Supplemental table 1. Clinical characteristics of the study population.

	German Cohort	Hyperglycemic clamp replication cohort
Gender (female / male)	1000 / 505	262/195
NGT / IFG / IGT / (IFG+IGT)	1098 / 151 / 146 / 110	265 (NGT) / 192 (IGT)
Age (y)	39 ±13	45 ±15
BMI (kg/m²)	28.5 ±7.8	26.0 ±4.5
Fasting glucose (mM)	5.09 ±0.54	5.26 ±0.93
Glucose, 120min OGTT (mM)	6.26 ±1.64	6.75 ±2.18
Fasting insulin (pM)	62.5 ±52.4	54.2 ±35.0

Data are given as means ±SD. BMI – body mass index; IFG – impaired fasting glucose; IGT – impaired glucose tolerance; NGT – normal glucose tolerance; OGTT – oral glucose tolerance test.

Supplemental table 2. Associations of *G6PC2* SNP rs560887 with anthropometrics, metabolic parameters, and insulin secretion in the overall cohort.

SNP	***G6PC2* SNP rs560887**			**P_{add}**
Genotype	**G/G**	**G/A**	**A/A**	
N OGTT (f/m)	718 (486/232)	646 (420/226)	140 (93/47)	-
Age (years)	39 (38-40)	39 (38-40)	40 (38-42)	0.8
BMI (kg/m²)	28.53 (27.94-29.12)	28.53 (27.93-29.14)	28.65 (27.48-29.82)	0.9
HbA1c (%)	5.33 (5.30-5.37)	5.30 (5.27-5.33)	5.26 (5.18-5.34)	0.06
Fasting glucose (mmol/l)	5.16 (5.12-5.20)	5.07 (5.02-5.11)	4.9 (4.83-4.99)	<0.0001
Glucose 120 min OGTT (mmol/l))	6.24 (6.12-6.37)	6.28 (6.15-6.41)	6.20 (5.94-6.46)	0.6
Fasting Insulin (pmol/l)	63.0 (58.9-67.1)	61.7 (57.9-65.5)	62.6 (54.5-70.7)	1.0
C-peptide 30 min OGTT (pmol/l)	2070 (2005-2136)	2034 (1964-2104)	1969 (1823-2117)	0.2
Insulinogenic index (x 10^{-9})	157 (146-168)	146 (136-156)	144 (108 -180)	0.3
HOMA-IR (mU x mmol x l^{-2})	2.48 (2.30-2.65)	2.37 (2.22-2.53)	2.33 (2.01-2.65)	0.4
OGTT-derived ISI (AU)	16.46 (15.67-17.25)	16.25 (15.48-17.03)	17.95 (15.69-20.20)	0.4
N Euglycemic clamp	233	228	50	
Clamp-derived ISI (AU)	0.084 (0.078-0.091)	0.086 (0.078-0.094)	0.090 (0.075-0.105)	0.7
N ivGTT	144	136	33	
First phase insulin secretion (ivGTT) pmol/ml	962 (858-1065)	903 (796-1009)	846 (629-1063)	0.06
N Hyperglycemic clamp	209	202	46	
First phase insulin secretion (pmol/l)	744 (687-806)	740 (678-808)	723 (633-827)	0.8
Second phase insulin secretion (pmol/l)	248 (230-268)	243 (223-265)	259 (229-293)	0.9

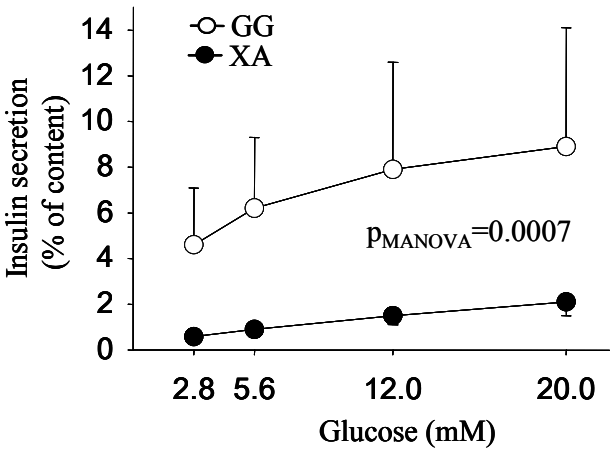
Data represent means (95 % CI). For statistical analysis, data were $\log_e$-transformed. BMI was adjusted for age and gender. HbA1c, glucose and insulin levels, HOMA IR and ISI (OGTT- and clamp-derived) were adjusted for gender, age, and BMI. Indices of insulin secretion were adjusted for gender, age, BMI and insulin sensitivity. Secretion indices of the hyperglycemic clamp studies were adjusted for age, gender, BMI, study center, glucose tolerance status and family relatedness.

HbA1c – glycosylated hemoglobin; HOMA-IR - homeostatic model assessment of insulin resistance; P_{add} – p-values for the additive inheritance model; BMI – body mass index; SNP – single nucleotide polymorphism.

Supplemental table 3. Distribution of G6PC2 rs560887 genotype in the hyperglycaemic clamp group

		G6PC2 SNPrs560887			
Independent studies		GG	GA	AA	Total
Hoorn	Count	68	57	14	139
	%	48.9%	41.0%	10.1%	100.0%
Utrecht	Count	32	37	8	77
	%	41.6%	48.1%	10.4%	100.0%
Germany	Count	53	49	16	118
	%	44.9%	41.5%	13.6%	100.0%
Twins	Count	56	59	8	123
	%	45.5%	48.0%	6.5%	100.0%
Total	Count	209	202	46	457
	%	45.7%	44.2%	10.1%	100.0%

Supplementary figure 1



*Supplemental figure 1***Glucose-induced insulin secretion in isolated human islets.** Batches of islets were stimulated with 2.8, 5.6, 12.0, 20.0 mM of glucose for 1 hour. Insulin concentration of the supernatant and insulin content of the islets were measured. Data are given as means. White circles: homozygous carriers of the major allele (N=2); black circles: heterozygous and homozygous carriers of the minor allele (N=3). Differences between groups were tested using MANOVA.

Supplemental Material and Methods

Participants: For the primary cohort, the participants were selected from the ongoing Tübingen Family Study (1). We selected 1,504 non-diabetic subjects for which complete OGTT data were available. Individuals on medication affecting glucose metabolism were excluded. Additional six subjects were excluded due to implausible insulin and/or glucose values. The clinical characteristics are shown in supplementary table 1. Informed written consent was obtained from all participants, and the local Ethics Committees approved the protocol.

A control group for replication purposes was formed using four independent studies from the Netherlands and Germany. The clinical characteristics of the 457 participants are given in supplementary table 1. Details of the studies and patient characteristics of the different subcohorts have been previously described (2).

Genotyping: DNA isolation from whole blood was done using a DNA isolation kit (NucleoSpin, Macherey & Nagel, Düren, Germany). Genotyping of the *G6PC2* SNP rs560887 was performed using the TaqMan assay (Applied Biosystems, Forster City, CA, USA) on a GeneAmp PCR system 7000. Genotyping success rate was 99.6 %. Genotypes were verified by bidirectional sequencing in 50 randomly selected subjects resulting in 100 % identical results. In the hyperglycemic clamp cohort, genotyping was performed using a Sequenom platform as previously described (2). G6PC2 rs560887 was equally distributed in the subcohorts of the hyperglycaemic clamp group (p=0.573, supplementary table 3).

OGTT: A 75-g OGTT was performed as described earlier with glucose, insulin and C-peptide measurements (3).

ivGTT and hyperinsulinemic-euglycemic clamp: A subset of 326 participants was studied by intravenous glucose tolerance test (ivGTT) with measurement of glucose and insulin. Clamp-derived insulin sensitivity was determined in another subgroup of 512 subjects by a

hyperinsulinemic-eugylcemic clamp with measurement of glucose and insulin. Both tests were performed as described earlier (3).

Hyperglycemic clamp: A replication cohort of 457 participants was studied using the hyperglycemic clamp technique. After a priming infusion of glucose to acutely raise blood glucose levels to 10 mmol/l, blood glucose levels were measured every 5 minutes and were kept constant at 10 mmol/l for 2 hours by using a variable glucose infusion. Insulin levels were measured at 2.5 minute intervals during the first 10 minutes and 20 minute intervals for the remainder of the clamp (18).

Analytical procedures: Plasma insulin and C-peptide were measured by commercial chemiluminescence assays for ADVIA Centaur (Siemens Medical Solutions, Fernwald, Germany). Blood glucose was measured using a bedside glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH, USA). Insulin levels during the hyperglycemic clamps were measured as previously described (2).

Calculations:

*Insulin secretion***:** From the OGTT, we calculated the insulinogenic index (IGI) as (Ins30-Ins0) /(Glc30-Glc0). First phase insulin secretion during the ivGTT was calculated as AUC (trapezoid method) using insulin levels 0, 2, 4, 6, 8, and 10 minutes after glucose bolus. First phase insulin secretion during the hyperglycaemic clamp was calculated as the sum of insulin levels during the first 10 min of the clamp. Second phase insulin secretion was determined as the mean of the insulin levels during the last 40 minutes of the clamp (80-120 min) (2).

Insulin sensitivity: $HOMA_{IR}$ (homeostasis model assessment of insulin resistance) was calculated as (Glc0 x Ins0 x 2) / 45. OGTT-derived insulin sensitivity index (ISI) was estimated as described earlier (3). ISI derived from euglycemic hyperinsulinemic clamp was calculated as mean infusion rate of exogenous glucose necessary to maintain euglycemia during the last 60 min of the hyperinsulinemic-euglycemic clamp divided by the steady-state insulin concentration.

Statistical analyses: Unless otherwise stated, data are given as unadjusted means ±SD. Non-normally distributed data were logarithmically transformed prior to statistical analysis. Differences between genotypes were tested using multivariate linear regression analysis in an additive model. P-values <0.05 were considered to indicate nominal associations, after correction for multiple comparisons taking into account the different techniques to assess insulin secretion (according to Bonferroni), P-values <0.0255 were considered statistically significant. Hardy-Weinberg equilibrium was tested using χ^2 test. Pairwise linkage disequilibrium (LD) (D', r^2) was determined using the Java linkage disequilibrium plotter (http://www.genepi.com.au/projects/jlin). In the replication cohort with hyperglycemic clamp, we used a generalized linear regression model (Generalized Estimating Equations) including age, sex, BMI, glucose tolerance status, family relatedness, and study centre for the analyses of 1st and 2nd phase insulin secretion. For statistical analysis, the JMP 7.0 statistical software package (SAS Institute, Cary, NC, USA) or SPSS version 16.0 (SPSS, Chicago, IL, USA) (for hyperglycaemic clamps) were used.

Power calculation: The OGTT cohort was sufficiently powered ($1-\beta \geq 0.8$) to detect effect sizes of 13% in the whole group, as well as 15% in the NGT subgroup and 25% in the IFG/IGT subgroup. The IVGTT cohort was sufficiently powered ($1-\beta \geq 0.8$) to detect effect sizes of 28% in the whole group, and 34% in the NGT subgroup and 49% in the IFG/IGT group. The clamp cohort was sufficiently powered ($1-\beta \geq 0.8$) to detect effect sizes of 23% in the whole group, and 35% in the NGT and 41% in the IFG/IGT group (all analyses in the dominant inheritance model, $\alpha=0.05$).

Human islet culture and incubation: Isolated human islets were obtained from the Laboratory of Isolation and Transplantation of Cells, University of Geneva/Faculty of Medicine (CMU) through the European Consortium for Islet Transplantation (ECIT). Human islets derived from five donors, from which three carried the minor allele of SNP rs560887

(two heterozygous, one homozygous), and two were homozygous carriers of the major allele. All donors were between 51 and 62 years old, their BMI was between 21.6 and 27.5 kg/m².

Human islets were purified by hand picking and cultured overnight in CMRL 1066 medium (Biochrom, Berlin, Germany) containing 5.5 mmol/l glucose supplemented with 10 % fetal calf serum, 2 mmol/l L-glutamine, and 10 mmol/l Hepes. The islets were preincubated for 30 minutes in modified Krebs-Ringer-bicarbonate buffer (KRB) containing: 135 mmol/l NaCl, 4.8 mmol/l KCl, 1.2 mmol/l $MgSO_4$, 1.2 mmol/l NaH_2PO_4, 4.8 mmol/l Na_2HPO_4, 5 mmol/l $NaHCO_3$, 2.6 mmol/l $CaCl_2$, 10 mmol/l Hepes, 0.5 mmol/l glucose, 0.05 % (w/v) BSA (fatty acid free, Sigma, St. Louis, MO, USA)

Thereafter, batches of 10 islets/500 µl were incubated for 1 h in KRB supplemented with 2.8, 5.6, 12.0, 20.0 mM glucose, respectively. Insulin concentrations in the supernatants and insulin content of the islets after extraction with acid ethanol (1.5 %/75 % (v/v) HCl/ethanol) were measured by radioimmunoassay (Linco Research, St. Charles, Missouri).

Online supplementary references

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