study Group

BABYDIAB: Anette G. Ziegler, M.D., Ezio Bonifacio Ph.D., Peter Achenbach, M.D., Christiane Winkler, Ph.D. Forschergruppe Diabetes e.V. and Institute of Diabetes Research, Helmholtz Zentrum München, German Research Center for Environmental Health, Munich-Neuherberg, Germany der TU München, Munich, Germany

DAISY: Marian Rewers, M.D., Ph.D., Brigitte I. Frohnert, M.D., Ph.D., Jill Norris, Ph.D., Andrea Steck, M.D., Kathleen Waugh, M.P.H., Liping Yu, M.D.; University of Colorado, Anschutz Medical Campus, Barbara Davis Center for Diabetes.

DEWIT: William A. Hagopian, M.D., Ph.D., Michael Killian, Rachel Hervey; Pacific Northwest Research Institute.

DiPiS: Åke Lernmark, Ph.D., Helena Elding Larsson, M.D., Ph.D., Markus Lundgren, M.D., Ph.D., Marlena Maziarz, Ph.D., Lampros Spiliopoulos, Josefin Jönsson. Department of Clinical Sciences Malmö, Lund University.

DIPP: [1]Riitta Veijola, M.D., Ph.D., [2]Jorma Toppari, M.D., Ph.D., [2]Jorma Ilonen, M.D., Ph.D., [3,4]Mikael Knip, M.D., Ph.D., [5]Heikki Hyöty, MD, PhD; [1]University of Oulu and Oulu University Hospital, [2]University of Turku and Turku University Hospital, [3]Tampere University Hospital, [4]University of Helsinki, [5]Tampere University.

IBM Research, Center for Computational Health: Vibha Anand, Ph.D., Mohamed Ghalwash, Ph.D., Kenney Ng, Ph.D., Zhiguo Li, Ph.D., B.C. Kwon, Ph.D., Harry Stavropoulos, M.S, Eileen Koski, M.Phil, Ashwani Malhotra, Ph.D., Shelley Moore, Jianying Hu, Ph.D.

T1DI Alumni: Jessica Dunne, Ph.D., Bin Liu, Ph.D., Ying Li, Ph.D.

JDRF: Olivia Lou, PhD., Frank Martin. Ph.D.

Study Sites, Newborn Screening and Recruitment

The BABYDIAB/BABYDIET study: is a prospective, longitudinal birth-cohort study in Germany (1,2). Between 1989 and 2000, BABYDIAB enrolled 1,650 offspring of mothers and/or fathers with type 1 diabetes. Between 2000 and 2006, BABYDIET enrolled 791 offspring or siblings of patients with type 1 diabetes. Participants were enrolled at birth and followed with type 1 diabetes-related autoantibody testing at age 9 months, at 2 years, and every 3 years thereafter. Those who became islet autoantibody-positive were subsequently followed six-monthly. A subgroup of 150 children participated in the BABYDIET dietary intervention study and had 3-monthly follow-up visits until the age of 3 years, and yearly thereafter (ClinicalTrials.gov Identifier: NCT01115621). The intervention (delay of exposure to gluten) failed to show an effect on islet autoimmunity or type 1 diabetes risk. After completion of the trial, participants were followed further in BABYDIAB/BABYDIET. Written informed consent was provided by participating families. BABYDIAB was approved by the Bavarian ethical committee (Bayerische Landesärztekammer; No. 95357) and the BABYDIET was approved by the ethics committee of the Ludwig-Maximilians-University, Munich, Germany (No. 329/00).

Diabetes Autoimmunity Study in the Young (DAISY): is a prospective, longitudinal study located in Denver, Colorado. Screening for genetic risk was conducted using cord blood collected from 32,114 general population infants born at Denver's St. Joseph hospital between 1994 and 2004. Excluded were extremely premature newborns and those with severe congenital malformation or disease. Upon completion of informed consent, cord blood samples were typed for type 1 diabetes -associated HLA-DR, DQ genotypes. (3) Children with high-risk, (DRB1*04-DQB1*03:02/DRB1*03) or moderate-risk (DRB1*04-DQB1*03:02/ DRB1*04-DQB1*03:02, DRB1*04-DQB1*03:02/DRX, DRB1*03/DRB1*03) or a specific low-risk (DRB1*04-DQB1*0302/DR2) genotypes were invited to participate in the follow-up protocol. (4) In 1993-95, first-degree relatives of individuals with type 1 diabetes were also enrolled, regardless of HLA genotype, if younger than 8 years. Starting in 1996, the eligibility for relatives was restricted to those younger than 4 years. Participants were enrolled into the follow-up study if informed consent was completed and at least one clinic visit, with type 1 diabetes-related autoantibody testing, was completed. All children enrolled at birth were tested at 9, 15, and 24 months of age and annually thereafter for islet autoantibodies. Children who were autoantibody positive were placed on an accelerated schedule in which they returned for a blood draw every 3 to 6 months.

Diabetes Evaluation in Washington Study (DEWIT): The DEW-IT study is a prospective, population-based observational study that HLA-screened 42,000 Washington State newborns born from 1995-2001 and 2008-2012 (5), under IRB approval and informed consent, using dried bloodspots from the Washington State Department of Health Newborn Screening Program. HLA-DQ genotyping utilized direct sequencing of exon 2 of each of DQA1 and DQB1. Individuals were eligible for further study if they had at least one of two high risk HLA haplotypes (DQA1*03-DQB1*03:02 and/or DQA1*05:01-DQB1*02:01) plus no protective haplotype (DQA1*01-DQB1*05:03, DQA1*01-DQB1*06:01, DQA1*01-DQB1*06:02, DQA1*01-DQB1*06:03, DQA1*05-

DQB1*0301, DQA1*03-DQB1*03:01, DQA1*02-DQB1*03:03 or DQA1*02-DQB1*02:02). Based on HLA genotyping, 14.2% of the screened population was deemed at increased risk for developing type 1 diabetes (estimated to contain 68% of future cases). Eligible children were offered periodic surveillance for three islet autoantibodies (GADA, IA2A and IAA) by mail-based or provider-based sampling, via testing in a DASP/IASP participating laboratory under CLIA certification using a DASP/IASP participating laboratory under CLIA certification using published methods (6). A total of 4,674 individuals were tested, with >10% having at least one islet autoantibody present. Children positive for any autoantibodies were asked to submit additional sample(s) for confirmation and closer surveillance. Children with no autoantibodies were followed less frequently. Web-based methods for contact, conveying antibody test results and education about early disease symptoms were also developed. The primary objective of DEW-IT has been to develop methods for population-based type 1 diabetes prediction that are accurate, cost-efficient and suitable to incorporate into the existing healthcare infrastructure. However, because all contact was remote (by phone and mail) the follow up sampling was often less frequent than in the other studies.

Diabetes Prediction in Skane (DiPiS): The DiPiS study is a prospective, longitudinal, population-based study in the southern part of Sweden. (7) Cord blood samples were obtained from 35,688 children born between September 2000 and August 2004 and analysed for type 1 diabetes HLA risk genotypes and cord-blood islet autoantibodies. HLA DQ genotypes were estimated as high-risk (DQA1*05-B1*02/DQB1*03:02 (6 points)), medium-risk (DQB1*03:02/DQB1*06:04 or DQB1*03:02/X (4 points)), low-risk (DQA1*05-B1*02/DQB1*03:04, DQA1*05-B1*02/DQB1*06:04, DQA1*05-B1*02/X (3 points)), neutral (2 points) or protective (0 points).(8) Based on prior observations other risk factors were included in the final risk score: first-degree relative (2 points), positive for cord blood islet autoantibodies (2 points), ABO incompatibility (1 point), febrile infection during pregnancy (1 point), presence of severe life event (1 point), high or low maternal age (1 point) as well as born large for gestational age (1 point). Parents were asked to participate if their child reached 3 points or more. A total of 7,826 families were invited to participate and follow-up samples from 4359 children have been included in the 15-year follow-up. Among the participating children, 233 (12.4 %) had a high-risk HLA genotype, 562 (30 %) medium-risk, 488 (26 %) low-risk, 524 (30 %) neutral, and 67 (3.6 %) had protective genotype. Initial follow up was performed yearly with islet autoantibodies and a yearly questionnaire regarding lifestyle, medical, psychological and dietary factors. Children who developed islet autoimmunity with two or more islet autoantibodies transferred to an intensive follow-up group and were followed every three months with islet autoantibodies, random blood glucose, HbA1c, height and weight and were offered a yearly oral glucose tolerance test.(9)

Diabetes Prediction and Prevention Study (DIPP): The DIPP study is a prospective, population-based birth cohort study launched in 1994 in Finland, where it is ongoing in Oulu, Tampere and Turku University Hospitals (10). In 1994-2009 a total of 145607 children were screened for HLA-DR/DQ associated susceptibility to type 1 diabetes from cord blood using sequence-specific oligonucleotide probes as previously described (11,12). The majority of the children eligible for follow-up carried either the high-risk HLA

genotype DQB1*02/*0302 or the moderate-risk genotypes DQB1*0302/x (x≠*02, *0301, *0602, or *0603). In addition, a few other HLA genotypes were included as detailed in the Supplementary Table 1 of Ilonen et al. (13) A total of 17572 children were eligible and 13204 were enrolled in the study after signing the informed consent. Children who had at least three follow-up visits (n= 11680) were included in the present study.
DIPP participants had follow-up visits at the Oulu and Tampere centers at 3, 6, 12, 18 and 24 months of age and annually thereafter while in the Turku center the visits occurred every 3 months until 2 years of age and biannually thereafter. Children with any positive islet autoantibody were followed 3-monthly until 15 years of age or the diagnosis of type 1 diabetes. Height and weight were measured, and venous blood sample was drawn at every visit. In addition, clinical data such as diet, diseases, medications and vaccinations were collected by using diaries and interviews during study visits. (14,15)

Measurement of islet autoantibodies

BABYDIAB/BABYDIET:

IAA, GADA, IA-2A, and ZnT8A were measured in venous blood samples by the Institute of Diabetes Research, Helmholtz Center Munich, using radiobinding assays (RBAs) and thresholds based on the upper 99th centile and Q-Q plots of results from control children as described elsewhere (1,16,17). IAA levels were measured using a competitive RBA. (18) Assays (laboratory 121) were evaluated by the Diabetes Antibody Standardization Program (DASP) and the Islet Autoantibody Standardization Program (IASP). (19–22)

DAISY:

All antibodies were measured, in duplicate, from frozen serum samples. Autoantibody detection assays have modified over the course of the 24 years study history. Methodology and time periods are described below for each autoantibody measured by the DAISY Study.

Glutamic acid decarboxylase antibodies (GADA) and Islet antigen-2 antibodies (IA-2A):

June 8, 1994-Feb. 16, 2010 autoantibodies to GAD65 were measured utilizing a modified radioassay with in vitro transcribed and translated human GAD65 labeled with [^{3}H]leucine. (23,24) (Results expressed as an index: (sample cpm-negative control cpm)/(positive control cpm-negative control cpm). Samples which test positive for autoantibodies are re-tested for confirmation, using a separately aliquoted sample from the same clinic visit blood draw.

Nov. 17, 1995-Feb. 16, 2010 autoantibodies to islet cell antigen 512 (IA-2) were measured utilizing a modified radioassay with in vitro transcribed and translated human ICA512bdc protein construct labeled with [^{35}S]methionine. (24,25) The GADA and IA-2A autoantibodies were measured in a combined assay. Samples which test positive for autoantibodies are re-tested for confirmation, using a separately aliquoted sample from the same clinic visit blood draw.

Feb. 22, 2010-present the GADA and IA-2A assays were standardized to be brought into alignment with Bristol, UK and Munich, Germany labs. (17)

Insulin autoantibodies (IAA):

March 15, 1994-Dec. 18, 1998 insulin autoantibodies (IAA) were measured using a quantitative radioimmunoassay, competitive insulin assay (cIAA), utilizing [^{125}I] labeled and unlabeled insulin (26) and precipitation with polyethylene glycol. This assay requires a substantial amount of serum, 600ul, so testing was not routinely repeated.

Jan. 27, 1999-present IAA is measured using a micro-IAA assay (mIAA), which utilizes ELISA techniques and Protein A-Sepharose with a much decreased volume of serum and reagents. (27) The assay is conducted in a 96-well format to allow for high-throughput analysis and may be repeated for confirmation of positive samples.

Zinc transporter 8 autoantibodies (ZnT8A)

Dec. 2010-present ZnT8 autoantibodies are measured for islet antibody positive participants. Detection of ZnT8 autoantibodies utilizes the radioimmunoassay similar to GADA and IA-2A, with modifications to allow for the ZnT8 probe. The ZnT8 probe is a synthetic molecule fusion cDNA that is transcribed in vitro and translated in the presence of [^{35}S]methionine. (28,29)

ZnT8 was measured in all ever islet antibody positive participants. If the sample was positive for ZnT8, frozen serum samples from previous clinic visits were tested for ZnT8. All islet antibody positive participants were also tested for ZnT8 antibodies going forward. In 2012, all participants who completed a blood draw at a clinic visit, were tested for ZnT8 antibodies.

DEWIT:

Whole Glutamate Decarboxyase (GAD65) or the intracellular portion of insulinoma antigen-2 (IA2) were biosynthetically radiolabeled in the presence of ^{35}S-methionine, while HPLC-purified ^{125}I-monoiodo-Tyr$_{A14}$-human insulin was purchased commercially (Perkin-Elmer, Hopkinton MA USA). Each antigen was incubated separately in triplicate wells with 2 to 5 uL of patient serum, then captured by Protein A, washed extensively to remove unbound antigen, and counted. Results were expressed using an index relative to specific positive control and negative control sera as described, with each cutoff defined as the 99th percentile of >200 individual healthy population controls. (6) When measured, ZnT8 antibodies were detected via an analogous procedure using biosynthetically ^{35}S-methionine radiolabeled antigen expressed from a construct comprising a hinged dimer containing the antigenic peptide loop of each of the aa325-Arg and the aa325-Trp alleles (JH6.2). Samples close to the cutoff, and those where triplicate well CV's were >30%, were reflexively repeated. The laboratory participates in a DASP/IASP proficiency testing, with DEW-IT assays exceeding mean performance of all participating laboratories for all 4 antigens over each of 12 consecutive workshops. The four autoantibodies assays are each CLIA certified (CLIA# 50D0982418).

DiPiS:

Autoantibodies to GAD65 and IA-2: Analysis in dried blood spots, serum or plasma

GAD65 autoantibodies (GADA) and IA-2 autoantibodies (IA2A) were analyzed in dried blood spots (DBS) with a radioligand binding assay (RBA). (49,50) GADA and IA2A levels were expressed as units per mL (U/mL) derived from the WHO standard 97/550.

The samples were considered to be positive if the IA2A levels were above 5 U/mL or the GADA levels were above 34 U/mL.

Autoantibodies to IAA

Analysis for IAA was first performed using a Noncompetitive method where serum samples (7 mL) were added to duplicate microplate wells and 125I was added and incubated on a shaker at 4°C for 48 h. PAS in a 40% slurry was added to a filter plate and washed three times with Tris buffer. Supermix scintillation solution was added to the wells after the plate had dried for 15 min. The radioactivity was measured in a ßcounter (Wallac Micro Beta Trilux; PerkinElmer). Positive samples for IAA were further analyzed using a competitive method. Serum samples were added to four wells in a 96-well plate. To these wells, 36 mL of 125I insulin with an activity of 60,000 cpm/well were added, with 0.072 IU (or 2 IU/mL) of unlabeled insulin (Actrapid; Novo Nordisk) added to the last two wells. The plates were incubated and examined under conditions similar to those described for the noncompetitive method. IAA levels were calculated as relative units and were related to positive controls. Positivity for IAA was set to 1.9 relative units. The competitive method was used to verify false-positive binding in the noncompetitive assay. In subsequent analysis, the competitive assay was used.

Autoantibodies to ZnT8

ZnT8 autoantibodies (ZnT8A) were analyzed in 5 µL of serum with RBA, as described previously. (30) Duplicate samples were incubated with equal amounts of the three radio-labelled ZnT8 R/W/Q variants. Every putative positive sample (cut-off >59 U/mL) was analyzed for each ZnT8 variant separately.

DIPP:

The islet autoantibodies were analyzed in the Research Laboratory, Department of Pediatrics, University of Oulu, Oulu, Finland except for ZnT8A, which were analyzed in the PEDIA laboratory, University of Helsinki. The biochemical autoantibodies IAA, GADA, IA-2A, and ZnT8A were analyzed with specific radiobinding assays. Classical islet cell antibodies (ICA) were measured by indirect immunofluorescence and ICA was used as the primary screening tool for the beta-cell autoimmunity in children born in 1994-2002. IAA, IA-2A and GADA were measured from all samples obtained from children who became ICA positive. DIPP children born 2003 or later were monitored for ICA, IAA, IA-2A and GADA at every visit. ZnT8A were measured from all samples of children who seroconverted to positivity for at least one other biochemical islet autoantibody (IAA, IA-2A, GADA). In addition, all five islet autoantibodies were measured in the first 1004 children followed in the DIPP study. The reference values for the IAA, GADA, and IA-2A assays were based on the 99th percentile of more than 370 non-diabetic Finnish children and adolescents.

Islet cell antibodies (ICA)

ICA were quantified by standard immunofluorescence on sections of frozen human pancreas, blood group O donor. Fluorescein-conjugated antihuman IgG (Sigma-Aldrich Corp., St. Louis, MO) was used for detection. The end-point dilution titers of the ICA-positive samples were recorded, and the results were expressed in Juvenile Diabetes Foundation units (JDF-U). The detection limit was 2.5 JDF-U.

Insulin Autoantibodies (IAA)

Serum levels of IAA were quantified with a microassay modified from the method described by Williams et al. (31,32) Protein A-Sepharose (Pharmacia Biotech, Uppsala, Sweden) was used for precipitation of antibody-antigen complexes after incubation of the serum samples with mono- [125I]TyrA14-human insulin (Amersham Biosciences, Little Chalfont, UK) for 72 h in the absence or presence of an excess of unlabeled insulin. The IAA titers representing specific binding were expressed in relative units (RU) based on a standard curve run on each plate. A subject was considered positive for IAA when the specific binding exceeded 99th percentile in 371 nondiabetic Finnish subjects.

Autoantibodies to GAD65 (GADA)

GADA were measured by a radiobinding assay as previously described (33). The results were expressed in relative units on the basis of a standard curve. The cut-off limit for positivity was at the 99th percentile for 373 nondiabetic Finnish children and adolescents.

Autoantibodies to islet antigen 2 (IA-2A)

IA-2A were quantified by a radiobinding assay as previously described by (34). Antibody titers were expressed in relative units based on a standard curve. The cut-off limit for positivity was defined by the 99th percentile in 374 healthy Finnish children and adolescents.

Autoantibodies to zinc transporter 8 (ZnT8A)

ZnT8A were analyzed by a radiobinding assay modified from the method by Wenzlau et al. (35) and Salonen et al (36). A35S-methionine-labelled chimeric recombinant plasmid (4.1) encoding the COOH-terminal region (aa268–369) of the ZnT8 aa325-Arg allele and the ZnT8 aa325-Trp allele was used. The detection limit was set based on the levels of 250 nondiabetic Finnish children and adolescents.

Based on the results from the Diabetes Autoantibody Standardization Program (DASP) and the Islet Autoantibody Standardization Program (IASP) in 2010–2016 (19,21,22), the disease sensitivities of the IAA, GADA, IA-2A, and ZnT8A radiobinding assays have been 36–62%, 64–88%, 62–72%, and 62–70, respectively, while the corresponding disease specificities have been 94-98%, 94-99%, 93-100%, and 99–100%, respectively. ICA assay sensitivity in 2016 IASP workshop was 84% and specificity 88%.

Technical Appendix

Foundational features: (Table S1)

There are nine static variables: **1) Sex:** male and female, **2) Race:** black, white, other, biracial **3) Ethnicity:** Hispanic or non-Hispanic**, 4) Birth Month, 5) Birth Year.** Subjects' day of birth was not shared in compliance with the GDPR/HIPPA rules and all dates were shifted (transformed) by individual sites according to their local policy. **6) Type 1 diabetes Family History Status:** as yes/no **7) First Degree relationship** (harmonized to Mother, Father, Sibling, Multiple) **8) Breastfeeding ever status:** as yes/no **9) HLA Genotype (class II) markers - DRB, DQA1, DQB1.**

There are 9 dynamic variables: **1) Clinical Age:** in Years (resolution to 1 decimal point) for each visit (including diagnosis visit) 2) **Height** (harmonized to centimeters)**, 3) Weight** (harmonized to Kilograms) **4) HbA1c** (harmonized to NGSP, US standard), **5) Random Glucose or Plasma Glucose**, **6) OGTT_0** (harmonized to mg/dL), **7**) **OGTT_120** (harmonized to mg/dL) **8) Islet autoantibody status**: as positive/negative for IAA – Insulin autoantibodies, GADA – GAD autoantibodies, IA2 – Insulinoma-associated antigen-2 antibodies. ZnT8 – Zinc transporter 8 autoantibodies (where available, these were measured after the study period from available samples if any only if one of the other three autoantibodies were positive) **9) Type 1 Diabetes Diagnosis Status:** as yes/no.

Harmonized dataset: (Table S2)

The ratio of female to male subjects are similar in each cohort, all cohorts have a majority of male subjects. However, each cohort differed in race/ethnicity distributions (due to geographic differences) as well as in many other aspects. The European sites (DIPP, DiPiS and BABYDIAB) are homogeneous in racial/ ethnicity distributions (data not shown) while the DAISY and DEW-IT (US sites) cohorts are racially diverse. First-degree relatives accounted for all of BABYDIAB participants - 57% and 38% had, respectively, affected mother or father. Within US sites, 44% of DAISY subjects are first-degree relatives – 13%, 16%, and 14% have, respectively affected mother, father or sibling. Only 7% of DiPiS and 9% of DEW-IT participants have an affected relative.

HLA risk groups in the harmonized dataset: (Table S3-S5)

Please refer to supplemental Table S5 for following details. In the harmonized dataset, 3368 (14%) subjects belong to group A, 11224 (46%) to group B, 3969 (16%) to group C, and 5847 (24%) to group D. There are 255 (~1%) of subjects who are not assigned an HLA group because of missing genotype (one haplotype or both missing and a missing allele). The proportion of subjects in the highest harmonized risk group (Group A) are similar in two US sites - DAISY, and DEW-IT (20%, 17% respectively) and in two EU sites - DiPiS and DIPP (12% and 14% respectively) but much lower (6%) in the BABYDIAB (Germany) site. Similarly, Group B proportion is similar in the two US sites - DAISY and DEWIT (47%, 45% respectively) but varies among the EU sites DIPP (66%), BABYDIAB (20%), DiPiS (5%). Overall Group C subject proportions in DIPP and DiPiS are smaller

than in other cohorts (12% each) vs. 20% in DAISY, 21% in BABYDIAB and 26% in DEW-IT. Lastly, among the EU sites, very few subjects in DIPP (8%) are in the lowest risk group, Group D, whereas for DiPiS they are the majority (71%) and sizeable for BABYDIAB (46%). For the US sites, both DAISY and DEW-IT have similar but smaller proportions (13% and 11% respectively) of Group D subjects. In the BABYDIAB cohort 6% of subjects remain unassigned to any group due to incomplete or missing HLA genotype.

Islet autoimmunity and risk of type 1 diabetes in following cohorts

A) Individual study cohorts: (Supplemental Figures S2(a-e))

BABYDIAB: After 15 years follow up from confirmed seroconversion, type 1 diabetes developed in 38% (95% CI 22-59%) of subjects with single autoantibody, in 78% (58-92%) of subjects with two autoantibodies, and 84% (68-95%) of subjects with at least three autoantibodies at seroconversion respectively (Supplemental Figure 1a) with overall $P < 0.0001$.

DAISY: After 15 years follow up from confirmed seroconversion, type 1 diabetes developed in 39% (95% CI 28-50%) of subjects with single autoantibody, 76% (58-85%) of subjects with two autoantibodies, and 92% (82-95%) of subjects with at least three autoantibodies at seroconversion respectively (Supplemental Figure 1b) with overall $P < 0.0001$.

DEW-IT: After ~15 years follow up from confirmed seroconversion, type 1 diabetes had developed in 22% (95% CI 12-40%) of subjects with single autoantibody, 68% (48-85%) of subjects with two autoantibodies, and 75% (52-92%) of subjects with at least three autoantibodies at seroconversion by 15 years respectively (Supplemental Figure 1c) with overall $P < 0.0001$.

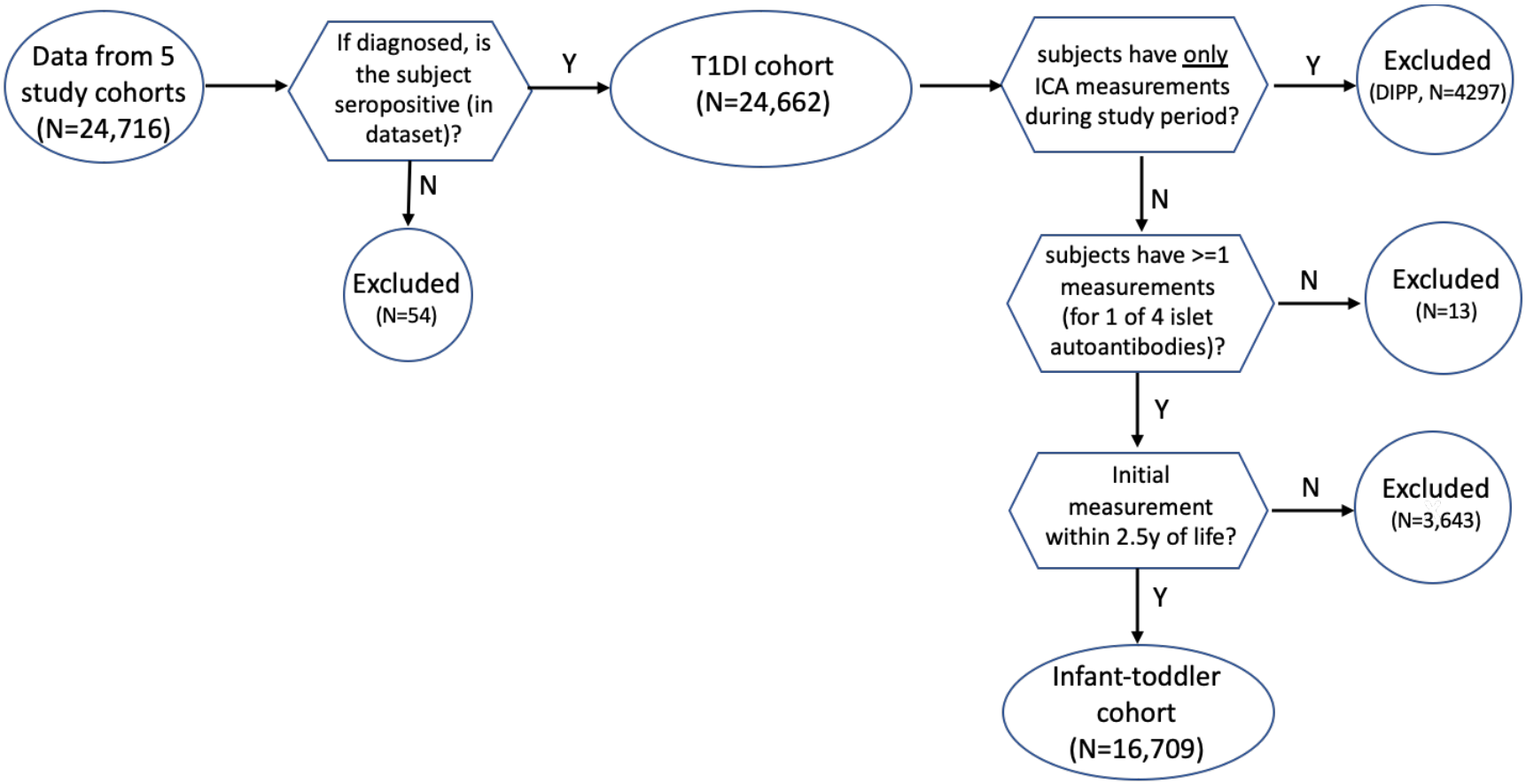
DiPiS: Since DiPiS started in early 2000s and are still following up subjects at age > 12, we report the incidence in the maximum follow up period for which we have data by number of autoantibodies at confirmed seroconversion. After follow-up of 10 years from seroconversion, type 1 diabetes had developed in 12% (95% CI 5-25%) of subjects with single autoantibody, 61% (35-85%) of subjects with two autoantibodies and 82% (65-92%) of subjects with at least three autoantibodies as these data were unavailable for computation of the incidence at 15 years (Supplemental Figure 1d) with overall $P < 0.0001$.

DIPP: After 15 years follow up from confirmed seroconversion, type 1 diabetes had developed in 45% (95% CI 38-50%) of subjects with single autoantibody, in 85% (75-91%) of subjects with two autoantibodies, and 91% (80-95%) of subjects with at least three autoantibodies by ~15 years as these data were unavailable for computation of the incidence at 15 years (Supplemental Figure 1e) with overall $P < 0.0001$.

B) Harmonized T1DI cohort (overall cohort): (Supplemental Figure S2(f))

After 15 years follow up from confirmed seroconversion, type 1 diabetes had developed in 40% (95% CI 35-45%) of subjects with single autoantibody, in 82% (75-88%) of subjects with two autoantibodies, and 90% (84-95%) of subjects with at least three autoantibodies (Supplemental Figure 2) with overall $P < 0.0001$.

Supplemental Figure S1 - Derivation of the infant-toddler cohort



Supplemental Table S1 − Data variables submitted by individual study cohorts

	Static variables – Subject-level*									Dynamic variables - Visit-level (follow up) †															
	Socio-Demo			Type1 Diabetes Family History		Diet	Genetic		Age at Diagno-sis	Growth		Glucose					Islet Autoantibodies†								Visit Age
												OGTT					GADA		IA2A		IAA		ZnT8A		
	DOBm	DOBy	Sex	FDR	Reln	BF ever	HLA loci	HLA Risk group		H	W	0	120	A1c	FG	RG	L	O	L	O	L	O	L	O	
BABYDIAB	✓	✓	✓	✓	✓	✓	DR DQA1 DQB1	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓
DAISY	✓	✓	✓	✓	✓	✓	DR	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
DEW-IT	✓	✓	✓	✓	✓		DR DQA1 DQB1	✓	✓	✓	✓						✓	✓	✓	✓	✓	✓	✓	✓	✓
DiPiS	✓		✓	✓	✓	✓	DQA1 DQB1	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
DIPP	✓	✓	✓				DR DQA1 DQB1	✓	✓	✓	✓						✓	✓	✓	✓	✓	✓	✓	✓	✓

* FDR: First Degree Relative of Type 1 diabetes, Reln: Relationship if any, BF ever: Breastfed ever (yes / no), H: Height, W: Weight
† L: Titer level of islet autoantibodies *(not harmonized as of this study)*, O: Binary output (0 or 1), FG: Fasting Glucose, RG: Random Glucose

Supplemental Table S2 − Characteristics of T1DI study cohort*†

Cohort	Sex (F)	Race				Hispanic	Breast fed ever (Y)	Fam Hx (Y) %, [Unknown Relationship n =]	FDR (Relationship) N %			
		W	B	O	Biracial				Father	Mother	Sibling or half-sib	Multiple
BABYDIAB (N=2376)	1158 49%						1787 75%	2376 100% [none]	895 38%	1344 57%	57 2%	80 3%
DAISY (N=2544)	1220 48%	2296 90%	62 2%	27 1%	139 6%	544 21%	2077 82%	1126 44% [n = 1]	414 16%	324 13%	362 14%	25 1%
DEW-IT (N=3756)	1803 48%	2713 72%	15 <1%	25 <1%	149 4%	161 4%		355 10% [none]	132 4%	89 2%	81 2%	43 1%
DiPiS (N=4360)	2071 48%						3520 81%	308 7% [n = 103]	149 3%	129 3%	25 <1%	5 <1%
DIPP (N=11680)	5252 45%	11680 100%						not available				

*All variables contain missing values, except Sex
† Contains all data, including for N=54 diagnosed cases with no follow up or positive islet autoantibodies during the study period which were excluded for all analyses

Supplemental Table S3 − Haplotype groups*

DR3-DQ2.5 includes	**DR4-DQ8.1 includes**	
(DR3)-DQA1*05-DQB1*02 (DR3)-DQA1*05:01-DQB1*02:01 DQ2 (Finland)	DRB1*04:01-DQA1*03-DQB1*03:02 DRB1*04:02-DQA1*03-DQB1*03:02 DRB1*04:03-DQA1*03-DQB1*03:02 DRB1*04:04-DQA1*03-DQB1*03:02 DRB1*04:05-DQA1*03-DQB1*03:02 DRB1*04:08-DQA1*03-DQB1*0304 DRB1*04:08-DQA1*03-DQB1*03:02 DRB1*04:13-DQA1*03-DQB1*03:02 (DR4)-DQA1*03:01-DQB1*03:02/4 (DR4)-DQA1*03-DQB1*03:02 (DR4/9)-DQA1*03:01-DQB1*03:02/3/4 DRB1*04:01-DQA1*03:01-DQB1*03:02 DRB1*04:04-DQA1*03:01-DQB1*03:02 DQA1*03:01-DQB1*03:02/3/4 (DR4)-DQA1*03:01-DQB1*03:02 (DR4)-DQA1*03:01-DQB1*03:02/4 (DR4)-DQA1*03-DQB1*03:02	
X (Neutral) includes	**Y (Protective) includes**	**Z (Highly Protective) includes**
(DR1)-DQA1*01:01-DQB1*05:01	(DR11/12/13)-DQA1*05-DQB1*03:01	(DR13/14/15/16)-DQA1*01-DQB1*06
(DR1/10)-DQA1*01-DQB1*05:01	(DR4)-DQA1*03-DQB1*03:01	(DR13)-DQA1*01:02-DQB1*06:09
(DR1/10)-DQA1*01-DQB1*05:01	(DR4)-DQA1*03-DQB1*03:01	(DR13)-DQA1*01-DQB1*06:09
(DR1/10)-DQB1*05:01	DRB1*04:01-DQA1*03-DQB1*03:01	(DR14)-DQA1*01:03-DQB1*0503
(DR4)-DQA1*030x-DQB1*04:01	DRB1*04:01/8-DQA1*03-DQB1*03:01	(DR14)-DQA1*01:04-DQB1*0503
(DR4)-DQA1*03:01-DQB1*04:01	DRB1*0408-DQA1*03-DQB1*03:01	(DR7)-DQA1*0201-DQB1*03:03
(DR4)-DQA1*03:02-DQB1*02:02	(DR11/13)-DQA1*05-DQB1*03:01/9	(DR15)-DQB1*06:02
(DR9)-DQA1*03:0x-DQB1*02:02	(DR11/13)-DQA1*05:01-DQB1*03:01	(DR13)-DQB1*06:03
(DR9)-DQA1*03:02-DQB1*03:03	(DR11/13) -DQA1*05-DQB1*03:01/9	(DR13)-DQB1*06:03
(DR9)-DQA1*03:01-DQB1*03:02	(DR11/13)-DQA1*05-DQB1*03:01	(DR13)-DQB1*06:09
(DR9)-DQA1*03-DQB1*03:03	(DR15)-DQB1*06:01	(DR14)-DQB1*05:03
(DR9)-DQA1*03:01-DQB1*03:04	(DR7)-DQA1*02:01-DQB1*02:02	(DR7)-DQA1*02:01-DQB1*03:03
(DR9)-DQA1*03:01-DQB1*03:03	(DR7)-DQA1*02:01-DQB1*02:01	(DR15)-DQA1*01-DQB1*06:03
(DR13)-DQA1*01:02-DQB1*06:04	(DR7)-DQA1*02:01-DQB1*02	(DR15)-DQA1*01-DQB1*06:02/3

(DR13)-DQB1*06:04	(DR7)-DQA1*02:01-DQB1*03:01?	(DR15)-DQA1*01:03-DQB1*06:03
(DR16)-DQA1*01:02-DQB1*05:02	(DR4)-DQA1*03-DQB1*03:01	(DR15)-DQA1*01:03-DQB1*06:02/3
(DR16)-DQB1*05:02	(DR3)-DQA1*05:01-DQB1*03:01?	(DR15)-DQA1*01:02-DQB1*06:02
(DR8)-DQA1*04:01-DQB1*04:02	DRB1*0403-DQA1*03-DQB1*03:02	DRB1*0407-DQA1*03-DQB1*03:01
(DR8)-DQB1*04	(DR4)-DQA1*03:01-DQB1*03:01	
(DR13/14/15/16)-DQA1*01-DQB1*05/06	DRB1*0407-DQA1*03-DQB1*03:02	
	(DR4)-DQA1*03-DQB1*03:01	
	(DR8)-DQA1*03-DQB1*03:01	
	(DR15)-DQA1*01:03-DQB1*06:01	

***** Based on odds ratios from Table 2 (37)

Supplemental Table S4 − HLA harmonization to 4 Risk Groups

Group A	**Group B**	**Group C**	**Group D**
DR3-DQ2.5/DR4-DQ8.1	DR-DQ8.1/DR-DQ8.1	DR-DQ8.1/Y	DR3-DQ2.5/Y
	DR-DQ8.1/X	DR3-DQ2.5/X	DR3-DQ2.5/Z
	DR3-DQ2.5/DR3-DQ2.5	DR-DQ8.1/X or Y or Z	DR-DQ8.1/Z
			X/Y
			Y/Y
			X/X X/Z Y/Z Z/Z
			DR3-DQ2.5/X or Y

Supplemental Table S5 − Summary of HLA risk group assignment

Cohort	Cohort Type	Cohort Subjects (N)	Group: A	Group: B	Group: C	Group: D	Unassigned*
BABYDIAB	Overall	2376	134 (6%)	484 (20%)	510 (22%)	1097 (46%)	151 (6%)
	Infant / Toddler	2346	131 (6%)	477 (20%)	504 (22%)	1092 (47%)	142 (6%)
DAISY	Overall	2544	502 (20%)	1196 (47%)	501 (20%)	338 (13%)	7 (<1%)
	Infant / Toddler	2170	466 (22%)	1057 (49%)	410 (19%)	230 (11%)	7 (<1%)
DEW-IT	Overall	3756	627 (17%)	1674 (45%)	994 (27%)	407 (11%)	54 (1.4%)
	Infant / Toddler	559	157 (28%)	310 (56%)	37 (7%)	50 (9%)	5 (<1%)
DiPiS	Overall	4360	515 (12%)	199 (5%)	533 (12%)	3111 (71%)	2 (<1%)
	Infant / Toddler	4353	514 (12%)	199 (5%)	532 (12%)	3106 (71%)	2 (<1%)
DIPP	Overall	11680	1603 (14%)	7694 (66%)	1440 (12%)	899 (8%)	44 (<1%)
	Infant / Toddler	7281	944 (13%)	4589 (63%)	1025 (14%)	701 (10%)	22 (<1%))
Totals† (N=24,716)	Overall	**24716**	**3381 (14%)**	**11247 (45%)**	**3978 (16%)**	**5852 (24%)**	**258 (1%)**
	Infant / Toddler	**16709**	**2212 (13%)**	**6632 (40%)**	**2508 (15%)**	**5179 (31%)**	**144 (1%)**

*Genotyping missing or indeterminate, †Contains all data, including for N=54 diagnosed cases with no follow up or positive islet autoantibodies during the study period which were excluded for all analyses

Supplemental Table S6 – Cumulative incidence of type 1 diabetes by HLA risk group and number of islet autoantibodies at seroconversion in <u>overall cohort</u> (N=1613)

HLA Class II	Num of islet antibodies Risk Group	T1D Incidence in 10 Years or as noted					
		Single (1) (95% CI) (N=1191)				Two (2) (95% CI) || (Fig S3b) @Seroconversion (N=319)	Three (3) (95% CI) (Fig S3c) @Seroconversion (N=103)
		@Sero conversion (Fig S3a)	@Seroconversion + 2 y (N=1009) (Fig S4a)				
		1*	S-0‡ (N=335)	S-S† (N=419)	S-M§ (N=255)		
DR4-DQ8/DR3-DQ2.5	A	51% (45-60%)	25% (5-48%)	40% (28-50%)	68% (55-75%)	72% (55-95%)	88% (75-95%)
DR4-DQ8/DR4-DQ8 DR4-DQ8/neutral (x) DR3-DQ2/DR3-DQ2	B	32% (28-38%)	5% (4-18%)	25% (15-30%)	55% (45-75%)	65% (50-85%)	92% (80-95%)
DR4-DQ8/protective (y) DR3-DQ2/neutral (x) DR4-DQ8/unknown	C	25% (15-35%)	5% (4-15%)	25% (5-30%)	95% (75-98%)	80% (50-90%)	60% (30-88%) *in 5y*

DR4-DQ8/highly protective (z) DR3-DQ2/protective (y) DR3-DQ2/highly protective (z) DR3-DQ2/unknown Neutral (x) /any except DR3 or 4 Protective (y) /any except DR3 or 4 Highly protective (z) /any except DR 3 or 4	**D**	12% (8-35%)	2% (0-10%)	2% (0-10%)	65% (45-85%)	70% (56-95%)	80% (55-95%)

* (**Group A vs D, Group A vs B, Group A vs C, Group B vs D: *P* < 0.0001**), (Group B vs C: ***P* = 0.04**), (Group C vs D: ***P* = 0.02**)
† (**Group A vs D *P* < 0.0001**), (Group B vs C: *P* = 0.01, **Group B vs D: *P* = 0.0001**) (Group A vs B: *P* = 0.048) (Group C vs A: ***P* = 0.0002**)
‡ (Group A vs B: *P* = 0.0013, Group A vs C: *P* = 0.015, Group A vs D: *P* = 0.008)
§ (Group A vs B: *P* = 0.03) (Group B vs C: *P* = 0.01)
| | (Group A vs B: *P* = 0.05)

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

Supplemental Table S7 − Risk of progression to type 1 diabetes among children with multiple islet autoantibodies (at seroconversion or thereafter) during follow-up in overall cohort (N=912)

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)
<= 2.08 years	228	62% [55%, 68%]	83% [77%, 89%]	92% [87%, 95%]	17.8
>2.08 and <= 4.13 years	228	42% [36%, 49%]	74% [67%, 80%]	87% [78%, 92%]	13.4
>4.13 and <= 8.16 years	228	31% [24%, 37%]	63% [55%, 70%]	75% [66%, 82%]	9.7
>8.16 and <= 21.2 years	228	27% [20%, 33%]	48% [35%, 59%]	56% [39%, 68%]	6.4
Total	912				11.8

Figure captions

Supplemental Figure S2 − Cumulative incidence of type 1 diabetes by the number of positive islet autoantibodies at seroconversion in individual studies and the combined (overall) cohort.

(a) BABYDIAB
(b) DAISY
(c) DEWIT
(d) DiPiS
(e) DIPP
(f) Combined cohort

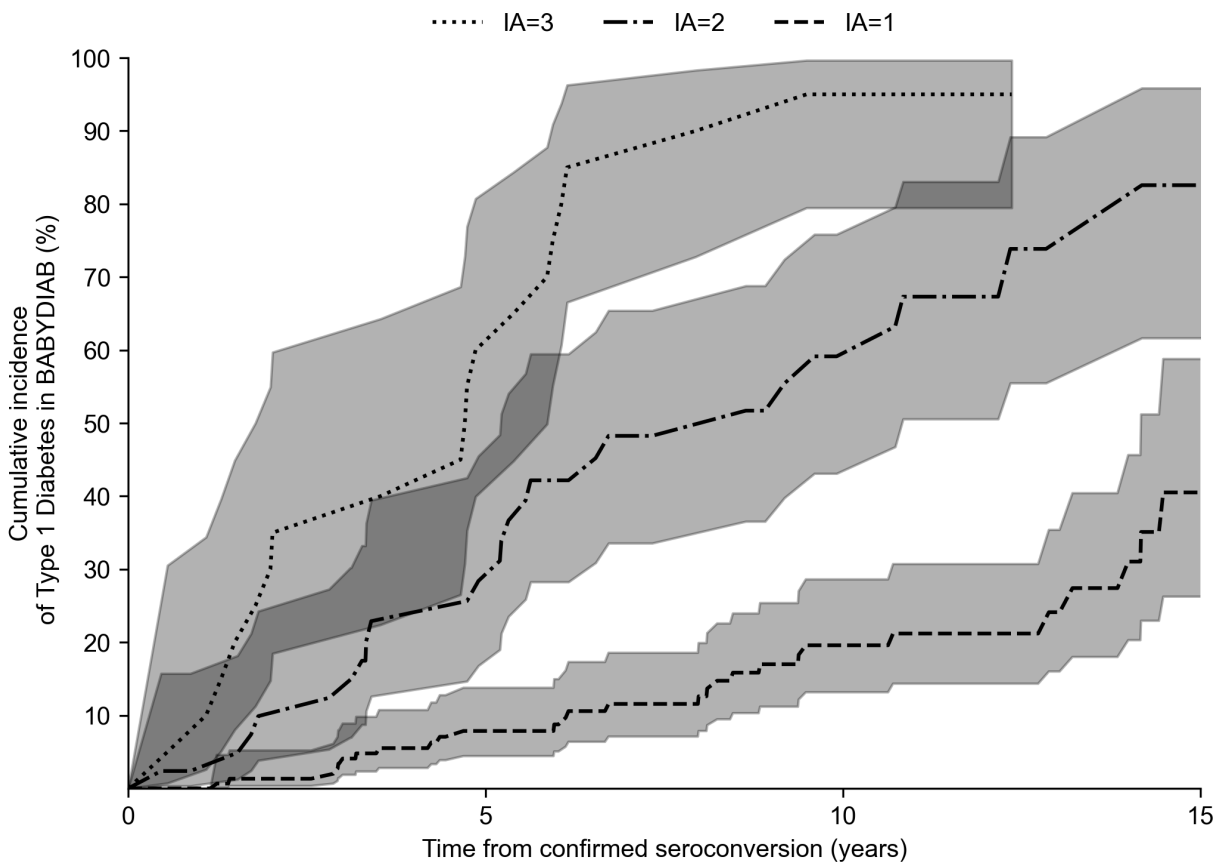
Supplemental Figure S3 − Cumulative incidence of type 1 diabetes by the number of autoantibodies at seroconversion and HLA risk group in the combined (overall) cohort.

(a) Single autoantibody
(b) Two autoantibodies
(c) Three autoantibodies

Supplemental Figure S4 − Cumulative incidence of type 1 diabetes by HLA risk group among subjects with stable single autoantibody (S-S).

(a) in the overall cohort
(b) in the infant-toddler cohort

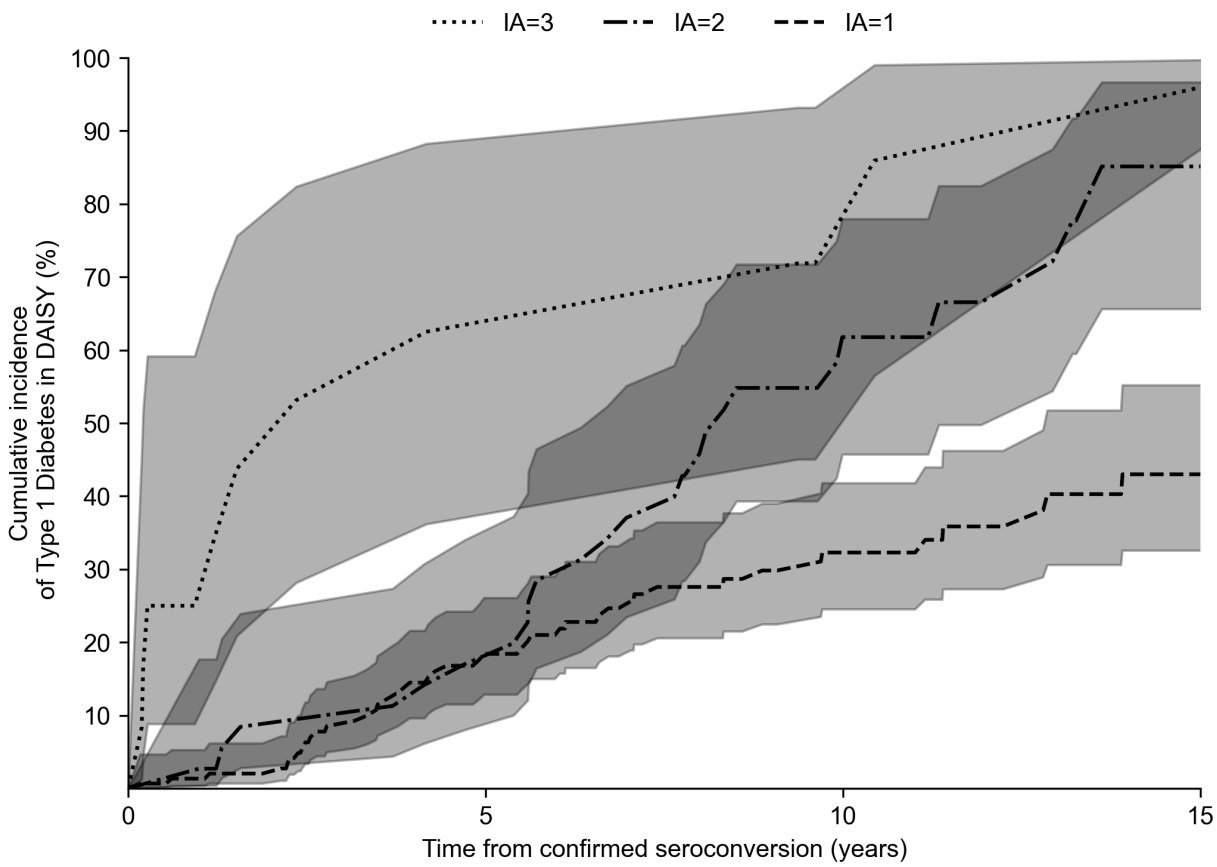
Supplemental Figure S5 − Cumulative incidence of type 1 diabetes by age quartile at development of multiple positivity in the infant-toddler cohort.



Number at risk

IA=3	20	8	1	0
IA=2	42	26	10	2
IA=1	158	113	56	8

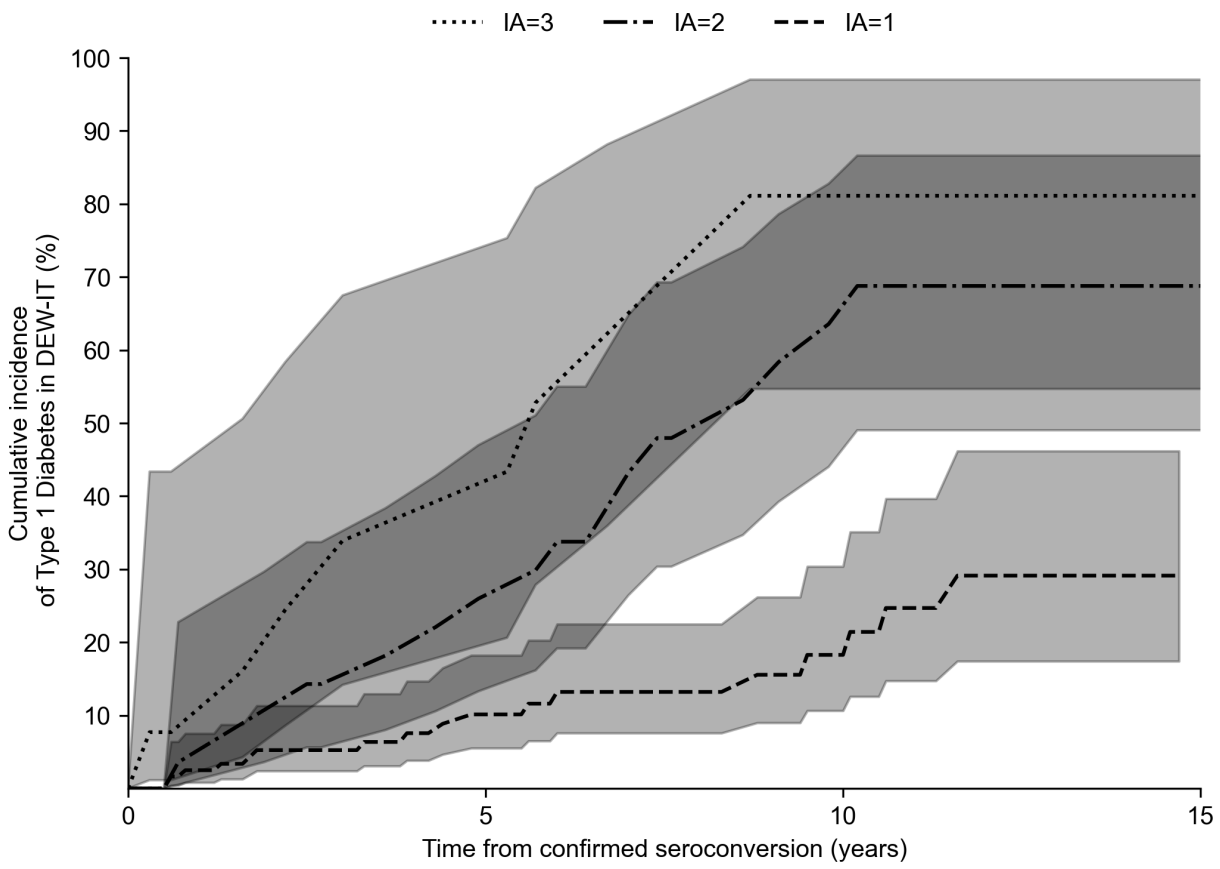
Figure S2(a) - Cumulative incidence of type 1 diabetes by the number of positive islet autoantibodies at seroconversion in BABYDIAB (N=220 subjects, median age @ seroconversion: 5.57 years)



Number at risk

IA=3	12	4	2	1
IA=2	37	29	11	1
IA=1	150	100	52	16

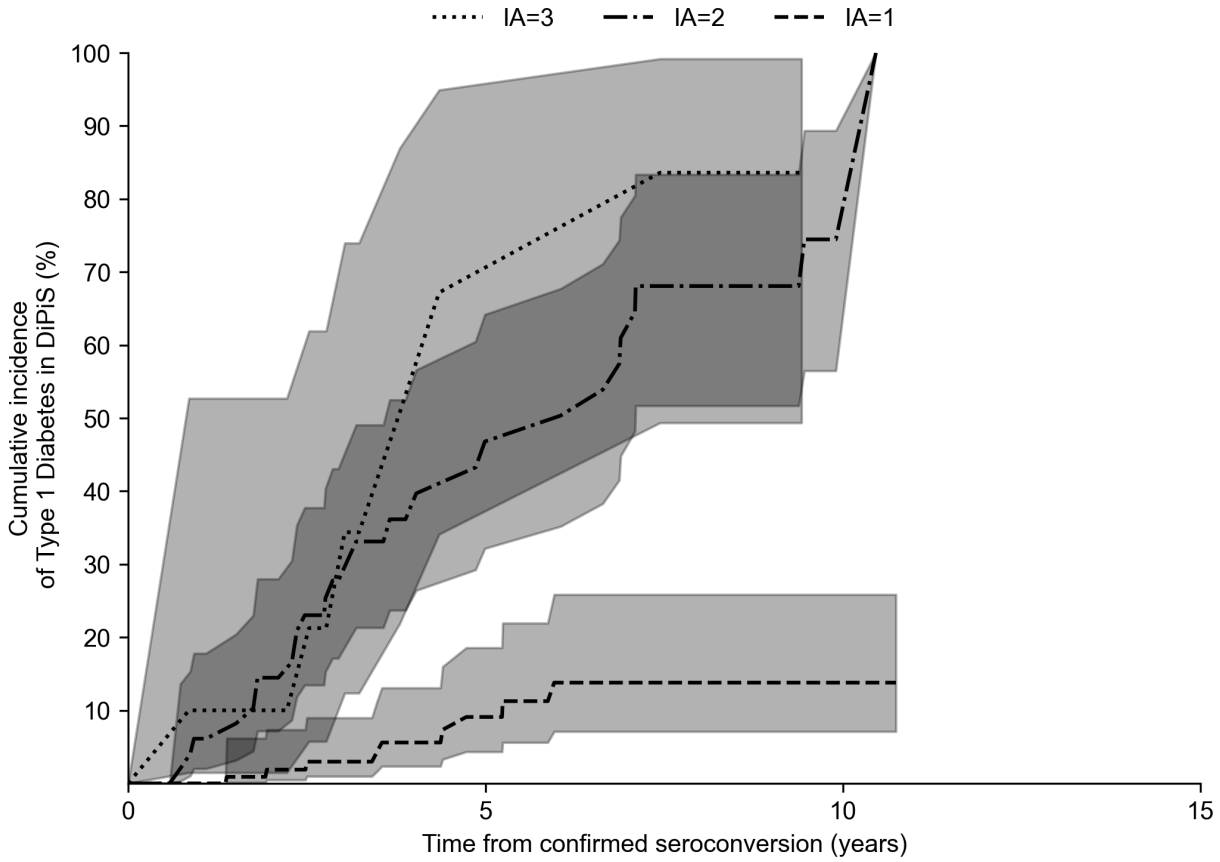
Figure S2(b) - Cumulative incidence of type 1 diabetes by the number of positive islet autoantibodies at seroconversion in DAISY (N=199 subjects, median age @ seroconversion: 5.96 years)



Number at risk

IA=3	13	7	2	1
IA=2	32	19	7	1
IA=1	128	66	27	0

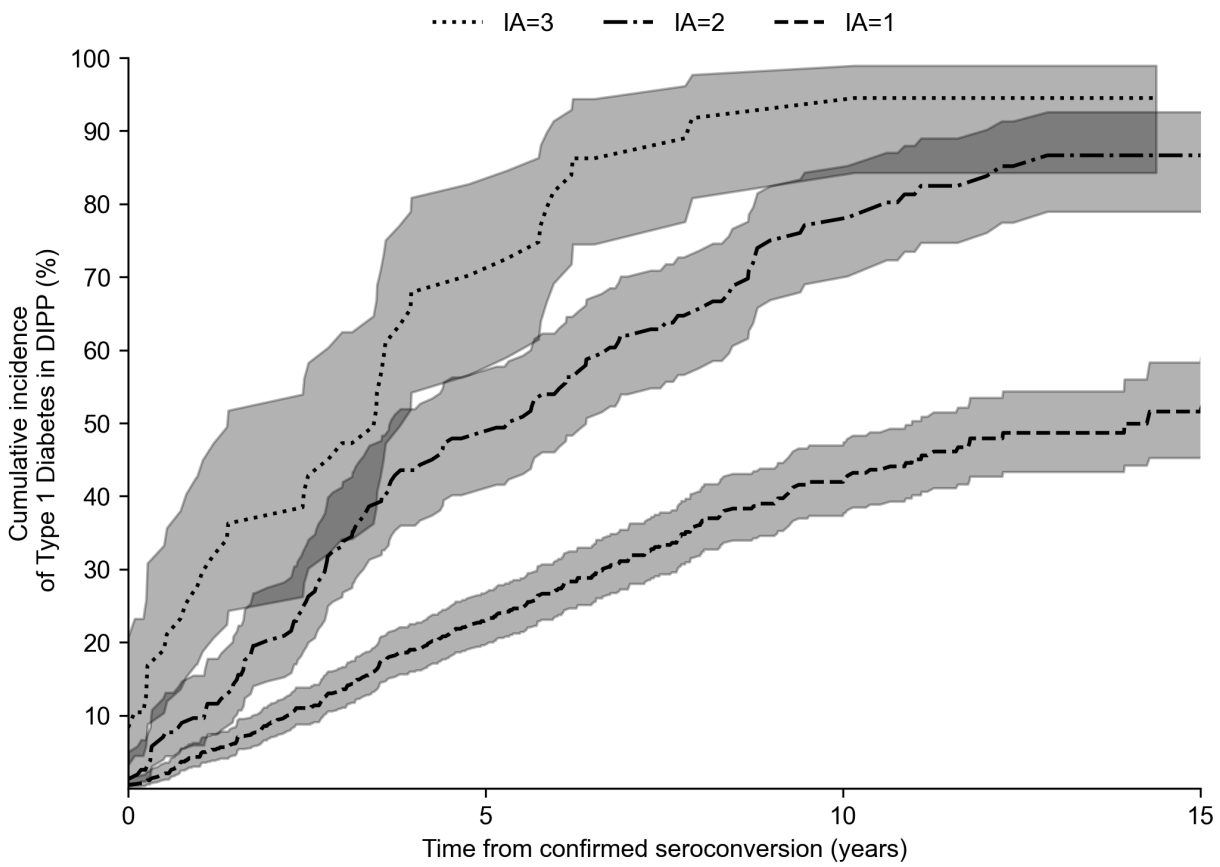
Figure S2(c) - Cumulative incidence of type 1 diabetes by the number of positive islet autoantibodies at seroconversion in DEW-IT (N=173 subjects, median age @ seroconversion: 7.5 years)



Number at risk

IA=3	10	2	0	0
IA=2	51	15	1	0
IA=1	123	46	1	0

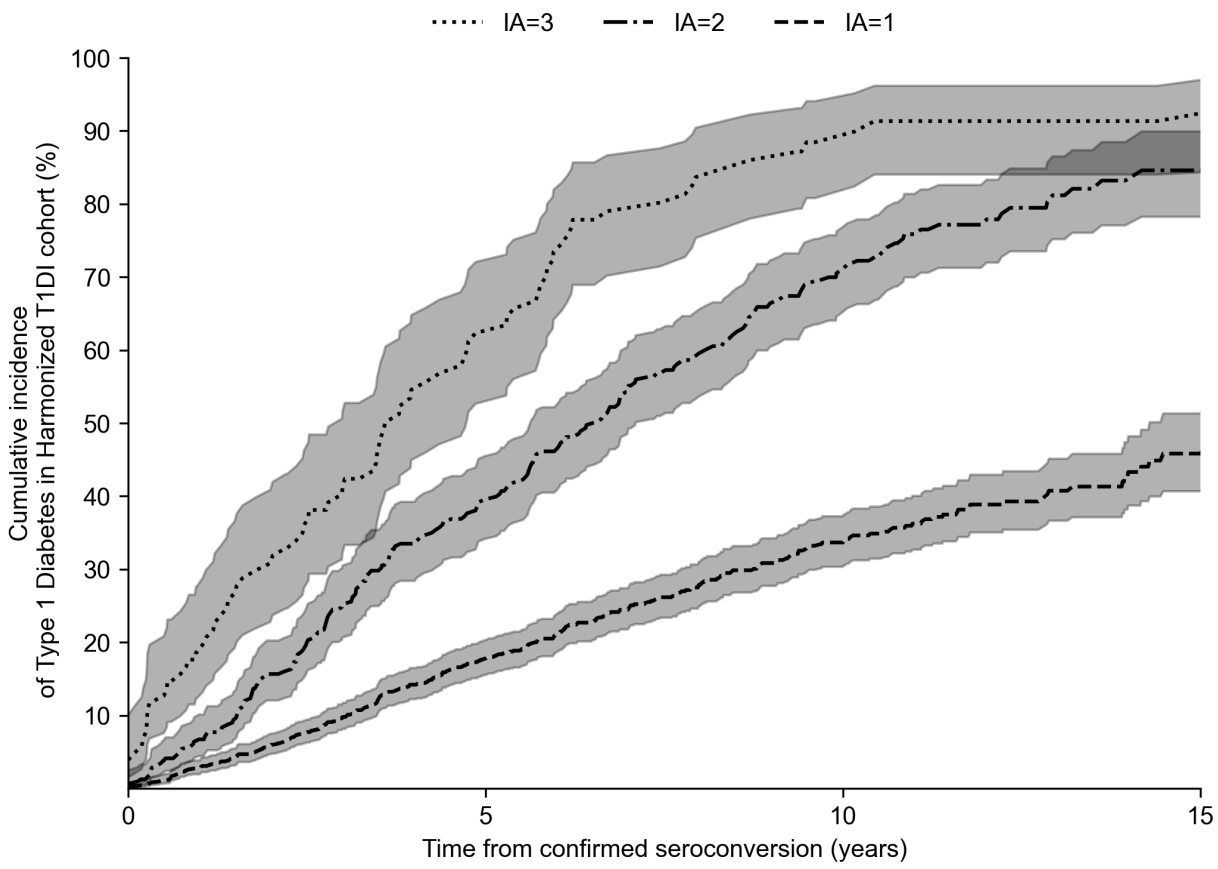
Figure S2(d) - Cumulative incidence of type 1 diabetes by the number of positive islet autoantibodies at seroconversion in DiPiS (N=184 subjects, median age @ seroconversion: 7.03 years)



Number at risk

IA=3	48	13	3	0
IA=2	157	69	22	3
IA=1	632	360	144	19

Figure S2(e) - Cumulative incidence of type 1 diabetes by the number of positive islet autoantibodies at seroconversion in DIPP (N=837 subjects, median age @ seroconversion: 3.04 years)



Number at risk

IA=3	103	34	8	2
IA=2	319	158	51	7
IA=1	1191	685	280	43

Figure S2(f) - Cumulative incidence of type 1 diabetes by the number of positive islet autoantibodies at seroconversion in the overall cohort (N=1613 subjects, median age @ seroconversion: 4.6 year

Figure S3-1

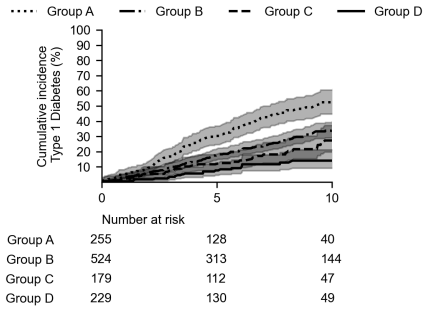


Figure S3-1(a)

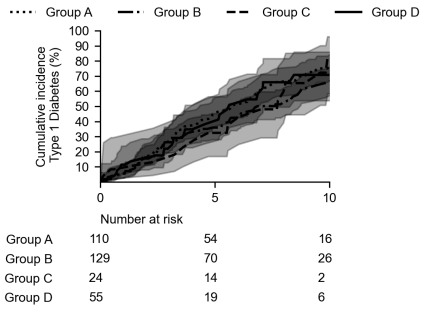


Figure S3-1(b)

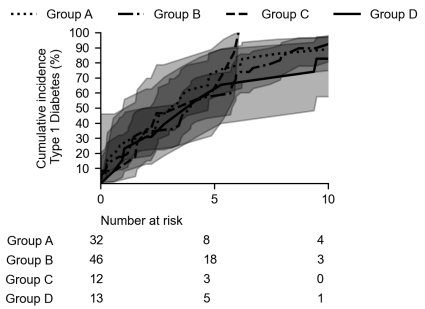


Figure S3-1(c)

Figure S3-2

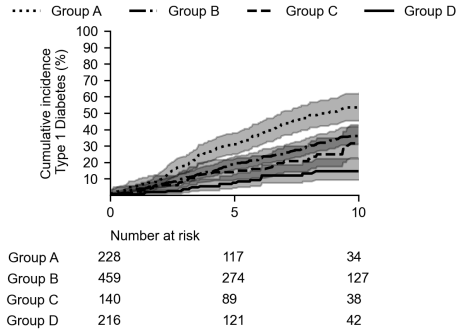


Figure S3-2(a)

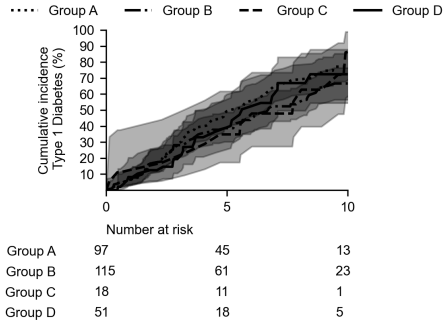


Figure S3-2(b)

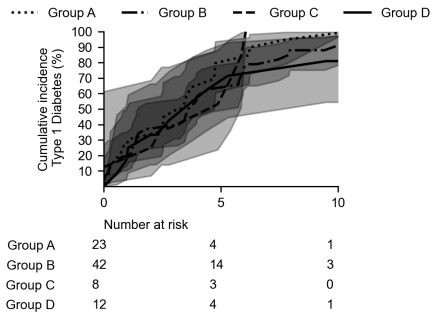
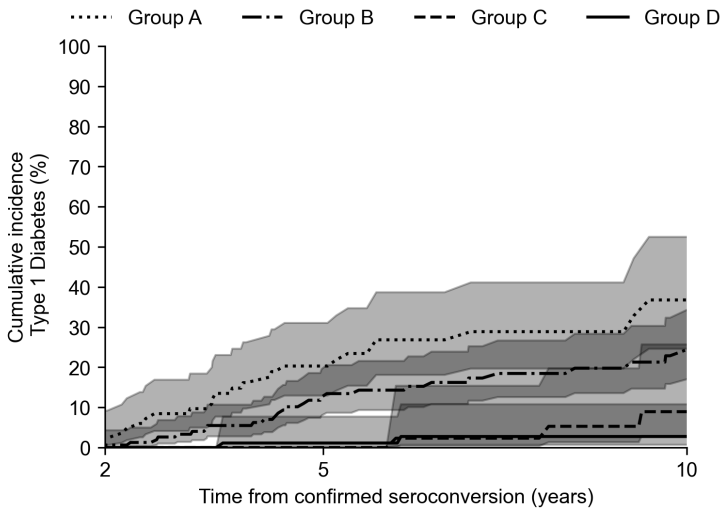


Figure S3-2(c)

Figures (a) – Single autoantibody, (b) – Two autoantibodies, (c) – Three autoantibodies

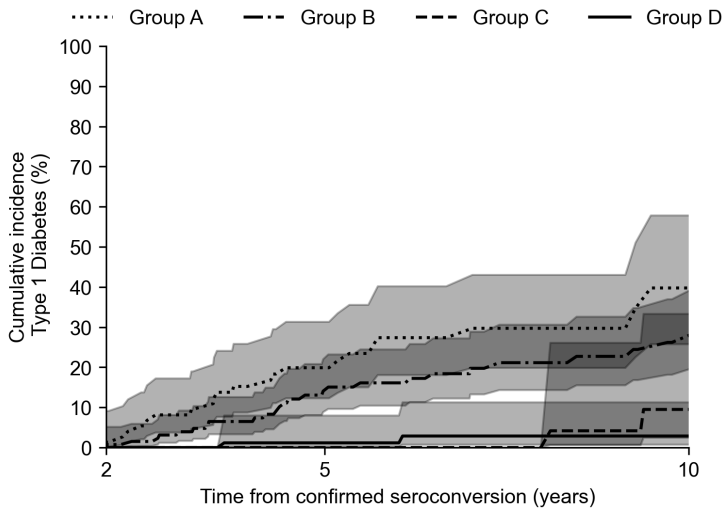
Supplemental Figure S3 − Cumulative incidence of type 1 diabetes by the number of autoantibodies at seroconversion and HLA risk group in (3-1) - the overall cohort (N=1613) and in (3-2) - the infant-toddler cohort (N=1413).

S4(a)



Number at risk			
Group A	82	52	15
Group B	161	106	48
Group C	63	50	21
Group D	108	70	24

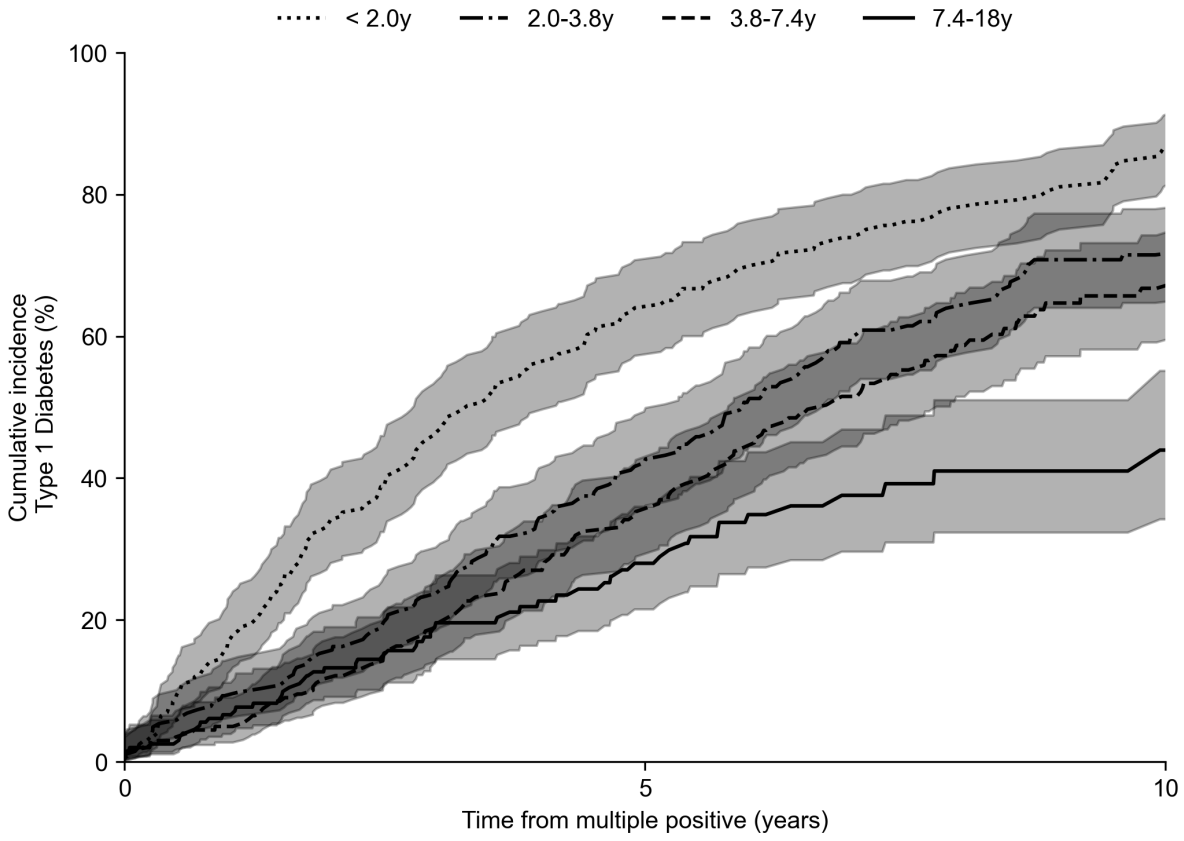
S4(b)



Number at risk			
Group A	74	46	11
Group B	134	86	41
Group C	39	33	15
Group D	104	67	22

Supplemental Figure S4 − Cumulative incidence of type 1 diabetes by HLA risk group among subjects with stable single autoantibody (S-S) in S4(a) - in the overall cohort and S4(b) - in the infant-toddler cohort.

Supplemental Figure S5 − Cumulative incidence of type 1 diabetes by age quartile at development of multiple positivity in the infant-toddler cohort.



Number at risk

< 2.0y	202	67	22
2.0-3.8y	205	111	38
3.8-7.4y	202	115	30
7.4-18y	203	78	19

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