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ARTICLE

No association of multiple type 2 diabetes loci with type 1 diabetes

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Abstract
Aims/hypothesis We used recently confirmed type 2 diabetes gene regions to investigate the genetic relationship between type 1 and type 2 diabetes, in an average of 7,606 type 1 diabetic individuals and 8,218 controls, providing >80% power to detect effects as small as an OR of 1.11 at a false-positive rate of 0.003.
Methods The single nucleotide polymorphisms (SNPs) with the most convincing evidence of association in 12 type 2 diabetes-associated gene regions, *PPARG*, *CDKAL1*, *HNF1B*, *WFS1*, *SLC30A8*, *CDKN2A–CDKN2B*, *IGF2BP2*, *KCNJ11*, *TCF7L2*, *FTO, HHEX–IDE* and *THADA*, were analysed in type 1 diabetes cases and controls. *PPARG* and *HHEX–IDE* were additionally tested for association in 3,851 type 1 diabetes families. Tests for interaction with HLA class II genotypes, autoantibody status, sex, and age-at-diagnosis of type 1 diabetes were performed with all 12 gene regions.
Results Only *PPARG* and *HHEX–IDE* showed any evidence of association with type 1 diabetes cases and controls (p=0.004 and p=0.003, respectively; p>0.05 for other SNPs). The potential association of *PPARG* was supported by family analyses (p=0.003; $p_{combined}=1.0\times10^{-4}$). No SNPs showed evidence of interaction with any covariate (p>0.05).
Conclusions/interpretation We found no convincing genetic link between type 1 and type 2 diabetes. An association of *PPARG* (rs1801282/Pro12Ala) could be consistent with its known function in inflammation. Hence, our results reinforce evidence suggesting that type 1 diabetes is a disease of the immune system, rather than being due to inherited defects in beta cell function or regeneration or insulin resistance.

Keywords Age-at-diagnosis · Association study · Autoantibodies · Genetics · *PPARG* · *SLC30A8* · Type 1 diabetes · Type 2 diabetes

Abbreviations

GWA	Genome-wide association
IA-2	Insulinoma-associated antigen 2
MAF	Minor allele frequency
SNP	Single nucleotide polymorphism
T1DGC	Type 1 Diabetes Genetics Consortium
TPO	Thyroid peroxidase
WTCCC	Wellcome Trust Case Control Consortium

Introduction

The possible relationship between type 1 and type 2 diabetes is a controversial subject. Formally, the two are considered to be aetiologically distinct. Type 1 diabetes is characterised by autoimmune destruction of pancreatic beta cells, resulting in a failure to produce insulin. Type 2 diabetes is caused by impaired beta cell function and capacity to secrete insulin, coupled to a decline in tissue sensitivity to insulin. Owing to similarities in their clinical manifestation, especially in a form of type 1 diabetes

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diagnosed in adulthood (latent autoimmune diabetes in adults), it has been suggested that type 1 and type 2 diabetes may share a common pathophysiological aetiology [1–3]. Therefore, we sought to investigate this hypothesis by investigating if genetic variants that predispose individuals to type 2 diabetes risk are associated with type 1 diabetes.

Currently, due mainly to the success of genome-wide association (GWA) studies, there are 18 confirmed type 2 diabetes loci: *PPARG*, *CDKAL1*, *HNF1B*, *WFS1*, *SLC30A8*, *CDKN2A–CDKN2B*, *IGF2BP2*, *KCNJ11*, *TCF7L2*, *FTO*, *HHEX–IDE*, *JAZF1*, *CDC123-CAMK1D*, *TSPAN8-LGR5*, *THADA*, *ADAMTS9*, *NOTCH2* and, most recently, *KCNQ1* [4–16] (Electronic supplementary material [ESM] Tables 1 and 2). Six of these regions were identified in a meta-analysis that combined three GWA studies, and were confirmed in an independent set of up to 14,157 cases and 43,209 controls, by Zeggini et al. [5] (ESM Table 2). The effects of the 18 chromosome regions on type 2 diabetes risk have ORs ranging from 1.09 to 1.37 using single nucleotide polymorphisms (SNPs) [4–16] (Table 1; ESM Tables 1 and 2).

We have previously evaluated the association of five type 2 diabetes gene regions with type 1 diabetes. The gene regions *KCNJ11*, *IRS1* and *PPARG* were analysed in 2,434 type 1 diabetes families, and the *PPARG* rs1801282 (Pro12Ala) variant was found to have a RR of 0.87, $p=0.008$ [17]. In two recent studies, *TCF7L2* and *FTO* showed no evidence of association with type 1 diabetes in approximately 6,000 cases and 7,000 controls [18, 19]. We have now expanded the size of our sample sets to maximise the statistical power of our study: we have, in theory, 89% power to find effects as small as an OR of 1.11 at an α level of 0.003 for the mean minor allele frequency (MAF) of 0.28 in 7,606 cases and 8,218 controls, assuming a multiplicative model. The present study, therefore, is a comprehensive analysis of 12 known type 2 diabetes loci, including tests of interactions with HLA class II genotypes, using the *HLA-DRB1*03*- and *HLA-DRB1*04*-tagging SNPs rs7454108 and rs2187668, age-at-diagnosis of type 1 diabetes, sex and autoantibody status, to evaluate whether type 1 and type 2 diabetes share a common genetic background.

Methods

Case–control samples An average of 7,606 British type 1 diabetes cases and 8,218 British controls, all of whom were of white ethnicity, were genotyped. Type 1 diabetes cases were recruited for the Juvenile Diabetes Research Foundation/Wellcome Trust Diabetes and Inflammation Laboratory's Genetic Resource Investigating Diabetes British, type 1 diabetes collection (http://www.childhood-diabetes.org.uk/grid.shtml, accessed 22 April 2009). Cases had been diagnosed with type 1 diabetes before 17 years of age (mean age at diagnosis 7.8 years). Control DNA samples came from the British 1958 Birth Cohort ($n=7,733$) and the Wellcome Trust Case Control Consortium's (WTCCC) UK Blood Service sample collection ($n=3,181$) [20]. Cases and controls were matched in terms of 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 [20, 21]. The appropriate ethics committees approved the collection of all DNA samples, and written consent was obtained from all individuals, or from the parents of individuals who were too young to provide consent.

Family samples One SNP in *PPARG* (rs1801282) and one in the *HHEX–IDE* region (rs1111875) were genotyped in 3,851 type 1 diabetes families of white European descent. The total comprised 593 multiplex (affected sib-pair) families from the Diabetes UK Warren I collection; 80 simplex families from Yorkshire, UK; 263 multiplex/simplex families from Northern Ireland; 331 multiplex families from the Human Biological Data Interchange, USA; 951 multiplex/simplex families from Finland; 410 simplex families from Romania; 357 simplex families from Norway and 866 affected sib-pair families made available through the Type 1 Diabetes Genetics Consortium (T1DGC; http://www.t1dgc.org, accessed 22 April 2009; http://www-gene.cimr.cam.ac.uk/todd/dna-refs.shtml, accessed 22 April 2009). Of the T1DGC families, 166 families were from the Asia–Pacific region, 422 families were from across Europe and 278 were from North America.

Genotyping Samples were genotyped by personnel blind to case–control status using the Taqman 5′ nuclease assay (Applied Biosystems, Warrington, UK) according to the manufacturer's protocol. To minimise error, two operators independently scored the genotypes. Previously published genotypes at *FTO*, *TCF7L2* and *PPARG* were also included [17–19] as a subset of our present dataset. The present study includes up to 3,363 additional type 1 diabetes cases and controls. A full description of genotypes common to the current and previously published data is provided in ESM Table 3. We genotyped a minimum of 5,896 cases and 7,101 controls and a maximum of 8,229 cases and 10,406 controls, depending

Table 1 Association analysis of type 2 diabetes loci in type 1 diabetes cases and controls

Gene region	SNP	Cases, *n*	Controls, *n*	Minor allele, Number of chromosomes (allele frequency)		OR (95% CI)[a]	*p* value	Power, %[b]	Power, %[c]	Type 2 diabetes[d]	
				Cases	Controls					OR	Ref.
TCF7L2	rs7903146 (C>T)[e]	5,896	7,322	3,395 (0.29)	4,260 (0.29)	0.99 (0.94–1.05)	0.744	81	100	1.37	[8, 15]
	rs12255372 (G>T)	7,776	8,847	4,421 (0.28)	5,125 (0.29)	0.97 (0.92–1.02)	0.235	92	100	1.52	[9]
CDKN2A–B	rs10811661 (T>C)	7,634	7,148	2,754 (0.18)	2,498 (0.17)	1.03 (0.97–1.09)	0.354	68	100	0.83	[4, 8, 15]
FTO	rs9939609 (T>A)[e]	7,655	7,182	6,098 (0.40)	5,644 (0.39)	1.03 (0.98–1.08)	0.252	92	100	1.17	[4, 8]
KCNJ11	rs5219 (C>T)	8,058	10,406	5,772 (0.36)	7,411 (0.36)	1.02 (0.97–1.06)	0.501	97	100	1.14	[8, 15]
HHEX–IDE	rs1111875 (G>A)	7,968	9,675	6,369 (0.40)	8,042 (0.42)	0.94 (0.90–0.98)	0.003	97	100	0.88	[4, 8, 15]
IGF2BP2	rs4402960 (G>T)	7,554	7,101	4,755 (0.31)	4,546 (0.32)	0.98 (0.93–1.03)	0.457	89	99	1.14	[4, 8, 15]
CDKAL1	rs7756992 (A>G)	7,714	7,200	4,185 (0.27)	3,893 (0.27)	1.00 (0.95–1.05)	0.957	86	100	1.20	[11]
SLC30A8	rs13266634 (C>T)	7,680	7,200	4,879 (0.32)	4,463 (0.31)	1.04 (0.99–1.09)	0.146	89	93	0.89	[4, 8, 15]
WFS1	rs10010131 (G>A)	7,745	9,259	6,173 (0.40)	7,564 (0.41)	0.96 (0.92–1.00)	0.065	96	96	0.90	[12]
HNF1B	rs7501939 (C>T)	7,712	7,217	6,249 (0.41)	5,800 (0.40)	1.01 (0.97–1.06)	0.554	93	85	1.10	[13]
PPARG	rs1801282 (C>G)	8,229	9,342	1,880 (0.11)	2,304 (0.12)	0.91 (0.85–0.97)	0.004	60	82	0.88	[4, 8, 15]
THADA	rs7578597 (T>C)	7,601	8,395	1,598 (0.11)	1,840 (0.11)	0.94 (0.88–1.01)	0.113	50	80	0.87	[5]
	rs17031005 (A>G)	7,256	8,760	1,481 (0.10)	1,878 (0.11)	0.94 (0.87–1.01)	0.078	50	80	NA	–

[a] The ORs and 95% CIs are for the minor allele using the common allele as the reference allele

[b] Power calculated for an OR of 1.11 at an α level of 0.003, assuming a multiplicative allelic effects model, given the allele frequency and number of type 1 diabetes cases and controls genotyped

[c] Power calculated for the size of effect detected in type 2 diabetes at an α level of 0.003, assuming a multiplicative allelic effects model, using the number of type 1 diabetes cases and controls genotyped

[d] The ORs are those reported in the literature for an association with type 2 diabetes

[e] New analyses of previously published data [18, 19] are included for information (ESM Table 3)

CDKN2A–B denotes *CDKN2A–CDKN2B*

on the number of samples available at the time the SNPs were genotyped. The average success rate for sample scoring was 96.77%, with a range of 95.20–97.75% across the 14 SNPs (ESM Table 4). The HLA classical loci, *HLA-DRB1* and *HLA-DQB1* were genotyped in a subset of type 1 diabetes case samples (n=3,312) as described previously [22]. Two HLA class II SNPs, rs7454108 and rs2187668, which tag the type 1 diabetes-predisposing DR3 and DR4 haplotypes, were also genotyped in the full case–control collection [23].

Autoantibody measurements Levels of to GAD, insulinoma-associated antigen 2 (IA-2) and thyroid peroxidase (TPO) were measured in plasma samples. TPO autoantibody was measured with a PLATO processor ELISA immunoassay (Phadia, Milton Keynes, UK), using recombinant TPO antigen standardised against the National Institute of Biological Standards and Controls standard serum 66/387. GAD and IA-2 autoantibodies were measured by the Department of Clinical Science at the University of Bristol (Bristol, UK), using a radioimmunoassay [24, 25]. The threshold value for the presence of autoantibodies was taken as 85 IU/ml for TPO (n=969 autoantibody-positive individuals), 6 WHO units/ml for IA-2 (n=1,521 autoantibody-positive individuals), and 14 WHO units/ml for GAD (n=1,305 autoantibody-positive individuals) [26, 27].

Table 2 Association of *PPARG* and *HHEX–IDE* in type 1 diabetes families

Gene region	SNP	Families, *n*	Allele or genotype	Transmitted[a], *n* (%)	Not transmitted[a], *n* (%)	RR (95% CI)	*p* value	$p_{combined}$ value[d]
PPARG	rs1801282	3,312	G	862 (45.0)	988 (55.0)	0.87 (0.80–0.95)	0.003[b]	1.0×10^{-4}
			C/C	3,139 (77.4)	9,176 (75.4)	1.00 (reference)		
			C/G	853 (21.0)	2,789 (22.9)	0.86 (0.78–0.95)		
			G/G	65 (1.6)	206 (1.7)	0.84 (0.62–1.15)	0.010[c]	
HHEX–IDE	rs1111875	3,229	A	1,728 (49.8)	1,742 (50.2)	0.99 (0.93–1.06)	0.812[b]	0.017
			G/G	1,283 (34.8)	3,855 (34.9)	1.00 (reference)		
			G/A	1,788 (48.6)	5,324 (48.2)	1.01 (0.92–1.10)		
			A/A	611 (16.6)	1,867 (16.9)	0.98 (0.85–1.12)	0.880[c]	

[a] Note that the frequencies of genotypes in the cases and pseudo-controls are given, as well as the transmission counts from the transmission disequilibrium test analysis

[b] The *p* value for association from the transmission disequilibrium test, which assumes a multiplicative model

[c] The *p* value for association from the 2 *df* conditional logistic regression model, which does not assume a specific mode of inheritance

[d] The *p* value obtained by combining the results of the case–control and family data set (please refer to the Methods for further details)

Statistical methods—association tests Statistical analyses were performed using Stata version 10 (http://www.stata.com, accessed 22 April 2009), using routines available from http://www-gene.cimr.cam.ac.uk/clayton/software/stata/ (accessed 22 April 2009). We calculated power assuming a multiplicative effects model and an α level of 0.003, as this is equivalent to an α level of 0.05 divided by 17, the number of gene regions considered in the present report. All SNP genotypes were in Hardy–Weinberg equilibrium in the controls ($p\geq0.07$) and the parents ($p\geq0.06$). SNPs were tested for association using logistic regression with disease status as the dependent variable and the SNP to be tested coded 0, 1 or 2, corresponding to counts of the minor allele, as the predictor variable. Geographical region was included in the logistic model as strata within which SNP associations could be tested. To test which inheritance model was appropriate, we compared a multiplicative allelic effects model with a model that did not assume a specific mode of inheritance using a likelihood ratio test. The multiplicative model was appropriate for all SNPs ($p\geq0.08$). No correction was made for multiple comparisons.

The SNPs were tested for association in the families using the transmission disequilibrium test (TDT), which assumes a multiplicative allelic effects model. To test whether the multiplicative allelic effects model was appropriate, we used a conditional logistic regression model to compare sets of offspring with matched pseudo-controls (which consisted of the genotypes that could have been transmitted to the offspring but were not). The SNPs were modelled assuming no specific mode of inheritance, and were compared with the allelic effects model with a likelihood ratio test [28]. The multiplicative model was appropriate for both SNPs ($p>0.4$). The *p* values from the association tests in the case–control set and families were combined using Fisher's method. At the *THADA* SNP, rs17031005, we pooled information across multiple studies using Woolf's estimate of the common OR [29] and a Wald test on 1 *df*.

Statistical methods—interaction tests We tested for non-multiplicative interaction effects between the SNPs and HLA class II genotypes in a case-only analysis. This test assumes that the HLA genotypes and the SNP of interest are conditionally independent in controls. The SNP genotype was entered into a regression model as the dependent variable, the HLA genotypes as the predictor variables, and geographical region as strata. Three different groupings of the HLA class II genotypes were considered, and these have been described in detail elsewhere [30]. The first approach grouped individuals into those positive or negative for the *HLA-DRB1*03/HLA-DRB1*04* genotype (where *HLA-DRB1*03/HLA-DRB*0403* are classed as *HLA-DRB1*03/HLA-DRB1*04*-negative, as were cases carrying the *HLA-DQB1*0301* allele). The second approach used genotype risk estimates produced by Koeleman et al. [31] to categorise individuals as being at a high-, medium- or low-risk of type 1 diabetes based on HLA status. Third, we used recursive partitioning to divide the individuals into type 1 diabetes cases or controls based on their HLA class II genotype [22]. As classical HLA genotyping data were not available for the majority of the type 1 diabetes sample set, the SNPs rs74544108 and rs2187668 [23], which are in strong linkage disequilibrium with the *HLA-DRB1*04* and *HLA-DRB1*03* alleles (with $r^2=0.78$ and $r^2=0.97$, respectively), were used as tags. The genotypes ($n=8{,}416$ cases) were coded as 3/3, 3/4, 3/X, 4/4, 4/X and X/X, with 3 and 4 corresponding to

*HLA-DRB1*03* and *HLA-DRB1*04*, respectively. X represented all other HLA class II alleles excluding the alleles *HLA-DRB1*03* and *HLA-DRB1*04*.

A case-only analysis was performed for age-at-diagnosis and sex, using genotype at the SNP of interest as the predictor variable, and sex (coded as a binary variable) or age-at-diagnosis (coded as a continuous variable) as the outcome variable in a regression model (logistic regression for sex and linear regression for age-at-diagnosis). We also tested all SNPs for interaction with age-at-diagnosis divided into quartiles (0–4, 5–7, 8–10 and 11–16 years), using multinomial logistic regression. Interactions between the autoantibodies and SNP genotype were also tested in the cases. Each autoantibody was coded as a binary trait, corresponding to the presence or absence of the autoantibody, and was used as the dependent variable in a logistic model, with genotype at the test locus as the independent variable [27].

Results

The results of the association tests are summarised in Table 1. We found no convincing evidence of association between the type 1 and type 2 diabetes candidate genes in the case–control dataset, with the possible exceptions of *PPARG* (rs1801282 p=0.004, OR [95% CI] 0.91 [0.85–0.97]) and *HHEX–IDE* (rs1111875, p=0.003, OR [95% CI] 0.94 [0.90–0.98]) (Table 1; ESM Table 4). Hence, these two SNPs were further genotyped in a minimum of 3,229 families. The *PPARG* SNP rs1801282 had already been genotyped in a subset (n=2,355) of these families [17] (ESM Table 3). By genotyping an additional 1,135 families the power for the detection of associations was increased.

The result we obtained for the SNP rs1801282 in the families was consistent with that obtained in the case–control collection (p=0.003, RR [95% CI] 0.87 [0.80–0.95]; Table 2; $p_{\text{combined}}=1\times10^{-4}$). We obtained no additional support for an association of *HHEX–IDE* (p=0.812, RR [95%CI] 0.99 [0.93–1.06]; Table 2) with type 1 diabetes in the families. Although none of these SNPs showed a convincing association with type 1 diabetes overall, we were interested in whether they may affect a subgroup of type 1 diabetes cases. Therefore, we tested for interaction between all the SNPs and HLA class II genotypes, autoantibody status, age-at-diagnosis and sex. We found no evidence of interaction effects between the HLA genotypes and the type 2 diabetes loci (p>0.02) (ESM Table 5). We also found no evidence for an interaction between any of the autoantibodies and the type 2 diabetes SNPs ($p\geq0.05$). This included the *SLC30A8* SNP rs13266634, which has been reported by Hutton and colleagues [32] to determine the specificity of ZnT8, a newly discovered type 1 diabetes autoantigen [33].

No evidence of interactions with age-at-diagnosis or sex with the SNP association with type 1 diabetes was found ($p\geq0.05$). Gohlke et al. [34] reported an association between early age-at-diagnosis (<5 years of age) and the *SLC30A8* SNP rs13266634, and Wenzlau et al. [33] showed that ZnT8 autoantibodies appear before 3 years of age. Therefore, we also tested for interactions between the type 2 diabetes loci and age-at-diagnosis divided into quartiles, which included a group of individuals under 4 years of age, and found no evidence of interactions between age at diagnosis and the type 2 diabetes loci over any of the four age groups (p>0.04; ESM Table 6), except for some evidence at *CDKN2A–CDKN2B* (rs10811661 p=0.008; ESM Table 6), which is unlikely to be a true result, taking into account the number of hypotheses tested.

Six recently reported type 2 diabetes gene regions, *JAZF1*, *CDC123–CAMK1D*, *TSPAN8–LGR5*, *THADA*, *ADAMTS9* and *NOTCH2*, identified by a meta-analysis published by Zeggini et al. [5], were also analysed for association with type 1 diabetes in a meta-analysis by Cooper et al. [35] (results for nine type 2 diabetes-associated SNPs, from the Cooper et al. type 1 diabetes meta-analysis are given in ESM Table 2). The Cooper et al. [35] meta-analysis combined GWA data (305,090 SNPs) for 1,964 British type 1 diabetes cases and 2,953 controls from the WTCCC study [20] with data for 1,601 US type 1 diabetes cases from the Genetics of Kidneys in Diabetes study and 1,704 US controls from the National Institute of Mental Health [35], and hence included up to 3,565 cases and 4,657 controls. Three SNPs with the most convincing evidence of association with type 2 diabetes from these six regions published by Zeggini et al. [5] were not included in the type 1 diabetes meta-analysis. However, substitutes in linkage disequilibrium with these type 2 diabetes SNPs (r^2>0.8 in the HapMap (http://www.hapmap.org, accessed 22 April 2009) Centre d'Etude du Polymorphisme Humain [CEPH] panel of 32 individuals) were included (ESM Table 2).

Only the *THADA* SNP rs17031005, which is in linkage disequilibrium with the type 2 diabetes-associated SNP rs7578597 in the *THADA* gene region (r^2=1), showed evidence of association with type 1 diabetes in the meta-analysis by Cooper et al. [35] ($p=4.79\times10^{-5}$; ESM Table 2). Therefore, we followed up this result by genotyping rs17031005 in 7,256 cases and 8,760 controls, and the type 2 diabetes-associated SNP rs7578597 in 7,601 British type 1 diabetes cases and 8,395 controls. The genotypes of samples, which were used by both the WTCCC and the present study, were compared at rs17031005 and revealed 99.9% concordance between the studies. We found no evidence of association with type 1 diabetes at rs17031005

(p=0.606) either in the 5,442 cases and 6,239 controls who were not included in the type 1 diabetes meta-analysis by Cooper et al. [35] or in the full dataset of 7,256 cases and 8,760 controls (p=0.078; Table 1). Similarly, rs7578597 was not associated with type 1 diabetes (p=0.113). As we found no association with type 1 diabetes, the type 1 diabetes meta-analysis result in the *THADA* gene region is likely to be a false-positive result [35] (ESM Table 2). Finally, a meta-analysis combining the 5,442 cases and 6,239 controls genotyped in the present study with the WTCCC and the US studies used by Cooper et al. [35] provided no additional support for association with type 1 diabetes at rs17031005 (p=0.003). Furthermore, we found no evidence of interactions between the *THADA* SNPs and HLA class II genotypes (p>0.05; ESM Table 5), age-at-diagnosis (p>0.05; ESM Table 6), sex or autoantibody status (p>0.05).

Discussion

We have investigated confirmed type 2 diabetes-associated SNPs for association with type 1 diabetes and interaction with sex, age-at-diagnosis, HLA class II genotypes and autoantibody status in a dataset larger than any previously published study. Yet, we have failed to find evidence of association between type 1 diabetes and these confirmed loci, with the possible exception of the *PPARG* rs1801282 SNP. As the controls represent the British population, we expect approximately 3.4% to have or to develop type 2 diabetes (http://www.diabetes.org.uk/Professionals/Information_resources/Reports/Diabetes-prevalence-2008/, accessed 22 April 2009). However, this only led to a very small loss of power (<1%). Indeed, at the *PPARG* SNP, we had 87% power to exclude an effect as small as an OR of 1.14 at an α level of 0.003 and a MAF of 0.12, while a recently published negative study analysing SNP rs2197423 in *PPARG* was powered to exclude effects with an OR of greater than 1.34 [36]. Hence, the present study was statistically well powered to detect effects as small as those reported for type 2 diabetes but was not powered to exclude effects with an OR of below 1.11 (Table 1).

The transcription factor PPARγ has been reported to have effects in type 2 diabetes, in the immune system, and in inflammation, as evidenced by its production in several types of immune cell. In macrophages, PPARγ regulates lipid metabolism and controls the inflammatory response, while in T cells it inhibits IL-2 secretion and can induce apoptosis. Type 2 diabetes treatments that act by binding to PPARγ, which belong to the thiazolidinedione class of drugs, have been investigated in the treatment of several autoimmune diseases. Human psoriasis cases showed reduced plaque formation when treated with pioglitazone [37]. PPARγ may have multiple targets in rheumatoid arthritis (RA), as it regulates the key RA mediators TNF-α, IL-1β and IL-6 [38]. Troglitazone reduced adjuvant-induced arthritis in the Lewis rat model [39], and rosiglitazone reduced colitis in a murine model of inflammatory bowel disease [38, 40]. More specifically, the larger of the two PPARγ isoforms (γ2) is the only one to express the region of the gene containing the rs1801282/Pro12Ala SNP. This isoform is predominantly expressed in fat cells. An increase in the production of the Ala12 variant of the γ2 isoform, which confers a 25% reduced risk of type 2 diabetes, is known to improve insulin sensitivity [41]. Improved insulin sensitivity could provide some protection against type 1 diabetes.

Wenzlau et al. [33] found that *SLC30A8*, which encodes the ZnT8 autoantigen, is highly expressed in the pancreas. They also reported that antibodies to ZnT8 are detectable at a very young age in type 1 diabetic patients, frequently before 3 years [33]. Wenzlau et al. [32] later identified rs13266634 as the SNP responsible for the type 1 diabetes autoimmune response to ZnT8 [32]. In contrast to the findings of Wenzlau et al. [32, 33] and Gohlke et al. [34], our results suggest that altering the specificity of the ZnT8 autoantibody via *SLC30A8* SNP rs13266634 does not alter the risk of type 1 diabetes, even in individuals diagnosed with type 1 diabetes at a very young age (0–4 years) (ESM Table 6), or by HLA class II genotype (ESM Table 5).

Whilst we have investigated SNPs in 12 of the type 2 diabetes-associated gene regions for association with type 1 diabetes, it is possible that these regions also contain type 1 diabetes causal variants that do not affect type 2 diabetes. However, this is not evident in the results from the meta-analysis of up to 3,565 type 1 diabetes cases and 4,657 controls [35]. Equally, as these SNPs explain less than 10% of genetic variation [42] in type 2 diabetes risk, there may be undiscovered type 2 diabetes loci associated with type 1 diabetes. Finally, as all our type 1 diabetic patients were paediatric cases, we cannot rule out the possibility that type 2 diabetes genes affect adult-onset type 1 diabetes. One recent report suggested that *TCF7L2* may be associated with latent autoimmune diabetes in adults, a form of type 1 diabetes that is diagnosed in individuals aged >35 years [2]. This result, however, may have been due to a small number of type 2 diabetes cases included in the study [43]. Our results, and those of a recent study of type 1 diabetes loci in type 2 diabetes cases and controls [44], nevertheless indicate that type 2 diabetes susceptibility loci do not make a substantial contribution to the risk of paediatric type 1 diabetes.

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