Identification of novel risk genes associated with type 1 diabetes mellitus using a genome-wide gene-based association analysis

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ABSTRACT

Aims/Introduction: Type 1 diabetes mellitus is a serious disorder characterized by destruction of pancreatic β-cells, culminating in absolute insulin deficiency. Genetic factors contribute to the susceptibility of type 1 diabetes mellitus. The aim of the present study was to identify more susceptibility genes of type 1 diabetes mellitus.

Materials and Methods: We carried out an initial gene-based genome-wide association study in a total of 4,075 type 1 diabetes mellitus cases and 2,604 controls by using the Gene-based Association Test using Extended Simes procedure. Furthermore, we carried out replication studies, differential expression analysis and functional annotation clustering analysis to support the significance of the identified susceptibility genes.

Results: We identified 452 genes associated with type 1 diabetes mellitus, even after adapting the genome-wide threshold for significance (P < 9.05E-04). Among these genes, 171 were newly identified for type 1 diabetes mellitus, which were ignored in single-nucleotide polymorphism-based association analysis and were not previously reported. We found that 53 genes have supportive evidence from replication studies and/or differential expression studies. In particular, seven genes including four non-human leukocyte antigen (HLA) genes (RASIP1, STRN4, BCA1 and MYL2) are replicated in at least one independent population and also differentially expressed in peripheral blood mononuclear cells or monocytes. Furthermore, the associated genes tend to enrich in immune-related pathways or Gene Ontology project terms.

Conclusions: The present results suggest the high power of gene-based association analysis in detecting disease-susceptibility genes. Our findings provide more insights into the genetic basis of type 1 diabetes mellitus.

INTRODUCTION

Type 1 diabetes mellitus is a serious disorder characterized by destruction of pancreatic β-cells, leading to absolute insulin deficiency. Type 1 diabetes mellitus arises from uncontrolled inflammatory processes, and accounts for 5–10% of total cases of diabetes worldwide¹. Diabetes mellitus is a major risk factor for micro- and macrovascular complications, and is associated with endothelial dysfunction, premature atherosclerosis and reduced capability of neovascularization in ischemic conditions. The increasing number of people developing diabetes might be associated with the changing environment in relation to diet and infection, but more with genetic factors² ³. Identification of genes predisposing to type 1 diabetes mellitus will increase our understanding of the genetic pathogenesis of type 1 diabetes mellitus, and contribute to the development of novel prevention and treatment of type 1 diabetes mellitus in the future.

Extensive evidence has shown that genetic factors play important roles in the development of type 1 diabetes mellitus³ ⁴ ⁵. However, identification of specific responsible genes and their variants has had limited success. The single-nucleotide polymorphism (SNP)-based genome-wide association studies (GWAS) have identified a long list of risk genes for type 1
diabetes mellitus. However, the traditional GWAS ignored a large number of loci with moderate effects, because of the stringent significance thresholds used.

Gene-based analysis takes a gene as a basic unit for association analysis. As this method can combine genetic information given by all the SNPs in a gene to obtain more informative results, it is being used as a novel method complementing SNP-based GWAS to identify disease susceptibility genes. Notably, this method can increase our chance of finding novel genes, which are usually ignored by SNP-based association analysis. In the present study, we presented a statistically robust gene-based GWAS, focusing on identifying ‘novel’ genes underlying susceptibility to type 1 diabetes mellitus.

**MATERIALS AND METHODS**

**Discovery Study Sample**

The initial discovery sample included 4,075 cases and 2,604 controls. The case data came from ‘UK Genetic Resource Investigating Diabetes’ (available at www.childhood-diabetes.org.uk/grid.shtml). The control participants came from the 1958 British Birth Cohort. Genotyping, data-quality filter and SNP-based association analysis were detailed in the original publication in Nat Genet, thus not elaborated here.

**Replication Study Sample**

Replication analyses were carried out in three independent study samples (Replication sample 1 [R1]: a total of 1,879 samples including 935 diabetic nephropathy cases and 944 normoalbuminuric controls; replication sample 2 [R2]: 486 trios including 223 affected trios and 263 unaffected trios; replication sample 3 [R3]: 685 white individuals with type 1 diabetes from the Diabetes Control and Complications Trial [DCCT]/Epidemiology of Diabetes Intervention and Complications [EDIC] study). Basic characteristics of the study participants, as well as the genotyping process, data quality control and SNP-based association analysis, have been detailed previously in the original publications, thus not elaborated here.

**Gene-Based Association Analysis**

Raw data used in the present gene-based GWAS analysis and replication studies are P-values from genome-wide SNP-based GWAS. The data were downloaded from the publicly available dbGaP database (accession number: phs0000180, phs000018, phs000086 and phs000088). Gene-based association analysis was carried out using Gene-based Association Test using Extended Simes procedure, and the resultant gene-based P-value is a measure of statistical significance. TheGene-based Association Test using Extended Simes procedure was modeled in KGG, a systematic biological Knowledge-based mining system for Genome-wide Genetic studies (available at http://bioinfo1.hku.hk:k13080/kggweb). The defined length of the extended gene region is from 5-kb upstream to 5-kb downstream of each gene.

**RESULTS**

**Discovery of Novel Genes Associated With Type 1 Diabetes Mellitus**

A total of 24,984 genes were analyzed in the initial gene-based GWAS. Three quantile–quantile plots for gene-based P-values, SNP-based P-values inside genes and SNP-based P-values outside genes are shown in Figure 1. We observed dramatic deviations at the tails of the distributions for the three plots. The deviation was much stronger for the plot of gene-based P-values than the other two plots, suggesting relatively higher power for gene-based association analysis.

The Manhattan plot of gene-level P-values across chromosomes is showed in Figure 2. As expected, the majority of these associations were mapped to the HLA region, whose physical location lies in chromosome 6p21. To adjust for multiple testing, we used the Benjamini–Hochberg procedure to control false discovery rate (FDR) of the genome-wide association tests. To obtain a FDR of 0.05 across the whole genome, the significance level for a gene-based test is 9.05E-04. Accordingly, 452 genes were statistically significant (Table S1).
In the original SNP-based GWAS analysis, according to the genome-wide \( P \)-value threshold of statistical significance (Bonferroni correction \( P < 9.94 \times 10^{-8} \)), a total of 699 SNPs showed significant associations. These 699 SNPs corresponded to 269 genes. Comparatively, the current gene-based study detected 183 additional candidate genes for type 1 diabetes mellitus that were undetected by the SNP-based association analysis. To discover whether any of the 183 genes had been reported in other previous association studies, we searched the Phenotype-Genotype Integrator (PheGenI; www.ncbi.nlm.nih.gov/gap/PheGenI/), a database archiving previous association results. We compared the 183 genes and the list of genes with significant SNP-based \( P \)-values \( (P < 1.0 \times 10^{-7}; \text{Table S1}) \). This comparison showed that just 12 among the total 183 genes were previously reported for an association with type 1 diabetes mellitus\(^7\)–\(^22\). The rest of the 171 ‘novel’ genes were first detected for type 1 diabetes mellitus by the present study.

**Confirmation of Type 1-Associated Genes by Replication Studies**

The results of the replication analyses are summarized in Table S1 \( (P < 5.0 \times 10^{-2}) \). For the 171 ‘novel’ genes, only one gene was replicated for associations with type 1 diabetes mellitus in study R1. In addition, eight genes were replicated in study R2 and 14 genes were replicated in study R3. In total, 23 of the 171 ‘novel’ genes (Table 1) were confirmed for their association with type 1 diabetes mellitus. For the replication studies, the significance level of \( P < 5.0 \times 10^{-2} \) was used.

**Differential Expression Analyses of Type 1 Diabetes Mellitus Associated Genes**

For the aforementioned 171 ‘novel’ genes, we used \( t \)-test to compare ribonucleic acid expression signals in PBMCs or monocytes between type 1 diabetes mellitus patients and healthy controls. We found that 37 genes, including 21 non-HLA genes (e.g. FAM46B, OLFLML3 and HIPK1), were differentially expressed between type 1 diabetes mellitus patients.
**Table 1**  'Novel' type 1 diabetes mellitus-associated genes with supplementary evidence

| Gene symbol | Chr. | Start position | Length | SNP† | Gene P-value | Replication P-value | Differential expression P-value |
|-------------|------|----------------|--------|------|--------------|---------------------|----------------------------------|
| RASIP1      | 19   | 49,218,842     | 30,128 | 6    | 4.83E-04     | 2.94E-02             | R2                              |
| STRN4       | 19   | 47,217,768     | 36,952 | 5    | 1.97E-04     | 2.67E-02             | R2                              |
| BCA1        | 16   | 75,257,928     | 49,023 | 8    | 9.32E-05     | 2.27E-02             | R2                              |
| FYN         | 6    | 111,977,485    | 222,142| 48   | 3.08E-04     | 4.69E-03             | R3                              |
| MYL2        | 12   | 111,343,623    | 19,781 | 5    | 4.61E-04     | 8.53E-03             | R3                              |
| HLA-J       | 6    | 29,668,748     | 13,985 | 1    | 3.49E-06     | 3.89E-02             | R3                              |
| PPP1R11     | 6    | 30,029,932     | 13,178 | 4    | 5.50E-06     | 2.99E-02             | R3                              |
| ITPR3       | 6    | 33,584,161     | 85,190 | 29   | 4.35E-05     | NS†                  |                                  |
| PLEKH4A1    | 10   | 124,129,220    | 67,646 | 11   | 9.32E-05     | NS†                  |                                  |
| A Kelly    | 12   | 14,513,611     | 143,086| 13   | 8.83E-05     | NS†                  |                                  |
| OR2B6       | 6    | 27,920,019     | 10,941 | 2    | 2.04E-06     | NS†                  |                                  |
| OR5V1       | 6    | 29,318,007     | 110,47 | 2    | 2.16E-06     | NA§                  |                                  |
| HIST1H4E    | 6    | 26,199,873     | 10,376 | 2    | 3.48E-06     | NA§                  |                                  |
| HIST1H2BF   | 6    | 26,194,787     | 10,429 | 1    | 6.65E-06     | NS†                  |                                  |
| GUSBL1      | 6    | 26,834,266     | 95,067 | 2    | 3.48E-07     | NA§                  |                                  |
| HMGB1       | 13   | 31,027,877     | 17,204 | 2    | 2.07E-04     | NA§                  |                                  |
| ZNF192      | 6    | 28,104,716     | 25,520 | 1    | 3.65E-06     | NS†                  |                                  |
| RING1       | 6    | 33,171,286     | 14,213 | 4    | 1.45E-04     | NS†                  |                                  |
| FAM46B      | 1    | 27,326,511     | 17,822 | 1    | 6.36E-05     | NA§                  |                                  |
| OLFL3       | 1    | 114,517,030    | 12,845 | 2    | 2.13E-04     | NS†                  |                                  |
| HIPK1       | 1    | 114,466,996    | 58,426 | 6    | 5.54E-04     | NS†                  |                                  |
| NSL1        | 1    | 212,894,495    | 75,644 | 12   | 5.48E-05     | NS†                  |                                  |
| IL10        | 1    | 206,935,948    | 14,891 | 8    | 2.28E-04     | NS†                  |                                  |
| TRIM27      | 6    | 28,865,779     | 30,989 | 3    | 8.88E-07     | NS†                  |                                  |
| NAPC2O2     | 12   | 6,598,298      | 47,834 | 10   | 1.36E-04     | NS†                  |                                  |
| PPP1R10     | 6    | 30,563,182     | 26,838 | 2    | 4.08E-04     | NS†                  |                                  |
| VPS52       | 6    | 33,213,049     | 31,613 | 2    | 6.73E-07     | NA§                  |                                  |
| PHF1        | 6    | 33,373,773     | 15,457 | 2    | 3.14E-04     | NA§                  |                                  |
| BAK1        | 6    | 33,535,323     | 17,747 | 7    | 5.18E-05     | NS†                  |                                  |
| IKZF3       | 17   | 37,916,198     | 109,243| 7    | 7.54E-05     | NS†                  |                                  |
| ZZF1        | 17   | 3,902,739      | 148,514| 23   | 3.55E-04     | NS†                  |                                  |
| GNS         | 12   | 65,102,222     | 56,004 | 4    | 6.65E-05     | NS†                  |                                  |
| ORMDL3      | 17   | 38,072,296     | 16,558 | 3    | 7.22E-04     | NS†                  |                                  |
| BRAP        | 12   | 112,074,950    | 53,840 | 3    | 1.16E-04     | NS†                  |                                  |
| CR1ZL1      | 21   | 34,956,647     | 62,513 | 3    | 3.72E-04     | NA§                  |                                  |
| SULT1A1     | 16   | 28,619,113     | 27,953 | 1    | 4.74E-04     | NA§                  |                                  |
| TMEM129     | 4    | 1,716,279      | 15,405 | 3    | 7.99E-04     | NS†                  |                                  |
| KZF1        | 7    | 50,439,231     | 38,668 | 15   | 6.66E-05     | 4.42E-02             | R1                              |
| MICA        | 6    | −1             | 0      | 3    | 7.52E-05     | 7.52E-05             | R2                              |
| PLBD1       | 12   | 14,651,597     | 74,194 | 11   | 3.17E-04     | 3.26E-02             | R2                              |
| DEX         | 16   | 11,017,748     | 23,509 | 3    | 8.72E-04     | 3.03E-03             | R3                              |
| SBK1        | 16   | 28,298,840     | 41,330 | 7    | 2.28E-05     | 1.37E-02             | R2                              |
| GCA         | 2    | 163,195,583    | 28,566 | 4    | 9.67E-06     | 4.16E-02             | R3                              |
| OR2B3       | 6    | 29,048,985     | 11,105 | 1    | 5.27E-07     | 3.31E-02             | R3                              |
| HGPSF2      | 6    | 29,963,782     | 12,246 | 1    | 3.49E-06     | 3.89E-02             | R3                              |
| HCP4P3      | 6    | 29,967,622     | 10,983 | 1    | 3.49E-06     | 3.89E-02             | R3                              |
| OR2U1P      | 6    | 29,225,436     | 11,420 | 5    | 1.05E-06     | 4.73E-02             | R3                              |
| VNR1R1P     | 6    | 26,262,313     | 10,651 | 1    | 6.19E-06     | 2.94E-02             | R3                              |
and controls (Table 2). For the differential expression study, the significance level of \( P < 5.0 \times 10^{-8} \) was used.

In short, through a gene-based association study, we identified 183 type 1 diabetes mellitus-associated genes that were insignificant in the original SNP-based association tests. Among the 183 genes, 171 genes are ‘novel’ genes identified for type 1 diabetes mellitus. Replication studies and/or differential expression studies further supported the significance of 53 genes to type 1 diabetes mellitus. In particular, four non-HLA genes (RASIP1, STRN4, BCAR1 and MYL2) and three HLA genes (FYNS, HLA-J and PPP1R11) were validated by both replication and differential expression studies.

Functional Annotation Clustering Analysis

Gene ontology analysis showed significant enrichment of 452 identified type 1 diabetes mellitus genes in particular biological terms and pathways. For example, we found a significant clustering (Bonferroni correction \( P = 1.50 \times 10^{-5} \)) of 33 genes (e.g. HLA-DQB1, HLA-DMB, HLA-DMA) directly involved in immune response (Table S2). These genes tend to enrich in immune-related KEGG pathways (including hsa04940: type I diabetes mellitus, hsa04612: antigen processing and presentation, hsa04672: intestinal immune network for immunoglobulin A production; Table S2).

DISCUSSION

Elucidation of the genetic basis of type 1 diabetes mellitus remains one of the huge challenges in the field of human genetics, which is largely because of the complex nature of the genetic determination for type 1 diabetes mellitus, including polygenetic determinations, gene-by-gene and gene-by-environment interactions. Thus far, most of the published GWAS studies reported the results of single-marker-based analysis, where each SNP was analyzed individually.\(^{6,21,23} \) Because of the large number of SNPs tested in a GWAS, stringent \( P \)-value thresholds for significance (typically \( P < 5.0 \times 10^{-8} \)) are used to control false positive findings. Consequently, a large number of SNPs with moderate effects are missed. The gene-based association test is an important supplementary method for the SNP-based association test, which combines genetic information given by all the SNPs in a gene, thus obtaining a more informative result.\(^{24} \) As we expected, the present gene-based association study identified more significant type 1 diabetes mellitus-susceptibility genes than the SNP-based test. Specifically, 171 ‘novel’ genes were identified for type 1 diabetes mellitus.

Compared with SNP/variant-based association tests, the gene-based association analyses have several distinct advantages. First, by combining the effects of all SNPs assigned to genes into a statistic analysis while correcting for linkage disequilibrium (LD), the gene-based analysis substantially alleviates the multiple-testing burden by reducing the number of tests in a GWAS. Second, the test unit is a gene, which is highly consistent across populations. Therefore, gene-based association analyses ignore confounding factors that are intrinsic in genetic variants-based association tests, such as allele frequencies, LD structure and heterogeneity across diverse human populations.\(^{25} \) Finally, the gene-based tests are also ideally suitable for a network (or pathway) approach to interpret findings from GWAS.\(^{26} \)

As previously reported,\(^{2,3,27,28} \) more than half of the significant association signals for type 1 diabetes mellitus were identified within chromosome 6p21, which is the most important region in the vertebrate genome with respect to infection and autoimmunity, and is crucial in adaptive and innate immunity. The present study also found that more than 50% of the identified ‘novel’ genes (100 HLA genes/171 novel genes) are located at the HLA region. However, among 53 type 1 diabetes mellitus-associated genes validated by replication studies and/or differential expression studies, 28 genes are non-HLA genes. So far, the roles of these non-HLA genes directly in the pathogenesis of type 1 diabetes mellitus are largely unknown, but some of these genes play important roles in immune response. For example, BCAR1 might mediate its diabetogenic impact through impaired \( \beta \)-cell function.\(^{29} \) IKZF1 and IKZF3 are members of the Ikaros family of zinc-finger proteins. The gene product is a transcription factor that is important in regulation...
Table 2 | Differential expression analyses for ‘novel’ genes in type 1 diabetes mellitus-related cells

| Sample | Target cells | Sample size | Platform | References | GSE NO. |
|--------|--------------|-------------|----------|------------|---------|
| S1     | PBMC         | 46:44       | [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array | 14 | GSE35725 |
| S2     | CD14+ Monocyte | 166       | Illumina HumanHT-12 V3.0 expression beadchip | 15 | GSE33440 |
| S3     | PBMC         | 9:10        | Phalanx Human One Array (version 4.3) | 16 | GSE29142 |

| Gene   | Probe ID   | t-test P-value | Gene   | Probe ID   | t-test P-value | Gene   | Probe ID   | t-test P-value |
|--------|------------|---------------|--------|------------|---------------|--------|------------|---------------|
| ATF7IP | 207728_at  | 2.10E-03      | FYN    | ILMN_1781207 | 2.64E-02     | HIPK1  | 14,914     | 3.56E-02 |
| BCA1   | 223116_at  | 4.17E-02      | BAK1   | ILMN_1805990 | 6.11E-03     | OLFML3 | 21,862     | 1.54E-02 |
| FAM46B | 229518_at  | 4.97E-02      | IL10   | ILMN_2073307 | 1.05E-02     | BAK1   | 22,739     | 1.60E-02 |
| FYN    | 1559101_at | 2.01E-03      | NCAPD2 | ILMN_1775008 | 1.40E-04     | PPP1R11 | 3.205     | 1.69E-02 |
| GUSL1  | 1555568_at | 1.40E-02      | NSL1   | ILMN_1739210 | 2.27E-02     | BRAP   | 12,945     | 4.45E-02 |
| HIST1H2B | 208490_x_at | 8.84E-03   | PHF1   | ILMN_1746968 | 6.88E-03     | CRYZL1 | 24,489     | 8.59E-03 |
| HIST1H2F | 206951_x_at | 4.29E-03   | PPP1R10 | ILMN_1659058 | 3.95E-02     | GNS    | 8137       | 1.83E-03 |
| HMGB1  | 200679_x_at | 1.99E-02      | PPP1R11 | ILMN_1747598 | 2.33E-03     | HLA-J  | 24,451     | 1.63E-02 |
| ITPR3  | 201187_s_at | 4.29E-03      | TRIM27 | ILMN_1655482 | 8.79E-03     | IKZF3  | 15,319     | 1.87E-04 |
| MYL2   | 209742_s_at | 7.58E-03      | VP552  | ILMN_1666632 | 1.32E-02     | ORMDL3 | 102,33     | 2.96E-02 |
| OLFML3 | 218162_at  | 7.24E-03      |        |             |               | SULT1A1 | 20,808     | 2.61E-02 |
| OR2B6  | 216522_at  | 2.65E-03      |        |             |               | TMEM129 | 17,978     | 4.31E-02 |
| OR5V1  | 234840_s_at | 3.07E-03      |        |             |               | ZZEF1  | 16,832     | 1.46E-03 |

Only the most significant probe was listed, even if more than one probe was tested or detected for a gene. GSE NO, Gene Expression Omnibus Number (www.ncbi.nlm.nih.gov/geo/); PBMC, peripheral blood mononuclear cells.
of B lymphocyte proliferation and differentiation\(^{30,31}\). \textit{PLEKHAI} (also known as TAPP1) encodes a pleckstrin homology domain-containing adapter protein. The interaction of TAPP1 adapter proteins with phosphatidylinositol (3, 4)-bisphosphate could regulate B cell activation and autoantibody production. Several studies suggest that TAPP1 might play roles in B and T cell activation, which are necessary and sufficient conditions for immune response\(^{32}\). \textit{MYL2} can regulate the coordinated rearrangements of the actin–myosin cytoskeleton, and facilitate early and late events in T cell activation and signal transduction\(^{33}\). High mobility group box-1 (HMGB1) is an important component of the immune response, which can activate immune cells involved in immune process\(^{34}\). Interleukin-10 (IL-10) is a cytokine with anti-inflammatory and immunomodulatory function, which can regulate the biological functions of B and T cells\(^{35}\). GCA is a causal factor in autoimmune pancreatic β-cell destruction\(^{36}\). The aforementioned evidence supports that \textit{BCAR1}, \textit{IKZF1}, \textit{IKZF3}, \textit{PLEKHAI}, \textit{MYL2}, \textit{HMGB1}, IL-10 and GCA might have functional relevance to diabetes mellitus or immune response. In addition, some previous studies suggested that IL-10, \textit{OMDL3} and \textit{FUT2} have been associated with type 1 diabetes mellitus\(^{7,37}\). Further studies are required to dissect the roles of these non-HLA genes in the pathogenesis of type 1 diabetes mellitus.

Our replication studies had a relatively low replication rate for the significant genes detected in the initial study. Small sample size (e.g., 486 trios subjects in R2 and 685 subjects in R3), and difference in demography (e.g., discover sample from the UK, whereas three replication study samples from New England, the USA and Canada) and genetic background could contribute to this. Another more important factor might be the difference in case identification. For R1, the case is s diabetic nephropathy patient. For R3, although the case is an individual with type 1 diabetes, the association analysis was carried out between the gene and type 1 diabetes-associated phenotype (E-selectin level in serum).

In conclusion, the findings presented in our study suggest high power for gene-based association analyses in detecting disease-susceptibility genes across the human genome. Our findings point to the involvement of new pathways in the pathogenesis of type 1 diabetes mellitus, and provide more insights into the genetic basis of type 1 diabetes mellitus.

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REFERENCES

1. Maahs DM, West NA, Lawrence JM, et al. Epidemiology of type 1 diabetes. \textit{Endocrinol Metab Clin North Am} 2010; 393: 481–497.
2. Noble JA, Valdes AM. Genetics of the HLA region in the prediction of type 1 diabetes. \textit{Curr Diab Rep} 2011; 116: 533–542.
3. Park Y, Eisenbarth GS. Genetic susceptibility factors of Type 1 diabetes in Asians. \textit{Diabetes Metab Res Rev} 2001; 171: 2–11.
4. Sugihara S. Genetic susceptibility of childhood type 1 diabetes mellitus in Japan. \textit{Pediatr Endocrinol Rev} 2012; 10 (Suppl. 1): 62–71.
5. Mosaad YM, Aaf FA, Metwally SS, et al. HLA-DQB1* alleles and genetic susceptibility to type 1 diabetes mellitus. \textit{World J Diabetes} 2012; 38: 149–155.
6. Paterson AD, Waggott D, Borjight AP, et al. A genome-wide association study identifies a novel major locus for glycemic control in type 1 diabetes, as measured by both A1C and glucose. \textit{Diabetes} 2010; 592: 539–549.
7. Barrett JC, Clayton DG, Concannon P, et al. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. \textit{Nat Genet} 2009; 416: 703–707.
8. Bui A, Martinez-Perez A, Perera-Lluna A, et al. A new gene-based association test for genome-wide association studies. \textit{BMC Proc} 2009; 3(Suppl. 7): S130.
9. Mueller PW, Rogus JJ, Cleary PA, et al. Genetics of Kidneys in Diabetes (GoKinD) study: a genetics collection available for identifying genetic susceptibility factors for diabetic nephropathy in type 1 diabetes. \textit{J Am Soc Nephrol} 2006; 177: 1782–1790.
10. Pezzolesi MG, Poznik GD, Mychaleckyj JC, et al. Genome-wide association scan for diabetic nephropathy susceptibility genes in type 1 diabetes. \textit{Diabetes} 2009; 586: 1403–1410.
11. Paterson AD, Lopes-Virella MF, Waggott D, et al. Genome-wide association identifies the ABO blood group as a major locus associated with serum levels of soluble E-selectin. \textit{Arterioscler Thromb Vasc Biol} 2009; 2911: 1958–1967.
12. Li MX, Gui HS, Kwan JS, et al. GATES: a rapid and powerful gene-based association test using extended Simes procedure. \textit{Am J Hum Genet} 2011; 883: 283–293.
13. Beyan H, Drexhage RC, van der Heul Nieuwenhuijsen L, et al. Monocyte gene expression profiles associated with childhood-onset type 1 diabetes and disease risk: a study of identical twins. \textit{Diabetes} 2010; 597: 1751–1755.
14. Levy H, Wang X, Kaldunski M, et al. Transcriptional signatures as a disease-specific and predictive inflammatory biomarker for type 1 diabetes. \textit{Genes Immun} 2012; 138: 593–604.
15. Irvine KM, Gallego P, An X, et al. Peripheral blood monocyte gene expression profile clinically stratifies
patients with recent-onset type 1 diabetes. Diabetes 2012; 615: 1281–1290.

16. Stechova K, Kolar M, Blatry R, et al. Healthy first degree relatives of patients with type 1 diabetes exhibit significant differences in basal gene expression pattern of immunocompetent cells compared to controls: expression pattern as predeterminant of autoimmune diabetes. Scand J Immunol 2011; 75: 210–219.

17. Sherman BT, da Huang W, Tan Q, et al. DAVID Knowledgebase: a gene-centered database integrating heterogeneous gene annotation resources to facilitate high-throughput gene functional analysis. BMC Bioinformatics 2007; 8: 426.

18. Tarone RE. A modified Bonferroni method for discrete data. Biometrics 1990; 462: 515–522.

19. Hochberg Y, Benjamini Y. More powerful procedures for multiple significance testing. Stat Med 1990; 97: 811–818.

20. Todd JA, Walker NM, Cooper JD, et al. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. Nat Genet 2007; 397: 857–864.

21. Cooper JD, Smyth DJ, Smiles AM, et al. Meta-analysis of genome-wide association study data identifies additional type 1 diabetes risk loci. Nat Genet 2008; 4012: 1399–1401.

22. Plagnol V, Howson JM, Smyth DJ, et al. Genome-wide association analysis of autoantibody positivity in type 1 diabetes cases. PLoS Genet 2011; 78: e1002216.

23. Hakonarson H, Qu HQ, Bradfield JP, et al. A novel susceptibility locus for type 1 diabetes on Chr12q13 identified by a genome-wide association study. Diabetes 2008; 574: 1143–1146.

24. Howard TD, Koppelman GH, Xu J, et al. Gene-gene interaction in asthma: IL4RA and IL13 in a Dutch population with asthma. Am J Hum Genet 2002; 701: 230–236.

25. Liu JZ, McRae AF, Nyholt DR, et al. A versatile gene-based test for genome-wide association studies. Am J Hum Genet 2010; 871: 139–145.

26. Baranzini SE, Galwey NW, Wang J, et al. Pathway and network-based analysis of genome-wide association studies in multiple sclerosis. Hum Mol Genet 2009; 1811: 2078–2090.

27. Bugawan TL, Klitz W, Alejandrino M, et al. The association of specific HLA class I and II alleles with type 1 diabetes among Filipinos. Tissue Antigens 2002; 596: 452–469.

28. Sheehy MJ, Scharf SJ, Rowe JR, et al. A diabetes-susceptible HLA haplotype is best defined by a combination of HLA-DR and -DQ alleles. J Clin Invest 1989; 833: 830–835.

29. Harder MN, Ribel-Madsen R, Justesen JM, et al. Type 2 diabetes risk alleles near BCAR1 and in ANK1 associate with decreased beta-cell function whereas risk alleles near ANKRD55 and GRB14 associate with decreased insulin sensitivity in the Danish Inter99 cohort. J Clin Endocrinol Metab 2013; 984: E801–E806.

30. Sun J, Matthias G, Mihatsch MJ, et al. Lack of the transcriptional coactivator OBF-1 prevents the development of systemic lupus erythematosus-like phenotypes in Aiolos mutant mice. J Immunol 2003; 1704: 1699–1706.

31. Hu SJ, Wen LL, Hu X, et al. IKZF1: a critical role in the pathogenesis of systemic lupus erythematosus? Mod Rheumatol 2013; 232: 205–209.

32. Landego I, Jayachandran N, Wullschleger S, et al. Interaction of TAPP adapter proteins with phosphatidylinositol (3,4,5)-bisphosphate regulates B-cell activation and autoantibody production. Eur J Immunol 2012; 4210: 2760–2770.

33. Liu X, Lindberg R, Xiao BG, et al. CD24 and myosin light polypeptide 2 are involved in prevention of experimental autoimmune encephalomyelitis by myelin basic protein-pulsed dendritic cells. J Neuroimmunol 2006; 1721–2: 137–144.

34. Klune JR, Dhupar R, Cardinal J, et al. HMGB1: endogenous danger signaling. Mol Med 2008; 147–8: 476–484.

35. Yin YW, Hu AM, Sun QQ, et al. Association between interleukin 10 gene -1082 A/G polymorphism and the risk of type 2 diabetes mellitus: a meta-analysis of 4250 subjects. Cytokine 2012; 622: 226–231.

36. Martinez A, Santiago JL, Cenit MC, et al. IFIH1-GCA-KCNH7 locus: influence on multiple sclerosis risk. Eur J Hum Genet 2008; 167: 861–864.

37. Smyth DJ, Cooper JD, Howson JM, et al. FUT2 nonsecretor status links type 1 diabetes susceptibility and resistance to infection. Diabetes 2011; 6011: 3081–3084.

SUPPORTING INFORMATION
Additional Supporting Information may be found in the online version of this article.

Table S1 | Information of 452 significant genes associated with type 1 diabetes mellitus.
Table S2 | (a) Enrichment of Gene Ontology (GO) term of the 452 identified genes. (b) Enrichment of Kyoto Encyclopedia of Genes and Genomes pathways of the 452 identified genes.