Original Article

Evaluation of Common Variants in the Six Known Maturity-Onset Diabetes of the Young (MODY) Genes for Association With Type 2 Diabetes

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An important question in human genetics is the extent to which genes causing monogenic forms of disease harbor common variants that may contribute to the more typical form of that disease. We aimed to comprehensively evaluate the extent to which common variation in the six known maturity-onset diabetes of the young (MODY) genes, which cause a monogenic form of type 2 diabetes, is associated with type 2 diabetes. Specifically, we determined patterns of common sequence variation in the genes encoding Gck, Ipf1, Tcf2, and NeuroD1 (MODY2 and MODY4–MODY6, respectively), selected a comprehensive set of 107 tag single nucleotide polymorphisms (SNPs) that captured common variation, and genotyped each in 4,206 patients and control subjects from Sweden, Finland, and Canada (including family-based studies and unrelated case-control subjects). All SNPs with a nominal *P* value <0.1 for association to type 2 diabetes in this initial screen were then genotyped in an additional 4,470 subjects from North America and Poland. Of 30 nominally significant SNPs from the initial sample, 8 achieved consistent results in the replication sample. We found the strongest effect at rs757210 in intron 2 of TCF2, with corrected *P* values <0.01 for an odds ratio (OR) of 1.13. This association was observed again in an independent sample of 5,891 unrelated case and control subjects and 500 families from the U.K., for an overall OR of 1.12 and a *P* value $<10^{-6}$ in >15,000 samples. We combined these results with our previous studies on HNF4α and TCF1 and explicitly tested for gene-gene interactions among these variants and with several known type 2 diabetes susceptibility loci, and we found no genetic interactions between these six genes. We conclude that although rare variants in these six genes explain most cases of MODY, common variants in these same genes contribute very modestly, if at all, to the common form of type 2 diabetes. *Diabetes* 56:685–693, 2007

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CEPH, Centre d'Etude du Polymorphisme Humain; LD, linkage disequilibrium; MODY, maturity-onset diabetes of the young; SNP, single nucleotide polymorphism.

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Common human diseases, like diabetes, cancer, and heart disease, are heritable, and yet to date only a fraction of their genetic predisposition has been explained. Positional cloning using linkage analysis in families has been successful in pinpointing the genes that cause Mendelian disorders, but it has been much less effective in identifying causative alleles in more common diseases. Many common diseases have rare Mendelian forms (rev. in 1). Studying the genes that cause these rare disorders has provided insight into the molecular biology of common diseases, often identifying novel proteins, pathways, and mechanisms. The extent to which the same genes that are responsible for monogenic cases also explain inherited risk of the more common form of each disease remains an open question.

Two genes with widely replicated association to the common form of type 2 diabetes, KIR6.2 and PPARγ, carry rare mutations that cause Mendelian disorders of glucose metabolism: neonatal diabetes (rev. in 2) and PPARγ ligand resistance syndrome (3), respectively. The Mendelian disease most phenotypically similar to typical type 2 diabetes is maturity-onset diabetes of the young (MODY),

an autosomal dominant condition characterized by early onset of hyperglycemia and a defect in the function of β-cells in the pancreas (4). Six genes are known to cause MODY (5–10), accounting for 2–5% of type 2 diabetes (4). Mutations in HNF4α, TCF1 (encoding Hnf1α), and TCF2 (encoding Hnf1β) cause abnormal regulation of transcription in β-cells, causing a defect in metabolic signaling of insulin secretion and/or β-cell mass (4). Two other transcription factors, Ipf1 and NeuroD1, cause MODY diabetes through aberrant transcriptional regulation of β-cell development and function (4). Glucokinase catalyzes the phosphorylation of glucose to form glucose-6-phosphate, the first and rate-limiting step in glycolysis (11). Mutations in the MODY genes cause β-cell dysfunction and diabetes through haploinsufficiency (12) or, rarely, through dominant-negative mechanisms (13,14). Given that most cases of monogenic diabetes are caused by mutations in these six genes, it is critical to know the extent to which more common variants contribute to the common form of type 2 diabetes.

One limitation of many complex disease studies is that they only consider association of individual alleles or haplotypes. However, susceptibility for multigenic diseases may be modulated by epistasis, or nonadditive gene-gene interactions (15). Consequently, failure to take into account interactions in an association study could lead to false negatives and impair reproducibility across studies. For example, if allele frequencies of interacting loci differ between studies, they can reduce power to detect the single-locus association in one population, leading to failure to replicate a true association (16). The six MODY genes represent an excellent candidate gene set for evaluating genetic interactions in type 2 diabetes: these genes are each known to harbor causal variants and are interconnected biologically, interacting through protein-protein interactions and by regulating one another's expression.

MODY genes have generated much interest as candidate genes for late-onset type 2 diabetes; thus far, however, associations of common variants within these genes have been negative or inconsistent. The most promising association is of common variants near the P2 promoter in HNF4α (17–20), although a number of studies have not been able to confirm these findings. Common variants in the MODY3 gene, TCF1, do not appear to influence risk of type 2 diabetes, based on several large studies (21,22).

By contrast, the other four MODY genes have not been extensively analyzed for association with type 2 diabetes, although there have been some reports on the coding and promoter variants in these genes. The G(−30)A variant upstream from the GCK promoter has been inconsistently associated with diabetes, but it has frequently been found to be associated with increased fasting glucose (23–33). There have also been several published nominal associations for variants in IPF1 with the common form of type 2 diabetes (34–37). The common A45T missense polymorphism in NEUROD1 has been evaluated by a number of groups, and a recent meta-analysis on this single nucleotide polymorphism (SNP) indicates that it does not play a role in the common form of type 2 diabetes (38). To date, very few polymorphisms in TCF2 have been evaluated for a role in the common form of type 2 diabetes, with no positive reports.

In summary, whereas the MODY genes have been resequenced in many diabetic patients, the published studies so far have not demonstrated a strong and consistent genetic effect of common variants in type 2 diabetes. However, these studies were typically modest in size and thus did not have the power to assess small effects of individual variants. In most cases, only the coding and immediately adjacent regions had previously been evaluated, leaving open the possibility that noncoding (presumably regulatory) variants may play a role. Finally, genetic interactions between these genes have not been evaluated.

To more comprehensively characterize the genotype-phenotype correlation between MODY genes and the common form of type 2 diabetes, we characterized patterns of common variation and linkage disequilibrium (LD) of GCK, IPF1, TCF2, and NEUROD1 in a reference panel and selected tag SNPs to capture the common variation at each locus. We then genotyped these markers in a large collection of patients with type 2 diabetes and control subjects. We combined these results with the genotype data from our previous studies on HNF4α and TCF1 (19,22) and tested for interactions among the six MODY loci.

RESEARCH DESIGN AND METHODS

Clinical samples. Informed consent was obtained from all participants. The phenotypic characteristics of stage 1 and 2 samples are described in Table 1. The characteristics of our samples have been described elsewhere (19,20,22,39,40,41). Plasma glucose (fasting and during an oral glucose tolerance test) was measured by a glucose oxidase method with a Beckman Glucose analyzer (Beckman Instruments, Fullerton, CA). All subjects included in this study are of Caucasian ancestry; specific matching schemes are described below.

Stage 1 clinical samples. Stage 1 clinical samples included: 321 type 2 diabetic trios; 1,189 siblings discordant for type 2 diabetes; two Scandinavian case-control samples containing 942 and 1,028 subjects, respectively; and 254 subjects from the Saguenay Lac–St. Jean region in Quebec. These case-control samples were individually matched for age, BMI, and geographic region (by study center). The type 2 diabetic patients met World Health Organization 1998 criteria for type 2 diabetes. In the trios and discordant sibling collections, severe impaired glucose tolerance was defined as >10.0 mmol/l at 120 min, with blood glucose ≥8.5 mmol/l. Age of onset was available for 641 individuals in this study, with a mean of 53.0 ± 11.7 years. The λ was 1.03, based on >500,000 genomewide markers, indicating essentially no population stratification in these samples.

Stage 2 clinical samples. The case-control samples from Genomics Collaborative were comprised of 2,452 individuals of U.S. Caucasian ancestry and 2,018 subjects from Poland. Each sample was individually matched for sex, age, and ethnicity/geographic origin (for three generations). Two studies indicate there is no substantial population stratification in these samples (42,43).

U.K. clinical samples. All type 2 diabetic subjects were unrelated U.K. Caucasian individuals who had diabetes defined either by World Health Organization criteria (44) or by being treated with medication for diabetes, and they were recruited from four sources: *1*) a young-onset collection (defined as ≥18 and ≤45 years at age of diagnosis), *2*) probands from type 2 diabetes sibships from the Diabetes U.K. Warren 2 repository described previously (45,46), *3*) a collection from the Warren 2 repository with type 2 diabetes diagnosis between 35 and 65 years, and *4*) a collection of subjects from the Oxagen resource, selected in the same way as the Warren 2 case subjects. Patients with high GAD autoantibody levels (>3 units [normal <0.5]) were excluded from the study. Control subjects were recruited from three sources: *1*) parents from a consecutive birth cohort (Exeter Family Study) with normal (<6.0 mmol/l) fasting glucose and/or normal A1C levels (<6%; Diabetes Control and Complications trial corrected) (45), *2*) U.K. Caucasians (HRC [Human Random Control]) obtained from the ECACC (European Cell Culture Collection), and *3*) a follow-up study of all people born in the U.K. during 1 week in 1958 (http://www.cls.ioe.ac.uk/Cohort/Ncds/mainncds.htm). All subjects from our family-based study were independent of those from the case-control study. Families fitting the following criteria were included: an affected proband with both parents, or one parent and at least one unaffected sibling.

Two different matching schemes were used with regard to BMI (BMI matched or not matched). This could potentially lead to false negatives; however, the following evidence indicates this is not the case in this study: *1*) no variant in this study had a significant association in all subsamples with one

TABLE 1
Clinical characteristics of patient samples

Sample	Sex (M/F)	Age (years)	BMI (kg/m^2)	Fasting plasma glucose (mmol/l)	A1C (%) or plasma glucose at 2-h OGTT (mmol/l)
GCI USA C/C					
Diabetes	644/582	63 ± 11	33 ± 7	9.8 ± 3.0	8.0 ± 3.1
NGT	644/582	61 ± 10	27 ± 5	5.1 ± 0.9	ND
GCI Poland C/C					
Diabetes	422/587	62 ± 10	30 ± 5	8.9 ± 4.0	7.9 ± 1.3
NGT	422/587	59 ± 7	26 ± 4	4.8 ± 1.2	ND
Scandinavian trios					
Probands	168/153	39 ± 9	27 ± 5	7.2 ± 2.6	8.5 ± 2.9
Parents	236/236	—	—	—	—
Sibships					
Diabetes/severe IGT sibling	280/329	65 ± 10	29 ± 5	9.3 ± 3.3	14.3 ± 5.6
NGT sibling	275/305	62 ± 10	26 ± 3	5.4 ± 0.4	6.0 ± 1.1
Scandinavia C/C					
Diabetes/severe IGT	252/219	60 ± 10	28 ± 5	9.8 ± 3.4	15.0 ± 5.3
NGT	254/217	60 ± 10	27 ± 4	6.2 ± 1.8	6.8 ± 2.8
Sweden C/C					
Diabetes	267/247	66 ± 12	28 ± 4	8.5 ± 2.5	6.5 ± 1.5
NGT	267/247	66 ± 12	28 ± 4	4.8 ± 0.7	ND
Canada C/C					
Diabetes	70/57	53 ± 8	29 ± 5	6.4 ± 1.8	12.8 ± 2.1
NGT	70/57	52 ± 8	29 ± 4	5.1 ± 0.6	6.1 ± 1.1

Data are n or the means ± SD. Plasma glucose was measured at baseline (fasting) and 2 h after an oral glucose tolerance test. C/C, case control; GCI, Genomics Collaborative; IGT, impaired glucose tolerance; ND, not determined; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test.

matching scheme and not the other, and *2*) tests of association between BMI and rs757210 and G(−30)A in all stage 1 control subjects demonstrated that these variants have no significant association with BMI (data not shown).

Genotyping. Genotyping for stage 1 and 2 samples was performed as previously described by primer extension of multiplex products with detection by matrix-assisted laser desorption ionization–time-of-flight mass spectroscopy using a Sequenom platform (47,48). For the U.K. samples, genotyping was outsourced to KBioscience (Herts, U.K.). rs757210 has a rare third allele; all results are given for A allele versus C+G.

Statistical analysis. To determine the association of each particular SNP with type 2 diabetes, we used simple χ^2 analysis in the case-control samples, the transmission disequilibrium test (49) for the trios, and the discordant allele test (50) for the stage 1 sibling pairs (using oldest unaffected sibling and a random affected sibling). The U.K. family study used SibTDT for the sibling pairs. Multimarker tests were evaluated using Haploview (51). Because this program cannot accommodate discordant sibling pairs, they were not included in the reported multimarker results. Results for the subsamples were combined using Mantel-Haenszel meta-analysis of the odds ratios (ORs) (52). Homogeneity among studies was tested using a Pearson r^2 goodness-of-fit test as previously described (52). Genetic models for rs757210 were evaluated in the five unrelated case-control samples in stage 1 and 2 using the program MODEL (http://pngu.mgh.harvard.edu/~purcell/model/model.html). Reported results are not corrected for multiple hypothesis testing.

False-positive report probability. False-positive report probability was calculated using Wacholder et al.'s (53) Excel spreadsheet showing input and output for false-positive report probability calculations. The prior probabilities used are listed in Table 4; the step 3 likely OR values entered were 1.05, 1.15, and 1.25 (only 1.15 is reported in Table 4). The OR and 95% CI values in Table 3 were used for the calculations in step 4.

Haplotype structure. To evaluate the haplotype structure of the GCK, IPF1, TCF2, and NEUROD1 regions, we selected SNPs from dbSNP and Celera based on an evenly spaced grid across the regions. These were genotyped in a multigenerational panel of 12 Centre d'Etude du Polymorphisme Humain (CEPH) pedigrees containing 96 chromosomes, with additional SNPs added based on the extent of LD. To be included in the LD maps, SNPs were required to attain a 75% genotyping percentage, be in Hardy-Weinberg equilibrium, and have a minor allele frequency >5%. Additionally, for the family-based studies, multiple errors in Mendelian inheritance were cause for exclusion. GCK had 62 passing SNPs, spanning 83.2 kb with an average marker spacing of one per 1.3 kb. IPF1 had 23 working SNPs, covering 44.2 kb and averaging one marker per 1.9 kb. There were 80 passing SNPs in the TCF2 region, spanning 76.3 kb with an average SNP spacing of one per 0.95 kb. There were 10 passing SNPs in NEUROD1, spanning 44.1 kb with an average marker spacing of one per 4.4 kb. Haplotype structures for the GCK, IPF1, TCF2, and NEUROD1 regions are shown in supplemental Appendix 1, which can be found in an online appendix (available at http://dx.doi.org/10.2337/db06-0202).

Tag SNPs. This study was performed over several years, and as dbSNP coverage improved and methods for tag SNP selection evolved, additional tag SNPs were added. The final tag SNP set was selected using the program Tagger (54). To determine how well the final tag SNP set captured variation in these regions, SNPs were evaluated for their correlation to one another in the CEPH samples described above. On the hypothesis that any of these variants could be a putative causal variant or proxy thereof, we selected a final set of tag SNPs and multimarker combinations (>5% frequency) that had an r^2 >0.8 to all of the markers typed in the CEPH panel. For all tests, tag SNPs, and correlation values, see supplemental Appendix 2.

Genetic interactions study. To assess whether any of the MODY variants created a genetic background in which the effect of a different MODY variant on phenotype could be observed, we performed two analyses. First, we used the set of 23 variants that showed a nominal single-marker association with type 2 diabetes. For each pair of variants A and B from this set, we first stratified the cohort based on genotype at variant A and then assessed the association of variant B with type 2 diabetes phenotype (χ^2 test) in each of the subcohorts defined by genotype at A (1,518 hypotheses tested in total). The second method used the fact that epistasis implies stronger LD between two interacting loci in case subjects compared with control subjects. The assumption of no LD in control subjects for unlinked genes allows for a case-only test (simply a test for LD in case subjects). To test for interactions, canonical correlation analysis (55) was used to measure the association between the sets of markers in two genes, testing the OR for the association between markers in each gene. Permutation was used to obtain empirical significance values for both methods.

RESULTS

Study design. A multistage study design offers a powerful approach to detect modest associations while minimizing the cost of genotyping large numbers of markers (56). Our study design is shown in Fig. 1. We started by evaluating LD patterns spanning each gene, including at least 20 kb upstream and downstream of the coding regions. A dense, evenly spaced grid of SNPs from each region was genotyped in a panel composed of 30 CEPH trios (for details

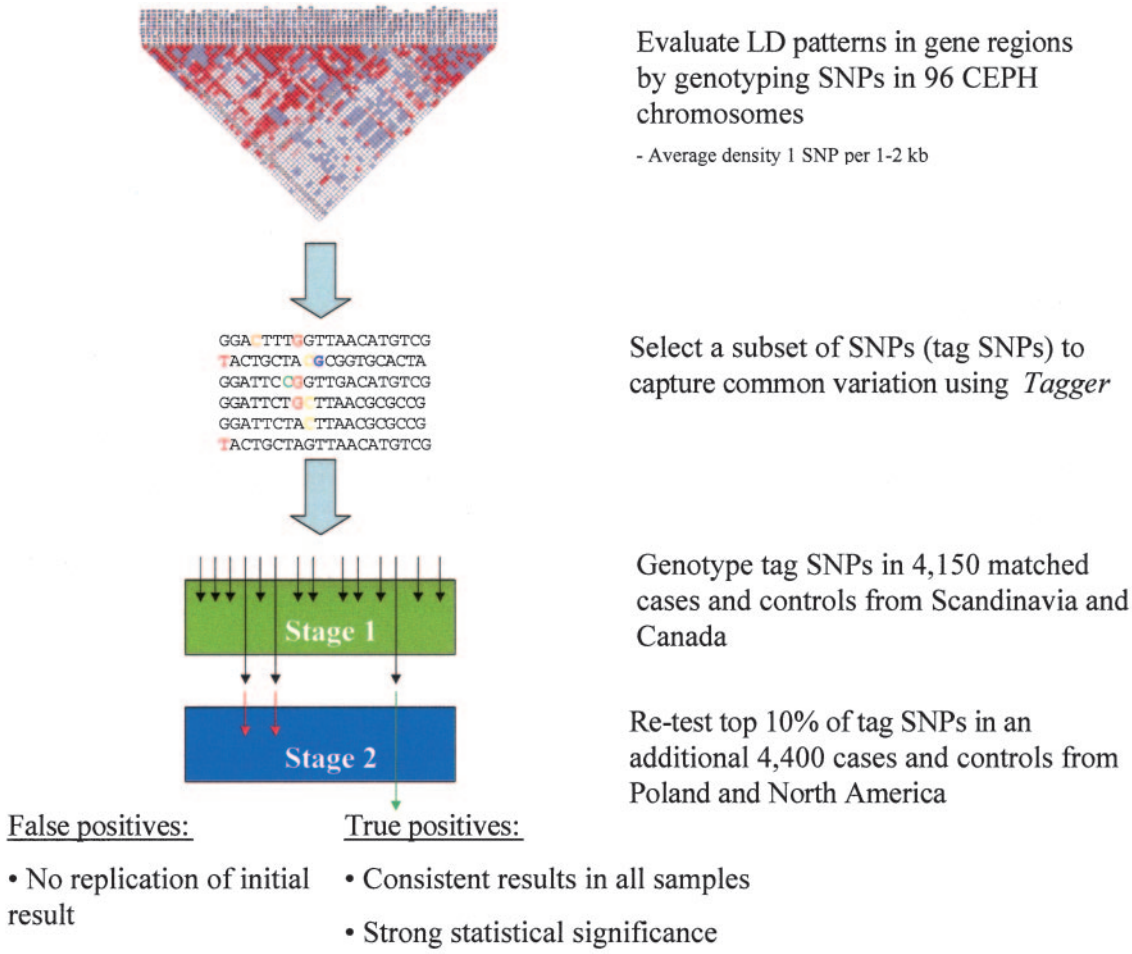

FIG. 1. A *P* value of 0.1 was selected as the threshold for stage 1. This value is liberal enough to allow SNPs with modest effects (and therefore weak statistical significance in a small sample size) to pass through, yet it excludes those SNPs that are unlikely to achieve significance in the combined sample.

see RESEARCH DESIGN AND METHODS and supplemental Appendix 1). SNP maps for HNF4α and TCF1 were reported previously (19,22). The IPF1 and NEUROD1 gene regions both showed extensive LD and limited haplotype diversity, indicating that most undiscovered SNPs are likely to be highly correlated to the SNPs already studied in these regions (48). The GCK and TCF2 regions showed more evidence of historical recombination, indicating that a higher number of tag SNPs are required to adequately capture the common variation within these genes.

We used the program Tagger (54) to analyze the CEPH LD maps, selecting a total of 135 tests comprised of 63 SNPs (from the four genes) to take forward into association studies. These tests included the common missense and previously implicated promoter variants. They provide an r^2 >0.8 to all other markers typed in the CEPH panels, indicating that they should provide strong power for both untyped reference panel SNPs and most undiscovered common SNPs when genotyped in the disease panel (supplemental Appendix 2).

Association results. The tag SNPs were genotyped in 4,206 type 2 diabetic patients and matched control subjects from Scandinavia and Canada (Table 1). For test details and complete association results, see supplemental Appendix 3. Including reanalysis of our previous data on HNF4α and TCF1 (19,22) (performed for consistency across the genes), we found a total of 30 tests that achieved a P value <0.1 in our stage 1 sample (Table 2). (Note that SNPs within genes are often correlated—for example, many of the nominally associated SNPs in TCF1 and TCF2 have high r^2 with one another.) We then genotyped the SNPs with $P < 0.1$ in stage 1 in a second independent sample of 4,470 subjects from Poland and North America. Eight SNPs attained nominally significant associations in the combined 8,676 individuals: rs730497—a perfect proxy for GCK G(−30)A—and seven SNPs in TCF2 (Table 3). The strongest statistical evidence was for rs3110641 (OR 1.16 [1.08–1.26], $P = 0.0002$) and rs757210 (OR 1.13 [1.06–1.21], $P = 0.0003$). Six of the TCF2 SNPs are located within ~1 kb of rs3110641 and are in LD with it (supplemental Appendix 1c). Therefore, it seems likely that the more modest associations observed in TCF2 may be attributable to those SNPs' correlation with the stronger signal at rs3110641.

To further evaluate the observed associations for rs3110641, rs757210, and GCK G(−30)A, we genotyped them in an additional 5,891 unrelated case and control subjects and 500 families from the U.K. The results from these samples did not support our hypothesis for rs3110641 (C allele OR of 1.04, $P = 0.33$), making it more likely that the initial observation may have been a false-positive. However, both samples did replicate the original effect seen for rs757210. The A allele had an OR of 1.10 ($P = 0.02$) in 3,640 control subjects and 2,251 case subjects

TABLE 2
Stage 1 association results with $P < 0.1$

Gene	Test	Allele	OR	*P* value
HNF4A	rs1884613	G	1.11 (0.97–1.27)	0.05
GCK	hCV329749	A	1.10 (0.99–1.19)	0.07
GCK	rs2284779	C	1.27 (1.01–1.59)	0.04
GCK	G(−30)A	A	1.15 (0.99–1.33)	0.07
	rs10259649, rs741038, rs730497, rs2908287			
GCK	rs2971667	TTCCA	1.09 (0.99–1.20)	0.08
TCF1	rs2649999	C	0.89 (0.78–1.01)	0.07
TCF1	rs2701175	A	0.87 (0.78–0.96)	0.006
TCF1	rs1920792	T	1.17 (1.06–1.30)	0.002
TCF1	GE117884_349	G	1.12 (1.01–1.25)	0.03
TCF1	GE117881_360	A	0.87 (0.79–0.97)	0.009
TCF1	rs1169289	G	0.90 (0.81–1.00)	0.04
TCF1	I27L	L	1.13 (1.01–1.25)	0.02
TCF1	rs1169292	C	0.91 (0.82–1.01)	0.08
TCF1	rs735396	A	0.89 (0.80–0.99)	0.03
TCF1	rs2258043, rs1920792	G	1.09 (0.99–1.21)	0.09
TCF1	GE117881_360	CA	0.87 (0.78–0.96)	0.007
TCF1	rs2649999, rs2244608, rs2701175	CT	0.90 (0.79–1.02)	0.1
TCF1	GE117881_360	CG	1.11 (1.00–1.23)	0.05
TCF1	rs2701175, rs1920792	CT	1.13 (1.01–1.26)	0.02
IPF1	YamadaG4, rs2297316, rs2293943	G	0.88 (0.77–1.00)	0.06
IPF1	YamadaG4, rs9512918	GT, DEL, C	0.87 (0.75–1.00)	0.04
TCF2	rs11263755	A	1.13 (0.99–1.28)	0.08
TCF2	rs2285741	A	0.91 (0.82–1.01)	0.08
TCF2	rs3110641	C	1.18 (1.04–1.34)	0.009
TCF2	rs1859211	C	0.88 (0.76–1.02)	0.10
TCF2	rs757210, rs2285741, rs10962	A	1.10 (0.99–1.23)	0.08
TCF2	rs3110641, rs10962, rs3110641	GGC	1.11 (0.99–1.23)	0.06
TCF2	rs6422978, rs11263755, rs2285741	GCA	1.14 (1.02–1.27)	0.02
TCF2	rs10962 rs3110641	AGGC	1.12 (1.00–1.24)	0.04
NEUROD1	A45T	T	0.91 (0.82–1.01)	0.06

Results given for all the MODY gene variants with tests with $P < 0.1$ in stage 1 samples. For all association results, see supplemental Appendix 3. All *P* values are two-tailed and uncorrected for multiple hypothesis testing. G(−30)A was tested by perfect proxy rs730497. HNF4α data is from Ref. 19; TCF1 data is from Ref. 22.

and, similarly, an OR of 1.13 ($P = 0.03$) in the family study ($n = 500$ trios and duos families). To determine the genetic model by which rs757210 influences diabetes risk, we analyzed rs757210 using MODEL. The results indicate that while the additive, multiplicative, and dominant models all have $P < 0.001$, the best-fitting model was the dominant model. There was no significant effect under a recessive model.

The GCK G(−30)A variant had no effect on type 2 diabetes risk in the U.K. samples (case/control OR of 0.96, family OR of 1.00). However, the GCK G(−30)A association is also supported by a number of other previous reports of diabetes/diabetes-related traits for this allele (23–33) and thus may be considered less likely to be a false positive than an SNP without such evidence.

We performed a meta-analysis of the current results and previously reported association results for rs757210 and G(−30)A with type 2 diabetes (Fig. 2). Both achieved overall statistical significance in the combined analysis; however, their overall effect on disease risk was very modest [rs757210: OR 1.12 [1.07–1.17], $P = 10^{-6}$; G(−30)A: OR 1.08, $P = 0.004$], although the effect was of similar size to other well-established type 2 diabetes genes such as P12A in PPARγ and E23K in KIR6.2.

Genetic interactions. To avoid false negatives caused by testing SNPs one at a time, we evaluated the possibility that combinations of MODY genes interact to influence type 2 diabetes. We tested pairwise combinations of genes in two ways. First, we took the SNPs with $P < 0.1$ in the initial sample and looked for significant single-marker effects of each marker by stratifying on the genotype at each of the 22 other markers. Second, we looked for genetic interactions at the level of the gene (as opposed to the allele), testing for correlation between unlinked loci in the case subjects. Both methods evaluated significance using permutation testing. We also included data from our group for the well-established type 2 diabetes susceptibility loci P12A in PPARγ, E23K in KIR6.2, and rs7903146 in TCF7L2 in our interaction tests (39,40,57). No significant interactions (threshold of $P < 0.05$ after permutation testing) between these nine candidate genes were observed using either approach.

DISCUSSION

Mendelian mutations in the MODY genes cause the vast majority of cases of monogenic type 2 diabetes, and a number of previous association studies have suggested

TABLE 3
Stage 2 association results with $P < 0.05$

Gene	SNP	Allele	Stage 1 OR	Stage 1 *P*	Stage 2 OR	Stage 2 *P*	Stage 3 OR	Stage 3 *P*	Combined OR	*P*
GCK	G(−30)A	A	1.15	0.07	1.11	0.07	0.96	0.43	1.04 (0.983–1.11)	0.21
TCF2	rs11263755	A	1.13	0.07	1.14	0.01	—	—	1.13 (1.04–1.23)	0.003
TCF2	rs2285741	A	0.91	0.08	0.95	0.13	—	—	0.94 (0.88–1.00)	0.05
TCF2	rs2689*	A	1.11	0.06	1.08	0.06	—	—	1.09 (1.02–1.17)	0.009
TCF2	rs3110641	C	1.18	0.008	1.15	0.003	1.04	0.33	1.10 (1.04–1.17)	0.0006
TCF2	rs3094513*	T	1.14	0.02	1.05	0.26	—	—	1.08 (1.01–1.16)	0.02
TCF2	rs3110640	C	1.12	0.04	1.04	0.32	—	—	1.07 (1.00–1.14)	0.04
TCF2	rs757210	A	1.10	0.08	1.14	0.001	1.11	0.005	1.12 (1.07–1.18)	5×10^{-6}

Results are given for the all tests with $P < 0.1$ in stage 1 samples and $P < 0.05$ in stage 2 samples. "Stage 3" indicates combined U.K. samples. For all stage 2 association results, see supplemental Appendix 3. All *P* values are two-tailed and uncorrected for multiple hypothesis testing. G(−30)A was tested by perfect proxy rs730497. Multiple attempts to genotype SNP rs7331478 (tested by rs2297316, rs2293943, YamadaG4, and rs9512918 in Table 2) failed, so we could not determine whether this SNP is associated in the larger sample. *Tested by multimarker comparison in stage 1.

that missense SNPs within these genes might be associated with common type 2 diabetes. Thus, before beginning this study, we estimated that the prior probability of at least one of these genes being involved in type 2 diabetes was quite high. Despite this, we did not find any major effects on type 2 diabetes in the MODY genes, and only a single variant in TCF2 (with a very modest OR) was reproduced across multiple large studies and achieved a high level of overall statistical significance.

We found it useful to use Wacholder et al.'s (53) Bayesian approach to communicating the posterior probability that these associations are false positives. Table 4 shows the false-positive report probability over a range of possible prior probabilities for the OR of 1.15 in our combined 8,676-subject study. The table indicates that if the reader assumes a relatively high a priori likelihood that common variants in MODY genes play a role in type 2 diabetes (1–10%), our best result (rs757210) has a low probability of being a false positive (<7%). GCK G(−30)A has a 6–42% false-positive probability, although one could argue that because G(−30)A is implicated in >10 diabetes-related studies, an even higher prior probability may be indicated for this particular SNP. Given the known role of MODY genes in type 2 diabetes, these relatively high prior probabilities do not seem at all unreasonable, and thus one or both of these associations may be real.

A concurrent study on the MODY genes in type 2 diabetes was performed by Bonnycastle et al. (58). They also concluded that there are no common variants in these genes that have large effects on type 2 diabetes risk, but, based on nominal associations, they generated hypotheses that several variants may confer modest effects. None of their leading hypotheses were associated in our samples; conversely, our best result (rs757210) showed a consistent but nonsignificant OR (1.10 [0.93–1.30]) in their 1,408 case and control subjects from Finland.

One possible reason for the varying association results is that our groups used different statistical criteria to declare association and differently powered samples. The modest effects seen in our study would provide little power in a study of the size performed by Bonnycastle et al. (58). Another possible explanation is cryptic population heterogeneity or an unknown genetic or environmental modifier that differs between the two samples (although we note that all positive associations genotyped by both reports fail a formal test for heterogeneity; also, both studies examined large numbers of Scandinavian samples). We note that all populations studied are of European ancestry, and therefore one cannot extrapolate this result to other populations of more diverse ancestry.

Over the past few years, inconsistent replication has obscured the role of several MODY genes in common type 2 diabetes. One possibility is that a failure to replicate a positive result may be caused by some unknown genetic interaction with an allele that varies in frequency between study populations, limiting the power to detect the association in the second sample. It is plausible that combinations of variants in risk genes could be required to consistently demonstrate an effect on disease. To test this hypothesis, we evaluated pairwise combinations of the MODY genes and three well-established type 2 diabetes genes, PPARγ, KIR6.2, and TCF7L2. Using two methods, we found no evidence that any of these nine genes interact, suggesting that the observed inconsistency in association results is likely due to false positives. However, it remains possible that there are critical interactions with untested loci or environmental factors, with the criteria for defining case or control status, or interaction(s) among gene variants that will only be apparent on consideration of three or more loci.

In our study of the six MODY genes, we identified several common variants that may be associated with the common form of type 2 diabetes, particularly rs757210 in TCF2, which has been replicated in >15,000 samples and achieved strong overall statistical significance. Two other widely replicated common type 2 diabetes genes, KIR6.2 and PPARγ, also cause rare Mendelian forms of diabetes (2,3), yet each of these three monogenic diabetes genes carries only a modest effect on risk of common type 2 diabetes (allelic OR <1.25). This finding that in aggregate the genes responsible for most cases of monogenic type 2 diabetes contribute little to the common form of the disease has important implications for many complex traits. If the common forms of disease are most often influenced by inherited variants in genes distinct from those that cause Mendelian forms, it will suggest that the underlying pathogenic mechanisms of common forms may not be as similar as initially hoped, and that study of Mendelian subtypes alone would not necessarily identify all of the pathways and genes required to understand the common forms of disease. Combined with the fact that many type 2 diabetes candidate genes with strong biological support have been demonstrated to have no effect on the common subtype of type 2 diabetes, these results indicate that a large portion of the genetic component of

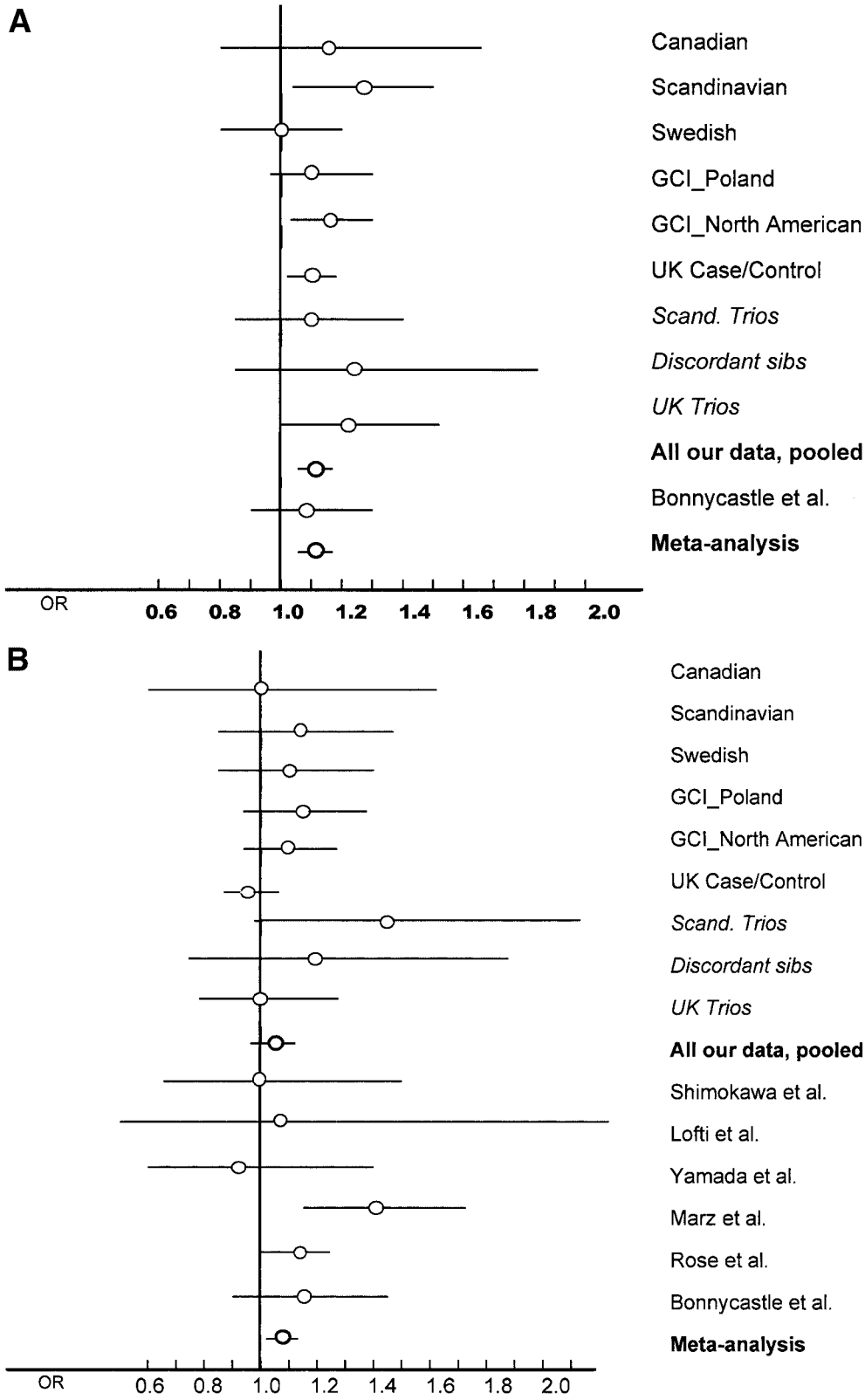

FIG. 2. *A*: TCF2 rs757210. *B*: GCK G(−30)A. For each study, the circle represents the estimated OR for the minor allele and the line indicates the extent of the 95% CI around this estimate. "Meta-analysis" indicates the combined data for all studies above it. Panel *B* includes previously published studies evaluating type 2 diabetes association between type 2 diabetic patients and normal glycemic control subjects. Family-based studies are listed in italics. GCI, Genomics Collaborative; Scand., Scandinavia.

type 2 diabetes risk may lie in genes and/or pathways not yet identified, thus requiring unbiased genomewide approaches. Whole-genome association studies (2) offer one such approach and should reveal other genes that harbor common variants that contribute significantly to risk of type 2 diabetes.

TABLE 4
False-positive report probability for associations

Prior	G(−30)A	rs11263755	rs2285741	rs2689	rs3110641	rs3094513	rs3110640	rs757210
0.25	2.1*	1.8*	22	5.2*	0.10*	10*	10*	0.21*
0.1	6.1*	5.2*	45	14*	0.29*	25	25	0.64*
0.01	42	38	90	65	3.1*	78	78	6.6*
0.001	88	86	99	95	24	97	97	42
0.0001	99	98	100	99	76	100	100	88
0.00001	100	100	100	100	97	100	100	99

Data are %. Table was prepared using Wacholder et al.'s (51) Excel spreadsheet for false-positive report probability calculations. For each variant, we inputted the OR and 95% CI listed in Table 3. *A false-positive report probability <20% (or a 20% false-positive rate) that the true OR is 1.15.

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