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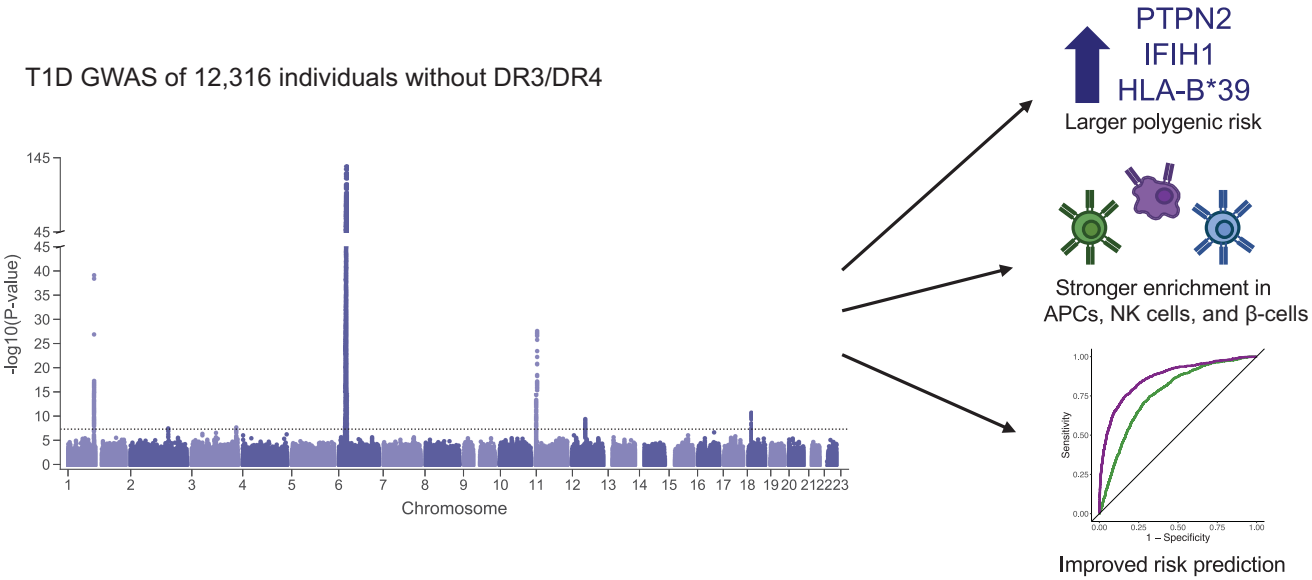

APC, antigen-presenting cell; GWAS, genome-wide association-study; NK, natural killer; T1D, type 1 diabetes.

ARTICLE HIGHLIGHTS

- **Why did we undertake this study?**
  More than 10% of individuals with type 1 diabetes (T1D) do not carry high-risk HLA-DR3 or -DR4 alleles, and the reasons why they develop T1D are not well understood.
- **What is the specific question we wanted to answer?**
  We aimed to characterize genetic risk of T1D and improve risk prediction in individuals without HLA-DR3 or -DR4 alleles.
- **What did we find?**
  We identified 18 T1D risk variants and distinct biological pathways in individuals without HLA-DR3 or -DR4 alleles and developed a risk score that significantly improved prediction of T1D in these individuals.
- **What are the implications of our findings?**
  This study reveals different T1D genetic risk and biological mechanisms in the absence of a high-risk HLA background and will help inform treatment and therapeutic discovery in these individuals.

Genetic Discovery and Risk Prediction for Type 1 Diabetes in Individuals Without High-Risk HLA-DR3/DR4 Haplotypes

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ORIGINAL ARTICLE

OBJECTIVE

More than 10% of patients with type 1 diabetes (T1D) do not have high-risk HLA-DR3 or -DR4 haplotypes with distinct clinical features, such as later onset and reduced insulin dependence. We aimed to identify genetic drivers of T1D in the absence of DR3/DR4 and improve prediction of T1D risk in these individuals.

RESEARCH DESIGN AND METHODS

We performed T1D association and fine-mapping analyses in 12,316 non-DR3/DR4 samples. Next, we performed heterogeneity tests to examine differences in T1D risk variants in individuals without versus those with DR3/DR4 haplotypes. We further assessed genome-wide differences in gene regulatory element and biological pathway enrichments between the non-DR3/DR4 and DR3/DR4 cohorts. Finally, we developed a genetic risk score (GRS) to predict T1D in individuals without DR3/DR4 and compared with an existing T1D GRS.

RESULTS

A total of 18 T1D risk variants in non-DR3/DR4 samples were identified. Risk variants at the MHC and multiple other loci genome wide had heterogeneity in effects on T1D dependent on DR3/DR4 status, and non-DR3/DR4 T1D had evidence for a greater polygenic burden. T1D-associated variants in non-DR3/DR4 were more enriched for regulatory elements and pathways involved in antigen presentation, innate immunity, and β-cells and depleted in T cells compared with DR3/DR4. A non-DR3/DR4 GRS outperformed an existing risk score GRS2 in discriminating non-DR3/DR4 T1D from no diabetes (area under the curve 0.867; $P = 7.48 \times 10^{-32}$) and type 2 diabetes (0.907; $P = 4.94 \times 10^{-44}$).

CONCLUSIONS

In total, we identified heterogeneity in T1D genetic risk dependent on high-risk HLA-DR3/DR4 haplotype, which uncovers disease mechanisms and enables more accurate prediction of T1D across the HLA background.

Type 1 diabetes (T1D) is an autoimmune disease characterized by the destruction of insulin-producing β-cells and which has complex etiology (1). T1D is highly polygenic with >90 known risk loci (2), and the largest genetic risk factors map to the MHC locus (3), which encodes cell surface receptors that present antigenic peptides to T cells (1). Haplotypes of the class II HLA-DR and -DQ genes *DRB1*0301-DQA1*0501-DQB1*0201*

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(DR3) and *DRB1*04:01/02/04/05/08-DQA1*03:01-DQB1*03:02/04* (DR4) (3) confer substantial risk of T1D and are detected in 90% of individuals of European ancestry (1). The DR3/DR4 haplotypes are thought to increase T1D risk by altering peptide binding and presentation to T cells (1).

The remaining 10% of individuals of European ancestry who develop T1D without DR3 or DR4 haplotypes have lower rates of T1D autoantibodies (4) and later onset of disease with lower insulin dependence, which can be misdiagnosed as type 2 diabetes (T2D) or latent autoimmune diabetes in adults (5–7), leading to mismanagement and complications (5). The preventive therapy teplizumab is most effective in at-risk individuals positive for DR4 and negative for DR3, and it is less established if it effectively delays onset in individuals without DR3/DR4 (8). Furthermore, existing genetic risk scores (GRSs) for T1D heavily weigh DR3/DR4 haplotypes due to their large effect, and T1D in individuals without DR3/DR4 may be poorly predicted by these scores. The *PTPN22* locus has been shown to interact with DR3/DR4 status (9–11), suggesting that genetic heterogeneity outside the MHC locus exists in individuals without DR3/DR4. There is increasing evidence that adult-onset T1D, which has a lower rate of DR3/DR4 (12), is more prevalent than previously estimated (13), further underscoring the need to understand T1D in the absence of DR3/DR4.

In this study, we performed genome-wide association analyses of T1D in individuals without DR3/DR4 to understand genetic drivers of T1D in the absence of DR3 or DR4 haplotypes. We then used association data to evaluate genetic risk, biological pathways and cell types, and risk prediction in non-DR3/DR4 T1D.

RESEARCH DESIGN AND METHODS

Subjects and Genotype Imputation

We compiled genotype data from 10,100 individuals with T1D and 19,623 control individuals of European ancestry from publicly available cohorts (Supplementary Table 1). T1D case cohorts were matched to control cohorts based on country of origin and genotype array where possible, as previously described (2). We applied the Haplotype Research Consortium imputation preparation program (version 4.2.9; https://www.well.ox.ac.uk/~wrayner/tools/) and used PLINK version 1.90 (14) to perform quality control prior to imputation to remove variants with a minor allele frequency <1%, with missing genotypes >5%, in violation of Hardy-Weinberg equilibrium (HWE) ($P < 1 \times 10^{-5}$ in the control cohort and $P < 1 \times 10^{-10}$ in the case cohort), and with a difference in allele frequency >0.2 compared with the Haplotype Research Consortium version r1.1 reference panel (15), as well as variants with allele ambiguity (2,14). Related individuals were removed based on identity by descent >0.2. We imputed genotypes for all samples into the Trans-Omics for Precision Medicine (TOPMed) version 2 and Michigan Multiethnic HLA reference panels (16,17). For the HLA reference panel, we note that classical HLA alleles and amino acids have binary encodings as A for absent or T for present (18). In genome-wide imputation, we removed variants with imputation accuracy r^2 <0.3. In HLA imputation, we removed variants with imputation accuracy r^2 <0.5 and SD in control allele frequency >0.055 across cohorts. Variants that passed quality control in all cohorts were tested for association.

Association Testing and Meta-Analysis

We categorized individuals based on DR3/DR4 status using two-field HLA alleles imputed from the Type 1 Diabetes Genetics Consortium (T1DGC) reference panel with SNP2HLA, which includes HLA-DQB1*02:02 not present in the Michigan HLA panel (19). DR3 was defined by HLA-DRB1*03:01-DQB1*02:01 and DR4 by HLA-DRB1*04:01/02/04/05/08-DQB1*03:02/04/02:02 (6). We excluded 23 individuals identified as without DR3/DR4 via SNP2HLA alleles but who had DR3 or DR4 tag single nucleotide polymorphisms (SNPs) (20,21). The non-DR3/DR4 group consisted of individuals without a DR3 or DR4 haplotype (DRX/DRX), and the DR3/DR4 group consisted of individuals with at least one DR3 or DR4 haplotype (DR3/DR4, DR3/DR3, DR4/DR4, DR3/DRX, DR4/DRX). In the non-DR3/DR4 group, 100 case and 300 control samples were removed prior to association analyses, and these samples were randomly selected from cohorts proportionate to the total cohort size. In both non-DR3/DR4 and DR3/DR4 groups, we tested variants for T1D association using Firth bias-corrected logistic regression in EPACTS (https://github.com/statgen/EPACTS). We tested variants with minor allele frequency >1% for the association, including covariates for sex and the first four genotype principal components (PCs). The PCs were included to account for population structure and were derived using PLINK with parameters –indep 50 52. Summary statistics were combined across cohorts in a fixed-effects inverse variance–weighted meta-analysis using METAL (22). The genomic inflation λ was 1.069 for non-DR3/DR4 and 1.098 for DR3/DR4. We used linkage disequilibrium (LD) score regression (LDSC) (23) to test for heritability excluding the MHC, using population prevalence of 1% and sample prevalence of 51% for DR3/DR4 and 10% for non-DR3/DR4.

Fine-Mapping Independent Signals

We identified 1-Mb regions around the lead variants of genome-wide significant loci. We performed conditional analysis at each locus using stepwise analysis iteratively including the most significant variant in the regression model and reperforming the meta-analysis until no significant variants remained. In conditional analyses, we used a locus-wide significance threshold of $P < 1 \times 10^{-5}$. We then performed Bayesian fine-mapping to create credible sets of likely causal variants for each signal (24). From the summary statistics, effect size, and SE were used to calculate the approximate Bayes factor (BF) for each variant in r^2 >0.1 with the lead variant. We calculated the probability of association for each variant by dividing the BF by the total sum of BFs. We then created 99% credible sets by including variants in descending order of probability of association until the cumulative posterior probability was at least 99% (Supplementary Table 12).

Assay for Transposase-Accessible Chromatin With Sequencing Peak Calling

Assay for transposase-accessible chromatin with sequencing data for 20 immune cell types in resting and stimulated conditions were obtained from the Gene Expression Omnibus database (accession no. GSE118189) (25) and processed to generate peak coordinates. Reads were aligned using STAR (26) to hg19, and duplicate reads, reads mapping to blacklisted regions from ENCODE, and reads

with mapping quality <30 were filtered. Peak calling was performed using MACS2 (27) on binary alignment map files further filtered for read pairs with insert size no larger than 140 bp (macs2 callpeak –nomodel –nolambda –keep-dup all –call-summits -f BAMPE -g hs –q 0.01), combining individual samples for each cell type and treatment, for a total of 40 distinct peak sets. We then used bedtools multiinter to obtain a consensus set of peaks and featureCounts (28) to obtain the peak counts in each cell type.

Genome-Wide Association Study Enrichment Analysis

For each group, we performed partitioned heritability LDSC to estimate genome-wide enrichment in immune cell–accessible chromatin (23,25). We used the summary statistics for each group excluding the MHC locus and formatted it for LDSC using the munge_sumstats.py script. We generated binary annotations from each accessible chromatin bed file and computed cell-specific LD enrichment scores for each risk cohort using the version 2.2 1000G baseline model. Additionally, we performed gene set enrichment analysis in the Gene Ontology, Kyoto Encyclopedia of Genes and Genomes, and Reactome pathways with the summary statistics for each group using MAGMA (29) with default parameters. We corrected for multiple tests in each group using the false discovery rate (FDR) and considered FDR <0.10 as significant and uncorrected $P <$ 0.05 as nominally significant.

Heterogeneity Tests

We tested for differences in T1D effect between non-DR3/DR4 and DR3/DR4 using merged PLINK files of Michigan HLA and TOPMed imputed variants for all 29,723 samples. We tested for heterogeneity in marginal effects on T1D using Breslow-Day (BD) tests with the -bd flag in PLINK (14). We performed BD tests for lead variants at the six non-MHC loci identified in non-DR3/DR4 samples, as well as lead variants at 83 additional known T1D risk loci (2). At MHC and other loci with multiple signals, we tested for heterogeneity in effects on T1D conditional or other known variants. We generated regression models with PLINK using the -glm interaction firth flag, including sex, the first four genotype PCs, DR3/DR4 status, and additional variants as covariates, and evaluated the interaction with DR3/DR4 status. For the 12 MHC signals identified in non-DR3/DR4 samples, we conditioned on preceding lead variants from stepwise regression. For the *IFIH1* and *PTPN2* loci, we conditioned on lead variants for all other known signals at the locus (14). For the HLA locus, we conditioned on HLA-DRB1*03:01, HLA-DQB1*02:01, HLA-DRB1*04:01, HLA-DRB1*04:02, HLA-DRB1*04:04, HLA-DRB1*04:05, HLA-DRB1*04:08, HLA-DQB1*03:02, and HLA-DQB1*03:04 to examine heterogeneity in 40 known two-field HLA risk alleles independent of these DR3 and DR4 alleles. To obtain the conditional effect of each variant within non-DR3/DR4 or DR3/DR4, we performed logistic regression separately for each group using EPACTS, including sex, the first four genotype PCs, and the same variants from the interaction tests above. We performed additional association analyses of non-DR3/DR4, including T1D with age of onset >17 or <17 years, T1D age of onset directly, and T1D interaction with sex with the glm function in PLINK, including the first four genotype PCs and (for age of onset analyses) sex as covariates. We corrected for multiple tests, considering FDR <0.10 as significant and uncorrected $P <$ 0.05 as nominally significant.

Generation and Statistical Analysis of the GRSs

We calculated GRS1 using SNP2HLA-imputed genotypes to define DR3 and DR4 tag SNPs and TOPMed variants for the remaining 28 signals (20). We calculated GRS2 by using TOPMed variants where possible (60 variants), Michigan HLA for rs116522341 and rs1281934, and the Michigan HLA proxies DQB1*06:02, B*18:01, DPB1*03:01, rs1611547, and rs114170382, for rs17843689, rs371250843, rs559242105, rs144530872, and rs149663102, respectively. For GRS2, we excluded individuals with ambiguous (>2) HLA-DR/DQ calls, in line with the published methods (30). For each of the signals identified in the non-DR3/DR4 group, we generated an 18-varaint GRS as the sum of all signals weighted by the β (*B*) for each effect allele (*X*) as follows (Supplementary Table 12):

$$GRS = \sum_{i=0}^{N} B_i X_i$$

We additionally leveraged 82 non-MHC T1D risk loci from a T1D genome-wide association study (GWAS) (2) weighted by effects in the non-DR3/DR4 GWAS to generate a 100-variant combined GRS. We determined the ability of GRS2, the 18-variant GRS, and the 100-variant GRS to distinguish non-DR3/DR4 T1D from either all control samples (DR3/DR4 + non-DR3/DR4) or non-DR3/DR4 control samples. We tested the ability of each GRS to discriminate T1D and nondiabetes using the area under the curve (AUC) of the receiver operating characteristic (ROC) statistics in pROC. We calculated the difference between GRS AUCs using the DeLong test and calculated the diagnostic criteria using the Youden index. We then generated percentiles for how many individuals in the non-DR3/DR4 T1D or control groups fall at various GRS thresholds. We calculated sensitivity at each GRS as true positive divided by (true positive plus false negative) and specificity as true negative divided by (true negative plus false positive). We further tested the ability to differentiate T1D from nondisease in the 100-variant non-DR3/DR4 T1D GRS using the independent test group of 100 case and 300 control samples removed prior to association analyses. We also compared the ability of each GRS to differentiate T1D from T2D using 1,999 individuals with T2D from the Wellcome Trust Case Control Consortium (WTCCC) study (31). After imputing the T2D genotypes into the TOPMed and Michigan HLA reference panels, we calculated the scores for each GRS as described above and derived ROC statistics comparing T1D with T2D. We additionally validated the performance of the 100-variant non-DR3/DR4 GRS to distinguish T1D from T2D using the 100-T1D case test group and all 1,999 T2D samples.

RESULTS

We performed T1D association analyses in individuals without high-risk DR3 or DR4 haplotypes (Fig. 1). We obtained genotype data for 29,723 individuals with T1D and control individuals from five European ancestry cohorts (Supplementary Table 1) and imputed genotypes into 308 million variants in the TOPMed version 2 and 56,310 variants in Michigan HLA reference panels (32,33). We partitioned individuals into 1,292 with T1D and 11,424 control subjects without a DR3 or DR4 haplotype (non-DR3/DR4 group) and 8,808 individuals with T1D and 8,199 control subjects with at

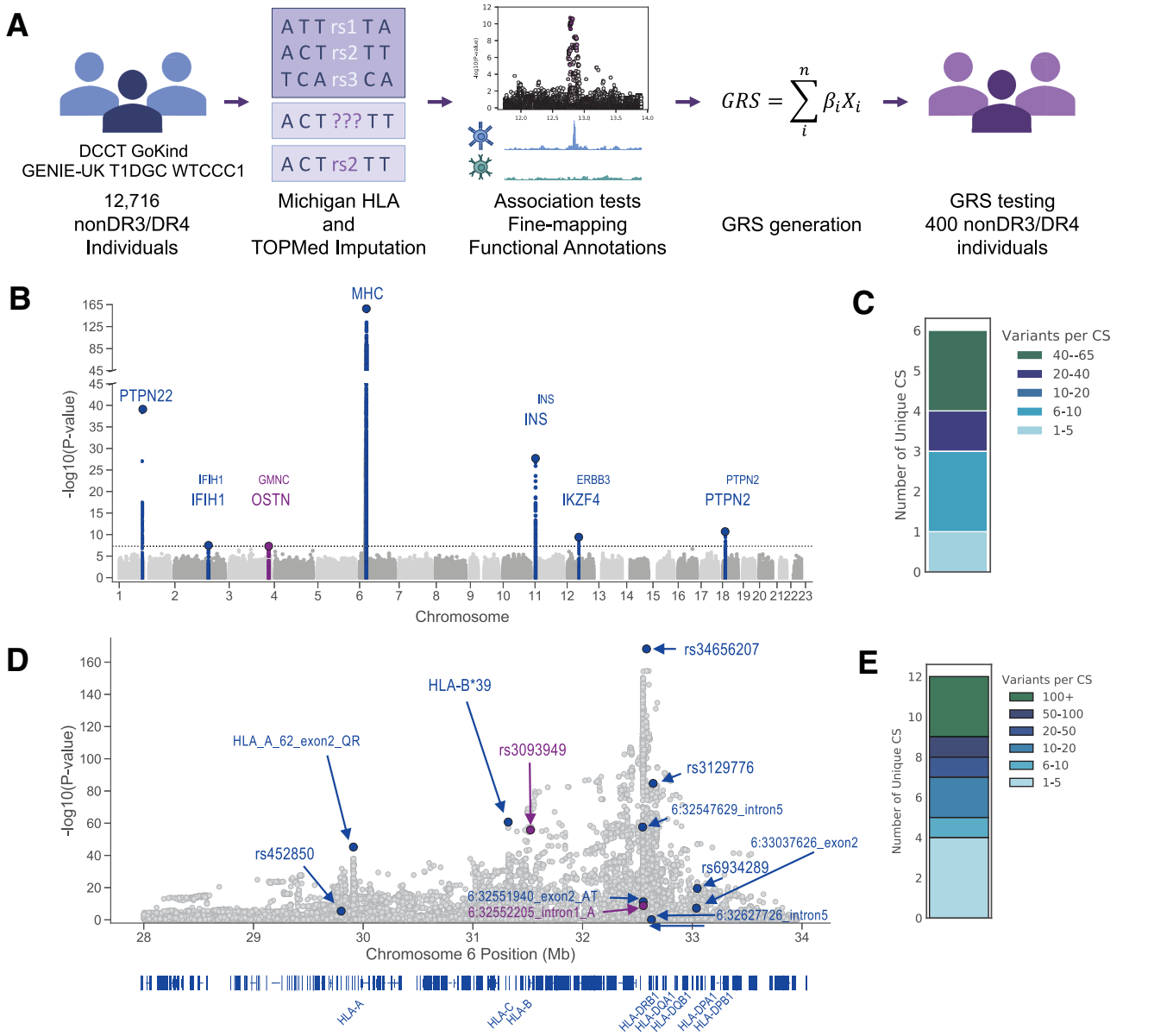
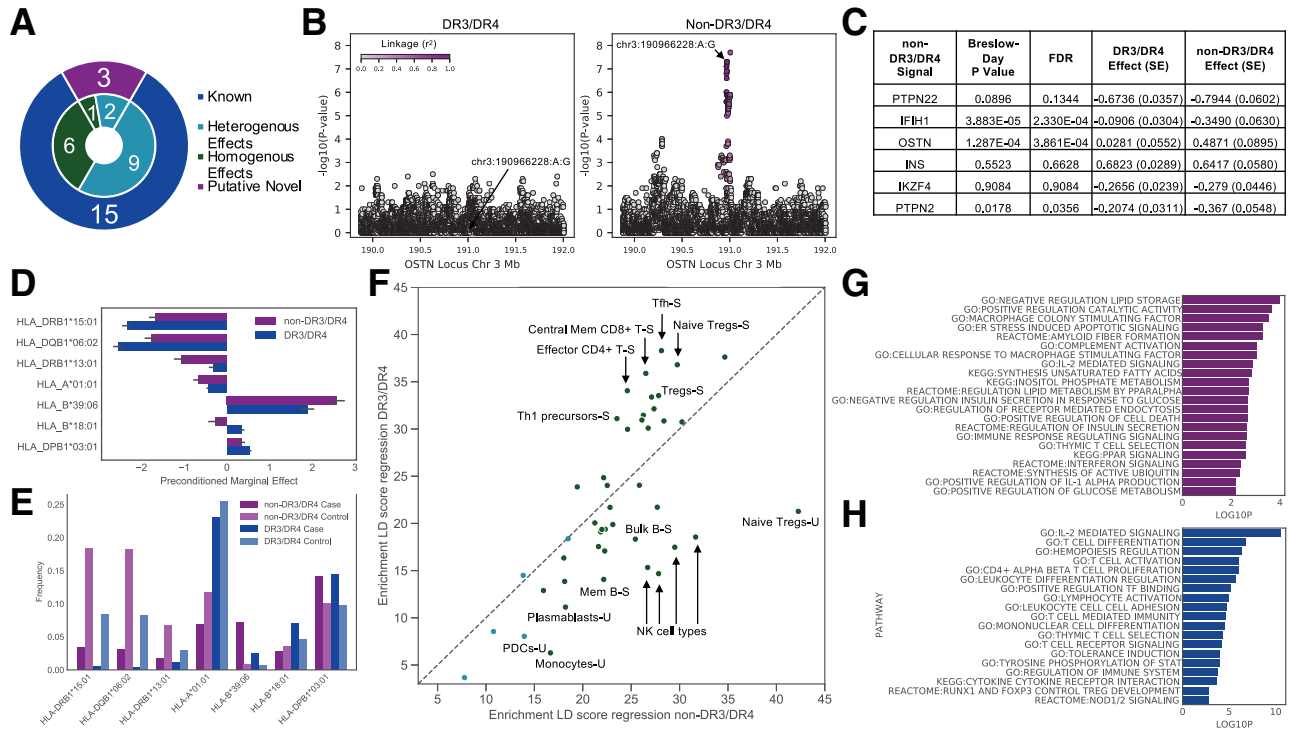


Figure 1—Genetic discovery in non-DR3/DR4 T1D. *A*: Overview of genetic discovery and risk prediction for T1D in individuals without DR3/DR4. *B*: Genome-wide T1D association ($\log_{10}$ *P* values from meta-analysis of *n* = 12,316 samples) in individuals without DR3/DR4 haplotypes. Known T1D loci are blue, and novel loci are purple. All loci are labeled with the nearest gene. *C*: Number of candidate causal variants in fine-mapped non-MHC T1D signals. *D*: T1D association at the MHC locus ($\log_{10}$ *P* values from marginal association in meta-analysis of *n* = 12,316 samples). Known signals are blue, and novel signals are purple. The location of class I and II HLA genes are shown on the bottom. *E*: Number of candidate causal variants in fine-mapped MHC signals. CS, credible set; DCCT, Diabetes Control and Complications Trial; GENIE, Genetics of Nephropathy, an International Effort; GoKinD, Genetics of Kidneys in Diabetes.

least one DR3 or DR4 haplotype (DR3/DR4 group) (Supplementary Table 1). We then tested for T1D association in the non-DR3/DR4 group, as well as in the DR3/DR4 group for comparison. Seven loci, including *PTPN22*, *INS*, *IFIH1*, *PTPN2*, *IKZF4*, *OSTN*, and MHC, had a genome-wide significant ($P < 5 \times 10^{-8}$) T1D association in non-DR3/DR4 (Fig. 1*B* and Supplementary Table 2). We identified 12 additional signals at locus-wide significance ($P < 1 \times 10^{-5}$) at the MHC locus through conditional association analysis (Research Design and Methods, Fig. 1*D*, and Supplementary Table 3). At each signal, we then generated a set of variants (termed credible set) most likely causal for T1D (Fig. 1*C* and *E* and Supplementary Table 4).

We compared the T1D effects of risk variants at these seven loci between the non-DR3/DR4 and DR3/DR4 groups using a BD test and considered tests significant at FDR <0.10 (Research Design and Methods). Of the six non-MHC loci, five are known to affect T1D risk, while *OSTN* was previously unreported (Fig. 2*A*). At the novel *OSTN* locus, the lead variant had significant (FDR <0.10) heterogeneity in effect on T1D between the non-DR3/DR4 and DR3/DR4 groups (b = 0.4871 vs. 0.0281; FDR = 3.86×10^{-4}) (Fig. 2*B*). We also observed significantly stronger effects in the non-DR3/DR4 group for lead variants at the *IFIHI* (b = −0.35 vs. −0.091; FDR = 2.33×10^{-4}) and *PTPN2* (b = −0.37 vs. −0.21, FDR = 0.0356) loci (Fig. 2*C*), both of which are implicated in β-cell function (34) (Supplementary Table 5). We further examined heterogeneity at



non-DR3/DR4 Signal	Breslow-Day P Value	FDR	DR3/DR4 Effect (SE)	non-DR3/DR4 Effect (SE)
PTPN22	0.0896	0.1344	-0.6736 (0.0357)	-0.7944 (0.0602)
IFIH1	3.883E-05	2.330E-04	-0.0906 (0.0304)	-0.3490 (0.0630)
OSTN	1.287E-04	3.861E-04	0.0281 (0.0552)	0.4871 (0.0895)
INS	0.5523	0.6628	0.6823 (0.0289)	0.6417 (0.0580)
IKZF4	0.9084	0.9084	-0.2656 (0.0239)	-0.279 (0.0446)
PTPN2	0.0178	0.0356	-0.2074 (0.0311)	-0.367 (0.0548)

Figure 2—Heterogeneity of genetic and biological mechanisms in non-DR3/DR4 and DR3/DR4 T1D. *A*: Number of known and novel signals with heterogeneity in effect on T1D depending on DR3/DR4 background. *B*: Locus plots of T1D association at the novel *OSTN* locus in DR3/DR4 (left) and non-DR3/DR4 (right). *C*: Non-MHC loci with genome-wide significant T1D association in non-DR3/DR4 association analyses with effect sizes and interaction analysis significance for difference in effect. *D*: Enrichment scores for DR3/DR4 and non-DR3/DR4 in stimulated and unstimulated immune cell regulatory elements. Dark green points are significant at FDR <0.10 in non-DR3/DR4 and DR3/DR4, while light blue points are significant by FDR in only DR3/DR4. *E*: Biological pathway enrichment in non-DR3/DR4 T1D. *F*: Biological pathway enrichment in DR3/DR4 T1D. *G*: T1D effect sizes of class I and II HLA alleles in each risk group after conditioning on nine DR3 and DR4 risk alleles. *H*: T1D case and control group frequencies of known HLA alleles in non-DR3/DR4 and DR3/DR4. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; Mem, memory; PDC, plasmacytoid dendritic cell; S, stimulated; TF, transcription factor; Tfh, T follicular helper cell; Th1, T helper 1 cell; Treg, regulatory T cell; U, unstimulated.

83 additional T1D loci (2), and although no further loci had significant heterogeneity, we observed more nominal evidence ($P < 0.05$) at nine loci. Among these, five, including *PRR15L*, *RAD51B*, *PRF1*, *PRKD2*, and 6q27, had larger effects in the non-DR3/DR4 group, including several implicated in immune cells and β-cells. By comparison, the four loci, including 14q32, *IL2RA*, *IL2*, and *CD69*, with smaller effects in the non-DR3/DR4 group largely affected T-cell function (Supplementary Fig. 1 and Supplementary Table 6).

We next determined whether individuals with T1D in the absence of HLA-DR3/DR4 may carry a greater burden of polygenic risk. We determined whether known T1D risk variants broadly had stronger effects in the non-DR3/DR4 group. A higher proportion (60.2%) of variants at 88 known T1D loci had a stronger effect in non-DR3/DR4, although this was not significant (binomial $P = 0.069$). We also identified a small increase in heritability for T1D in non-DR3/DR4 compared with DR3/DR4 (h^2 [SE] 0.2846 [0.0795] vs. 0.2693 [0.0428]). Finally, there was a significant increase in the ability to distinguish T1D from control in non-DR3/DR4 compared with DR3/DR4 (AUC 0.752 vs. 0.733; $P = 0.0238$) using a risk score derived from only non-MHC variants (2). The ability to distinguish T1D from control in non-DR3/DR4 was further improved when using effects derived from the non-DR3/DR4 T1D GWAS (AUC 0.768; $P = 1.66 \times 10^{-5}$). Overall, these results support a modest increase in polygenic contribution to T1D risk in the absence of high-risk DR3/DR4 haplotypes.

We next investigated whether variants within the MHC locus contribute to heterogeneity between non-DR3/DR4 and DR3/DR4 (Research Design and Methods). Of the 12 MHC signals identified in non-DR3/DR4 T1D, 8 exhibited significant heterogeneity (FDR <0.10) (Fig. 2*A* and Supplementary Table 7). Most signals were linked to known T1D alleles, except for two apparent novel signals at HLA-DRB1*09:01-DQA1*03:01-DQB1*03:03 and B*44:02 (Fig. 2*A* and Supplementary Table 8). Among a larger set of 40 known T1D MHC alleles, 22 two-field class I and class II alleles had significant heterogeneity (FDR <0.10) (Research Design and Methods, Fig. 2*D* and *E*, and Supplementary Table 9). In class I, HLA-B*39:06 had increased risk in non-DR3/DR4 (FDR = 0.027), HLA-A*01:01 was more protective in non-DR3/DR4 (FDR = 0.015), and HLA-B*18:01 had opposed effects in non-DR3/DR4 and DR3/DR4 (FDR = 6.31×10^{-6}) (Fig. 2*D* and *E*). In class II, DRB1*15:01-DQB1*06:02 was more protective in DR3/DR4 (DRB1*15:01 FDR = 1.91×10^{-7}, DQB1*6:02 FDR = 1.51×10^{-6}) (Fig. 2*D* and *E*), DRB1*13:01-DQB1*06:03 was more protective in non-DR3/DR4 (FDR = 0.027) (Fig. 2*A*, *D*, and *E*), and DPB1*03:01 had increased risk in DR3/DR4 (FDR = 0.085) (Fig. 2*D* and *E*). Together this reveals numerous MHC alleles with heterogeneous effects on T1D in a non-DR3/DR4 background.

Individuals without DR3/DR4 haplotypes have typically later onset of T1D; thus, we examined whether heterogeneity in T1D risk between non-DR3/DR4 and DR3/DR4 was consistent across age of onset. As all T1D ages of onset were <40 years, we were unable to examine non-DR3/DR4 heterogeneity in older-onset T1D. We compared the effects of non-DR3/DR4 signals in each cohort, including WTCCC and T1DGC where all case individuals had an age of onset of <17 years (Supplementary Table 1). Among loci significant in non-DR3/DR4 that also exhibited heterogeneity, we observed no difference in T1D effects in WTCCC and T1DGC compared with the other cohorts with an age of onset up to 40 years (Cochran $Q > 0.05$) (Supplementary Table 2 and Supplementary Fig. 2). Similarly, none of these loci showed an association with T1D stratified by age of onset of 17 years or T1D age of onset directly (all $P > 0.05$). Signals with heterogeneity in non-DR3/DR4 were largely distinct from those with an age-dependent association from a previous study (6), and HLA-B*39:06 had a larger effect in non-DR3/DR4 yet was associated with younger onset (Supplementary Fig. 1). As sex also impacts T1D, we determined whether the T1D association in non-DR3/DR4 interacted with sex and found no significant evidence (all $P > 0.05$). This supports that heterogeneity in non-DR3/DR4 is distinct from T1D age-of-onset– and sex-dependent effects.

We next determined whether there were broader differences in T1D association patterns between non-DR3/DR4 and DR3/DR4. We first tested whether T1D risk variants showed different enrichment for gene regulatory elements in specific immune cell types (23,25). In non-DR3/DR4, there was significant enrichment (FDR <0.10) of T1D variants in regulatory elements for B cells, mature natural killer (NK) cells, and unstimulated regulatory T cells (Fig. 2*F* and Supplementary Table 10). By comparison, in DR3/DR4, T1D variants were most enriched (FDR <0.10) in regulatory elements for T-cell populations (Supplementary Table 10). We next determined whether T1D risk variants were enriched for genes involved in different biological pathways (Fig. 2*G* and *H* and Supplementary Table 11). T1D in DR3/DR4 had significant enrichment (FDR <0.10) for T-cell–related pathways, including IL-2 signaling, and T-cell differentiation, activation, and proliferation (Fig. 2*H*). In non-DR3/DR4, the strongest enrichments, although not significant after FDR correction, were for regulation of lipid storage, macrophage-stimulating factor, stress-induced apoptotic signaling, and complement pathways (Fig. 2*G*). Collectively, this suggests a greater antigen-presenting, innate immune, and β-cell contribution and lower T-cell contribution to T1D in non-DR3/DR4.

Finally, we evaluated the ability of GRSs to predict T1D in individuals without DR3/DR4. More than 35% of individuals without DR3/DR4 T1D were below the 5th percentile for T1D in the published GRS2 (30), and only 7.5% were above the 50th percentile used to classify T1D with high specificity. In line with this, GRS2 had less ability to distinguish individuals without DR3/DR4 T1D from all control subjects (AUC 0.764) (Fig. 3*A* and *E*) compared with all individuals with T1D (AUC 0.927) (30). We generated a non-DR3/DR4–specific T1D GRS using the 18 risk signals in our non-DR3/DR4 GWAS (Research Design and Methods and Supplementary Table 12). This GRS improved discrimination of individuals without DR3/DR4 T1D from all control subjects compared with GRS2 (AUC 0.835; $P = 6.30 \times 10^{-15}$) (Fig. 3*A*, *E*, and *F*). We next generated a larger 100-variant non-DR3/DR4 T1D GRS that included 82 additional T1D risk loci (Research Design and Methods and Supplementary Table 12), which further discriminated T1D from control (AUC 0.867; $P = 7.48 \times 10^{-32}$) (Fig. 3*B*, *E*, and *G*). When subsets of individuals with T1D and control subjects were compared with individuals without DR3/DR4, the 100-variant non-DR3/DR4 GRS improved discrimination of T1D over both the 18-variant GRS (AUC 0.894; $P = 5.70 \times 10^{-26}$) (Fig. 3*C*, *F*, and *G*) and GRS2 (AUC 0.878; $P = 0.026$) (Fig. 3*D*, *E*, and *G*). We further confirmed the ability of the 100-variant non-DR3/DR4 GRS to predict T1D using a test set of 100 T1D and 300 control non-DR3/DR4 samples excluded from association tests (AUC 0.887) (Supplementary Fig. 3*A* and *B*).

Since individuals with T1D and no DR3/DR4 tend to have a later onset and a lower dependence on insulin therapy, leading to a potential misdiagnosis of T2D (5,6,12), we next evaluated the ability of GRS to differentiate T1D from T2D (Supplementary Fig. 4). In GRS2 there was limited ability to distinguish non-DR3/DR4 T1D from all T2D (AUC 0.759) (Supplementary Fig. 4*A* and *E*). In contrast, our non-DR3/DR4 GRS strongly discriminated non-DR3/DR4 T1D from T2D compared with GRS2 (18-variant AUC 0.890 [$P = 1.51 \times 10^{-32}$]; 100-variant AUC 0.907 [$P = 4.94 \times 10^{-44}$]) (Supplementary Fig. 4*A*, *B*, and *E*–*G*). When subsets of individuals with T1D and T2D were compared with individuals without DR3/DR4, the 100-variant non-DR3/DR4 GRS again improved on both the 18-variant GRS (AUC 0.920 and 0.905, respectively; $P = 0.0775$) (Supplementary Fig. 4*C*, *F*, and *G*) and GRS2 (AUC 0.876; $P = 1.89 \times 10^{-6}$) (Supplementary Fig. 4*D*, *E*, and *G*). The predictions were consistent when using a test set of 100 T1D samples (AUC 0.906) (Supplementary Fig. 3*C* and *D*). The ability to distinguish non-DR3/DR4 T1D from T2D appeared largely driven by the improved estimation of effects at the MHC locus (T1D vs. T2D HLA AUC 0.885), as the extensive HLA combinations in GRS2 still do not enable differentiation of non-DR3/DR4 T1D from T2D (T1D vs. T2D GRS2 HLA AUC 0.698).

We finally determined the diagnostic value of a GRS for T1D in a non-DR3/DR4 background. Individuals with T1D and no DR3/DR4 on the published GRS2 scale do not meet minimum requirements for diagnostic feasibility (maximum Youden index <0.5). When GRS2 is compared with only non-DR3/DR4 and rescaled (Table 1), the 50th percentile of T1D had a specificity of 95.4%, and the score had improved diagnostic ability (maximum Youden index = 0.609). The 18-variant non-DR3/DR4 GRS at the 50th percentile for T1D had a specificity of 95.2% and a maximum Youden index of 0.607. Furthermore, the 100-variant non-DR3/DR4 GRS had improved specificity (96.9%) at the 50th percentile for T1D and a higher maximum Youden index of 0.644. In addition, while GRS2 had a limited ability to distinguish T1D from T2D (maximum Youden index = 0.414), the 100-variant non-DR3/DR4 GRS had diagnostic value (maximum Youden index >0.5) in differentiating individuals with T1D and no DR3/DR4 T1D from individuals with T2D as well as all control subjects. Overall, our results provide a GRS that can likely be adopted in a clinical setting to distinguish non-DR3/DR4 T1D from both nondiabetes and T2D.

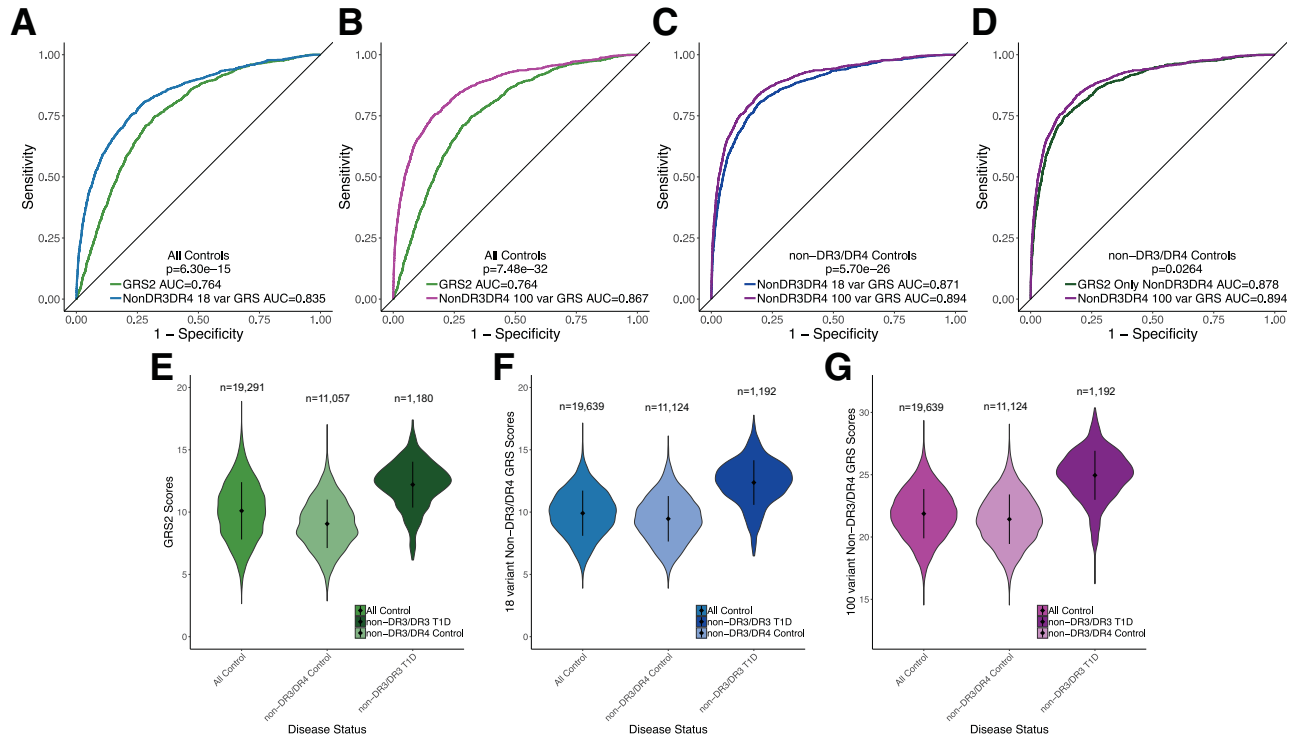


Figure 3—Genetic risk prediction in individuals with T1D and no DR3/DR4. ROC curves showing the ability of GRSs to differentiate T1D from nondiabetes in non-DR3/DR4 cohort and corresponding violin plots. The AUCs are shown for each GRS, and the *P* values comparing predictive ability of the GRSs are calculated using the DeLong test. *A*: GRS2 compared with the 18-variant non-DR3/DR4 GRS in the non-DR3/DR4 T1D group vs. all (non-DR3/DR4 and DR3/DR4) control subjects. *B*: GRS2 compared with the 100-variant non-DR3/DR4 T1D GRS in non-DR3/DR4 T1D group vs. all control subjects. *C*: The 18-variant non-DR3/DR4 T1D GRS compared with the 100-variant non-DR3/DR4 T1D GRS in non-DR3/DR4 T1D and control groups. *D*: GRS2 subset of the non-DR3/DR4 T1D and control groups compared with the 100-variant non-DR3/DR4 T1D GRS in the non-DR3/DR4 T1D and control groups. *E*–*G*: Violin plots for scores in all control subjects, control subjects without DR3/DR4, and individuals with T1D are depicted for GRS2 (*E*), 18-variant non-DR3/DR4 T1D GRS (*F*), and 100-variant non-DR3/DR4 T1D GRS (*G*). The number of samples differs between GRSs due to ambiguous HLA-DR/DQ alleles leading to several sample exclusions in GRS2. var, variant.

CONCLUSIONS

In the absence of high-risk DR3/DR4 haplotypes, we observed heterogeneity in T1D risk for class I and class II MHC alleles as well as other loci genome wide. Genes at loci with larger effects in non-DR3/DR4, such as *PTPN2*, *IFIH1*, *PRR15L*, and *RAD51B*, are implicated in inflammatory signaling (34,35) and survival (36) in immune cells and β-cells. We also identified a novel T1D locus in non-DR3/DR4 near *OSTN*, which has been linked to diabetic cardiomyopathy (37). Conversely, loci such as *IL2RA*, *IL2*, and *CD69* with a reduced effect in non-DR3/DR4 impact T-cell function and activation (38). Genomic annotations and molecular pathways related to B cells, NK cells, and β-cells were also more enriched in non-DR3/DR4 T1D, and T cells less enriched, compared with DR3/DR4. These results suggest that mechanisms of T1D in a non-DR3/DR4 background may depend more on inflammation and β-cell dysfunction than T-cell activation (5–7). Given modest evidence for a higher polygenic burden in non-DR3/DR4 T1D, these individuals may require additional polygenic risk to develop T1D.

Based on previous GRSs, many individuals with T1D and no DR3/DR4 would not have been predicted to develop T1D. The GRS reported here enables accurate discrimination of T1D from nondiabetes and T2D in individuals without DR3/DR4. To improve accessibility, variants in the non-DR3/DR4 GRS are all derived from publicly available imputation panels. While the subset of individuals that develop T1D without DR3/DR4 exhibit later onset and lower insulin dependence, they have highly similar genetic risk profiles to DR3/DR4 T1D and not T2D. Accurate prediction of T1D in non-DR3/DR4 will help avoid misdiagnosis of T2D and prevent ketoacidosis and future complications. While the non-DR3/DR4 T1D background has generally later onset, it can occur at any age, and our results suggest that the effects of variants on T1D risk in a non-DR3/DR4 background are consistent across age of onset. Furthermore, our GRS may have value in discriminating T1D in populations in which DR3/DR4 is uncommon.

The ability to distinguish at-risk individuals is also critical for determining eligibility for clinical trials and therapies. The preventive therapy teplizumab modulates T-cell activity and is most efficacious in individuals with HLA-DR4 (8), and several clinical trials aiming to preserve β-cell function at onset preferentially recruit individuals with DR3/DR4 (39,40). Given evidence for differences in disease mechanisms, particularly a less prominent contribution from T cells, alternate therapies may be needed to prevent T1D in individuals without DR3/DR4. In addition, as autoantibodies are seen at lower rates in non-DR3/DR4 T1D, additional biomarkers are needed for this group (4).

There are several notable limitations of our study. First, as the non-DR3/DR4 GRS was tested in samples from the same cohorts, which are not fully independent, the accuracy of the GRS requires validation in other cohorts. Second, all individuals with

Table 1—Sensitivity, specificity, diagnostic value, and scales for each T1D GRS

	T1D centile	Nondisease centile	Sensitivity (%)	Specificity (%)	Youden index
Non-DR3/DR4–only GRS2					
8.86	5	47.6	95.00	47.63	0.4263
10.33	15	73.7	85.00	73.72	0.5872
11.20	25	85.3	75.00	85.35	0.6035
11.77	35	91.3	65.00	91.27	0.5627
12.40	50	95.4	50.00	95.41	0.4541
13.39	75	98.9	25.00	98.85	0.2385
14.85	95	99.9	5.000	99.95	0.0978
11.23**	25.2	86.0	74.8	86.0	0.6086
18-variant non-DR3/DR4 GRS					
9.067	5	42.0	94.97	41.95	0.3691
10.59	15	72.5	84.98	72.60	0.5758
11.36	25	84.5	75.00	84.53	0.5953
11.85	35	90.3	65.02	90.26	0.5527
12.50	50	95.2	50.00	95.20	0.4519
13.55	75	98.8	25.00	98.78	0.2377
15.08	95	99.9	5.034	99.87	0.04908
11.05**	19.4	80.1	80.6	80.1	0.6068
100-variant non-DR3/DR4 GRS					
21.25	5	46.9	94.97	46.90	0.4187
23.06	15	78.7	84.98	78.68	0.6366
23.84	25	88.4	75.00	88.41	0.6341
24.43	35	93.6	65.02	93.56	0.5858
25.11	50	96.9	50.00	96.90	0.4689
26.28	75	99.3	25.00	99.33	0.2433
27.82	95	99.9	5.034	99.96	0.0450
23.21**	16.4	80.8	83.6	80.8	0.6439

** Score with the maximum Youden index.

T1D in this study were younger than 40 years, and as many T1D diagnoses occur after age 40, similar studies are needed to establish genetic heterogeneity in older-onset T1D. In addition, several cohorts consisted of individuals with T1D selected for diabetes complications, which may result in biases in the case population. Finally, larger and more ancestrally diverse sample sizes in association analyses will help establish the full extent of heterogeneity in T1D genetic risk due to HLA background, including within previously defined endotypes.

Our findings are in line with a growing body of literature (6,41) supporting T1D as a heterogeneous disease consisting of subtypes with distinct pathophysiological features. More broadly, stratifying by high-risk genetic background may be an effective strategy to discover genetic and mechanistic heterogeneity in other complex diseases.

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