

ORIGINAL ARTICLE

Evidence That HLA Class I and II Associations With Type 1 Diabetes, Autoantibodies to GAD and Autoantibodies to IA-2, Are Distinct

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OBJECTIVE—A major feature of type 1 diabetes is the appearance of islet autoantibodies before diagnosis. However, although the genetics of type 1 diabetes is advanced, the genetics of islet autoantibodies needs further investigation. The primary susceptibility loci in type 1 diabetes, the HLA class I and II genes, are believed to determine the specificity and magnitude of the autoimmune response to islet antigens. We investigated the association of glutamic acid decarboxylase autoantibodies (GADA) and insulinoma-associated antigen-2 autoantibodies (IA-2A) with the HLA region.

RESEARCH DESIGN AND METHODS—Associations of GADA and IA-2A with *HLA-DRB1*, *HLA-DQB1*, *HLA-B*, *HLA-C*, *HLA-A*, *MICA*, and 3,779 single nucleotide polymorphisms (SNPs) were analyzed in 2,531 childhood-onset case subjects (median time since diagnosis 5 years). All analyses were adjusted for age-at-diagnosis and duration of diabetes.

RESULTS—GADA and IA-2A were associated with an older age-at-diagnosis ($P < 10^{-15}$). For GADA, the primary association was with *HLA-DQB1* ($P = 9.00 \times 10^{-18}$), with evidence of a second independent effect in the HLA class I region with SNP, rs9266722 ($P = 2.84 \times 10^{-6}$). *HLA-DRB1* had the strongest association with IA-2A ($P = 1.94 \times 10^{-41}$), with *HLA-A*24* adding to the association, albeit negatively ($P = 1.21 \times 10^{-16}$). There was no evidence of association of either IA-2A or GADA with the highly type 1 diabetes predisposing genotype, *HLA-DRB1*03:04*.

CONCLUSIONS—Despite genetic association of type 1 diabetes and the islet autoantibodies localizing to the same HLA class II genes, *HLA-DRB1* and *HLA-DQB1*, the effects of the class II alleles and genotypes involved are quite different. Therefore, the presence of autoantibodies is unlikely to be causal, and their role in pathogenesis remains to be established. *Diabetes* 60:2635–2644, 2011

Islet autoantibodies, specifically glutamic acid decarboxylase autoantibodies (GADA), insulinoma-associated antigen-2 autoantibodies (IA-2A), and insulin autoantibodies (IAA), are commonly found in type 1 diabetic case subjects at time of diagnosis, with >90% of patients

positive for one or more of IA-2A, IAA, or GADA (1–3). Autoantibodies can appear months or even years before diagnosis of disease, with the probability of developing disease increasing with the number of persistent islet autoantibodies present (3–8). Consequently, they can be used to predict the onset of type 1 diabetes in unaffected relatives of type 1 diabetic case subjects (6,9). The major histocompatibility complex (MHC) region, specifically the HLA class II genes, are the primary genetic loci associated with type 1 diabetes (10,11). Recently, a combination of high throughput, dense, single nucleotide polymorphism (SNP) and classical HLA typing has allowed further mapping of the MHC region, revealing independent type 1 diabetes associations at the *HLA-B* and *HLA-A* loci, involving multiple alleles, including *HLA-B*39*, *HLA-B*18*, *HLA-A*24*, *HLA-A*01*, and *HLA-A*11* (10,11). Autoantibody expression has also been correlated with HLA class II alleles. However, once unaffected relatives of type 1 diabetic patients were positive for two or more autoantibodies, HLA class II risk genotypes did not add to the prediction of type 1 diabetes diagnosis (6,12).

It seems reasonable to assume that the genetics of islet autoantibodies may be shared in part with the genetics of type 1 diabetes. Others have investigated the association of specific type 1 diabetes-associated HLA class II and I alleles and genotypes with GADA and IA-2A, but these have predominantly been confined to small sample sets. GADA has been shown to be associated with the *HLA-DRB1*03:HLA-DQB1*02* haplotype (13,14), whereas *HLA-DRB1*04* was shown to be negatively correlated with GADA (14). The largest study to date did not obtain evidence of association between the HLA class II and I genes and GADA (15). The *HLA-DRB1*04:HLA-DQB1*03:02* haplotype has been reported to be positively associated with IA-2A, whereas there is a reported negative association of *HLA-DRB1*03:HLA-DQB1*02* with IA-2A (13–16). Both *HLA-DRB1*07:HLA-DQA1*02:01* (or 03:01) and *HLA-DRB1*09:HLA-DQA1*03:01* haplotypes have been reported to be associated with increased prevalence of IA-2A in case subjects with type 1 diabetes, which the authors concluded was attributable to the *HLA-DQA1* gene driving the association of these haplotypes with IA-2A (17). *HLA-A*24* has also been negatively associated with IA-2A (15). However, this was not shown to be independent of the primary HLA class II autoantibody associations nor has it been independently replicated. To date, a comprehensive analysis of the association of these islet autoantibodies with the MHC region, in a large well-powered sample of type 1 diabetes, has not been reported. Here we have fine-mapped, using over 3,700 SNPs and classical HLA genotypes, the association of GADA and IA-2A in the extended MHC region in up to ~2,500 type 1 diabetic case subjects.

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RESEARCH DESIGN AND METHODS

Subjects. All type 1 diabetic case subjects were recruited for the Juvenile Diabetes Research Foundation/Wellcome Trust Diabetes and Inflammation Laboratory's Genetic Resource Investigating Diabetes British, type 1 diabetes collection (www.childhood-diabetes.org.uk/grid.shtml). Case subjects were diagnosed with type 1 diabetes under age 17 years (mean age-at-diagnosis = 7.6 years) and on average had diabetes for 6.8 years (Table 1). The appropriate ethics committees approved the collection of all DNA samples, and written consent was obtained from all individuals or parents of individuals who were too young to consent.

Genotyping. The *HLA-DRB1*, *HLA-DQB1*, *HLA-A*, *HLA-B*, and *HLA-C* genes were typed at four-digit resolution using Dynal RELI SSO assays (Invitrogen, Paisley, U.K.). A subset of 2,960 case subjects was genotyped at *HLA-DRB1* and *HLA-DQB1* using Roche Molecular Systems SSO reverse dot blot technology. As described previously, the *MICA-STR* was genotyped using PCR followed by capillary electrophoresis of the amplified product (18); the 2,420 SNPs from the Type 1 Diabetes Genetics Consortium (T1DGC) genome-wide association (GWA) study were genotyped using the Illumina 550 K Infinium platform (19), and 1,687 SNPs from the Wellcome Trust Case-Control Consortium GWA study were genotyped using the Affymetrix GeneChip Human Mapping 500 K Array set (20). Three hundred and twenty-eight SNPs that passed quality control were common to both platforms. Follow-up and tag SNPs were genotyped blind to case-control status using the Taqman 5' nuclease assay (Applied Biosystems, Warrington, U.K.) according to manufacturer's protocols. Two operators scored genotypes. *HLA-DQA1* genotypes were not determined.

Autoantibodies. Presence of each of the autoantibodies (IA-2A, GADA) in the type 1 diabetic case subjects was tested using plasma stored in aliquots at -80°C . Autoantibodies to GAD and IA-2 were measured in the Department of Clinical Sciences at North Bristol, University of Bristol, between 2006 and 2007, using a radioimmunoassay (21,22). In the 2007 Proficiency Evaluation of the Diabetes Antibody Standardization Program (23), the GADA assay achieved 94% sensitivity with 96% specificity, whereas the IA-2 assay sensitivity was 70% sensitivity with 96% specificity. Presence of GADA and IA-2A was taken as above 14 and 6 World Health Organization units/mL, respectively, which corresponds to the 97.5th percentile of the distribution of these autoantibodies in 2,800 school children from Oxford, U.K. (21,22). We did not measure IA-2 since most case subjects were recruited after diagnosis and antibodies to exogenous insulin are produced.

Statistical analyses. All statistical analyses were carried out in STATA (www.stat.com) or R (24). Associations with autoantibodies were tested using logistic regression models with autoantibody status (positive/negative) as the outcome variable and genotype at the test locus and any covariates (sex, duration, age-at-diagnosis; see RESULTS) as predictors. Inclusion of the covariates accounted for their effects, such that association of genotype with autoantibody positivity was tested, rather than, for example, association of genotype with decline in autoantibody positivity (as a result of disease duration). A model with and without the genotype variable was compared using a likelihood ratio test to assess significance. Stepwise logistic regression was used to test for independent associations (25). Haplotypes were generated using the R library *haplo.stats* (available from www.r-project.org) and analyzed by logistic regression using the posterior probabilities as weights and robust variance estimates to account for the dependency between haplotype

assignments. Despite being genotyped at four-digit resolution, to maximize statistical power, analyses of the classical loci were confined to two-digit resolution, except for *HLA-DQB1*0301* and *HLA-DQB1*0302*.

RESULTS

Autoantibodies. GADA were measured in 2,530 type 1 diabetic case subjects, 1,274 (50.4%) of whom were found to have levels of GADA above the 97.5th percentile for the reference population (RESEARCH DESIGN AND METHODS) and were defined as GADA positive. IA-2A were measured in 2,521 type 1 diabetic case subjects, 1,475 (58.5%) of whom had levels above the 97.5th percentile for the reference population (RESEARCH DESIGN AND METHODS) and were defined as IA-2A positive. Of the 2,520 case subjects tested for both IA-2A and GADA, 788 (31.3%) were positive for both autoantibodies and 1,958 (77.7%) were positive for at least one of IA-2A and GADA.

As expected, GADA positivity and IA-2A positivity were both found to be correlated with a shorter duration of diabetes ($P = 9.80 \times 10^{-14}$ and 8.65×10^{-22} , respectively, having accounted for age-at-diagnosis effects; Table 1). In addition, GADA positivity and IA-2A positivity were also correlated with a later age-at-diagnosis ($P = 3.72 \times 10^{-29}$ and 7.22×10^{-20} , respectively, having accounted for duration of diabetes; Supplementary Data). More females were GADA positive than males ($P = 2.91 \times 10^{-12}$; Table 1). Hence, age-at-diagnosis and duration of disease were included as covariates in the statistical models testing for association at IA-2A and GADA; in addition, sex was included for GADA.

GADA associations. In total, 3,779 SNPs between 25 and 33 Mb on chromosome 6p21 containing the extended MHC region, and the *HLA-DQB1*, *HLA-DRB1*, *HLA-B*, *HLA-C*, *HLA-A*, and *MICA(STR)* genes were analyzed for association with GADA. In type 1 diabetes, the strongest association signal was with the HLA class II region (10,11), which was also found to have the strongest signal for association with GADA positivity (Fig. 1A and Table 2). The strongest association with GADA positivity was with *HLA-DQB1* ($P = 9.00 \times 10^{-18}$), but *HLA-DRB1* was also convincingly associated ($P = 3.10 \times 10^{-17}$; Table 2). Indeed, the two SNPs most associated with GADA positivity (rs502055, located at 32.579 Mb between *HLA-DRB1* and *HLA-DQA1*, $P = 6.53 \times 10^{-11}$, and rs2187668, located at 32.606 Mb in intron 1 of *HLA-DQA1*, $P = 1.28 \times 10^{-9}$; Fig. 1A) were in linkage

TABLE 1

Distribution of phenotypes in type 1 diabetic case subjects overall and for each autoantibody subgroup

Phenotype	All case subjects	GADA		IA-2A	
		Positives	Negatives	Positives	Negatives
N	2,531	1,274	1,256	1,475	1,046
Age-at-diagnosis (years)					
Mean (SD)	7.6 (4.0)	8.5 (3.9)	6.6 (3.9)	8.3 (3.7)	6.6 (4.2)
Median	7	9	6	8	6
Age (years)					
Mean (SD)	14.4 (8.3)	14.1 (7.1)	14.8 (9.3)	13.7 (6.8)	15.4 (10.0)
Median	13	13	13	13	13
Duration (years)					
Mean (SD)	6.8 (7.8)	5.5 (6.5)	8.2 (8.7)	5.4 (6.1)	8.8 (9.4)
Median	5	3.4	6	4	6
Sex					
Male, N (%)	1,316 (52)	579 (45)	737 (59)	779 (53)	530 (51)
Female, N (%)	1,215 (48)	695 (55)	519 (41)	696 (47)	516 (49)

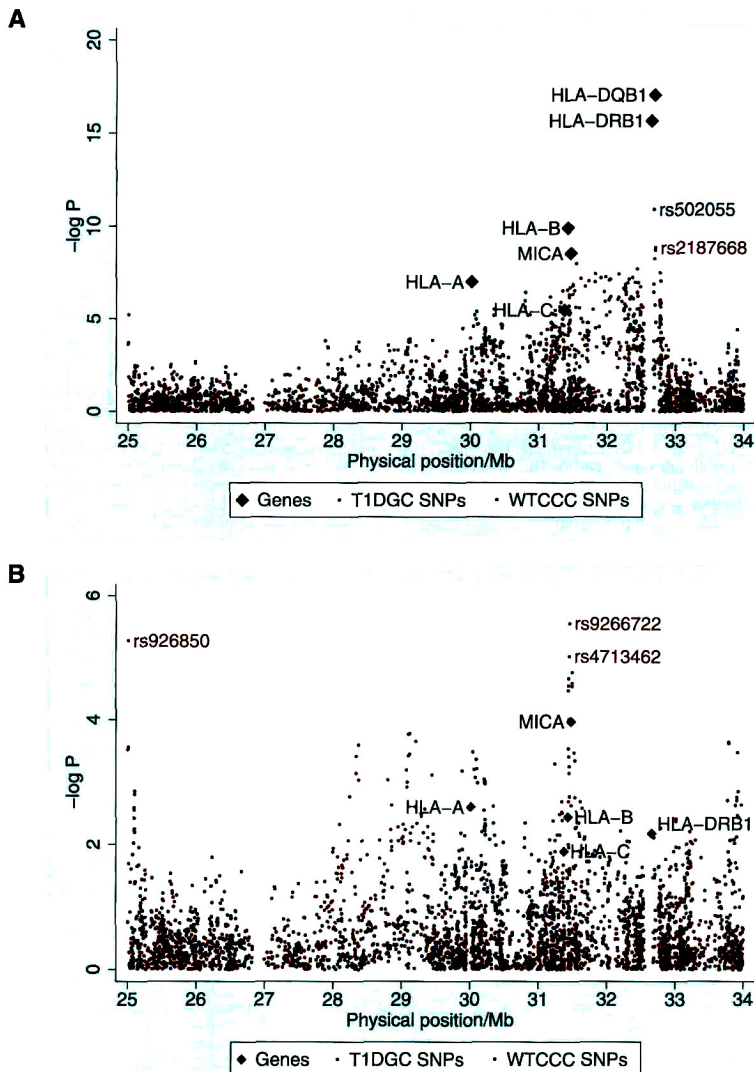


FIG. 1. GADA positivity association map. A: Association of SNPs used in the T1DGC Illumina experiment (using ~1,218 type 1 diabetic case subjects) and the WTCCC Affymetrix experiment (using ~1,056 case subjects) and the classical loci (using between 2,408 and 1,275 type 1 diabetic case subjects; Table 2) with GADA positivity. The top SNPs from both the WTCCC and T1DGC experiments are in linkage disequilibrium with HLA-DRB1*03 ($r^2 = 0.85$ and $D' = 1.00$ with rs502055 and $r^2 = 1.00$ and $D' = 1.00$ with rs2187668). B: Association of SNPs and genes with GADA positivity conditional on HLA-DQB1 genotypes.

TABLE 2

Association of the HLA genes with GADA positivity in type 1 diabetic case subjects

Gene	Physical position†	Number of samples		<i>P</i>	<i>P</i> addition of locus to <i>HLA-DQB1</i>	<i>P</i> addition of locus to <i>HLA-DQB1</i> and rs9266722
		GADA positive	GADA negative			
<i>HLA-DQB1</i>	32,273,871–33,498,585	1,195	1,213	$9.00 \times 10^{-18*}$	NA	NA
<i>HLA-DRB1</i>	32,485,163–32,557,625	1,194	1,212	$3.10 \times 10^{-17*}$	0.0391	0.0050
<i>HLA-B</i>	31,237,115–31,324,989	774	776	1.26×10^{-10}	0.0036	0.0422
<i>HLA-C</i>	31,236,526–31,323,369	667	608	3.48×10^{-6}	0.0130	0.0209
<i>MICA (STR)</i>	31,367,561–31,384,016	1,206	1,196	3.09×10^{-9}	1.08×10^{-4}	0.3385
<i>HLA-A</i>	29,909,037–29,913,661	876	853	$1.06 \times 10^{-10*}$	0.0025	0.0045

*The multiplicative allelic effects model was not an appropriate approximation ($P = 5.66 \times 10^{-4}$ for *HLA-DQB1*, $P = 0.0011$ for *HLA-DRB1*, $P = 0.0015$ for *HLA-A*); hence the *P* value for the genotype effects model is reported. †Physical positions are from GRCh37 (www.tlbase.org).

disequilibrium (LD) with the type 1 diabetes susceptible allele, *HLA-DQB1*02* ($g^2 > 0.69$) and were in strong LD with the *HLA-DRB1*03* type 1 diabetes susceptible allele ($r^2 > 0.85$).

There is very little to distinguish the effects of *HLA-DRB1* and *HLA-DQB1* on GADA positivity ($P = 0.002$ for addition of *HLA-DQB1* to *HLA-DRB1* and $P = 0.04$ for addition of *HLA-DRB1* to *HLA-DQB1*; Table 2), such that either *HLA-DRB1* or *HLA-DQB1* is sufficient to model the association, but both are not required. This contrasts with type 1 diabetes in which both genes are independently associated with disease and add to the association of each other ($P < 3 \times 10^{-240}$, Supplementary Data and Supplementary Tables 1 and 2). We examined which specific class II alleles and genotypes were involved in the GADA association and whether these were consistent with the type 1 diabetes associations. Despite having full four-digit HLA typing, owing to the rarity of many four-digit alleles, the HLA loci were analyzed at two-digit resolution, except at *HLA-DQB1* for the *HLA-DQB1*0301* and **0302* alleles, which are known to have opposite effects in type 1 diabetes (odds ratio [OR] [95% CI] = 4.21 [3.46–5.13] and 0.44 [0.36–0.54], respectively; data not shown).

At *HLA-DQB1*, the 02/02 genotype was most associated with GADA positivity (OR [95% CI] = 2.32 [1.71–3.13]; Table 3), whereas at *HLA-DRB1*, as expected owing to

LD, the 03/03 genotype was most associated with GADA positivity (OR [95% CI] = 2.60 [1.86–3.65]; Table 4). These findings, and that *HLA-DRB1*03* was convincingly associated with GADA positivity ($P = 4.80 \times 10^{-17}$; Supplementary Table 4), agree with previous reports in the literature (13,14). Unexpectedly, the highly type 1 diabetes susceptible *HLA-DQB1*02/0302* genotype (Supplementary Data and Supplementary Table 1) had a comparable frequency in GADA positives (0.38) and GADA negatives (0.40; Table 3). Similarly, the most type 1 diabetes-associated genotype at *HLA-DRB1*, *HLA-DRB1*03/04* (Supplementary Tables 2 and 3), was not associated with GADA (OR = 1.23 [0.90–1.68] using 04/04 as reference [Table 4]). There was also no suggestion of an *HLA-DRB1*03/04* synergic effect with GADA ($P = 0.19$, compared with $P < 7 \times 10^{-520}$ in type 1 diabetes; Supplementary Data). Both the *HLA-DQB1*0302/0302* genotype and the *HLA-DRB1*04/04* genotype are highly susceptible for type 1 diabetes (Supplementary Tables 1 and 2), but these genotypes were not associated with GADA positivity (Tables 3 and 4). This is different from type 1 diabetes in which all *HLA-DQB1*0302/X* and *HLA-DRB1*04/X* genotypes confer susceptibility (Supplementary Tables 1 and 2). *HLA-DQB1*0302* and *HLA-DRB1*04* alleles were only (negatively) associated with GADA positivity when they were with specific type 1 diabetes low risk alleles (*06 or *04 at

TABLE 3

Association of *HLA-DQB1* genotypes with GADA positivity in type 1 diabetic case subjects

Genotype	N (frequency)		OR [95% CI]	
	GADA positives	GADA negatives	Unconditional	
02/02	197 (0.16)	83 (0.07)	2.32 [1.71–3.13]	2.76 [1.77–4.32]
02/0303	28 (0.02)	13 (0.01)	2.31 [1.15–4.63]	2.76 [1.28–5.95]
02/04	24 (0.02)	16 (0.01)	1.70 [0.87–3.34]	2.03 [0.96–4.30]
0302/0301	60 (0.05)	29 (0.02)	1.55 [0.96–2.52]	1.85 [1.03–3.32]
02/05	74 (0.06)	69 (0.06)	1.09 [0.74–1.59]	1.30 [0.78–2.14]
02/0302	451 (0.38)	482 (0.40)	1.00 (reference)	1.19 [0.81–1.75]
Rares	56 (0.05)	52 (0.04)	0.88 [0.57–1.33]	1.04 [0.61–1.78]
02/0301	40 (0.03)	41 (0.03)	0.86 [0.53–1.39]	1.02 [0.57–1.83]
0302/0302	67 (0.06)	74 (0.06)	0.84 [0.57–1.23]	1.00 (reference)
02/06	28 (0.02)	37 (0.03)	0.61 [0.35–1.03]	0.72 [0.39–1.35]
0301/05	25 (0.02)	39 (0.03)	0.55 [0.32–0.95]	0.66 [0.35–1.24]
0302/05	76 (0.06)	130 (0.11)	0.54 [0.39–0.75]	0.65 [0.41–1.03]
0302/04	19 (0.02)	46 (0.04)	0.44 [0.25–0.78]	0.52 [0.27–1.02]
0302/0303	10 (0.01)	23 (0.02)	0.43 [0.19–0.95]	0.51 [0.22–1.21]
0302/06	40 (0.03)	79 (0.07)	0.39 [0.26–0.60]	0.47 [0.27–0.80]

Effect estimates were adjusted for the known covariates: sex, duration of disease, and age-at-diagnosis, whereas genotype frequencies were not. OR are reported with 95% CI using the most common, neutrally associated, genotype *HLA-DQB1*02/0302* as reference and also the less common neutral genotype *HLA-DQB1*0302/0302* as reference. Rare genotypes (13 in total) were grouped at 0.01 frequency.

TABLE 4

Association of *HLA-DRB1* genotypes with GADA positivity in type 1 diabetic case subjects

Genotype	N (frequency)		OR [95% CI]			
	GADA positives	GADA negatives	Unconditional		Conditional on <i>HLA-DQB1</i>	
03/03	163 (0.14)	63 (0.05)	2.60 [1.86–3.65]	3.20 [2.11–4.85]	3.89 [2.10–7.18]	12.24 [2.68–55.86]
03/07	37 (0.03)	16 (0.01)	1.81 [0.96–3.40]	2.22 [1.13–4.36]	2.60 [1.17–5.78]	7.80 [1.62–37.47]
03/04	420 (0.35)	441 (0.36)	1.00 (reference)	1.23 [0.90–1.68]	1.00 (reference)	3.14 [1.29–7.66]
03/13	31 (0.03)	31 (0.03)	0.81 [0.47–1.41]	1.00 [0.55–1.82]	1.20 [0.51–2.84]	3.42 [0.84–14.03]
04/07	64 (0.05)	63 (0.05)	0.95 [0.64–1.41]	1.17 [0.74–1.85]	0.92 [0.62–1.37]	2.92 [1.15–7.39]
04/11	22 (0.02)	8 (0.01)	2.20 [0.94–5.15]	2.70 [1.12–6.55]	1.33 [0.50–3.58]	2.10 [0.79–5.63]
01/03	57 (0.05)	53 (0.04)	1.08 [0.70–1.65]	1.33 [0.81–2.17]	1.27 [0.66–2.43]	1.39 [0.49–3.97]
03/09	20 (0.02)	11 (0.01)	1.85 [0.85–4.00]	2.27 [1.01–5.10]	1.68 [0.64–4.40]	1.66 [0.32–8.60]
04/08	22 (0.02)	48 (0.04)	0.46 [0.26–0.80]	0.56 [0.31–1.03]	0.25 [0.09–0.75]	1.02 [0.14–7.52]
Rares	96 (0.08)	114 (0.09)	0.77 [0.56–1.06]	0.94 [0.63–1.41]	0.63 [0.36–1.12]	1.04 [0.53–2.02]
04/04	108 (0.09)	115 (0.09)	0.81 [0.59–1.12]	1.00 (reference)	0.52 [0.29–0.93]	1.00 (reference)
03/08	16 (0.01)	11 (0.01)	1.47 [0.65–3.34]	1.81 [0.77–4.25]	1.23 [0.39–3.90]	0.87 [0.20–3.71]
01/04	93 (0.08)	155 (0.13)	0.53 [0.39–0.72]	0.66 [0.45–0.97]	0.39 [0.20–0.76]	0.86 [0.32–2.31]
04/13	45 (0.04)	83 (0.07)	0.40 [0.26–0.60]	0.49 [0.30–0.79]	0.42 [0.19–0.94]	0.74 [0.31–1.75]

Effect estimates were calculated accounting for the known covariates: sex, duration of disease, and age-at-diagnosis, whereas genotype frequencies were not. Rare genotypes (39 in total) were grouped at 0.01 frequency. *HLA-DRB1**0403 and *0407 alleles have opposite effects in type 1 diabetes to the remaining *HLA-DRB1**04 alleles; however, they are of such low frequency in the case subjects that an extra subtype was not warranted and so are included with the *HLA-DRB1**04 allele. The apparent association of certain *HLA-DRB1* genotypes following conditioning on *HLA-DQB1* are likely to be attributable to effects outside of the class II region (see main text) and all have very large 95% CIs reflecting uncertainty in the model.

HLA-DQB1 and *01 or *13 at *HLA-DRB1*; Tables 3 and 4), which contrasts other reports (13,14).

Haplotypes of *HLA-DQB1*–*HLA-DRB1* were also tested for association with GADA (Supplementary Table 5) and reflected the unconditional associations observed (Tables 3 and 4). The strongest association with GADA positivity was with the *HLA-DQB1**02:*HLA-DRB1**03/*HLA-DQB1**02:*HLA-DRB1**03 homozygous genotype (OR [95% CI] = 2.62 [1.87–3.67]; Supplementary Table 5); *HLA-DQB1**0302:*HLA-DRB1**04/*HLA-DQB1**05:*HLA-DRB1**01, *HLA-DQB1**0302:*HLA-DRB1**04/*HLA-DQB1**0402:*HLA-DRB1**08, and *HLA-DQB1**0302:*HLA-DRB1**04/*HLA-DQB1**06:*HLA-DRB1**13 were negatively associated with GADA positivity. In contrast, *HLA-DQB1**0302:*HLA-DRB1**04/*HLA-DQB1**05:*HLA-DRB1**01 and *HLA-DQB1**0302:*HLA-DRB1**04/*HLA-DQB1**0402:*HLA-DRB1**08 were associated with a significant increase in risk of type 1 diabetes (Supplementary Table 1). *HLA-B**18 is associated with increased risk of type 1 diabetes, whereas *HLA-B**08 is not (10,11). However, although *HLA-B**08:*HLA-DRB1**03:*HLA-DQB1**02 could well be driving the association of *HLA-DRB1**03 and *HLA-DQB1**02 with GADA positivity, the effect could not strictly be distinguished from *HLA-B**18:*HLA-DRB1**03:*HLA-DQB1**02 or *HLA-B**X:*HLA-DRB1**03:*HLA-DQB1**02 since the 95% CIs overlapped (Supplementary Table 6). The extended DR3 haplotype, *HLA-A**01:*HLA-B**08:*HLA-DRB1**03, was also associated with GADA positivity ($P = 1.24 \times 10^{-6}$; OR = 1.75 [1.40–2.19]).

The association with GADA positivity extended into the class I region, and included *HLA-B*, *HLA-C*, *HLA-A*, and *MICA* (Table 2 and Fig. 1A). Given the strong LD in the region, these associations were likely to be attributable to the *HLA* class II effects. *HLA-DQB1* was the most associated locus with GADA and so was used to test whether there were additional associations that were outside and independent of the *HLA* class II region. Having conditioned on *HLA-DQB1*, there remained some evidence of association around *MICA* ($P = 1.08 \times 10^{-4}$) including two SNPs, rs9266722 and rs4713462, both at 31.35 Mb ($P = 2.84 \times 10^{-6}$

and 9.66×10^{-6} ; Fig. 1B), which were in LD with each other ($D' = 0.98$; $r^2 = 0.36$). The genotyping of rs9266722 was checked and extended to the full collection with GADA measures (an additional 1,124 samples) to maximize power. The genotyping was 99.90% consistent, and the association of rs9266722 remained in the 2,320 samples tested for association with GADA conditional on *HLA-DQB1* ($P = 4.84 \times 10^{-6}$). The SNP, rs9266722, was in LD with *HLA-B**15 ($r^2 = 0.58$; $D' = 0.93$) and the *MICA*-STR*5 allele ($r^2 = 0.48$; $D' = 0.79$). However, the association with *MICA* did not remain once rs9266722, which lies in the pseudo-gene *HLA-S* located between *HLA-B* and *MICA*, was in the model ($P = 0.33$). Even the *MICA*-STR*5 allele alone did not add to the association of rs9266722 ($P = 0.16$). Conversely, rs9266722 did not add convincingly to the association of the *MICA*-STR*5 ($P = 0.017$). Likewise, the effects of *HLA-B* and rs9266722 could not be distinguished; *HLA-B* did not improve the association of *HLA-DQB1* and rs9266722 ($P = 0.04$; Table 2) and rs9266722 did not improve the association of *HLA-DQB1* and *HLA-B* ($P = 0.13$). At *HLA-A*, although no alleles were in LD with rs9266722 ($r^2 > 0.04$), the effect of rs9266722 could not be distinguished from *HLA-A* ($P = 0.0045$ for the addition of *HLA-A* to a model including *HLA-DQB1* and rs9266722; $P = 0.0039$ for the addition of rs9266722 to a model including *HLA-DQB1* and *HLA-A*). More samples, therefore, are needed to decipher the association with GADA positivity in the class I region.

Once the effect of *HLA-DQB1* was accounted for, as well as the association discussed in the class I region, evidence for an additional association with GADA positivity was found with the intronic SNP, rs926850 in *FAM65B* at 25.0 Mb ($P = 3.15 \times 10^{-5}$; Fig. 1B). To increase power, this SNP was genotyped in an additional 1,101 case subjects and further tested for association with GADA. The evidence did not improve, becoming relatively unconvincing ($P = 0.0067$), and hence, associations with GADA were confined to the *HLA* class II region and a second independent effect in the class I region.

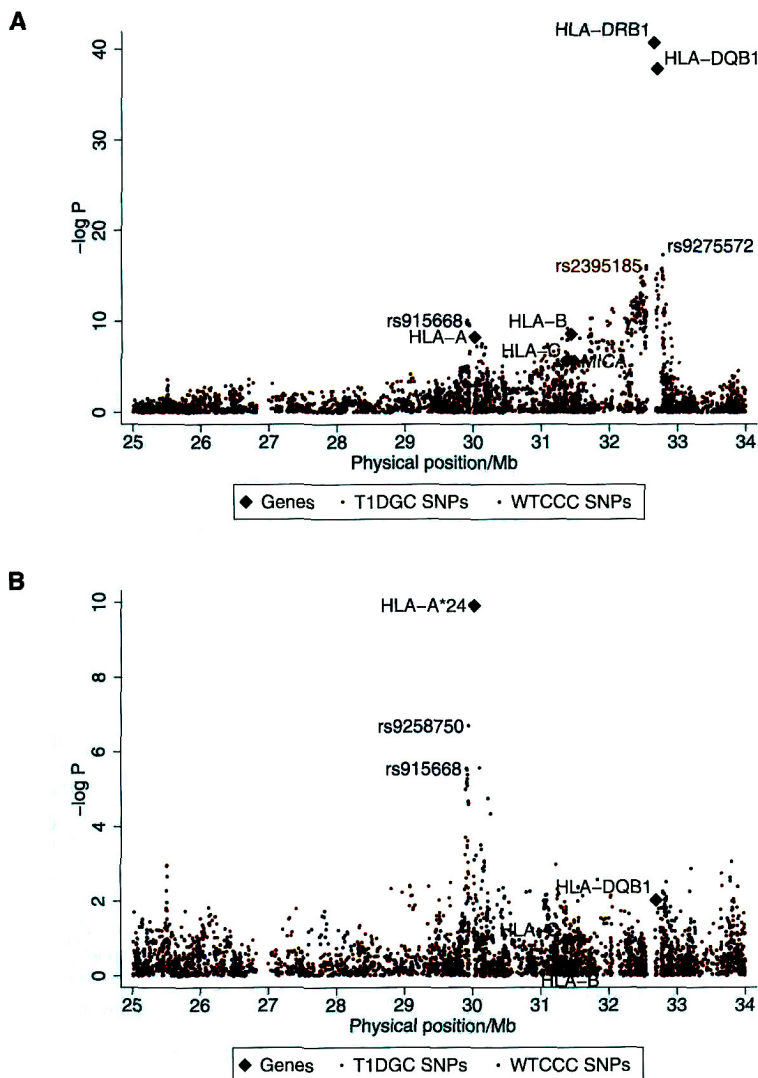


FIG. 2. IA-2A positivity association map. **A:** Association of SNPs used in the T1DGC Illumina experiment (using ~1,215 type 1 diabetic case subjects) and the WTCCC array experiment (using ~1,051 case subjects) with IA-2A and the classical loci (using between 2,399 and 1,268 type 1 diabetic case subjects). The most associated SNP, rs9275572, is in LD with HLA-DRB1*03 ($r^2 = 0.72$, $D' = 1.00$). **B:** Association of SNPs and classical genes with IA-2A, conditional on HLA-DRB1 alleles. The most associated SNP, rs9258750, is in LD with the HLA-A*24 allele ($r^2 = 0.55$, $D' = 0.99$).

TABLE 5

Association of the HLA genes with IA-2A positivity in type 1 diabetic case subjects

Gene	Physical position*	Number of samples		<i>P</i>	<i>P</i> addition of locus to <i>HLA-DRB1</i>	<i>P</i> addition of locus to <i>HLA-DRB1</i> and <i>HLA-A*24</i>
		IA-2A positive	IA-2A negative			
<i>HLA-DQB1</i>	32,273,871–33,498,585	1,394	1,005	1.34×10^{-38}	0.0094	0.0320
<i>HLA-DRB1</i>	32,485,163–32,557,625	1,390	1,007	1.94×10^{-41}	NA	NA
<i>HLA-B</i>	31,237,115–31,324,989	893	647	2.69×10^{-9}	0.5518	0.8631
<i>HLA-C</i>	31,236,526–31,323,369	752	516	2.42×10^{-6}	0.1120	0.1290
<i>MICA</i>	31,367,561–31,384,016	1,410	983	2.67×10^{-6}	0.2250	0.6640
<i>HLA-A</i>	29,909,037–29,913,661	1,005	716	5.50×10^{-9}	6.13×10^{-6}	NA

*Physical positions are from GRCh37 (www.tlbase.org).

IA-2A associations. Convincing evidence of the association with IA-2A was obtained in both the HLA class I and II regions ($P < 3 \times 10^{-6}$; Fig. 2A and Table 5). *HLA-DRB1* had the strongest evidence for association with IA-2A positivity ($P = 1.94 \times 10^{-41}$; Fig. 2A and Table 5). However, in contrast with the association with type 1 diabetes, there was no evidence of deviation from a multiplicative allelic effects model ($P > 0.2$ for the class I and II genes). The most associated SNP, rs9275572 ($P = 5.07 \times 10^{-17}$; Fig. 2A), which was genotyped in the WTCCC experiment (and by the T1DGC, $P = 5.9 \times 10^{-16}$), was also in the class II region, between the pseudo-genes *MTC03P1* and *HLA-DQB3*. It was in strong LD with *HLA-DRB1*03* ($r^2 = 0.72$; $D' = 1.00$).

The *HLA-DRB1*03* allele (and the *HLA-DQB1*02* allele) was negatively associated with IA-2A positivity (OR [95% CI] = 0.44 [0.34–0.58]) for *HLA-DRB1*03* using *HLA-DRB1*0404* as reference (Table 6). In contrast, *HLA-DRB1*0401* (and its associated *HLA-DQB1* allele, *HLA-DQB1*0302*) were strongly associated with IA-2A positivity (OR = 1.72 [1.34–2.20]; Table 6), a major similarity to type 1 diabetes susceptibility (Supplementary Table 2). Interestingly, because the effects of *HLA-DRB1*0401* and *HLA-DRB1*03* were in opposite directions, the most susceptible type 1 diabetes genotype, *HLA-DRB1*03/04*, was not

associated with IA-2A, having a frequency of 0.331 in the IA-2A positive case subjects and 0.356 in IA-2A negatives (OR = 1.07 [0.74–1.54]) using as a reference the non-*HLA-DRB1*03*, non-*HLA-DRB1*04* homozygous genotype, which had a frequency of 0.064 in IA-2A positives and negatives (Supplementary Table 7). The rare allele *HLA-DRB1*09* was associated with IA-2A positivity. However, unlike another report of only 618 samples (17), we found that the type 1 diabetes protective allele, *HLA-DRB1*07* (Supplementary Table 2), was negatively correlated with IA-2A (OR = 0.65 [0.45–0.93]; Table 6). The highly type 1 diabetes protective allele, *HLA-DQB1*0301*, however, was associated with IA-2A positivity (Table 6).

In addition to the evidence of association in the class II region, the HLA class I region also showed evidence of association with IA-2A positivity (Fig. 2A and Table 5). The SNP, rs915668 near *HLA-A*, was the most associated in that region ($P = 5.71 \times 10^{-10}$). However, these class I associations with IA-2A positivity could be attributable to LD with HLA class II alleles, and, hence, we tested the class I variants conditional on class II to ensure they are independent of the primary class II effect. A model including the *HLA-DRB1* alleles was only improved by *HLA-A* ($P = 6.13 \times 10^{-6}$; Table 5) and SNPs in the region adjacent to *HLA-A* (Fig. 2B). No evidence of additional effects at the

TABLE 6

HLA-DRB1 and *HLA-DQB1* allelic associations with IA-2A positivity in type 1 diabetic case subjects

Allele	<i>HLA-DRB1</i>				<i>HLA-DQB1</i>			
	N (frequency)		OR [95% CI]		N (frequency)		OR [95% CI]	
	IA-2A positives	IA-2A negatives	Unconditional	Conditional on <i>HLA-A*24</i>	IA-2A positives	IA-2A negatives	Unconditional	
09	47 (0.02)	19 (0.01)	1.85 (1.02–3.36)	1.22 [0.60–2.49]	0301	250 (0.09)	59 (0.03)	3.09 [2.13–4.49]
0401	903 (0.32)	413 (0.21)	1.72 (1.34–2.20)	1.47 [1.08–1.99]	0303	67 (0.02)	26 (0.01)	2.55 [1.51–4.29]
11	42 (0.02)	16 (0.01)	1.35 (0.68–2.64)	1.40 [0.61–3.24]	0302	1,087 (0.39)	629 (0.31)	1.89 [1.49–2.41]
04	50 (0.02)	13 (0.01)	1.20 (0.33–1.76)	1.34 [0.86–2.07]	05	284 (0.10)	198 (0.10)	1.00 [reference]
0402	35 (0.01)	19 (0.01)	NA		0402	61 (0.02)	61 (0.03)	0.85 [0.55–1.31]
0405	58 (0.02)	46 (0.02)	NA		06	119 (0.04)	97 (0.05)	0.74 [0.52–1.04]
0404	229 (0.08)	166 (0.08)	1.00 (reference)	1.00 (reference)	02	920 (0.33)	940 (0.47)	0.71 [0.57–0.88]
Rare	57 (0.02)	37 (0.02)	0.82 (0.50–1.34)	0.74 [0.36–1.52]				
01	271 (0.10)	174 (0.09)	0.73 (0.54–0.98)	0.64 [0.44–0.92]				
07	141 (0.05)	96 (0.05)	0.65 (0.45–0.93)	0.56 [0.35–0.87]				
08	64 (0.02)	64 (0.03)	0.53 (0.34–0.83)	0.59 [0.34–1.01]				
13	113 (0.04)	94 (0.05)	0.50 (0.34–0.73)	0.47 [0.29–0.75]				
03	770 (0.28)	857 (0.43)	0.44 (0.34–0.58)	0.35 [0.26–0.49]				

Effect estimates were adjusted for the known covariates, duration of disease, and age-at-diagnosis, whereas the listed allele frequencies were not. Rare: alleles 10, 12, 14, 15, 16, and 0403 had a frequency of less than 0.5% and so were combined. NA, not available (the 04 alleles were combined, except 0404, which was neutral, and 0401, which was associated).

other genes and SNPs tested was obtained (Fig. 2B and Table 5). The class II independent effect in the class I region with IA-2A was largely attributable to HLA-A*24 ($P = 1.21 \times 10^{-10}$) for the association of HLA-A*24 alone conditional on *HLA-DRB1*). Despite being associated with susceptibility to type 1 diabetes (10), HLA-A*24 was negatively associated with IA-2A positivity (OR [95% CI] = 0.44 [0.34–0.57]) using all other non-HLA-A*24 alleles at *HLA-A* as reference. Strikingly, haplotype analyses showed that HLA-A*24 significantly reduced the positive IA-2A association of HLA-DRB1*04 from (OR [95% CI] = 2.02 [1.49–2.73]) for the HLA-A*02.HLA-DRB1*04 haplotype to a neutral effect for the HLA-A*24.HLA-DRB1*04 haplotype (OR = 0.94 [0.63–1.40]; Supplementary Table 8). Although HLA-DRB1*03 was negatively associated with IA-2A (Table 6), the association was significantly stronger in the presence of HLA-A*24 on the same haplotype (OR = 0.55 [0.39–0.77] HLA-A*02.HLA-DRB1*03 compared with OR = 0.25 [0.16–0.38] for HLA-A*24.HLA-DRB1*03; Supplementary Table 8). Note, however, that HLA-DRB1*03 remained negatively associated with IA-2A positivity even after conditioning on HLA-A*24 (OR = 0.35 [0.26–0.49] using HLA-DRB1*0404 as reference; Table 6).

The HLA-A*01.HLA-B*08.HLA-DRB1*03 extended haplotype was also negatively associated with IA-2A positivity (OR [95% CI] = 0.59 [0.44–0.78]), and again presence of HLA-A*24 increased this association (OR = 0.26 [0.13–0.52]; Supplementary Table 9). The negative association of HLA-DRB1*03 was, as expected given the lack of independent association at *HLA-B*, unaltered whether it was on the HLA-B*08.HLA-DRB1*03 haplotype or the HLA-B*18.HLA-DRB1*03 haplotype (OR = 0.29 and 0.34, respectively; Supplementary Table 10).

DISCUSSION

We have observed striking associations of islet autoantibodies with HLA alleles and haplotypes. Because levels of autoantibodies reduce after diagnosis of disease (26), there will be an increased false-negative rate for IA-2A and GADA compared with at time of diagnosis. A faster decline in GADA and/or IA-2A positivity in the younger onset case subjects, which we had limited power to detect, may be possible. However, we have adjusted for diabetes duration in our analyses, so the genetic associations we report were not attributable to decline in autoantibody levels (Supplementary Data). Although the false-negative rate reduces the power of our study to detect genetic associations, it does not affect the significant associations obtained. We cannot comment on genetic associations with autoantibody positivity prediagnosis since we only measured autoantibodies at a single time point at or after diagnosis. Some case subjects will have been transiently positive for autoantibodies. However, we were interested in the genetic association of these autoantibodies among case subjects, not in using them for prediction of type 1 diabetes onset; hence this will not affect our study since the samples were positive at the time of measurement. Indeed, our findings are highly consistent with other studies of IA-2A and GADA (13–16), and 30% of our case subjects were sampled within 2 years of diagnosis.

Our data show that although both GADA and IA-2A are very strongly associated with type 1 diabetes, and risk of all three localizes to the MHC region, the alleles and genotypes involved differ between autoantibodies and type 1 diabetes. Our major novel finding is that even though the HLA-DRB1*03/04 (and the HLA-DQB1*02/0302) genotype

is highly predisposing for type 1 diabetes, it has very little effect on positivity for GADA or IA-2A, a finding that is also reflected in the 434 case subjects recruited within 1 year of diagnosis (frequency of HLA-DRB1*03/04 was 0.28 on GADA positives, 0.26 in GADA negatives, 0.33 in IA-2A positives, 0.35 in IA-2A negatives). Furthermore, HLA-DRB1*03/04 was not associated with decline in GADA or IA-2A positivity with disease duration (Supplementary Data). HLA-DRB1*03/04 has its strongest effects in children with the youngest age-at-diagnosis. In contrast, in our type 1 diabetic case subjects, both positivity for IA-2A and GADA were associated with an older age-at-diagnosis (Table 1) such that case subjects with the youngest age-at-diagnosis were less likely to be autoantibody positive. This agrees with other studies that also found GADA positivity was associated with an older age-at-diagnosis (13,27).

There are a number of possible explanations for these surprising findings. One possibility is that perhaps HLA-DRB1*03/04 recognizes a yet undiscovered, highly diabetogenic autoantigen, which accelerates disease progression. This leads to a younger age-at-diagnosis, with GADA and IA-2A produced as a result of disease pathogenesis, but not having caused disease. It is believed that HLA-DRB1*03/04 genotype susceptibility is attributable to a HLA DQ trans-dimer molecule (28), and it presumably binds certain preproinsulin peptides, including from the signal peptide sequence (29,30). The HLA-DRB1*03/04 genotype susceptibility is directed toward a potent, highly pathogenic anti-preproinsulin response. Knip et al. (14) have also shown that HLA-DQB1*02/0302 is associated with IAA positivity. IAA has also been shown to be more common in young onset type 1 diabetic case subjects and is often the first autoantibody to appear before age 3 years (8). Functional masking has been observed for HLA class I molecules in a mouse model of multiple sclerosis in which HLA-A3 and HLA-A2 have opposite functional effects on autoreactive CD8+ T cells (31). So, another explanation for the lack of HLA-DRB1*03/04 association with IA-2A is that there is functional antigen competition between the HLA-DRB1*03 and HLA-DRB1*04 associated haplotypes, with one favoring one autoantigen response and the other competing or suppressing that response. Together these alleles maintain an equilibrium that manifests as no association of HLA-DRB1*03/04 with autoantibody positivity. Our results do not support a simple model in which HLA-DRB1*03/04 drives early and rapid β -cell destruction, effectively lowering the amount of GAD and IA-2 antigens available to the autoimmune response, since a negative association of this genotype would be expected.

The positive association of the type 1 diabetes protective allele, HLA-DQB1*0301, with IA-2A positivity was also unexpected. However, in contrast with HLA-DRB1*03/04, which is associated with a younger age-at-diagnosis, both HLA-DQB1*0301 and IA-2A positivity (Table 1) were associated with an older age-at-diagnosis ($P = 4.48 \times 10^{-13}$ and 7.22×10^{-20} , respectively). The frequency of HLA-DQB1*0301 doubled from 0.04 in type 1 diabetes under 4 years to 0.08 in over 11 years.

The inverse associations of the classical alleles with type 1 diabetes compared with IA-2A and GADA are further illustrated by our results. HLA-DRB1*03 is associated with risk of type 1 diabetes, and also of GADA positivity, but is negatively associated with IA-2A positivity. HLA-DRB1*04 is highly predisposing for type 1 diabetes, is only associated with GADA (negatively) in the presence of HLA-DRB1*01 or *13, and is positively associated with IA-2A. Both HLA-A*24

and HLA-B*39 have been shown to independently confer susceptibility to type 1 diabetes (10,11). Yet HLA-A*24 was negatively associated with IA-2A positivity (in agreement with another report [15]), whereas HLA-B*39 was not associated with either GADA or IA-2A independently of the class II effects. Consequently, as suggested previously (32,33), autoantibody positivity does not necessarily reflect what is occurring at the T-cell level.

Overall, given that there are these strong differences in the genetics of type 1 diabetes and of IA-2A and GADA, these data suggest that GADA and IA-2A may not be the first consequence of the autoimmune attack against β -cells (34), and their exact role in pathogenesis remains to be established.

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J.M.M.H. researched and analyzed data and wrote the manuscript. H.S. helped with DNA preparation and autoantibody data. D.J.S. genotyped SNPs. N.M.W. performed data management. K.A.C. and P.J.B. measured autoantibodies. J.A.T. researched and analyzed data and wrote the manuscript. All authors reviewed and edited the manuscript.

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Effects of age-at-diagnosis and duration of diabetes on GADA and IA-2A positivity

Duration of diabetes was inversely correlated with age-at-diagnosis ($\rho=-0.13$). However, as backward stepwise regression was used to test which covariates were required when testing for associations with autoantibody positivity, GADA and IA-2A positivity were both associated with an older age-at-diagnosis as well as a longer duration of disease. The effects attributable to both covariates (and also to sex with GADA) were accounted for in the models testing for genetic associations, (Methods) to ensure the associations obtained with autoantibody positivity were due to genotype.

This can be illustrated by comparison of autoantibody positivity by age-at-diagnosis in those cases with the shortest duration of disease, to cases with the longest duration of disease. Within the cases having diabetes for ≤ 2 yrs, GADA positivity was 53% in cases with an age-at-diagnosis of ≤ 4 yrs, 57% in cases diagnosed between 5-7 years of age, 69% in those diagnosed between 8-11 years and 70% in those diagnosed between 12-16 years of age. So there was, as expected, a clear trend, that in the cases with the shortest duration of diabetes, GADA positivity was associated with an older age-at-diagnosis. A similar trend was also seen with IA-2A positivity, with 59% of cases diagnosis at ≤ 4 yrs positive for IA-2A compared to 71% of those diagnosed between 12-16 years.

Comparison with cases that have had diabetes for over 8 years, reveals the same trend, that autoantibody positivity is correlated with an older age-at-diagnosis (despite an overall lower frequency of autoantibody positivity). 33% of cases diagnosed at ≤ 4 yrs of age were GADA positive; 36% of cases diagnosed between 5-7 years of age were GADA positive; 46% of cases diagnosed between 8-11 years were GADA positive and 50% of cases diagnosed between 12-16 years were GADA positive. Again the same trend was observed for IA-2A, with 29% of those diagnosed at ≤ 4 yrs positive for IA-2A compared to 52% of those diagnosed between 12-16 years of age.

HLA class II associations with type 1 diabetes

In order to fully understand the associations of the class II genes, *HLA-DRB1* and *HLA-DQB1* with the autoantibodies GADA and IA-2A, it is necessary to re-visit the associations of these genes with type 1 diabetes.

We used control DNA samples from the British 1958 Birth Cohort ($n=5,395$). Cases and controls were matched, using place of recruitment and place of birth respectively, for each of 12 geographical regions across Great Britain (Southern England, South-western England, South-eastern England, Eastern England, London, Midlands, Wales, North-eastern England, North Midlands, East and West Ridings, Northern England and Scotland) to minimise bias in our association results owing to varying disease prevalence and allele frequencies across Great Britain.

Despite being genotyped in a set of 6,227 type 1 diabetes cases and 5,395 geographically matched controls (6,176 cases and 5,225 controls genotyped at both loci) there remained many genotypes that were rare (< 0.01 frequency in either cases or controls). So at *HLA-DQB1*, all the 05 subtypes were grouped together as a single allele, HLA-DQB1*05. HLA-DQB1*0601 was grouped with the rare allele HLA-DQB1*0602. For rare genotypes all the 06 subtypes were grouped into a single two-digit allele, HLA-DQB1*06 (*i.e.* 0301/060X and 060X/060X). Similarly at *HLA-DRB1* the alleles, 12, 16 and 1001 were grouped as they had a frequency of less than 0.01. Genotypes were also grouped, as so many rare genotypes (<0.01 frequency) were generated which leads to inestimable parameters in the statistical model (see footnote to Supplementary Table 1 and 2 for details). Logistic regression using disease status

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as dependent variable and genotype (or allele) as independent variable was used to test for association with type 1 diabetes. Note, geographical region was included as a confounder to account for variation in disease incidence and allele/genotype frequency across Great Britain (refer to methods of main text). A genotype effects model (which does not assume a specific mode of inheritance) was compared to a multiplicative allelic effects model. The multiplicative model, as is well known, was not an appropriate approximation ($P < 7 \times 10^{-20}$) (10; 11).

HLA-DQB1 and *HLA-DRB1* genotypes were both strongly associated with type 1 diabetes ($P = 0$; Supplementary Table 1 and 2), further more, they had convincing independent effects on diabetes risk ($P = 2.91 \times 10^{-240}$, for addition of *HLA-DQB1* genotypes to *HLA-DRB1* genotypes; $P = 1.28 \times 10^{-240}$, for addition of *HLA-DRB1* genotypes to *HLA-DQB1* genotypes). Unexpectedly, a joint model including both *HLA-DRB1* and *HLA-DQB1*, showed that the *HLA-DQB1**0302/0302 genotype was more strongly associated with type 1 diabetes than *HLA-DQB1**02/0302 (Supplementary Table 1). In contrast, the *HLA-DRB1**03/04 genotypes were the most associated with type 1 diabetes after conditioning on *HLA-DQB1* (08/0401 had a larger OR but the 95% CIs overlapped those for 03/04 and this merely reflects the uncertainty in the effect estimate of the 08/0401 genotype as it is rare). Interestingly, the *HLA-DRB1**03/03 genotype which has an unconditional OR (4.57 [3.53 -5.92]; Supplementary Table 2) similar to *HLA-DRB1**0401/0401 (OR = 4.79 [3.39-6.76]; Supplementary Table 2), following conditioning on *HLA-DQB1*, the susceptibility is significantly reduced with the 95% CI overlapping 1.00. Similarly, the effects of *HLA-DRB1**07/09, and *03/11 were reduced, while *03/13 is increased after conditioning on *HLA-DQB1*.

Effects of duration of diabetes on genetic associations with autoantibody positivity

As most cases were tested for autoantibodies after diagnosis, autoantibody positivity would be less than had all cases been tested for autoantibodies at time of diagnosis; we estimate the false negative rate to be less than 20%. This effect of duration on autoantibody positivity has been accounted for in the statistical analysis by including duration as a covariate in the regression models. Here we illustrate this with reference to the *HLA-DRB1**03/04 genotype.

First consider whether or not *HLA-DRB1**03/04 is associated with GADA positivity. This can be tested in a logistic regression model, using GADA positivity as the outcome variable and *HLA-DRB1**03/04 genotype as independent variable. However, GADA is associated with increasing age-at-diagnosis, and *HLA-DRB1**03/04 is also associated with age-at-diagnosis, *i.e.* age-at-diagnosis is a confounder to be included in the model. GADA positivity has a sex bias (more females are GADA positive than males) so sex is included as a covariate. Using a likelihood ratio test, this model ($\text{GADA} \sim \text{age-at-diagnosis} + \text{sex} + \text{DR3/4}$) could be compared to a model without DR3/4, to determine whether or not DR3/4 is associated with GADA positivity. However, with this test, association of genotype with decline in GADA positivity with diabetes duration, could be detected but interpreted as association of GADA positivity with *HLA-DRB1**03/04 genotype. Therefore, to account for decline in GADA positivity with diabetes duration, duration was included as a covariate. No evidence of association of GADA positivity with *HLA-DRB1**03/04 genotype was obtained, $P = 0.33$. As the duration variable was included, decline in autoantibody positivity with disease duration was not tested.

Association of the *HLA-DRB1**03/04 genotype with decline in GADA positivity with increasing duration of disease can be tested. We do not have the appropriate study design for this and so the study (across individuals) does not have much statistical power to detect this, but we can apply the test. Using an interaction term in the logistic regression model with GADA positivity as the outcome variable, the

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independent variables are then HLA-DRB1*03/04 genotype, age-at-diagnosis, sex, duration of disease, and the *interaction* of HLA-DRB1*03/04 with duration. This interaction term allows us to test the genotype dependence of decline in GADA positivity with duration of disease by comparing this model to a model without the interaction term using a likelihood ratio test. This was not significant, $P = 0.15$. So there was no association of genotype with decline in GADA positivity in our cohort with a median disease duration of 5 years.

Note however, whether or not there is a genotype dependence of decline in autoantibody positivity with duration of disease *a priori* is unknown, so for all tests the duration variable was included to ensure we are testing association of GADA positivity with genotype without being confounded by decline in GADA positivity with diabetes duration.

Using the same methods, there was no evidence of a HLA-DRB1*03/04 genotype dependence on decline in IA-2A positivity with duration of diabetes, $P = 0.08$. Furthermore, HLA-DRB1*03/04 was not associated with IA-2A positivity $P=0.92$.

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Supplementary Table 1. Association of *HLA-DQB1* with type 1 diabetes. Odds ratios (ORs) are reported for a model that only includes *HLA-DQB1* (unconditional model) and also for the model conditional on *HLA-DRB1* genotypes.

Genotype	N (frequency)		Unconditional		Conditional on <i>HLA-DRB1</i>	
	Cases	Controls	OR [95% CI]		OR [95% CI]	
0302/0302	404 (0.065)	54 (0.010)	0.77 [0.56-1.06]	7.94 [5.76-10.93]	1.92 [1.24-2.97]	5.53 [3.08-9.93]
02/02	680 (0.110)	341 (0.064)	0.21 [0.17-0.25]	2.17 [1.79-2.63]	1.33 [0.66-2.69]	3.84 [1.85-7.99]
0302/0604	141 (0.023)	43 (0.008)	0.33 [0.23-0.47]	3.37 [2.32-4.90]	1.11 [0.63-1.96]	3.20 [1.69-6.07]
02/0302	2,382 (0.384)	247 (0.047)	1.00 [reference]	10.28 [8.47-12.48]	1.00 [reference]	2.89 [1.75-4.76]
0302/0609	14 (0.002)	7 (0.001)	0.25 [0.10-0.64]	2.58 [1.01-6.57]	0.98 [0.33-2.91]	2.82 [0.92-8.70]
0302/05	514 (0.083)	158 (0.030)	0.34 [0.27-0.42]	3.46 [2.75-4.35]	0.72 [0.45-1.15]	2.07 [1.22-3.52]
0302/0603	96 (0.016)	61 (0.012)	0.17 [0.12-0.24]	1.72 [1.20-2.45]	0.57 [0.33-1.00]	1.65 [0.88-3.11]
0302/04	132 (0.021)	25 (0.005)	0.51 [0.33-0.80]	5.25 [3.33-8.28]	0.53 [0.28-1.03]	1.54 [0.77-3.07]
02/04	115 (0.019)	53 (0.010)	0.22 [0.16-0.32]	2.29 [1.60-3.29]	0.46 [0.27-0.77]	1.32 [0.78-2.22]
05/05	48 (0.008)	110 (0.021)	0.04 [0.03-0.06]	0.45 [0.31-0.65]	0.44 [0.22-0.86]	1.27 [0.62-2.57]
0302/0601	36 (0.006)	149 (0.028)	0.03 [0.02-0.04]	0.26 [0.18-0.39]	0.36 [0.18-0.72]	1.04 [0.51-2.14]
02/0303	103 (0.017)	157 (0.030)	0.07 [0.05-0.09]	0.68 [0.51-0.91]	0.36 [0.21-0.61]	1.03 [0.61-1.76]
02/0604	144 (0.023)	72 (0.014)	0.22 [0.16-0.30]	2.21 [1.61-3.05]	0.35 [0.19-0.66]	1.02 [0.50-2.05]
02/05	390 (0.063)	414 (0.078)	0.10 [0.08-0.12]	1.00 [reference]	0.35 [0.21-0.57]	1.00 [reference]
0302/0301	228 (0.037)	230 (0.043)	0.10 [0.08-0.13]	1.06 [0.84-1.34]	0.33 [0.23-0.47]	0.95 [0.57-1.59]
05/04	29 (0.005)	36 (0.007)	0.09 [0.05-0.14]	0.88 [0.52-1.47]	0.26 [0.13-0.51]	0.75 [0.38-1.48]
0302/0303	74 (0.012)	85 (0.016)	0.09 [0.06-0.12]	0.88 [0.62-1.24]	0.24 [0.16-0.36]	0.69 [0.39-1.23]
0301/05	172 (0.028)	293 (0.055)	0.06 [0.05-0.08]	0.64 [0.51-0.81]	0.14 [0.09-0.23]	0.41 [0.24-0.70]
0303/Y	34 (0.006)	231 (0.044)	0.02 [0.01-0.02]	0.15 [0.10-0.23]	0.13 [0.08-0.22]	0.38 [0.22-0.65]
0301/0301	65 (0.011)	183 (0.035)	0.04 [0.03-0.05]	0.39 [0.28-0.53]	0.11 [0.07-0.17]	0.31 [0.18-0.55]
02/0301	218 (0.035)	485 (0.092)	0.05 [0.04-0.06]	0.49 [0.39-0.60]	0.09 [0.07-0.11]	0.25 [0.15-0.40]
05/0601	30 (0.005)	395 (0.075)	0.01 [0.01-0.01]	0.08 [0.05-0.12]	0.08 [0.04-0.15]	0.23 [0.12-0.44]
0301/06	53 (0.009)	435 (0.082)	0.01 [0.01-0.02]	0.13 [0.09-0.18]	0.07 [0.04-0.11]	0.19 [0.11-0.34]
02/0609	10 (0.002)	31 (0.006)	0.03 [0.02-0.07]	0.36 [0.17-0.75]	0.06 [0.02-0.15]	0.16 [0.06-0.45]
0301/0303	17 (0.003)	123 (0.023)	0.01 [0.01-0.02]	0.14 [0.08-0.24]	0.05 [0.03-0.08]	0.14 [0.07-0.28]
02/0603	31 (0.005)	138 (0.026)	0.02 [0.02-0.04]	0.26 [0.17-0.39]	0.04 [0.02-0.08]	0.12 [0.05-0.26]
02/0601	12 (0.002)	354 (0.067)	0.004 [0.002-0.006]	0.04 [0.02-0.07]	0.04 [0.01-0.12]	0.12 [0.04-0.35]
0402/X	7 (0.001)	50 (0.009)	0.01 [0.01-0.03]	0.14 [0.06-0.31]	0.04 [0.02-0.11]	0.12 [0.05-0.30]

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0301/04	13 (0.002)	39 (0.007)	0.04 [0.02-0.07]	0.39 [0.20-0.75]	0.04 [0.01-0.10]	0.11 [0.04-0.30]
06/06	6 (0.001)	296 (0.056)	0.002 [0.001-0.005]	0.02 [0.01-0.05]	0.02 [0.01-0.05]	0.06 [0.02-0.15]

To reduce the number of rare genotype combinations, and create a model which had parameters that were estimable, some alleles and genotypes were grouped together. X and Y are used to represent a group of rare genotypes: X = all 05 alleles, all 06 alleles and 0402; Y = X and 0303.

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Supplementary Table 2. Association of *HLA-DRB1* with type 1 diabetes. Odds ratios (ORs) are reported for a model that only includes *HLA-DRB1* (unconditional model) and also for the model conditional on *HLA-DQB1* genotypes.

Genotype	N (frequency)		Unconditional		Conditional on <i>HLA-DQB1</i>	
	Cases	Controls	OR [95% CI]		OR [95% CI]	
08/0401	113 (0.018)	17 (0.003)	0.73 [0.43-1.26]	6.12 [3.57-10.49]	2.12 [0.86-5.26]	12.43 [5.10-30.26]
03/04 *	1,629 (0.263)	183 (0.034)	1.00 [reference]	8.33 [6.67-10.40]	1.00 [reference]	5.86 [4.32-7.94]
03/0404	575 (0.093)	64 (0.012)	1.04 [0.77-1.41]	8.68 [6.39-11.79]	0.70 [0.50-0.96]	4.07 [2.79-5.94]
0401/0401	257 (0.041)	51 (0.010)	0.58 [0.41-0.81]	4.79 [3.39-6.76]	0.69 [0.43-1.12]	4.05 [2.67-6.15]
01/0401	520 (0.084)	153 (0.029)	0.38 [0.30-0.48]	3.17 [2.48-4.04]	0.68 [0.42-1.11]	3.98 [2.58-6.15]
03/13	183 (0.030)	162 (0.030)	0.13 [0.10-0.18]	1.12 [0.86-1.47]	0.59 [0.32-1.12]	3.48 [1.87-6.47]
4/04 **	107 (0.017)	33 (0.006)	0.37 [0.24-0.57]	3.12 [2.04-4.79]	0.50 [0.29-0.87]	2.96 [1.82-4.81]
03/R	204 (0.033)	97 (0.018)	0.23 [0.17-0.30]	1.89 [1.41-2.54]	0.45 [0.29-0.71]	2.65 [1.69-4.17]
13/401	220 (0.036)	128 (0.024)	0.20 [0.15-0.26]	1.63 [1.24-2.14]	0.37 [0.22-0.61]	2.15 [1.35-3.45]
0401/07	249 (0.040)	175 (0.033)	0.17 [0.13-0.21]	1.38 [1.07-1.78]	0.35 [0.26-0.47]	2.06 [1.48-2.86]
01/03	304 (0.049)	186 (0.035)	0.18 [0.14-0.23]	1.52 [1.19-1.95]	0.32 [0.19-0.55]	1.89 [1.13-3.18]
03/03	546 (0.088)	116 (0.022)	0.55 [0.43-0.71]	4.57 [3.53 -5.92]	0.25 [0.12-0.52]	1.48 [0.72-3.07]
07/09	158 (0.026)	246 (0.046)	0.07 [0.06-0.09]	0.61 [0.47-0.79]	0.22 [0.14-0.34]	1.27 [0.81-1.97]
Rares	322 (0.052)	302 (0.057)	0.12 [0.10-0.15]	1.00 [reference]	0.17 [0.13-0.23]	1.00 [reference]
01/04 ***	119 (0.019)	96 (0.018)	0.15 [0.11-0.20]	1.21 [0.88-1.66]	0.16 [0.09-0.28]	0.95 [0.58-1.56]
03/11	26 (0.004)	127 (0.024)	0.02 [0.02-0.04]	0.20 [0.13-0.31]	0.15 [0.09-0.25]	0.89 [0.53-1.51]
0401/0404	117 (0.017)	78 (0.015)	0.17 [0.12-0.24]	1.41 [1.01-1.97]	0.13 [0.08-0.21]	0.76 [0.50-1.16]
11/T	108 (0.017)	450 (0.085)	0.03 [0.02-0.04]	0.23 [0.17-0.30]	0.12 [0.08-0.17]	0.69 [0.50-0.95]
13/U	114 (0.018)	495 (0.093)	0.03 [0.02-0.03]	0.22 [0.17-0.29]	0.09 [0.06-0.14]	0.52 [0.33-0.81]
01/S	90 (0.015)	537 (0.101)	0.02 [0.01-0.03]	0.16 [0.12-0.21]	0.07 [0.04-0.13]	0.40 [0.23-0.71]
03/15	9 (0.002)	211 (0.040)	0.005 [0.003-0.010]	0.04 [0.02-0.08]	0.07 [0.02-0.22]	0.40 [0.12-1.29]
01/07	36 (0.006)	201 (0.038)	0.02 [0.01-0.03]	0.17 [0.12-0.25]	0.04 [0.02-0.08]	0.25 [0.14-0.44]
15/V	52 (0.008)	698 (0.131)	0.009 [0.006-0.012]	0.07 [0.05-0.10]	0.04 [0.02-0.07]	0.24 [0.13-0.42]

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03/07	129 (0.021)	228 (0.043)	0.07 [0.05-0.09]	0.55 [0.42-0.72]	0.04 [0.02-0.07]	0.21 [0.11-0.43]
14/W	14 (0.002)	168 (0.032)	0.01 [0.01-0.17]	0.08 [0.05-0.14]	0.02 [0.01-0.04]	0.11 [0.05-0.21]
07/07	4 (0.001)	123 (0.023)	0.004 [0.001-0.010]	0.03 [0.01-0.08]	0.003 [0.001-0.009]	0.02 [0.01-0.05]

* includes 03/0401, 03/0405 and 03/4 where 4 represents any 04 subtype with ambiguous 4 digit resolution.

** 4/04, where 4 represents any 04 subtype with ambiguous 4 digit resolution, includes 4/4, 4/0401, 4/0403, 4/404, 4/405

*** 01/04 includes 04 subtypes other than 0401.

03/R includes 03/08, 03/09, 03/12, 03/16 and 03/1001; 01/S includes 01/01, 01/11, 01/13, 01/14, 01/15, 01/12, 01/16 and 01/1001; 11/T includes 11/11, 11/13, 11/14, 11/15, 11/04 (all 04 subtypes); 13/U includes 13/12, 13/16, 13/1001, 13/13, 13/14, 13/15, 13/0402, 13/404, 13/405, 13/07, 13/09, 13/4 (ambiguous 04 subtypes); 15/V includes 4/15, 15/12, 15/16, 15/1001, 15/15, 15/0401, 15/0403, 15/0404, 15/0405, 15/07, 15/09; 14/W includes 14/12, 14/16, 14/1001, 14/14, 14/15, 14/401, 14/403, 14/404, 14/07, 14/09, 4/14. Rares are all remaining genotypes with a frequency of less than 0.01 in cases and controls combined.

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Supplementary Table 3. Type 1 diabetes association of *HLA-DRB1* coded using the classical alleles into DR3/4/X genotypes in 6,205 type 1 diabetes cases and 5,329 controls. DR3 denotes all *HLA-DRB103 alleles, DR4 denotes all *HLA-DRB1**04 except *HLA-DRB1**0403, *HLA-DRB1**0407 which were included in the DRX group of all other *HLA-DRB1* alleles and included *HLA-DQB1**0301.**

Allele/Genotype	Cases N(freq)	Controls N(freq)	OR [95% CI]	P
DR3	4,106 (0.33)	1,432 (0.13)	6.59 [6.03-7.21]	
DR4	4,795 (0.39)	1,648 (0.15)	9.67 [8.78-10.65]	
DRX	3,509 (0.28)	7,578 (0.71)	1.00 [reference]	
3/4	2,123 (0.34)	140 (0.03)	11.56 [9.59-13.93]	
4/4	430 (0.07)	62 (0.01)	5.21 [3.94-6.88]	
3/3	545 (0.09)	115 (0.02)	3.70 [2.98-4.60]	
4/X	1,812 (0.29)	1,384 (0.26)	1.00 [reference]	
3/X	893 (0.14)	1,062 (0.20)	0.64 [0.57-0.72]	
X/X	402 (0.06)	2,566 (0.48)	0.12 [0.11-0.14]	
				0

Supplementary Table 4 GADA association with *HLA-DRB1* coded as DR3/4/X (where X denotes any non *HLA-DRB103 and any non *HLA-DRB1**04 allele but includes *HLA-DRB1**0403 and *0407). There was no evidence for a DR3/4 interaction effect ($P = 0.19$). The effect of *HLA-DRB1* (coded as DR3/4/X) with GADA was primarily due to DR3, $P = 4.80 \times 10^{-17}$. The association with DR4 did not differ to that of DRX. Following analyses conditioned on the class I effect marked by rs9266722, DR3 was still convincingly associated with GADA positivity.**

Genotype or allele	N (Frequency)		Single locus	P	Conditional on rs9266722	
	GADA positives	GADA negatives	OR [95% CI]		OR [95% CI]	P
DR4	843 (0.353)	1,012 (0.417)	0.88 [0.74-1.05]		0.96 [0.80-1.15]	
DR3	909 (0.381)	693 (0.286)	1.69 [1.45-1.98]		1.77 [1.51-2.07]	
DRX	636 (0.266)	719 (0.297)	1.00 [reference]		1.00 [reference]	
				1.92×10^{-16}		5.62×10^{-16}
3/3	162 (0.14)	63 (0.05)	2.57 [1.83-3.61]		2.48 [1.76-3.50]	
3/X	186 (0.16)	144 (0.12)	1.21 [0.92-1.59]		1.11 [0.84-1.47]	
3/4	399 (0.33)	423 (0.35)	1.00 [reference]		1.00 [reference]	
X/X	71 (0.06)	83 (0.07)	0.74 [0.51-1.06]		0.65 [0.45-0.94]	
4/4	68 (0.06)	90 (0.07)	0.71 [0.49-1.02]		0.74 [0.51-1.07]	
4/X	308 (0.26)	409 (0.34)	0.65 [0.53-0.81]		0.63 [0.50-0.78]	

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Supplementary Table 5. Genotype associations with GADA positivity of the *HLA-DQB1.HLA-DRB1* haplotypes. Only the most common alleles and genotypes could be analyzed due to the sparsity of data, hence all rare alleles and genotypes (< 0.01 frequency) were grouped together.

Genotype DQ.DR / DQ.DR	OR [95% CI]	
02.03 / 02.03	2.62 [1.87-3.67]	3.31 [2.05-5.35]
02.03 / 0303.rares	2.34 [0.98-5.57]	2.96 [1.17-7.52]
02.03 / 02.07	1.55 [0.82-2.93]	1.96 [0.96-4.03]
0302.04 / 0301.rares	1.48 [0.69-3.17]	1.87 [0.81-4.31]
02.03 / 04.08	1.46 [0.60-3.58]	1.85 [0.71-4.82]
0302.04 / 0301.04	1.32 [0.66-2.63]	1.67 [0.77-3.60]
02.03 / 05.01	1.17 [0.76-1.79]	1.48 [0.86-2.55]
02.03 / 0301.04	1.17 [0.57-2.42]	1.48 [0.67-3.30]
02.03 / 0302.04	1.00 [reference]	1.27 [0.85-1.88]
02.07 / 0302.04	0.95 [0.62-1.48]	1.21 [0.69-2.10]
02.07 / 0301.04	0.89 [0.38-2.07]	1.12 [0.45-2.79]
Rares	0.83 [0.62-1.11]	1.05 [0.68-1.64]
0302.04 / 0302.04	0.79 [0.53-1.17]	1.00 [reference]
02.03 / 06.13	0.65 [0.37-1.16]	0.83 [0.43-1.61]
0301.04 / 05.01	0.58 [0.33-1.01]	0.73 [0.38-1.41]
0302.04 / 05.01	0.54 [0.38-0.75]	0.68 [0.42-1.10]
0302.04 / 0402.08	0.44 [0.25-0.76]	0.56 [0.29-1.06]
0302.04 / 06.13	0.36 [0.23-0.58]	0.46 [0.26-0.81]

Supplementary Table 6. *HLA-B.HLA-DRB1.HLA-DQB1* haplotype associations with GADA. Haplotype ORs are given relative to the two most common haplotypes, *HLA-B*08.HLA-DRB1*03.HLA-DQB1*02* and *HLA-B*X.HLA-DRB1*04.HLA-DQB1*0302*. Haplotypes below 0.01 frequency were grouped together and labeled as “Rares”. Alleles other than *HLA-B*08*, *18, and *15 at *HLA-B* or *HLA-DRB1*03*, *04 and *01 at *HLA-DRB1* are denoted by X.

Haplotype	GADA positives	GADA negatives	OR [95% CI]	
08.03.02	0.268	0.183	1.00 [reference]	2.21 [1.69-2.89]
X.X.0301	0.018	0.008	1.03 [0.50-2.12]	2.27 [1.08-4.79]
08.04.0302	0.019	0.009	0.89 [0.49-1.63]	1.97 [1.10-3.55]
X.03.02	0.067	0.049	0.88 [0.62-1.24]	1.95 [1.36-2.80]
18.03.02	0.056	0.049	0.72 [0.50-1.03]	1.59 [1.08-2.35]
Rares			0.67 [0.47-0.96]	1.49 [1.01-2.19]
X.X.02	0.036	0.041	0.63 [0.41-0.96]	1.38 [0.89-2.15]
X.04.0301	0.044	0.044	0.56 [0.39-0.81]	1.25 [0.87-1.79]
X.04.0302	0.212	0.248	0.45 [0.35-0.59]	1.00 [reference]
X.X.06	0.059	0.079	0.41 [0.29-0.57]	0.90 [0.63-1.29]
18.04.0302	0.014	0.017	0.40 [0.22-0.74]	0.89 [0.50-1.60]
X.01.05	0.062	0.093	0.36 [0.26-0.51]	0.80 [0.57-1.14]
15.04.0302	0.081	0.112	0.36 [0.26-0.50]	0.79 [0.59-1.05]
15.01.05	0.004	0.012	0.23 [0.11-0.49]	0.52 [0.24-1.12]
15.X.06	0.004	0.011	0.21 [0.11-0.43]	0.47 [0.23-0.98]

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Supplementary Table 7. IA-2A association with *HLA-DRB1* coded as DR3/4/X (where X denotes any non *HLA-DRB103 and any non *HLA-DRB1**04 allele but includes *HLA-DRB1**0403 and *0407). There was no convincing evidence of deviation from a multiplicative allelic effects model ($P = 0.0042$ without conditioning on *HLA-A* and $P = 0.059$ with conditioning on *HLA-A**24).**

Genotype or allele	N (Frequency)		Single locus			Conditional on <i>HLA-A</i> *24	
	IA-2A positives	IA-2A negatives	OR [95% CI]	OR [95% CI]	<i>P</i>	OR [95% CI]	<i>P</i>
DR4	1,197 (0.43)	646 (0.32)		1.75 [1.45-2.10]		1.65 [1.32-2.07]	
DR3	749 (0.27)	850 (0.42)		0.56 [0.48-0.65]		0.48 [0.40-0.58]	
DRX	834 (0.30)	518 (26)		1.00 [reference]		1.00 [reference]	
					8.59×10^{-38}		1.94×10^{-31}
3/3	78 (0.06)	147 (0.15)	0.36 [0.23-0.55]	0.33 [0.24-0.46]		0.33 [0.22-0.48]	
3/X	133 (0.10)	197 (0.20)	0.48 [0.32-0.71]	0.44 [0.34-0.59]		0.45 [0.32-0.63]	
3/4	460 (0.33)	359 (0.36)	1.07 [0.74-1.54]	1.00 [reference]		1.00 [reference]	
X/X	90 (0.06)	64 (0.06)	1.00 [reference]	0.93 [0.65-1.35]		1.35 [0.85-2.13]	
4/4	108 (0.08)	47 (0.05)	1.96 [1.19-3.21]	1.83 [1.24-2.70]		2.34 [1.44-3.80]	
4/X	521 (0.37)	193 (0.19)	2.11 [1.45-3.07]	1.97 [1.57-2.47]		2.23 [1.70-2.94]	

*HLA-DQB1**02/0302, which is highly predisposing to type 1 diabetes, was not associated with IA-2A positivity ($P = 0.47$; 532 IA-2A positive cases (38%) carried the *HLA-DQB1**02/0302 genotype, while 397 cases (40%) who were not IA-2A positive had the *HLA-DQB1**02/0302 genotype).

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Supplementary Table 8. IA-2A association with *HLA-A.HLA-DRB1* haplotypes.

<i>HLA-A.HLA-DRB1</i> haplotype	Frequency		OR [95% CI]	
	IA-2A positives	IA-2A negatives		
X.04	0.222	0.143	3.80 [2.96-4.87]	2.21 [1.59-3.06]
02.04	0.204	0.134	3.47 [2.66-4.52]	2.02 [1.49-2.73]
02.08	0.009	0.003	2.67 [1.22-5.85]	1.56 [0.70-3.43]
X.08	0.009	0.008	2.28 [1.09-4.81]	1.32 [0.61-2.87]
X.X	0.118	0.085	1.88 [1.46-2.42]	1.09 [0.82-1.46]
02.X	0.084	0.081	1.72 [1.31-2.26]	1.00 [reference]
24.04	0.042	0.050	1.61 [1.14-2.27]	0.94 [0.63-1.40]
X.03	0.222	0.314	1.00 [reference]	0.58 [0.44-0.76]
02.03	0.047	0.079	0.95 [0.72-1.24]	0.55 [0.39-0.77]
24.X	0.028	0.041	0.91 [0.62-1.33]	0.53 [0.36-0.79]
24.03	0.011	0.039	0.42 [0.29-0.62]	0.25 [0.16-0.38]
24.08	0.004	0.022	0.40 [0.19-0.81]	0.23 [0.11-0.49]

X~ any non HLA-A*24, or HLA-A*02 allele at *HLA-A* and any non-*HLA-DRB1**04, *03, *08 allele at *HLA-DRB1* and included HLA-DQB1*0301, HLA-DRB1*0403, *0407.

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Supplementary Table 9. IA-2A association with *HLA-A.HLA-B.HLA-DRB1* haplotypes. Rare haplotypes were grouped to form the reference group.

A.B.DRB1 haplotype	Frequency		OR [95% CI]	
	IA-2A positives	IA-2A negatives		
01.X.04	0.035	0.020	2.66 [1.63-4.33]	2.61 [1.63-4.18]
X.X.04	0.171	0.103	2.59 [1.89-3.56]	2.54 [1.92-3.36]
01.08.04	0.017	0.010	2.28 [1.21-4.28]	2.23 [1.20-4.16]
02.X.04	0.187	0.138	2.01 [1.47-2.75]	1.97 [1.47-2.64]
X.X.01	0.046	0.027	1.47 [0.93-2.31]	1.44 [0.94-2.21]
02.X.X	0.058	0.037	1.33 [0.89-1.98]	1.30 [0.91-1.86]
X.X.X	0.043	0.041	1.07 [0.70-1.63]	1.00 [reference]
Rares	0.092	0.093	1.00 [reference]	1.00 [reference]
24.X.04	0.024	0.030	0.94 [0.61-1.47]	0.92 [0.60-1.41]
02.08.03	0.026	0.026	0.86 [0.52-1.44]	0.85 [0.52-1.38]
X.18.03	0.034	0.041	0.84 [0.55-1.27]	0.82 [0.55-1.22]
02.X.01	0.025	0.034	0.76 [0.48-1.20]	0.74 [0.48-1.15]
24.X.01	0.010	0.008	0.73 [0.35-1.52]	0.71 [0.35-1.47]
X.08.03	0.028	0.040	0.66 [0.42-1.05]	0.65 [0.42-1.00]
X.X.03	0.027	0.034	0.64 [0.41-1.00]	0.62 [0.41-0.95]
01.08.03	0.128	0.197	0.60 [0.44-0.82]	0.59 [0.44-0.78]
02.X.03	0.017	0.035	0.51 [0.31-0.83]	0.50 [0.31-0.80]
02.18.03	0.007	0.015	0.49 [0.24-1.02]	0.48 [0.23-1.00]
24.X.X	0.012	0.029	0.44 [0.24-0.81]	0.43 [0.24-0.77]
24.39.X	0.008	0.021	0.39 [0.20-0.76]	0.39 [0.20-0.74]
24.08.03	0.005	0.021	0.27 [0.13-0.54]	0.26 [0.13-0.52]

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Supplementary Table 10 IA-2A association with *HLA-DRB1.HLA-B* haplotypes. The haplotypes listed without an OR were grouped together and labeled “Rares” as they were individually too rare to estimate effects in the regression model. ORs are reported using the most common haplotype as reference (HLA-B*X.HLA-DRB1*04) and also using the neutral rare haplotypes as reference.

<i>HLA-B.HLA-DRB1</i> haplotype	Frequency		OR [95% CI]	
	IA-2A positives	IA-2A negatives		
18.04	0.020	0.008	2.24 [1.17-4.29]	1.07 [0.58-1.95]
X.04	0.417	0.295	2.10 [1.61-2.74]	1.00 [reference]
08.04	0.018	0.014	1.94 [1.08-3.50]	0.93 [0.53-1.61]
39.04	0.013	0.014	1.43 [0.74-2.74]	0.68 [0.37-1.25]
X.1	0.085	0.068	1.10 [0.79-1.52]	0.52 [0.37-0.74]
X.X	0.121	0.111		
18.X	0.011	0.003		
08.X	0.005	0.005		
08.01	0.003	0.006		
18.01	0.000	0.003		
39.01	0.009	0.012		
39.03	0.003	0.010		
Rares	0.152	0.150	1.00 [reference]	0.48 [0.36-0.62]
18.03	0.046	0.064	0.71 [0.49-1.02]	0.34 [0.23-0.49]
X.3	0.050	0.074	0.60 [0.42-0.86]	0.29 [0.20-0.41]
08.03	0.185	0.283	0.60 [0.47-0.78]	0.29 [0.22-0.37]
39.X	0.014	0.030	0.58 [0.34-1.00]	0.28 [0.16-0.48]

X denotes, any allele other than HLA-B*18, *08 or *39 at HLA-B and any allele other than HLA-DRB1*04, HLA-DRB1*03 or HLA-DRB1*01 at HLA-DRB1.