

The Prevalent Glu23Lys Polymorphism in the Potassium Inward Rectifier 6.2 (KIR6.2) Gene Is Associated With Impaired Glucagon Suppression in Response to Hyperglycemia

Otto Tschritter, Michael Stumvoll, Fausto Machicao, Martin Holzwarth, Melanie Weisser, Elke Maerker, Anna Teigeler, Hans Häring, and Andreas Fritsche

Genetic factors play an important role in the pathogenesis of type 2 diabetes. The relevance to type 2 diabetes of the common polymorphism Glu23Lys in the potassium inward rectifier 6.2 (KIR6.2) gene is still controversial. The aim of this study was to assess whether this polymorphism influences β -cell function, α -cell function, or insulin action. We therefore studied 298 nondiabetic subjects using an oral glucose tolerance test (OGTT) and 75 nondiabetic subjects using a hyperglycemic clamp (10 mmol/l) with additional glucagon-like peptide (GLP)-1 and arginine stimulation. The prevalence of the Lys allele was $\sim 37\%$, and the Lys allele was associated with higher incremental plasma glucose during the OGTT ($P = 0.03$, ANOVA). Neither first- nor second-phase glucose-stimulated C-peptide secretion was affected by the presence of the polymorphism; nor were maximal glucose-, GLP-1-, or arginine-induced C-peptide secretion rates; nor was insulin sensitivity (all $P > 0.7$). However, the relative decrease in plasma glucagon concentrations during the 10 min after the glucose challenge was reduced in carriers of the Lys allele ($10 \pm 3\%$ decrease from baseline in Lys/Lys, $18 \pm 2\%$ in Glu/Lys, and $20 \pm 2\%$ in Glu/Glu; $P = 0.01$, ANOVA). In conclusion, our findings suggest that the common Glu23Lys polymorphism in KIR6.2 is not necessarily associated with β -cell dysfunction or insulin resistance but with diminished suppression of glucagon secretion in response to hyperglycemia. Our findings thus confirm its functional relevance for glucose metabolism in humans. *Diabetes* 51:2854–2860, 2002

Genetic factors are clearly important for the main pathogenic mechanisms leading to common type 2 diabetes, β -cell dysfunction, and impaired insulin action (1,2). However, the precise nature of the genetic lesions responsible for β -cell

dysfunction of common type 2 diabetes remains to be identified. Plausible candidate genetic variants that could affect insulin secretion include the potassium inward rectifier 6.2 (KIR6.2) gene.

The protein encoded by this gene represents a potassium channel and, together with the sulfonylurea receptor (SUR1), plays an essential role in insulin secretion by the β -cell (3). In humans, some genetic variants of KIR6.2 have been identified. Only one resulting amino acid polymorphism (Glu23Lys) has a sufficiently high prevalence to play a significant role in common type 2 diabetes (4). The data regarding the association of this polymorphism with type 2 diabetes are controversial. Some studies found no association with an increased diabetes risk (5–7), including one recent report using transmission disequilibrium testing. On the other hand, two recent reports including a meta-analysis could demonstrate an association of the Glu23Lys polymorphism with type 2 diabetes (8,9).

Impairment in β -cell function would appear to be the prime mechanism by which this polymorphism results in glucose intolerance. To date, only one study has examined the influence of the KIR6.2 Glu23Lys polymorphism on insulin secretion with an appropriate test for β -cell function. In healthy Danish subjects studied with an intravenous glucose tolerance test and tolbutamide challenge, no difference in insulin secretion could be detected between carriers of the polymorphism and wild-type subjects (5).

It is of note that KIR6.2 is expressed not only in pancreatic β -cells but also in muscle and pancreatic α -cells (10–12), tissues with proven relevance for glucose homeostasis. Conceivably, functional alterations of KIR6.2 as a consequence of the Glu23Lys polymorphism may also affect insulin-stimulated glucose disposal and glucagon secretion. Therefore, the aim of the present study was to assess the influence of the Glu23Lys polymorphism in KIR6.2 on glucose tolerance, β -cell function, α -cell function, and insulin action in a large population of nondiabetic German volunteers.

RESEARCH DESIGN AND METHODS

Subjects. We studied 298 nondiabetic subjects (characteristics are shown in Table 1) by a standard oral glucose tolerance test (OGTT) (13). A subset of 135 subjects had also undergone a euglycemic clamp (14). Furthermore, we studied 75 nondiabetic subjects (characteristics are shown in Table 2) by a modified hyperglycemic clamp with additional glucagon-like peptide (GLP)-1 and arginine administration (15). The subjects were unrelated and had tested

From the Department of Endocrinology, Metabolism and Pathobiochemistry, Medizinische Klinik, Eberhard-Karls-Universität, Tübingen, Germany.

Address correspondence and reprint requests to Professor Dr. med. Hans-Ulrich Häring, Medizinische Universitätsklinik, Otfried-Müller-Str. 10, D-72076 Tübingen, Germany. E-mail: hans-ulrich.haering@med.uni-tuebingen.de.

Received for publication 18 January 2002 and accepted in revised form 4 June 2002.

AUCinc, incremental area under the curve; GLP, glucagon-like peptide; IGT, impaired glucose tolerance; K_{ATP} channel, ATP-sensitive K^+ channel; KIR6.2, potassium inward rectifier 6.2; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; SUR1, sulfonylurea receptor; WHR, waist-to-hip ratio.

TABLE 1
Characteristics of carriers of 0, 1, or 2 Lys alleles in codon 23 of KIR6.2 who underwent the OGTT

	Glu/Glu	Glu/Lys	Lys/Lys	<i>P</i> (ANOVA)	<i>P</i> Glu/Glu vs. X/Lys (<i>t</i> test)	<i>P</i> Glu/Glu vs. Lys/Lys (<i>t</i> test)
<i>n</i> (M/F)	111 (37/74)	151 (52/99)	36 (10/26)	0.75	1.00	0.68
IGT/NGT	16/95	19/132	7/29	0.58	1.00	0.44
Age (years)	39 ± 1	38 ± 1	37 ± 2	0.49	0.24	0.37
BMI (kg/m ²)	28.0 ± 0.7	26.8 ± 0.5	27.0 ± 1.1	0.37	0.16	0.52
WHR	0.87 ± 0.01	0.87 ± 0.01	0.85 ± 0.01	0.49	0.56	0.24
Fasting plasma glucose (mmol/l)	5.1 ± 0.1	5.1 ± 0.04	5.1 ± 0.1	0.79	0.50	0.78
Fasting plasma insulin (pmol/l)	60 ± 5	60 ± 4	54 ± 7	0.81	0.83	0.57
Fasting plasma glucagon (ng/l)	63 ± 3	67 ± 2	66 ± 4	0.47	0.23	0.57
2-h plasma glucose (mmol/l)	5.9 ± 0.1	6.1 ± 0.1	6.3 ± 0.3	0.38	0.23	0.23
2-h plasma insulin (pmol/l)	354 ± 30	362 ± 23	357 ± 55	0.98	0.85	0.96
2-h glucagon (ng/l)	51 ± 2	54 ± 2	55 ± 3	0.20	0.08	0.14
AUCinc glucose (mmol · l ⁻¹ · h)*	221 ± 14	235 ± 12	262 ± 25	0.05	0.04	0.02
Insulin sensitivity index*†	17.7 ± 1.0	17.2 ± 0.9	17.4 ± 1.8	0.14	0.05	0.41

Data are means ± SE. *P* values are unadjusted for multiple comparisons. *Adjusted for BMI, age, and WHR. †Calculated from the OGTT.

negative for GAD; their genotype was unknown at the time of recruitment. Our recruitment mechanisms in general include newspaper ads and word-of-mouth proposing diabetes screens. Hyperglycemic clamps are proposed to everyone undergoing an OGTT and performed in those who agree to participate. The study protocol was approved by the ethics committee of the University of Tübingen. Before the study, informed written consent was obtained from all participants.

Genotyping. Detection of Glu23Lys was done by direct sequencing. PCR products were sequenced bidirectionally using an dye terminator cycle sequencing ready reaction kit (ABI PRISM 310; Applied Biosystems, Foster City, CA). The nucleotide substitution at codon 23 was detected by the following primers: sense 5'-GAA TAC GTG CTG ACA CGC CT-3' and antisense 5'-CAG CTG CAC AGG AAG AAG GAC AT-3'. We found frequencies of 37% in the OGTT group and 38% in the clamp group for the Lys allele. The genotype distributions were in Hardy-Weinberg equilibrium ($P = 0.4$ for OGTT and $P = 0.6$ for clamp, χ^2 test). The rare allelic frequency in the subjects was not different between subjects with normal and impaired glucose tolerance (NGT and IGT) in both groups ($P = 0.6/0.5$, χ^2 test).

Hyperglycemic clamp. After an overnight fast and after baseline samples had been obtained, a hyperglycemic clamp was performed as previously described (15,16). An intravenous bolus of 20% glucose over 1 min was given to instantaneously raise blood glucose to 10 mmol/l (bolus dose [mg] = body weight [kg] × desired increase in blood glucose [mg/dl] × 1.5). Subsequently, a glucose infusion was adjusted to maintain blood glucose at 10 mmol/l. After 120 min, GLP-1 [human GLP-1(7-36) amide; Poly Peptide, Wolfenbüttel, Germany] was given as a primed-continuous infusion (0.6 pmol/kg; 1.5 pmol · kg⁻¹ · min⁻¹) during the next 80 min. At 180 min, a bolus of 5 g arginine hydrochloride (Pharmacia & Upjohn, Erlangen, Germany) was injected over 45 s while the GLP-1 infusion was continued.

Analytical procedures. Blood glucose was determined with a HemoCue blood glucose photometer (HemoCue AB, Ängelholm, Sweden) at 5-min intervals. Samples for insulin (Microparticle Enzyme Immunoassay; Abbott Laboratories, Tokyo, Japan), C-peptide (radioimmunoassay; Byk-Sangtec, Dietzenbach, Germany), and glucagon (radioimmunoassay; Linco Research, St. Charles, MO) were taken at -30, -15, 0, 2.5, 5, 7.5, 10, 20, 40, 60, 80, 100, 120, 125, 130, 140, 150, 160, 170, 180, 182.5, 185, 187.5, 190, and 200 min.

Calculations. Insulin sensitivity (insulin sensitivity index) from the hyperglycemic clamp was determined by relating the glucose infusion rate to the plasma insulin concentration during the second hour (15). Insulin sensitivity was estimated from the OGTT using the composite index proposed by Matsuda and DeFronzo (17). Phases of insulin secretion based on insulin and C-peptide concentrations during the hyperglycemic clamp were calculated as follows: first phase, mean of 2.5–10 min; second phase, mean of 80–120 min; first GLP phase, mean of 125–130 min; GLP plateau, mean of 160–180 min; and maximal insulin secretion, mean of 182.5–190 min (15). Insulin secretion rates during the hyperglycemic clamp were calculated by deconvolution from C-peptide concentrations using standard kinetic parameters from the literature as previously described (18,19). Suppression of glucagon by hyperglycemia during the clamp studies was determined as glucagon levels relative to baseline (mean of two measurements during a 30-min baseline period) during the 10 min (mean of measurement at 2.5, 5, 7.5, and 10 min) after the glucose challenge. Suppression of glucagon during the OGTT was calculated as the difference between glucagon concentrations at times 0 and 120 min. This difference was divided by the change in glucose concentration during the 120 min of the OGTT to determine the glucagon suppression relative to increase (in mol/l) of glucose concentration (20).

Statistical analysis. Parameters were logarithmically transformed to approximate linearity if necessary. The secretion indexes of the hyperglycemic

TABLE 2
Characteristics of carriers of 0, 1, or 2 Lys alleles in codon 23 of KIR6.2 who underwent the hyperglycemic clamp

	Glu/Glu	Glu/Lys	Lys/Lys	<i>P</i> (ANOVA)	<i>P</i> Glu/Glu vs. X/Lys (<i>t</i> test)	<i>P</i> Glu/Glu vs. Lys/Lys (<i>t</i> test)
<i>n</i> (M/F)	31 (12/19)	31 (17/14)	13 (7/6)	0.40	0.18	0.36
IGT/NGT	7/24	5/26	4/9	0.55	0.83	0.57
Age (years)	38 ± 3	39 ± 2	41 ± 3	0.77	0.59	0.48
BMI (kg/m ²)	25.1 ± 1.0	26.1 ± 0.7	24.8 ± 0.8	0.57	0.56	0.84
WHR	0.84 ± 0.01	0.86 ± 0.01	0.86 ± 0.02	0.37	0.16	0.33
Fasting plasma glucose (mmol/l)	5.1 ± 0.1	5.0 ± 0.1	5.1 ± 0.2	0.68	0.55	0.98
Fasting plasma insulin (pmol/l)	56 ± 7	61 ± 11	45 ± 7	0.58	0.98	0.32
Fasting plasma glucagon (ng/l)	68 ± 3	68 ± 4	60 ± 4	0.33	0.58	0.13
AUCinc glucose (mmol · l ⁻¹ · h)*	196 ± 30	298 ± 26	297 ± 74	0.06	0.02	0.17
Insulin sensitivity index†	0.15 ± 0.02	0.15 ± 0.02	0.17 ± 0.03	0.76	0.51	0.51

Data are means ± SE. *P* values are unadjusted for multiple comparisons. *Adjusted for BMI and age. †Calculated from the hyperglycemic clamp.

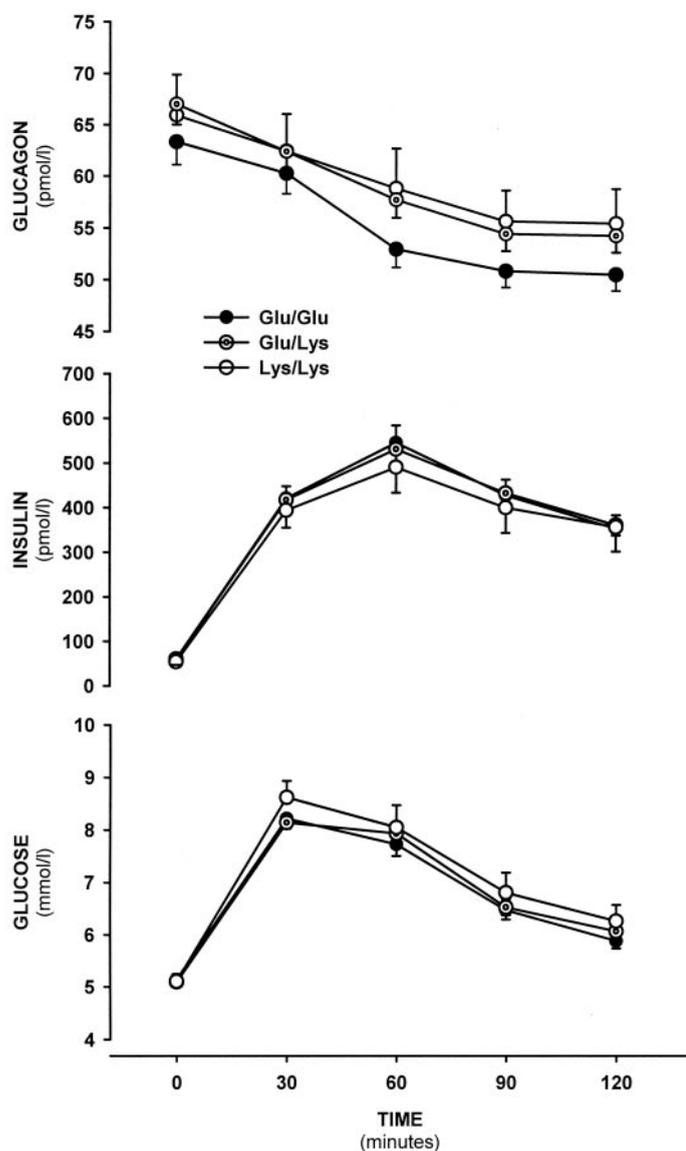


FIG. 1. Blood glucose, insulin, and glucagon levels during an OGTT in 298 subjects of the OGTT group. AUC for glucagon (adjusted for BMI), $P = 0.03$ Glu/Glu vs. X/Lys; AUC for insulin, $P = 0.8$ Glu/Glu vs. X/Lys; AUCinc for glucose (adjusted for BMI, age, and WHR), $P = 0.04$ Glu/Glu vs. X/Lys.

clamp were adjusted for insulin sensitivity, BMI, age, and sex. For some statistical comparisons with the wild type (Glu/Glu), subjects heterozygous (Glu/Lys) and homozygous (Lys/Lys) for the mutation were combined and referred to as X/Lys. Comparisons between genotypes were made on adjusted values using the unpaired Student's t test (Glu/Glu versus X/Lys) or ANOVA for the three genotype groups. A P value of <0.05 was considered to be statistically significant. The statistical software package JMP (SAS Institute, Cary, NC) was used.

In the present study, the statistical tests were not corrected for multiple comparisons. One polymorphism was examined for three different traits (insulin secretion, insulin sensitivity, and glucagon suppression). The issue of whether it is desirable to correct for multiple comparisons has been controversial, because the overall false-positive rate can be preserved only at the cost of failing to detect a true effect (21). The extent to which similar results will be shown in other studies can aid the interpretation of the plausibility of the present findings.

RESULTS

Glucose. In the OGTT group, the Lys allele of the KIR6.2 Glu23Lys polymorphism was associated with an increased

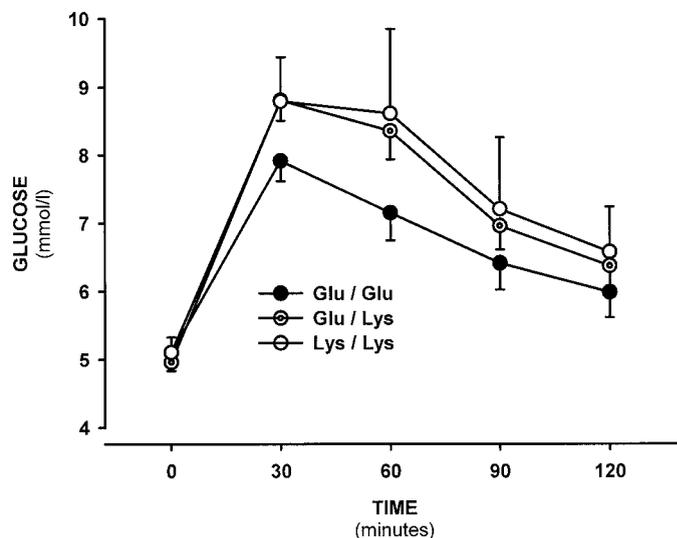


FIG. 2. Blood glucose levels during an OGTT in 75 subjects of the hyperglycemic clamp group. AUCinc for glucose (adjusted for BMI, age, and WHR), $P = 0.02$ Glu/Glu vs. X/Lys.

incremental area under the curve (AUCinc) for glucose during a standard OGTT (240 ± 11 vs. 221 ± 14 $\text{mmol} \cdot \text{l}^{-1} \cdot \text{h}$; $P = 0.037$) (Fig. 1). Analogously, in the clamp group, there was an increased AUCinc for glucose in carriers of the Lys allele during the OGTT (298 ± 27 vs. 196 ± 30 $\text{mmol} \cdot \text{l}^{-1} \cdot \text{h}$; $P = 0.019$) (Fig. 2).

Insulin secretion. As shown in Fig. 3 and Table 3, insulin secretion during the modified hyperglycemic clamp was not different between carriers and noncarriers of the polymorphism. Plasma C-peptide concentrations after acute (first-phase) glucose stimulation were not different between Glu/Glu ($1,311 \pm 114$ pmol/l) and X/Lys ($1,308 \pm 85$ pmol/l; $P = 0.98$). Plasma C-peptide concentrations after prolonged (second-phase) glucose stimulation were also not different between Glu/Glu ($2,463 \pm 135$ pmol/l) and X/Lys ($2,472 \pm 159$ pmol/l; $P = 0.97$). Furthermore, C-peptide and insulin secretion in response to other secretagogues used in the modified hyperglycemic clamp were also not different between the genotypes (Table 3). In particular, the combined glucose, GLP-1, and arginine stimulus—the best parameter for maximal insulin secretion—was not different between Glu/Glu ($10,869 \pm 614$ pmol/l) and X/Lys ($10,727 \pm 812$ pmol/l; $P = 0.90$).

Insulin sensitivity. Insulin sensitivity estimated from the OGTT (17) was 17.7 ± 1.1 units in Glu/Glu and 17.3 ± 0.7 units in X/Lys. With values adjusted for BMI, waist-to-hip ratio (WHR), and age, there was a tendency toward decreased insulin sensitivity in subjects with X/Lys ($P = 0.052$) (Table 1). Insulin sensitivity measured during the hyperglycemic clamp was not different between Glu/Glu (0.15 ± 0.02 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ per pmol/l) and X/Lys (0.16 ± 0.02 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ per pmol/l; $P = 0.72$) (Table 2). Insulin sensitivity measured during the euglycemic clamp was also not different between Glu/Glu (0.11 ± 0.01 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ per pmol/l) and X/Lys (0.11 ± 0.01 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ per pmol/l; $P = 0.76$).

Glucagon. In the subjects studied by OGTT only, the glucagon area under the curve was significantly greater in X/Lys ($7,070 \pm 174$ $\text{ng} \cdot \text{l}^{-1} \cdot \text{h}$) compared with Glu/Glu ($6,631 \pm 194$ $\text{ng} \cdot \text{l}^{-1} \cdot \text{h}$; $P = 0.027$) after adjustment for

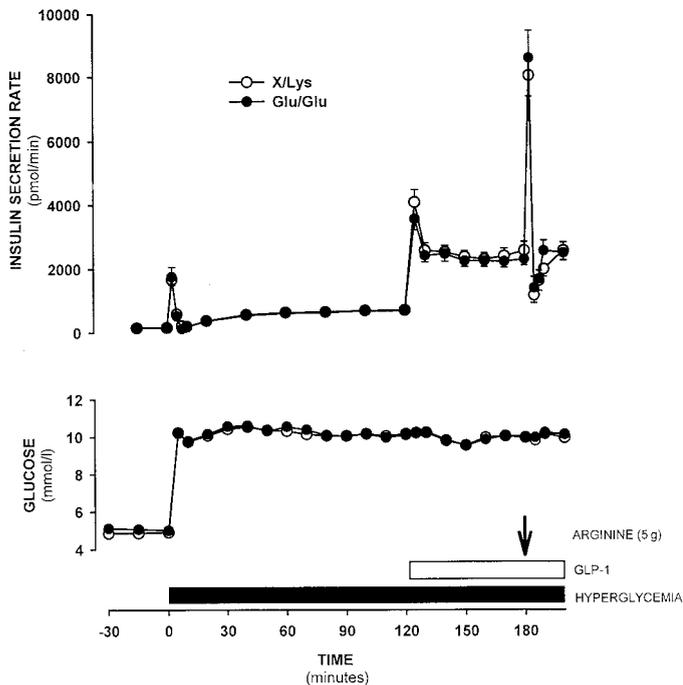


FIG. 3. Blood glucose and insulin secretion rates during a modified hyperglycemic clamp in subjects without (Glu/Glu) and with (X/Lys) the Glu23Lys polymorphism in KIR6.2.

BMI (Fig. 1). The reduction of glucagon during the OGTT was not different between Glu/Glu and X/Lys (12.8 ± 1.6 vs. 12.3 ± 1.3 ng/l; $P = 0.8$). However, the reduction in glucagon in relation to the increase in glucose was reduced in carriers of the Lys allele (9.1 ± 5.2 ng/mmol in X/Lys vs. 54.4 ± 24.5 in Glu/Glu; $P = 0.03$).

In the subjects studied by hyperglycemic clamp, the relative decrease in plasma glucagon concentrations during the 10 min after the glucose challenge was reduced in carriers of the Lys allele ($10 \pm 3\%$ decrease from baseline in Lys/Lys, $18 \pm 2\%$ in Glu/Lys, and $20 \pm 2\%$ in Glu/Glu; $P = 0.01$, ANOVA) (Fig. 4). The absolute glucagon levels during the GLP-1 and arginine infusion in the modified hypergly-

cemic clamp were not different between carriers and noncarriers of the Lys allele (data not shown).

DISCUSSION

In this study, we attempted to identify a mechanism by which the Glu23Lys polymorphism in the KIR6.2 gene may affect glucose homeostasis and possibly the risk of type 2 diabetes. We found that this common polymorphism is associated with diminished α -cell suppression in response to hyperglycemia but not with β -cell dysfunction or insulin resistance.

ATP-sensitive K^+ channels (K_{ATP} channels) are essential for glucose-mediated insulin secretion in the β -cell (22,23). KIR6.2 knockout mice and mice expressing a dominant-negative mutation of KIR6.2 in the pancreas have impaired insulin secretion (24,25). Therefore, we hypothesized that carriers of the Glu23Lys mutation may also exhibit impaired insulin secretion. In the present study, however, we found no evidence for a contribution of KIR6.2 to the biological variation of β -cell function. The insulin secretory response did not differ between carriers and noncarriers of the polymorphism, neither to glucose during a classic hyperglycemic clamp nor to other secretagogues such as GLP-1 or arginine. The modified hyperglycemic clamp used in this study was previously shown to produce a greater insulin secretory response than any other in vivo assessment of insulin secretion (15). Therefore, the maximal secretory response to the combined stimuli—glucose, GLP-1, and arginine—may come closest to an in vivo measurement of total functional β -cell capacity. The data presented here make it unlikely that the Glu23Lys polymorphism in the KIR6.2 gene affects insulin secretion, not even under circumstances of extremely increased insulin demand such as insulin resistance.

Nevertheless, we could demonstrate a higher AUC_{inc} for glucose during an OGTT in nondiabetic carriers of the Lys allele among the 75 subjects undergoing the hyperglycemic clamp, as well as in the larger group of ~ 300 patients studied with only an OGTT. This is, in essence,

TABLE 3

Plasma C-peptide and insulin concentrations during the different phases of the modified hyperglycemic clamp for carriers of 0, 1, or 2 Lys alleles in codon 23 of KIR6.2

	Glu/Glu	Glu/Lys	Lys/Lys	<i>P</i> (ANOVA)	<i>P</i> Glu/Glu vs. X/Lys (<i>t</i> test)	<i>P</i> Glu/Glu vs. Lys/Lys (<i>t</i> test)
C-peptide (pmol/l)						
First phase	1,311 \pm 114	1,314 \pm 83	1,295 \pm 215	1.00	0.98	0.94
Second phase	2,463 \pm 135	2,473 \pm 158	2,471 \pm 395	1.00	0.97	0.98
GLP, first phase	5,493 \pm 302	5,885 \pm 431	5,889 \pm 932	0.77	0.47	0.60
GLP, second phase	8,590 \pm 449	9,063 \pm 852	8,473 \pm 1,099	0.85	0.74	0.91
Maximum	10,869 \pm 614	10,912 \pm 1,029	10,285 \pm 1,287	0.91	0.90	0.65
Insulin (pmol/l)						
First phase	241 \pm 41	216 \pm 23	253 \pm 73	0.82	0.76	0.89
Second phase	302 \pm 49	283 \pm 37	319 \pm 104	0.91	0.89	0.87
GLP, first phase	1,651 \pm 238	1,752 \pm 222	1,813 \pm 448	0.92	0.70	0.73
GLP, second phase	3,700 \pm 490	3,689 \pm 528	3,452 \pm 873	0.96	0.90	0.79
Maximum	5,512 \pm 626	5,566 \pm 704	5,065 \pm 1,137	0.92	0.91	0.71
Glucagon (relative to baseline)						
First phase	0.80 \pm 0.02	0.82 \pm 0.02	0.90 \pm 0.03	0.01	0.06	0.01

Data are means \pm SE. *P* values are unadjusted for multiple comparisons.

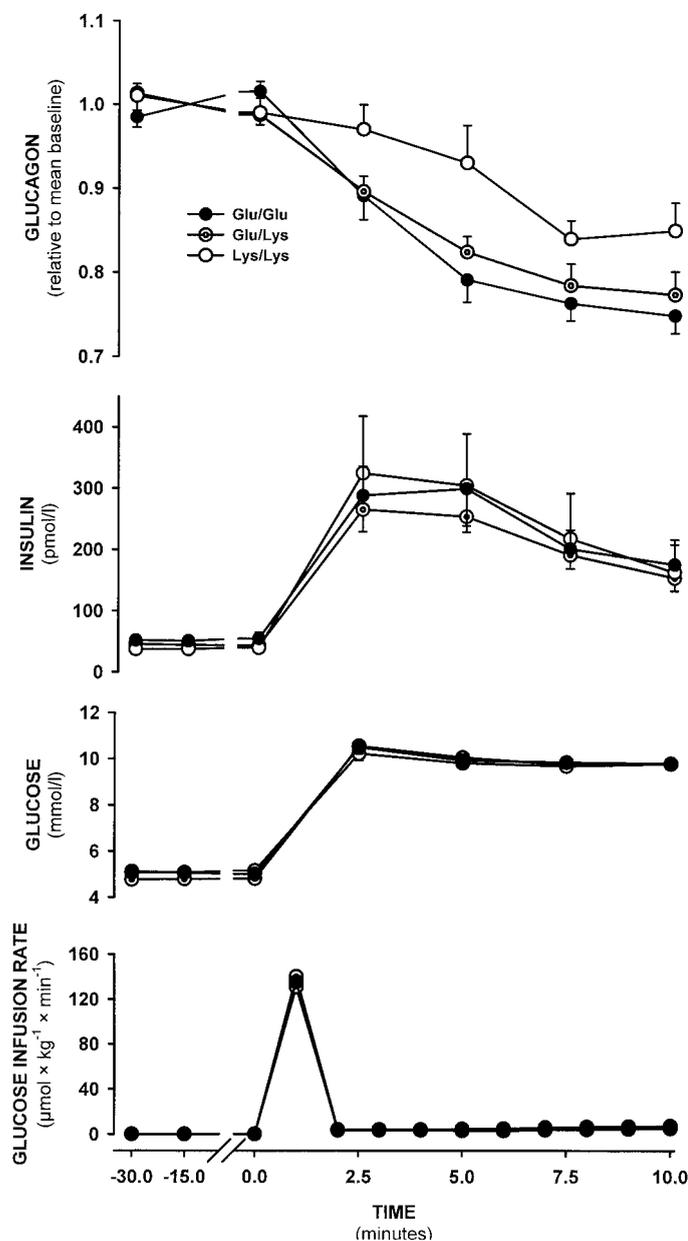


FIG. 4. Blood glucose, plasma insulin, and plasma glucagon levels (relative to baseline) during the first phase of the hyperglycemic clamp in subjects without (Glu/Glu), heterozygous for (Glu/Lys), and homozygous for (Lys/Lys) the Glu23Lys polymorphism in KIR6.2. The glucagon suppression in response to hyperglycemia was significantly different between the genotypes ($P = 0.01$, ANOVA, unadjusted for multiple comparisons). Plasma glucose and insulin concentrations and glucose infusion rates were not different (all $P > 0.5$).

consistent with results of a recent subanalysis from the U.K. Prospective Diabetes Study (8) and a meta-analysis including 521 diabetic and 367 control subjects (9) showing an association of this polymorphism with type 2 diabetes. The question remained what the underlying mechanism might be leading to IGT.

Although the KIR6.2 channel is also expressed in skeletal muscle tissue, we could not demonstrate any effect of the polymorphism on insulin-stimulated glucose disposal measured under euglycemic and hyperglycemic clamp conditions. In the subjects studied by OGTT, however, the estimated insulin sensitivity showed a weak association of the Lys allele with impaired insulin sensitivity. This is at

variance with results of a study demonstrating an association of the homozygous Lys allele with increased insulin sensitivity (5). Nevertheless, considering a study in KIR6.2 knockout mice also showing increased insulin sensitivity (25), it appears very unlikely that impaired insulin sensitivity is responsible for the association of the Lys allele with impaired glucose tolerance.

On the other hand, under our experimental conditions of similar elevation of blood glucose to a level of 10 mmol/l and similar plasma insulin levels in wild-type, heterozygous, and homozygous genotypes, we could demonstrate a reduced suppression of plasma glucagon levels in the carriers of the Lys allele. Insufficient suppression of glucagon is known to contribute to the impairment of glucose tolerance (20,26,27). Thus, based on our observations, it appears possible that the association of the Glu23Lys polymorphism with diabetes among other mechanisms involves impaired hyperglycemia-induced glucagon suppression. However, the effect on reduced glucagon suppression is mainly present in the homozygous carriers of the mutation. Nevertheless, in view of the weak net effect on overall glucose homeostasis in our nondiabetic population, it is unlikely that this polymorphism alone has a main effect on the risk of type 2 diabetes, but it may well be a contributing factor.

Notably, the reduced glucagon suppression found in the hyperglycemic clamp experiments was replicated in the OGTT group when expressed per increase in glucose. However, the results from the OGTT should be interpreted with caution because glucose levels were different between the genotype groups.

How can a mutant KIR6.2 channel be involved in glucose-regulated glucagon secretion? Studies in KIR6.2 knockout mice demonstrate that α -cells were widely distributed in pancreatic islets and increased in number whereas β -cells were reduced in number due to increased apoptosis (28). Therefore, the K_{ATP} channels seem to be involved in cell survival and differentiation of the endocrine pancreas. The altered islet architecture in these mice may lead to impaired intraislet communication between glucose, insulin, and glucagon (29), with resulting alterations in glucose sensing of the α -cell. Alternatively, the glucose-sensing process may be impaired at the level of the brain. KIR6.2 is also expressed in the brain, mainly in regions where glucose-responsive neurons are located, such as the ventromedial hypothalamus (30). Studies in KIR6.2 knockout mice showed functional α -cells in isolated pancreatic islets, whereas glucagon secretion was impaired during systemic hypoglycemia or isolated neuroglycopenia (31). This points to an important role of the KIR6.2 channel in central nervous glucose sensing and maintenance of glucose homeostasis. It is possible that the Glu23Lys polymorphism in KIR6.2 leads to alteration in brain glucose sensing, not only during hypoglycemia but also during hyperglycemia, and results in impaired glucagon suppression in response to hyperglycemia. In keeping with this hypothesis, recent observations in children with SUR1 mutations associated with congenital hyperinsulinism suggest that reduced activity of the SUR1/ K_{ATP} channel complex causes β -cell insensitivity to glucose (32). It is possible that similar mechanisms are operative in α -cells

or hypothalamic neurons and underlie the glucose insensitivity suggested by our findings.

Finally, Schwanstecher et al. (33) recently demonstrated functional effects of this polymorphism in a transfected mammalian cell line. The Glu23Lys polymorphism in KIR6.2 enhanced the open probability of K_{ATP} channels and reduced their ATP sensitivity. Although this was not examined in pancreatic α - or β -cells or hypothalamic neurons, it is possible that the increase in ATP concentration required to suppress the channel activity results in either an increased threshold concentration for insulin release or an increased sensing threshold for hyperglycemic glucose concentrations. If the latter mechanism were operative in α -cells or hypothalamic neurons, reduced suppression of glucagon secretion would be expected.

In conclusion, our results from the modified hyperglycemic clamp strongly suggest that the Glu23Lys polymorphism in KIR6.2 is not associated with β -cell dysfunction even under conditions of excessive β -cell secretory demand. We provide preliminary evidence that the association of this polymorphism with increased blood glucose levels found in this and some other studies may be explained by diminished suppression of glucagon secretion in response to hyperglycemia. The provisional findings of this study have to be proven in further human genotype/phenotype association studies or studies with transgenic animals.

ACKNOWLEDGMENTS

This study was supported by a research grant from the Deutsche Forschungsgemeinschaft (Fr 1561/1-1) and the European Community (QLRT-1999-00674). M.S. is currently supported by a Heisenberg grant of the Deutsche Forschungsgemeinschaft.

We thank all the research volunteers for their participation. We gratefully acknowledge the excellent technical assistance of Isolde Riedlinger.

REFERENCES

- Gerich JE: The genetic basis of type 2 diabetes mellitus: impaired insulin secretion versus impaired insulin sensitivity. *Endocr Rev* 19:491-503, 1998
- Weyer C, Bogardus C, Mott DM, Pratley RE: The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *J Clin Invest* 104:787-794, 1999
- Inagaki N, Gono T, Clement JP 4th, Namba N, Inazawa J, Gonzalez G, Aguilar Bryan L, Seino S, Bryan J: Reconstitution of IKATP: an inward rectifier subunit plus the sulfonylurea receptor. *Science* 270:1166-1170, 1995
- Inoue H, Ferrer J, Warren-Perry MG, Zhang Y, Millns H, Turner R, Elbein SC, Hampe CL, Suarez BK, Inagaki N, Seino S, Permutt MA: Sequence variants in the pancreatic islet β -cell inwardly rectifying K^+ channel Kir6.2 (Bir) gene. *Diabetes* 46:502-507, 1997
- Hansen L, Echwald SM, Hansen T, Urhammer SA, Clausen J, 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
- 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
- Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl M-C, Nemesh J, Lane CD, Schaffner SF, Bolk A, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L, Lander ES: The common PPAR gamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 26:76-80, 2000
- Gloyn AL, Hashim Y, Ashcroft SJH, Ashfield R, Wiltshire S, Turner RC: 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
- 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
- Spruce AE, Standen NB, Stanfield PR: Voltage-dependent ATP-sensitive potassium channels of skeletal muscle membrane. *Nature* 316:736-738, 1985
- Suzuki M, Fujikura K, Inagaki N, Seino S, Takata K: Localization of the ATP-sensitive K^+ channel subunit Kir6.2 in mouse pancreas. *Diabetes* 46:1440-1444, 1997
- Ronner P, Matschinsky FM, Hang TL, Epstein AJ, Buettger C: Sulfonylurea-binding sites and ATP-sensitive K^+ channels in α -TC glucagonoma and β -TC insulinoma cells. *Diabetes* 42:1760-1772, 1993
- World Health Organization: *WHO Expert Committee on Diabetes Mellitus. Second Report.* Geneva, Switzerland, 1980 (Tech. Rep. Ser. no. 646)
- Stumvoll M, Wahl HG, Löblein K, Becker R, Machicao F, Jacob S, Häring H: The Pro12Ala polymorphism in the peroxisome proliferator activated receptor γ 2 gene is associated with increased antilipolytic insulin sensitivity. *Diabetes* 50:876-881, 2001
- Fritsche A, Stefan N, Hardt E, Schützenauer S, Häring H, Stumvoll M: A novel hyperglycemic clamp for characterization of islet function in humans: assessment of three different secretagogues, maximal insulin response and reproducibility. *Eur J Clin Invest* 30:411-418, 2000
- Fritsche A, Stefan N, Hardt E, Häring H, Stumvoll M: Characterisation of beta-cell dysfunction of impaired glucose tolerance: evidence for impairment of incretin-induced insulin secretion. *Diabetologia* 43:852-858, 2000
- Matsuda A, DeFronzo R: Insulin sensitivity indices obtained from oral glucose tolerance testing. *Diabetes Care* 22:1462-1470, 1999
- Eaton RP, Allen RC, Schade DS, Erickson KM, Standefer J: Prehepatic insulin production in man: kinetic analysis using peripheral connecting peptide behavior. *J Clin Endocrinol Metab* 51:520-528, 1980
- Van Cauter E, Mestrez F, Sturis J, Polonsky KS: Estimation of insulin secretion rates from C-peptide levels: comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* 41:368-377, 1992
- Ahren B, Larsson H: Impaired glucose tolerance (IGT) is associated with reduced insulin-induced suppression of glucagon concentrations. *Diabetologia* 44:1998-2003, 2001
- Rothman KJ: No adjustments are needed for multiple comparisons. *Epidemiology* 1:43-46, 1990
- Ashcroft FM, Porks P, Smith PA, Ammala C, Bokvist K, Rorsman P: Stimulus-secretion coupling in pancreatic beta cells. *J Cell Biochem* 55:54-65, 1994
- Miki T, Nagashima K, Seino S: The structure and function of the ATP-sensitive K^+ channel in insulin-secreting pancreatic β -cells. *J Mol Endocrinol* 22:113-123, 1999
- Miki T, Tashiro F, Iwanaga T, Nagashima K, Yoshitomi H, Aihara H, Nitta Y, Gono T, Inagaki N, Miyazaki J, Seino S: Abnormalities of pancreatic islets by targeted expression of a dominant-negative K_{ATP} channel. *Proc Natl Acad Sci U S A* 94:11969-11973, 1997
- Miki T, Nagashima K, Tashiro F, Kotake K, Yoshitomi H, Tamamoto A, Gono T, Iwanaga T, Miyazaki J, Seino S: Defective insulin secretion and enhanced insulin action in K_{ATP} channel-deficient mice. *Proc Natl Acad Sci U S A* 95:10402-10406, 1998
- Mitrakou A, Kelley D, Mookan M, Veneman T, Pangburn T, Reilly T, Gerich J: Role of reduced suppression of glucose production and diminished early insulin release in impaired glucose tolerance. *N Engl J Med* 326:22-29, 1992
- Larsson H, Berglund G, Ahren B: Glucose modulation of insulin and glucagon secretion is altered in impaired glucose tolerance. *J Clin Endocrinol Metab* 80:1778-1782, 1995
- Miki T, Iwanaga T, Nagashima K, Ihara Y, Seino S: Roles of ATP-sensitive K^+ channels in cell survival and differentiation in the endocrine pancreas. *Diabetes* 50:S48-S51, 2001
- Unger RH: Glucagon physiology and pathophysiology in the light of new advances. *Diabetologia* 28:574-578, 1985
- Levin BE, Dunn-Meynell AA, Routh VH: Brain glucosensing and the K_{ATP} channel. *Nat Neurosci* 4:459-460, 2001
- Miki T, Liss B, Minami K, Shiuchi T, Saraya A, Kashima Y, Horiuchi M,

- Ashcroft F, Minokoshi Y, Roeper J, Seino S: ATP-sensitive K^+ channels in the hypothalamus are essential for the maintenance of glucose homeostasis. *Nat Neurosci* 4:507-512, 2001
32. Grimberg A, Ferry RJ, Kelly A, Koo-McCoy S, Polonsky K, Glaser B, Permutt MA, Aguilar-Bryan L, Stafford D, Thornton PS, Baker L, Stanley CA: Dysregulation of insulin secretion in children with congenital hyperinsulinism due to sulfonylurea receptor mutations. *Diabetes* 50:322-328, 2001
33. Schwanstecher C, Meyer U, Schwanstecher U: KIR6.2 polymorphism predisposes to type 2 diabetes by inducing overactivity of pancreatic β -cell ATP-sensitive K^+ channels. *Diabetes* 51:875-879, 2002