

ORIGINAL ARTICLE

Non-HLA type 1 diabetes genes modulate disease risk together with HLA-DQ and islet autoantibodies

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The possible interrelations between human leukocyte antigen (HLA)-DQ, non-HLA single-nucleotide polymorphisms (SNPs) and islet autoantibodies were investigated at clinical onset in 1–34-year-old type 1 diabetes (T1D) patients ($n=305$) and controls ($n=203$). Among the non-HLA SNPs reported by the Type 1 Diabetes Genetics Consortium, 24% were supported in this Swedish replication set including that the increased risk of minor *PTPN22* allele and high-risk HLA was modified by GAD65 autoantibodies. The association between T1D and the minor AA+AC genotype in *ERBB3* gene was stronger among IA-2 autoantibody-positive patients (comparison $P=0.047$). The association between T1D and the common insulin (AA) genotype was stronger among insulin autoantibody (IAA)-positive patients (comparison $P=0.008$). In contrast, the association between T1D and unidentified 26471 gene was stronger among IAA-negative (comparison $P=0.049$) and IA-2 autoantibody-negative (comparison $P=0.052$) patients. Finally, the association between *IL2RA* and T1D was stronger among IAA-positive than among IAA-negative patients (comparison $P=0.028$). These results suggest that the increased risk of T1D by non-HLA genes is often modified by both islet autoantibodies and HLA-DQ. The interactions between non-HLA genes, islet autoantibodies and HLA-DQ should be taken into account in T1D prediction studies as well as in prevention trials aimed at inducing immunological tolerance to islet autoantigens.

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INTRODUCTION

Autoimmune (type 1) diabetes arises after a chronic prodrome of islet autoimmunity associated with a progressive loss of the pancreatic islet beta cells (for reviews, see refs 1,2). The islet autoimmunity may be on-going months to years before the clinical diagnosis of diabetes.^{3–5} The onset of hyperglycemia is the likely final step in a prolonged disease process that eventually results in insufficient beta-cell function to maintain normal blood glucose. The beta-cell autoimmunity that develops long before the clinical onset is becoming better understood through longitudinal investigations from birth of children at increased genetic risk for type 1 diabetes (T1D).^{3,5–8} The apparent two-step pathogenesis of autoimmune (type 1) diabetes complicates the dissection of both the genetic etiology and the actual pathogenesis. The first step is perceived as an event(s) that triggers autoantibodies against one or several of the islet autoantigens, insulin, GAD65, islet antigen-2 (IA-2) or ZnT8.^{8–10} The first autoantibody appears to be against either insulin, GAD65 or both.¹¹ Insulin autoantibodies (IAAs) tend to appear in human leukocyte antigen (HLA)-DQ8 children with a peak during the first 2 years of life. GAD65 autoantibodies (GADAs) as the first autoantibody tend to appear in HLA-DQ2 children with a peak around 5 years of age.¹¹ The second step represents the time period from seroconversion to the clinical onset of diabetes. The length of the second step may be dependent not only on the

number of islet autoantibodies^{8,12,13} but also on HLA and non-HLA genes^{14–16} as well as environmental factors.¹⁷

HLA-DQ on the short arm of chromosome 6 is the most important gene for T1D.^{18,19} Although certain DQ haplotypes are strongly associated with T1D, it remains to be fully established whether the principal association is with the risk for islet autoimmunity (step one), progress to clinical onset (step two) or both. It is important to distinguish between the association of HLA-DQ with the ability of an individual to mount an immune response to certain islet autoantigens and that between HLA-DQ and the loss of beta cells that would be consistent with the clinical diagnosis of diabetes.^{20,21}

At the time of clinical diagnosis, a major proportion of beta cells have been lost in a process involving macrophages or dendritic cells as potential antigen-presenting cells as well as both T and B lymphocytes.¹ In the absence of reliable and reproducible cellular tests,²² the autoimmune pathogenesis and progression to clinical onset is best reflected by autoantibodies against insulin, GAD65, IA-2 and ZnT8.^{23–26} Also, at the time of clinical diagnosis, autoantibodies against GAD65 are primarily associated with HLA-DQ2,²¹ IA-2 with DR4 subtypes rather than with DQ8,^{24,27} insulin with DQ8 (ref. 21) and ZnT8 with DQ6.4.^{9,28} We also observed that IA-2 autoantibodies are negatively associated with DQ2.^{21,28} Although the associations between HLA-DQ and islet autoantibodies may be mechanistically related to peptide presentations by DQ heterodimers (for reviews, see

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refs 29,30), non-HLA genes also contribute to the risk for islet autoimmunity, progression to clinical onset of diabetes or both.^{14,31–33} In a previous investigation of a Swedish population-based case-control study of 1–34-year-old T1D patients and controls it was observed that both *INS* and *DQ8* contributed to age-dependent risk for T1D.²¹ The previously reported association between *PTPN22* and T1D³⁴ was not only supported in our Swedish case-control study but also extended to demonstrate that the largest fold increase in risk of GADA-positive T1D associated with high-risk *PTPN22* allele was observed among those in the low-risk HLA-DQ group.¹⁴

During the course of investigating possible interrelations between HLA-DQ, non-HLA genes and islet autoantibodies,^{14,21} the T1D Genetics Consortium (T1DGC)³⁵ completed Genome Wide Association Studies (GWAS) and reported at least 50 loci that conferred risk for T1D.^{15,16} An opportunity emerged from an invitation of the T1DGC to type as a replication set the Swedish cohort of patients and controls.²¹ As all of the T1D patients and controls in this population-based case-control study stemmed from 1985 to 1989, thereby preceding the T1DGC effort, the first aim of the present study was to examine to what extent the T1D-associated non-HLA genes and loci reported by the T1DGC in much larger, and heterogeneous, datasets could be replicated in a

smaller data set from Sweden. The second aim was to find supporting evidence for our previous result that the increased risk of T1D associated with the minor (T) *PTPN22* allele was modified by both HLA-DQ and autoantibodies against GAD65.¹⁴ The third aim was to estimate the associations between T1D and each of the non-HLA genes, stratified by HLA-DQ, as well as that between the non-HLA genes and autoantibodies against any of the islet autoantigens, GAD65, IA-2, or insulin as well as islet cell (cytoplasmic) antibody (ICA), all measured within days after the clinical onset of diabetes.²¹

RESULTS

T1D and HLA as well as non-HLA genes

The T1DGC reported a total of 30 single-nucleotide polymorphism (SNPs) where the minor allele was related to increased risk for T1D. In the present data set we found supporting evidence for seven (*DQA1*, *DQA2*, *PTPN22*, *C1QTNF6*, *HORMAD2*, *730217* and *ERBB3*) genes of the minor allele non-HLA genes reported by the T1DGC (7/30, 23%; Table 1a). Among the total of 21 genes where the T1DGC reported the major allele to be associated with T1D, five (*26471*, *INS*, *BACH2*, *IL2RA* and *SCAP2*) were nominally statistically significantly associated with T1D in our study

Table 1a. Summary of estimated OR (95% CI) of being diagnosed with type 1 diabetes for subjects with the risk carrying minor allele (including those with a heterozygous genotype mm+Mm) compared with subjects with the major genotype (MM), adjusting for age, sex, region of birth and HLA risk category (original)

Original HLA risk categories										
Gene	SNP	Risk carrying minor allele			Major allele			Total n for current SNP	OR (95% CI)	P-value
		Allele	Patients	Controls	Allele	Patients	Controls			
<i>HLA-DQA1</i>	rs2187668	A	142	50	G	136	140	468	2.51 (1.55, 4.05)	1.72e-4
<i>PTPN22</i>	rs2476601	T	103	44	C	167	141	455	2.02 (1.24, 3.29)	0.005
<i>C1QTNF6</i>	rs229541	T	206	119	C	70	73	468	1.87 (1.17, 3.01)	0.009
<i>HLA-DQA2</i>	rs7454108	C	187	52	T	91	135	465	2.39 (1.18, 4.87)	0.016
<i>HORMAD2</i>	rs5753037	T	174	107	C	106	83	470	1.58 (1.01, 2.46)	0.043
<i>730217</i>	rs4900384	G	166	101	A	118	89	474	1.57 (1.01, 2.43)	0.046
<i>ERBB3</i>	rs2292239	A	178	109	C	101	81	469	1.58 (1.01, 2.48)	0.047
<i>SH2B3</i>	rs3184504	T	217	136	C	61	56	470	1.51 (0.92, 2.49)	0.106
<i>442263</i>	rs10499194	T	115	73	C	159	118	465	1.32 (0.85, 2.06)	0.220
<i>CTRB</i>	rs7202877	G	72	36	T	210	155	473	1.32 (0.78, 2.24)	0.294
<i>TAGAP</i>	rs1738074	G	228	151	A	44	41	464	1.27 (0.73, 2.22)	0.398
<i>645481</i>	rs10517086	A	137	86	G	144	102	469	1.20 (0.78, 1.84)	0.412
<i>PTPN2</i>	rs478582	C	205	138	T	74	52	469	1.22 (0.75, 1.98)	0.425
<i>IL18RAP</i>	rs917997	A	112	73	G	162	114	461	1.19 (0.76, 1.86)	0.442
<i>IFIH1</i>	rs1990760	A	238	157	G	32	26	453	1.29 (0.67, 2.50)	0.449
<i>CCR5</i>	rs333	DEL	13	11	INS	48	40	112	1.57 (0.49, 5.06)	0.451
<i>SMARCE1</i>	rs7221109	T	155	102	C	127	89	473	1.17 (0.76, 1.80)	0.479
<i>UBASH3A</i>	rs3788013	A	182	116	C	101	75	474	1.17 (0.75, 1.82)	0.494
<i>IL2</i>	rs2069762	G	122	78	T	160	113	473	1.15 (0.75, 1.77)	0.521
<i>COBL</i>	rs4948088	A	25	16	C	254	173	468	1.28 (0.60, 2.75)	0.523
<i>C6orf173</i>	rs9388489	G	194	129	A	85	59	467	1.14 (0.72, 1.82)	0.570
<i>GAB3</i>	rs2664170	G	114	81	A	165	107	467	1.11 (0.71, 1.73)	0.651
<i>IL2RA</i>	rs2104286	G	125	85	A	149	106	465	1.07 (0.69, 1.66)	0.758
<i>IL21</i>	rs6822844	T	91	60	G	188	130	469	1.06 (0.67, 1.68)	0.799
<i>SIRPG</i>	rs2281808	T	161	108	C	120	83	472	1.06 (0.68, 1.63)	0.805
<i>C10orf59</i>	rs10509540	C	135	88	T	148	105	476	1.05 (0.68, 1.62)	0.835
<i>KIF5A</i>	rs1678542	C	188	130	G	92	61	471	1.04 (0.66, 1.65)	0.860
<i>IL10</i>	rs3024505	T	80	59	C	213	137	489	1.04 (0.65, 1.66)	0.873
<i>GSDML</i>	rs2290400	A	200	130	G	82	57	469	1.02 (0.64, 1.64)	0.928
<i>GLIS3</i>	rs7020673	C	208	137	G	72	47	464	1.02 (0.62, 1.67)	0.950

Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism. The number of patients and controls in the 'risk carrying minor allele' columns count subjects with either the mm or Mm genotype. The number of patients and controls in the 'major allele' column count subjects with the MM genotype. The SNPs are sorted by the P-value associated with the OR (95% CI). The corresponding results where the major allele carries risk are summarized in Table 1b. Last, the results adjusted for HLA risk categories based on DQ genotypes can be found in the Supplementary Tables 1A and 1B.

Table 1b. Summary of estimated OR (95% CI) of being diagnosed with type 1 diabetes for subjects with the risk carrying major allele (MM) compared with subjects with the minor genotype (including those with a heterozygous genotype mm+Mm), adjusting for age, sex, region of birth and HLA risk category (original)

Original HLA risk category										
Gene	SNP	Risk carrying major allele			Minor allele			Total n for current SNP	OR (95% CI)	P-value
		Allele	Patients	Controls	Allele	Patients	Controls			
26471	rs4788084	G	108	42	A	171	147	468	2.33 (1.44, 3.77)	0.001
INS	rs689	A	195	96	T	82	93	466	2.00 (1.28, 3.11)	0.002
IL2RA	rs12722495	A	222	149	G	41	39	451	1.90 (1.09, 3.32)	0.025
SCAP2	rs7804356	T	195	116	C	90	74	475	1.65 (1.04, 2.61)	0.034
BACH2	rs11755527	C	56	27	G	218	160	461	1.89 (1.05, 3.43)	0.035
FHOD3	rs2644261	T	147	89	C	131	92	459	1.52 (0.97, 2.37)	0.067
RGS1	rs2816316	T	190	122	G	89	68	469	1.48 (0.93, 2.34)	0.096
DKFZp667F0711	rs947474	A	205	130	G	74	63	472	1.43 (0.90, 2.28)	0.127
CD69	rs4763879	G	76	41	A	136	92	345	1.34 (0.79, 2.29)	0.280
IL2	rs2069763	G	93	53	T	179	138	463	1.29 (0.81, 2.05)	0.292
CTLA4	rs3087243	C	120	74	T	151	116	461	1.26 (0.80, 1.98)	0.311
TMSB4X	rs5979785	T	186	112	C	90	72	460	1.26 (0.79, 2.00)	0.337
PRKD2	rs425105	A	212	142	G	67	46	467	1.20 (0.72, 2.01)	0.477
PTPN2	rs45450798	G	180	125	C	98	69	472	1.16 (0.74, 1.81)	0.527
CD226	rs763361	C	87	56	T	193	136	472	1.15 (0.72, 1.85)	0.550
ZFP36L1	rs1465788	G	143	93	A	134	96	466	1.08 (0.70, 1.66)	0.718
RBM17	rs11594656	T	185	121	A	94	69	469	1.07 (0.68, 1.69)	0.761
IL7R	rs6897932	C	166	110	T	113	79	468	1.07 (0.69, 1.67)	0.765
442263	rs6920220	G	155	106	A	122	86	469	1.07 (0.69, 1.64)	0.773
CLEC16A	rs12708716	A	138	97	G	125	89	449	1.06 (0.68, 1.64)	0.811
CTSH	rs3825932	C	116	84	T	162	109	471	1.01 (0.65, 1.57)	0.961

Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism. The number of patients and controls in the 'risk carrying major allele' columns count subjects with the MM genotype. The number of patients and controls in the 'minor allele' column count subjects with either the mm or Mm genotype. The SNPs are sorted by the P-value associated with the OR (95% CI). The corresponding results where the minor allele carries risk are summarized in Table 1a. Last, the results adjusted for HLA risk categories based on DQ genotypes can be found in the Supplementary Tables 1A and 1B.

(5/21, 24%; Table 1b). In the analysis adjusted for HLA based on DQ genotypes, *DQA1*, *PTPN22* and *C1QTNF6* were nominally statistically significantly associated with T1D in our study among the genes with the minor allele being the risk carrying allele for T1D (Supplementary Table 1A, Supplementary Figure 1A). Last, among the genes with the major allele associated with T1D, *26471*, *INS*, *BACH2* and *IL2RA* were found to be nominally statistically significantly associated with T1D in our study (Supplementary Table 1B, Supplementary Figure 1B). The estimates based on our data set for all 51 SNPs reported by the T1DGC are summarized in Tables 1a and 1b, Supplementary Tables 1A and 1B and Supplementary Figures 1A and 1B.

PTPN22, low-risk HLA-DQ and GADA

We found that the minor (T) allele of *PTPN22* contributed to an increase in risk of T1D, primarily in patients with neutral risk HLA-DQ (bottom panel of Table 2). This result, based on $n=508$ subjects, is consistent with our previous result for *PTPN22* based on $n=1240$ subjects (top panel of Table 2).¹⁴ Both, our current data set ($n=508$) and the data set used for the previous *PTPN22* analysis ($n=1240$) are subsets of the original matched case-control study ($n=1708$). This comparison between the prior¹⁴ and current results for *PTPN22* demonstrates that the reduced sample size available for typing of the remaining T1DGC identified SNPs was sufficient to find supporting evidence of a nominally statistically significant increase in T1D risk among carriers of the minor *PTPN22* allele.

In addition, in the original data set, among GADA-positive patients, the OR was 2.83 (95% CI=2.00–3.99), whereas in GADA-negative patients, the OR was 1.41 (95%CI=0.98–2.04; P for

comparison=0.007). Furthermore, the OR of association between *PTPN22* (CT+TT) and GADA-positive patients declined with increasing HLA risk category from 6.12 to 1.54 ($P=0.003$); no such change was detected in GADA-negative patients ($P=0.722$; P for comparison=0.001).¹⁴ In the current study, based on the analysis adjusted for age, sex, region of birth and HLA risk category the OR of association between *PTPN22* (CT+TT) and GADA-positive T1D patients (OR=2.98 (1.69, 5.26); $P=1.68e-4$) was higher compared with that for GADA-negative T1D patients (OR=1.17 (0.62, 2.22); $P=0.635$) and this difference in association was nominally statistically significant (P for comparison=0.028; Table 3, Figure 1).

Results were very similar for the analysis based on the T1DGC data set adjusting for age, sex, region of birth and HLA risk category based on DQ genotypes (Supplementary Table 2, Figure 1).

ERBB3 gene and IA-2A

The association between the carrier status of the minor AA+AC genotype in the *ERBB3* gene and T1D was modified by islet antigen-2 autoantibody (IA-2A; P for comparison=0.047), adjusting for age, sex, region of birth and HLA risk category (Table 4, Figure 1). The OR of association between *ERBB3* (AA+AC) and IA-2A-positive T1D (OR=1.79 (1.02, 3.13); $P=0.042$) was higher compared with IA-2A-negative T1D (OR=1.28 (0.74, 2.21); $P=0.378$; P for comparison=0.047; Table 4, Figure 1).

Similar observation was made based on analysis using HLA risk categories based on DQ genotypes (Supplementary Table 3, Figure 1).

Table 2. Estimated ORs and 95% CIs of being diagnosed with T1D for subjects with PTPN22(CT+TT) genotype compared with subjects with PTPN22(CC) genotype overall and stratified by HLA risk category adjusting for age, sex and region of birth

HLA risk category ^a	PTPN22 WT (CC)		PTPN22 MUT (CT+TT)		Fold increase (MUT/WT) OR (95% CI)
	nP/nC	OR (95% CI)	nP/nC	OR (95% CI)	
<i>PTPN22 data set (n = 1240)¹⁴</i>					
All patients	444/404	1.00 (reference)	268/124	2.11 (1.57, 2.83)	2.11 (1.57, 2.83)
Low	44/194	1.00 (reference)	32/62	2.27 (1.32, 3.92)	2.27 (1.32, 3.92)
Neutral	110/143	3.52 (2.32, 5.34)	77/37	9.64 (5.74, 16.17)	2.74 (1.71, 4.38)
High	290/67	20.65 (13.44, 31.72)	159/25	29.26 (17.06, 50.19)	1.42 (0.86, 2.34)
<i>Current study (n = 508^b)</i>					
All patients	167/141	Reference	103/44	2.02 (1.24, 3.29)	2.02 (1.24, 3.29)
Low	15/67	Reference	8/21	1.61 (0.59, 4.41)	1.61 (0.59, 4.41)
Neutral	43/48	4.34 (2.13, 8.85)	37/13	13.30 (5.63, 31.43)	3.06 (1.43, 6.58)
High	109/26	19.76 (9.56, 40.83)	58/10	27.88 (11.41, 68.12)	1.41 (0.63, 3.16)

Abbreviations: CI, confidence interval; MUT, mutant; OR, odds ratio; TD1, type 1 diabetes; WT, wild type. ^aLow = {(DQB1*0602 or DQB1*0603), (DQB1*0301 but not DQB1*02, DQB1*0302, DQB1*0604)}, neutral = {(DQA1*0501-B1*0201 but not DQA1*0301-B1*0302), other genotypes (not appearing in low or high categories)}, high = {(DQA1*0501-B1*0201 and DQA1*0301-B1*0302), (DQA1*0301-B1*0302 but not DQA1*0501-B1*0201)}. ^bn = 455 of these have rs2476601 (PTPN22 SNP) information ascertained by the T1DGC.

Table 3. Top: estimated ORs and 95% CIs of being diagnosed with T1D for subjects with the variant (minor) genotype compared with subjects with the major genotype and with low-risk HLA haplotype (reference group), adjusting for age, sex and region of birth

HLA risk category	PTPN22 (CC)		PTPN22 (CT+TT)		Fold increase	
	nP/nC	OR (95% CI)	nP/nC	OR (95% CI)	OR (95% CI)	
Low	167/141	Reference	103/44	2.02 (1.24, 3.29)	2.02 (1.24, 3.29)	
Neutral	43/48	4.34 (2.13, 8.85)	37/13	13.30 (5.63, 31.43)	3.06 (1.43, 6.58)	
High	109/26	19.76 (9.56, 40.83)	58/10	27.88 (11.41, 68.12)	1.41 (0.63, 3.16)	
Patient autoantibody	PTPN22 (CC)		PTPN22 (CT+TT)		P-value	P-value for comparison
	nP/nC	OR (95% CI)	nP/nC	OR (95% CI)		
GADA –	74/139	Reference	31/44	1.17 (0.62, 2.22)	0.635	0.028
GADA+	88/139	Reference	70/44	2.98 (1.69, 5.26)	1.68e-4	
ICA –	42/141	Reference	23/44	1.57 (0.78, 3.17)	0.211	0.346
ICA+	124/141	Reference	80/44	2.41 (1.42, 4.10)	0.001	
IAA-	96/136	Reference	64/42	1.92 (1.11, 3.30)	0.019	0.563
IAA+	69/136	Reference	37/42	2.20 (1.13, 4.26)	0.020	
IA-2A –	70/139	Reference	47/41	2.17 (1.21, 3.88)	0.009	0.377
IA-2A+	86/139	Reference	43/41	2.26 (1.21, 4.23)	0.011	

Abbreviations: CI, confidence interval; GADA, GAD65 autoantibody; IA-2A, islet antigen-2 autoantibody; ICA, Islet cell (cytoplasmic) antibody; OR, odds ratio; TD1, type 1 diabetes. Low HLA risk = {(DQB1*0602 or DQB1*0603), (DQB1*0301 but not DQB1*02, DQB1*0302, DQB1*0604)}. Neutral HLA risk = {(DQA1*0501-B1*0201 but not DQA1*0301-B1*0302), Other genotypes (not appearing in low or high categories)}. High HLA risk = {(DQA1*0501-B1*0201 and DQA1*0301-B1*0302), (DQA1*0301-B1*0302 but not DQA1*0501-B1*0201)}. The fold increase column summarizes the estimated OR (95% CI) of being diagnosed with T1D for carriers of the variant allele compared with non-carriers, for subjects in a given HLA risk category. The P-value = 0.005 for the model without interaction (top row), P-value = 0.016 for the model with interaction of PTPN22 and HLA risk category, and the P-value for trend = 0.705. Bottom: estimated OR and 95% CI of being diagnosed with autoantibody-positive T1D (or autoantibody-negative T1D) for subjects with the minor genotype compared with those with the major genotype, adjusted for age, sex, region of birth and HLA risk category.

Gene 26471, IAA and IA-2A

The OR of association between the common GG genotype in the 26471 gene and T1D was OR = 2.33 (1.44, 3.77); $P = 0.001$ (Table 5, Figure 1).

A strong relationship between gene 26471 and T1D was observed in the analysis using the HLA risk categories based on DQ genotypes. The overall OR of being diagnosed with T1D for those with the GG genotype compared with subjects with the AA+AG genotype was 2.79 (1.73, 4.50); $P = 2.46e - 5$; Supplementary Table 4, Figure 1).

Furthermore, the results suggest that the association was stronger between IAA-negative T1D and 26471 gene (OR = 2.74 (1.6, 4.66); $P = 2.17e - 4$) compared with IAA-positive T1D and the 26471 gene (OR = 1.51 (0.78, 2.92); $P = 0.223$; P for comparison = 0.049). Similarly for IA-2A, where the association appeared stronger between IA-2A-negative T1D and 26471 gene (OR = 2.97 (1.66, 5.30), $P = 2.45e - 4$) compared with IA-2A-positive T1D and the 26471 gene (OR = 1.47 (0.81, 2.69), $P = 0.205$; P for comparison = 0.052; Table 5, Figure 1). These results were

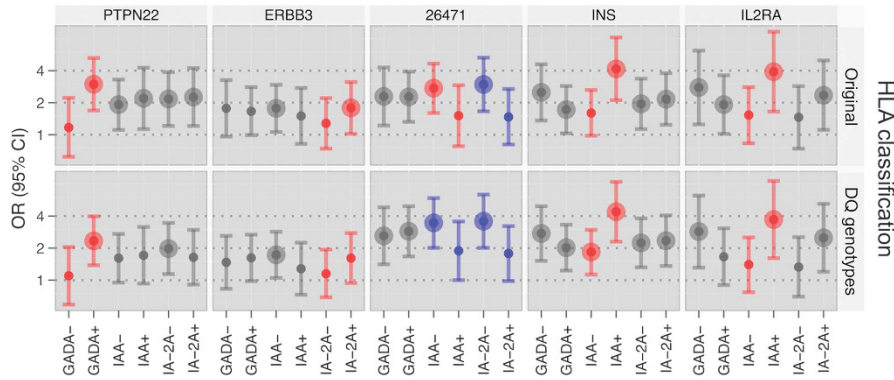


Figure 1. Summary of the estimated OR (dot) and 95% confidence intervals (whiskers) of the association between a given SNP and autoantibody-positive (-negative) T1D, adjusting for age, sex, region of birth and HLA risk category (original or one based on DQ genotypes). The red pairs indicate that the association between a given SNP and autoantibody-positive T1D was nominally statistically significantly different from the association between that SNP and autoantibody-negative T1D at 0.05 level (comparison P -value < 0.05), the blue pairs indicate the same at 0.10 level (comparison P -value < 0.10). The halos indicate that the OR is nominally statistically significantly different from one. Select SNPs are presented here, see Supplementary Figures 1A and 1B for a corresponding summaries of all SNPs.

Table 4. Top: estimated odds ratios (ORs) and 95% confidence intervals (95% CIs) of being diagnosed with T1D for subjects with the variant (minor) genotype compared with subjects with the major genotype and with low-risk HLA haplotype (reference group), adjusting for age, sex and region of birth

HLA risk category	ERBB3 (CC)		ERBB3 (AA+AC)		Fold increase	
	nP/nC	OR (95% CI)	nP/nC	OR (95% CI)	OR (95% CI)	
Low	101/81	Reference	178/109	1.58 (1.01, 2.48)	1.58 (1.01, 2.48)	
Neutral	11/35	Reference	14/56	0.86 (0.34, 2.14)	0.86 (0.34, 2.14)	
High	22/26	3.19 (1.28, 7.96)	58/36	5.72 (2.54, 12.89)	1.79 (0.86, 3.72)	
	68/20	11.25 (4.77, 26.53)	106/17	22.65 (9.45, 54.30)	2.01 (0.98, 4.16)	

Patient autoantibody	ERBB3 (AA)		ERBB3 (AA+AC)		P-value	P-value for comparison
	nP/nC	OR (95% CI)	nP/nC	OR (95% CI)		
GADA –	41/79	Reference	65/108	1.77 (0.96, 3.26)	0.065	0.577
GADA+	57/79	Reference	109/108	1.66 (0.99, 2.80)	0.056	
ICA –	21/81	Reference	43/109	1.80 (0.90, 3.59)	0.095	0.860
ICA+	80/81	Reference	134/109	1.54 (0.95, 2.51)	0.081	
IAA –	55/78	Reference	114/106	1.77 (1.06, 2.95)	0.029	0.250
IAA+	45/78	Reference	61/106	1.50 (0.82, 2.75)	0.189	
IA-2A –	52/79	Reference	67/106	1.28 (0.74, 2.21)	0.378	0.047
IA-2A+	45/79	Reference	89/106	1.79 (1.02, 3.13)	0.042	

Abbreviations: CI, confidence interval; GADA, GAD65 autoantibody; IA-2A, islet antigen-2 autoantibody; ICA, Islet cell (cytoplasmic) antibody; OR, odds ratio; TD1, type 1 diabetes. Low HLA risk = {(DQB1*0602 or DQB1*0603), (DQB1*0301 but not DQB1*02, DQB1*0302, DQB1*0604)}. Neutral HLA risk = {(DQA1*0501-B1*0201 but not DQA1*0301-B1*0302), Other genotypes (not appearing in low or high categories)}. High HLA risk = {(DQA1*0501-B1*0201 and DQA1*0301-B1*0302), (DQA1*0301-B1*0302 but not DQA1*0501-B1*0201)}. The fold increase column summarizes the estimated OR (95% CI) of being diagnosed with T1D for carriers of the variant allele compared with non-carriers, for subjects in a given HLA risk category. The P -value = 0.047 for the model without interaction (top row), P -value = 0.056 for the model with interaction of ERBB3 and HLA risk category, and the P -value for trend = 0.176. Bottom: the estimated OR and 95% CI of being diagnosed with autoantibody-positive T1D (or autoantibody-negative T1D) for subjects with the minor genotype compared with those with the major genotype, adjusted for age, sex, region of birth and HLA risk category.

supported by the analysis using the HLA risk categories based on DQ genotypes (Supplementary Table 4, Figure 1).

Major insulin gene AA genotype and IAA

The OR of association between the common AA genotype in the *INS* gene and T1D was significant (OR = 2.00 (1.28, 3.11); $P = 0.002$). In addition, the OR of association between the insulin gene and IAA-positive T1D patients (OR = 4.16 (2.11, 8.21); $P = 4.03 \times 10^{-5}$) was higher compared with that for IAA-negative T1D patients (OR = 1.6 (0.98, 2.63); $P = 0.060$) and this difference in association was nominally statistically significant (P for comparison = 0.008; Table 6, Figure 1).

The results were very similar in the analysis using the HLA risk categories based on DQ genotypes (Supplementary Table 5, Figure 1).

Major IL2RA AA genotype and IAA

The OR of association between the common AA genotype in the *IL2RA* gene and T1D was significant (OR = 1.90 (1.09, 3.33); $P = 0.025$). In addition, the OR of association between *IL2RA* and IAA-positive T1D patients (OR = 3.91 (1.65, 9.28); $P = 0.002$) was higher compared with that for IAA-negative T1D patients (OR = 1.53 (0.83, 2.80); $P = 0.171$) and this difference in association

Table 5. Top: estimated odds ratios (ORs) and 95% confidence intervals (95% CIs) of being diagnosed with T1D for subjects with the variant (major) genotype compared with subjects with the minor genotype and with low-risk HLA haplotype (reference group), adjusting for age, sex and region of birth

HLA risk category	26471 (AA+AG)		26471 (GG)		Fold increase	
	nP/nC	OR (95% CI)	nP/nC	OR (95% CI)	OR (95% CI)	
Low	171/147	Reference	108/42	2.33 (1.44, 3.77)	2.33 (1.44, 3.77)	
Neutral	17/74	Reference	9/17	2.29 (0.86, 6.11)	2.29 (0.86, 6.11)	
High	42/45	4.39 (2.20, 8.79)	41/16	12.55 (5.63, 27.99)	2.86 (1.38, 5.91)	
	112/28	18.22 (9.13, 36.38)	58/9	32.22 (13.07, 79.45)	1.77 (0.77, 4.04)	
Patient autoantibody	26471 (AA+AG)		26471 (GG)		P-value for	P-value comparison
	nP/nC	OR (95% CI)	nP/nC	OR (95% CI)		
GADA –	67/144	Reference	39/42	2.29 (1.22, 4.29)	0.010	0.669
GADA+	98/144	Reference	67/42	2.27 (1.32, 3.92)	0.003	
ICA –	42/147	Reference	24/42	1.92 (0.96, 3.82)	0.064	0.728
ICA+	128/147	Reference	84/42	2.56 (1.52, 4.32)	4.06e – 4	
IAA –	97/141	Reference	72/41	2.74 (1.60, 4.66)	2.17e – 4	0.049
IAA+	72/141	Reference	33/41	1.51 (0.78, 2.92)	0.223	
IA-2A –	67/143	Reference	53/41	2.97 (1.66, 5.30)	2.45e – 4	0.052
IA-2A+	90/143	Reference	44/41	1.47 (0.81, 2.69)	0.205	

Abbreviations: CI, confidence interval; GADA, GAD65 autoantibody; IA-2A, islet antigen-2 autoantibody; ICA, Islet cell (cytoplasmic) antibody; OR, odds ratio; TD1, type 1 diabetes. Low HLA risk = {(DQB1*0602 or DQB1*0603), (DQB1*0301 but not DQB1*02, DQB1*0302, DQB1*0604)}. Neutral HLA risk = {(DQA1*0501-B1*0201 but not DQA1*0301-B1*0302), Other genotypes (not appearing in low or high categories)}. High HLA risk = {(DQA1*0501-B1*0201 and DQA1*0301-B1*0302), (DQA1*0301-B1*0302 but not DQA1*0501-B1*0201)}. The fold increase column summarizes the estimated OR (95% CI) of being diagnosed with T1D for carriers of the variant allele compared with non-carriers, for subjects in a given HLA risk category. The P-value = 0.001 for the model without interaction (top row), P-value = 0.002 for the model with interaction of 26471 and HLA risk category, and the P-value for trend = 0.636. Bottom: estimated OR and 95% CI of being diagnosed with autoantibody-positive T1D (or autoantibody-negative T1D) for subjects with variant (major) genotype compared with those with the minor genotype, adjusted for age, sex, region of birth and HLA risk category.

Table 6. Top: estimated odds ratios (ORs) and 95% confidence intervals (95% CIs) of being diagnosed with T1D for subjects with the (major) genotype compared with subjects with the minor genotype and with low-risk HLA haplotype (reference group), adjusting for age, sex and region of birth

HLA risk category	INS (AT+TT)		INS (AA)		Fold increase	
	nP/nC	OR (95% CI)	nP/nC	OR (95% CI)	OR (95% CI)	
Low	82/93	Reference	195/96	2.00 (1.28, 3.11)	2.00 (1.28, 3.11)	
Neutral	8/49	Reference	17/40	2.45 (0.94, 6.37)	2.45 (0.94, 6.37)	
High	27/25	6.76 (2.64, 17.31)	56/36	10.03 (4.18, 24.02)	1.48 (0.74, 2.97)	
	47/19	15.74 (6.13, 40.47)	122/20	38.36 (15.51, 94.85)	2.44 (1.18, 5.01)	
Patient autoantibody	INS (AT+TT)		INS (AA)		P-value	P-value for comparison
	nP/nC	OR (95% CI)	nP/nC	OR (95% CI)		
GADA –	29/92	Reference	77/94	2.50 (1.36, 4.59)	0.003	0.228
GADA+	53/92	Reference	111/94	1.72 (1.03, 2.87)	0.037	
ICA –	24/93	Reference	43/96	1.43 (0.75, 2.71)	0.280	0.269
ICA+	58/93	Reference	151/96	2.22 (1.36, 3.62)	0.001	
IAA –	59/91	Reference	109/91	1.60 (0.98, 2.63)	0.060	0.008
IAA+	21/91	Reference	83/91	4.16 (2.11, 8.21)	4.03e-5	
IA-2A –	35/91	Reference	84/93	1.95 (1.13, 3.37)	0.017	0.792
IA-2A+	42/91	Reference	92/93	2.16 (1.24, 3.79)	0.007	

Abbreviations: CI, confidence interval; GADA, GAD65 autoantibody; IA-2A, islet antigen-2 autoantibody; ICA, Islet cell (cytoplasmic) antibody; OR, odds ratio; TD1, type 1 diabetes. Low HLA risk = {(DQB1*0602 or DQB1*0603), (DQB1*0301 but not DQB1*02, DQB1*0302, DQB1*0604)}. Neutral HLA risk = {(DQA1*0501-B1*0201 but not DQA1*0301-B1*0302), other genotypes (not appearing in low or high categories)}. High HLA risk = {(DQA1*0501-B1*0201 and DQA1*0301-B1*0302), (DQA1*0301-B1*0302 but not DQA1*0501-B1*0201)}. The fold increase column summarizes the estimated OR (95% CI) of being diagnosed with T1D for carriers of the variant allele compared with non-carriers, for subjects in a given HLA risk category. The P-value = 0.002 for the model without interaction (top row), P-value = 0.009 for the model with interaction of INS and HLA risk category, and the P-value for trend = 0.861. Bottom: estimated OR and 95% CI of being diagnosed with autoantibody-positive T1D (or autoantibody-negative T1D) for subjects with variant (major) genotype compared with those with the minor genotype, adjusted for age, sex, region of birth and HLA risk category.

Table 7. Top: estimated odds ratios (ORs) and 95% confidence intervals (95% CIs) of being diagnosed with T1D for subjects with the variant (major) genotype compared with subjects with the minor genotype and with low-risk HLA haplotype (reference group), adjusting for age, sex and region of birth

HLA risk category	IL2RA (AG+GG)		IL2RA (AA)		Fold increase	
	nP/nC	OR (95% CI)	nP/nC	OR (95% CI)	OR (95% CI)	
Low	41/39	Reference	222/149	1.90 (1.09, 3.33)	1.90 (1.09, 3.33)	
Neutral	3/13	Reference	21/78	1.27 (0.32, 4.94)	1.27 (0.32, 4.94)	
High	13/14	4.63 (1.05, 20.46)	65/46	7.21 (1.91, 27.26)	1.56 (0.66, 3.68)	
	25/12	10.46 (2.43, 45.00)	136/25	27.48 (7.14, 105.81)	2.63 (1.15, 6.00)	

Patient autoantibody	IL2RA (AG+GG)		IL2RA (AA)		P-value	P-value for comparison
	nP/nC	OR (95% CI)	nP/nC	OR (95% CI)		
GADA –	14/39	Reference	86/146	2.78 (1.25, 6.18)	0.012	0.472
GADA+	26/39	Reference	130/146	1.92 (1.02, 3.62)	0.044	
ICA –	13/39	Reference	50/149	1.51 (0.68, 3.37)	0.312	0.227
ICA+	28/39	Reference	171/149	2.14 (1.16, 3.96)	0.015	
IAA –	31/38	Reference	126/143	1.53 (0.83, 2.80)	0.171	0.028
IAA+	10/38	Reference	92/143	3.91 (1.65, 9.28)	0.002	
IA-2A –	21/36	Reference	92/147	1.46 (0.74, 2.86)	0.272	0.194
IA-2A+	16/36	Reference	110/147	2.35 (1.11, 4.99)	0.026	

Abbreviations: CI, confidence interval; GADA, GAD65 autoantibody; IA-2A, islet antigen-2 autoantibody; ICA, Islet cell (cytoplasmic) antibody; OR, odds ratio; TD1, type 1 diabetes. Low HLA risk = {(DQB1*0602 or DQB1*0603), (DQB1*0301 but not DQB1*02, DQB1*0302, DQB1*0604)}. Neutral HLA risk = {(DQA1*0501-B1*0201 but not DQA1*0301-B1*0302), other genotypes (not appearing in low or high categories)}. High HLA risk = {(DQA1*0501-B1*0201 and DQA1*0301-B1*0302), (DQA1*0301-B1*0302 but not DQA1*0501-B1*0201)}. The fold increase column summarizes the estimated OR (95% CI) of being diagnosed with T1D for carriers of the variant allele compared with non-carriers, for subjects in a given HLA risk category. The P-value = 0.025 for the model without interaction (top row), P-value = 0.042 for the model with interaction of IL2RA and HLA risk category, and the P-value for trend = 0.300. Bottom: estimated OR and 95% CI of being diagnosed with autoantibody-positive T1D (or autoantibody-negative T1D) for subjects with variant (major) genotype compared with those with the minor genotype, adjusted for age, sex, region of birth and HLA risk category.

was nominally statistically significant (P for comparison = 0.028; Table 7, Figure 1).

The results were very similar in the analysis using the HLA risk categories based on DQ genotypes (Supplementary Table 6, Figure 1).

None of the other non-HLA genetic polymorphisms reported by T1DGC as nominally statistically significantly associated with T1D showed a relationship with T1D, T1D stratified by HLA-DQ or islet autoantibody-specific T1D in our data set (Supplementary Tables 7A–13B).

DISCUSSION

Supporting evidence for at least 24% of the non-HLA genes previously identified in the T1DGC^{15,16} to be associated with T1D was found in the present study of 305 T1D patients and 203 controls. The rank order of the genes associated with T1D was consistent with recent reports based on T1DGC data.^{16,18} More importantly, the second aim to test whether the interrelation between low-risk HLA-DQ and *PTPN22*, and that between *PTPN22* and GADA-specific T1D¹⁴ was successfully verified in this smaller data set. Also, the observations that the major allele (A) of *INS* interacts with high-risk HLA, and that it is associated with IAA-positive T1D, are consistent with previous findings.^{21,33,36,37} Additional major findings from our analyses were that: (1) *ERBB3* increased the risk of IA-2A-positive T1D; (2) the risk carrying G allele of 26471 was associated with IAA-negative and IA-2A-negative T1D; (3) the risk carrying major allele A of *IL2RA* was associated with IAA-positive T1D and appears to double the risk of T1D among subjects in the high-risk HLA group. These interactions at the time of the clinical diagnosis of T1D support the view that the T1D pathogenesis is highly heterogeneous. Thus, the statistical approach used in the previous¹⁴ and in the present study should prove useful when larger numbers of T1D patients are studied.

In the analyses stratified by the HLA risk category (Table 2, top panels of Tables 3–7) we show not only the fold increase for each HLA risk category but also the OR of the combined effect of HLA risk category and the SNP compared with the reference (low-risk HLA and non-risk allele of a given SNP). Results presented that way clearly show the importance of HLA in the disease process, and that a fold increase of, for example, two attributed to a given SNP in a given HLA risk category has more severe consequences for patients in the high-risk compared with low-risk HLA. It will be equally important to apply the present approach for studying progression to T1D in children who have developed islet autoimmunity, defined as having developed persistent islet autoantibodies, which is the first sign of a beta-cell destructive process that after months to years may result in a clinical diagnosis of diabetes.

The *PTPN22* T variant leads to a substitution of an arginine for a tryptophan residue at position 620 in the *PTPN22* protein.³⁸ The mutation is thought to allow T cells to remain functionally active for a longer period of time. The OR of association between *PTPN22*T and GADA-positive T1D declined with increasing HLA risk, whereas there was no such change in the GADA-negative T1D patients.¹⁴ Our finding that the association between *PTPN22*T and T1D was modified by GADA and HLA, whether risk categories or actual genotypes were used to stratify subjects by risk of T1D, suggests that it will be important to explore antigen presentation of GAD65 on HLA heterodimers in relation to T-cell function. At present, we do not have enough information to determine whether our results are due to a triggering mechanism of islet autoimmunity (step one), progression to clinical diagnosis of diabetes or both. Taken together, our results suggest that the altered T-cell function conferred by the Arg620Trp substitution was exclusively associated with GADA-positive T1D. Hence, the *PTPN22* polymorphism appears to be more important to the risk for seroconversion to GADA than IAA. This observation should be

taken into account in designing studies to follow children genetically predisposed to T1D until the development of the first islet autoantibody, be it GADA, IAA, IA-2A or, perhaps, ZnT8A.^{8,39}

The *ERBB3* gene is coding for a tyrosine kinase expressed as a cell surface growth factor receptor widely expressed in many developing mammalian tissues, including in the intestinal tract.⁴⁰ The risk of being diagnosed with IA-2A-positive T1D was increased in individuals with the minor (C) allele of *ERBB3* gene. Longitudinal studies have suggested that IA-2A is rarely the first autoantibody to appear at the time of seroconversion to islet autoimmunity.^{8,39,41,42,43} Screening for IA-2A and ZnT8A among parents and siblings was found to be a more cost-effective way to predict clinical onset than employing the entire battery of islet autoantibody tests.⁴³ We speculate that the minor *ERBB3* C allele may be a useful marker for this type of IA-2A-related progression to clinical onset.

A more complex relationship was observed between for the rare allele of the yet unknown gene *26471* and IAA-negative T1D as well as possibly IA-2A-negative T1D. These interactions provide additional evidence suggesting that the T1D disease pathogenesis is highly heterogeneous.

The Swedish 1985–1989 data set has been previously analyzed to evaluate the relationship between the HLA and islet autoantibodies,²¹ the *GIMAP5* gene and IA-2 autoantibodies,⁴⁴ the interaction between HLA-DQ, GADAs and *PTPN22* (ref. 14) and identification of *ITPR3* gene as a possible non-HLA gene increasing the risk of diabetes.⁴⁵ Although the number of subjects was reduced due to prior extensive analyses, especially the use of larger DNA quantities in the pre-PCR era,^{46,47} it was still possible to detect hitherto not previously recognized interactions: *ERBB3* and IA-2A, *26471* and IAA, and possibly IA-2A, as well as *IL2RA* and IAA.

The samples which did not have an adequate amount of DNA required by the T1DGC seemed to represent a random sample compared with our most recent study of this 1985–1989 Swedish population-based case–control study.¹⁴ Our sample size was large enough to support our previous findings stratifying on HLA risk group, as well as in relation to autoantibody-specific T1D. However, it was too small for us to try to consider the association between autoantibody-specific T1D and T1DGC identified SNPs stratifying by the HLA risk group, as we were able to do in our previous analysis.¹⁴ In our analysis, we were able to find supporting evidence for 24% of the SNPs identified by the T1DGC as nominally statistically significantly associated with T1D. Our inability to find evidence of an association between T1D and the remaining SNPs could be the result of a lack of power to detect smaller effect sizes detected in the T1DGC studies. Alternatively, the lack of replication for some SNPs could be due to the differences in the Swedish population studied by us and the multinational and more heterogeneous population studied by the T1DGC. It would be interesting to determine which T1DGC SNPs could be replicated in similar datasets from other countries. A larger Swedish data set would allow us to test not only whether other SNPs could be replicated, but also whether the association of any of *ERBB3*, gene *26471*, *INS* or *IL2RA* SNPs and islet autoantibodies are modified by the HLA risk group, as we previously reported for *PTPN22*, GADA-positive T1D and low-risk HLA.¹⁴

In future studies with larger cohorts, such as the on-going BDD study of consecutively diagnosed 1–18-year-old patients now reaching >4000 patients,^{9,28} should prove useful to further dissect the possible interaction between the T1DGC identified SNPs, HLA-DQ and islet autoantibodies. Also, further investigations are needed to determine whether the T1DGC identified SNPs are primarily associated with the risk for islet autoantibodies in individuals with certain HLA-DQ rather than with the clinical onset of T1D. In the future, when selecting individuals for primary prevention trials, it will be important to understand whether the T1DGC identified SNPs and HLA-DQ need to be considered as risk

factors of islet autoimmunity. Furthermore, could it be possible that for some non-HLA genes there is no association with T1D, and that such genes are risk factors for progression to T1D only in those subjects already positive for islet autoantibodies?

We conclude that in the present case–control study we found supporting evidence for the association between T1D and about 24% of the SNPs reported by the T1DGC.¹⁶ We also found supporting evidence that the minor *PTPN22* allele contributed to an increased T1D risk primarily in patients with neutral risk HLA-DQ. In addition, we report three novel findings. First, the *ERBB3* gene and T1D association was modified by IA-2A. Second, the association between T1D and the *26471* gene was stronger both in IAA-negative and IA-2A-negative T1D patients. Third, the common *INS* genotype was associated with T1D patients positive for IAA but its association with T1D did not appear to be modified by HLA-DQ. Fourth, the association between IAA-positive T1D and *IL2RA* was nominally statistically significantly stronger than that between IAA-negative T1D and *IL2RA*, but we did not detect an interaction with HLA risk categories based on DQ genotypes. The SNPs evaluated in this study are likely to be of most value in studying the Swedish population, as the frequencies of genetic polymorphisms vary among populations. However, our analysis and results demonstrating that non-HLA genes affect T1D risk differently depending on the presence of islet autoantibodies and HLA risk categories are likely to be universal. Thus, these interactions should be studied in other populations and taken into account when subjects are randomized into both primary and secondary prevention trials, especially when autoantigens will be used to halt the progression to clinical onset of T1D.

METHODS

Subjects

The present study was carried out in a subset of subjects from a matched case–control study of Swedish T1D patients ($n=1006$) and controls ($n=702$) matched based on age, gender and region of birth, as previously described in detail.^{14,21,47–49} Because prior extensive analyses of the original set of DNA samples,^{14,21,44,45} the number of available DNA samples to fulfill the requirement of the T1DGC for SNP analysis on the Illumina (Illumina, Inc., San Diego, CA, USA) typing system had diminished (Supplementary Table 14). Contingent on available DNA, in the present study, it was possible to fully genotype 305 T1D patients and 203 controls. The National Ethics Board approved the study and all patients and controls gave informed consent.

To make the maximum use of available data, all subjects with sufficient amount of DNA for genetic typing were used in an unmatched analysis ($n=508$), similarly to the previously reported data set ($n=1240$) where *PTPN22* was the SNP of interest¹⁴ (Supplementary Table 14). To verify the appropriateness of this approach, we compared the results of conditional logistic regression analysis of the 148 subjects in matched sets with results of ordinary logistic regression analysis with adjustment for age, sex and region of birth. As the regression coefficients were very similar (data not shown), we decided to account for the matching using adjustment rather than to perform a matched analysis.

On the basis of the HLA classification used in the DiPIS study,⁵⁰ we defined three HLA risk categories (high, neutral and low) to account for the confounding effects of HLA.¹⁴ The HLA risk categories (referred to as the original HLA risk classification in the text) were defined as follows: low = {(DQB1*0602 or DQB1*0603), (DQB1*0301 but not DQB1*02, DQB1*0302, DQB1*0604)}, neutral = {(DQA1*0501-B1*0201 but not DQA1*0301-B1*0302), other genotypes (not appearing in low or high categories)}, high = {(DQA1*0501-B1*0201 and DQA1*0301-B1*0302), (DQA1*0301-B1*0302 but not DQA1*0501-B1*0201)}.¹⁴

We also introduce a second HLA risk classification based on DQ genotypes,^{28,51} where DQ2/8 and DQ8/8 are defined as high risk, DQ2/X as neutral, and all other DQ genotypes are defined as low risk.

We defined autoantibody-positive and -negative subjects based on GADA, IAA, IA-2A and ICA using previously defined cutoffs.²¹

The main analysis and results described in this paper are based on 508 subjects, 305 T1D patients and 203 controls (Supplementary Table 14), referred to as the Swedish T1DGC (S-T1DGC) data set.

HLA typing

HLA typing of DQA1 and DQB1 was performed by PCR amplification of the second exon of the genes followed by dot blot hybridizations of sequence-specific oligo probes and by restriction fragment-length polymorphism using DR- and DQ-based probes to establish haplotype.^{21,46,52} HLA-DQ types were available on all 508 subjects in the S-T1DGC data set.

Islet autoantibody analyses

Data were available on autoantibodies against insulin, GAD65 and IA-2. All serum and plasma samples have been expended in prior studies and it has therefore not been possible to determine the presence of the recently described ZnT8 transporter autoantibodies.^{53,54}

Insulin autoantibodies

IAs as well as antibodies to insulin were measured by radiobinding assay using acid-charcoal extraction and cold insulin displacement as described⁵⁵ IAA-Δ percent results were available on 196 of the 203 patients (96.6%) and 299 of the 305 control subjects (98.0%) in the S-T1DGC data set.

GAD65 autoantibodies

Autoantibodies to radiolabeled human Mr 65 000 GAD65 (GADA) were quantified by fluid-phase immunoprecipitation assay using fluorographic densitometry as described,^{56,57} except that, for 15–35-year old, GAD65 was radiolabeled by coupled *in vitro* transcription and translation, as described.^{58,59} The two assay formats correlate well, and autoantibody levels are expressed as GADA index,⁵⁹ using the WHO–Juvenile Diabetes Foundation (JDF) standard⁶⁰ as the positive control. GADA measurements were available on 200 of the 203 patients (98.5%) and 297 of the 305 control subjects (97.4%) in the S-T1DGC data set.

Islet antigen-2 autoantibodies

Antibodies to IA-2 (refs 61,62) were measured by radiobinding immunoassay.⁶³ *In vitro* translation with³⁵ S-methionine yielded a 46-kDa IA-2 polypeptide highly precipitable by diabetes sera. Radiobinding assays used protein A-Sepharose to separate antibody bound from free IA-2, and autoantibody levels are expressed as an IA-2A index,⁵⁹ using the WHO–JDF standard as a positive control.⁶⁰ IA-2A measurements were available on 198 of the 203 patients (97.5%) and 277 of the 305 control subjects (90.8%) in the S-T1DGC data set.

Islet cell (cytoplasmic) antibodies

ICAs were determined by indirect two-color immunofluorescence using blood group O frozen human pancreas.⁶⁴ The same pancreas was used throughout the study. Two independent observers evaluated coded slides. Samples were titrated in doubling dilutions to determine end points for conversion into JDF units⁶⁵ as described.⁶⁴ ICA of 0 JDF units corresponded to no detectable antibody. ICA measurements were available on 203 of the 203 patients (100%) and 304 of the 305 control subjects (99.7%) in the S-T1DGC data set.

Statistical analyses

The analyses performed for the S-T1DGC SNPs were based on those performed in ref. 14 for the rs2476601 SNP in PTPN22. Using the dbSNP database (<http://www.ncbi.nlm.nih.gov/SNP/>), we identified the common allele 'M' (referred to as ancestral or major) and the rare (minor) allele 'm' for each SNP. Regardless of whether the risk carrying allele was common or rare, we combined the 'mm' genotype with the 'Mm' genotype due to low numbers of subjects in the 'mm' group for most SNPs. In future studies, it will be important to extend the SNP analysis to the three SNP genotypes. It cannot be excluded that mode of inheritance of a heterozygous or homozygous predisposing allele may affect the combined interaction between a SNP, HLA-DQ and islet autoantibodies.

The variables in our data set were coded as follows. For each SNP, if 'M' was the risk carrying allele, 'MM' was coded as 1 and 'Mm+mm' as 0; if 'm' was the risk carrying allele, 'mm+Mm' was coded as 1 and 'MM' as 0; age as a continuous variable, region as unordered categorical, and the autoantibody status for GADA, IAA, IA-2A and ICA as binary. HLA haplotypes were grouped into low-, neutral and high-risk HLA, and these HLA risk categories were encoded in two ways, as an ordered categorical variable (low, neutral

and high), and as a linear effect (–1, 0, 1). The outcome, or the case–control (T1Dcc) status, was coded as a binary variable (case = 1, control = 0).

In the analysis, we considered all T1D cases, as well as what we refer to as 'an autoantibody-positive (or autoantibody-negative) T1D', which were subgroups of T1D patients who were positive (or negative) for a given autoantibody, regardless of their status with respect to other autoantibodies. In this way, we created eight additional data subsets. In each of these, we included all of the controls (regardless of their autoantibody status), and one of the following: autoantibody-positive T1D cases, autoantibody-negative T1D cases, in which autoantibody was one of GADA, IAA, IA-2A or ICA. Hence, autoantibody status is used to divide T1D cases into subsets, as is commonly performed in RA and other autoimmune diseases.¹⁴

For SNPs with 'M' being the risk carrying allele, all measures of association refer to the OR of association for subjects with the 'MM' genotype and T1D compared with subjects with the 'Mm+mm' genotype. For SNPs with 'm' being the risk carrying allele, all measures of association refer to the ORs of association in subjects with the 'mm+Mm' genotype and T1D compared with subjects with the 'MM' genotype.

For each SNP in the T1DGC data set, we performed the following analysis: step 1—we estimated the OR of association between the risk carrying allele of a given SNP (rSNP) and T1D by fitting a logistic regression model with T1D as the outcome, with the SNP as the main effect, and adjusting for age, sex, region (design variables) and the HLA risk category. Step 2—to estimate the OR in each of the HLA risk categories, we fit a model similar to that in step 1, but with an added interaction term between the HLA and the SNP. Step 3—to determine whether the association between the rSNP and T1D was associated with the appearance of autoantibodies, we fit the models described in step 1 to the autoantibody-positive T1D data set (note: all controls were included in all subsets of the data), similarly for autoantibody-negative T1D data set, in which autoantibody was one of GADA, IAA, IA-2A or ICA. Step 4—to test whether the association between the rSNP and T1D was nominally statistically significant, we used the Wald's test with one degree of freedom (d.f.); similarly for autoantibody-positive T1D and autoantibody-negative T1D, in which autoantibody was one of GADA, IAA, IA-2A or ICA. Step 5—to compare the estimated ORs of association between the rSNP and autoantibody-positive T1D and that between the rSNP and autoantibody-negative T1D, we used a Wald's test with d.f. = 1 based on estimates of mean and variance from polytomous logistic regression.⁶⁶ It allowed us to model an outcome with three levels: controls, autoantibody-positive T1D cases, and autoantibody-negative T1D cases, thus accounting for the correlation between the analyses involving autoantibody-positive T1D and autoantibody-negative T1D, in which the correlation was due to the fact that the same set of controls was used in the pair of analyses for a given autoantibody. Step 6—we considered whether there was a linear trend in the interaction effect of HLA risk category on the association between the rSNP and T1D. To accomplish that, we fit the models described in steps 2 and 3, adjusting for HLA risk (coded as a categorical variable as before), but now using the HLA coded as linear effect in the interaction term. We used a likelihood ratio test with d.f. = 1 to test the appropriateness of the linear HLA effect assumption. We evaluated the appropriateness of our assumptions using residual plots for the models we fitted.

All of these analyses were performed for all SNPs for both HLA risk classifications. Thus, there were two tests performed for each type of analysis for each SNP, due to adjustment for each of the two HLA risk classifications. We report the results of all analyses (in the manuscript and Supplementary Information), with the *P*-values not adjusted for multiple comparisons, for that reason we refer to *P*-values as 'nominally statistically significant'.

All statistical analyses were performed in R v.2.12.1 (<http://www.r-project.org>). Data and the R code used in the analyses are available upon request.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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THE SWEDISH CHILDHOOD DIABETES REGISTER, THE DIABETES INCIDENCE IN SWEDEN STUDY GROUP AND THE TYPE 1 DIABETES GENETICS CONSORTIUM

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