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A type 1 diabetes genetic risk score predicts progression of islet autoimmunity and development of type 1 diabetes in individuals at risk

Short running title: Genetic risk score in type 1 diabetes prediction

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Abbreviations: SNP: Single Nucleotide Polymorphism; PTP: Pathway to Prevention, OGGT: Oral Glucose Tolerance Test, GAD65: Glutamic Acid Decarboxylase 65, IA-2A: Insulinoma-Associated Antigen 2, ZnT8: Zinc transporter 8, ICA: Islet Cell Antibodies, AUC: Area under the curve.

Key Words: Type 1 diabetes, genetics, risk, score, prediction, TrialNet, autoantibodies, HLA, non-HLA, DPTRS, age, SNP

ABSTRACT

Objective: We tested the ability of a type 1 diabetes (T1D) genetic risk score (GRS) to predict progression of islet autoimmunity and T1D in at-risk individuals.

Research Design and Methods: We studied the 1,244 TrialNet Pathway to Prevention study participants (non-diabetic, autoantibody-positive (Ab+) relatives of patients) who were genotyped with Illumina ImmunoChip (median [range] age at initial autoantibody determination=11.1 years [1.2-51.8], 48% male, 80.5% non-Hispanic White; median follow-up=5.4 years). Of 291 participants with single Ab+ at screening, 157 converted to multiple Ab+, and 55 developed diabetes. Of 953 participants with multiple Ab+ at screening, 419 developed diabetes. We calculated the T1D GRS from 30 T1D-associated SNPs. We used multivariable Cox regression models, time-dependent ROC curves and AUC measures to evaluate prognostic utility of T1D GRS, age, sex, Diabetes Prevention Trial-1 Risk Score (DPTRS), Ab+ number or type, HLA DR3/DR4-DQ8 status, and race/ethnicity. We used recursive partitioning analyses to identify cut-points in continuous variables.

Results: Higher T1D GRS significantly increased the rate of progression to T1D adjusting for DPTRS, age, Ab+ number, sex, and ethnicity (HR=1.29 for a 0.05 increase, 95%CI=1.06-1.6, P=0.011). Progression to T1D was best predicted by a combined model with GRS, Ab+ number, DPTRS and age (7-year time-integrated AUC=0.79, 5-year AUC=0.73). Higher GRS was significantly associated with increased progression rate from single to multiple Ab+ after adjusting for age, Ab+ type, ethnicity, and sex (GRS>0.295, HR=2.27, 95%CI=1.47-3.51, P=0.0002).

Conclusion: The T1D GRS independently predicts progression to T1D, and improves prediction along T1D stages in Ab+ relatives.

Early identification of individuals at risk for type 1 diabetes (T1D) allows study of the biology of the preclinical stages of T1D, and inclusion of those at highest T1D risk in T1D monitoring and prevention trials. Current prediction models for T1D use immunologic and metabolic markers but these change during disease progression and reflect advanced stages in the autoimmune process (1-8) whereas genetic predictors are time-independent and may be assessed only once at study entry. T1D has a significant heritable risk as evidenced by studies of monozygotic twins demonstrating rates of concordance >50% for disease, higher with younger age at diagnosis of the index twin (9; 10). Approximately 50% of this heritability is attributable to the HLA region (11), with another >50 loci making smaller contributions to disease risk (reviewed in (12-14)). Recently, Oram et al. developed and validated a T1D genetic risk score (T1D GRS) that incorporates HLA and non-HLA T1D-associated SNPs (15) and was discriminative of T1D from type 2 diabetes, monogenic diabetes and controls (16). In this study, we tested the prognostic utility of the T1D GRS for differentiating rates of progression of islet autoimmunity and development of clinical T1D in autoantibody-positive relatives of individuals with T1D.

RESEARCH DESIGN AND METHODS

Participants

TrialNet is a NIH-funded international network that aims to prevent T1D (17). TrialNet Pathway to Prevention (PTP) is an observational study that prospectively follows at-risk first or second-degree relatives of patients with T1D for development of islet autoimmunity and clinical T1D (18). This study included TrialNet PTP participants who had ≥1 positive persistently detectable islet autoantibody and had been genotyped using the Illumina ImmunoChip (n=1,244). Study participants gave informed consent and the study was approved by ethics committees at each site.

Procedures

Participants were initially screened for autoantibodies to glutamic acid decarboxylase (GAD65), insulin (microinsulin antibody assay, mIAA) and insulinoma-associated antigen 2 (IA-2A). If any of these were positive, autoantibodies to zinc transporter 8 (ZnT8) and islet cell antibodies (ICA) were tested. Participants were monitored with autoantibody testing, hemoglobin A1c (HbA1c) and oral glucose tolerance test (OGTT) at 6- or 12-month intervals depending on estimated risk (19). T1D was diagnosed in participants with: i) symptomatic hyperglycemia, defined as fasting plasma glucose ≥7.0 mmol/L, 2-hour plasma glucose after 75g oral glucose ≥11.1 mmol/L, a random plasma glucose ≥11.1 mmol/L or a HbA1C ≥ 6.5%; or ii) asymptomatic hyperglycemia documented on two separate occasions. Islet autoantibody (18) and C-peptide (20) assays have been previously described. HLA genotyping was performed as previously described (21). Illumina ImmunoChip genotyping was performed at the Center for Public Health Genomics, University of Virginia. The Diabetes Prevention Trial-1 (DPT-

1) Risk Score is a diabetes risk score derived from ICA-positive individuals and validated in TrialNet that combines BMI, age, glucose, and C-peptide (2; 22). We stratified our analysis by a metabolic DPT-1 Risk Score of ≤7 or >7 based on previous work (23).

T1D GRS

The T1D GRS was calculated from 30 variants known to be associated with T1D (Supplementary Table S2), ranked and weighed by published odds ratios as previously described (15). We drew ORs for each SNP from the largest available meta-analysis study that used T1Dbase (https://www.t1dbase.org/page/Welcome/display). Twenty-nine of these variants were directly genotyped whereas rs11755527 was imputed using IMPUTE2 (r^2=0.99997). rs2187668 and rs7454108 were used to determine HLA DR haplotype (24). The T1D GRS threshold that was previously shown to optimally discriminate T1D from T2D was 0.280 (15). T1D GRS percentiles in a reference T1D population (25) are provided to allow comparisons between different genetic scores. The same methods were used to calculate a 10-SNP score using the top 10 T1D-associated SNPs (Supplementary Table S2), which account for most of the genetic risk. We assessed the predictive power of both the 10 SNP and 30 SNP scores.

Statistical Analyses

We used summary statistics and graphical analyses to assess the distributions and characteristics of the clinical and metabolic measures as well as the T1D GRS, overall and by subgroup. Comparisons between subgroups were made using primarily nonparametric approaches, e.g. Wilcoxon rank sum or Kruskal-Wallis tests, and the chi-square or Fisher exact tests, as appropriate.

Kaplan-Meier methods were used to evaluate the time-to-event distributions for time to progression to T1D and time from single to multiple autoantibody positivity overall and in subgroups (see Supplementary Table S3 for definitions). Cox proportional hazards models were used to test the prognostic influence of these measures on these outcomes in univariate and multivariable settings. Models were adjusted for age, sex, and race/ethnicity. For models of time to conversion from single to multiple autoantibodies, we also adjusted for autoantibody type (i.e. GAD65, insulin or IA-2A). For time-to-T1D models, we additionally adjusted for DPT-1 Risk Score and the number of positive autoantibodies present at screening. T1D GRS, age, and DPT-1 Risk Score were each evaluated as continuous and dichotomized factors. We assessed whether T1D GRS added predictive power independently over HLA DR3/DR4-DQ8 status by including DR3/DR4-DQ8 in initial multivariate analyses; the HLA DR3/DR4-DQ8 variable was then removed from the final models due to the overlap between the two variables (i.e., HLA DR3/DR4-DQ8 is included in the T1D GRS) causing collinearity. Recursive partitioning analyses (risk-stratification method based on classification and regression trees) were used to identify variables and associated cut-points that best differentiated outcome-specific risk (*rpart* package in R) (26). To obtain stable hazard ratio (HR) estimates reflecting meaningful unit changes in the continuous 30-SNP T1D GRS measure, we multiplied this measure by a constant (x20) when included as a continuous factor in models. All reported HRs for continuous T1D GRS measures reflect this multiplier and reflect HRs associated with an increase of 0.05 in the T1D GRS.

The predictive accuracy of models for time to progression to multiple autoantibodies and to T1D was evaluated for T1D GRS (or HLA), islet autoantibody

number, age, and DPT-1 Risk Score using time-dependent AUC analyses (*survAUC* in R*).* Time-integrated AUC measures were calculated for each model in addition to year-specific AUCs on subjects with complete data for the multivariable models, consistent with standard AUC goodness-of-fit measures. In addition, to evaluate if GRS added more to our prognostic models than HLA, we directly compared the GRS vs. HLA models as well as when combined with clinical factors (DPT-1 Risk Score, age, autoantibody number). Time-integrated AUC estimates were limited to 7 years given that the 3rd quartile for follow-up in event-free participants in the overall cohort was just over 7 years. Predictive accuracy between models was compared at major time points and reflect comparisons of estimated 5-year AUCs unless stated otherwise (*timeROC* package in R).

RESULTS

Characteristics of TrialNet participants in this study (N=1,244) are presented in Supplementary Table S4. The median age at autoantibody determination was 11.1 years (range 1.2-51.8), 48% were male, 81% non-Hispanic White, and 90% first-degree relatives of a patient with T1D. The estimated median follow-up was 5.4 years [95%CI=5.0-5.8 years]. Of the 291 participants positive for a single antibody, 157 progressed to multiple autoantibody positivity and 55 developed T1D. Of the 953 participants who had multiple antibodies when initially screened, 419 developed T1D.

Overall, the 30-SNP T1D GRS ranged from 0.138 to 0.341 (median=0.272, corresponding to the 38^{th}-39^{th} percentiles in the reference T1D population (25)). The median T1D GRS for single and multiple autoantibody positive subjects were 0.266 (30^{th} percentile; range=0.138-0.341) and 0.274 (41st percentile; range=0.169-0.328), respectively.

The T1D GRS is an independent predictor of clinical T1D in islet autoantibody positive relatives

The T1D GRS was a significant predictor of risk and rate of progression to T1D in continuous univariate analysis (HR=1.7, 95% CI: 1.43-2.0; P<0.0001) as well as after adjustment for other risk factors (Supplementary Table S5). Of note, with inclusion of the T1D GRS in the multivariable model, HLA DR3/DR4-DQ8 was no longer significant (HR=1.06, 95% CI: 0.79-1.41, P=0.71, data not shown). The best predictive model of progression to T1D, with a 7-year time-integrated AUC of 0.794, included GRS, the

metabolic DPT-1 Risk Score, age at autoantibody determination and number of positive autoantibodies (Supplementary Table S5). The GRS remained a significant predictor in this model (HR=1.29, 95%CI: 1.06-1.56; P=0.009). Since we observed a significant interaction between T1D GRS and DPT-1 Risk Score (P=0.001) as well as between GRS and autoantibody number (P=0.001), next we also analyzed models of progression to T1D stratified by these features.

Interaction and stratified analyses revealed that GRS is best able to further differentiate T1D risk in those participants with a baseline metabolic DPT-1 Risk Score ≤7.0 (N=716, which represents 63% of 1136 participants with DPT-1 Risk Score data available at baseline) (HR=1.66, 95%CI:1.18-2.34, P=0.003) even after adjusting for age, autoantibody number, sex, ethnicity, and DPT-1 Risk Score (Supplementary Table S5b). Although those with a DPT-1 Risk Score >7 had a higher T1D GRS than those with DPT-1 Risk Score ≤7 (0.274 (0.026) vs. 0.268 (0.028), P=0.002), the GRS did not further stratify the risk of T1D in participants who had already developed metabolic abnormalities, as reflected by a DPT-1 Risk Score>7.0 (HR=1.07, 95%CI=0.81-1.41, P=0.64).

Since ICA and GAD65 autoantibodies may overlap [NEED REF BUT WE'RE AT MAX ALLOWED], we performed sensitivity analyses with the 167 (out of 1244) subjects who were only positive for ICA and GAD65 in this cohort and observed that their classification as positive for one versus two autoantibodies yielded similar results and consistent estimates.

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Multivariable recursive partitioning models identified variable cut-points and five risk clusters (Figure 1 and Supplementary Figure 1). The optimal cut-point for GRS in relation to time to progression to T1D was 0.250. DPT-1 Risk Score >7 identified the

highest risk group, while in those with DPT-1 Risk Score ≤7, the risk of T1D could be further stratified according to autoantibody number and T1D GRS. To assess the improvement of T1D prediction when including T1D GRS with established predictors, we calculated time-dependent ROC curves integrated across all time points (iAUC) and standard ROC curves for the 2- and 5-year time point. For the overall at-risk cohort with complete data on these factors (N=1106, 415 events), the iAUCs were 0.57 for T1D GRS, 0.53 for HLA, 0.59 for autoantibody number, 0.59 for age, and 0.774 for DPT-1 Risk Score. The iAUC for the final composite risk model (i.e. T1D GRS, metabolic DPT-1 Risk Score, age, and autoantibody number) was 0.794. Given that we identified that GRS has the most prognostic utility in participants with DPT-1 Risk Scores ≤7, we also evaluated the time-dependent ROC and AUC measures in those with complete data on these factors (N=696, 132 T1D events). In this subset, we found similar patterns of iAUC for these factors. We observed that the model with GRS combined with the "clinical" variables (i.e., DPT-1 Risk Score, age and autoantibody number) had significantly better prediction accuracy than the model with HLA combined with the clinical variables, although this was significant at earlier time points (i.e. ROC and AUC estimates for up to 3 years). For example, the 2-year AUC for the clinical+HLA model was 0.78 vs. 0.82 for the clinical+GRS model ($p<0.0001$; Figure 2). Similarly, we observed that, in the participants with lower metabolic risk, the T1D prediction model that combined GRS in addition to the clinical variables DPT-1 Risk Score, age and autoantibody number performed significantly better than HLA DR3/DR4-DQ8 in addition to the clinical variables (iAUC: 0.60 vs. 0.53; $p=0.007$).

The T1D GRS is an independent predictor of progression from multiple islet autoantibody positivity to T1D

There were 953 participants who were identified as having multiple autoantibody positivity at screening and 157 additional participants who developed multiple positive autoantibodies during follow-up, for a total of 1,110 multiple autoantibody positive participants in our cohort. After adjusting for age and DPT-1 Risk Score, the T1D GRS was a significant independent prognostic factor for time to progression to T1D as a continuous (P=0.015) and as a dichotomized variable (cut-point=0.250, P=0.017, Supplementary Table S6). Among multiple autoantibody positive participants with lower metabolic DPT-1 Risk Score, high T1D GRS was a significant factor in multivariable analysis (T1D GRS≥0.250, HR=2.07, 95%CI=1.21-3.55, P=0.008) (Supplementary Table S6b). Five-year T1D-free rate estimates were 89% for those with a low T1D GRS (<0.250) versus 77% in participants with high T1D GRS (≥0.250). The risk of progressing from multiple islet autoantibody positivity to T1D could be stratified by the composite grouping of DPT-1 Risk Score, age and T1D GRS (Figure 3). Time-to-event ROC and AUC analyses demonstrated that the addition of GRS to the model with age and DPT-1 Risk Score improved the prediction model for T1D in a similar manner to that seen in all autoantibody positive participants with DPT-1 Risk Scores ≤7 (2-year AUC: clinical+HLA=0.68 vs. clinical+GRS=0.73; p<0.0001). Interestingly, the GRS improved the prediction afforded by HLA DR3/DR4-DQ8 alone (iAUC: 0.647 vs. 0.564, respectively; p=0.006).

The T1D GRS is an independent predictor of progression of islet autoimmunity

In our cohort, 157 of the 291 single autoantibody positive participants progressed to multiple islet autoantibody positivity. Elevated T1D GRS was associated with progression from singe to multiple autoantibody positivity, where an increase of 0.050 (e.g. from 0.225 to 0.275) significantly increased risk by 50% (HR=1.49, 95%CI=1.1-2.05, P=0.015) after adjustment for age, sex, ethnicity, and autoantibody type (Supplementary Table S7).

Recursive partitioning identified 0.295 (69-70th percentiles) as the optimal cut-point to discriminate individuals with the highest rate of progression from single to multiple autoantibody positivity. Single autoantibody positive participants whose T1D GRS exceeded 0.295 had above two times higher risk of autoantibody progression (HR=2.27, 95%CI=1.47-3.51, P=0.0002) even adjusting for age, autoantibody type, sex, and ethnicity.

We observed a potential interaction between T1D GRS and age at first autoantibody determination (P=0.052). In participants younger than 35 years (n=229), after adjusting for age, sex, ethnicity, and autoantibody type, T1D GRS was a significant predictor of progression to multiple Ab+, both as a continuous (HR=1.65, 95%CI=1.15-2.37, P=0.0065) and dichotomous variable, with a cut-point of 0.295 (HR=2.57, 95%CI=1.6-4.13, P=0.0001) but also 0.250 (HR=1.68, 95%CI=1.07-2.64, P=0.023). On the other hand, in older participants (≥35 years of age when classified as single autoantibody positive), who were at much lesser risk of T1D overall, the T1D GRS did not significantly inform the risk and prognosis for progression to multiple autoantibody positivity after adjusting for autoantibody type and sex (age was not significant and thus

was excluded from the model) although the numbers were relatively smaller (n=62, HR=0.86, 95%CI=0.25-2.96, P=0.81) (Figure 4).

In time-dependent ROC analysis, the T1D GRS alone delivered an iAUC of 0.55 compared to 0.53, 0.52 and 0.53 for age, autoantibody type and HLA DR3/DR4-DQ8 heterozygosity, respectively. The iAUC of a multivariable model that combined age, autoantibody type and T1D GRS was 0.581.

A reduced 10-SNP T1D GRS performed similarly to the T1D GRS in predicting islet autoimmunity progression and T1D

We evaluated the performance of a T1D GRS based on the top 10 SNPs (listed in (15)) (T1D GRS-10), using the same analytic approach as for the 30-SNP measure. In multivariable analysis, the T1D GRS-10 predicted progression to T1D in all subjects (HR=1.16 for each increase by 0.10 in score, 95%CI=1.03-1.31, P=0.014) and in the subgroup of multiple positive autoantibody subjects (HR=1.15, 95%CI= 1.02-1.30, P=0.024). Similarly to the 30 SNP score, the 10-SNP GRS was only a significant factor in those with the metabolic DPT-1 Risk Score <7 (P=0.0026). T1D GRS-10 also predicted progression from single to multiple autoantibody positivity after adjusting for age, sex, ethnicity, and autoantibody type (HR=1.26 for a 0.1 increase in T1D GRS-10, 95%CI=1.03-1.55, P=0.026). The overall predictive power of GRS-10 was similar to that of GRS-30 (iAUC=0.575).

CONCLUSIONS

We studied 1,244 initially non-diabetic, islet autoantibody positive relatives of patients with T1D and demonstrated that the T1D GRS is an independent predictor of progression of islet autoimmunity and development of clinical T1D. The T1D GRS improved current prediction models by stratifying risk among individuals who were either single or multiple autoantibody positive. We demonstrated that the combined modeling of the T1D GRS, which includes HLA and non-HLA factors, in addition to autoantibody and metabolic data offers better prediction of T1D in at-risk relatives. This approach could increase our ability to predict T1D in relatives of patients, as well as screen and select participants for natural history studies and intervention trials.

This study adds to the recent expanding literature on the applicability of genetic information in the prediction of T1D. The T1D GRS used in the present study was originally developed and validated to distinguish T1D and type 2 diabetes in the Wellcome Trust Case Control Consortium (n=3,887) and in a cohort defined by insulin insufficiency (15). The score was also able to discriminate T1D and maturity-onset diabetes in the young (MODY) and, in neonatal diabetes, individuals with monogenic neonatal diabetes (16). Our present findings extend the use of the T1D GRS to prediction of T1D in relatives at risk. There have been previous attempts to develop genetic scores that integrate genetic information to improve the prediction of T1D (reviewed in (27)). In particular, it was shown that the combination of HLA and non-HLA genetic factors increases the power of the T1D predictive model (28-31). Winkler et al (29) developed a genetic score using logistic regression and Bayesian feature selection of T1D Genetic Consortium to define a set of 10 SNPS, including HLA, that identified risk of T1D in first-degree relatives from

the BABYDIAB study. Our score, although generated from a log-additive model, contains very similar genetic information so it is not surprising that the results are consistent. A key additional benefit of our T1D GRS is the inclusion of SNPs tagging other significant HLA risk alleles, e.g. HLA DRB1*15, DRB1*57 and A24. Specifically, DRB1*15:01 (linked to DQB1*06:02) is common in Caucasians and confers strong genetic protection against T1D (21). A score generated by merging the Winkler (29; 30) and Oram (15) scores has recently been proposed to identify newborns from the general population who will develop islet autoimmunity and T1D. In this study, Bonifacio et al. (32) demonstrated that, even in a subset of individuals with high risk HLA genotypes from the TEDDY study, a T1D genetic score predicted development of autoantibodies. While different characteristics in each cohort (e.g. age, background risk of T1D, proportion of individuals with a relative with T1D) may require adaptations of the T1D GRS, the concept of combining genetic information into a single factor will greatly improve its utility for prediction and trial design. By virtue of being a number, the T1D GRS facilitates incorporation of complex genetic information into prediction models. Importantly, selecting appropriate cut-points will optimize the use of the T1D GRS for different goals.

The T1D GRS significantly added predictive power to the current variables used to stratify T1D risk in the TrialNet PTP study. The measurement of autoantibodies and differences in risk associated with autoantibody positivity are well described (33) as well as age and metabolic data (34-36). The fact that the T1D GRS was not a predictor in those with DPT-1 Risk Score >7 demonstrates that, when metabolic abnormalities develop, measures that evaluate these directly become most predictive and, consequently, the role of genetics in risk assessment diminishes. However, the majority

of individuals entering TrialNet PTP have a low DPT-1 Risk Score; in this group, the addition of T1D GRS to the currently established predictors (i.e. age, autoantibody number, DPT-1 Risk Score) can best add predictive power and assist in stratification for prevention trials. In the present study, multivariate modeling of autoantibody status, DPT-1 Risk Score, age and additional demographic factors still leaves the T1D GRS as a significant independent predictor of progression. This observation supports the assessment of all of these features, either in a combined model or a sequential approach, at entry to the TrialNet PTP and other similar studies. Previous studies have shown conflicting results on the ability of genetic factors, age, autoantibody and metabolic data to predict T1D (36; 37). Some of the differences in the role of genetics could be due to the challenges to capture genetic information; an advantage of the T1D GRS is that it includes SNPs tagging other significant HLA risk alleles, e.g. HLA DRB1*15, DRB1*57 and A24, in addition to non-HLA SNPs. Supporting this notion, in the present study, the T1D GRS was superior to HLA DR3/DR4-DQ8 alone to predict progression to T1D. Since the T1D GRS further stratified T1D risk beyond that associated with autoantibody number in individuals with low DPT-1 Risk Score, it is plausible that applying the T1D GRS earlier in life would allow discrimination of the individuals who will develop a high DPT-1 Risk Score and T1D.

The unique longitudinal follow up and monitoring of the TrialNet PTP study also allowed us to further investigate the contribution of the GRS to pre-clinical stages of T1D. Progression from single to multiple autoantibody positivity was independently predicted by the T1D GRS in participants younger than 35 years of age. Because our cohort included a relatively limited number of individuals ≥35 years old, larger studies will be

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required to better assess the applicability of the GRS in this age group. We had previously observed the protective effect of age on progression to T1D in at-risk adults with a threshold of 35 years of age (38) and the influence of age on the effect of another genetic factor, namely type 2 diabetes-associated *TCF7L2* variants on T1D progression (39). Interestingly, despite having been originally discovered in studies in childhood diabetes, the T1D GRS was able to identify more adult than childhood T1D cases in a recent study of T1D in UK Biobank (40). These results and those from the present study suggest that the genetic factors that regulate the progression of islet autoimmunity may slightly differ by age and further support the emerging notion that age is a key factor in the heterogeneity of T1D pathogenesis. The importance of age in progression through T1D stages is also highlighted by its significant and strong influence in the multivariable models even after adjustment for DPT-1 Risk Score, which includes age as well.

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We tested the predictive power of a restricted set of the top 10 SNPs from our score (15), which proved to contain the vast majority of predictive power in the T1D GRS. This is unsurprising due to the high weights of HLA and the top SNPs in the score. These results may be relevant to large scale studies where the cost of the T1D GRS per individual may be important.

The study limitations include that it evaluated the performance of T1D GRS only in autoantibody-positive relatives of people with T1D, although recent data (32) suggest that the T1D GRS will be a significant predictor in general population cohorts as well. We tested the T1D GRS and derived score cutoffs within the 1,244 TrialNet participants who had ImmunoChip data; we anticipate that expanding SNP analysis to the whole cohort will validate the current findings. Similarly, TrialNet is a cohort of >80% non-Hispanic

whites and, although we were able to control for race/ethnicity, the T1D GRS needs to be specifically tested in other races and possibly modified according to genetic differences. Finally, it is possible that newly discovered variants, better capture of known HLA variants, stage-specific variants (e.g. progression from single to multiple autoantibody positivity) or longer follow-up of the cohort (allowing us to assess whether the rate of progression and its factors change with time) could improve the understanding of the long-term predictive power of the T1D GRS.

In summary, the T1D GRS is a strong independent predictor of progression of islet autoimmunity and to clinical T1D in the TrialNet PTP study. Multivariate modeling suggests that the combination of islet autoantibody measurements, DPT-1 Risk Score, age and T1D GRS into a prediction model may improve assessment of T1D risk. This study, in addition to recent positive analyses in BABYDIAB (29)), DAISY (31) and TEDDY (32), suggest that future T1D prediction studies are likely to use a genetic score, such as the T1D GRS, at enrollment. These findings warrant further investigations on the use of the T1D GRS for early assessment T1D risk, particularly in longitudinal studies.

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Author Contributions: M.J.R. designed the study, interpreted the data and wrote the manuscript. S.G. contributed to study design, analyzed the data, contributed to data interpretation, and reviewed/edited the manuscript. A.K.S., S.S., J.M.W., M.W., P.A., J.S.,

M.A. and A.P. contributed to data interpretation and manuscript review/edits. R.O. contributed to study design, reviewed data, contributed to data interpretation, and reviewed and edited the manuscript. M.J.R., S.G., A.K.S., J.M.W., P.A., J.S., M.A. and A.P. are members of the T1D TrialNet Study Group (Supplementary Table S1). MJ Redondo is the guarantor of this article and takes full responsibility for the work as a whole, including the study design, access to data, and the decision to submit and publish the manuscript.

FIGURE LEGENDS

Figure 1. Time to T1D in initially non-diabetic, islet autoantibody-positive relatives of patients, by DPT-1 Risk Score (≤7 vs. >7), number of positive autoantibodies (i.e. single vs multiple autoantibody positivity), and T1D GRS (<0.250 vs. ≥0.250) (P<0.0001). While the T1D GRS did not further increase the predictive ability in the group with DPT-1 Risk Score >7, which already had high risk of T1D, it was able to stratify risk in individuals with DPT-1 Risk Score <7, with either single positive autoantibody or multiple positive autoantibodies. Abbreviations: DPT-1: Diabetes Prevention Trial-1. Ab+: autoantibody-positive. T1D: Type 1 diabetes. GRS: Genetic risk score

Figure 2. Comparison of two-year AUC for models to predict progression to T1D in participants with DPT-1 Risk Scores ≤7. The clinical model (i.e., DPT-1 Risk Score, age and islet autoantibody number) in addition to HLA had a 2-year AUC of 0.78, compared to 0.82 for the clinical model in addition to GRS (p<0.0001). Abbreviations: Ab+: autoantibody-positive. DPT-1 Risk Score: Diabetes Prevention Trial-1 Risk Score. T1D: Type 1 diabetes. GRS: Genetic risk score

Figure 3. Time to T1D in multiple islet autoantibody positive relatives, by DPT-1 Risk Score (≤7 vs. >7), age (<10 vs. ≥10 years) and T1D GRS (<0.250 vs. ≥0.250) (P=0.0001). While the T1D GRS did not further increase the predictive ability in

participants with DPT-1 Risk Score >7, it did stratify risk in individuals with DPT-1 Risk Score <7, aged <10 years or ≥10 years. Abbreviations: Ab+: autoantibody-positive. DPT-1 Risk Score: Diabetes Prevention Trial Risk Score. T1D: Type 1 diabetes. GRS: Genetic risk score

Figure 4. Time from single to multiple islet autoantibody positivity in relatives of patients, by age (<35 vs. ≥35 years) and T1D GRS group (<0.295 vs. ≥0.295) (P=0.0001). While the T1D GRS did not further increase the predictive ability in participants aged ≥35 years, it was able to stratify risk in individuals aged <35 years. Abbreviations: DPT-1 Risk Score: Diabetes Prevention Trial Risk Score. MA+: Multiple autoantibody positive. SA+: Single autoantibody positive (confirmed). T1D: Type 1 diabetes. GRS: Genetic risk score

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