Transcription Factor 7-Like 2 (*TCF7L2*) Gene Polymorphism and Progression From Single to Multiple Autoantibody Positivity in Individuals at Risk for Type 1 Diabetes

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EPIDEMIOLOGY/HEALTH SERVICES RESEARCH

OBJECTIVE

The type 2 diabetes–associated alleles at the *TCF7L2* locus mark a type 1 diabetes phenotype characterized by single islet autoantibody positivity as well as lower glucose and higher C-peptide measures. Here, we studied whether the *TCF7L2* locus influences progression of islet autoimmunity, from single to multiple (≥2) autoantibody positivity, in relatives of patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS

We evaluated 244 participants in the Type 1 Diabetes TrialNet Pathway to Prevention study with confirmed single autoantibody positivity at screening and Immunochip single nucleotide polymorphism data (47.5% male; median age 12.8 years, range 1.2–45.9; 90.2% white). We analyzed risk allele frequency at *TCF7L2* rs4506565 (in linkage disequilibrium with rs7903146). Altogether, 62.6% participants carried ≥1 risk allele. Univariate and multivariable Cox proportional hazards models and Kaplan-Meier statistical methods were used.

RESULTS

During follow-up (median 5.2 years, range 0.2–12.6), 62% of the single autoantibody–positive participants developed multiple autoantibody positivity. In the overall cohort, the *TCF7L2* locus did not significantly predict progression to multiple autoantibody positivity. However, among single GAD65 autoantibody–positive participants (n = 158), those who carried ≥1 risk allele had a lower rate of progression to multiple autoantibody positivity (hazard ratio [HR] 0.65, P = 0.033) than those who did not, after adjustment for HLA risk haplotypes and age. Among subjects who were either IA-2 or insulin autoantibody positive only, carrying ≥1 *TCF7L2* risk allele was not a significant factor overall, but in overweight or obese participants, it increased the risk of progression to multiple autoantibody positivity (HR 3.02, P = 0.016) even with adjustment for age.

CONCLUSIONS

The type 2 diabetes–associated *TCF7L2* locus influences progression of islet autoimmunity, with differential effects by autoantibody specificity and interaction by obesity/overweight.

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

See accompanying article, p. 2441.

The heterogeneity of type 1 diabetes is a barrier to effective prevention and treatment of the disease. This disorder is likely the common result of several different etiologic and pathogenic mechanisms (1) as suggested by the phenotypic diversity of the disease (2). Defining clusters of cases with similar, actionable characteristics could accelerate the discovery of successful interventions.

Transcription factor 7-like 2 *(TCF7L2)* single nucleotide polymorphisms (SNPs) are strongly associated with type 2 diabetes risk, with about 30% increase per risk allele (3), and have been functionally linked to impaired insulin secretion, defects in incretin- and glucose-induced glucagon suppression, abnormal insulin processing, and increased hepatic glucose release during fasting (4–6). *TCF7L2* variants also influence insulin sensitivity (7–9) and β-cell development (10,11). Similar findings have been demonstrated across different races (12,13). More recently, this locus has been shown to influence glucose metabolism in response to glipizide and metformin in healthy volunteers (14). The region contains several SNPs that are in strong linkage disequilibrium (http://gvs.gs.washington.edu/GVS). In U.S. control individuals, the frequencies of heterozygous and homozygous carriers of the at-risk allele were 40.6% and 7.9%, respectively (3).

We previously reported that *TCF7L2* SNP rs7903146 is more frequent in patients with type 1 diabetes expressing a single positive autoantibody than in those with multiple autoantibody positivity and, more recently, that individuals with type 1 diabetes and *TCF7L2* genetic variants have distinct metabolic abnormalities, namely, lower glucose and higher C-peptide measures at the onset of diabetes compared with participants with type 1 diabetes without a *TCF7L2* risk allele (15–17). A potential hypothesis to explain these observations is that individuals who develop autoimmune diabetes but have only mild signs of islet autoimmunity, e.g., single autoantibody positivity, may carry a second diabetogenic factor, such as a *TCF7L2* genetic variant that could impair insulin secretion and/or action. However, since *TCF7L2* is also involved in the differentiation and function of β-cells (10,11,18), which are the target of the autoimmune attack that starts in the preclinical phases of type 1 diabetes, it could also modify the progression of islet autoimmunity. Here, we aimed to understand whether the *TCF7L2* locus influences the conversion from single to multiple islet autoantibody positivity in individuals at risk for type 1 diabetes.

RESEARCH DESIGN AND METHODS

Participants

Type 1 Diabetes TrialNet is a National Institutes of Health (NIH)-funded, international network of centers with the mission to prevent type 1 diabetes and stop its progression (19). The observational arm of the TrialNet Pathway to Prevention (PTP) study (TN01; clinical trial reg. no. NCT00097292, ClinicalTrials.gov) screens first- or second-degree relatives of patients with type 1 diabetes and prospectively monitors islet autoantibody–positive individuals without diabetes for progression of islet autoimmunity and development of this disease (20). Here, we restricted analyses to those participants who had Immunochip data and were classified as confirmed single autoantibody positive on the same autoantibody on two consecutive autoantibody tests obtained within 1 year or less. Two subjects were excluded due to being classified as having a single islet cell autoantibody (ICA), while an additional 10 subjects were excluded because they were diagnosed with diabetes during the screening phase, close to the timing of their confirmation as single autoantibody positive (mean 52 days, range 18–97). After these exclusions, 244 subjects were included in the final data set. All study participants provided informed consent, and the study was approved by the responsible ethics committee at each study site.

Procedures

All subjects were screened for autoantibodies to glutamic acid decarboxylase (GAD65), insulin (microinsulin autoantibody [mIAA] assay, used by the TrialNet PTP study since 2004), and insulinoma-associated antigen 2 (IA-2). If any of these were positive, autoantibodies to zinc transporter 8 (ZnT8A) and ICA were also tested. Oral glucose tolerance tests were conducted with oral glucose administration at a dose of 1.75 g/kg (maximum 75 g). C-peptide (ng/mL) and glucose (mg/dL) levels were measured at fasting and at −10, 0, 30, 60, 90, and 120 min poststimulation.

The assays for islet autoantibody and C-peptide determinations were performed as previously described (20,21). HLA genotyping was conducted by the Type 1 Diabetes Genetics Consortium Laboratories as previously described (22). The Illumina Immunochip was used to genotype *TCF7L2* rs7901695 and rs4506565 at the Center for Public Health Genomics at the University of Virginia. The Immunochip is a custom array of 186,000 SNPs selected from regions of the genome robustly associated with autoimmune diseases.

Statistical Analyses

HLA DR3 was defined as DRB1*03:01-DQA1*05:01-DQB1*02:01 and HLA DR4-DQ8 was defined as DQA1*03:01-DQB1*03:02 with DRB1*04:01, *04:02, or *04:05. We analyzed frequencies of *TCF7L2* rs4506565 and rs7901695, which were in nearly complete linkage disequilibrium (R^2 of 0.913 and 0.909, respectively, in a population of European ancestry) with rs7903146 (http://gvs.gs.washington.edu/GVS). We examined ordered outcomes for numbers of minor (risk) alleles (0, 1, or 2) and found high concordance (99.0%) between the two *TCF7L2* SNPs, rs4506565 and rs7901695 (95% CI 0.98–1.00). Minor allele frequencies were evaluated as an ordered variable as well as dichotomized, i.e., carrier (one or two minor alleles) versus noncarrier (no minor alleles), and also homozygous for the minor allele (two minor alleles) versus not (no or one minor allele). All analyses were conducted for each of these SNPs, although the results presented focus on one (rs4506565, minor allele: T) given the concordance of the results. Hardy-Weinberg equilibrium was verified for the full sample.

All clinical and metabolic factors were summarized and evaluated using descriptive statistics. Time to conversion to multiple autoantibody positivity was defined as the time from confirmed single autoantibody–positive status (i.e., the first time point date) to the time that a subject was identified as having multiple positive autoantibodies. Univariate and multivariable Cox proportional hazards models and Kaplan-Meier methods were used to evaluate factors and their influence on time to conversion to multiple autoantibody positivity. Variable selection models based on all subsets regression analyses were used to identify significant risk factors as well as interactions in relation to risk of

progression to multiple positive autoantibodies (*glmulti* R package). Race and ethnicity were evaluated by univariate analyses, and since they were not significantly associated with the outcomes of interest and the percentage of nonwhite and/or Hispanic subjects in this cohort was very small, this factor was not included in the multivariable analysis.

This study defined BMI categories according to age. For subjects <20 years old, BMI percentiles were calculated based on the age- and sex-based reference values of the U.S. Centers for Disease Control and Prevention (CDC) using the *childsds* package in the R statistical program. Based on CDC criteria, overweight was classified as a BMI at or above the 85th percentile and below the 95th percentile, and obese was classified as a BMI equal to or greater than the 95th percentile. For those ≥20 years old, standard CDC thresholds for BMI were used to classify subjects as overweight (≥25 to <30 kg/m^2) or obese (≥30 kg/m^2).

Cut-point analyses were conducted using recursive partitioning algorithms (http://CRAN.R-project.org/package=rpart) (23). Statistical significance was determined if $P < 0.05$, except for tests of interactions where those with $P < 0.10$ were explored further. All analyses were conducted using the statistical program R version 3.4.3 for Windows (https://CRAN.R-project.org).

RESULTS

Participant characteristics and the comparison between those who carried ≥1 risk allele at the *TCF7L2* locus and those who did not are illustrated in Table 1. The median age was 12.8 years (range 1.22–45.9). Most (81%) of participants were non-Hispanic white. Only 19 individuals were homozygous for the minor allele for rs4506565, and therefore their characteristics were not summarized separately. During the course of follow-up, 151 subjects (62%) progressed to multiple positive autoantibodies, and the median follow-up on participants who had not yet progressed was 5.2 years (range 0.2–12.6).

In the overall cohort, the *TCF7L2* locus was not a significant risk factor for conversion to multiple autoantibody positivity in the univariate (hazard ratio [HR] 0.83, 95% CI 0.6–1.14, $P = 0.24$) or the multivariable setting adjusting for age, HLA status, and specific type of positive autoantibody (HR 0.81, 95% CI 0.59–1.12, $P = 0.21$). However, variable selection modeling revealed significant interactions between being positive for GAD65 autoantibody and carrying ≥1 risk allele (vs. carrying none; HR 0.53, 95% CI 0.35–0.82, $P = 0.004$) as well as having a high-risk HLA haplotype (i.e., DR3-DQ2 and/or DR4-DQ8 vs. neither one; HR 1.67, 95% CI 1.1–2.51, $P = 0.014$). Therefore, we explored further the relationships between carrying ≥1 risk allele and other factors with conversion from single to multiple autoantibody positivity in two separate strata, namely, participants with GAD65 autoantibody positivity versus those who were GAD65 autoantibody negative (i.e., their single positive autoantibody was either IA-2 or

Table 1—Baseline characteristics of study participants, overall and by subgroup, i.e., participants with ≥1 risk allele versus none at the *TCF7L2* locus

Characteristic	All subjects, *N* = 244	≥1 risk allele, *N* = 135	No risk alleles, *N* = 109	*P* value
Sex				0.93
Female	128 (52.5)	70 (51.9)	58 (53.2)	
Male	116 (47.5)	65 (48.1)	51 (46.8)	
Age at confirmed single Ab+ (years)				0.50
Median	12.8	13.45	12.5	
Range	1.22–45.9	1.22–45.9	1.3–45.7	
Islet Ab+ type				0.71
GAD65	158 (64.8)	87 (64.4)	71 (65.1)	
IA-2	17 (6.7)	11 (8.1)	6 (5.5)	
mIAA	69 (28.3)	37 (27.4)	32 (29.4)	
Race				0.46
White	220 (96.5)	119 (96)	101 (97.1)	
Black/African American	3 (1.3)	2 (1.6)	1 (1.0)	
Asian	1 (0.4)	1 (0.8)	0 (0.0)	
Multiracial	4 (1.8)	2 (1.6)	2 (1.9)	
Unknown/missing	16	1	5	
Ethnicity				0.45
Hispanic/Latino	23 (9.7)	12 (9.0)	11 (10.4)	
Non-Hispanic/Latino	214 (90.3)	121 (91.0)	93 (89.4)	
Unknown/missing	7	2	5	
Fasting C-peptide (ng/mL)				0.71
Median	1.52	1.56	1.49	
Range	0.38–7.3	0.38–5.13	0.43–7.3	
Missing	2	2	0	
C-peptide AUC				0.10
Median	649.9	628.5	681.8	
Range	197.8–3,089.4	197.8–1,652.3	242.4–3,089.4	
Missing	12	9	3	
OGTT status				0.44
Normal	196 (81.7)	105 (79.5)	91 (84.6)	
Abnormal	44 (18.3)	27 (20.5)	17 (15.7)	
Missing time point	4	3	1	
HLA DR3 and DR4				0.21
No	217 (92.3)	117 (90.0)	100 (95.2)	
Yes	18 (7.7)	13 (10.0)	5 (4.8)	
Missing	9	5	4	
HLA DR3 and/or DR4				0.999
No	74 (31.5)	41 (31.5)	33 (31.4)	
Yes	161 (68.5)	89 (68.5)	72 (68.6)	
Missing	9	5	4	
BMI category				0.033
Normal	131 (58.2)	78 (61.9)	53 (53.5)	
Overweight	48 (21.3)	18 (14.3)	28 (28.3)	
Obese	46 (20.4)	30 (23.8)	18 (18.2)	
Missing	19	9	10	

Data are *n* or *n* (%) unless otherwise indicated. *P* values are given for the comparison of the characteristic between groups. Continuous measures were compared using Wilcoxon rank sum test. Categorical variables were compared using χ^2 tests (or Fisher exact tests if cell sizes too small). Ab+, autoantibody positivity; AUC, area under the curve; OGTT, oral glucose tolerance test.

mIAA). We found that among participants who were positive for GAD65 autoantibody, carrying ≥1 risk allele at the *TCF7L2* locus was an independently significant, negative factor of progression from single to multiple autoantibody positivity; the risk of progression in carriers of ≥1 risk allele at the *TCF7L2* locus was significantly lower than in noncarriers (HR 0.65, 95% CI 0.44–0.97, $P = 0.033$) after adjustment for age and HLA. In addition, our analyses showed that carrying at least one of the two highest risk HLA haplotypes (i.e., HLA DR3-DQ2 and/or HLA DR4-DQ8) significantly increased the risk of progression in this group (HR 1.66, 95% CI 1.02–2.7, $P = 0.04$) (Table 2). Among individuals who were negative for GAD65 autoantibodies (i.e., were positive for IA-2 or mIAA), neither the *TCF7L2* locus (HR 0.74, $P = 0.46$) nor type 1 diabetes–associated HLA risk alleles (HR 1.1, $P = 0.78$) were significant in the multivariable model. However, in the GAD65 autoantibody–negative subjects, there was a significant interaction between carrying ≥1 minor allele at the *TCF7L2* locus and being overweight/obese (HR 3.84, $P = 0.03$). Specifically, in GAD65 autoantibody–negative subjects who were not overweight or obese, there was no significant effect of *TCF7L2* carrier status on their progression risk (HR 0.72, $P = 0.45$). On the contrary, GAD65 autoantibody–negative subjects who were overweight or obese had significantly greater risk of progression to multiple positive autoantibodies versus noncarriers (HR 3.02, 95% CI 1.23–7.45, $P = 0.016$) after adjustment for age (Table 2). HLA was not a significant factor in this subgroup ($P = 0.66$), and, with adjustment for HLA, the effect of *TCF7L2* carrier status was still significant (HR 2.88, $P = 0.023$). These same results held even when focusing only on participants who were non-Hispanic white ($n = 198$). For the GAD65-positive non-Hispanic white subjects ($n = 123$), carrying ≥1 risk allele was a significant factor of progression even after adjusting for age (HR 0.64, $P = 0.045$). In the non-Hispanic white participants who were GAD65 negative ($n = 75$), we saw the same type of interaction effect between carrier status and being overweight or obese; further, there was a more pronounced effect of carrier status on time to multiple autoantibody positivity in overweight/obese non-GAD65 autoantibody–positive subjects (HR 4.29, 95% CI 1.48–12.45, $P = 0.007$).

We then determined the risk and rate of progression from single to multiple autoantibody positivity in the four groups defined by carrying ≥1 risk allele or none and by initially being single positive for GAD65 autoantibody or not (Fig. 1). The distributions of time to multiple positivity between these groups were significantly different ($P = 0.017$). We observed the highest risk and rate of progression to multiple autoantibody positivity in the participants who were initially single GAD65 autoantibody positive and did not carry any risk allele at the *TCF7L2* locus (5-year risk 76%, 95% CI 62–84). In contrast, those who were GAD65 positive but carried ≥1 risk allele had lower risk (5-year risk 68%, 95% CI 55–77), although this pairwise comparison at the specific time point of 5 years was not statistically significant (Supplementary Table 2). Among participants initially single IA-2 or mIAA (not GAD65) autoantibody positive, those who carried ≥1 risk allele and were overweight/obese ($n = 18$) had a 5-year risk of progression to multiple positivity of 83% (95% CI 53–94), which is significantly higher than that of the other three groups combined (HR 3.17, 1.66–6.05, $P = 0.0005$) with adjustment for age (Fig. 2 and Supplementary Table 3).

CONCLUSIONS

We studied 244 TrialNet participants with confirmed positivity for a single islet autoantibody and found that among those initially positive only for GAD65 autoantibody, participants carrying ≥1 risk allele at the *TCF7L2* locus had a 44% lower risk and rate of progression to multiple islet autoantibodies than individuals carrying no risk alleles. Furthermore, among single GAD65 autoantibody–positive participants, those lacking a minor allele at the *TCF7L2* locus but carrying a type 1 diabetes–associated HLA haplotype (i.e., DR3-DQ2 or DR4-DQ8) were at the highest risk of progression to multiple autoantibody positivity. In participants who initially had single positive mIAA or IA-2 (not GAD65) autoantibody and were obese or overweight, carrying ≥1 risk allele at the *TCF7L2* locus marked a faster progression to multiple autoantibody positivity.

Our findings that the type 2 diabetes–associated *TCF7L2* locus is associated with lower rate of progression of islet autoimmunity among single GAD65 autoantibody–positive participants, particularly if type 1 diabetes HLA haplotypes

Table 2—Multivariable analyses of time of conversion from confirmed single GAD65 or non-GAD65 autoantibody (i.e., either mIAA or IA-2 autoantibody) positivity to multiple autoantibody positivity

Time from confirmed single GAD65 autoantibody to multiple autoantibody positivity

Factor	HR (95% CI)	*P* value
TCF7L2 rs4506565_T variant (1 or 2 minor alleles vs. 0)	0.65 (0.44–0.97)	0.033
Age at single GAD65 autoantibody positivity	0.99 (0.98–1.006)	0.26
HLA DR3-DQ2 and/or DR4-DQ8	1.66 (1.02–2.7)	0.04

Time from either mIAA or IA-2 autoantibody to multiple autoantibody positivity stratified by whether or not participants were overweight/obese*

	Not overweight/obese ($n = 51$, 25 events)		Overweight/obese ($n = 33$, 22 events)	
Factor	HR (95% CI)	*P* value	HR (95% CI)	*P* value
TCF7L2 rs4506565_T variant (1 or 2 minor alleles vs. 0)	0.80 (0.36–1.79)	0.59	3.02 (1.23–7.45)	0.016
Age at single mIAA or IA-2 autoantibody positivity	0.97 (0.93–1.01)	0.14	0.97 (0.94–0.99)	0.008

*HLA was removed from the model for being statistically nonsignificant.

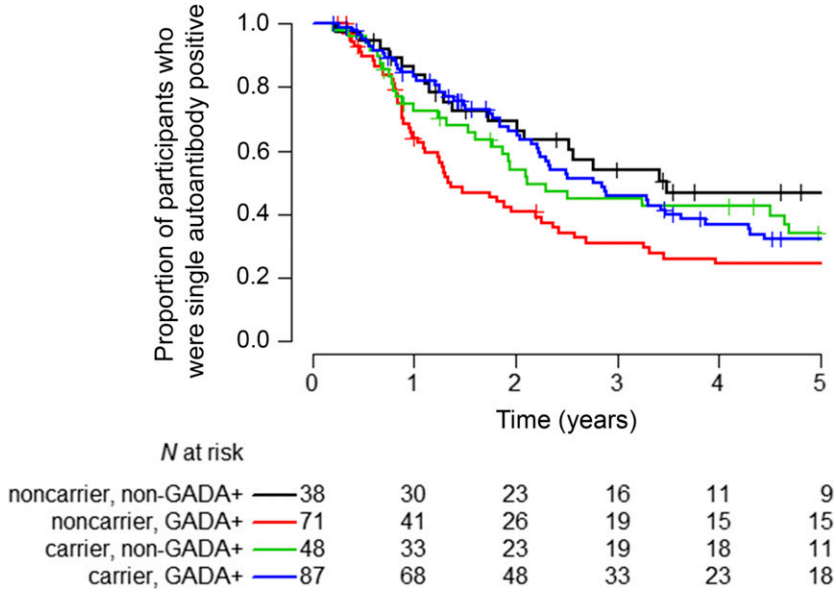

Figure 1—Time to conversion from confirmed single autoantibody positivity to multiple autoantibody positivity in relatives of patients with type 1 diabetes (n = 244), by *TCF7L2* carrier status and type of islet autoantibody positive (GAD65 autoantibody positive [GADA+] vs. not). P value = 0.017. The numbers at the bottom indicate the number of participants still followed at each time point.

are absent, suggest that these individuals may be protected to a certain degree from the spreading of the autoimmune response. It is possible that autoantibody-positive individuals with the *TCF7L2* risk allele may have the metabolic abnormalities that characterize type 2 diabetes and may develop diabetes that displays characteristics intermediate between type 1 and type 2 diabetes. Supporting the latter hypothesis, in a previous study, we demonstrated that TrialNet participants with new-onset autoimmune type 1 diabetes who carried the *TCF7L2* risk allele were more likely to have a single positive autoantibody and had lower glucose and higher C-peptide measures as compared with participants who did not carry any risk allele at the *TCF7L2* locus (17). Our present results suggest that their islet autoimmunity is less likely to progress from single to multiple autoantibody positivity. A recent study found that the type 2 diabetes–associated *HNF1A* locus is also more common in individuals with latent autoimmune diabetes in adults (LADA) (24), which also highlights the genetic underpinnings of the phenotypic heterogeneity of autoimmune diabetes (2).

One possible explanation for our findings that GAD65-positive participants who carry ≥1 risk allele at the *TCF7L2* locus are less likely to progress to multiple autoantibody positivity is that *TCF7L2* is involved in β-cell differentiation. The expression of dominant-negative *TCF7L2* in murine β-cells led to abnormalities of β-cell gene expression, specifically for Ccnd1, Ccnd2, Irs1, Irs2, Ins1, Ins2, and Mafa (25). In a recent study, inactivation of pancreatic pericytic *TCF7L2* in transgenic mice was associated with impaired expression of genes involved in β-cell function and maturation (18). It is conceivable that these abnormalities prevent the β-cell from being recognized by the immune system and therefore the islet autoimmunity process slows down. Studies to test this hypothesis may include demonstrating protection from diabetes after introduction of a comparable *TCF7L2* mutation into a suitable murine model.

The interaction between the *TCF7L2* locus and single GAD65 autoantibody (but not other autoantibody types) as well as the observation that the type 1 diabetes–associated HLA risk haplotypes influenced progression of single to multiple autoantibody positivity only among GAD65-positive individuals and not in GAD65-negative participants is consistent with emergent literature that the first type of islet autoantibody to appear marks a distinct type 1 diabetes phenotype. Specifically, children participating in the TEDDY (The Environmental Determinants of Diabetes in the Young) study who developed first positivity for GAD65 autoantibodies, as opposed to mIAA, were older, more likely to have HLA DR3/DR3, less likely to have DR4/DR8,

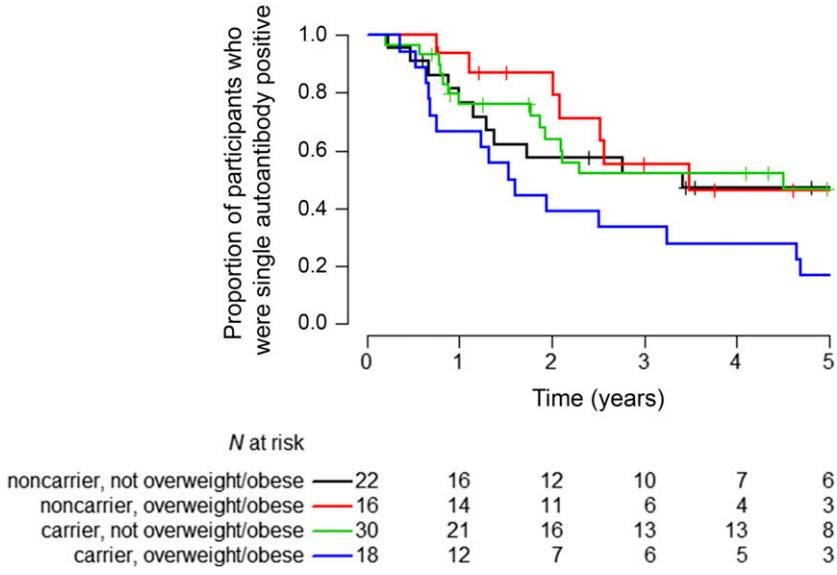

Figure 2—Time to conversion from confirmed single autoantibody positivity to multiple autoantibody positivity in non-GAD65 autoantibody–positive relatives of patients with type 1 diabetes (n = 86), by *TCF7L2* carrier status and overweight/obese status (four-group comparison, P = 0.046). The subgroup of participants who carried ≥1 risk allele at the *TCF7L2* locus and were overweight/obese was statistically different from the other three groups combined (P = 0.0005) with adjustment for age and HLA. The numbers at the bottom indicate the number of participants still followed at each time point.

and less likely to progress to multiple autoantibody positivity (26). These findings and the current study suggest that the diabetogenic mechanisms in individuals who develop GAD65 as their first islet autoantibody may be different from those in individuals who develop mIAA or IA-2 as their first autoantibody.

Interestingly, in participants with mIAA or IA-2 as first autoantibody, who have type 1 diabetes–associated characteristics more frequently than participants with GAD65 as first autoantibody (26), carrying ≥1 risk allele at the *TCF7L2* locus was associated with increased progression of islet autoimmunity in the presence of elevated BMI. Obese and overweight individuals with single mIAA or IA-2 autoantibodies were more likely to progress to multiple autoantibody positivity in the presence of ≥1 risk allele. We have previously demonstrated that elevated BMI accelerates the diagnosis of type 1 diabetes, with differences by age (27,28). Obesity-induced adipokines have been proposed to contribute to the development of other autoimmune diseases (29–31), although these results need confirmation and the mechanism is still unclear. Previous studies have demonstrated an association between *TCF7L2* SNPs and BMI (32) as well as their interaction upon diabetes risk (33,34). A possible mediator of these relationships is ACSL5, a protein that influences fatty acid metabolism and whose expression is regulated by *TCF7L2* (7). Further studies are needed to understand the potential role of adipokines as mediators of the interaction between obesity and the *TCF7L2* locus upon progression of islet autoimmunity.

One of the limitations of the study is the relative small size of some of the groups; for example, that of participants who were initially single IA-2 or mIAA autoantibody positive, carried ≥1 risk allele, and were overweight/obese. However, TrialNet is the largest study on the natural history of islet autoimmunity and type 1 diabetes development in at-risk individuals who have been prospectively followed with extensive immunologic, metabolic, and genetic characterization. We find some compelling exploratory findings worth evaluating further in a larger cohort. Since TrialNet participants are not selected by HLA types, we were able to study their effect on the outcomes of interest. In particular, we observed that carrying high-risk HLA haplotypes (i.e., HLA DR3-DQ2 and/or HLA DR4-DQ8) significantly increased the risk of progression among GAD65-positive participants, while that was not the case among GAD65-negative participants. Since there could be falsely positive islet autoantibody test results, as with any other test, and there may be fluctuation in autoantibody titers, we only considered eligible subjects those who had a single positive autoantibody on two consecutive evaluations that were at most 1 year apart. This strategy was implemented in the TrialNet study design and in our analysis to minimize the number of false positives. With persistent autoantibody positivity, these individuals are very likely to have true islet autoimmunity.

Taken together, our findings demonstrate that the type 2 diabetes–associated *TCF7L2* risk allele marks a lower risk of progression to multiple autoantibody positivity among single GAD65 autoantibody–positive participants, particularly if type 1 diabetes risk HLA haplotypes are absent. In single mIAA or IA-2 autoantibody participants, the *TCF7L2* risk allele interacts with obesity/overweight to mark faster progression to multiple autoantibody positivity. These findings suggest that the *TCF7L2* locus plays a role in the development of islet autoimmunity in subsets of individuals and support an interrelationship between genetic, immunologic, and metabolic factors in the pathogenesis of autoimmune type 1 diabetes.

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