*Brief Genetics Report*

# $K_{IR}6.2$ Polymorphism Predisposes to Type 2 Diabetes by Inducing Overactivity of Pancreatic β-Cell ATP-Sensitive $K^+$ Channels

**Christina Schwanstecher, Ulrike Meyer, and Mathias Schwanstecher**

**E23K, a common single nucleotide polymorphism in $K_{IR}6.2$, the pore-forming subunit of pancreatic β-cell ATP-sensitive $K^+$ channels, significantly enhanced open probability of these channels, thus reducing their sensitivity toward inhibitory $ATP^{4-}$ and increasing the threshold concentration for insulin release. Previous association studies and high allelic frequency suggest this effect to critically inhibit secretion and play a major role in pathogenesis of common type 2 diabetes. Based on evidence for functional relevance of E23K in both the heterozygous (E/K; with E in position 23 of $K_{IR}6.2$ in one allele and K in the other) and homozygous (K/K; with K in position 23 of $K_{IR}6.2$ in both alleles) genotype, we propose a model in which enhanced susceptibility to type 2 diabetes is associated with evolutionary advantage of the E/K state. *Diabetes* 51:875–879, 2002**

Type 2 diabetes is generally perceived as a polygenic disorder, with disease development being influenced by both hereditary and environmental factors (1). Genes encoding for key components of insulin secretion and glucose metabolism pathways have been widely considered as targets for defects in type 2 diabetes. 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 (2).

In pancreatic β-cells, ATP-sensitive $K^+$ ($K_{ATP}$) channels critically control insulin secretion by coupling metabolism to electrical activity (3). Recent advances resulted in cloning of these channels and elucidation of their subunit composition (4). The β-cell channels are assembled, with tetradimeric stoichiometry, from two structurally distinct subunits: an inwardly rectifying $K^+$ channel subunit ($K_{IR}6.2$), forming the pore, and the regulatory sulfonylurea receptor subunit-1 (SUR1). While hypoglycemic sulfonylureas (e.g., glibenclamide) exert their effects on channel activity by interaction with SUR1, there is strong evidence that the receptor site for inhibitory $ATP^{4-}$ is formed by $K_{IR}6.2$.

Three common missense single nucleotide polymorphisms (SNPs) have been observed in $K_{IR}6.2$ (E23K, L270V, and I337V) (5–9), and their potential impact in type 2 diabetes led us to analyze their functional relevance. Whereas L270V and I337V were without effect on the properties of reconstituted human SUR1/$K_{IR}6.2$ channels (including expression rate, single channel conductance, spontaneous open probability [$P_O$], and nucleotide and drug sensitivities [results not shown]), E23K markedly affected channel gating, significantly reducing the time spent in long interburst closed states (17 ± 3% for SUR1/the mutant isoform of $K_{IR}6.2$ with K instead of E in position 23 [$K_{IR}6.2_{E23K}$] vs. 54 ± 6% for wild-type channels, $n = 10$ each, $P < 0.001$) (Fig. 1*A* and *B*), thus producing a 1.6-fold increase of $P_O$ ($P_O = 0.66 \pm 0.05$ for SUR1/$K_{IR}6.2_{E23K}$ vs. 0.41 ± 0.04 for wild-type channels, $n = 10$ each, $P < 0.001$). The increase of $P_O$ was confirmed by noise analysis (patches with 100–500 channels; $P_O = 0.85 \pm 0.04$ for SUR1/$K_{IR}6.2_{E23K}$ vs. 0.58 ± 0.03 for wild-type channels, $n = 10$ each, $P < 0.001$) and determination of the maximal increment of the spontaneous patch current ($\Delta I_{max}$; $P_O = 0.77 \pm 0.04$ for SUR1/$K_{IR}6.2_{E23K}$ vs. 0.52 ± 0.02 for wild-type channels, $n = 10$ each, $P < 0.001$) (Fig. 1*C*). Importantly, a model for the heterozygous genotype (E/K, with E in position 23 of $K_{IR}6.2$ in one allele and K in the other; coexpression of $K_{IR}6.2_{E23K}$ and the wild-type isoform of $K_{IR}6.2$ [$K_{IR}6.2_{wt}$], with a cDNA ratio of 1:1) (Fig. 1*C*) resulted in intermediate $P_O$ values (0.67 ± 0.02 and 0.61 ± 0.02 from noise analysis and measurement of $\Delta I_{max}$, respectively; $n = 10$; $P < 0.01$ for comparison with pure wild-type channels each).

Consistent with the idea that nucleotide-induced channel inhibition results from interaction with the interburst closed states (10,11), E23K decreased sensitivity toward inhibitory $ATP^{4-}$ in the models for the heterozygous (E/K) and homozygous (K/K; with K in position 23 of $K_{IR}6.2$ in both alleles) genotype by 1.4- and 2.2-fold, respectively ($IC_{50} = 12.4 \pm 0.3$ μmol/l for coexpression of $K_{IR}6.2_{E23K}$ with $K_{IR}6.2_{wt}$ and 19.7 ± 0.5 μmol/l for pure SUR1/

From the Institute of Pharmacology and Toxicology, University of Braunschweig, Braunschweig, Germany.

Address correspondence and reprint requests to Dr. M. Schwanstecher, Institut für Pharmakologie und Toxikologie, Universität Braunschweig, Mendelssohnstraβe 1, 38106 Braunschweig, Germany. E-mail: m.schwanstecher@tu-bs.de.

Received for publication 18 December 2000 and accepted in revised form 29 November 2001.

DMEM-HG, Dulbecco's modified Eagle's medium–high glucose; $IC_{R2}$, ATP concentration that suppresses spontaneous open probability to the threshold for insulin secretion (0.02); $\Delta I_{max}$, maximal increment of the spontaneous patch current; $K_{ATP}$, ATP-sensitive $K^+$ channel; $K_{IR}6.2_{E23K}$, mutant isoform of $K_{IR}6.2$, with K instead of E in position 23; $K_{IR}6.2_{wt}$, wild-type isoform of $K_{IR}6.2$; $P_O$, spontaneous open probability; SNP, single nucleotide polymorphism; SUR1, regulatory sulfonylurea receptor subunit-1.

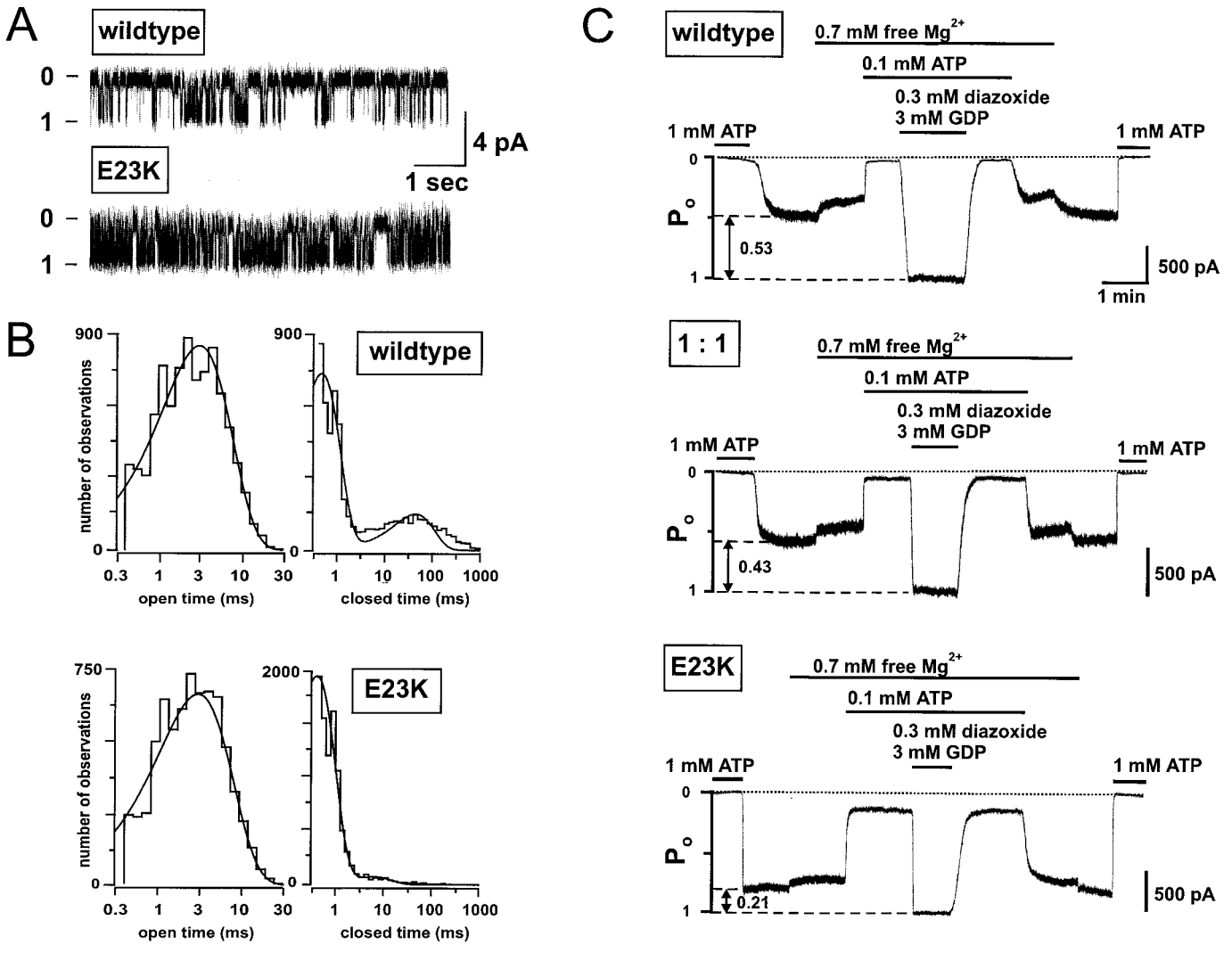


**FIG. 1. E23K induces overactivity of SUR1/$K_{IR}$6.2 channels by reducing the time spent in interburst closed states. *A*: Representative single-channel currents recorded from inside-out patches of COS-1 cells transiently coexpressing human SUR1 with wild-type ($K_{IR}6.2_{wt}$) or mutant $K_{IR}6.2$ ($K_{IR}6.2_{E23K}$) (absence of nucleotides). 1 and 0 indicate the open and closed channel levels, respectively. *B*: Open and closed time distributions from the records shown in part A. Whereas open time distributions did not differ significantly ($T_O$ = 3.1 or 3.0 ms for wild-type and mutant channels, respectively), E23K induced a loss of the longer closed events (wild-type channels: $T_{C1}$ = 0.47 ms, $A_1$ = 83%, $T_{C2}$ = 45 ms, and $A_2$ = 17%; mutant channels: $T_{C1}$ = 0.40 ms, $A_1$ = 97%, $T_{C2}$ = 6.7 ms, and $A_2$ = 3%), where $T_O$ is the time constant of the open state; $T_{C1}$, $T_{C2}$, $A_1$, and $A_2$ are time constants or relative fractions of short (intraburst) or long (interburst) closed states, respectively. *C*: E23K reduces the maximal increment of the patch current ($\Delta I_{max}$, see RESEARCH DESIGN AND METHODS). Representative traces recorded from inside-out patches of COS-1 cells transiently coexpressing human SUR1 with $K_{IR}6.2_{wt}$, $K_{IR}6.2_{E23K}$, or $K_{IR}6.2_{wt}$ plus $K_{IR}6.2_{E23K}$ (cDNA ratio of 1:1) as indicated. Patches with 100–500 channels were exposed to nucleotides, $Mg^{2+}$, and diazoxide as shown above the records. Dashed lines indicate spontaneous and maximal channel activity, with maximal activity ($P_O$ = 1) being defined by the simultaneous addition of 0.3 mmol/l diazoxide and 3 mmol/l GDP. The distance between these lines corresponds to $\Delta I_{max}$ (double-headed arrow). In these single experiments, calculation of spontaneous $P_O$ from $\Delta I_{max}$ (see RESEARCH DESIGN AND METHODS) gave the following values: 0.47 (wild type), 0.57 (1:1 ratio), and 0.79 (E23K).**

$K_{IR}6.2_{E23K}$ channels vs. 8.9 ± 0.2 μmol/l for pure wild-type channels; $n$ = 15 each; $P < 0.01$ or $<0.001$ for comparison with wild-type channels, respectively) (Fig. 2*A* and *B*).

Stimulation of insulin secretion requires reduction of the $P_O$ of pancreatic β-cell $K_{ATP}$ channels to values <0.02 (12). Both increased spontaneous $P_O$ values (Fig. 1), and reduced ATP sensitivity (Fig. 2*A*) contributed to a rightward shift of the corresponding ATP concentration ($IC_{R2}$, defined as the ATP concentration that suppresses $P_O$ to the threshold for insulin secretion [0.02]) through E23K (Fig. 2*B*). This shift amounted to 1.5- or 3.1-fold in the models for the E/K and K/K state, respectively ($IC_{R2}$ = 154 ± 6 μmol/l for coexpression of $K_{IR}6.2_{E23K}$ with $K_{IR}6.2_{wt}$ and 303 ± 11 μmol/l for pure SUR1/$K_{IR}6.2_{E23K}$ channels vs. 98 ± 4 μmol/l for pure wild-type channels, $n$ = 15 and $P < 0.001$ for comparison with wild-type channels each). The effect of E23K on the $IC_{R2}$ value was not altered by additional introduction of L270V and/or I337V (Fig. 2*C*).

All results obtained with the human isoforms were confirmed in channels reconstituted from mouse $K_{IR}6.2$ plus hamster SUR1 (results not shown). Effects of E23K on $P_O$ and ATP sensitivity were consistent with previous reports on the role of the $K_{IR}6.2$ $NH_2$ terminus (10,11,13).

Results indicate that E23K in $K_{IR}6.2$ significantly affects properties of pancreatic β-cell $K_{ATP}$ channels, increasing spontaneous $P_O$ and reducing ATP sensitivity in models for the heterozygous (E/K) and homozygous (K/K) state (Figs. 1 and 2). Both effects contribute to a rightward shift of the ATP concentration required to suppress channel

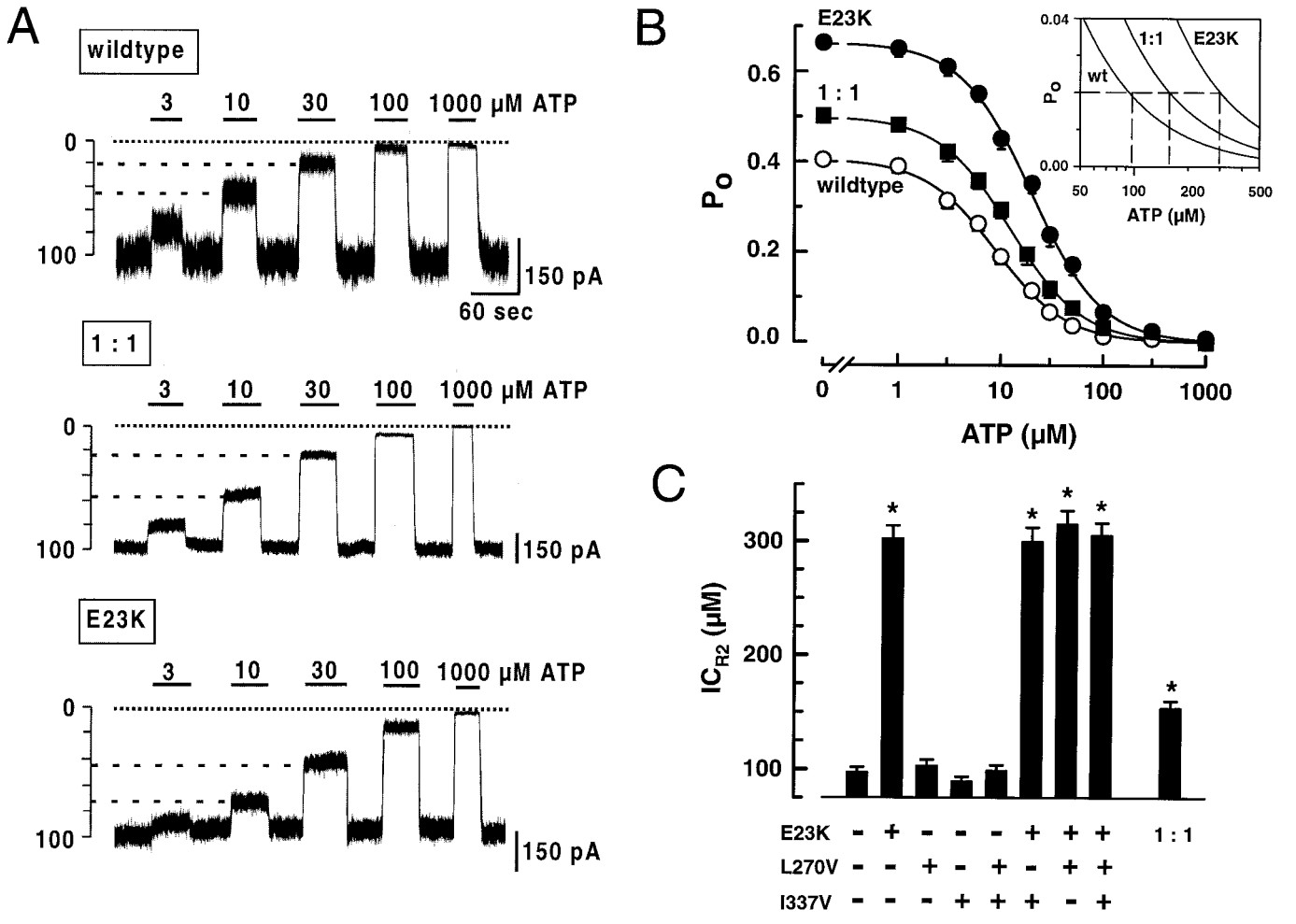


**FIG. 2. E23K increases the ATP concentration required to suppress $P_O$ to 0.02. *A*: ATP sensitivity of wild-type or mutant SUR1/$K_{IR}6.2$ channels transiently expressed in COS-1 cells (1:1, cDNA ratio of $K_{IR}6.2_{wt}$ and $K_{IR}6.2_{E23K}$; see also Fig. 1*C*). Representative currents were recorded from inside-out patches exposed to $ATP^{4-}$ as shown above the records. Dashed lines indicate residual channel activity (%) in the presence of 10 or 30 μmol/l $ATP^{4-}$. $IC_{50}$ values in these registrations were 7.9 μmol/l (wild type), 11.7 μmol/l (1:1 ratio), and 24.6 μmol/l (E23K). *B*: Concentration-inhibition curves from records as shown in *A*. Results were normalized to spontaneous $P_O$ values determined in single-channel records ($K_{IR}6.2_{wt}$ = 0.41, $K_{IR}6.2_{E23K}$ = 0.66; see text). The equivalent value for the 1:1 coexpression (0.50) was approximated as described under RESEARCH DESIGN AND METHODS. Data are shown as means ± SE (if larger than symbol size) of $n$ = 15 independent experiments. $IC_{50}$ values for ATP and Hill coefficients were: 8.9 ± 0.2 μmol/l, 1.26 ($K_{IR}6.2_{wt}$, ○); 12.4 ± 0.3 μmol/l, 1.25 ($K_{IR}6.2_{wt}$ + $K_{IR}6.2_{E23K}$, cDNA ratio of 1:1, ■); and 19.7 ± 0.5 μmol/l, 1.28 ($K_{IR}6.2_{E23K}$, ●). The inset shows a magnified view of the $y$-axis for $P_O$ ≤0.04. The horizontal line represents $P_O$ = 0.02. Intersections with the concentration-inhibition curves indicate the ATP concentration ($IC_{R2}$) at which residual activity reaches 2% of maximal channel activity. $IC_{R2}$ values were 98 ± 4 μmol/l ($K_{IR}6.2_{wt}$), 154 ± 6 μmol/l ($K_{IR}6.2_{wt}$ + $K_{IR}6.2_{E23K}$, cDNA ratio of 1:1), and 303 ± 11 μmol/l ($K_{IR}6.2_{E23K}$). *C*: The effect of E23K on $IC_{R2}$ values was not altered by additional introduction of L270V and/or I337V into human $K_{IR}6.2$. $IC_{R2}$ values for ATP were calculated based on determination of $P_O$ in single-channel registrations (see Fig. 1*A*) and concentration-inhibition curves as shown in *A* and *B* (1:1 coexpression of $K_{IR}6.2_{wt}$ and $K_{IR}6.2_{E23K;}$ see *A* and *B*). *$P$ < 0.01 for comparison with the wild type ($n$ = 8–16).**

activity to the threshold for insulin secretion ($IC_{R2}$) (Fig. 2*B*), implying that E23K might induce a critical inhibition of release.

This idea is strongly supported by recent reports that demonstrate that lowering ATP sensitivity by no more than 3.6-fold induces severe neonatal diabetes in transgenic mice (3), and that reveal clear association of homozygous E23K (K/K) with type 2 diabetes in Caucasians (overall $\chi^2$ = 21.3 with 1 df, $P$ < 0.000004) (8,9). Particularly, the latter studies suggest that because of its high allelic prevalence (weighted averaged estimate in Caucasians = 34%) (5–9), E23K is of considerable importance in the pathogenesis of common type 2 diabetes.

Additional evidence in favor of E23K derives from reinterpretation of published association studies (5–8,14–16), as follows:

- Partial absence of coupling with E23K led to a lack of detectable association of homozygous I337V with type 2 diabetes (8), arguing against localization of the disease locus downstream (i.e., in the 3′ direction) codon 337 of the $K_{IR}6.2$ gene.
- Besides codon 270, there is no additional common missense SNP within the $K_{IR}6.2$ gene (5–8), excluding $K_{IR}6.2$ as potential site for an alternative causative variant.
- At 7.5 kb from the 5′ end of the $K_{IR}6.2$ gene, a common missense SNP in codon 1369 of the SUR1 gene was found to be in linkage disequilibrium with E23K in $K_{IR}6.2$ (>77%) (6). In another population, however, linkage could not be confirmed (8,16), and thus, similar to the conclusion drawn above, localization of the causative variant upstream (i.e., in the 5′ direction) codon 1369 in the SUR1 gene seems unlikely.

- Regions coding for the COOH terminus of SUR1 (exons 33–39) did not enclose any further common SNPs with potential functional relevance (14,16); hence, so far the only alternative candidate chromosomal section left would be that in-between the genes for SUR1 and $K_{IR}6.2$, encompassing the promoter region of $K_{IR}6.2$ (17). Modulation of $K_{IR}6.2$ expression, however, is presumably not involved because isolated targeted overexpression of $K_{IR}6.2$ does not lead to enhanced formation of functional channels (3).

We conclude that E23K promotes development of type 2 diabetes by increasing the threshold ATP concentration, thus inducing overactivity of pancreatic β-cell $K_{ATP}$ channels and inhibiting insulin secretion. This model is consistent with the postulated critical role of an inborn secretory defect in the genesis of type 2 diabetes (1).

The conclusion that E23K may induce a discrete inhibition of insulin secretion is supported by a study in healthy young adults (7). Here, E23K was associated with either direct (slightly reduced glucose-stimulated release) or indirect (elevated insulin sensitivity or glucose effectiveness) (examples in 18,19) evidence for decreased secretion. However, because most of these effects did not reach statistical significance, definite proof of reduced secretion will require additional, more detailed clinical studies.

High allelic frequency of E23K in Caucasians (see above) with similar values in all populations screened (5–9) suggests that E23K represents a balanced polymorphism, conferring selectionary advantage through fine-tuning of insulin secretion in heterozygotes. By reducing glucose uptake in muscle and fat, discrete inhibition of release might result in favorable substrate supply for tissues with insulin-independent uptake, and hence high frequency of the E/K state (~45%) (5–9) might have evolved as an adaptation to the human brain. Notably, this model implies increased susceptibility to type 2 diabetes as an inherent price for the evolutionary benefit of the heterozygous state, and thus E23K provides evidence in support of the "thrifty genotype" hypothesis (20). However, diverging from this concept, predisposition might have evolved as a response to altered tissue demands rather than periodic famine.

Early studies (5–7) were unable to reveal the impact of E23K because they were not designed to detect a trait with a relative risk as low as 2, based on small quantitative changes of channel properties. Specifically, the number of probands in initial association studies (5–7) were too low to reach statistical significance, and functional analysis was restricted to assessment of end points of metabolic channel control (5). The latter presumably prompted failure to detect relevance of the polymorphism because E23K affects neither maximal nucleoside diphosphate–induced channel activation (i.e., metabolic inhibition through 3 mmol/l azide [5]) nor maximal ATP-mediated closure (i.e., millimoles per liter of resting ATP levels [5]) (Fig. 1*C*).

A recent family-based study failed to replicate association between E23K and type 2 diabetes (21). However, this contradictory result is presumably explained by weak predisposition through the heterozygous state plus specific selection of probands with type 2 diabetes. The study did not focus on the typical type 2 diabetic patient but was mainly grounded on younger probands with prediabetic irregularities of glucose homeostasis. Yet here, discrete inhibition of insulin release is expected to be balanced by compensatory mechanisms (e.g., increased insulin sensitivity or glucose effectiveness; see above).

The present report is the first to identify the subtle variation of $K_{ATP}$ channel function that is induced by E23K in $K_{IR}6.2$. We infer that this functionally relevant SNP plays an important role in the pathogenesis of common type 2 diabetes and propose a model in which predisposition results as an implicit price for advantage associated with the heterozygous state.

## RESEARCH DESIGN AND METHODS

**Materials.** All chemicals were obtained from the sources described elsewhere (22).

**Molecular biology.** Point mutations were introduced into human (GenBank Q14654) or mouse (GenBank D50581) $K_{IR}6.2$ by standard molecular biology techniques, and constructs were sequenced to verify PCR fidelity before transfection. For analysis of combinations of the polymorphisms (Fig. 2*C*), mutations were sequentially introduced into the same $K_{IR}6.2$ cDNA.

**Electrophysiology.** Transfections were performed as described (22). COS-1 cells were cultured in Dulbecco's modified Eagle's medium–high glucose (DMEM-HG) (10 mmol/l glucose) supplemented with 10% FCS, plated at a density of $8 \times 10^4$ cells/dish (35 mm), and allowed to attach overnight. For analysis of single-channel kinetics (Fig. 1*A* and *B*), 2 μg of pECE-human SUR1 (GenBank NP_000343) complementary DNA and 2 μg of pECE-human $K_{IR}6.2$ complementary DNA were mixed and used to transfect six 35-mm plates. Amounts of complementary DNA used in transfections for patches with >100 channels (Figs. 1*C* and 2) were 10-fold higher (20 μg each) than those for measurement of single-channel kinetics. In controls we used pECE-hamster SUR1 (GenBank A56248) and pECE-mouse $K_{IR}6.2$ instead of the human isoforms. For transfection the cells were incubated for 4 h in a Tris-buffered salt solution containing DNA (5–10 μg/ml) plus DEAE-dextran (1 mg/ml), 2 min in HEPES-buffered salt solution plus DMSO (10%), and 4 h in DMEM-HG plus chloroquine (100 μmol/l). Cells were then returned to DMEM-HG plus 10% FCS. Experiments in the inside-out configuration of the patch-clamp technique were performed 1–2 days after transfection at room temperature. Membrane patches were clamped at −50 mV. The intracellular bath solution contained (in mmol/l): 140 KCl, 2 $CaCl_2$, 10 EDTA, and 5 HEPES (pH 7.15). The pipette solution contained (in mmol/l): 140 KCl, 2.6 $CaCl_2$, 1.2 $MgCl_2$, and 10 HEPES (pH 7.4).

Open and closed time distributions in single-channel registrations (Fig. 1*B*) were analyzed using pCLAMP Software version 6.0 (Axon Instruments, Union City, CA). $T_O$ is the time constant of the open state; $T_{C1}$, $T_{C2}$, $A_1$, and $A_2$ are time constants or relative fractions of short (intraburst) or long (interburst) closed states, respectively. The fraction of time spent in long interburst closed states ($P_{C2}$) was calculated with $P_{C2} = (T_{C2} \times A_2)/(T_{C1} \times A_1 + T_{C2} \times A_2) \times (1 - P_O)$, where $P_O$ is the mean $P_O$ (in the absence of drugs and nucleotides) calculated from single-channel current registrations (see below).

For calculation of $P_O$ from single-channel currents, only records without superimposed openings were accepted. $P_O$ was calculated by dividing the total time the channel spent in the open state by the sample time (30 s). For assessment of $P_O$ by noise analysis (10) or determination of the maximal current increment ($\Delta I_{max}$) (Fig. 1*C*), patches with multiple channels were used. Density of $K_{ATP}$ channels per patch ranged from 100 to 500. Estimates of $P_O$ were not affected by this variation. In coexpression experiments ($K_{IR}6.2_{wt}$ plus $K_{IR}6.2_{E23K}$ with a cDNA ratio of 1:1) (Fig. 1*C*), patches with >150 channels were chosen to attain an acceptable frequency of each channel species (22). $P_O$ was calculated with $P_O = 1 - [\sigma^2/(i \times I)]$ or $P_O = I/(I + \Delta I_{max})$, respectively, where $I$ is the mean spontaneous patch current (in the absence of drugs and nucleotides), $\sigma^2$ is the variance, $i$ is the single channel current, and $\Delta I_{max}$ is the increment of the patch current induced by the simultaneous addition of 0.3 mmol/l diazoxide plus 3 mmol/l GDP (Fig. 1*C*).

For analysis of ATP sensitivity (Fig. 2), patches were chosen with little "run-down" over the measuring period, and effects were corrected for this loss of channel activity by use of linear interpolation. Artifacts caused by incomplete wash-out or slow reversibility were excluded by making sure that experiments with stepwise decrease of the $ATP^{4-}$ concentration yielded $EC_{50}$ values and slope factors identical to those from experiments with stepwise increases of the concentration. Variation of channel densities (see above) did not affect percentage of sensitive channels, $EC_{50}$ values, or Hill coefficients

(Fig. 2*B*). Curves shown in Fig. 2*B* were normalized to $P_O$ values calculated from single-channel registrations because we felt these estimates to be more accurate than determinations by noise analysis or measurement of $\Delta I_{max}$ (see above). The equivalent value for the 1:1 coexpression, $P_{O\text{-}S(1:1)}$, was approximated from the values obtained in patches with multiple channels as follows: $P_{O\text{-}S(1:1)} = P_{O\text{-}S(wt)} + [(P_{O\text{-}S(E23K)} - P_{O\text{-}S(wt)}) \times (P_{O\text{-}M(1:1)} - P_{O\text{-}M(wt)})/(P_{O\text{-}M(E23K)} - P_{O\text{-}M(wt)})]$, where $P_{O\text{-}S(wt)}$ and $P_{O\text{-}S(E23K)}$ are the $P_O$ estimates from single-channel registrations for pure wild-type (0.41) and E23K (0.66) channels, respectively, and $P_{O\text{-}M(wt)}$, $P_{O\text{-}M(1:1)}$, and $P_{O\text{-}M(E23K)}$ are the arithmetic means of corresponding values from noise analysis and determination of $\Delta I_{max}$ (0.55, 0.64, and 0.81, respectively; see text).

**Data.** Data analysis and statistics were performed as described (22). Results are shown as records from representative single experiments (Figs. 1 and 2*A*) or as the means ± SE ($n$ = 8–16). $IC_{R2}$ values were estimated by fitting the function $P_O = 1/[1+([ATP]/IC_{50})^n]$ to the data of each single experiment, where $IC_{50}$ is the half-maximally inhibitory ATP-concentration and $n$ is the Hill coefficient. $P$ values were calculated by the Mann-Whitney $U$ test with correction for multiple comparisons or $\chi^2$ statistics based on data from Table 2 in ref. 8 and Table 4 in ref. 9.


## ACKNOWLEDGMENTS

This work was supported by grants from the Deutsche Forschungsgemeinschaft (to M.S. and C.S.).

We are grateful to Drs. Lydia Aguilar-Bryan and Joseph Bryan (Baylor College of Medicine, Houston, TX) for providing us with the human isoforms of SUR1 and $K_{IR}6.2$ and to Drs. Heinz dal Ri (University of Göttingen, Göttingen, Germany) and Colin Nichols (Washington University School of Medicine, St. Louis, MO) for helpful discussion. We thank Haide Fürstenberg, Ursula Herbort-Brand, Claudia Ott, Beate Pieper, Carolin Rattunde, and Petra Weber for their excellent technical assistance.


## REFERENCES


1. Polonsky KS, Sturis J, Bell GI: Non-insulin-dependent diabetes mellitus—a genetically programmed failure of the beta cell to compensate for insulin resistance. *N Engl J Med* 334:777–783, 1996
2. Permutt MA, Chiu K, Ferrer J, Glaser B, Inoue H, Nestorowicz A, Stanley CA, Tanizawa Y: Genetics of type II diabetes. *Recent Prog Horm Res* 53:201–216, 1998
3. Koster JC, Marshall BA, Ensor N, Corbett JA, Nichols CG: Targeted overactivity of β cell $K_{ATP}$ channels induces profound neonatal diabetes. *Cell* 100:645–654, 2000
4. Aguilar-Bryan L, Clement JPIV, Gonzalez G, Kunjilwar K, Babenko A, Bryan J: Toward understanding the assembly and structure of $K_{ATP}$ channels. *Physiol Rev* 78:227–245, 1998
5. Sakura H, Wat N, Horton V, Millns H, Turner RC, Ashcroft FM: Sequence variations in the human Kir6.2 gene, a subunit of the beta-cell ATP-sensitive K-channel: no association with NIDDM in white Caucasian subjects or evidence of abnormal function when expressed in vitro. *Diabetologia* 39:1233–1236, 1996
6. Inoue H, Ferrer J, Warren-Perry M, Zhang Y, Millns H, Turner RC, Elbein SC, Hampe CL, Suarez BK, Inagaki N, Seino S, Permutt MA: Sequence variants in the pancreatic islet beta-cell inwardly rectifying $K^+$ channel Kir6.2 (Bir) gene: identification and lack of role in Caucasian patients with NIDDM. *Diabetes* 46:502–507, 1997
7. Hansen L, Echwald SM, Hansen T, Urhammer SA, Clausen JO, Pedersen O: Amino acid polymorphisms in the ATP-regulatable inward rectifier Kir6.2 and their relationships to glucose- and tolbutamide-induced insulin secretion, the insulin sensitivity index, and NIDDM. *Diabetes* 46:508–512, 1997
8. Hani EH, Boutin P, Durand E, Inoue H, Permutt MA, Velho G, Froguel P: Missense mutations in the pancreatic islet beta cell inwardly rectifying $K^+$ channel gene (KIR6.2/BIR): a meta-analysis suggests a role in the polygenic basis of type II diabetes mellitus in Caucasians. *Diabetologia* 41:1511–1515, 1998
9. Gloyn AL, Hashim Y, Ashcroft SJ, Ashfield R, Wiltshire S, Turner RC: UK Prospective Diabetes Study (UKPDS 53): association studies of variants in promoter and coding regions of beta-cell ATP-sensitive K-channel genes SUR1 and Kir6.2 with type 2 diabetes mellitus (UKPDS 53). *Diabet Med* 18:206–212, 2001
10. Koster JC, Sha Q, Shyng S-L, Nichols CG: ATP inhibition of $K_{ATP}$ channels: control of nucleotide sensitivity by the N-terminal domain of the Kir6.2 subunit. *J Physiol* 515:19–30, 1999
11. Babenko AP, Gonzalez G, Bryan J: The N-terminus of $K_{IR}6$: 2 limits spontaneous bursting and modulates the ATP-inhibition of $K_{ATP}$ channels. *Biochem Biophys Res Commun* 255:231–238, 1999
12. Schwanstecher C, Dickel C, Ebers I, Lins S, Zünkler BJ, Panten U: Diazoxide-sensitivity of the adenosine 5′-triphosphate-dependent $K^+$ channel in mouse pancreatic beta-cells. *Br J Pharmacol* 107:87–94, 1992
13. Tucker SJ, Gribble FM, Proks P, Trapp S, Ryder TJ, Haug T, Reimann F, Ashcroft FM: Molecular determinants of $K_{ATP}$ channel inhibition by ATP. *EMBO J* 17:3290–3296, 1998
14. Inoue H, Ferrer J, Welling CM, Elbein SC, Hoffman M, Mayorga R, Warren-Perry M, Zhang Y, Millns H, Turner R, Province M, Bryan J, Permutt MA, Aguilar-Bryan L: Sequence variants in the sulfonylurea receptor (SUR) gene are associated with NIDDM in Caucasians. *Diabetes* 45:825–831, 1996
15. Hani EH, Clement K, Velho G, Vionnet N, Hager J, Philippi A, Dina C, Inoue H, Permutt MA, Basdevant A, North M, Demenais F, Guy-Grand B, Froguel P: Genetic studies of the sulfonylurea receptor gene locus in NIDDM and in morbid obesity among French Caucasians. *Diabetes* 46:688–694, 1997
16. Hansen T, Echwald SM, Hansen L, Moller AM, Almind K, Clausen JO, Urhammer SA, Inoue H, Ferrer J, Bryan J, Aguilar-Bryan L, Permutt MA, Pedersen O: Decreased tolbutamide-stimulated insulin secretion in healthy subjects with sequence variants in the high-affinity sulfonylurea receptor gene. *Diabetes* 47:598–605, 1998
17. Ashfield R, Ashcroft SJ: Cloning of the promoters for the beta-cell ATP-sensitive K-channel subunits Kir6.2 and SUR1. *Diabetes* 47:1274–1280, 1998
18. Tripathy D, Carlsson AL, Lehto M, Isomaa B, Tuomi T, Groop L: Insulin secretion and insulin sensitivity in diabetic subgroups: studies in the prediabetic and diabetic state. *Diabetologia* 43:1476–1483, 2000
19. Henriksen JE, Levin K, Thye-Ronn P, Alford F, Hother-Nielsen O, Holst JJ, Beck-Nielsen H: Glucose-mediated glucose disposal in insulin-resistant normoglycemic relatives of type 2 diabetic patients. *Diabetes* 49:1209–1218, 2000
20. Neel JV: The "thrifty genotype" in 1998 (Review). *Nutr Rev* 57:S2–S9, 1999
21. Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L, Lander ES: The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 26:76–80, 2000
22. Markworth E, Schwanstecher C, Schwanstecher M: $ATP^{4-}$ mediates closure of pancreatic beta-cell ATP-sensitive potassium channels by interaction with 1 of 4 identical sites. *Diabetes* 49:1413–1418, 2000