Diabetes and insulin secretion: whither $K_{\text{ATP}}$?

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Nichols, C. G., and J. C. Koster. Diabetes and insulin secretion: whither $K_{\text{ATP}}$? Am J Physiol Endocrinol Metab 283: E403–E412, 2002; 10.1152/ajpendo.00168.2002.—The critical involvement of ATP-sensitive potassium ($K_{\text{ATP}}$) channels in insulin secretion is confirmed both by the demonstration that mutations that reduce $K_{\text{ATP}}$ channel activity underlie many if not most cases of persistent hyperinsulinemia, and by the ability of sulfonylureas, which inhibit $K_{\text{ATP}}$ channels, to enhance insulin secretion in type II diabetics. By extrapolation, we contend that mutations that increase $\beta$-cell $K_{\text{ATP}}$ channel activity should inhibit glucose-dependent insulin secretion and underlie, or at least predispose to, a diabetic phenotype. In transgenic animal models, this prediction seems to be borne out. Although earlier genetic studies failed to demonstrate a linkage between $K_{\text{ATP}}$ mutations and diabetes in humans, recent studies indicate significant association of $K_{\text{ATP}}$ channel gene mutations or polymorphisms and type II diabetes. We suggest that further efforts to understand the involvement of $K_{\text{ATP}}$ channels in diabetes are warranted.

ATP-sensitive potassium channels; pancreas; Kir6.2; SUR1

METABOLITE REGULATION OF INSULIN SECRETION

In the pancreas, the ATP-sensitive potassium ($K_{\text{ATP}}$) channel is proposed to be a critical link in glucose-induced insulin release from pancreatic $\beta$-cells (Fig. 1A) (6, 10, 62). According to this paradigm, during the fed state, when glucose metabolism is increased, pancreatic $K_{\text{ATP}}$ channels are inhibited by a high intracellular ATP-to-ADP concentration ratio ($[\text{ATP}]/[\text{ADP}]$). This depolarizes the plasma membrane, which leads to $\text{Ca}^{2+}$ entry through voltage-dependent $\text{Ca}^{2+}$ channels, or VDCC, thereby stimulating insulin secretion. A rise in circulating serum insulin, in turn, leads to an increased glucose uptake in the periphery and a compensatory drop in blood glucose. Conversely, a falling intracellular $[\text{ATP}]/[\text{ADP}]$ during the fasting state is presumed to relieve inhibition of $K_{\text{ATP}}$ channels, resulting in membrane hyperpolarization and cessation of insulin release. Sulfonylurea drugs remain in use as major hypoglycemic agents in the treatment of type II diabetes (37). These agents cause insulin secretion and act by inhibiting $K_{\text{ATP}}$ channel activity through the regulatory SUR1 subunit (2), which emphasizes the central role of the $K_{\text{ATP}}$-dependent pathway in regulation of insulin secretion. However, this pathway is modulated by so-called $K_{\text{ATP}}$-independent mechanisms, and it is important to bear in mind that glucose and other nutrient metabolites, as well as incretins, act as “gain modulators” at various additional stages of the insulin secretory process and thereby can enhance the signal through the $K_{\text{ATP}}$-dependent pathway (3).

According to the model, alterations in the metabolic signal, in the responsiveness of the $K_{\text{ATP}}$ channel to metabolites, or in the number of active $K_{\text{ATP}}$ channels should lead to altered release of insulin. Increased metabolic flux, increased $K_{\text{ATP}}$ sensitivity to inhibitory nucleotides, or reduced density of $K_{\text{ATP}}$ channels should all lead to abnormally low $K_{\text{ATP}}$ activity and relative hyperinsulinism (HI). Conversely, decreased metabolic flux, decreased $K_{\text{ATP}}$ sensitivity to inhibitory nucleotides, or increased density of $K_{\text{ATP}}$ channels should all lead to abnormally high $K_{\text{ATP}}$ activity and relative hypoinsulinism and a predisposition to non-insulin-dependent diabetes mellitus (NIDDM) (Fig. 1A). The purpose of this prospective is to summarize the clear picture that is emerging regarding the causal role of decreased $K_{\text{ATP}}$ channel activity in HI and to marshal the accumulating evidence from both animal and human studies in support of the second postulate, that relative $K_{\text{ATP}}$ overactivity may be a potent causal factor in NIDDM.
MOLECULAR BASIS OF THE K<sub>ATP</sub> CHANNEL

K<sub>ATP</sub> channels are generated as a complex of four pore-forming Kir6.2 subunits, each of which is associated with a sulfonylurea receptor (SUR1) subunit (Fig. 1B) (7). Kir6.2 subunits surround the central ion-conducting pore, and nucleotide inhibition results from the binding of ATP to specific regions in the cytoplasmic domains of Kir6.2. However, systematic mutagenesis of the Kir6.2 subunit has demonstrated that residues throughout the subunit can affect the ATP sensitivity of the channel allosterically (14, 18, 57, 85, 87, 94–96). Although the native K<sub>ATP</sub> channel is inhibited by micromolar ATP, ATP sensitivity can be almost completely abolished by individual point mutations. SUR1 is a member of the ATP binding cassette, or ABC, family of membrane proteins, each of which contains two classical nucleotide binding folds (NBFs) (39). Biochemical and electrophysiological experiments have demonstrated that nucleotide hydrolysis at both NBFs is involved in K<sub>ATP</sub> channel stimulation by MgADP and by potassium channel-opening drugs such as diazoxide (8, 25, 86, 97). Thus, the net determinant of physiological activity is the combined effect of ATP inhibition through Kir6.2 and the counteracting effects of ATP hydrolysis and MgADP binding in the NBFs of SUR1 (86) (Fig. 1B).

K<sub>ATP</sub> OVER- OR UNDERACTIVITY AS A CAUSAL MECHANISM OF DIABETES AND HYPERINSULINISM

Persistent hyperinsulinemia is caused by underactive K<sub>ATP</sub> channels. The relatively rare but severe disease known as nesidioblastosis, or persistent HI, results from maintained insulin secretion in the face of low blood glucose (41). Untreated, this disease causes severe hyperinsulinemia, which can lead to severe hypoglycemia and brain damage if not treated promptly. The mechanism of this disease is believed to be due to the absence or dysfunction of functional K<sub>ATP</sub> channels in pancreatic beta cells, which normally serve to terminate insulin secretion when blood glucose levels are high. In the absence of functional K<sub>ATP</sub> channels, insulin secretion continues unabated even with low blood glucose, leading to hyperinsulinemia.
vere brain damage in neonates. Very few, and rather coarse, treatments are available, namely, glucose infusion, administration of the drug diazoxide, and eventually 75–100% pancreatectomy, the latter inevitably leading to later-onset diabetes (41). Recent efforts have defined mutations of SURI and Kir6.2 subunits that are linked to HI (65, 66, 92), and most cases of HI involve defects in SURI or Kir6.2 (84). These mutations typically result in reduced or abolished channel activity, predicted to cause maintained β-cell depolarization and persistent insulin secretion. These important advances establish a clear link between KATP channel defects and the HI disease, and the different phenotypes that result from different mutations have begun to give some insight into the variability of treatment efficacies, such as the variable efficacy of the KATP channel-opening drug diazoxide (65, 66).

Diabetes can be caused by relative KATP overactivity: glucokinase mutations are causal in HI and diabetes. One form of maturity onset diabetes of the young (MODY2) is frequently associated with reduced glucokinase activity (16, 22, 29, 78, 100, 101). Glucokinase catalyzes the conversion of glucose to glucose 6-phosphate, the first reaction of glycolysis, and reduced glucokinase activity will reduce the glycolytic flux, hence lowering ATP/ADP, increasing KATP channel activity, and thereby reducing insulin secretion (68, 77). In direct contrast, some forms of HI have been shown to result from an enhanced activity of glucokinase (23). Overactive glucokinase will therefore increase glycolytic flux, providing a stronger inhibitory signal (i.e., elevated ATP/ADP) to the KATP channel, and hence increasing insulin secretion for a given glucose level.

Thus, in the case of glucokinase, it seems clear that human disease mutations that render KATP channels underactive (reduced or absent KATP channels themselves, or secondary to glucokinase overactivity) cause HI as a result of uncontrolled insulin secretion. Flipping the coin, there is also clear evidence that human disease mutations that render KATP channels overactive (secondary to glucokinase underactivity) in MODY2 cause diabetes as a result of reduced insulin secretion. The missing piece in this otherwise very simple yin-yang picture is a lack of evidence from early studies for overactive KATP mutations causing diabetes. However, as we will consider, animal studies suggest that even mildly overactive KATP channels may significantly affect insulin secretion, and from reconsideration of human patient studies there clearly emerges a probable link.

GENETIC MANIPULATION OF KATP IN MICE: MODELS OF HI AND DIABETES?

Knockout and dominant-negative models of hyperinsulinemia. Although it is quite clear that underactive KATP channels cause HI in humans, there has been variable success at generating mouse models for HI by knockout of the SURI or Kir6.2 gene (61, 83). In particular, a lack of overt HI and hypoglycemia has reduced their apparent relevance.

Miki et al. (61) first generated transgenic mice expressing a dominant-negative mutant of Kir6.2 (Kir6.2[G132S]) in β-cells under control of the insulin promoter. This mutation abolishes KATP currents, causing elevation of intracellular calcium concentration ([Ca2+]i). Neonatal transgenic mice exhibit relatively high levels of serum insulin despite hypoglycemia, thus resembling HI in humans, but they rapidly develop hyperglycemia and reduced insulin secretion. Adult mice show enhanced β-cell apoptosis and reduced number of β-cells. Both Kir6.2 and SURI genes have subsequently been knocked out by homologous recombination, and quite similar phenotypes result (60, 83). In both cases, the mice show a transient hypoglycemia as neonates, glucose-dependent insulin secretion is greatly reduced or abolished, and older animals are glucose intolerant (61, 83). Abnormally elevated insulin-to-glucose ratios were really only observed in the 1st day of life for SUR1–/– mice, and by day 5, the situation had reversed to a hyperglycemic phenotype. Certain incretins can bypass the KATP channel to induce insulin secretion more directly, and even though glucose-induced insulin secretion is greatly reduced or abolished in these knockout animals, in each case there is minimal impairment of glucose tolerance, and blood glucose is normal in young animals.

Thus these various knockout animals reiterate the expected cellular phenotypes (i.e., abolition of KATP channels and elevated [Ca2+]i) that are expected to underlie HI. However, in no case was persistent HI observed, and rapid reversal of any transient neonatal hypoglycemia resulted in a hyperglycemic or diabetic phenotype. The reasons for the lack of correlation between the mouse and human phenotypes with HI are not entirely clear. Although temporally uncorrelated, there is evidence that HI patients may cross over to a diabetic phenotype in later life, although this has generally been attributed to the near-total pancreatectomy that is acutely required to treat the neonatal symptoms (56). However, there are recent studies indicating that nonsurgically treated HI patients may become diabetic in later life (26, 40). Conceivably, β-cell death [as observed in mice expressing Kir6.2 dominant-negative Kir6.2 constructs (61)], coupled with a decreased glucose-dependent insulin release [as demonstrated in both knockout mice (60, 83) and in SUR1–/– HI patients (26)], may underlie a later-onset diabetes.

Severe diabetes in mice expressing overactive β-cell KATP channels with reduced ATP sensitivity. KATP dependence of insulin secretion could be blocked either by abolition of KATP channel activity (as we have described) or by raising channel activity to a constant, unregulated level. Mutations that make channel activity high may therefore be expected to cause a primary hypoinsulinemic diabetes. To test this prediction, we generated Kir6.2[ΔN30]-green fluorescein protein (GFP) transgenic mice (52). The transgene construct contains a deletion of 30 amino acids from the NH2 terminus and a COOH-terminal GFP tag. In cell lines, the ΔN30 deletion reduces the ATP sensitivity of the
expressed $K_{\text{ATP}}$ channel by $\sim$10-fold. The phenotype of the mice is striking and appears to dramatically confirm a critical requirement for $K_{\text{ATP}}$ closure in order for insulin secretion to occur (52). All progeny from four of the founders developed severe hyperglycemia, hypoinsulinemia, and ketoacidosis. Almost all died within the first 5 days of birth, most likely as a result of dehydration combined with ketoacidosis. We attempted back-crossing onto various mouse strains, but in all cases, neonatal lethality of the transgene was observed.

$K_{\text{ATP}}$ channels in transgenic β-cells had reduced ATP sensitivity (Fig. 1C) compared with control, and there were no morphological abnormalities at the earliest stages of the disease. Together, these results indicate that the single relevant defect is likely to be a failure of insulin release due to increased $K_{\text{ATP}}$ channel activity. Most striking about this conclusion is that the change in ATP sensitivity in isolated β-cell channels is only about fourfold. However, as illustrated in Fig. 1D, the relevant region of the ATP-sensitivity curve is at the very foot of activation (10), and in the physiological (i.e., millimolar) range of [ATP], the relative current through transgenic channels is expected to be significantly elevated.

Mild impairment of glucose tolerance in mice expressing lower levels of Kir6.2[ΔN30] transgene: a window to a late-onset model of $K_{\text{ATP}}$-induced diabetes? In marked contrast to the four severely diabetic lines we have described (52), progeny from a fifth (D-line) founder carrying the Kir6.2[ΔN30] transgene developed apparently normally, with normal blood glucose levels, and were fertile (B. Marshall, J. C. Koster, and C. G. Nichols, unpublished observations). Analysis of isolated islets from these progeny mice reveals undetectable levels of green fluorescence in most islet cells, but invariably one or a few β-cells (<2%) show an intense green fluorescence. These fluorescing cells express $K_{\text{ATP}}$ channels with ATP insensitivity in the range expected for channels including Kir6.2[ΔN30] subunits. The extent of electrical coupling between β-cells is incompletely understood (50, 64), but it is possible that expression of ATP-insensitive $K_{\text{ATP}}$ channels in only a few cells might contribute to suppression of excitability throughout the islet. Glucose-dependent insulin secretion does not seem to be significantly impaired in these mice; however, it is possible that, under some conditions, residual $K_{\text{ATP}}$ channel activity of just a few β-cells in the islet may suppress excitability. We examined the possibility that this activity might be a latent determinant of diabetes induced by diet. Paired transgenic and nontransgenic D-line littermates were fed a high-fat diet for 12 mo. Diabetogenicity of this regimen was significantly more severe in the transgenic mice than in the littermate controls as assessed by more significantly impaired glucose tolerance. Although the underlying pancreatic defect remains to be clearly established, these data are an indication that even very mild $K_{\text{ATP}}$ overactivity may actually predispose to a diabetic phenotype. Coupled with the severe consequences of about fivefold reduction of ATP sensitivity of $K_{\text{ATP}}$ channels (52), it may be expected that very subtle $K_{\text{ATP}}$ overactivity in the human pancreas may predispose to a diabetic phenotype (further discussion follows).

OTHER EXPERIMENTAL MODELS OF INHERITED DIABETES AND HI: WHAT EMERGES FROM GENETIC MODELS?

NIDDM results from effective exhaustion of the pancreatic β-cell and nonresponsiveness to elevated glucose. This end result could be caused by a whole host of factors, ensuring that no single therapeutic approach would be successful for treatment and that no model for induction would suffice to fully explain the disease process. Many transgenic mice expressing different proteins under insulin promoter control in β-cells have now been generated to examine the specific consequences of altered gene expression in β-cells. Table 1 summarizes the results of a large number of studies and illustrates some common traits that arise from manipulation of gene expression in β-cells. We can roughly group the phenotypes of the mice into four classes: 1) progressive β-cell disappearance, often with lymphocyte infiltration, and insulin-dependent diabetes mellitus; 2) HI and hypoglycemia; 3) mild phenotypes, often with normoglycemia; 4) reduced insulin secretion without, or preceding, loss of β-cells.

In many or most cases, the phenotype is readily explained by the known actions of the proteins involved. There are actually few studies in which transgene expression leads to nonspecific β-cell destruction and diabetes, and only three or four models (in class 4) that reiterate the phenotype that we observed in Kir6.2[ΔN30]-GFP transgenic mice (52). Specifically, of all the studies considered in Table 1, only homozygous glucokinase knockouts and calmodulin-overexpressing mice show profound neonatal hyperglycemia and hypoinsulinemia, with normal, or near-normal, morphology and insulin content. Two separate glucokinase knockout mouse lines both showed severe perinatal diabetes, with death occurring within 1 wk (27, 91). The study of Sakura et al. (77) demonstrated that the electrical activity of isolated β-cells from knockout animals was completely normal, with the single exception that inhibition of $K_{\text{ATP}}$ channels and consequent generation of action potentials (and hence insulin secretion) in response to elevated glucose were completely abolished. Given the similarity of the HI disease resulting from either glucokinase overactivity (23) or $K_{\text{ATP}}$ underactivity (47), the phenotypic identity between these glucokinase knockout mice (77) and our Kir6.2[ΔN30]-GFP mice (52) provides important support for the argument that the profound neonatal diabetes, with normal islet architecture and insulin content, is due to $K_{\text{ATP}}$ channel overactivity and not to nonspecific protein overproduction.

LINKAGE BETWEEN $K_{\text{ATP}}$ MUTATIONS AND TYPE II DIABETES?

Numerous control-based genetic studies in the past five years have focused on the possible association of
polymorphisms in \( K_{\text{ATP}} \) and the development of type II (NIDDM) diabetes in distinct human populations. Multiple initial linkage studies of highly polymorphic markers near the \( Kir6.2 \) and \( SUR1 \) gene loci (located 4.5 kb apart on the human chromosome 11p15.1) failed to implicate \( K_{\text{ATP}} \) as a primary diabetogene in various type II diabetic populations (42, 43, 45, 89, 103). However, given the multifactorial and complex nature of the disorder, a subordinate role in a subgroup of type II diabetic subjects or in other ethnic groups could not be precluded. More recently, numerous population-based studies have investigated the association of genetic variants within the \( Kir6.2 \) and \( SUR1 \) genes with an increased susceptibility to type II diabetes in distinct ethnic subgroups. A summary of the more common \( K_{\text{ATP}} \) variants identified and of their linkage with type II diabetes is presented in Table 2.

**SUR1 polymorphisms.** A majority of the identified polymorphisms (Table 2) map to the larger \( SUR1 \) gene, with fewer localized to the pore-forming \( Kir6.2 \). These sequence variants include numerous missense and silent mutations, an intronic nucleotide transversion, as well as an intronic nucleotide insertion. Notably, linkage disequilibrium studies of the \( SUR1 \) gene have implicated the intronic nucleotide transversion [intron 16 (\(-3t\rightarrow c\))] with an increased susceptibility to type II diabetes in various cohorts (combined Utah and United Kingdom group of Ref. 43) and in different Caucasian

<table>
<thead>
<tr>
<th>Gene(s)</th>
<th>Promoter</th>
<th>Manipulation</th>
<th>Reference Nos.</th>
<th>Phenoctype</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \beta )-Cell disruption, type I diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class I MHC</td>
<td>RIP</td>
<td>TG</td>
<td>(4, 58)</td>
<td>IDDM in males, ( \beta )-cell disappearance</td>
</tr>
<tr>
<td>Class II MHC</td>
<td>HIP</td>
<td>TG</td>
<td>(80)</td>
<td>IDDM, ( \beta )-cell disappearance</td>
</tr>
<tr>
<td>NOS2</td>
<td>IP</td>
<td>TG</td>
<td>(90)</td>
<td>IDDM, ( \beta )-cell disappearance</td>
</tr>
<tr>
<td>SV40 oncogenes</td>
<td>RIP-II</td>
<td>TG</td>
<td>(1, 32)</td>
<td>( \beta )-Cell tumors, autoimmune diabetes</td>
</tr>
<tr>
<td>IL-2</td>
<td>RIP-II</td>
<td>TG</td>
<td>(17)</td>
<td>IDDM, ( \beta )-cell disappearance, lymphocyte mediated</td>
</tr>
<tr>
<td>Islet amyloid polypeptide</td>
<td>RIP-II</td>
<td>TG</td>
<td>(11, 46)</td>
<td>Normoglycemia, elevated insulin content, IDDM, ( \beta )-cell disappearance in homozygous</td>
</tr>
<tr>
<td>( \gamma )-Interferon</td>
<td>IP</td>
<td>TG</td>
<td>(80, 81)</td>
<td>IDDM, ( \beta )-cell disappearance, lymphocyte mediated</td>
</tr>
<tr>
<td>( \alpha )-Interferon</td>
<td>TG</td>
<td>TG</td>
<td>(68)</td>
<td>IDDM, ( \beta )-cell disappearance, lymphocyte mediated</td>
</tr>
<tr>
<td>H-ras oncogene</td>
<td>IP</td>
<td>TG</td>
<td>(15)</td>
<td>IDDM in males, ( \beta )-cell disappearance</td>
</tr>
</tbody>
</table>

**Table 1. Phenotypic consequences of transgenic protein expression in mouse \( \beta \)-cells**

<table>
<thead>
<tr>
<th>Gene(s)</th>
<th>Promoter</th>
<th>Manipulation</th>
<th>Reference Nos.</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexokinase</td>
<td>IP</td>
<td>TG</td>
<td>(19)</td>
<td>Hyperinsulinemia, hypoglycemia diabetic rescue</td>
</tr>
<tr>
<td>VIP</td>
<td>IP</td>
<td>TG</td>
<td>(48)</td>
<td>Hyperinsulinemia, hypoglycemia</td>
</tr>
<tr>
<td>Placental lactogen</td>
<td>RIP-II</td>
<td>TG</td>
<td>(99)</td>
<td>Hyperinsulinemia, hypoglycemia</td>
</tr>
<tr>
<td>GLUT2</td>
<td>Lentivirus/RIP-I</td>
<td>TG</td>
<td>(30, 93)</td>
<td>Rescue of Glut2/−/−</td>
</tr>
<tr>
<td>CGRP</td>
<td>RIP</td>
<td>TG</td>
<td>(49)</td>
<td>Diabetic rescue</td>
</tr>
<tr>
<td>PTHrP</td>
<td>IP</td>
<td>TG</td>
<td>(72)</td>
<td>Hyperplasia, hyperinsulinemia, hypoglycemia, diabetic rescue</td>
</tr>
<tr>
<td>IGF-II</td>
<td>RIP-1</td>
<td>TG</td>
<td>(12)</td>
<td>Hyperinsulinemia, mild hyperglycemia, fat-fed diabetes</td>
</tr>
<tr>
<td>TNF-( \alpha )</td>
<td>IP</td>
<td>TG</td>
<td>(70, 71)</td>
<td>Hyperinsulinemia, hypoglycemia, diabetic rescue</td>
</tr>
</tbody>
</table>

**Mild phenotypes**

| \( \beta \)2 microglobulin | IP       | TG           | (5)           | Normal to mild depletion of insulin, to diabetes |
| Cholera toxin               | HIP      | TG           | (102)         | Mild hyperglycemia, hypoinsulinemia              |
| EGF, KGF                    | HIP      | TG           | (54)          | Islet proliferation                              |
| \( IL-4 \)                  | HIP      | TG           | (63)          | Rescue of autoimmune diabetes                    |
| \( IL-6 \)                  | RIP      | TG           | (9)           | Hyperplasia, inflammation, normoglycemia         |
| \( Ca/Zn SOD \)             | IP       | TG           | (55)          | Protection against alloxan diabetes              |
| IFNY                     | TG       | (28)          |               | Protection against streptozotocin diabetes        |
| \( \alpha_2 \)-Adrenoceptor | HIP      | TG           | (13)          | Normoglycemia, hyperglycemic response to \( \alpha_2 \)-agonist |
| Kir6.2 (dom-neg)            | HIP      | TG(−)        | (61)          | Normoglycemia                                   |
| Kir6.2 (dom-neg)            | RIP-II   | TG(−)        | (53)          | Normoglycemia                                   |
| Reduced insulin secretion   | RIP-I    | TG           | (52)          | Lethal neonatal diabetes that precedes \( \beta \)-cell disruption |
| Kir6.2 (ATP-insens)         | RIP-II   | TG           | (20, 21, 75)  | Lethal neonatal diabetes that precedes \( \beta \)-cell disruption |
| Calmodulin (inactive)       | RIP-II   | TG           | (74)          | Neonatal diabetes, due to reduced insulin secretion |
| \( V \)-gated K channel     | IP       | TG           | (69)          | Reduced insulin secretion                        |
| Glucokinase                 | KO       |               | (27, 73, 77)  | Lethal neonatal diabetes that precedes \( \beta \)-cell disruption |
| Glucokinase                 | AS       | Reduction    | (27, 77)      | Reduced insulin secretion                        |
| Glut2/−/−                   | KO       |               | (31)          | Neonatal diabetes, due to reduced insulin secretion |
| Glut2/−/−                   | AS       | Reduction    | (98)          | Hypoinsulinemia, hyperglycemia                    |
| Liver PFK                   | TG       |               | (51)          | Reduced insulin secretion                        |

IP, insulin promoter; RIP, rat IP; HIP, human IP; KO, knockout; Het, heterozygous; AS, antisense, TG, transgenic.

**Table 2.**
Table 2. Association of $K_{ATP}$ polymorphisms with type II diabetes

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Variants Examined</th>
<th>Reference Nos.</th>
<th>Diabetes Link</th>
</tr>
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<tbody>
<tr>
<td><strong>SUR1 polymorphisms</strong></td>
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<tr>
<td>Utah (N. European) and UKPDS</td>
<td>exon 18 T759T (ACC ACT) intron 16 (−3t c), exon 33 S1369A</td>
<td>(43)</td>
<td>exon 18 T759T intron 16 (−3t c)</td>
</tr>
<tr>
<td>French Caucasian</td>
<td>exon 18 T759T (ACC ACT) intron 16 (−3t c), exon 33 S1369A</td>
<td>(34)</td>
<td>exon 18 T759T</td>
</tr>
<tr>
<td>Japanese</td>
<td>exon 6 R275Q, exon 12 V560M exon 20 D811N, exon 21 R835C intron 16 (−3c t), exon 33 S1369A</td>
<td>(67)</td>
<td>None</td>
</tr>
<tr>
<td>Danish Caucasian</td>
<td>exon 14 D673N, intron 16 (−3c t) exon 18 T759T (ACC ACT), exon 33 S1369A</td>
<td>(36)</td>
<td>exon 18 T759T</td>
</tr>
<tr>
<td>Finnish Caucasian</td>
<td>exon 14 K649K (AAG-AAA) intron 16 (−3c t) exon 18 T759T (ACC ACT), exon 21 L829L (CTG TTG), exon 31 R1273R (AGA AGG), exon 33 S1369A</td>
<td>(76)</td>
<td>exon 18 T759T intron 16 (−3c t)</td>
</tr>
<tr>
<td>Dutch Caucasian</td>
<td>intron 16 (−3c t) exon 18 T759T (ACC ACT)</td>
<td>(38)</td>
<td>intron 16 (−3c t)</td>
</tr>
<tr>
<td>DIF, UKPDS</td>
<td>−522 bp insertion (SUR1 prom.) exon 18 T759T (ACC ACT), intron 16 (−3c t), exon 33 S1369A</td>
<td>(24)</td>
<td>None</td>
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<tr>
<td>French</td>
<td>intron 16 (−3t c)</td>
<td>(59)</td>
<td>intron 16 (−3t c)</td>
</tr>
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<td><strong>Kir6.2 polymorphism</strong></td>
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<tr>
<td>Utah (N. European) and UKPDS</td>
<td>E10K, E23K, L270V, I337V, L267L (CTC-CTG), A190A (GCT-GCC)</td>
<td>(42)</td>
<td>None</td>
</tr>
<tr>
<td>Danish Caucasian</td>
<td>E226K, L270V, I337V, L267L (CTC-CTG), A190A (GCT-GCC), K361K (AAG-AAA)</td>
<td>(35)</td>
<td>Combination of compound heterozygous E23K with heterozygous L270V</td>
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<tr>
<td>French Caucasian</td>
<td>E23K, L270V, I337V</td>
<td>(33)</td>
<td>E23K (KK homozygosity)</td>
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<tr>
<td>UKPDS, DIF</td>
<td>E23K</td>
<td>(24)</td>
<td>E23K (KK homozygous in UKPDS)</td>
</tr>
</tbody>
</table>

Amino acids in boldface identify mutations that localize to the NBFs (nucleotide binding folds) in the SUR1 subunit. UKPDS, United Kingdom Prospective Diabetes Study; DIF, Diabetes in Families Study.

(33, 59) and Japanese (67) populations. Consistent with a possible role in β-cell dysfunction, the intron 16 (−3t→c) variant has recently been shown to be associated with impaired second-phase insulin secretion during hyperglycemic clamp in both normal and impaired glucose-tolerant Dutch subjects (38). In addition, a combined at-risk genotype of the intron 16 (−3c→t) variant and the missense mutation in exon 18 T759T (ACC-ACT) is coupled with a 50% reduction in serum C-peptide and a 40% reduction in serum insulin responses upon tolbutamide injection in normoglycemic subjects (36). Alone, the exon 18 T759T silent mutation is also linked with type II diabetes as well as morbid obesity in a French Caucasian cohort (34). Although not a coding region mutation, these findings suggest that the intron 16 (−3t→c) variant, located in a splice acceptor site, is associated with a functional change in the $K_{ATP}$ channel in the β-cell, possibly through an effect on the stability or splicing of the mRNA product. Alternatively, the polymorphism could be in linkage disequilibrium with nearby sequence variants within the SUR1 gene or flanking genes that directly underlie the β-cell dysfunction. The latter explanation now appears likely, as both intron 16 (−3t→c) (34, 43, 59) and (−3c→t) (24, 36, 38, 67, 76) transversions have been shown to be significantly associated with type II diabetes in various cohorts (43). RT-PCR analysis encompassing intron 16 showed no aberrant mRNA splicing, and the genotype does not uniformly cosegregate with type II diabetes in the various study populations, inconsistent with a role as a major diabetogenic polymorphism. Thus, as with the SUR1 silent mutation at exon 18 (T759T), the increased susceptibility to type II diabetes observed with the intron 16 (−3t→c) genotype likely reflects a linkage disequilibrium with diabetogenic variant(s) located within the SUR1 gene or in flanking genes.

As we have outlined, the paradigm of glucose-induced insulin release predicts that $K_{ATP}$ overactivity (which would occur if the channel had decreased sensitivity to inhibitory ATP or an increased sensitivity to stimulatory MgADP) should lead to suppressed insulin release due to impaired glucose sensing by the β-cell. Missense mutations, which change amino acid sequence, are of particular relevance with respect to channel function. Of the six missense mutations in SUR1 identified, three (exon 20 D811N, exon 21 R835C, and exon 33 S1369A) are localized to the NBFs of SUR1 (Fig. 1B, shown in bold in Table 2). Given the critical regulatory role of the NBFs in $K_{ATP}$ channel function (8, 25, 86, 97), these mutations are likely to alter channel activity. However, neither the D811N nor the R835C mutations reportedly alter sensitivity to inhibitory ATP or activation by metabolic inhibition when expressed in transfected mammalian cells (67). Although these data do not preclude subtle changes in channel function that could underlie β-cell dysfunction, they seem to indicate that both the D811N and
R835C mutations may be in linkage disequilibrium with a genetic variant either at or near the SUR1 locus, a disequilibrium that contributes to the inherited basis of type II diabetes.

Kir6.2 polymorphisms. Mutations in the ATP-sensing Kir6.2 subunit that reduce sensitivity of the channels to inhibitory ATP are predicted to maintain the hyperpolarized membrane potential of the β-cell despite an increased [ATP/ADP] and, thereby, block the depolarization-dependent rise in $[\text{Ca}^{2+}]_i$, necessary to stimulate insulin release. This is confirmed in the Kir6.2[ΔN30] mouse model, in which overactive $\text{K}_{\text{ATP}}$ channels abolish insulin secretion, leading to an overt diabetic phenotype (52). In this case, the observation that a modest fourfold decrease in ATP sensitivity of the channels can result in such a profound diabetic phenotype raises the intriguing possibility that similarly ATP-insensitive mutants of $\text{K}_{\text{ATP}}$ may underlie diabetes in the human population.

As shown in Table 2, several linkage studies have identified both missense and silent mutations in the Kir6.2 gene. Of these the E23K, I337V, and L270V have been shown to be significantly associated with type II diabetes, either alone [E23K in both a French Caucasian population (33) and the UKPDS cohort (42)] or in combination, as was observed with the compound homozygous E23K/I337V with heterozygous L270V [Danish Caucasian population (35)]. The point mutation (E23K), originally considered a common polymorphism without association to diabetes (35, 42, 79), is of particular interest. A subsequent meta-analysis (33), combining all four studies of various Caucasian populations and an independent study of UK populations (24), reveals strong association of homozygous E23K with type II diabetes ($\chi^2 = 12.9, P < 0.00033$). Homozygous E23K (frequency in the overall population = 12%) increases the odds ratio (the estimate for the genotypic relative risk) to 2.14, thus accounting for 11% of the contribution of both environmental and genetic factors and may differ between populations. Nevertheless, there are significant unexplored possible mechanisms by which genetic mutations of $\text{K}_{\text{ATP}}$ genes or the chromosomal regions controlling $\text{K}_{\text{ATP}}$ expression may contribute causally to diabetes.

Previous functional analyses failed to show a significant effect of any one of the E23K, I337V, or L270V mutations on sensitivity of reconstituted $\text{K}_{\text{ATP}}$ channels expressed in *Xenopus* oocytes to either metabolic inhibition or to the sulfonylurea tolbutamide, suggesting that gross channel activity is unaltered (79). However, detailed analyses of single-channel properties and sensitivities to the regulatory nucleotides were not made. Recent electrophysiological measurements have now demonstrated a modest but significant effect of the Kir6.2 (E23K) mutation on channel activity with respect to both open probability (i.e., the percentage of the time the channel exists in an open state vs. a closed state) and ATP sensitivity (82). When coexpressed with SUR1, the Kir6.2 (E23K/E23K) genotype resulted in a halving of the sensitivity to inhibitory ATP and an increase of open probability (82). Because $\text{K}_{\text{ATP}}$ channels operate physiologically at the very foot of the activation curve (Fig. 1D) (10), the net effect of such a shift in sensitivity will be to increase current by about fourfold at 500 μM ATP, thereby potentially significantly shifting the physiological glucose dependence of activity to higher glucose concentrations, and to inhibit insulin secretion.

Given our findings that similarly overactive β-cell $\text{K}_{\text{ATP}}$ channels underlie profound neonatal hyperglycemia in a transgenic mouse model of diabetes (52), these data raise the intriguing possibility that the K/K genotype may contribute to impaired insulin secretion in human populations. Thus the transgenic animal studies and the electrophysiological studies of E23K imply a compelling mechanism by which inherited changes in $\text{K}_{\text{ATP}}$ activity can contribute to development of the disorder.

**PERSPECTIVES AND PROSPECTS**

Although more studies are necessary to establish a direct effect on insulin secretion, recent work suggests that mutations in $\text{K}_{\text{ATP}}$ or regulatory proteins that result in subtle increase of channel activity can cause diabetes in animals and may contribute to an increased susceptibility to type II diabetes in human populations. Relative $\text{K}_{\text{ATP}}$ channel overactivity could be generated either by changing the nucleotide responsiveness of channels, by changing the metabolic signal itself, or simply by increasing the density of otherwise normal $\text{K}_{\text{ATP}}$ channels. Only very recently have noncoding regions of these genes begun to be examined for possible diabetes association (24), and significant regions of the genes have yet to be examined. Undoubtedly, the etiology of type II diabetes is likely to involve a complex contribution of both environmental and genetic factors and may differ between populations. Nevertheless, there are significant unexplored possible mechanisms by which genetic mutations of $\text{K}_{\text{ATP}}$ genes or the chromosomal regions controlling $\text{K}_{\text{ATP}}$ expression may contribute causally to diabetes.

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