Prognostic Classification Factors Associated With Development of Multiple Autoantibodies, Dysglycemia, and Type 1 Diabetes—A Recursive Partitioning Analysis

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OBJECTIVE

To define prognostic classification factors associated with the progression from single to multiple autoantibodies, multiple autoantibodies to dysglycemia, and dysglycemia to type 1 diabetes onset in relatives of individuals with type 1 diabetes.

RESEARCH DESIGN AND METHODS

Three distinct cohorts of subjects from the Type 1 Diabetes TrialNet Pathway to Prevention Study were investigated separately. A recursive partitioning analysis (RPA) was used to determine the risk classes. Clinical characteristics, including genotype, antibody titers, and metabolic markers were analyzed.

RESULTS

Age and GAD65 autoantibody (GAD65Ab) titers defined three risk classes for progression from single to multiple autoantibodies. The 5-year risk was 11% for those subjects >16 years of age with low GAD65Ab titers, 29% for those ≤16 years of age with low GAD65Ab titers, and 45% for those subjects with high GAD65Ab titers regardless of age. Progression to dysglycemia was associated with islet antigen 2 Ab titers, and 2-h glucose and fasting C-peptide levels. The 5-year risk is 28%, 39%, and 51% for respective risk classes defined by the three predictors. Progression to type 1 diabetes was associated with the number of positive autoantibodies, peak C-peptide level, HbA_{1c} level, and age. Four risk classes defined by RPA had a 5-year risk of 9%, 33%, 62%, and 80%, respectively.

CONCLUSIONS

The use of RPA offered a new classification approach that could predict the timing of transitions from one preclinical stage to the next in the development of type 1 diabetes. Using these RPA classes, new prevention techniques can be tailored based on the individual prognostic risk characteristics at different preclinical stages.

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**A complete list of the Type 1 Diabetes TrialNet Study Group can be found in the Supplementary Data online.*

Both immunologic and metabolic markers have been used in genetically at-risk individuals (particularly relatives of individuals in whom type 1 diabetes [T1D] has been diagnosed) to predict in whom T1D will develop. Numerous natural history studies (1–7) have shown that the manifestation of multiple islet cell antibodies (ICAs) and an abnormal oral glucose tolerance test (OGTT) result reflect disease progression and carry increasing levels of T1D risk. It has also been recognized that β-cell dysfunction and metabolic disarrangement mark the preclinical stage of T1D (8,9). It follows, therefore, that the risk factors associated with the transition from one stage to the next, such as from single to multiple islet autoantibody positivity, from multiple islet autoantibody positivity to dysglycemia, and from dysglycemia to T1D onset, might be different across the spectrum of disease progression, and a better recognition of them might enable a better appreciation of levels of risk and may even suggest opportunities for targeted interventions that might alter the disease process. Moreover, strategic preventive measures that identify and treat preclinical pathological changes, and consequently interrupt disease progression, can be introduced and tested at each preclinical stage along the natural history of the disease. A thorough appreciation of the prognostic risk factors relevant to disease progression is needed, as well as an approach for risk classifications to determine the individual's risk of progression to the next stage.

In the current study, we investigate three distinct cohorts of subjects from the Type 1 Diabetes TrialNet Pathway to Prevention Study (TNPTP) in an attempt to determine the factors that can predict progression to multiple positive autoantibodies from a single autoantibody, progression to dysglycemia from multiple positive autoantibodies, and progression to the onset of T1D from dysglycemia by a recursive partitioning analysis (RPA). To our knowledge, this is the first study to address the RPA results focused specifically on T1D-related risk classification.

RESEARCH DESIGN AND METHODS

Subjects

The TNPTP is one of the largest ongoing prospective studies with the objective to refine information on the pathogenesis and natural history of T1D and to facilitate the assessment and recruitment of individuals who might qualify for T1D prevention trials. In the TNPTP study, relatives of individuals with T1D are screened for the presence of pancreatic islet autoantibodies (GAD65 autoantibody [GAD65Ab], islet antigen 2 antibody [IA-2A], and microinsulin autoantibody [mIAA]). Those individuals positive for at least one autoantibody are then followed longitudinally for the development of additional islet autoantibodies (including ICA and zinc transporter 8 autoantibody [ZnT8Ab]), dysglycemia, and T1D. The details on the screening and follow-up schemes were described in a previous publication (10). Between 2001 and January 2015, a total of 144,295 eligible relatives were screened for the presence of pancreatic islet autoantibodies. From these screened subjects, we derived three distinct cohorts for this analysis. The three cohorts define subjects at different preclinical stages. Cohort 1 focuses on subjects with only one islet autoantibody, cohort 2 focuses on the subjects with two or more islet autoantibodies, and cohort 3 focuses on the subjects with dysglycemia. Table 1 shows the detailed criteria for the eligibility for inclusion in the three cohorts. All subjects (and/or their parents) signed a written consent form approved by the human subjects committee at the participating study site.

Laboratory Measures

HLA Typing

HLA genotyping was performed at eight loci to four-digit resolutions by the Type 1 Diabetes Genetics Consortium laboratories. HLA-DQA1 and DQB1 alleles were amplified by PCR with the use of sequence-specific probes (11). In this study, a high-risk HLA genotype was defined as having DR3 or DR4 present. DR3 is the combination of 0301/0501/0201 (DRB1/DQA1/DQB1), and DR4 is the combination of 04xx/0301/0201, 04xx/0301/0301, 04xx/0301/0302, or 04xx/0301/0304.

Autoantibody Assay

Cytoplasmic ICA positivity was determined on frozen sections of human pancreas by indirect immunofluorescence at the University of Florida (Gainesville, FL). Samples were considered positive at 10 JDFU.

GAD65Abs, IA-2As, mIAAs, and ZnT8Abs were measured by radioimmunoassay in the TrialNet Core laboratory at the Barbara Davis Center for Childhood Diabetes (BDC) (Denver, CO). Prior to June 2010, GAD65Abs and IA-2As were tested in a combined assay using 3H-leucine–labeled GAD65 and ^{35}S-methionine–labeled ICA512, with results expressed as a local BDC index. Since June 2010, the laboratory has performed the harmonized GAD65 (GAD65H) and harmonized IA-2A (IA-2AH) assays for the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Consortia. The harmonized results are expressed in NIDDK units per milliliter that were derived from standard curves made up of dilutions of common positive and negative NIDDK working calibrators (12). The BDC local and harmonized assays correlate well. In 2,170 TrialNet natural history study samples, positive/negative status by different assays was 96% concordant for GAD65Ab and 95% concordant for IA-2A, based on unpublished data. Autoantibody positivity was defined using threshold indexes per units of GAD65 $\geq$0.032, GAD65H $\geq$20 NIDDK units/mL, ICA512 $\geq$0.049, IA-2AH $\geq$5 NIDDK units/mL, mIAA $\geq$0.01, and ZnT8Ab $\geq$0.02.

Glucose Tolerance Test

An OGTT was administered to assess glycemic status. The dose of oral glucose was 1.75 g/kg (maximum 75 g of carbohydrate). Blood samples were obtained for C-peptide and glucose measurements in the fasting state and then 30, 60, 90, and 120 min later. Peak C-peptide level was the maximum point of all C-peptide measurements. The area under the curve for C-peptide was calculated using the trapezoid rule.

Dysglycemia is defined based on abnormal oral glucose tolerance: fasting plasma glucose levels $\geq$110 mg/dL (6.1 mmol/L) and $<$126 mg/dL (7 mmol/L); or 2-h plasma glucose levels $\geq$140 mg/dL (7.8 mmol/L) and $<$200 mg/dL (11.1 mmol/L); or 30, 60, and 90 min plasma glucose levels during OGTT of $\geq$140 mg/dL (7.8 mmol/L) and $<$200 mg/dL (11.1 mmol/L).

Diagnosis of Diabetes

Diabetes was diagnosed according to the following American Diabetes Association criteria: *1*) presence of unequivocal hyperglycemia including acute metabolic decompensation (diabetic ketoacidosis) and *2*) fasting plasma glucose

Table 1—Subject inclusion/exclusion criteria for cohorts

Cohort 1: single autoantibody	Cohort 2: multiple autoantibodies	Cohort 3: dysglycemia
1) Single-autoantibody positivity of mIAA, GAD65Ab, or IA-2A at initial screening and confirmed positivity of the same type of autoantibody on another occasion	*1*) Two or more positive autoantibodies of mIAA, GAD65Ab, IA-2A, ICA, or ZnT8Ab at any screening or follow-up visit	*1*) One or more positive autoantibodies of mIAA, GAD65Ab, IA-2A, ICA, or ZnT8Ab at the time of abnormal OGTT results
2) Normal baseline OGTT result	*2*) Normal baseline OGTT result	*2*) At least one abnormal OGTT result

level of ≥126 mg/dL (7 mmol/L); 2-h plasma glucose level during an OGTT of ≥200 mg/dL (11.1 mmol/L); or random plasma glucose level of ≥200 mg/dL (11.1 mmol/L) accompanied by symptoms of polyuria, polydipsia, and/or weight loss. The criteria in criterion 2 must be met on two consecutive tests (13).

Statistical Methods

The outcome was the development of persistent multiple antibodies defined as detection on two occasions of at least two of the five islet autoantibodies (GAD65Ab, mIAA, IA-2A, ZnT8Ab, and ICA) in cohort 1, the development of dysglycemia (at least one abnormal OGTT result) in cohort 2, and the development of T1D in cohort 3. An RPA technique was used to establish prognostic groups (14–16). This technique is a nonparametric methodology that creates a decision tree with respect to prognostic factors and their interactions, which are most important in determining the outcome of interests. Kaplan-Meier statistics were used by RPA to estimate the time to the event. A group of subjects (a node) would split into child nodes if the log-rank statistic was significant for any variable beyond the 0.05 probability level. The significance level was adjusted for the number of multiple comparisons by the Bonferroni method (17). Each splitting resulted in the definition of two more homogeneous subgroups; that is, subjects in the same subgroup have a similar level of risk for the outcome. The variables considered as prognostic factors for the RPA model included the following: age, BMI *z* score, metabolic indicators (fasting glucose, 2-h glucose, area under the curve for C-peptide, peak C-peptide from a 2-h OGTT, and HbA_{1c}), and autoantibody titers (ICA, mIAA, ICA512, IA-2AH, GAD65, GAD65H, and ZnT8Ab). These were entered as continuous variables. The titer values were standardized in the model to ensure a common scale (18). A standardized deviation score indicates its difference from the mean of the original autoantibody titers in number of SDs (of the original titers) derived from subjects in each cohort. That is, the standardized titers from either harmonized or nonharmonized assays are rescaled to have a mean of 0 and an SD of 1. As the subjects may not have both harmonized and nonharmonized assays for GAD65Ab and IA-2A, the RPA model would adapt to the missing titer value through the use of a surrogate measure (the standardized deviation score from another assay). Race (nonwhite vs. white), sex (male vs. female), relationship to the family member with T1D (first degree vs. second degree), and HLA genotype (non–high risk vs. high risk) were entered as dichotomized variables in the models. In the cohort 1 analysis, the type of positive autoantibody was also included in the RPA model. In the cohort 2 and cohort 3 analyses, the number of positive autoantibodies was also included in the model. Terminal node groups were tested by the log-rank test to determine whether any two nodes were similar enough in survival to be merged. The final classification was made by amalgamating terminal node subsets with a similar survival profile into distinct classes.

All *P* values were two-sided. SAS version 9.2 (SAS Institute, Cary, NC) was used to assess the baseline characteristics and survival analysis. Recursive partitioning was implemented in R-project using the Party package developed by the R-Project (19).

RESULTS

A total of 1,073 TNPTP subjects are included in cohort 1, 1,826 subjects are included in cohort 2, and 1,444 subjects are included in cohort 3. The baseline demographics and clinical characteristics of three study cohorts are summarized in Table 2.

Development of Multiple Positive Autoantibodies in Cohort 1

Of 1,073 subjects in cohort 1, multiple positive autoantibodies in mIAA, GAD65Ab, IA-2A, ICA, or ZnT8Ab persistently developed in 147 subjects. The median follow-up time in this cohort is 2 years (interquartile range [IQR] 0.83–3.68). A recursive decision tree was used to select the prognostic factors that are associated with progression from initial confirmed detection of a single-islet autoantibody to confirmed detection of at least one additional autoantibody. A total of five nodes was produced, resulting in three terminal nodes (Supplementary Fig. 1*A*). The three risk groups were defined by the following two significant variables: GAD65Ab titer and age at the initial detection of a single autoantibody (Table 3*A*). The 5-year risks of the development of multiple autoantibodies were 11%, 29%, and 45% for three risk groups defined by RPA. Compared with the low-risk group, the hazard ratio was 2.71 (95% CI 1.62–4.53) for the intermediate-risk group and 4.68 (2.98–7.36) for the high-risk group ($P < 0.001$). The time from the initial detection of a single autoantibody to confirmed detection of at least one additional antibody by these risk groups is depicted in Supplementary Fig. 1*B*.

Since TrialNet used two different assays for GAD65Ab and IA-2A, we repeated the analysis using antibody positivity in lieu of titers (Supplementary Fig. 4). Three risk groups emerged with very similar 5-year risks.

Development of Dysglycemia in Cohort 2

With a median follow-up time of 1.6 years, dysglycemia developed in 426 subjects with multiple positive autoantibodies with at least one abnormal OGTT result. The overall 5-year cumulative risk is ~40% in this cohort.

The recursive partitioning decision tree is composed with four terminal nodes (Supplementary Fig. 2*A*). Four risk groups were defined by the following three dominant variables: 2-h glucose level at a threshold of 110 mg/dL, fasting C-peptide level at a threshold of 1.24 ng/mL, and ICA512 titer at a threshold of 0.025.

Table 2—Demographic and clinical characteristics of cohorts at baseline

	Cohort 1: single autoantibody (*N* = 1,073)		Cohort 2: multiple autoantibodies (*N* = 1,826)		Cohort 3: dysglycemia (*N* = 1,444)	
Characteristics at baseline	*n*	Summary data	*n*	Summary data	*n*	Summary data
Age, median (IQR), years	1,070	17.00 (9.00–36.00)	1,823	11.00 (6.00–16.00)	1,442	13.00 (9.00–33.00)
BMI, median (IQR), kg/m^2	973	22.39 (17.76–26.76)	1,630	18.55 (16.13–23.30)	1,282	21.39 (16.96–27.25)
Race						
White	883	82.29%	1,606	87.95%	1,265	87.60%
African American	23	2.14%	53	2.90%	33	2.29%
Other	164	15.29%	166	9.09%	145	10.04%
Unknown	3	0.28%	1	0.05%	1	0.07%
Sex						
Male	421	39.53%	913	50.00%	682	47.23%
Female	644	60.47%	904	49.51%	757	52.42%
Unknown	8	0.75%	9	0.49%	5	0.35%
Relationship to patients with T1D						
Sibling	403	37.56%	1,062	58.16%	751	52.01%
Offspring	183	17.05%	372	20.37%	251	17.38%
Parent	357	33.27%	226	12.38%	325	22.51%
Second-degree relative	118	11.00%	139	7.61%	92	6.37%
Unknown	12	1.12%	27	1.48%	25	1.73%
HLA genotype: DR risk group						
DR3/DR3	63	5.87%	75	4.11%	75	5.19%
DR3/DR4	123	11.46%	333	18.24%	288	19.94%
DR3/X	282	26.28%	283	15.50%	271	18.77%
DR4/DR4	53	4.94%	146	8.00%	117	8.10%
DR4/X	314	29.26%	543	29.47%	461	31.93%
Other	194	18.08%	176	9.64%	141	9.76%
Unknown	44	4.10%	270	14.79%	91	6.30%
Immunological factors						
ICA titer , median (IQR), JDRF units	1,056	0.00 (0.00–0.00)	1,800	20.00 (0.00–160.00)	1,418	0.00 (5.00–160.00)
mIAA titer, median (IQR)	1,073	0.002 (0.000–0.008)	1,824	0.007 (0.002–0.025)	1,444	0.004 (0.001–0.017)
GAD65 titer, median (IQR)	715	0.048 (0.001–0.160)	1,376	0.162 (0.049–0.522)	991	0.106 (0.024–0.414)
GAD65H titer, median (IQR), NIDDK units/mL	478	62.00 (24.00–187.00)	953	284.00 (76.00–623.00)	794	162.00 (41.00–533.00)
ICA512 titer, median (IQR)	715	0.033 (0.006–0.677)	1,376	0.019 (0.001–0.568)	991	0.023 (0.001–0.685)
IA-2AH titer, median (IQR), NIDDK units/mL	478	0.001 (−0.004 to 0.010)	953	0.000 (0.000–129.000)	794	1.500 (0.000–217.000)
ZnT8Ab titer, median (IQR)	609	0.001 (−0.002 to 0.003)	1,043	0.021 (0.001–0.209)	585	0.011 (0.001–0.243)
ICA positive, *n* (%)	0	0.00%	975	53.40%	635	43.98%
mIAA positive, *n* (%)	258	24.04%	850	46.55%	487	33.73%
GAD65Ab positive, *n* (%)	757	70.55%	1,621	88.77%	1,173	81.23%
IA-2A positive, *n* (%)	57	5.31%	840	46.00%	650	45.01%
ZnT8Ab positive, *n* (%)	0	0.00%	523	28.64%	267	18.49%

Continued on p. 1040

Table 2—Continued

Characteristics at baseline	Cohort 1: single autoantibody (*N* = 1,073)		Cohort 2: multiple autoantibodies (*N* = 1,826)		Cohort 3: dysglycemia (*N* = 1,444)	
	n	Summary data	*n*	Summary data	*n*	Summary data
Number of positive autoantibodies, %						
1	1,073	100.00	0	0.00	534	36.98
2	0	0.00	1,008	55.2	344	23.82
3	0	0.00	421	23.06	282	19.53
4	0	0.00	278	15.22	219	15.17
5	0	0.00	119	6.52	65	4.50
Metabolic factors, mean (SD)						
Fasting glucose, mg/dL	1,073	89.41 (7.77)	1,826	88.43 (8.17)	1,444	92.76 (10.94)
2-h glucose, mg/dL	1,073	104.71 (19.07)	1,826	105.98 (18.85)	1,444	150.73 (24.27)
Fasting C-peptide, ng/mL	1,071	1.79 (0.90)	1,820	1.52 (0.77)	1,442	1.85 (1.06)
Peak C-peptide, ng/mL	1,071	8.48 (3.56)	1,820	7.18 (3.22)	1,442	8.92 (4.20)
HbA_{1c}, %	1,068	5.04 (0.31)	1,814	5.05 (0.31)	1,362	5.19 (0.35)

Cohort 1, baseline is the time of the initial detection of single-autoantibody positivity; cohort 2, baseline is the time of the initial detection of multiple positive autoantibodies; cohort 3, baseline is the time of initial dysglycemia; GAD65Ab positivity was based on either GAD65 titer or GAD65H titer. IA-2A positivity was based on either ICA512 titer or IA-2AH titer. The 5-year risk was the cumulative risk from a Kaplan-Meier estimate.

Pairwise comparison between any two risk groups revealed that the risk of the group in node 4 is similar to the risk of the group in node 7 ($P = 0.37$). As a consequence, further risk classification combined these two groups into a single, intermediate class (Table 3*B*). The cumulative 5-year risks were 28%, 41%, and 51%, respectively, for three risk classes. Compared with the low-risk class, the hazard ratio was 1.78 (95% CI 1.37–2.32) for the intermediate-risk class and 2.68 (2.07–3.48) for the high-risk class (Table 3). The time from initial detection of multiple positive autoantibodies to dysglycemia by three risk classes is shown in Supplementary Fig. 2*B*.

Changing from antibody titers to positivity did not affect the three risk groups except that mIAA positivity replaced fasting C-peptide in distinguishing between intermediate and high risk (Supplementary Fig. 5).

Risk of Onset of T1D (Cohort 3)

T1D developed in a total of 414 subjects with dysglycemia during further follow-up. The median follow-up time between dysglycemia and T1D was 1.8 years (IQR 0.8–3.6). The overall 5-year risk was 42%. Five groups are defined by five RPA terminal nodes (Supplementary Fig. 3*A*). The risk in node 3 did not differ from the group in node 9, who had more than one positive autoantibody and peak C-peptide greater than 8.35 ng/mL ($P = 0.81$). The subjects in node 3 and node 9 thus could be considered as having similar risk and were combined into an intermediate-risk class. Consequently, RPA defined four final risk classes. The log-rank test confirmed the significant risk differences among four RPA classes ($P < 0.001$) (Supplementary Fig. 3*B*). Four risk classes had 5-year risks of 9%, 33%, 62%, and 80%, respectively (Table 3*C*). Compared with the low-risk class, the hazard ratio was 4.85 (95% CI 2.95–7.95) for the intermediate-risk class, 9.05 (5.45–15.03) for the high-risk class, and 21.84 (13.40–35.59) for the very high-risk class (Table 3).

Changing from antibody titers to positivity did not affect the four risk groups except that IA-2A positivity further distinguished between low and high risk (Supplementary Fig. 6).

Table 3—Risk classifications defined by RPA for cohorts 1, 2, and 3

Cohort	Class	Classification risk factors (thresholds)	5-year risk	Hazard ratio (95% CI)
A: Cohort 1: single autoantibody: Outcome, progression to persistent multiple positive autoantibodies				
RPA classification	Low risk	Age >16 years and GAD65Ab titer (GAD65) ≤0.126		
	Intermediate risk	Age ≤16 years and GAD65Ab titer (GAD65) ≤0.126		
	High risk	GAD65Ab titer (GAD65) >0.126		
5-year risk*	Low risk		11%	Reference
	Intermediate risk		29%	2.71 (1.62–4.53)
	High risk		45%	4.68 (2.98–7.36)
B: Cohort 2: multiple autoantibodies: Outcome, progression to dysglycemia				
RPA classification	Low risk	2-h glucose ≤110 mg/dL and IA-2A titer (ICA512) ≤0.025		
	Intermediate risk	2-h glucose ≤110 mg/dL and IA-2A titer (ICA512) >0.025; or 2-h glucose >110 mg/dL and fasting C-peptide ≤1.235 ng/mL		
	High risk	2-h glucose >110 mg/dL and fasting C-peptide >1.235 ng/mL		
5-year risk*	Low risk		28%	Reference
	Intermediate risk		39%	1.78 (1.37–2.32)
	High risk		51%	2.68 (2.07–3.48)
C: Cohort 3: dysglycemia: Outcome, progression to T1D				
RPA classification	Low risk	Single autoantibody at dysglycemia and age >16 years at dysglycemia		
	Intermediate risk	Single autoantibody at dysglycemia and age ≤16 years at dysglycemia or multiple autoantibodies and peak C-peptide >8.35 ng/mL at dysglycemia		
	High risk	Multiple autoantibodies and peak C-peptide ≤8.35 ng/mL and HbA_{1c} ≤5.1 at dysglycemia		
	Very high risk	Multiple autoantibodies and peak C-peptide ≤8.35 ng/mL and HbA_{1c} >5.1 at dysglycemia		
5-year risk*	Low risk		9%	Reference
	Intermediate risk		33%	4.85 (2.95–7.95)
	High risk		62%	9.05 (5.45–15.03)
	Very high risk		80%	21.84 (13.40–35.59)

*Cumulative risk from Kaplan-Meier estimate.

CONCLUSIONS

In many prognostic factor studies, multivariate analyses using the Cox proportional hazards model are applied to identify independent prognostic factors. However, the coefficient estimates derived from the Cox proportional hazards model may be biased as a result of violating assumptions of independence. In the previous studies (20,21) that examined the relationship between metabolic and immunologic risk factors among at-risk subjects, the results showed the significant correlations among some of the risk factors. As well, in previous studies (22–25) some autoantibodies have been shown to be age related. Moreover, the cutoff values for defining risk classes by summing the Cox regression coefficients for a given set of prognostic factors for each individual subject are often arbitrary. RPA classification is a useful tool that could prioritize the prognostic factors and divide the subjects into distinctive groups. RPA has an advantage over the proportional hazards model in identifying prognostic factors because it does not require risk factor independence and, as a nonparametric technique, makes no requirement on the underlying distributions of the variables considered. Hence, it relies on fewer modeling assumptions. Also, because the method is designed to divide subjects into groups based on the length of survival, it defines groupings for risk classification, whereas Cox regression models do not. Moreover, there is no need to explicitly include covariate interactions because of the recursive splitting structure of tree model construction.

This is the first study that characterizes the risk factors associated with the transition from one preclinical stage to the next following a recommended staging classification system (9). The tree-structured prediction model reveals that the risk parameters are not the same across each transition. Baseline age and GAD65Ab titers dominated the influence on the transition from single to multiple autoantibodies. Two-hour glucose levels, IA-2A titers, and fasting C-peptide levels are the major risk factors that influence progression to dysglycemia among the subjects in the multiple–positive autoantibodies cohort, and progression from dysglycemia to the onset of T1D was determined by the number of positive autoantibodies, peak C-peptide level, HbA_{1c}, or age at dysglycemia.

Based on the RPA classification, the subjects at younger age and with higher GAD65Ab titer are at higher risk for progression to multiple positive autoantibodies from a single autoantibody (seroconversion). Approximately 70% of subjects with a single autoantibody were positive for GAD65Ab, much higher than for insulin autoantibody (24%) and IA-2A (5%). Our study results are consistent with those of others (22–24) in that seroconversion is age related. Previous studies in infants and children at an early age have shown that progression from single to two or more autoantibodies occurs more commonly in children $<$5 years of age. In our study, the majority of subjects were older, with a median age of 24 years, and 75% of them were between 15 and 41 years old at the time of initial detection of a single positive autoantibody. This indicates that progression is not limited to early childhood (25). The subjects $\leq$16 years of age had almost triple the 5-year risk compared with subjects $>$16 years of age at the same GAD65Ab titer level. Hence, not all individuals with a single islet autoantibody can be thought of as being at low risk for disease progression.

Interestingly, age was not significantly related to the development of dysglycemia, but it was related to the development of both multiple autoantibodies and T1D onset. Furthermore, higher fasting C-peptide levels ($>$1.24 ng/mL) were associated with an increased risk of dysglycemia among those with a higher 2-h glucose level ($>$110 mg/dL). Higher basal C-peptide level has been shown (26–28) to be associated with an increased risk of the development of T1D in previous studies. One reason could be that the subjects with a higher basal C-peptide level require a higher postprandial C-peptide level. Our data have shown that the subjects with a higher basal C-peptide level had a lower ratio of peak C-peptide to fasting C-peptide than those subjects with a lower basal C-peptide level. When the postprandial C-peptide level could not be met, the glucose intolerance thus occurs. The subjects with higher basal C-peptide levels were also observed to have higher BMI values, which may have led to the increased insulin resistance and further jeopardizes glucose tolerance. A previous longitudinal study (6,29,30) has shown that IA-2A titers increase during the years preceding the diagnosis of T1D. Our study confirms this, particularly in relation to progression to dysglycemia from multiple autoantibody positivity.

Our results support the evidence that autoantibody titers are strong predictors at each transition leading to T1D development. The risk of the development of multiple autoantibodies was significantly increased when the GAD65Ab titer level was elevated, and the risk of the development of dysglycemia was increased when the IA-2A titer level increased. These indicate that better risk prediction on the timing of transitions can be obtained by evaluating autoantibody titers. The results also suggest that an autoantibody titer should be carefully considered in planning prevention trials for T1D in addition to the number of positive autoantibodies and the type of autoantibody. The thresholds derived by RPA are directly related to the outcome of interests in each cohort. Therefore, the prevention trials that are targeted for the specific cohort can gain efficiency.

The specific association of GAD65Ab and IA-2A with different risk cohorts may also indicate that they may be related to different stages of the pathogenic processes occurring before disease onset.

By the time of dysglycemia, the manifestation of multiple autoantibody positivity increased the risk of the development of T1D. As prediabetes progresses, metabolic risk factors seem to have more and more influence on the disease progression. The combination of lower peak C-peptide levels and elevated HbA_{1c} levels constituted a very high-risk subgroup, demonstrating an 80% 5-year T1D risk. Age was a significant risk factor to distinguish between low-risk and intermediate-risk groups. It may suggest that age had a more important prediction role only before metabolic deterioration occurred.

These RPA risk groups serve an important purpose in being able to recognize and convey to individuals a level of risk associated with the progression of disease. They may also serve to identify groups for

intervention strategies designed to halt disease progression and, using the risk factors, suggest targets for that intervention.

Individuals are grouped by risk level, but the group may be heterogeneous in terms of immunogenic factors and other characteristics, so it should be noted that individuals within a risk group may respond differently to any particular intervention. The study population is a mixture of children and adults, and thus the findings may not be representative of young children. Furthermore, all subjects in these study cohorts were relatives of individuals with T1D, and thus the research findings here may not be generalizable to the general population. Future external validation should be performed for the purpose of replicating these studies in similar and/or other settings. In the future studies, it is recommended to integrate extragenetic factors (such as full HLA typing and gene expression data) in the model for prospective, more optimal prediction and classification.

This is the first study that identifies the risk factors associated with the timing of transitions from one preclinical stage to the next in the development of T1D. Based on RPA risk parameters, we identify the characteristics of groups with similar 5-year risks for advancing to the next preclinical stage. It is clear that individuals with one or more autoantibodies or with dysglycemia are not homogeneous with regard to the risk of disease progression. Also, there are differences in risk factors at each stage that are associated with increased risk of progression. The potential benefit of identifying these groups allows for a more informed discussion of diabetes risk and the selective enrollment of individuals into clinical trials whose risk more appropriately matches the potential benefit of an experimental intervention. Since the risk levels in these groups are substantial, their definition makes possible the design of more efficient trials with target sample sizes that are feasible, opening up the field of prevention to additional at-risk cohorts.

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