CLINICAL STUDY

Co-localisation of the Kir6.2/SUR1 channel complex with glucagon-like peptide-1 and glucose-dependent insulinotrophic polypeptide expression in human ileal cells and implications for glycaemic control in new onset type 1 diabetes

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Abstract

Objective: The ATP-dependent K^+-channel (K_{ATP}) is critical for glucose sensing and normal glucagon and insulin secretion from pancreatic endocrine α- and β-cells. Gastrointestinal endocrine L- and K-cells are also glucose-sensing cells secreting glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotrophic polypeptide (GIP) respectively. The aims of this study were to 1) investigate the expression and co-localisation of the K_{ATP} channel subunits, Kir6.2 and SUR1, in human L- and K-cells and 2) investigate if a common hyperactive variant of the Kir6.2 subunit, Glu23Lys, exerts a functional impact on glucose-sensing tissues *in vivo* that may affect the overall glycaemic control in children with new-onset type 1 diabetes.

Design and methods: Western blot and immunohistochemical analyses were performed for expression and co-localisation studies. Meal-stimulated C-peptide test was carried out in 257 children at 1, 6 and 12 months after diagnosis. Genotyping for the Glu23Lys variant was by PCR-restriction fragment length polymorphism.

Results: Kir6.2 and SUR1 co-localise with GLP-1 in L-cells and with GIP in K-cells in human ileum tissue. Children with type 1 diabetes carrying the hyperactive Glu23Lys variant had higher HbA_{1C} at diagnosis (coefficient = 0.61%, $P = 0.02$) and 1 month after initial insulin therapy (coefficient = 0.30%, $P = 0.05$), but later disappeared. However, when adjusting HbA_{1C} for the given dose of exogenous insulin, the dose-adjusted HbA_{1C} remained higher throughout the 12 month study period (coefficient = 0.42%, $P = 0.03$).

Conclusions: Kir6.2 and SUR1 co-localise in the gastrointestinal endocrine L- and K-cells. The hyperactive Glu23Lys variant of the K_{ATP} channel subunit Kir6.2 may cause defective glucose sensing in several tissues and impaired glycaemic control in children with type 1 diabetes.

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Introduction

The pancreatic endocrine α- and β-cells are glucose-sensing cells equipped with an ATP-sensitive K^+-channel (K_{ATP} channel), consisting of two subunits, SUR1 and Kir6.2. Apart from the α- and β-cells, the intestinal endocrine L- and K-cells are also glucose-sensing tissues secreting either of the incretin hormones, glucagon-like peptide-1 (GLP-1) or glucose-dependent insulinotrophic peptide (GIP), in response to glucose. The majority of K-cells are in the proximal part of the small intestine whereas the majority of L-cells are

found in the distal part of the small intestine. It is assumed, from mRNA studies in a murine L-cell like cell line, GLUTag (1), that the K_{ATP} channel subunits are also co-expressed in L- and K-cells *in vivo*. The K_{ATP} channel subunits Kir6.2 and SUR1 have, however, not been identified at the protein level in primary human tissues from the proximal or distal gut.

Rare mutations in the *Kir6.2* gene, causing various degrees of hyperactivity of the channel complex, associate with non-autoimmune diabetes syndromes including transient neonatal diabetes and permanent neonatal diabetes alone or with developmental delay

and epilepsy (2–4). In contrast, mutations that cause loss of channel activity associate with persistent hyperinsulinaemic hypoglycaemia of infancy (5, 6). A common variant, Glu23Lys, of the Kir6.2 subunit (*KCNJ11*) was the first naturally occurring variant of Kir6.2 *in vitro* that was found to induce a hyperactive state of the K_{ATP} channel complex (7) and reducing its sensitivity to the inhibitory action of ATP. In healthy subjects, the Glu23Lys variant associates with an impaired post glucose-load release of insulin (8, 9) and with a weaker suppression of glucagon secretion in response to hyperglycaemia (10). It has therefore been speculated that this reduction in β-cell function and increased α-cell activity in Glu23Lys carriers may explain the association of this variant with type 2 diabetes (8, 9).

Although the *KCNJ11* is a β-cell gene, the Glu23Lys polymorphism does not seem to increase the risk of developing type 1 diabetes (11), but its effect on disease progression and residual β-cell function in patients with newly onset type 1 diabetes has not been investigated. Type 1 diabetes is characterised by the progressive loss of insulin producing β-cells due to an autoimmune attack, and is estimated to retain 10% of the β-cell mass at the time of diagnosis (12). In parallel with the effect in impaired β-cell function (8, 9), the Glu23Lys variant also seems to have an effect on α-cell function (10). But as glucagon secretion from the α-cells may effectively be regulated by an intraislet paracrine insulin release from the β-cells, it is not possible to distinguish the effect of Glu23Lys in primary defective α-cell function from an indirect effect caused by impaired intraislet insulin release. This influence of intraislet insulin release can better be controlled by studying newly onset type 1 diabetes with progressive loss of β-cells and, therefore, offers ideal conditions for an *in vivo* study of glucose-induced glucagon secretion (13). The aims of the present study, therefore, were to 1) investigate the expression and co-localisation of the K_{ATP} channel subunits, Kir6.2 and SUR1, in human small intestine (L- and K-cells); 2) investigate *in vivo* if a common hyperactive variant, Glu23Lys, of the Kir6.2 subunit exerts a functional impact on postprandial glucose sensing in α-, β-, L- and K-cells; and 3) relate these effects to the overall glycaemic control in children with newly onset type 1 diabetes.

Subjects and methods

Subjects

This observational study was conducted in 18 centres representing 15 countries in Europe and Japan. Clinical information on demographics and anthropometry, insulin regimen as well as blood samples for centralised measurement of HbA$_{1c}$ (0, 1, 3, 6, 9, 12 months after diagnosis) and boost-stimulated C-peptide, GLP-1, GIP

and glucagon 1, 6, 12 months after diagnosis were collected prospectively. All children aged less than 16 years with newly diagnosed diabetes between August 1999 and December 2000, presenting to the paediatric departments of the participating centres, were eligible for the study. Exclusion criteria were: suspected non-type-1 diabetes (MODY, secondary diabetes etc), decline of enrolment into the study by patients or parents, and patients initially treated outside of the centres for more than 5 days. There were no significant differences with respect to gender distribution, age, anthropometric data, HbA$_{1c}$ at diagnosis, ethnicity or family history of diabetes between patients included and patients not included into the study (data not shown). Only four patients from Japan were included.

The diagnosis of type 1 diabetes was according to the World Health Organisation criteria.

The cohort included 131 girls and 126 boys, 85% of the patients were white Caucasian, age at clinical diagnosis was 9.1 ± 3.7 years (mean $\pm$ S.E.M.), BMI 16.5 ± 3.2 kg/m^2 (mean $\pm$ S.E.M.) and HbA$_{1c}$ $11.2 \pm 2.1\%$ (mean $\pm$ S.E.M.) at the time of diagnosis. Diabetic ketoacidosis was present in 20.6% of the cases at the time of diagnosis (HCO$_3 \leq 15$ mmol/l and/or pH < 7.30).

The study was performed according to the criteria of the Helsinki II Declaration and was approved by the local ethics committee in each centre. All patients, their parents or guardians gave informed consent.

Methods: sample preparation and western blot analysis of Kir6.2, SUR1 and GLP-1 on primary human ileum tissue

The primary antibodies used for western blot analysis were: polyclonal rabbit anti-GLP-1 code GLP2135 diluted 1:10 000 (14), polyclonal goat anti-Kir6.2 code N-18 diluted 1:200 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), polyclonal rabbit anti-SUR1 code H-18 diluted 1:200 (Santa Cruz Biotechnology).

The human ileum tissue sample was obtained at surgery for intestinal obstruction (male, 76 years). Pancreas and kidney were taken from a male Sprague Dawley rat anaesthetised with pentobarbital and perfused transcardially with 300 ml ice-cold Na$^+$-Krebs buffer (mM): NaCl 119, NaHCO$_3$ 15, KCl 4.6, CaCl$_2$ 1.5, NaH$_2$PO$_4$ 1.2, MgCl$_2$ 1.2 and glucose 5.5.

All tissues were immediately frozen on dry ice and stored at -80 °C until further processing. The frozen sample was first ground on dry ice using mortar and pestle and further homogenised with a mechanical stirrer in 50–100 μl of ice-cold lysis buffer (10 mM Tris (pH = 7.4), 50 mM β-glycerophosphate (disodium salt), 100 μM Na$_3$VO$_4$, 0,5% deoxycholate, 1 mM EGTA, 1 mM EDTA, 1 mM NaF, 20 mM Na$_4$P$_2$O$_7$ and 1% Triton X-100), containing a cocktail of protease inhibitors (1 mM dithiothreitol, 20 μM pepstatin, 20 μM leupeptin, 0,1 U/ml aprotinin and 1 mM phenylmethylsulphonyl fluoride). The samples were

centrifuged at 10 000 g for 10 min at 4 °C and the supernatants were collected and stored at −80 °C until further processing.

The protein content of the samples was determined using a Bio-Rad DC protein assay (Bio-Rad). Prior to protein separation by SDS–PAGE, the protein samples were diluted 1:1 with a loading buffer and heated at 95 °C for 5 min. Each protein sample was separated in a 10% SDS-polyacrylamide gel. The separated proteins were then transferred to a PVDF membrane (Bio-Rad). The PVDF blot was blocked by 5% non-fat dry milk in Tris-buffered saline (TBS) with 1% Tween-20 (TBS-T) for 1 h at room temperature. Blots were cut into half according to the expected molecular weights of the target antigens. The antibodies were diluted in TBS-T containing 3% non-fat dry milk and the upper half was incubated with the SUR1 primary antibody and the lower half was incubated with the Kir6.2 primary antibody and the blots were left overnight at 4 °C. The PVDF blots were then washed three times, 5 min each in TBS-T. The secondary antibodies, all conjugated to horseradish peroxidase, were diluted in TBS-T and incubated with the PVDF blot for 1 h at room temperature. The PVDF blots were then washed three times, 5 min each in TBS-T, before they were processed for analysis using an Enhanced ChemiLuminiscence detection kit (Pierce). The blots were scanned by an electronically cooled charge-coupled camera system (Fujifilm LAS-1000; Fujifilm, Tokyo 107-0052, Japan) and the chemifluorescent images were captured and stored digitally.

Immunohistochemistry analysis of Kir6.2/SUR1 and GLP-1 co-localisation on human ileum and pancreas sections

All immunohistochemistry analysis was carried out on formalin-fixed material. Human intestinal and pancreatic tissue ($n=6$) were obtained from archival material collected during the 1970s according to the contemporary ethical guidelines.

The antibodies used for immunohistochemistry were: monoclonal mouse anti-GLP-1 antibody code GLPb-17F1A37B22C4 (F1) diluted 1:3000, generous gift from Novo Nordisk, polyclonal rabbit anti-GLP-1 antibody code GLP2135 diluted 1:10 000 (14), polyclonal rabbit anti-GIP code GIP98171 diluted 1:3000 (15), polyclonal goat anti-Kir6.2 code N-18 diluted 1:3000 (Santa Cruz Biotechnology), polyclonal goat-anti SUR1 code C-16 diluted 1:1000 (Santa Cruz Biotechnology) and monoclonal mouse anti-insulin antibody code HUI 001 (Novo Nordisk, 2880 Bagsvaerd, Denmark) diluted 1:5000.

Prior to the immunohistochemical stainings the tissue sections were dewaxed and subjected to antigen retrieval by microwave irradiation in 0.5 mM EGTA buffer pH 9.0.

For double labelling experiments with the antibody combination GLP-1 and Kir6.2, GLP-1 and SUR1, GIP and Kir6.2, GIP and SUR1, insulin and Kir6.2, and insulin and SUR1, the tissue sections were incubated overnight with the primary antibodies at 4 °C. Bound goat antibodies were visualised using biotinylated anti goat antibody (1:200, The Binding Site), streptavidin coupled to peroxidase and biotinylated tyramide using the Perkin–Elmer TSA-indirect kit as described by the manufacturer (Perkin–Elmer) and finished by incubation with streptavidin coupled to Texas Red (Amersham). Bound mouse (F1 and HUI 001) or rabbit (98171 and 2135) antibodies were visualised using digoxigenin-coupled anti mouse/anti rabbit antibody (both 1:25, Chemicon, Billerica, MA, USA) and fluorescein-labelled anti digoxigenin antibody (1:200, Immunoresearch Laboratories, Suffolk, UK).

C-peptide, glucagon, GIP and GLP-1

Residual β-cell function (C-peptide), glucagon, GIP and GLP-1 release after a Boost-test (6 ml/kg (max: 360 ml) of Boost/Sustacal (Mead Johnson, Evansville, IN, USA; 237 ml = 8 fl oz contains 33 g carbohydrate, 15 g protein and 6 g fat, a total of 240 kcal) (16) was followed 1, 6 and 12 months after diagnosis in 257 children with newly diagnosed type 1 diabetes. Blood was drawn 90 min after ingestion of the Boost drink.

Serum samples were labelled and frozen at −20 °C until shipment on dry ice. C-peptide, glucagon, GIP and GLP-1 were analysed centrally. Samples were thawed only once for RIA determination.

Plasma C-peptide was analysed by a fluoroimmunometric assay (AutoDELFIA C-peptide. Analytical sensitivity: better than 4.97 pmol/l CV 5%).

Glucagon, GIP and GLP-1 concentrations in plasma were all measured after extraction of plasma with 70% ethanol (vol/vol, final concentration). The glucagon RIA was directed against the C-terminus of the glucagon molecule (antibody code no. 4305) and therefore mainly measures glucagon of pancreatic origin (17). For the GIP RIA (18) we used the C-terminal directed antiserum R 65, which cross reacts fully with human GIP but not with the so-called GIP 8000, whose chemical nature and relationship to GIP secretion is uncertain. The antiserum reacts equally with intact GIP and GIP 3-42, the primary metabolite. Human GIP and 125-I human GIP (70 MBq/nmol) were used for standards and tracer. The plasma concentrations of GLP-1 were measured (19) against standards of synthetic GLP-1 7-36amide using antiserum code no. 89 390, which is specific for the amidated C-terminus of GLP-1 and therefore mainly reacts with GLP-1 of the intestinal origin. The assay reacts equally with intact GLP-1 and with GLP-1 3-36amide, the primary metabolite. Because of the rapid and intravascular conversion of GLP-1 to their primary metabolites, it is essential to determine both the intact hormone and the metabolite for estimation of the rate of secretion of these hormones. For both assays, sensitivity was below

1 pmol/l, intraassay coefficient of variation below 6% at 20 pmol/l, and recovery of standard, added to plasma before extraction, about 100% when corrected for losses inherent in the plasma extraction procedure.

Clinical biochemistry

Glycaemic control as assessed by HbA_{1c} was determined 0, 1, 3, 6, 9 and 12 months after diagnosis. HbA_{1c} was measured by ion-exchange HPLC (normal reference range 4.1–6.4%; Steno Diabetes Centre, Gentofte, Denmark) as previously described [20].

Insulin dose- adjusted HbA_{1c}

As HbA_{1c} is influenced by the insulin dose, HbA_{1c} and insulin dose cannot be considered separately. A unified suggestion in which both parameters were included, was found by multiple regression analysis with the logarithm of C-peptide as the dependent variable and gender, age, HbA_{1c} and daily insulin dose (U/kg bodyweight) as independent variables at 1, 6 and 12 months. The multiple regression analysis showed that there was a negative correlation between stimulated C-peptide and HbA_{1c} and insulin dose. A combined expression of insulin dose-adjusted HbA_{1c} was defined: actual $HbA_{1c} + (4 \times insulin\ dose\ (U/Kg/24\ h))$. A calculated dose-adjusted HbA_{1c} below 9% corresponded to an estimated maximal C-peptide level above 300 pmol/l and was used to define clinical remission.

Genotyping

The analysis of the Glu23Lys polymorphism of the *Kir6.2* gene (*KCNJ11*) was performed by PCR on genomic DNA with the forward primer: 5′-GACTCTGCAGT-GAGGCCCTA-3′ and the reverse primer: 5′-ACGTTG-CAGTTGCCTTTCTT. The PCR was carried out in a volume of 50 μl containing 50 ng genomic DNA, 1X ammoniumbuffer, 0.5 μmol/l of each primer, 1.0 mmol/l $MgCl_2$, 0.2 mmol/l dNTP and 1 U AmpliTaq DNA polymerase (Promega). The cycling conditions were: 3 min at 95 °C followed by 35 cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s, with a final step at 72 °C for 9 min. The PCR products were digested with 5 U of BanII (New England Biolabs, Beverly, MA, USA) overnight at 37 °C, separated by gel electrophoresis on 3% NuSieve 3:1 gels (BioWhittaker Molecular Applications, Rockland, ME, USA) and finally visualised by ethidium bromide staining.

Statistical methods

Most evaluations are based on the normal distribution (GLP-1, GIP and glucagon) but C-peptide was considered on the logarithmic scale. C-peptide, HbA_{1c} adjusted for insulin dose, GLP-1 and glucagon values were analysed by means of multiple regression with gender, age, standard bicarbonate at onset, and the Glu23Lys variants as independent factors in a compound symmetric repeated measurement model allowing for random subject effects. A P value < 0.05 was considered statistically significant.

Results

Kir6.2 and SUR1 expression and localisation in primary human ileum tissue

Western blot analysis Expression of both the pore-forming and regulatory subunits of the K_{ATP} channel, Kir6.2 and SUR1, was found in primary tissue from human ileum (Fig. 1, lane 4, 5 and 7). The two bands for Kir6.2 detection in ileum represent one glycosylated form and one non-glycosylated form of the Kir6.2 subunit. The relatively large size for GLP-1 detection is due to the antibody recognition-site which is the large proglucagon fragment, explaining the positive signal in the pancreas sample (Fig. 1, lane 2). The relatively weak signal of the GLP-1 protein in the ileum sample is explained by the much dispersed distribution of the L-cells through the ileum tissue, as seen on Fig. 2.1b and 2.2b.
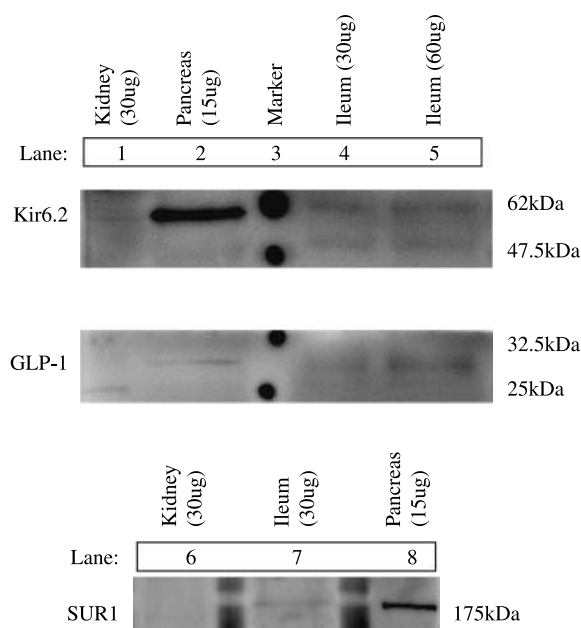

Figure 1 Western blot analysis of Kir6.2/SUR1/GLP-1 protein expression in human ileum tissue as compared with rat kidney (negative control) and rat pancreas (positive control). Molecular weights of the protein marker bands are shown to the right of the images.

Immunohistochemical analysis

GLP1/Kir6.2/SUR1 Antibody staining demonstrated that the Kir6.2/SUR1 complex co-localised with the GLP-1-expressing cells (L-cells) in the distal part of the small intestine (Fig. 2.1a–c and 2.2a–c).

GIP/Kir6.2/SUR1 Antibody staining demonstrated that the Kir6.2/SUR1 complex co-localised with the GIP-expressing cells (K-cells) in the proximal part of the gut (Fig. 2.5a–c and 2.6a–c).

Pancreas/Kir6.2/SUR1 The α-cells and the β-cells in the pancreas exhibited Kir6.2/SUR1 staining co-localising with glucagon (the GLP-1 antibody also recognises proglucagon expression, data not shown) and insulin (Fig. 2.3a–c and 2.4a–c). The β-cells did not recognise the proglucagon antibody, emphasising the specificity of the antibodies (data not shown). The insulin negative but Kir6.2/SUR1 positive cells (colouring red) in the periphery of the islets correspond to Kir6.2/SUR1 α-cells (Fig. 2.3c and 2.4c).

In vivo *study of the functional impact of the Glu23Lys variant on α-, β-, L- and K-cells*

Genotyping The Lys allele frequency was 32.1% and the genotype frequencies of Glu23Glu, Glu23Lys and Lys23Lys were 46, 44 and 10% respectively (Table 1). The genotypes were in Hardy-Weinberg equilibrium.

Kir6.2 genotype and residual β-cell function Mean boost-stimulated C-peptide concentrations decreased throughout the study period (1–12 months), but the Glu23Lys variant had no effect on the residual β-cell function (Fig. 3).

Kir6.2 genotype and postprandial GLP-1, GIP and glucagon release Mean Boost-stimulated GLP-1 and glucagon concentrations increased during the first 12 months after diagnosis of type 1 diabetes (Figs 4 and 5). Carriers of the Glu23Lys and Lys23Lys variants showed a weak trend for lower meal-stimulated GLP-1 release than
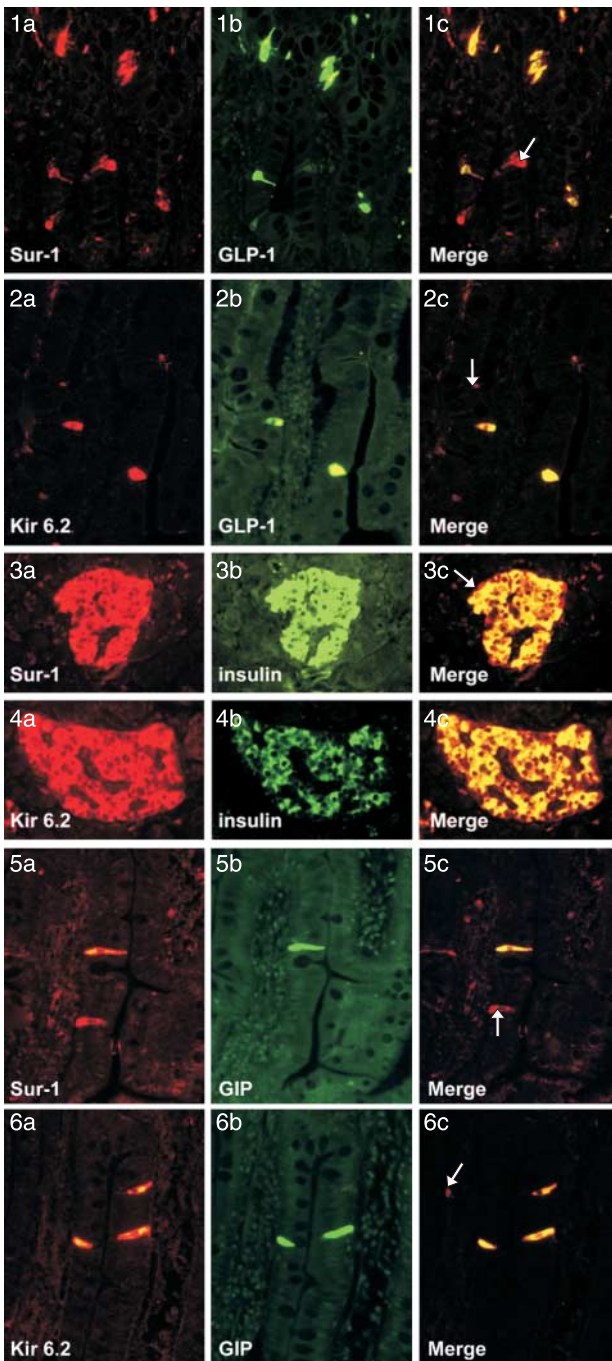

Figure 2 Immunohistochemical staining of Kir6.2 and SUR1 subunits in the human gut. Figure 2.1 distal part of human small intestine (a) SUR1 antibody stains red, (b) GLP-1 antibody stains green, (c) merged picture, yellow staining shows co-expression of SUR1 and GLP-1 in human L-cells, arrow indicates cell only stained for SUR1 antibody. Figure 2.2 distal part of human small intestine (a) Kir6.2 antibody stains red, (b) GLP-1 antibody stains green, (c) merged picture, yellow staining shows co-expression of Kir6.2 and GLP-1 in human L-cells, arrow indicates cell only stained for Kir6.2 antibody. Figure 2.3 human pancreatic islets (a) SUR1 antibody stains red, (b) insulin antibody stains green, (c) merged picture, yellow staining shows co-expression of SUR1 and insulin in human β-cells, arrow indicates α-cell only stained for SUR1 antibody. Figure 2.4 human pancreatic islets (a) Kir6.2 antibody stains red, (b) insulin antibody stains green, (c) merged picture, yellow staining shows co-expression of Kir6.2 and insulin in human β-cells. Figure 2.5 proximal part of human small intestine (a) SUR1 antibody stains red, (b) GIP antibody stains green, (c) merged picture, yellow staining shows co-expression of SUR1 and GIP in human K-cells, arrow indicates cell only stained for SUR1 antibody. Figure 2.6 proximal part of human small intestine (a) Kir6.2 antibody stains red, (b) GIP antibody stains green, (c) merged picture, yellow staining shows co-expression of Kir6.2 and GIP in human K-cells, arrow indicates cell only stained for Kir6.2 antibody. 200× magnification.

**Table 1** Age, gender, height, weight and genotype frequencies of the Glu23Lys variant on the *Kir6.2* gene.

	Glu23Glu	**Glu23Lys**	**Lys23Lys**
Number	118 (46%)	113 (44%)	26 (10%)
Age (years)	9.4 ($\pm$0.3)	9.0 ($\pm$0.4)	8.6 ($\pm$0.8)
Gender (M/F)	59/59	52/61	15/11
Height (cm)	137 ($\pm$2)	133 ($\pm$2)	137 ($\pm$6)
Weight (kg)	33.6 ($\pm$1.5)	30.4 ($\pm$1.2)	30.6 ($\pm$3.2)

Values are presented as means $\pm$ S.E.M.

carriers of the Glu23Glu genotype (2.60 pmol/l reduction, $P = 0.12$, dominant model, Fig. 4), whereas the homozygous carriers of the Lys23Lys variant tended to have higher meal-stimulated glucagon release than patients carrying the Glu23Glu and Glu23Lys genotypes (1.76 pmol/l reduction, $P = 0.07$, recessive model, Fig. 5); neither of these analyses reach statistical significance. The GIP concentration increased during the study period, but there was no trend of association with the Glu23Lys variant ($P = 0.81$, Fig. 6).

***Impact of the Glu23Lys variant on the overall glycaemic control in patients with newly onset type 1 diabetes*** Patients carrying the Glu23Lys and Lys23Lys genotypes (dominant model) had significantly higher HbA$_{1c}$ (0.61%, $P = 0.02$) at time of diagnosis compared with the Glu23Glu. This effect of 23Lys on HbA$_{1c}$ (HbA$_{1c}$ reflects glycaemic control 4–6 weeks before actual value) was still evident 1 month after diagnosis (coefficient = 0.30%, $P = 0.05$) but disappeared 3, 6, 9 and 12 months after diagnosis. When we adjusted (linear model) for the individual insulin doses, the effect reappeared and the 23Lys carriers had again higher insulin dose-adjusted HbA$_{1C}$ (coefficient = 0.42%, $P = 0.03$) throughout the whole study period (adjusted for age, BMI and gender; Fig. 7).
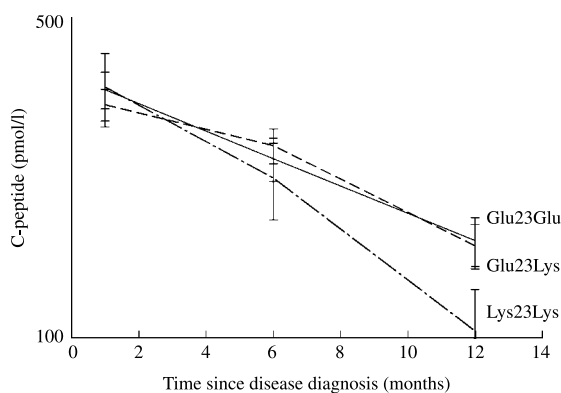
Figure 3 Meal-stimulated C-peptide (pmol/l, arithmetic means $\pm$ S.E.M.) 12 months after diagnosis distributed according to genotypes of the Glu23Lys variant on the *Kir6.2* gene, Glu23Glu, Glu23Lys and Lys23Lys.
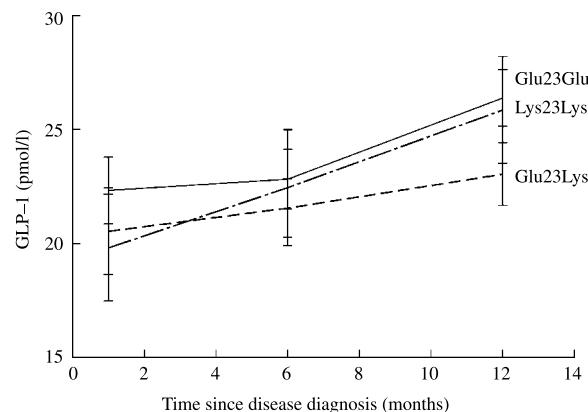
Figure 4 GLP-1 secretion (pmol/l, arithmetic means $\pm$ S.E.M.) 12 months after diagnosis according to genotypes of the Glu23Lys variant on the *Kir6.2* gene, Glu23Glu, Glu23Lys and Lys23Lys.

The poorer glycaemic control could not be explained by lower daily insulin dose (Fig. 8) as the Kir6.2 genotype did not associate with daily dose of exogenous insulin (U/kg/24 h).

Discussion

In pancreatic β-cells, K$_{ATP}$ channels are critical in regulating normal glucose-stimulated insulin secretion (21) and the same channels are present in α-cells where they are involved in glucagon secretion (22). The localisation of the K$_{ATP}$ channel subunits, Kir6.2 and SUR1, to the gastrointestinal endocrine L- and K-cells indeed suggests that the GLP-1 and GIP secretion from these cells is regulated through the same glucose-sensing machinery as is present in the α- and β-cells. Another site of glucose-sensing activity related to the gastrointestinal tract is the so-called hepatoportal glucose sensor (23–25). Although the specific cells of this sensor have not been identified, the glucose-sensing characteristics depend on the expression of GLUT2,
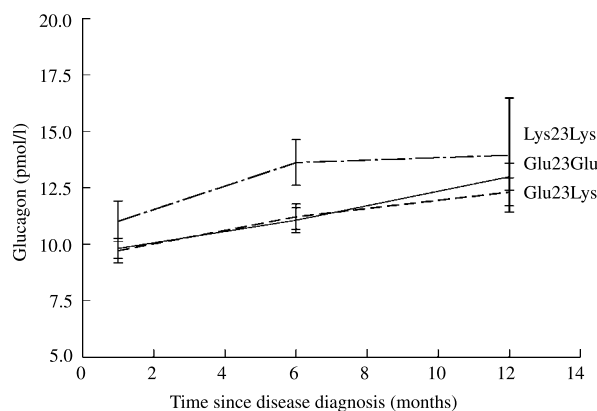
Figure 5 Glucagon secretion (pmol/l, arithmetic means $\pm$ S.E.M.) 12 months after diagnosis according to genotypes of the Glu23Lys variant on the *Kir6.2* gene, Glu23Glu, Glu23Lys and Lys23Lys.
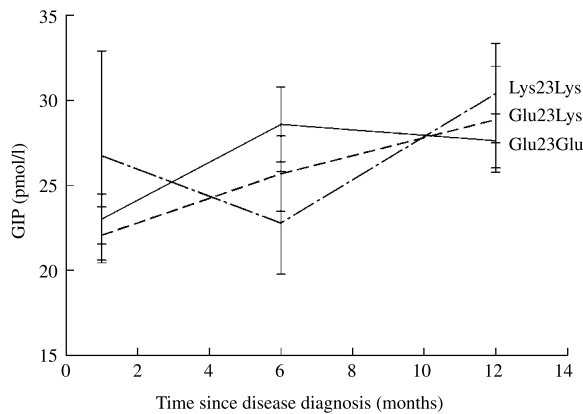
Figure 6 GIP secretion (pmol/l, arithmetic means ± s.e.m.) 12 months after diagnosis according to genotypes of the Glu23Lys variant on the *Kir6.2* gene, Glu23Glu, Glu23Lys and Lys23Lys.

Figure 8 Insulin dose (U/kg per 24 h, arithmetic means ± s.e.m.) 12 months after diagnosis according to genotype of the Glu23Lys variant on the *Kir6.2* gene, Glu23Glu, Glu23Lys and Lys23Lys.

glucokinase, GLP-1 receptor activity and afferent Kir6.2/SUR1 expressing glucose-sensing enteric neurons (26). The effect of the hepatoportal glucose sensor is to initiate an insulin-independent glucose uptake in skeletal muscle when portal glucose load increases (23). This insulin-independent mechanism of glucose clearance would be predicted to depend on a normal function of the Kir6.2/SUR1 complex, and consequently a hyperactive Kir6.2, like the 23Lys variant investigated in our study, would be predicted to reduce the activity of the hepatoportal sensor, to reduce the peripheral glucose uptake and impair glycaemic control.

Indeed, we found that, during insulin deficiency (acute diagnosis of type 1 diabetes), carriers of the 23Lys variant of Kir6.2 had higher HbA_{1c} at diagnosis and after 1 month, possibly secondary to an impaired hepatoportal glucose sensing. During exogenous insulin supplementation the negative effect of the 23Lys variant is compensated, as the difference in HbA_{1c} disappears after 3, 6, 9 and 12 months. However, when the glycaemic efficacy of the exogenous insulin is accounted
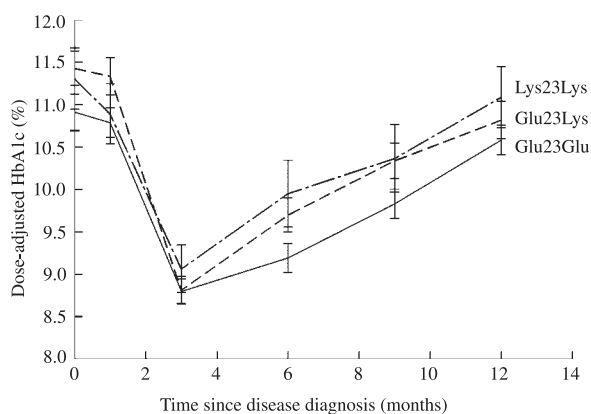

Figure 7 Insulin dose-adjusted HbA_{1c} (%, arithmetic means ± s.e.m.) 12 months after diagnosis according to genotypes of the Glu23Lys variant on the *Kir6.2* gene, Glu23Glu, Glu23Lys and Lys23Lys. $P = 0.03$.
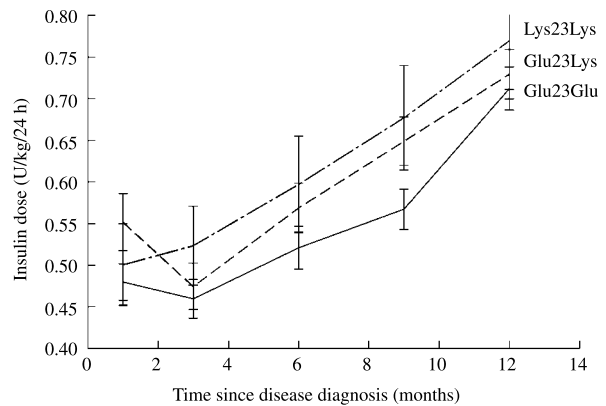
for, by adjusting the HbA_{1c} for the insulin dose, the negative effect of the 23Lys reappears and is again associated with higher insulin-dose HbA_{1c} and now throughout the whole study period. In subjects with sufficient/significant β-cell function the negative effect of the 23Lys on the hepatoportal glucose sensor will be difficult to visualise as it will be compensated by the endogenous insulin production, and it requires difficult and invasive procedures to estimate the endogenous insulin production on a 24 h basis. However, a reduction in hepatoportal glucose sensing and impaired insulin-independent peripheral glucose uptake will exert a stress to the β-cells in addition to peripheral insulin resistance, so reduced hepatoportal glucose sensing may also contribute to the association of the 23Lys variant of Kir6.2 with type 2 diabetes (8–10).

We also looked for direct evidence of a more mechanistic effect for the 23Lys variant in glucose-sensing cells (other than β-cells) like the glucagon-producing α-cells and the GLP-1/GIP producing intestinal L- and K-cells, because the residual β-cell mass in type 1 diabetes is likely too small to reveal the effect of the 23Lys variant on insulin secretion. It has previously been demonstrated *in vivo* that homozygous Lys23Lys hyperactive K_{ATP} channels carriers have increased glucagon release during a hyperglycaemic clamp compared with heterozygous and wild type carriers (10). We could not directly confirm this finding, although we found that the homozygous carriers of the Lys23Lys showed a trend in the same direction with a 10% elevation of postprandial glucagon levels ($P = 0.07$). Similarly, we could not confirm a dominant negative effect of the 23Lys allele previously reported on glucose-induced insulin secretion (8), despite GLP-1 and GIP secretion being regulated through the same glucose-sensing machinery as is present in β-cells. Again, we merely found a weak trend of 2.6 pmol/l lower meal-stimulated GLP-1 level in 23Lys carriers ($P = 0.12$), but not the same trend with regard to the GIP levels. This could, however, be explained by the late time point for blood sampling (90 min after

Boost intake) in our study, which is more sensitive for estimating GLP-1 rather than GIP release as the GIP-secreting K-cells reside in the proximal part of the small intestine, whereas the GLP-1 secreting L-cells occupy the distal part.

The present study is the first, to our knowledge, to investigate the co-expression of Kir6.2 with GLP-1 in human tissue and the relationship *in vivo* between a common hyperactive K_{ATP} channel (Lys allele), glycaemic control and meal-stimulated GLP-1 and glucagon release in patients with insulin-treated diabetes. Postprandial GLP-1 release was recently studied in five children with rare hyperactive Kir6.2 mutations before and after start of oral antidiabetic treatment with sulfonylureas, and also here GLP-1 release appeared to be unaffected (27).

Although only trends, we cannot, however, exclude that higher postprandial glucagon and lower GLP-1 levels in 23Lys carriers do not add-on to an already reduced hepatoportal glucose sensor and further impair systemic glucose regulation. An additional central (neuronal) mechanism could also contribute to elevated glucagon level among the patients homozygous for the Glu23Lys variant, as studies in Kir6.2$^{-/-}$ mice show that K_{ATP} channels in the hypothalamus are important for glucose sensing and the activation of the K_{ATP} channels is critical for glucagon secretion and food intake when the brain glucose level falls (28). Finally, lower GLP-1 levels may lead to a more rapid gastric emptying rate in the Glu23Lys carriers and result in increased postprandial glucose excursions.

Our data show that the L- and K-cells are equipped with the same K_{ATP} channel complex as α- and β-cells indicating shared glucose-sensing regulatory machinery for these cell types in humans. The association of the 23Lys allele of the Kir6.2 with impaired glycaemic control in type 1 diabetes may suggest that a hepatoportal glucose sensor exists also in humans and need to be replicated in other prospective studies of glycaemic control in type 1 diabetes patients.

Acknowledgements

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