Islet Autoantibody Levels Differentiate Progression Trajectories in Individuals with Presymptomatic Type 1 Diabetes

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ABSTRACT

Our previous data-driven analysis of evolving patterns of islet autoantibodies (IAbs) against insulin (IAA), glutamic acid decarboxylase (GADA) and islet antigen 2 (IA-2A) discovered three trajectories characterized by either multiple IAbs (TR1), IAA (TR2), or GADA (TR3) as the first appearing autoantibodies. Here we examined the evolution of IAb levels within these trajectories in 2,145 IAb-positive participants followed from early life and compared those who progressed to type 1 diabetes (n=643) to those remaining undiagnosed (n=1,502). Using thresholds determined by 5-year diabetes risk, four levels were defined for each IAb and overlayed onto each visit. In diagnosed participants, high IAA levels were seen in TR1 and TR2 at ages <3 years, whereas IAA remained at lower levels in the undiagnosed. Proportions of dwell times (total duration of follow-up at a given level) at the four IAb levels differed between the diagnosed and undiagnosed for GADA and IA-2A in all three trajectories ($p<0.001$), but for IAA dwell times differed only within TR2 ($p<0.05$). Overall, undiagnosed participants more frequently had low IAb levels and later appearance of IAb than diagnosed participants. In conclusion, while it has been long appreciated that the number of autoantibodies is an important predictor of type 1 diabetes, consideration of autoantibody levels within the three autoimmune trajectories improved differentiation of IAb positive children who progressed to type 1 diabetes from those who did not.

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

Development of islet autoantibodies (IAb) precedes the clinical diagnosis of type 1 diabetes. The presence or absence (positivity/negativity) of IAb, their age at appearance, and number of IAb are known to predict the risk of clinical disease (1,2). The longitudinal IAb patterns are, however, heterogeneous and these patterns may reflect distinct disease subtypes and different pathways to clinical diagnosis (3–7).

Previous prospective studies following participants with increased genetic risk for type 1 diabetes have identified different initiation patterns of islet autoimmunity: insulin autoantibodies (IAA), antibodies against GAD65 (GADA), or antibodies against islet antigen 2 (IA-2A) as the first appearing IAb (8–10). IAA first or GADA first are two main patterns at initiation of islet autoimmunity and have been associated with DR4 and DR3 HLA haplotypes, respectively, and with different ages at first positivity. A third pattern is multiple IAb, most often both IAA and GADA, appearing simultaneously at seroconversion.

The Type 1 Diabetes Intelligence (T1DI) cohort has combined IAb data from five prospectively-followed study cohorts following a total of 24,662 unique participants (2). Our recent data-driven analyses using a Continuous-Time Hidden Markov Model (CT-HMM) and the presence or absence of IAA, GADA and/or IA-2A as well as age of observation discovered three main autoimmune trajectories: predominantly multiple IAb (TR1), IAA (TR2) or GADA (TR3) as the first appearing autoantibodies (11,12). Of note, each trajectory consisted of multiple component states that are manifested by distinct islet autoantibody probabilities and ages at event. For each trajectory the initial state is essentially autoantibody negative (e.g., TR2-0) and the following states are numbered sequentially and describe the evolution of autoantibody profile in that trajectory. For example, TR2-1 represents component state 1 of trajectory 2 (TR2) which predominantly includes children with a high probability of IAA as the first appearing IAb. Further, the trajectories were associated with varying ages at first IAb appearance as well as timing and overall risk of progression to type 1 diabetes.

Several studies have shown that beyond the presence or absence of the various IAb, the level of IAb plays an important role in prediction of type 1 diabetes (13–21). Further, there is heterogeneity amongst the IAb in regards to the association of antibody level and progression risk (15). To examine the role of IAb levels in the combined T1DI cohort, we have previously harmonized IAb levels originally measured in the five T1DI studies. These harmonized IAb levels effectively stratified 5-year progression to type 1 diabetes in this large multinational cohort (22).

Here, we sought to expand on previous observations to visualize and determine how autoantibody levels differ within the three trajectories and between those who have progressed to diabetes and those who have not. To refine the trajectories and their component states, we categorized the intensity of the antibody response of IAA, GADA, and IA-2A into four IAb level groups (L0: negative antibody; L1: low positive antibody level; L2: medium positive antibody level; L3: highest positive antibody level), respectively, and analyzed the evolution of these IAb levels in each trajectory. Since most participants who develop autoimmunity follow one of the three trajectories, we specifically compared participants who were diagnosed with type 1 diabetes during the follow-up to those who remained undiagnosed at the end of their follow-up.

RESEARCH DESIGN AND METHODS

Study Population and Trajectories

The Type 1 Diabetes Intelligence (T1DI) cohort has combined data from 24,662 unique individuals who participated in five prospectively-followed study cohorts, from Finland (DIPP), Germany (BABYDIAB), Sweden (DiPiS), and USA (DAISY, DEW-IT) (2). Out of the five original studies, DAISY, DEW-IT, DiPiS, and DIPP used HLA genotype as inclusion criterion by considering children with high-risk, moderate risk, or specific lower risk HLA genotype eligible for follow-up as described in detail in Anand et al. (2). In addition, BABYDIAB and DAISY recruited newborns with first-degree relatives with type 1 diabetes for follow-up. From the T1DI cohort, we analyzed 2,145 participants (42,209 visits) who had two or more visits and any IAb positivity at least once (11,12,23). Supplementary Table 1 shows the number of samples by participants' age and Supplementary Table 2 presents the sampling intervals in the five prospective studies. In our previous analysis, we discovered three islet autoimmunity progression trajectories and their component states in a data-driven way using a Continuous-Time Hidden Markov Model (CT-HMM). In that work, each trajectory was characterized by the predominant autoantibody pattern observed in the first positive serum sample of the study participants as follows: multiple islet autoantibodies first (TR1), IAA first (TR2), or GADA first (TR3), each including specific states of transition (11,12). Each individual may enter the trajectory in any state at any age but can only stay at the same state or proceed to the next state in transition. In this cohort, 643 (30%) participants (11,566 visits) were diagnosed with type 1 diabetes by the end of their observation period, hereafter referred to as diagnosed, and 1,502 participants (30,643 visits) remained undiagnosed at the end of their observation period, hereafter referred to as undiagnosed. The development of clinical onset of type 1 diabetes was ascertained following the American Diabetes Associations criteria (24).

The median age of the diagnosed participants at the last observation, which represents the age at diagnosis, was 7.62 years (IQR, 4.19 to 11.22), while the median age of the undiagnosed participants at the last observation was 12.87 years (IQR, 9.29 to 15.42). The median follow-up time of all participants was 11.6 years (IQR, 6.64 to 14.47). The model assigned each participant exclusively to one of the three trajectories as defined above. Table 1 shows the description of the study cohort.

Islet Autoantibody Levels

Previous work harmonized IAb levels as multiples of upper limit of normal (mULN) to facilitate combined analysis (22). Autoantibody level measurements were converted into mULN by dividing the measurement by the positivity threshold level for the corresponding assay. Positive autoantibody test results will have a value ≥1.0 and negative autoantibody test results will have a value <1.0. The continuous values (mULN) were then categorized into four level groups (Table 2).

The threshold values between L1 and L2 were the autoantibody type-specific thresholds that effectively stratified 5-year progression to type 1 diabetes at the confirmatory visit (22). The threshold values between L2 and L3 were specified as the levels corresponding to the 75th percentile of the respective autoantibody-positive cohort.

Data Visualization and Statistical Analysis Methods

We used an interactive data visualization method called DPVis (25) to characterize the islet autoantibody levels in the three trajectories. Using this method, we visualized each participant visit having an autoantibody level by overlaying a color-labelled dot corresponding to IAb level onto the three trajectories. We also visualized the proportion of the four islet autoantibody levels (L0, L1, L2, L3) that the participants belonged to over their observation periods using stacked bar charts. Then, we visualized the islet autoantibody levels of individual participants within their observations as parallel bar charts. These charts depict the major trends and

differences among the four levels of islet autoantibodies of individual participants within the three trajectories. We then computed “dwell time”, the proportion of the total duration of follow-up spent at a given level, by the four islet autoantibody levels per trajectory and analyzed differences between the diagnosed and undiagnosed participants within each trajectory by using Chi-square tests. We further sorted participants by the maximum islet autoantibody level each participant achieved over their observation period. In particular, , we stratified individuals by the maximum levels (L0, L1, L2, L3) of IA-2A that each participant achieved, because high IA-2A levels have been associated with rapid progression from autoimmunity to overt type 1 diabetes. Then, we analyzed differences in dwell times in different GADA and IAA levels between the diagnosed and undiagnosed participants within each trajectory by using Chi-square tests. Finally, we compared the diabetes-free survival rates of young children with single IAb positivity and different IAb levels (L1, L2, and L3), following the screening protocols recommended in prior studies (26–28).

Data and Resource Availability

The data that support the findings of this study are not publicly available because they were used under license for the current study only. Data are, however, available upon reasonable request with permission from the originating sites whose representatives are William Hagopian (DEW-IT), Markus Lundgren (DiPiS), Marian Rewers (DAISY), Riitta Veijola (DIPP) and Anette Ziegler (BABYDIAB).

RESULTS

Overall differences in islet autoantibody levels between the diagnosed and the undiagnosed participants

In order to investigate the differences between individuals who were diagnosed or not diagnosed during the study period, we separated the two groups of participants into different panels in each figure. Further, for both groups, each individual was categorized into one of three trajectories (TR1, TR2, and TR3), where the individual could appear in one or more states (e.g., TR1-0, TR1-1, and TR1-2). Here we first describe the layout of the visualized data, and then present detailed descriptions for each trajectory and analysis.

Using the DPVis method (Figure 1), the three autoimmune trajectories and their component states were visualized for the diagnosed and undiagnosed participants. In the visualization, each individual visit was color-coded to denote the four levels of GADA, IAA, or IA-2A. Figure 2 illustrates the normalized proportions of the four autoantibody levels for each islet autoantibody at all visits categorized by age. Supplementary Figure 1 shows the distribution of the four islet autoantibody levels for all visits at all ages. Figure 3 visualizes the length of time that each individual participant spent at one of the four islet autoantibody levels ("dwell time") as marked with the four colors across their observation period. Table 3 quantitatively compares the proportion of these dwell times at the four autoantibody levels between the diagnosed and undiagnosed participants. Since IA-2A positivity is known to predict relatively rapid progression to type 1 diabetes (20,22,29–31), we further stratified participants by the maximum IA-2A levels they reached during the observation period and compared the dwell times at the four levels of IAA and GADA between the diagnosed and undiagnosed participants (Figure 4, Table 4 and Supplementary Figure 2). Similarly, Supplementary Figure 3 illustrates the dwell times at various islet autoantibody levels in individuals stratified by maximum IAA or GADA level. Supplementary Figures 4, 5, and 6 show the cumulative

diabetes-free survival rates of children with single IAb positivity at the age of 2 and 6 years with different IAb levels (L1, L2, and L3).

Overall, the evolution of IAb levels in each of the three trajectories appears different between the diagnosed and undiagnosed participants. The IAb levels detected at the age of 2 or 6 years among those who had single IAb positivity can stratify T1D risk. Details of these differences are presented below.

Islet autoantibody levels in Trajectory 1 (TR1: predominantly multiple IAb first)

In TR1, high levels of IAA appeared more prevalent in the diagnosed participants than the undiagnosed ones (Figures 1 and 2). The most prominent pattern was that high IAA levels were seen among the diagnosed participants at early ages, younger than three years of age, whereas among the undiagnosed IAA remained mostly at low levels regardless of age. Among the diagnosed, 59% of visits that were categorized to TR1-1 and occurred between 1 and 2 years of age reached the highest level (L3) for IAA. Among the undiagnosed, only 8% of TR1-1 visits in the same age range reached L3 for IAA. In TR1, the proportion of visits with L3 of IA-2A or GADA appeared similar between the diagnosed and undiagnosed participants across their ages (Figure 2).

In TR1, there were differences in the distribution of dwell times in the four different autoantibody levels between the diagnosed and undiagnosed participants for GADA and IA-2A, but not for IAA (Figure 3 and Table 3). For GADA, the diagnosed participants spent significantly more time with GADA positivity across the three positive levels combined, compared to the undiagnosed (29% vs 5%, respectively; $p < 0.001$; Table 3). However, the proportions of dwell times among the three positive GADA levels were similar. For IAA, both the overall time of antibody positivity as well as the proportions of dwell times among the three positive levels were similar between the diagnosed and the undiagnosed. For IA-2A, the diagnosed participants stayed positive significantly longer than the undiagnosed participants

(43% vs 6%, respectively; $p < 0.001$; Table 3), but as with GADA, the proportions of dwell times among the three positive IA-2A levels were similar.

To investigate the interplay of IA-2A and other autoantibodies in TR1, we compared the diagnosed and the undiagnosed who reached four different levels of IA-2A. We found weakly significant differences in the proportion of dwell times in the four IAA levels (Table 4; $p < 0.05$), with the diagnosed spending more time in higher levels. There were, however, no significant differences in the proportion of dwell times between the diagnosed and the undiagnosed in any of the four GADA levels.

Islet autoantibody levels in Trajectory 2 (TR2: predominantly IAA first)

Similar to TR1, in the diagnosed participants in TR2, high levels of IAA were more prevalent than in the undiagnosed, particularly at early ages. Figure 2 and Supplementary Figure 1 show that among the diagnosed, 55% of observations that were categorized to TR2-1 and occurred between 1 and 2 years of age reached the highest level (L3) for IAA. Among the undiagnosed, only 31% of observations that were categorized to TR2-1 at the same age range reached L3 for IAA.

Table 3 shows that in TR2 there were significant differences in the distribution of dwell times in the four different autoantibody levels between the diagnosed and undiagnosed for GADA ($p < .001$), IAA ($p < .05$), and IA-2A ($p < .001$). For GADA, the diagnosed participants spent significantly more time with GADA positivity across the three positive levels combined, compared to the undiagnosed (46% vs 15%, respectively; $p < 0.001$; Table 3), but the distribution across positive levels was similar. For IAA, unlike IAA levels in TR1, the diagnosed participants spent significantly more time with IAA positivity than the undiagnosed (55% vs 36%; $p < 0.05$; Table 3), but again the distribution across positive levels was similar. For IA-2A, the diagnosed participants stayed at positive levels significantly longer than the

undiagnosed participants (46% vs 9%, respectively; $p < 0.001$; Table 3), with no noticeable difference in the distribution of the three positive levels.

In TR2, there were significant differences in dwell times in the four GADA levels between the diagnosed and undiagnosed for those who remained negative for IA-2A (L0) or reached L1 or L2 (Table 4; $p < .001$, $p < .001$, $p < .05$, respectively). However, no noticeable difference was found in the distribution of dwell times across positive GADA levels among those at L3 of IA-2A. Significant differences were observed in dwell times of the four IAA levels between the diagnosed and undiagnosed who remained negative for IA-2A (L0) or reached L1 or L3 ($p < .01$, $p < .001$, $p < .05$, respectively), with the diagnosed spending more time in higher IAA levels.

Islet autoantibody levels in Trajectory 3 (TR3: predominantly GADA first)

Similar to the other two trajectories, in TR3, high levels of GADA were more prevalent among the diagnosed compared to the undiagnosed, particularly at early ages. Figure 2 and Supplementary Figure 1 show that among the diagnosed participants categorized to TR3-1, the proportions of observations at high GADA levels (L3) at age 1 to 2 years and 2 to 3 years (43% and 50%, respectively) were higher compared to the undiagnosed (3% and 17%, respectively). In TR3, there were significant differences in the distribution of dwell times in the four different autoantibody levels between the diagnosed and the undiagnosed participants for GADA and IA-2A (Table 3; $p < .001$, $p <.001$, respectively), but not for IAA. For GADA, the diagnosed participants spent significantly more time with GADA positivity (57%), compared to 26% of the undiagnosed (Table 3; $p < 0.001$), but the distribution among positive levels was similar. For IA-2A, the diagnosed participants stayed at positive levels significantly longer than the undiagnosed (30% vs 6%, respectively; $p < 0.001$), again, with no noticeable difference in distribution among positive levels.

In TR3, there were significant differences in dwell times in the four GADA levels between the diagnosed and undiagnosed for those who remained negative for IA-2A (L0) or reached L1 or L2 (Table 4; $p < .001$, $p < .001$, $p < .01$), with the diagnosed spending more time in higher GADA levels. There were also significant differences in dwell times in the four IAA levels between the diagnosed and the undiagnosed participants who reached IA-2A level of L1 ($p < .001$), but the distribution in positive IAA levels were similar between the diagnosed and undiagnosed.

Survival Analyses

Survival analyses showed differences in progression to type 1 diabetes between IAb levels, among participants who had single IAb positivity at the age of 2 years. Altogether 206 participants had single GADA positivity at age 2, and those with GADA L2 or L3 progressed faster to diabetes than those with GADA L1 ($P < 0.001$; Supplementary Fig.4). There were no statistically significant differences in progression rate between participants with GADA L2 and L3. A total of 327 participants had single IAA positivity at age 2 and those with IAA L3 progressed faster to diabetes than those with IAA L1 ($P < 0.001$; Supplementary Fig. 5). Participants with IAA L2 progressed only marginally faster to diabetes than those with IAA L1 ($P = .056$). There were no statistically significant differences in progression rate between participants with IAA L2 and L3. Positivity for single IA-2A was observed in 50 participants at the age of 2 years. The participants with IA-2A L2 and L3 progressed faster to diabetes than those with IA-2A L1 ($P < 0.01$; Supplementary Fig. 6). No statistically significant differences in progression rate were observed between participants with IA-2A L2 and L3.

At the age of 6 years, 253 participants had single GADA positivity. Participants with GADA L2 progressed faster to diabetes than those with GADA L1 ($P = 0.012$; Supplementary Fig. 4) but no differences in progression rate were observed between participants with GADA L2 and

L3 or with GADA L1 and L3. A total of 148 participants had single IAA positivity at the age of 6 years but no differences in progression rates among participants with IAA L1, L2, and L3 were observed (Supplementary Fig. 5). Single IA-2A positivity was present in 92 participants at age 6 years and participants with IA-2A L3 progressed faster to diabetes than those with IA-2A L1 ($P = 0.014$; Supplementary Fig. 6). There were no statistically significant differences in progression rates between participants with IA-2A L2 and L3 or with IA-2A L1 and L2.

We also conducted survival analyses for participants who had multiple IAb positivity at the ages of 2 and 6 years and compared diabetes-free survival rates between different IAb levels. However, no statistically significant differences in progression rates were found between the IAb levels.

DISCUSSION

The present study refined previously described islet autoantibody trajectories by adding information about autoantibody levels to explore differential patterns between individuals who do or do not progress to type 1 diabetes within the observation time. Overall, each trajectory showed unique islet autoantibody level transition patterns. In each trajectory the undiagnosed participants showed generally similar patterns to the diagnosed with two notable differences: i) their age at transition from negativity to positivity was delayed; and ii) they had a higher proportion of participants who only reached a low-positive IAb level (L1). In sum, the undiagnosed participants had generally lower IAb levels and later appearance of IAb than the diagnosed participants

In particular, when at-risk participants had a single positivity at the age of two years, the higher level (L2/3) of GADA, IAA or IA-2A was associated with faster progression to diabetes in comparison to the lower level of positivity (L1). The participants with single IAA or single GADA positivity at the age of two years were likely to belong to TR2 (predominantly IAA only first) or TR3 (predominantly GADA only first), based on the previous findings (12). Therefore, IAb levels at an early age can be informative with respect to the main IAb trajectories and associated risks of progression to type 1 diabetes.

The major strength of this study is the visualization of the islet autoantibody trajectories enriched with autoantibody levels. This approach provided unique data-driven insights to islet autoantibody levels within the main autoimmune trajectories. Data visualizations can help both identify and understand patterns that cannot be easily summarized statistically, thereby facilitating the process of generating new hypotheses. For example, in the predominantly GADA-initiated trajectory (TR3), the distribution of positive GADA levels was conspicuously similar between the diagnosed and undiagnosed (Figure 2), but the undiagnosed initiated the

positive level later and persisted longer before transition to the higher levels (Figure 3). Another strength is the very large multinational cohort of children who were at increased risk for type 1 diabetes either because of positive family history or having HLA-conferred risk for the disease.

Differences in the original cohort studies were, however, a clear limitation. To overcome this, special attention was paid to harmonization of the autoantibody levels and HLA risk groups (2,22). Another limitation is the small number of participants in some of the trajectory-related states (Figure 2). In addition, information on ZnT8A was not available for our analyses, and should be added in analyses of future studies, because ZnT8A may be the first autoantibody to appear and the analysis could be further refined (32,33). Moreover, autoantibodies to tetraspanin-7 may further contribute to our understanding of the implications of varying autoimmune trajectories of type 1 diabetes (34). Since the population in the cohort is young, the findings need to be validated using data from older individuals in order to be generalizable. In addition to immunophenotyping with autoantibody patterns, it should also be noted that metabolic assessment can be especially useful to identify approaching stage 3 type 1 diabetes. Moreover, combining metabolic data with islet autoantibody trajectory information might further improve individual risk assessment and thereby help to refine selection criteria for intervention trials.

Multiple studies have reported the autoantibody-specific initiation of islet autoimmunity (8,9) and evolution of islet autoantibody pattern based on positivity or negativity (35–38). Our recent data-driven analysis, also based on binary autoantibody categories, demonstrated the longitudinal profile of the three main patterns of islet autoimmunity (12). Here we have enhanced this approach by including IAA, GADA and IA-2A levels, which helps to further distinguish future progressors from those who remain healthy. Endotypes of islet autoimmunity have thus far been characterized by the first-appearing autoantibodies (4,39,40). However, it is

apparent that the longitudinal evolution of islet autoantibodies together with their levels can better define the putative endotypes. This paper provides a novel approach of analyzing the dynamic patterns of autoantibody levels and comparing the dwell times of the three autoantibodies at a given level between the diagnosed and undiagnosed participants.

This analysis utilized data from prospective studies with IAb information from frequently sampled longitudinal visits beginning from early ages. In contrast, IAb screening programs may identify autoantibody positive children at any age without knowledge of prior IAb history. Thus, it is important to have refined understanding of trajectories and the significance of dynamic IAb levels in order to predict individual risk of progression. Better understanding of risk may have important implications for future research and interventions.

In conclusion, visualization of islet autoimmunity progression using IAb levels, order of appearance, and trajectories, can enhance insights to type 1 diabetes pathogenesis. It has been long appreciated that the number of autoantibodies is an important predictor of type 1 diabetes; this study further refines the main trajectories using the dynamic patterns of autoantibody levels. Furthermore, these data show that not only positivity for a single IAb observed at an early age but also the IAb level can be used for risk stratification for type 1 diabetes. In the future, Artificial Intelligence approaches to analyze these trends in the complex datasets may allow these patterns to be better translated to prediction of progression to diabetes in children. The findings in this paper need to be validated in an independent cohort. Future work also needs to correlate the observed trajectories with changes in beta cell function and glycemia in order to test whether longitudinal IAb patterns should influence individual risk assessment and selection criteria for intervention trials.

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Tables

Table 1. Distribution of undiagnosed and diagnosed participants in three trajectories over sex, seroconversion age, and diagnosis age.

		Diagnosed			Undiagnosed		
		TR1 (n=256)	**TR2** (n=273)	**TR3** (n=114)	**TR1** (n=483)	**TR2** (n=257)	**TR3** (n=762)
Sex	Male	155 (61%)	146 (53%)	52 (46%)	283 (59%)	145 (56%)	409 (54%)
	Female	101 (39%)	127 (47%)	62 (54%)	200 (41%)	112 (44%)	353 (46%)
Age of Seroconversion*		2.51 (1.51 to 4.2)	1.79 (1.04 to 3.13)	4.05 (2.3 to 6.01	4.98 (2.02 to 8.03)	6.0 (2.42 to 9.18)	6.5 (3.99 to 9.62)
Age of Diagnosis*		4.07 (1.88 to 7.06)	3.85 (1.74 to 6.75)	5.68 (2.92 to 9.08)	-	-	-

* The ages are shown in median (25th percentile to 75th percentile).

Table 2. Four islet autoantibody levels of multiples of upper limit of normal (mULN) for the three islet autoantibodies: GADA, IAA, IA-2A.

	GADA	**IAA**	**IA-2A**
L0	< 1.0	< 1.0	< 1.0
L1	1.0 – 5.3	1.0 – 3.5	1.0 – 2.4
L2	5.4 – 20.7	3.6 – 5.4	2.5 – 235.1
L3	≥ 20.8	≥ 5.5	≥ 235.2

Table 3. The proportion of dwell times (total duration of follow-up at a given level) in percentage by the four islet autoantibody levels (L0, L1, L2, L3) per trajectory and diagnosis for each autoantibody: (a) GADA, (b) IAA, (c) IA-2A.

(a) GADA

Trajectory	Diagnosis	N	GADA-L0	GADA-L1	GADA-L2	GADA-L3
TR1***	**D**	256	71.5	13.9	9.0	5.6
	UD	483	95.5	2.4	1.0	1.1
TR2***	**D**	273	53.7	16.1	18.3	11.9
	UD	257	84.9	5.6	4.7	4.7
TR3***	**D**	114	43.1	19.9	21.9	15.1
	UD	762	74.3	13.9	6.9	4.9

(b) IAA

Trajectory	Diagnosis	N	IAA-L0	IAA-L1	IAA-L2	IAA-L3
TR1	**D**	256	76.4	14.4	3.1	6
	UD	483	86.9	10.9	1.2	1
TR2*	**D**	273	45.1	27.3	8.8	18.9
	UD	257	64.3	21	4.5	10.3
TR3	**D**	114	91.5	7.2	0.5	0.7
	UD	762	98	1.8	0.1	0.1

(c) IA-2A

Trajectory	Diagnosis	N	IA2A-L0	IA2A-L1	IA2A-L2	IA2A-L3
TR1***	**D**	256	56.7	1.3	29.4	12.5
	UD	483	93.9	0.4	4.7	0.9
TR2***	**D**	273	53.8	2.2	33.5	10.5
	UD	257	91	0.5	6.9	1.6
TR3***	**D**	114	69.7	1.6	23.2	5.5
	UD	762	94.3	0.8	3.9	1

Chi-square tests show significant differences in the proportions between diagnosis within each trajectory: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 4. The proportion of dwell times (total duration of follow-up at a given level) in percentage for GADA and IAA for participants stratified by the islet autoimmunity trajectory they followed (TR1, TR2, or TR3), diagnosis (D=diagnosed, UD=undiagnosed) and the maximumIA-2A level (L0, L1, L2, or L3) that each participant achieved during observation.

Trajectory	IA-2A level	Type 1 diabetes	N	GADA Levels				P	IAA Levels				P
				L0	L1	L2	L3		L0	L1	L2	L3	
TR1	**L0**	**D**	13	96.5	3.5	0	0	n.s.	83.2	12.8	0.4	3.6	n.s.
		UD	411	98.6	1.2	0.1	0.1		86.2	11.3	1.4	1.1	
	L1	**D**	8	92.7	5.1	0.4	1.8	n.s.	95.5	2.2	0.4	1.9	*
		UD	6	93.9	4.7	1.4	0		87.6	11.7	0	0.7	
	L2	**D**	105	66.5	18.3	9.8	5.4	n.s.	72.2	14.5	4.8	8.5	*
		UD	40	82	6.6	7.7	3.6		86.8	11.4	1.3	0.4	
	L3	**D**	103	64.7	15.3	10.4	9.5	n.s.	72.6	19.4	2.4	5.6	n.s.
		UD	25	69.4	13.5	4.5	12.7		82.9	14.3	2.2	0.6	
TR2	**L0**	**D**	69	57.8	14.8	16.5	11	***	43.9	28.9	5.7	21.6	**
		UD	206	94.6	2.6	1.8	1		67.3	18.1	4.3	10.4	
	L1	**D**	9	52.5	3.4	26.7	17.4	***	35	29.6	16.2	19.1	***
		UD	8	86	14	0	0		64.4	29.2	0	6.4	
	L2	**D**	111	53.5	19.8	16.4	10.4	*	36.2	29.7	12.9	21.2	n.s.
		UD	27	34.6	18.7	22.4	24.3		42	35	8.6	14.3	
	L3	**D**	74	40.5	17.9	25.3	16.3	n.s.	46.4	28.3	8.8	16.5	*
		UD	16	36.9	19.7	18.6	24.8		50.3	39.9	4.2	5.6	
TR3	**L0**	**D**	34	43.9	23.7	23.4	9.1	***	90.2	9.2	0.1	0.5	n.s.
		UD	590	77.4	15.6	4.9	2.2		98.7	1.1	0.1	0.1	
	L1	**D**	5	43.2	20.9	12	23.8	***	69	17.2	2.6	11.2	***
		UD	52	85.3	5.1	3.2	6.4		98.5	1.3	0.2	0	
	L2	**D**	43	37.6	18.5	28	15.8	**	92.3	6.5	0.8	0.4	n.s.
		UD	80	60.4	9.4	16.4	13.8		95.3	4.4	0.1	0.2	
	L3	**D**	29	31	23.9	22.5	22.6	n.s.	94.1	5.7	0.1	0.1	n.s.
		UD	39	38.5	14.9	23.6	23		94	5.5	0.1	0.4	

Proportions of dwell times at different GADA and IAA levels between the diagnosed and undiagnosed were analyzed by Chi-square tests: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure legends

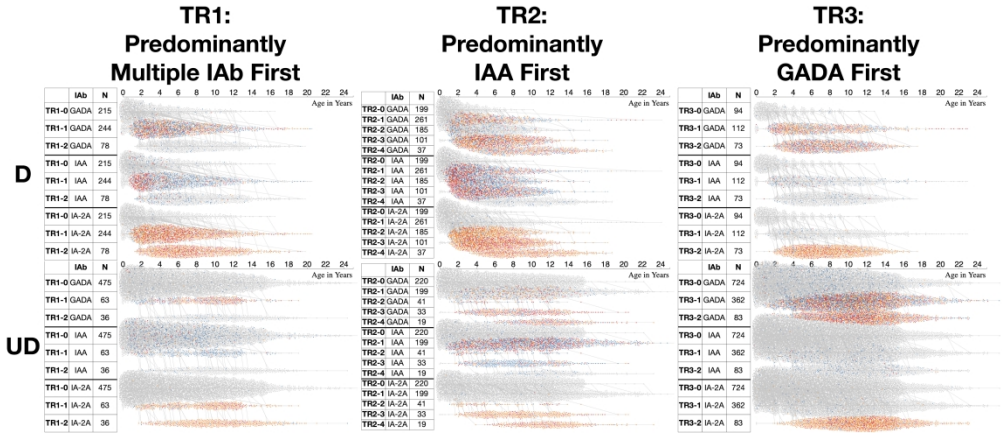
Figure 1. Visualization of the entire data set by trajectory and islet autoantibody level comparing diagnosed and undiagnosed individuals and illustrating the differences. Three autoimmune trajectories and their component states overlaid with autoantibody levels towards type 1 diabetes. The diagram includes three subfigures summarizing the three respective trajectories and their component states overlaid with the islet autoantibody levels, (a) TR1: Predominantly Multiple IAb First, (b) TR2: Predominantly IAA First, (c) TR3: Predominantly GADA First. Each subfigure consists of two plots (top, bottom), the top plot shows trajectories for the diagnosed (D) and the bottom one shows those for the undiagnosed (UD). The table on the left includes three columns 1) component state label, 2) IAb type (GADA, IAA, or IA-2A), 3) the total number of participants per state (row). The waterfall chart on the right shows visits (dots) colored according to the IAb level (L0: Gray, L1: Blue, L2: Orange, L3: Red). Y-axis represents component states and x-axis represents age of participants in years. In TR1, most diagnosed children advance from TR1-0 (IAb negative) to TR1-1 (Multiple IAb positive) and TR1-2 (IA-2A positive). The distributions of autoantibody levels over age show higher proportion of IAA L3 (red) in early age of the diagnosed participants when compared to the undiagnosed participants. In TR2, the diagnosed participants frequently have IAA L3 (red) in early age across all positive states, whereas the undiagnosed participants have fewer IAA positive visits and those with L3 are spread across ages. In TR3, both the diagnosed and the undiagnosed participants advance to IAb positive states: TR3-1 and TR3-2, but the timing is later in the undiagnosed.

Figure 2. Summary of islet autoantibody levels at each visit by age comparing diagnosed and undiagnosed individuals. Normalized proportions of autoantibody levels over age are depicted. The diagram shows 48 panels (6 rows, 8 columns) summarizing the normalized

proportion of autoantibody levels over participants' age. Component panels represent the diagnosed and undiagnosed groups for each of the eight IAb positive states (TR1-1, TR1-2, TR2-1, TR2-2, TR2-3, TR2-4, TR3-1, TR3-2) and three IAb types (GADA, IAA, IA-2A). For example, TR1-1 indicates the first positive component state of trajectory TR1, predominantly multiple islet autoantibodies first. Each panel includes a stacked bar chart that shows the proportion of visits in percentage (y-axis), which are broken down into stacks of four IAb levels (L0: Gray, L1: Blue, L2: Orange, L3: Red), over ages of participants (x-axis). We excluded visits with no autoantibody measurement and age ranges with less than 10 observations. In TR1-1, TR2-1, and TR2-2, the proportion of the highest IAA level (L3) at early ages (<2y) tends to be higher for the diagnosed participants than for the undiagnosed. In TR3-1, the proportion of the highest GADA level (L3) at early ages (<2y) appears higher for the diagnosed compared to the undiagnosed participants.

Figure 3. Development of autoantibody levels and dwell times for individual participants sorted by duration by follow-up. The diagram includes six panels (2 rows and 3 columns) summarizing the dwell time of individual participants at each autoantibody level (L0: Gray, L1: Blue, L2: Orange, L3: Red) for three islet autoantibodies (GADA, IAA, IA-2A) over their ages (x-axis) per trajectory (column) and per diagnosis (row). In each panel, we sorted participants (horizontal bars) by their age at last observation with increasing order from top to bottom. Overall, the undiagnosed participants have longer follow-up time as seen in the horizontal length of bars across the board. Most of the diagnosed participants tend to show dynamic changes of autoantibody levels and longer dwell times at higher levels over the follow-up period compared to the undiagnosed participants. In all trajectories an evolution to high levels of IA-2A frequently precedes diagnosis.

Figure 1. Development of autoantibody levels and dwell times for individual participants sorted by their maximum IA-2A level. The diagram includes six panels (2 rows: Diagnosed, Undiagnosed; 3 columns: TR1, TR2, TR3) summarizing the dwell time of individual participants at each autoantibody level (L0: Gray, L1: Blue, L2: Orange, L3: Red) for three islet autoantibodies (GADA, IAA, IA-2A) over their ages per trajectory per diagnosis. In each panel, participants in each trajectory (column) are sorted by the maximum level of IA-2A with increasing order from top to bottom. More than a half of diagnosed participants across the three trajectories reach high IA-2A levels (L2, L3) during follow-up. On the other hand, a majority of undiagnosed participants across the three trajectories stay IA-2A negative (L0) during follow-up.



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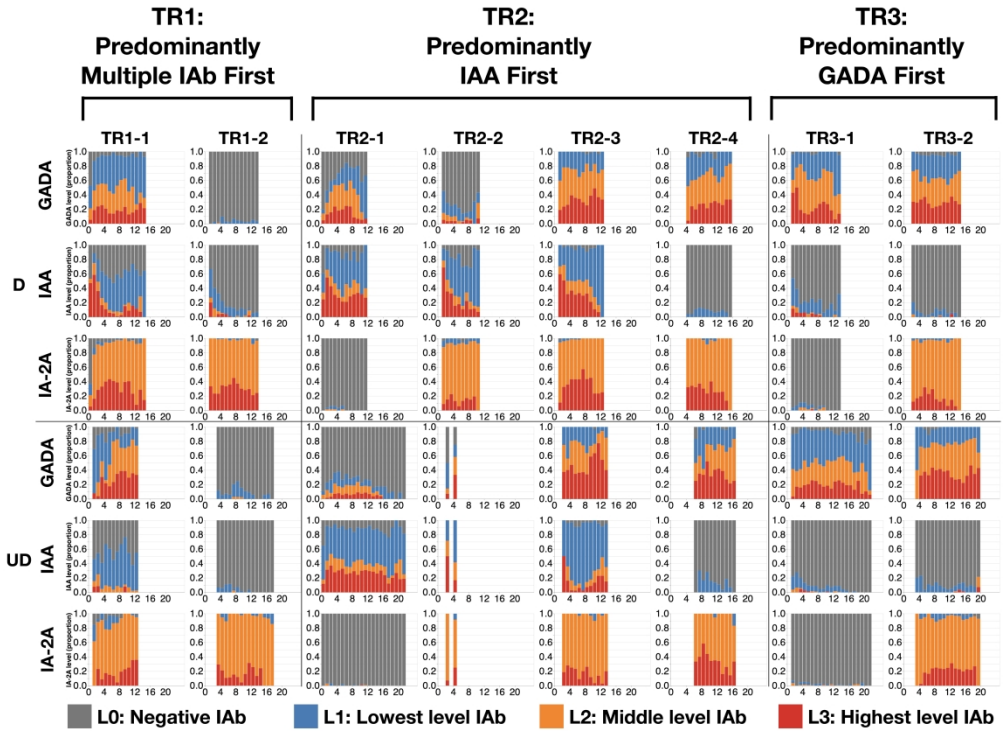

Summary of islet autoantibody levels at each visit by age comparing diagnosed and undiagnosed individuals. Normalized proportions of autoantibody levels over age are depicted. The diagram shows 48 panels (6 rows, 8 columns) summarizing the normalized proportion of autoantibody levels over participants' age. Component panels represent the diagnosed and undiagnosed groups for each of the eight IAb positive states (TR1-1, TR1-2, TR2-1, TR2-2, TR2-3, TR2-4, TR3-1, TR3-2) and three IAb types (GADA, IAA, IA-2A). For example, TR1-1 indicates the first positive component state of trajectory TR1, predominantly multiple islet autoantibodies first. Each panel includes a stacked bar chart that shows the proportion of visits in percentage (y-axis), which are broken down into stacks of four IAb levels (L0: Gray, L1: Blue, L2: Orange, L3: Red), over ages of participants (x-axis). We excluded visits with no autoantibody measurement and age ranges with less than 10 observations. In TR1-1, TR2-1, and TR2-2, the proportion of the highest IAA level (L3) at early ages (<2y) tends to be higher for the diagnosed participants than for the undiagnosed. In TR3-1, the proportion of the highest GADA level (L3) at early ages (<2y) appears higher for the diagnosed compared to the undiagnosed participants.

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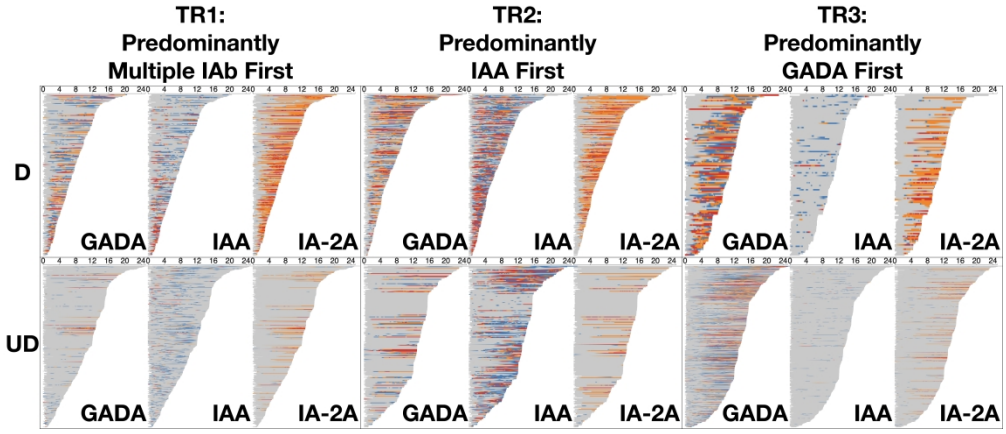

Development of autoantibody levels and dwell times for individual participants sorted by duration by follow-up. The diagram includes six panels (2 rows and 3 columns) summarizing the dwell time of individual participants at each autoantibody level (L0: Gray, L1: Blue, L2: Orange, L3: Red) for three islet autoantibodies (GADA, IAA, IA-2A) over their ages (x-axis) per trajectory (column) and per diagnosis (row). In each panel, we sorted participants (horizontal bars) by their age at last observation with increasing order from top to bottom. Overall, the undiagnosed participants have longer follow-up time as seen in the horizontal length of bars across the board. Most of the diagnosed participants tend to show dynamic changes of autoantibody levels and longer dwell times at higher levels over the follow-up period compared to the undiagnosed participants. In all trajectories an evolution to high levels of IA-2A frequently precedes diagnosis.

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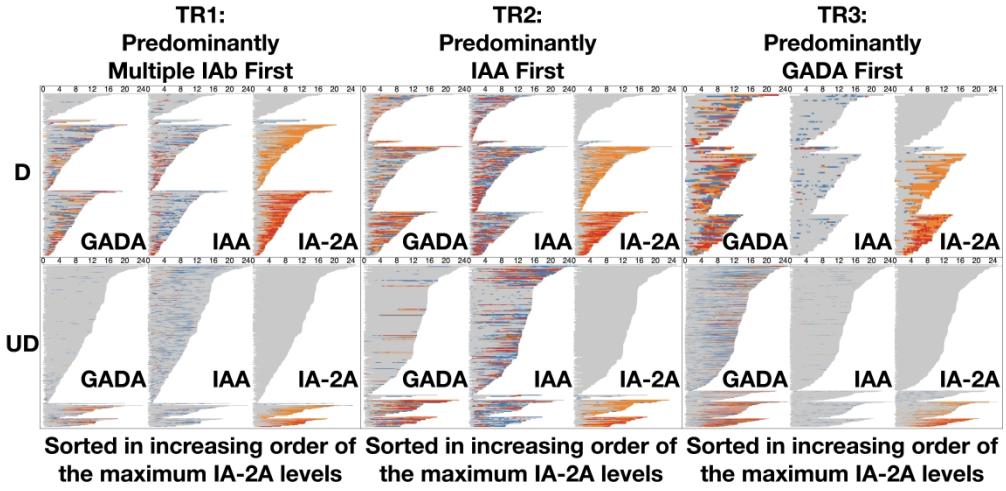

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Supplementary Material

Islet Autoantibody Levels Differentiate Progression Trajectories in Individuals with Presymptomatic Type 1 Diabetes

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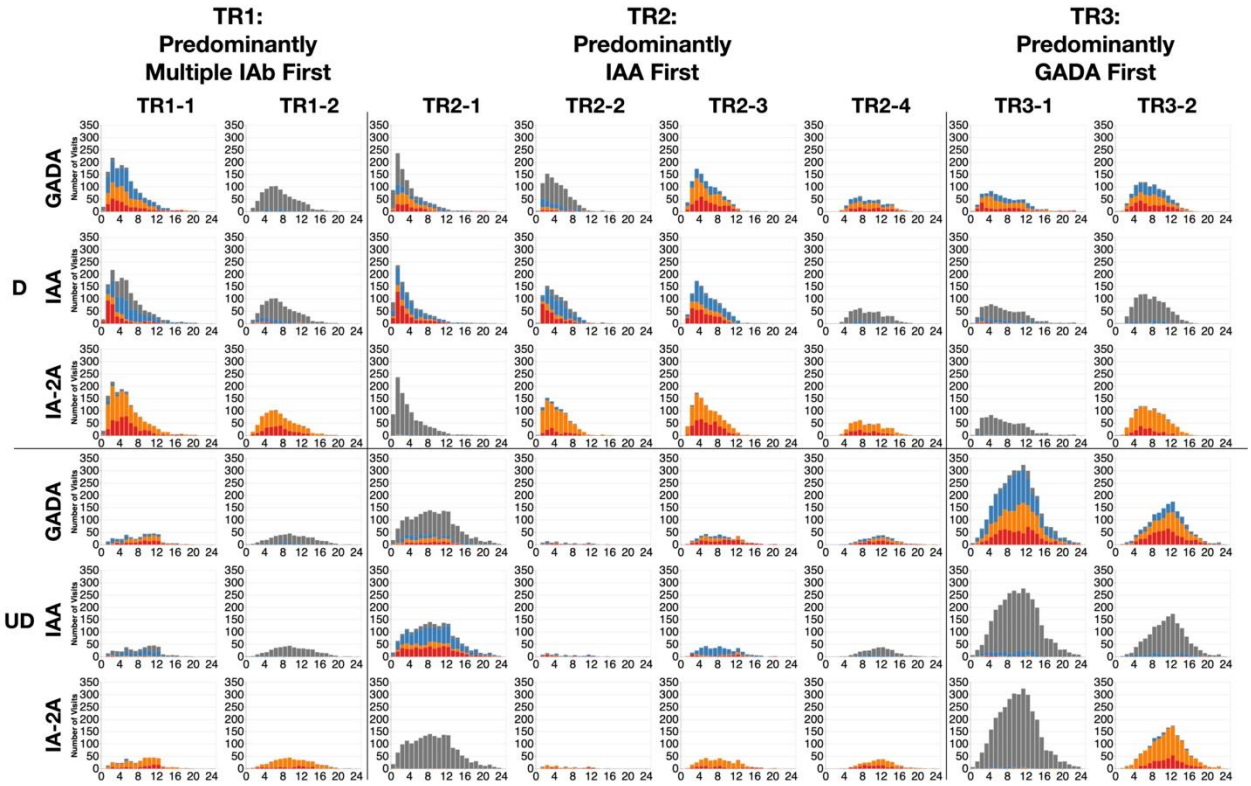
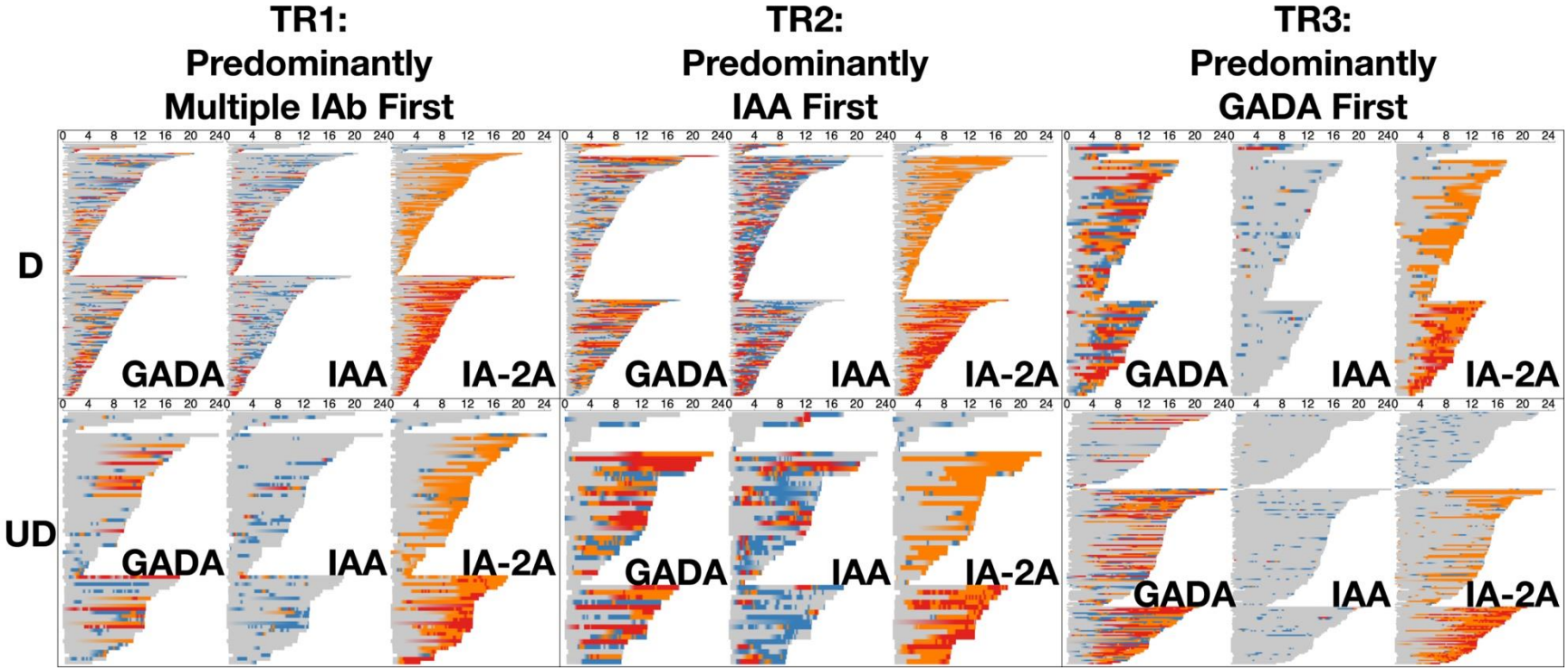
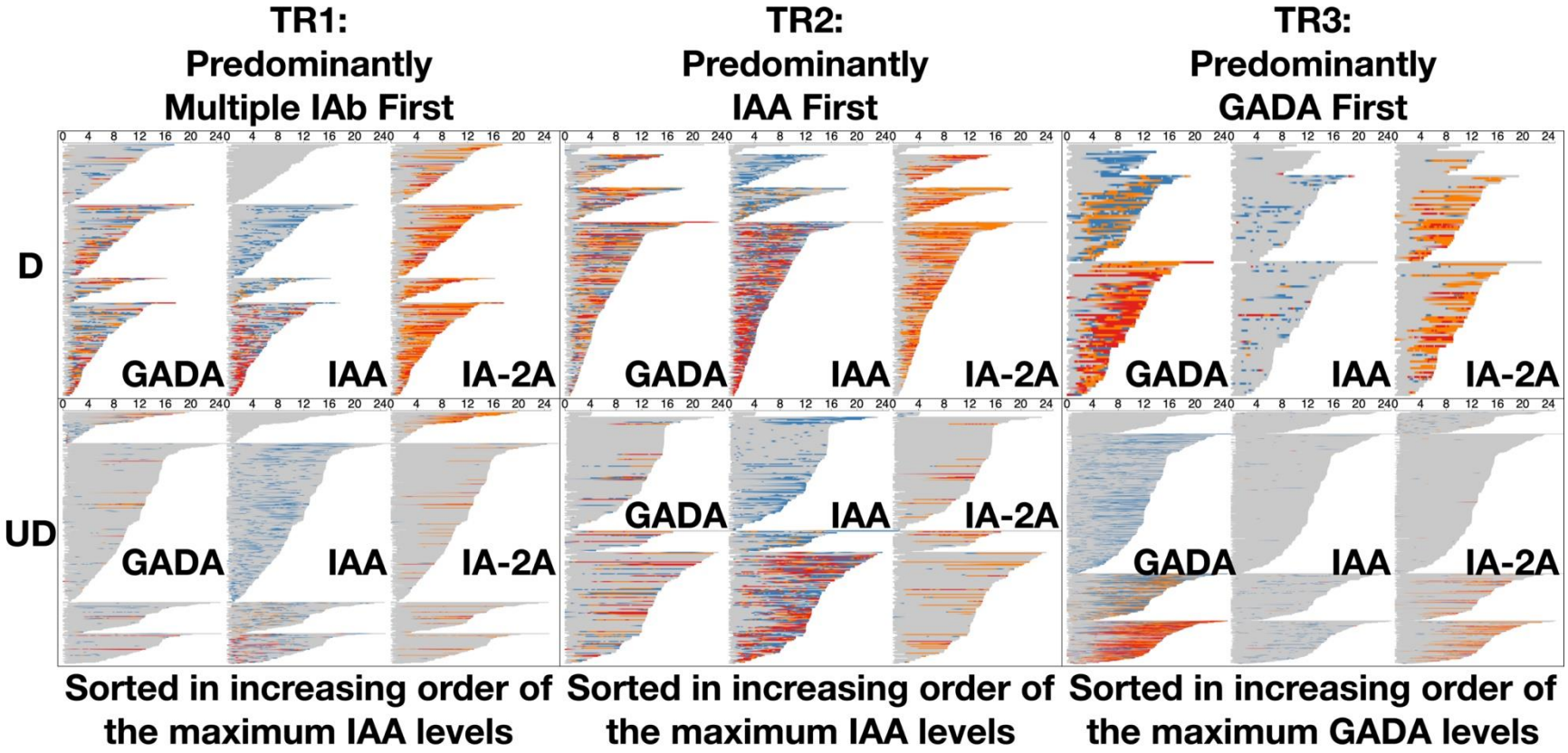
Supplementary material includes:
Supplementary Figures 1-6
Supplementary Tables 1-2



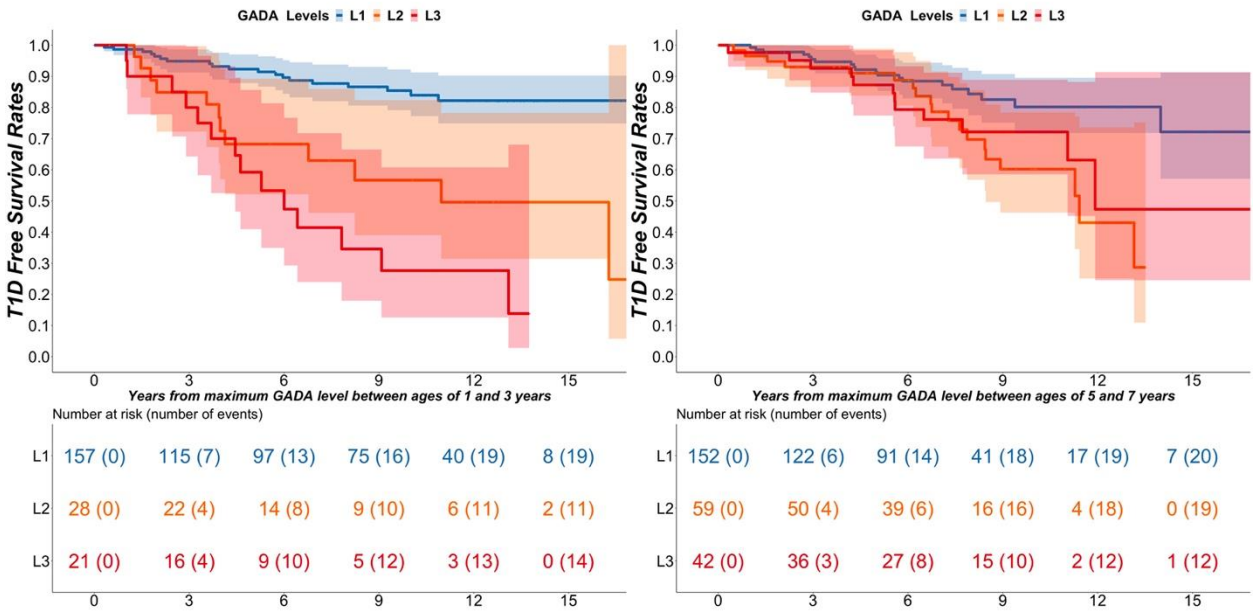
Supplementary Figure 1. 1. Proportions of autoantibody levels at each visit by age comparing diagnosed and undiagnosed individuals. The diagram shows 48 panels (6 rows, 8 columns) summarizing the proportion of autoantibody levels over participants' age per 8 IAb positive states (TR1-1, TR1-2, TR2-1, TR2-2, TR2-3, TR2-4, TR3-1, TR3-2) and the IAb type (GADA, IAA, IA-2A). For example, TR1-1 indicates the first positive component state of trajectory TR1, predominantly multiple islet autoantibodies first. Each panel includes a stacked bar chart that shows the total number of visits (y-axis), which are broken down into stacks of four autoantibody levels and missing data IAb levels (L0: Gray, L1: Blue, L2: Orange, L3: Red), over ages of participants (x-axis).



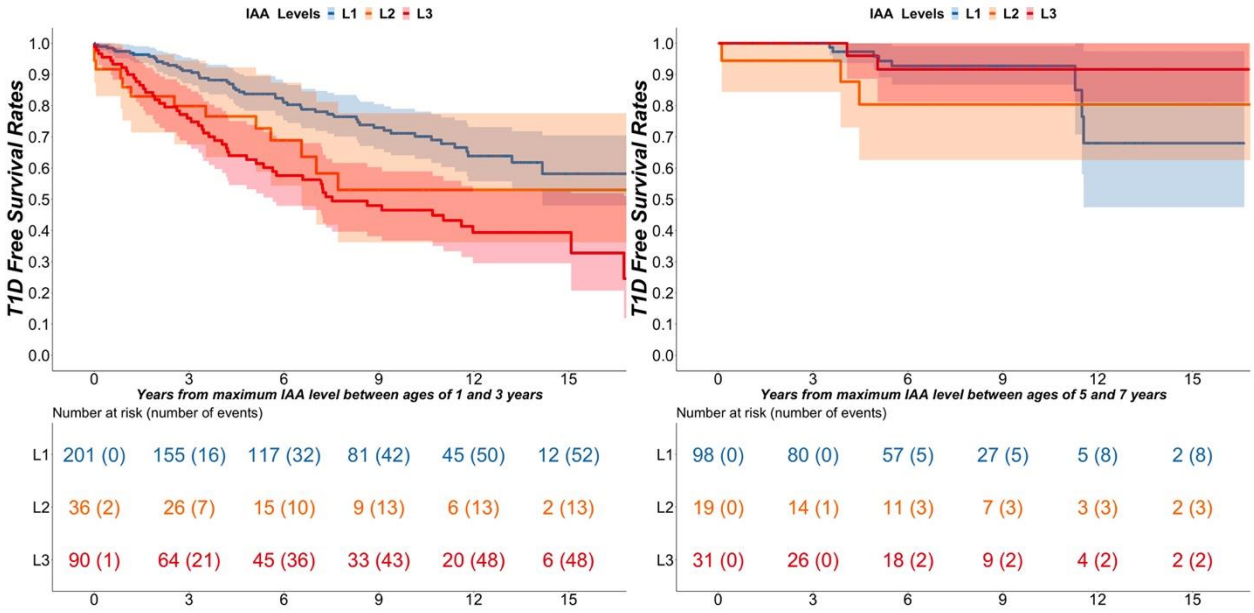
Supplementary Figure 2. 2. Development of islet autoantibody levels for individual sorted by maximum IA-2A level. The diagram includes six panels (2 rows and 3 columns) summarizing the dwell time of individual participants at each autoantibody level (L0: Gray, L1: Blue, L2: Orange, L3: Red) for three islet autoantibodies (GADA, IAA, IA-2A) over their ages (x-axis) per trajectory (column) and per diagnosis (row). In each panel, we sorted participants (bars) by the maximum level of IA-2A with increasing level from top to bottom. We excluded those who only reached L0 of IA-2A from this figure.



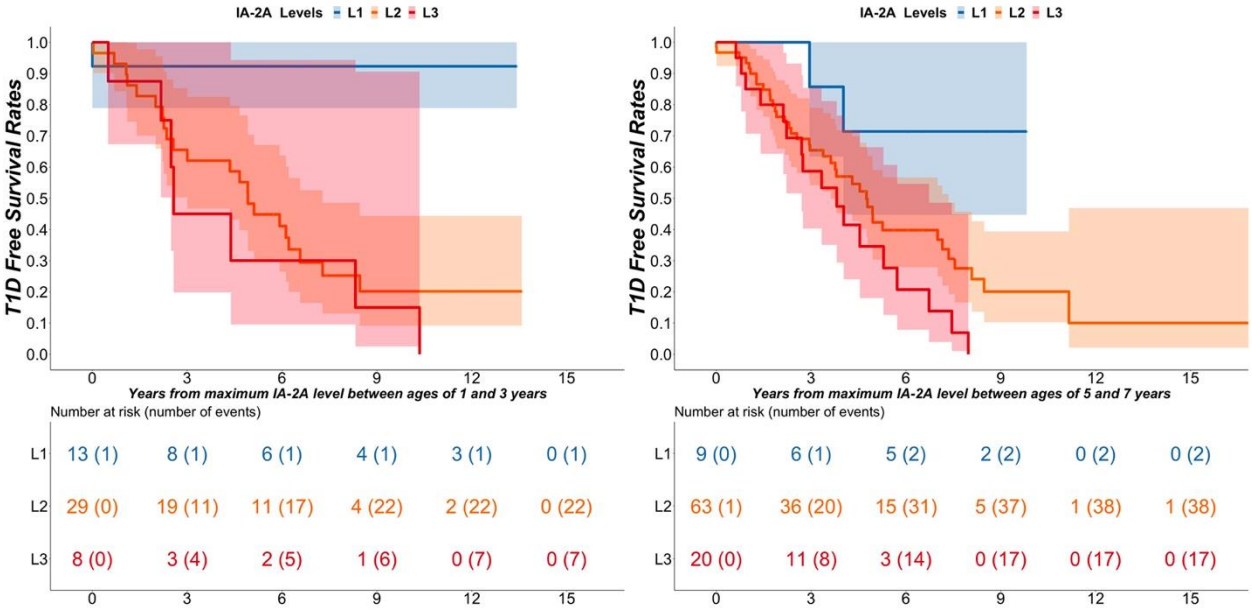
Supplementary Figure 3. 3 Development of islet autoantibody levels for individual participants sorted by maximum IAA or GADA level. The diagram includes six panels (2 rows and 3 columns) summarizing the dwell time of individual participants at each autoantibody level (L0: Gray, L1: Blue, L2: Orange, L3: Red) for three islet autoantibodies (GADA, IAA, IA-2A) over their ages (x-axis) per trajectory (column) and per diagnosis (row). Participants in each trajectory (column) are sorted by the maximum level of IAA for TR1 and TR2, and of GADA for TR3 with increasing level from top to bottom.



Supplementary Figure 4. Diabetes-free survival curves (mean and 95% confidence intervals) of the participants who had GADA only positivity at the age of 2 years (Left) and 6 years (Right), stratified by GADA positivity levels (L1, L2, L3). Participants with GADA L2 and L3 at age 2 years progressed faster to diabetes than those with GADA L1 ($p < .001$). At age 6 years, participants with GADA L2 progressed faster to diabetes than those with GADA L1 ($p = .012$).



Supplementary Figure 5. Diabetes-free survival curves (mean and 95% confidence intervals) of the participants who had IAA only positivity at the age of 2 years (Left) and 6 years (Right), stratified by IAA positivity levels (L1, L2, L3). Participants with IAA L3 at age 2 years progressed faster to diabetes than those with IAA L1 ($p < .001$). Participants with IAA L2 at age 2 progressed marginally faster to diabetes than those with IAA L1 ($p = .056$). No differences were observed in the progression rates between participants with IAA L1, L2, or L3 at age 6 years.



Supplementary Figure 6. Diabetes-free survival curves (mean and 95% confidence intervals) of the participants who had IA-2A only positivity at the age of 2 years (Left) and 6 years (Right), stratified by IA-2A positivity levels (L1, L2, L3). Participants with IA-2A L2 and L3 at age 2 years progressed faster to diabetes than those with IA-2A L1 ($p < .01$). Participants with IA-2A L3 at age 6 years progressed faster to diabetes than those with IA-2A L1 ($p = .014$).

Supplementary Table 1. The number of samples analyzed for islet autoantibodies at various ages of the 2,145 autoantibody positive participants.

Age	All	Diagnosed	Undiagnosed
0 - 1	4126	1381	2745
1 - 2	4180	1397	2783
2 - 3	3667	1280	2387
3 - 4	3361	1170	2191
4 - 5	3182	1089	2093
5 - 6	3043	1011	2032
6 - 7	2717	836	1881
7 - 8	2523	690	1833
8 - 9	2436	590	1846
9 - 10	2256	494	1762
10 - 11	2084	415	1669
11 - 12	1985	341	1644
12 - 13	1739	237	1502
13 - 14	1283	162	1121
14 - 15	1041	102	939
15 - 16	695	57	638
16 - 17	370	50	320
17 - 18	268	26	242
18 - 19	212	18	194
19 - 20	132	13	119
20 - 21	110	7	103
21 - 22	69	5	64
22 - 23	49	5	44
23 - 24	23	0	23
24 - 25	13	1	12
25 - 26	2	0	2
26 - 27	4	0	4

Supplementary Table 2. Demographic data and sampling intervals of the 2,145 autoantibody positive participants in the five study sites.

Study Sites	Number of Female Participants	Number of Male Participants	Sampling Intervals in Years Median (IQR)	Number of Participants in TR1	Number of Participants in TR2	Number of Participants in TR3
BABYDIAB	123	124	0.57 (0.22 to 1.10)	70 (28.3%)	91 (36.8%)	86 (34.8%)
DAISY	127	153	0.55 (0.31 to 1.00)	105 (37.5%)	52 (18.6%)	123 (43.9%)
DEW-IT	25	37	0.50 (0.30 to 1.17)	35 (56.5%)	15 (24.2%)	12 (19.4%)
DIPIS	196	183	0.86 (0.23 to 1.06)	127 (33.5%)	57 (15.0%)	195 (51.5%)
DIPP	484	693	0.31 (0.25 to 0.51)	402 (34.2%)	315 (26.8%)	460 (39.1%)