

Clinical Research Article

Genome-Wide Association Analysis of Pancreatic Beta-Cell Glucose Sensitivity

Harshal A. Deshmukh,^{1,*} Anne Lundager Madsen,^{2,*} Ana Viñuela,³ Christian Theil Have,² Niels Grarup,² Andrea Tura,⁴ Anubha Mahajan,^{5,6} Alison J. Heggie,¹ Robert W. Koivula,^{6,7} Federico De Masi,⁸ Konstantinos K. Tsigos,⁸ Allan Linneberg,^{9,10} Thomas Drivsholm,^{9,11} Oluf Pedersen,² Thorkild I. A. Sørensen,^{12,13} Arne Astrup,¹⁴ Anette A. P. Gjesing,² Imre Pavo,¹⁵ Andrew R. Wood,¹⁶ Hartmut Ruetten,¹⁷ Angus G. Jones,¹⁸ Anitra D. M. Koopman,¹⁹ Henna Cederberg,²⁰ Femke Rutters,¹⁹ Martin Ridderstrale,²¹ Markku Laakso,²² Mark I. McCarthy,²³ Tim M. Frayling,¹⁶ Ele Ferrannini,²⁴ Paul W. Franks,^{6,7,25,26} Ewan R. Pearson,²⁷ Andrea Mari,⁴ Torben Hansen,² and Mark Walker¹

¹Translational and Clinical Research Institute, Newcastle University, Newcastle upon Tyne, NE2 4HH UK; ²Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, DK-2100 Copenhagen, Denmark; ³Department of Genetic Medicine and Development, University of Geneva Medical School, 1211, Geneva; ⁴Institute of Neuroscience, National Research Council, Corso Stati Uniti 4, 35127 Padua, Italy; ⁵Wellcome Centre for Human Genetics, Nuffield Department of Medicine, University of Oxford, Oxford, OX3 7BN, UK; ⁶Oxford Centre for Diabetes, Endocrinology and Metabolism, Radcliffe Department of Medicine, University of Oxford, Oxford, OX3 7LE, UK; ⁷Department of Clinical Sciences, Genetic and Molecular Epidemiology Unit, Skåne University Hospital Malmö, Lund University, 205 02 Malmö, Sweden; ⁸Integrative Systems Biology Group, Department of Health Technology, Technical University of Denmark (DTU), Kemitorvet, Building 208, 2800 Kgs. Lyngby, Denmark; ⁹Center for Clinical Research and Disease Prevention, Bispebjerg and Frederiksberg Hospital, The Capital Region, DK-2000 Copenhagen, Denmark; ¹⁰Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, DK-2200 Denmark; ¹¹Section of General Practice, Institute of Public Health, Faculty of Health Sciences, University of Copenhagen, Øster Farimagsgade 5, DK-1014 Copenhagen, Denmark; ¹²Novo Nordisk Foundation Centre for Basic Metabolic Research (Section of Metabolic Genetics), Faculty of Health and Medical Sciences, University of Copenhagen, DK-2100 Copenhagen, Denmark; ¹³Department of Public Health (Section of Epidemiology), Faculty of Health and Medical Sciences, University of Copenhagen, 1014 Copenhagen, Denmark; ¹⁴Department of Nutrition, Exercise and Sports (NEXS), Faculty of Science, University of Copenhagen, 1958 Copenhagen, Denmark; ¹⁵Eli Lilly Regional Operations Ges.m.b.H., Koelblgasse 8–10, 1030 Vienna, Austria; ¹⁶Genetics of Complex Traits, University of Exeter Medical School, University of Exeter, Exeter EX2 5DW, UK; ¹⁷Diabetes Division, Sanofi-Aventis Deutschland GmbH, Frankfurt, 65926 Frankfurt am Main, Germany; ¹⁸NIHR Exeter Clinical Research Facility, University of Exeter Medical School, Exeter EX2 5DW, UK; ¹⁹Department of Epidemiology and Biostatistics, VUMC, de Boelelaan 1089a, 1081 HV, Amsterdam, the Netherlands; ²⁰Department of Endocrinology, Abdominal Centre, Helsinki University Hospital, 00014 Helsinki, Finland; ²¹Department of Clinical Sciences, Diabetes & Endocrinology Unit, Lund University, Skåne University Hospital Malmö, CRC, 91-12, 205 02, Malmö, Sweden; ²²Institute of Clinical

Medicine, Internal Medicine, University of Eastern Finland, 70210 Kuopio, Finland; ²³Oxford Centre for Diabetes Endocrinology and Metabolism, University of Oxford, Oxford, OX3 7LJ, UK; ²⁴CNR Institute of Clinical Physiology, 56124 Pisa, Italy; ²⁵Department of Nutrition, Harvard TH Chan School of Public Health, Boston, Massachusetts 02115; ²⁶Department of Public Health and Clinical Medicine, Umeå University, 901 87 Umeå, Sweden; ²⁷Division of Population Health & Genomics, School of Medicine, University of Dundee, Dundee DD1 9SY, UK

ORCID numbers: 0000-0002-3859-3118 (H. A. Deshmukh); 0000-0001-5526-1070 (N. Grarup); 0000-0003-3466-5900 (A. Tura); 0000-0002-2526-9810 (M. Walker).

*These authors contributed equally to the study

Abbreviations: ACE model, model using additive genetic influence, shared environment (household effect), and the unique environment; AE model, model using additive genetic influence and the unique environment; BMI, body mass index; eQTL, expression quantitative trait loci; GWAS, genome-wide association study; HbA1c, glycated hemoglobin A1c; IGF2, insulin-like growth factor 2; IMI DIRECT, Innovative Medicines Initiative Diabetes Research on Patient Stratification; IVGTT, intravenous glucose challenge; MAF, minor allele frequency; MMTT, mixed meal tolerance test; OGTT, oral glucose tolerance test; RISC, Relationship between Insulin Sensitivity and Cardiovascular disease study; SE, standard error; SNP, single nucleotide polymorphism.

Received: 31 May 2020; Accepted: 14 September 2020; First Published Online: 18 September 2020; Corrected and Typeset: 3 November 2020.

Abstract

Context: Pancreatic beta-cell glucose sensitivity is the slope of the plasma glucose-insulin secretion relationship and is a key predictor of deteriorating glucose tolerance and development of type 2 diabetes. However, there are no large-scale studies looking at the genetic determinants of beta-cell glucose sensitivity.

Objective: To understand the genetic determinants of pancreatic beta-cell glucose sensitivity using genome-wide meta-analysis and candidate gene studies.

Design: We performed a genome-wide meta-analysis for beta-cell glucose sensitivity in subjects with type 2 diabetes and nondiabetic subjects from 6 independent cohorts (n = 5706). Beta-cell glucose sensitivity was calculated from mixed meal and oral glucose tolerance tests, and its associations between known glycemia-related single nucleotide polymorphisms (SNPs) and genome-wide association study (GWAS) SNPs were estimated using linear regression models.

Results: Beta-cell glucose sensitivity was moderately heritable (h^2 ranged from 34% to 55%) using SNP and family-based analyses. GWAS meta-analysis identified multiple correlated SNPs in the *CDKAL1* gene and *GIPR-QPCTL* gene loci that reached genome-wide significance, with SNP rs2238691 in *GIPR-QPCTL* (P value = 2.64×10^{-9}) and rs9368219 in the *CDKAL1* (P value = 3.15×10^{-9}) showing the strongest association with beta-cell glucose sensitivity. These loci surpassed genome-wide significance when the GWAS meta-analysis was repeated after exclusion of the diabetic subjects. After correction for multiple testing, glycemia-associated SNPs in or near the *HHEX* and *IGF2B2* loci were also associated with beta-cell glucose sensitivity.

Conclusion: We show that, variation at the *GIPR-QPCTL* and *CDKAL1* loci are key determinants of pancreatic beta-cell glucose sensitivity.

Key Words: Glucose intolerance, diabetes progression, beta-cell function, incretin, mathematical model

Decreased insulin secretion secondary to impaired pancreatic beta-cell function is an essential element in the development of abnormal glucose tolerance and type 2 diabetes. Using a progressive, stepped intravenous glucose infusion, a dose-response curve can be generated for insulin secretion rates against

plasma glucose levels. In cross-sectional studies, the slope of this curve (termed beta-cell glucose sensitivity) progressively decreases from normal to impaired glucose tolerance, and through to type 2 diabetes (1). An analogous dose-response relationship can be derived from standard oral glucose and

mixed meal tolerance tests (OGTT and MMTT, respectively) using C-peptide kinetic analysis to measure insulin secretion rates (2). This approach offers several advantages. First, it assesses beta-cell glucose sensitivity under conditions that reflect daily living in contrast to intravenous glucose-based methods that exclude the incretin system. Second, it is independent of potential confounders, such as hepatic insulin clearance, that can influence circulating insulin levels and impact on measures of beta-cell function that examine changes in insulin levels in response to a glucose challenge.

In line with the studies using intravenous glucose infusion, we have shown that the model-based beta-cell glucose sensitivity decreases with progressive glucose intolerance using cross-sectional data (3). Crucially, beta-cell glucose sensitivity was a strong, independent predictor of deteriorating glucose tolerance (4) and the development of type 2 diabetes (5) in longitudinal follow-up studies of people free from diabetes. Furthermore, beta-cell glucose sensitivity, together with a model-derived measure of whole-body insulin sensitivity, was found to completely replace the classical clinical risk factors (such as obesity and plasma glucose concentrations) as predictors of deteriorating glucose tolerance (4). In view of the emerging importance of beta-cell glucose sensitivity as a predictor of deteriorating glucose tolerance, we conducted a genome-wide analysis to understand the genetic basis of this phenotype.

The aims of this study were to define the heritability of beta-cell glucose sensitivity and to perform genome-wide association and candidate gene (known diabetes and glycemic risk loci) association analyses for beta-cell glucose sensitivity across a range of glucose tolerance.

Methods

Cohort description

The discovery cohorts were 2 multicenter prospective cohort studies within the Innovative Medicines Initiative Diabetes Research on Patient Stratification (IMI DIRECT) Consortium (6), which were specifically designed to address the molecular basis to glycemic deterioration. The IMI DIRECT cohorts include detailed information and biomaterials suitable for the analysis of genetic and nongenetic biomarkers for glycemic deterioration before and after the onset of type 2 diabetes. Cohort 2.1 ($n = 2233$) enrolled people with normal and dysregulated, but not diabetic, glucose homeostasis based on HbA1c (5.7%–6.4%, 40–48 mmol/mol) and OGTT, while cohort 2.2 consisted of those ($n = 784$) who had been recently diagnosed with type 2 diabetes at the time of enrollment. The study design and sample selection are previously described (7).

The data for the replication analyses (which has been meta-analyzed with the discovery cohorts) came from 4

independent cohorts, consisting of a mix of volunteers spanning a range from normal glucose tolerance to type 2 diabetes. These were the Relationship between Insulin Sensitivity and Cardiovascular disease (RISC) study (8), the ADIGEN study (9) the 1936-cohort (10) and the Family study (11). The RISC study is a prospective study of 1276 men and women with normal glucose tolerance of European ancestry, aged from 30 to 60 years, from 20 centers in 13 European countries (8). The ADIGEN study was a follow-up examination at around the age of 50 years of 2 groups of young men assessed for military service at around 19 years of age between 1943 and 1977 in the metropolitan area of Copenhagen (Denmark); 1 group were the most obese in that population ($n = 248$) and the control group ($n = 320$) was a random selection of 0.5% of that population. The study was designed to investigate frequent functional genetic variants that influence the development of obesity. The 1936-cohort is a population-based prospective age-specific cohort that consists of 1198 Danish subjects born in 1936, who were resident in municipalities nearby Glostrup Hospital (Denmark) in 1976. In the present study, we included subjects participating in the 20-year follow-up in 1996 (12). The purpose of the study was to follow and examine the association between insulin sensitivity and the development of cardiovascular diseases. The Family study consists of approximately 95 families from the Copenhagen area, including a total of 533 individuals, of whom 336 individuals were included in the present study. Families were recruited if one parent had type 2 diabetes. The study was designed to identify genetic loci influencing glucose homeostasis using linkage methods in families with type 2 diabetes. OGTT and MMTT were conducted as previously described (7–11). Briefly, following an overnight fast, blood was sampled at baseline (0 minutes) and at 30-minute intervals for 2 hours following the oral glucose/meal challenge. Blood was assayed for plasma glucose, insulin, and C-peptide at a central quality control laboratory for each cohort.

Glucose sensitivity measurement

Beta-cell function was assessed from the OGTT and MMTT (see Table 1) using a model that describes the relationship between insulin secretion and glucose concentration, which has been described in detail previously (2, 3). Glucose sensitivity measures were determined from the baseline OGTT and MMTT data for each cohort. Glucose sensitivity is the mean slope over the observed glucose range of the model-determined dose-response that relates insulin secretion to glucose concentration during the OGTT or MMTT. As shown in previous studies (13), glucose sensitivity reflects both intrinsic beta-cell function, as tested by intravenous glucose infusion, and the effects of incretin hormones. All analyses were conducted by 3 operators supervised by A. Mari.

Table 1. Demographic Characteristics and Key Metabolic Parameters of the Study Population for the GWAS Meta-analysis of Beta-Cell Glucose Sensitivity (n = 5706)

	IMI DIRECT 2.1 (n = 2233)	IMI DIRECT 2.2 (n = 784)	RISC study (n = 1276)	1936 birth cohort (n = 622)	ADIGEN study (n = 455)	Family study (n = 336)
Age, years, mean (\pm SD)	62.2 (\pm 6.50)	61.9 (\pm 7.91)	44.06 (\pm 8.34)	60.5 (\pm 0.46)	48.9 (\pm 5.81)	44.7 (\pm 13.3)
Ethnicity	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian
Women, %	24%	43%	54%	53%	0%	56%
% with diabetes	0%	100%	0%	6%	10%	9.5%
BMI, mean (\pm SD)	28.1(\pm 4.01)	30.49 (\pm 4.99)	25.68 (\pm 4.13)	26.7 (\pm 3.99)	30.2 (\pm 6.78)	26.8 (\pm 4.90)
Oral challenge	OGTT	MMTT	OGTT	OGTT	OGTT	OGTT
Glucose sensitivity, pmol min ⁻¹ m ⁻² mmol/L ⁻¹ , (median \pm SD)	97.7 (\pm 1.6)	69.1 (\pm 1.8)	107.1 (\pm 1.8)	85.1 (\pm 66)	69.1 (\pm 43.6)	61.6 (\pm 40.7)
Platform	Illumina Human- Core array	Illumina Human- Core array	Affymetrix	Illumina HumanCore Exome-24 BeadChip	Human610-Quad v.1.0 BeadChip	Illumina HumanCore Exome-24 BeadChip
pHWE exclusion	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Imputation software	IMPUTE	IMPUTE	IMPUTE	IMPUTE	IMPUTE	IMPUTE
GWAS software	SNPTEST	SNPTEST	SNPTEST	SNPTEST	SNPTEST	Mixed model using GCTA version 1.91.2
NCBI Build for imputation	GRCh38	GRCh38	GRCh38	GRCh38	GRCh38	GRCh38

Abbreviations: BMI, body mass index; pHWE, *P* value for Hardy-Weinberg equilibrium; IMI DIRECT, Innovative Medicines Initiative Diabetes Research on Patient Stratification; RISC, Relationship between Insulin Sensitivity and Cardiovascular disease study.

Genotyping and imputation methods

Pre-imputation quality control was standardized across all the 6 cohorts with minor allele frequency cutoff of 0.01, and sample and single nucleotide polymorphism (SNP) call rate of >0.98. Genotype imputation involved a 2-step process: (i) the genotypes to be imputed were “pre-phased” (a statistical method is applied to genotype data to infer the underlying haplotypes of each individual) using SHAPEIT (14); and (ii) IMPUTE (15) was then used to combine the inferred haplotypes with a reference panel of haplotypes and impute the unobserved genotypes in each sample using the 1000 Genomes Phase 3 (October 2014 release). Imputation was carried out in chunks of 1 Mb with a 500-kb buffer region. Imputed variants in each nonoverlapping part of each chunk were concatenated into per chromosome files.

Statistical analysis

Heritability estimation. Narrow-sense heritability for glucose sensitivity was estimated using the GCTA software (16) and the directly genotyped markers from the 2 IMI

DIRECT cohorts. We then estimated univariate heritability of glucose sensitivity by the restricted maximum likelihood method in GCTA (with sex and age at baseline included as covariates). The heritability thus estimated is also known as “narrow-sense” or “chip” heritability which is an indicator of additive genetic contribution from all SNPs. We estimated the heritability of beta-cell glucose sensitivity in the Family study using the tool SOLAR (17). We used 2 different models to estimate the heritability of the trait in the Family study. The first model includes the additive genetic influence and the unique environment (AE model). The second model uses the additive genetic influence, the shared environment (household effect), and the unique environment (ACE model).

Candidate gene selection and analysis. For candidate gene analysis, we selected 155 SNPs associated with type 2 diabetes and glycemic and insulin-related traits reported in previous studies (18, 19). This SNP set was used by a recent GWAS of first-phase insulin secretion, measured by intravenous glucose tolerance tests (20). Linear regression models adjusted for age, sex, body mass

index (BMI), first 3 principal components of ancestry, and study center were used to test the association of each SNP with glucose sensitivity. For this analysis, a P value ≤ 0.0003 ($0.05/\text{number of tests}$) was considered statistically significant.

Genome-wide association analysis. We performed genome-wide association study (GWAS) of beta-cell glucose sensitivity in 5706 individuals of European descent. In the primary analyses, glucose sensitivity measures were fitted in a linear regression model with age, gender, and study center (for RISC and DIRECT studies, which were conducted at multiple centers), BMI, and the first 3 principal components for race/ethnicity (derived from EIGENSTRAT) included as covariates. We also ran the analysis without adjustment for BMI in an attempt to identify loci associated with glucose sensitivity via adiposity. The glucose sensitivity was normalized by \log_{10} transformation. To account for imputation uncertainty, we used the 1000 Genomes imputation allele dosage in linear models.

Meta-analysis of genome-wide association studies. We used the METAL program (21) to meta-analyze individual studies by combining the study-specific P values across studies taking sample size and direction of effect into account. In total, 8 978 282 SNPs passed quality control (minor allele frequency [MAF] 2% in individual cohorts; imputation quality >0.3 in MACH or >0.4 in IMPUTE) and were included in the meta-analysis. METAL was also used to assess heterogeneity across the 3 cohorts for the top signals.

eQTL analyses. To identify potential effector transcripts mediating the activity of the top associated variants, we extracted cis-expression quantitative trait loci (eQTLs) information available from each of the top associated SNPs in 43 GTEx tissues (22) and pancreatic islets (23). Since these studies reported multiple genes associated with each SNP, we selected the most strongly associated eQTLs per SNP that generated a nominal P value, before calculating a corrected P value with the $p.adjust$ function in R and using the Benjamini-Hochberg method.

Results

Demographic and key metabolic characteristics of the study population

Table 1 summarizes the demographic characteristics of the study population ($n = 5706$). All the participating cohorts comprised individuals of both genders, except the ADIGEN study which recruited just males.

Heritability estimates for beta-cell glucose sensitivity

The SNP-based heritability (narrow-sense heritability) of beta-cell glucose sensitivity in the combined discovery cohort, type 2 diabetes, and prediabetes population ($n = 3017$) after adjustments for age, sex, and BMI was 34% ($h^2 = 0.34 [\pm 0.09]$ P value = 2.33×10^{-10}).

In the Family study, after adjustments for age, sex, and BMI and using the inverse transformed phenotype in an AE model, we obtained a heritability of 55% (standard error [SE] 13%; P value = 7.12×10^{-9}). Analysis with the ACE model did not change the result, with zero shared environmental effect (C). The heritability was also calculated using only people with normal glucose tolerance ($n = 252$) with the same parameters. Both the AE model and the ACE model (with zero variance explained by shared environment) gave a heritability of 52% (SE 12%; P value = 2.73×10^{-8}). Thus, the heritability did not seem to be affected by altered glucose tolerance.

Genome-wide association study and meta-analysis

Figs. 1 and 2 show the Manhattan and Q-Q plots, respectively, for the GWAS meta-analysis across the 6 cohorts. The Q-Q plots for individual cohorts are shown in Supplementary Figure 1 (6). The GWAS meta-analysis showed multiple correlated SNPs on chromosome 6 in the *CDKAL1* gene locus and on chromosome 19 in the *GIPR-QPCTL* gene region reaching the accepted level of significance for GWAS with P value $< 10^{-8}$ (all significant SNPs listed in Supplementary Table 1 (6)). Greatest significance was seen for SNP rs2238691 (Z-score = -5.953 , P value = 2.64×10^{-9}) within the *GIPR-QPCTL* region and for rs9368219 (Z-score -5.9 , P value = 3.15×10^{-9}) in the *CDKAL1* region (**Figs 3 and 4**, respectively). The effect estimates for these SNP were comparable across the replication and discovery cohorts. The SNPs with a P value between $>10^{-8}$ and $<10^{-7}$ are summarized in Supplementary Table 2 (6).

To further explore the top associated variants, we extracted cis-expression quantitative trait loci (cis-eQTLs) associations from 43 GTEx tissues (22) and pancreatic islets from the InsPIRE study (23). Cis-eQTLs analysis is used to explore candidate genes mediating the activity of GWAS variants. By extracting the most significant eQTLs per tissue for each of the SNPs included in Supplementary Table 1 (6), we found that the most strongly associated eQTLs were all expressed in pancreatic islets (Supplementary Table 3 (6)), although no individual eQTL was significant after correction for multiple testing.

In view of the potential secondary metabolic effects of the diabetic state on pancreatic beta-cell function, the

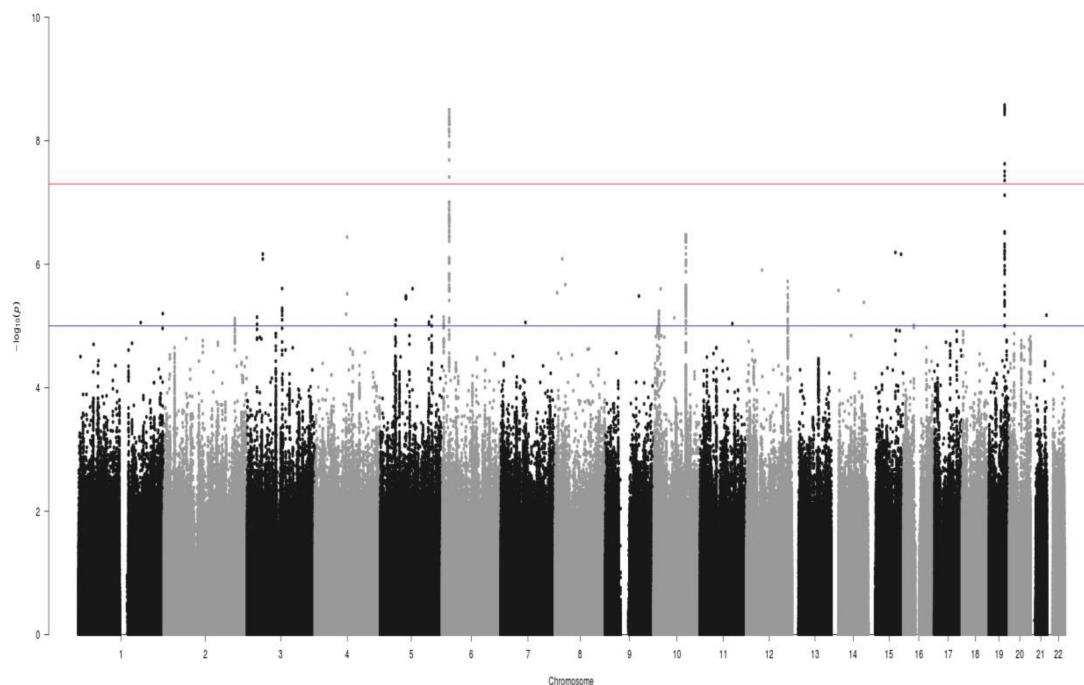


Figure 1. Manhattan plot of genome-wide P values of association for beta-cell glucose sensitivity: horizontal upper and lower lines represent the suggestive genome-wide significance thresholds of $p < 10^{-7}$ and $p < 10^{-5}$, respectively.

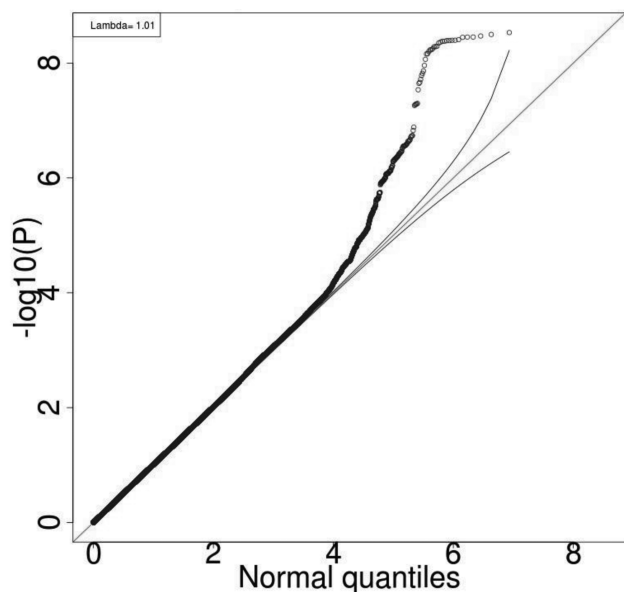


Figure 2. Q-Q plot of genome-wide P values of association for beta-cell glucose sensitivity of the observed versus expected P values given the number of statistical tests performed for beta-cell glucose sensitivity.

GWAS meta-analysis was repeated in nondiabetic subjects ($n = 4544$) after excluding the IMI DIRECT 2.2 cohort and the known diabetic patients in the other cohorts listed in [Table 1](#). There was no change in the SNPs that achieved genome-wide significance ([Supplementary Table 4 \(6\)](#)), although the top SNPs identified in each of the *CDKAL1*

and *GIPR-QPCTL* regions were different (rs1040558 and rs35541137, respectively).

Candidate gene association tests

[Table 2](#) shows the association of known glycated hemoglobin A1c (HbA1c), glycemic traits (fasting and 2-hour plasma glucose) and type 2 diabetes SNPs with beta-cell glucose sensitivity ($P < 0.01$). After correction for multiple testing, type 2 diabetes-associated SNPs in or near *HHEX*, *CDKAL1*, *IGF2B2*, fasting glucose-associated SNPs in or near *CDKAL1* and *IGF2BP2*, and a 2-hour glucose post-OGTT-associated SNP at the *GIPR* locus were all associated with beta-cell glucose sensitivity. The association of these SNPs was directionally consistent with the expected underlying biology. For instance, the “T” allele at rs1111875 in the *HHEX* locus is protective for diabetes and is associated with higher beta-cell glucose sensitivity. This SNP was nominally associated with the expression of the *MARK2P9* gene in pancreatic islets (P value = 6.35×10^{-3}), but not with eQTLs in other tissues ([Supplementary Table 5 \(6\)](#)). Other variants included in the analysis were also significant cis-eQTLs in pancreatic islets, this being the only tissue with significant eQTLs after multiple testing for the candidate SNPs listed in [Table 2](#). The associations of the 155 known loci for type 2 diabetes HbA1c and other glycemic traits with beta-cell glucose sensitivity are summarized in [Supplementary Table 6 \(6\)](#). The heterogeneity

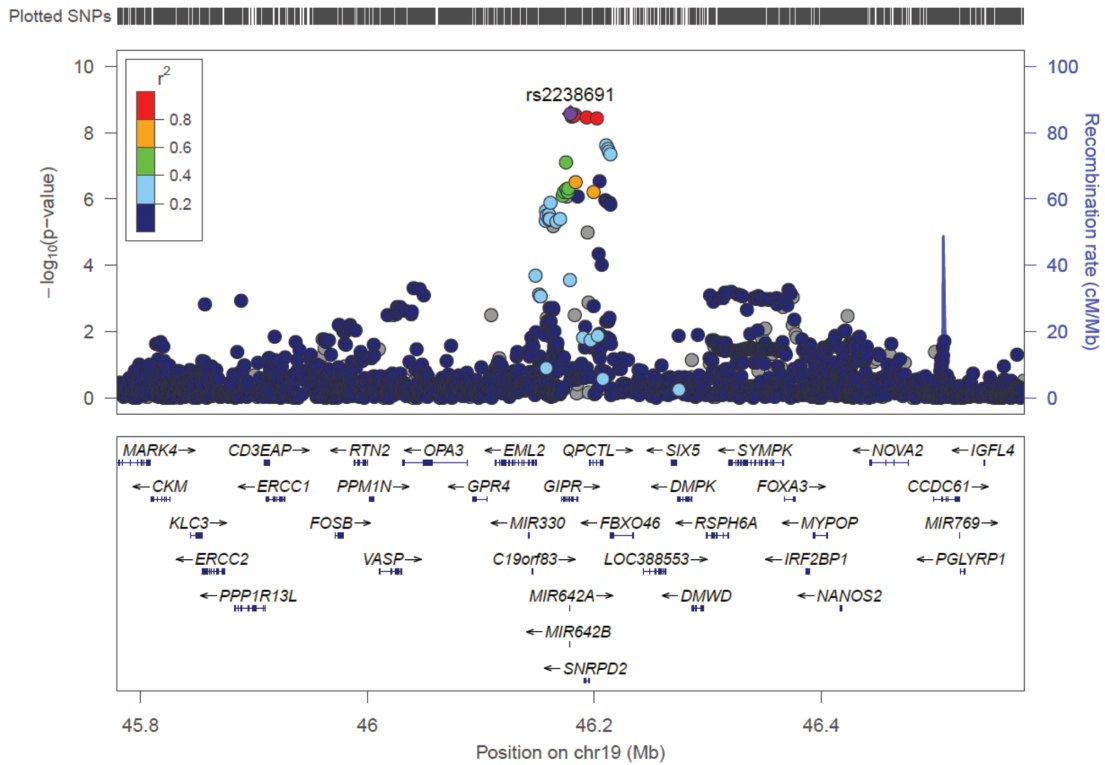


Figure 3. Regional association plot of *GIPR-QPCTL* gene region. Plot produced in Locus Zoom with the most strongly associated SNP (rs2238691) shown as the purple diamond.

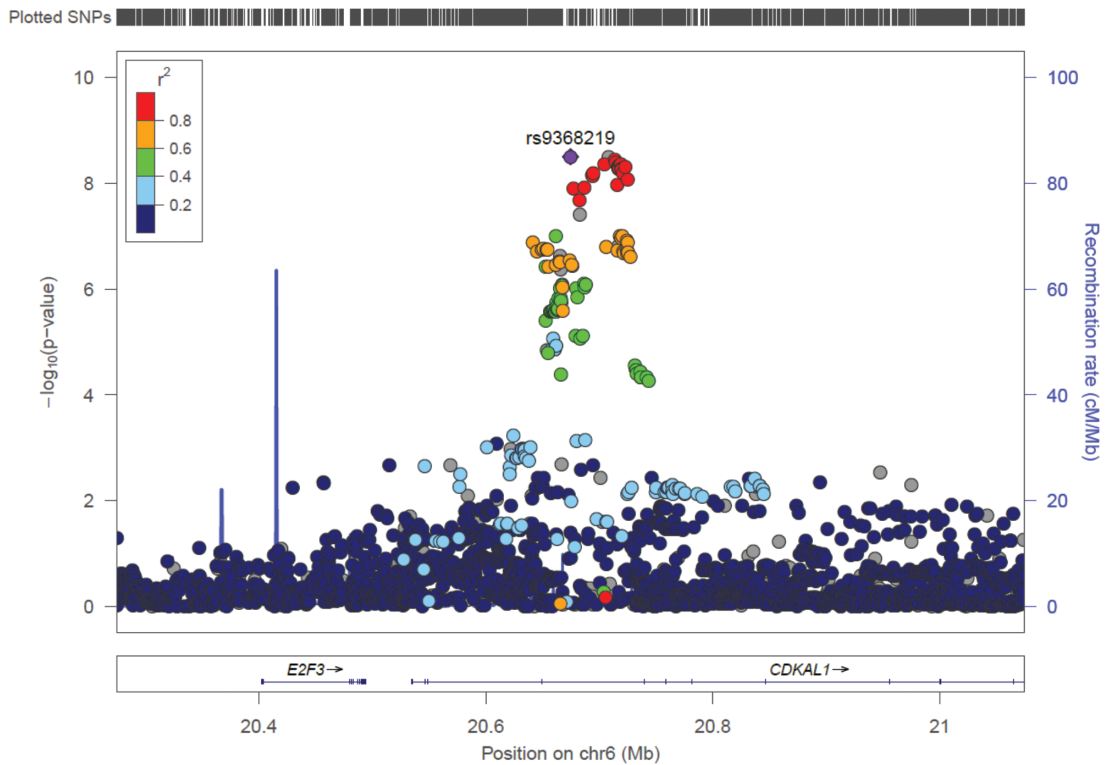


Figure 4. Regional association plot of *CDKAL1* gene region. Plot produced in LocusZoom with the most strongly associated SNP (rs9368219) shown as the purple diamond.

Table 2. Association of the Known SNPs for Type 2 Diabetes, HbA1c, and Glycemic Traits With Beta-Cell Glucose Sensitivity

Phenotype	CHR	SNP	Position	Effect allele	Z-score	P value	Gene
2-hr glucose	19	rs11672660	46180184	t	-5.92	3.21E-09	<i>GIPR</i>
Fasting glucose	6	rs9368222	20686996	a	-4.907	9.25E-07	<i>CDKAL1</i>
T2D	6	rs7756992	20679709	a	4.902	9.47E-07	<i>CDKAL1</i>
T2D	10	rs1111875	94462882	t	4.661	3.15E-06	<i>HHEX</i>
Fasting glucose	3	rs7651090	185513392	a	3.753	1.75E-04	<i>IGF2BP2</i>
T2D	3	rs4402960	185511687	t	-3.751	1.76E-04	<i>IGF2BP2</i>
FGFproinsulin	11	rs11603334	72432985	a	3.366	7.62E-04	<i>ARAP1</i>
T2D	11	rs1552224	72433098	a	-3.366	7.63E-04	<i>ARAP1</i>
Fasting glucose	13	rs11619319	28487599	a	3.16	1.58E-03	<i>PDX1</i>
Fasting glucose	2	rs560887	169763148	t	-3.097	1.95E-03	<i>G6PC2</i>
T2D	7	rs849135	28196413	a	2.926	3.43E-03	<i>JAZF1</i>
HbA1c	2	rs552976	169791438	a	-2.902	3.71E-03	<i>ABCB11</i>
Fasting glucose	7	rs6943153	50791579	t	2.85	4.37E-03	<i>GRB10</i>
T2D	19	rs8108269	46158513	t	2.794	5.20E-03	<i>GIPR</i>
Fasting glucose	11	rs174550	61571478	t	-2.769	5.63E-03	<i>FADS1</i>
Fasting glucose	11	rs174576	61603510	a	2.607	9.15E-03	<i>FADS1</i>

Phenotype refers to the phenotype reported to be associated with this SNP.

Associations reaching Bonferroni equivalents of $P < 0.05$ are in bold. Base pair position build-37.p13.

Abbreviations: CHR, chromosome; HbA1c, glycated hemoglobin A1c; SNP, single nucleotide polymorphism; T2D, type 2 diabetes.

in the effect sizes for the top SNPs in Table 2 across the cohorts was not significant (Supplementary Table 7 (6)).

The same candidate gene analyses were repeated in just the nondiabetic subjects. The associations between SNPs and beta-cell glucose sensitivity observed in the whole cohort (Table 2) remained in the nondiabetic subjects (Supplementary Table 8 (6)), albeit at generally lesser degrees of statistical significance.

For the known SNPs associated with type 2 diabetes and glycemic traits ($P < 0.01$), we examined the overlap between their association with beta-cell glucose sensitivity in our study and the early peak insulin response to an intravenous glucose challenge (IVGTT) as previously reported (19). As shown in Supplementary Figure 2 (6), SNPs in *GIPR*, *G6PC2*, *JAZF1*, and *FADS1* are associated with pancreatic beta-cell glucose sensitivity but not with early insulin response during the IVGTT, while SNPs in *ABCB11*, *CDKAL1*, *IGF2BP2*, *ARAP1*, *HHEX*, *PDX1*, and *GRB10* show an association with both phenotypes. Conversely, variants in *MNTR1B* and *TCF7L2* were strongly associated with the early insulin response during the IVGTT, but variation in these type 2 diabetes susceptibility loci was not associated with pancreatic beta-cell glucose sensitivity after correction for multiple testing.

Discussion

This is the first study to conduct a genome-wide association meta-analysis of pancreatic beta-cell glucose sensitivity. The key finding is that variation at the *CDKAL1* and *GIPR-QPCTL* regions showed the strongest associations with beta-cell glucose sensitivity in the entire study cohort,

and when the analysis was limited to nondiabetic subjects. These findings were corroborated by the candidate gene analyses, which also found a strong association between known variants at the *HHEX* locus and beta-cell glucose sensitivity. In addition, we observed that pancreatic islet eQTLs clustered with the top GWAS SNPs, while multiple variants from the candidate gene SNPs were individually associated with islet eQTLs.

We have previously shown that type 2 diabetes risk variants in *CDKAL1* and *HHEX* were associated with decreased beta-cell glucose sensitivity in nondiabetic individuals (24); however, these associations have not been tested in a larger cohort that includes subjects with abnormal glucose tolerance. Lyssenko and colleagues reported similar findings in a large longitudinal cohort study but measured the acute insulin response to an oral glucose load rather than beta-cell glucose sensitivity (25).

CDKAL1 represents one of the strongest signals of association with type 2 diabetes across diverse ancestries, with minimal heterogeneity in allelic effects between populations (26-30). The role of *CDKAL1* in pancreatic beta-cell function remains to be fully defined. However, it is strongly expressed in human adult pancreatic islets relative to other tissues (31), and *CDKAL1* gene deletion is accompanied by modestly impaired insulin secretion during high-fat feeding in mice (28). There is emerging evidence that *CDKAL1* encodes a methyltransferase that regulates tRNA^{Lys} function and proinsulin synthesis in pancreatic beta cells (32).

A large GWAS (33) identified the *GIPR-QPCTL* (rs10423928) locus to be associated with 2-hour blood

glucose levels after an oral glucose challenge. This study also showed that *GIPR* had strong specific mRNA expression in the sorted pancreatic beta cells, supporting the role of *GIPR* in insulin secretion. The *GIPR-QPCTL* (rs10423928) locus is in linkage disequilibrium with rs2238691 identified in our study ($r^2 = 41\%$ in HapMap CEU Population and $r^2 = 99\%$ in our study) suggesting that these 2 GWAS identified the same signal. Gastric inhibitory polypeptide (GIP) along with glucagon-like peptide-1 (GLP-1) are incretin hormones that serve to amplify the insulin secretory response after food ingestion, and *GIPR* plays a key role in this process. As previously reported, the beta-cell glucose sensitivity is in part influenced by the effects of incretin hormones (13). Interestingly, variation at the GLP-1 receptor gene locus was not associated with beta-cell glucose sensitivity in this analysis.

A recent study reported a similar approach to investigate the genetic basis of the early insulin response to an IVGTT (20). The strongest associations were in or near the *MTNR1B* and *CDKAL1* loci. Taken with our findings, the evidence highlights a critical role for *CDKAL1* in the regulation of pancreatic insulin secretion (Supplementary Figure 2 (6)). Intriguingly, although variation in *TCF7L2* has been identified as the strongest common genetic determinant of type 2 diabetes, it was not a significant determinant of beta-cell glucose sensitivity but was associated with the early insulin response to an IVGTT (20).

We show that the known loci for HbA1c, such as *ABCB11*, and the established type 2 diabetes loci, *IGF2BP2*, and *ARAP1*, could mediate their effect on type 2 diabetes risk by their action on pancreatic beta-cell glucose sensitivity. Previous literature has shown the association of these loci with glucose homeostasis and cis-eQTLs active in pancreatic islets (23). Insulin-like growth factor 2 (*IGF2*) mRNA-binding protein 2 (*IGF2BP2*) belongs to a family of IGF2 mRNA-binding proteins that play an important role in pancreatic development (34), while *IGF2BP2* mRNA levels are associated with glucose and insulin homeostasis (35).

A limitation of our study is its modest sample size of 5706 European samples for a GWAS study, which constrains our ability to detect associations with low-frequency variants. Another potential concern is that beta-cell glucose sensitivity was determined from OGTT and MMTT. However, the MMTT was used only in the DIRECT 2.2 cohort and GWAS analyses were first conducted separately within the individual cohorts, and then the cohort specific *P* values were meta-analyzed. Furthermore, when the DIRECT 2.2 cohort was excluded as part of the analysis of the nondiabetic subjects, variation in *CDKAL1* and

GIPR-QPCTL regions remained the strongest determinants of beta-cell glucose sensitivity.

Clearly, the measurement of pancreatic beta-cell glucose sensitivity as a predictor of type 2 diabetes would be impractical in the clinical setting. The identification, therefore, of clinically applicable biomarkers of beta-cell glucose sensitivity is attractive, and as a step toward this goal we have explored the genetic architecture of this pancreatic beta-cell phenotype.

In summary, *CDKAL1* and *GIPR-QPCTL* loci showed the strongest associations with beta-cell glucose sensitivity by genome-wide and candidate gene-based approaches, and these associations were independent of diabetes status.

Acknowledgments

Financial Support: The work leading to this publication was funded by the Innovative Medicines Initiative Joint Undertaking under grant agreement n°115317 (DIRECT), resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007–2013) and EFPIA companies' in-kind contribution. We acknowledge the support of the DIRECT Consortium (6) European Union's Horizon 2020 research and innovation program (grant agreement No 667191), the Novo Nordisk Foundation Center for Basic Metabolic Research which is an independent Research Center based at the University of Copenhagen, Denmark and partially funded by an unconditional donation from the Novo Nordisk Foundation (www.cbmr.ku.dk) (Grant number NNF18CC0034900.), and the Danish Diabetes Academy, which is funded by the Novo Nordisk Foundation, grant number NNF17SA0031406

The ADIGEN study was supported by grants from The Danish Medical Research Council, The Danish National Research Foundation, and The Velux Foundation. We thank Professor George Davey Smith, Bristol University, for his contribution to the male-GOYA GWAS.

The Family study was supported by grants from the Danish Medical Research Council; the University of Copenhagen; an EEC grant (BMH4CT950662); the Velux Foundation; the Lundbeck Foundation Centre of Applied Medical Genomics for Personalized Disease Prediction and Care (LUCAMP; www.lucamp.org); the European Foundation for the Study of Diabetes (EFSD) and the Danish Diabetes Association. [Genetics DIRECT]

The 1936-cohort acknowledges support of the Danish Heart Foundation, and the Danish Medical Research Council financially supported the 1996/97 investigation

ERP holds a Wellcome Trust New Investigator Award (102820/Z/13/Z). The work undertaken by P.W.F was supported in part by ERC-2015-CoG_NASCENT_681742.

Additional Information

Correspondence and Reprint Requests: Prof Mark Walker, Translational and Clinical Research Institute (Diabetes), The Medical School, Newcastle University, Framlington Place, Newcastle upon Tyne, NE2 4HH. E-mail: mark.walker@ncl.ac.uk; Prof Torben Hansen, Novo Nordisk Foundation Center for Basic Metabolic

Research, Faculty of Health and Medical Sciences, University of Copenhagen, Maersk Tower, Blegdamsvej 3B, 07-8-26, DK-2200, Copenhagen N, Denmark. E-mail: torben.hansen@sund.ku.dk; Dr Andrea Mari, Institute of Neuroscience, National Research Council, Corso Stati Uniti 4, 35127 Padova, Italy. E-mail: andrea.mari@cnr.it.

Disclosure Summary: The authors declare that there are no conflicts of interest related to this manuscript.

Data Availability: The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

References

- Byrne MM, Sturis J, Sobel RJ, Polonsky KS. Elevated plasma glucose 2 h postchallenge predicts defects in beta-cell function. *Am J Physiol*. 1996;270(4 Pt 1):E572-E579.
- Mari A, Schmitz O, Gastaldelli A, Oestergaard T, Nyholm B, Ferrannini E. Meal and oral glucose tests for assessment of beta-cell function: modeling analysis in normal subjects. *Am J Physiol Endocrinol Metab*. 2002;283(6):E1159-E1166.
- Ferrannini E, Gastaldelli A, Miyazaki Y, Matsuda M, Mari A, DeFronzo RA. beta-Cell function in subjects spanning the range from normal glucose tolerance to overt diabetes: a new analysis. *J Clin Endocrinol Metab*. 2005;90(1):493-500.
- Walker M, Mari A, Jayapaul MK, Bennett SM, Ferrannini E. Impaired beta cell glucose sensitivity and whole-body insulin sensitivity as predictors of hyperglycaemia in non-diabetic subjects. *Diabetologia*. 2005;48(12):2470-2476.
- Tura A, Grassi A, Winhofer Y, et al. Progression to type 2 diabetes in women with former gestational diabetes: time trajectories of metabolic parameters. *PLoS One*. 2012;7(11):e50419.
- Deshmukh H, Walker M. Glucosens Supplementary material. *figshare*. Deposited May 31, 2020. https://figshare.com/articles/Glucosens_SUPPL_INFO_22_5_2020_docx/12401978.
- Koivula RW, Heggie A, Barnett A, et al.; DIRECT Consortium. Discovery of biomarkers for glycaemic deterioration before and after the onset of type 2 diabetes: rationale and design of the epidemiological studies within the IMI DIRECT Consortium. *Diabetologia*. 2014;57(6):1132-1142.
- Hills SA, Balkau B, Coppack SW, et al.; Report prepared on behalf of the 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(3):566-570.
- Black E, Holst C, Astrup A, et al. Long-term influences of body-weight changes, independent of the attained weight, on risk of impaired glucose tolerance and Type 2 diabetes. *Diabet Med*. 2005;22(9):1199-1205.
- Drivsholm T, Ibsen H, Schroll M, Davidsen M, Borch-Johnsen K. Increasing prevalence of diabetes mellitus and impaired glucose tolerance among 60-year-old Danes. *Diabet Med*. 2001;18(2):126-132.
- Gjesing AP, Ekstrom CT, Eiberg H, et al. Fasting and oral glucose-stimulated levels of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are highly familial traits. *Diabetologia*. 2012;55(5):1338-1345.
- Drivsholm T, Eplöv LF, Davidsen M, et al. Representativeness in population-based studies: a detailed description of non-response in a Danish cohort study. *Scand J Public Health*. 2006;34(6):623-631.
- Muscelli E, Mari A, Casolaro A, et al. Separate impact of obesity and glucose tolerance on the incretin effect in normal subjects and type 2 diabetic patients. *Diabetes*. 2008;57(5):1340-1348.
- Delaneau O, Marchini J, Zagury JF. A linear complexity phasing method for thousands of genomes. *Nat Methods*. 2011;9(2):179-181.
- 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(6):e1000529.
- Yang J, Lee SH, Goddard ME, Visscher PM. Genome-wide complex trait analysis (GCTA): methods, data analyses, and interpretations. *Methods Mol Biol*. 2013;1019:215-236.
- Williams JT, Van Eerdewegh P, Almasy L, Blangero J. Joint multipoint linkage analysis of multivariate qualitative and quantitative traits. I. Likelihood formulation and simulation results. *Am J Hum Genet*. 1999;65(4):1134-1147.
- 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(9):981-990.
- 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, et al. Genome-wide trans-ancestry meta-analysis provides insight into the genetic architecture of type 2 diabetes susceptibility. *Nat Genet*. 2014;46(3):234-244.
- Wood AR, Jonsson A, Jackson AU, et al. A genome-wide association study of IVGTT-based measures of first-phase insulin secretion refines the underlying physiology of type 2 diabetes variants. *Diabetes*. 2017;66(8):2296-2309.
- Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics*. 2010;26(17):2190-2191.
- Consortium GT, Laboratory DA, Coordinating Center -Analysis Working G, Statistical Methods groups-Analysis Working G, Enhancing Gg, Fund NIHC, et al. Genetic effects on gene expression across human tissues. *Nature*. 2017;550(7675):204-213.
- Viñuela A, Varshney A, van de Bunt M, et al. Genetic variant effects on gene expression in human pancreatic islets and their implications for T2D. *Nat Commun*. 2020;11(1):4912.
- Pascoe L, Tura A, Patel SK, et al.; RISC Consortium; U.K. Type 2 Diabetes Genetics Consortium. Common variants of the novel type 2 diabetes genes CDKAL1 and HHEX/IDE are associated with decreased pancreatic beta-cell function. *Diabetes*. 2007;56(12):3101-3104.
- Lyssenko V, Jonsson A, Almgren P, et al. Clinical risk factors, DNA variants, and the development of type 2 diabetes. *N Engl J Med*. 2008;359(21):2220-2232.
- Liang J, Zhu Y, Liu XK, et al. Protective effects of an obesity-associated polymorphism (CDKAL1 rs9356744) on prediabetes: the cardiometabolic risk in Chinese (CRC) study. *Exp Clin Endocrinol Diabetes*. 2018;126(9):540-545.

27. Noury AE, Azmy O, Alsharnoubi J, Salama S, Okasha A, Gouda W. Variants of CDKAL1 rs7754840 (G/C) and CDKN2A/2B rs10811661 (C/T) with gestational diabetes: insignificant association. *BMC Res Notes*. 2018;**11**(1):181.
28. Okamura T, Yanobu-Takanashi R, Takeuchi F, et al. Deletion of CDKAL1 affects high-fat diet-induced fat accumulation and glucose-stimulated insulin secretion in mice, indicating relevance to diabetes. *PLoS One*. 2012;**7**(11):e49055.
29. Tarnowski M, Malinowski D, Pawlak K, Dziedziejko V, Safranow K, Pawlik A. GCK, GCKR, FADS1, DGKB/TMEM195 and CDKAL1 gene polymorphisms in women with gestational diabetes. *Can J Diabetes*. 2017;**41**(4):372-379.
30. Tuerxunyiming M, Mohemaiti P, Wufuer H, Tuheti A. Association of rs7754840 G/C polymorphisms in CDKAL1 with type 2 diabetes: a meta-analysis of 70141 subjects. *Int J Clin Exp Med*. 2015;**8**(10):17392-17405.
31. Zeggini E, Weedon MN, Lindgren CM, et al. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science*. 2007;**316**(5829):1336-1341.
32. Wei FY, Suzuki T, Watanabe S, et al. Deficit of tRNA(Lys) modification by Cdkal1 causes the development of type 2 diabetes in mice. *J Clin Invest*. 2011;**121**(9):3598-3608.
33. Saxena R, Hivert MF, Langenberg C, et al.; GIANT consortium; MAGIC investigators. Genetic variation in GIPR influences the glucose and insulin responses to an oral glucose challenge. *Nat Genet*. 2010;**42**(2):142-148.
34. Bell JL, Wächter K, Mühleck B, et al. Insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs): post-transcriptional drivers of cancer progression? *Cell Mol Life Sci*. 2013;**70**(15):2657-2675.
35. Chen BH, Hivert MF, Peters MJ, et al. Peripheral blood transcriptomic signatures of fasting glucose and insulin concentrations. *Diabetes*. 2016;**65**(12):3794-3804.