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 ~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 ± 3% decrease from baseline in Lys/Lys, 18 ± 2% in Glu/Lys, and 20 ± 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...
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 CGG CAT ACA CCG CTG-3′ and antisense 5′-CCG CTG CAC AGG AAG AAC GAC AT-3′. We found frequencies of 37% in the subjects was not distributed was in Hardy-Weinberg equilibrium (P=0.9). The rare allelic frequency in the subjects was not distributed was in Hardy-Weinberg equilibrium (P=0.9). The rare allelic frequency in the subjects was not distributed was in Hardy-Weinberg equilibrium (P=0.9).

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

<table>
<thead>
<tr>
<th>Glu/Glu</th>
<th>Glu/Lys</th>
<th>Lys/Lys</th>
<th>P (ANOVA)</th>
<th>P Glu/Glu vs. X/Lys (t test)</th>
<th>P Glu/Glu vs. Lys/Lys (t test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (M/F)</td>
<td>111 (37/74)</td>
<td>151 (52/99)</td>
<td>36 (10/26)</td>
<td>0.75</td>
<td>1.00</td>
</tr>
<tr>
<td>IGT/NGT</td>
<td>15/3</td>
<td>19/12</td>
<td>7/29</td>
<td>0.58</td>
<td>1.00</td>
</tr>
<tr>
<td>Age (years)</td>
<td>7/2</td>
<td>37/2</td>
<td>2.0</td>
<td>0.49</td>
<td>0.24</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>28.0 ± 7</td>
<td>26.8 ± 0.5</td>
<td>27.0 ± 1.1</td>
<td>0.37</td>
<td>0.16</td>
</tr>
<tr>
<td>WHR</td>
<td>0.87 ± 0.01</td>
<td>0.87 ± 0.01</td>
<td>0.85 ± 0.01</td>
<td>0.49</td>
<td>0.56</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>5.1 ± 0.1</td>
<td>5.1 ± 0.04</td>
<td>5.1 ± 0.1</td>
<td>0.79</td>
<td>0.50</td>
</tr>
<tr>
<td>Fasting plasma insulin (pmol/l)</td>
<td>60.5</td>
<td>60.4</td>
<td>54.7</td>
<td>0.81</td>
<td>0.83</td>
</tr>
<tr>
<td>Fasting plasma glucagon (ng/l)</td>
<td>63.3</td>
<td>67.2</td>
<td>66.4</td>
<td>0.47</td>
<td>0.23</td>
</tr>
<tr>
<td>2-h plasma glucose (mmol/l)</td>
<td>5.9 ± 0.1</td>
<td>6.1 ± 0.1</td>
<td>6.3 ± 0.3</td>
<td>0.38</td>
<td>0.23</td>
</tr>
<tr>
<td>2-h plasma insulin (pmol/l)</td>
<td>354 ± 30</td>
<td>362 ± 23</td>
<td>357 ± 55</td>
<td>0.98</td>
<td>0.85</td>
</tr>
<tr>
<td>2-h glucagon (ng/l)</td>
<td>51.2</td>
<td>54.2</td>
<td>55 ± 3</td>
<td>0.20</td>
<td>0.08</td>
</tr>
<tr>
<td>AUCinc glucose (mmol·h·l-1)</td>
<td>221 ± 14</td>
<td>235 ± 12</td>
<td>262 ± 25</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>Insulin sensitivity index*†</td>
<td>17.7 ± 1.0</td>
<td>17.2 ± 0.9</td>
<td>17.4 ± 1.8</td>
<td>0.14</td>
<td>0.05</td>
</tr>
</tbody>
</table>

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

**Analytical procedures.** Blood glucose was determined with a HemoCue blood glucose photometer (HemoCue AB, Angelholm, Sweden) at 5-min intervals. Samples for insulin, microplate enzyme immunosay (Abbott Laboratories, Tokyo, Japan), C-peptide (radioimmunoassay; Byk-Sangtec, Dietzenbach, Germany), and glucagon (radioimmunoassay; Linco Research, St. Charles, MO) were taken at 0, 15, 0, 2.5, 5, 7.5, 10, 20, 30, 60, 80, 100, 120, 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 GIP phase, mean of 125-130 min; GIP 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 clamp were calculated as follows:

1. **Parameters were logarithmically transformed to approximate linearity if necessary.**
2. The secretion indexes of the hyperglycemic clamp were calculated as follows: first phase, mean of 2.5-10 min; second phase, mean of 80-120 min; first GIP phase, mean of 125-130 min; GIP 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).
In the OGTT group, the Lys allele of the KIR6.2

RESULTS

values using the unpaired Student
referred to as X/Lys. Comparisons between genotypes were made on adjusted
(0.15 ± 0.02 μmol·kg⁻¹·min⁻¹ per pmol/l) and X/Lys (0.16 ± 0.02 μmol·kg⁻¹·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 μmol·kg⁻¹·min⁻¹ per pmol/l) and X/Lys (0.11 ± 0.01 μmol·kg⁻¹·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 ± 174 ng·l⁻¹·h) compared with Glu/Glu (6.631 ± 194 ng·l⁻¹·h; P = 0.027) after adjustment for
In Lys/Lys, the GLP-1 and arginine infusion in the modified hyperglycemic clamp did not 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.

In this study, we attempted to identify a mechanism by which the Glu23Lys polymorphism in the KIR6.2 gene may affect 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 AUCinc 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**

<table>
<thead>
<tr>
<th>Glu/Glu</th>
<th>Glu/Lys</th>
<th>Lys/Lys</th>
<th>P (ANOVA)</th>
<th>P Glu/Glu vs. X/Lys (t test)</th>
<th>P Glu/Glu vs. Lys/Lys (t test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-peptide (pmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First phase</td>
<td>1,311 ± 114</td>
<td>1,314 ± 83</td>
<td>1,295 ± 215</td>
<td>1.00</td>
<td>0.98</td>
</tr>
<tr>
<td>Second phase</td>
<td>2,463 ± 135</td>
<td>2,473 ± 158</td>
<td>2,471 ± 295</td>
<td>0.99</td>
<td>0.97</td>
</tr>
<tr>
<td>GLP, first phase</td>
<td>5,493 ± 302</td>
<td>5,885 ± 431</td>
<td>5,889 ± 932</td>
<td>0.77</td>
<td>0.47</td>
</tr>
<tr>
<td>GLP, second phase</td>
<td>8,590 ± 449</td>
<td>9,063 ± 852</td>
<td>8,473 ± 1,099</td>
<td>0.85</td>
<td>0.74</td>
</tr>
<tr>
<td>Maximum</td>
<td>10,869 ± 614</td>
<td>10,912 ± 1,029</td>
<td>10,285 ± 1,287</td>
<td>0.91</td>
<td>0.90</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First phase</td>
<td>241 ± 41</td>
<td>216 ± 23</td>
<td>253 ± 73</td>
<td>0.82</td>
<td>0.76</td>
</tr>
<tr>
<td>Second phase</td>
<td>302 ± 49</td>
<td>283 ± 37</td>
<td>319 ± 104</td>
<td>0.91</td>
<td>0.89</td>
</tr>
<tr>
<td>GLP, first phase</td>
<td>1,651 ± 238</td>
<td>1,752 ± 222</td>
<td>1,813 ± 448</td>
<td>0.92</td>
<td>0.70</td>
</tr>
<tr>
<td>GLP, second phase</td>
<td>3,700 ± 490</td>
<td>3,689 ± 528</td>
<td>3,452 ± 873</td>
<td>0.96</td>
<td>0.90</td>
</tr>
<tr>
<td>Maximum</td>
<td>5,512 ± 626</td>
<td>5,566 ± 704</td>
<td>5,065 ± 1,137</td>
<td>0.92</td>
<td>0.91</td>
</tr>
<tr>
<td>Glucagon (relative to baseline)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First phase</td>
<td>0.80 ± 0.02</td>
<td>0.82 ± 0.02</td>
<td>0.90 ± 0.03</td>
<td>0.01</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Data are means ± SE. P values are unadjusted for multiple comparisons.

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.
consistent 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 β-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 altered β-cell function during systemic 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, Schwantechter 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 KATP 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.

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