



Excess BMI Accelerates Islet Autoimmunity in Older Children and Adolescents

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OBJECTIVE

Sustained excess BMI increases the risk of type 1 diabetes (T1D) in autoantibody-positive relatives without diabetes of patients. We tested whether elevated BMI also accelerates the progression of islet autoimmunity before T1D diagnosis.

RESEARCH DESIGN AND METHODS

We studied 706 single autoantibody-positive pediatric TrialNet participants (ages 1.6–18.6 years at baseline). Cumulative excess BMI (ceBMI) was calculated for each participant based on longitudinally accumulated BMI \geq 85th age- and sex-adjusted percentile. Recursive partitioning analysis and multivariable modeling defined the age cut point differentiating the risk for progression to multiple positive autoantibodies.

RESULTS

At baseline, 175 children (25%) had a BMI \geq 85th percentile. ceBMI range was -9.2 to 15.6 kg/m² (median -1.91), with ceBMI ≥ 0 kg/m² corresponding to persistently elevated BMI \geq 85th percentile. Younger age increased the progression to multiple autoantibodies, with age cutoff of 9 years defined by recursive partitioning analysis. Although ceBMI was not significantly associated with progression from single to multiple autoantibodies overall, there was an interaction with ceBMI ≥ 0 kg/m², age, and HLA ($P = 0.009$). Among children ≥ 9 years old without HLA DR3-DQ2 and DR4-DQ8, ceBMI ≥ 0 kg/m² increased the rate of progression from single to multiple positive autoantibodies (hazard ratio 7.32, $P = 0.004$) and conferred a risk similar to that in those with T1D-associated HLA haplotypes. In participants < 9 years old, the effect of ceBMI on progression to multiple autoantibodies was not significant regardless of HLA type.

CONCLUSIONS

These data support that elevated BMI may exacerbate islet autoimmunity prior to clinical T1D, particularly in children with lower risk based on age and HLA. Interventions to maintain normal BMI may prevent or delay the progression of islet autoimmunity.

The global rise in incidence of type 1 diabetes (T1D) has intensified efforts to identify modifiable risk factors in order to prevent or delay onset of clinical diabetes (1). Although there are several genetic loci for T1D susceptibility, heritability does not completely predict appearance of islet autoantibodies or disease development (2), highlighting the role of other influences such as the environment (3).

The parallel rise in obesity (4,5) and T1D incidence suggests a potential link between elevated body weight and T1D progression (6–9). The accelerator hypothesis proposes that obesity-induced insulin resistance triggers the autoimmune-mediated β -cell

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destruction that characterizes T1D (10). Additionally, obesity-induced insulin resistance may also accelerate clinical onset of T1D by increasing insulin needs in those with already compromised insulin secretory capacity, as was previously proposed by Libman et al. (11,12). The threshold hypothesis proposes that diabetes develops when the combination of genetic and environmental diabetogenic factors exceeds a threshold (13). We have recently demonstrated that longitudinal, sustained elevation in BMI, measured as cumulative excess BMI (ceBMI), increases the progression to T1D in adult (14) and pediatric (15) islet autoantibody-positive participants of the TrialNet Pathway to Prevention (PTP) cohort. These data suggest that lifestyle modifications may slow the progression to T1D. However, the mechanism is unclear.

While increased insulin resistance may lead to hyperglycemia and diagnosis of stage 3 clinical diabetes, we hypothesized that ceBMI may exacerbate the autoimmune process in preclinical stages of T1D. In order to understand the mechanism by which ceBMI increases the risk of T1D, we herein explored its influence on conversion from single to multiple autoantibody positivity in pediatric participants of the TrialNet PTP cohort. These data may reveal a therapeutically modifiable risk factor, with both genetic and environmental influences, and potential for intervention prior to the development of T1D.

RESEARCH DESIGN AND METHODS

Subjects

The TrialNet PTP cohort was established in 2001 and has previously been described (16). Briefly, first-degree relatives (ages 1–45 years) and second- or third-degree relatives (ages 1–20 years) without diabetes of individuals with T1D were enrolled and screened for presence of pancreatic islet autoantibodies. Islet autoantibody status was assessed according to the Diabetes Antibody Standardization Program. Participants were tested first for the presence of GAD65 (17), insulin (18), or islet-antigen 2 (IA-2/islet cell autoantibody [ICA]512) autoantibodies (19), and if positive, they were tested for ICA (20). Measurement of zinc transporter 8 (ZnT8) autoantibodies (21) was initiated with samples stored since 2004 and was consistently measured in the PTP cohort starting in 2012. Confirmed autoantibody-positive individuals

were observed longitudinally with either semiannual or annual monitoring, which included measurement of height and weight, and oral glucose tolerance testing (OGTT).

A total of 182,145 participants in the PTP study were screened from 2004 through August 2017, of whom 2,382 were determined to be single autoantibody positive and were monitored for progression to development of multiple autoantibody positivity. The current analysis focuses on the 1,224 participants who were <18 years old when identified as single autoantibody positive; 766 of these had two or more evaluations for BMI. For avoidance of confounding by weight loss that precedes T1D

the BMI value corresponding to the 85th percentile for age- and sex-adjusted BMI for each subject at the time of each study visit. ceBMI has been used previously as a measure of persistent elevation of BMI beyond the overweight threshold (24,25). The weighted sums of the differences between the actual BMI and BMI corresponding to the 85th percentile for the sex and age at that evaluation were calculated using the method described by Lee et al. (25) and Bouchard et al. (24). Briefly, a ceBMI score was calculated by summing the difference calculated at each BMI assessment while accounting for the irregular timing between evaluations (Eq. 1):

$$ceBMI_{lyrsj} = \sum_{i=0}^m \frac{(BMI_{t_i} - 85^{th} \text{ percentile BMI}_{t_i}) + (BMI_{t_{i+1}} - 85^{th} \text{ percentile BMI}_{t_{i+1}})}{2} \times \left(\frac{\text{number of days between } t_i \text{ and } t_{i+1}}{365.25} \right) \tag{1}$$

diagnosis, further exclusions included BMI data collected within 6 months of diabetes diagnosis as well as any subjects who were determined to be autoantibody positive >2 years prior to their first BMI evaluation. To facilitate consistent and valid calculations of BMI percentiles, and use of Centers for Disease Control and Prevention (CDC) criteria to define overweight and obesity, we restricted our analysis cohort to only include participants with at least two BMI measurements meeting the above criteria before 20 years of age. Our resulting cohort consisted of a total of 706 subjects (Supplementary Fig. 1).

Laboratory and Anthropometric Measurements

At each study visit, a standard protocol OGTT was performed and HbA_{1c} was obtained. Glucose was measured using the glucose oxidase method (22). Diabetes was diagnosed according to American Diabetes Association criteria (fasting glucose ≥126 mg/dL, random glucose ≥200 mg/dL, 2-h OGTT ≥200 mg/dL) (23), which must have been met on two occasions. An HbA_{1c} ≥6.5% could be used as part of confirmatory testing (16).

BMI was calculated as weight in kilograms divided by the square of height in meters. The CDC 2000 growth charts (www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas.htm) were used to obtain

where *ceBMI_{lyrsj}* is ceBMI-years for subject *j* in units kg/m²*years and *m* is the number of BMI evaluations for subject *j*. *t_i* is the time *i* (i.e., some future time point after baseline), and *t_i+1* is the time *i*+1, which is the next time point after *t_i*. We further annualized *ceBMI_{lyrsj}* to accommodate the irregular timing of BMI assessment in relation to the time at multiple autoantibody positivity, or censoring (Eq. 2):

$$ceBMI_j = \frac{ceBMI_j}{\frac{t_m - t_0}{365.25}} \tag{2}$$

where *ceBMI_j* is a value representing the annual average ceBMI in kg/m² for subject *j* over the number of years subject *j* had *m* BMI evaluations, *t_m* is time in days at the last BMI measurement, and *t₀* is the time of first BMI evaluation.

Statistical Considerations

Categorical variables were compared among groups by Pearson χ² tests or Fisher exact tests when cell sizes were insufficient. The majority of continuous variables summarized had skewed distributions and were compared between groups using nonparametric tests (Wilcoxon rank sum tests or Kruskal-Wallis tests, depending on the number of groups). Nonparametric Spearman rank correlation tests were used to assess correlation between continuous measures at baseline.

Analyses of BMI were based on age- and sex-adjusted BMI percentiles. As such, baseline underweight status was defined as <5th percentile, normal weight between the 5th and 85th percentile, overweight \geq 85th percentile, and obesity \geq 95th percentile. ceBMI was analyzed both as a continuous measure and a dichotomized measure. ceBMI \geq 0 kg/m² indicated an individual's BMI on average \geq 85th percentile for their sex and age during the observation period.

The main clinical outcome for analysis was time to conversion from single to multiple autoantibody positivity, defined as the time from the first BMI evaluation with single autoantibody positivity to the date of appearance of multiple positive autoantibodies. Those who did not convert from single to multiple autoantibody positivity were censored at their last date of follow-up. Kaplan-Meier methods were used to assess differences in the time to conversion to multiple autoantibody positivity between groups of interest, and Cox proportional hazards models were used to assess the influence and significance of continuous and categorical variables. Assumptions for proportionality of hazards were tested for in these models. Given the known existence of risk factors and their potentially confounding effects, all time-to-event analyses were adjusted for age and sex as well as other factors as appropriate. To assess possible cut points for age at autoantibody positivity presentation in terms of their influence and stratification of risk on time to the appearance of multiple autoantibodies, we utilized recursive partitioning analyses (26) (rpart package in R) as previously described (14,15). A model-based and iterative approach, specifically, recursive partitioning analyses, was used to identify the "optimal" cut point of the marker that best discriminated the outcome of interest, i.e., time to progression to multiple positive autoantibodies.

Overall, inferential tests were two sided, with *P* values <0.05 considered to be statistically significant. For interaction terms, *P* values <0.1 were considered sufficient for further exploration and evaluation of relationships given the sample size and number of events. All analyses were conducted in the statistical program R (version 3.5.1 for Windows).

RESULTS

Demographics

A total of 706 single-positive-autoantibody pediatric subjects between the ages of

2 and 18 years at the first BMI evaluation from the TrialNet PTP study were included in these analyses (Table 1). Of these, 144 subjects (20%) progressed to multiple autoantibody positivity during the observation time. The median age at the first visit with BMI data available was 10.2 years (range 1.6–18.6), and the median BMI percentile at their first evaluation was 61.9% (interquartile range 84.6–35.1). At baseline, 12% of participants were overweight (BMI \geq 85th to <95th percentile) and 13% were obese (\geq 95th percentile). Supplementary Table 2 summarizes BMI percentile by subgroups.

There was a spectrum of ceBMI from -9.2 to 15.6 kg/m² (median -1.91 kg/m² [interquartile range -3.6 to 0.23]). In this pediatric cohort, 26.8% (189 of 706) had ceBMI values \geq 0 kg/m² representing sustained excess BMI above the CDC threshold defining elevated BMI (overweight or obesity). There were no significant differences in age at first visit between those who were persistently overweight or obese compared with those of normal ceBMI (median ages 9.4 and 9.9 years, respectively, *P* = 0.97). Similarly, no significant differences in distribution of males to females based on ceBMI \geq 0 kg/m² vs. <0 kg/m² (*P* = 0.78) and islet autoantibody type (*P* = 0.85) or differences in HLA haplotype distribution (i.e., having DR3-DQ2 and/or DR4-DQ8, DR3-DQ2, or DR4-DQ8; all *P* \geq 0.80). We did find a higher proportion of Hispanic/Latino individuals among those who had a ceBMI \geq 0 kg/m² compared with subjects with a ceBMI <0 kg/m² (26% vs. 13%, respectively, *P* = 0.0001).

Age and HLA Type Are Inherent Characteristics That Influence the Progression From Single to Multiple Autoantibody Positivity

Older age at first autoantibody presentation was a significant protective risk factor for the development of multiple autoantibody positivity. For every year of age, the associated rate of progression to multiple autoantibody positivity was reduced by 7% (hazard ratio [HR] 0.93, 95% CI: 0.89–0.97; *P* = 0.0007), after adjustment for ceBMI \geq 0 kg/m², sex, autoantibody type, and T1D-associated HLA haplotype. As expected, carrying at least one of the high-risk HLA haplotypes (i.e., DR3-DQ2 and DR4-DQ8) also had a significant impact on the progression from single to multiple positive autoantibodies.

Based on that same multivariable model, individuals with HLA DR3-DQ2 and/or DR4-DQ8 had a nearly twofold increased risk of developing multiple autoantibodies compared with those with neither DR3-DQ2 nor DR4-DQ8 (HR 1.86, 95% CI 1.23–2.82; *P* = 0.003), persistent after adjustments for sex, autoantibody type, and ceBMI \geq 0 kg/m².

ceBMI Influences Risk of Developing Multiple Islet Autoantibodies in an Age-Specific Manner

Using recursive partitioning cut point analyses, we identified the age cut point of \geq 9 years at autoantibody presentation as a threshold that best discriminated the rate of progression from single to multiple positive autoantibodies, with those \geq 9 years old having a reduced rate compared with those younger than 9 years of age even with adjustments for other factors listed above (HR 0.51, *P* < 0.0001). Within each of the age subgroups (i.e., \geq 9 and <9 years), age as a continuous variable was no longer a significant influence on the likelihood of progression to multiple positive autoantibodies. The presence of HLA DR3-DQ2 and/or DR4-DQ8 corresponded to a significantly increased rate of progression to multiple autoantibody positivity in individuals \geq 9 years of age (HR 2.27; *P* = 0.019) and, although not reaching statistical significance, it showed a similar directional impact in children <9 years of age at autoantibody presentation (HR 1.65; *P* = 0.059) even after adjustment for additional risk factors.

In the overall cohort, ceBMI was not independently associated with progression from single to multiple autoantibodies as a continuous measure (HR 1.03, 95% CI 0.99–1.07; *P* = 0.19) or dichotomized at the overweight threshold (ceBMI \geq 0 kg/m²) after adjustment for age, HLA type, autoantibody subtype, and sex (HR 1.18, 95% CI 0.82–1.68; *P* = 0.37). Race or ethnicity were not significant in the analysis with the overall cohort or in the age subgroups, and thus they were not included in the final models. We observed evidence of a significant interaction effect between ceBMI (\geq 0 kg/m² vs. not) and age (\geq 9 years vs. not) (*P* = 0.02); specifically, ceBMI increased the rate of progression to multiple positive autoantibodies for individuals \geq 9 years of age. For each 1 kg/m² increase in continuous ceBMI

Table 1—Demographics and baseline characteristics of PTP subjects

Characteristic	All subjects N = 706	ceBMI <0 kg/m ² N = 517	ceBMI ≥0 kg/m ² N = 189	P
Age at single Ab+ (years), median (range)	9.8 (1.2–17.9)	9.9 (1.2–17.9)	9.4 (1.6–17.6)	0.97
Sex				0.78
Male	347 (49.2)	252 (48.7)	95 (50.3)	
Female	359 (50.8)	265 (51.3)	94 (49.7)	
Ab type				0.85
GADA+	460 (65.1)	340 (65.8)	120 (63.5)	
IA-2A+	33 (4.7)	24 (4.6)	9 (4.8)	
IAA+	213 (30.2)	153 (29.6)	60 (31.7)	
Race				0.67
White	577 (81.7)	425 (82.2)	152 (80.4)	
Nonwhite/unknown	129 (18.3)	92 (17.8)	37 (19.6)	
Ethnicity				0.0001
Hispanic/Latino	118 (16.7)	69 (13.3)	49 (25.9)	
Non-Hispanic/Latino	564 (79.9)	430 (83.2)	134 (70.9)	
Unknown/missing	24 (3.4)	18 (3.5)	6 (3.2)	
HLA haplotype				
DR3 and/or DR4-DQ8				0.92
No	205 (29.1)	151 (29.3)	54 (28.6)	
Yes	499 (70.9)	364 (70.7)	135 (71.4)	
DR3-DQ2				0.99
No	408 (58.0)	298 (57.9)	110 (58.2)	
Yes	296 (42.0)	217 (42.1)	79 (41.8)	
DR4-DQ8				0.80
No	432 (61.4)	318 (61.7)	114 (60.3)	
Yes	272 (38.6)	197 (38.3)	75 (39.7)	
Missing	2	2	0	
Baseline BMI percentile				<0.0001
Underweight (<5%)	26 (3.7)	24 (4.6)	2 (1.0)	
Normal (≥5%, <85%)	505 (71.5)	468 (90.5)	37 (19.6)	
Overweight (≥85%, <95%)	84 (11.9)	20 (3.9)	64 (33.9)	
Obese (≥95%)	91 (12.9)	5 (1.0)	86 (45.5)	
Baseline ceBMI (kg/m ²)				<0.0001
Median	−1.92	−2.6	2.54	
Range	−9.2 to 15.7	−9.2 to 15.7	−5.7 to 14.2	
Baseline BMI category				<0.0001
Normal (<0 kg/m ²)	531 (75.2)	492 (95.2)	39 (20.6)	
Overweight (≥0 kg/m ²)	175 (24.8)	25 (4.8)	150 (79.4)	<0.0001

Data are n (%) unless otherwise indicated. Ab, autoantibody; GADA, GAD autoantibody; IA-2A, IA-2 autoantibody; IAA, insulin autoantibody.

measure, the risk increased by 7% (HR 1.07, 95% CI 1.01–1.13; $P = 0.02$). When we dichotomized at ceBMI ≥0 kg/m², we again found that participants who were 9 years old or older at single autoantibody presentation were at greater risk if they were persistently overweight or obese, with a near twofold increased rate of developing multiple positive autoantibodies compared with those who on average remained below the overweight threshold over time (HR 1.92, 95% CI 1.12–3.29; $P = 0.018$) (Fig. 1 and Supplementary Table 3). In children <9 years of age at autoantibody presentation, the influence of ceBMI was not significant either as continuous (HR 0.98, 95% CI 0.92–1.05; $P = 0.57$) or as dichotomized (HR 0.81, 95% CI 0.5–1.32; $P = 0.40$) measure (Fig. 1 and Supplementary Table 3). Of note,

although an age of 9 years was identified through the recursive partitioning analysis, the same patterns persisted with other age cut points in the peri-pubertal period (e.g., 8, 10, 12, 14 years). At each of these ages, the effect of ceBMI on the risk of progression was minimal in younger children but more pronounced in older youth.

ceBMI Is Important for Rate of Multiple Autoantibody Progression in Participants With Less Aggressive Inherent Risk Factors

We additionally found a significant three-way interaction between age, ceBMI, and HLA type ($P = 0.009$), leading to further stratification to determine the effect of ceBMI in each of the four subgroups based on HLA status (carrying DR3-DQ2 and/or

DR4-DQ8 vs. none) and age (≥9 vs. <9 years old). As shown in Table 2, having HLA DR3-DQ2 and/or DR4-DQ8 was significantly associated with an increased rate of progression to multiple autoantibody positivity in participants ≥9 years of age compared with the absence of DR3-DQ2 or DR4-DQ8 haplotypes. Importantly, in individuals ≥9 years old without either of the high-risk DR3-DQ2 or DR4-DQ8 HLA haplotypes, having ceBMI ≥0 kg/m² was a significant factor in differentiating risk of progression to multiple autoantibodies (HR 7.32, 95% CI 1.88–28.5; $P = 0.004$) (Table 2). In fact, ceBMI ≥0 kg/m² appeared to best differentiate the likelihood of progression in participants without the T1D-associated HLA haplotypes, and their risk of progression became similar to that of the

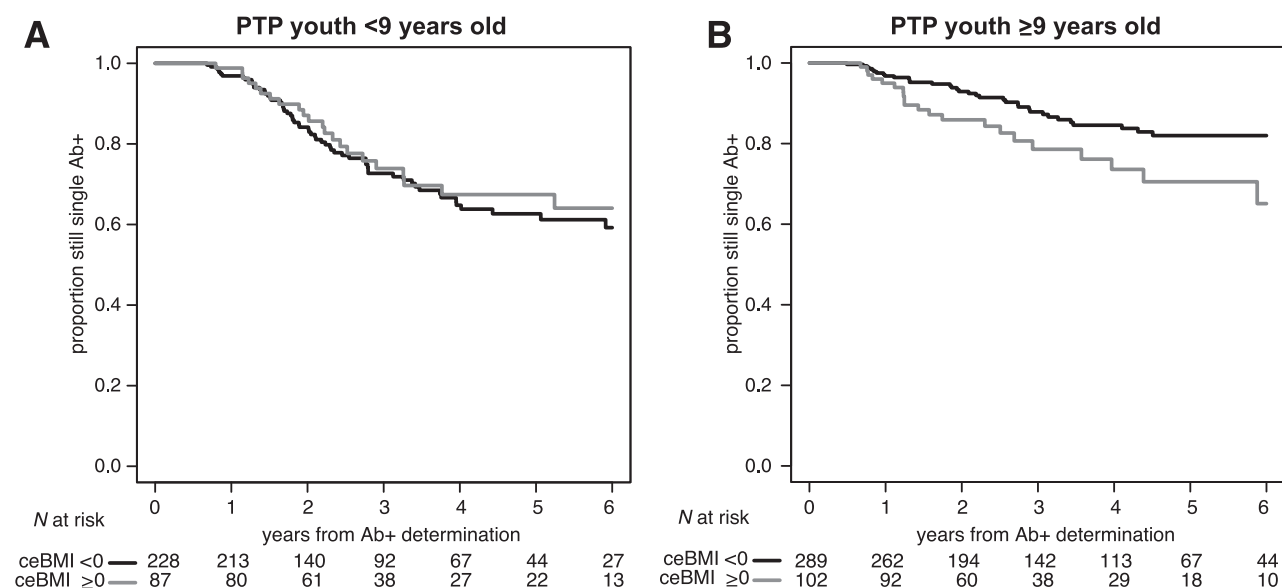


Figure 1—ceBMI increased progression to multiple autoantibody positivity in an age-specific manner. ceBMI above the age- and sex-adjusted 85th percentile (i.e., ceBMI ≥ 0 kg/m²) did not increase risk in subjects < 9 years of age (HR 0.81, 95% CI 0.5–1.31; $P = 0.39$) (A) but was influential in those ≥ 9 years of age at first autoantibody presentation, where ceBMI ≥ 0 kg/m² (i.e., being persistently obese or overweight) almost doubled the risk of progression from single to multiple autoantibody positivity (HR 1.93, 95% CI 1.13–3.31; $P = 0.017$) (B).

participants who did have T1D-associated HLA haplotypes (Fig. 2). In contrast, ceBMI ≥ 0 kg/m² was not a significant factor for risk of progression to multiple autoantibody positivity in those older children (≥ 9 years) with DR3-DQ2 and/or DR4-DQ8 (HR 1.38, 95% CI 0.32–2.59, $P = 0.31$). In children < 9 years of age, ceBMI did not influence the progression to multiple autoantibodies, regardless of HLA type (Table 2). Supplementary Table 3 illustrates additional comparisons.

CONCLUSIONS

T1D is now recognized as a spectrum of diseases with heterogeneous preclinical stages prior to the onset of symptomatic hyperglycemia. While T1D prevention trials focus on halting the development of clinical onset of T1D (stage 3), there

is also great interest in arresting the development of multiple autoantibody positivity, which marks entry into stage 1 of T1D. In the TrialNet PTP cohort, the presence of HLA DR3-DQ2 and/or DR4-DQ8 haplotypes and younger age are inherent, independent risk factors for progressing from single to multiple positive autoantibodies (27–29). We have demonstrated that ceBMI above the threshold for overweight/obese increases the risk for multiple autoantibody development in youth > 9 years old who do not have HLA DR3-DQ2 or DR4-DQ8, thus diminishing the protective influence of older age and absence of high-risk HLA haplotypes. Our findings that ceBMI had influence in some subgroups but not in others (causing lack of detectable effect on the overall cohort) highlight the

recognized heterogeneity of T1D and the importance of stratified analysis.

Investigations into the role of BMI in T1D pathophysiology have not been consistent. Many of these prior prospective studies limited analyses to one measurement of BMI (e.g., birth weight, weight at cohort enrollment) without considering the interval values (30–32). Our previous analysis of longitudinal BMI measurements demonstrated that sustained elevation in BMI increased the risk of T1D onset in both pediatric and adult subjects followed in the TrialNet PTP cohort (14,15). The mechanism of this influence may be related to insulin resistance in the setting of compromised β -cell function (11,12). This current study proposes that obesity-induced exacerbation of autoimmunity may be an additional mechanism by which longitudinal elevation in

Table 2—ceBMI increased risk of progression to multiple autoantibody positivity in older children with lower-risk HLA haplotype

Age	HLA haplotype	n	HR (95% CI)	P	ceBMI (kg/m ²)	n	HR (95% CI)	P
< 9 years	Neither DR3 nor DR4-DQ8	87	Ref	0.068	< 0	65	Ref	0.15
					≥ 0	22	0.34 (0.08–1.49)	
	DR3 and/or DR4-DQ8	226	1.62 (0.96–2.73)	< 0	161	Ref	0.87	
				≥ 0	65	0.96 (0.57–1.61)		
≥ 9 years	Neither DR3 nor DR4-DQ8	118	Ref	0.018	< 0	86	Ref	0.004
					≥ 0	32	7.32 (1.88–28.5)	
	DR3 and/or DR4-DQ8	273	2.28 (1.15–4.53)	< 0	203	Ref	0.31	
				≥ 0	70	1.38 (0.32–2.59)		

Ref, reference.

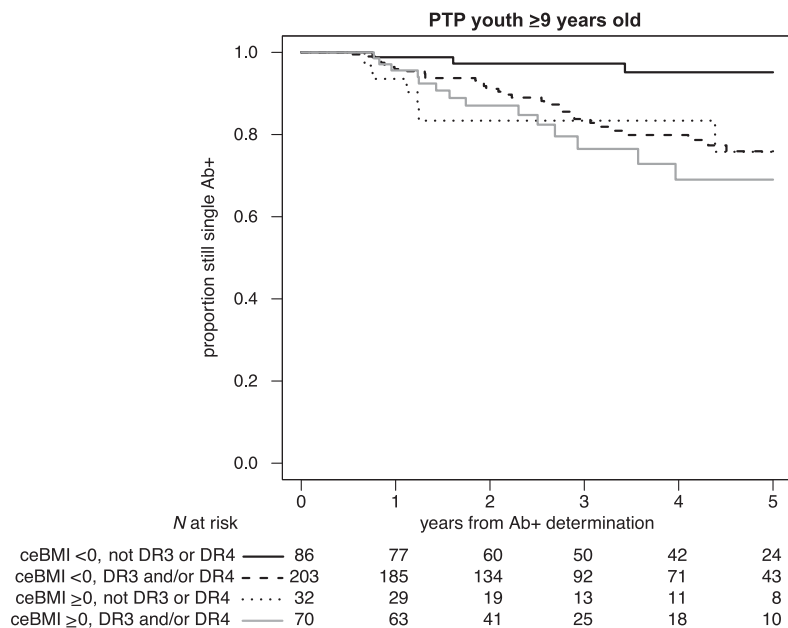


Figure 2—ceBMI influenced progression to multiple autoantibody positivity in subjects ≥ 9 years of age at first autoantibody progression with a lower-risk HLA haplotype (i.e., neither DR3 nor DR4-DQ8): In this subset of children, ceBMI ≥ 0 kg/m² (i.e., being persistently obese or overweight) increased by more than sevenfold the risk of progression to multiple autoantibody positivity (HR 7.32, 95% CI 1.88–28.5; $P = 0.004$), and their risk was similar to that in the children who had the high-risk HLA haplotypes. In contrast, ceBMI did not further increase the progression to multiple autoantibodies in children with high-risk HLA haplotypes.

BMI contributes to the preclinical stages of T1D. The accelerator hypothesis first promoted by Wilkin and colleagues (6,10) proposed that both type 1 and type 2 diabetes have a common origin in obesity-induced insulin resistance, while our data suggest that elevated BMI is associated with an acceleration in the progression of islet autoimmunity in certain subgroups of individuals.

The appearance of multiple islet autoantibodies has been shown to be a significant milestone for T1D development, with 70% of individuals progressing to clinical diabetes (stage 3) within 10 years of seroconversion (33). Of note, older age is a protective factor from progression to clinical T1D (2,26). Given that only a minority of single antibody subjects develop T1D (27), identifying ceBMI as modifiable risk factor, with both genetic and environmental causes, that influences the conversion from single to multiple autoantibodies in the PTP cohort has clinical implications, as it could be targeted to prevent or delay progression to clinical T1D. Importantly, subjects ≥ 9 years old without HLA DR3 or DR4, who should have less aggressive autoimmunity and lower incidence of multiple autoantibody conversion, may acquire high risk of entering stage 1 of T1D if BMI is sustained above

the overweight/obese threshold. Thus, in this subgroup with lower intrinsic risk (i.e., older and lacking high-risk HLA haplotypes), risk factors such as elevated BMI may play a more critical role in initiating the first preclinical phase of T1D.

There is growing literature that suggests that obesity-induced inflammation may be a contributing factor leading to the development of autoimmune disease (34,35). Elevated levels of the proinflammatory adipokine leptin have been implicated in several autoimmune diseases, such as rheumatoid arthritis, lupus, and inflammatory bowel disease, among others. In a study by Marzullo et al. (36), positivity for thyroid peroxidase antibodies was associated with elevated leptin levels independent of BMI status. The levels of the anti-inflammatory adipokine, adiponectin, were found to be similar in patients with T1D compared with healthy subjects, but monocytes from T1D subjects had reduced adiponectin receptors, blunting adiponectin inhibition of T-cell proliferation (37). Moreover, adiponectin has been shown to have direct effects on the β -cells, promoting function and survival (38), further highlighting the adipose tissue as an active participant in T1D pathophysiology, although the nature of the associations is still unclear.

The potential relationship between obesity-induced inflammation and autoimmune disease, including islet autoimmunity, could also underlie our previous observations that the type 2 diabetes-associated *TCF7L2* genetic variant, which may be involved in fatty acid metabolism (39), accelerates antigen spreading in individuals with a single positive ICA512/IA-2 or insulin autoantibody who are obese or overweight (40). In this smaller study of pediatric and adult TrialNet participants who had available ImmunoChip data, the effect of ceBMI or age strata was not evaluated. In addition, this genetic variant is associated with single autoantibody expression at the onset of clinical T1D (41). In these individuals with only mild islet autoimmunity, *TCF7L2* variants, via β -cell dysfunction, insulin resistance, and diabetogenic interaction with obesity (39) could precipitate the progression to clinical T1D, reflecting a complex interplay between metabolic risk variants and autoimmunity in the setting of T1D (41). Taken together, these findings suggest that type 2 diabetes-related mechanisms (e.g., genetics, obesity/overweight) not only can add to the risk of clinical diabetes in individuals with evidence of islet autoimmunity but also interact with the autoimmune process (reviewed in ref. 42). When the combination (and interaction) of diabetogenic factors exceeds a certain threshold, clinical diabetes develops, as proposed by the threshold hypothesis (13). This concept could justify therapeutic strategies that address type 2 diabetes-related mechanisms, when present, as adjuvants to immunomodulatory therapies aiming to prevent or delay T1D progression.

A major strength of this study is the size of the study cohort followed longitudinally at regular intervals using a standard protocol, which allows for sophisticated analysis including recursive partitioning to identify specific subgroups who are susceptible to the effects of ceBMI. The inclusion of subjects carrying low-, intermediate-, and high-risk HLA class genotypes in the PTP cohort allowed us to identify ceBMI as a modifiable risk factor for autoantibody progression in older individuals who would be considered to have a less aggressive autoimmune profile based on HLA subtype. Our study does, however, have some limitations. The TrialNet PTP cohort is not followed from birth, and therefore the duration of a single

positive autoantibody is unknown. Additionally, some individuals are followed for a short period of time, which may have influenced these results. We do note an increased proportion of Hispanic/Latino subjects among those with a $\text{ceBMI} \geq 0 \text{ kg/m}^2$, which is consistent with higher prevalence of elevated BMI among Hispanic children from the general population (5), and while we did accommodate HLA subtype in our analysis, there may be other genetic contributors. Per TrialNet study protocol, ZnT8 autoantibodies were not measured in one-third of the participants, with ~20–25% (i.e., 8% of the entire cohort) expected to be positive. Some of the subgroups analyzed in this unique cohort of participants were relatively small in size; thus, results should be confirmed in the future. Finally, the optimal cut points identified for age that influence progression to multiple positive autoantibodies are data derived using recursive partitioning analysis and may not be broadly applicable to other populations.

We provide evidence that sustained elevation in BMI may at least in part contribute to T1D pathophysiology through an acceleration of the autoimmune process, particularly in those without high-risk factors, in addition to the known effect of increasing β -cell demand. Future studies are required to identify signaling molecules responsible for obesity-induced autoimmunity specifically in T1D, but our data demonstrate the important interaction of diverse risk factors in T1D, e.g., age, HLA subtype, and BMI. Targeted lifestyle interventions to maintain BMI <85th percentile may help prevent or delay the progression to multiple autoantibody positivity and could halt the growing incidence of clinical stage 3 T1D.

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