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A genome-wide association study of IVGTT-based measures of first phase insulin secretion refines the underlying physiology of type 2 diabetes variants

Mechanisms of type 2 diabetes genetic risk factors

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ABSTRACT

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 non-diabetic individuals from 10 studies. We aimed to refine the mechanisms of 178 known associations between common variants and glycaemic 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*, *IGFBP2*, *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 glycaemic 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 beta-cell function and insulin secretion (2; 3). Oral-based measures of insulin secretion do not distinguish between mechanisms involving gut hormone signaling, e.g. incretin pathways, and mechanisms intrinsic to islet cell function or mass.

Compared to 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 five to ten minutes following glucose stimulation. Intravenous measures include the intravenous glucose tolerance test (IVGTT) and hyperglycaemic clamp. Family studies have shown that first phase insulin response, as measured by IVGTT is one of the most highly heritable glycaemic 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. This study classified 16 variants into groups: nine were labelled as “beta-cell”, two as “hyperglycaemia”, four as “insulin resistance” and one as “insulin processing” (based on proinsulin measures) (2). 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 mins 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 secretion with two aims - first, to refine the underlying physiology of known type 2 diabetes and glycaemic trait variants; 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 glycaemic traits, and third, we focused on characterizing the most recent lists of known type 2 diabetes and glycaemic 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 (IRASFS (16), TRIPOD (17), BETAGENE (18)), 6 studies of 2,900 individuals of European ancestry (EUGENE2 (19), RISC (20), Hyperglycemic clamp consortium (HCC) (13), YOUTH92 (21), FAMILY (21) and FUSION (22)) and one 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 (QC) and intravenous measurement techniques for studies included 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 ten studies each used a version of the IVGTT test. FUSION, Youth92, FAMILY and TRIPOD used tolbutamide-modified IVGTTs, IRASFS and BETAGENE 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 minute intervals and kept constant at 10 mmol/l for at least two hours via continuous variable glucose infusions (13).

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 minutes, or if a measure at 10 minutes was not available, during the first 8 minutes, using the trapezium equation (26), with a minimum of insulin values at 0, 2, 4, 6, and 8 minutes 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) minutes, 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 Hyperglycaemic clamps see t'Hart et al. (13)).

Disposition index

Disposition index (DI) was calculated as the product of AIR and insulin sensitivity index 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.

Oral glucose tolerance test measures of insulin secretion as a comparison

Corrected insulin response (CIR) was based on oral glucose tolerance tests. 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. : Corrected Insulin Response (CIR) = $(100 \times \text{insulin at 30 min}) / (\text{glucose at 30 min} \times (\text{glucose at 30 min} - 3.89))$ (3).

Genotyping and imputation

Genotyping and imputation within studies

Details on the genotyping platform used and genotype quality control procedures employed for each study are presented in **Supplementary Table 3**. All GWAS cohorts were genotyped using commercially available Affymetrix (Affymetrix, Inc., Santa Clara, CA, USA), or Illumina (Illumina, Inc. San Diego, CA, USA) 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 ~39M SNPs and indels from 2184 haplotypes available from the 1000 Genomes Project Phase 1 - version 3 (35)). Due to 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 residualised phenotype to create a normal distribution. This process is important to reduce false positive results when testing 1000s 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 using 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 (MAC) filter of $MAC > 5$ and genomic control correction to the input files prior to meta-analysis. Variants with a meta-analysis P -value $< 5 \times 10^{-8}$ were considered to be genome-wide

significant. All genome-wide statistics are available on our website (url: <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 cases and 114,981 controls (39) and for non-Europeans this included variants at GWAS significance across a trans-ethnic study meta-analysis of 26,488 cases and 83,964 controls (40).

Glycaemic 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 glycaemic or insulin related trait, including fasting glucose, fasting insulin, 2-hour insulin, HbA1C, and proinsulin. We also selected an additional 5 variants associated with fasting glycaemic traits identified by an earlier meta-analysis that fell 250kb 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 CDKALI.

Results are represented in tables 1-5 and figures 1-4. The two strongest association signals represented known type 2 diabetes loci, those in or near *MTNR1B* and *CDKALI* (**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 *CDKALI* with peak insulin response ($P=1.5\times 10^{-12}$) and AIR ($P=1.5\times 10^{-9}$). The peak insulin 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 acute insulin response (17 variants were associated with both peak insulin and AIR at $P<0.05$) (**Table 1**). Peak Insulin 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 these are discussed below [*NOTCH2*, *PPARG* and *GCCI*]. 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 - eight were previously classified as “beta-cell”, one as “hyperglycaemia” (*MTNIRB*) and one as “insulin processing” (*ARAP1/STARD10*) (2). We did not detect any evidence that the variant previously labelled as “beta-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 eleven other variants we detected, those in the *HNF1A*, *IGFBP2*, *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 through an insulin secretory mechanism including lower first phase insulin response. Our findings provide new evidence that type 2 diabetes variants in the loci labelled 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 eight 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 eight 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 glycaemic traits. These traits consisted of those analysed by the Meta-Analysis of Glucose and Insulin Consortium (MAGIC) and included fasting glucose and insulin, proinsulin, HbA1c, and 2-hour 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) 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 glycaemic trait alleles are associated with lower insulin secretion rate

For a subset of 1,268 non-diabetic individuals we had a measure of insulin secretion rate by C-peptide deconvolution (27; 30). For ten known variants, the type 2 diabetes or glycaemic trait risk allele was associated with lower insulin secretion rate at $P < 0.05$. These analyses highlighted two 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 ten variants previously associated with a glycaemic trait and associated with insulin secretion rate in our study, two were not known type 2 diabetes variants – those in *TMPRSS6* (HbA1C 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 insulin secretion rates.

We identified sixteen variants with apparently paradoxical effects on type 2 diabetes risk, glycaemic traits, and first phase insulin secretion or insulin secretion rate. These included three (*PPARG*, *FTO*, *TET2*) with known primary effects on insulin resistance (43) or BMI and three (*ARAP1*, *PCSK1*, *MADD*) (44) with known primary effects on proinsulin. Whilst the effects on insulin secretion were similar when correcting for insulin resistance and BMI the associations with disposition index tended to be weaker (**Tables 4 and 5**).

DISCUSSION

By performing a large genome-wide association study 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 glycaemic 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 as well as insulin resistance when considering 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 IV 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 disposition index, 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 *CDKALI* 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 beta-cell function. These include *IGF2BP2*, *C2CD4A*, *FAF1*, *PTPRD*, *AP3S2*, *NF1B*, *MAEA*,

KCNK16, and *LPP*. Of the nine variants previously labeled as “beta-cell” by Dimas et al., eight were associated with first phase insulin secretion, the exception being that in the *THADA* gene. Based on 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 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 ~6.6kb 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 *CDKALI*, 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, multi-ethnic, type 2 diabetes study of 26,488 cases and 83,964 controls. 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 beta cell function, as is seen in *MODY2*. 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, glycaemic 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

disposition index rather than peak insulin or acute insulin response, 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*, *PSCK1*) (44). The exceptions were the alleles in *GRB10* and *G6PC2*, where correcting for insulin resistance or using disposition index 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 glycaemia which may explain these apparent paradoxical results and suggest that effects of hyperglycemia may override genetic effects observed in healthy volunteers (47).

Known type 2 diabetes or glycaemic trait alleles associated with lower insulin secretion rate

For a subset of 1,268 non-diabetic individuals we had a measure of insulin secretion rate by C-peptide deconvolution, a measure of insulin secretion that accounts for hepatic insulin clearance (29; 30). Eighteen known variants were nominally associated with insulin secretion rate at $P < 0.05$ – 10 where the type 2 diabetes or glycaemic trait risk allele was associated with lower insulin secretion rate, and 8 where the risk allele was associated with higher insulin secretion rate. These analyses highlighted two 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 had shown 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 insulin secretion rate are either likely to reflect the need for higher insulin secretion to remain non-diabetic 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 (**Fig. 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 to 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 6kb 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 *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 IV glucose given its effect on type 2 diabetes and in comparison to 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 beta-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 insulin secretion rate, (that 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 (**Fig. 3 & 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 present study. Third, our data are also consistent with previous data on OGTT-based measures that show the variant at *MTNIRB* has a disproportionately large effect on insulin secretion and fasting glucose levels compared to its effect on type 2 diabetes, possibly as a result of an additional effect on insulin action (58).

Our study had 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 characterised the most recent catalogue of variants associated with type 2 diabetes and glycaemic traits.

The limitations were 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.

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

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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.

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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, Knowles JW, Magi R, Hivert MF, Benazzo A, Rybin D, Jackson AU, Stringham HM, Song C, Fischer-Rosinsky A, Boesgaard TW, Grarup N, Abbasi FA, Assimes TL, Hao K, Yang X, Lecoeur C, Barroso I, Bonnycastle LL, Bottcher Y, Bumpstead S, Chines PS, Erdos MR, Graessler J, Kovacs P, Morken MA, Narisu N, Payne F, Stancakova A, Swift AJ, Tonjes A, Bornstein SR, Cauchi S, Froguel P, Meyre D, Schwarz PE, Haring HU, Smith U, Boehnke M, Bergman RN, Collins FS, Mohlke KL, Tuomilehto J, Quertemous T, Lind L, Hansen T, Pedersen O, Walker M, Pfeiffer AF, Spranger J, Stumvoll M, Meigs JB, Wareham NJ, Kuusisto J, Laakso M, Langenberg C, Dupuis J, Watanabe RM, Florez JC, Ingelsson E, McCarthy MI, Prokopenko I, Investigators M: Impact of type 2 diabetes susceptibility variants on quantitative glycemic traits reveals mechanistic heterogeneity. *Diabetes* 2014;63:2158-2171
3. Prokopenko I, Poon W, Magi R, Prasad BR, Salehi SA, Almgren P, Osmark P, Bouatia-Naji N, Wierup N, Fall T, Stancakova A, Barker A, Lagou V, Osmond C, Xie W, Lahti J, Jackson AU, Cheng YC, Liu J, O'Connell JR, Blomstedt PA, Fadista J, Alkayyali S, Dayeh T, Ahlqvist E, Taneera J, Lecoeur C, Kumar A, Hansson O, Hansson K, Voight BF, Kang HM, Levy-Marchal C, Vatin V, Palotie A, Syvanen AC, Mari A, Weedon MN, Loos RJ, Ong KK, Nilsson P, Isomaa B, Tuomi T, Wareham NJ, Stumvoll M, Widen E, Lakka TA, Langenberg C, Tonjes A, Rauramaa R, Kuusisto J, Frayling TM, Froguel P, Walker M, Eriksson JG, Ling C, Kovacs P, Ingelsson E, McCarthy MI, Shuldiner AR, Silver KD, Laakso M, Groop L, Lyssenko V: A central role for GRB10 in regulation of islet function in man. *PLoS genetics* 2014;10:e1004235
4. Gjesing AP, Hornbak M, Allin KH, Ekstrom CT, Urhammer SA, Eiberg H, Pedersen O, Hansen T: 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, Wang J, Ma X, Hou X, Lu J, Yu W, Jiang F, Bao Y, Xiang K, Jia W: 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, Norris JM, Saad MF, Mitchell BD, Rotter JI, Langefeld CD, Wagenknecht LE, Bergman RN, Insulin Resistance Atherosclerosis Study Family S: 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. *American journal of human genetics* 1997;60:651-656
8. Simonis-Bik AM, Nijpels G, van Haften TW, Houwing-Duistermaat JJ, Boomsma DI, Reiling E, van Hove EC, Diamant M, Kramer MH, Heine RJ, Maassen JA, Slagboom PE, Willemsen G, Dekker JM, Eekhoff EM, de Geus EJ, t Hart LM: 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, Schafer SA, Kirchhoff K, Kantartzis K, Guthoff M, Silbernagel G, Stefan N, Haring HU, Fritsche A: 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, Langefeld CD, Ziegler J, Haffner SM, Bryer-Ash M, Norris JM, Taylor KD, Haritunians T, Rotter JI, Chen YD, Wagenknecht LE, Bowden DW, Bergman RN: 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, Hsu F, Haffner SM, Fingerlin T, Norris JM, Chen YI, Rich SS, Haritunians T, Taylor KD, Bergman RN, Rotter JI, Bowden DW: 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, Reiling E, Nijpels G, Heine RJ, Maassen JA, Machicao F, Schafer SA, Haring HU, t Hart LM, van Haefen TW: Variants of CDKAL1 and IGF2BP2 affect first-phase insulin secretion during hyperglycaemic clamps. *Diabetologia* 2008;51:1659-1663

13. t Hart LM, Simonis-Bik AM, Nijpels G, van Haefen TW, Schafer SA, Houwing-Duistermaat JJ, Boomsma DI, Groenewoud MJ, Reiling E, van Hove EC, Diamant M, Kramer MH, Heine RJ, Maassen JA, Kirchhoff K, Machicao F, Haring HU, Slagboom PE, Willemsen G, Eekhoff EM, de Geus EJ, Dekker JM, Fritsche A: 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-Ostapchouk JV, van Haefen TW, Landman GW, Reiling E, Kleefstra N, Bilo HJ, Klungel OH, de Boer A, van Diemen CC, Wijmenga C, Boezen HM, Dekker JM, van 't Riet E, Nijpels G, Welschen LM, Zavrelova H, Bruin EJ, Elbers CC, Bauer F, Onland-Moret NC, van der Schouw YT, Grobbee DE, Spijkerman AM, van der AD, Simonis-Bik AM, Eekhoff EM, Diamant M, Kramer MH, Boomsma DI, de Geus EJ, Willemsen G, Slagboom PE, Hofker MH, t Hart LM: 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, Wang N, Guo X, Taylor KD, Fingerlin TE, Norris JM, Buchanan TA, Xiang AH, Haritunians T, Ziegler JT, Williams AH, Stefanovski D, Cui J, Mackay AW, Henkin LF, Bergman RN, Gao X, Gauderman J, Varma R, Hanis CL, Cox NJ, Highland HM, Below JE, Williams AL, Burt NP, Aguilar-Salinas CA, Huerta-Chagoya A, Gonzalez-Villalpando C, Orozco L, Haiman CA, Tsai MY, Johnson WC, Yao J, Rasmussen-Torvik L, Pankow J, Snively B, Jackson RD, Liu S, Nadler JL, Kandeel F, Chen YD, Bowden DW, Rich SS, Raffel LJ, Rotter JI, Watanabe RM, Wagenknecht LE: 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, Ellsworth DL, Haffner SM, Langefeld CD, Mitchell BD, Norris JM, Rewers M, Saad MF, Stamm E, Wagenknecht LE, Rich SS: 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. *Controlled clinical trials* 1998;19:217-231

18. Watanabe RM, Allayee H, Xiang AH, Trigo E, Hartiala J, Lawrence JM, Buchanan TA: 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, Stancakova A, Zilinskaite J, Vanttinen M, Hansen T, Marini MA, Hammarstedt A, Jansson PA, Sesti G, Smith U, Pedersen O, Laakso M, Stefan N, Fritsche A, Haring HU: 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, Coppack SW, Dekker JM, Mari A, Natali A, Walker M, Ferrannini E, Group E-RS: 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, Hansen L, Echwald SM, Ferrer J, Pedersen O: 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, Ghosh S, Hauser ER, Eriksson J, Nylund SJ, Kohtamaki K, Toivanen L, Vidgren G, Tuomilehto-Wolf E, Ehnholm C, Blaschak J, Langefeld CD, Watanabe RM, Magnuson V, Ally DS, Hagopian WA, Ross E, Buchanan TA, Collins F, Boehnke M: 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, Guo T, Bian L, Ossowski V, Wiedrich K, Sutherland J, Wiedrich C, Mahke D, Huang K, Abdussamad M, Traurig M, Weil EJ, Nelson RG, Bennett PH, Knowler WC, Bogardus C, Baier LJ: 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, Palmer CD, Sidore C, Chines PS, Burt NP, Fuchsberger C, Li Y, Erdmann J, Frayling TM, Heid IM, Jackson AU, Johnson T, Kilpelainen TO, Lindgren CM, Morris AP, Prokopenko I, Randall JC, Saxena R, Soranzo N, Speliotes EK, Teslovich TM, Wheeler E, Maguire J, Parkin M, Potter S, Rayner NW, Robertson N, Stirrups K, Winckler W, Sanna S, Mulas A, Nagaraja R, Cucca F, Barroso I, Deloukas P, Loos RJ, Kathiresan S, Munroe PB, Newton-Cheh C, Pfeufer A, Samani NJ, Schunkert H, Hirschhorn JN, Altshuler D, McCarthy MI, Abecasis GR, Boehnke M: The metabochip, a custom genotyping array for genetic studies of metabolic, cardiovascular, and anthropometric traits. *PLoS genetics* 2012;8:e1002793
25. Mari A, Tura A, Natali A, Anderwald C, Balkau B, Lalic N, Walker M, Ferrannini E, Investigators R: Influence of hyperinsulinemia and insulin resistance on in vivo beta-cell function: their role in human beta-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, Shapiro ET, Tillil H, Beebe C, Galloway JA, Frank BH, Karrison T, Van Cauter E: Quantitative study of insulin secretion and clearance in normal and obese subjects. *The Journal of clinical investigation* 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. *Genetic epidemiology* 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 genetics* 2009;5:e1000529
35. Genomes Project C, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, Marchini JL, McCarthy S, McVean GA, Abecasis GR: A global reference for human genetic variation. *Nature* 2015;526:68-74
36. Kang HM, Sul JH, Service SK, Zaitlen NA, Kong SY, Freimer NB, Sabatti C, Eskin E: Variance component model to account for sample structure in genome-wide association studies. *Nature genetics* 2010;42:348-354
37. Zhou X, Stephens M: Genome-wide efficient mixed-model analysis for association studies. *Nature genetics* 2012;44:821-824
38. Uh HW, Beekman M, Meulenbelt I, Houwing-Duistermaat JJ: Genotype-Based Score Test for Association Testing in Families. *Statistics in biosciences* 2015;7:394-416
39. Morris AP, Voight BF, Teslovich TM, Ferreira T, Segre AV, Steinthorsdottir V, Strawbridge RJ, Khan H, Grallert H, Mahajan A, Prokopenko I, Kang HM, Dina C, Esko T, Fraser RM, Kanoni S, Kumar A, Lagou V, Langenberg C, Luan J, Lindgren CM, Muller-Nurasyid M, Pechlivanis S, Rayner NW, Scott LJ, Wiltshire S, Yengo L, Kinnunen L, Rossin EJ, Raychaudhuri S, Johnson AD, Dimas AS, Loos RJ, Vedantam S, Chen H, Florez JC, Fox C, Liu CT, Rybin D, Couper DJ, Kao WH, Li M, Cornelis MC, Kraft P, Sun Q, van Dam RM, Stringham HM, Chines PS, Fischer K, Fontanillas P, Holmen OL, Hunt SE, Jackson AU, Kong A, Lawrence R, Meyer J, Perry JR, Platou CG, Potter S, Rehnberg E, Robertson N, Sivapalaratnam S, Stancakova A, Stirrups K, Thorleifsson G, Tikkanen E, Wood AR, Almgren P, Atalay M, Benediktsson R, Bonnycastle LL, Burtt N, Carey J, Charpentier G, Crenshaw AT, Doney AS, Dorkhan M, Edkins S, Emilsson V, Eury E, Forsen T, Gertow K, Gigante B, Grant GB, Groves CJ, Guiducci C, Herder C, Hreidarsson AB, Hui J, James A, Jonsson A, Rathmann W, Klopp N, Kravic J, Krjutskov K, Langford C, Leander K, Lindholm E, Lobbens S, Mannisto S, Mirza G, Muhleisen TW, Musk B, Parkin M, Rallidis L, Saramies J, Sennblad B, Shah S, Sigurethsson G, Silveira A, Steinbach G, Thorand B, Trakalo J, Veglia F, Wennauer R, Winckler W, Zabaneh D, Campbell H, van Duijn C, Uitterlinden AG, Hofman A, Sijbrands E, Abecasis GR, Owen KR, Zeggini E, Trip MD, Forouhi NG, Syvanen AC, Eriksson JG, Peltonen L, Nothen MM, Balkau B, Palmer CN, Lyssenko V, Tuomi T, Isomaa B, Hunter DJ, Qi L, Wellcome Trust Case Control C, Meta-Analyses of G, Insulin-related traits Consortium I, Genetic Investigation of ATC, Asian Genetic Epidemiology Network-Type 2 Diabetes C, South Asian Type 2 Diabetes C, Shuldiner AR, Roden M, Barroso I, Wilsgaard T, Beilby J, Hovingh K, Price JF, Wilson JF, Rauramaa R, Lakka TA, Lind L, Dedoussis G, Njolstad I, Pedersen NL, Khaw KT, Wareham NJ, Keinanen-Kiukaanniemi SM, Saaristo TE, Korpi-Hyovalti E, Saltevo J, Laakso M, Kuusisto J, Metspalu A, Collins FS, Mohlke KL, Bergman RN, Tuomilehto J, Boehm BO, Gieger C, Hveem K, Cauchi S, Froguel P, Baldassarre D, Tremoli E, Humphries SE, Saleheen D, Danesh J, Ingelsson E, Ripatti S, Salomaa V, Erbel R, Jockel KH, Moebus S, Peters A, Illig T, de Faire U, Hamsten A, Morris AD, Donnelly PJ, Frayling TM, Hattersley AT, Boerwinkle E, Melander O, Kathiresan S, Nilsson PM, Deloukas P, Thorsteinsdottir U, Groop LC, Stefansson K, Hu F, Pankow JS, Dupuis J, Meigs JB, Altshuler D, Boehnke M, McCarthy MI, Replication DIG, Meta-analysis C: Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. *Nature genetics* 2012;44:981-990
40. Replication DIG, Meta-analysis C, Asian Genetic Epidemiology Network Type 2 Diabetes C, South Asian Type 2 Diabetes C, Mexican American Type 2 Diabetes C, Type 2 Diabetes Genetic Exploration by Next-generation sequencing in multi-Ethnic Samples C,

Mahajan A, Go MJ, Zhang W, Below JE, Gaulton KJ, Ferreira T, Horikoshi M, Johnson AD, Ng MC, Prokopenko I, Saleheen D, Wang X, Zeggini E, Abecasis GR, Adair LS, Almgren P, Atalay M, Aung T, Baldassarre D, Balkau B, Bao Y, Barnett AH, Barroso I, Basit A, Been LF, Beilby J, Bell GI, Benediktsson R, Bergman RN, Boehm BO, Boerwinkle E, Bonnycastle LL, Burt N, Cai Q, Campbell H, Carey J, Cauchi S, Caulfield M, Chan JC, Chang LC, Chang TJ, Chang YC, Charpentier G, Chen CH, Chen H, Chen YT, Chia KS, Chidambaram M, Chines PS, Cho NH, Cho YM, Chuang LM, Collins FS, Cornelis MC, Couper DJ, Crenshaw AT, van Dam RM, Danesh J, Das D, de Faire U, Dedoussis G, Deloukas P, Dimas AS, Dina C, Doney AS, Donnelly PJ, Dorkhan M, van Duijn C, Dupuis J, Edkins S, Elliott P, Emilsson V, Erbel R, Eriksson JG, Escobedo J, Esko T, Eury E, Florez JC, Fontanillas P, Forouhi NG, Forsen T, Fox C, Fraser RM, Frayling TM, Froguel P, Frossard P, Gao Y, Gertow K, Gieger C, Gigante B, Grallert H, Grant GB, Grrop LC, Groves CJ, Grundberg E, Guiducci C, Hamsten A, Han BG, Hara K, Hassanali N, Hattersley AT, Hayward C, Hedman AK, Herder C, Hofman A, Holmen OL, Hovingh K, Hreidarsson AB, Hu C, Hu FB, Hui J, Humphries SE, Hunt SE, Hunter DJ, Hveem K, Hydrie ZI, Ikegami H, Illig T, Ingelsson E, Islam M, Isomaa B, Jackson AU, Jafar T, James A, Jia W, Jockel KH, Jonsson A, Jowett JB, Kadowaki T, Kang HM, Kanoni S, Kao WH, Kathiresan S, Kato N, Katulanda P, Keinänen-Kiukaanniemi KM, Kelly AM, Khan H, Khaw KT, Khor CC, Kim HL, Kim S, Kim YJ, Kinnunen L, Klopp N, Kong A, Korpi-Hyovalti E, Kowlessur S, Kraft P, Kravic J, Kristensen MM, Krithika S, Kumar A, Kumate J, Kuusisto J, Kwak SH, Laakso M, Lagou V, Lakka TA, Langenberg C, Langford C, Lawrence R, Leander K, Lee JM, Lee NR, Li M, Li X, Li Y, Liang J, Liju S, Lim WY, Lind L, Lindgren CM, Lindholm E, Liu CT, Liu JJ, Lobbens S, Long J, Loos RJ, Lu W, Luan J, Lyssenko V, Ma RC, Maeda S, Magi R, Mannisto S, Matthews DR, Meigs JB, Melander O, Metspalu A, Meyer J, Mirza G, Mihailov E, Moebus S, Mohan V, Mohlke KL, Morris AD, Muhleisen TW, Muller-Nurasyid M, Musk B, Nakamura J, Nakashima E, Navarro P, Ng PK, Nica AC, Nilsson PM, Njolstad I, Nothen MM, Ohnaka K, Ong TH, Owen KR, Palmer CN, Pankow JS, Park KS, Parkin M, Pechlivanis S, Pedersen NL, Peltonen L, Perry JR, Peters A, Pinidiyapathirage JM, Platou CG, Potter S, Price JF, Qi L, Radha V, Rallidis L, Rasheed A, Rathman W, Rauramaa R, Raychaudhuri S, Rayner NW, Rees SD, Rehnberg E, Ripatti S, Robertson N, Roden M, Rossin EJ, Rudan I, Rybin D, Saaristo TE, Salomaa V, Saltevo J, Samuel M, Sanghera DK, Saramies J, Scott J, Scott LJ, Scott RA, Segre AV, Sehmi J, Sennblad B, Shah N, Shah S, Shera AS, Shu XO, Shuldiner AR, Sigurdsson G, Sijbrands E, Silveira A, Sim X, Sivapalaratnam S, Small KS, So WY, Stancakova A, Stefansson K, Steinbach G, Steinthorsdottir V, Stirrups K, Strawbridge RJ, Stringham HM, Sun Q, Suo C, Syvanen AC, Takayanagi R, Takeuchi F, Tay WT, Teslovich TM, Thorand B, Thorleifsson G, Thorsteinsdottir U, Tikkanen E, Trakalo J, Tremoli E, Trip MD, Tsai FJ, Tuomi T, Tuomilehto J, Uitterlinden AG, Valladares-Salgado A, Vedantam S, Veglia F, Voight BF, Wang C, Wareham NJ, Wennauer R, Wickremasinghe AR, Wilsgaard T, Wilson JF, Wiltshire S, Winckler W, Wong TY, Wood AR, Wu JY, Wu Y, Yamamoto K, Yamauchi T, Yang M, Yengo L, Yokota M, Young R, Zabaneh D, Zhang F, Zhang R, Zheng W, Zimmet PZ, Altshuler D, Bowden DW, Cho YS, Cox NJ, Cruz M, Hanis CL, Kooner J, Lee JY, Seielstad M, Teo YY, Boehnke M, Parra EJ, Chambers JC, Tai ES, McCarthy MI, Morris AP: Genome-wide trans-ancestry meta-analysis provides insight into the genetic architecture of type 2 diabetes susceptibility. *Nature genetics* 2014;46:234-244

41. Manning AK, Hivert MF, Scott RA, Grimsby JL, Bouatia-Naji N, Chen H, Rybin D, Liu CT, Bielak LF, Prokopenko I, Amin N, Barnes D, Cadby G, Hottenga JJ, Ingelsson E, Jackson AU, Johnson T, Kanoni S, Ladenvall C, Lagou V, Lahti J, Lecoeur C, Liu Y, Martinez-Larrad MT, Montasser ME, Navarro P, Perry JR, Rasmussen-Torvik LJ, Salo P, Sattar N, Shungin D, Strawbridge RJ, Tanaka T, van Duijn CM, An P, de Andrade M,

Andrews JS, Aspelund T, Atalay M, Aulchenko Y, Balkau B, Bandinelli S, Beckmann JS, Beilby JP, Bellis C, Bergman RN, Blangero J, Boban M, Boehnke M, Boerwinkle E, Bonnycastle LL, Boomsma DI, Borecki IB, Bottcher Y, Bouchard C, Brunner E, Budimir D, Campbell H, Carlson O, Chines PS, Clarke R, Collins FS, Corbaton-Anchuelo A, Couper D, de Faire U, Dedoussis GV, Deloukas P, Dimitriou M, Egan JM, Eiriksdottir G, Erdos MR, Eriksson JG, Eury E, Ferrucci L, Ford I, Forouhi NG, Fox CS, Franzosi MG, Franks PW, Frayling TM, Froguel P, Galan P, de Geus E, Gigante B, Glazer NL, Goel A, Groop L, Gudnason V, Hallmans G, Hamsten A, Hansson O, Harris TB, Hayward C, Heath S, Hercberg S, Hicks AA, Hingorani A, Hofman A, Hui J, Hung J, Jarvelin MR, Jhun MA, Johnson PC, Jukema JW, Jula A, Kao WH, Kaprio J, Kardia SL, Keinänen-Kiukaanniemi S, Kivimaki M, Kolcic I, Kovacs P, Kumari M, Kuusisto J, Kyvik KO, Laakso M, Lakka T, Lannfelt L, Lathrop GM, Launer LJ, Leander K, Li G, Lind L, Lindstrom J, Lobbens S, Loos RJ, Luan J, Lyssenko V, Magi R, Magnusson PK, Marmot M, Meneton P, Mohlke KL, Mooser V, Morken MA, Miljkovic I, Narisu N, O'Connell J, Ong KK, Oostra BA, Palmer LJ, Palotie A, Pankow JS, Peden JF, Pedersen NL, Pehlic M, Peltonen L, Penninx B, Pericic M, Perola M, Perusse L, Peyser PA, Polasek O, Pramstaller PP, Province MA, Raikonen K, Rauramaa R, Rehnberg E, Rice K, Rotter JI, Rudan I, Ruukonen A, Saaristo T, Sabater-Lleal M, Salomaa V, Savage DB, Saxena R, Schwarz P, Seedorf U, Sennblad B, Serrano-Rios M, Shuldiner AR, Sijbrands EJ, Siscovick DS, Smit JH, Small KS, Smith NL, Smith AV, Stancakova A, Stirrups K, Stumvoll M, Sun YV, Swift AJ, Tonjes A, Tuomilehto J, Trompet S, Uitterlinden AG, Uusitupa M, Vikstrom M, Vitart V, Vohl MC, Voight BF, Vollenweider P, Waeber G, Waterworth DM, Watkins H, Wheeler E, Widen E, Wild SH, Willems SM, Willemsen G, Wilson JF, Witteman JC, Wright AF, Yaghoobkar H, Zelenika D, Zemunik T, Zgaga L, Replication DIG, Meta-analysis C, Multiple Tissue Human Expression Resource C, Wareham NJ, McCarthy MI, Barroso I, Watanabe RM, Florez JC, Dupuis J, Meigs JB, Langenberg C: A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycemic traits and insulin resistance. *Nature genetics* 2012;44:659-669

42. Prasad RB, Lessmark A, Almgren P, Kovacs G, Hansson O, Oskolkov N, Vitai M, Ladenvall C, Kovacs P, Fadista J, Lachmann M, Zhou Y, Sonestedt E, Poon W, Wollheim CB, Orho-Melander M, Stumvoll M, Tuomi T, Paabo S, Koranyi L, Groop L: 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, Wheeler E, Montasser ME, Luan J, Magi R, Strawbridge RJ, Rehnberg E, Gustafsson S, Kanoni S, Rasmussen-Torvik LJ, Yengo L, Lecoeur C, Shungin D, Sanna S, Sidore C, Johnson PC, Jukema JW, Johnson T, Mahajan A, Verweij N, Thorleifsson G, Hottenga JJ, Shah S, Smith AV, Sennblad B, Gieger C, Salo P, Perola M, Timpson NJ, Evans DM, Pourcain BS, Wu Y, Andrews JS, Hui J, Bielak LF, Zhao W, Horikoshi M, Navarro P, Isaacs A, O'Connell JR, Stirrups K, Vitart V, Hayward C, Esko T, Mihailov E, Fraser RM, Fall T, Voight BF, Raychaudhuri S, Chen H, Lindgren CM, Morris AP, Rayner NW, Robertson N, Rybin D, Liu CT, Beckmann JS, Willems SM, Chines PS, Jackson AU, Kang HM, Stringham HM, Song K, Tanaka T, Peden JF, Goel A, Hicks AA, An P, Muller-Nurasyid M, Franco-Cereceda A, Folkersen L, Marullo L, Jansen H, Oldehinkel AJ, Bruinenberg M, Pankow JS, North KE, Forouhi NG, Loos RJ, Edkins S, Varga TV, Hallmans G, Oksa H, Antonella M, Nagaraja R, Trompet S, Ford I, Bakker SJ, Kong A, Kumari M, Gigante B, Herder C, Munroe PB, Caulfield M, Antti J, Mangino M, Small K, Miljkovic I, Liu Y, Atalay M, Kiess W, James AL, Rivadeneira F, Uitterlinden AG, Palmer CN, Doney AS, Willemsen G, Smit JH, Campbell S, Polasek O, Bonnycastle LL, Hercberg S, Dimitriou M, Bolton JL, Fowkes GR, Kovacs P, Lindstrom J, Zemunik T, Bandinelli S, Wild SH, Basart HV, Rathmann W, Grallert H, Replication DIG, Meta-analysis C, Maerz W,

Kleber ME, Boehm BO, Peters A, Pramstaller PP, Province MA, Borecki IB, Hastie ND, Rudan I, Campbell H, Watkins H, Farrall M, Stumvoll M, Ferrucci L, Waterworth DM, Bergman RN, Collins FS, Tuomilehto J, Watanabe RM, de Geus EJ, Penninx BW, Hofman A, Oostra BA, Psaty BM, Vollenweider P, Wilson JF, Wright AF, Hovingh GK, Metspalu A, Uusitupa M, Magnusson PK, Kyvik KO, Kaprio J, Price JF, Dedoussis GV, Deloukas P, Meneton P, Lind L, Boehnke M, Shuldiner AR, van Duijn CM, Morris AD, Toenjes A, Peyser PA, Beilby JP, Korner A, Kuusisto J, Laakso M, Bornstein SR, Schwarz PE, Lakka TA, Rauramaa R, Adair LS, Smith GD, Spector TD, Illig T, de Faire U, Hamsten A, Gudnason V, Kivimaki M, Hingorani A, Keinanen-Kiukaanniemi SM, Saaristo TE, Boomsma DI, Stefansson K, van der Harst P, Dupuis J, Pedersen NL, Sattar N, Harris TB, Cucca F, Ripatti S, Salomaa V, Mohlke KL, Balkau B, Froguel P, Pouta A, Jarvelin MR, Wareham NJ, Bouatia-Naji N, McCarthy MI, Franks PW, Meigs JB, Teslovich TM, Florez JC, Langenberg C, Ingelsson E, Prokopenko I, Barroso I: Large-scale association analyses identify new loci influencing glycemic traits and provide insight into the underlying biological pathways. *Nature genetics* 2012;44:991-1005

44. Strawbridge RJ, Dupuis J, Prokopenko I, Barker A, Ahlqvist E, Rybin D, Petrie JR, Travers ME, Bouatia-Naji N, Dimas AS, Nica A, Wheeler E, Chen H, Voight BF, Taneera J, Kanoni S, Peden JF, Turrini F, Gustafsson S, Zabena C, Almgren P, Barker DJ, Barnes D, Dennison EM, Eriksson JG, Eriksson P, Eury E, Folkersen L, Fox CS, Frayling TM, Goel A, Gu HF, Horikoshi M, Isomaa B, Jackson AU, Jameson KA, Kajantie E, Kerr-Conte J, Kuulasmaa T, Kuusisto J, Loos RJ, Luan J, Makrilakis K, Manning AK, Martinez-Larrad MT, Narisu N, Nastase Mannila M, Ohrvik J, Osmond C, Pascoe L, Payne F, Sayer AA, Sennblad B, Silveira A, Stancakova A, Stirrups K, Swift AJ, Syvanen AC, Tuomi T, van 't Hooft FM, Walker M, Weedon MN, Xie W, Zethelius B, Consortium D, Consortium G, Mu TC, Consortium CA, Consortium CD, Ongen H, Malarstig A, Hopewell JC, Saleheen D, Chambers J, Parish S, Danesh J, Kooner J, Ostenson CG, Lind L, Cooper CC, Serrano-Rios M, Ferrannini E, Forsen TJ, Clarke R, Franzosi MG, Seedorf U, Watkins H, Froguel P, Johnson P, Deloukas P, Collins FS, Laakso M, Dermitzakis ET, Boehnke M, McCarthy MI, Wareham NJ, Groop L, Pattou F, Gloyn AL, Dedoussis GV, Lyssenko V, Meigs JB, Barroso I, Watanabe RM, Ingelsson E, Langenberg C, Hamsten A, Florez JC: 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, Eiberg H, Grarup N, Jorgensen T, Ekstrom CT, Pedersen O, Hansen T: 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. *Nature genetics* 1997;17:138-139

47. Heni M, Ketterer C, Hart LM, Ranta F, van Haefen TW, Eekhoff EM, Dekker JM, Boomsma DI, Nijpels G, Kramer MH, Diamant M, Simonis-Bik AM, Heine RJ, de Geus EJ, Schafer SA, Machicao F, Ullrich S, Thamer C, Stefan N, Staiger H, Haring HU, Fritsche A: The impact of genetic variation in the G6PC2 gene on insulin secretion depends on glycemia. *The Journal of clinical endocrinology and metabolism* 2010;95:E479-484

48. Sparso T, Andersen G, Albrechtsen A, Jorgensen T, Borch-Johnsen K, Sandbaek A, Lauritzen T, Wasson J, Permutt MA, Glaser B, Madsbad S, Pedersen O, Hansen T: 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, Chen L, McGinty JA, French PM, Rutter GA: 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 metabolism* 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, McGinty JA, French PM, Ferrer J, Thorens B, Hodson DJ, Rutter GA, Da Silva Xavier G: Selective disruption of Tcf7l2 in the pancreatic beta cell impairs secretory function and lowers beta cell mass. *Human molecular genetics* 2015;24:1390-1399
53. Boj SF, van Es JH, Huch M, Li VS, Jose A, Hatzis P, Mokry M, Haegebarth A, van den Born M, Chambon P, Voshol P, Dor Y, Cuppen E, Fillat C, Clevers H: 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, Del Guerra S, Orho-Melander M, Almgren P, Sjogren M, Ling C, Eriksson KF, Lethagen AL, Mancarella R, Berglund G, Tuomi T, Nilsson P, Del Prato S, Groop L: Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. *The Journal of clinical investigation* 2007;117:2155-2163
55. Schafer SA, Tschritter O, Machicao F, Thamer C, Stefan N, Gallwitz B, Holst JJ, Dekker JM, Hart LM, Nijpels G, van Haften TW, Haring HU, Fritsche A: Impaired glucagon-like peptide-1-induced insulin secretion in carriers of transcription factor 7-like 2 (TCF7L2) gene polymorphisms. *Diabetologia* 2007;50:2443-2450
56. Kirchhoff K, Machicao F, Haupt A, Schafer SA, Tschritter O, Staiger H, Stefan N, Haring HU, Fritsche A: 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, Barroso I, Gribble FM, Savage DB, Ong KK, O'Rahilly S, Wareham NJ: 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, Kravic J, Krus U, Taneera J, Isomaa B, Tuomi T, Renstrom E, Groop L, Lyssenko V: Effects of common genetic variants associated with type 2 diabetes and glycemic traits on alpha- and beta-cell function and insulin action in humans. *Diabetes* 2013;62:2978-2983
59. Cho YS, Chen CH, Hu C, Long J, Ong RT, Sim X, Takeuchi F, Wu Y, Go MJ, Yamauchi T, Chang YC, Kwak SH, Ma RC, Yamamoto K, Adair LS, Aung T, Cai Q, Chang LC, Chen YT, Gao Y, Hu FB, Kim HL, Kim S, Kim YJ, Lee JJ, Lee NR, Li Y, Liu JJ, Lu W, Nakamura J, Nakashima E, Ng DP, Tay WT, Tsai FJ, Wong TY, Yokota M, Zheng W, Zhang R, Wang C, So WY, Ohnaka K, Ikegami H, Hara K, Cho YM, Cho NH, Chang TJ, Bao Y, Hedman AK, Morris AP, McCarthy MI, Consortium D, Mu TC, Takayanagi R, Park KS, Jia W, Chuang LM, Chan JC, Maeda S, Kadowaki T, Lee JY, Wu JY, Teo YY, Tai ES, Shu XO, Mohlke KL, Kato N, Han BG, Seielstad M: Meta-analysis of genome-wide association studies identifies eight new loci for type 2 diabetes in east Asians. *Nature genetics* 2012;44:67-72
60. Tsai FJ, Yang CF, Chen CC, Chuang LM, Lu CH, Chang CT, Wang TY, Chen RH, Shiu CF, Liu YM, Chang CC, Chen P, Chen CH, Fann CS, Chen YT, Wu JY: A genome-wide association study identifies susceptibility variants for type 2 diabetes in Han Chinese. *PLoS genetics* 2010;6:e1000847

Table 1. Type 2 diabetes risk alleles associated with lower first phase insulin response, AIR and or peak insulin ($P<0.05$), from a total of 76 analysed. Betas represent per allele effects in standard deviations. Associations reaching Bonferroni equivalents of $P<0.05$ are emboldened.

Locus	OGTT/fasting*	Lead SNP	Chr†	Position‡	Risk Allele	Peak insulin			Acute insulin response		
						Beta§	SE	P-value	Beta§	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

*Association ($P < 0.05$) with OGTT based measure of insulin secretion or fasting glucose, as reported by (1) Prokopenko et al (CIR), (2) this study (CIR_{BMI+SI adjusted}) and as

Dimas et al classified: HG=hyperglycemic, BC=beta cell ,UC=unclassified, N/A=not available in Dimas et al; indicated according to their classification HG:

hyperglycaemic reduced beta-cell function after glucose stimulation, BC: defective beta-cell function, PROINS: decreased proinsulin, UC: unclassified. †Chromosome.

‡Base-pair position build-37. §Betas represent standard deviation effects per risk allele.

Table 2. Fasting glucose raising alleles associated with lower first phase insulin response but not identified as a type 2 diabetes risk allele. Betas represent per allele effects in standard deviations. Associations reaching Bonferroni equivalents of $P < 0.05$ are emboldened.

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

*Chromosome. †Base-pair position build-37. ‡Betas represent standard deviation effects per risk allele.

Table 3. Known type 2 diabetes and glycaemic trait variants associated with insulin secretion rate (ISR). N = 1,268. Betas represent per allele effects in standard deviations. Associations reaching Bonferroni equivalents of $P < 0.05$ are emboldened.

Locus	Trait*	Classification†	Association Pattern‡	Lead SNP	Chr§	Position	Risk Allele	ISR		
								Beta¶	SE	P-value
<i>MTNR1B</i>	T2D / FG	HG	1,2,3,4	rs10830963	11	92,708,710	G	-0.232	0.043	9.01E-08
<i>CDKAL1</i>	T2D / FG	BC	1,2,3,4	rs7756992	6	20,679,709	G	-0.232	0.045	1.91E-07
<i>CDKN2A/B</i>	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>	HbA1C	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

*Associated trait: T2D=type 2 diabetes, FG=fasting glucose; †Classification by Dimas et al: HG=hyperglycemic, BC=beta cell, UC=unclassified, N/A=not available

in Dimas et al; ‡Code relating to significance of association across phenotypes and datasets: 1=associated at $P < 0.05$ with peak insulin in our data, 2=associated at

$P < 0.05$ with acute insulin response in our data, 3=associated with CIR in Prokopenko et al., 4=associated at $P < 0.05$ with CIRBMI+SI adjustment in our data.

§Chromosome. ||Base-pair position build-37; ¶Betas represent standard deviation effects per effect allele.

Table 4. Apparently paradoxical associations between known glycaemic variants and first phase insulin secretion. Betas represent per allele effects in standard deviations. Associations reaching Bonferroni equivalents of $P < 0.05$ are emboldened.

Locus	Known Trait*	Lead SNP	Chr†	Position‡	Effect Allele	Peak insulin				Acute insulin response				DI¶
						Beta§	SE	P-value	P-value (BMI+SI)	Beta§	SE	P-value	P-value (BMI+SI)	P-value (BMI)
<i>G6PC2</i>	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
<i>GRB10</i>	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
<i>OR4S1/PTPRJ#</i>	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
<i>MADD</i>	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
<i>PCSK1</i>	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
<i>PPARG</i>	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
<i>ARAP1</i>	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
<i>FTO</i>	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
<i>GCC1</i>	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
<i>NOTCH2</i>	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

*Trait previously associated with SNP. †Chromosome. ‡Base-pair position build-37. §Betas represent standard deviation effects per effect allele.||P-value after BMI+SI adjustment.

FG=fasting glucose, Fproinsulin=fasting proinsulin, T2D=type 2 diabetes, FI-adjBMI=fasting insulin adjusted for BMI ¶DI = Disposition Index adjusted for BMI; #*PTPRJ* is the nearest non-olfactory receptor gene in the locus.

Table 5. Apparently paradoxical associations between known glycaemic variants and insulin secretion rate. Betas represent per allele effects in standard deviations.

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

*Associated trait: FG=fasting glucose,Fproinsulin=fasting proinsulin,T2D=type 2 diabetes, FG=fasting glucose,FI=fasting insulin; †Classification by Dimas et al:

UC=unclassified, N/A=not available; ‡Code relating to significance of association across phenotypes and datasets: 1=associated at $P<0.05$ with peak insulin in our data,

2=associated at $P<0.05$ with acute insulin response in our data, 3=associated with CIR in Prokopenko et al., 4=associated at $P<0.05$ with CIRBMI+SI adj in our data.

§Chromosome. ||Base-pair position build-37. ¶Betas represent standard deviation effects per effect allele; #P-value after ISR adjustment for BMI.

FIGURE LEGENDS

Figure 1. IVGTT (peak insulin response) based first phase insulin secretion versus OGTT based insulin secretion (corrected insulin response) for known type 2 diabetes variants. Units = standard deviation. 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 ($P > 0.05$).

Figure 2. Insulin secretion rate versus OGTT based insulin secretion (corrected insulin response) for known type 2 diabetes variants. Units = standard deviation. 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 ($P > 0.05$).

Figure 3. IVGTT (peak insulin response) based first phase insulin secretion versus type 2 diabetes risk (OR = Odds ratio), for known type 2 diabetes variants. Y-axis units = standard deviation. (Type 2 diabetes odds ratios 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 ($P > 0.05$).

Figure 4. Insulin secretion rate versus type 2 diabetes risk for known type 2 diabetes variants. Y-axis units = standard deviation. 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 ($P > 0.05$).