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Role of Type 1 Diabetes–Associated SNPs on Risk of Autoantibody Positivity in the TEDDY Study

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The Environmental Determinants of Diabetes in the Young (TEDDY) study prospectively follows 8,677 children enrolled from birth who carry HLA-susceptibility genotypes for development of islet autoantibodies (IA) and type 1 diabetes (T1D). During the median follow-up time of 57 months, 350 children developed at least one persistent IA (GAD antibody, IA-2A, or micro insulin autoantibodies) and 84 of them progressed to T1D. We genotyped 5,164 Caucasian children for 41 non-HLA single nucleotide polymorphisms (SNPs) that achieved genome-wide significance for association with T1D in the genome-wide association scan meta-analysis conducted by the Type 1 Diabetes Genetics Consortium. In TEDDY participants carrying high-risk HLA genotypes, eight SNPs achieved significant association to development of IA using time-to-event analysis ($P < 0.05$), whereof four were significant after adjustment for multiple testing ($P < 0.0012$): rs2476601 in *PTPN22* (hazard ratio [HR] 1.54 [95% CI 1.27–1.88]), rs2292239 in *ERBB3* (HR 1.33 [95% CI 1.14–1.55]), rs3184504 in *SH2B3* (HR 1.38 [95% CI 1.19–1.61]), and rs1004446 in *INS* (HR 0.77 [0.66–0.90]). These SNPs were also significantly associated with T1D in particular: rs2476601 (HR 2.42 [95% CI 1.70–3.44]). Although genes in the HLA region remain the most important genetic risk factors for T1D, other non-HLA genetic factors

contribute to IA, a first step in the pathogenesis of T1D, and the progression of the disease.

Although HLA accounts for approximately one-half of type 1 diabetes (T1D) risk (1), there remains substantial residual genetic risk, likely attributed to single nucleotide polymorphisms (SNPs) in genes outside the HLA region. In addition, nongenetic (environmental) factors contribute to the disease pathogenesis since the concordance rate between monozygotic twins is significantly less than 100% (for T1D between 13–65%) (2,3).

The Type 1 Diabetes Genetics Consortium (T1DGC) recently conducted a large genome-wide association scan (GWAS) meta-analysis (~7,000 T1D cases and ~9,000 controls) that identified 46 non-HLA (SNPs) in 40 loci that were robustly associated with T1D, the majority of which were replicated in a separate large collection (4,5). Some of these SNPs have previously been studied in other smaller long-term follow-up studies of newborn children. In BABYDIAB, a study that followed children to mothers or fathers with T1D from birth, some of these SNPs were related to the progression from autoantibody positivity to

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T1D (6). In the Diabetes Autoimmunity Study in the Young (DAISY), a study that followed children both from the general population (GP) as well as first-degree relatives (FDRs) of T1D patients, three SNPs (*PTPN22*, *UBASH3A*, and *C1QTNF6*) were associated with the development of islet autoantibodies (IA) and T1D, while SNPs in *PTPN22* were associated only with the risk of developing autoantibodies and those in *INS* and rs10517086 were only associated with T1D (7,8).

The Environmental Determinants of Diabetes in the Young (TEDDY) study is an international prospective study that enrolled newborn children during 5 years into a 15-year follow-up with a coordinated protocol. The TEDDY study attempts to identify the interplay between environmental factors and genetic susceptibility. T1D is marked by at least two stages before the β -cell function is deranged and the blood glucose starts to rise. In the first subclinical stage, autoantibodies can be identified (9) while the decrease in endogenous insulin production is still subclinical. In the later stages, blood glucose levels can be affected, observed by performing an oral glucose tolerance test (10). In this later stage, an infiltration of cytotoxic T-cells into the islets may eventually occur (11). The primary outcome of TEDDY is the development of persistent confirmed islet autoimmunity that is assessed every 3 months, and the secondary outcome is the diagnosis of T1D as defined by the American Diabetes Association (12).

In this article, our primary aim was to determine whether the non-HLA SNPs previously shown to be associated with T1D conferred an increased risk for IA (in participants with high-risk HLA genotypes), which is an initial step in the progression to T1D.

RESEARCH DESIGN AND METHODS

Subjects

During a period starting on 1 September 2004 and ending on 28 February 2010, a total of 421,047 newborn children in Finland, Sweden, Germany, and the U.S. (CO, GA, and WA) were screened for high-risk genotypes for T1D as

previously described (13,14). The high-risk genotypes for subjects from the GP were as follows: DR3/4, DR4/4, DR4/8, and DR3/3 (Table 1).

The original blood sample for HLA screening was obtained either as cord blood in the maternity clinic or as dry blood spot on day 3 to 4. When the child was eligible, the family was contacted by a study nurse and invited to participate in the follow-up study with blood sampling for analysis of autoantibodies (GAD antibody [GADA], IA-2A, and micro insulin autoantibodies [mIAA]) every 3 months during the first 4 years and biannually thereafter. A confirmatory blood sample for DNA to be used in HLA and other (non-HLA) SNPs was drawn at the 9-month visit. This study was performed according to the principles expressed in the Declaration of Helsinki. Written informed consents were obtained for all study participants from a parent or primary caretaker, separately, for genetic screening and participation in prospective follow-up. The study was approved by local institutional review boards and is monitored by an external evaluation committee formed by the National Institutes of Health.

Study Outcome: Islet Autoimmunity and T1D

The primary outcome was the development of persistent confirmed IA assessed every 3 months. IA were confirmed if identified in both reference laboratories. Persistent autoimmunity was defined by the presence of confirmed IA (GADA, IA-2A, or mIAA) on two or more consecutive visits. Date of persistent confirmed IA was defined as the draw date of the first of two consecutive samples at which the child was confirmed as positive for a specific autoantibody (or any autoantibody). As children can be born with maternal IA, positive results due to maternal transmission were excluded when defining the child's IA status. In order to distinguish maternal autoantibodies from IA in the child, the IA status of the mother was measured when the child was aged 9 months if the child was IA positive at 3 or 6 months of age. The child's IA status

Table 1—High-risk HLA genotypes constituting the criteria for eligibility for FDRs and children from the GP into the TEDDY study

Code in TEDDY	Genotypes	Abbreviation	GP
A	DRB1*04-DQA1*03-DQB1*03:02/DRB1*03-DQA1*05-DQB1*02:01	DR3/4	Yes
B	DRB1*04-DQA1*03-DQB1*03:02/DRB1*04-DQA1*03-DQB1*03:02	DR4/4†	Yes
C	DRB1*04-DQA1*03-DQB1*03:02/DRB1*08-DQA1*04-DQB1*04:02	DR4/8	Yes
D	DRB1*03-DQA1*05-DQB1*02:01/DRB1*03-DQA1*05-DQB1*02:01	DR3/3	Yes
E	DRB1*04-DQA1*03-DQB1*03:02/DRB1*04-DQA1*03-DQB1*02:02	DR4/4†	No
F	DRB1*04-DQA1*03-DQB1*03:02/DRB1*01-DQA1*01-DQB1*05:01	DR4/1	No
G	DRB1*04-DQA1*03-DQB1*03:02/DRB1*13-DQA1*01-DQB1*06:04	DR4/13	No
H	DRB1*04-DQA1*03-DQB1*03:02/DRB1*04-DQA1*03-DQB1*03:04	DR4/4	No
I	DRB1*04-DQA1*03-DQB1*03:02/DRB1*09-DQA1*03-DQB1*03:03	DR4/9	No
J	DRB1*03-DQA1*05-DQB1*02:01/DRB1*09-DQA1*03-DQB1*03:03	DR3/9	No

Genotypes A, B, C, and D confer GP eligibility but exclude DRB1*04:03. Genotypes A through J confer eligibility to an FDR of a diabetic patient. †Where DQB1*03:02 is noted, either allele or DQB1*03:04 is allowed, see Hagopian et al. (14).

was determined based on both maternal and child IA. If maternal autoantibodies were present, the child was not considered persistently IA positive unless the child had a negative sample prior to the first positive sample. All samples positive for IA and 5% of samples negative for IA were retested for confirmation in both reference laboratories. In the U.S., all sera were assayed at the Barbara Davis Center for Childhood Diabetes at the University of Colorado, Denver; in Europe, all sera were assayed at the University of Bristol, U.K. GADA, IA-2A, and mIAA were all measured using radioimmunobinding assays (15–18).

HLA Typing

Genotype screening was performed using either a dried blood spot punch or a small volume whole blood lysate specimen format (14,19). After PCR amplification of exon 2 of the HLA class II gene (DRB1, DQA1, or DQB1), alleles were identified either by direct sequencing, oligonucleotide probe hybridization, or other genotyping techniques. Additional typing to certify specific DR-DQ haplotypes was specified for each clinical center. Confirmation of the HLA genotypes was performed by the central HLA Reference Laboratory at Roche Molecular Systems, Oakland, CA, on the eligible subjects at 9 months of age.

SNPs

SNPs were genotyped by the Center for Public Health Genomics at the University of Virginia, using the Illumina ImmunoChip. The ImmunoChip is a custom genotyping array of SNPs selected from regions of the genome robustly associated with autoimmune disease. The final selection of SNPs for 12 autoimmune diseases was determined by the ImmunoChip Consortium. We genotyped all individuals on the ImmunoChip, a custom array of ~186,000 SNPs developed for fine mapping loci with statistically robust evidence of genome-wide association with autoimmune disease (including T1D). For purposes of this targeted interrogation of autoimmunity, only 41 SNPs previously shown to be associated with T1D risk from Barrett et al. (4) were analyzed. The 9-month TEDDY sample was used for SNP genotyping, having had DNA extracted by Roche Molecular Systems (Pleasanton, CA). Quality control (QC) steps to ensure high quality of the reported SNPs included the removal of subjects due to low call rate (>5% SNPs missing) and discordance with reported sex and prior genotyping. Secondly, SNPs were removed from analysis due to low call rate (<95%), Hardy-Weinberg equilibrium P value $<10^{-6}$ (except for chromosome 6 due to HLA eligibility requirements), as well as being monomorphic or an insertion-deletion.

The 46 SNPs presented by the T1DGC GWAS meta-analysis (4) and the *INS* SNP (rs1004446) from the T1DGC linkage analysis (20) were selected for this article. Four of the GWAS SNPs (4) were not available on the ImmunoChip (rs11755527 [*BACH2*], rs12444268 [chromosome 16], and rs917997 [*IL18RAP*]) or did not pass QC (rs2664170 [*Xq28*]). Since all subjects were entered into the TEDDY study based on high-risk HLA genotypes, no SNPs in the

HLA region were included in this analysis. Finally, rs1678536 (in a region with multiple genes) was excluded, as it was not included in the meta-analysis by Barrett et al. (4). A total of 41 SNPs were included in the statistical analyses (Tables 2 and 5).

Study Population

Of those newborns enrolled in TEDDY, the SNP QC steps resulted in a total of 7,023 subjects with SNP data on 176,586 SNPs. To permit more generalizable interpretation of the genetic associations, we included non-Hispanic Caucasians from the U.S. sites and all subjects from the European sites. Only subjects who were identified without a T1D-FDR were included, leaving 5,546 subjects. After including one subject per family carrying one of the TEDDY HLA genotypes, we performed principal components analysis for the U.S. subjects, using EIGENSTRAT software (21) to assess population heterogeneity. After applying principal components analysis, a total of 5,164 TEDDY children remained (male/female = 1.06).

Statistical Analyses

The primary analysis was to examine the association of the non-HLA SNPs with the development of persistent confirmed IA. For each SNP, the minor allele frequency (MAF) was determined in the study population and used in the analysis. This assumes that the risk is associated with the minor allele, even though the actual allele that has the lower frequency may differ across populations. Time to persistent confirmed IA was defined as the age when the first confirmed positive sample was drawn, and the right censored time was the age when the last negative sample was drawn. A Cox proportional hazard model was used after adjusting for HLA, country, and sex. Additionally, the first two principal components for the U.S. children and a constant for the European children were used to adjust for potential population stratification. The primary outcome was the first appearance for any type of persistent confirmed IA, and stratified analysis by HLA genotype was also performed.

Secondary outcomes were time to persistent confirmed GADA alone, mIAA alone, and time to T1D. Other appearances of first autoantibody were not performed because of low numbers. Time to T1D was defined as the age of diagnosis, and the right censored time was the age when the last clinic visit occurred. Finally, multiple Cox regression analysis for the primary outcome and T1D was performed including the SNPs that showed significant associations in the primary analysis.

The strength and directions of associations were denoted by hazard ratios (HRs) with 95% CIs. P values less than 0.05 were considered as statistically significant. Significance after Bonferroni correction was achieved when P values were less than 0.0012 (0.05/41). Statistical analyses were performed using SAS (version 9.3, SAS Institute, Cary, NC). QC analyses were performed using PLINK (<http://pngu.mgh.harvard.edu/purcell/plink/>) (22).

Table 2—Primary statistical analysis

Chromosome	SNP	Gene of interest	Minor allele	MAF	Autoantibody-positive subjects	P†
					(n = 350) vs. autoantibody-negative subjects (n = 4,814)*	
1p13.2	rs2476601	PTPN22	A	0.1113	1.54 (1.27–1.88)	0.00002
1p31.3	rs2269241	PGM1	C	0.2294	0.95 (0.80–1.14)	0.61488
1q31.2	rs2816316	RGS1	C	0.1768	1.24 (1.03–1.49)	0.02159
1q32.1	rs3024505	IL10	A	0.1568	1.03 (0.84–1.26)	0.76514
2p25.1	rs1534422	0	G	0.4574	1.00 (0.86–1.16)	0.95979
2q24.2	rs1990760	IF1H1	C	0.3976	0.98 (0.84–1.14)	0.78124
2q33.2	rs3087243	CTLA4	A	0.3978	0.97 (0.84–1.13)	0.73136
3p21.31	rs11711054	CCR5	G	0.3011	1.05 (0.89–1.23)	0.56956
4p15.2	rs10517086	0	A	0.2889	1.21 (1.04–1.42)	0.01623
4q27	rs4505848	IL2	G	0.3813	1.00 (0.86–1.17)	0.99731
5p13.2	rs6897932	IL7R	T	0.2896	0.92 (0.78–1.09)	0.34873
6q22.32	rs9388489	C6orf173	G	0.4476	1.04 (0.90–1.21)	0.57597
6q25.3	rs1738074	TAGAP	T	0.4131	0.87 (0.75–1.01)	0.06579
6q23.3	rs2327832	TNFAIP3	G	0.2120	0.90 (0.74–1.08)	0.24535
7p12.1	rs4948088	COBL	A	0.0442	0.62 (0.40–0.97)	0.03827
7p15.2	rs7804356	SKAP2	C	0.2282	0.86 (0.72–1.03)	0.11118
9p24.2	rs7020673	GLIS3	C	0.4939	1.01 (0.87–1.17)	0.88838
10p15.1	rs12251307	IL2RA	T	0.1262	0.95 (0.76–1.19)	0.66028
10p15.1	rs11258747	PRKCQ	T	0.2409	1.11 (0.94–1.32)	0.22364
10q23.31	rs10509540	RNLS	C	0.2666	1.00 (0.85–1.18)	0.99217
11p15.5	rs7111341	INS	T	0.2659	0.88 (0.74–1.05)	0.14362
11p15.5	rs1004446	INS	A	0.3759	0.77 (0.66–0.90)	0.00111
12p13.31	rs4763879	CD69	A	0.3789	1.10 (0.94–1.28)	0.23606
12q13.2	rs2292239	ERBB3	T	0.3271	1.33 (1.14–1.55)	0.00024
12q24.12	rs3184504	SH2B3	T	0.4590	1.38 (1.19–1.61)	0.00002
14q24.1	rs1465788	ZFP36L1, C14orf181	T	0.2874	0.90 (0.77–1.07)	0.23562
14q32.2	rs4900384	C14orf64	G	0.3247	1.01 (0.86–1.18)	0.93607
15q25.1	rs3825932	CTSH	T	0.3470	0.87 (0.74–1.01)	0.07539
16p11.2	rs4788084	IL27	T	0.4432	0.96 (0.83–1.11)	0.5755
16p13.13	rs12708716	CLEC16A	G	0.3430	0.84 (0.72–0.99)	0.03435
16q23.1	rs7202877	CTRB2	G	0.1115	1.12 (0.89–1.40)	0.33022
17p13.1	rs16956936	2 genes	T	0.1187	1.02 (0.82–1.28)	0.84991
17q12	rs2290400	ORMDL	T	0.4718	0.93 (0.80–1.08)	0.33644
17q21.2	rs7221109	CCR7	T	0.3710	1.03 (0.88–1.20)	0.73972
18p11.21	rs1893217	PTPN2	G	0.1641	0.99 (0.81–1.20)	0.90474
18q22.2	rs763361	CD226	T	0.4799	1.08 (0.93–1.26)	0.29751
19q13.32	rs425105	PRKD2	C	0.1585	1.07 (0.88–1.31)	0.48067
20p13	rs2281808	SIRPG	T	0.3420	0.94 (0.80–1.10)	0.42631
21q22.3	rs11203203	UBASH3A	A	0.3462	1.05 (0.90–1.23)	0.54469
22q12.2	rs5753037	HORMAD2	T	0.3604	1.09 (0.93–1.27)	0.30521
22q13.1	rs229541	C1QTNF6	A	0.4096	1.08 (0.93–1.25)	0.3356

Replication of 41 newly identified T1D risk loci from the T1DGC in 350 autoantibody-positive subjects vs. 4,814 autoantibody-negative subjects enrolled in the TEDDY study. Subjects eligible for TEDDY carried high-risk HLA DR-DQ genotypes. The MAF for the respective SNP was calculated from the results from all subjects. Proportional hazards models included HLA category, sex, and country for European sites as covariates. For U.S. sites, the principal components were estimated from our data from the U.S. population to be included in the model as covariates for the two major principal components. The principal components were used to adjust for heterogeneity within the Caucasian population in the U.S. sites. FDRs and members from the same family were omitted from the analyses. Risk was estimated as HRs and 95% CIs. The factors indicating nominal significant risk or protection are indicated in bold. *HR (95% CI). † χ^2 -P values remained significant after Bonferroni correction for multiple comparison of 41 SNPs if less than 0.0012.

RESULTS

As of 31 July 2013, 6.8% (350/5,164; male/female = 1.43) developed any type of persistent confirmed IA. Of those, 150 children developed GADA alone and 130 developed mIAA alone, while 48 developed both. A total of 84 subjects had progressed to T1D, but 3 of them were not identified with persistent confirmed IA. The median follow-up time was 57 months (interquartile range 42–75).

Risk for Autoantibody Positivity in TEDDY Using T1DGC GWAS SNPs Associated With T1D

Proportional hazard modeling identified eight SNPs as nominally associated with time to IA in this population of children born with high-risk HLA genotypes (Table 2). Five SNPs had a minor allele that conferred increased risk, and three SNPs had the minor allele associated with decreased risk. The five SNPs with minor alleles associated with increased risk were *PTPN22* (HR 1.54 [95% CI 1.27–1.88]; $P = 0.00002$), *RGS1* (HR 1.24 [95% CI 1.03–1.49]; $P = 0.02159$), a region on chromosome 4p15.2 without any known genes (HR 1.21 [95% CI 1.04–1.42]; $P = 0.01623$), *ERBB3* (HR 1.33 [95% CI 1.14–1.55]; $P = 0.00024$), and *SH2B3* (HR 1.38 [95% CI 1.19–1.61]; $P = 0.00002$). The SNPs where the minor allele was associated with decreased risk were *COBL* (HR 0.62 [95% CI 0.40–0.97]; $P = 0.03827$), *INS* (HR 0.77 [95% CI 0.66–0.90]; $P = 0.00111$), and *CLEC16A* (HR 0.84 [95% CI 0.72–0.99]; $P = 0.03435$) (Table 2). When all eight nominally significant SNPs were included in a multiple Cox regression analysis ($n = 5,164$), they all remained significant ($P < 0.05$) (Table 3). After Bonferroni correction for the number of SNPs tested, only four remained significant (*PTPN22*, $P = 0.00002$; *ERBB3*, $P = 0.00024$; *SH2B3*, $P = 0.00002$; and *INS*, $P = 0.00111$).

T1DGC SNPs and Risk for Autoantibody Positivity Among Participants With Specific Genotypes

The highest HRs were found among participants carrying the DR3/3 genotype ($n = 1,161$). Four SNPs were

nominally associated with risk for IA ($n = 40$; 3.4%) in this group: *CCR7* (HR 1.75 [95% CI 1.12–2.74]; $P = 0.0138$), *SH2B3* (HR 1.73 [95% CI 1.10–2.71]; $P = 0.01686$), *TNFAIP3* (HR 1.70 [95% CI 1.05–2.75]; $P = 0.02938$), and *CD226* (HR 1.56 [95% CI 1.00–2.42]; $P = 0.04822$) (Fig. 1A). None of the SNPs was associated with decreased risk for IA in this group.

In participants with HLA-DR3/4 genotype ($n = 2,081$), four T1D-associated SNPs had minor alleles that were nominally associated with increased risk of IA ($n = 188$; 9.0%): *PTPN22* (HR 1.44 [95% CI 1.09–1.90]; $P = 0.01067$), *ERBB3* (HR 1.32 [95% CI 1.07–1.62]; $P = 0.00893$), *RGS1* (HR 1.31 [95% CI 1.02–1.67]; $P = 0.032$), and a region on chromosome 4p15.2 (HR 1.29 [95% CI 1.05–1.60]; $P = 0.01785$). One of the remaining 37 SNPs had a minor allele associated with decreased risk of IA: *SIRPG* (HR 0.79 [95% CI 0.63–0.98]; $P = 0.03098$) (Fig. 1B).

Among participants with HLA-DR4/4 or HLA-DR4/8 genotypes ($n = 1,922$), three SNPs had a minor allele that nominally indicated increased risk of IA ($n = 122$; 6.3%): *SH2B3* (HR 1.65 [95% CI 1.27–2.13]; $P = 0.00015$), *PTPN22* (HR 1.63 [95% CI 1.19–2.24]; $P = 0.00238$), and *ERBB3* (HR 1.37 [95% CI 1.06–1.78]; $P = 0.01659$). One SNP was identified as having a minor allele that was associated with decreased risk of IA in this group: *INS* (HR 0.68 [95% CI 0.51–0.89]; $P = 0.00544$) (Fig. 1C).

Analysis of the interaction between each SNP and HLA for persistent confirmed IA identified two SNPs as significant: *CCR7* (increased risk among DR3/3; $P = 0.0138$) and *SIRPG* (decreased risk among DR3/4 [$P = 0.0310$] and increased risk among DR4/8 [$P = 0.0372$]). Only one SNP remained significantly associated with IA after Bonferroni correction in any of the three different HLA categories (*SH2B3* [$P = 0.00015$] among DR4/4 or DR4/8). A summary of the nominally significant associations for IA analysis in relation to HLA category is shown in Table 4.

Table 3—Multiple Cox regression analysis including the factors that showed significant associations to autoantibody positivity when analyzed univariately

Chromosome	SNP	Gene	Autoantibody-positive subjects ($n = 350$) vs. autoantibody-negative subjects ($n = 4,814$)*	P
1p13.2	rs2476601	<i>PTPN22</i>	1.54 (1.26–1.87)	<0.0001
1q31.2	rs2816316	<i>RGS1</i>	1.25 (1.04–1.51)	0.0185
4p15.2	rs10517086	0	1.22 (1.04–1.43)	0.0142
7p12.1	rs4948088	<i>COBL</i>	0.62 (0.40–0.97)	0.0346
11p15.5	rs1004446	<i>INS</i>	0.77 (0.65–0.90)	0.0010
12q13.2	rs2292239	<i>ERBB3</i>	1.31 (1.12–1.52)	0.0005
12q24.12	rs3184504	<i>SH2B3</i>	1.38 (1.19–1.61)	<0.0001
16p13.13	rs12708716	<i>CLEC16A</i>	0.84 (0.71–0.98)	0.0324

The analysis was corrected for HLA category, sex, and country for European sites as covariates. For U.S. sites, the principal components were estimated from our data from the U.S. population to be included in the model as a covariate for the two major principal components. The principal components were used to adjust for heterogeneity within the Caucasian population in the U.S. sites. The factors indicating nominal significant risk or protection are indicated in bold. *HR (95% CI).

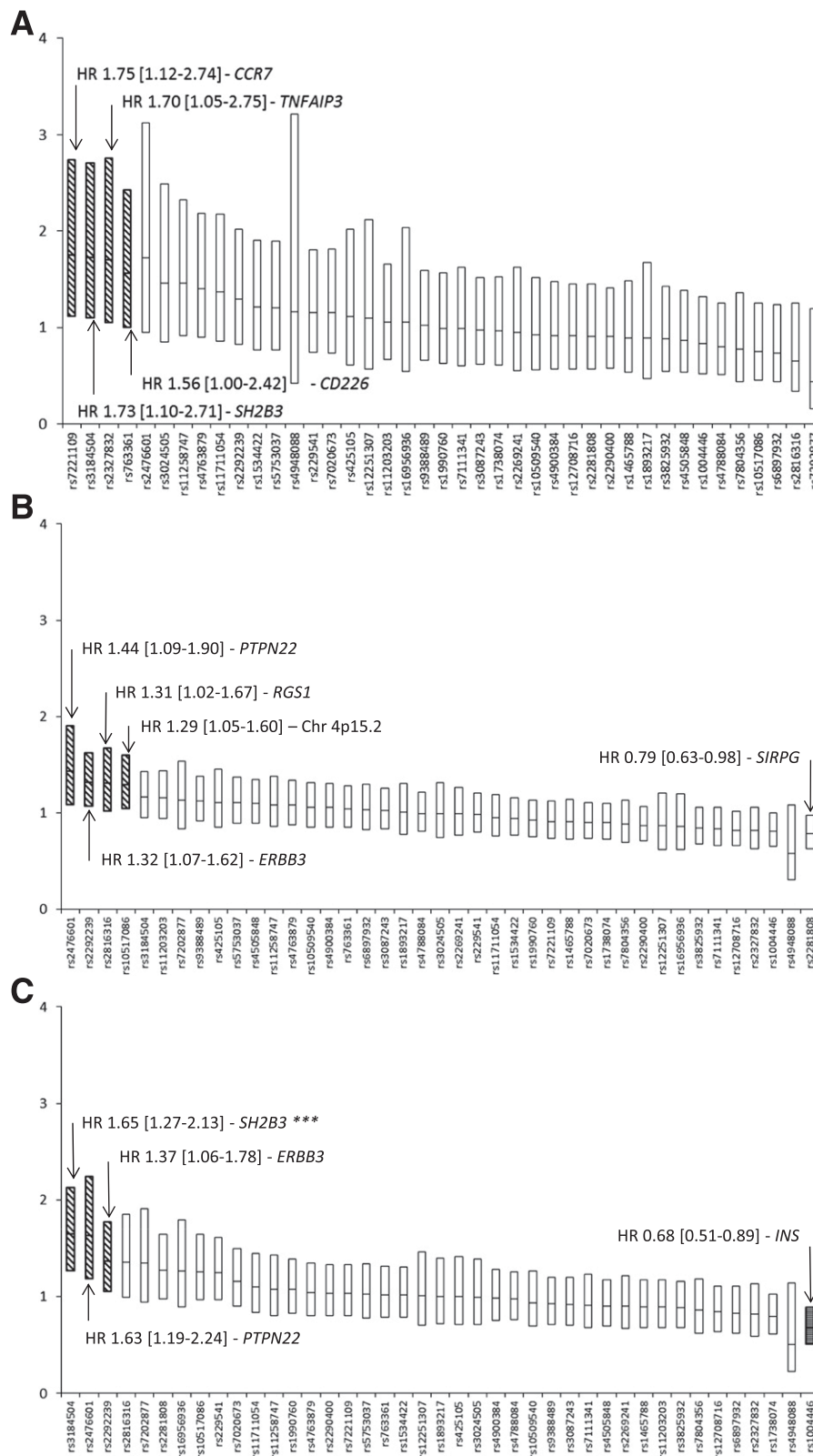


Figure 1—Children were enrolled from the GP in the TEDDY study based on their high-risk HLA genotypes, and SNPs were analyzed using the ImmunoChip ($n = 5,164$). The figure illustrates a summary of the 41 SNPs replicated from the study by Barrett et al. (4). SNPs associated with nominally significant increased risk for autoantibody positivity are indicated by upward diagonal lines, and SNPs associated with protection from autoantibody positivity are indicated by horizontal lines. The upper 95% CI is indicated by the upper bar of the box, the lower 95% CI is indicated by the lower bar of the box, and the HR is indicated by a solid bar in the middle of the box. HRs were different from 1.0, but only rs3184504 in *SH2B3* ($P = 0.00015$) among the DR4/4 or DR4/8 carriers were significant after Bonferroni correction. A: HRs and 95% CIs in 40 autoantibody-positive subjects and 1,121 autoantibody-negative subjects carrying the DRB1*03-DQA1*05-

Association Between SNPs and Specific IA

All eight SNPs that showed nominal association ($P < 0.05$) with positivity for any IA were tested for association with the two most common IA, mIAA ($n = 150$) and GADA ($n = 130$). Two SNPs were significantly associated with the risk of developing mIAA first as well as developing GADA first (*ERBB3* and *SH2B3*). Another two SNPs, *PTPN22* and a region on chromosome 4p15.2, were significantly associated with the risk of developing only mIAA first. One SNP (*COBL*) was significantly associated with decreased risk for mIAA as the first autoantibody (Table 4).

Association of T1DGC GWAS Meta-Analysis SNPs Among Children With High-Risk HLA Genotypes in TEDDY

Eight SNPs previously reported as attaining genome-wide association for T1D were nominally associated with time to T1D in this study of high-risk HLA genotyped participants in TEDDY. These SNPs included four where the minor allele was associated with increased risk: *PTPN22* (HR 2.42 [95% CI 1.70–3.44]; $P = 1.01 \times 10^{-6}$), *CCR5* (HR 1.40 [95% CI 1.02–1.92]; $P = 0.03629$), *ERBB3* (HR 1.53 [95% CI 1.13–2.08]; $P = 0.0066$), and *SH2B3* (HR 1.40 [95% CI 1.03–1.89]; $P = 0.03083$). Two SNPs in high linkage disequilibrium with the *INS* gene on chromosome 11p were associated with T1D, where minor alleles conferred decreased risk, rs7111341 (HR 0.57 [95% CI 0.38–0.85]; $P = 0.00646$) and rs1004446 (*INS*; HR 0.63 [95% CI 0.45–0.88]; $P = 0.0072$). Two other SNPs that also indicated decreased risk were C14orf64 (HR 0.71 [95% CI (0.50–1.00)]; $P = 0.04973$) and *CTSH* (HR 0.68 [95% CI 0.48–0.95]; $P = 0.02483$) (Table 5). When all eight significant SNPs were included in a multiple Cox regression analysis ($n = 5,164$), all except C14orf64 remained significant ($P < 0.05$) (Table 6). Only one SNP remained significant after Bonferroni correction (*PTPN22*; $P = 1.01 \times 10^{-6}$).

DISCUSSION

We conclude that although HLA is still the most important factor for prediction of T1D, other genetic factors (some within the immune system) may contribute to early development of IA and progression of the disease. In this study with a population where all subjects carried any of four high-risk HLA genotypes, we found eight SNPs previously reported as associated with T1D as nominally associated with the risk of developing autoantibodies in TEDDY (*PTPN22*, *RGS1*, a region on chromosome 4p15.2, *COBL*, *INS*, *ERBB3*, *SH2B3*, and *CLEC16A*) across all high-risk HLA categories. After inclusion in multiple Cox regression analysis, all eight remained significant ($P <$

0.05). However, after Bonferroni correction, only four remained significant (*PTPN22*, *ERBB3*, *SH2B3*, and *INS*). The SNP that attained the highest risk for autoantibody positivity was *PTPN22* since it conferred ~50% increased risk (HR 1.54 [95% CI 1.27–1.88]), which was replicated in all HLA categories except DR3/3. *SH2B3* was associated with ~40–70% increased risk for IA, which is higher but consistent with previously reported risk for T1D of ~30% (23–25), except in DR3/4 carriers, where it was not significant. *ERBB3* conferred ~30% increased risk for IA, which is similar to previously reported increased risk for T1D (23–25), except in the DR3/3 carriers, where it did not reach significance. In addition, *PTPN22*, *ERBB3*, *SH2B3*, and *INS* were also significantly associated with time to T1D.

The SNP with the highest association for both IA ($P < 0.001$) and T1D ($P < 0.001$) was *PTPN22*, a gene coding for a protein tyrosine phosphatase involved in T-cell receptor signaling (26). This association to both IA as well as T1D has been demonstrated earlier (27). In DAISY, *PTPN22*, and another two SNPs in *UBASH3A* and *C1QTNF6* were predictive for IA as well as T1D (7,8). *PTPN22* is expressed in lymphoid tissue such as fetal liver, spleen, tonsils, and B- and T-cells and has been extensively studied. The variant C1858T encodes for a variant of *PTPN22* (rs2476601) where tryptophan is substituted by arginine (R620W) (26). This less common variant of *PTPN22* confers a more active enzyme that inhibits T-cell receptor signaling (26) and has been associated with other autoimmune diseases besides T1D (28), for example, systemic lupus erythematosus (29) and rheumatoid arthritis (30). Moreover, *PTPN22* (R620W) has been reported to be associated with development of IA in children exposed to cow's milk formula early in childhood (31) and also with development of thyroid peroxidase autoantibodies and Graves disease in patients with T1D (25). Carriers of the TT variant of *PTPN22* have previously shown a strong association with GADA positivity at onset of T1D, particularly among subjects with low risk or neutral HLA genotypes (32).

The *ERBB3* locus was also associated with IA. *ERBB3* is a member of the family of intracellular receptors of protein tyrosine kinases that control cell proliferation and differentiation and may have a mechanism similar to that of *PTPN22* variants (33).

The *SH2B3* locus, which was significantly associated with time to IA after Bonferroni correction, expresses an adaptor protein involved in intracellular signaling to downregulate B- and T-cell proliferation (34). Carriers of the minor allele of rs3184504 (*SH2B3*) are protected against bacterial infections (35).

DQB1*02:01/DRB1*03-DQA1*05-DQB1*02:01 (DR3/3) HLA genotype ($n = 1,161$). B: HRs and 95% CIs in 188 autoantibody-positive subjects and 1,893 autoantibody-negative subjects carrying the DRB1*04-DQA1*03-DQB1*03:02/DRB1*03-DQA1*05-DQB1*02:01 (DR3/4) HLA genotype ($n = 2,081$). C: HRs and 95% CIs in 122 autoantibody-positive subjects and 1,800 autoantibody-negative subjects carrying either DRB1*04-DQA1*03-DQB1*03:02/DRB1*04-DQA1*03-DQB1*03:02 (DR4/4) or DRB1*04-DQA1*03-DQB1*03:02/DRB1*08-DQA1*04-DQB1*04:02 (DR4/8) HLA genotypes ($n = 1,922$). ***, indicates significance after Bonferroni correction.

Table 4—Secondary statistical analyses

Chromosome	SNP	Gene of interest	HLA combined (n = 5,164) any IA (n = 350)*	DR3/3 (n = 1,161) any IA (n = 40)*	DR4/4 and DR4/8 (n = 1,922) any IA (n = 122)*	DR3/4 (n = 2,081) any IA (n = 188)*	mIAA as first (n = 150)*	GADA as first (n = 130)*
SNPs significant for time to any autoantibody across all HLA genotypes								
	1p13.2	PTPN22	1.54 (1.27–1.88)	1.72 (0.95–3.12)	1.63 (1.19–2.24)	1.44 (1.09–1.90)	1.67 (1.25–2.22)	1.23 (0.86–1.77)
	1q31.2	RGST1	1.24 (1.03–1.49)	0.65 (0.34–1.25)	1.36 (1.00–1.85)	1.31 (1.02–1.67)	1.30 (0.99–1.72)	1.07 (0.78–1.47)
	4p15.2	0	1.21 (1.04–1.42)	0.75 (0.45–1.25)	1.26 (0.96–1.64)	1.29 (1.05–1.60)	1.36 (1.07–1.72)	1.06 (0.81–1.37)
	7p12.1	COBL	0.62 (0.40–0.97)	1.16 (0.42–3.21)	0.51 (0.23–1.14)	0.58 (0.31–1.09)	0.44 (0.20–0.99)	1.06 (0.59–1.90)
	11p15.5	INS	0.77 (0.66–0.90)	0.83 (0.52–1.32)	0.68 (0.51–0.89)	0.81 (0.65–1.00)	0.83 (0.65–1.05)	0.82 (0.64–1.06)
	12q13.2	ERBB3	1.33 (1.14–1.55)	1.29 (0.83–2.02)	1.37 (1.06–1.78)	1.32 (1.07–1.62)	1.29 (1.03–1.63)	1.30 (1.01–1.67)
	12q24.12	SH2B3	1.38 (1.19–1.61)	1.73 (1.10–2.71)	1.65 (1.27–2.13)	1.17 (0.95–1.43)	1.41 (1.12–1.77)	1.49 (1.17–1.91)
	16p13.13	CLEC16A	0.84 (0.72–0.99)	0.91 (0.57–1.45)	0.84 (0.64–1.11)	0.82 (0.66–1.02)	0.85 (0.67–1.09)	0.82 (0.63–1.07)
Other SNPs not significant for time to any autoantibody across all HLA genotypes								
	6q23.3	<i>TNFAIP3</i>	0.90 (0.74–1.08)	1.70 (1.05–2.75)	0.82 (0.59–1.13)	0.81 (0.63–1.06)	0.93 (0.70–1.24)	0.73 (0.53–1.01)
	16q23.1	<i>CTRB2</i>	1.12 (0.89–1.40)	0.43 (0.16–1.19)	1.34 (0.95–1.91)	1.13 (0.83–1.53)	1.39 (1.02–1.90)	1.01 (0.69–1.48)
	17q12	<i>ORMDL</i>	0.93 (0.80–1.08)	0.90 (0.58–1.40)	1.04 (0.81–1.33)	0.87 (0.71–1.06)	1.12 (0.89–1.40)	0.76 (0.59–0.97)
	17p13.1	2 genes	1.02 (0.82–1.28)	1.05 (0.54–2.03)	1.26 (0.89–1.79)	0.86 (0.62–1.20)	1.37 (1.00–1.86)	0.74 (0.48–1.12)
	17q21.2	<i>CCR7</i>	1.03 (0.88–1.20)	1.75 (1.12–2.74)	1.03 (0.80–1.33)	0.91 (0.73–1.13)	0.96 (0.76–1.21)	1.09 (0.85–1.40)
	18q22.2	<i>CD226</i>	1.08 (0.93–1.26)	1.56 (1.00–2.42)	1.02 (0.79–1.31)	1.04 (0.85–1.28)	0.95 (0.76–1.21)	1.01 (0.79–1.29)
	20p13	<i>SIRPG</i>	0.94 (0.80–1.10)	0.91 (0.57–1.45)	1.27 (0.98–1.65)	0.79 (0.63–0.98)	1.07 (0.84–1.36)	0.99 (0.77–1.28)

Summary of associations to autoantibody positivity, time to any autoantibody (n = 350) across HLA categories (n = 5,164), split by HLA categories and by specific autoantibodies. The factors indicating nominal significant risk or protection are indicated in bold. *HR (95% CI).

Table 5—Primary statistical analysis

Chromosome	SNP	Gene of interest	Minor allele	MAF	Subjects with T1D (n = 84) vs. all other subjects (n = 5,080)*	P†
1p13.2	rs2476601	PTPN22	A	0.1113	2.42 (1.70–3.44)	1.01 × 10⁻⁶
1p31.3	rs2269241	<i>PGM1</i>	C	0.2294	1.04 (0.73–1.49)	0.81755
1q31.2	rs2816316	<i>RGS1</i>	C	0.1768	0.91 (0.60–1.37)	0.64423
1q32.1	rs3024505	<i>IL10</i>	A	0.1568	0.97 (0.64–1.48)	0.89277
2p25.1	rs1534422	<i>0</i>	G	0.4574	1.06 (0.78–1.44)	0.72504
2q24.2	rs1990760	<i>IF1H1</i>	C	0.3976	1.08 (0.79–1.46)	0.63795
2q33.2	rs3087243	<i>CTLA4</i>	A	0.3978	1.20 (0.88–1.63)	0.24179
3p21.31	rs11711054	CCR5	G	0.3011	1.40 (1.02–1.92)	0.03629
4p15.2	rs10517086	<i>0</i>	A	0.2889	1.25 (0.91–1.72)	0.16686
4q27	rs4505848	<i>IL2</i>	G	0.3813	0.98 (0.71–1.34)	0.88061
5p13.2	rs6897932	<i>IL7R</i>	T	0.2896	1.09 (0.79–1.52)	0.59696
6q22.32	rs9388489	<i>C6orf173</i>	G	0.4476	0.98 (0.72–1.33)	0.89509
6q25.3	rs1738074	<i>TAGAP</i>	T	0.4131	0.85 (0.62–1.15)	0.29345
6q23.3	rs2327832	<i>TNFAIP3</i>	G	0.2120	1.26 (0.89–1.79)	0.19154
7p12.1	rs4948088	<i>COBL</i>	A	0.0442	0.68 (0.28–1.66)	0.39871
7p15.2	rs7804356	<i>SKAP2</i>	C	0.2282	0.75 (0.51–1.12)	0.15947
9p24.2	rs7020673	<i>GLIS3</i>	C	0.4939	1.18 (0.87–1.60)	0.28837
10p15.1	rs12251307	<i>IL2RA</i>	T	0.1262	1.07 (0.68–1.68)	0.76958
10p15.1	rs11258747	<i>PRKCQ</i>	T	0.2409	1.20 (0.85–1.68)	0.3033
10q23.31	rs10509540	<i>RNLS</i>	C	0.2666	0.98 (0.70–1.38)	0.901
11p15.5	rs7111341	INS	T	0.2659	0.57 (0.38–0.85)	0.00646
11p15.5	rs1004446	INS	A	0.3759	0.63 (0.45–0.88)	0.0072
12p13.31	rs4763879	<i>CD69</i>	A	0.3789	0.95 (0.69–1.30)	0.72972
12q13.2	rs2292239	ERBB3	T	0.3271	1.53 (1.13–2.08)	0.0066
12q24.12	rs3184504	SH2B3	T	0.4590	1.40 (1.03–1.89)	0.03083
14q24.1	rs1465788	<i>ZFP36L1, C14orf181</i>	T	0.2874	0.88 (0.63–1.24)	0.47921
14q32.2	rs4900384	C14orf64	G	0.3247	0.71 (0.50–1.00)	0.04973
15q25.1	rs3825932	CTSH	T	0.3470	0.68 (0.48–0.95)	0.02483
16p11.2	rs4788084	<i>IL27</i>	T	0.4432	0.88 (0.65–1.19)	0.41201
16p13.13	rs12708716	<i>CLEC16A</i>	G	0.3430	0.89 (0.64–1.23)	0.47246
16q23.1	rs7202877	<i>CTRB2</i>	G	0.1115	0.85 (0.52–1.40)	0.52287
17p13.1	rs16956936	<i>2 genes</i>	T	0.1187	1.19 (0.77–1.84)	0.42846
17q12	rs2290400	<i>ORMDL</i>	T	0.4718	0.78 (0.58–1.06)	0.11691
17q21.2	rs7221109	<i>CCR7</i>	T	0.3710	1.11 (0.82–1.52)	0.50186
18p11.21	rs1893217	<i>PTPN2</i>	G	0.1641	0.93 (0.62–1.40)	0.7313
18q22.2	rs763361	<i>CD226</i>	T	0.4799	1.02 (0.75–1.38)	0.90741
19q13.32	rs425105	<i>PRKD2</i>	C	0.1585	1.00 (0.66–1.51)	0.9864
20p13	rs2281808	<i>SIRPG</i>	T	0.3420	0.81 (0.58–1.13)	0.21733
21q22.3	rs11203203	<i>UBASH3A</i>	A	0.3462	1.06 (0.77–1.46)	0.74207
22q12.2	rs5753037	<i>HORMAD2</i>	T	0.3604	1.20 (0.87–1.65)	0.26825
22q13.1	rs229541	<i>C1QTNF6</i>	A	0.4096	0.92 (0.67–1.25)	0.59161

Replication of 41 newly identified T1D risk loci from the T1DGC in 84 subjects with T1D vs. 5,080 autoantibody-negative subjects enrolled in the TEDDY study. Subjects eligible for TEDDY carried high-risk HLA DR-DQ genotypes. The MAF for the respective SNP was calculated from the results from all subjects. Proportional hazards models included HLA category, sex, and country for European sites as covariates. For U.S. sites, the principal components were estimated from our data from the U.S. population to be included in the model as a covariate for the two major principal components. The principal components were used to adjust for heterogeneity within the Caucasian population in the U.S. sites. FDRs and members from the same family were omitted from the analyses. Risk was estimated as HRs and 95% CIs. The factors indicating nominal significant risk or protection are indicated in bold. *HR (95% CI). † χ^2 -P values remained significant after Bonferroni correction for multiple comparison of 41 SNPs if less than 0.0012.

Table 6—Multiple Cox regression analysis including the factors that showed significant associations to T1D when analyzed univariately

Chromosome	SNP	Gene	Subjects with T1D (<i>n</i> = 84) vs. all other subjects (<i>n</i> = 5,080)*	<i>P</i>
1p13.2	rs2476601	<i>PTPN22</i>	2.49 (1.74–3.55)	<0.0001
3p21.31	rs11711054	<i>CCR5</i>	1.36 (1.00–1.86)	0.0499
11p15.5	rs7111341	<i>INS</i>	0.62 (0.41–0.94)	0.0250
11p15.5	rs1004446	<i>INS</i>	0.67 (0.47–0.95)	0.0236
12q13.2	rs2292239	<i>ERBB3</i>	1.47 (1.08–2.00)	0.0140
12q24.12	rs3184504	<i>SH2B3</i>	1.44 (1.06–1.96)	0.0185
14q32.2	rs4900384	C14orf64	0.72 (0.51–1.02)	0.0619
15q25.1	rs3825932	<i>CTSH</i>	0.68 (0.49–0.96)	0.0269

The analysis was corrected for HLA category, sex, and country for European sites as covariates. For U.S. sites, the principal components were estimated from our data from the U.S. population to be included in the model as a covariate for the two major principal components. The principal components were used to adjust for heterogeneity within the Caucasian population in the U.S. sites. The factors indicating nominal significant risk or protection are indicated in bold. *HR (95% CI).

The *INS*-VNTR (and its surrogate SNP rs689) has long been associated with T1D risk. We identified the minor allele A for rs1004446 in *INS* as significantly protective for the development of IA in the entire group and nominally among DR4/4 or DR4/8 carriers and for T1D. The minor allele A of rs1004446 in *INS* conferred 23–32% decreased risk for IA but was not significantly associated in subjects with DR3/3 or DR3/4 genotypes. The minor allele A of rs1004446 in *INS* has previously been shown to be protective for T1D (25). The other *INS* SNP in our study (rs7111341) was in weak linkage disequilibrium with rs689 ($r^2 = 0.26$). The minor allele T of rs7111341 in *INS* was associated with reduced risk for the progression to T1D. The long class III *INS*-VNTR is correlated to the rare variant T of rs689 (36). Earlier studies of *INS*-VNTR have shown that class III *INS*-VNTR confers protection for the development of T1D (37,38). In BABYDIAB, it was found that the class III *INS*-VNTR was protective for the development of IA before 2 years of age and T1D before 6 years of age in carriers of the high-risk HLA-DR3/DR4-DQ8 or DR4-DQ8/DR4-DQ8 genotypes (39).

There appears to be a genetic heterogeneity related to the appearance of the first autoantibody. The first autoantibody to appear in most subjects was mIAA (43%; 150/350). Five of eight SNPs that were associated with the appearance of any autoantibody (*PTPN22*, a region on chromosome 4p15.2, *COBL*, *ERBB3*, and *SH2B3*) were replicated for mIAA as the first autoantibody. The second most common autoantibody to appear as the first autoantibody was GADA (37%; 130/350), which was significantly associated with two of the eight SNPs (*ERBB3* and *SH2B3*).

In addition to the most prominent risk factor for IA and T1D, *PTPN22*, two other SNPs were significantly associated with increased risk for IA and nominally for T1D: *ERBB3* and *SH2B3*. One SNP, rs1004446 in *INS*, showed consistent decreased risk for both IA (significant) and T1D (nominal). Four SNPs achieved nominally significant

HR for IA but not for T1D: *RGS1*, a region on chromosome 4p15.2, *COBL*, and *CLEC16A* (Tables 2 and 5). These findings could be due to the fact that the group that had developed T1D was much smaller compared with the group of subjects that had developed IA and that an association with T1D may be found as future cases of T1D develop. On the other hand, four SNPs showed nonsignificant associations with autoantibody positivity but achieved nominally significant HR for T1D: rs7111341 in *INS*, C14orf64, *CTSH* (reduced risk), and *CCR5* (increased risk). The finding that some SNPs might not be predictive of IA but predictive of T1D could perhaps reflect an environmental exposure associated with progression from IA to T1D.

The major strength of the current study is the use of IA as an end point in time to event analyses. Additional strengths of this study are that we have unrelated subjects without autoantibodies or T1D from the same countries in the same age ranges as the subjects with outcome events. One limitation of the current study is the relatively low number of autoantibody-positive subjects and subjects with T1D raising concerns for statistical power. Nevertheless, this approach of screening children with genetic susceptibility for T1D followed from birth has prompted us to screen almost half a million newborn babies in four different countries. The screening procedure at birth and the close monitoring of autoantibodies every third month for the first 4 years and biannually thereafter is unique in the field of diabetes research. Therefore, we think that the advantages of this approach far outweigh the limitation in power. Besides, the proportional HR identifies risk for time to event in this longitudinal study, thereby increasing the power compared with cross-sectional case-control studies. A current limitation is that we cannot draw conclusions about the importance of these SNPs as predictive factors at ages above 9 years since all our subjects are currently below this threshold. Another limitation on this study is

that our data only include subjects from the GP, and therefore we do not know whether these findings will be applicable also for FDRs.

A fundamental difference between this population and previous publications reporting on risk or protective SNPs for T1D is that all the children enrolled in this study are carriers of high-risk HLA genotypes for T1D. Moreover, our population consisting of autoantibody-positive subjects, as well as T1D cases, are younger compared with most previous publications since the subjects are younger than 9 years of age. Previous studies reporting the risk of non-HLA genetic factors on the risk of IA are limited to FDRs (40) or included a limited number of GP subjects (7), whereas the subjects included in the current study are all from the GP.

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Author Contributions. C.T. conceived the study, interpreted data, and wrote the manuscript. D.H. and H.-S.L. analyzed data and reviewed/edited the manuscript. W.H., B.A., and S.S.R. contributed to study design and performance, discussion, and reviewed/edited the manuscript. Å.L., O.S., M.R., A.Z., D.S., and J.K. contributed to study design and performance and discussion. S.O.-G. and W.-M.C. contributed to study performance and discussion. J.T. and J.I. contributed to discussion. J.M. and A.K.S. reviewed/edited the manuscript. J.-X.S. contributed to study design and performance and reviewed/edited the manuscript. D.H. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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