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

Maria S. Remedi, Jonathan B. Friedman, and Colin G. Nichols

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 $\beta$ 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 $\beta$ 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 $\beta$ 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 $\beta$ 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 $\beta$ cell $K_{ATP}$ channels: we previously demonstrated development of profound neonatal diabetes in mice constitutively expressing Kir6.2-GOF mutations in pancreatic $\beta$ 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 $\beta$ 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 $\beta$ 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...
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\(_{\text{ATP}}\) 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 MRCR. 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 DNase1 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 (6x) 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 not dialyzed on 0.1 µM 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 micronection services were provided by the Washington University Mouse Genetics Core. Kir6.1[WT] knockouts (Kir6.1 KO) 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 florescent microscope (LSM 510 laser-scanning confocal microscope; ZEISS). Images were taken with 20× objectives using the same settings for both control and transgenic islets.

$^{86}$Rb$^+$ efflux experiments
Isolated islets were preincubated for 6 h with $^{86}$Rb$^+$ (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). $^{86}$Rb$^+$ 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 nifedpine to block Ca$^{2+}$ 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. $^{86}$Rb 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

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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 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
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 \(\beta\) cell secretion directly. Given the likely low level of Kir6.1 in the \(\beta\) cell, under normal conditions, WT Kir6.1 is unlikely to play a major role, and we detected no significant effects of Kir6.1.KO on blood glucose (Kir6.1.KO: 108 ± 2.02 mg/dl and WT: 119 ± 2.59 mg/dl), glucose tolerance, or \(K_{\text{ATP}}\) conductance assessed by Rb efflux (not depicted).
Kir6.1-GOF mouse islets show decreased responsivity 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 a 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)}
was slightly lower in BAC-Kir6.1[GD] islets (0.029 ± 0.010 min$^{-1}$) than in control islets (0.042 ± 0.004 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 Kir6.1KO 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 descending 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-Kir6.1[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).
Our data show that deliberate expression of Kir6.1-GOF mutations in β cells can result in their ready incorporation into functional β cell KATP 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 KATP 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 KATP conductance and hence of insulin secretion.

Diabetic mice and patients with KATP-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 transferring 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 KATP channels has potentially important implications for KATP 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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REFERENCES


