Type 2 diabetes is generally perceived as a polygenic disorder, with disease development being influenced by both hereditary and environmental factors. However, despite intensive investigations, little progress has been made in identifying the genes that impart susceptibility to the common late-onset forms of the disease. E23K, a common single nucleotide polymorphism in KIR6.2, the pore-forming subunit of pancreatic β-cell ATP-sensitive K+ (K_{ATP}) channels, significantly enhances the spontaneous open probability of these channels, and thus modulates sensitivities toward inhibitory and activatory adenine nucleotides. Based on previous association studies, we present evidence that with an estimated attributable proportion of 15% in Caucasians, E23K in KIR6.2 appears to be the most important genetic risk factor for type 2 diabetes yet identified. Diabetes 51 (Suppl. 3):S358–S362, 2002

ROLE OF K_{ATP} CHANNELS IN INSULIN SECRETION

Plasma insulin concentrations are normally determined by a feedback system that is controlled mainly by the level of plasma glucose (1). The overall activity of the pancreatic β-cell is set by the sensitivity of peripheral tissues to the action of insulin, with insulin-resistant subjects having increased plasma insulin levels and secretion rates. Insulin secretion is also elicited in response to amino and fatty acids; the extent of this response is modified by neural (e.g., autonomic tone) and hormonal (e.g., glucagon, glucagon-like peptide) factors. Glucose, however, is the dominating factor in controlling insulin secretion.

Glucose enters the β-cell by facilitated diffusion, and its phosphorylation by glucokinase to glucose-6-phosphate determines the rate of glycolysis and the rate of pyruvate generation (Fig. 1) (2,3). Thus the rate of glycolysis will increase with blood glucose. In β-cells, pyruvate is the main product of glycolysis (4) and, compared to other cell types, an unusually high proportion of glucose-derived pyruvate enters the mitochondrial tricarboxylic acid (TCA) cycle (2).

Subsequent oxidative metabolism generates the trigger for insulin secretion (5). Electron transfer from the TCA cycle to the respiratory chain by NADH and reduced flavin adenine dinucleotide (FADH2) initiates the production of ATP, which is delivered to the cytosol. Here the rise of the ATP-to-ADP ratio causes a reduction in plasma membrane K+ conductance, resulting in depolarization of the membrane (6). Hence voltage-sensitive Ca^{2+} channels are opened that are similar to those expressed in other excitable cells. This is the critical step by which glucose stimulates insulin secretion, as the increase in cytosolic Ca^{2+} is the main trigger for exocytosis (6,7).

The decrease in K^{+} conductance results from closure of the ATP-sensitive potassium (K_{ATP}) channels (6). These channels dominate the resting membrane potential in β-cells and act as transducers of glucose-induced metabolic changes into electrical activity. Their central role in the stimulation of insulin secretion can easily be demonstrated using channel modulators (e.g., tolbutamide, diazoxide) that do not interfere with glucose metabolism (8).

The significance of membrane-potential control is illustrated by the syndrome of persistent hyperinsulinemic hypoglycemia of infancy (PHHI). PHHI is most frequently caused by mutations in one of the two subunits of the K_{ATP} channel, the regulatory sulfonylurea receptor subunit 1 (SUR1) or the inwardly rectifying potassium channel subunit (Kir6.2), resulting in permanent depolarization of the β-cell and uncontrolled hypersecretion of insulin (9). However, most PHHI patients show discrete glucose-induced insulin secretion above the constitutively elevated basal rate (10). This observation argues in favor of K_{ATP} channel–independent effects of glucose, which is capable of stimulating a partial secretory response under conditions of clamped, elevated cytosolic Ca^{2+} (8). Thus closure of K_{ATP} channels by ATP generated in the mitochondria appears to be the most critical step in insulin secretion, with other metabolic factors enhancing the secretory response (11).

ROLE OF CYTOSOLIC NUCLEOTIDES IN K_{ATP} CHANNEL CONTROL

K_{ATP} channels are assembled with a tetradimeric stoichiometry from two structurally distinct subunits: Kir6.2, which forms the pore, and SUR1 (12,13). While hypoglycemic sulfonylureas (e.g., glimepiride, tolbutamide) and potassium channel openers (e.g., diazoxide) exert their effects on channel activity by interaction with SUR1, there

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E_{max}, maximal effect; f_{K}, frequency of the allele with K in position 23 of Kir6.2 in Caucasians; IC_{50}, half-maximal inhibitory concentration value; K_{ATP}, ATP-sensitive potassium channel; KIR6.2, inwardly rectifying potassium channel subunit; OR, odds ratio; PHHI, persistent hyperinsulinemic hypoglycemia of infancy; P_{op}, open probability; PPAR-γ, peroxisome proliferator–activated receptor-γ; SNP, single nucleotide polymorphism; SUR1, regulatory sulfonylurea receptor subunit 1; TCA, tricarboxylic acid.

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EFFECT OF E23K, A COMMON POLYMORPHISM IN KIR6.2, ON NUCLEOTIDE SENSITIVITY OF PANCREATIC K<sub>ATP</sub> CHANNELS

Three common missense single nucleotide polymorphisms (SNPs) have been observed in KIR6.2: E23K, L270V, and I337V (33–37). Their potential impact in type 2 diabetes led us to analyze their functional relevance (38). Whereas L270V and I337V did not have an effect on the properties of reconstituted human SUR1/K<sub>ir6.2</sub> channels, substitution of a lysine (K) for a glutamic acid (E) in position 23 (E23K) markedly affected channel gating by significantly reducing the time spent in long interburst closed states, thus producing a distinct increase in the spontaneous open probability (P<sub>o</sub>). Importantly, an in vitro model for the heterozygous genotype (E2K, with E in position 23 of KIR6.2 in one allele and K in the other) resulted in intermediate P<sub>o</sub> values. Consistent with the idea that nucleotide-induced channel inhibition results from interaction with the interburst closed states (39,40), E23K decreased sensitivity toward inhibitory ATP<sup>−</sup> in the models for the heterozygous and homozygous genotypes (K/K, with K in position 23 of K<sub>ir</sub>6.2 in both alleles). Stimulation of insulin secretion requires reduction of the P<sub>o</sub> of pancreatic β-cell K<sub>ATP</sub> channels to values <0.02 (25,41). Both the increased spontaneous P<sub>o</sub> values and the reduced ATP sensitivity contributed to a rightward shift of the corresponding ATP concentration (IC<sub>R2</sub> value) through E23K (38).

These experiments demonstrated that E23K is functionally relevant in significantly modulating spontaneous P<sub>o</sub> and ATP sensitivity (38). However, they did not show how channel properties are affected in intact cells, as here additional factors other than the cytosolic concentration of ATP are involved in channel control. Because MgADP appears particularly important among these factors, channel properties were analyzed in the simultaneous presence of activatory and inhibitory nucleotides (30). These experiments indicated that besides reducing sensitivity toward inhibitory ATP, E23K in KIR6.2 increases the sensitivity of pancreatic β-cell K<sub>ATP</sub> channels for activation through nucleoside diphosphates (30).

E23K AND TYPE 2 DIABETES

The role of E23K in type 2 diabetes has been assessed in five population-based studies of Caucasians (33–37). Whereas the earlier studies with modest sample sizes failed to detect the predisposition (33–35), a fourth study (36) that included the results of the first three studies in a meta-analysis showed a clear association of the homozygous state (K in both alleles). This finding was confirmed in a recent analysis (37), yielding an overall P value for the association of 4 × 10<sup>−6</sup> (38). The functional significance of E23K was much weaker in an in vitro model for the heterozygous genotype (38), suggesting that in this state, predisposition to type 2 diabetes should be more discrete. Although tending toward an increased risk (33–37), the overall P for association with the heterozygous state consistently remained insignificant (P > 0.05).

Hence, the clear functional relevance and the association of homozygous E23K with type 2 diabetes suggest that by critically affecting the nucleotide sensitivity of K<sub>ATP</sub> channels in pancreatic β-cells, this polymorphism induces...
a discrete inhibition of insulin secretion, thereby predisposing to the disease (38). This model is consistent with the postulated critical role of an inborn secretory defect in the genesis of type 2 diabetes (42).

The conclusion that E23K predisposes to type 2 diabetes by inducing overactivity of pancreatic β-cell K<sub>ATP</sub> channel activity and thereby inhibiting insulin secretion is strongly supported by a recent study in transgenic mice (43). Those authors demonstrated that discrete targeted reduction of the channels’ ATP sensitivity in pancreatic β-cells is sufficient to induce severe neonatal diabetes in mice. The model is also consistent with a study in healthy young adults indicating that both the homozygous and the heterozygous state were associated with either direct or indirect evidence for reduced glucose-induced insulin secretion (35). However, because KIR6.2 expression is not restricted to pancreatic β-cells (12,13), other effects (e.g., altered glucose sensing in the brain) might still contribute to predisposition.

We then estimated the relative attributable risk of E23K in Caucasians by calculating the odds ratio (OR). In published studies (33–37), ORs for the homozygous genotype ranged from 1.8 to 2.7 (Fig. 2A), yielding a weighted average of 2.11 (P < 4 × 10<sup>−6</sup>). Values for the heterozygous genotype varied from 0.79 to 1.9 (Fig. 2B), with a mean of 1.12 for the combination of all studies (P > 0.05). Allelic frequencies of E23K were very similar in the populations screened, ranging from 30 to 38% (Fig. 2C), with a weighted average of 34%. Consistent with Hardy-Weinberg equilibrium, genotypic frequencies averaged at 42 (E/E, homozygous genotype with E in position 23 of KIR6.2 in both alleles), 47 (E/K), and 11% (K/K).

Risk and frequency estimates were used to calculate the proportion of type 2 diabetic patients that would not develop the disease if all subjects had the lower-risk E/E genotype. Taking the calculated average values of 2.11 for the K/K and 1.12 for the E/K genotype, we found that in Caucasians, 10 and 5% of disease cases were attributable to occurrence of these genotypes, respectively. Thus the combination of all published data suggests that in this ethnic group, 15% of type 2 diabetic cases might be attributable to K<sub>ATP</sub> channel overactivity induced by the E23K allele of KIR6.2.

**FIG. 2.** Estimated risk (OR) and allelic frequency (f<sub>K</sub>) for E23K in KIR6.2 (with 95% confidence intervals). UK1, UK2, UK3, DK, and Fr refer to the data from previous case-control studies among Caucasian groups (33, 34, 37, 35, 36, respectively). “All” refers to the pooled data, with the dashed lines indicating the 95% confidence interval for these data. A and B: Odds ratios for the homozygous (K/K) and heterozygous (E/K) genotypes, respectively. C: Frequency of the KIR6.2 allele coding for K instead of E in position 23 based on allele counts in the control groups (f<sub>K</sub>).

**FIG. 3.** Potency of tolbutamide in inhibiting human SUR1/KIR6.2<sub>wt</sub> or SUR1/KIR6.2<sub>E23K</sub> channels. Channels were reconstituted in COS-1 cells by co-expression of human SUR1 with human KIR6.2<sub>wt</sub> or KIR6.2<sub>E23K</sub> as indicated. Representative currents recorded at −50 mV from inside-out patches exposed to nucleotides and tolbutamide, as shown by the lines above the records. ADP (0.3 mmol/l) was added to enhance maximal tolbutamide-induced inhibition (55). The IC<sub>50</sub> values were 3.0 μmol/l (wild-type) and 5.3 μmol/l (E23K). For further methodological details, see refs. 38 or 55.

**POTENTIAL ROLE OF E23K IN EVOLUTION**

The high allelic prevalence of E23K (f<sub>K</sub>) in Caucasians with similar values in all populations screened (Fig. 2C) suggests that E23K represents a balanced polymorphism that confers selectionary advantage through fine-tuning of insulin secretion in heterozygotes (38). By reducing glucose uptake in muscle and fat, discrete inhibition of release might result in favorable substrate supply for tissues with insulin-independent uptake; hence, high frequency of the E/K state might have evolved as an adaptation to the human brain. Importantly, this model implies increased susceptibility to type 2 diabetes as the inherent price for the evolutionary benefit of the heterozygous state, and thus E23K provides evidence in support of the “thrifty genotype” hypothesis (44). However, diverging from this...
concept, predisposition might have evolved as a response to altered tissue demands rather than to periodic famine (38).

Recently, we argued that tetrameric K\textsubscript{ATP} channel stoichiometry should confer protection against diabetic dysregulation resulting from heterozygous mutations in K\textsubscript{IR}6.2 (45). The lower OR for the E/K state supports this idea (Fig. 2A and B). E23K, however, sheds additional light on the impact of channel stoichiometry. While on the one hand this architecture protects against diabetes, on the other hand it imposes a hurdle if evolution seeks to reduce ATP sensitivity. This is due to the dominance of the K\textsubscript{IR} isofrom with higher affinity (45). Hence, gaining selectionary benefit requires mutational changes to be strong enough to significantly decrease sensitivity in the heterozygous state, which would imply potentiated reduction in homozygotes to be likely to confer disadvantage. Specifically, an allelic frequency of E23K \((f_{E}) < 50\%\) supports this notion, suggesting a selectionary drawback of K/K versus E/E and thus that E/K is advantageous over both homozygous states. Because the latter is the basis for evolutionary persistence of a functionally relevant SNP, E23K might be the consequence of pressure toward a reduction of insulin secretion combined with multimeric channel structure.

High frequency of E23K in Caucasians points toward a high age of the variant (i.e., \(-10^{5}\) years) (46), suggesting that analysis in other ethnic groups will unveil worldwide occurrence. Importantly, a similar allelic frequency (34.3\%) has been reported in the Japanese population (47).

### POLYGENIC BASIS OF TYPE 2 DIABETES

Type 2 diabetes is generally perceived as a polygenic disorder, with disease development being influenced by both hereditary and environmental factors (1,11,42). Genes encoding for key components of insulin secretion and glucose metabolism pathways have been widely considered as targets for defects in type 2 diabetes, and many associations have been reported (49). Besides E23K, three other associations have been confirmed in independent samples: a genetic variation in Calpain 10 (50), a silent SNP in codon 759 of SUR1 (51–53), and a common polymorphism (P12A) in peroxisome proliferator–activated receptor-\(\gamma\) (PPAR-\(\gamma\)) (49,54). Whereas Calpain 10 and the silent SNP in codon 759 of SUR1 were associated with higher risk for type 2 diabetes (50–53), P12A in PPAR-\(\gamma\) was reported to be protective (49,54). Estimates of the weighted attributable proportions in Caucasians are 4, 5, and \(-6.8\%\), respectively. Thus, with an estimated attributable proportion of 15\%, E23K in K\textsubscript{IR}6.2 appears to be the most important genetic risk factor yet identified.

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