



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

Andrew R. Wood,¹ Anna Jonsson,² Anne U. Jackson,³ Nan Wang,^{4,5} Nienke van Leewen,⁶ Nicholette D. Palmer,⁷ Sayuko Kobes,⁸ Joris Deelen,⁹ Lorena Boquete-Vilarino,¹ Jussi Paananen,¹⁰ Alena Stančáková,¹⁰ Dorret I. Boomsma,¹¹ Eco J.C. de Geus,¹¹ Elisabeth M.W. Eekhoff,¹² Andreas Fritsche,^{13,14,15} Mark Kramer,¹² Giel Nijpels,¹⁶ Annemarie Simonis-Bik,¹² Timon W. van Haeften,¹⁷ Anubha Mahajan,¹⁸ Michael Boehnke,³ Richard N. Bergman,¹⁹ Jaakko Tuomilehto,^{20,21,22,23} Francis S. Collins,²⁴ Karen L. Mohlke,²⁵ Karina Banasik,^{2,18,26} Christopher J. Groves,²⁷ Mark I. McCarthy,^{18,27,28} Diabetes Research on Patient Stratification (DIRECT), Ewan R. Pearson,²⁹ Andrea Natali,³⁰ Andrea Mari,³¹ Thomas A. Buchanan,^{4,5,32} Kent D. Taylor,^{33,34} Anny H. Xiang,³⁵ Anette P. Gjesing,² Niels Grarup,² Hans Eiberg,³⁶ Oluf Pedersen,² Yii-Derr Chen,³³ Markku Laakso,³⁷ Jill M. Norris,³⁸ Ulf Smith,³⁹ Lynne E. Wagenknecht,⁴⁰ Leslie Baier,⁸ Donald W. Bowden,⁷ Torben Hansen,² Mark Walker,⁴¹ Richard M. Watanabe,^{4,5,32} Leen M. 't Hart,^{6,42,43} Robert L. Hanson,⁸ and Timothy M. Frayling¹

Diabetes 2017;66:2296–2309 | <https://doi.org/10.2337/db16-1452>

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

¹Genetics of Complex Traits, Institute of Biomedical and Clinical Science, University of Exeter Medical School, Royal Devon and Exeter Hospital, Exeter, U.K.

²Novo Nordisk Foundation Center for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

³Department of Biostatistics and Center for Statistical Genetics, University of Michigan, Ann Arbor, MI

⁴Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA

⁵Diabetes & Obesity Research Institute, Keck School of Medicine, University of Southern California, Los Angeles, CA

⁶Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, the Netherlands

⁷Department of Biochemistry, Wake Forest School of Medicine, Winston-Salem, NC

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

⁸Phoenix Epidemiology and Clinical Research Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Phoenix, AZ

⁹Max Planck Institute for Biology of Ageing, Cologne, Germany

¹⁰Institute of Biomedicine, University of Eastern Finland, Kuopio, Finland

¹¹Department of Biological Psychology, VU University, Amsterdam, the Netherlands

¹²Diabetes Center, Internal Medicine Unit, VU University Medical Center, Amsterdam, the Netherlands

¹³Division of Endocrinology, Diabetology, Angiology, Nephrology and Clinical Chemistry, Department of Internal Medicine IV, University of Tübingen, Tübingen, Germany

¹⁴Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Center Munich, University of Tübingen, Tübingen, Germany

¹⁵German Center for Diabetes Research (DZD e.V.), Tübingen, Germany

¹⁶EMGO+ Institute for Health and Care Research, VU University Medical Center, Department of General Practice, Amsterdam, the Netherlands

¹⁷Department of Internal Medicine, Utrecht University Medical Center, Utrecht, the Netherlands

¹⁸Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, U.K.

¹⁹Diabetes & Obesity Research Institute, Cedars-Sinai Medical Center, Los Angeles, CA

²⁰Department of Health, National Institute for Health and Welfare, Helsinki, Finland

²¹Dasman Diabetes Institute, Dasman, Kuwait

²²Department of Clinical Neurosciences and Preventive Medicine, Danube University Krems, Krems, Austria

²³Saudi Diabetes Research Group, King Abdulaziz University, Jeddah, Saudi Arabia

²⁴Medical Genomics and Metabolic Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD

²⁵Department of Genetics, University of North Carolina, Chapel Hill, NC

²⁶Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Copenhagen, Denmark

²⁷Oxford Centre for Diabetes, Endocrinology & Metabolism, University of Oxford, Oxford, U.K.

²⁸Oxford Biomedical Research Centre, National Institute for Health Research, Churchill Hospital, Oxford, U.K.

²⁹School of Medicine, University of Dundee, Dundee, U.K.

³⁰Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy

³¹Institute of Neuroscience, National Research Council, Padova, Italy

³²Department of Physiology and Biophysics, Keck School of Medicine, University of Southern California, Los Angeles, CA

³³Institute for Translational Genomics and Population Sciences, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA

³⁴Department of Pediatrics, University of California, Los Angeles, Los Angeles, CA

³⁵Department of Research and Evaluation, Kaiser Permanente Southern California, Pasadena, CA

³⁶Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark

³⁷Department of Medicine, University of Eastern Finland and Kuopio University Hospital, Kuopio, Finland

³⁸Department of Epidemiology, Colorado School of Public Health, University of Colorado Denver, Aurora, CO

³⁹Lundberg Laboratory for Diabetes Research, Department of Molecular and Clinical Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

⁴⁰Wake Forest School of Medicine, Winston-Salem, NC

⁴¹Institute of Cellular Medicine, Newcastle University, Newcastle, U.K.

⁴²Section of Molecular Epidemiology, Leiden University Medical Center, Leiden, the Netherlands

⁴³Department of Epidemiology and Biostatistics, EMGO+ Institute for Health and Care Research, VU University Medical Center, Amsterdam, the Netherlands

Corresponding authors: Timothy M. Frayling, t.m.frayling@exeter.ac.uk; Robert L. Hanson, rhanson@phx.niddk.nih.gov; Leen M. 't Hart, lmthart@lumc.nl; Richard M. Watanabe, rwatanab@usc.edu; Mark Walker, mark.walker@newcastle.ac.uk; Torben Hansen, torben.hansen@sund.ku.dk; and Donald W. Bowden, dbowden@wfubmc.edu.

Received 30 November 2016 and accepted 2 May 2017.

This article contains Supplementary Data online at <http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db16-1452/-/DC1>.

A.R.W., A.J., A.U.J., N.W., and N.v.L. contributed equally to this work.

© 2017 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. More information is available at <http://www.diabetesjournals.org/content/license>.

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): $CIR = (100 \times \text{insulin at 30 min}) / (\text{glucose at 30 min} \times [\text{glucose at 30 min} - 3.89])$.

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 pro-insulin. 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 ($P = 3.3 \times 10^{-17}$), 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

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

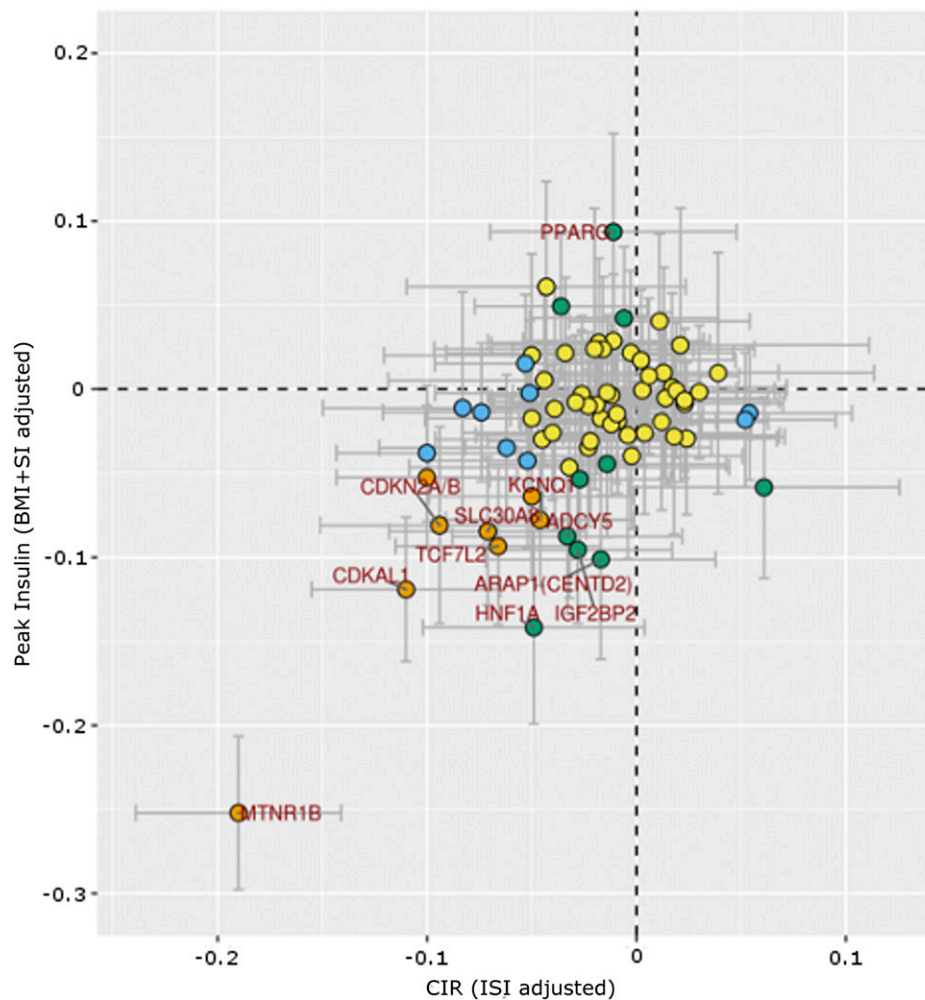


Figure 1—IVGTT (peak insulin response)-based first-phase insulin secretion vs. OGTT-based insulin secretion (CIR) for known type 2 diabetes variants. Data are SD. Orange circles, SNP associated with both peak insulin response and CIR ($P < 0.05$); green circles, SNP associated with peak insulin response ($P < 0.05$); 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]

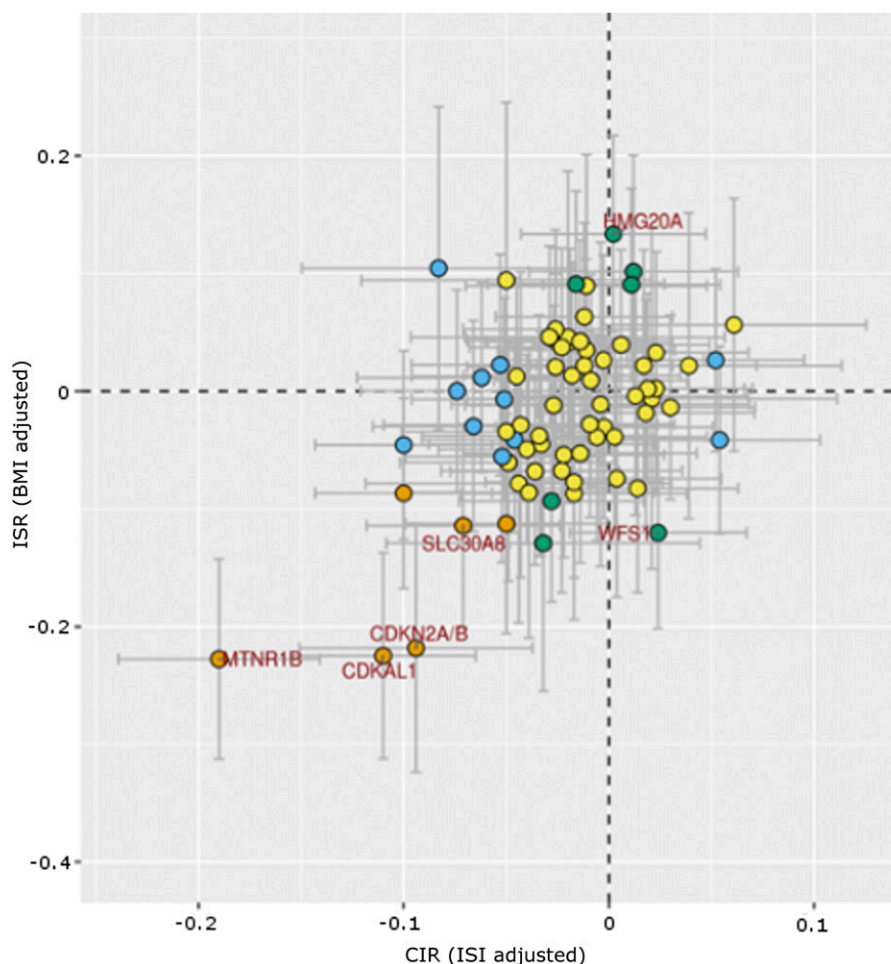


Figure 2—ISR- vs. OGTT-based insulin secretion (CIR) for known type 2 diabetes variants. Data are SD. Orange circles, SNP associated with both ISR and CIR ($P < 0.05$); green circles, SNP associated with ISR ($P < 0.05$); blue circles, SNP associated with CIR ($P < 0.05$); yellow circles, SNP not associated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

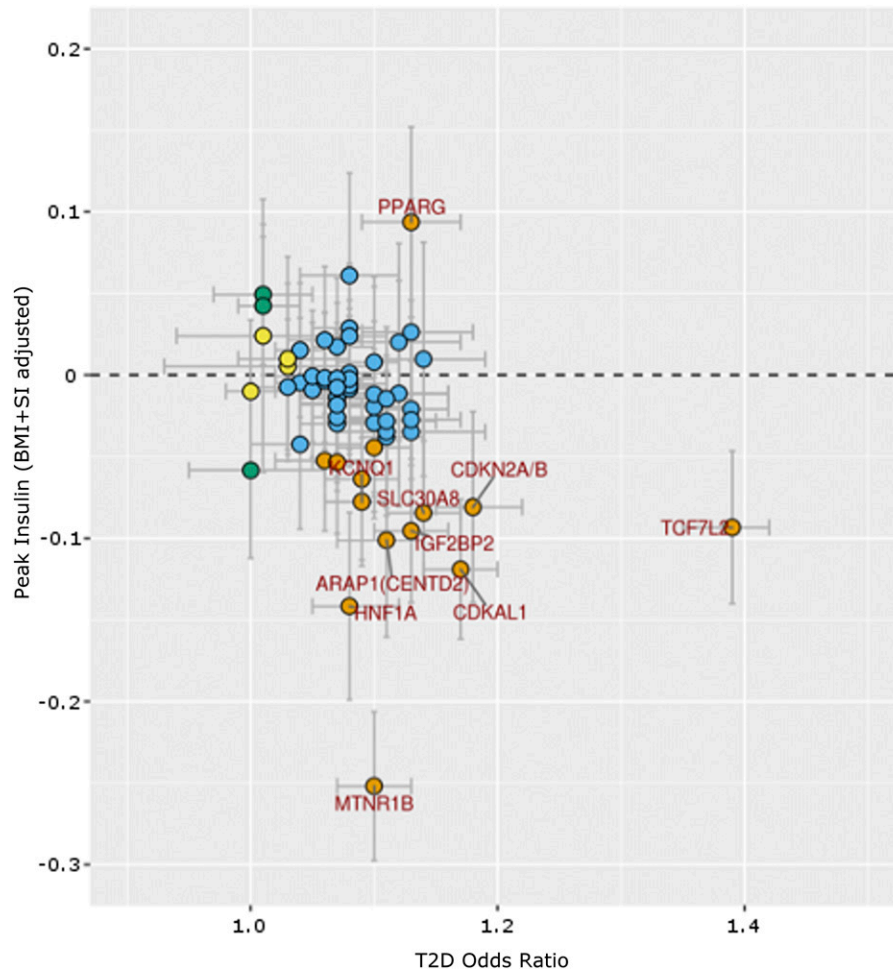


Figure 3—IVGTT (peak insulin response)-based first-phase insulin secretion vs. type 2 diabetes risk (OR) for known type 2 diabetes variants. The y-axis data are SD. Type 2 diabetes ORs are from Morris et al. (39), and some were reported from previous studies of East Asians (59,60). Orange circles, SNP 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

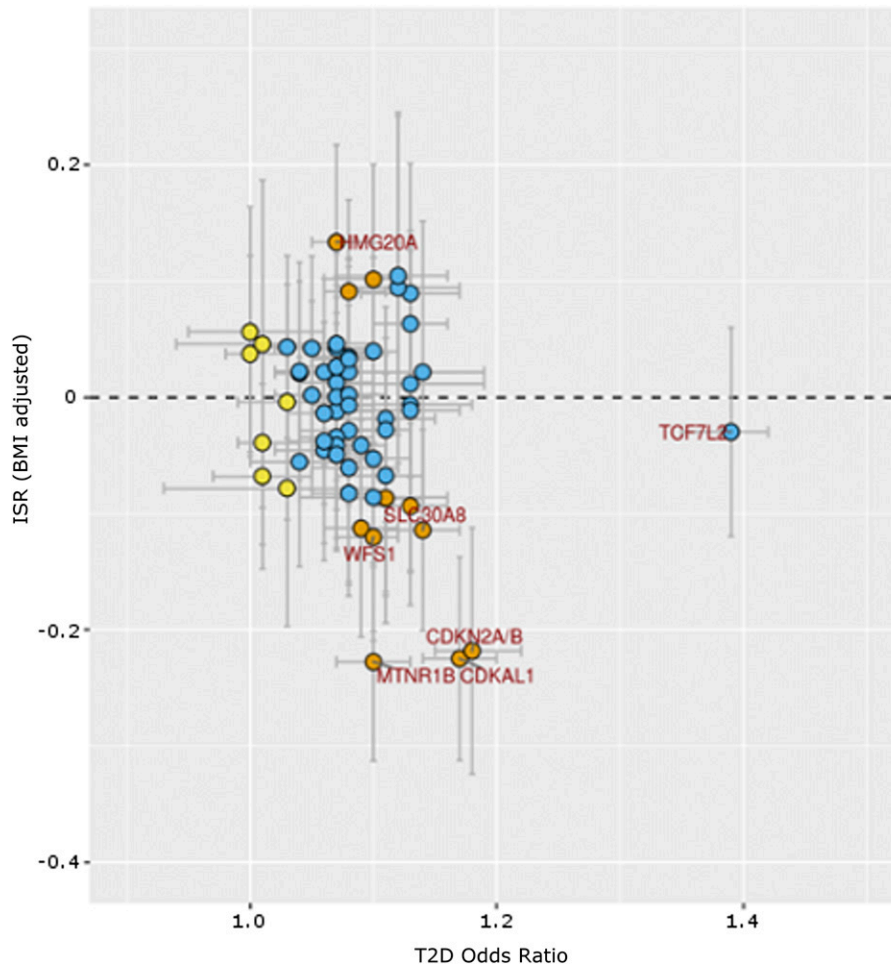


Figure 4—ISR vs. type 2 diabetes risk for known type 2 diabetes variants. The y-axis data are SD. Orange circles, SNP associated with both ISR and type 2 diabetes risk ($P < 0.05$); green circles, SNP associated with ISR ($P < 0.05$); blue circles, SNP associated with type 2 diabetes risk ($P < 0.05$); 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

Locus	Lead SNP	Chr	Position†	Effect allele	Peak insulin response			AIR		
					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., *HMG2*) 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

Locus	Trait	Classification†	Association pattern‡	Lead SNP	Chr	Position	Risk allele	ISR		
								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
<i>WFS1</i>	T2D	UC	—	rs4458523	4	6,289,986	G	-0.124	0.041	0.003
<i>SLC30A8</i>	T2D/FG	BC	1,2,3	rs3802177	8	118,185,025	G	-0.122	0.044	0.006
<i>TMPRSS6</i>	HbA _{1c}	N/A	—	rs855791	22	37,462,936	A	-0.114	0.042	0.006
<i>PDX1</i>	FG	N/A	1,2,4	rs11619319	13	28,487,599	G	-0.129	0.049	0.008
<i>ANK1</i>	T2D	N/A	3	rs516946	8	41,519,248	C	-0.113	0.048	0.018
<i>HHEX/IDE</i>	T2D	BC	1,2,3	rs1111875	10	94,462,882	C	-0.086	0.041	0.037
<i>IGF2BP2</i>	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

Locus	Known trait*	Lead SNP	Chr	Position†	Effect allele	Peak insulin response						AIR		DI‡	
						β	SE	P value	P value (BMI + S)	β	SE	P value	P value (BMI + S)	P value (BMI)	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	0.018
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	0.033	0.252
OR451/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	0.013
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	0.013	0.302
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	0.027	0.253
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	0.068	0.272
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	0.253
FTO	BMI	rs1421085	16	53,800,954	C	0.038	0.022	0.084	0.688	0.048	0.022	0.031	0.951	0.068	0.272
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	0.272
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	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. §P value after BMI + SI adjustment. ¶P value after BMI + SI adjustment. #PTPRJ is the nearest nonfactory 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.

Funding. A.R.W. and T.M.F. are supported by a European Research Council grant (SZ-245 50371-GLUCOSEGENES-FP7-IDEAS-ERC), R.L.H., S.K., and L.B. are

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)#	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	
ADAMT59	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 AIF in our data; 3, associated with CIR in Prokopenko et al. (3). ||Base pair position build-37. #P value after ISR adjustment for BMI.

supported by the Intramural Research Program of the National Institute of Diabetes and Digestive and Kidney Diseases. This work was in part funded by the Innovative Medicines Initiative Joint Undertaking under grant agreement No. 115317 (Diabetes Research on Patient Stratification [DIRECT]), resources of which are composed of financial contributions from the European Union's Seventh Framework Programme (FP7/2007-2013), European Federation of Pharmaceutical Industries and Associations companies' in-kind contribution (<http://www.direct-diabetes.org/>), and the Netherlands Organisation for Health Research and Development (Priority Medicines Elderly Program 113102006). The Netherlands Twin Register, part of the HCC cohort, is supported by the European Research Council (grant 230374); by Biobanking and BioMolecular resources Research Infrastructure—The Netherlands, a research infrastructure financed by the Dutch government (NWO 184.021.007); and the Netherlands Organisation for Scientific Research (NWO 480-04-004 and NWO/SPI 56-464-14192). The RISC study was supported by European Union grant QLG1-CT-2001-01252. The current study was supported by the National Institutes of Health for IRASFS (HL060944, HL061019, HL060919, and HG007112) and by the National Institute of Diabetes and Digestive and Kidney Diseases for the Genetics Underlying Diabetes in Hispanics (GUARDIAN) Consortium (DK085175). The provision of genotyping data was supported in part by the National Institutes of Health grant UL1-TR-000124 (Clinical and Translational Science Institute) and National Institute of Diabetes and Digestive and Kidney Diseases grant DK063491. Computing resources were provided in part by the Wake Forest School of Medicine Center for Public Health Genomics. Support for FUSION was provided by National Institutes of Health grants R01-DK062370 (to M.B.), R01-DK072193 (to K.L.M.), and intramural project number 1Z01-HG000024 (to F.S.C.). Genotyping was conducted by the Johns Hopkins University Genetic Resources Core Facility SNP Center at the Center for Inherited Disease Research, with support from National Institutes of Health contract N01-HG-65403.

Duality of Interest. The RISC study was supported by AstraZeneca. The initial genotyping of the RISC samples was funded by Merck & Co, Inc. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. A.R.W., A.J., A.U.J., N.W., N.v.L., D.W.B., T.H., M.W., R.M.W., L.M.t., R.L.H., and T.M.F. designed the study. A.R.W., A.J., A.U.J., N.W., N.v.L., N.D.P., J.D., L.B.-V., J.P., and A.P.G. performed study-specific data processing and analyses. A.R.W., A.J., A.U.J., N.W., and N.v.L. performed central processing and statistical analysis of the study-specific analyses provided. S.K., A.S., D.I.B., E.J.C.d.G., E.M.W.E., A.F., M.K., G.N., A.S.-B., T.W.v.H., A.Mah., M.B., R.N.B., J.T., F.S.C., K.L.M., K.B., C.J.G., M.I.M., E.R.P., A.N., A.Mar., T.A.B., K.D.T., A.H.X., N.G., H.E., O.P., Y.-D.C., M.L., J.M.N., U.S., L.E.W., L.B., D.W.B., T.H., M.W., R.M.W., L.M.t., R.L.H., and T.M.F. assisted in the provision of study-specific data prior to statistical analysis. A.R.W., A.J., A.U.J., N.W., N.v.L., T.H., M.W., R.M.W., L.M.t., R.L.H., and T.M.F. were involved in co-writing the first manuscript draft. N.D.P., D.I.B., T.A.B., A.Mar., and A.N. commented on and edited the draft manuscript. T.M.F. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Perry JR, Frayling TM. New gene variants alter type 2 diabetes risk predominantly through reduced beta-cell function. *Curr Opin Clin Nutr Metab Care* 2008; 11:371–377
2. Dimas AS, Lagou V, Barker A, et al.; MAGIC Investigators. Impact of type 2 diabetes susceptibility variants on quantitative glycemic traits reveals mechanistic heterogeneity. *Diabetes* 2014;63:2158–2171
3. Prokopenko I, Poon W, Mägi R, et al. A central role for GRB10 in regulation of islet function in man. *PLoS Genet* 2014;10:e1004235
4. Gjesing AP, Hornbak M, Allin KH, et al. High heritability and genetic correlation of intravenous glucose- and tolbutamide-induced insulin secretion among non-diabetic family members of type 2 diabetic patients. *Diabetologia* 2014;57:1173–1181
5. Hu C, Zhang R, Wang C, et al. Variants from GIPR, TCF7L2, DGKB, MADD, CRY2, GLIS3, PROX1, SLC30A8 and IGF1 are associated with glucose metabolism in the Chinese. *PLoS One* 2010;5:e15542

6. Rich SS, Bowden DW, Haffner SM, et al.; Insulin Resistance Atherosclerosis Study Family Study. Identification of quantitative trait loci for glucose homeostasis: the Insulin Resistance Atherosclerosis Study (IRAS) Family Study. *Diabetes* 2004;53:1866–1875
7. Sakul H, Pratley R, Cardon L, Ravussin E, Mott D, Bogardus C. Familiality of physical and metabolic characteristics that predict the development of non-insulin-dependent diabetes mellitus in Pima Indians. *Am J Hum Genet* 1997;60:651–656
8. Simonis-Bik AM, Nijpels G, van Haeften TW, et al. Gene variants in the novel type 2 diabetes loci CDC123/CAMK1D, THADA, ADAMTS9, BCL11A, and MTNR1B affect different aspects of pancreatic beta-cell function. *Diabetes* 2010;59:293–301
9. Staiger H, Machicao F, Schäfer SA, et al. Polymorphisms within the novel type 2 diabetes risk locus MTNR1B determine beta-cell function. *PLoS One* 2008;3:e3962
10. Rich SS, Goodarzi MO, Palmer ND, et al. A genome-wide association scan for acute insulin response to glucose in Hispanic-Americans: the Insulin Resistance Atherosclerosis Family Study (IRAS FS). *Diabetologia* 2009;52:1326–1333
11. Palmer ND, Langefeld CD, Ziegler JT, et al. Candidate loci for insulin sensitivity and disposition index from a genome-wide association analysis of Hispanic participants in the Insulin Resistance Atherosclerosis (IRAS) Family Study. *Diabetologia* 2010;53:281–289
12. Groenewoud MJ, Dekker JM, Fritsche A, et al. Variants of CDKAL1 and IGF2BP2 affect first-phase insulin secretion during hyperglycaemic clamps. *Diabetologia* 2008;51:1659–1663
13. Hart LM, Simonis-Bik AM, Nijpels G, et al. Combined risk allele score of eight type 2 diabetes genes is associated with reduced first-phase glucose-stimulated insulin secretion during hyperglycemic clamps. *Diabetes* 2010;59:287–292
14. van Vliet-Ostaptchouk JV, van Haeften TW, Landman GW, et al. Common variants in the type 2 diabetes KCNQ1 gene are associated with impairments in insulin secretion during hyperglycaemic glucose clamp. *PLoS One* 2012;7:e32148
15. Palmer ND, Goodarzi MO, Langefeld CD, et al. Genetic variants associated with quantitative glucose homeostasis traits translate to type 2 diabetes in Mexican Americans: The GUARDIAN (Genetics Underlying Diabetes in Hispanics) Consortium. *Diabetes* 2015;64:1853–1866
16. Henkin L, Bergman RN, Bowden DW, et al. Genetic epidemiology of insulin resistance and visceral adiposity. The IRAS Family Study design and methods. *Ann Epidemiol* 2003;13:211–217
17. Azen SP, Peters RK, Berkowitz K, Kjos S, Xiang A, Buchanan TA. TRIPOD (Troglitazone In the Prevention Of Diabetes): a randomized, placebo-controlled trial of troglitazone in women with prior gestational diabetes mellitus. *Control Clin Trials* 1998;19:217–231
18. Watanabe RM, Allayee H, Xiang AH, et al. Transcription factor 7-like 2 (TCF7L2) is associated with gestational diabetes mellitus and interacts with adiposity to alter insulin secretion in Mexican Americans. *Diabetes* 2007;56:1481–1485
19. Staiger H, Stancáková A, Zilinskaite J, et al. A candidate type 2 diabetes polymorphism near the HHEX locus affects acute glucose-stimulated insulin release in European populations: results from the EUGENE2 study. *Diabetes* 2008;57:514–517
20. Hills SA, Balkau B, Coppock SW, et al.; EGIR-RISC Study Group. The EGIR-RISC STUDY (The European group for the study of insulin resistance: relationship between insulin sensitivity and cardiovascular disease risk): I. Methodology and objectives. *Diabetologia* 2004;47:566–570
21. Hansen T, Ambye L, Grarup N, et al. Genetic variability of the SUR1 promoter in relation to beta-cell function and type II diabetes mellitus. *Diabetologia* 2001;44:1330–1334
22. Valle T, Tuomilehto J, Bergman RN, et al. Mapping genes for NIDDM. Design of the Finland-United States Investigation of NIDDM Genetics (FUSION) Study. *Diabetes Care* 1998;21:949–958
23. Hanson RL, Muller YL, Kobes S, et al. A genome-wide association study in American Indians implicates DNER as a susceptibility locus for type 2 diabetes. *Diabetes* 2014;63:369–376
24. Voight BF, Kang HM, Ding J, et al. The metabochip, a custom genotyping array for genetic studies of metabolic, cardiovascular, and anthropometric traits. *PLoS Genet* 2012;8:e1002793
25. Mari A, Tura A, Natali A, et al.; RISC Investigators. Influence of hyperinsulinemia and insulin resistance on in vivo β -cell function: their role in human β -cell dysfunction. *Diabetes* 2011;60:3141–3147
26. Matthews JN, Altman DG, Campbell MJ, Royston P. Analysis of serial measurements in medical research. *BMJ* 1990;300:230–235
27. Hovorka R, Soons PA, Young MA. ISEC: a program to calculate insulin secretion. *Comput Methods Programs Biomed* 1996;50:253–264
28. Polonsky KS, Given BD, Hirsch L, et al. Quantitative study of insulin secretion and clearance in normal and obese subjects. *J Clin Invest* 1988;81:435–441
29. Hovorka R, Koukkou E, Southerden D, Powrie JK, Young MA. Measuring pre-hepatic insulin secretion using a population model of C-peptide kinetics: accuracy and required sampling schedule. *Diabetologia* 1998;41:548–554
30. Van Cauter E, Mestrez F, Sturis J, Polonsky KS. Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* 1992;41:368–377
31. Pacini G, Bergman RN. MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsiveness from the frequently sampled intravenous glucose tolerance test. *Comput Methods Programs Biomed* 1986;23:113–122
32. Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genet Epidemiol* 2010;34:816–834
33. Fuchsberger C, Abecasis GR, Hinds DA. minimac2: faster genotype imputation. *Bioinformatics* 2015;31:782–784
34. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet* 2009;5:e1000529
35. Auton A, Brooks LD, Durbin RM, et al.; 1000 Genomes Project Consortium. A global reference for human genetic variation. *Nature* 2015;526:68–74
36. Kang HM, Sul JH, Service SK, et al. Variance component model to account for sample structure in genome-wide association studies. *Nat Genet* 2010;42:348–354
37. Zhou X, Stephens M. Genome-wide efficient mixed-model analysis for association studies. *Nat Genet* 2012;44:821–824
38. Uh HW, Beekman M, Meulenbelt I, Houwing-Duistermaat JJ. Genotype-based score test for association testing in families. *Stat Biosci* 2015;7:394–416
39. Morris AP, Voight BF, Teslovich TM, et al.; Wellcome Trust Case Control Consortium; Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) Investigators; Genetic Investigation of ANthropometric Traits (GIANT) Consortium; Asian Genetic Epidemiology Network–Type 2 Diabetes (AGEN-T2D) Consortium; South Asian Type 2 Diabetes (SAT2D) Consortium; DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. *Nat Genet* 2012;44:981–990
40. Mahajan A, Go MJ, Zhang W, et al.; DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium; Asian Genetic Epidemiology Network Type 2 Diabetes (AGEN-T2D) Consortium; South Asian Type 2 Diabetes (SAT2D) Consortium; Mexican American Type 2 Diabetes (MAT2D) Consortium; Type 2 Diabetes Genetic Exploration by Nex-generation sequencing in multi-Ethnic Samples (T2D-GENES) Consortium. Genome-wide trans-ancestry meta-analysis provides insight into the genetic architecture of type 2 diabetes susceptibility. *Nat Genet* 2014;46:234–244
41. Manning AK, Hivert MF, Scott RA, et al.; DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium; Multiple Tissue Human Expression Resource (MUTHER) Consortium. A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycemic traits and insulin resistance. *Nat Genet* 2012;44:659–669
42. Prasad RB, Lessmark A, Almgren P, et al. Excess maternal transmission of variants in the THADA gene to offspring with type 2 diabetes. *Diabetologia* 2016;59:1702–1713
43. Scott RA, Lagou V, Welch RP, et al.; DIAbetes Genetics Replication and Meta-analysis (DIAGRAM) Consortium. Large-scale association analyses identify new loci influencing glycemic traits and provide insight into the underlying biological pathways. *Nat Genet* 2012;44:991–1005

44. Strawbridge RJ, Dupuis J, Prokopenko I, et al.; DIAGRAM Consortium; GIANT Consortium; MuTHER Consortium; CARDIoGRAM Consortium; C4D Consortium. Genome-wide association identifies nine common variants associated with fasting proinsulin levels and provides new insights into the pathophysiology of type 2 diabetes. *Diabetes* 2011;60:2624–2634
45. Gjesing AP, Ribel-Madsen R, Harder MN, et al. Genetic and phenotypic correlations between surrogate measures of insulin release obtained from OGTT data. *Diabetologia* 2015;58:1006–1012
46. Stoffers DA, Ferrer J, Clarke WL, Habener JF. Early-onset type-II diabetes mellitus (MODY4) linked to IPF1. *Nat Genet* 1997;17:138–139
47. Heni M, Ketterer C, Hart LM, et al. The impact of genetic variation in the G6PC2 gene on insulin secretion depends on glycemia. *J Clin Endocrinol Metab* 2010;95:E479–E484
48. Sparsø T, Andersen G, Albrechtsen A, et al. Impact of polymorphisms in WFS1 on prediabetic phenotypes in a population-based sample of middle-aged people with normal and abnormal glucose regulation. *Diabetologia* 2008;51:1646–1652
49. da Silva Xavier G, Mondragon A, Sun G, et al. Abnormal glucose tolerance and insulin secretion in pancreas-specific Tcf7l2-null mice. *Diabetologia* 2012;55:2667–2676
50. McCarthy MI, Rorsman P, Gloyn AL. TCF7L2 and diabetes: a tale of two tissues, and of two species. *Cell Metab* 2013;17:157–159
51. Nobrega MA. TCF7L2 and glucose metabolism: time to look beyond the pancreas. *Diabetes* 2013;62:706–708
52. Mitchell RK, Mondragon A, Chen L, et al. Selective disruption of Tcf7l2 in the pancreatic β cell impairs secretory function and lowers β cell mass. *Hum Mol Genet* 2015;24:1390–1399
53. Boj SF, van Es JH, Huch M, et al. Diabetes risk gene and Wnt effector Tcf7l2/TCF4 controls hepatic response to perinatal and adult metabolic demand. *Cell* 2012;151:1595–1607
54. Lyssenko V, Lupi R, Marchetti P, et al. Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. *J Clin Invest* 2007;117:2155–2163
55. Schäfer SA, Tschritter O, Machicao F, et al. Impaired glucagon-like peptide-1-induced insulin secretion in carriers of transcription factor 7-like 2 (TCF7L2) gene polymorphisms [published corrections appear in *Diabetologia* 2009;52:557 and *Diabetologia* 2008;51:208]. *Diabetologia* 2007;50:2443–2450
56. Kirchhoff K, Machicao F, Haupt A, et al. Polymorphisms in the TCF7L2, CDKAL1 and SLC30A8 genes are associated with impaired proinsulin conversion. *Diabetologia* 2008;51:597–601
57. Loos RJ, Franks PW, Francis RW, et al. TCF7L2 polymorphisms modulate proinsulin levels and beta-cell function in a British European population. *Diabetes* 2007;56:1943–1947
58. Jonsson A, Ladenvall C, Ahluwalia TS, et al. Effects of common genetic variants associated with type 2 diabetes and glycemic traits on α - and β -cell function and insulin action in humans. *Diabetes* 2013;62:2978–2983
59. Cho YS, Chen CH, Hu C, et al.; DIAGRAM Consortium; MuTHER Consortium. Meta-analysis of genome-wide association studies identifies eight new loci for type 2 diabetes in east Asians. *Nat Genet* 2011;44:67–72
60. Tsai FJ, Yang CF, Chen CC, et al. A genome-wide association study identifies susceptibility variants for type 2 diabetes in Han Chinese. *PLoS Genet* 2010;6:e1000847