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ARTICLE

Ethnic differences in progression of islet autoimmunity and type 1 diabetes in relatives at risk

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Abstract

Aims/hypothesis We hypothesised that progression of islet autoimmunity and type 1 diabetes mellitus differs among races/ethnicities in at-risk individuals.

Methods In this study, we analysed the data from the Type 1 Diabetes TrialNet Pathway to Prevention Study. We studied 4873 non-diabetic, autoantibody-positive relatives of individuals with type 1 diabetes followed prospectively (11% Hispanic, 80.9% non-Hispanic white [NHW], 2.9% non-Hispanic black [NHB] and 5.2% non-Hispanic other [NHO]). Primary outcomes were time from single autoantibody positivity confirmation to multiple autoantibody positivity, and time from multiple autoantibody positivity to type 1 diabetes mellitus diagnosis.

Results Conversion from single to multiple autoantibody positivity was less common in Hispanic individuals than in NHW individuals (HR 0.66 [95% CI 0.46, 0.96], $p = 0.028$) adjusting for autoantibody type, age, sex, Diabetes Prevention Trial Type 1 Risk Score and *HLA-DR3-DQ2/DR4-DQ8* genotype. In participants who screened positive for multiple autoantibodies ($n = 2834$), time to type 1 diabetes did not differ by race/ethnicity overall ($p = 0.91$). In children who were <12 years old when multiple autoantibody positivity was determined, being overweight/obese had differential effects by ethnicity: type 1 diabetes risk was increased by 36% in NHW children (HR 1.36 [95% CI 1.04, 1.77], $p = 0.024$) and was nearly quadrupled in Hispanic children (HR 3.8 [95% CI 1.6, 9.1], $p = 0.0026$). We did not observe this interaction in participants who were ≥12 years old at determination of autoantibody positivity, although this group size was limited. No significant differential risks were observed between individuals of NHB and NHW ethnicity.

Conclusions/interpretation The risk and rate of progression of islet autoimmunity were lower in Hispanic compared with NHW at-risk individuals, while significant differences in the development of type 1 diabetes were limited to children <12 years old and were modified by BMI.

Keywords Diabetes in childhood · Genetics of type 1 diabetes · Prediction and prevention of type 1 diabetes · Weight regulation and obesity

A complete list of the Type 1 Diabetes TrialNet Study Group can be found in the electronic supplementary material.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00125-018-4660-9) contains peer-reviewed but unedited supplementary material, which is available to authorised users.

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Research in context

What is already known about this subject?

- Type 1 diabetes is a disease continuum with variable progression along well-defined stages
- A wealth of data has been generated on genetic, immunological and metabolic risk factors that enable us to predict type 1 diabetes risk
- A limited number of studies show that there are significant racial/ethnic differences in genetic, immunological, metabolic and clinical characteristics, as well as in incidence and prevalence, of type 1 diabetes

What is the key question?

- Does progression of islet autoimmunity and type 1 diabetes differ among races/ethnicities in at-risk individuals?

What are the new findings?

- Conversion from single to multiple autoantibody positivity was less common in Hispanic individuals than non-Hispanic white individuals after adjusting for confounding factors
- Overweight or obese participants who were less than 12 years old at autoantibody presentation displayed significantly increased rates of type 1 diabetes compared with lean participants. This increase was more pronounced in Hispanic (nearly fourfold increase) than in non-Hispanic white participants (increase by only 36%)

How might this impact on clinical practice in the foreseeable future?

- These important findings can be used in the identification of different type 1 diabetes phenotypes and may potentially lead to inclusion of race/ethnicity in prediction models of the progression of islet autoimmunity and type 1 diabetes

Abbreviations

BMI%ile	BMI percentile
DPTRS	Diabetes Prevention Trial–Type 1 Risk Score
GAD65	Glutamic acid decarboxylase 65
IA-2	Islet antigen 2
ICA	Islet cell autoantibody
mIAA	Micro-insulin autoantibody
NHW	Non-Hispanic white
NHB	Non-Hispanic black
NHO	Non-Hispanic other
NIH	National Institutes of Health
PTP	Pathway to Prevention
ZnT8	Zinc transporter 8

Introduction

Type 1 diabetes mellitus is a chronic autoimmune condition characterised by beta cell destruction leading to insulin deficiency. Studies of the natural history and pathogenesis of type 1 diabetes have shown that it is a disease continuum with variable progression along well-defined stages: presymptomatic beta cell autoimmunity with normoglycaemia; presymptomatic beta cell autoimmunity with dysglycaemia and symptomatic beta cell autoimmunity with dysglycaemia [1]. A wealth of data has been generated on genetic, immunological and metabolic risk factors that enable us to predict type 1 diabetes risk and design studies to intervene early in the autoimmune process, before the onset of symptoms. In genetically susceptible children positive for multiple autoantibodies, the 10 year risk of developing type 1 diabetes is 70%, with lifetime risk reaching 100% [2]. A predictive score (the Diabetes Prevention Trial–Type 1 Risk Score [DPTRS]) has been proposed to estimate the type 1 diabetes risk in at-risk individuals [3].

The growing public health impact of studies examining racial/ethnic differences is underscored by recent data demonstrating that the increase in type 1 diabetes incidence disproportionally affects racial and ethnic minorities [4]. However, much of the knowledge on type 1 diabetes pathogenesis stems from studies primarily conducted in the non-Hispanic white (NHW) population and generalisability to other races/ethnicities has not been established. The incidence of type 1 diabetes in children varies by race/ethnicity (e.g. the SEARCH study reported 27, 19 and 14.8 new incidences per 100,000 person-years in 2012, respectively, in NHW, non-Hispanic black [NHB] and Hispanic participants [4]). While a limited number of studies showed that there are significant racial/ethnic differences in genetic, immunological, metabolic and clinical characteristics [5–13], the risk and rate of progression of islet autoimmunity and type 1 diabetes development have not been compared among different racial/ethnic groups. A full understanding of these and associated factors may inform the design of future prediction models and prevention trials and, eventually, clinical care.

Type 1 Diabetes TrialNet is a National Institutes of Health (NIH)-funded international consortium of clinical research

centres aiming to prevent or delay type 1 diabetes. Relatives of individuals with type 1 diabetes are offered screening for the presence of islet autoantibodies and, if positive, enrolment in the Pathway to Prevention (PTP) study; if eligible, participation in prevention studies is offered [14]. Inclusion of an increasing number of individuals of minority racial/ethnic background provided us with a unique opportunity to compare the natural course prior to development of type 1 diabetes in those of Hispanic and NHW ethnicity.

We hypothesised that the progression of islet autoimmunity and type 1 diabetes significantly differs among races/ethnicities in at-risk individuals. This study aimed to compare the rates and risk factors of progression of islet autoimmunity and type 1 diabetes development among races/ethnicities in at-risk individuals.

Methods

Design and settings

We analysed data from the Type 1 Diabetes TrialNet PTP study. The TrialNet PTP study screened relatives of individuals with type 1 diabetes with the aim of identifying participants for monitoring and/or prevention studies. First-degree relatives (1–45 years old) and second-degree relatives (1–20 years old) of individuals with type 1 diabetes were eligible for PTP screening; of note, due to rescreen guidelines and allowable timeframes, PTP participants could be identified as autoantibody positive after 45 years of age. Eligible relatives were tested for the presence of islet autoantibodies, including glutamic acid decarboxylase 65 (GAD65) autoantibody, islet antigen 2 (IA-2) autoantibody and micro-insulin autoantibody (mIAA), followed by islet cell autoantibody (ICA) if they were positive for ≥1 autoantibody(ies) at the initial screening test [15]. Additionally, zinc transporter 8 (ZnT8) autoantibody measurement was performed consistently from 2012 onwards in participants with ≥1 positive autoantibody(ies) at the initial screening test [16]. Participants who were negative for all tested autoantibodies were eligible for yearly rescreening until 18 years of age. Participants confirmed positive for a single autoantibody on a consecutive visit within 1 year were defined as single confirmed autoantibody positive. Those positive for two or more autoantibodies at any screening or follow-up were defined as multiple autoantibody positive. Single confirmed autoantibody-positive and multiple autoantibody-positive participants were offered enrolment in 'monitoring' and, if eligible and interested, prevention studies. Baseline risk assessments included OGTT, HbA_{1c} measurement and HLA typing. Participants positive for multiple autoantibodies were monitored semi-annually throughout the PTP; single confirmed autoantibody-positive participants were monitored semi-annually until 2012, and then annually. This monitoring includes OGTT, HbA_{1c} measurement and autoantibody testing. Details of the screening and follow-up processes have previously been described [15, 16]. All participants and/or their parents provided written informed consent and assent, as appropriate, approved by local Institutional Review Boards.

Inclusion and exclusion criteria

Between 22 March 2004 and 31 July 2017, 182,145 relatives were screened in the TrialNet PTP study at 21 clinical centres and approximately 100 collaborating clinical sites in the USA, Canada, UK, Finland, Italy, Germany, Australia and New Zealand. A total of 5703 autoantibody-positive participants who had at least one follow-up visit were identified in the TrialNet PTP–Monitoring Cohort. Exclusion criteria included fasting blood glucose <2.8 mmol/l or ≥7 mmol/l, 2 h OGTT blood glucose ≥11.1 mmol/l, type 1 diabetes at first monitoring visit, missing fasting or 2 h OGTT blood glucose data and, for the current analysis, unknown or missing ethnicity data. Individuals with glucose <2.8 mmol/l were excluded because of the potential for data quality problems and those with fasting glucose ≥7 mmol/l and 2 h OGTT glucose ≥11.1 mmol/l were excluded because of suspected type 1 diabetes.

Race and ethnicity categorisation

Race and ethnicity categories were based on self-report and on standard NIH classifications and definitions [17]. Individuals who listed more than one race were categorised as multiracial. We evaluated individuals based on these NIH-defined groups and also on composite race/ethnicity groups. Specifically, participants were assigned to one of the following four racial/ethnic groups: Hispanic, NHW, NHB and non-Hispanic other [NHO]. Non-Hispanic multiracial participants were included in the 'NHO' category.

Anthropometric measures and laboratory analyses

BMI BMI was calculated using data from the first monitoring visit. BMI percentiles (BMI%iles) were calculated for all participants ≥2 years old. For adults over 20 years old, BMI%iles were calculated by imputing 20 as their age to be able to evaluate BMI%iles as a continuous measure across all participants. Classification of an individual as overweight was defined as a BMI ≥85th but <95th percentile, and obesity was defined as a BMI ≥95th percentile adjusting for age and sex according to Centers for Disease Control and Prevention criteria. Because of the very limited number of underweight participants, all participants with a BMI <85th percentile were considered to be lean.

HLA typing HLA genotyping was performed at TrialNet HLA Laboratory at the Barbara Davis Center, which receives whole blood from clinical sites and extracts DNA using the AutoGen QuickGene-610 instrument. In this analysis, participants were classified by the presence or absence of the highest risk genotype (i.e. *DR3-DQ2* [*DRB1*03:01–DQA1*05:01–DQB1*02:01*] and *DR4-DQ8* [*DQA1*03:01–DQB1*03:02* with *DRB1*04:01*, *DRB1*04:02* or *DRB1*04:05*]). Further information on HLA typing is provided in electronic supplemental material (ESM) Methods.

Autoantibody assays GAD65, IA-2, mIAA and ZnT8 autoantibodies were measured by radioimmunoassay in the TrialNet Core Laboratory at the Barbara Davis Center for Childhood Diabetes in Denver, CO, USA. During the 2015 Islet Autoantibody Standardization Program Workshop, respective sensitivities and specificities were 52% and 100% for mIAA, 82% and 99% for GAD65 autoantibody, 72% and 100% for IA-2 autoantibody and 70% and 97% for ZnT8 autoantibody [18]. ICA positivity was tested by indirect immunofluorescence in the Diagnostic Referral Laboratories at the University of Florida. An ICA value greater than 5 Juvenile Diabetes Foundation units was considered positive [19].

OGTT The glycaemic status of the participants was tested with an OGTT (oral glucose dose 1.75 g/kg, maximum 75 g) after an overnight fast. C-peptide (nmol/l) and glucose (mmol/l) measurements were performed in the fasted state and at 30, 60, 90 and 120 min. The trapezoid method was used to calculate the AUC (nmol/l × min) for C-peptide.

Diagnosis of diabetes Diabetes was diagnosed according to TrialNet Natural History Study of the Development of Type 1 Diabetes Protocol (TrialNet Protocol TN01): fasting plasma glucose ≥7.0 mmol/l, 2 h plasma glucose during an OGTT ≥11.1 mmol/l, a random plasma glucose ≥11.1 mmol/l with symptoms of hyperglycaemia or presence of unequivocal hyperglycaemia including acute metabolic decompensation (diabetic ketoacidosis). The first three criteria were required to be met on two occasions, with a strong preference that at least one of the two testing occasions included an OGTT. HbA_{1c} level ≥48 mmol/mol (≥6.5%) from a laboratory that used The National Glycohemoglobin Standardization Program certified assay standardised to The Diabetes Control and Complications Trial was also accepted as a confirmatory criterion.

DPTRS A metabolic risk score was calculated for each individual based on a model including $\log_e$-BMI, age, $\log_e$-fasting C-peptide and post-challenge glucose and C-peptide sums from 2 h OGTT at baseline assessment [3]. This score was used to compare races/ethnicities both as continuous variable and dichotomised variable (<6.5 and ≥6.5). For the purpose of this analysis, we used <6.5 and ≥6.5 to define low and high DPTRS, respectively, based on the previously published differential diabetes risk [20].

Statistical methods

Descriptive analyses were used to summarise characteristics across all participants as well as within single confirmed autoantibody- and multiple autoantibody-positive cohorts. Characteristics were compared between the race/ethnicity composite groups using Kruskal–Wallis tests for continuous variables and χ^2 tests for categorical/dichotomised factors, where Fisher exact tests were used as appropriate with small numbers in subset groups. At-risk individuals who enrolled in prevention trials were censored at the time of their entry into the trial. Primary outcomes for these analyses were time to multiple positive autoantibodies in single confirmed autoantibody positive participants and time to type 1 diabetes diagnosis in multiple autoantibody-positive participants. Time to multiple positive autoantibodies was defined as the time from single confirmed autoantibody positive determination to the time when two or more positive autoantibodies were identified. Time to progression to type 1 diabetes diagnosis was defined as the time from participants being identified as having multiple positive autoantibodies to the time when they were diagnosed with type 1 diabetes. Those who had not progressed at their last follow-up visit were censored at that time point. The two analysis cohorts were not mutually exclusive, as single confirmed autoantibody-positive participants who subsequently converted to multiple positive autoantibodies were included in the multiple autoantibody-positive cohort after that time. Kaplan–Meier methods were used to estimate the proportion of participants who had not had an event (e.g. development of type 1 diabetes) by a certain time. Estimated event rates were also calculated using cumulative incidence analyses. Univariate and multivariable Cox regression models were used to evaluate prognostic utility of the various markers and factors in relation to time to progression to multiple positive autoantibodies or type 1 diabetes diagnosis for the single confirmed and multiple positive autoantibody cohorts, respectively. Cumulative incidences of events of interest were graphed and also adjusted for identified factors of interest using the methodology of Therneau et al [21] and Nieto et al [22]. Multivariable models employed a hybrid variable selection approach based on backwards selection and all subsets regression approaches. Age at autoantibody determination was evaluated as a continuous measure; however, an optimal cut-point for age was identified using recursive partitioning analyses (rpart package in R) [23], which uses a tree-based method and iteratively evaluates all possible cut-points of age that best differentiated participants' prognosis in relation to, for instance, time to progression to type 1 diabetes [24, 25]. Statistical significance was determined at $p < 0.05$.

All analyses were performed using the statistical program R version 3.4.1 for Windows [26].

Results

A total of 4873 TrialNet PTP participants, comprising 11% Hispanic, 80.9% NHW, 2.9% NHB and 5.2% NHO, were followed prospectively. At screening, 2039 participants (42%) were single confirmed autoantibody positive while 2834 (58%) were positive for multiple autoantibodies. Median follow-up for single to multiple autoantibody conversion was 1.9 years (interquartile range 0.7–4.2 years) and for progression to type 1 diabetes was 1.0 years (interquartile range 0.4–2.9 years) in event-free participants. A total of 363/2039 (18%) participants progressed from single to multiple autoantibody positivity. Across all 4873 participants, 591 (12%) progressed to type 1 diabetes during follow-up (65 single confirmed and 526 multiple autoantibody positive) (Fig. 1). The estimated cumulative rate of type 1 diabetes incidence at 5 years was 39% (95% CI 36, 42) in the multiple autoantibody-positive cohort and 26.4% (95% CI 24, 29) across all autoantibody-positive participants.

We observed differences in the distributions of baseline characteristics between the race/ethnicity groups (Table 1). The pairwise comparisons of baseline characteristics for NHB vs NHW, and NHO vs NHW, were not included because there were no significant differences in primary outcomes (i.e. time to multiple positive autoantibodies in single confirmed autoantibody-positive participants and time to type 1 diabetes in participants with multiple positive autoantibodies) between these respective groups.

In progression from single confirmed to multiple positive autoantibodies, we found that Hispanic ethnicity, age at screening, sex, DPTRS and *HLA-DR3-DQ2/DR4-DQ8* were significant factors affecting time to progression (ESM Table 1). Hispanic ethnicity was significantly associated with a protective effect for conversion to multiple positive autoantibodies (HR 0.66 [95% CI 0.46, 0.96], $p = 0.028$; ESM Table 1) after adjustment for positive autoantibody type, age, sex, DPTRS, obesity and *HLA-DR3-DQ2/DR4-DQ8* genotype (Fig. 2). This lower likelihood of Hispanic participants to progress to multiple positive autoantibody status compared with NHW participants was also observed in those who did not have *HLA-DR3-DQ2/DR4-DQ8*, even after adjustment for potential confounders (HR 0.63 [95% CI 0.42, 0.94], $p = 0.024$).

In participants who were positive for multiple autoantibodies at screening, time to type 1 diabetes did not differ significantly by race/ethnicity in the overall cohort ($p = 0.91$), or between Hispanic vs NHW participants after adjusting for age, sex, DPTRS, number of autoantibodies, *HLA-DR3-DQ2/DR4-DQ8* genotype and BMI (ESM Table 2). Cut-point analyses identified 12 years as the appropriate cut-off age in relation to time to progression to type 1 diabetes. In the overall cohort, there was a significant three-way interaction between age, being overweight/obese vs not, and Hispanic vs NHW ($p = 0.006$; ESM Table 2). Stratified analyses showed that in children <12 years old, ethnicity (Hispanic vs NHW) was a significant effect modifier on the effects of being overweight/obese on cumulative incidence and rate of progression to type 1 diabetes ($p = 0.025$). In children <12 years old at the time of multiple autoantibody determination, although it was not significantly different, lean Hispanic participants appeared to have a lower rate of progression to type 1 diabetes (HR 0.65 [95% CI 0.36, 1.17], $p = 0.15$; Fig. 3) than lean NHW participants after adjusting for sex, number of autoantibodies, DPTRS and *HLA-DR3-DQ2/DR4-DQ8*. However, in this age group, the state of being overweight/obese increased the risk of type 1 diabetes by 36% in NHW children (HR 1.36 [95% CI 1.04, 1.77], $p = 0.024$;

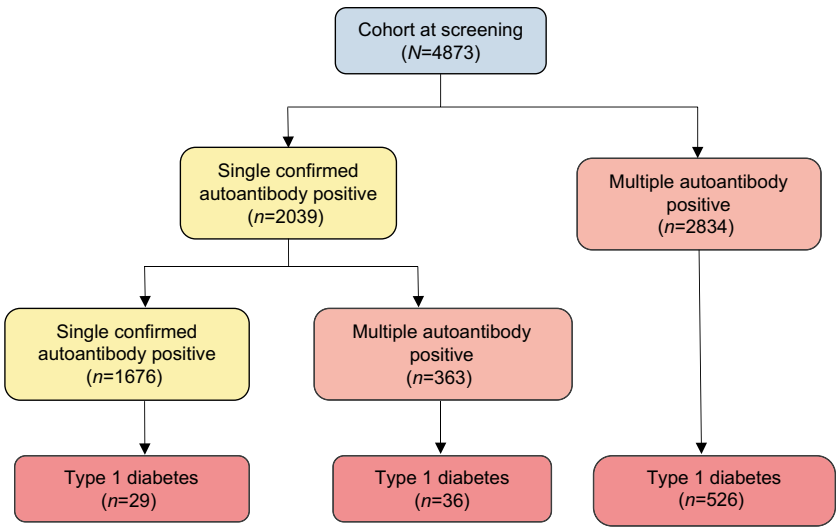

Fig. 1 Flow-diagram of islet autoimmunity progression and type 1 diabetes development in TrialNet PTP participants

Table 1 Baseline characteristics at screening by race and ethnicity groups ($N = 4873$)

Characteristic	Hispanic	NHW	NHB	NHO	*p* value (Overall)	*p* value (Hispanic vs NHW)
All participants						
No.	537	3940	143	253		
Age						
Median, years	12.4	12.0	11.2	11.7	0.51	0.19
Range, years	1.3–48.7	1.06–51.8	2.6–45.7	1.7–46		
Missing data, *n*	0	0	1	0		
Sex						
Female, *n* (%)	303 (57)	2001 (51)	84 (59)	144 (57)	0.006	0.02
Male, *n* (%)	230 (43)	1934 (49)	59 (41)	107 (43)		
Missing data, *n*	4	5	0	2		
Autoantibody status at screening, *n* (%)						
Single confirmed positive	280 (52)	1585 (40)	48 (34)	126 (50)	<0.00001	<0.00001
Multiple positive	257 (48)	2355 (60)	95 (66)	127 (50)		
1	280 (52)	1585 (40)	48 (34)	126 (50)	<0.00001	<0.00001
2	113 (21)	1003 (26)	46 (33)	68 (27)		
3	63 (12)	606 (15)	23 (16)	25 (10)		
4	45 (8)	462 (12)	13 (9)	21 (8)		
5	32 (6)	236 (6)	11 (8)	9 (4)		
Missing at least one autoantibody titre, *n*	4	48	2	4		
DPTRS						
Median	5.85	6.18	6.13	6.04	<0.0001	<0.00001
Range	0.78–10.1	0.49–10.1	3.5–9.1	1.3–9.76		
<6.5, *n* (%)	383 (75)	2289 (61)	89 (67)	155 (67)	<0.0001	<0.00001
≥6.5, *n* (%)	126 (25)	1460 (39)	43 (33)	77 (33)		
Missing, *n*	28	191	11	21		
BMI percentile						
Median	81.1	67.8	76.4	69.0	<0.0001	<0.0001
Range	0–99.8	0–100	2.0–99.6	0–99.2		
Normal/lean, *n* (%)	286 (55)	2682 (70)	75 (56)	164 (67)	<0.0001	<0.0001
Overweight, *n* (%)	98 (19)	612 (16)	24 (18)	49 (20)		
Obese, *n* (%)	139 (26)	534 (14)	36 (27)	32 (13)		
Missing data, *n*	14	112	8	8		
Subgroup 1: Single confirmed autoantibody positive ($n = 2039$)						
No.	280	1585	48	126		
Antibody type, *n* (%)						
GAD65	195 (69.6)	1118 (70.5)	38 (79)	89 (70.6)	0.12	0.21
IA-2	7 (2.5)	76 (4.8)	1 (2)	1 (0.8)		
mIAA	78 (27.9)	391 (24.7)	9 (19)	36 (28.6)		
HLA-DR3-DQ2/DR4-DQ8						
Both *DR3-DQ2* and *DR4-DQ8* present, *n* (%)	19 (7)	202 (13)	0	11 (10)	0.001	0.009
Not both *DR3-DQ2* and *DR4-DQ8* present, *n* (%)	252 (93)	1306 (87)	46 (100)	104 (90)		
DR3-DQ2 and/or *DR4-DQ8* present, *n* (%)	152 (56)	1142 (76)	29 (63)	80 (70)	<0.0001	<0.0001
Neither present, *n* (%)	119 (44)	366 (24)	17 (37)	35 (30)		
Missing data, *n*	9	77	2	11		
Fasting C-peptide						
Median, nmol/l	0.59	0.53	0.62	0.52	0.015	0.0015
Range, nmol/l	0.13–1.93	0.02–2.50	0.17–1.27	0.06–3.38		
Missing data, *n*	0	3	0	0		

Table 1 (continued)

Characteristic	Hispanic	NHW	NHB	NHO	*p* value (Overall)	*p* value (Hispanic vs NHW)
Mean C-peptide AUC						
Median, nmol/l × min	2.19	1.98	1.9	2.0	0.0007	<0.0001
Range, nmol/l × min	0.31–8.23	0.28–8.27	0.72–3.91	0.40–8.77		
Missing data, *n*	8	42	2	9		
BMI percentile						
Median	82.9	71.0	89.1	66.5	<0.0001	<0.0001
Range	0.45–99.8	0–100	2.8–99.6	0–99.1		
Normal/lean, *n* (%)	139 (51)	1036 (67.1)	20 (44.4)	85 (70)	<0.0001	<0.0001
Overweight, *n* (%)	52 (19)	253 (16.4)	11 (24.4)	23 (19)		
Obese, *n* (%)	81 (30)	254 (16.5)	14 (31.1)	14 (11)		
Missing data, *n*	8	42	3	4		
Subgroup 2: Multiple autoantibody positive (*n* = 2834)						
No.	257	2355	95	127		
HLA-DR3-DQ2/DR4-DQ8						
Both *DR3-DQ2* and *DR4-DQ8* present, *n* (%)	58 (23)	532 (24)	11 (12)	21 (18)	0.03	0.99
Not both *DR3-DQ2* and *DR4-DQ8* present, *n* (%)	194 (77)	1688 (76)	80 (88)	97 (82)		
DR3-DQ2 and/or *DR4-DQ8* present, *n* (%)	183 (73)	1867 (84)	67 (74)	93 (79)	<0.0001	<0.0001
Neither present, *n* (%)	69 (27)	353 (16)	24 (26)	25 (21)		
Missing data, *n*	5	135	4	9		
Fasting C-peptide						
Median, nmol/l	0.49	0.45	0.41	0.44	0.046	0.01
Range, nmol/l	0.11–1.71	0.05–2.48	0.08–1.24	0.10–1.61		
Missing data, *n*	0	5	0	0		
Mean C-peptide AUC						
Median, nmol/l × min	1.75	1.621	1.72	1.63	0.012	0.003
Range, nmol/l × min	0.36–7.49	0.16–7.89	0.48–3.53	0.68–4.71		
Missing data, *n*	10	52	2	6		
BMI percentile						
Median	79.3	65.9	73.5	72.1	0.0001	0.0006
Range	0–99.7	0–100	1.98–99.6	0.6–99.2		
Normal/lean, *n* (%)	147 (59)	1646 (72)	55 (61)	79 (64)	<0.0001	<0.0001
Overweight, *n* (%)	46 (18)	359 (16)	13 (14)	26 (21)		
Obese, *n* (%)	58 (23)	280 (12)	22 (24)	18 (15)		
Missing data, *n*	6	70	5	4		

ESM Table 3), while the risk was almost quadrupled in Hispanic participants (HR 3.8 [95% CI 1.6, 9.1], $p = 0.0026$; ESM Table 3), even after adjusting for sex, number of autoantibodies, DPTRS and *HLA-DR3-DQ2/DR4-DQ8* (Fig. 3). In participants ≥12 years old at multiple autoantibody determination, there were no significant differences among Hispanic vs NHW ethnicities. However, we observed a significant interaction between the BMI%ile as a continuous measure and Hispanic ethnicity ($p = 0.012$). Although BMI%ile was not a significant factor in NHW participants ≥12 years old (HR 0.997 [95% CI 0.991, 1.004], $p = 0.38$), we noted that it had a significant effect (HR 0.96 [95% CI 0.94, 0.99], $p = 0.007$) in Hispanic participants ≥12 years old even with the relatively limited number of participants in that group. However, with only 13 events in this restricted cohort, we consider this an interesting and hypothesis-generating observation that warrants further investigation.

To further assess the role and effect modification by Hispanic ethnicity on progression to type 1 diabetes, we also evaluated this outcome in the overall cohort of all autoantibody-positive participants ($n = 4873$). Findings were similar after adjusting for the number of autoantibodies and other potential confounders (Fig. 4, ESM Tables 4 and 5). No significant differential risks were observed between NHB and NHW ethnic groups.

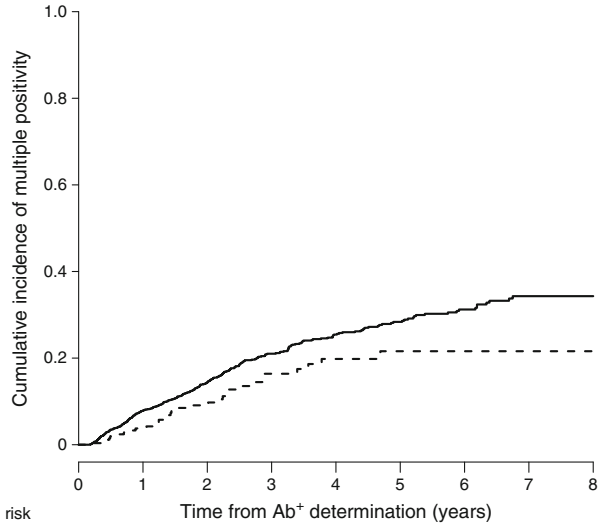

Fig. 2 Cumulative incidence of multiple autoantibody positivity in Hispanic vs NHW individuals in the TrialNet PTP cohort with single confirmed autoantibody positivity at screening ($p = 0.01$). Curves are adjusted for autoantibody type, age, sex, DPTRS and *HLA-DR3-DQ2/DR4-DQ8* status. Ab$^+$, autoantibody positive. Solid line, NHW; dashed line, Hispanic

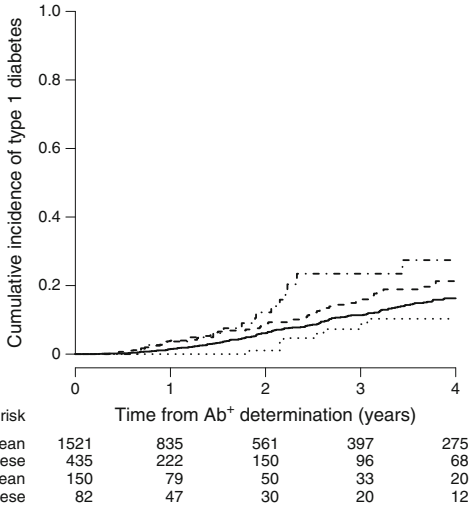

Fig. 4 Cumulative incidence of type 1 diabetes in TrialNet PTP participants <12 years old in the whole at-risk cohort (i.e. both single confirmed and multiple autoantibody-positive cohort), by ethnicity (i.e. Hispanic vs NHW) and BMI groups. Curves are adjusted for sex, number of autoantibodies, DPTRS and *HLA-DR3-DQ2/DR4-DQ8* status. Ab$^+$, autoantibody positive. Solid line, lean NHW; dashed line, overweight/obese NHW; dotted line, lean Hispanic; dash–dotted line, overweight/obese Hispanic

Discussion

In our study of the role of race and ethnicity in relation to the progression of islet autoimmunity and type 1 diabetes development in at-risk individuals from the TrialNet PTP cohort, we found that conversion from single to multiple autoantibody positivity was less common in Hispanic than in NHW individuals after adjustment for autoantibody type, age, sex, DPTRS, obesity and *HLA-DR3-DQ2/DR4-DQ8*. Although time from multiple autoantibody positivity to diagnosis of type 1 diabetes did not differ by race/ethnicity in the overall cohort, we found that Hispanic ethnicity as well as age (specifically <12 or ≥12 years) were significant effect modifiers on the influence of BMI%ile on rates of progression to type 1 diabetes. In children <12 years old at multiple autoantibody determination, an overweight or obese status was a significant factor for progression to type 1 diabetes in both Hispanic and NHW participants; however, this risk was much more pronounced in Hispanic participants than in NHW participants (respective HRs 3.8 and 1.36) even adjusting for sex, number of autoantibodies, DPTRS and *HLA-DR3-DQ2/DR4-DQ8*. There was also an indication that Hispanic ethnicity could modify the effects of BMI%ile in participants ≥12 years old at autoantibody determination.

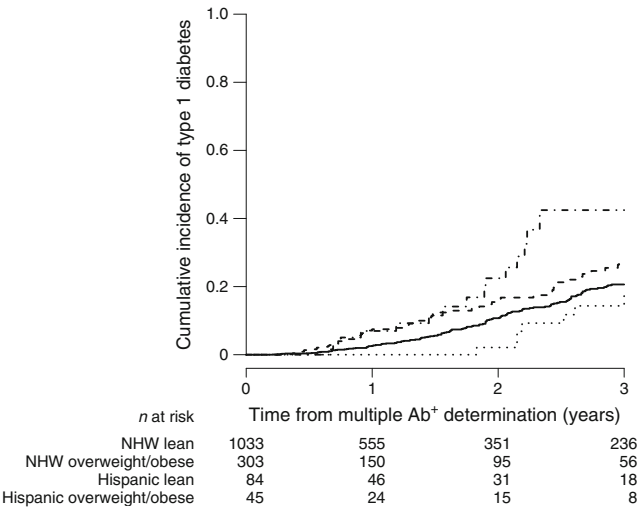

Fig. 3 Cumulative incidence of type 1 diabetes in TrialNet PTP participants <12 years old with multiple positive autoantibodies, by ethnicity (i.e. Hispanic vs NHW) and BMI groups. Curves are adjusted for sex, number of autoantibodies, DPTRS, and *HLA DR3-DQ2/DR4-DQ8* status. Ab$^+$, autoantibody positive. Solid line, lean NHW; dashed line, overweight/obese NHW; dotted line, lean Hispanic; dash–dotted line, overweight/obese Hispanic

Single to multiple autoantibody conversion is a crucial step in islet autoimmunity progression and type 1 diabetes risk [1]. After adjustment for confounding factors, we observed that progression to multiple autoantibody positivity was less common in Hispanic than in NHW participants. The slower progression in Hispanic individuals suggests a lower frequency of predisposing characteristics beyond those that we adjusted for (e.g. additional type 1 diabetes-linked HLA haplotypes or non-HLA genetic factors, or environmental factors). Further research is warranted to understand the basis of this ethnic difference and its impact on type 1 diabetes prediction models as well as type 1 diabetes prevention efforts. Finally, the lower relative ratio of type 1 diabetes development in single

confirmed autoantibody-positive vs multiple autoantibody-positive participants in our cohort is similar to previous findings [1] and underscores the role of multiple islet autoantibody positivity in progression to type 1 diabetes [2].

Although there was no difference in time to type 1 diabetes diagnosis in the multiple autoantibody-positive cohort between the races/ethnicities overall, Hispanic participants appeared to be less likely to progress to type 1 diabetes than the NHW participants when considering lean children aged <12 years after adjustment for sex, number of autoantibodies, DPTRS and *HLA-DR3-DQ2/DR4-DQ8*. Differences in additional type 1 diabetes-associated HLA haplotypes and/or non-HLA genetic factors, as well as epigenetic or environmental factors, may explain the lower relative risk and rate of progression in this group. The significant role of Hispanic ethnicity on modifying the effects of BMI in this age group was best illustrated through the greater detrimental effect of elevated BMI in Hispanic vs NHW children aged <12 years. This phenomenon may be related to the limitation of BMI in estimating per cent body fat and adiposity, which disproportionally affect US Hispanic individuals [27]. Accordingly, BMI has been shown to increase the risk of cardiovascular disease in Hispanic individuals more than in non-Hispanic individuals [28]. Higher adiposity and consequently higher insulin resistance for a given BMI%ile in the Hispanic individuals might have contributed to this disproportional detrimental effect.

Similarly, the lack of clear ethnic differences in progression in individuals aged ≥12 years could be due to lower statistical power because of a smaller sample size or fewer incidences of progression in individuals ≥12 years old, as expected since it is known that older age is associated with slower progression from multiple autoantibody positivity to type 1 diabetes [2, 29, 30]. The ethnic/racial differences in progression to type 1 diabetes in older individuals may be identified in future studies in larger cohorts with a longer observation period. On the contrary, others have reported that high-risk autoantibody profiles (presence of IA2 and/or ZnT8) and genetic factors (HLA class I and non-HLA genes), but not age, are independent risk factors in progression from multiple autoantibody positivity to type 1 diabetes [31–34].

Our data provide insight into the impact of race/ethnicity in type 1 diabetes progression and may be valuable for the design of predictive models and prevention trials. Future studies aimed at identifying factors (e.g. genetic, epigenetic, environmental, etc.) contributing to slower progression in Hispanic individuals will advance our understanding of the natural history of type 1 diabetes and may have a significant impact on the prevention of type 1 diabetes. Such analyses may also allow for the determination of categorical diabetes subtypes with important therapeutic implications.

We observed that NHW individuals were more likely to have multiple positive autoantibodies when compared with Hispanic individuals, similar to the findings of a previous study conducted in individuals with newly diagnosed type 1 diabetes [35]. The higher incidence of islet autoimmunity observed in NHW individuals could be due to the different distribution of type 1 diabetes-associated HLA haplotypes/genotypes, with increased frequency of susceptibility [6] and decreased frequency of protective types [36] when compared with Hispanic ethnicity.

Counselling family members regarding type 1 diabetes risk is an integral part of modern diabetes care [37]. If confirmed, our findings highlight that the differential effect of race/ethnicity may need to be taken into consideration when counselling at-risk family members. Seroconversion from single confirmed to multiple positive autoantibodies (i.e. presymptomatic phase of type 1 diabetes) was less common in Hispanic participants (vs NHW participants) in this study. Additionally, type 1 diabetes risk appeared to be lower in Hispanic children younger than 12 years without elevated BMI, and in this group, elevated BMI was more detrimental in Hispanic than NHW participants with regard to type 1 diabetes risk. Hence, during counselling of Hispanic families with at-risk children <12 years old, it might be advisable to include the warning that being overweight/obese increases the children's risk for progression to type 1 diabetes to a much greater extent than the risk in NHW children. This knowledge may encourage Hispanic families to make healthy lifestyle changes. Furthermore, this finding has important public health implications due to the higher prevalence of overweight/obesity status [38] and greater annual rate of increase in type 1 diabetes incidence in Hispanic compared with NHW individuals [4].

Our study had the following limitations: lack of adjustment for other HLA [39, 40] and non-HLA loci [40, 41] that are known to be associated with type 1 diabetes development despite adjustment for the presence of the highest risk genotype; the observational nature of the study allowed identification of associations without implications on causality and the smaller number of participants in minority groups other than Hispanic limited our ability to delineate racial/ethnic differences in those groups. Longer follow-up of the participants will increase the precision of the estimates of progression. Because of the design of the TrialNet PTP study, the duration of positivity of single or multiple autoantibodies prior to screening is not known. However, the racial differences in the proportion of multiple autoantibody-positive individuals at screening (60% in NHW vs 48% in Hispanic individuals, Table 1) reflects the differential rates of early progression. Thus, the difference in progression from single to multiple autoantibody positivity between Hispanic and NHW participants could have been more robust if participants were followed since birth in a different study design. Our study focused on racial/ethnic differences in progression of islet autoantibodies (from single to multiple) and development of type 1 diabetes; analyses of differences in the first

appearance of autoantibodies will require cohorts that follow individuals from birth. Additionally, caution should be taken in applying our data to the determination of type 1 diabetes risk in the general population, as our study participants were relatives of individuals with type 1 diabetes and thus were at increased risk for the development of type 1 diabetes.

The major strengths of this study were the relatively large sample size, including a significant number of Hispanic individuals, and the availability of comprehensive type 1 diabetes predictive data (e.g. *HLA-DR3-DQ2/DR4-DQ8*, DPTRS, etc.). These characteristics enabled us to compare the races/ethnicities for outcome measures while adjusting for confounding factors.

In conclusion, progression of islet autoimmunity, from single to multiple positive autoantibodies, was less common in Hispanic than in NHW individuals, while differences in progression from multiple autoantibodies to type 1 diabetes were limited to children <12 years old and were modified by BMI. Further research is warranted to investigate factors playing a role in the racial/ethnic heterogeneity of type 1 diabetes pathogenesis. Better insight into these factors will allow for adequate counselling of at-risk individuals and for the development of prediction models and design of prevention trials.

Data availability The datasets generated during and/or analysed during the current study are available in the NIDDK Central Repository.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement All authors are members of the Type 1 Diabetes TrialNet Study Group and, as such, contributed to the data used in this article. MT and MJR designed the study. All authors contributed to result interpretation. SMG performed statistical analyses. MT wrote the initial draft and edited the manuscript. SMG, HR, IL, DAB and MJR reviewed and edited the manuscript. MJR is the guarantor of this work, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors approved the final version of the manuscript.

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