Supplementary Appendix

Supplement to Ng et al., Islet autoantibody type-specific titer thresholds improve stratification of risk of progression to type 1 diabetes in children

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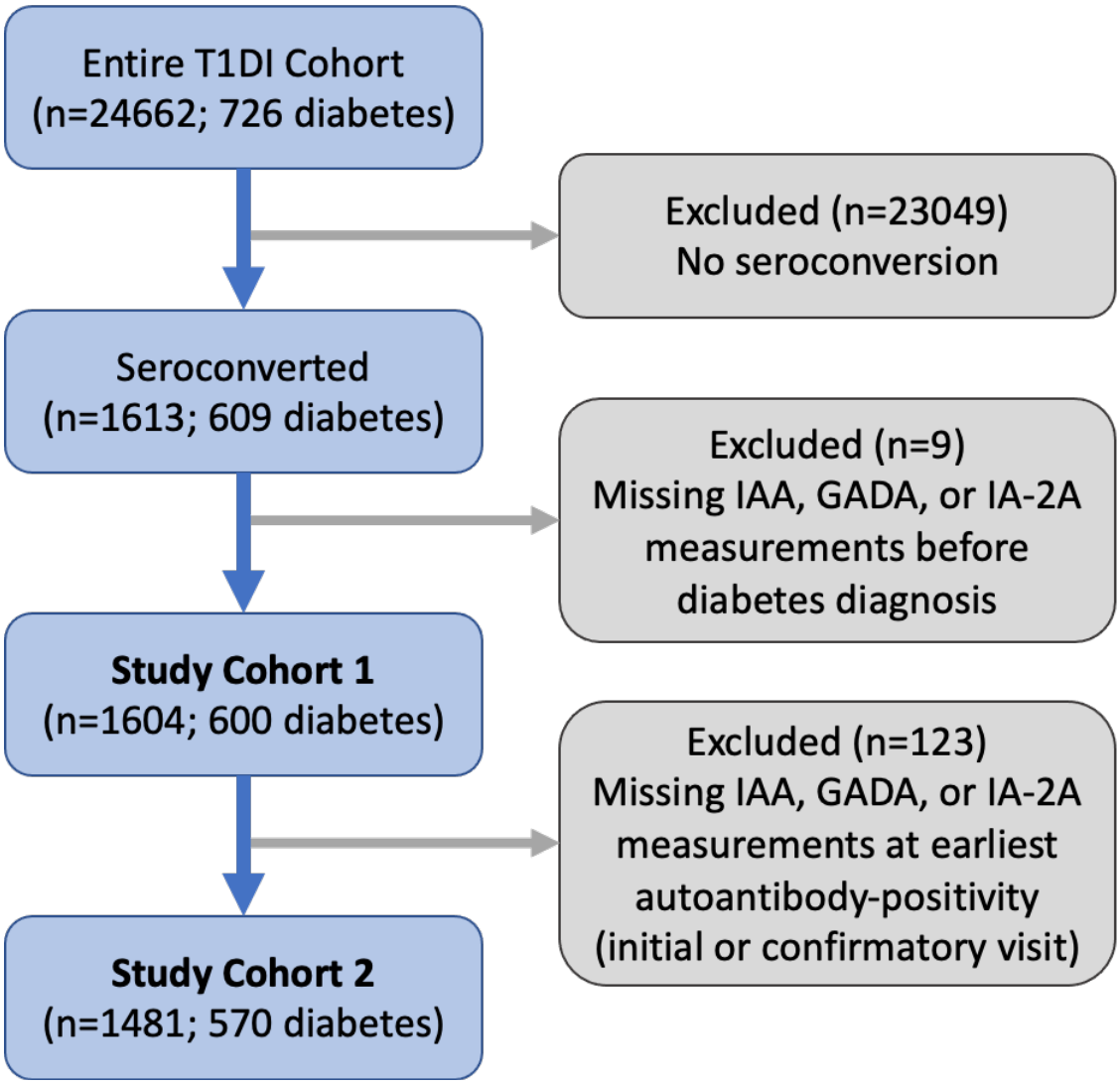
Characteristic	Count (%) or Mean ±SD		
	All (n=1604)	**Developed Diabetes (n=600)**	**Did Not Develop Diabetes (n=1004)**
Female Sex – no. (%)	717 (44.7)	264 (44.0)	453 (45.1)
Age – yr			
IAA Initial Visit	5.0 ±4.0	3.4 ±2.9	6.5 ±4.3
Min-Max Age at Initial Visit	0.3 – 22.2	0.3 – 22.2	0.3 – 20.5
GADA Initial Visit	5.6 ±4.0	3.9 ±3.0	6.9 ±4.2
Min-Max Age at Initial Visit	0.0 – 23.3	0.0 – 18.3	0.0 – 23.3
IA-2A Initial Visit	5.3 ±3.7	4.2 ±3.1	7.3 ±4.1
Min-Max Age at Initial Visit	0.3 – 17.5	0.6 – 16.8	0.3 – 17.5
IAA Confirmatory Visit	5.4 ±4.1	3.8 ±2.9	7.0 ±4.4
Min-Max Age at Confirmatory Visit	0.5 – 22.3	0.5 – 22.3	0.5 – 20.6
GADA Confirmatory Visit	6.1 ±4.1	4.3 ±3.0	7.5 ±4.3
Min-Max Age at Confirmatory Visit	0.5 – 23.9	0.7 – 18.6	0.5 – 23.9
IA-2A Confirmatory Visit	5.7 ±3.9	4.6 ±3.2	7.8 ±4.2
Min-Max Age at Confirmatory Visit	0.4 – 19.4	0.7 – 18.7	0.4 – 19.4
Data Source – no. (%)			
BABYDIAB	220 (13.7)	74 (12.3)	146 (14.5)
DAISY	199 (12.4)	81 (13.5)	118 (11.8)
DEW-IT	173 (10.8)	42 (7.0)	131 (13.0)
DIPIS	184 (11.5)	42 (7.0)	142 (14.1)
DIPP	828 (51.6)	361 (60.2)	467 (46.5)
HLA Risk Group – no. (%)			
A	393 (24.5)	219 (36.5)	174 (17.3)
B	696 (43.4)	264 (44.0)	432 (43.0)
C	214 (13.3)	58 (9.7)	156 (15.5)
D	296 (18.4)	58 (9.7)	238 (23.7)
Missing	5 (0.3)	1 (0.2)	4 (0.4)
Autoantibody titer at Confirmatory Visit – mULN			
IAA	7.5 ±15.8	9.6 ±17.6	5.3 ±13.6
GADA	15.7 ±80.1	20.4 ±91.5	12.0 ±69.5
IA-2A	90.7 ±145.7	104.0 ±144.7	65.3 ±144.4

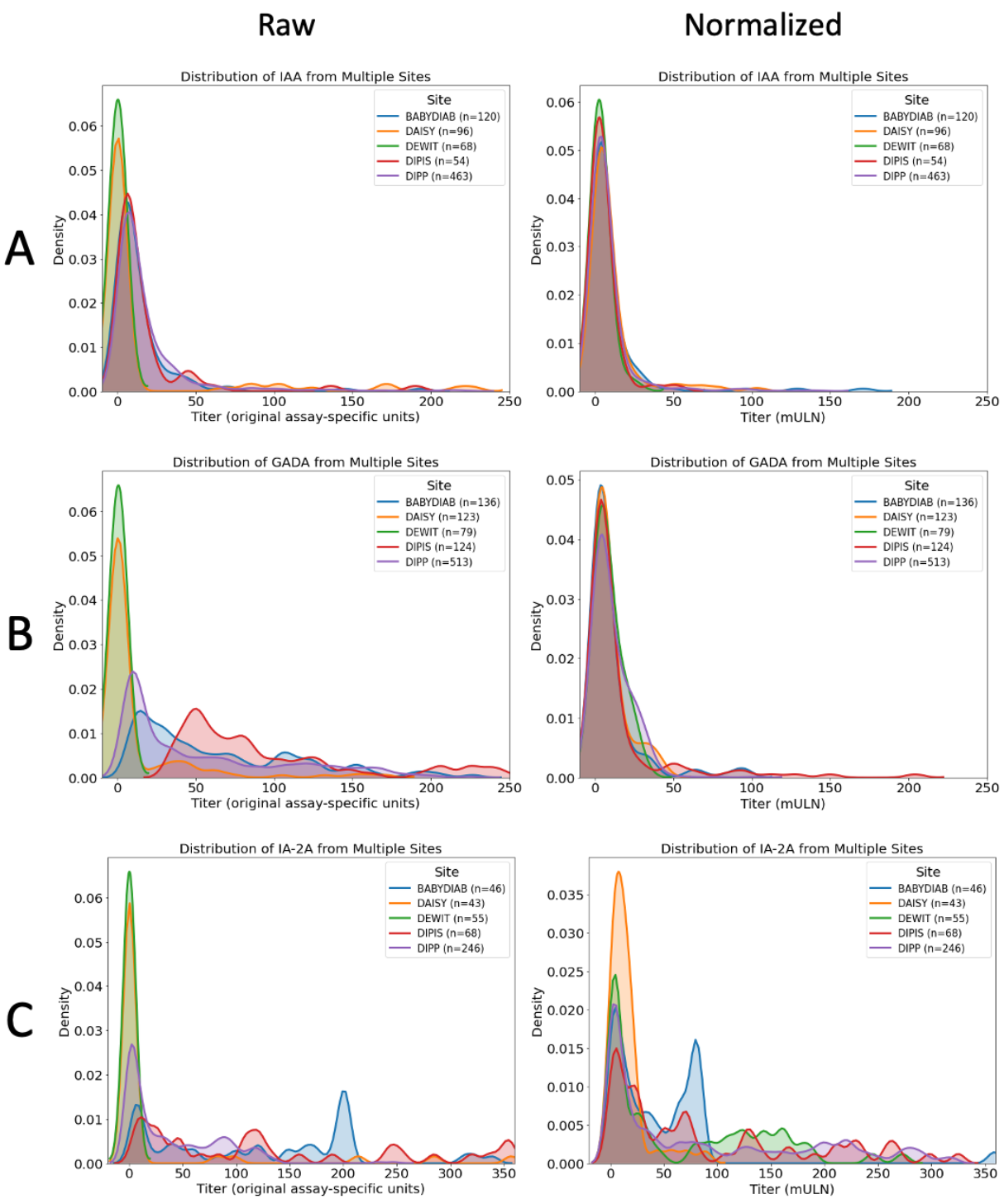
Supplemental Table S1: Key characteristics of the Study Cohort 1 population.
Plus–minus values are means ±SD. Percentages may not total to 100 because of rounding. Autoantibody-positive percentages may not total to 100 due to multiple positivity.
Abbreviations: HLA, human leukocyte antigen; GADA, glutamic acid decarboxylase autoantibodies; IAA, insulin autoantibodies; IA-2A, insulinoma-associated antigen-2 autoantibodies; mULN, multiples of upper limit of normal.

Characteristic	Count (%) or Mean ±SD		
	All (n=1481)	Developed Diabetes (n=570)	Did Not Develop Diabetes (n=911)
Female Sex – no. (%)	659 (45.5)	251 (44.0)	408 (44.8)
Age – yr			
Earliest autoantibody positivity (initial visit)	5.5 ±4.1	3.6 ±2.9	6.7 ±4.3
Min-Max age at earliest positivity (initial visit)	0.3 – 23.3	0.3 – 16.8	0.3 – 23.3
Earliest autoantibody positivity (confirmatory visit)	6.0 ±4.3	4.0 ±3.0	7.2 ±4.5
Min-Max age at earliest positivity (confirmatory visit)	0.5 – 23.9	0.5 – 18.7	0.5 – 23.9
Data Source – no. (%)			
BABYDIAB	216 (14.6)	71 (12.5)	145 (15.9)
DAISY	196 (13.2)	79 (13.9)	117 (12.8)
DEW-IT	173 (11.7)	42 (7.4)	131 (14.4)
DIPIS	69 (4.7)	17 (3.0)	52 (5.7)
DIPP	827 (55.9)	361 (63.3)	466 (51.1)
HLA Risk Group – no. (%)			
A	357 (24.1)	204 (35.8)	153 (16.8)
B	684 (46.2)	260 (45.6)	424 (46.5)
C	199 (13.4)	54 (9.5)	145 (15.9)
D	236 (15.9)	51 (8.9)	185 (20.3)
Missing	5 (0.3)	1 (0.2)	4 (0.4)
Autoantibody positive at earliest positivity (confirmatory visit) – no. (%)			
IAA	848 (57.3)	406 (71.2)	442 (48.5)
GADA	916 (61.9)	416 (73.0)	500 (54.9)
IA-2A	446 (30.1)	303 (53.1)	143 (15.7)
Autoantibody titer at earliest positivity (confirmatory visit) – mULN			
IAA	4.6 ±12.8	7.6 ±16.0	2.8 ±9.9
GADA	9.4 ±62.9	13.5 ±72.7	6.8 ±55.9
IA-2A	23.9 ±74.4	48.2 ±91.8	8.7 ±56.0

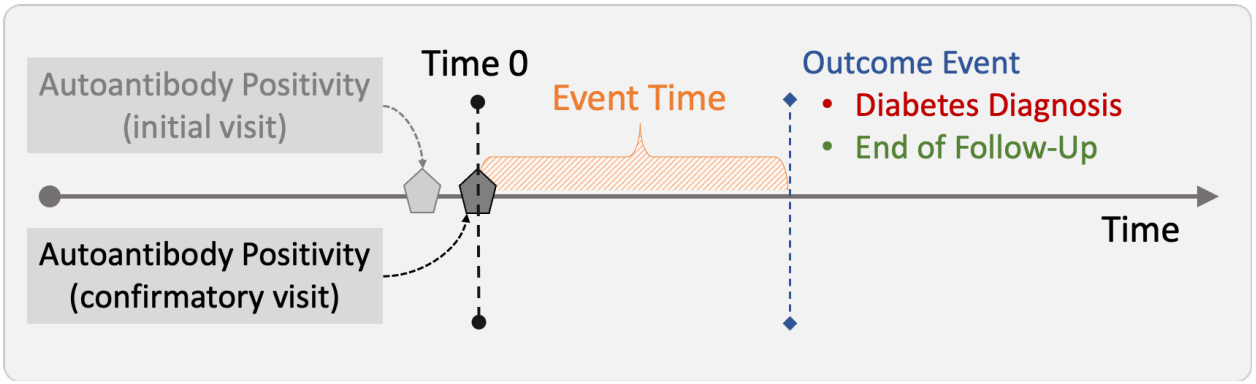
Supplemental Table S2: Key characteristics of the Study Cohort 2 population.
Plus–minus values are means ±SD. Percentages may not total to 100 because of rounding.
Autoantibody-positive percentages may not total to 100 due to multiple positivity.
Abbreviations: HLA, human leukocyte antigen; GADA, glutamic acid decarboxylase autoantibodies; IAA, insulin autoantibodies; IA-2A, insulinoma-associated antigen-2 autoantibodies; mULN, multiples of upper limit of normal.



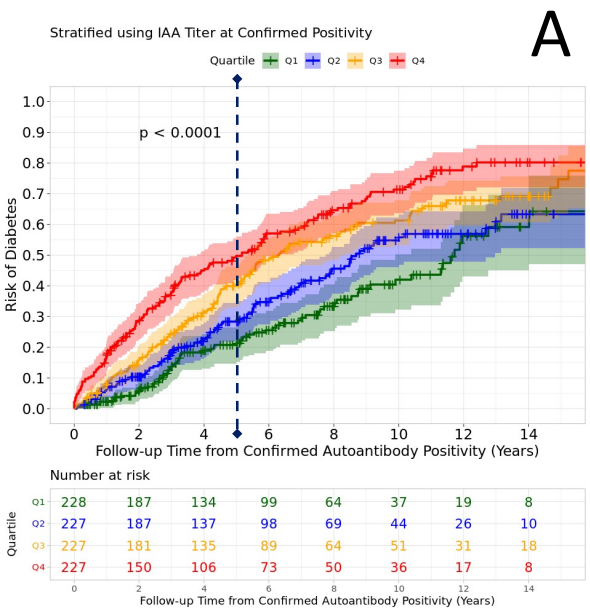
Supplemental Figure S1: Study cohort selection flowchart. IAA indicates insulin autoantibodies; GADA, glutamic acid decarboxylase autoantibodies; and IA-2A, insulinoma-associated antigen-2 autoantibodies.

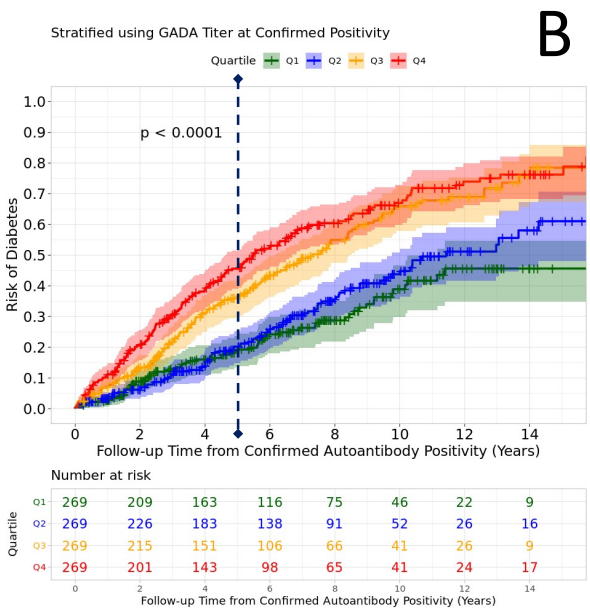


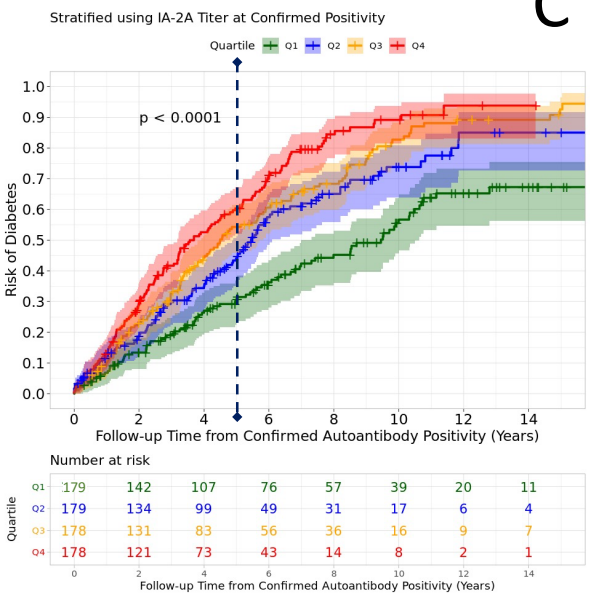
Supplemental Figure S2: Distributions of the raw (in original assay-specific units) and normalized (in multiples of upper limit of normal – mULN – units) titer levels from the confirmatory visit for positivity to IAA (A), GADA (B), and IA-2A (C), across the five studies. BABYDIAB reported high IA-2A levels outside standard curve as 201 units. IAA indicates insulin autoantibodies; GADA, glutamic acid decarboxylase autoantibodies; and IA-2A, insulinoma-associated antigen-2 autoantibodies.



Supplemental Figure S3: Illustration of the relative temporal relationships between the initial visit for autoantibody positivity, the confirmatory visit for autoantibody positivity (time 0), and the outcome event: either type 1 diabetes diagnosis or end of follow up (censored).





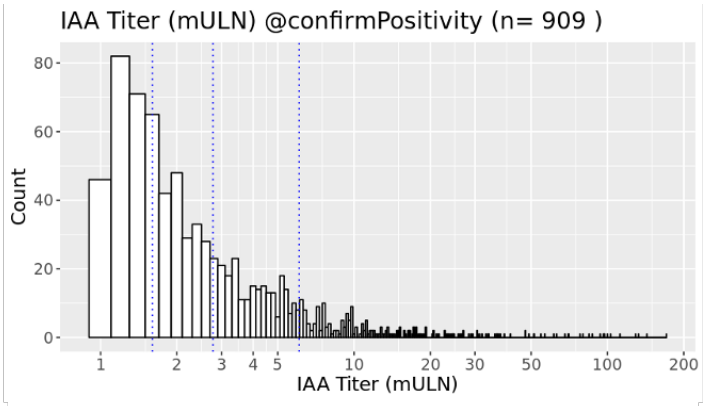


D

	IAA (n=909) p<0.0001		GADA (n=1076) p<0.0001		IA-2A (n=714) p<0.0001	
Quartile	5-year Risk (%)	95% CI	5-year Risk (%)	95% CI	5-year Risk (%)	95% CI
1	21.4	15.2 27.1	19.3	13.8 24.4	30.7	23.1 36.7
2	28.3	21.7 34.4	20.2	14.8 25.3	44.1	36.7 51.4
3	39.9	32.9 46.3	36.4	29.9 42.2	54.3	45.8 61.4
4	49.7	42.5 56.1	45.6	39.0 51.5	60.4	52.1 67.3

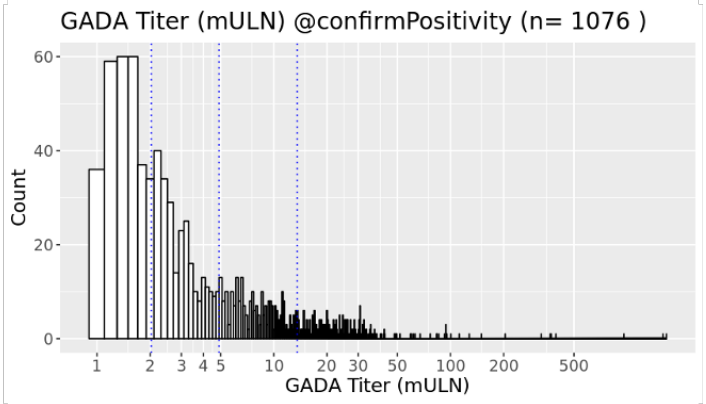
Supplemental Figure S4: Progression to diabetes from the time of the confirmatory visit for positivity of IAA (A), GADA (B), and IA-2A (C). Stratification is based on quartiles of autoantibody-positive values, at the confirmatory visit for positivity to the specific autoantibody. The dashed vertical line marks the 5-year follow-up time point. (D) The 5-year diabetes risk estimates and 95% CIs for the quartile strata for each of the three autoantibody types. IAA indicates insulin autoantibodies; GADA, glutamic acid decarboxylase autoantibodies; and IA-2A, insulinoma-associated antigen-2 autoantibodies.

A



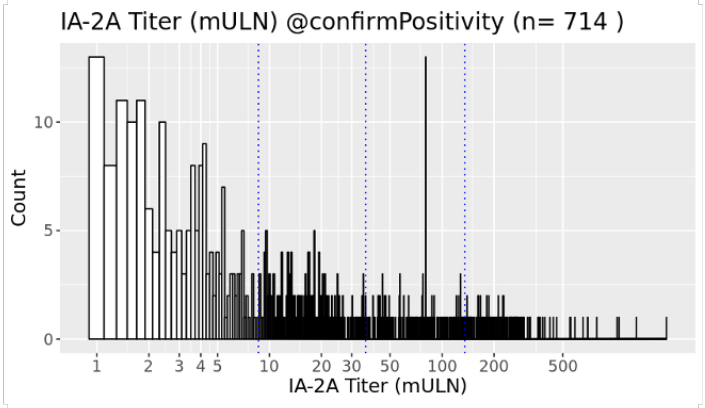
	0%	25%	50%	75%	100%
IAA Titer	1	1.6	2.775	6.075	170.86667

B



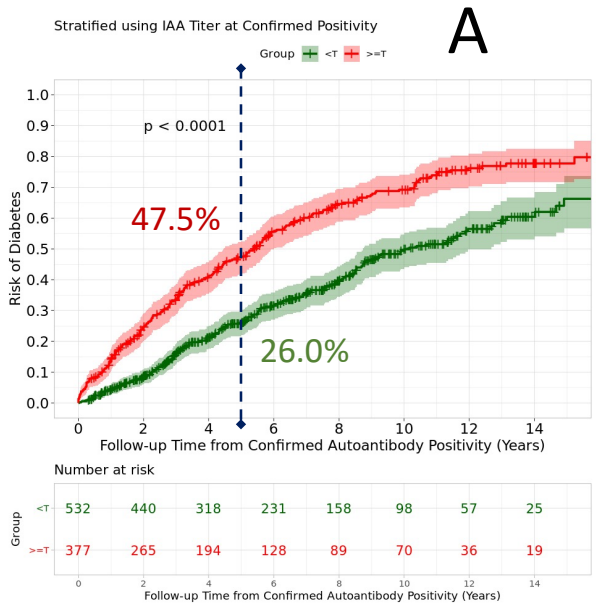
	0%	25%	50%	75%	100%
GADA Titer	1	2.0276225	4.89904	13.5538375	1668.5

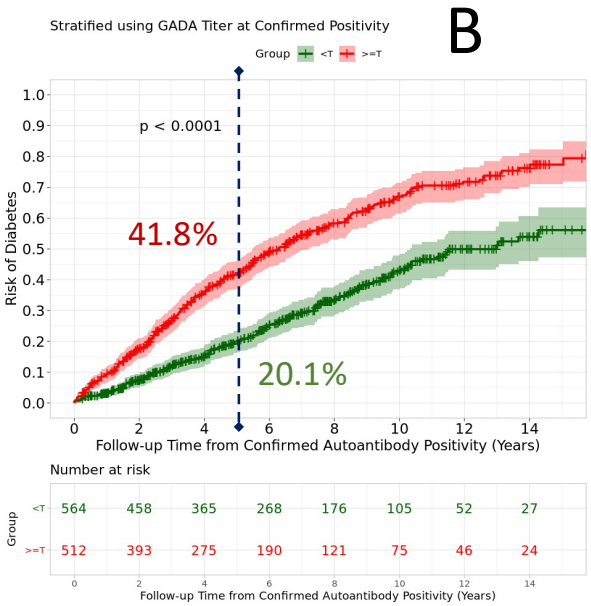
C

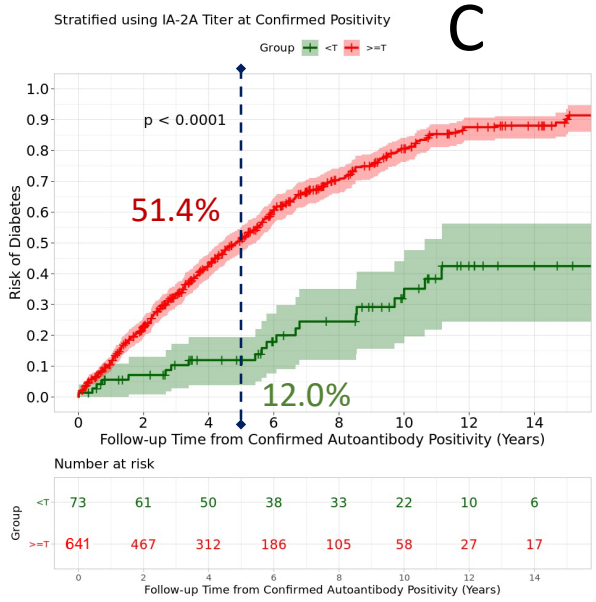


	0%	25%	50%	75%	100%
IA-2A Titer	1	8.6285725	36.05	135.25595	1995.4

Supplemental Figure S5: Histogram distributions and quartile thresholds of autoantibody titer levels, at the confirmatory visit for positivity to IAA (A), GADA (B), and IA-2A (C). IAA indicates insulin autoantibodies; GADA, glutamic acid decarboxylase autoantibodies; IA-2A, insulinoma-associated antigen-2 autoantibodies; mULN, multiples of upper limit of normal.







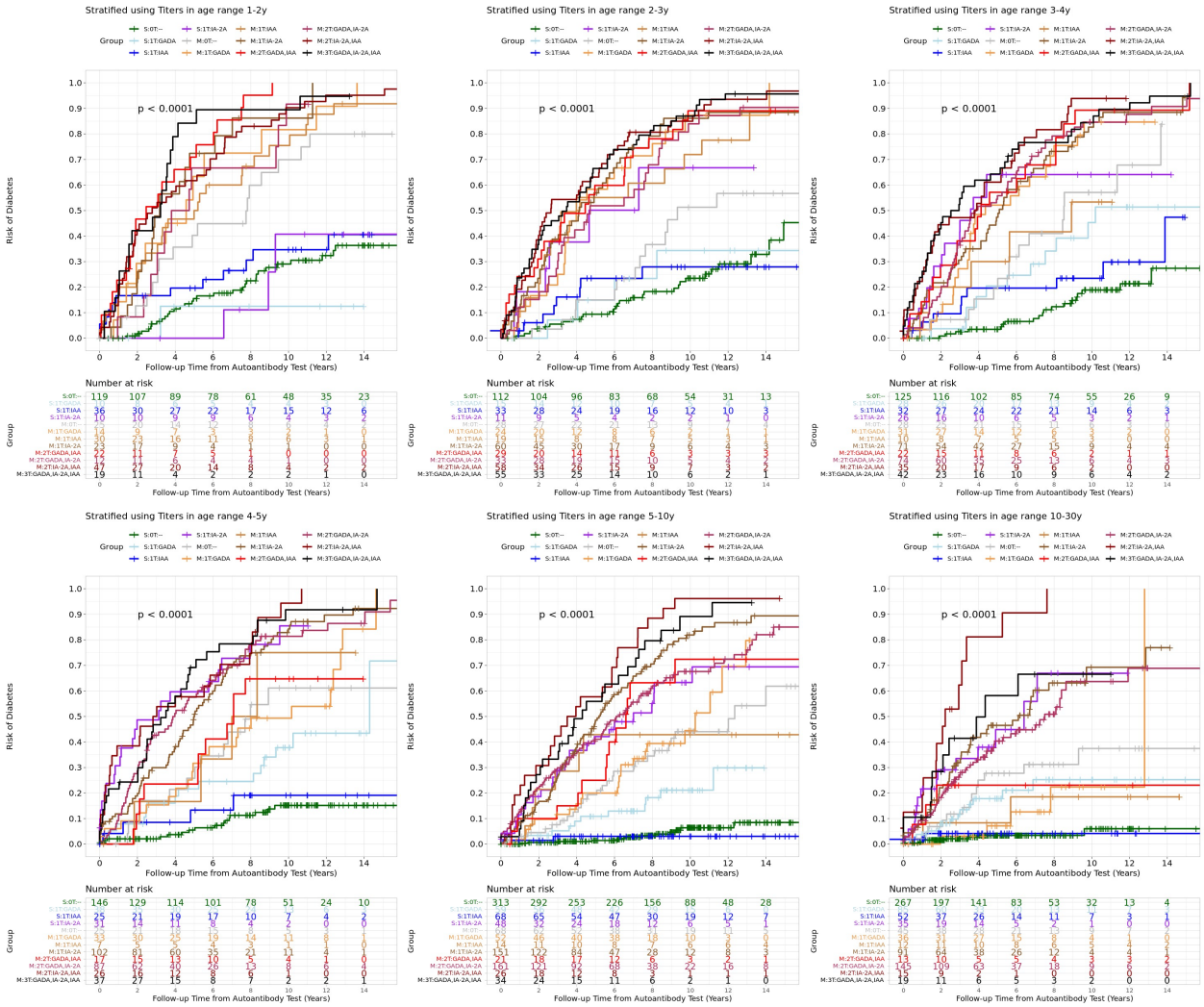
D

	IAA (n=909) p<0.0001		GADA (n=1076) p<0.0001		IA-2A (n=714) p<0.0001	
Group	5-year Risk (%)	95% CI	5-year Risk (%)	95% CI	5-year Risk (%)	95% CI
< T below threshold	26.0	21.8 29.9	20.1	16.4 23.7	12.0	3.8 19.4
>= T at/above threshold	47.5	42.0 52.5	41.8	37.1 46.5	51.4	47.1 55.4

Supplemental Figure S6: Progression to diabetes from the time of the confirmatory visit for positivity, to IAA (A), GADA (B), and IA-2A (C). Stratification is based on the autoantibody-specific thresholds (T_{IAA} = 3.6 mULN, T_{GADA} = 5.4 mULN, and T_{IA-2A} = 2.5 mULN) applied to the autoantibody titer levels from the confirmatory visit for positivity to the specific autoantibody. The dashed vertical line marks the 5-year follow-up time point. (D) The 5-year diabetes risk estimates and 95% CIs for the below threshold (<T) and at/above threshold (>=T) strata for each of the three autoantibody types. IAA indicates insulin autoantibodies; GADA, glutamic acid decarboxylase autoantibodies; IA-2A, insulinoma-associated antigen-2 autoantibodies; mULN, multiples of upper limit of normal.

Models	Variable	Cox Model				
		Coefficient	Hazard Ratio (95% CI)	P-Value	Sig	Concordance (SE)
Model 1 coxph(formula = Surv(stime_confirm, T1D_Dx) ~ strata(DS_BABYDIAB + DS_DAISY + DS_DEWIT + DS_DIPIS) + HLA_GROUP_C + HLA_GROUP_B + HLA_GROUP_A + SEX_Male + Age_earliest_confirmatory_visit + IAA_positive_confirm + GADA_positive_confirm + IA2A_positive_confirm + tt(GADA_positive_confirm), data = data, tt = function(x, t, ...) x*t)	HLA_GROUP_C	0.4681	1.6 (1.08-2.35)	1.77E-02	*	0.753 (0.01)
	HLA_GROUP_B	0.3347	1.4 (1.03-1.89)	3.11E-02	*	
	HLA_GROUP_A	0.7896	2.2 (1.62-3)	5.49E-07	***	
	SEX_Male	-0.0939	0.91 (0.77-1.08)	2.72E-01		
	Age_earliest_confirmatory_visit	-0.0889	0.91 (0.89-0.94)	1.05E-08	***	
	IAA_positive_confirm	0.7438	2.1 (1.74-2.55)	2.87E-14	***	
	GADA_positive_confirm	0.3554	1.43 (1.05-1.95)	2.48E-02	*	
	IA2A_positive_confirm	1.3688	3.93 (2.95-5.23)	2.00E-16	***	
	tt(GADA_positive_confirm)	0.0618	1.06 (1.01-1.12)	2.23E-02	*	
	tt(IA2A_positive_confirm)	-0.0555	0.95 (0.9-0.99)	2.68E-02	*	
Model 2 coxph(formula = Surv(stime_confirm, T1D_Dx) ~ strata(DS_BABYDIAB + DS_DAISY + DS_DEWIT + DS_DIPIS) + HLA_GROUP_C + HLA_GROUP_B + HLA_GROUP_A + SEX_Male + Age_earliest_confirmatory_visit + IAA_positive_confirm + GADA_positive_confirm + IA2A_positive_confirm + **IAA_titer_confirm + GADA_titer_confirm + IA2A_titer_confirm +** **tt(IAA_titer_confirm),** data = data, tt = function(x, t, ...) x * t)	HLA_GROUP_C	0.4203	1.52 (1.03-2.24)	3.30E-02	*	0.777 (0.01)
	HLA_GROUP_B	0.2715	1.31 (0.97-1.78)	8.12E-02	.	
	HLA_GROUP_A	0.6975	2.01 (1.47-2.74)	1.12E-05	***	
	SEX_Male	-0.0387	0.96 (0.81-1.14)	6.52E-01		
	Age_earliest_confirmatory_visit	-0.0710	0.93 (0.9-0.96)	1.37E-05	***	
	IAA_positive_confirm	0.0108	1.01 (0.77-1.34)	9.39E-01		
	GADA_positive_confirm	-0.1897	0.83 (0.59-1.15)	2.64E-01		
	IA2A_positive_confirm	0.1331	1.14 (0.78-1.67)	4.91E-01		
	IAA_titer_confirm	0.3114	1.37 (1.24-1.51)	4.57E-10	***	
	GADA_titer_confirm	0.1637	1.18 (1.11-1.25)	8.86E-08	***	
	IA2A_titer_confirm	0.1583	1.17 (1.1-1.24)	2.30E-07	***	
	tt(IAA_titer_confirm)	-0.0172	0.98 (0.97-1)	1.15E-02	*	

Supplemental Table S3: Multivariable Cox proportional hazards regression models to analyze the association between autoantibodies at the earliest confirmatory visit and type 1 diabetes risk. The regression formula, variables, coefficients, hazard ratios, 95% confidence intervals, P-values, significance indicators, and concordance (standard error) from the fitted models are shown. The models were adjusted for HLA risk group, sex, age at the earliest confirmatory visit and stratified by study site. Model 1 uses the autoantibody positivity indicators for IAA, GADA, IA-2A, at the earliest confirmatory visit, as the primary predictors. Time varying coefficients for GADA positivity and IA-2A positivity were used to handle violations of the proportional hazard assumption (assessed via the Schoenfeld test). Model 2 adds log normalized autoantibody titers for IAA, GADA, IA-2A, at the earliest confirmatory visit to Model 1 as the primary predictors. Time varying coefficients for IAA titer were used to handle violation of the proportional hazard assumption. The *tt(...)* function indicates the constructed time dependent covariates which are interactions of the predictor and survival time used for estimating the time varying coefficients.



Supplemental Figure S7: The risk of type 1 diabetes in subjects who developed confirmed-positive islet autoantibodies, stratified by single or multiple autoantibody positivity and the combination of IAA, GADA, IA-2A titers above thresholds (T_{IAA} = 3.6 mULN (multiples of upper limit of normal), T_{GADA} = 5.4 mULN, T_{IA-2A} = 2.5 mULN) for screening at different age ranges. The strata are:

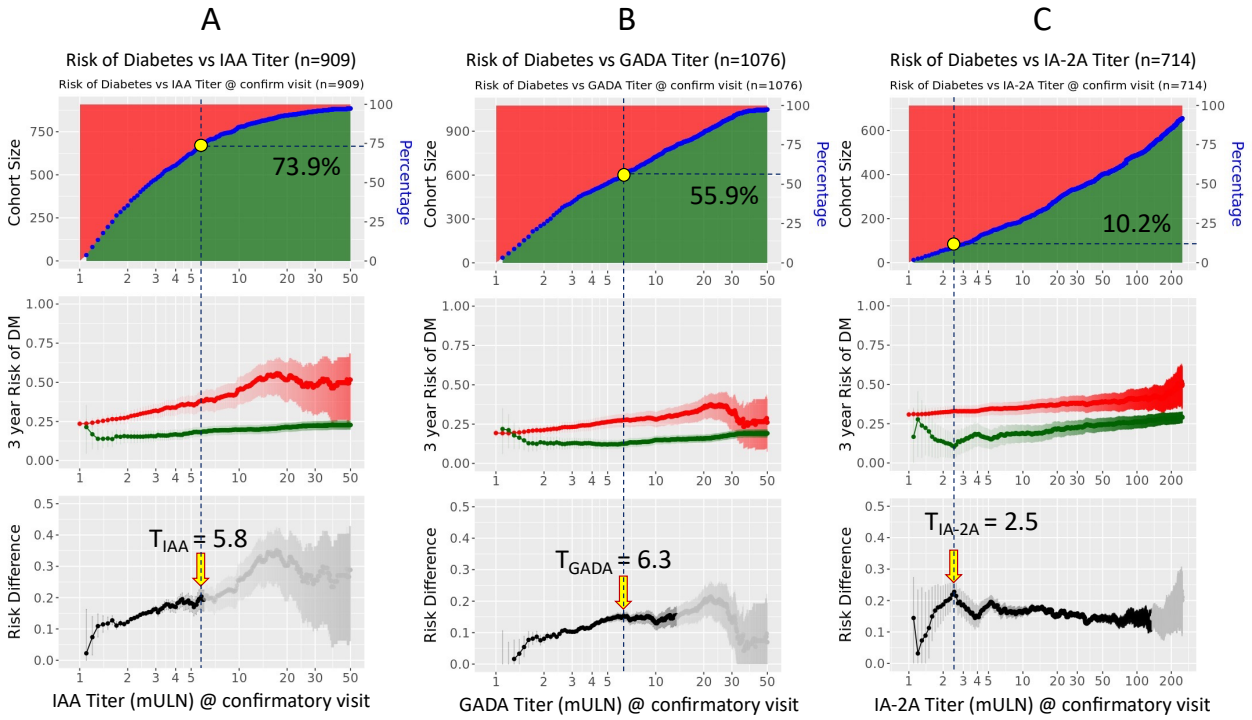
- S:0T:-- = single positive, no autoantibodies above titer threshold
- S:1T:GADA = single positive, one (GADA) above titer threshold
- S:1T:IAA = single positive, one (IAA) above titer threshold
- S:1T:IA-2A = single positive, one (IA-2A) above titer threshold
- M:0T:-- = multiple positive, no autoantibodies above titer threshold
- M:1T:GADA = multiple positive, one (GADA) above titer threshold
- M:1T:IAA = multiple positive, one (IAA) above titer threshold
- M:1T:IA-2A = multiple positive, one (IA-2A) above titer threshold
- M:2T:GADA,IAA = multiple positive, two (GADA, IAA) above titer threshold
- M:2T:GADA,IA-2A = multiple positive, two (GADA, IA-2A) above titer threshold
- M:2T:IA-2A,IAA = multiple positive, two (IA-2A, IAA) above titer threshold
- M:3T:GADA,IA-2A,IAA = multiple positive, all three above titer threshold

Age Range (years)	Strata with 5-year diabetes risk ≥ 50%	Composite high-risk criteria	Number of Children	Number of children that progressed to diabetes within 5 years	Number of high risk children identified	Positive Predictive Value	Negative Predictive Value	Sensitivity	Specificity
1-2	M:3T:GADA,IA-2A,IAA M:2T:IA-2A,IAA M:2T:GADA,IA-2A M:2T:GADA,IAA M:1T:IA-2A M:1T:IAA M:1T:GADA	Multiple positive with ≥ 1 autoantibody ≥ specific threshold titer	1111	189	167	65.3%	91.0%	56.3%	93.6%
2-3	M:3T:GADA,IA-2A,IAA M:2T:IA-2A,IAA M:2T:GADA,IA-2A M:2T:GADA,IAA M:1T:IA-2A M:1T:IAA M:1T:GADA S:1T:IA-2A	Multiple positive with ≥ 1 autoantibody ≥ specific threshold titer OR IA-2A ≥ specific threshold titer	1181	223	289	59.3%	93.3%	74.4%	87.5%
3-4	M:3T:GADA,IA-2A,IAA M:2T:IA-2A,IAA M:2T:GADA,IA-2A M:2T:GADA,IAA M:1T:GADA S:1T:IA-2A	Multiple positive with ≥ 2 autoantibodies ≥ specific threshold titer OR Multiple positive with GADA ≥ specific threshold titer OR Single positive with IA-2A ≥ specific threshold titer	1088	207	231	57.5%	90.4%	62.7%	88.3%
4-5	M:3T:GADA,IA-2A,IAA M:2T:IA-2A,IAA M:2T:GADA,IA-2A M:1T:IA-2A S:1T:IA-2A	IA-2A ≥ specific threshold titer	1083	206	283	56.5%	92.9%	74.3%	85.6%
5-10	M:3T:GADA,IA-2A,IAA M:2T:IA-2A,IAA	Multiple positive with IAA and IA-2A ≥ specific threshold titer	1326	232	60	54.9%	82.7%	13.9%	97.3%
10+	M:3T:GADA,IA-2A,IAA M:2T:IA-2A,IAA	Multiple positive with IAA and IA-2A ≥ specific threshold titer	1006	157	35	62.3%	80.5%	11.8%	98.1%

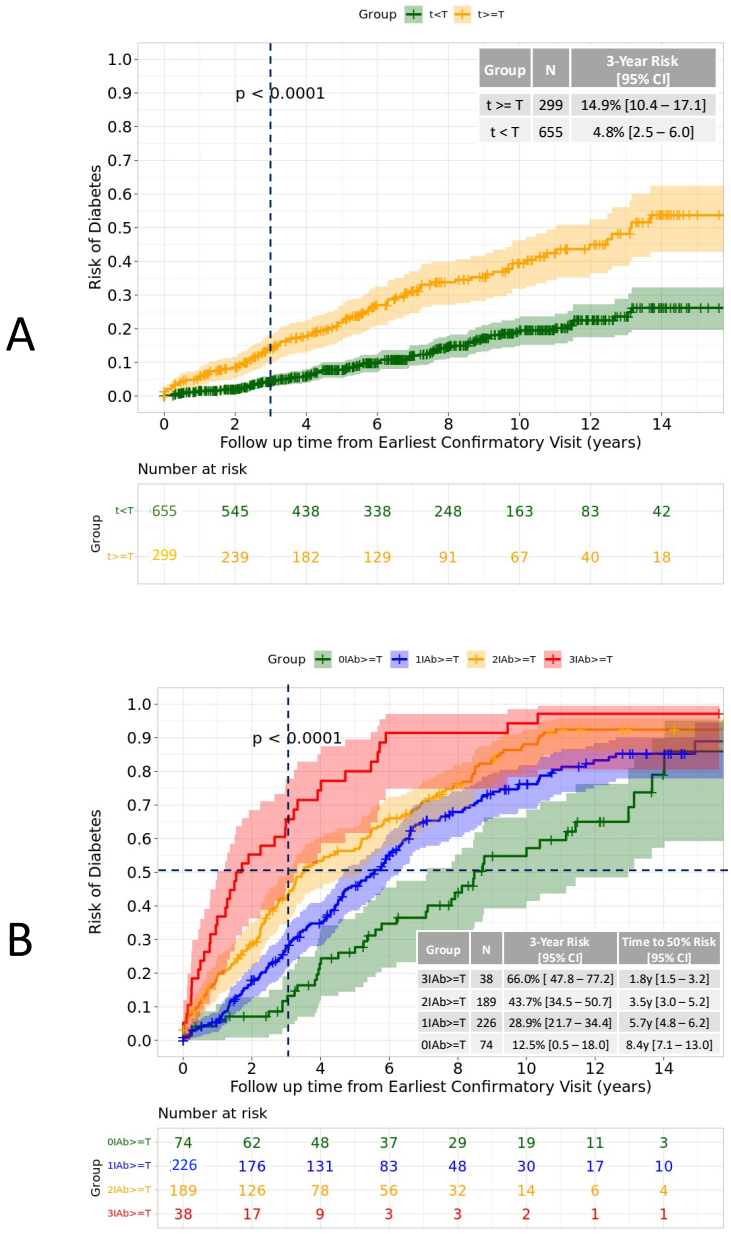
Supplemental Table S4: Description and performance of the high diabetes risk strata.
For each age range group, the following information is shown:

- All strata with 5-year diabetes risk >= 50%.
- The composite high-risk criteria defined by combining the criteria of the separate strata.
- The total number of children, number that progressed to diabetes within 5 years, and the number of high-risk children identified using the high diabetes risk stratum.
- The inverse probability of censoring weighted (IPCW) positive predictive value, negative predictive value, sensitivity, and specificity.

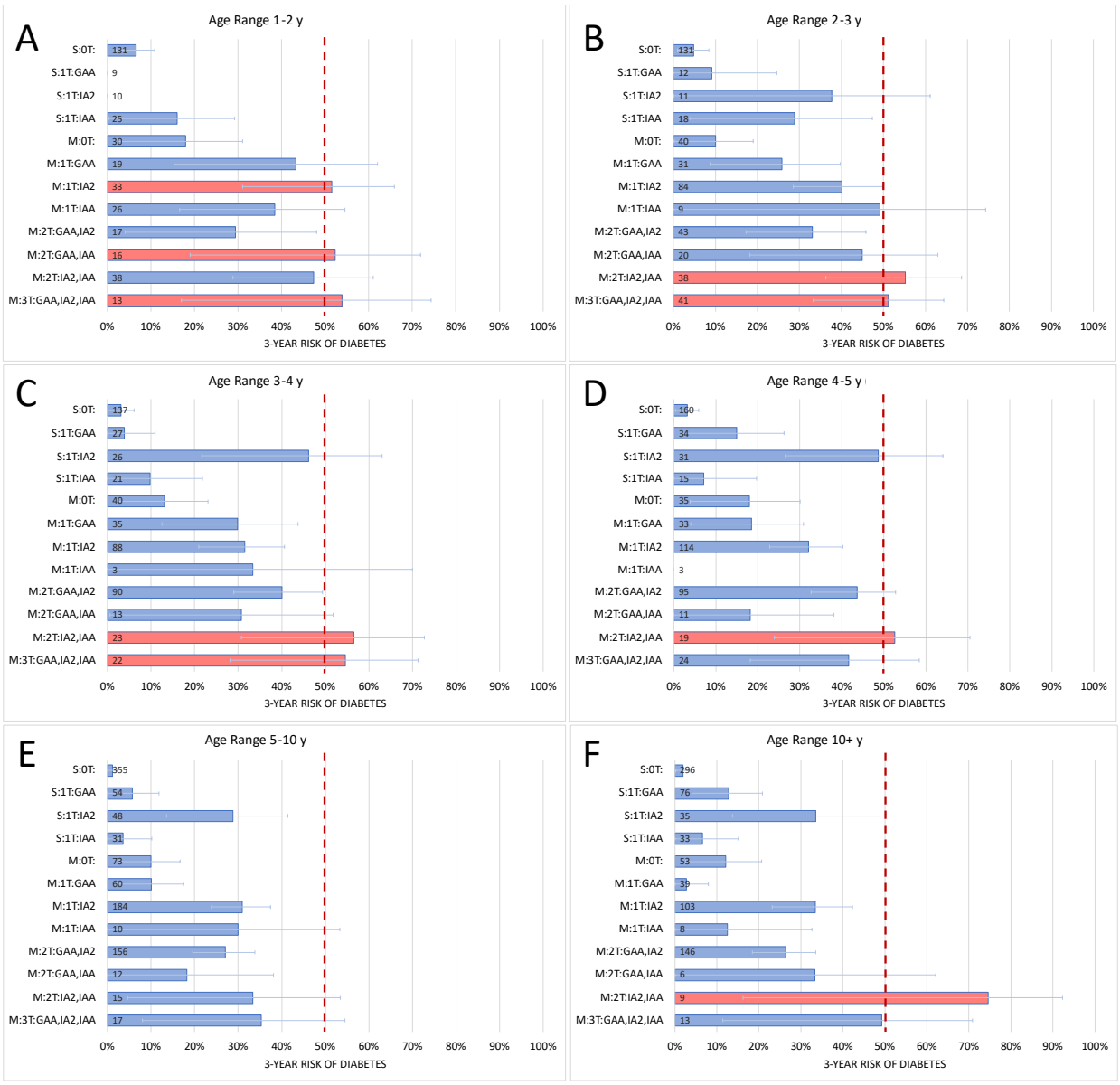
IAA indicates insulin autoantibodies; GADA, glutamic acid decarboxylase autoantibodies; and IA-2A, insulinoma-associated antigen-2 autoantibodies.



Supplemental Figure S8: Identifying autoantibody type-specific titer thresholds for IAA (A), GADA (B), and IA-2A (C) using a 3-year risk of diabetes outcome. Top panel: The size of the red cohort (titer ≥ threshold) and the green cohort (titer < threshold) for each autoantibody titer threshold level. Middle panel: 3-year risk of diabetes and 95% confidence intervals from the time of the confirmatory visit for autoantibody positivity for the red and green cohorts for each titer threshold level. Bottom panel: The difference in the 3-year diabetes risk between the red and green cohorts for each titer threshold level. An arrow marks the lowest titer threshold level where there is a maximum risk difference between the cohorts and the threshold covers up to 75% of the cohort (T_{IAA} = 5.8 mULN, T_{GADA} = 6.3 mULN, and $T_{IA\text{-}2A}$ = 2.5 mULN). The percentile of children who tested positive for the respective autoantibody corresponding to the final titer threshold is highlighted in the top panel (T_{IAA} → 73.9%, T_{GADA} → 55.9%, and $T_{IA\text{-}2A}$ → 10.2%). IAA indicates insulin autoantibodies; GADA, glutamic acid decarboxylase autoantibodies; IA-2A, insulinoma-associated antigen-2 autoantibodies; mULN, multiples of upper limit of normal; DM, diabetes mellitus.



Supplemental Figure S9: Progression to diabetes from the time of the earliest confirmatory visit in children with single and multiple autoantibody positivity. Stratification is based on the autoantibody titer measured at the earliest confirmatory visit and the identified autoantibody type-specific titer thresholds (T_{IAA}= 5.8 mULN, T_{GADA}= 6.3 mULN, $T_{IA\text{-}2A}$ = 2.5 mULN) from Supplemental Figure S8. (A) Single autoantibody-positive children are partitioned into two groups: those with autoantibody titer below threshold (t < T) and those with titer at-or-above threshold (t >= T). (B) Multiple autoantibody-positive children are partitioned into four mutually exclusive groups: those with no autoantibody titer at-or-above threshold (0IAb >= T), those with one autoantibody titer at-or-above threshold (1IAb >= T), those with two autoantibody titers at-or-above threshold (2IAb >= T), and those with all three autoantibody titers at-or-above threshold (3IAb >= T). The dashed vertical line marks the 3-year follow-up time point. IAA indicates insulin autoantibodies; GADA, glutamic acid decarboxylase autoantibodies; IA-2A, insulinoma-associated antigen-2 autoantibodies; mULN, multiples of upper limit of normal.



Supplemental Figure S10: The 3-year risk of type 1 diabetes and 95% confidence intervals in subjects who developed confirmed-positive islet autoantibodies, stratified by single or multiple autoantibody positivity and the combination of IAA, GADA, IA-2A titers above thresholds (T_{IAA}= 5.8 mULN, T_{GADA}= 6.3 mULN, $T_{IA\text{-}2A}$ = 2.5 mULN) for screening at different age ranges (A: 1-2.0y, B: 2-3.0y, C: 3-4.0y, D: 4-5.0y, E: 5-10.0y, F: 10+ y). The 12 strata are the same as those described in Figure 3. The number of subjects in each stratum is shown at the base of each bar. The dashed vertical red lines mark the 50% 3-year risk of diabetes level. Strata that exceed that risk level are classified as "high-risk" and shaded red. IAA indicates insulin autoantibodies; GADA, glutamic acid decarboxylase autoantibodies; IA-2A, insulinoma-associated antigen-2 autoantibodies; mULN, multiples of upper limit of normal.

Section S1: Islet autoantibody titer normalization

As summarized in the supplement for [1], each study used different assays to measure the islet autoantibodies: IAA, GADA, and IA-2A. The threshold for positivity (i.e., the upper limit of normal) is assay dependent and was determined by each study, usually as the 99th percentile of their normal, healthy, nondiabetic, control test subject population. In BABYDIAB, very high IA-2A values that were outside the standard curve (>200 units) were reported as 201 units in years 2001-2009. Each of the studies employed rigorous quality control procedures to control for drift in the assays, and their laboratories have participated with satisfactory results in all proficiency workshops of the Diabetes Autoantibody Standardization program (DASP) [2–4] and the Islet Autoantibody Standardization program (IASP) [5]. The results of these workshops demonstrated that the different laboratories had excellent discrimination between type 1 diabetic and control sera, high sensitivities, and high specificities. More importantly, the results demonstrated good concordance between the different laboratories in the ranking of samples by IAA, GADA, and IA-2A levels (which is an important prerequisite to be able to compare titers across studies).

The different laboratory assays report autoantibody titer measurements in terms of either "indices" or "arbitrary/relative units." Index titers are computed based on negative and positive control samples using the formula:

$$t_i = (t_s - t_-)/(t_+ - t_-)$$

where t_s is the (mean) titer measurement of the unknown subject sample, t_+ is the (mean) titer measurement of the positive control sample, and t_- is the (mean) titer measurement of the negative control sample. The original titer measurements are usually in *cpm* (counts per minute) or *od* (optical density).

Arbitrary or relative units were computed using several methods [3]. One approach uses a formula based on one reference standard sample, and is very similar to the index formula above:

$$t_r = N\ (t_s - t_-)/(t_+ - t_-)$$

where t_s is the (mean) titer measurement of the unknown subject sample, t_+ the (mean) titer measurement of the positive control sample, t_- the (mean) titer measurement of the negative control sample, and N a constant used to scale the units relative to a positive reference with an arbitrary value of N units. Another approach uses a "standard curve," based on multiple standard samples by constructing a regression curve for the titer measurement (cpm or od) versus the assigned reference in units/ml, for each of the known standard samples. This regression curve can then be used to convert the titer measurement of the unknown samples (in cpm or od) into the desired reference relative units (in units/ml):

$$t_r = at_s + b$$

where t_s is the (mean) titer measurement of the unknown subject sample, a the slope, and b the intercept (i.e., $t_r = b$ when $t_s = 0$) of the fitted regression.

Since the titer measurements for the same autoantibody, from different assays, with different index and relative units, are not directly comparable, we converted the autoantibody titer measurements into multiples of upper limit of normal (mULN), by dividing the subject titer value, t, by the positivity threshold level, T, for the corresponding assay:

$$t_{mULN} = \frac{t}{T}$$

Positive autoantibody test results will have a value ≥ 1 and negative autoantibody test results will have a value < 1. For a given assay, both t and T are in the same units. Taking the ratio of the two quantities removes some of the underlying variations across assays and allows us to compare the titers. In the case of index units (t_i, T_i), we have:

$$t_{mULN} = \frac{t_i}{T_i} = \frac{(t_s - t_-)/(t_+ - t_-)}{(T - t_-)/(t_+ - t_-)} = \frac{(t_s - t_-)}{(T - t_-)}$$

In the denominator, $T \gg t_-$, since the positivity threshold T, computed as the upper limit of normal or 99th percentile of the normal control test subject population, will be much larger than t_-, the (mean) titer measurement of the negative control sample (which will be small and around the 50th percentile (median) of the normal control test subject population). In the numerator, for subjects that are autoantibody positive (which is the case that we are interested in), $t_s \geq T$, and, as a result, $t_s \gg t_-$. In this case, we can approximate t_{mULN} as:

$$t_{mULN} \approx \frac{t_s}{T}$$

In the case of relative units (t_r, T_r) using a single standard, we have:

$$t_{mULN} = \frac{t_r}{T_r} = \frac{N(t_s - t_-)/(t_+ - t_-)}{N(T - t_-)/(t_+ - t_-)} = \frac{(t_s - t_-)}{(T - t_-)}$$

Again, with $T \gg t_-$ and $t_s \gg t_-$, when the subject is autoantibody positive, we have:

$$t_{mULN} \approx \frac{t_s}{T}$$

For relative units (t_r, T_r) derived using a standard curve from multiple standards, we have:

$$t_{mULN} = \frac{t_r}{T_r} = \frac{at_s + b}{aT + b}$$

Since $t_r = b$ when $t_s = 0$, we expect b to be a small value, especially when compared to the values of T and t_s, when the subject is autoantibody positive. In this case, we have $T \gg b$ and $t_s \gg b$, and can approximate t_{mULN} as:

$$t_{mULN} \approx \frac{at_s}{aT} = \frac{t_s}{T}$$

Although not perfect, by converting the autoantibody titers into multiples of upper limit of normal (mULN), we obtain measurements that are more comparable for our use case

(autoantibody positivity) and given reasonable assumptions (the values of b and t_- are small and near 0).

Distributions of the raw (in original assay-specific index or arbitrary units) and normalized (in mULN units) titer levels, from the confirmatory visit for positivity to IAA, GADA, and IA-2A for Study Cohort 1 (Table 1), are shown in Supplemental Figure S2. With the original raw titer levels, there is a large difference in the dynamic range, and there is little overlap across study sites. However, with the mULN normalized titer levels, there is a much narrower dynamic range, and more significant overlap across study sites.

Section S2: The T1DI Study Group

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