Shared Genetic Basis for Type 1 Diabetes, Islet Autoantibodies, and Autoantibodies Associated With Other Immune-Mediated Diseases in Families With Type 1 Diabetes

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Type 1 diabetes (T1D) is a polygenic autoimmune disease that is often present with autoantibodies directed against pancreatic islet proteins. Many genetic susceptibility loci are shared with other autoimmune or immune-mediated diseases that also cosegregate in families with T1D. The aim of this study was to investigate whether susceptibility loci identified in genome-wide association studies (GWAS) of T1D were also associated with autoantibody positivity in individuals with diabetes. Fifty single nucleotide polymorphisms (SNPs) were genotyped in 6,556 multiethnic cases collected by the Type 1 Diabetes Genetics Consortium (T1DGC). These were tested for association with three islet autoantibodies—against autoantibodies to GAD (GADA), IA-2 (IA-2A), and zinc transporter 8 (ZnT8A)—and autoantibodies against thyroid peroxidase (TPOA) in autoimmune thyroid disease, gastric parietal cells (PCA) in autoimmune gastritis, transglutaminase (TGA) in celiac disease, and 21-hydroxylase (21-OHA) in autoimmune hypoadrenalism. In addition to the MHC region, we identify SNPs in five susceptibility loci (*IFIH1, PTPN22, SH2B3, BACH2*, and *CTLA4*) as significantly associated with more than one autoantibody at a false discovery rate less than 5%. *IFIH1*/2q24 demonstrated the most unrestricted association, as significant association was demonstrated for PCA, TPOA, GADA, 21-OHA, and IA-2A. In addition, 11 loci were significantly associated with a single autoantibody.

Type 1 diabetes (T1D) is a chronic disease that results from an immune-mediated destruction of the insulin-producing β-cells in the pancreatic islets of Langerhans. The specific β-cell destruction is caused by a complex interplay between multiple risk-conferring genes and environmental factors. To date, genome-wide association studies (GWAS) have identified more than 40 susceptibility loci for T1D (1). Except for the MHC region on chromosome 6p21, which contributes approximately 50% of the genetic risk, each of the loci identified through GWAS has modest individual effect on the total genetic risk for T1D. Autoantibodies directed against islet antigens are often detected at the onset of disease in affected individuals and are a distinctive signature of the autoimmune process that takes place at the initiation of the disease. Autoantibodies against insulin protein (IAA), IA-2 (IA-2A), GAD (GADA), and zinc transporter 8 (ZnT8A) have also been demonstrated to predict the onset of T1D in individuals at high genetic risk of the disease (2,3). Apart from the MHC region (4,5), very few studies have comprehensively studied the genetic causes of autoantibody development in T1D. Two GWAS have identified the *FCRL3*/1q23 locus as a susceptibility locus for IA-2A (6) and ZnT8 (7) positivity in a European population.

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A number of genetic susceptibility loci are shared between T1D and other immune-mediated and autoimmune diseases (8), such as celiac disease, rheumatoid arthritis, and autoimmune thyroid disease (AITD). It is also known that some of these diseases commonly coexist in individuals with T1D and cosegregate in families with T1D. The Type 1 Diabetes Genetics Consortium (T1DGC) has collected the largest number of affected offspring families with T1D. In addition to genetic markers and measurements of islet autoantibodies (GADA, IA-2A, and ZnT8A), the T1DGC Autoantibody Workshop sample collection has also been investigated for the presence of other autoimmune diseases. Measurements of other autoimmune disease–associated autoantibodies in individuals with T1D were made available for analysis through the T1DGC Autoantibody Laboratory. In order to investigate the possible genetic overlap between T1D and autoantibody positivity, we analyzed the association between single nucleotide polymorphisms (SNPs) in 50 T1D risk loci identified through recent GWAS and GADA, IA-2A, and ZnT8A, as well as autoantibodies against thyroid peroxidase (TPOA) (detected in AITD), gastric parietal cells (PCA) (autoimmune gastritis), transglutaminase (TGA) (celiac disease), and 21-hydroxylase (21-OHA) (autoimmune hypoadrenalism).

RESEARCH DESIGN AND METHODS

Participants With T1D
A total of 7,077 T1D cases from 4,134 families were available from the T1DGC and collected by the four T1DGC regional networks. A total of 975 cases came from the Asia-Pacific Network, 2,480 from the European Network, 3,299 from the North American Network, and 323 from the U.K. Network. Caucasian (non-Hispanic white) ethnicity was self-reported in 84.0% of the cases, 10.4% were of Black or African American ethnicity, 5.4% were of Asian ethnicity, and 0.3% was of Native American, Native Alaskan, Hawaiian or Pacific Islander ethnicity. All T1DGC protocols and consents were approved by the individual institutional review boards. Informed consent (and assent) was obtained from members of all participating families. Inclusion criteria have been described previously (9). Fifty percent of the cases were female. The

median age at diagnosis was 9 years (range 0–52 years), and the median disease duration at blood sampling was 7 years (range 0–63 years). Approximately 25% of the samples were taken within 3 years of diagnosis of T1D. A subset of cases were tested for ZnT8 ($N = 1,504$), with a disease duration less than 2 years with a median age at onset in those affected individuals of 11 years.

Autoantibody Measurements
Autoantibodies were measured in serum that had been stored at $-80°C$. GADA, IA-2A, TPOA, TGA, and 21-OHA were measured by radiobinding assays (outlined in Akolkar et al. [10]) in two laboratories (Bristol, U.K., and Aurora, CO). PCA and ZnT8A were measured as described in Wenzlau et al. (11). Quality-control methods and results for the autoantibody measurements are described in Akolkar et al. (10).

Genotyping and Quality Control
Of the 7,077 T1D cases with autoantibody measurements, a total of 6,556 were genotyped for 50 disease-risk SNPs selected from published GWAS loci. The SNPs were genotyped using the TaqMan 5′ nuclease assay (Applied Biosystems) according to the manufacturer's protocol. All SNPs had a genotyping success rate >90%, with 45/50 SNPs >95%. The mean genotyping success rate per individual was 95.7%. Genotype frequencies were tested for deviation from Hardy-Weinberg equilibrium. Three SNPs (rs2476601 in *PTPN22*, rs689 in *INS*, and rs9388489 in *CENPW*) exhibited statistically significant deviations from Hardy-Weinberg expectations ($P < 0.001$). These SNPs were not excluded from analysis, as disease association can cause genotype frequencies to deviate in affected offspring families.

Statistical Analysis
The statistical analyses were performed using the R (www.r-project.org) package functions. In R, we used the geepack (12) function to perform logistic regression analysis while controlling for family relatedness. This approach used the generalized estimating equations (GEE) (13) method. Family ID was used to identify clusters and an exchangeable working correlation matrix and robust variance was used to test for association using the Wald test. Autoantibody positivity was coded as a binary phenotype.

In addition to family relatedness, the logistic regression models were adjusted for self-reported (primary) ethnicity. Ethnicity was coded as a factor with four levels (1 = Caucasian, 2 = black or African American, 3 = Asia, 4 = Native Indian/Alaskan or Pacific Islander). Covariates, including age at diabetes onset, duration of diabetes, and sex, were included in the models if significantly associated with the autoantibody trait. SNP genotypes were included in the model under the assumption of additive allelic effects, coded 0, 1, or 2 for the number of minor alleles at a site. The estimated odds ratios (ORs) for autoantibodies were calculated for a 10-year difference in onset and duration, except for the ZnT8A that was measured in subjects with duration of diabetes less than 2 years. A false discovery rate (FDR)-adjusted P value below 0.05 (FDR <0.05) was used as the threshold for statistical significance. Despite only 50 SNPs tested, the threshold for genome-wide significance is defined as $P < 5.0 \times 10^{-8}$.

RESULTS
Of the 6,556 T1D subjects who had genotyping data available, 44.5% were positive for GADA, 46.6% were positive for IA-2A, 25.6% were positive for TPOA, 20.0% were positive for PCA, 7.4% were positive for TGA, and 1.6% were positive for 21-OHA autoantibodies. In the subset with ZnT8A measured, 1,335 cases were genotyped and 58.2% were positive.

Positivity for GADA was associated with later age at diabetes onset (OR 1.90, $P < 2.0 \times 10^{-16}$), shorter duration of disease (OR 0.67, $P < 2.0 \times 10^{-16}$), and greater prevalence of female sex (OR 1.52, $P = 5.6 \times 10^{-16}$). Positivity for IA-2A and ZnT8A was only associated with a shorter duration of diabetes (IA-2A: OR 0.52, $P < 2 \times 10^{-16}$; ZnT8A: OR 0.76, $P = 8.7 \times 10^{-5}$). Positivity for TPOA and PCA was associated with a later age at onset (TPOA: OR 1.23, $P = 1.0 \times 10^{-7}$; PCA: OR 1.27, $P = 1.5 \times 10^{-8}$), longer diabetes duration (TPOA: OR 1.22, $P = 4.4 \times 10^{-12}$; PCA: OR 1.37, $P < 2 \times 10^{-16}$), and female sex (TPOA: OR 2.22, $P < 2 \times 10^{-16}$; PCA: OR 1.72, $P < 2.0 \times 10^{-16}$). TGA positivity was associated with an earlier age at onset (OR 0.56, $P = 5.7 \times 10^{-11}$), shorter disease duration (OR 0.83, $P = 1.3 \times 10^{-3}$), and female sex (OR 1.31, $P = 4.8 \times 10^{-3}$). 21-OHA positivity was only associated

Table 1—Significant autoantibody associations at T1D risk loci

Locus	Chr.	SNP	Allele	GADA OR	GADA P	IA-2A OR	IA-2A P	ZnT8A OR	ZnT8A P	TPOA OR	TPOA P	PCA OR	PCA P	TGA OR	TGA P	21-OHA OR	21-OHA P
PTPN22	1	rs2476601	C>T*	1.1	0.07	0.83	**0.0007**			1.33	**6.70E-07**	1.32	**7.00E-06**			1.62	**0.0058**
IL10	1	rs3024505	C*>T			1.16	**0.015**										
IFIH1	2	rs1990760	A*>G	0.88	**0.0034**	1.11	**0.0076**			0.82	**1.00E-05**	0.76	**1.40E-08**				
CTLA4	2	rs3087243	G*>A			0.90	**0.0048**					0.91	0.04	1.27	**0.0007**		
IL2	4	rs2069762	T*>G			0.91	0.023									1.82	**0.0009**
HLA	6	rs2187668	G>A*	1.54	**<2E-16**	0.6	**<2E-16**							1.95	**<2E-16**	1.42	0.03
HLA	6	rs7454108	T>C*			2.27	**<2E-16**	1.48	**0.00011**	1.24	**4.40E-05**	1.33	**2.00E-06**			2.12	**7.40E-06**
BACH2	6	rs11755527	C>G*			0.89	**0.002**			1.14	**0.0022**	0.84	**0.0026**			1.4	0.022
SKAP2	7	rs7804356	T*>C														
IL2RA	10	rs12722495	A*>G	0.83	0.023							0.78	**0.0093**				
INS	11	rs689	A*>T			1.12	0.033					1.07	**0.0025**				
CD69	12	rs4763879	G>A*							1.17	**4.20E-04**						
ERBB3	12	rs2292239	C>A*			0.91	0.017			1.13	**0.0063**						
SH2B3	12	rs3184504	C>T*	1.07	0.0725	1.08	0.06	1.3	**0.003**	1.13	**0.003**	1.22	**4.30E-05**	1.16	0.046		
CTSH	15	rs3825932	T*>C														
IL27	16	rs4788084	G*>A			1.15	**0.0005**	1.23	0.014	0.86	**0.0016**						
SIRPG	20	rs2281808	C*>T													1.32	0.059
UBASH3A	21	rs3788013	C>A*			0.93	0.052			1.16	**0.0012**					1.39	0.025

The allele with * confers risk of T1D. OR, calculated for the minor allele. P values in boldface type pass an FDR of 5%. Chr., chromosome.

with a longer diabetes duration (OR 1.31, $P = 3.5 \times 10^{-4}$).

After adjusting for covariates, self-reported ethnicity also had an effect on autoantibody positivity. Compared with Caucasians, individuals of black or African American ethnicity had a slightly higher risk of positivity for GADA (OR 1.29, $P = 0.013$) but significantly lower risk of positivity for TPOA (OR 0.31, $P = 1.4 \times 10^{-12}$) and TGA (OR 0.36, $P = 1.0 \times 10^{-4}$). Individuals of Asian ethnicity had a significantly lower risk of positivity for IA-2A (OR 0.27, $P < 2.0 \times 10^{-16}$) and ZnT8A (OR 0.29, $P = 3.7 \times 10^{-6}$) compared with Caucasians. Although the Native American/Alaskan or Pacific Islander ancestry group was small (only 20 subjects), subjects of these ethnicities had higher risk for TGA positivity (OR 4.71, $P = 5.2 \times 10^{-3}$) compared with Caucasians.

The significant associations with T1D-associated SNPs for the seven autoantibodies are shown in Table 1. The logistic regression models were adjusted for family relatedness, self-reported ethnicity, and significant covariates. Eighteen of the 50 loci exhibited significant (FDR <5%) evidence of association with at least one autoantibody. Two associated MHC region SNPs, rs2187668 and rs7454108, are proxies for the predisposing HLA-DRB1*03 (DR3) and HLA-DRB1*04 (DR4) alleles, respectively (14,15). Both SNPs were associated strongly with autoantibody positivity. HLA-DR3 (rs2187668-A) was positively associated with GADA and TGA but negatively associated with IA-2A. HLA-DR4 (rs7454108-C) was positively associated with IA-2A, ZnT8A, PCA, TPOA, and 21-OHA.

For those T1D-associated SNPs not in the MHC region, only a SNP in the IFIH1/2q24 locus was significantly associated with GADA positivity. The major (A) allele of IFIH1 rs1990760 confers both risk of T1D and positivity for GADA. In contrast, six SNPs in non-MHC loci were associated with IA-2A positivity, residing in PTPN22/1p13, IL10/1q32, IFIH1/2q24, CTLA4/2q33, BACH2/6q15, and IL27/16p11. For SNPs associated with IA-2A positivity, the allele associated with risk of T1D was associated with protection from IA-2A positivity. The only exception was the A allele of CTLA4 rs3087243, which confers protection from T1D and is associated with IA-2A

positivity. The ZnT8A was tested in 1,335 subjects and only the *CTSH*/15q25 SNP was associated with positivity for ZnT8A among the non-MHC SNPs.

Positivity for TPOA was detected in ~25% of cases, whereas PCA was detected in ~20% of cases. Positivity for PCA and TPOA was associated with a SNP in *IFIH1*/2q24, with the T1D risk-conferring major allele (A) of rs1990760 positively associated with both autoantibodies. A SNP in *PTPN22*/1p13 locus demonstrated association with TPOA and PCA, as did a SNP in *SH2B3*/12q24. For both *PTPN22* and *SH2B3* loci, the T1D risk allele also was associated with positivity for TPOA and PCA. In addition to the shared loci, TPOA was associated with SNPs in four additional non-MHC loci—*BACH2*/6q15, *CD69*/12p13, *ERBB3*/12q13, and *SIRPG*/20p13. Non-MHC SNP associations with PCA were observed in *SKAP2*/7p15, *IL2RA*/10p15, *INS*/11p15, and *UBASH3A*/21q22. For all SNPs in these T1D risk loci, the risk allele was associated with autoantibody positivity. The only exception was in the *INS* locus for which the T1D *INS* rs689 protective allele (T) was associated with positivity for PCA.

Positivity for TGA was detected in 482 cases and the only significantly associated non-MHC SNP was found in the T1D risk locus *CTLA4*/2q33. The protective minor allele (A) of *CTLA4* rs3087243 was associated with positivity for TGA. Positivity for 21-OHA was only detected in 104 cases, yet SNPs in two non-MHC loci demonstrated significant association, *PTPN22*/1p13 and *IL2*/4q27, both of which conferred risk of T1D and autoantibody positivity (Fig. 1).

CONCLUSIONS

We demonstrate that there are genetic components that contribute to the risk of T1D and, at the same time, contribute to the development of autoantibodies associated with immune-mediated diseases. These shared genetic factors are replicated from the previous GWAS of autoantibody positivity in European subjects, such as the association for IA-2A with SNPs in the *IL27* and *IFIH1* loci; the association of TPOA with SNPs in the *PTPN22*, *BACH2* and *SH2B3* loci; and the association of PCA with SNPs in the *IFIH1* locus (6). At the same time, our results do not replicate other loci reported in the GWAS, yet they suggest novel loci that could contribute to autoantibody positivity and therefore should be investigated in independent populations.

Five non-MHC T1D risk loci (*IFIH1*, *PTPN22*, *SH2B3*, *BACH2*, and *CTLA4*)

contained SNPs that were consistently and robustly associated with positivity for more than one autoantibody. These loci are known to confer risk of several autoimmune disorders, in varying degrees, including T1D (1), AITD (6,16), and celiac disease (17,18). The *IFIH1* SNP rs1990760 was associated with positivity for PCA, TPOA, and GADA, yet it was associated with protection from IA-2A. *IFIH1* has not previously been associated with the risk of autoimmune gastritis, but association has been reported in AITD (19), although convincing evidence is lacking from large GWAS (20,21). *IFIH1* encodes a viral dsRNA-activated apoptosis protein with a role in host protection and clearance of virus infected cells (22). Enterovirus infections have been linked to T1D in epidemiological studies (23) and *IFIH1* has, therefore, been suggested as a link between viral triggers and autoimmunity in T1D (24). The fact that SNPs in *IFIH1* are associated with autoimmune diseases with no established link to virus infections and the strong effect of *IFIHI* on autoantibody positivity demonstrated in this study suggest that *IFIH1* may have additional functions specific for autoimmunity. In pancreatic β-cells, *IFIH1* has been shown to regulate local release of inflammatory mediators after dsRNA stimulation and contribute to apoptosis (25). Thus, *IFIH1* could have cell-specific function that relates the immune system to selective target cells and contributes to autoimmunity.

PTPN22, *SH2B3*, *BACH2*, and *CTLA4* all have reported function associated with the adaptive immune response, such as regulation of T-cell receptor (TCR) signaling, T-cell activation, or the antibody response. The *PTPN22* SNP was associated with positivity for TPOA, PCA, and 21-OHA but with protection from IA-2A positivity. The *PTPN22* gene encodes a lymphoid-specific intracellular phosphatase involved in the negative regulation of T-cell activation (26). The risk-associated allele is a gain-of-function variant that controversially results in a reduction of TCR signaling and activation (27). This same variant has been associated with the risk of AITD (28) and autoimmune hypoadrenalism (29,30) but not with autoimmune gastritis. Although *IFIH1* and *PTPN22* have relatively modest effects on the risk of positivity for islet autoantibodies, they
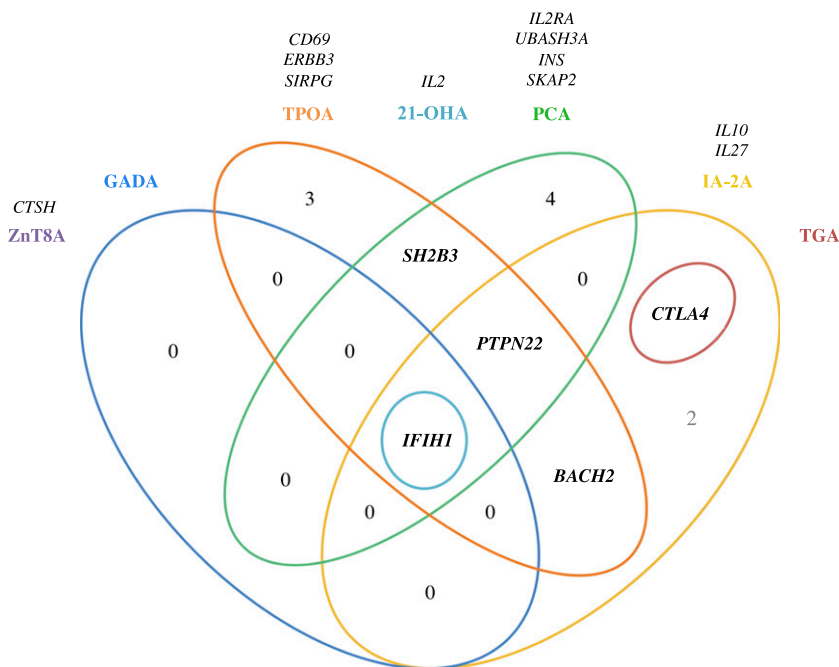

Figure 1—A schematic picture of the overlapping and autoantibody-specific significantly associated T1D risk loci reported in this study. The five loci that are shared between more than one autoantibody are showed in bold (*IFIH1*, *PTPN22*, *SH2B3*, *BACH2*, *CTLA4*), while the 11 autoantibody-specific loci are listed above the respective autoantibody.

both contribute to later development of markers for autoimmune hypoadrenalism, AITD, and autoimmune gastritis in T1D, especially in females.

SH2B3 SNP was associated with positivity for both TPOA and PCA; however, in our analyses, there was only marginal significance of the association with GADA (6) (*P* = 0.07). The *SH2B3* gene encodes an adaptor protein involved in T-cell activation by regulating TCR signaling (31). The *SH2B3* locus also confers susceptibility to celiac disease (18) but has not been associated with AITD or autoimmune gastritis.

A SNP in *BACH2* was associated with positivity for TPOA but with protection from IA-2A positivity, a finding that has not been reported previously. *BACH2* encodes a transcriptional repressor/activator involved in regulation of antibody response (32) and has, among others, been associated with the risk of AITD (20) and celiac disease (17,18). Recently, BACH2 was also identified as a regulator of β-cell apoptosis (33).

The SNP in the *CTLA4* locus was associated with IA-2A and TGA, results that have not been reported previously. Variants in the *CTLA4* locus have been associated consistently with susceptibility to other immune-mediated conditions, including celiac disease (18) and AITD (34,35). The *CTLA4* gene encodes a major negative regulator of T-cell activation (36). The major allele (G) of the *CTLA4* rs3087243 SNP confers risk of T1D and celiac disease and is the only risk allele that was associated with positivity for IA-2A; however, it was associated with protection from TGA autoantibodies. This differential effect highlights the degree of complexity in which the exact pathogenic mechanisms may differ between autoimmune diseases.

Eleven non-MHC SNPs demonstrated significant association with only a single autoantibody. For IA-2A, we replicate the association with *IL27* (6) and also report an association with *IL10*. For TPOA, we report novel associations with SNPs in *CD69*, *ERBB3*, and *SIRPG*. PCA positivity was associated with SNPs in *SKAP2*, *IL2RA*, *INS*, and *UBASH3A*. 21-OHA positivity was associated with SNPs in *IL2*, while positivity of ZnT8A was associated with a SNP in *CTSH*. Most of these genes have been associated with immune regulation, e.g., T-cell activation and signaling. The single-autoantibody specificity

association with single SNPs/genes also suggests target organ-specific mechanisms of autoimmunity.

HLA-associated alleles in the MHC are, not surprisingly, the major determinants of the risk for autoimmune disease as well as autoantibody development. Although the coverage of the MHC region in these data were limited, we replicated the association for HLA-DR3 with GADA positivity as well as the positive association of HLA-DR4 with IA-2A (4,5,37). Positivity for ZnT8A was associated with HLA-DR4 but not HLA-DR3, which might reflect a true lack of association or the lower power given the smaller subset of individuals that were tested. All nonislet autoantibodies demonstrated a positive association with HLA-DR4, with the exception of TGA that was associated with HLA-DR3. The HLA-DR3-DQ2 haplotype is strongly associated with celiac disease (38) and is required for presentation of dietary wheat epitopes that have been modified by tissue transglutaminase to T cells in the intestine (39). For TPOA and PCA, the association with SNPs in the MHC is weaker than that observed for SNPs in *PTPN22* and *IFIH1*. This interesting result may be explained by the genotyped SNPs failing to "tag" the most important HLA alleles. While risk of AITD and autoimmune hypoadrenalism is primarily associated with HLA-DR3 (40–42), autoantibody positivity was strongly associated with HLA-DR4, suggesting a difference in genetic control between disease susceptibility and development of autoimmunity.

Our study has lower power to detect associations of T1D-associated SNPs with 21-OHA, TGA, and ZnT8A due to the relatively low number of cases that were autoantibody positive. We cannot eliminate the possibility of an association with other T1D risk loci for these autoantibodies, especially for TGA, given the documented overlap between the genetic risk for T1D and celiac disease. Further, a majority of the subjects in this study come from a multiethnic collection of affected offspring trios. Although the data analysis controlled for relatedness among siblings and primary ethnicity, it is possible that the test statistics are inflated by residual stratification in the data. The effect sizes of the replicated loci, however, correspond with those reported in the European case-control cohort (6), suggesting that

the test statistic inflation may be low. The lack of correspondence for some loci could also be explained by differences in effect sizes in a family-based cohort compared with a case-control–based cohort, as has previously been suggested for T1D (1).

These analyses are consistent with recent reports that demonstrate a relationship between alleles that confer risk of autoimmune disease (e.g., T1D) and IA-2A positivity. IA-2A positivity was associated consistently with the protective alleles for all SNPs in T1D loci, with the exception of *CTLA4*, and exhibited an inverse relationship with risk of positivity for other autoantibodies. This paradoxical relationship is also apparent for variants in the MHC region, where the HLA-DR3 and HLA-DR4 alleles confer strong protection versus risk of IA-2A, whereas the heterozygous DR3/DR4 genotype confers the highest risk of T1D. These findings support that the islet autoantibodies are not, in themselves, pathogenic in T1D but are a consequence of the immune-mediated destruction of the β-cells. The association of *FCRL3* SNPs with IA-2A and ZnT8A but not with T1D supports this observation (6). In conclusion, the major loci reported here associate with multiple autoantibodies. Interestingly, four of the five loci showing significant association with multiple autoantibody positivity are also associated with seronegative autoinflammatory conditions, but here the main genetic signal is discordant with that of T1D (*PTPN22, IFIH1,* and *BACH2* for inflammatory bowel disease and *SH2B3* for ankylosing spondylitis) (43). These results highlight putative causal defects in T-cell activation and regulatory T cells, which may lead to defective control over B-cell tolerance and thereby contribute to initiation as well as maintenance of autoimmunity.

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