KCNJ11 E23K affects diabetes risk and is associated with the Disposition index: results of two independent German cohorts

Antje Fischer¹, Eva Fisher, PHD², Matthias Möhlig, MD¹, Matthias Schulze, DRPH², Kurt Hoffmann, PHD², Martin O. Weickert, MD¹, Rita Schueler¹, Martin Osterhoff, PHD¹, Andreas F.H. Pfeiffer, MD¹, Heiner Boeing, PHD², Joachim Spranger, MD¹

¹German Institute of Human Nutrition Nuthetal and Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin, ²German Institute of Human Nutrition Potsdam-Rehbrücke, Epidemiology, Nuthetal, Germany

Correspondence:
Joachim Spranger, MD
Clinical Nutrition,
German Institute of Human Nutrition Potsdam-Rehbrücke
Arthur-Scheunert-Allee 114-116
14558 Nuthetal
Germany
Email: joachim.spranger@charite.de

Received for publication 19 June 2007 and accepted in revised form 16 September 2007.
Various cross-sectional studies suggested that a polymorphism (E23K) within the ATP-sensitive potassium channel KCNJ11 gene is associated with type 2 diabetes (1). However, only two prospective studies have addressed the relation between KCNJ11 E23K and type 2 diabetes and these studies were intervention trials based on individuals with IFG/IGT (2-4). With respect to functional effects, recent studies were inconsistent to demonstrate a relation of the polymorphism with markers of insulin secretion (3; 5; 6) although in vitro studies clearly suggested a defect in insulin secretion (7-9). One study proposed a relation to glucagon response, while insulin secretion itself was not affected (10). However, the relation between polymorphism and insulin secretion might have been masked in some of those studies by differences of insulin sensitivity and detailed analysis might thus require consideration of insulin sensitivity of the study participants, such as given in the disposition index (11).

We here investigated the effect of KCNJ11 E23K on diabetes risk within a prospective case-cohort study (n=2,945) of the EPIC-Potsdam cohort. We additionally tested the association with diabetes in a second, independent cross-sectional study, the MetabolicSyndrome BerlinPotsdam (MeSyBePo)-study with 1,891 subjects was investigated. Details of recruitment and phenotyping (Case/Control: age 59.57/50.63 years; ♀55.5%/69.1%; ♂44.5%/30.9%; BMI 32.22/28.67) of this study participants were also published recently (16). In all participants of MesyBepo a 75g oral glucose tolerance test (OGTT) with insulin measurements was performed. Only individuals with normal glucose tolerance (n=1,070) or confirmed Type 2 Diabetes mellitus (n=324) were considered for analysis of the association with type 2 diabetes. The association between the polymorphism and the Disposition Index was investigated only in individuals with NGT, IFG or IGT, since accepted markers of insulin sensitivity and secretion have been shown to be unreliable in patients with type 2 diabetes mellitus, especially in patients with anti-diabetic treatment. A euglycemic hyperinsulinemic clamp was performed in a subset of 56 healthy controls. In these participants, the Insulin Sensitivity Index (ISI) according to Stumvoll (ISI was calculated as: $0.157 - 4.576 \times 10^{-5} \text{Ins}_{120} - 0.00519 \text{Gluc}_{90} - 0.000299 \text{Ins}_{0}$) correlated best to the M-value ($r=0.591; p<0.001$) of the clamps (17). Therefore ISI was subsequently used to estimate insulin sensitivity in the total cohort. As the characteristics of our study cohort were basically comparable to those of Stumvoll's cohort, the ratio of AUC_{Insulin} / AUC_Glucose, which performed best in Stumvoll's study compared to a hyperglycemic clamp, was used to estimate insulin secretion. Correspondingly the disposition index (DI) was calculated as the product of ISI and

**Research Design and Methods:**

We designed a case–cohort study within the EPIC-Potsdam study, a prospective cohort involving 27,548 Caucasian volunteers mainly aged 35–65 years from the general population (12-14). 2,263 individuals were randomly selected for a subcohort. Because the subcohort is representative of the entire cohort at baseline, 68 incident cases belonged to the sub-cohort and 682 incident cases were identified in the remainder of the total cohort, the latter classified as ‘external’ cases (external cases/random sub-cohort: age 54.59/49.50 years; ♀283/1394, ♂399/869; BMI 30.41/25.95, mean follow up 7 years) (15). We used all incident cases (internal and external cases) in Cox regression models accounting for the case-cohort design.

For confirmation of genetic association and additional analysis of diabetes-associated subtraits, the cross-sectional MetabolicSyndrome BerlinPotsdam (MeSyBePo)-study with 1,891 subjects was investigated. Details of recruitment and phenotyping (Case/Control: age 59.57/50.63 years; ♀55.5%/69.1%; ♂44.5%/30.9%; BMI 32.22/28.67) of this study participants were also published recently (16). In all participants of MesyBepo a 75g oral glucose tolerance test (OGTT) with insulin measurements was performed. Only individuals with normal glucose tolerance (n=1,070) or confirmed Type 2 Diabetes mellitus (n=324) were considered for analysis of the association with type 2 diabetes. The association between the polymorphism and the Disposition Index was investigated only in individuals with NGT, IFG or IGT, since accepted markers of insulin sensitivity and secretion have been shown to be unreliable in patients with type 2 diabetes mellitus, especially in patients with anti-diabetic treatment. A euglycemic hyperinsulinemic clamp was performed in a subset of 56 healthy controls. In these participants, the Insulin Sensitivity Index (ISI) according to Stumvoll (ISI was calculated as: $0.157 - 4.576 \times 10^{-5} \text{Ins}_{120} - 0.00519 \text{Gluc}_{90} - 0.000299 \text{Ins}_{0}$) correlated best to the M-value ($r=0.591; p<0.001$) of the clamps (17). Therefore ISI was subsequently used to estimate insulin sensitivity in the total cohort. As the characteristics of our study cohort were basically comparable to those of Stumvoll's cohort, the ratio of AUC_{Insulin} / AUC_Glucose, which performed best in Stumvoll's study compared to a hyperglycemic clamp, was used to estimate insulin secretion. Correspondingly the disposition index (DI) was calculated as the product of ISI and
AUC_{Insulin} / AUC_{Glucose} (11; 17; 18). Both studies have been approved by the local ethic authorities. Genotyping was performed by Taqman-technology (HT7900 System; ABI, Foster City, CA, USA). Details of genotyping will be given by the authors upon request.

Data were analyzed using SPSS (SPSS Inc., Chicago, IL, USA, version 12.0) and SAS (SAS Institute, Cary, NC, USA, version 9.1). General linear model was calculated to analyse the effects of the polymorphism on continuous variables after adjustment for confounders (age, sex, BMI). Relative risks (RRs) were calculated using Cox proportional hazards regression modified according to the Barlow method in EPIC-Potsdam. Unconditional logistic regression analysis was performed to estimate odds ratios in MeSyBePo. Multiplicative interaction terms between the genotype and age, sex or BMI, respectively, were used to analyze potential interactions of these variables and disease risk. A two-tailed α-error below 5% was considered to be significant.

Results
In both studies, the KCNJ11 E23K polymorphism was found in Hardy-Weinberg-Equilibrium. In the prospective EPIC-Potsdam cohort, the polymorphism was associated with increased relative risk for type 2 diabetes after adjustment for age, gender and BMI (Table 1A), although point estimates reached no statistical significance. Results were not indicative for effect modification with age or BMI. However, in gender specific sub analyses we found a significant association among women [RR (95% CI): KK 1.92 (1.21-3.04)], while no association was observed among men (RR (95% CI): KK 0.96 (0.60-1.53); p for interaction: 0.098). The association between KCNJ11 E23K and prevalent Type 2 Diabetes was significant in the MeSyBePo cohort [OR (95%CI): EK 1.32 (0.97-1.79); KK 1.85 (1.18-2.91)] (Table 1B). However, in some contrast to the results in the EPIC-cohort, comparable point estimates were found among women [OR (95% CI): KK 1.85 (1.03-3.35)] and men (OR (95% CI): KK 1.86 (0.92-3.76)). Interestingly, analysis of subtraits in this second cohort additionally demonstrated an effect of the polymorphism on the disposition index. Thus, individuals with the EE-genotype had a higher Disposition index than those with EK or KK-genotype (crude model: EE 3.66±0.02, EK 3.45±0.02, KK 3.36±0.03; p=0.003). This relation remained significant after adjustment for age, sex and BMI (Table 1C).

Conclusions
The here presented data from one prospective observational study and one cross-sectional study basically confirm that KCNJ11 E23K is associated with an increased risk of type 2 diabetes in Caucasian individuals, particularly among women. A potential gender-specific effect should be further investigated in subsequent studies or meta-analyses. The association with the disposition index suggested that consideration of insulin sensitivity may be required to elucidate effects of KCNJ11 E23K on insulin secretion and may partially explain controversial results of previous studies.

Some limitations of this study should be mentioned. Although other polymorphisms in KCNJ11 and ABCC8 might be relevant in the relation to type 2 diabetes, this study focused exclusively on KCNJ11 E23K. First, a considerable number of previous cross-sectional studies demonstrated associations between this polymorphism and type 2 diabetes. Second, in vitro data suggested a functional relevance of this polymorphism. Third, covering the KCNJ11 and the associated ABCC8 gene with haplotype tagging SNPs would have required considerably larger cohorts to avoid an underpowered study. Although we aimed to investigate a clear a-priori defined hypothesis, we cannot exclude that other polymorphisms in the genomic region of KCNJ11 might confer the here described relation between KCNJ11 E23K and type 2 diabetes.
Taken together, the here presented data further support a role of KCNJ11 E23K in the pathogenesis of type 2 diabetes mellitus in Caucasian individuals.

Acknowledgements:
JS is supported by the BMBF (PTJ-BIO/0313847A) and a Heisenberg-Professorship of the Deutsche Forschungsgemeinschaft (DFG SP716/2-1). This project has been funded by the BMBF (01GSO487) within the framework of NGFN2 (Neuronetz Adipositas).
†Kurt Hoffmann deceased during the review process of the manuscript and the authors would like to dedicate this manuscript to our colleague and friend.
References:


TABLE 1
Relation between KCNJ11 E23K and diabetes in the prospective EPIC-Potsdam cohort (A), the cross-sectional MeSyBePo cohort (B) and association with the Disposition Index in the cross-sectional MeSyBePo cohort (C). The subcohort of EPIC-Potsdam was representative for the entire cohort and thus included 68 incident cases. Those were considered as cases in Cox regression models accounting for the case-cohort design together with the “external” incident cases of the remainder cohort.

<table>
<thead>
<tr>
<th>A</th>
<th>Genotype</th>
<th>OddsRatio (95%CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n_{subcohort}/n_{case})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>adjustment</td>
<td>age, gender, BMI</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EE (913/257)</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EK (1052/326)</td>
<td>1.13 (0.90-1.42)</td>
<td>0.281</td>
</tr>
<tr>
<td></td>
<td>KK (298/99)</td>
<td>1.25 (0.91-1.71)</td>
<td>0.163</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B</th>
<th>Genotype</th>
<th>OddsRatio (95%CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n_{control}/n_{case})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>adjustment</td>
<td>age, gender, BMI</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EE (444/121)</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EK (492/156)</td>
<td>1.32 (0.97-1.79)</td>
<td>0.080</td>
</tr>
<tr>
<td></td>
<td>KK (134/47)</td>
<td>1.85 (1.18-2.91)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C</th>
<th>Genotype</th>
<th>Mean (±SE)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>age, gender, BMI</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EE (627)</td>
<td>3.64 (±0.02)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EK (730)</td>
<td>3.46 (±0.02)</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>KK (210)</td>
<td>3.38 (±0.03)</td>
<td></td>
</tr>
</tbody>
</table>