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Variants of *GCKR* Affect both Beta Cell and Kidney Function in Patients with Newly Diagnosed Type 2 Diabetes. The Verona Newly Diagnosed Type 2 Diabetes Study (VNDS). 2.

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Experimental Design

The Verona Newly Diagnosed Type 2 Diabetes Study (VNDS) is an ongoing study aiming at building a biobank of patients with newly diagnosed type 2 diabetes. As of Jan 1 2002, all patients referred to the Division of Endocrinology and Metabolic Diseases of University of Verona School of Medicine, whose diabetes has been diagnosed in the last six months, are asked to participate in this research. The clinical evidence on which the diagnosis of type 2 diabetes has been made is reviewed and the diagnosis confirmed (1). Patients are drug-naïve or, if already treated with antidiabetic drugs, undergo a treatment washout of at least one week before metabolic tests are performed. Among the exclusion criteria are age>75 years, non-Italian ancestry, insulin treatment, presence of anti-GAD antibodies, malignancies, and any condition severely impairing liver and/or kidney function. In this study, we report the data collected in 509 patients, whose characteristics are summarized in Table 1.

All subjects consumed a weight-maintaining diet containing 200-250 g of carbohydrate/day for at least three days before studies. Body weight was stable in all subjects for at least 1 month before studies. No subject participated in any heavy exercise. Each subject gave informed written consent before participating in the research, which was approved by the Human Investigation Committee of the Verona City Hospital. Measurements of standard clinical phenotypes were collected in all patients. Metabolic tests were carried out on two separate days in random order. On both days, patients were admitted to the Metabolic Clinic Research Center at 07:30 after an overnight fast. All studies were carried out in a quiet, temperature controlled (22° C) room.

On one day an OGTT (75 g) was performed to assess beta cell function. For ethical reasons, the OGTT was not performed in patients presenting with FPG greater than 15 mmol/l. During the entire test patients were sitting in a comfortable cardiac chair. One teflon (21 g) venous catheter was inserted into an antecubital vein for blood sampling and kept patent with heparinized normal saline solution. After a 30' rest to establish baseline and after collecting a 20 cc blood sample for leukocyte DNA extraction, at time = 0' subjects ingested 75 g of glucose in 300 ml of water over 5 min. Blood samples to measure glucose, C-peptide and insulin concentrations were collected at times -10', 0', +15', +30', +45', +60', +90', +120', +150', +180', +210' and +240'. In some patients further blood samples were collected at +270' and +300'. Urines were collected to measure glycosuria.

On a separate day, a euglycemic insulin clamp was performed to assess insulin sensitivity (2). During the entire test patients were lying in bed. One teflon catheter was introduced into an antecubital vein for the infusion of test substances. Another teflon catheter was placed retrogradely into a wrist vein for sampling arterialized venous blood, according to the "hot box" technique. After a 30' rest in bed to establish baseline, indirect calorimetry (at least 40') was performed as previously described, for a companion study (1). At the end of calorimetric measures, baseline blood samples were collected and a standard euglycemic insulin (intravenous prime: 4.8 $nmol \cdot min^{-1} \cdot m^{-2}$ BSA; continuous infusion: 240 $pmol \cdot min^{-1} \cdot m^{-2}$ BSA) clamp was performed (1). Plasma glucose was allowed to decline until it reached 5.5 mmol/l, after which glucose clamping started with a glucose concentration goal of 5 mmol/l. The duration of the glucose clamp was at least of 120', but it was prolonged, if and as needed, to ensure at least 60' of insulin clamp at euglycemia in each patient. Timed blood samples were collected to measure hormone and substrate levels. In the last 45' of the clamp indirect calorimetry was repeated to assess substrate oxidation and energy production rates for a companion study. Urines were collected to measure urea excretion rate.

In both metabolic tests, all blood samples were collected in pre-chilled tubes and readily spun at 1,500 g. Plasma and serum specimens were stored at –80° C.

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Mathematical Modeling of Beta Cell Function

The analysis of the glucose and C-peptide curves during the OGTT follows the general strategy described in previous publications (3; 4) with some modifications and builds upon previous works from other laboratories (5; 6). The kinetics of C-peptide is described with a two-compartment model, in which the two pools (1 and 2) exchange with each other and the irreversible loss of the hormone is from pool 1, the same where C-peptide concentration is measured. C-peptide kinetic parameters are computed according to the equations by Van Cauter et al. (7).

Herein are the equations describing the model of glucose induced insulin secretion during an OGTT:

$$dcp_1(t)/dt = ISR(t) + cp_2 \cdot k_{12} - (k_{01} + k_{21}) \cdot cp_1 \quad (Eq.1)$$

where ISR = insulin secretion rate, cp_1 = C-peptide mass in the sampling (accessible) compartment, cp_2 = C-peptide mass in the remote compartment, k_{12} and k_{21} = rate constants of the exchange between the two C-peptide compartments, and k_{01} = rate constant of the irreversible loss of C-peptide from the accessible compartment. Note that the values of the volume of distribution of C-peptide pool 1 (accessible compartment), k_{12}, k_{21}, and k_{01} are computed according to the equations by Van Cauter et al. (7).

$$ISR(t) = BSR + DSR(t) + PSR(t) \quad (Eq.2)$$

where BSR = basal insulin secretion rate, DSR = insulin secretion rate due to the derivative (or dynamic) component, and PSR = insulin secretion rate due the proportional (or static) component.

$$BSR = CP_{ss} \cdot V_1 \cdot k_{01} \quad (Eq.\ 3)$$

where CP_{ss} is basal C-peptide concentration and V_1 is the volume of the accessible compartment of C-peptide.

From the modeling viewpoint, DSR(t) and PSR(t) are the components which in intravenous glucose tolerance tests or hyperglycemic clamps describe classical first phase insulin secretion and second phase insulin secretion, respectively. Furthermore, from a physiological viewpoint, the sum of BSR and PSR(t) describes the relationship linking glucose concentration and insulin secretion rate, in the absence of the derivative component (DSR).

DSR(t) and PSR(t) are mathematically defined as follows:

$$DSR(t) = X1(t) \cdot \tau^{-1} \quad (Eq.\ 4)$$

$$dX1(t)/dt = \sigma 1 \cdot [dG(t)/dt]/[\log(1.1+t)] - X1(t) \cdot \tau^{-1} \quad \text{if } dG(t)/dt > 0 \quad (Eq.\ 5)$$

$$dX1(t)/dt = - X1(t) \cdot \tau^{-1} \quad \text{if } dG(t)/dt \leq 0 \quad (Eq.\ 6)$$

where $\sigma 1$ = glucose sensitivity of derivative control of insulin secretion, G = plasma glucose concentration, X1 = C-peptide (insulin) mass made available for the derivative component of insulin secretion, τ = time constant of the derivative component of insulin secretion, and the term $\log(1.1 + t)$ accomodates the time-associated decline of $\sigma 1$ documented in humans during a hyperglycemic stimulus (8).

$$PSR(t) = X2(t) \cdot \delta^{-1} \quad (Eq.\ 7)$$

$$dX2(t)/dt = \sigma 2 \cdot [G(t) - \theta] - X2(t) \cdot \delta^{-1} \quad (Eq.\ 8)$$

where $\sigma 2$ = glucose sensitivity of the proportional component of insulin secretion, X2 = C-peptide (insulin) mass made available for the proportional component of insulin secretion, δ = time constant of the proportional component of insulin secretion, θ = glucose threshold above which the beta-cell responds with the proportional component of insulin secretion to plasma glucose concentration.
This model was implemented in the SAAM 1.2 software (SAAM Institute, Seattle, WA) (9) to estimate its unknown parameters. Numerical values of the unknown parameters were estimated by using nonlinear least squares. Weights were chosen optimally, i.e., equal to the inverse of the variance of the measurement errors, which were assumed to be additive, uncorrelated, with zero mean, and a coefficient of variation (CV) of 6-8%. The unknown parameters of the model are: CP_{ss}, $\sigma 1$, τ, $\sigma 2$, δ, and θ. They were estimated with good precision, as shown by their CVs (online-only appendix table 1)
A good fit of the model to data was obtained as shown by the table of the weighted residuals (online-only appendix table 2).

There are two main physiological outputs of the model:

1. derivative control (units: $[pmol \cdot m^{-2}$ BSA$] \cdot [mmol \cdot l^{-1} \cdot min^{-1}]^{-1}$): it is the amount of insulin secreted in response to a rate of glucose increase of 1 mmol/l per min which lasts for 1 minute;
2. stimulus-response curve linking glucose concentration (x axis) to insulin secretion rate (y axis): as explained above, it is the sum of BSR and PSR. With the purpose of avoiding artifactual increases in the power of statistical analyses, we used the stimulus-response curve at the pre-determined glucose concentrations of 5.5, 8.0, 11.0, 15.0 and 20.0 mmol/l.

References

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Supplementary Table 1. Coefficients of variation of the beta cell model parameters. CP_{ss}= basal C-peptide concentration; $\sigma 1$ = parameter regulating glucose sensitivity of derivative control of insulin secretion, τ = time constant of derivative control of insulin secretion, $\sigma 2$ = glucose sensitivity of proportional control of insulin secretion, δ = time constant of proportional control of insulin secretion, θ: glycemic threshold of proportional control of insulin secretion.

	Coefficients of Variation (%)	
Model Parameter	**Median**	**I.Q. Range**
CP_{ss}	11.5	7.6 – 19.9
$\sigma 1$	39.3	24.4 – 77.2
τ	60.3	57.9 – 61.2
$\sigma 2$	16.5	12.3 – 23.0
δ	33.2	21.6 – 68.6
θ	13.7	8.2 – 23.3

Supplementary Table 2. Weighted residuals of the model fit to the C-peptide data of the OGTT. Data are means±SD. The weighted residuals are a quantitative point-by-point assessment of the goodness-of-fit of the model to the data: a theorically perfect fit should generate weighted residuals with mean 0 and SD of 1.

	C-Peptide weighted residuals									
Time	15’	30’	45’	60’	90’	120’	150’	180’	210’	240’
Mean	-0.397	+0.118	+0.199	+0.267	+0.115	-0.026	+0.142	+0.015	+0.026	+0.107
SD	1.03	1.14	1.197	1.263	1.302	1.213	1.319	1.268	1.277	1.164

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Supplementary Table 3. Influence of rs6717980 genotype on clinical and metabolic variables of patients with newly diagnosed type 2 diabetes in the VNDS. Beta and SE are the multivariate model estimates of the change and its standard deviation, respectively, in the phenotypic trait associated to the presence of one minor allele. Reported p values were obtained after adjusting for age, gender and BMI.

*Variables were log-transformed before statistical analysis to approximate a gaussian distribution; the coefficient beta and its S.E. refer to log-transformed values.

		rs6717980			Additive model		
Phenotype	**N**	**AA**	**AG**	**GG**	**BETA**	**SE**	***P* Value**
Minor allele (G) frequency, %		**0.32**					
Number (M/F)	501 (333/168)	225	229	47			
Age (yrs)	501	59 [52-65]	59 [51.5±65]	59 [52-65]	-2.25	0.70	0.71
BMI* (kg/m^2)	500	29.4 [26.0-33.4]	29.6 [27.1-33.2]	28.4 [26.7-32.0]	-0.002*	0.01*	0.87
Fasting P-glucose (mmol/l)	493	7.0 [6.1-7.8]	7.1 [6.2-8.4]	7.0 [6.1-7.8]	0.14	0.13	0.29
2hr P-glucose (mmol/l)	482	13.1 [10.4-16.2]	13.7 [11.0-16.5]	13.1 [10.0-16.5]	0.20	0.29	0.50
Fasting insulin* (mU/l)	430	11.8 [7.8-16.9]	11.8 [7.5-16.3]	10.1 [7.4-16.1]	-0.02*	0.04*	0.56
2hr insulin* (mU/l)	436	64.7 [42.3-106.9]	64.6 [38.7-90.0]	59.7 [34.5-108]	-0.02*	0.05*	0.65
Insulinogenic Index (mU/mmol)*	396	3.8 [2.2-7.1]	3.8 [2.0-6.3]	4.2 [2.0-7.5]	-0.04*	0.07*	0.56
CIR_{120}*(mUxL/mmol)	436	0.54 [0.26-1.4]	0.46 [0.20±1.1]	0.58 [1.2-1.4]	-0.08*	0.09*	0.39
HbA1c	487	6.6 [6.1-7.3]	6.8 [6.2-7.7]	6.6 [6.0-7.1]	0.03*	0.10*	0.79
Triglycerides* (mmol/l)	492	1.43 [1.0-2.0]	1.4 [1.0-2.0]	1.4 [1.0-2.1]	0.01*	0.04*	0.78
HDL-cholesterol* (mmol/l)	490	1.13 [0.97-1.33]	1.2 [0.97-1.4]	1.1 [0.96-1.4]	0.04*	0.02*	0.06
Cholesterol (mmol/l)	491	4.9 [4.3-5.5]	5 [4.4-5.8]	5.2 [4.3-6.0]	0.14	0.07	**0.04**
SBP (mmHg)	488	135 [124-150]	140 [120-150]	130 [128-150]	0.70	1.24	0.57
DBP (mmHg)	488	80 [80-90]	85 [80-90]	80 [80-90]	0.06	0.66	0.93
Insulin Sensitivity* (µmol/min/m^2 BSA)	490	599 [379-819]	499 [308-768]	623 [456-779]	-0.04*	0.04*	0.35
eGFR (ml/min/1.73m^2)	471	81.9 [71.0-94.1]	80.3 [70.2-92.9]	79 [66.6-88.2]	-1.84	1.35	0.18
U-Alb/Creatinine* (mg/mmol)	427	0.7 [0.4-2.6]	0.7 [0.4-2.5]	0.8 [0.5-3.2]	0.02*	0.07*	0.77
Serum Creatinine (µmol/L)	471	78 [67-87]	80 [69-88]	81 [73-87]	1.01	1.01	0.31

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Supplementary Table 4. Influence of rs1049817 genotype on clinical and metabolic variables of patients with newly diagnosed type 2 diabetes in the VNDS. Beta and SE are the multivariate model estimates of the change and its standard deviation, respectively, in the phenotypic trait associated to the presence of one minor allele. Reported p values were obtained after adjusting for age, gender and BMI.

*Variables were log-transformed before statistical analysis to approximate a gaussian distribution; the coefficient beta and its S.E. refer to log-transformed values.

§ Analysis performed under recessive model.

		rs1049817			Additive model		
Phenotype	**N**	**AA**	**AG**	**GG**	**BETA**	**SE**	***P* Value**
Minor allele (G) frequency, %		**0.35**					
Number (M/F)	502 (337/165)	201	246	55			
Age (yrs)	502	60 [51.5-65]	60 [52-65]	58 [52-65]	-0.25	0.69	0.72
BMI* (kg/m^2)	501	29.4 [26.1-33.4]	29.3 [26.6-32.8]	29.4 [27.5-34.4]	0.002*	0.01*	0.87
Fasting P-glucose (mmol/l)	493	7. 1[6.1-7.9]	7.2 [6.2-8.3]	7.0 [6.1-7.7]	-0.02	0.13	0.89
2hr P-glucose (mmol/l)	482	13.3 [10.9-16.2]	13.8 [10.7-16.6]	12.4 [10.0-15.4]	-0.09	0.29	0.76
Fasting insulin* (mU/l)	430	11.4 [7.6-16.4]	1.5 [7.5-16.2]	11.1 [8.8-17.3]	0.03*	0.04*	0.46
2hr insulin* (mU/l)	436	61.7 [40.1-106.8]	61.4 [37.6-88.0]	81.5 [49.2-144.5]	0.05*	0.05*	0.30
Insulinogenic Index* (mU/mmol)	395	3.7 [2.2-7.4]	3.7 [1.9-6.3]	4.5 [2.5-7.5]	0.07*	0.07*	0.32
CIR_{120}*(mUxL/mmol^2)	421	0.54 [0.25-1.3]	0.4 [0.2-11]	0.80 [0.35-1.53]	0.05*	0.09*	0.54
HbA1c (%)	487	6.7 [6.1-7.4]	6.7 [6.2-7.6]	6.5 [6.0-7.1]	-0.02	0.09	0.81
Triglycerides* (mmol/l)	493	1.45 [1.0-2.1]	1.32 [1.0-1.9]	1.6 [1.1-2.3]	0.006*	0.04*	0.09
HDL-cholesterol* (mmol/l)	490	1.1 [1.0- 1.4]	1.2 [0.97-1.4]	1.1 [0.92-1.33]	0.01*	0.02*	0.59
Cholesterol (mmol/l)	492	4.8 [4.3- 5.6]	5.1 [4.4-5.6]	5.1 [4.3-5.5]	0.07	0.07	0.30
SBP (mmHg)	490	137 [125-147]	140 [120-150]	130 [120-150]	0.41	122	0.74
DBP (mmHg)	490	85 [80-90]	85 [80-90]	80 [76-90]	-0.56	0.64	0.38
Insulin Sensitivity* (μmol/min/m^2 BSA)	491	551 [355-846]	567 [367-780]	565 [292-760]	-0.03*	0.04*	0.52
eGFR (ml/min/1.73m^2)	470	82.6 [72.1-94.9]	81.3 [70.4-94.8]	75 [65.3-85.3]	-3.35 **-9.20**	1.30 **2.69**	**0.010** **0.001**§
U-Alb/Creatinine* (mg/mmol)	430	0.80 [0.4-2.7]	0.7 [0.4-2.3]	0.80 [0.4-2.4]	0.02*	0.07*	0.76
Serum Creatinine (μmol/L)	470	77 [67.3-86.0]	79 [67-88]	85 [75-94]	2.40 **6.63**	0.97 **2.00**	**0.013** **0.001**§

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Supplementary Table 5. Influence of rs65476256 genotype on clinical and metabolic variables of patients with newly diagnosed type 2 diabetes in the VNDS. Beta and SE are the multivariate model estimates of the change and its standard deviation, respectively, in the phenotypic trait associated to the presence of one minor allele. Reported p values were obtained after adjusting for age, gender and BMI. *Variables were log-transformed before statistical analysis to approximate a gaussian distribution; the coefficient beta and its S.E. refer to log-transformed values.
§ Analysis performed under recessive model.

		rs65476256			Additive model		
Phenotype	**N**	**GG**	**GA**	**AA**	**BETA**	**SE**	***P* Value**
Minor allele (A) frequency, %			**0.34**				
Number (M/F)	509 (342/167)	219	237	53			
Age (yrs)	509	59 [51-65]	60 [52.5-65.5]	57 [51-65]	0.19	0.68	0.78
BMI* (kg/m^2)	508	29.6 [26.1-33.4]	29.3 [267-32.9]	29.3 [27.3-33.5]	0.001*	0.01*	0.96
Fasting P-glucose (mmol/l)	500	7.1 [6.2-7.9]	7.1 [6.2-8.3]	7.0 [6.1-7.8]	-0.05	0.12	0.70
2hr P-glucose (mmol/l)	488	13.7 [11-16.2]	13.8 [10.6-16.6]	12.1 [9.8-15.4]	-0.27	0.28	0.35
Fasting insulin* (mU/l)	434	11.3 [7.3-15.8]	11.5 [7.6-16.6]	11.3 [8.4-17.8]	0.04*	0.04*	0.27
2hr insulin* (mU/l)	440	61.4 [38.9-102.3]	61.4 [38.2-88.5]	84.7 [47.1-145.0]	0.07*	0.05*	0.15
Insulinogenic Index* (mU/mmol)	400	3.7 [2.2-6.3]	3.6 [1.9-6.6]	4.8 [2.7-85.8]	0.09*	0.07*	0.20
CIR_{120}*(mUxL/mmol^2)	440	0.50 [0.24-1.26]	0.40 [0.2-1.1]	0.9 [0.38-1.60]	0.10*	0.08*	0.22
HbA1c (%)	494	6.7 [6.2-7.6]	6.7 [6.2-7.6]	6.5 [6.0-6.9]	-0.1	0.09	0.28
Triglycerides* (mmol/l)	500	1.41 [1.03-2.1]	1.3 [1.0-1.9]	1.6 [1.0-2.3]	0.02*	0.04*	0.51
HDL-cholesterol* (mmol/l)	497	1.14 [1.0-1.35]	1.15 [0.97-1.38]	1.1 [0.9-1.4]	0.003*	0.02*	0.87
Cholesterol (mmol/l)	499	4.8 [4.3-5.6]	5.0 [4.4-5.7]	5.1 [4.3-5.5]	0.08	0.07	0.22
SBP (mmHg)	496	135 [124-144]	140 [125-150]	130 [120-150]	0.70	1.21	0.56
DBP (mmHg)	496	84 [80-90]	85 [80-90]	80 [76-90]	-0.33	0.64	0.60
Insulin Sensitivity* (µmol/min/m^2 BSA)	498	556 [351-828]	562 [367-780]	518 [289-743]	-0.02*	0.04*	0.56
eGFR (ml/min/1.73m^2)	477	82.5 [72.1-94.9]	80.8 [70.0-93.5]	75.2 [66.8-85.8]	-3.24 **-8.72**	1.30 **2.77**	**0.013** **0.002**§
U-Alb/Creatinine* (mg/mmol)	435	08 [0.4-2.8]	0.6 [0.4-1.9]	0.8 [0.4-2.3]	-0.02*	0.07*	0.81
Serum Creatinine (µmol/L)	477	77 [68-86]	79 [68-87]	85 [74-94]	2.14 6.25	0.97 2.10	**0.028** **0.003**§

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Supplementary Table 6. Influence of rs780094 genotype on clinical and metabolic variables of patients with newly diagnosed type 2 diabetes in the VNDS. Beta and SE are the multivariate model estimates of the change and its standard deviation, respectively, in the phenotypic trait associated to the presence of one minor allele. Reported p values were obtained after adjusting for age, gender and BMI.

*Variables were log- transformed before statistical analysis to approximate a gaussian distribution; the coefficient beta and its S.E. refer to log-transformed values.

§ Analysis performed under recessive model.

		rs780094			Additive model		
Phenotype	**N**	**GG**	**GA**	**AA**	**BETA**	**SE**	***P* Value**
Minor allele (A) frequency, %		**0.47**					
Number (M/F)	506 (339/167)	136	263	107			
Age (yrs)	506	59 [50-64]	59 [53-65]	60 [52-65]	-0.61	0.65	0.34
BMI* (kg/m^2)	505	29.7 [27.2-32.8]	29.3 [26.4-33.6]	29.4 [26.2-32.9]	0.007*	0.01*	0.49
Fasting P-glucose (mmol/l)	501	7.0 [6.2-7.9]	7.0 [6.2-7.9]	7.3 [6.0-8.2]	-0.01	012	0.92
2hr P-glucose (mmol/l)	487	12.6 [10.4-15.6]	13.5 [10.3-16.4]	14.3 [11.3-16.8]	-0.55	0.27	**0.045**
Fasting insulin* (mU/l)	436	12.1 [7.8-16-8]	11.1 [6.8-7.6]	11.5 [7.6-16.9]	-0.01*	0.04*	0.75
2hr insulin* (mU/l)	439	69.6 [39.7-104.5]	60.2 [38.6-90.1]	61.7 [36.9-107.6]	-0.04*	0.05*	0.40
Insulinogenic Index (mU/mmol)*	402	4.3 [2.4-7.4]	3.6 [2.0-6.0]	3.4 [1.9-7.4]	-0.12*	0.07*	0.08
$CIR_{120'}$ *(mUxL/mmol^2)	439	0.6 [0.2-1.4]	0.5 [0.2-1.3]	0.4 [0.2-0.8]	-0.15*	0.08*	0.06
HbA1c	492	6.6 [6.1-7.4]	6.7 [6.2-7.6]	6.7 [6.2-7.5]	-0.06	0.09	0.52
Triglycerides* (mmol/l)	497	1.4 [1.0-1.9]	1.4 [1.0-2.0]	1.4 [1.0-2.2]	0.04*	0.03*	0.27
HDL-cholesterol* (mmol/l)	494	1.1 [1.0-1.4]	1.2 [1.0-1.4]	1.1 [1.0-1.3]	-0.007*	0.02*	0.72
Cholesterol (mmol/l)	496	5.0 [4.3-5.6]	5.0 [4.3-5.7]	4.3 [4.8-5.5]	-0.03	0.07	0.70
SBP (mmHg)	493	130 [120-140]	140 [125-150]	140 [130-149]	-1.32	1.15	0.25
DBP (mmHg)	493	81 [80-90]	83 [80-90]	85 [80-90]	-0.06	0.61	0.93
Insulin Sensitivity* (µmol/min/m^2 BSA)	495	572 [372-769]	602 [356-820]	492 [345-819]	-0.04*	0.04*	0.35
eGFR (ml/min/1.73m^2)	474	79.0 [68.3-87.6]	81.3 [70.6-94.9]	83.9 [72.6-97.3]	**2.56** 5.06	**1.23** 1.92	**0.038** **0.009**§
U-Alb/Creatinine* (mg/mmol)	431	0.8 [0.4-2.1]	0.7 [0.4-2.5]	1.0 [0.4-3.1]	0.03*	0.07*	0.63
Serum Creatinine (µmol/L)	474	81 [69-90]	78 [68-87]	77 [67-87]	-1.78 -3.33	0.91 1.43	0.052 **0.021**§

SUPPLEMENTARY DATA

Supplementary Table 7. Influence of rs2384628 genotype on clinical and metabolic variables of patients with newly diagnosed type 2 diabetes in the VNDS. Beta and SE are the multivariate model estimates of the change and its standard deviation, respectively, in the phenotypic trait associated to the presence of one minor allele. Reported p values were obtained after adjusting for age, gender and BMI.

*Variables were log-transformed before statistical analysis to approximate a gaussian distribution; the coefficient beta and its S.E. refer to log-transformed values.

		rs2384628			Additive model		
Phenotype	**N**	**CC**	**CA**	**AA**	**BETA**	**SE**	***P* Value**
Minor allele (A) frequency, %		**0.46**					
Number (M/F)	501 (338/163)	139	266	96			
Age (yrs)	501	59 [50-66]	60 [62-65]	59 [52-66]	0.55	0.67	0.41
BMI* (kg/m^2)	500	29.4 [26.1-33.3]	29.3 [26.6-32.9]	29.7 [26.9-33.6]	-0.003*	0.01*	0.81
Fasting P-glucose (mmol/l)	492	7.0 [6.3-7.8]	7.2 [6.2-8.3]	7.0 [3.0-7.8]	-0.07	0.12	0.56
2hr P-glucose (mmol/l)	481	13.7 [11.1-16.2]	13.8 [10.8-16.5]	12.4 [10.0-15.7]	-0.29	0.27	0.29
Fasting insulin* (mU/l)	428	11.4 [6.8-17.2]	11.5 [7.8-16.1]	11.5 [7.7-16.8]	0.02*	0.04*	0.52
2hr insulin* (mU/l)	435	61.7 [45.6-106.8]	60.8 [33.3-89.1]	76.6 [44.9-136.5]	0.04*	0.05*	0.39
Insulinogenic Index (mU/mmol)*	394	3.8 [2.3-6.9]	3.6 [1.6-6.9]	3.9 [2.6-6.2]	0.01*	0.07*	0.89
CIR_{120},* (mUxL/mmol^2)	435	0.5 [0.3-1.1]	0.4 [0.2-1.1]	0.7 [0.3-1.4]	0.10*	0.08*	022
HbA1c	486	6.7 [6.2-7.4]	6.8 [6.2-7.6]	6.5 [6.1-7.1]	-0.10	0.90	0.28
Triglycerides* (mmol/l)	492	1.5 [1.1-2.0]	1.4 [1.0-2.0]	1.5 [1.0-2.1]	0.003*	0.003*	0.93
HDL-cholesterol* (mmol/l)	489	1.1 [1.0-1.3]	1.1 [1.0-1.4]	1.1 [1.0-1.4]	0.007*	0.02*	0.69
Cholesterol (mmol/l)	491	4.7 [4.2-5.5]	5.0 [4.4-5.6]	5.1 [4.4-5.5]	0.12	0.07	0.08
SBP (mmHg)	488	136 [124-144]	140 [120-150]	130 [125-150]	0.20	1.17	0.87
DBP (mmHg)	488	85 [80-90]	84 [80-90]	80 [80-90]	-0.48	0.62	0.44
Insulin Sensitivity* (µmol/min/m^2 BSA)	490	563 [366-851]	556 [367.792]	565 [289-773]	-0.04*	0.04*	0.37
eGFR (ml/min/1.73m^2)	469	81.6 [71.6-93.1]	81.4 [72.0-96.1]	76.1 [66.9-92.1]	-1.25	1.28	0.33
U-Alb/Creatinine* (mg/mmol)	431	0.8 [0.4-3.2]	0.7 [0.4-1.6]	1.1 [0.4-3]	0.04*	0.07*	0.52
Serum Creatinine (µmol/L)	469	77 [68-87]	79 [68-87]	81 [69-93]	0.90	0.95	0.34

SUPPLEMENTARY DATA

Supplementary Table 8. Influence of rs8731 genotype on clinical and metabolic variables of patients with newly diagnosed type 2 diabetes in the VNDS. Beta and SE are the multivariate model estimates of the change and its standard deviation, respectively, in the phenotypic trait associated to the presence of one minor allele. Reported p values were obtained after adjusting for age, gender and BMI.

*Variables were log-transformed before statistical analysis to approximate a gaussian distribution; the coefficient beta and its S.E. refer to log-transformed values.

§ Analysis performed under recessive model.

		rs8731			Additive model		
Phenotype	**Number**	**GG**	**GC**	**CC**	**BETA**	**SE**	***P* Value**
Minor allele (C) frequency, %		**0.21**					
Number (M/F)	496 (332/164)	314	157	25			
Age (yrs)	496	59 [52-65]	59 [52-65]	59 [49-65]	-1.04	0.77	0.18
BMI* (kg/m^2)	495	29.3 [26.4-32.9]	29.6 [26.7-33.7]	30.4 [26.5-33.3]	0.02*	0.01*	0.07
Fasting P-glucose (mmol/l)	490	7.1 [6.1-8.3]	7.1 [6.2-7.7]	6.7 [6.1-7.7]	-0.09	0.15	0.54
2hr P-glucose (mmol/l)	478	13.3 [10.6-16.4]	13.7 [10.4-15.5]	12.0 [10.4-17.0]	-0.37	0.33	0.26
Fasting insulin* (mU/l)	426	11.1 [7.5-16.2]	11.5 [7.6-16.6]	13.8 [9.4-18.3]	-0.006*	0.04*	0.89
2hr insulin* (mU/l)	431	63 [36.1-100.5]	62.4 [42.2-95.1]	79.4 [47.0-137.0]	0.01*	0.06*	0.81
Insulinogenic Index (mU/mmol)*	393	3.7 [2.0-6.5]	4.1 [2.3-7.3]	4.4 [2.6-12.1]	0.04*	0.08*	0.66
CIR_{120},*(mUxL/mmol^2)	429	0.5 [0.2-1-1]	0.5 [0.2-1.4]	1.1 [0.3-1.6]	0.12*	0.10*	0.23
HbA1c	487	6.6 [6.1-7.5]	6.8 [6.2-7.5]	6.5 [6.0-8.1]	0.03	0.11	0.77
Triglycerides*(mmol/l)	487	1.4 [1.0-2.1]	1.4 [1.0-2.0]	1.4 [1.0-1.9]	-0.09*	0.04*	**0.022**
HDL-cholesterol* (mmol/l)	485	1.1 [1.0-2.1]	1.1 [1.0-1.4]	1.1 [0.9-1.2]	0.01*	0.02*	0.62
Cholesterol (mmol/l)	486	5.0 [4.4-5.4]	5.0 [4.3-5.8]	4.4 [4.2-5.6]	-0.05	0.08	0.53
SBP (mmHg)	484	140 [128-150]	134 [120-150]	130 [115-140]	-2.66	1.38	**0.05**
DBP (mmHg)	484	84 [80-90]	85 [80-90]	80 [80-90]	-0.32	0.73	0.66
Insulin Sensitivity* (µmol/min/m^2 BSA)	487	546 [346-804]	602 [370-800]	602 [411-775]	0.08* **0.1**	0.05* **0.06**	0.06 **0.037**§
eGFR (ml/min/1.73m^2)	466	80.9 [69.7-93.6]	79.8 [71.0-91.2]	83.5 [75.5-101.3]	0.48	1.53	0.75
U-Alb/Creatinine* (mg/mmol)	426	0.8 [0.4-2.7]	0.7 [0.4-1.7]	0.5 [0.4-2.7]	-0.12*	0.08*	0.13
Serum Creatinine (µmol/L)	466	79 [68-88]	80 [68-87]	73[67-86]	-0.44	1.14	0.71

SUPPLEMENTARY DATA

Supplementary Table 9. Number of patients with newly diagnosed type 2 diabetes of the VNDS in each cell of the *GCKR* score. The *GCKR* score was computed by counting 1 per each rs6717980 G allele and 1 per each rs2384682 C allele carried by each subject. The *GCKR* score could range from a minimum of 0 (a carrier of neither rs6717980 G alleles nor rs2384682 C alleles) to a maximum of 4 (a carrier of both GG in rs6717980 and CC in rs2384682).

Genotype Score	rs6717980 G=0 A=1	rs2384628 A=0 C=1	N
0	GG	AA	27
1	GG	AC	151
	GA	AA	
2	GG	CC	196
	GA	AC	
	AA	AA	
3	GA	CC	68
	AA	AC	
4	AA	CC	3

SUPPLEMENTARY DATA

Supplementary Table 10. Statistically independent effects of *GCKR* score (P = 0.012) on the stimulus (glucose)-response (insulin secretion rate) curve (proportional control) of beta cell in the VNDS patients. Data were analyzed by multivariate ANOVA for repeated measures; covariates of the multivariate model: body mass index (BMI; P = 0.014), glomerular filtration rate computed by the MDRD formula (eGFR; P = 0.0001), age (P = 0.17) and gender (male=0, female=1) (P = 0.06).

Dependent Variable	Parameter	BETA	SE	P Value
Insulin Secretion Rate at plasma glucose 5.5 mmol/l (pmol·min^{-1}·m^{-2} BSA)	Intercept	107	34.5	0.002
	BMI	+4.76	0.58	0.0001
	eGFR	-0.66	0.16	0.0001
	Age	-0.53	0.31	0.09
	Gender	-0.72	6.3	0.91
	GCKR Score	-1.72	3.53	0.63
Insulin Secretion Rate at plasma glucose 8.0 mmol/l (pmol·min^{-1}·m^{-2} BSA)	Intercept	267	62.9	0.0001
	BMI	+4.17	1.07	0.0001
	eGFR	-1.45	0.29	0.0001
	Age	-0.70	0.57	0.22
	Gender	+7.43	11.5	0.52
	GCKR Score	-11.3	6.43	0.08
Insulin Secretion Rate at plasma glucose 11.0 mmol/l (pmol·min^{-1}·m^{-2} BSA)	Intercept	461	117	0.0001
	BMI	+4.35	1.98	0.03
	eGFR	-2.52	0.54	0.0001
	Age	-0.17	1.05	0.87
	Gender	+34.0	21.3	0.11
	GCKR Score	-31.9	11.9	0.008
Insulin Secretion Rate at plasma glucose 15.0 mmol/l (pmol·min^{-1}·m^{-2} BSA)	Intercept	635	206	0.002
	BMI	+6.60	3.49	0.06
	eGFR	-3.97	0.95	0.0001
	Age	+1.28	1.86	0.49
	Gender	+72.3	37.5	0.06
	GCKR Score	-54.1	21.0	0.01
Insulin Secretion Rate at plasma glucose 20.0 mmol/l (pmol·min^{-1}·m^{-2} BSA)	Intercept	881	328	0.008
	BMI	+9.13	5.56	0.10
	eGFR	-5.76	1.52	0.0001
	Age	+2.73	2.97	0.36
	Gender	+123	59.9	0.04
	GCKR Score	-80.8	33.6	0.02