Follow-Up Analysis of Genome-Wide Association Data Identifies Novel Loci for Type 1 Diabetes

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OBJECTIVE—Two recent genome-wide association (GWA) studies have revealed novel loci for type 1 diabetes, a common multifactorial disease with a strong genetic component. To fully utilize the GWA data that we had obtained by genotyping 563 type 1 diabetes probands and 1,146 control subjects, as well as for 483 case subject–parent trios, using the Illumina HumanHap550 BeadChip, we designed a full stage 2 study to capture other possible association signals.

RESEARCH DESIGN AND METHODS—From our existing datasets, we selected 982 markers with P < 0.05 in both GWA cohorts. Genotyping these in an independent set of 636 nuclear families with 974 affected offspring revealed 75 markers that also had P < 0.05 in this third cohort. Among these, six single nucleotide polymorphisms in five novel loci also had P < 0.05 in the Wellcome Trust Case-Control Consortium dataset and were further tested in 1,303 type 1 diabetes probands from the Diabetes Control and Complications Study.

In stage 2, we selected markers from the same linkage disequilibrium blocks at 16p13 and 2q31-q32 for additional cohorts (stage 2) of only a small number of the results of the GWA genotyping (stage 1) or replication in additional cohorts (stage 2) of a large number of statistical GWA candidates in several independent cohorts has revealed additional loci that are associated with type 1 diabetes. The two genes at these respective loci, USH3A and BACH2, are both biologically relevant to autoimmunity.

CONCLUSIONS—Evaluation of a large number of statistical GWA candidates in several independent cohorts has revealed additional loci that are associated with type 1 diabetes. The two genes at these respective loci, USH3A and BACH2, are both biologically relevant to autoimmunity.

Type 1 diabetes is a multifactorial disease with a strong genetic component that results from autoimmune destruction of the pancreatic β-cells. The major type 1 diabetes susceptibility locus, mapping to the HLA class II genes at 6p21 (1) and encoding highly polymorphic antigen-presenting proteins, accounts for almost 50% of the genetic risk for type 1 diabetes (2). Several other loci with more modest effects are known, but they do not account for the remaining portion of the risk.

The recent development of high-throughput single nucleotide polymorphism (SNP) genotyping array technologies has enabled us (3) and others (4) to perform high-density genome-wide association (GWA) studies in search of the remaining type 1 diabetes loci. We recently reported the outcome of our GWA for type 1 diabetes in a large pediatric type 1 diabetic cohort of European descent (3); in addition to confirming previously identified loci, we observed highly significant and replicated association with KIAA0350 (now renamed CLEC16A [C-type lectin domain family 16 member A]). Subsequent follow-up of our data also revealed a locus on 12q13 (5). In parallel and independently, the Wellcome Trust Case Control Consortium (WTCCC) (4) also demonstrated replicated (6) association to the same linkage disequilibrium blocks at 16p13 and 12q13 along with two additional loci on 12q24 and 18p11.

The results that we have reported thus far were of loci that achieved statistical significance on the basis of the results of the GWA genotyping (stage 1) or replication in additional cohorts (stage 2) of only a small number of the most promising loci. Here, we describe the results of a full evaluation of all statistical candidates from the GWA phase.
RESEARCH DESIGN AND METHODS

Study populations

Type 1 diabetes cohort from Canada. The Canadian cohort consisted of 1,120 nuclear family trios (one affected child and two parents) and 267 independent type 1 diabetes cases, collected in pediatric diabetes clinics in Montreal, Toronto, Ottawa, and Winnipeg. The median age at onset is 8 years with lower and upper quartiles at 4.6 and 11 years, respectively. All patients were diagnosed under the age of 18 years and treated with insulin since diagnosis, and none have stopped treatment for any reason since. Disease diagnosis was based on these clinical criteria rather than any laboratory tests. Ethnic backgrounds were of mixed European descent, with the largest single subset (409 families) being French Canadian. The Research Ethics Board of the Montreal Children’s Hospital and other participating centers approved the study, and written informed consent was obtained from all subjects. Approval for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Complications and Interventions type 1 diabetes cohort. The Diabetes Control and Complications Trial was a multicenter randomized clinical trial to determine the effect of intensive insulin treatment with respect to reduced development and progression of retinopathy and nephropathy complications in patients with type 1 diabetes (7,8). A total of 1,441 subjects with type 1 diabetes were recruited from 29 centers across North America into the DCCT between 1983 and 1989; they were between 13 and 39 years of age, and 53% were male. They were recruited into two cohorts: the primary prevention cohort consisted of 726 subjects with no retinopathy, an albumin excretion rate <20 μg/min, and diabetes duration of 1–5 years and was studied to determine whether intensive therapy prevented the development of diabetic retinopathy in patients with no retinopathy. The secondary intervention cohort consisted of 715 subjects who had nonproliferative retinopathy, an albumin excretion rate <20 μg/min, and diabetes duration of 1–15 years and was studied to determine whether intensive therapy would affect the progression of early retinopathy (7). Approval for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Complications and Interventions (DCCT/EDIC) genetics study was provided by the Research Ethics Board of the Hospital for Sick Children, Toronto.

The Illumina 1M assay was genotyped on all available probands. To detect and remove outliers due to population stratification from the majority of self-reported white probands, Eigenstrat (9) was used to select probands by sequential analysis. After exclusions of outliers, there were 1,363 DCCT/EDIC probands (695 male and 668 female), with mean ± SD age of type 1 diabetes diagnosis 21 ± 8 years (range 0–38).

Control subjects from Philadelphia. The control group used to match with the DCCT/EDIC cases included 2,024 children with self-reported Caucasian
Table 2

| Chr | SNP   | Position | Trans: untrans | OR | TDT P | Aff allele frequency | Ctrl allele frequency | P | OR |
|-----|-------|----------|----------------|----|-------|---------------------|----------------------|---|----|
| 1   | rs1983853 | 85,083,780 | 202:254 | 0.8 | 0.015 | 0.121               | 0.151                | 0.021 | 0.779 |
| 1   | rs1230661 | 113,987,113 | 456:331 | 1.378 | 8.36 x 10^-6 | 0.267 | 0.216 | 8.75 x 10^-4 | 1.324 |
| 1   | rs4839305 | 114,035,394 | 482:354 | 1.362 | 9.5 x 10^-6 | 0.3 | 0.25 | 0.002 | 1.285 |
| 1   | rs1217407 | 114,195,271 | 505:395 | 1.278 | 2.46 x 10^-4 | 0.298 | 0.244 | 7.42 x 10^-4 | 1.316 |
| 1   | rs12566340 | 114,221,851 | 492:379 | 1.298 | 1.29 x 10^-4 | 0.288 | 0.237 | 0.0015 | 1.299 |
| 1   | rs7529353 | 114,221,985 | 474:354 | 1.339 | 3.04 x 10^-3 | 0.294 | 0.242 | 9.75 x 10^-4 | 1.309 |
| 1   | rs2358994 | 114,230,984 | 398:287 | 1.387 | 2.2 x 10^-5 | 0.232 | 0.175 | 7.11 x 10^-5 | 1.426 |
| 1   | rs7520320 | 114,336,816 | 211:169 | 1.249 | 0.031 | 0.136 | 0.107 | 0.013 | 1.315 |
| 1   | rs12029644 | 114,338,303 | 222:172 | 1.291 | 0.012 | 0.136 | 0.105 | 0.008 | 1.343 |
| 2   | rs2111485 | 162,818,782 | 417:500 | 0.834 | 0.0061 | 0.393 | 0.333 | 0.027 | 0.849 |
| 2   | rs1990760 | 162,832,297 | 422:518 | 0.815 | 0.0017 | 0.398 | 0.343 | 0.048 | 0.864 |
| 2   | rs10241611 | 204,429,997 | 534:462 | 1.156 | 0.023 | 0.439 | 0.397 | 0.02 | 1.191 |
| 2   | rs926169 | 204,430,967 | 531:459 | 1.135 | 0.048 | 0.414 | 0.387 | 0.0094 | 1.212 |
| 2   | rs2317267 | 204,449,111 | 502:472 | 1.176 | 0.014 | 0.358 | 0.321 | 0.028 | 1.184 |
| 6   | rs3757247 | 91,014,184 | 545:482 | 1.13 | 0.049 | 0.504 | 0.455 | 0.0075 | 2.126 |
| 9   | rs10758593 | 4,282,083 | 535:462 | 1.17 | 0.015 | 0.492 | 0.426 | 2.97 x 10^-4 | 1.303 |
| 9   | rs10758594 | 4,285,583 | 535:456 | 1.17 | 0.012 | 0.513 | 0.451 | 6.66 x 10^-4 | 1.282 |
| 9   | rs10758595 | 4,285,583 | 535:456 | 1.17 | 0.012 | 0.425 | 0.492 | 2.6 x 10^-4 | 0.764 |
| 9   | rs10758596 | 4,285,583 | 535:456 | 1.17 | 0.012 | 0.365 | 0.306 | 4.62 x 10^-4 | 1.308 |
| 9   | rs10758597 | 4,285,583 | 535:456 | 1.17 | 0.012 | 0.486 | 0.41 | 2.96 x 10^-5 | 1.358 |
| 9   | rs10758598 | 4,285,583 | 535:456 | 1.17 | 0.012 | 0.487 | 0.414 | 5.67 x 10^-5 | 1.343 |
| 9   | rs10758599 | 4,285,583 | 535:456 | 1.17 | 0.012 | 0.254 | 0.354 | 4.38 x 10^-9 | 0.622 |
| 9   | rs10758600 | 4,285,583 | 535:456 | 1.17 | 0.012 | 0.405 | 0.462 | 0.0016 | 0.792 |
| 9   | rs10758601 | 4,285,583 | 535:456 | 1.17 | 0.012 | 0.458 | 0.388 | 8.39 x 10^-5 | 1.356 |
| 9   | rs10758602 | 4,285,583 | 535:456 | 1.17 | 0.012 | 0.379 | 0.303 | 9.89 x 10^-6 | 1.402 |
| 15  | rs8035957 | 36,625,556 | 423:342 | 1.24 | 0.0034 | 0.304 | 0.263 | 0.011 | 1.225 |
| 16  | rs12931878 | 10,949,695 | 362:446 | 0.812 | 0.0031 | 0.161 | 0.225 | 1.01 x 10^-5 | 0.657 |
| 16  | rs1035089 | 10,955,851 | 517:451 | 1.146 | 0.034 | 0.48 | 0.42 | 8.25 x 10^-4 | 1.277 |
| 16  | rs1330041 | 10,996,309 | 291:345 | 0.844 | 0.032 | 0.172 | 0.246 | 1.01 x 10^-6 | 0.637 |
| 16  | rs725613 | 11,077,184 | 397:465 | 0.854 | 0.021 | 0.3 | 0.39 | 3.24 x 10^-7 | 0.672 |
| 16  | rs2041670 | 11,082,153 | 384:444 | 0.865 | 0.037 | 0.265 | 0.345 | 2.01 x 10^-6 | 0.682 |
| 16  | rs1763553 | 11,149,407 | 319:387 | 0.824 | 0.01 | 0.202 | 0.279 | 1.30 x 10^-6 | 0.655 |
| 21  | rs9976767 | 42,709,459 | 571:504 | 1.13 | 0.041 | 0.474 | 0.437 | 0.038 | 1.164 |

Continued on following page
The six SNPs indicated in bold type represent novel associations deemed appropriate for further investigation. For the joint analysis of the three discovery cohorts, the two family cohorts were pooled for the TDT analysis. The TDT results were combined with those of the case-control cohort by weighted z scores. Combined P values for the three cohorts are shown, together with the gene in which the markers resides or to which they are nearest. P values are two sided in each instance. *Gene not previously implicated in type 1 diabetes. Aff allele freq, minor allele frequency in affected individuals; Ctrl allele freq, minor allele frequency in unaffected individuals; TIDGC, Type 1 Diabetes Genetics Consortium; Transuntrans, transmitted/transmitted allele ratio.

data of the WTCCC set was near perfect in all cases (supplementary Table 1). As shown in Table 2, 33 markers met the P < 0.05 threshold across all four cohorts. Although the bulk of them mapped to known loci (PTPN22 [14], 12q13, KIAA0350 [3,6], IL2RA [15–17], CTLA4 [18], and IFIH1 [19]), six SNPs in five loci were completely novel. These were tested in an additional case-control cohort consisting of 1,303 type 1 diabetes probands from the DCCT/EDIC study and an independent dataset of 1,673 control subjects from Philadelphia who had been genotyped on the Illumina 1M and HumanHap550K BeadChips, respectively.

Two signals replicated in this fifth independent cohort (Table 3), and the P values were significant after correction for testing six markers (five independent loci). They map to UBAH3A (ubiquitin-associated and SH3 domain-containing protein A) and BACH2 (broad complex-tramtrack-bric-a-brac [BTB] and cap 'n' collar [CNC] homology 2). Table 4 shows that rs9876767 is in fact significant at the genome-wide level when all five cohorts utilized were combined (\(P = 2.33 \times 10^{-8}\)).

**DISCUSSION**

Taken together, our full second-stage approach and combined meta-analysis have revealed additional loci associated with type 1 diabetes. Clearly the risks are relatively modest compared with previously described associations, and it was only with this sample size at our disposal that we could detect and establish these signals as true positives through an independent validation effort.

UBASH3A is the only gene in its corresponding region of linkage disequilibrium. Mice lacking Sts2 (the mouse homologue for UBAH3A) have been shown to be normal in all respects, including T-cell function (20). Mice lacking both Sts1 and Sts2 do have increased splenocyte numbers and are hyper-responsive to T-cell receptor stimulation. It has been suggested that STS1 and STS2 are critical regulators of the signaling pathways that control T-cell activation (20).

BACH2 is also the only gene at its corresponding region of linkage disequilibrium. The gene product is a member of the small Maf family, which consists of basic region
leucine zipper proteins that function either as transcriptional activators or repressors depending on the proteins with which they heterodimerize. Muto et al. (21) found that \( \text{Bach}^2\) mice had relatively high levels of serum IgM but low levels of IgA and IgG subclasses. The \( \text{Bach}^2\) mice have also been reported to present with deficient T-cell–independent and T-cell–dependent IgG responses, leading the authors to conclude that \( \text{Bach}^2\) was a regulator of the antibody response.

It should also be noted that rs1983853 yielded a nominally significant association with type 1 diabetes in all of the cohorts but did not survive correction for multiple testing in the final validation attempt in the Toronto dataset. This SNP resides in endothelial differentiation gene 7 (\( \text{EDG7} \), formerly \( \text{LPA3} \)), which has been implicated in mechanisms of embryo implantation (22). The SNPs on \( \text{GLIS3} \) and \( \text{RASGRP1} \) were not validated. They may have been false positives in the earlier stages; alternatively, lack of replication in DCCT/EDIC may be due to different and/or weaker genetic risk determinants in this cohort with late age of onset of type 1 diabetes. This question must be addressed in future studies. The GLI-similar 3 (\( \text{GLIS3} \)) gene plays important roles in the development of pancreatic \( \beta \)-cells. Mutations in this gene cause a rare syndrome with neonatal diabetes and congenital hypothyroidism (23). The RAS guanyl releasing protein 1 (\( \text{RASGRP1} \)) gene has important roles in immune regulation, and it has been suggested that it contributes to the autoimmunity of systemic lupus erythematosus (24).

In addition to our findings, what we failed to find deserves comment. In addition to the findings described above, our study confirmed another interesting locus, rs17696736 (\( \text{C12orf30} \)) at 12q24, reported in the WTCCC study (4,6). Our GWA family cohort suggested type 1 diabetes association with \( \text{P} = 0.011 \); however, limited by the sample size, our GWA case-control cohort did not show statistical significance (\( \text{P} > 0.05 \)). To validate the type 1 diabetes association, we genotyped rs17696736 using the Sequenom iPLEX assay (Sequenom, Cambridge, MA) in the 1,120 Canadian families and the 549 Type 1 Diabetes Genetics Consortium families. The call rate of rs17696736 genotyping was 99.8%, and no Mendelian error was found. With the family-based association test (25), we confirmed the type 1 diabetes association with \( \text{P} = 8.00 \times 10^{-7} \), minor G allele frequency 0.452, and OR 1.276. However, given the very thorough coverage of European genetic variation by the Hap550 and the power of our aggregate sample size, it is very unlikely that we missed more than a very small number of common variants with an effect size approaching that of the \( \text{INS} \) (minor allele frequency 0.2 and OR 0.5; each of our three discovery cohorts has \( >99.9\% \) power to detect it at \( \alpha = 0.05 \) level) or \( \text{PTPN22} \) (minor allele frequency 0.1 and OR 1.8; each of our three discovery cohorts has \( >99.0\% \) power to detect it at \( \alpha = 0.05 \) level) loci.

Undoubtedly, larger sample sizes and meta-analysis of all available GWA data will discover an increasing number of loci with decreasing effect sizes, which are unlikely to explain the remaining familial clustering of type 1 diabetes. Such explanation should be sought, it appears, in rare variants, the detection of which is now coming within reach with the use of high-throughput methods for sequencing and for detecting structural variation.

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**TABLE 3**

Validation results for the six SNPs of interest selected from the discovery process in the DCCT/EDIC type 1 diabetes probands and CHOP control subjects.

| Chr | SNP | Position | Gene | Aff allele freq | Ctrl allele freq | OR (95% CI) | \( P \) |
|-----|-----|----------|------|----------------|-----------------|-------------|------|
| 1   | rs1983853 | 85,083,780 | \( \text{EDG7} \) | 0.132 | 0.153 | 0.842 (0.726–0.976) | 0.022 |
| 6   | rs3757247 | 91,014,184 | \( \text{BACH2} \) | \textbf{0.497} | \textbf{0.463} | \textbf{1.144 (1.033–1.268)} | \textbf{0.010} |
| 9   | rs10758593 | 4,282,083 | \( \text{GLIS3} \) | 0.429 | 0.426 | 1.013 (0.913–1.124) | 0.81 |
| 9   | rs10758594 | 4,285,583 | \( \text{GLIS3} \) | 0.434 | 0.443 | 0.963 (0.869–1.068) | 0.48 |
| 15  | rs8035957 | 36,625,556 | \( \text{RASGRP1} \) | 0.270 | 0.261 | 1.047 (0.932–1.176) | 0.44 |
| 21  | rs9976767 | \textbf{42,709,459} | \( \text{UBASH3A} \) | \textbf{0.474} | \textbf{0.436} | \textbf{1.165 (1.051–1.292)} | \textbf{0.0036} |

The two SNPs that successfully replicated are presented in bold type. Minor allele frequencies, \( P \) values, and ORs are shown together with the gene in which the markers resides or to which they are nearest. \( P \) values are two-sided in each instance. Aff allele freq, allele frequency in affected individuals; Chr, chromosome; Ctrl allele freq, allele frequency in unaffected individuals.

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**TABLE 4**

Meta-analysis of the five cohorts.

| Chr | SNP | Position | Gene | Allele | OR (95% CI) | \( P \) |
|-----|-----|----------|------|--------|-------------|------|
| 1   | rs1983853 | 85,083,780 | \( \text{EDG7} \) | A | 0.833 (0.773–0.898) | 1.87 \times 10^{-6} |
| 6   | rs3757247 | 91,014,184 | \( \text{BACH2} \) | A | 1.134 (1.078–1.193) | 1.25 \times 10^{-6} |
| 9   | rs10758593 | 4,282,083 | \( \text{GLIS3} \) | A | 1.131 (1.074–1.190) | 2.64 \times 10^{-6} |
| 9   | rs10758594 | 4,285,583 | \( \text{GLIS3} \) | A | 1.114 (1.058–1.172) | 3.51 \times 10^{-5} |
| 15  | rs8035957 | 36,625,556 | \( \text{RASGRP1} \) | C | 1.144 (1.080–1.211) | 3.92 \times 10^{-6} |
| 21  | rs9976767 | 42,709,459 | \( \text{UBASH3A} \) | C | 1.155 (1.098–1.215) | 2.33 \times 10^{-8} |

\( P \) values and ORs are shown together with the relevant allele for each of the six SNPs.
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