Global expression profiling of glucose-regulated genes in pancreatic islets of spontaneously diabetic Goto-Kakizaki rats

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Abstract

The spontaneously diabetic Goto-Kakizaki (GK) rat is frequently used as a model for human type 2 diabetes. Selective loss of glucose-sensitive insulin secretion is an early pathogenetic event in human type 2 diabetes, and such a defect also typifies islets from the GK rat. We investigated whether expression of specific glucose-regulated genes is disturbed in islets from GK rats when compared with Wistar rats. Large-scale gene expression analysis using Affymetrix microarrays and qRT-PCR measurements of mRNA species from normal and diabetic islets were performed after 48 h of culture at 3 or 20 mM glucose. Of the 2020 transcripts differentially regulated in diabetic GK islets when compared with controls, 1033 were up-regulated and 987 were down-regulated. We identified significant changes in islet mRNAs involved in glucose sensing, phosphorylation, incretin action, glucocorticoid handling, ion transport, mitogenesis, and apoptosis that clearly distinguish diabetic animals from controls. Such markers may provide clues to the pathogenesis of human type 2 diabetes and may be of predictive and therapeutical value in clinical settings in efforts aiming at conferring β -cell protection against apoptosis, impaired regenerative capacity and functional suppression occurring in diabetes.

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Introduction

In human type 2 diabetes, loss of glucose-sensitive insulin secretion is an early pathogenetic event (Malaisse 1994, Zimmet *et al.* 2001). The pancreatic islet β -cell serves as a unique fuel-sensing organ and is the sole producer of insulin, the key hormone in maintenance of normoglycemia, whose exocytosis is tightly controlled by the ambient plasma glucose concentrations (MacDonald 1990, Sjöholm 1998). In contrast to most other cells, in which signaling is controlled through plasma membrane receptor-operated processes, the β -cell senses subtle fluctuations in plasma glucose concentration and translates these into finely tuned changes in insulin exocytosis (MacDonald 1990, Sjöholm 1998).

As the lesions intrinsic to the β -cell causing the selective loss of glucose sensitivity remain elusive, the aim of this study was to investigate whether expression of specific genes regulated by glucose is disturbed in islets from spontaneously diabetic Goto-Kakizaki (GK) rats when compared with normoglycemic Wistar rats. The GK rat is a non-obese animal model for type 2 diabetes (Goto *et al.* 1976, 1988). This widely employed model exhibits diabetes-related phenotypes such as hyperglycemia, glucose intolerance, insulin resistance, and a deficient insulin response to glucose *in vivo* and *in vitro*. There seems to be a selective loss of

glucose-sensitive insulin secretion (Kimura *et al.* 1982, Goto *et al.* 1988, Portha *et al.* 1991, Ostenson *et al.* 1993, Abdel-Halim *et al.* 1994) due to multiple defects in the insulin stimulus-secretion coupling. (For a review, see Portha 2005.)

Our results reveal substantial qualitative and quantitative differences in glucose-regulated islet gene expression between healthy and diabetic rats that may have implications for our understanding of the etiology and treatment of human type 2 diabetes.

Materials and methods

Materials

Collagenase A was obtained from Roche. Culture medium RPMI-1640, fetal calf serum, L-glutamine, benzylpenicillin, and streptomycin were from Flow Laboratories (Irvine, UK). Rat insulin ELISA kit was from Mercodia (Uppsala, Sweden).

Islet preparation and insulin secretion

Pancreatic islets were isolated by collagenase digestion (Sandler *et al.* 1987) from male diabetic GK and control Wistar rats, ~3 months old, purchased from Taconic Europe (barrier EBU 202, Bomholt site, Ry, Denmark).

The GK inbred model was developed by Tohoku University in 1975. Aarhus University Hospital in Denmark received stock in 1994. M&B A/S (now Taconic Europe) received stock from Aarhus in 1997. Glucose at 3 mM was chosen as a low-glucose comparator, non-stimulatory to both Wistar and GK islets, used in studying high glucose effects on islet gene expression in both rat strains. At the end of the culture period, batches of 500 islets were transferred to Eppendorf tubes with 0.5 ml Trizol (Invitrogen), in which the islets were homogenized and then snap-frozen in liquid nitrogen.

Insulin secretion and islet insulin content were measured as described (Sandler *et al.* 1987).

Monitoring of glycemia

Non-fasting glucose levels were monitored in intracardiac blood in normal Wistar and diabetic GK rats by measurement of glucose concentration using a glucose meter (Ascensia Contour, Bayer HealthCare), immediately after killing. The test principle used by this device is electrochemical biosensor technology using glucose oxidase. The strip uses the enzyme glucose oxidase to produce an electrical current that will stimulate a chemical reaction.

Isolation of islet RNA

Total RNA was isolated from islet tissue that was stored in Trizol at -70 °C using standard techniques. Briefly, tissues were homogenized, chloroform was added and the tubes were vigorously mixed for 15 s, and then allowed to sit for 2–3 min at room temperature before the tubes were centrifuged at 11 900 *g* for 15 min at 4 °C. The upper aqueous layer was removed to a new tube and isopropanol alcohol was added, the tubes were mixed, incubated at room temperature for 10 min, and centrifuged at 11 900 *g* for 10 min at 4 °C. The pellets were washed with 75% ethanol and centrifuged at 7500 *g* for 5 min at 4 °C. The pellets were dried for 10 min, 100 µl RNase free water was added and incubated at 50 °C for 10 min. Isolated total RNA was further purified using an RNeasy minicolumn (Qiagen 74104) by suspending the RNA pellet in 350 µl buffer RLT with 2-mercaptoethanol, followed by addition of 250 µl 100% ethanol. The samples were applied to the column and centrifuged for 15 s at ≥8000 *g*, with this step repeated with the flow-through. The column was washed twice with 500 µl buffer RPE centrifugation, and then dried by centrifugation for 2 min at maximum speed. The bound RNA was eluted using 30 µl of 65 °C RNase-free water and centrifugation.

Genome wide RNA transcript profiling

The integrity of total RNA was confirmed by a microfluidics lab-on-a-chip analysis using a RNA 6000 Nano LabChip device and an Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). The GeneChip Rat Expression Array 230A (Affymetrix Inc., Santa Clara, CA, USA) was used, which contains 15 866 different probe sets interrogating primarily annotated genes. Processing of total RNA samples and GeneChip experiments were carried out essentially as recommended by Affymetrix. Ten micrograms of labeled cRNA was hybridized for ~16 h at 45 °C to an expression probe array. The array was then washed, stained with streptavidin-R-phycoerythrin (SAPE, Molecular Probes; Eugene, OR, USA) and the signal amplified using a biotinylated goat anti-streptavidin antibody (Vector Laboratories, Burlingame, CA, USA) followed by a final staining with SAPE. Liquid handling steps were performed using Affymetrix GeneChip Fluidics Workstation 400. The array was then scanned twice using a confocal laser scanner (GeneArray Scanner 2500; Agilent Technologies) at an excitation wavelength of 488 nm and emission recorded at 570 nm, resulting in a scanned image. The scanned image was converted into numerical values of the signal intensity (Signal) and categorical expression level measurement (Absolute Call) using the Affymetrix Microarray Analysis Suite 5.0 software (MAS 5.0; Affymetrix Inc., Santa Clara, CA, USA). The software scaled the average intensity of all genes of each chip to a target intensity arbitrarily set at 150 units.

The quality control data for all microarrays used yielded values that were considered acceptable. High similarity of the scaling factors applied to each individual GeneChip array served as a first indicator of an optimal GeneChip experiment. Second, a background average value of ~65 indicated low unspecific binding. Third, a 3' to 5' signal ratio less or equal to 3 of the glyceraldehyde dehydrogenase gene or the β -actin gene indicated that the starting RNA had not undergone degradation and that the cDNA synthesis was optimal. Finally, the number of genes called 'present' was characteristic for the given tissue thus indicating an optimal recovery of the contained information.

To determine which genes were glucose responsive, genes were considered induced or suppressed if the average fold change was greater or equal to 1.1. The differentially regulated transcripts were annotated and then clustered according to functional annotation. A choice of twofold change limit of significance for the expressed transcripts could underestimate, and thereby miss, the true impact of small but biologically relevant and qualitatively important gene expression changes (Yao *et al.* 2004, Choe *et al.* 2005). Also, a twofold change, commonly used in the literature, is dubious to

Table 1 Glycemia in non-diabetic Wistar rats and diabetic GK rats

	Blood glucose (mmol/l)
Non-diabetic Wistar rats	8.4 ± 0.3
Diabetic GK rats	17.2 ± 3.1*

Non-fasting glucose levels were measured in intracardiac blood by glucose oxidase immediately after killing. Values are means ± s.e.m. of six experiments. *Denotes $P < 0.05$ for a chance difference versus non-diabetic Wistar rats using Student's *t*-test.

generate optimal results, taking into account that a factor of 2 can have different significance influenced by expression levels (Baldi & Long 2001, Mutch *et al.* 2002). Nevertheless, by including a quite large number of arrays (6 and 9) we have reduced random errors when compared with similar experiments, in which fewer numbers of samples have been used. Cluster analyses were performed as described in GeneSpring (Agilent Technologies), NetAffx (Affymetrix) and other databases.

cDNA synthesis

RNA was denatured for 10 min at 65 °C and immediately chilled on ice. First strand cDNA synthesis was performed in a 20 µl reaction mixture containing 2 µg total RNA in a solution of 10 µl, 4 µl 5× reverse transcriptase buffer (Invitrogen), 10 mM deoxynucleoside triphosphate, 1 µl random hexamer primers (100 pmol/µl), 1.5 µl dithiothreitol (Amersham Pharmacia Biotech; 100 mmol/l), and 1 µl RT1 reverse transcriptase (200 U/µl; Invitrogen). The reagents were mixed and incubated at 37 °C for 45 min. cDNA solutions were incubated for 5 min at 95 °C to inactivate reverse transcriptase and then stored at -20 °C.

Quantitative real-time RT-PCR

Expression of selected genes (Table 13) was performed from total RNA using an ABI Prism 9400 PCR machine (PE Applied Biosystems, Foster City, CA, USA). These genes were selected based on fold change in expression,

GO results, and/or potential roles in diabetes. The amount was adjusted to the endogenous reference gene β-actin. The selected genes and β-actin primers and hybridization probes (Perkin–Elmer Biosystems, Warrington, UK) were used. Probes were labeled at the 5' end with the reporter dye molecule 6-carboxy-fluorescein and at the 3' end with the quencher dye molecule 6-carboxytetramethylrhodamine. Results are expressed relative to β-actin with and the ratio of different groups (WH/WL, growth hormone/GL, and GL/WL). Therefore, the final value indicates an increase or decrease in mRNA for selected genes.

Statistical analysis

Results presented are derived from islets of individual rats, isolated on different days, unless otherwise stated. Means ± s.e.m. were calculated and groups of data were compared using Student's *t*-test for paired or unpaired data. Differences were considered statistically significant when $P < 0.05$.

Results

Diabetic phenotype in GK rats

Non-fasting glucose levels monitored in intracardiac blood in normal Wistar and diabetic GK rats, as expected, were significantly higher in diabetic GK rats relative to normal Wistar rats (Table 1).

Loss of glucose-sensitive insulin secretion in islets from GK rats

As shown in Table 2, insulin secretion at a high glucose concentration (20 mM), relative to 3 mM glucose, was stimulated 4.8-fold in non-diabetic Wistar rat islets ($P < 0.001$) but was not significantly augmented in islets from diabetic GK rats. In contrast, the islet insulin content was 40% higher in GK rats than in normal Wistar rats. This has been previously reported in GK islets after culture in widely different glucose concentrations (Metz *et al.* 1999).

Table 2 Glucose-sensitive insulin secretion in islets from non-diabetic Wistar rats and diabetic GK rats

	Insulin secretion (ng/islet per 60 min)	Insulin secretion (ng/islet per 60 min)	Islet insulin content (ng/islet)
	3 mM glucose	20 mM glucose	
Non-diabetic Wistar rats	5.56 ± 0.4	26.4 ± 2.8*	66.6 ± 3.6
Diabetic GK rats	6.67 ± 0.6	7.8 ± 0.5†	93.2 ± 2.1‡

Isolated islets were preincubated for 45 min at 3 mM glucose and then exposed for 60 min to the indicated glucose concentrations. Insulin in incubation buffers and islet insulin contents were measured by ELISA. Values are means ± s.e.m. of six experiments. *Denotes $P < 0.001$ for a chance difference versus Wistar islets in low glucose, †Denotes $P < 0.001$ for a chance difference versus Wistar islets in high glucose, ‡denotes $P < 0.001$ for a chance difference versus Wistar islets, using Student's *t*-test.

Microarray analysis of global glucose-regulated gene expression changes in islets from normal Wistar and diabetic GK rats

To gain insight into the molecular events underlying the selective loss of glucose-sensitive insulin secretion in islets from GK rats, we performed genome-wide oligonucleotide microarray analyses of glucose-stimulated islets from normal Wistar and diabetic GK rats. Of the 15 866 transcripts analyzed by microarray, 2020 were differentially expressed by comparing samples prepared from diabetic GK rat islets and normal Wistar rats, cultured in either 3 mM or 20 mM glucose.

The functional grouping of the differentially expressed transcripts shows that 1033 of these 2020 transcripts were up-regulated and 987 were down-regulated. A great proportion of these glucose responsive genes and expressed sequence tags are involved in metabolism, signaling, transport, apoptosis, transcription, proliferation, and immune response (Fig. 1). The experimental setup of the current work, two days *in vitro* culture, was deliberately designed in an attempt to minimize remaining influence of *in vivo* glucotoxicity or other manifestations of the diabetic state on islet function. However, we cannot completely exclude the possibility of remaining minor influences of *in vivo* glucotoxicity

Figure 1 (A) Functional clusters of genes up-regulated by high glucose in diabetic GK rats vs. non-diabetic Wistar rats. (B) Functional clusters of genes up-regulated by low glucose in diabetic GK rats versus non-diabetic Wistar rats. (C) Functional clusters of genes down-regulated by high glucose in diabetic GK rats versus non-diabetic Wistar rats. (D) Functional clusters of genes down-regulated by low glucose in diabetic GK rats versus non-diabetic Wistar rats.

Table 3 Islet transcripts up-regulated by high versus low glucose in diabetic GK rats

	Accession no.	Gene name	Fold change
Function Transport	AB052294	ATP-binding cassette, sub-family C (CFTR/MRP), member 8 (Abcc8)	1.5
	U44897	Potassium inwardly rectifying channel, subfamily J, member 11 (Kcnj11)	1.5
	NM_053685	Hyperpolarization-activated cyclic nucleotide-gated potassium channel 3 (Hcn3)	1.4
	NM_013186	Potassium voltage gated channel, Shab-related subfamily, member 1 (Kcnb1)	1.8
	X92069	Purinergic receptor P2X, ligand-gated ion channel, 5 (P2rx5)	1.6
	AA685184	Sodium channel, voltage-gated, type III, beta (Scn3b)	1.5
	AW532988	Hyperpolarization activated cyclic nucleotide-gated potassium channel 2 (Hcn2)	1.4
	NM_012878	Surfactant associated protein D (Sftpd)	1.5
	AI547447	Potassium voltage gated channel, Shaw-related subfamily, member 3 (Kcnc3)	1.8
	NM_012663	Vesicle-associated membrane protein 2 (Vamp2)	1.6
	M83681	RAB3D, member RAS oncogene family (Rab3d)	1.6
	AA943569	Synaptotagmin 1 (Syt1)	1.3
	NM_031344	Fatty acid desaturase 2 (Fads2)	1.4
	AA848820	15-hydroxyprostaglandin dehydrogenase (Hpgd)	1.7
	BI277460	Phosphoenolpyruvate carboxykinase 1 (Pck1)	1.4
Metabolism	Y11321	Forkhead box E1 (thyroid transcription factor 2) (Foxe1)	1.5
	L23863	POU domain, class 2, transcription factor 3 (Pou2f3)	1.5
	AI639457	GTP cyclohydrolase 1 (Gch)	1.5
	AW143805	Guanine nucleotide binding protein, alpha q polypeptide (Gnaq)	1.4
	AW533214	Rabaptin 5 (Rabep1)	1.3
Apoptosis	NM_012714	Gastric inhibitory polypeptide receptor (Gipr)	2.3
	NM_053777	Mitogen activated protein kinase 8 interacting protein (Mapk8ip)	1.2
	X92069	Purinergic receptor P2X, ligand-gated ion channel, 5 (P2rx5)	1.6
	AI234096	Protein kinase, cAMP dependent regulatory, type I, alpha (Prkar1a)	1.1
	AA799421	Protein kinase C, epsilon (Prkce)	1.2
Signal transduction	NM_053856	Secretogranin III (Scg3)	1.7
	NM_133522	Somatostatin receptor 3	1.3
	NM_019128	Internexin, alpha (Inexa)	1.9
	NM_012630	Prolactin receptor (Prlr)	1.3
	AW143798	Cyclin D1 (Ccnd1)	1.2
Development	AF106659	Ubiquitin specific protease 2 (Usp2)	1.3
	L07578	Casein kinase 1, delta (Csnk1d)	1.4
	NM_019349	Serine/threonine kinase 2 (Slk)	1.5
Catalytic activity			

or other manifestations of the diabetic state on islet gene expression.

Detailed descriptions of a selection of the differentially expressed transcripts, classified according to function and average fold change, are listed in Tables 3–12.

Differential expression of a subset of genes confirmed with qRT-PCR

In order to validate the qualitative changes in gene expression revealed by the microarray analyses, the expression of selected genes was also confirmed by real-time qRT-PCR. The same expression pattern as that identified by the microarray analyses was found by real-time qRT-PCR for 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD-1) (NM_017080), serum- and

glucocorticoid-inducible kinase 1 (SGK-1) (NM_019232), insulin-like growth factor binding protein 3 (IGFBP-3) (NM_012588), and ApoE (J02582; Table 13), whose expression levels were all significantly changed ($P < 0.05$). Insulin-2 was not captured as differentially expressed in the microarray analyses, using the selected cut off values, but was significantly more up-regulated by high glucose in islets from normal Wistar than from diabetic GK rats in the qRT-PCR analysis. Microarrays seem to offer a smaller dynamic range when compared with other platforms. This means that changes observed in other platforms may not be accurately reflected by microarray expression levels. The poor correlation between microarray and qRT-PCR may be due to the inherent sensitivity limits of a PCR-based approach (qRT-PCR) in comparison with a hybridization-based approach

Table 4 Islet transcripts down-regulated by high versus low glucose in diabetic GK rats

	Accession no.	Gene name	Fold change
Function			
	Metabolism		
	NM_019370	Ectonucleotide pyrophosphatase/phosphodiesterase 3 (Enpp3)	1.6
	NM_133298	Glycoprotein (transmembrane) nmb (Gpnm)	1.6
	NM_012907	Apolipoprotein B editing complex 1 (Apobec1)	2.5
	NM_053963	Matrix metalloproteinase 12 (Mmp12)	2.5
	AA849399	Cathepsin Z (Ctsz)	2
	NM_017320	Cathepsin S (cathepsin S)	2.5
	L12458	Lysozyme (Lyz)	2.5
Apoptosis	NM_019232	Serum- and glucocorticoid-inducible kinase 1 (Sgk)	1.6
	NM_133416	B-cell leukemia/lymphoma 2 related protein A1 (Bcl2a1)	2
	NM_012580	Heme oxygenase (decycling) 1 (Hmox1)	1.3
	J02582	Apolipoprotein E (ApoE)	2
	BG666928	BH3 interacting domain death agonist (Bid)	1.3
	AF279911	Bcl2-associated death promoter (Bad)	1.1
	NM_133307	Protein kinase C, delta (Prkcd)	1.3
Signal transduction	NM_053619	Complement component 5, receptor 1 (C5r1)	2
	A1009808	Ectonucleoside triphosphate diphosphohydrolase 1 (Entpd1)	1.6
	NM_032060	Complement component 3a receptor 1 (C3ar1)	4
	NM_013185	Hemopoietic cell kinase (Hck)	2
	NM_130421	Lymphocyte cytosolic protein 2 (Lcp2)	2
	A1178808	Interleukin 2 receptor, gamma (severe combined immuno-deficiency) (Il2rg)	1.6
Catalytic activity	NM_031811	Transaldolase 1 (Taldo1)	1.4
	NM_022592	Transketolase (Tkt)	1.3
	AF154349	Legumain (Lgmn)	1.4
	A1232474	Cathepsin L (Ctsl)	1.3
	A1409046	Cathepsin C (Ctsc)	1.4
Response to stimulus	NM_053843	Fc gamma receptor II beta	2
	A1171966	Major histocompatibility complex, class II, DM beta	2
	B1301490	Major histocompatibility complex, class II, DM alpha	1.6
	NM_017196	Allograft inflammatory factor 1 (Aif1)	2.5
	B1282932	Lymphocyte antigen 68 (C1qr1)	1.6

(microarray). Also, differences in sequence, their placement along the target transcript and feature design may all contribute to discrepancies in results obtained by the two methods used. All these reasons could explain why the insulin-2 gene was not captured as differently expressed in the microarray data but in qRT-PCR analysis.

Discussion

In human type 2 diabetes, loss of glucose-sensitive insulin secretion is an important pathogenetic event (Malaisse 1994, Zimmet *et al.* 2001). The change in β -cell phenotype involves a selective loss of glucose-stimulated insulin secretion even in the very earliest stages of disease progression. The molecular basis for this functional defect remains elusive, but also constitutes an inviting target for attempts to intervene against β -cell failure and outbreak of type 2 diabetes. In the present work, we have attempted to identify such targets using genome-wide transcription profiling of glucose-regulated genes in isolated pancreatic islets from normal Wistar rats and diabetic GK rats. In this

substrain of GK rats of this age, β -cell density and relative volume of islet endocrine cells are not different from age- and sex-matched Wistar rats (Ohneda *et al.* 1993, Guenifi *et al.* 1995). Our results reveal substantial qualitative and quantitative differences in glucose-regulated islet gene expression between healthy and diabetic rats that may have implications for our understanding of the etiology of human type 2 diabetes.

Key elements in steroid metabolism were abnormally expressed in diabetic GK islets (Table 7); e.g. 11 β -HSD-1 (NM_017080; Davani *et al.* 2000, Duplomb *et al.* 2004, Ortsater *et al.* 2005) and SGK-1 (NM_019232; Ullrich *et al.* 2005) which are important effectors of glucocorticoid handling in the β -cell and known to influence glucose-sensitive insulin secretion. In rodents, 11 β -HSD-1 (NM_017080) converts inactive 11-dehydrocorticosterone into active corticosterone. The mRNA and activity of 11 β -HSD-1 (NM_017080) have been shown to be up-regulated in islets from hyperglycemic mice (Davani *et al.* 2000, Ortsater *et al.* 2005) and the Zucker Diabetic Fatty rat (Duplomb *et al.* 2004) when compared with their normoglycemic counterparts. The β cell may be extra susceptible to glucocorticoid excess, since both iatrogenic Cushing syndrome and steroid-induced diabetes in animal

Table 5 Islet transcripts up-regulated by high glucose in diabetic GK rats versus high glucose in non-diabetic Wistar rats

	Accession no.	Gene name	Fold change
Function			
	Transport		
	NM_133521	F-box only protein 32 (<i>Fbxo32</i>)	1.3
	NM_031720	Deiodinase, iodothyronine, type II Solute carrier family 25 (mitochondrial carrier, brain), member 14 (<i>Dio2 Slc25a14</i>)	1.3
	AI547447	Potassium voltage gated channel, Shaw-related subfamily, member 3 (<i>Kcnc3</i>)	1.5
	AI009597	FXFD domain-containing ion transport regulator 3	1.4
	NM_017049	Solute carrier family 4, member 3 (<i>Slc4a3</i>)	1.2
	U39555	Solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1 (<i>Slc1a1</i>)	1.2
	AA945624	NAD(P)H dehydrogenase, quinone 2	1.2
	BG373779	Desmuslin (<i>Dmn</i>)	1.6
Metabolism	BI296640	Epsin 2 (<i>Epn2</i>)	1.2
	NM_052798	Zinc finger protein 354A (<i>Znf354a</i>)	1.4
	BM386917	Inosine triphosphatase (nucleoside triphosphate pyrophosphatase) (<i>Itpa</i>)	1.3
	AI229183	DnaJ (Hsp40) homolog, subfamily A, member 2 (<i>Dnaja2</i>)	1.4
	NM_012692	Cytochrome P450 IIA1 (hepatic steroid hydroxylase IIA1) gene (<i>Cyp2a1</i>)	1.6
	BI295900	Dihydrolipoamide S-acetyltransferase (E2 component of pyruvate dehydrogenase complex; <i>Dlat</i>)	1.3
	NM_053864	Valosin-containing protein (<i>Vcp</i>)	1.1
	NM_057137	Phenylalkylamine Ca ²⁺ antagonist (emopamil) binding protein (<i>Ebp</i>)	1.2
	Signal transduction		
	NM_017139	Preproenkephalin, related sequence (<i>Penk-rs</i>)	1.5
Signal transduction	AI233208	Calcium regulated heat stable protein 1 (<i>Carhsp1</i>)	1.2
	AI234096	Protein kinase, cAMP dependent regulatory, type I, alpha (<i>Prkar1a</i>)	1.1
	Cell cycle		
Cell cycle	BE097028	Phospholipase C, beta 1 (<i>Plcb1</i>)	1.1
	U95920	Pericentriolar material 1 (<i>Pcm1</i>)	1.2

models are associated with loss of glucose-stimulated insulin secretion, and steroid immunosuppressive treatment adversely affects islet transplantation outcome (Shapiro *et al.* 2000). Glucocorticoids decrease insulin gene expression (Philippe *et al.* 1992), increase glucose-6-phosphatase, phosphoenolpyruvate carboxykinase (PEPCK) activity and glucose cycling (Ling *et al.* 1998, Davani *et al.* 2004), and induce β -cell apoptosis (Weinhaus *et al.* 2000), all events occurring in GK rat islets (Portha 2005). Since the human insulin gene contains glucocorticoid-sensitive transcriptional elements (Fernandez-Mejia *et al.* 1999), it may be susceptible to islet overproduction of glucocorticoids. Our findings may also be of therapeutical significance, given the attention currently paid to pharmacological 11 β -HSD-1 inhibitors in clinical trials against type 2 diabetes (Seckl & Walker 2001).

β -cell susceptibility to glucocorticoids may also be relevant in the natural unfolding of diabetes, since mice overexpressing the glucocorticoid receptor restricted to the β -cell develop early β -cell failure, glucose intolerance and later in life overt diabetes (Ling *et al.* 1998, Davani *et al.* 2004). Humans with impaired β -cell function (low insulin responders) are predisposed to become overtly diabetic during glucocorticoid therapy (Wajngot *et al.* 1992).

Another gene involved in transducing glucocorticoid effects, which was found to be abnormally regulated by

glucose in diabetic GK islets, is SGK-1 (NM_019232; Table 7). SGK-1, whose expression is increased by glucocorticoids (Ullrich *et al.* 2005), appears to be instrumental in directly regulating insulin secretion negatively, since the suppression of glucose-sensitive insulin secretion normally evoked by dexamethasone was lost in islets from SGK-1 (NM_019232) knockout mice (Ullrich *et al.* 2005). In islets, SGK-1 (NM_019232) up-regulates the activity of voltage-activated K⁺ channels (K_v) thereby reducing Ca²⁺ entry and insulin secretion (Ullrich *et al.* 2005). Since intracellular Ca²⁺ handling appears intrinsically disturbed in GK rat islets (Kato *et al.* 1996, Varadi *et al.* 1996), and can be deranged by glucocorticoid treatment (Lambillotte *et al.* 1997), it seems that SGK-1 (NM_019232) also may be of both pathogenetic and therapeutical interest in type 2 diabetes and steroid diabetes.

As for the differential expression of K_v in our present study, it was found that the delayed rectifier $K_{v2.1}$ (*Kcnb1*; NM_013186) subtype was preferentially up-regulated by high glucose in islets from diabetic GK rats (Table 3). Since this is the dominant K_v in rodent and human islets, contributing to some 85% of their steady-state outward current, it negatively regulates Ca²⁺ dynamics and insulin secretion (MacDonald & Wheeler 2003). Hence, the up-regulation of $K_{v2.1}$

Table 6 Islet transcripts down-regulated by high glucose in diabetic GK rats versus high glucose in non-diabetic Wistar rats

	Accession no	Gene name	Fold change
Function			
	Transport	NM_022534 Transcobalamin 2 (Tcn2)	1.2
Metabolism	NM_030834	Monocarboxylate transporter (Slc16a3)	1.2
	NM_017288	Sodium channel, voltage-gated, type I, beta polypeptide (Scn1b)	1.2
	D87247	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (Pfkfb3)	1.4
	BI294137	Hexokinase 2 (Hk2)	1.4
	NM_012497	Aldolase C, fructose-biphosphate (Aldoc)	1.6
	NM_013190	Phosphofructokinase, liver, B-type (Pfkfb)	1.2
	BI283882	Glucose phosphate isomerase (Gpi)	1.2
	NM_022268	Liver glycogen phosphorylase (Pygl)	1.6
	AY081195	Monoglyceride lipase (Mgll)	1.4
	AA849399	Cathepsin Z (Ctsz)	1.6
	AB020480	SNF1-like kinase (Snf1lk)	1.4
	NM_019236	Hairy and enhancer of split 2 (<i>Drosophila</i>) (Hes2)	1.4
	NM_022627	Protein kinase, AMP-activated, beta 2 non-catalytic subunit (Prkab2)	1.4
	Apoptosis	NM_053420 BCL2/adenovirus E1B 19 kDa-interacting protein 3 (Bnip3)	1.4
Signal transduction	NM_012488	Alpha-2-macroglobulin (A2m)	1.6
	NM_053826	Pyruvate dehydrogenase kinase 1 (Pdk1)	1.4
	NM_022441	Activin A receptor type II-like 1 (Acvr1l1)	1.4
	AB020967	Tribbles homolog 3 (<i>Drosophila</i> ; Trib3)	1.4
	Al178808	Interleukin 2 receptor, gamma (severe combined immunodeficiency; Il2rg)	1.4
Development	NM_013085	Plasminogen activator, urokinase (Plau)	1.6
	NM_012588	Insulin-like growth factor binding protein 3 (Igfbp3)	1.6
	NM_019370	Ectonucleotide pyrophosphatase/phosphodiesterase 3 (Enpp3)	1.4
	NM_133298	Glycoprotein (transmembrane) nmb (Gpnmb)	1.6
	NM_019329	Contactin 3 (Cntn3)	1.4
Response to stimulus	NM_012580	Heme oxygenase (decycling) 1 (Hmox1)	1.4
	NM_031051	Macrophage migration inhibitory factor (Mif)	1.1
	BI284218	Solute carrier family 2 (facilitated glucose transporter), member 1 (Slc2a1)	1.4
Cell adhesion	BI282122	EGL nine homolog 1 (<i>C. elegans</i> ; Egn1)	1.4
	NM_133298	Glycoprotein (transmembrane) nmb (Gpnmb)	1.6
	NM_017190	Myelin-associated glycoprotein (Mag)	1.4
Cell-cell signaling	AI009159	Synapsin III (Syn3)	1.6
Homeostasis	BI277505	Phosphoglucosmutase 1 (Pgm1)	1.4

(NM_013186) in GK islets is likely to contribute to a more hyperpolarized state of the diabetic β -cells, thereby suppressing glucose-stimulated insulin secretion. Since this is exactly what typifies the GK rat islets (Table 2; Hughes *et al.* 1998), it is possible that $K_{v}2.1$ overexpression is an intrinsic β -cell defect that contributes to its loss of glucose sensitivity in the GK rat. To our knowledge, this is the first report describing $K_{v}2.1$ (Kcnb1; NM_013186) overexpression in diabetic islets and makes $K_{v}2.1$ an attractive target for antidiabetic drugs.

Among other ion transporters of strong pathophysiological and therapeutic interest that were aberrantly expressed in GK rat islets, were the sulfonylurea receptor (SUR1/Abcc8 (AB052294)) and its associated inwardly rectifying ATP-sensitive K^{+} channel (Kir6.2/Kcnj11 (U44897)). Both were found to be up-regulated by high glucose in GK rat islets (Table 3) but not in Wistar, again features that would be expected to contribute to attenuation of glucose-sensitive insulin

secretion in GK rat islets. Consistent with such a scenario are also previous reports describing that nonsense mutations in the SUR1/Abcc8 (AB052294) or Kir6.2/Kcnj11 (U44897) genes result in hyperinsulinism and hypoglycemia due to unregulated over-secretion of insulin (Nestorowicz *et al.* 1997, Verkarre *et al.* 1998).

Among other genes dysregulated in GK islets, we found up-regulation of the membrane receptor CD36 (NM_031561; Table 11). This facilitates the major fraction of long-chain FA uptake in a variety of tissues, including the β -cell (Noushmehr *et al.* 2005). In human β -cells, CD36 was found to specifically mediate the inhibitory effects of FA on insulin secretion (Noushmehr *et al.* 2005). Interestingly, CD36 (NM_031561) was also recently found to be up-regulated by high glucose and associated with accelerated atherosclerosis in humans (Griffin *et al.* 2001) thus forming a possible link between diabetes and atherosclerosis. CD36 (NM_031561) functions as a transporter for oxidized

Table 7 Islet transcripts up-regulated by low glucose in diabetic GK rats versus low glucose in non-diabetic Wistar rats

	Accession no	Gene name	Fold change
Function Transport	BM392280	Solute carrier family 29 (nucleoside transporters), member 3 (Slc29a3)	1.5
	BF417032	Transferrin receptor (Tfrc)	1.3
	NM_023101	Vesicle transport through interaction with t-SNAREs homolog 1A (yeast; Vti1a)	1.3
Metabolism	NM_023950	RAB7, member RAS oncogene family (Rab7)	1.2
	NM_031718	RAB2, member RAS oncogene family (Rab2)	1.1
	NM_134334	Cathepsin D (Ctsd)	1.6
	NM_031973	Dipeptidylpeptidase 7 (Dpp7)	1.4
	NM_012980	Matrix metalloproteinase 11 (Mmp11)	1.6
	NM_031811	Transaldolase 1 (Taldo1)	1.4
	NM_057188	Guanosine monophosphate reductase (Gmpr)	1.5
	NM_031095	Renin binding protein (Renbp)	1.4
	BG673255	Phosphate cytidyltransferase 1, choline, alpha isoform (Pcyt1a)	1.4
	D00252	Glutamate oxaloacetate transaminase 1 (Got1)	1.4
	U32497	Purinergic receptor P2X, ligand-gated ion channel 4 (P2rx4)	1.4
	AA799614	Sirtuin (silent mating type information regulation 2 homolog) 2 (S. cerevisiae; Sirt2)	1.3
Apoptosis	J02582	Apolipoprotein E (ApoE)	2.4
	BM986220	Amyloid beta (A4) precursor protein (App)	1.1
	AF279911	Bcl2-associated death promoter (Bad)	1.2
	M15481	Insulin-like growth factor 1 (Igf1)	2.5
	AA998057	V-akt murine thymoma viral oncogene homolog 1 (Akt1)	1.2
	NM_019232	Serum- and glucocorticoid-inducible kinase 1 (Sgk)	1.8
	NM_024134	DNA-damage inducible transcript 3 (Ddit3)	1.4
Signal transduction	NM_054011	SH3-domain binding protein 5 (BTK-associated; Sh3bp5)	1.4
	BE111972	Transforming growth factor, beta receptor 1 (Tgfb1)	1.5
Development		11 β -hydroxysteroid dehydrogenase type 1 (Hsd11b1)	1.5
		Interleukin 4 receptor (Il4r)	1.4
		Major histocompatibility complex, class II, DM alpha	1.9
Response to stimulus	NM_133380	Lymphocyte protein tyrosine kinase (Lck)	1.2
	BI301490	V-maf musculoaponeurotic fibrosarcoma oncogene family, protein G (avian; Mafg)	1.2
Homeostasis	AB050011		

LDL-cholesterol (Okajima *et al.* 2005). These findings, in conjunction with previous reports showing that oxidized LDL-cholesterol suppresses insulin gene transcription and promotes β -cell death (Cnop *et al.* 2002, Okajima *et al.* 2005), raises the possibility that CD36 (NM_031561) up-regulation might be involved in β -cell dysfunction. Although GK rats are not hyperlipidemic (Zhou *et al.* 1995), the increased CD36 (NM_031561) expression may promote functional suppression and possibly lipoapoptosis as part of glucolipotoxicity (Koyama *et al.* 1998, Prentki *et al.* 2002). In accordance with such a scenario, previous reports indicate that endogenous FA catabolism is greater in GK islets than in Wistar islets (Sener *et al.* 1993), and that high-fat feeding impedes glucose-sensitive insulin secretion in islets from GK rats while not affecting Wistar islets (Briaud *et al.* 2002).

Several genes regulating glucose metabolism were also found to be dysregulated in GK rat islets. In Wistar islets, several transcripts encoding glycolytic enzymes were up-regulated by glucose (Table 9), thus confirming findings in islet cell lines (Roche *et al.* 1997) indicating

an intact islet adaptive response to hyperglycemia. In contrast, these glycolytic genes were down-regulated by high glucose in diabetic islets (Table 6), suggesting impaired glycolytic signaling that may contribute to β -cell failure in GK rats (Mertz *et al.* 1996). These genes were also found to be down-regulated in islets from patients with type 2 diabetes (Gunton *et al.* 2005). Impaired glucose metabolism might negatively impact other islet functions than solely secretion, such as insulin gene expression and β -cell proliferation, both events strongly induced by glucose (Nielsen *et al.* 1985, Sjöholm 1997). Impaired insulin gene expression and β -cell proliferation characterize not only GK islets (Portha 2005) but also islets from type 2 diabetic humans (Butler *et al.* 2003, Del Guerra *et al.* 2005). (PEPCK/PCK1; BI277460) was induced by high glucose in diabetic GK islets (Table 3). This gluconeogenic enzyme is normally not expressed in islets (MacDonald *et al.* 1992), thus effectively preventing gluconeogenesis through conversion of oxaloacetate to phosphoenolpyruvate. Its induction by high glucose (and endogenous glucocorticoids) in diabetic islets may contribute to glucose futile cycling, consuming ATP in

Table 8 Islet transcripts down-regulated by low glucose in diabetic GK rats versus low glucose in non-diabetic Wistar rats

	Accession no	Gene name	Fold change
Function			
	Transport		
	AA944965	Group specific component (Gc)	1.4
	AF120492	ATPase, Cu + + transporting, beta polypeptide (Atp7b)	1.4
	NM_053311	ATPase, Ca + + transporting, plasma membrane 1 (Atp2b1)	1.1
Metabolism	L06821	Nucleoporin 153kD (Nup153)	1.4
	BG381386	Huntingtin interacting protein 1 related (Hip1r)	1.4
	BF404569	Glutamate receptor, ionotropic, kainate 5 (Grik5)	1.4
	AF280967	Prostaglandin E synthase (Ptges)	2
	NM_031344	Fatty acid desaturase 2 (Fads2)	1.4
	U03389	Prostaglandin-endoperoxide synthase 2 (Ptgs2)	2
	NM_031976	Protein kinase, AMP-activated, beta 1 non-catalytic subunit (Prkab1)	1.3
	AF332142	Chloride ion pump-associated 55 kDa protein (Pcyox1)	1.4
	AF221622	Thyroglobulin (Tg)	1.4
	BG664123	Cytochrome P450, subfamily 51 (Cyp51)	1.2
Apoptosis	NM_013065	Protein phosphatase 1, catalytic subunit, beta isoform (Ppp1cb)	1.2
	AA800031	Four and a half LIM domains 2 (Fhl2)	1.4
	AF205779	Glucocorticoid modulatory element binding protein 2 (Gmeb2)	1.2
	AW143805	Guanine nucleotide binding protein, alpha q polypeptide (Gnaq)	1.6
Signal transduction	NM_133317	Transducer of ERBB2, 1 (Tob1)	1.4
	NM_032069	Glutamate receptor interacting protein 1 (Grip1)	1.4
	NM_030851	Bradykinin receptor B1 (Bdkrb1)	1.4
	AF026530	Stathmin-like 4 (Stmn4)	1.6
	AI177031	ER transmembrane protein Dri 42 (Ppap2b)	1.4
	AW143805	Guanine nucleotide binding protein, alpha q polypeptide (Gnaq)	1.6
		Interleukin 2 receptor, beta chain (Il2rb)	1.4
		Pleiotrophin (Ptn)	1.4
Cell cycle	NM_017066	DNA primase, p49 subunit	1.4
	BE108911	Cyclin D1 (Ccnd1)	1.2
	X75207	Troponin T2, cardiac (Tnnt2)	1.4
Development	NM_012676	Pleiotrophin (Ptn)	1.4
	NM_017066	Spondin 2, extracellular matrix protein (Spon2)	1.4
	AA801238	Claudin 11 (Cldn11)	1.6
	BG673439	Tropomyosin 3, gamma (Tpm3)	1.4
	NM_057208	Microtubule-associated protein 2 (Mtap2)	1.6
	BG374290	Interferon regulatory factor 1 (Irf1)	1.2
Response to stimulus	NM_012591		

these islets (Ostenson *et al.* 1993). Interestingly, PEPCK/PCK1 (BI277460) was recently proposed as a candidate gene for human type 2 diabetes (Cao *et al.* 2004).

Several important effectors of protein phosphorylation and exocytosis were also dysregulated in GK islets. The AMP-activated protein kinase (AMPK; Prkab2, Prkab1; NM_022627, NM_031976), proposed as a novel β -cell glucose sensor (da Silva Xavier *et al.* 2003, Tsuboi *et al.* 2003, Leclerc & Rutter 2004) was down-regulated in diabetic islets (Table 12) consistent with reports of reduced AMPK (Prkab2, Prkab1; NM_022627, NM_031976) activity in human islets from type 2 diabetic subjects (Del Guerra *et al.* 2005). Protein kinase C (PKC) isoforms were also differentially expressed; PKC- δ /Prkcd (NM_133307) being down-regulated by high glucose in GK islets (Table 4), whereas PKC- ϵ /Prkce (AA799421) showed the opposite response (Table 3). PKC- δ /Prkcd (NM_133307) has previously been linked to β -cell apoptosis (Carpenter *et al.* 2002, Eitel *et al.* 2003), so its suppression in GK islets (recently confirmed; Warwar *et al.* 2006) could represent a defence effort against β -cell

demise. Conversely, the up-regulation in GK islets of PKC- ϵ /Prkce (AA799421), known to be required for short-term insulin secretion (Hoy *et al.* 2003; Mendez *et al.* 2003), may represent an effort to compensate for the impaired insulin secretion by the diabetic islets. Phospholipase C β 1 (PLC- β 1; BE097028) was up-regulated by high glucose in GK (but not Wistar) islets (Table 5), an event that could contribute to impaired insulin secretion in GK islets since overexpression of PLC- β 1 (BE097028) in insulin-secreting cells reportedly inhibits insulin release (Ishihara *et al.* 1999). Somatostatin receptor subtype 3, whose expression has been reported in normal rat islets (Ludvigsen *et al.* 2004), was found to be up-regulated by high glucose in diabetic GK islets (Table 3). This could indicate an enhanced sensitivity of diabetic islets to the suppressive influences of paracrine somatostatin on insulin secretion.

Several critical factors controlling cell proliferation and apoptosis were also aberrantly expressed in GK islets. Cyclin D1 (Ccnd1; X75207), driving cells from G1 into cell cycle S-phase, was down-regulated in diabetic

Table 9 Islet transcripts up-regulated by high versus low glucose in non-diabetic Wistar rats

	Accession no	Gene name	Fold change
Function			
Transport	U13253	Fatty acid binding protein 5, epidermal (Fabp5)	1.1
	AA901341	Solute carrier family 2 (facilitated glucose transporter), member 3 (Slc2a3)	1.4
Metabolism	BI283882	Glucose phosphate isomerase (Gpi)	1.2
	BI294137	Hexokinase 2 (Hk2)	1.3
	AI713204	Monoglyceride lipase (Mgll)	1.6
	NM_012497	Aldolase C, fructose-biphosphate (Aldoc)	1.4
	NM_133298	Glycoprotein (transmembrane) nmb (Gpnmb)	1.6
	NM_012580	Heme oxygenase (decycling) 1 (Hmox1)	1.4
	NM_020308	A disintegrin and metalloproteinase domain 15 (metargidin; Adam15)	1.3
	D83508	Early growth response 2 (Egr2)	1.4
Apoptosis	BM388843	Tissue inhibitor of metalloproteinase 2 (Timp2)	1.3
	NM_012588	Insulin-like growth factor binding protein 3 (Igfbp3)	1.4
	NM_022207	Unc-5 homolog B (C. elegans; Unc5b)	1.2
Signal transduction	AA851740	Interleukin 6 signal transducer (Il6st)	1.2
Response to stimulus	NM_012488	Alpha-2-macroglobulin (A2m)	1.7
	BI301490	Major histocompatibility complex, class II, DM alpha	1.5
Homeostasis	NM_019232	Serum- and glucocorticoid-inducible kinase 1 (Sgk)	1.5
	J02582	Apolipoprotein E (ApoE)	2
	BI277505	Phosphoglucomutase 1 (Pgm1)	1.3
	NM_031648	FXFD domain-containing ion transport regulator 1 (Fxyd1)	1.2

GK islets as compared with non-diabetic controls (Tables 8 and 12). Since cyclin D1 (X75207) is stimulated by known β -cell mitogens (Friedrichsen *et al.* 2006), it appears essential for postnatal β -cell growth (Kushner *et al.* 2005) and its overexpression increases human β -cell proliferation by tenfold (Cozar-Castellano *et al.* 2004); increase in D-type cyclin activity can be harnessed to advantage in promoting β -cell proliferation that is inadequate in islets from GK rats (Portha 2005) and type 2 diabetic patients (Bonner-Weir & Weir 2005; Butler *et al.* 2003). Caspase-6, involved in β -cell apoptosis (Thomas *et al.* 2001), was up-regulated in GK islets as compared with Wistar islets (Table 11). IGFBP-3 (NM_012588), recently proposed

as a novel mediator of β -cell apoptosis (Shim *et al.* 2004), was up-regulated by high glucose (Table 9) and might contribute to β -cell glucoapoptosis. Apolipoprotein E (ApoE; J02582), a known constituent of islet amyloid deposits in both rodents and man (Powell *et al.* 2003), was up-regulated in GK islets (Table 7). ApoE (J02582) has been suggested to promote amyloidogenesis by stabilizing amyloid fibrils (Kahn *et al.* 1999), found in 90% of type 2 diabetic patients' post-mortem (Opie 1901) and also in GK islets (Leckstrom *et al.* 1996). An association between ApoE (J02582) gene polymorphisms and type 2 diabetes has also been noted (Vidal *et al.* 2003). It is thus possible that all these proteins up-regulated (caspase-6, IGFBP-3, and ApoE)

Table 10 Islet transcripts down-regulated by high versus low glucose in non-diabetic Wistar rats

	Accession no	Gene name	Fold change
Function			
Transport	NM_133317	Transducer of ERBB2, 1 (Tob1)	1.4
	AF166267	Kinesin light chain 3 (Klc3)	1.4
Metabolism	BG664123	Cytochrome P450, subfamily 51 (Cyp51)	1.2
	NM_031599	Eukaryotic translation initiation factor 2 alpha kinase 3 (Eif2ak3)	1.2
	BE108911	DNA primase, p49 subunit	1.2
	BM385445	Topoisomerase (DNA) 2 alpha (Top2a)	1.4
	AI705393	Fragile X mental retardation syndrome 1 homolog (Fmr1)	1.4
	NM_053358	Single stranded DNA binding protein 3 (Ssd3)	1.4
	BF290483	General transcription factor II I repeat domain-containing 1 (Gtf2ird1)	1.2
	NM_017077	Forkhead box A3 (Foxa3)	1.2

Table 11 Islet transcripts up-regulated in diabetic GK rats versus non-diabetic Wistar rats

	Accession no	Gene name	Fold change
Function Transport	NM_023101	Vesicle transport through interaction with t-SNAREs homolog 1A (yeast; Vti1a)	1.2
	AF202115	Ceruloplasmin (Cp)	1.3
	NM_053637	Syntaxin binding protein 3 (Stxbp3)	1.2
	AA943122	Transmembrane protein 9 (predicted; Tmem9_predicted)	1.2
Metabolism	BI295900	Dihydrolipoamide S-acetyltransferase (E2 component of pyruvate dehydrogenase complex; Dlat)	1.2
	NM_019383	ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit d (Atp5h)	1.1
Apoptosis	AW528459	POU domain, class 2, transcription factor 1 (Pou2f1)	1.2
	AF051335	Reticulon 4 (Rtn4)	1.5
	NM_031775	Caspase 6 (Casp6)	1.2
Signal transduction	NM_019191	MAD homolog 2 (<i>Drosophila</i> ; Madh2)	1.1
	NM_023950	RAB7, member RAS oncogene family (Rab7)	1.1
	NM_012823	Annexin A3 (Anxa3)	1.4
Development	AF236130	Disabled homolog 2 (<i>Drosophila</i>) interacting protein (Dab2ip)	1.2
	NM_017154	Xanthine dehydrogenase hypothetical gene (Xdh)	1.2
	NM_057132	Ras homolog gene family, member A (Rhoa)	1.2
Cell adhesion	BF398112	Smoothened homolog (<i>Drosophila</i> ; Smo)	1.2
	NM_031561	CD36 antigen (collagen type I receptor, thrombospondin receptor)-like 2 (Scarb2)	1.2

Table 12 Islet transcripts down-regulated in diabetic GK rats versus non-diabetic Wistar rats

	Accession no	Gene name	Fold change
Function Transport	NM_031738	Solute carrier family 29 (nucleoside transporters), member 2 (Slc29a2)	1.3
Metabolism	NM_017245	Eukaryotic translation elongation factor 2 (Eef2)	1.1
	U19516	Eukaryotic translation initiation factor 2B, subunit 5 epsilon (Eif2b5)	1.1
	NM_012497	Aldolase C, fructose-biphosphate (Aldoc)	1.2
	NM_053291	Phosphoglycerate kinase 1 (Pgk1)	1.1
	U03389	Prostaglandin-endoperoxide synthase 2 (Ptgs2)	1.6
	NM_022627	Protein kinase, AMP-activated, beta 2 non-catalytic subunit (Prkab2)	1.4
	NM_031976	Protein kinase, AMP-activated, beta 1 non-catalytic subunit (Prkab1)	1.1
	NM_053623	Acyl-CoA synthetase long-chain family member 4 (Acsl4)	1.1
	NM_030997	VGF nerve growth factor inducible (Vgf)	1.7
	BG378885	High mobility group AT-hook 1 (Hmga1)	1.3
	BM387190	Transcription factor 19	1.3
	NM_053826	Pyruvate dehydrogenase kinase 1 (Pdk1)	1.3
	AB020480	SNF1-like kinase (Snf1lk)	1.2
Apoptosis	X75207	Cyclin D1 (Cnd1)	1.3
	BE113385	Casein kinase 1, alpha 1 (Csnk1a1)	1.1
	NM_031138	Ubiquitin-conjugating enzyme E2B, RAD6 homolog (<i>S. cerevisiae</i> ; Ube2b)	1.1
	AW143805	Guanine nucleotide binding protein, alpha q polypeptide (Gnaq)	1.4
Signal transduction	AI177031	ER transmembrane protein Dri 42 (Ppap2b)	1.4
	NM_030846	Growth factor receptor bound protein 2 (Grb2)	1.1
	AI103954	ADP-ribosylation factor-like 5 (Arl5)	1.1
Cell adhesion	BM384639	Aggrecan 1 (Agc1)	1.4
	NM_017190	Myelin-associated glycoprotein (Mag)	1.2
	NM_019329	Contactin 3 (Cntn3)	1.4

Table 13 Validation by qRT-PCR of glucose-regulated islet transcripts identified by microarray analysis

		Fold change
Gene name		
Serum- and glucocorticoid-inducible kinase 1 (Sgk)	Up-regulated by high versus low glucose in non-diabetic Wistar rats	1.71 ± 0.5* †
Apolipoprotein E (ApoE)		1.11 ± 0.4* †
Insulin-like growth factor binding protein 3 (Igfbp3)		2.79 ± 0.4* †
Insulin-2		11.70 ± 1.3*
Insulin-2	Down-regulated by high versus low glucose in diabetic GK rats	0.60 ± 4.8*, †
Insulin-2	Down-regulated by high glucose in diabetic GK rats versus high glucose in non-diabetic Wistar rats	0.02 ± 0.4*, †
Insulin-2	Down-regulated by low glucose in diabetic GK rats versus low glucose in non-diabetic Wistar rats	0.32 ± 0.1* †
11β-hydroxysteroid dehydrogenase type 1 (Hsd11b1)	Up-regulated by low glucose in diabetic GK rats versus low glucose in non-diabetic Wistar rats	1.73 ± 0.4* †
Apolipoprotein E (ApoE)		1.92 ± 0.3* †

Results are means ± S.E.M. of three experiments. *Denotes $P < 0.05$ for a chance difference versus controls using Student's unpaired *t*-test. †Denotes correlation between microarray and qRT-PCR is significant at the $P < 0.01$ level.

may contribute to the increased apoptosis known to occur in β-cells from GK rats (Koyama *et al.* 1998; Portha 2005) and patients with type 2 diabetes (Federici *et al.* 2001, Butler *et al.* 2003).

Expression of the glucose-dependent insulinotropic polypeptide receptor (GIPr; NM_012714) was approximately twofold lower in GK low glucose when compared with GK high glucose. This suggests a tonic reduction of incretin signaling in GK islets, potentially a major contributor to the islet defect in this diabetes model. Since the incretin signaling/sensing is an important physiologic regulator of glucose-stimulated insulin secretion and β-cell development, this impairment would be consistent with the reduced cyclin D1 (X75207) and increased apoptosis genes. The insulinotropic response to GIP (NM_012714) in β-cells is also grossly impaired in diabetic patients (Vilsboll *et al.* 2003).

Recent studies have suggested an inflammatory contribution to the pathogenesis of type 2 diabetes (Donath *et al.* 2005, Kolb & Mandrup-Poulsen 2005, Homo-Delarche *et al.* 2006). Major histocompatibility complex, class II gene (MHC class II; A1171966, B1301490) is an immune gene associated with type 2 diabetes (Acton *et al.* 1994). This gene was up-regulated in GK islets when compared with Wistar islets at 3 mM glucose and down-regulated by high glucose (20 mM) in GK islets in our study (Tables 4 and 7).

As expected, the insulin gene was robustly up-regulated (11.7-fold) in response to high glucose in islets from non-diabetic rats (Table 13), whereas glucose failed to influence insulin gene expression in islets from GK rats. Similar results were obtained in *Psammomys obesus*, another animal model of type 2 diabetes, by Leibowitz *et al.* (2002). However, the insulin content was significantly higher in islets from diabetic rats when compared with normal rats, suggesting that glucose-induced proinsulin biosynthesis

might predominantly be controlled at the translational level and also by stabilization of proinsulin mRNA rather than at the transcriptional level (Webb *et al.* 2000).

In conclusion, we identified significant changes in several islet mRNAs involved in glucose sensing, phosphorylation, incretin action, glucocorticoid handling, ion transport, mitogenesis, and apoptosis that clearly distinguish diabetic animals from controls. Such markers may provide clues to the pathogenesis of human type 2 diabetes and may be of predictive and therapeutical value in clinical settings in efforts aiming at conferring β-cell protection against apoptosis, impaired regenerative capacity, and functional suppression occurring in diabetes.

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