

# Variations in Insulin Secretion in Carriers of the E23K Variant in the KIR6.2 Subunit of the ATP-Sensitive K<sup>+</sup> Channel in the $\beta$ -Cell

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**An association between type 2 diabetes and genetic variation in the KIR6.2 gene has been reported in several populations. Based on in vitro studies with cell lines expressing the Glu<sup>23</sup>Lys (E23K) mutation, it was recently suggested that this mutation might result in altered insulin secretion. We have examined glucose-stimulated insulin secretion in relation to this KIR6.2 gene variant in two independent Dutch cohorts. Subjects with normal ( $n = 65$ ) or impaired ( $n = 94$ ) glucose tolerance underwent 3-h hyperglycemic clamps at 10 mmol/l glucose. We did not observe significant differences in first- or second-phase insulin secretion between carriers and noncarriers of the gene variant in either of the study populations (all  $P > 0.45$ ). Furthermore, we found no evidence for a significant interaction with disease-associated gene variants in the sulfonylurea receptor (*SUR1*) gene. We conclude that the E23K mutation in the KIR6.2 gene is not associated with detectable alterations in glucose-stimulated insulin secretion in two independent populations from the Netherlands. *Diabetes* 51:3135–3138, 2002**

**T**he ATP-sensitive K<sup>+</sup> channel in the  $\beta$ -cell is an important regulator of insulin secretion. The channel is composed of two subunits in a heterooctameric complex of four sulfonylurea receptor (*SUR1*) subunits and four KIR6.2 subunits (1,2). Previously, it has been shown that mutations in the gene encoding the sulfonylurea receptor subunit are associated with type 2 diabetes and reduced insulin secretion (3,4). Associations between mutations in the pore forming KIR6.2 subunit and type 2 diabetes have also been de-

scribed (4–9). Recent biochemical studies showed that cell lines expressing the KIR6.2 E23K mutation have a significantly higher open probability, reducing the sensitivity toward inhibitory ATP and thereby increasing the stimulation threshold of insulin secretion (10). Therefore, it was suggested that carriers of this gene variant might have reduced insulin secretion in vivo.

In this study, we have analyzed glucose-stimulated insulin secretion in relation to the E23K mutation in the KIR6.2 gene in two populations: individuals with normal glucose tolerance (NGT), part of whom had a positive family history, and individuals with impaired glucose tolerance (IGT), who are both at increased risk of type 2 diabetes. Insulin secretion was assessed with a 3-h hyperglycemic clamp at 10 mmol/l glucose in all subjects. The NGT and IGT groups were sampled from two independent populations to reduce the chance of false-positive associations.

The clinical characteristics and the distribution of the different genotypes are shown in Tables 1 and 2, respectively. All genotype distributions were in Hardy-Weinberg equilibrium and comparable with those reported in other Caucasian populations (data not shown) (4–9).

Both first- and second-phase glucose-stimulated insulin secretion were not significantly different between the three genotypes in either group (all  $P > 0.45$ , Table 2 and Fig. 1A and B). Analyzing the data assuming a recessive or dominant effect of the mutation also did not alter the results (i.e., EE/EK versus KK or EE versus EK/KK). Correction for age, sex, and BMI or separate testing of individuals with or without a first-degree relative with diabetes did not affect our results. Remarkably, we observed in both of our populations the lowest levels of glucose-induced insulin secretion in heterozygous carriers of the mutation (Fig. 1). However, the differences in second-phase insulin secretion were not significantly different from both other genotypes ( $P = 0.26$  and  $P = 0.21$  for E/K versus E/E + K/K in NGT and IGT subjects, respectively). A combined analysis of both the NGT and IGT groups reduced the  $P$  value to 0.09 (with age, sex, and BMI as covariates and glucose tolerance status as a dummy variable).

The insulin sensitivity indexes, as calculated from the hyperglycemic clamp, were not significantly different between carriers and noncarriers (Table 2). We have also calculated the disposition index for all of our participants (11,12). However, we did not observe any significant

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Received for publication 23 May 2002 and accepted in revised form 17 July 2002.

Additional information can be found in an online appendix at <http://diabetes.diabetesjournals.org>.

DI, disposition index; IGT, impaired glucose tolerance; K<sub>ATP</sub>, ATP-sensitive K<sup>+</sup> channel; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; SUR1, sulfonylurea receptor.

TABLE 1  
Clinical characteristics of subjects with NGT and IGT according to *KIR6.2* E23K genotype

	NGT			IGT		
	E/E	E/K	K/K	E/E	E/K	K/K
<i>n</i> (M/F)	26 (10/16)	32 (4/28)	7 (1/6)	34 (14/20)	50 (26/24)	10 (5/5)
Age (years)	44.3 ± 1.3	46.6 ± 1.1	47.6 ± 1.7	57.5 ± 1.3	57.7 ± 1.0	54.0 ± 2.8
BMI (kg/m <sup>2</sup> )	26.6 ± 0.9	24.9 ± 0.5	26.2 ± 1.5	28.7 ± 0.7	28.2 ± 0.5	29.1 ± 1.1
Fasting plasma glucose (mmol/l)	4.5 ± 0.1	4.7 ± 0.1	4.4 ± 0.2	6.6 ± 0.1	6.6 ± 0.1	6.6 ± 0.2
Fasting plasma insulin (pmol/l)	32 (24–42)	32 (24–40)	34 (24–64)	70 (61–113)	61 (45–94)	62 (48–108)

Data are means ± SE or median (interquartile range). E, glutamic acid; K, lysine. Differences in glucose and insulin levels between the genotypes were tested by ANOVA;  $P > 0.1$  for all comparisons with or without correction for age, sex, and BMI.

differences (Table 2). Furthermore, we did not find evidence for an interaction between this variant and obesity or other significant associations with diabetes-related parameters (Table 2, data not shown).

Also, the possibility that the E23K mutation has an effect on insulin secretion in an epistatic interaction with gene variants in the *SUR1* gene was examined. A total of 26 subjects with NGT and 44 subjects with IGT had a heterozygous or homozygous combination of both mutant alleles (i.e., E/K or K/K and  $-3c/t$  or  $-3t/t$  for *SUR1* exon 16 [nt  $-3$ ]). However, we were unable to demonstrate significant epistasis between the E23K variant and the previously identified exon 16 (or the exon 18 T760T) variant in the *SUR1* gene in both our study populations (all  $P > 0.1$ , Online Appendix 1) (3,13). However, we did observe a trend toward increased insulin secretion in homozygous carriers of both wild-type alleles (E/E and  $-3c/c$ , respectively,  $P \geq 0.1$ , Online Appendix 1). Larger cohorts are needed to confirm this observation. There was also no excess linkage disequilibrium between the E23K variant and the previously identified exon 16  $-3c \rightarrow t$  or exon 18 T760T variants in the *SUR1* gene in both populations (Online Appendix 2).

We conclude that the E23K variant in the *KIR6.2* gene does not lead to a detectable change in glucose-stimulated insulin secretion in subjects with NGT and IGT selected from two independent populations in the Netherlands. Furthermore, we could not demonstrate significant epistatic interactions of the variant with disease-associated gene variants in the other subunit (*SUR1*) of the ATP-sensitive K<sup>+</sup> channel (K<sub>ATP</sub>).

In both of our populations, the lowest levels of glucose-

induced insulin secretion were observed in heterozygous carriers of the mutation (Fig. 1). However, the differences compared with the other genotypes were not significant. This might be due to power limitations of the study, which was designed to detect differences of at least 20–40% with 90% power. Even if we are dealing with a real difference in insulin secretion, which would only be detectable by studying much larger cohorts, the magnitude of this difference in secretion is expected to be relatively small (~10–15% lower than the E/E-carrier control population). This effect is relatively small compared with the overall (rather large) variance in insulin secretion in the study population (Table 2). A similar situation, with the strongest reductions in insulin secretion in the heterozygous carriers, is seen with the exon 16 (nt  $-3$ ) mutation in the *SUR1* gene (3,14, and L.M. 't Hart, unpublished observations). The precise interpretation of these observations on glucose metabolism needs to be clarified.

It could be that differences in insulin secretion between different genotypes are masked by differences in insulin sensitivity between the different genotype groups. Therefore, we have calculated a disposition index (DI) according to Bergman et al. (11). Previously, it was shown that the DI is reduced in subjects with IGT and type 2 diabetes (15). However, the absence of significant difference in the DI among the three genotypes within each group (NGT and IGT) further supports our data regarding the effect of the E23K mutation on insulin secretion.

Recently, biochemical studies with COS-1 cell lines expressing the *KIR6.2* E23K mutant showed that the channels composed from either one or two mutant alleles exhibited altered functionality (10). The authors suggested

TABLE 2  
Hyperglycemic clamp results in subjects with NGT and IGT according to *KIR6.2* E23K genotype

	NGT			IGT		
	E/E	E/K	K/K	E/E	E/K	K/K
<i>n</i> (M/F)	26 (10/16)	32 (4/28)	7 (1/7)	34 (14/20)	50 (26/24)	10 (5/5)
First-phase insulin secretion (pmol/l)	1,707 (1,200–2,783)	1,659 (1,101–2,181)	1,698 (774–2,550)	600 (200–1,084)	593 (290–1,035)	370 (161–1,556)
Second-phase insulin secretion (pmol/l)	325 (248–473)	286 (211–390)	302 (262–522)	314 (169–428)	199 (131–347)	235 (132–382)
Glucose metabolized (mg · kg <sup>-1</sup> · min <sup>-1</sup> )	10.5 (7.9–13.0)	8.8 (6.4–10.5)	8.1 (7.4–13.4)	5.6 (3.5–7.1)	4.9 (4.0–6.2)	5.7 (5.0–7.9)
Insulin sensitivity index 100 × (mg · kg <sup>-1</sup> · min <sup>-1</sup> / pmol/l)	2.9 (1.8–5.1)	3.1 (2.3–4.0)	2.0 (1.4–5.1)	1.6 (1.0–2.8)	2.2 (1.4–3.0)	2.3 (1.3–4.8)
DI (mg · kg <sup>-1</sup> · min <sup>-1</sup> )	54.0 (33.5–73.0)	52.8 (36.0–66.1)	33.3 (18.6–56.5)	8.9 (3.9–16.4)	11.6 (6.8–22.4)	23.5 (3.1–40.5)

Data are means ± SE or median (interquartile range). E, glutamic acid; K, lysine. Differences between the genotypes were tested by ANOVA,  $P > 0.1$  for all comparisons with or without correction for age, sex, and BMI.

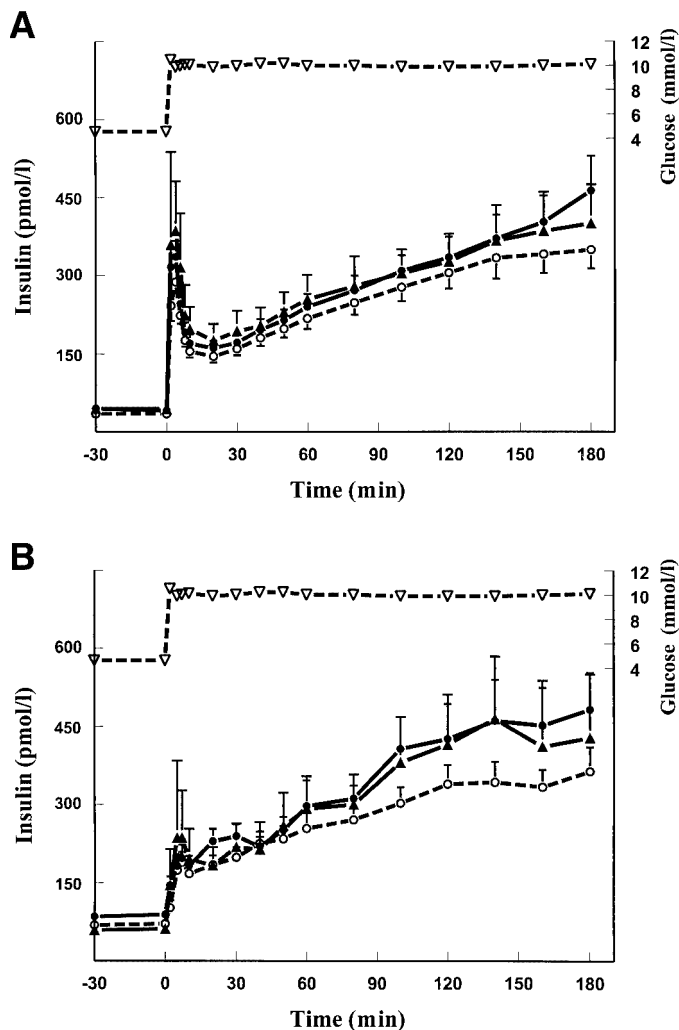


FIG. 1. Glucose and insulin profiles during a 3-h hyperglycemic clamp at 10 mmol/l glucose according to the KIR6.2 E23K genotype. Data are expressed as means  $\pm$  SE. **A:** subjects with NGT ( $n = 65$ ). **B:** subjects with IGT ( $n = 94$ ).  $\nabla$ , mean glucose levels in each subject group;  $\bullet$ , insulin levels in homozygous wild-type subjects (EE);  $\circ$ , heterozygotes (E/K); and  $\blacktriangle$ , homozygous mutant subjects (KK).

that this defect might result in a reduction of glucose-stimulated insulin secretion in individuals carrying this particular mutation. Their hypothesis is supported by observations in carriers of *SUR1* gene mutations. It was previously shown that these gene variants are associated with type 2 diabetes (4) and reduced insulin secretion (3,4). However, we are unable to detect a comparable effect in carriers of the E23K mutation. We have applied the hyperglycemic clamp at 10 mmol/l glucose to stimulate insulin secretion in subjects known to be at increased risk to develop type 2 diabetes (i.e., first-degree relatives of type 2 diabetic individuals [16,17] and subjects with IGT [18]). If the E23K mutation would have an effect on glucose-stimulated insulin secretion, we feel it would be most prominent in these at-risk individuals. Other defects in insulin secretion, such as the (dis)ability of the pancreas to respond to smaller increases in glucose, may be missed by our hyperglycemic clamp set up. A previous *in vivo* study investigating the E23K mutation in 346 healthy young Danish individuals using intravenous glucose tolerance testing also found no significant differences in glu-

cose or tolbutamide-stimulated insulin secretion, which is further support for our data (7). This implies that despite the observed alterations in the *in vitro* expression studies, this gene defect does not lead to detectable changes in glucose-induced insulin secretion *in vivo* of a considerable magnitude. A similar lack of *in vivo* dysfunction is seen in heterozygous carriers of an inactivating mutation in *SUR1*, causing congenital hyperinsulinemia (19). Also, these subjects, carrying 50% nonfunctional SUR1 subunits, were completely normal with respect to insulin secretion and insulin action. Together, these observations suggest that pancreatic  $\beta$ -cells are somehow able to compensate for changes in the functionality of mutant channels.

Previously, it has also been suggested that the E23K variant might be the gene variant responsible for the associations seen with nonfunctional variants in the *SUR1* gene. The absence of linkage disequilibrium, however, excludes the E23K variant as the mutation responsible for the insulin secretion defects seen in association with the nonfunctional exon 16-3t variant of *SUR1* in our study groups (3,13). There was also no support for the hypothesis that both mutations display their effects in an epistatic interaction, suggesting that the associations between mutations in the *SUR1* gene and type 2 diabetes and insulin secretion defects are independent of the E23K genotype. Due to the limitations in sample size and power, we cannot fully exclude the presence of (subtle) interactions between both variants.

We conclude that the E23K variant in the KIR6.2 gene does not lead to a detectable change in glucose-stimulated insulin secretion in both normal and impaired glucose tolerant subjects selected from two independent populations in the Netherlands.

## RESEARCH DESIGN AND METHODS

Caucasian subjects with NGT ( $n = 65$ ) and IGT ( $n = 94$ ) were participants of two distinct study cohorts in the Netherlands. Subjects with NGT were partially selected as first-degree relatives of type 2 diabetic subjects ( $n = 44$ ). The other part of this cohort consists of matched control subjects without a family member with known diabetes (16). Glucose tolerance status was confirmed by oral glucose tolerance test (OGTT) in all subjects according World Health Organization (1985) criteria (20).

Subjects with IGT were selected based on two separate OGTTs (measurements at baseline and after 2 h). They were included in this study if the mean postload glucose level was between 8.6 and 11.1 mmol/l (details of both study groups are described elsewhere [3,16,21,22]). Informed consent was obtained from all participants in the studies, and the local medical ethics committee approved the protocol. Participants were analyzed for the presence of the KIR6.2 E23K variant as previously described (6).

**Hyperglycemic clamp.** All subjects underwent 3-h hyperglycemic clamps at 10 mmol/l glucose before genotyping. The glucose level of 10 mmol/l was chosen on the basis of our previous observations in healthy volunteers, regarding the half-maximally stimulating blood glucose level for first- and second-phase glucose-induced insulin secretion (23). Presumably, changes in  $\beta$ -cell function can best be picked up around these blood glucose levels. First-phase insulin secretion was calculated as area under the curve during the first 10 min of the clamp. Second-phase insulin secretion was calculated as the average insulin level during the third hour minus basal. Details of the clamp procedure were as previously described (16,21). We assessed the insulin sensitivity index with the hyperglycemic clamps as the amount of glucose metabolized (expressed as milligrams per kilograms per minute) divided by average plasma insulin level of the third hour (expressed as picomoles per liter). In previous studies it has been shown that insulin sensitivity as assessed in this manner gives results similar to those of assessments with the gold standard, the hyperinsulinemic-euglycemic clamp (24). The DI was calculated as the product of first-phase insulin secretion and insulin sensitivity (11).

**Statistical analysis.** Statistical analysis was performed with SPSS version 10.0 software (SPSS, Chicago, IL). All data are presented as means  $\pm$  SE or

median with interquartile range. ANOVA or Mann-Whitney *U* test was used for general comparisons between the different genotypes. Variables were log transformed before analysis if necessary. Adjustments for age, sex, and BMI were done in separate analyses for all parameters. A priori power calculations indicate that our study design would allow the detection of a difference in first- or second-phase insulin secretion between 20 and 40% with 90% power, dependent upon the model applied (recessive or dominant, data not shown). Results were regarded significant at  $P \leq 0.05$ .

## ACKNOWLEDGMENTS

This work was supported by grants from the European Economic Community (BMH4-CT-98-3084) and the Diabetes Fonds Nederland.

The authors would like to thank the participants for their cooperation.

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