Association Analysis of Type 2 Diabetes Loci in Type 1 Diabetes

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OBJECTIVE—To search for a possible association of type 1 diabetes with 10 validated type 2 diabetes loci, i.e., *PPARG*, *KCNJ11*, *WFS1*, *HNF1B*, *IDE/HHEX*, *SLC30A8*, *CDKAL1*, *CDKN2A/B*, *IGF2BP2*, and *FTO/RPGRIP1L*.

RESEARCH DESIGN AND METHODS—Two European population samples were studied: *1*) one case-control cohort of 514 type 1 diabetic subjects and 2,027 control subjects and *2*) one family cohort of 483 complete type 1 diabetic case-parent trios (total 997 affected). A total of 13 tag single nucleotide polymorphisms (SNPs) from the 10 type 2 diabetes loci were analyzed for type 1 diabetes association.

RESULTS—No association of type 1 diabetes was found with any of the 10 type 2 diabetes loci, and no age-at-onset effect was detected. By combined analysis using the Wellcome Trust Case-Control Consortium type 1 diabetes data, SNP rs1412829 in the *CDKN2A/B* locus bordered on significance ($P = 0.039$) (odds ratio 0.929 [95% CI 0.867–0.995]), which did not reach the statistical significance threshold adjusted for 13 tests ($\alpha = 0.00385$).

CONCLUSIONS—This study suggests that the type 2 diabetes loci do not play any obvious role in type 1 diabetes genetic susceptibility. The distinct molecular mechanisms of the two diseases highlighted the importance of differentiation diagnosis and different treatment principles. ***Diabetes*** **57:1983–1986, 2008**

Type 1 and type 2 diabetes both result from the metabolic consequences of inadequate insulin effect and have similar complications but appear to be due to completely distinct pathogenetic mechanisms. Type 1 diabetes results from autoimmune β-cell destruction leading to insulin deficiency (1), whereas type 2 diabetes is the end point of a progressive insulin secretory defect on a background of insulin resistance (1). Both diseases are of multifactorial etiology, in which genetic predisposition plays a critical role and behaves as a complex trait. The risk to case-siblings relative to the general population is estimated to be as high as 4- to 6-fold in type 2 diabetes and 15-fold in type 1 diabetes (2).

Despite the difference in the basic pathogenetic processes for each type, an overlap in genetic predisposition has been proposed (3) and is quite plausible. For example, not all individuals with evidence of β-cell autoimmunity will develop clinical type 1 diabetes, a situation in which the factors responsible for impaired β-cell function and survival in type 2 diabetes may tip the balance (3). The role of inflammation in type 2 diabetes is increasingly recognized (4) and suggests another common link.

Of the known type 1 diabetes–associated loci, the insulin gene (*INS*) has been examined, and no type 2 diabetes association was found (5). A parent-specific association of *INS* has been (5) but has not been replicated by another study. Recently, we (6) and others (7) examined the major type 2 diabetes gene *TCF7L2* for possible type 1 diabetes association and found none. However, there has been no systematic examination of locus overlap between the two diseases; this gap in our understanding of diabetes has become more important with the proliferation of solidly replicated loci as a result of genome-wide association (GWA) studies enabled by recent technical breakthroughs. For type 2 diabetes, 11 loci have been validated involving *PPARG* (peroxisome proliferator–activated receptor γ), *KCNJ11* (potassium inwardly rectifying channel, subfamily J, member 11), *TCF7L2* (transcription factor 7-like 2), *WFS1* (Wolfram syndrome 1), and *HNF1B* (hepatocyte nuclear factor 1 homeobox B) and 6 novel type 2 diabetes–associated loci identified by GWA studies, i.e., *IDE/HHEX*, *SLC30A8*, *CDKAL1*, *CDKN2A/B*, *IGF2BP2*, and *FTO/RPGRIP1L* (8–13). The purpose of this study was to scrutinize data from our recent GWA study of type 1 diabetes in order to search for possible evidence of associated type 2 diabetes susceptibility loci.

RESEARCH DESIGN AND METHODS

Subjects and genotyping. As described in our previous report (14), two European-descent samples were studied. The first consisted of 514 type 1 diabetic subjects and 2,027 control subjects (representing the addition of 969 healthy control subjects to the set described by Hakonarson et al. [14] in order to increase statistical power) and 483 complete type 1 diabetes family trios (affected child and both parents). The average age at onset of the type 1 diabetic children was mean ± SD 7.89 ± 4.05. The median age was 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. Ethnic backgrounds were of mixed European descent. All samples were genotyped on the Illumina Infinium II HumanHap550 array (Illumina, San Diego, CA). The Research Ethics Board of the Montreal Children's Hospital, the Research Ethics Board of the Children's Hospital of Philadelphia, and other participating centers approved the study, and written informed consent was obtained from all subjects.

Type 2 diabetes–associated single nucleotide polymorphisms. As shown in Table 1, 13 type 2 diabetes–associated single nucleotide polymorphisms (SNPs) from the 10 type 2 diabetes loci were selected for the type 1 diabetes

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TABLE 1
Type 2 diabetes–associated SNPs for the type 1 diabetes analysis

Locus	Chr.	Reported type 2 diabetes association (ref)	HumanHap500 marker	HapMap CEU r^2	Genotyping success (%)	MendErr	HW *P*
PPARG	3p25	rs1801282 (15)	rs2197423	1.000	99.9	0	0.803
IGF2BP2	3q27.2	rs4402960 (10,11,13)	rs4402960	—	98.7	0	0.932
		rs1470579 (13)		0.978			
WFS1	4p16	rs10010131 (16)	rs10012946	1.000	99.6	0	0.045
CDKAL1	6p22.3	rs10946398 (10)	rs4712523	1.000	99.9	0	0.275
		rs7754840 (10,11,13)					
CDKAL1	6p22.3	rs7756992 (12)	rs7756992	—	99.9	0	0.270
SLC30A8	8q24.11	rs13266634 (9–13)	rs13266634	—	99.6	0	0.291
CDKN2A/B	9p21	rs564398 (10)	rs1412829	0.965	99.9	0	0.533
CDKN2A/B	9p21	rs10811661 (10,11,13)	rs2383208	1.000	99.9	0	0.875
IDE/HHEX	10q23.33	rs1111875 (9–11,13)	rs1111875	—	100.0	0	0.552
		rs5015480 (10)		0.958			
IDE/HHEX	10q23.33	rs7923837 (9)	rs7923837	—	100.0	0	0.856
KCNJ11	11p15	rs5215 (8,10)	rs5215	—	99.7	0	0.067
FTO	16q12.2	rs8050136 (10,11)	rs8050136	—	99.9	0	0.664
HNF1B	17cen-q21.3	rs7501939 (17)	rs7501939	—	99.7	0	0.599
		rs757210 (18)		0.811			

HW, Hardy-Weinberg equilibrium test of the control group in the case-control cohort.

analysis. They represent either the SNP originally reported as type 2 diabetes associated or a near-perfect proxy. The *PPARG* SNP rs1801282 is located at the first coding exon of PPAR-γ2 and causes the amino acid change Pro12Ala. The two *CDKAL1* SNPs rs4712523 and rs7756992 have an $r^2 = 0.747$. The two *CDKN2A/B* SNPs rs1412829 and rs2383208 have an $r^2 = 0.002$. The two *IDE/HHEX* SNPs rs1111875 and rs7923837 have an $r^2 = 0.744$. The type 2 diabetes association of *KCNJ11* was found from a non-synonymous SNP Glu23Lys (rs5219) at first. The rs5219 has an $r^2 = 0.9$ with rs5215 (8) and therefore is well tagged by rs5215 in GWA studies (8,10). All of the 13 SNPs have a genotyping success rate ≥98.7% and no Mendelian error in the 483 family trios. Only the *WFS1* SNP rs10012946 showed borderline nominal significance of divergence from Hardy-Weinberg equilibrium in the control group, which did not reach the significance threshold adjusted for 13 tests ($\alpha = 0.00385$).

Statistical methods. For the case-control cohort, the potential population stratification was corrected using the Eigenstrat algorithm (19) implemented in the Eigensoft version 2.0 software (http://genepath.med.harvard.edu/~reich/Software.htm). By the principal components analysis of population structure (19), 42 case and 130 control subjects were identified and removed as outliers. Therefore, 472 type 1 diabetic case and 1,897 control subjects were analyzed for genetic association. For the family cohort, the transmission disequilibrium test was performed using the Haploview software (www.broad.mit.edu/personal/jcbarret/haploview). For a joint analysis of the two cohorts, we combined the two z scores weighted by the sample sizes. According to the statistical power calculation for a case-control study with unequal sample sizes proposed by Fleiss et al. (20), the case-control cohort of 472 type 1 diabetic case and 1,897 control subjects has the statistical power equivalent to 756 case vs. 756 control subjects. The family cohort of 483 complete type 1 diabetic trios has the statistical power equivalent to 483 case vs. 483 control subjects. The joint Z score was calculated as:

$$z = \sqrt{\frac{756}{756 + 483}}z_1 + \sqrt{\frac{483}{756 + 483}}z_2$$

where Z_1 is of the case-control cohort and Z_2 is of the family cohort. Each Z score is equivalent to the square root of the respective χ^2 value. A protective or undertransmitted minor allele corresponds to a negative Z score, whereas a risk or overtransmitted minor allele corresponds to a positive Z score.

RESULTS AND DISCUSSION

As shown by our analysis (Table 2), none of the 13 SNPs from the 10 type 2 diabetes loci show statistically significant association. These 13 SNPs have a minor allele frequency range from 0.116 to 0.397. The statistical power of this study to detect an association from each SNP is shown in Fig. 1. Our study had sufficient power to detect an association with OR ≥1.20 for each SNP with different allele frequency. To further increase statistical power, we performed a combined analysis using the publicly available Wellcome Trust Case-Control Consortium (WTCCC) data (supplementary Table 1 [available in an online appendix at http://dx.doi.org/10,2337/db08-0270]). The WTCCC tested 2,000 type 1 diabetic case and 3,000 control subjects for 500 k SNPs (*Affymetrix* GeneChip) (8). As shown by the association analysis (Table 3), 12 of the 13 SNPs did not show statistical significance in either the WTCCC data alone or the combined analysis with our dataset. SNP rs1412829 in the *CDKN2A/B* locus met the significance threshold of $\alpha = 0.00385$ in the WTCCC data ($P = 0.002$) (OR 0.879 [95% CI 0.810–0.954]) but not in the combined analysis ($P = 0.039$) (0.929 [0.867–0.995]). *CDKN2A* and *CDKN2B* encode two specific inhibitors of cyclin-dependent kinase 4 (CDK4), i.e., $p16^{INK4a}$ and $p15^{INK4b}$, respectively. CDK5 and CDK4 play important roles in β-cell function and proliferation (10), and, as such, the locus is a reasonable functional candidate. Study of much larger cohorts will be needed to evaluate the possibility of a very weak effect in type 1 diabetes.

Our study suggests that the type 2 diabetes loci do not play any obvious role in type 1 diabetes genetic susceptibility. These known type 2 diabetes genes are mainly involved in two mechanisms, i.e., pancreatic β-cell function and peripheral insulin sensitivity. To explore whether these genes may promote the early onset of type 1 diabetes by impairing insulin secretion or insulin sensitivity, we also investigated the age-at-onset difference of different genotypes for each type 2 diabetes SNP marker. As shown by the one-way ANOVA test of age at onset of three genotypes for each SNP (Table 2), no SNP has an obvious effect on the type 1 diabetes age at onset. Unlike type 2 diabetes, type 1 diabetes typically has an acute onset that can be reliably defined.

Both type 1 and type 2 diabetes are complex diseases. With the rapid technological development of functional genomics, distinct molecular mechanisms of the two diseases are being recognized, establishing the basis of

TABLE 2
Type 1 diabetes association analysis

Name	MA	MAF	Eigenstrat χ^2	*Z* CaCo*	*Z* TDT†	*Z* (*P*)‡	Age-at-onset *F* (*P*)§
rs2197423	A	0.116	0.364	−0.604	−0.705	−0.912 (0.362)	0.648 (0.523)
rs4402960	A	0.330	0.354	−0.595	−1.732	−1.546 (0.122)	0.512 (0.600)
rs10012946	A	0.362	0.103	−0.321	−0.332	−0.458 (0.647)	1.048 (0.371)
rs4712523	C	0.340	0.898	−0.948	−0.045	−0.769 (0.442)	0.756 (0.470)
rs7756992	C	0.296	0.040	−0.201	0.105	−0.091 (0.927)	0.781 (0.458)
rs13266634	A	0.292	0.624	−0.790	−0.249	−0.773 (0.440)	0.429 (0.652)
rs1412829	C	0.370	1.572	1.254	−0.327	0.775 (0.438)	0.614 (0.542)
rs2383208	C	0.176	2.312	1.521	0.445	1.466 (0.143)	0.159 (0.853)
rs1111875	A	0.392	0.082	0.287	0.000	0.224 (0.823)	0.549 (0.578)
rs7923837	A	0.370	0.035	0.188	0.617	0.532 (0.595)	0.421 (0.656)
rs5215	C	0.347	2.946	1.716	0.651	1.747 (0.081)	0.430 (0.651)
rs8050136	A	0.397	0.458	0.677	0.338	0.740 (0.459)	0.255 (0.775)
rs7501939	A	0.396	0.154	0.392	0.000	0.306 (0.759)	0.186 (0.906)

*Z score of the case-control cohort. †Z score of the family cohort. ‡Combined Z value and P value. §One-way ANOVA test of age-at-onset difference of three genotypes. MA, minor allele; MAF, minor allele frequency.

different approaches for developing novel preventive or therapeutic strategies for type 1 and type 2 diabetes. In addition, this study highlights the importance of differentiation diagnosis of adult-onset type 1 diabetes from type 2 diabetes. Because type 1 diabetes does not share common genetic susceptibility with type 2 diabetes, it is important to manage different treatment for adult type 1 diabetic patients. Some issues remain for further studies on genetic mechanisms of type 1 and type 2 diabetes. The type 1 diabetes association of *CDKN2A/B* needs to be confirmed by an independent study with a large sample size. Assuming a multiplicative effects model, an OR of 0.929, and a minor allele frequency of 0.452, a study with 5,790 case and 5,790 control subjects has 80% statistical power to replicate the association at $\alpha = 0.05$. Both our study and the WTCCC study focused on pediatric-onset type 1 diabetes, and the possibility remains that type 2 diabetes loci may have some effect in adult-onset cases. Finally, the involvement of type 1 diabetes loci in type 2 diabetes genetics needs further investigation, the testing of which will require accurate phenotyping within the clinical spectrum of type 2 diabetes. For example, it will be interesting to

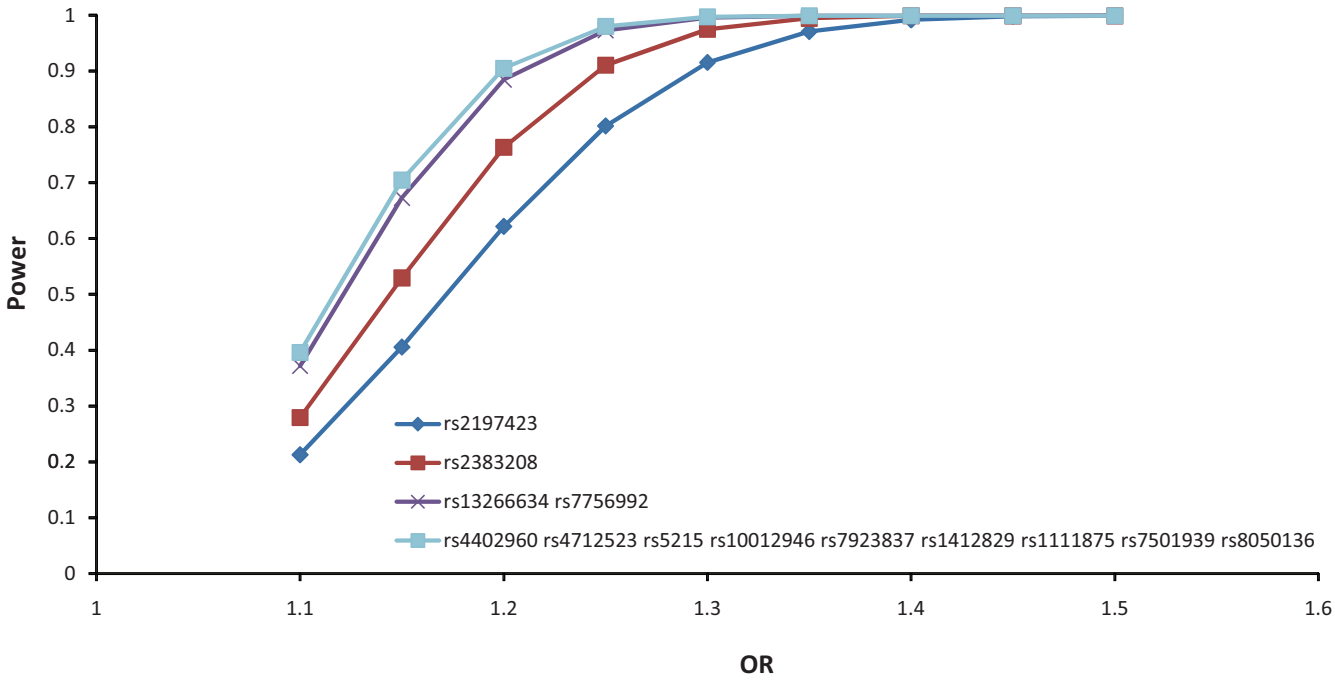

FIG. 1. The statistical power of this study to detect genetic associations with different minor allele frequencies (MAFs) at $\alpha = 0.05$ level. The *PPARG* SNP rs2197423 has an MAF = 0.116; the *CDKN2A/B* SNP rs2383208 has an MAF = 0.176; the *SLC30A8* SNP rs13266634 and the *CDKAL1* SNP rs7756992 have similar MAFs, average 0.294; the other nine SNPs, rs4402960, rs4712523, rs5215, rs10012946, rs7923837, rs1412829, rs1111875, rs7501939, and rs8050136, have similar MAFs, average 0.364. x-axis, OR value; y-axis, statistical power.

TABLE 3
Combined analysis of the WTCCC data and our data

Name	WTCCC MA	WTCCC MAF	*Z* (*P*) WTCCC	*Z* (*P*) combined*
rs2197423	A	0.124	0.047 (0.962)	−0.494 (0.621)
rs4402960	A	0.320	−1.075 (0.282)	−1.775 (0.076)
rs10012946	A	0.402	−0.753 (0.452)	−0.878 (0.380)
rs4712523	C	0.319	−0.075 (0.940)	−0.510 (0.610)
rs7756992	C	0.277	1.052 (0.293)	0.801 (0.423)
rs13266634	A	0.285	1.588 (0.112)	0.838 (0.402)
rs1412829	C	0.452	−3.094 (0.002)	−2.060 (0.039)
rs2383208	C	0.162	1.173 (0.241)	1.808 (0.071)
rs1111875	A	0.411	−1.273 (0.203)	−0.903 (0.366)
rs7923837	A	0.381	−1.367 (0.172)	−0.800 (0.424)
rs5215	C	0.354	0.951 (0.342)	1.792 (0.073)
rs8050136	A	0.398	−0.765 (0.444)	−0.190 (0.850)
rs7501939	A	0.421	−0.207 (0.836)	0.010 (0.992)

*The WTCCC data were combined with our data by weighted *Z* scores. The WTCCC 2,000 case and 3,000 control subjects have the statistical power equivalent to 2,400 case vs. 2,400 control subjects.

study all type 1 diabetes loci in the subset of insulin-resistant, non–insulin-treated, adult-onset cases that are positive for islet autoantibodies (21).

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