The candidate genes TAF5L, TCF7, PDCD1, IL6 and ICAM1 cannot be excluded from having effects in type 1 diabetes

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Abstract

Background: As genes associated with immune-mediated diseases have an increased prior probability of being associated with other immune-mediated diseases, we tested three such genes, IL23R, IRF5 and CD40, for an association with type 1 diabetes. In addition, we tested seven genes, TAF5L, PDCD1, TCF7, IL12B, IL6, ICAM1 and TBX21, with published marginal or inconsistent evidence of an association with type 1 diabetes.

Methods: We genotyped reported polymorphisms of the ten genes, nonsynonymous SNPs (nsSNPs) and, for the IL12B and IL6 regions, tag SNPs in up to 7,888 case, 8,858 control and 3,142 parent-child trio samples. In addition, we analysed data from the Wellcome Trust Case Control Consortium genome-wide association study to determine whether there was any further evidence of an association in each gene region.

Results: We found some evidence of associations between type 1 diabetes and TAF5L, PDCD1, TCF7 and IL6 (ORs = 1.05 – 1.13; P = 0.0291 – 4.16 × 10^-4). No evidence of an association was obtained for IL12B, IRF5, IL23R, ICAM1, TBX21 and CD40, although there was some evidence of an association (OR = 1.10; P = 0.0257) from the genome-wide association study for the ICAM1 region.

Conclusion: We failed to exclude the possibility of some effect in type 1 diabetes for TAF5L, PDCD1, TCF7, IL6 and ICAM1. Additional studies, of these and other candidate genes, employing much larger sample sizes and analysis of additional polymorphisms in each gene and its flanking region will be required to ascertain their contributions to type 1 diabetes susceptibility.
Background

Type 1 diabetes is a chronic autoimmune disease with a complex pathogenesis involving multiple genetic and environmental factors. Before the advent of genome-wide association (GWA) studies, disease loci were primarily sought through the testing of candidate genes, selected based usually upon limited prior information about the function of the gene and the pathogenic mechanisms of disease. The candidate gene approach has been successful in finding disease loci, but as only relatively small numbers of genes have been studied, few true positive associations have been found, despite numerous studies and enormous effort [1]. Only five type 1 diabetes loci with compelling evidence had been identified before the advent of GWA studies: the HLA class II genes on chromosome 6p21 [2]; the insulin gene (INS) on 11p15 [3]; CTLA4 on 2q33 [4]; PTPN22 on 1q13 [5,6]; and, IL2RA/CD25 on 10p15 [7,8]. Another five type 1 diabetes loci with convincing evidence have so far been identified by GWA studies in chromosome regions 2q24.3 [9], 12q24, 12q13, 16p13 and 18p11 [10,11].

Previously, we noted that, with the exception of INS [12], the type 1 diabetes loci contain polymorphisms that have been associated with susceptibility to other immune-mediated diseases, such as Graves’ disease (GD) and systemic lupus erythematosus (SLE), suggesting the existence of shared disease loci [13]. In this study, we tested three genes, namely, IL23R, IRF5 and CD40, which have been associated with other immune-mediated diseases (Table 1), including Crohn’s disease (CD), psoriasis, SLE and GD, for an association with type 1 diabetes. In addition, we tested seven genes, namely, ICAM1, TAF5L, PDCD1, TCF7, IL12B, IL6 and TBX21, with marginal or inconsistent evidence of an association with type 1 diabetes (Table 1). PDCD1 has also been associated with SLE and Rheumatoid Arthritis (RA). We genotyped reported polymorphisms, nonsynonymous SNPs (nsSNPs) and tag SNPs for the IL12B and IL6 regions in up to 7,888 case, 8,858 control and 3,142 parent-child trio samples. In addition, we used Wellcome Trust Case Control Consortium (WTCCC) [10] GWA study data to determine whether there was any further evidence of an association with type 1 diabetes in the linkage disequilibrium (LD) blocks containing the reported polymorphisms of interest.

Methods

Subjects

Type 1 diabetes families were white European or of white European descent, with two parents and at least one affected child comprising DNA samples from up to 918 Finnish multiplex/simplex families [14], 456 multiplex Diabetes UK Warren 1 families [15], 278 multiplex Human Biological Data Interchange (HBDI) families [16], 80 Yorkshire simplex families, 263 Belfast multiplex/simplex [17], 360 Norwegian simplex families [18] and 240 Romanian simplex families [19]. The single SNPs from TA5FL and IL12B, two SNPs from PDCD1 and five SNPs from IL6 were genotyped in all the families. The TCF7 SNP was genotyped in Warren, Yorkshire, Belfast and Romanian families. The TBX21 SNP was genotyped in Warren, Yorkshire, HBDI, Belfast and Romanian families. The CD40 SNP was genotyped in Warren and HBDI families.

The type 1 diabetes cases (maximum 7,888) [20], the British 1958 Birth Cohort (maximum 8,858) [21] and the UK Blood Services controls (1,500) have been described previously [6,10]. All cases and controls are white European. All DNA samples were collected after approval from the relevant research ethics committees and written informed consent was obtained from the participants.

Additional file 1, Table S1 contains a summary of the samples genotyped for each gene.

SNP identification and genotyping

IL12B is among the genes re-sequenced by the University of Washington: the Fred Hutchinson Cancer Research Centre (UW-FHCRC) Variation Discovery Resource [22]. The IL12B SeattleSNPs encompass the introns and exons between 1.7 kb 5’ of exon 2 to 2 kb 3’ of the untranslated exon 8 of IL12B. In this region, they detected 33 polymorphisms in their set of 23 DNA samples from Centre d’Etude du Polymorphisme Humain (CEPH) parents of European descent. As the untranslated exon 1 and 5’ region beyond it were not sequenced, we re-sequenced, a further 2.9 kb 5’, including exon 1 and the known promoter in the same 23 CEPH parents used by SeattleSNPs. This identified a further four polymorphisms including the CTCTAA/CG complex promoter deletion insertion (DIP) (rs17860508), incorrectly described previously as a 4 bp deletion [23].

IL6 was also resequenced by the SeattleSNP Project.

SNPs were genotyped using either Taqman MGB chemistry (Applied Biosystems) or Invader Biplex Assay (Third Wave Technologies, Madison). The D5S2941 microsatellite was genotyped using PCR and evaluated size differences using an ABI 3700 capillary sequencer.

Wellcome Trust Case Control Consortium

We used data from the WTCCC GWA study [10] to determine whether there was any evidence of an association with type 1 diabetes in the LD blocks containing the polymorphisms of interest. LD blocks were defined using Haploview [24] and HapMap Project [25] data for the 60 Centre d’Etude du Polymorphisme Humain (CEPH) par-
| Gene and polymorphism | Candidacy | Previous disease associations |
|-----------------------|-----------|-----------------------------|
| **TAF5L on 1q42** C744A (rs3753886) | TAF5L encodes a protein that is a component of the PCAF histone acetylase complex. It may participate in basal transcription, serve as a coactivator, function in promoter recognition or modify general transcription factors to facilitate complex assembly and transcription initiation. | **T1D:** *Chistiakov et al* [35] 247 Russian cases and 258 controls, derived OR for C allele = 0.69 (0.52–0.92), *P* = 0.013. |
| **PDCD1 on 2q37** 7146G>A (rs11568821) 872C>T (rs2227981) | Belongs to the B7-CD28 superfamily. These molecules play a critical role in the development of the immune response by controlling T cell numbers, through a fine balance of stimulation and negative regulation, which is essential for the prevention of autoimmunity. | **SLE, RA and T1D:** *Prokunina et al* [58] SLE: 7146G>A: 443 families and 520 cases of European, Mexican and African-American decent, RR for A allele = 2.6 (1.6–4.4), *P* = 1.0 × 10⁻⁷ for Europeans and RR for A allele = 1.18 (0.99–1.41), *P* = 0.05. *Lin et al* [59] 872C>T: 98 SLE cases and 100 controls, 84 RA cases and 135 controls, OR for T allele = 3.32 (1.78–6.21), *P* < 0.0001, no association in SLE. *Nielsen et al* [36] T1D: 7146G>A: 192 Danish cases and 155 controls, OR for A allele = 1.92 (1.1–3.3), *P* = 0.02. |
| **TCF7 on 5q31** Pro19Thr (rs5742913) | Role as a transcription factor in regulating key immune response elements and its involvement in T-cell development in the thymus. | **T1D:** *Noble et al* [38] 282 USA families, RR = 1.21, *P*<sub>TDT</sub> = 0.12. A allele transmitted from fathers, RR = 1.79, *P*<sub>ESP</sub> = 0.03. To male offspring, RR = 0.91 (0.67–1.24), *P*<sub>ESP</sub> = 0.04. To low HLA risk (non DR3/DR4) offspring, RR = 1.35, *P*<sub>TDT</sub> = 0.007, *P*<sub>ESP</sub> = 0.04. Early onset offspring, *P*<sub>ESP</sub> = 0.09. |
| **IL12B on 5q33.3** A1159C (rs3212227) Microsatellite D5S2941 | This gene, encoding the p40 subunit of IL-12, drives the differentiation of T lymphocytes into the Th1 subset, which is characterized by the production of cytokines that promote cell-mediated immunity. | **T1D:** *Morahan et al* [49] 249 Eastern Australian multiplex families, RR for A allele = 1.40 (1.11–1.77), *P* = 0.0025. 235 Australian simplex families, RR for A allele = 1.84 (1.32–2.56), *P* = 0.00014. *Davoodi-Sermiromi et al* [41] 364 USA multiplex families, RR for C allele = 1.23 (0.97–1.56), *P* = 0.08. haplotype: allele 2 (D5S2941) and 1159C, *P* = 0.02. *Windsor et al* [40] 648 cases and 246 controls – Western Australia, OR allele C = 1.6 (1.2–2.0), *P* = 0.001. Excess of heterozygotes in cases ≥ 16 yrs diagnosis, OR = 1.8(1.2–2.7), *P* = 0.005 and ≥ 26 yrs diagnosis, OR = 2.4 (1.5–3.8), *P* = 0.0002. Five studies failed to find an association: *Dahlman et al* [60] 2,873 families from UK, USA, Finland, Romania *McCormack et al* [61] 120 cases, 330 controls and 307 families from Northern Ireland *Nistico et al* [62] 470 cases and 544 controls from Italy. *Bergholdt et al* [63] 337 Danish simplex and 795 European and American multiplex families. *Johansson et al* [64] from Norway. |
| **IL6 on 7p21** 174G>C (rs1800795) | IL6 may contribute to the gradual destruction of the pancreatic beta cells, due to its regulatory role in inflammation and the immune response. | **T1D:** *Kristiansen et al* [43] 253 Danish families, RR allele C = 1.26 (1.01–1.57), *P*<sub>TDT</sub> = 0.04. Parent-female-trios: RR for C allele = 1.72 (1.25–2.36), *P* = 6.5 × 10⁻⁴. Parent-male-trios: RR for C allele = 0.91 (0.67–1.24), *P* = 0.58. Age-onset males = 14.04 yrs (11.62–16.46) and females = 8.81 yrs (6.60–11.01), interaction model *P* = 0.002. *Gillespie et al* [44] UK 1,230 cases. IL6-174CC less frequent in females diagnosed after, than in those diagnosed before, 10 years, *P* = 0.016. *Jahromi et al* [65] 257 cases and 120 newborn controls, UK, OR for C allele = 0.54 (0.39–0.74), derived *P* = 7.8 × 10⁻⁵. 53 parent-child trios RR for C allele = 0.83 (0.48–1.43). *Eerligh et al* [66] 206 Dutch simplex families, no association. |
| Gene | Chromosome | SNP | Description | T1D | SLE | IBD and psoriasis |
|------|------------|-----|-------------|-----|-----|-------------------|
| ICAM1 | 19p13 | G241R (rs1799969) | ICAM-1, upregulated during inflammation and hyperglycemia, is involved in the attachment of lymphocytes to the endothelium as well as in the priming of effector T cells. | Kristiansen et al. [45] 253 Danish families, no association with K469E. Nejentsev et al [67] 3,695 families from Finland, UK, USA, Norway & Romania, RR = 0.91 (0.83–0.99) P = 0.030. 446 families from Bart’s-Oxford study RR = 0.60 (0.40–0.89) P = 0.006. Ma et al. [68] 432 cases and 187 controls from Sweden. Tested five SNPs, rs281432 and rs5498 had P-values of 0.026 and <0.001 respectively when genotype model assumed. |  |  |
| TBX21 | 17q21 | His33Gln (rs2240017) | TBX21 has a role in the complex regulation of T lymphocyte responses, as a master-regulator of Th1 cytokine IFN-γ gene expression. |  |  |  |
| IL23R | 1p31 | Arg381Gln (rs11209026) | The protein encoded pairs with the receptor molecule IL12RB1/IL12Rbeta1, and both are required for IL23A signaling. This protein associates constitutively with Janus kinase 2 (JAK2), and also binds to transcription activator STAT3 in a ligand-dependent manner. |  |  | Duerr et al [69] CD: 547 non-Jewish cases and 548 controls, OR = 0.26 (0.15–0.43), P = 5.05 × 10⁻⁴. 401 Jewish cases and 433 controls, OR = 0.45 (0.27–0.73), P = 7.95 × 10⁻⁴. 883 IBD families, P = 1.32 × 10⁻¹⁰. Cargill et al [54] Psoriasis: White North American 1446 cases and 1432 controls, OR = 0.63 (0.50–0.79), P = 1.89 × 10⁻⁴. |  |
| IPEX | 7q32.1 | -3835 (rs2004640) | Member of a family of transcriptional factors that controls inflammatory and immune responses. | Sigurdsson et al [70], 679 cases and 438 controls from Sweden, Finland and Iceland, P = 2.4 × 10⁻⁷. Graham et al [71] 1,661 cases and 2,508 controls from USA, Spain, Sweden and Argentina, OR = 1.47 (1.36–1.60), P = 4.2 × 10⁻²¹. |  |  |
| CD40 | 20q12 | Kozak (rs1883832) | CD40 and CD40L signalling is known to play an important role in the immune response. The proteins are expressed in a variety of cell types and ligation causes cells to produce inflammatory cytokines and cellular adhesion molecules. | Tomer et al [48] 154 cases and 118 controls – Caucasian, OR for CC genotype = 1.6, P = 0.048. Kim et al [73] 132 cases and 164 controls from Korea, OR for CC genotype = 1.93 (1.21–3.09), P = 0.019. Mukai et al [74] 324 cases and 229 controls from Japan: late onset GD, decrease in TT allele (P = 0.011). Two studies failed to find an association: Houston et al [75] 451 cases and 446 controls from UK. Heward et al [76] 780 cases and 785 controls from UK. |  |  |

T1D = type 1 diabetes, SLE = systemic lupus erythematosus, RA = Rheumatoid Arthritis, IBD = Inflammatory Bowel disease, CD = Crohn’s disease and GD = Graves’ disease.
ents. We used the four gamete rule [26] for defining LD blocks within Haploview. We note that the 2,000 case and 3,000 (1,500 from the British 1958 Birth Cohort and 1,500 from the UK Blood Services) control samples used by the WTCCC, where also genotyped in this study. We required WTCCC SNPs to have a minor allele frequency (MAF) ≥ 0.05, a call rate ≥ 0.99 and no extreme deviation from Hardy-Weinberg equilibrium (HWE) ($\chi^2 \leq 25$) [27].

**Statistical Analysis**

All statistical analyses were performed in either Stata [28] or R [29] statistical systems. Additional routines may be downloaded [30].

All unaffected parent and control genotypes were assessed for, and found to be in Hardy-Weinberg equilibrium ($P > 0.05$). SNPs genotyped in the family collection were analysed using the transmission/disease-equilibrium test [31] and, after estimating pseudo-controls [32], conditional logistic regression, respectively modelling allelic relative risks (RRs; a one-degree-of-freedom (df) test) and genotype RRs (a two-df test). In the case and pseudo-control analysis, we consider the transmitted pair of alleles as the ‘case’ and the other three possible pairs of transmitted alleles as “pseudo-controls” in a matched case-control study [32]. The one-df test assumes multiplicative allelic effects and the two-df test assumes no specific mode of inheritance, for example, in the analysis of TCF7 C883A, genotype risks of C/A and A/A were modelled relative to the C/C genotype.

In the case-control collection, we performed similar tests using logistic regression models, stratified by 12 broad geographical regions (Southwestern; Southern; Southeastern; London; Eastern; Wales; Midlands; North Midlands; Northwestern; East and West Riding; Northern; and, Scotland), to allow for geographic variation in allele frequencies across Great Britain [27].

The tag SNPs were selected and analysed using a multilocus test, as previously described [7,33]. Qu et al. [8] recently reported that the family-based multilocus test was not confined to heterozygous parents, which compromised the protection against population stratification. This is incorrect, as described in the studies by Chapman et al. [33] and Lowe et al. [34], only transmissions from heterozygous parents contribute to the test.

**Results**

**TAF5L**

We genotyped one TAF5L SNP (C744A; rs3753886), which has previously been associated with type 1 diabetes [35] (Table 1). We found inconsistent evidence of an association between type 1 diabetes and C744A in the case-control and family collections. In 7,497 case and 7,496 control genotypes, we obtained marginal evidence of an association ($P = 7.32 \times 10^{-3}$; OR for allele A = 1.07, 95% CI 1.02–1.12; Table 2). Although, in 2,645 parent-child trio genotypes, there was borderline evidence of an association ($P = 0.0519$), the risk for the minor allele (RR for allele A = 0.92, 95% CI 0.85–0.99; Table 3) was going in the opposite direction to that in the case-control collection.

We found borderline evidence of an association in the WTCCC, which had two SNPs with a MAF ≥ 0.05 in the 5 kb LD block containing C744A. The lowest $P$-value was for C744A ($P = 0.0561$).

**PDCD1**

We genotyped two PDCD1 SNPs (7146G>A and 872C>T), which have previously been associated with type 1 diabetes [36] and RA respectively [37] (Table 1). We found inconsistent evidence of an association between type 1 diabetes and 7146G>A in the case-control and family collections (Tables 2 and 3). In 7,888 case and 8,858 control genotypes, we obtained limited evidence of an association ($P = 0.0102$; OR for allele A = 1.10, 95% CI = 1.02–1.17). In 3,125 parent-child trio genotypes, no evidence of an association was found ($P = 0.498$). For the second SNP, 872C>T, no evidence of an association was found in either collection (Tables 2 and 3).

There were no WTCCC SNPs with a MAF ≥ 0.05 in the 12 kb LD block containing 7146G>A. We note that 7146G>A was not included in either HapMap or the WTCCC study.

**TCF7**

We genotyped one TCF7 SNP (C883A; rs5742913), which had previously been associated with type 1 diabetes [38] (Table 1). Despite obtaining no evidence of an overall association, Noble et al. [38] proceeded to analyse subgroups, defined by up to three criteria, in which they found an excess of the A allele transmitted: from fathers; to male offspring; to low HLA risk (non-DR3/DR4) offspring; and, to early onset offspring (Table 1). We found some evidence of an association between type 1 diabetes and C883A in the case-control and family collections (Tables 2 and 3). In 7,434 case and 8,637 control genotypes, we obtained marginal evidence of an association ($P = 0.0561$). For the second SNP (872C>T), no evidence of an association was found in either collection (Tables 2 and 3).

We obtained limited evidence of an association ($P = 4.16 \times 10^{-4}$; OR for allele A = 1.13, 95% CI = 1.06–1.22). In 1,556 parent-child trio genotypes, no evidence of an association was found ($P = 0.608$). In addition, we found no evidence of an association between type 1 diabetes and C883A when comparable subgroup analyses, as described by Noble et al. [38], were performed (see Additional file 1, Table S2). We also performed a case-only regression analysis of cases and affected offspring from the case-control and family collections respectively, which showed no con-
Table 2: Association analyses in type 1 diabetes cases and controls.

| Alleles | Genotypes |
|---------|-----------|
| TAF5L   | C744A (rs3753886) |
|         | C | A | CC | CA | AA |
| 7,497 cases | 7,457 (49.7) | 7,537 (50.3) | 1,827 (24.4) | 3,803 (50.7) | 1,867 (24.9) |
| 7,496 controls | 7,671 (51.2) | 7,321 (48.8) | 1,928 (25.7) | 3,815 (50.9) | 1,753 (23.4) |
| OR (95% CI) | 1.00 (reference) | 1.07 (1.02–1.12) | 1.00 (reference) | 1.05 (0.97–1.14) | 1.14 (1.04–1.25) |
| P        | 7.32 × 10⁻³ | 0.0244 |

| PDCD1   | 7146G>A (rs11568821) |
|---------|---------------------|
|         | G | A | GG | GA | AA |
| 7,888 cases | 13,966 (88.3) | 1,810 (11.7) | 6,196 (78.6) | 1,574 (20.0) | 118 (1.5) |
| 8,858 controls | 15,846 (88.5) | 1,870 (11.5) | 7,088 (80.0) | 1,670 (18.9) | 100 (1.1) |
| OR (95% CI) | 1.00 (reference) | 1.10 (1.02–1.17) | 1.00 (reference) | 1.08 (1.00–1.17) | 1.32 (1.00–1.74) |
| P        | 0.0102 | 0.0268 |

| PDCD1   | 872C>T (rs2227981) |
|---------|---------------------|
|         | C | T | CC | CT | TT |
| 5,758 cases | 6,699 (58.2) | 4,817 (41.8) | 1,956 (34.0) | 2,787 (48.4) | 1,015 (17.6) |
| 7,289 controls | 8,440 (57.9) | 6,138 (42.1) | 2,461 (33.8) | 3,518 (48.3) | 1,310 (18.0) |
| OR (95% CI) | 1.00 (reference) | 1.00 (0.95–1.06) | 1.00 (reference) | 1.00 (0.92–1.08) | 0.97 (0.87–1.07) |
| P        | 0.586 | 0.760 |

| TCF7    | Pro19Thr (rs5742913) |
|---------|---------------------|
|         | C | A | CC | CA | AA |
| 7,434 cases | 13,022 (87.6) | 1,846 (12.4) | 5,709 (76.8) | 1,604 (21.6) | 121 (1.6) |
| 8,637 controls | 15,355 (88.9) | 1,919 (11.1) | 6,826 (79.0) | 1,703 (19.7) | 108 (1.3) |
| OR (95% CI) | 1.00 (reference) | 1.13 (1.06–1.22) | 1.00 (reference) | 1.13 (1.04–1.22) | 1.32 (1.01–1.73) |
| P        | 0.134 | 1.91 × 10⁻³ |

| IL12B   | A1159C (rs3212227) |
|---------|---------------------|
|         | A | C | AA | AC | CC |
| 4,321 cases | 6,898 (79.8) | 1,744 (20.2) | 2,748 (63.6) | 1,402 (32.5) | 171 (4.0) |
| 4,711 controls | 7,588 (80.5) | 1,834 (19.5) | 3,050 (64.7) | 1,488 (31.6) | 173 (3.7) |
| OR (95% CI) | 1.00 (reference) | 1.06 (0.98–1.14) | 1.00 (reference) | 1.06 (0.96–1.16) | 1.13 (0.90–1.42) |
| P        | 0.134 | 0.324 |

| IL6     | 174G>C (rs1800795) |
|---------|---------------------|
|         | G | C | GG | GC | CC |
| 7,785 cases | 8,726 (56.0) | 2,928 (33.1) | 4,312 (48.7) | 1,612 (18.2) |
| 8,852 controls | 10,168 (57.4) | 2,456 (31.6) | 3,814 (49.0) | 1,515 (19.5) |
| OR (95% CI) | 1.00 (reference) | 1.05 (1.01–1.10) | 1.00 (reference) | 1.04 (0.97–1.12) | 1.11 (1.01–1.21) |
| P        | 0.0291 | 0.0879 |
Table 2: Association analyses in type 1 diabetes cases and controls. (Continued)

| Gene       | SNP          | G  | A  | GG | GA  | AA |
|------------|--------------|----|----|----|-----|----|
| ICAM1      | G241R (rs1799969) | 5,776 cases | 10,257 (88.8) | 1,295 (11.2) | 4,553 (78.8) | 1,151 (19.9) | 72 (1.3) |
|            |              | 6,094 controls | 10,787 (88.5) | 1,401 (11.5) | 4,787 (78.6) | 1,213 (19.9) | 94 (1.54) |
|            |              | OR (95% CI) | 1.00 (reference) | 0.96 (0.89–1.05) | 1.00 (reference) | 1.00 (0.91–1.10) | 0.74 (0.54–1.02) |
|            |              | 6,094 controls | 10,787 (88.5) | 1,401 (11.5) | 4,787 (78.6) | 1,213 (19.9) | 94 (1.54) |
|            |              | OR (95% CI) | 1.00 (reference) | 0.96 (0.89–1.05) | 1.00 (reference) | 1.00 (0.91–1.10) | 0.74 (0.54–1.02) |
| TBX21      | His33Gln (rs2240017) | 4,342 cases | 8,456 (97.4) | 228 (2.6) | 4,116 (94.8) | 224 (5.2) | 2 (0.1) |
|            |              | 4,763 controls | 9,293 (97.6) | 233 (2.4) | 4,533 (95.2) | 227 (4.7) | 3 (0.1) |
|            |              | OR (95% CI) | 1.00 (reference) | 1.08 (0.89–1.30) | 1.00 (reference) | 1.08 (0.88–1.31) | 1.10 (0.16–7.54) |
| IL23R      | Arg381Gln (rs11209026) | 6,087 cases | 11,413 (93.7) | 761 (6.3) | 5,343 (87.8) | 727 (11.9) | 17 (0.3) |
|            |              | 6,303 controls | 11,776 (93.4) | 830 (6.6) | 5,499 (87.2) | 778 (12.3) | 26 (0.4) |
|            |              | OR (95% CI) | 1.00 (reference) | 0.93 (0.84–1.03) | 1.00 (reference) | 0.95 (0.85–1.06) | 0.67 (0.36–1.26) |
| IRF5       | -3835 (rs2004640) | 5,657 cases | 5,834 (51.6) | 5,480 (48.4) | 1,487 (26.3) | 2,860 (50.6) | 1,310 (23.2) |
|            |              | 6,044 controls | 6,160 (51.0) | 5,928 (49.0) | 1,581 (26.2) | 2,998 (49.6) | 1,465 (24.2) |
|            |              | OR (95% CI) | 1.00 (reference) | 0.98 (0.93–1.03) | 1.00 (reference) | 1.03 (0.94–1.12) | 0.96 (0.86–1.06) |
| CD40       | Kozak (rs1883832) | 4,392 cases | 6,624 (75.4) | 2,160 (24.6) | 2,500 (56.9) | 1,624 (37.0) | 268 (6.1) |
|            |              | 4,702 controls | 7,132 (75.8) | 2,272 (24.2) | 2,680 (57.0) | 1,772 (37.7) | 250 (5.3) |
|            |              | OR (95% CI) | 1.00 (reference) | 1.03 (0.96–1.10) | 1.00 (reference) | 0.97 (0.89–1.07) | 1.18 (0.97–1.42) |

OR = odds ratio for the minor allele, 95% CI = 95% confidence interval.
sistent evidence of an association in the WTCCC, which had two SNPs with a MAF ≥ 0.05 in the 66 kb LD block containing C883A. The SNP with the lowest P-value was rs756699 (P = 0.694). We note that C883A was not included in either HapMap or the WTCCC study.

**IL12B**

IL12B has been reported to be associated with type 1 diabetes in some but not all studies (Table 1). Therefore, we investigated the contribution of IL12B to type 1 diabetes susceptibility, as thoroughly as possible, by genotyping a panel of tag SNPs due to its association with type 1 diabetes in some but not all studies (Table 1). We detected an additional two, extremely rare, alleles: allele 3 (ten repeat units) and allele 4 (seven repeat units), both found at frequency of <0.001. In 1,327 case and 1,160 control genotypes, we obtained no evidence of an association between type 1 diabetes and D5S2941 allele 2 (P = 0.114). Davoodi-Semiromi et al. also reported an association between type 1 diabetes and the D5S2941 allele 2-1159C haplotype (P = 0.02) and suggested the possibility that the causal variant remained ungenotyped and elsewhere in IL12B [41]. Consequently, we also analysed this haplotype using an EM algorithm-based routine to assign haplotypes to cases and controls, which were then analysed using a linear model weighted by the posterior haplotype probabilities for each case or control. Again we found no association with disease for this haplotype in 1,298 case and 1,111 control genotypes (P = 0.195).

To investigate the possibility of a polymorphism associated with type 1 diabetes in IL12B that we, or others, have not yet genotyped, we adopted an linkage disequilibrium (LD) mapping approach using tag SNPs (Table 4). We combined resequencing data in 23 CEHPI parents from the SeattleSNP project [22] with inhouse resequencing of the untranslated exon 1 and 3, not resequenced in the SeattleSNP project (Methods). In the combined resequencing data, we identified 38 polymorphisms, comprising 34 SNPs (four SNPs provided by the in house resequencing), three deletion-insertion polymorphisms (DIPs) and the microsatellite DSS2941 (see Additional file 1, Table S5). Six tag SNPs were selected (minimum $R^2 = 0.80$) from 25 SNPs with a minor allele frequency (MAF) ≥ 0.10. The set of tag SNPs was genotyped in the case-control collection and analysed using a multilocus test, which tests for an association between type 1 diabetes and the tag SNPs due

### Table 3: Association analyses in type 1 diabetes families.

| Transmission/disequilibrium test | Parental MAF | Minor allele | RR (95% CI) | P |
|----------------------------------|-------------|-------------|-------------|---|
| Transmitted | Untransmitted |
| TAF5L A1159C (rs3212227) | 2.645 | 46.8 | 1,299 | 1,400 | 0.92 | 0.85–0.99 | 0.0519 |
| PDCD1 7146G>A (rs11568821) | 3.125 | 9.3 | 516 | 538 | 0.96 | 0.85–1.08 | 0.498 |
| PDCD1 872C>T (rs2227981) | 2.190 | 42.5 | 1,111 | 1,043 | 1.07 | 0.98–1.16 | 0.143 |
| TCF7 C883A (rs5742913) | 1,556 | 11.2 | 327 | 314 | 1.04 | 0.89–1.21 | 0.608 |
| IL12B A1159C (rs3212227) | 3,015 | 18.9 | 949 | 933 | 1.02 | 0.93–1.12 | 0.712 |
| IL6 174G>C (rs1800795) | 2,803 | 47.0 | 1,411 | 1,360 | 1.04 | 0.97–1.12 | 0.333 |
| TBX21 His33Gln (rs2240017) | 1,989 | 2.7 | 111 | 100 | 1.11 | 0.85–1.45 | 0.449 |
| CD40 174G>C (rs1800795) | 2,803 | 47.0 | 1,411 | 1,360 | 1.04 | 0.97–1.12 | 0.333 |

MAF = minor allele frequency, RR = relative risk for minor allele, 95% CI = 95% confidence interval.
We note that A1159C was not included in the WTCCC; we can conclude that this association is a false positive. Based on the perfect LD between rs6859018 and A1159C, 1) with A1159C in 60 CEPH parents. Consequently, 1) with A1159C in 60 CEPH parents. Consequently, 1) with A1159C in 60 CEPH parents. Consequently, 1) with A1159C in 60 CEPH parents. Consequently, 1) with A1159C in 60 CEPH parents.

As 

IL6

has been reported to be associated with type 1 diabetes [43] (Table 1), we sought to replicate this association by genotyping IL6-174G>C (rs1800795) and a set of tag SNPs for the IL6 region. We found inconsistent evidence of an association with type 1 diabetes and IL6-174G>C in the case-control and family collections (Tables 2 and 3). In 7,785 case and 8,852 control genotypes, we obtained limited evidence of an association with type 1 diabetes ($P = 0.0291$; OR for allele $C = 1.05$, 95% CI = 1.01–1.10). In 2,803 parent-child trio genotypes, no evidence of an association was found ($P = 0.333$). As Kristiansen et al. [43] found evidence that the IL6-174C allele was only associated with type 1 diabetes in female offspring (Table 1), we analysed IL6-174G>C by sex. In the cases and controls, we obtained limited evidence of an association in males ($P = 0.0378$), but not in females (see Additional file 1, Table S6a) and in the families, we found no evidence of an association in either male or female type 1 diabetes offspring (see Additional file 1, Table S6b).

Previously, Gillespie et al. [44] found frequency differences in IL6-174G>C genotypes between males and females diagnosed ages > 10 years. Consequently, we conducted a similar case-only analysis using a multinomial logistic regression model to adjust for broad geographical region within Great Britain and for population of the cases and affected offspring, respectively. We found no evidence of genotype differences between 5,700 male and 5,292 female cases and affected offspring ($P = 0.370$) and when analysed by age-at-diagnosis (see Additional file 1, Table S7).

To test for an association between type 1 diabetes and the IL6 region, we adopted a LD mapping approach. We used SeattleSNP resequencing data in 23 CEPH parents, four tag SNPs were selected (minimum $R^2 = 0.80$) from twelve SNPs with a MAF ≥ 0.10 (see Additional file 1, Table S8). We found no evidence for association in the case-control collection (multilocus test $P = 0.236$). However, in the family collection, limited evidence of an association was found (multilocus test $P = 0.0231$) (Table 4).

We found no evidence of an association in the WTCCC, which had six SNPs with a MAF ≥ 0.05 in the 14 kb LD block containing A1159C. The only associated SNP was rs6859018 ($P = 0.0274$), which is in perfect LD ($r^2 = 1$) with A1159C in 60 CEPH parents. Consequently, based on the perfect LD between rs6859018 and A1159C, we can conclude that this association is a false positive. We note that A1159C was not included in the WTCCC study.

**Table 4: Tag SNP results for IL6 and IL12B regions.**

| Exons | Sequencing source                  | Polymorphisms | Tag SNPs | Families | Cases and controls |
|-------|-----------------------------------|---------------|----------|----------|-------------------|
| IL12B | 8                                 | 33 (from Seattle) | 6        | N/A      | 0.940             |
|       |                                   | 25 had MAF ≥ 0.10 |          |          | 1,590 cases and 1,748 controls |
| IL6   | 6                                 | 49             | 4        | 0.0231   | 3,320 parent-child trios |
|       |                                   | 12 had MAF ≥ 0.10 |          |          | 3,486 cases and 3,783 controls |

We selected tag SNPs from SNPs with a MAF ≥ 0.10. MAF = minor allele frequency.

to LD with one or more causal variants [33]. The multilocus test $P$-value was 0.940, providing no evidence of an association between type 1 diabetes and the IL12B region (Table 4). We note that rs17860508, the promoter DIP CTCCTAA/CG, was selected as a tag SNP. Previously, this polymorphism had been associated with asthma susceptibility [23] and IgE levels [42], but we found no association with type 1 diabetes (4,367 case and 4,714 control genotypes, $P = 0.878$).

Finally, we tested for an association between type 1 diabetes and two rare nsSNPs (rs3213096 and rs3213119). Although the MAF was 0.022 for both nsSNPs in the SeattleSNPs CEPH sequencing panel, in the case-control collection, rs321096 had a much lower MAF of 0.0035 in controls and consequently, we had no power in a collection of 4,367 case and 4,714 control genotypes, $P = 0.0941$.

We found limited evidence of an association in the WTCCC, which had six SNPs with a MAF ≥ 0.05 in the 14 kb LD block containing A1159C. The only associated SNP was rs6859018 ($P = 0.0274$), which is in perfect LD ($r^2 = 1$) with A1159C in 60 CEPH parents. Consequently, based on the perfect LD between rs6859018 and A1159C, we can conclude that this association is a false positive. We note that A1159C was not included in the WTCCC study.

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| IL6   | 6                                 | 49             | 4        | 0.0231   | 3,320 parent-child trios |
|       |                                   | 12 had MAF ≥ 0.10 |          |          | 3,486 cases and 3,783 controls |

We selected tag SNPs from SNPs with a MAF ≥ 0.10. MAF = minor allele frequency.
ICAM1
We genotyped one ICAM1 SNP (G241R; rs1799969), which has previously been associated with type 1 diabetes [45] (Table 1). However, no evidence of an association was found in 5,776 cases and 6,094 controls (P = 0.382; Table 2).

We found some evidence of an association in the WTCCC, which had two SNPs with a MAF ≥ 0.05 in the 15 kb LD block containing G241R. The most associated SNP was rs892188 (P = 0.0257; OR for allele T = 1.10, 95% CI = 1.01–1.19), located just over 2 kb upstream of the 3' UTR of ICAM5, which has low LD (r² = 0.274) with G241R. We note that G241R was not included in the WTCCC study.

TBX21
We genotyped one TBX21 SNP (His33Gln; rs2240017), which had previously been associated with type 1 diabetes in a Japanese case and control collection [46] (Table 1). No evidence for association in either collection was found (Tables 2 and 3). We note that the G (Gln) allele frequency in controls was 0.024 (Table 2) and in parents 0.027, considerably lower than reported in Japanese by Sasaki et al. [46], but similar to that found in other European populations (MAF = 0.030) [47].

We found no evidence of an association in the WTCCC, which had one SNP with a MAF ≥ 0.05 in the 14 kb LD block containing His33Gln. The SNP, rs2240017, had a P-value of 0.131. We note that His33Gln was not included in the WTCCC study.

IL23R
We genotyped one IL23R SNP (Arg381Gln; rs11209026), which has previously been associated with IBD and psoriasis (Table 1). In 6,087 cases and 6,303 controls, we found no evidence of an association with type 1 diabetes (P = 0.183; Table 2).

The only WTCCC SNP with a MAF ≥ 0.05 in a 15 kb LD block containing Arg381Gln was Arg381Gln (P = 0.857).

IRF5 and CD40
We genotyped two SNPs from IRF5 and CD40 (-3835/rs2004640 and 168A>G/rs1883832, respectively), which have previously been associated with SLE and GD respectively (Table 1). The CD40 -168A>G SNP has been reported to disrupt the Kozak consensus sequence necessary for efficient translation [48]. No evidence of an association was found for either SNP (Tables 2 and 3).

We found no evidence of an association in the WTCCC, which had no SNPs with a MAF ≥ 0.05 in the 5 kb LD block containing -3835 and 168A>G was not contained within a block. Neither -3835 nor 168A>G were included in the WTCCC study.

Discussion
In this study, we have tested ten candidate genes for an association with type 1 diabetes using large case-control and family collections. We did obtain some evidence, albeit inconsistent between collections, of an association with TAF5L (C744A), PDCD1 (7146G>A), TCF7 (C883A) and IL6 (IL6-174G>C, rs2069849 and the IL6 region). Although TAF5L (C744A; rs3753886), PDCD1 (7146G>A; rs11568821), TCF7 (C883A; rs5742913) and IL6 (IL6-174G>C; rs1800795) have previously been associated with type 1 diabetes, the possibility remains that these associations are false positives. However, the findings reported here maybe the result of the case-control collection having more power than the family collection to detect SNPs with relatively small effects in type 1 diabetes or being in weak LD with the causal locus. Consequently, additional studies will be required to ascertain the contribution of TAF5L, PDCD1, TCF7 and IL6 to type 1 diabetes susceptibility. The case-control collection (8,000 cases and 8,000 controls) provided about 60% power to detect an OR of 1.2 for a MAF of 0.10 at a P-value of about 1 × 10⁻⁶; and about 96% power for a MAF of 0.20. The family collection (3,125 parent-child trios) provided less power to detect an OR of 1.2, after increasing the P-value to 1 × 10⁻³, there was about 45.1% power for a MAF of 0.10 and about 81.4% power for a MAF of 0.20.

We did not obtain any evidence of an association between type 1 diabetes and either ICAM1, IL12B or TBX21, all of which had previously been associated with type 1 diabetes [45,46,49]. However, limited evidence of an association with rs892188, located in the LD block containing the reported SNP in ICAM1, was provided by the WTCCC. Additional studies will be required to ascertain the contribution of rs892188 to type 1 diabetes susceptibility. The previous reports of disease associations may well have been false positives, which have to be expected given: the low prior probability, even for candidate genes [1,50], of detecting a true causal locus of complex disease; the frequent use of relatively small sample sizes and of nominal levels of significance; and, the large numbers of SNPs tested for an association with type 1 diabetes. It is interesting to note that when, by chance, a true positive result is found, as in the case for PTPN22 Arg620Trp SNP in type 1 diabetes [5], it is replicated by many groups, very rapidly [6,39,51,52], although this has a large effect approaching an OR = 2.

Finally, we did not obtain any evidence of an association between type 1 diabetes and IL23R, IRF5 or CD40, all of which have previously been associated with other immune-mediated diseases including, most recently,
**IL23R** in Crohn’s disease[10,53] and in psoriasis [54]. Lack of association of IL23R, IRF5 and CD40 with type 1 diabetes helps to delineate pathogenic mechanisms between type 1 diabetes and other immune-mediated diseases, especially when the associations reported for the other diseases are highly likely to be true positive results. Nevertheless, for these and other loci, it is possible that one or more of these genes could have allelic heterogeneity in which one allele predisposes to certain autoimmune diseases and a second allele at a different location in the gene predisposes to another. This possibility necessitates the continued investigation of further polymorphisms within each gene.

**Conclusion**
The functional candidate gene approach has now been superseded by GWA studies, which are detecting major susceptibility loci [10,11,53,55]. Most of the confirmed loci from GWA studies have ORs ≤ 1.3, consistent with an L-shaped distribution of allelic effect sizes (that is, a small number of genes with large effects and a large number of genes with small effects) [1,11]. TAF5L, PDCD1, TCF7, IL6 and ICAM1 may be amongst the numerous loci with small effects in type 1 diabetes. We note that genes found with small effects on disease may have much larger effects in subgroups of phenotypically defined cases. For example, CTLA4 genotypes has a small effect overall in type 1 diabetes (OR = 1.20, 95% CI 1.13–1.27), but subclassification of cases with or without the thyroid peroxidase autoantibodies revealed an increased effect (OR = 1.49, 95% CI 1.29–1.72) for cases with autoantibodies (without autoantibodies OR = 1.16, 95% CI 1.10–1.24) [56].

**Competing interests**
The author(s) declare that they have no competing interests.

**Authors’ contributions**
JDC contributed to data analysis and drafted the manuscript. DJS drafted the manuscript and worked on IL23R, IRF5 and CD40 studies. RB drafted the manuscript and worked on TCF7 and TBX21 studies. FP drafted the manuscript and worked on the IL12B study. KD worked on the TAF5L study. LMG drafted the manuscript and worked on the IL6 study. JM drafted the manuscript and worked on the PDCD1 study. LRZ worked on PDCD1, TCF7 and ICAM1 studies. AV drafted the manuscript and worked on the TCF7 study. NMW managed the data. JAT participated in the conception, design and coordination of the studies, data analysis and drafted the manuscript. All authors read and approved the final manuscript.

**Additional material**

**Additional file 1**
Supplementary Tables. Summary tables of additional analyses conducted. Click here for file [http://www.biomedcentral.com/content/supplementary/1471-2350-8-71-S1.doc]

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**References**
1. Wang WY, Barratt BJ, Clayton DG, Todd JA: Genome-wide association studies: theoretical and practical concerns. Nat Rev Genet 2005, 6:109-118.
2. Cucca F, Lampis R, Congia M, Angius E, Nutland S, Bain SC, Barnett AH, Todd JA: A correlation between the relative predisposition of MHC class II alleles to type 1 diabetes and the structure of their proteins. Hum Mol Genet 2001, 10:2025-2037.
3. Barratt BJ, Payne F, Lowe CE, Herrmann R, Healy BC, Harold D, Con-cannon P, Gharani N, McCarthy MI, Olavesen MG, McCormack R, Guja C, Ioncescu-Tirgoviste C, Undlien DE, Ronningen KS, Gillespie KM, Tuomilehto-Wolf E, Tuomilehto J, Bennett ST, Clayton DG, Cordell HJ, Todd JA: Remapping the insulin gene/IDDM2 locus in type 1 diabetes. Diabetes 2004, 53:1884-1889.
4. Ueda H, Howson JM, Esposito L, Heward H, Chamberlain G, Rainbow DB, Hunter KM, Smith AN, Di Genova G, Herr MH, Dahlman I, Payne F, Smyth D, Lowe C, Twells RC, Howlett S, Healy B, Nutland S, Rance HE, Everett V, Smink LJ, Lam AC, Cordell HJ, Walker NM, Bordin C, Helme J, Motzo C, Cucca F, Hess JF, Metzker ML, Rogers J, Gregory S, Allahabadia A, Nithyananthan R, Tuomilehto-Wolf E, Tuomilehto J, Bingley P, Gillespie KM, Undlien DE, Ronningen KS, Guja C, Iouncescu-Tirgoviste C, Savage DA, Maxwell AP, Carson DJ, Patterson CC, Franklyn JA, Clayton DG, Peterson LB, Wicker LS, Todd JA, Gough SC: Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature 2003, 423:506-511.
5. Bertini N, Musunucci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M, MacMurray J, Meloni GF, Lucarelli P, Pellecchia M, Eisenbarth GS, Comings D, Mustelin T: A functional variant of lymphoid tyrosine phosphatase is associated with type 1 diabetes. Nat Genet 2004, 36:337-338.
6. Smyth D, Cooper JD, Collins JE, Heward JM, Franklyn JA, Howson JM, Vella A, Nutland S, Rance HE, Maier L, Barratt BJ, Guja C, Ionescu-Tirgoviste C, Savage DA, Dunger DB, Widmer B, Strachan DP, Ring SM, Walker N, Clayton DG, Twells RC, Gough SC, Todd JA: Repli-
cation of an association between the lymphoid tyrosine phosphatase locus (LYP/PTPN12) with type 1 diabetes, and evidence for its role as a general autoimmune locus. Diabete 2004, 53:3020-3023.

7. Vella A, Cooper JD, Lowe CE, Walker N, Nutland S, Widmer B, Jones R, Ring SM, McArdle W, Pembrey ME, Strachan DP, Dunga DB, Twells RC, Clayton DG, Todd JA: Localization of a type 1 diabetes locus in the IL2RA/CDC25 region by use of tag single-nucleotide polymorphisms. Am J Hum Genet 2005, 76:773-779.

8. Qu HQ, Montpetit A, Ge B, Hudson TJ. Polychronakos C: Toward Further Mapping of the Association Between the IL2RA Locus and Type 1 Diabetes. Diabetes 2007, 56:1174-1176.

9. Smyth DJ, Cooper JD, Bailey R, Field S, Burren O, Smink LJ, Guja C, Ionescu-Tirgoviste C, Widmer B, Dunga DB, Savage DA, Walker NM, Clayton DG, Todd JA: A genome-wide association study of nonsynonymous SNPs identifies a type 1 diabetes locus in the interferon-induced helicase (IFIH1) region. Nat Genet 2007, 39:587-590.

10. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007, 447:661-678.

11. Todd JA, Walker NM, Cooper JD, Smyth DJ, Downes K, Plagnol V, Baird R, Nejentsev S, Field SF, Payne F, Lowe CE, Szczesny JS, Haffner JP, Zeiteis L, Yang JH, Vella A, Nutland S, Stevens HE, Schulenburg H, Coleman G, Maisuria M, Meadows W, Smink LJ, Healy B, Burren OS, Lam AA, Ovington NR, Allen J, Adlem E, Leung HT, Wallace C, Howson JM, Guja C, Ionescu-Tirgoviste C, Simmonds MJ, Heward JM, Gough SC, Dunga DB, Wicker LS, Clayton DG: Robust associations of four new chromosomal regions from genome-wide analyses of type 1 diabetes. Nat Genet 2007, 39:857-864.

12. Tait KF, Collins JE, Heward JM, Eaves I, Snook H, Franklyn J, Barnett AH, Todd JA, Maranian M, Compston A, Sawyer S, Gough SC. Evidence for a Type 1 diabetes-specific mechanism for the insulin gene-associated IDDM2 locus rather than a general influence on autoimmunity. Diabet Med 2004, 21:267-270.

13. Smyth DJ, Howson JM, Payne F, Maier LM, Bailey R, Holland K, Lowe CE, Cooper JD, Hulme JS, Vella A, Dahlan I, Lam AC, Nutland S, Walker NM, Twells RC: Type 1 diabetes in Northern Ireland 1989–1994: low incidence in areas with highest population density and most household IDDM in Northern Ireland. Hum Immunol 2004, 65:1770-1773.

14. Tuomilehto J, Lounamaa R, Tuomilehto-Wolf E, Reunanen A, Virtala E, Korpela R, Alavaipio P, Knekt P, Akerblom HK: Epidemiology of childhood diabetes mellitus in Finland—background of a nationwide study of type 1 (insulin-dependent) diabetes mellitus. The Childhood Diabetes in Finland (Dime) Study Group. Diabetologia 1992, 35:70-76.

15. Bain SC, Todd JA, Barnett AH: The British Diabetic Association – Warren repository. Autoimmunity 1990, 7:83-85.

16. Lernmark A: Human cell lines from families available for diabetes research. Diabetologia 1991, 34:61.

17. Patterson CC, Carson DJ, Hadden DR: Epidemiology of childhood IDDM in Northern Ireland 1989–1994: low incidence in areas with highest population density and most household crowding. Northern Ireland Diabetes Study Group. Diabetologia 1999, 39:1063-1069.

18. Udallien DE, Akselsen HE, Joner G, Dahl-Jorgensen K, Asgnaes O, Sovik O, Thorsbye E, Ronningen KS. No difference in the parental origin of susceptibility HLA class II haplotypes among Norwegian patients with insulin-dependent diabetes mellitus. Am J Hum Genet 1995, 57:1511-1514.

19. Ionescu-Tirgoviste C, Guja C, Herr M, Cucca E, Welsh K, Bunce M, Mathioudakis S, Todd JA: Low frequency of HLA DRB1*03 – DQB1*02 and DQB1*0302 haplotypes in Romania is consistent with the country’s low incidence of Type 1 diabetes. Diabetologia 2001, 44(Suppl 3):B60-66.

20. Cazes TD: [http://www.eurekam.cn/ukr/index/gridm.html].

21. Power C, Elliott J: Cohort profile: 1958 British birth cohort (National Child Development Study). Int J Epidemiol 2006, 35:34-41.

22. SeattleSNPs: [http://pga.mbi.washington.edu].

23. Morahan G, Huang D, Wu M, Holt BJ, White GP, Kendall GE, Sly PD, Holt PG: Association of IL12B promoter polymorphism with severity of atopic and non-atopic asthma in children. Lancet 2002, 360:455-459.

24. Barret JC, Fry B, Maller J, Daly MJ: Haplovew: analysis and visualization of LD and haplotype maps. Bioinformatics 2005, 21:263-265.

25. The International HapMap Project. Nature 2003, 426:789-796.

26. Wang N, Akery M, Zhang K, Chakraborty R, Jin L: Distribution of recombination crossovers and the origin of haplotype blocks: the interplay of population history, recombination, and mutation. Am J Hum Genet 2002, 71:1227-1234.

27. Clayton DG, Walker NM, Smyth DJ, Pask R, Cooper JD, Maier LM, Smink LJ, Lam AC, Ovington NR, Stevens HE, Nutland S, Howson JM, Faham M, Moorhead M, Jones HB, Falkowski M, Hardenbol P, Willis TD, Todd JA: Population structure, differential bias and genomic control in a large-scale, case-control association study. Nat Genet 2005, 37:1243-1246.

28. Sta: [http://www.statas.com/].

29. R: [http://www.r-project.org/].

30. Additional routines: [http://www.gene.cimr.cam.ac.uk/clayton sof/].

31. Spielman RS, Ewens WJ: The TDT and other family-based tests for linkage disequilibrium and association. Am J Hum Genet 1996, 59:983-989.

32. Cordell HJ, Clayton DG: A unified stepwise regression procedure for evaluating the relative effects of polymorphisms within a gene using case/control or family data: application to HLA in type 1 diabetes. Am J Hum Genet 2002, 70:124-141.

33. Chapman JM, Cooper JD, Todd JA, Clayton DG: Detecting disease associations due to linkage disequilibrium using haplotype tagging: a class of tests and the determinants of statistical power. Hum Hered 2003, 56:18-31.

34. Lowe CE, Cooper JD, Chapman JM, Barratt BJ, Twells RC, Green EA, Savage DA, Guja C, Ionescu-Tirgoviste C, Tuomilehto-Wolf E, Tuomilehto J, Todd JA, Clayton DG: Cost-effective analysis of candidate genes using hSNPs: a staged approach. Genes Immun 2005, 4:301-305.

35. Chistikova DA, Chernisheva A, Savost’anov KV, Turakulov RI, Kuravea TL, Dedov II, Nosikov VV: The TAF5L gene on chromosome 1q42 is associated with type 1 diabetes in Russian affected families. Autoimmunity 2003, 35:283-293.

36. Nielsen C, Hansen D, Hubsy S, Jacobsen BB, Lilleveg ST: Association of a putative regulatory polymorphism in the PD-1 gene with susceptibility to type 1 diabetes. Tissue Antigens 2003, 62:492-497.

37. Prokunina L, Padyukov L, Bennet A, de Faire U, Wilman B, Prince J, Allredsson L, Klareskog L, Aralon-Qricemle R: Association of the PD-1.3A allele of the PDCC1 gene in patients with rheumatoid arthritis negative for rheumatoid factor and the shared epitope. Arthritis Rheum 2004, 50:1770-1773.

38. Noble JA, White AM, Lazerzoni LC, Valdes AM, Mirel DB, Reynolds R, Gade A, Peltz G, Erich HA: A polymorphism in the TCF7 gene, C883A, is associated with type 1 diabetes. Diabetologia 2003, 52:1579-1585.

39. Ladner MB, Bottini N, Valdes AM, Noble JA: Association of the single nucleotide polymorphism C1858T of the PTPN22 gene with type 1 diabetes. Hum Immunol 2005, 66:50-64.

40. Windsor L, Morahan G, Huang D, McCann V, Jones T, James I, Christiansen FT, Price P: Alleles of the IL12B 3'UTR associate with late onset of type 1 diabetes. Hum Immunol 2004, 65:1432-1436.

41. Davoodi-Semirani A, Yang J. She JX, IL-12p40 is associated with type 1 diabetes in Caucasian-Americans. Diabetes 2002, 51:1579-1581.

42. Ladner MB, Bottini N, Valdes AM, Noble JA: Association of the single nucleotide polymorphism C1858T of the PTPN22 gene with type 1 diabetes. J Allergy Clin Immunol 2003, 1245-1248.

43. Khoo SK, Hayden CM, Roberts M, Horak E, de Klerk N, Zhang G, Robertson CF, Goldblatt J, Le Souef P: Associations of the IL12B promoter polymorphism in longitudinal data from asthmatic patients 7 to 42 years of age. J Allergy Clin Immunol 2004, 113:475-481.

44. Kristiansen OP, Larsen ZM, Lykkesoer AU, Larsen AE, Poulsen T: Association of a functional 17beta-estradiol sensitive IL6-174G/C promoter polymorphism with early-onset type 1 diabetes in females. Hum Mol Genet 2003, 12:1101-1110.

45. Cappel KM, Nolsoe RL, Christiansen OP, Bingley PJ, Mandrup-Poulten T: The interleukin adhe-
sion molecule-1 K649E polymorphism in type 1 diabetes. *Immunogenetics* 2000, 52:107-111.

46. Sakaai Y, Ibara K, Matsumura N, Kohno H, Nagafuchi S, Kuroamura R, Kusuhara K, Takeya R, Hoey T, Sumimoto H, Hara T: Identification of a novel type I diabetes susceptibility gene, T-beta.

47. Chung HT, Kim LH, Park BL, Lee JH, Park HS, Choi BW, Hong SJ, Cho SC, Kim JJ, Park CS, Shin HD: Association analysis of novel TBNX21 variants with asthma phenotypes. *Hum Mutat* 2003, 22:257.

48. Tomer Y, Concepcion E, Greenberg DA: A C/T single-nucleotide polymorphism in the region of the CD40 gene is associated with Graves’ disease. *Thyroid* 2002, 12:113.

49. Morahan G, Huang D, Ymer SI, Cancilla MR, Stephen K, Dadbadhao P, Werther G, Tait BD, Harrison LC, Colman PG: Linkage disequilibrium of a type I diabetes susceptibility locus with a regulatory IL2B allele.

50. Nat Genet 2001, 27:218-221.

51. McInnes IB, Lewis CM, Deloukas P, Forbes A, Sanderson J, Jewell DP, Satsangi J, Talmud PJ, Schurmann NC, Walters GR, Walker NM, Watkins NA, Winzer T, Jones RW, Hamshere ML, Holmans PA, Jones IR, Kirov G, Moskvina V, Nikolov BS, Lucock MF, Krueger GG, Begovich AB: The polymorphism in the promoter region of the gene for interleukin-6 is associated with susceptibility to type 2 diabetes mellitus.

52. J Med Genet 2004, 41:594-599.

53. Johansson S, Lie BA, Thorsby E, Undlien DE: The polymorphism in the 3′ untranslated region of IL2B has a negligible effect on the susceptibility to develop type 1 diabetes in Norway. *Immunogenetics* 2001, 53:603-605.

54. Jahromi MM, Millward BA, Demaine AG: A polymorphism in the promoter region of the gene for interleukin-6 is associated with susceptibility to type 2 diabetes mellitus.

55. Efendic S, Mackay AJ, Rowland IR, Effros RM, Morgan MA, Reicherts HD, Mulligan WM, Wyke B, McClellan JM: Polymorphisms in cytokines and metabolic genes as additional genetic markers for susceptibility to develop type 1 diabetes. *Immunogenetics* 2004, 56:36-40.

56. Nejentsev S, Guja C, McCormack R, Cooper J, Howson JM, Nutland S, Rance H, Walker N, Undlien D, Ronningen KS, Tuomilehto-Wolf E, Tuomilehto J, Ionescu-Tirgoviste C, Gale EA, Bingley PJ, Gillespie KM, Savage DA, Carson DJ, Patterson CC, Maxwell AP, Todd JA: Association of intercellular adhesion molecule-1 gene with type 1 diabetes. *Diabetes* 2004, 53:1627-1632.

57. Ma J, Mollsten A, Prazny M, Falhammer H, Brismar K, Dahlquist G, Efendic S, Gu HF: Genetic influences of the intercellular adhesion molecule-1 (ICAM-1) gene polymorphisms in development of Type 1 diabetes and diabetic nephropathy. *Diabet Med* 2007, 24:149-1650.

58. Duerr RH, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, Steinthard AH, Abraham C, Regueiro M, Griffiths A, Dassopoulos T, Bitton A, Yang H, Targan S, Datta LW, Kistner EO, Schumm LP, Lee A, Gregersen PK, Barmada MM, Rotter JL, Nicolae DL, Cho JH: A Genome-Wide Association Study Identifies IL23R as an Inflammatory Bowel Disease Gene. *Science* 2006.

59. Sigurdsson S, Nordmark G, Goring HH, Lindroos K, Wiman AC, Alarcon-Riquelme ME: Regulatory polymorphisms in PDCD1 is associated with susceptibility to systemic lupus erythematosus in humans. *Nat Genet* 2002, 32:666-669.

60. Lin SC, Yen JH, Tsai JJ, Tsai WC, Ou TT, Liu HW, Chen CJ: Association of a programmed death 1 gene polymorphism with the development of rheumatoid arthritis, but not systemic lupus erythematosus. *Arthritis Rheum* 2004, 50:770-775.

61. Dahlman I, Eaves IA, Kosoy R, Morrison VA, Heward J, Gough SC, Allahabadia A, Franklyn JA, Tuomilehto J, Tuomilehto-Wolf E, Cucca F, Guja C, Ionescu-Tirgoviste C, Stevens H, Carr P, Nutland S, McKinney P, Shield JP, Wang W, Cordell HJ, Walker N, Todd JA, Concan- non P: Parameters for reliable results in genetic association studies in common disease.

62. Nat Genet 2002, 30:149-150.

63. McCormack RM, Maxwell AP, Carson DJ, Patterson CC, Middleton D, Savage DA: The IL2B 3′ untranslated region DNA polymorphism is not associated with early-onset type 1 diabetes. *Imm Gen 2002, 3:433-435.

64. Nistico L, Giorgi G, Giordano M, Galgani A, Petrone A, D’Alfonso S, Federici M, Di Mario U, Pozzilli P, Buzzetti R, Cascino I: IL2B polymorphism and type 1 diabetes in the Italian population: a case-control study. *Hum Immunol* 2002, 63:928-934.

65. Bergholdt R, Ghandil P, Johannesen J, Kristiansen OP, Kockum I, Luthman H, Ronningen KS, Nerup J, Julier C, Pociot F: Genetic and functional evaluation of an interleukin-12 polymorphism (IDDM18) in families with type 1 diabetes. *J Med Genet* 2004, 41:328-334.

66. Johansson S, Lie BA, Thorsby E, Undlien DE: The polymorphism in the 3′ untranslated region of IL2B has a negligible effect on the susceptibility to develop type 1 diabetes in Norway. *Immunogenetics* 2001, 53:603-605.

67. Duerr RH, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, Steinthard AH, Abraham C, Regueiro M, Griffiths A, Dassopoulos T, Bitton A, Yang H, Targan S, Datta LW, Kistner EO, Schumm LP, Lee A, Gregersen PK, Barmada MM, Rotter JL, Nicolae DL, Cho JH: A Genome-Wide Association Study Identifies IL23R as an Inflammatory Bowel Disease Gene. *Science* 2006.

68. Sigurdsson S, Nordmark G, Goring HH, Lindroos K, Wiman AC, Alarcon-Riquelme ME: Regulatory polymorphisms in PDCD1 is associated with susceptibility to systemic lupus erythematosus in humans. *Nat Genet* 2002, 32:666-669.

69. Lin SC, Yen JH, Tsai JJ, Tsai WC, Ou TT, Liu HW, Chen CJ: Association of a programmed death 1 gene polymorphism with the development of rheumatoid arthritis, but not systemic lupus erythematosus. *Arthritis Rheum* 2004, 50:770-775.

70. Dahlman I, Eaves IA, Kosoy R, Morrison VA, Heward J, Gough SC, Allahabadia A, Franklyn JA, Tuomilehto J, Tuomilehto-Wolf E, Cucca F, Guja C, Ionescu-Tirgoviste C, Stevens H, Carr P, Nutland S, McKinney P, Shield JP, Wang W, Cordell HJ, Walker N, Todd JA, Concan- non P: Parameters for reliable results in genetic association studies in common disease.

71. Nat Genet 2002, 30:149-150.

72. McCormack RM, Maxwell AP, Carson DJ, Patterson CC, Middleton D, Savage DA: The IL2B 3′ untranslated region DNA polymorphism is not associated with early-onset type 1 diabetes. *Imm Gen 2002, 3:433-435.

73. Nistico L, Giorgi G, Giordano M, Galgani A, Petrone A, D’Alfonso S, Federici M, Di Mario U, Pozzilli P, Buzzetti R, Cascino I: IL2B polymorphism and type 1 diabetes in the Italian population: a case-control study. *Hum Immunol* 2002, 63:928-934.

74. Bergholdt R, Ghandil P, Johannesen J, Kristiansen OP, Kockum I, Luthman H, Ronningen KS, Nerup J, Julier C, Pociot F: Genetic and functional evaluation of an interleukin-12 polymorphism (IDDM18) in families with type 1 diabetes. *J Med Genet* 2004, 41:328-334.

75. Johansson S, Lie BA, Thorsby E, Undlien DE: The polymorphism in the 3′ untranslated region of IL2B has a negligible effect on the susceptibility to develop type 1 diabetes in Norway. *Immunogenetics* 2001, 53:603-605.

76. Duerr RH, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, Steinthard AH, Abraham C, Regueiro M, Griffiths A, Dassopoulos T, Bitton A, Yang H, Targan S, Datta LW, Kistner EO, Schumm LP, Lee A, Gregersen PK, Barmada MM, Rotter JL, Nicolae DL, Cho JH: A Genome-Wide Association Study Identifies IL23R as an Inflammatory Bowel Disease Gene. *Science* 2006.

77. Sigurdsson S, Nordmark G, Goring HH, Lindroos K, Wiman AC, Alarcon-Riquelme ME: Regulatory polymorphisms in PDCD1 is associated with susceptibility to systemic lupus erythematosus in humans. *Nat Genet* 2002, 32:666-669.

78. Lin SC, Yen JH, Tsai JJ, Tsai WC, Ou TT, Liu HW, Chen CJ: Association of a programmed death 1 gene polymorphism with the development of rheumatoid arthritis, but not systemic lupus erythematosus. *Arthritis Rheum* 2004, 50:770-775.
gene is associated with Graves' disease in Koreans. *Thyroid* 2003, 13:919-925.

74. Mukai T, Hiromatsu Y, Fukutani T, Ichimura M, Kaku H, Miyake I, Yamada K. A C/T polymorphism in the 5' untranslated region of the CD40 gene is associated with later onset of Graves' disease in Japanese. *Endocr J* 2005, 52:471-477.

75. Houston FA, Wilson V, Jennings CE, Owen CJ, Donaldson P, Perros P, Pearce SH. Role of the CD40 locus in Graves' disease. *Thyroid* 2004, 14:506-509.

76. Heward JM, Simmonds MJ, Carr-Smith J, Foxall H, Franklyn JA, Gough SC. A single nucleotide polymorphism in the CD40 gene on chromosome 20q (GD-2) provides no evidence for susceptibility to Graves' disease in UK Caucasians. *Clin Endocrinol (Oxf)* 2004, 61:269-272.

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