A Genome-Wide Association Study of IVGTT-Based Measures of First-Phase Insulin Secretion Refines the Underlying Physiology of Type 2 Diabetes Variants

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Understanding the physiological mechanisms by which common variants predispose to type 2 diabetes requires large studies with detailed measures of insulin secretion and sensitivity. Here we performed the largest genome-wide association study of first-phase insulin secretion, as measured by intravenous glucose tolerance tests, using up to 5,567 individuals without diabetes from 10 studies. We aimed to refine the mechanisms of 178 known associations between common variants and glycemic traits and identify new loci. Thirty type 2 diabetes or fasting glucose–raising alleles were associated with a measure of first-phase insulin secretion at $P < 0.05$ and provided new evidence, or the strongest evidence yet, that insulin secretion, intrinsic to the islet cells, is a key mechanism underlying the associations at the *HNF1A*, *IGF2BP2*, *KCNQ1*, *HNF1B*, *VPS13C/C2CD4A*, *FAF1*, *PTPRD*, *AP3S2*, *KCNK16*, *MAEA*, *LPP*, *WFS1*, and *TMPRSS6* loci. The fasting glucose–raising allele near *PDX1*, a known key insulin transcription factor, was strongly associated with lower first-phase insulin secretion but has no evidence for an effect on type 2 diabetes risk. The diabetes risk allele at *TCF7L2* was associated with a stronger effect on peak insulin response than on C-peptide–based insulin secretion rate, suggesting a possible additional role in hepatic insulin clearance or insulin processing. In summary, our study provides further insight into the mechanisms by which common genetic variation influences type 2 diabetes risk and glycemic traits.

Common genetic variants associated with type 2 diabetes are more likely to be associated with insulin secretion than insulin resistance (1). Studies of genetic variation and insulin secretion have been largely limited to fasting glucose– or oral glucose tolerance test (OGTT)–based measures of β-cell function and insulin secretion (2,3). Oral-based measures of insulin secretion do not distinguish between mechanisms

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involving gut hormone signaling, e.g., incretin pathways, and mechanisms intrinsic to islet cell function or mass.

Compared with OGTT-based measures, intravenous-based measures provide a more accurate measure of first-phase insulin secretion, with initial release of insulin peaking in the first 5–10 min following glucose stimulation. Intravenous measures include the intravenous glucose tolerance test (IVGTT) and hyperglycemic clamp. Family studies have shown that first-phase insulin response as measured by IVGTT is one of the most highly heritable glycemic measures (4–9), but genetic studies of intravenous-based measures of insulin secretion have examined limited numbers of variants or been performed in single studies (8,10–14), with the exception of a recent meta-analysis performed in Hispanic Americans (15).

Two studies have examined the effects of known type 2 diabetes variants in large meta-analyses of studies with OGTT data. A study of 23,443 individuals with OGTT-based measures of insulin secretion and insulin resistance, with a subset of 4,180 individuals with clamp-based measures of insulin resistance, examined 36 known type 2 diabetes variants (2). This study classified 16 variants into groups: nine were labeled as "β-cell," two as "hyperglycemia," four as "insulin resistance," and one as "insulin processing" (based on proinsulin measures). This analysis left 20 variants as "unclassified," which may include those that do not operate through these mechanisms as defined or may reflect a lack of power to distinguish mechanisms when the type 2 diabetes risk effect is relatively weak. A second study performed a six-study genome-wide association study (GWAS) meta-analysis of OGTT-based measures of insulin secretion, including the corrected insulin response (CIR; insulin response corrected to glucose at 30 min during an OGTT) (3). This study provided genome-wide data from 10,831 individuals and identified a signal in *GRB10* but otherwise did not identify any variants not previously identified as type 2 diabetes variants.

Here we performed a meta-analysis–based GWAS of intravenous-based measures of glucose-stimulated insulin secretion. We used several measures of first-phase insulin

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secretion with two aims: first, to refine the underlying physiology of known type 2 diabetes and glycemic trait variants, and second, to identify novel variants associated with first-phase insulin secretion. Our study provides an advance to previous studies in several ways. First, it is the largest GWAS meta-analysis of intravenous-based measures of glucose-stimulated insulin secretion. Second, we used imputation from the 1000 Genomes Project to capture a wider range of genetic variation than previous GWAS of glycemic traits. Third, we focused on characterizing the most recent lists of known type 2 diabetes and glycemic trait variants. Using more than 5,500 individuals with intravenous measures of first-phase insulin secretion, we show that most variants previously associated with insulin secretion, as measured by OGTT, operate through a primary islet cell–based mechanism, and we provide new insight into the mechanisms of several variants where previous data had been unclear.

RESEARCH DESIGN AND METHODS

Study Samples

The meta-analysis consisted of a total of 10 studies and a maximum of 5,567 individuals, with the full number available depending on the phenotype. These studies represented several different ethnic groups, with 3 studies of 2,346 Hispanic (Insulin Resistance Atherosclerosis Family Study [IRASFS] [16], Troglitazone In the Prevention Of Diabetes [TRIPOD] [17], BetaGene [18]), 6 studies of 2,900 individuals of European ancestry (European Network on Functional Genomics of Type 2 Diabetes [EUGENE2] [19], Relationship between Insulin Sensitivity and Cardiovascular Disease [RISC] [20], hyperglycemic clamp cohorts [HCC] [13], YOUTH 92 [21], FAMILY [21], and Finland-United States Investigation of NIDDM Genetics [FUSION] [22]), and 1 study of 332 Pima Indians (23). All studies were genotyped with a GWAS chip except the 413 HCC participants and a subset of 328 of 554 individuals from the FUSION study who were typed with the MetaboChip (24). Full descriptive characteristics, study design, sample size, sample quality control, and intravenous measurement techniques for the included studies are provided in Supplementary Tables 1–3. All participants provided written informed consent, and the studies were approved by the respective local research ethics committees or institutional review boards.

Phenotypes

The 10 studies each used a version of the IVGTT test. FUSION, YOUTH 92, FAMILY, and TRIPOD used tolbutamide-modified IVGTTs, and IRASFS and BetaGene used insulin-modified IVGTTs. In the RISC study, the IVGTT was conducted at the end of an isoglycemic clamp as previously reported (25). In the HCC study, participants underwent a hyperglycemic clamp after an overnight fast. After the priming glucose bolus, blood glucose was measured at 2- to 2.5-min intervals and kept constant at 10 mmol/L for at least 2 hours via continuous variable glucose infusions (13). The EUGENE2 study used a 0.3 g/kg body weight glucose bolus and the Pima Indian study used a 25-g glucose bolus.

Peak Insulin Response

Peak insulin response was measured as peak insulin minus baseline insulin. The peak insulin time point was determined for each study, according to the time point having the highest average insulin value across all individuals.

Acute Insulin Response

Acute insulin response (AIR) was measured as the incremental area under the insulin curve during the first 10 min, or if a measure at 10 min was not available, during the first 8 min, using the trapezium equation (26), with a minimum of insulin values at 0, 2, 4, 6, and 8 min during the IVGTT. Incremental insulin was calculated by subtracting the fasting insulin level.

Insulin Secretion Rate

Insulin secretion rate (ISR) was estimated from measured serum C-peptide concentrations at 0,2,4,6,8 (RISC) and 0, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, and 19 (FAMILY) min, using the ISEC software (27,28), which calculates the secretion rate based on predefined C-peptide kinetic parameters from each individual's weight, height, age, sex, and clinical status (glucose tolerance and obesity status) determined in a population-based study (29,30). ISR provides an estimate of the rate of insulin secretion prior to hepatic insulin clearance.

Insulin Sensitivity

We used the MINMOD software (31) to calculate insulin sensitivity or a method suitable to the study (e.g., for hyperglycemic clamps, see 't Hart et al. [13]).

Disposition Index

Disposition index (DI) was calculated as the product of AIR, and insulin sensitivity index was calculated using the MINMOD software (31). DI differs from peak insulin response and AIR because it is not a pure test of insulin secretion but takes into account the level of background insulin resistance.

OGTT Measures of Insulin Secretion as a Comparison

CIR was based on OGTT. To compare IVGTT-based results to OGTT-based results, we calculated the CIR in a subset of 2,523 individuals from five of our studies. Calculation was the same as that used and described by Prokopenko et al. (3): CIR = (100 × insulin at 30 min)/(glucose at 30 min × [glucose at 30 min − 3.89]).

Genotyping and Imputation

Genotyping and Imputation Within Studies

Details on the genotyping platform used and genotype quality control procedures used for each study are presented in Supplementary Table 3. All GWAS cohorts were genotyped using commercially available Affymetrix (Santa Clara, CA) or Illumina (San Diego, CA) genotyping arrays. To facilitate meta-analyses for each trait, studies performed genotype imputation using MACH (32), MINIMAC (33), or IMPUTE (34) to impute up to a common set of variants. All studies (except the Pima study) imputed up to ~39 million

single nucleotide polymorphisms (SNPs) and indels from 2,184 haplotypes available from the 1000 Genomes Project Phase 1, version 3 (35). Because of the relative difference in ancestry between the Pima cohort and the samples within the 1000 Genomes reference panel, imputation in Pima was based on 532 haplotypes derived from whole-genome sequencing efforts within the Pima study.

MetaboChip Genotyping

Additional studies genotyped on the Illumina MetaboChip without subsequent imputation but with available phenotype data were also incorporated into the meta-analysis. Details of these studies can be found in Supplementary Tables 1–3.

Statistical Analysis

Phenotype Transformations

Each trait was adjusted for age, sex, and study-specific covariates as necessary (Supplementary Table 2) by adding them to a regression model and using the residuals as the phenotype. We then inverse normalized this residualized phenotype to create a normal distribution. This process is important to reduce false-positive results when testing thousands of rarer variants. Analyses were repeated adjusting for BMI and insulin sensitivity. To account for population stratification, studies also adjusted for principal components or if running association testing outside of a linear mixed-model framework.

Association Analysis

Additive association analysis for each trait was carried out using MACH2QTL (32), SOLAR (for IRASFS), or linear mixed models, as implemented in EMMAX (36), GEMMA (37), or QTassoc (38) (Supplementary Table 3). For each trait and adjustment combination, we performed a fixed-effects meta-analysis based on standard errors, as implemented in METAL (http://csg.sph.umich.edu/abecasis/Metal). We applied a variant minor allele count filter of >5 and genomic control correction to the input files prior to meta-analysis. Variants with a meta-analysis $P < 5 \times 10^{-8}$ were considered to be genome-wide significant. All genome-wide statistics are available on our website (http://www.t2diabetesgenes.org/data/).

Selection of Known Variants and Previous Traits

Type 2 Diabetes

We selected 76 variants identified by GWAS as associated with type 2 diabetes. For European studies, these were based on a GWAS + MetaboChip meta-analysis of 34,840 case and 114,981 control subjects (39), and for non-Europeans, this included variants at GWAS significance across a *trans*-ethnic study meta-analysis of 26,488 case and 83,964 control subjects (40).

Glycemic and Insulin-Related Traits

We selected variants representing 65 signals listed in the supplementary or main tables of Prokopenko et al. (3) as associated with a glycemic or insulin-related trait, including fasting glucose, fasting insulin, 2-h insulin, HbA_{1c}, and proinsulin. We also selected an additional five variants associated with fasting glycemic traits identified by an earlier meta-analysis that fell 250 kb outside of the 65 signals (41).

RESULTS

Several Variants Are Associated With Intravenous-Based Measures of First-Phase Insulin Secretion at Genome-Wide Significance, Including *MTNR1B* and *CDKAL1*

Results are represented in Tables 1–5 and Figs. 1, 2, 3, and 4. The two strongest association signals represented known type 2 diabetes loci, those in or near *MTNR1B* and *CDKAL1* (Table 1). The known signal at *MTNR1B* was associated with peak insulin response ($P = 1.3 \times 10^{-24}$), AIR ($P = 3.7 \times 10^{-21}$), and DI ($P = 3.3 \times\times 10^{-17}$), and *CDKAL1* with peak insulin response ($P = 1.5 \times 10^{-12}$) and AIR ($P = 1.5 \times 10^{-9}$). The peak insulin response and AIR results were very similar after adjusting for BMI and/or SI (Supplementary Table 4). In addition, we identified a few novel genome-wide associations that require further validation and replication; these associations were either rare variants (*REG3G*), only present in a specific ethnic group (*CHST1*), or sensitive to covariates used (*BLVRA/MRPS24*) (Supplementary Table 5). We tested these novel variants for an association with type 2 diabetes in the DIAbetes Genetics Replication and Meta-analysis (DIAGRAM) GWAS (40), but none of the SNPs were associated with type 2 diabetes at $P < 0.05$.

Twenty-one Known Type 2 Diabetes Risk Alleles Are Associated With Lower First-Phase Insulin Response

Of 76 type 2 diabetes known risk alleles, 21 were associated (at $P < 0.05$) with reduced first-phase insulin secretion as measured by peak insulin response or AIR (17 variants were associated with both peak insulin response and AIR at $P < 0.05$) (Table 1). Peak insulin response and AIR associations tended to be very similar for all variants (Table 1). This number of risk alleles associated with reduced insulin secretion at $P < 0.05$ is far more than the 2–3 expected by chance. Three additional type 2 diabetes risk alleles were associated with higher first-phase insulin response and are discussed below (*NOTCH2*, *PPARG*, and *GCC1*). Results were similar when adjusting for BMI and insulin sensitivity (Supplementary Table 4). These 21 variants included 10 previously classified as having a clear role in insulin secretion, 8 were previously classified as "β-cell," 1 as "hyperglycemia" (*MTNR1B*), and 1 as "insulin processing" (*ARAP1/STARD10*) (2). We did not detect any evidence that the variant previously labeled as "β-cell" in the *THADA* gene was associated with first-phase insulin response. We were not able to account for the potential parent of origin effect at THADA (42), but neither were the previous largest OGTT-based studies. Of the 11 other variants we detected, those in the *HNF1A*, *IGF2BP2*, and *KCNQ1* genes had previously been associated with at least one measure of insulin secretion or fasting glucose, and our data now strengthens the evidence that these variants increase type 2 diabetes risk

Table 1—Type 2 diabetes risk alleles associated with lower first-phase insulin response, AIR, or peak insulin response ($P < 0.05$), from a total of 76 analyzed

						Peak insulin response			AIR		
Locus	OGTT/fasting*	Lead SNP	Chr	Position‡	Risk allele	β	SE	*P* value	β	SE	*P* value
MTNR1B	**HG (1,2)**	**rs10830963**	**11**	**92,708,710**	**G**	**−0.235**	**0.023**	**1.34E-24**	**−0.218**	**0.023**	**3.65E-21**
CDKAL1	**BC (1,2)**	**rs7756992**	**6**	**20,679,709**	**G**	**−0.152**	**0.022**	**1.50E-12**	**−0.131**	**0.022**	**1.45E-09**
HNF1A	**UC (1,2)**	**rs12427353**	**12**	**121,426,901**	**G**	**−0.141**	**0.029**	**1.07E-06**	**−0.136**	**0.029**	**3.15E-06**
IGF2BP2	**UC**	**rs4402960**	**3**	**185,511,687**	**T**	**−0.101**	**0.022**	**4.90E-06**	**−0.091**	**0.022**	**4.65E-05**
TCF7L2	**BC (1)**	**rs7903146**	**10**	**114,758,349**	**T**	**−0.105**	**0.024**	**7.39E-06**	**−0.103**	**0.024**	**1.43E-05**
ARAP1 (CENTD2)	**PROINS (2)**	**rs1552224**	**11**	**72,433,098**	**A**	**−0.128**	**0.030**	**1.92E-05**	**−0.140**	**0.030**	**3.54E-06**
SLC30A8	**BC (1)**	**rs3802177**	**8**	**118,185,025**	**G**	**−0.089**	**0.022**	**5.88E-05**	**−0.090**	**0.022**	**5.94E-05**
ADCY5	**BC (2)**	**rs11717195**	**3**	**123,082,398**	**T**	**−0.092**	**0.023**	**8.43E-05**	−0.078	0.023	8.33E-04
KCNQ1	**UC (1,2)**	**rs163184**	**11**	**2,847,069**	**G**	**−0.075**	**0.020**	**1.52E-04**	**−0.082**	**0.020**	**3.73E-05**
C2CD4A	N/A (1,2)	rs7163757	15	62,391,608	C	−0.072	0.022	8.05E-04	−0.071	0.022	9.34E-04
HHEX/IDE	BC (1)	rs1111875	10	94,462,882	C	−0.061	0.020	0.003	−0.058	0.020	0.005
CDKN2A/B	**BC (1)**	**rs10811661**	**9**	**22,134,094**	**T**	−0.087	0.029	0.003	**−0.102**	**0.030**	**6.00E-04**
FAF1	N/A	rs17106184	1	50,909,985	G	−0.104	0.039	0.008	−0.091	0.039	0.020
PTPRD	N/A (1)	rs17584499	9	8,879,118	T	−0.063	0.027	0.020	−0.062	0.027	0.023
PROX1	BC	rs2075423	1	214,154,719	G	−0.050	0.022	0.021	−0.060	0.022	0.006
AP3S2	N/A (2)	rs2028299	15	90,374,257	C	−0.057	0.026	0.026	−0.052	0.026	0.045
HNF1B	UC (1)	rs4430796	17	36,098,040	G	−0.045	0.022	0.039	−0.066	0.022	0.003
MAEA	N/A (2)	rs6815464	4	1,309,901	C	−0.059	0.031	0.060	−0.063	0.031	0.043
KCNK16	N/A	rs1535500	6	39,284,050	T	−0.041	0.022	0.060	−0.045	0.022	0.041
DGKB	BC	rs17168486	7	14,898,282	T	−0.044	0.024	0.061	−0.050	0.024	0.034
LPP	N/A	rs6808574	3	187,740,523	C	−0.039	0.023	0.090	−0.048	0.023	0.040

β represents per allele effects in SD. Associations reaching Bonferroni equivalents of $P < 0.05$ are in bold typeface. Chr, chromosome. *Association ($P < 0.05$) with OGTT-based measure of insulin secretion or fasting glucose, as reported by: 1, Prokopenko et al. (3) (CIR) and 2, this study ($CIR_{BMI + SI\ adjusted}$), as classified by Dimas et al. (2): HG, hyperglycemia-reduced β-cell function after glucose stimulation; BC, defective β-cell function; PROINS, decreased proinsulin; UC, unclassified; N/A, not available. ‡Base pair position build-37.

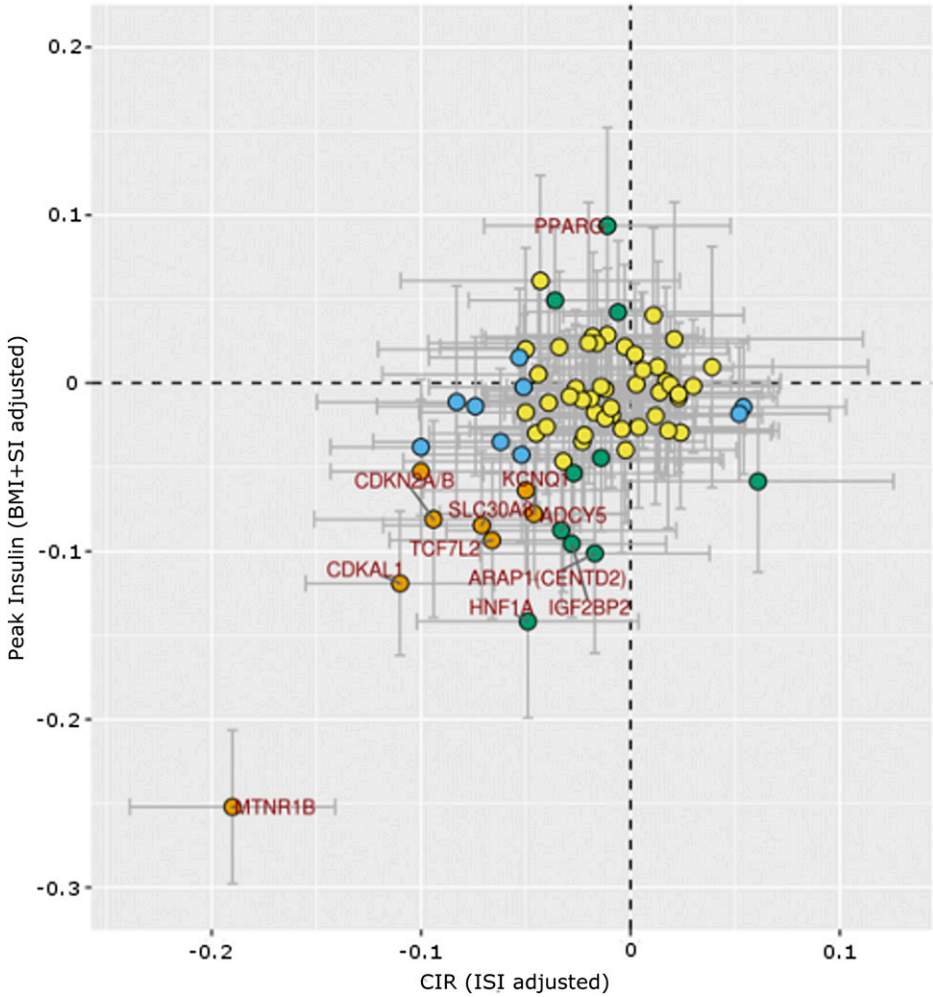

Figure 1—IVGTT (peak insulin response)-based first-phase insulin secretion vs. OGTT-based insulin secretion (CIR) for known type 2 diabetes variants. Data are SD. Orange circles, SNP associated with both peak insulin response and CIR ($P < 0.05$); green circles, SNP associated with peak insulin response ($P < 0.05$); blue circles, SNP associated with CIR ($P < 0.05$); yellow circles; SNP not associated with either trait ($P > 0.05$). ISI, insulin sensitivity index.

through an insulin secretory mechanism, including lower first-phase insulin response. Our findings provide new evidence that type 2 diabetes variants in the loci labeled as in or near the *HNF1B*, *VPS13C/C2CD4A*, *FAF1*, *PTPRD*, *AP3S2*, *KCNK16*, *MAEA*, and *LPP* genes alter type 2 diabetes risk through mechanisms that include first-phase insulin secretion. Although none of these 8 reached Bonferroni-corrected levels of significance, we would only expect 2–3 of 76 type 2 diabetes risk alleles to be associated with lower insulin secretion at $P < 0.05$, suggesting most of these 8 variants operate through insulin secretion mechanisms (Table 1).

Six Variants Associated With Higher Fasting Glucose but Not Type 2 Diabetes Are Associated With Lower First-Phase Insulin Secretion

We next examined 70 known variants associated with intermediate glycemic traits. These traits consisted of those analyzed by the Meta-Analyses of Glucose and Insulin-Related Traits Consortium (MAGIC) and included fasting glucose and insulin, proinsulin, HbA_{1c}, and 2-h post-OGTT glucose levels. These variants partially overlap those associated with type 2 diabetes. We identified 6 variants not in the type 2 diabetes list where the fasting glucose–raising allele was associated with first-phase insulin secretion before (Table 2) and after correcting for BMI and insulin sensitivity (Supplementary Table 6). Fasting glucose–raising alleles in or near the *PDX1*, *DNLZ*, *CRY2*, *GLIS3*, *PROX1*, and *ADRA2A* genes were associated with lower first-phase insulin secretion at $P < 0.05$. We next examined published data from the DIAGRAM consortium to establish whether or not these alleles were associated with type 2 diabetes but had not reached genome-wide significance—only the allele at *PDX1* was not nominally associated with type 2 diabetes ($P > 0.05$) in Morris et al. (39). All five of the other alleles associated with higher fasting glucose and lower first-phase insulin were associated with a higher risk of type 2 diabetes with P values of 0.03 (*CRY2*, odds ratio [OR]

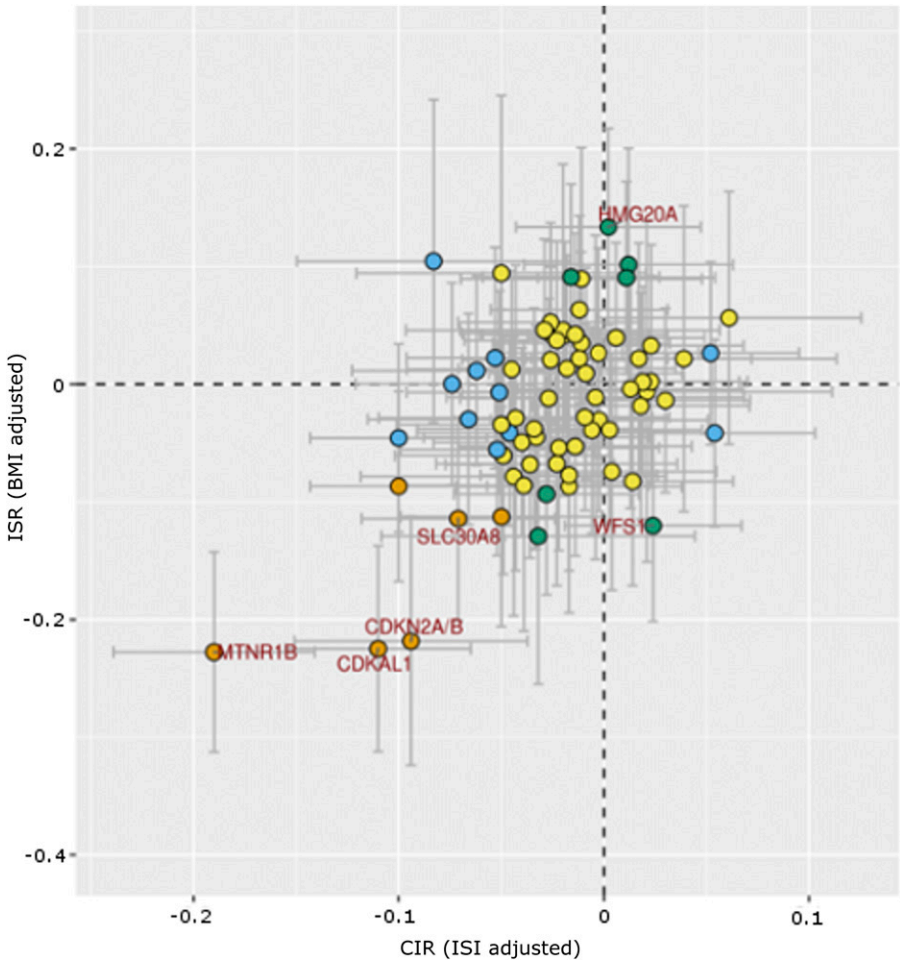

Figure 2—ISR- vs. OGTT-based insulin secretion (CIR) for known type 2 diabetes variants. Data are SD. Orange circles, SNP associated with both ISR and CIR ($P < 0.05$); green circles, SNP associated with ISR ($P < 0.05$); blue circles, SNP associated with CIR ($P < 0.05$); yellow circles, SNP not associated with either trait ($P > 0.05$). ISI, insulin sensitivity index.

1.03), 0.001 (*ADRA2A*, OR 1.06), 0.0001 (*GLIS3*, OR 1.04), 0.0001 (*DNLZ*, OR 1.06), and 1×10^{-7} (*PROX1*, OR 1.06) (39).

Ten Known Type 2 Diabetes or Glycemic Trait Alleles Are Associated With Lower ISR

For a subset of 1,268 individuals without diabetes, we had a measure of ISR by C-peptide deconvolution (27,30). For 10 known variants, the type 2 diabetes or glycemic trait risk allele was associated with lower ISR at $P < 0.05$. These analyses highlighted 2 variants that had no clear underlying physiological profile based on previous OGTT data or our own peak insulin response or AIR analyses—those in *WFS1* and *TMPRSS6* (Table 3). Of these 10 variants previously associated with a glycemic trait and associated with ISR in our study, 2 were not known type 2 diabetes variants—those in *TMPRSS6* (HbA_{1c}-raising allele associated with lower ISR) and *PDX1* (fasting glucose–raising allele associated with lower ISR). The *TMPRSS6* allele, like the *PDX1* allele, was not nominally associated with type 2 diabetes in the DIAGRAM study ($P > 0.05$).

Sixteen Variants Where the Type 2 Diabetes Risk Allele Is Apparently Paradoxically Associated With Higher Insulin Secretion or ISRs

We identified 16 variants with apparently paradoxical effects on type 2 diabetes risk, glycemic traits, and first-phase insulin secretion or ISR. These included 3 (*PPARG*, *FTO, TET2*) with known primary effects on insulin resistance (43) or BMI and 3 (*ARAP1*, *PCSK1*, *MADD*) (44) with known primary effects on proinsulin. Although the effects on insulin secretion were similar when correcting for insulin resistance and BMI, the associations with DI tended to be weaker (Tables 4 and 5).

DISCUSSION

By performing a large GWAS of first-phase IVGTT-based insulin secretion, we provide new insights into the likely mechanisms by which some of the known type 2 diabetes and glycemic trait variants affect glucose homeostasis. Our results complement those from OGTT-derived measures of insulin secretion and emphasize the need to consider first-phase, second-phase, and C-peptide–derived measures of insulin secretion and insulin resistance when considering

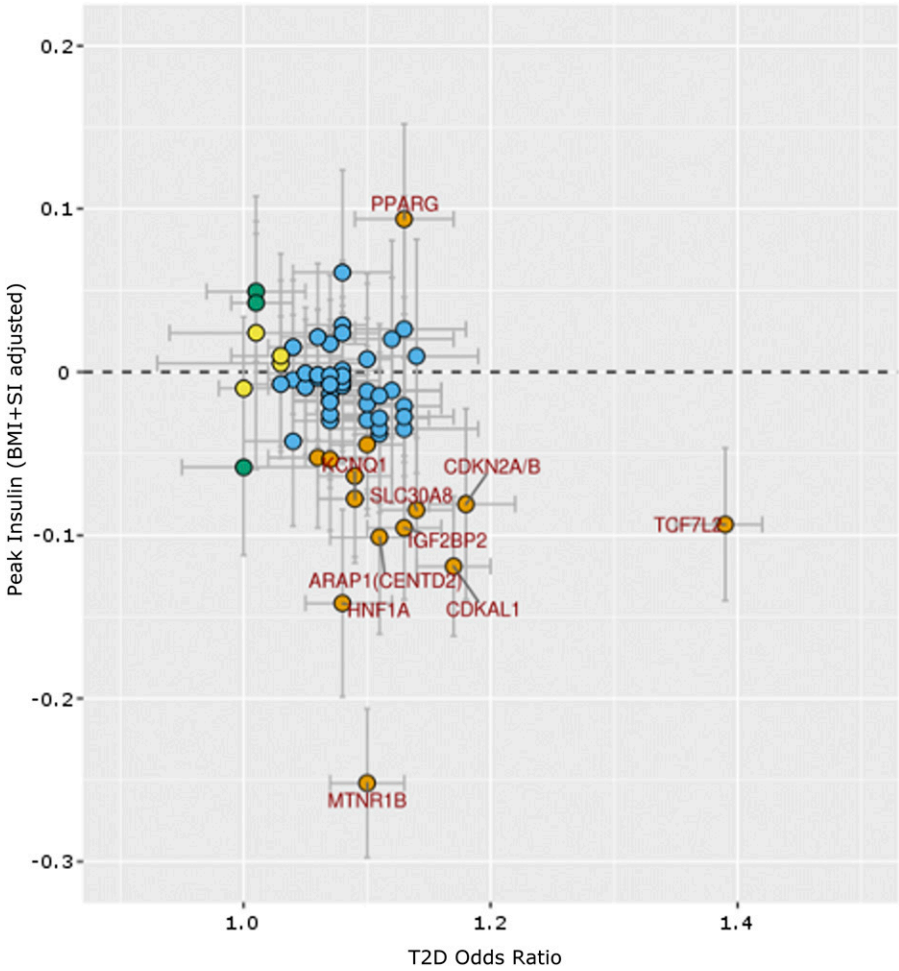

Figure 3—IVGTT (peak insulin response)-based first-phase insulin secretion vs. type 2 diabetes risk (OR) for known type 2 diabetes variants. The *y*-axis data are SD. Type 2 diabetes ORs are from Morris et al. (39), and some were reported from previous studies of East Asians (59,60). Orange circles, SNP associated with both peak insulin response and type 2 diabetes risk ($P < 0.05$); green circles, SNP associated with peak insulin response ($P < 0.05$); blue circles, SNP associated with type 2 diabetes risk ($P < 0.05$); yellow circles, SNP not associated with either trait ($P > 0.05$). T2D, type 2 diabetes.

the likely function of type 2 diabetes–associated alleles. We provide details of 178 previously described associations in Supplementary Table 8. We did not identify any robust associations between new variants and IVGTT-based measures of insulin secretion and so we focus this discussion on the known variants. The lack of novel variants is perhaps not surprising given the large studies of type 2 diabetes performed and relative power, and the likelihood that any variant with a strong effect on first-phase insulin secretion is likely to have been associated with type 2 diabetes or an OGTT-based measure of insulin secretion. Previous family studies have also shown strong genetic overlaps between OGTT-derived CIR and IVGTT-derived AIR (45).

Known Type 2 Diabetes Risk Alleles Are Associated With Lower First-Phase Insulin Secretion in Response to Intravenous Glucose

We found that 21 of the alleles previously associated with higher type 2 diabetes risk are also associated with lower insulin secretion during IVGTT at $P < 0.05$. Associations were similar with DI, which corrects for insulin sensitivity. Those with the strongest effects, and the only ones reaching genome-wide significance, were those in or near the *MTNR1B* and *CDKAL1* genes. In addition to classifications based on OGTT-derived measures (2), we can now also classify a number of previously unclassified loci as being involved in β-cell function. These include *IGF2BP2*, *C2CD4A*, *FAF1*, *PTPRD*, *AP3S2*, *NF1B*, *MAEA*, *KCNK16*, and *LPP*. Of the nine variants previously labeled as "β-cell" by Dimas et al. (2), eight were associated with first-phase insulin secretion, the exception being that in the *THADA* gene. On the basis of the analysis of Dimas et al., this variant is more likely to operate on fasting glucose rather than stimulated glucose tolerance.

A Common Allele Upstream of *PDX1* Is Associated With Higher Fasting Glucose and Lower First-Phase Insulin Secretion but Not Type 2 Diabetes

We identified six alleles that were associated with lower first-phase insulin secretion that were previously associated

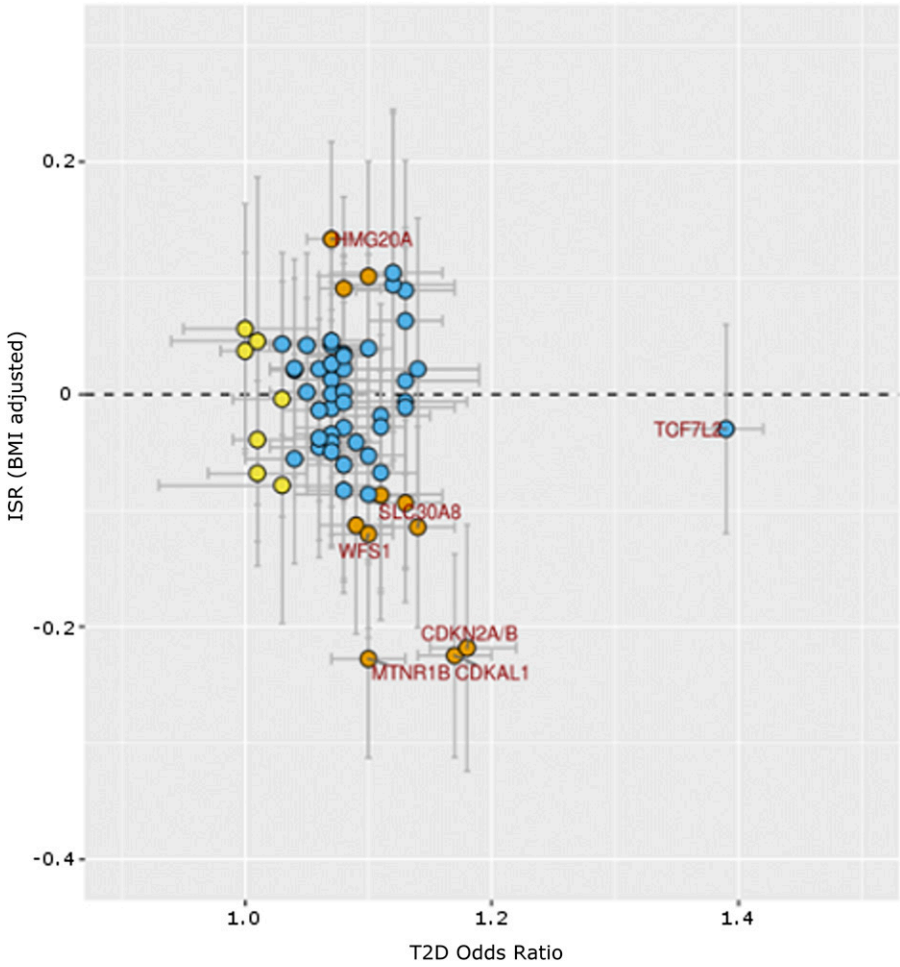

Figure 4—ISR vs. type 2 diabetes risk for known type 2 diabetes variants. The *y*-axis data are SD. Orange circles, SNP associated with both ISR and type 2 diabetes risk ($P < 0.05$); green circles, SNP associated with ISR ($P < 0.05$); blue circles, SNP associated with type 2 diabetes risk ($P < 0.05$); yellow circles, SNP not associated with either trait ($P > 0.05$). T2D, type 2 diabetes.

with higher fasting glucose levels but were not associated, at genome-wide significance, with type 2 diabetes risk. These include those in or near *PDX1*, *DNLZ*, *CRY2*, *GLIS3*, *PROX1*, and *ADRA2A* genes. Five of these six variants are nominally associated with type 2 diabetes risk in the expected direction. The exception is the allele ~6.6 kb upstream of *PDX1*, a gene in which mutations cause maturity-onset diabetes of the young (46). This allele has the third strongest association with first-phase insulin secretion in our study, after those in *MTNR1B* and *CDKAL1*, and ahead of those in known type 2 diabetes loci, such as *TCF7L2*, *SLC30A8*, *IGF2BP2*, *CDKN2A/B*, and *HHEX/IDE*, but was not associated with type 2 diabetes in the most recent, multiethnic, type 2 diabetes study of 26,488 case and 83,964 control subjects. One explanation for this apparently paradoxical association is that the *PDX1* allele causes a stable resetting of glucose tolerance but does not lead to deterioration in β-cell function, as is seen in maturity-onset diabetes of the young 2. We also note that it is not associated with oral-based measures of insulin secretion (3).

Variants With Apparently Paradoxical Effects on Type 2 Diabetes Risk, Glycemic Traits, and First-Phase Insulin Secretion

We identified 16 variants with an apparently paradoxical effect on at least one measure of insulin secretion and type 2 diabetes risk—the type 2 diabetes risk allele was associated with higher insulin secretion. Many of these associations were much weaker when using DI rather than peak insulin response or AIR, suggesting the association with higher insulin secretion is a compensatory mechanism for higher background insulin resistance (*FTO*, *PPARG*) (43) or less efficient insulin processing (*MADD*, *ARAP1*, *PCSK1*) (44). The exceptions were the alleles in *GRB10* and *G6PC2*, where correcting for insulin resistance or using DI did not appreciably weaken the association. At both these loci, previous studies have noted the paradoxical associations between the allele associated with higher fasting glucose and higher OGTT-based insulin secretion (3). It was also previously shown that the effect of the *G6PC2* gene was dependent on glycemia, which may explain these apparent paradoxical results and suggests that effects of

Table 2—Fasting glucose–raising alleles associated with lower first-phase insulin response but not identified as a type 2 diabetes risk allele

					Peak insulin response			AIR		
Locus	Lead SNP	Chr	Position†	Effect allele	β	SE	*P* value	β	SE	*P* value
PDX1	**rs11619319**	**13**	**28,487,599**	**G**	**−0.106**	**0.023**	**2.54E-06**	**−0.115**	**0.023**	**3.74E-07**
DNLZ	**rs3829109**	**9**	**139,256,766**	**G**	**−0.088**	**0.022**	**5.77E-05**	**−0.089**	**0.022**	**4.83E-05**
CRY2	rs11607883	11	45,839,709	G	−0.047	0.020	0.017	−0.055	0.020	0.005
GLIS3	rs10814916	9	4,293,150	C	−0.044	0.020	0.029	−0.046	0.020	0.023
PROX1	rs340874	1	214,159,256	C	−0.041	0.020	0.039	−0.056	0.020	0.006
ADRA2A	rs11195502	10	113,039,667	C	−0.069	0.035	0.052	−0.079	0.036	0.026

β represents per allele effects in SD. Associations reaching Bonferroni equivalents of $P < 0.05$ are in bold typeface. Chr, chromosome. †Base pair position build-37.

hyperglycemia may override genetic effects observed in healthy volunteers (47).

Known Type 2 Diabetes or Glycemic Trait Alleles Associated With Lower ISR

For a subset of 1,268 individuals without diabetes, we had a measure of ISR by C-peptide deconvolution, a measure of insulin secretion that accounts for hepatic insulin clearance (29,30). Eighteen known variants were nominally associated with ISR at $P < 0.05$; 10 where the type 2 diabetes or glycemic trait risk allele was associated with lower ISR, and 8 where the risk allele was associated with higher ISR. These analyses highlighted 2 variants that had no clear underlying physiological profile based on previous data or our own peak insulin response or AIR analyses, those in *WFS1* and *TMPRSS6,* although one large study showed the *WFS1* allele as associated with oral-based measures of insulin secretion (48). The statistical confidence of these associations was not strong and further studies are needed to confirm them. The diabetes risk alleles associated with higher ISR are either likely to reflect the need for higher insulin secretion to remain without diabetes given a primary effect on insulin resistance (e.g., *HMGA2*) or insulin processing (e.g., *PCSK1*) or need further data to support the findings.

Alleles With Disproportionate Effects on Different Traits

We compared the effects of known variants across different traits (Figs. 1–4). Previous studies have highlighted that some known type 2 diabetes variants appear to have disproportionately small or large effects on type 2 diabetes risk compared with their effects on fasting glucose or insulin secretion (2). Here we highlight how measures of first-phase insulin secretion help refine these comparisons. Several variants are noteworthy. First, our most notable finding is that of the common variant 6 kb upstream of *PDX1*, which is the third most strongly associated locus with first-phase insulin secretion (peak insulin) but there is no evidence it affects type 2 diabetes risk even in the latest very large type 2 diabetes case-control study (40). Unlike the alleles in or near

Table 3—Known type 2 diabetes and glycemic trait variants associated with ISR

								ISR		
Locus	Trait	Classification†	Association pattern‡	Lead SNP	Chr	Position‖	Risk allele	β	SE	*P* value
MTNR1B	**T2D/FG**	**HG**	**1,2,3,4**	**rs10830963**	**11**	**92,708,710**	**G**	**−0.232**	**0.043**	**9.01E-08**
CDKAL1	**T2D/FG**	**BC**	**1,2,3,4**	**rs7756992**	**6**	**20,679,709**	**G**	**−0.232**	**0.045**	**1.91E-07**
CDKN2A/B	**T2D/FG**	**BC**	**1,2,3**	**rs10811661**	**9**	**22,134,094**	**T**	**−0.224**	**0.054**	**3.46E-05**
WFS1	T2D	UC	—	rs4458523	4	6,289,986	G	−0.124	0.041	0.003
SLC30A8	T2D/FG	BC	1,2,3	rs3802177	8	118,185,025	G	−0.122	0.044	0.006
TMPRSS6	HbA_{1c}	N/A	—	rs855791	22	37,462,936	A	−0.114	0.042	0.006
PDX1	FG	N/A	1,2,4	rs11619319	13	28,487,599	G	−0.129	0.049	0.008
ANK1	T2D	N/A	3	rs516946	8	41,519,248	C	−0.113	0.048	0.018
HHEX/IDE	T2D	BC	1,2,3	rs1111875	10	94,462,882	C	−0.086	0.041	0.037
IGF2BP2	T2D/FG	UC	1,2	rs4402960	3	185,511,687	T	−0.091	0.044	0.037

N = 1,268. β represents per allele effects in SD. Associations reaching Bonferroni equivalents of $P < 0.05$ are in bold typeface. Chr, chromosome; FG, fasting glucose; T2D, type 2 diabetes. †Classification by Dimas et al. (2): HG, hyperglycemic; BC, β-cell; UC, unclassified; N/A, not available. ‡Code relating to significance of association across phenotypes and data sets: 1, associated at $P < 0.05$ with peak insulin response in our data; 2, associated at $P < 0.05$ with AIR in our data; 3, associated with CIR in Prokopenko et al. (3); 4, associated at $P < 0.05$ with $CIR_{BMI + SI}$ adjustment in our data. ‖Base pair position build-37.

Table 4—Apparently paradoxical associations between known glycemic variants and first-phase insulin secretion

						Peak insulin response				AIR				DI¶
Locus	Known trait*	Lead SNP	Chr	Position‡	Effect allele	β	SE	*P* value	*P* value (BMI + SI)‖	β	SE	*P* value	*P* value (BMI + SI)‖	*P* value (BMI)
G6PC2	FG	rs560887	2	169,763,148	C	**0.061**	**0.024**	**0.012**	**6.70E-04**	**0.074**	**0.024**	**0.002**	**8.25E-05**	**1.1E-04**
GRB10	FG	rs6943153	7	50,791,579	T	0.069	0.021	8.47E-04	0.003	0.066	0.021	0.002	0.003	0.018
OR4S1/PTPRJ#	FG	rs1483121	11	48,333,360	G	0.114	0.036	0.002	0.001	0.111	0.036	0.002	0.002	0.033
MADD	Fproinsulin	rs10501320	11	47,293,799	G	0.084	0.029	0.003	**4.12E-04**	0.090	0.029	0.002	**4.82E-04**	0.252
PCSK1	Fproinsulin	rs6235	5	95,728,898	G	**0.096**	**0.025**	**1.09E-04**	0.002	**0.104**	**0.025**	**2.98E-05**	**4.35E-04**	0.013
PPARG	FI-adjBMI	rs17036328	3	12,390,484	T	0.096	0.029	9.42E-04	0.012	**0.110**	**0.029**	**1.64E-04**	0.005	0.302
ARAP1	Fproinsulin	rs11603334	11	72,432,985	A	**0.128**	**0.030**	**1.96E-05**	8.55E-04	**0.140**	**0.030**	**3.60E-06**	**7.45E-05**	0.027
FTO	BMI	rs1421085	16	53,800,954	C	0.038	0.022	0.084	0.688	0.048	0.022	0.031	0.951	0.253
GCC1	T2D	rs6467136	7	127,164,958	G	0.052	0.022	0.015	0.024	0.050	0.022	0.021	0.033	0.068
NOTCH2	T2D	rs10923931	1	120,517,959	T	0.066	0.032	0.039	0.057	0.053	0.032	0.1	0.212	0.272

β represents per allele effects in SD. Associations reaching Bonferroni equivalents of $P < 0.05$ are in bold typeface. Chr, chromosome; FG, fasting glucose; FI-adjBMI, fasting insulin adjusted for BMI; Fproinsulin, fasting proinsulin; T2D, type 2 diabetes. *Trait previously associated with SNP. ‡Base pair position build-37. ‖*P* value after BMI + SI adjustment. ¶DI adjusted for BMI. #*PTPRJ* is the nearest nonolfactory receptor gene in the locus.

G6PC2 and *GRB10*, the allele at *PDX1* associated with lower insulin secretion and was also associated with higher fasting glucose. Second, the common variant in *TCF7L2* appears to have a disproportionately small effect on first-phase insulin secretion in response to intravenous glucose given its effect on type 2 diabetes and in comparison with other variants. This observation is consistent with the effect of this variant on OGTT-based measures of insulin secretion (2). There is emerging evidence that *TCF7L2* influences diabetes risk through mechanisms involving multiple tissues (49–52), including a possible role on hepatic glucose production (53) in addition to direct effects at the pancreatic β-cell (49,52). One possibility is that the *TCF7L2* risk allele also affects insulin clearance, a possibility consistent with our observation that the allele has a weaker effect on ISR (which uses C-peptide as the main measure of insulin secretion, and so excludes any effects on hepatic insulin clearance from the insulin secretion measure) than peak insulin response (Figs. 3 and 4). Another possibility is that the effect of the *TCF7L2* risk allele on diabetes risk additionally depends on impaired incretin action (54,55) and impaired proinsulin processing (56,57), mechanisms not directly assessed in the current study. Third, our data are also consistent with previous data on OGTT-based measures that show the variant at *MTNR1B* has a disproportionately large effect on insulin secretion and fasting glucose levels compared with its effect on type 2 diabetes, possibly as a result of an additional effect on insulin action (58).

Strengths and Limitations

Our study has several strengths and limitations. Although our sample size of ~5,500 subjects is modest relative to previous OGTT-based measures, we have used the largest sample size yet for an intravenous-based measure of insulin secretion. Furthermore, we have characterized the most recent catalog of variants associated with type 2 diabetes and glycemic traits.

The limitations are that we had a mixed ancestry set of studies, although results in Europeans were very similar, suggesting that the known common variants have limited, if any, heterogeneous effects across different ethnic groups. Some of the associations we observed only reached nominal levels of statistical confidence, and further analyses are needed, ideally in even larger sample sizes, to characterize the approximately 50% of known variants with no clear mechanism.

Conclusions

Our study provides further insight into the mechanisms by which common genetic variation influences type 2 diabetes risk and glycemic traits, and it further supports the notion that many established genetic variants for type 2 diabetes risk confer increased risk through an effect on β-cell function.

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Table 5—Apparently paradoxical associations between known glycemic variants and ISR

Locus	Trait	Classification†	Association pattern‡	Lead SNP	Chr	Position‖	Risk allele	ISR			DI	
								β	SE	*P* value	*P* value (BMI)#	*P* value (BMI)#
GRB10	FG	N/A	3	rs6943153	7	50,791,579	T	0.143	0.045	0.001	8.25E-04	0.018
HMG20A	T2D	N/A	—	rs7178572	15	77,747,190	G	0.136	0.043	0.001	0.002	0.969
OR4S1/PTPRJ	FG	N/A	—	rs1483121	11	48,333,360	G	0.232	0.086	0.007	0.003	0.033
PCSK1	Fproinsulin	N/A	—	rs6235	5	95,728,898	G	0.108	0.044	0.015	0.02	0.013
TMEM163	T2D	N/A	2	rs6723108	2	135,479,980	T	0.094	0.042	0.024	0.03	0.854
ADAMTS9	T2D	UC	—	rs6795735	3	64,705,365	C	0.088	0.040	0.028	0.02	0.428
IKBKAP	FG	N/A	—	rs16913693	9	111,680,359	T	0.246	0.117	0.036	0.05	0.404
KLHDC5	T2D	N/A	—	rs10842994	12	27,965,150	C	0.104	0.050	0.038	0.04	0.291
TET2	FI	N/A	—	rs9884482	4	106,081,636	C	0.082	0.041	0.048	0.03	0.788

β represents per allele effects in SD. Chr, chromosome; FG, fasting glucose; FI; fasting insulin; Fproinsulin, fasting proinsulin; T2D, type 2 diabetes. †Classification by Dimas et al. (2): UC, unclassified; N/A, not available. ‡Code relating to significance of association across phenotypes and data sets: 2, associated at $P < 0.05$ with AIR in our data; 3, associated with CIR in Prokopenko et al. (3). ‖Base pair position build-37. #*P* value after ISR adjustment for BMI.

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