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The Glu23Lys polymorphism in *KCNJ11* and impaired hypoglycaemia awareness in patients with type 1 diabetes

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Abstract Impaired awareness of hypoglycaemia affects approximately 25% of all patients with type 1 diabetes (T1D). Duration of diabetes and tight glycaemic control represent the main risk factors for hypoglycaemia unawareness. However, even among patients with good glycaemic control and longstanding T1D, awareness of hypoglycaemia may be intact. Genetic factors might explain some of this remaining variability, and genes involved in central glucose sensing should represent plausible candidates. Some evidence indicates that ventromedial hypothalamus glucose-responsive neurons require the potassium inward rectifier (KIR) 6.2 subunit of the K_{ATP} channel to sense glucose. Therefore, the effects of the Glu23Lys polymorphism in the *KCNJ11* (*KIR6.2*) gene (potassium inwardly rectifying channel, subfamily J, member 11) on impaired hypoglycaemia awareness in 217 patients with T1D were studied. Hypoglycaemia awareness status was determined using standardized questionnaires. Genotyping of the Glu23-Lys in a cohort of T1D subjects was done using the TaqMan allelic discrimination assay (frequency of the Lys-allele = 0.35; $P = 0.57$ for Hardy–Weinberg equilibrium). The study confirms that diabetes duration, C-peptide, and HbA_{1c} represent risk factors for impaired hypoglycaemia awareness. However, no significant effect of the Glu23Lys polymorphism on impaired hypoglycaemia awareness was observed with or without adjustment for age, diabetes duration, C-peptide, and HbA_{1c} . Even though the study provides a relatively large dataset, it is possible that small differences may have been missed.

Keywords Genetics · Type 1 diabetes · Hypoglycaemia unawareness · Potassium inwardly rectifying channel

Introduction

Impaired awareness of hypoglycaemia is a major limiting factor in managing type 1 diabetes (T1D) (Cryer 1994) and occurs in about 25% of patients (Gerich et al. 1991), leading to a sixfold increased risk of severe hypoglycaemia due to the loss of autonomic warning symptoms before the development of neuroglycopenia (Gold et al. 1994). Several risk factors for impaired awareness of hypoglycaemia have been identified, including long duration of diabetes, tight glycaemic control (i.e., low haemoglobin A1c), and repeated episodes of hypoglycaemia (Clarke et al. 1991; Gerich et al. 1991; DCCT 1993; Amiel 1994; Cryer 1994; Mookan et al. 1994). However, even among patients with good glycaemic control and longstanding T1D, awareness of hypoglycaemia may be intact. Although the role of genetics in regulating hypoglycaemia awareness has not been systematically investigated, it is possible that genetic factors explain some of this remaining variability (Pedersen-Bjergaard et al. 2003). Some people may be equipped with a higher glucose threshold below which autonomic symptoms develop. This would allow symptoms to show at higher glucose concentrations and decrease the likelihood for true hypoglycaemia, thus protecting from development of impaired hypoglycaemia awareness. Conversely, individuals with an intrinsically lower threshold are more prone to developing symptomatic hypoglycaemia.

Because the central nervous system has a critical role in sensing hypoglycaemia (Amiel 1994), genes involved in central glucose sensing should represent plausible candidates. One molecular correlate for the hypothalamic glucose sensor is the potassium inward rectifier (KIR) 6.2 subunit of the K_{ATP} channel, which is encoded by *KCNJ11* (potassium inwardly rectifying

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channel, subfamily J, member 11). While this gene may be better known for its role in beta cell function (Riedel et al. 2005), there is good evidence that glucose-responsive neurons of the ventromedial hypothalamus (VMH) also require the KIR6.2 subunit for glucose sensing (Miki et al. 2001). Miki et al. (2001) showed that mice with deletion of the Kir6.2 pore-forming subunit of the K_{ATP} channel (*Kir6.2^{-/-}*) both lack functional glucose-responsive (GR) neurons in the VMH and show severely impaired neurohumoral counterregulatory responses. In *Kir6.2^{-/-}* mice, the brain-mediated glucagon component of the counterregulatory response to hypoglycaemia is attenuated, and the ability to correct hypoglycaemia is impaired. These findings indicate that VMH GR neurons require the Kir6.2 subunit of the K_{ATP} channel to sense glucose (Levin et al. 2001).

A common polymorphism (Glu23Lys) exists in this gene, which in cell models has been shown to alter the channel's responsiveness to glucose (Schwanstecher et al. 2002). We therefore studied whether this polymorphism is associated with the presence or absence of impaired awareness of hypoglycaemia.

Materials and methods

Subjects

The protocol was approved by the Ethical Committee of the University of Leipzig School of Medicine. All patients gave written informed consent to participate in the study. We recruited 217 patients with T1D of at least 2 years' duration. Participants were treated at the Clinic Lippe-Detmold, a large city hospital, on an in- and outpatient basis. The study included a clinical examination, laboratory analyses, and detailed questionnaires specifically addressing awareness of hypoglycaemia, current health, co-medication, medical history, compli-

cations of diabetes, and lifestyle factors such as dietary habits, physical activity, smoking, alcohol consumption, and psychosocial conditions.

Patients had either typical T1D (*N*=164) or latent autoimmune diabetes in adults (LADA; *N*=53). T1D was defined by young onset (age < 30 years), severe hyperglycaemia or ketoacidosis, respectively, and insulin treatment from the time of diagnosis. Patients were classified as having LADA if they had been treated with insulin within 6 months of diagnosis, if they were anti-GAD positive, and if their fasting C-peptide level was < 0.1 nmol/l.

Assessment of hypoglycaemia awareness status

At present, there is no consensus on how to classify hypoglycaemia awareness. Therefore, all participants completed one questionnaire developed by Clarke et al. (1995) and a second one based on the Edinburgh Hypoglycaemia Scale (Gold et al. 1994; McAulay et al. 2001) to assess their previous experiences of hypoglycaemia and their self-estimated state of hypoglycaemic awareness. These methods can be applied to all patients and have already been used by others (Gold et al. 1994; Clarke et al. 1995; McAulay et al. 2001; Pedersen-Bjergaard et al. 2003). Because some episodes of severe hypoglycaemia may not be recalled by the diabetic patients themselves (Jorgensen et al. 2003), our participants were asked to answer the questionnaires in the presence of their partners or relatives. One hundred and fifty-four subjects filled out the questionnaires in the presence of their partners or relatives. Episodes of severe hypoglycaemia were defined as events of hypoglycaemia requiring intravenous glucose or glucagon injection. According to the questionnaire by Clarke et al. (1995), we obtained a score that can be translated into a dichotomous variable: intact vs. impaired hypoglycaemia

Table 1 Clinical characteristics of all participants (total) and according to *KCNJ11* genotypes (Glu/Glu, Glu/Lys, Lys/Lys)

	Total (<i>N</i> =217)	Glu/Glu (<i>N</i> =91)	Glu/Lys (<i>N</i> =102)	Lys/Lys (<i>N</i> =24)	<i>P</i>
Demography					
Age (years)	41 ± 17	41 ± 18	40 ± 17	42 ± 15	0.87
<i>M/F</i>	114/104	42/49	58/44	14/10	0.28 ^a
Clinical characteristics					
Body mass index (kg/m ²)	24 ± 4	24 ± 4	25 ± 3	24 ± 4	0.32
Duration of diabetes (years)	18 ± 14	19 ± 15	17 ± 13	21 ± 12	0.16
Daily insulin (IU)	47.4 ± 18.6	46.4 ± 18.4	48.1 ± 18.6	49.1 ± 19.7	0.98
C-peptide (ng/ml)					
< 0.01	180 (83%)	73 (80%)	84 (82%)	22 (92%)	0.42 ^a
> 0.01	38 (17%)	18 (20%)	18 (18%)	2 (8%)	
% HbA _{1c}	7.8 ± 1.6	8.0 ± 1.6	7.4 ± 1.4	8.5 ± 2.0	0.004
Hypoglycaemia awareness score [based on Clarke et al. (1995)]	2.26 ± 3.72	2.42 ± 4.38	2.29 ± 3.36	1.46 ± 2.23	0.53
Hypoglycaemia awareness (<i>N</i>)					
Normal	132 (61%)	54 (59%)	61 (60%)	17 (71%)	
Impaired	86 (39%)	37 (41%)	41 (40%)	7 (29%)	0.57 ^a

^a χ^2 test

Data are mean ± SD; *P*-values for comparisons between genotypic groups by ANOVA statistics

awareness. Briefly, for each subject a score was obtained characterising the hypoglycaemia awareness status. Subjects with a score <2 were considered as having normal awareness and those with a score >3 as having impaired hypoglycaemia awareness.

The second questionnaire was based on the Edinburgh Hypoglycaemia Scale; a simple seven-point scale has been validated to assess autonomic, neuroglycopenic, and nonspecific symptoms of hypoglycaemia, with 1 meaning that symptoms are not present at all, and 7 meaning that a great number of symptoms are present (Gold et al. 1994; McAulay et al. 2001). Awareness of hypoglycaemia was defined as normal if no subjective alteration in warning symptoms was reported and if predominantly autonomic warning symptoms were associated with the onset of acute hypoglycaemia. In contrast, patients with impaired awareness of hypoglycaemia experienced predominantly neuroglycopenic symptoms and scored between 1 and 2 with regard to autonomic warning symptoms.

The assessment of the status of hypoglycaemia awareness was in congruence between the two questionnaires applied in our study.

Genotyping of the Glu23Lys polymorphism

Genotyping of the Glu23Lys polymorphism in a cohort of subjects with T1D ($N=217$) was done using the TaqMan allelic discrimination assay (Assays-on-Demand, SNP Genotyping Products: C_11654065_10; Applied Biosystems). The TaqMan genotyping reaction was amplified on a GeneAmp PCR system 9700 (95°C for 15 min, 95°C for 15 s, and 60°C for 1 min, for 38 cycles), and fluorescence was detected on an ABI PRISM 7000 sequence detector (Applied Biosystems).

Statistics

Standard descriptive and comparative statistics (χ^2 -test, ANOVA) were used to characterise and compare clinical parameters in different genotypic groups. Univariate and logistic regression analyses were used to calculate

the effects of investigated factors on impaired hypoglycaemia awareness in 217 subjects with T1D, which was reported as the relative risk (with 95% CI) of impaired hypoglycaemia awareness. The analysis was also carried out in a subgroup of 154 patients who filled out the questionnaires in the presence of their relatives or partners. Data were analysed using the SPSS software package, version 11.5 (SPSS, Chicago, IL, USA).

Results and discussion

We genotyped the Glu23Lys polymorphism in a cohort of 217 subjects with T1D who had been recruited for hypoglycaemia awareness status [Table 1; frequency of the A-allele (Lys)=0.35]. The genotype distribution was consistent with Hardy–Weinberg equilibrium ($P=0.57$). In the univariate analyses, significant risk factors for impaired awareness of hypoglycaemia were C-peptide status, %HbA_{1c}, diabetes duration, and age. In a multivariate analysis duration of diabetes, plasma C-peptide concentration and low HbA_{1c} remained independent determinants of impaired awareness of hypoglycaemia (Table 2), confirming previous studies. No significant effect of the Glu23Lys polymorphism on hypoglycaemia awareness status was observed with or without adjustment for age, diabetes duration, C-peptide, and HbA_{1c} (Table 2). When the analysis was performed without subjects having LADA, the results remained unchanged. Similarly, no significant effect of the variant was found in a subgroup of patients ($N=154$) who filled out the questionnaires to assess hypoglycaemia awareness status in the presence of their partners or relatives [$P=0.761$; relative risk and 95% CI=0.908 (0.486–1.694)].

Although evidence from experimental models supports the role of Kir6.2 in K_{ATP} channel activity and glucose responsiveness of VMH neurons, a common genetic polymorphism in the *KCNJ11* with functional relevance does not seem to play a role in the risk of developing impaired hypoglycaemia awareness. This probably has to do with the presence of other glucosensing mechanisms not requiring K_{ATP} channels. For example, in mice lacking *Kir6.2*, the counterregulatory response is not totally abolished (Miki et al. 2001).

Table 2 Analyses of the influence of *KCNJ11* genotypes (Glu23Lys) and other factors on impaired hypoglycaemia awareness in subjects with type 1 diabetes

Variable	Univariate analysis relative risk (95% CI)	<i>P</i>	Multivariate analysis relative risk (95% CI)	<i>P</i>
KCNJ11 genotype (additive model)	0.845 (0.557–1.282)	0.429	0.651 (0.375–1.130)	0.127
KCNJ11 genotype (dominant model)	1.668 (0.661–4.210)	0.278	2.248 (0.658–7.684)	0.196
KCNJ11 genotype (recessive model)	0.990 (0.942–1.041)	0.703	1.575 (0.771–3.215)	0.212
C-peptide (negative vs. positive)	5.40 (2.016–14.465)	0.001	5.56 (1.744–17.723)	0.004
% HbA _{1c}	0.679 (0.552–0.837)	<0.0001	0.611 (0.450–0.830)	0.002
Duration of diabetes (>20 years vs. <10 years)	4.239 (2.161–8.316)	<0.0001	2.944 (1.144–7.574)	0.025
Age	1.034 (1.016–1.052)	<0.0001	1.003 (0.977–1.029)	0.844
Sex	1.365 (0.790–2.358)	0.264	–	–

Therefore, it is likely that polymorphisms in the *KCNJ11* are insufficient for affecting the function of the K_{ATP} channel to the extent necessary to result in changes in hypoglycaemia awareness in subjects with T1D.

It is worth noting, however, that even though we provided a relatively large dataset, we were clearly underpowered to detect small differences. To detect a 25% difference in the proportion of impaired awareness to the awareness of hypoglycaemia between the genotype groups, we had a power of approximately 70% ($\alpha=0.05$). Smaller effects are likely to be missed by this type of study.

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