

Diabetes induced by gain-of-function mutations in the Kir6.1 subunit of the K_{ATP} channel

Maria S. Remedi,^{1,2,3} Jonathan B. Friedman,² and Colin G. Nichols^{2,3}

¹Department of Medicine, ²Department of Cell Biology and Physiology, and ³Center for the Investigation of Membrane Excitability Diseases, Washington University School of Medicine, St. Louis, MO 63110

Gain-of-function (GOF) mutations in the pore-forming (Kir6.2) and regulatory (SUR1) subunits of K_{ATP} channels have been identified as the most common cause of human neonatal diabetes mellitus. The critical effect of these mutations is confirmed in mice expressing Kir6.2-GOF mutations in pancreatic β cells. A second K_{ATP} channel pore-forming subunit, Kir6.1, was originally cloned from the pancreas. Although the prominence of this subunit in the vascular system is well documented, a potential role in pancreatic β cells has not been considered. Here, we show that mice expressing Kir6.1-GOF mutations (Kir6.1[G343D] or Kir6.1[G343D,Q53R]) in pancreatic β cells (under rat-insulin-promoter [Rip] control) develop glucose intolerance and diabetes caused by reduced insulin secretion. We also generated transgenic mice in which a bacterial artificial chromosome (BAC) containing Kir6.1[G343D] is incorporated such that the transgene is only expressed in tissues where Kir6.1 is normally present. Strikingly, BAC-Kir6.1[G343D] mice also show impaired glucose tolerance, as well as reduced glucose- and sulfonylurea-dependent insulin secretion. However, the response to K^+ depolarization is intact in Kir6.1-GOF mice compared with control islets. The presence of native Kir6.1 transcripts was demonstrated in both human and wild-type mouse islets using quantitative real-time PCR. Together, these results implicate the incorporation of native Kir6.1 subunits into pancreatic K_{ATP} channels and a contributory role for these subunits in the control of insulin secretion.

INTRODUCTION

In the pancreatic β cell, ATP-sensitive K^+ (K_{ATP}) channels play a critical role in coupling membrane excitability to glucose-stimulated insulin secretion, and thereby maintaining blood glucose in a narrow range. Gain-of-function (GOF) mutations in the pore-forming Kir6.2 subunit or in the regulatory sulfonylurea receptor 1 (SUR1) subunit have been identified as the commonest cause of human neonatal diabetes mellitus (NDM; Gloyn et al., 2004b; Vaxillaire et al., 2004; Sperling, 2006; Hamilton-Shield, 2007; Polak and Cavé, 2007). Normally, increased glucose metabolism elevates intracellular [ATP]/[ADP] ratio inhibiting K_{ATP} channels, leading to membrane depolarization, opening of voltage-dependent Ca^{2+} channels, and an increase in intracellular [Ca^{2+}], which, in turn, triggers insulin secretion (Nichols, 2006). Antidiabetic sulfonylurea drugs promote insulin secretion by binding to the SUR1 subunit of the K_{ATP} channel, independently of the metabolic state of the cell (Ashcroft and Gribble, 1999). Sulfonylurea drugs have been widely used in type 2 diabetes treatment and, recently, successfully used to treat NDM patients with K_{ATP} -GOF mutations (Pearson et al., 2006).

There are two Kir6 subunit-encoding genes (*KCNJ8*, Kir6.1 and *KCNJ11*, Kir6.2). Kir6.2 is a major

component of pancreatic β cell K_{ATP} channels: we previously demonstrated development of profound neonatal diabetes in mice constitutively expressing Kir6.2-GOF mutations in pancreatic β cells (Koster et al., 2000; Remedi et al., 2009). These mice rapidly develop severe diabetes followed by the development of secondary consequences of systemic diabetes, including marked loss of insulin content and β cell mass (Girard et al., 2009; Remedi et al., 2009; Wang et al., 2014), consequences that are completely avoided by maintenance of normoglycemia at disease onset (Remedi et al., 2009, 2011; Benninger et al., 2011) and dramatically reversed by intensive insulin therapy (Wang et al., 2014).

Kir6.1 was originally cloned from the pancreas (Inagaki et al., 1995), but neither the presence of Kir6.1-containing K_{ATP} channels in the β cell nor any-contribution to insulin secretion has been established. Given the high sequence similarity between Kir6.1 and Kir6.2, and based on the development of severe diabetes in Kir6.2-GOF mice (Girard et al., 2009; Remedi et al., 2009), we hypothesized that pancreatic expression of GOF mutations in the Kir6.1 subunit of the channel would also decrease insulin secretion. To probe this, we generated transgenic mice expressing Kir6.1 mutations

Correspondence to Maria S. Remedi: mremedi@wustl.edu

Abbreviations used: BAC, bacterial artificial chromosome; GOF, gain-of-function; GTT, glucose tolerance test; KO, knockout; NDM, neonatal diabetes mellitus; qRT-PCR, quantitative real-time PCR.

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under Cre-recombinase control to specifically express the transgene in the tissue of interest. Single mutant Kir6.1[G343D], double mutant Kir6.1[G343D,Q53R], and WT Kir6.1 transgenic mice (Li et al., 2013) were crossed with rat-insulin-promoter Cre mice (Rip-Cre [Herrera, 2002]) to generate pancreatic β cell-specific Rip-Kir6.1[GD], Rip-Kir6.1[GD,QR], and Rip-Kir6.1[WT] mice, respectively. Single mutant Rip-Kir6.1[GD] mice show glucose intolerance, and double mutant Rip-Kir6.1[GD,QR] mice develop as severe diabetes as do Rip-Kir6.2-GOF transgenic mice. We also expressed Kir6.1 mutants in bacterial artificial chromosomes (BACs) in order to express the transgene only in tissues where the Kir6.1 gene is normally present. Strikingly, BAC-Kir6.1[GD] mice show similar glucose intolerance to Rip-Kir6.1[GD] mice. The results have important implications for the molecular basis of pancreatic K_{ATP} channels and for the potential role of *KCNJ8* (Kir6.1) gene variants in insulin secretory control and the development of diabetes.

MATERIALS AND METHODS

Human islets and study approval

Isolated cadaver-derived human islets were obtained from the Integrated Islet Distribution Program (IIDP) sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases and the JDRF-sponsored Islets for Research Program at Washington University (JDRF-31-2008-382). The IIDP uses only cadaver donors that have consented to research. The Washington University Medical Center (WUMC) Human Studies Committee (HSC) Institutional Review Board (IRB) approved all studies involving the use of isolated cadaver-derived human islets (approval number: 93-0068). The IRB exempted the study from HIPAA compliance based on the regulatory definition of human subject. Review date: 7/8/2004; review committee: 08 MRCC. Human islet donor data: four independent organ donors, two males and two females; mean age 35 yr, mean purity 85.8%, and mean viability 92.9%.

Quantitative real-time PCR (qRT-PCR) analysis

Mouse islets were isolated and immediately processed for RNA isolation. Upon arrival, human islets were collected by centrifugation, cleaned, and hand-selected in CMRL media under a stereomicroscope (SMZ745 system; Nikon) and immediately processed for RNA isolation to perform qRT-PCR. Cellular RNA was isolated using the RNeasy Mini kit (QIAGEN), and DNA was removed using DNaseI RNase-free solution (QIAGEN). cDNA was prepared from RNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems), and qRT-PCR was performed using StepOne software 2.3 and TaqMan primers (Applied Biosystems). Actin was used as a reference gene.

Generation of CX1-Kir6.1[WT], CX1-Kir6.1[GD], CX1-Kir6.1[GD,QR], BAC-Kir6.1[WT], and BAC-Kir6.1[GD] transgenic mice

Inducible Kir6.1-GOF transgenes were generated by subcloning WT, Kir6.1[Gly343Asp] (G343D), and Kir6.1[Gly343Asp,Gln53Arg] (G343D,Q53R)-poly-A cDNAs downstream of the CX-1 promoter in the pBS-CX1-LEL vector (a gift from G. Owens, University of Virginia, Charlottesville, VA), which contains the chicken β -actin (CX1) promoter, followed by the eGFP coding region (including a stop codon), flanked by two loxP sites, to create CX1-Kir6.1[WT], CX1-Kir6.1[G343D], and CX1-Kir6.1[G343D,Q53R] transgenic constructs (Li et al., 2013). 6–11 founder mice carrying (1) Kir6.1[WT], Kir6.1[G343D], or Kir6.1[G343D,Q53R] were generated and bred to homogeneity by multiple (6 \times) backcrosses to C57BL/6J mates as previously described (Li et al., 2013). These mice were crossed with rat-insulin-promoter Cre (Rip-Cre) mice to generate pancreatic β cell-specific Rip-Kir6.1[GD], Rip-Kir6.1[GD,QR], and Rip-Kir6.1[WT] mice.

A mouse BAC containing the Kir6.1 genomic locus (BAC ID# RP23-159E3) was used to generate the Kir6.1[WT] and Kir6.1[G343D] BAC transgenes via galK-based homologous recombination in bacteria. Positive clones from the first round of recombineering were subjected to a second round of recombineering to remove the galK gene, leaving behind only the desired mutations. Positive recombinants were identified via directional PCR, restriction digest, and sequencing. Mutant and WT BAC DNA were prepared using a modified QIAGEN maxi-prep, which was dot dialyzed on 0.1 μ M EMD Millipore filters using BAC injection buffer. The Washington University Transgenic Vectors Core generated the Kir6.1[WT] and Kir6.1[G343D] BAC transgenes, and microinjection services were provided by the Washington University Mouse Genetics Core. Kir6.1^{-/-} (Kir6.1 knockout [Kir6.1KO]) mice, originally generated by Miki et al. (2002), were obtained as a gift from S. Seino (Kobe University Graduate School of Medicine, Kobe, Japan).

Animal study approval

All procedures complied with the standards for the care and use of animal subjects as stated in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication No. 85-23, revised 1996).

Blood glucose

Tail blood was assayed for glucose content using a Glucometer Elite XI meter (Bayer Corp). The limit of detection was 600 mg/dl, and glucose at or above this level was recorded as 600 mg/dl but considered to be a lower limit of the true value.

Glucose tolerance test (GTT)

Intraperitoneal GTT was performed in 12-h fasted 7-wk-old mice by injection of a bolus of glucose (1.5 g/kg body weight). Blood was taken at different times and assayed for glucose content as above.

Islet isolation

8-wk-old mice were anesthetized with 0.2 ml isoflurane and killed by cervical dislocation. The bile duct was cannulated and perfused with Hank's balanced salt solution (Sigma-Aldrich) containing collagenase (0.3 mg/ml, Collagenase Type XI; Sigma-Aldrich). Pancreata were removed and digested for 5 min at 37°C, hand shaken, and washed three times in cold Hanks' solution. Islets were isolated by hand under a dissecting SMZ745 microscope, and pooled islets were maintained overnight in CMRL-1066 (5.6 mM glucose) culture medium (GIBCO) supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 µg/ml streptomycin.

Insulin secretion

After overnight incubation in low glucose (5.6 mM) CMRL-1066 medium, islets (10 per well in 12-well plates) were preincubated in glucose-free CMRL-1066 plus 3 mM glucose and then incubated for 60 min at 37°C in CMRL-1066 plus different glucose concentrations, 1 µM glibenclamide, or 30 mM KCl, as indicated. After the incubation period, the medium was removed and assayed for insulin release. Insulin secretion was measured using a Rat Insulin radioimmunoassay according to manufacturer's procedure (RIA; EMD Millipore; Remedi et al., 2009). Plasma insulin was measured using Singulex Erenna method (similar to insulin enzyme-linked immunosorbent assay) at the Core Laboratory for Clinical Studies, Washington University in St. Louis. Experiments were repeated in triplicate.

Isolated islet fluorescence imaging

Freshly isolated islets from 8-wk-old mice were imaged for eGFP, a reporter of transgene expression, using an inverted fluorescent microscope (LSM 510 laser-scanning confocal microscope; ZEISS). Images were taken with 20× objectives using the same settings for both control and transgenic islets.

⁸⁶Rb⁺ efflux experiments

Isolated islets were preincubated for 6 h with ⁸⁶Rb⁺ (rubidium chloride 1.5 mCi/ml; GE Healthcare). Loaded islets were placed in microcentrifuge tubes (30 per group) and washed twice with RPMI-1640 medium at 37°C (Sigma-Aldrich). ⁸⁶Rb⁺ efflux was assayed by replacing the bathing solution with Ringer's solution with metabolic inhibitor (MI), with or without 1 µM glibenclamide. MI solution contained 2.5 mg/ml oligomycin and 1 mM 2-deoxyglucose, together with 10 mM tetraethylammonium to block voltage-gated K⁺ channels and

10 µM nifedipine to block Ca²⁺ entry. The other solutions were different glucose concentrations (1, 7, and 20 mM glucose). The bathing solution was replaced with fresh solution every 5 min over a 50-min period and counted in a scintillation counter. ⁸⁶Rb efflux in the presence of glucose and drugs were fit by a single exponential, in which the rate constant (*k*) is proportional to the K⁺ (Rb⁺)-conductance in the islet membranes.

Statistics

Data are presented as mean ± SEM. Differences among groups were tested using unpaired *t* test to assess significance. Differences were assumed to be significant in each case if *P* < 0.05, and nonsignificant differences are not indicated in the figures.

RESULTS

Kir6.1 is expressed in mouse and human pancreatic islets

Although Kir6.1 subunits are prominent components of vascular K_{ATP} channels (Flagg et al., 2010), the cDNA was originally cloned from pancreatic islets (Inagaki et al., 1995), but there was little consideration of the presence of this subunit in insulin-secreting cells. The latter studies suggest that Kir6.1 is naturally expressed in islets, raising the possibility that Kir6.1 subunits might contribute to native islet K_{ATP} function. WT and Kir6.1KO mouse islets were subjected to qRT-PCR. As predicted, robust Kir6.1 signals, in addition to Kir6.2 signals, were detected in mouse WT islets (Fig. 1 A), but there was no Kir6.1 signal detectable (at up to 50 cycles) in Kir6.1KO islets (Fig. 1 B), confirming the validity of the Kir6.1 signal in WT islets. Expression of Kir6.2 was not different between WT and Kir6.1KO islets (Fig. 1 B). Importantly, human islets obtained from cadaveric organ donors also showed a robust Kir6.1 transcript level (Fig. 1 C). Thus, the Kir6.1 subunit of the K_{ATP} channel is natively present in both mouse and human pancreatic islets.

β cell expression of ATP-insensitive Kir6.1-containing K_{ATP} channels

To study the effects of Kir6.1 subunits of the K_{ATP} channel in vivo, we have generated novel mice that express either the Kir6.1 WT (Kir6.1 [WT]) or a Kir6.1-GOF, single mutant G343D (Kir6.1 [GD]) or double mutant G343D/Q53R (Kir6.1 [GD,QR]) transgenes under Cre-recombinase control (Li et al., 2013). Global Cx1 Kir6.1 [WT], Kir6.1 [GD], and Kir6.1 [GD,QR] mice express GFP fluorescence ubiquitously in the whole body, but after tissue-specific Cre-recombination, these mice lose fluorescence only in the tissue of interest (Li et al., 2013). Specific expression in pancreatic β cells was achieved by crossing Kir6.1 transgenic mice with Rip-Cre mice. Fig. 2 shows marked loss of fluorescence in

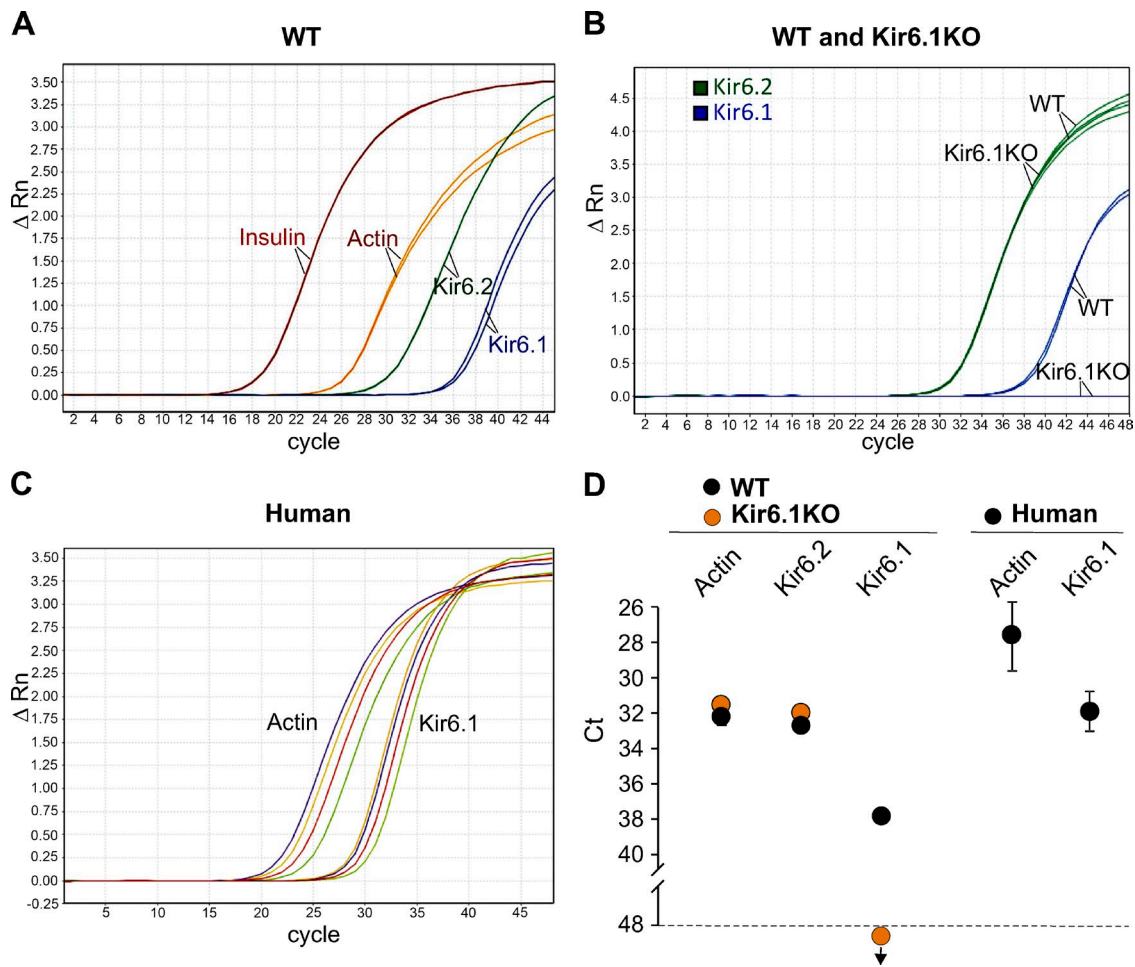


Figure 1. Kir6.1 transcript is present in primary human and mouse islets. qRT-PCR in mouse and human islets. (A and B) Representative traces of transcript amplification versus cycle number from mouse islets. (A) Insulin is represented in red, Kir6.2 in green, and Kir6.1 in blue. Actin was used as housekeeping gene and is shown in orange. Same color lines are duplicates from the same sample. (B) Comparative amplification traces for Kir6.2 (green) and Kir6.1 (blue) genes in WT and Kir6.1KO islets ($n = 5$ mice of each genotype, each sample was run in duplicates). Kir6.2 transcripts are present at similar levels in both WT and Kir6.1KO islets, but Kir6.1 transcript is only detected in WT islets and not in Kir6.1KO islets. (C) Each color represents traces for Kir6.1 transcript and the corresponding actin transcript in islets from independent human cadaveric organ donors. (D) Mean crossing threshold (Ct) for transcripts from mouse and human islets. Data represent mean \pm SEM, $n = 4$ independent batches of human islets and $n = 5$ mice from each genotype. Samples were run in duplicates.

pancreatic islets from double transgenic Rip-Kir6.1[WT] (A), Rip-Kir6.1[GD] (B), and Rip-Kir6.1[GD,QR] (C; as an indication of transgene expression), compared with bright fluorescent islets from single Kir6.1[WT], Kir6.1[GD], and Kir6.1[GD,QR] transgenic mice (lacking recombination and absence of transgene expression). Littermate control mice in all experiments below include pooled WT, single transgenic Rip-Cre, and single transgenic Kir6.1[GD] or Kir6.1[GD,QR] mice. In each case, no significant differences were found between these genotypes when independently tested.

Diabetes in Kir6.1-GOF mutant mice

GOF mutations in the Kir6.1 subunit of the K_{ATP} channel result in either glucose intolerance (Rip-Kir6.1[GD]) or frank diabetes (Rip-Kir6.1[GD,QR]),

mimicking the consequences of weak or more significant expression of Kir6.2-GOF transgenes (Koster et al., 2000, 2005; Remedi et al., 2009). 7-wk-old double transgenic Rip-Kir6.1[GD] mice showed higher fed glucose than littermate control mice (Fig. 2 B). They also demonstrate increased fasting blood glucose and a mildly impaired glucose tolerance, accompanied by reduced circulating plasma insulin after glucose challenge with respect to control littermates (Fig. 3 B). Importantly, however, 2 out of 10 Rip-Kir6.1[GD] mice showed diabetes with dramatically high fasting blood glucose levels and a marked impairment of glucose tolerance (Fig. 2 B). Strikingly, all Rip-Kir6.1[GD,QR] mice developed severe diabetes (Fig. 2 C) with markedly impaired glucose tolerance, as the result of significantly reduced plasma insulin levels in response to

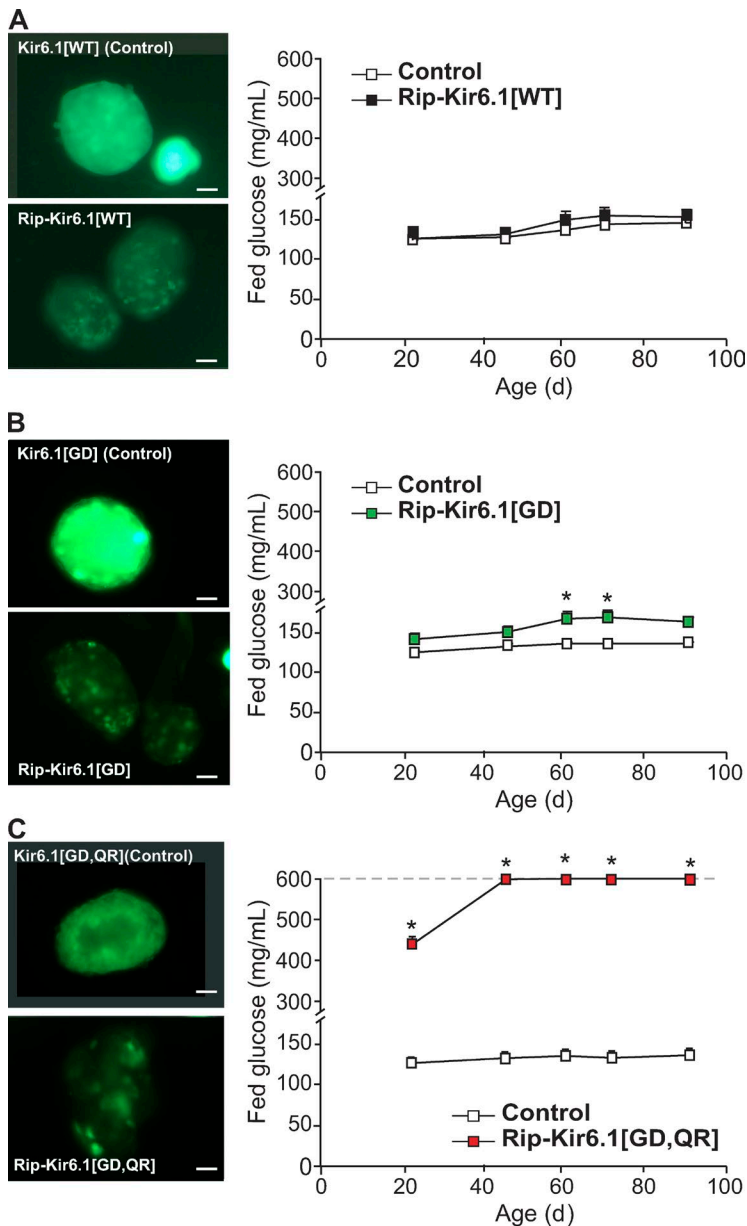


Figure 2. Increased blood glucose in Kir6.1-GOF mice. (A–C, left) Representative intrinsic GFP fluorescence in freshly isolated islets from transgenic mice. High fluorescence in transgenic islets indicates the presence of the Kir6.1 transgene (Kir6.1[x], Control), and loss of fluorescence in transgenic β cells within islets after Cre-recombination (Rip-Kir6.1[x]) indicates actual expression of the transgene specifically in these cells. Bars, 10 μ m. (right) Fed blood glucose over time after weaning in Rip-Kir6.1[WT] (A, black), single mutant Rip-Kir6.1[GD] (B, green), and double mutant Rip-Kir6.1[GD,QR] (C, red) with respect to their control littermates (white). Data represent mean \pm SEM, $n = 8$ –10 animals in each group. Significant differences (*, $P < 0.05$) between transgenic and control littermate mice are indicated (t test).

glucose challenge (Fig. 3 C). Importantly, however, and as expected, overexpression of WT Kir6.1 subunits in Rip-Kir6.1[WT] mice did not result in increased blood glucose or glucose intolerance (Figs. 2 A and 3A), suggesting that impaired glucose tolerance and diabetes are promoted by Kir6.1-GOF and are not an artifactual consequence of overexpressed transgenic protein levels per se.

We have also generated BAC Kir6.1 transgenic (BAC-Kir6.1[GD]) and WT (BAC-Kir6.1[WT]) mice (see Materials and methods). Because the BAC constructs are expected to contain the native regulatory elements of the Kir6.1 gene, with no exogenous promoter, the transgene should be expressed under native promoter control, and hence only in tissues where the Kir6.1 subunit

is normally present. Strikingly, BAC-Kir6.1[GD] mice show higher blood glucose levels, reduced circulating insulin in fed conditions (Fig. 4 A), and impaired glucose tolerance with reduced plasma insulin at 30 min, compared with BAC-Kir6.1[WT] mice (Fig. 4 B). The similar phenotype of BAC-Kir6.1[GD] mice to Rip-Kir6.1[GD] mice implicates native Kir6.1 in controlling glucose tolerance and, as shown below, most likely by regulating β cell secretion directly. Given the likely low level of Kir6.1 in the β cell, under normal conditions, WT Kir6.1 is unlikely to play a major role, and we detected no significant effects of Kir6.1KO on blood glucose (Kir6.1KO: 108 ± 2.02 mg/dl and WT: 119 ± 2.59 mg/dl), glucose tolerance, or K_{ATP} conductance assessed by Rb efflux (not depicted).

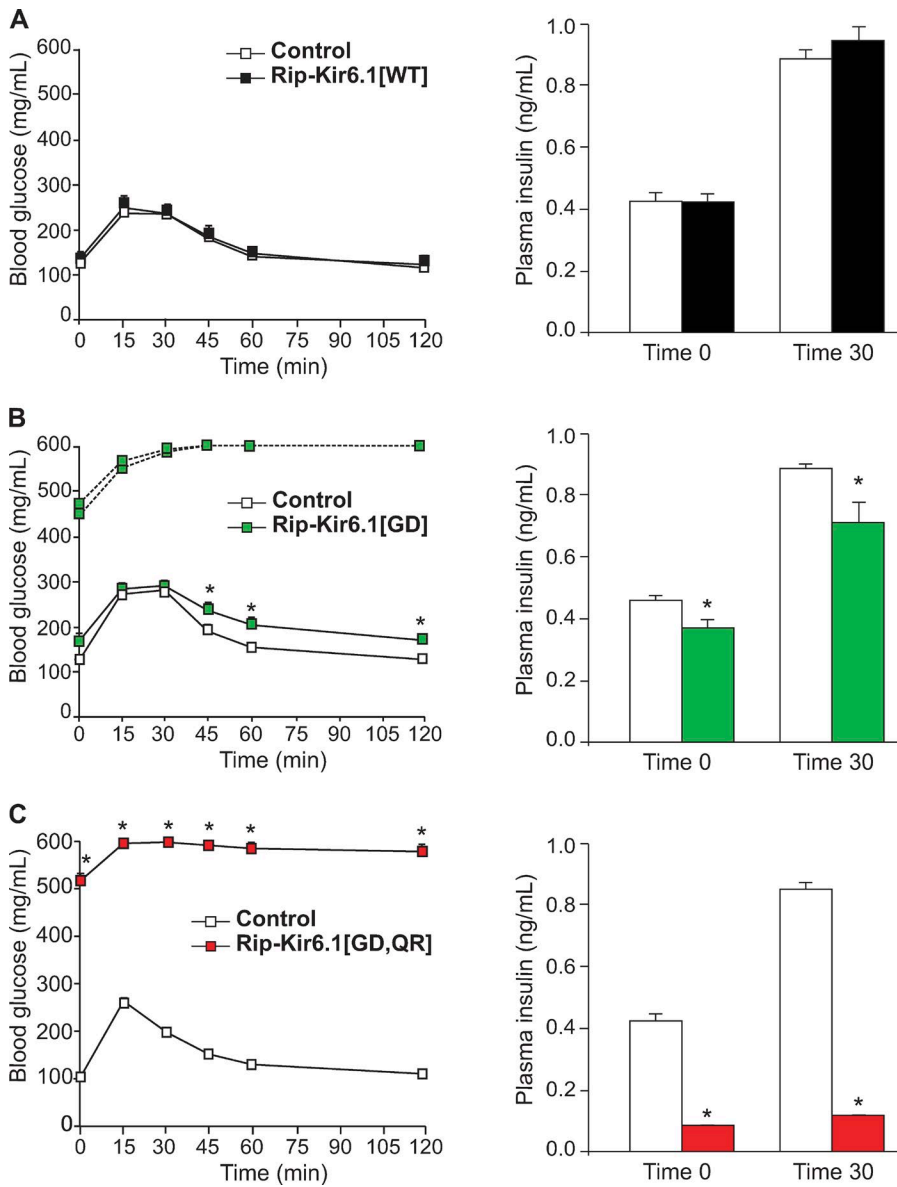


Figure 3. Glucose tolerance in Kir6.1 transgenic mice. (A–C) GTT on 12-h fasted (left) and plasma insulin (right) in Rip-Kir6.1[WT] (A, black), Rip-Kir6.1[GD] (B, green), and Rip-Kir6.1[GD,QR] (C, red) and control littermates at 7 wk of age. Blood glucose concentration versus time after injection of 1.5 g/kg glucose. Plasma insulin was determined in fasted mice (time 0) and 30 min after glucose challenge during GTT. Data represent mean \pm SEM, $n = 8$ –10 animals in each group. For Rip-Kir6.1[GD], 2 of 10 animals, with fasting blood glucose >300 mg/dl, are shown individually (dashed line), and the remaining 8 of 10 animals, with fasting blood glucose <200 mg/dl, are shown as mean data (solid line). Significant differences (*, $P < 0.05$) between transgenic and control littermate mice are indicated (t test).

Kir6.1-GOF mouse islets show decreased responsiveness to glucose challenge but maintained KCl response

Consistent with hyperglycemia in BAC-Kir6.1[GD] mice shown above, there was also a modest but significant reduction in glucose-dependent insulin secretion from isolated BAC-Kir6.1[GD] islets, with respect to controls (Fig. 5). Importantly, however, BAC-Kir6.1[GD] still responded to glibenclamide, although with an smaller response, and responded with similar maximal secretion in response to K^+ depolarization (KCl; Fig. 5), indicating no marked impairment of secretory processes downstream of the electrical signal. As expected, BAC-Kir6.1[WT] mice showed similar glucose- and glibenclamide-dependent secretion to control littermate mice (Fig. 5).

Kir6.1-GOF islets exhibit a reduction in total K_{ATP} channel density but sensitivity to glibenclamide drugs
K conductance in intact BAC-Kir6.1[WT] and BAC-Kir6.1[GD] transgenic was assessed by $^{86}Rb^+$ efflux in the presence of different [glucose] and glibenclamide. Fig. 6 A shows representative sample fluxes from both genotypes. Fluxes were fit by single exponentials and reciprocal rate constants (normalized to 1 mM glucose) are plotted in Fig. 6 B. In BAC-Kir6.1[WT] islets, the flux is glucose sensitive, being almost completely reduced to basal (i.e., flux in glibenclamide) in the presence of 20 mM glucose. In contrast, the BAC-Kir6.1[GD] flux is markedly less glucose sensitive, with substantial glibenclamide-sensitive (i.e., K_{ATP} -dependent) flux still present even at 20 mM glucose (Fig. 6 B). Interestingly, the absolute maximum rate constant (in 1 mM glucose)

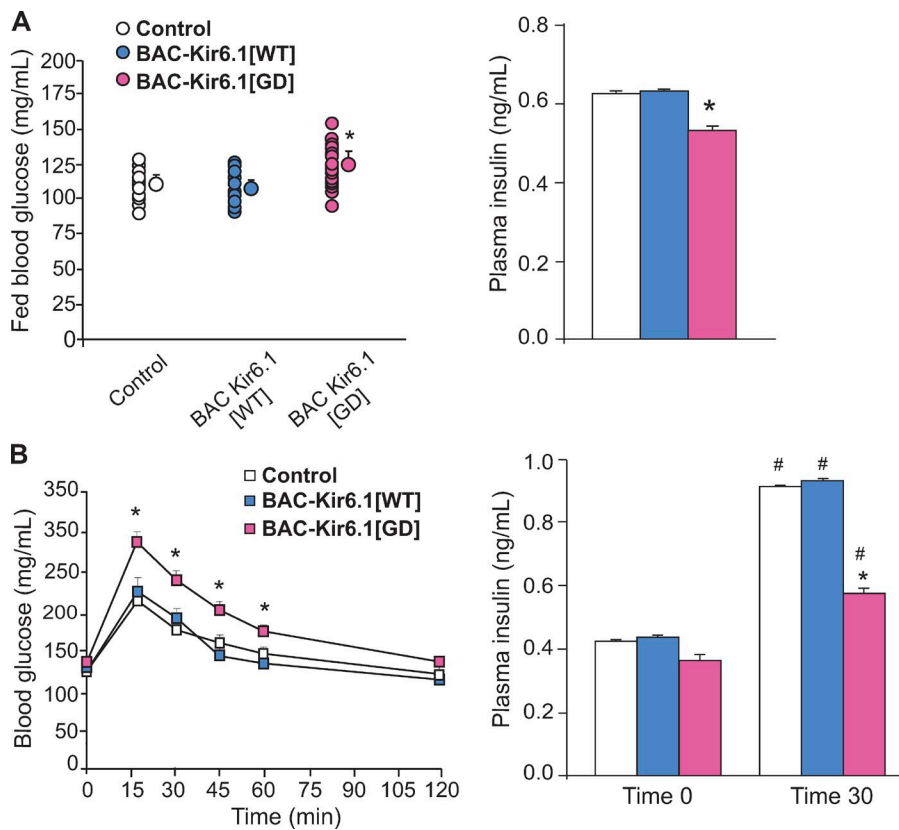


Figure 4. Fed blood glucose and glucose tolerance in BAC-Kir6.1 mice. (A) Fed blood glucose (left) and plasma insulin (right) in BAC-Kir6.1[WT] (blue), BAC-Kir6.1[GD] (pink), and control littermate (white) mice. (B) Glucose (left) and insulin levels (right) at time 0 (fasting) and 30 min after glucose challenge in BAC-Kir6.1[WT] (blue), BAC-Kir6.1[GD] (pink), and control littermate (white) mice. Data represent mean \pm SEM, $n = 10$ – 15 animals in each group. Significant differences (*, $P < 0.05$) between transgenic and control littermate mice under the same condition and (#, $P < 0.05$) on the same genotype mice under different conditions are indicated. Nonsignificant differences are not indicated.

was slightly lower in BAC-Kir6.1[GD] islets ($0.029 \pm 0.010 \text{ min}^{-1}$) than in control islets ($0.042 \pm 0.004 \text{ min}^{-1}$), which may reflect reduced maximal K_{ATP} conductance as a result of lower single channel current in Kir6.1-containing channel pores (Nichols, 2006).

DISCUSSION

Kir6.1-containing channels are present in both human and mouse islets: Implications for human diabetes
 Although Kir6.1 subunits (encoded by *KCNJ8*) are prominent components of vascular K_{ATP} channels (Flagg et al., 2010), the Kir6.1 cDNA was originally cloned from pancreatic islets (Inagaki et al., 1995), yet there has been little subsequent consideration of any role or even the likely presence of this subunit in insulin-secreting cells. Our expression analysis shows that Kir6.1 transcript is clearly present in both human and mouse islets (Fig. 1) and, importantly, that there is no Kir6.1 transcript detected in Kir6.1.KO mouse islets and no effect of Kir6.1 deletion on Kir6.2 transcript levels (Fig. 1). Other experiments (supplementary data in Pagliuca et al. [2014]) have also found *KCNJ8* gene expression in human β cells isolated from cadaveric donors and in β cells generated from human pluripotent stem cells. Furthermore, transcriptome data from 10 human cadaveric organ donors show that *KCNJ8* (Kir6.1, ranked 5,410) was second only to *KCNJ11* (Kir6.2, ranked 3,486) as the highest expressed Kir subunit in β cells (genes ranked in descend-

ing order according to magnitude of expression, i.e., insulin was ranked 1; Segerstolpe et al., 2016). Together, these studies highlight the presence of Kir6.1 in pancreatic β cells and raise the exciting possibility that alterations in Kir6.1 expression or channel properties could affect insulin secretion and thereby play a modulatory role in human diabetes.

Diabetes induced by GOF mutation in the Kir6.1 subunit of the K_{ATP} channel

In the pancreatic β cell, K_{ATP} channels are the critical link between glucose metabolism and insulin secretion, highlighted by the fact that GOF mutations in the pore-forming Kir6.2 and regulatory SUR1 subunits of the K_{ATP} channel (K_{ATP} -GOF) are the main cause of human NDM (Gloyn et al., 2004a,b; Vaxillaire et al., 2004; Massa et al., 2005). Mice expressing Kir6.2-GOF mutations specifically in pancreatic β cells reiterate key features of NDM (Koster et al., 2000; Girard et al., 2009; Remedi et al., 2009). Here, we have shown that pancreatic expression of a severely ATP-insensitive Kir6.1 mutation in Rip-Ki6.1[GD,QR] mice also results in reduced circulating plasma insulin and development of severe diabetes, whereas expression of a mildly ATP-insensitive mutation in Rip-Kir6.1[GD] mice leads to development of glucose intolerance, comparable with the phenotypes of strong and mild GOF mutations in Kir6.2 in mice and humans (Koster et al., 2000, 2006; Gloyn et al., 2004b; Flanagan et al., 2007; Remedi et al., 2009, 2011; Villareal et al., 2009).

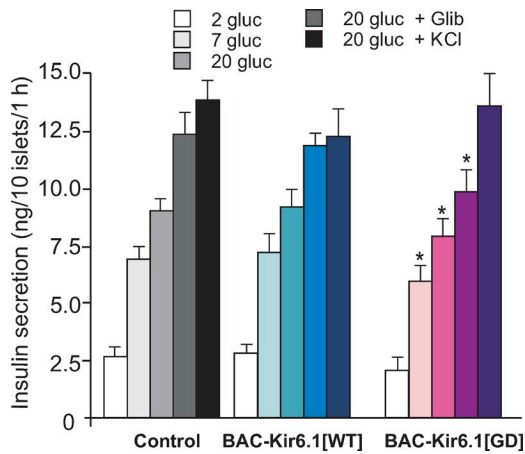


Figure 5. Impaired glucose-dependent insulin secretion in BAC-Kir6.1[GD] transgenic mice. Glucose-dependent insulin secretion on isolated islets from BAC-Kir6.1[GD] and BAC-Kir6.1[WT] mice. Batches of 10 islets were statically preincubated in low glucose and then incubated in various glucose concentrations, or in glibenclamide, or in KCl for 1 h. Data represent mean \pm SEM, $n = 4$ –5 mice in each group. Significant differences (*, $P < 0.05$) between transgenic and control littermates under the same condition are indicated (t test).

Our data show that deliberate expression of Kir6.1-GOF mutations in β cells can result in their ready incorporation into functional β cell K_{ATP} channels and hence in the modulation of insulin secretion. One potential caveat is that transgenic expression of mutant subunit proteins may result in gross islet abnormalities, but the fact that no significant differences were found between Rip-Kir6.1[WT] mice and littermate control mice (Figs. 2 A and 3A) suggests no major abnormalities as a result of transgene overexpression per se. More importantly, BAC-Kir6.1-GOF transgenic mice (but not BAC-Kir6.1[WT] transgenic mice), which express the transgene under control of the native promoter, also show higher glucose levels and reduced circulating plasma insulin in fed conditions (Fig. 4 A). BAC-Kir6.1-GOF show glucose intolerance with decreased insulin secretion 30 min after glucose challenge (Fig. 4 B) and a specific reduction in glucose-sensitive insulin secretion in vitro (Fig. 5). The overall K_{ATP} conductance in BAC-Kir6.1-GOF transgenic islets exhibits reduced glucose sensitivity, with substantial glibenclamide-sensitive Rb flux still present at high (20 mM) [glucose] (Fig. 6). These results imply that not only are Kir6.1 subunits normally expressed in the islet but that GOF mutations can reduce the glucose dependence of K_{ATP} conductance and hence of insulin secretion.

Diabetic mice and patients with K_{ATP} -GOF mutations respond to sulfonylurea drugs

The high sensitivity of Kir6.2/SUR1 channels to sulfonylurea inhibition has led to a dramatic change in therapy for NDM, with most patients successfully trans-

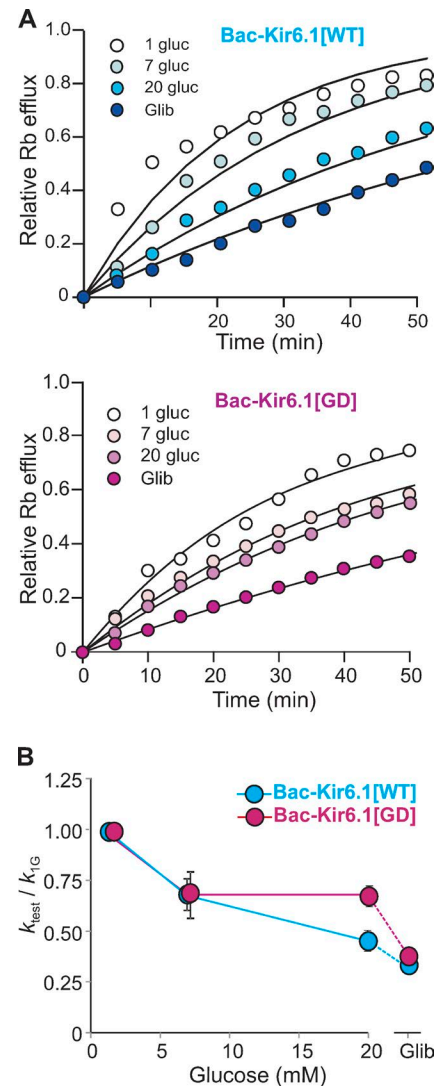


Figure 6. Reduced glucose sensitivity of K_{ATP} conductance in Kir6.1-GOF islets. (A) Glucose- and glibenclamide-dependent Rb⁺ effluxes from BAC-Kir6.1[WT] (blue) and BAC-Kir6.1[GD] (pink) islets. (B) Rate constant for Rb⁺ efflux at each condition (k_{test}), normalized to rate constant in 1 mM glucose (k_{1G}), in BAC-Kir6.1[WT] and BAC-Kir6.1[GD] islets. Data represent mean \pm SEM, $n = 4$ mice ($n > 50$ islets per sample) in each group.

ferring from injected insulin therapy to sulfonylurea tablets (Hattersley and Ashcroft, 2005; Pearson et al., 2006; Masia et al., 2007; Wambach et al., 2010) or even being initiated on sulfonylurea therapy (Wambach et al., 2010; Marshall et al., 2015). However, many Kir6.2-GOF mutations also exhibit reduced sensitivity to sulfonylureas (Koster et al., 2005), which may contribute to the generally observed requirement for relatively high doses of sulfonylureas in treatment of NDM patients (Hattersley and Ashcroft, 2005; Pearson et al., 2006). The involvement of Kir6.1 subunits in β cell K_{ATP} channels has potentially important implications for K_{ATP} pharmacologies in the treatment of diabetes and the possibility that isoform-specific blockers of

these channels (Wellman et al., 1999) may need to be considered in the future. Although no mutations in the Kir6.1-encoding gene (*KCNJ8*) located on chromosome 12p12 have yet been identified in diabetic patients, multiple studies have suggested linkage to diabetes mapping to chromosome 12p (Ng et al., 2004; Wiltshire et al., 2004). Further studies will be necessary to confirm any linkage between Kir6.1 and glucose intolerance or diabetes.

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