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SHORT COMMUNICATION

A mutation in *KCNJ11* causing human hyperinsulinism (Y12X) results in a glucose-intolerant phenotype in the mouse

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Abstract
Aims/hypothesis We identified a mouse with a point mutation (Y12STOP) in the *Kcnj11* subunit of the K_{ATP} channel. This point mutation is identical to that found in a patient with congenital hyperinsulinism of infancy (HI). We aimed to characterise the phenotype arising from this loss-of-function mutation and to compare it with that of other mouse models and patients with HI.
Methods We phenotyped an *N*-ethyl-*N*-nitrosourea-induced mutation on a C3H/HeH background (*Kcnj11*^{Y12STOP}) using intraperitoneal glucose tolerance testing to measure glucose and insulin plasma concentrations. Insulin secretion and response to incretins were measured on isolated islets.
Results Homozygous male and female adult *Kcnj11*^{Y12STOP} mice exhibited impaired glucose tolerance and a defect in insulin secretion as measured in vivo and in vitro. Islets had an impaired incretin response and reduced insulin content.
Conclusions/interpretation The phenotype of homozygous *Kcnj11*^{Y12STOP} mice is consistent with that of other *Kcnj11*-knockout mouse models. In contrast to the patient carrying this mutation homozygously, the mice studied did not have hyperinsulinaemia or hypoglycaemia. It has been reported that HI patients may develop diabetes and our mouse model may reflect this clinical feature. The *Kcnj11*^{Y12STOP} model may thus be useful in further studies of K_{ATP} channel function in various cell types and in investigation of the development of hyperglycaemia in HI patients.

Keywords ENU · Hyperinsulinism · K_{ATP}-channel · *Kcnj11* · *Kir6.2*

Abbreviations

ENU	*N*-Ethyl-*N*-nitrosourea
GIP	Glucose-dependent insulinotropic peptide
GLP-1	Glucagon-like peptide 1
HI	Hyperinsulinism of infancy
IPGTT	Intraperitoneal glucose tolerance test

Introduction

Inactivating mutations in the gene encoding Kir6.2 (*KCNJ11*) result in familial hyperinsulinism of infancy (HI). Conversely, activating *KCNJ11* mutations cause neonatal diabetes mellitus [1].

Mouse models of HI have been generated by genetic deletion of *Kcnj11* or expression of a dominant negative *Kcnj11* transgene, either in the whole animal or specifically in the beta cell, as reviewed by Seino et al. [2]. Global knockout of *Kcnj11* produced beta cell depolarisation, an increase in basal intracellular calcium concentration ($[Ca^{2+}]_i$) and a loss of insulin secretion in response to glucose or the K_{ATP} channel blocker tolbutamide [3]. Neonates exhibited transient hypoglycaemia, consistent with predictions from studies on HI patients. Unexpectedly, adult mice exhibited mild glucose intolerance, due to an enhanced insulin

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sensitivity [3]. Interestingly, mice in which *Kcnj11* was deleted heterozygously hypersecreted insulin and showed enhanced glucose tolerance as adults [4].

When a dominant negative *Kcnj11* mutation (G132S) was targeted to the beta cell, neonatal mice exhibited high serum insulin and hypoglycaemia, but developed severe diabetes (due to loss of beta cell mass) as adults [5]. In contrast, mice carrying a different beta cell-specific dominant negative mutation (GYG132-134 to AAA; AAA-TG) exhibited hyperinsulinism as adults [6]; however, about 30% of their beta cells had normal K_{ATP} channel density. These results suggest that incomplete loss of beta cell K_{ATP} function in vivo leads to hyperinsulinism and a complete loss to eventual diabetes.

We identified a *Kcnj11* tyrosine to stop codon (*Kcnj11*^{Y12STOP}) mutation in a mouse mutagenised by *N*-ethyl-*N*-nitrosourea (ENU). This mutation was also found homozygously in a patient with familial HI [7] and shown to abolish K_{ATP} channel activity when expressed heterologously. The patient was only 3.7 years old at the time of publication and was unresponsive to diazoxide (as expected if he lacked functional K_{ATP} channels). Thus, a near-total pancreatectomy was performed to control his hyperinsulinism [7]. Both parents of this patient carried the mutation in the heterozygous state, but were asymptomatic.

Methods

Animals Mice were kept in accordance with UK Home Office welfare guidelines, project license restrictions and approval by local Ethics Committee. Mice were supplied by MRC Harwell, Harwell Science and Innovation Campus, Harwell, UK.

ENU genotype-driven screen The Harwell ENU-DNA archive was screened for mutations in *Kcnj11* as described previously [8]. *Kcnj11*^{Y12STOP} animals were generated using frozen sperm samples from the BALB/c × C3H/HeH F1 founder and C3H/HeH eggs [8].

Intraperitoneal glucose tolerance test and OGTT Intraperitoneal glucose tolerance test (IPGTT) and OGTT were carried out according to the EMPReSS protocols for both (http://empress.har.mrc.ac.uk/viewempress/pdf/ESLIM_004_001.pdf) using 2 g glucose/kg body weight. Blood was collected under a local anaesthetic. Plasma insulin was assayed using ELISA kits (Ultra-sensitive; Mercodia, Uppsala, Sweden). Plasma glucose was measured using a glucose analyser (GM9; Analox, London, UK).

Insulin tolerance test Animals were fasted for 5 h and a blood sample taken before interperitoneal injection of 1 IU insulin per kg of mouse body weight. Subsequent blood samples were taken at 15, 30, 45, 60 and 90 min.

Insulin secretion assay Islets were isolated by liberase digestion and handpicking, as detailed in Electronic supplementary material (ESM Methods), Insulin secretion assay. Insulin secretion from isolated islets (five islets per well) was measured during 1 h static incubations. Each assay was carried out in triplicate.

Other methods For details on other methods, see ESM Methods Immunoblotting, ESM Methods Immunohistochemistry, ESM Methods Islet area and ESM Methods Quantitative RT-PCR.

Results

We identified a T36A mutation resulting in a missense amino acid change from tyrosine to a stop at codon 12 (Y12STOP, *Kcnj11*^{Y12STOP}) of the 390 amino acid protein. RNA was prepared from isolated islets and sequenced to confirm that the mutation is expressed (ESM Fig. 1).

Kcnj11^{Y12STOP} heterozygotes were indistinguishable from wild-type littermates in IPGTTs at 12 and 20 weeks of age (Fig. 1a, b; ESM Fig. 2a, c). In contrast, glucose tolerance was strongly impaired in homozygous mutant mice (Fig. 1; ESM Fig. 2). Similar results were observed in an OGTT (ESM Fig. 3). Homozygous *Kcnj11*^{Y12STOP} mice secreted significantly less insulin during an IPGTT than wild-type or heterozygous littermates at 12 and 20 weeks of age (Fig. 1c, d; ESM Fig. 2b, d). Insulin tolerance tests showed that homozygous mice were relatively more insulin-sensitive than wild-type or heterozygous mice (Fig. 1e).

Insulin secretion was measured in islets isolated from 20-week-old mice (Fig. 2a). Homozygous islets showed significantly elevated basal insulin secretion (at 2 mmol/l glucose) and secreted less insulin at 20 mmol/l glucose compared with wild-type or heterozygous islets. Tolbutamide elicited similar insulin secretion in all groups of islets (Fig. 2a).

In wild-type islets, addition of glucagon-like peptide 1 (GLP-1) or glucose-dependent insulinotropic peptide (GIP) further stimulated insulin secretion induced by 20 mmol/l glucose, namely by 4.9-fold for GLP-1 and 3.7-fold for GIP (Fig. 2b, c). Homozygous mutant islets showed a markedly impaired response compared with wild-type islets, the increase in insulin secretion being only 2.8-fold for GLP-1 and 1.59-fold for GIP (Fig. 2b, c).

Insulin content was substantially lower in islets isolated from 13-week homozygous *Kcnj11*^{Y12STOP} mice than in their wild-type littermates, whether measured by ELISA (ESM Fig. 4a) or by immunoblotting of islet proteins for

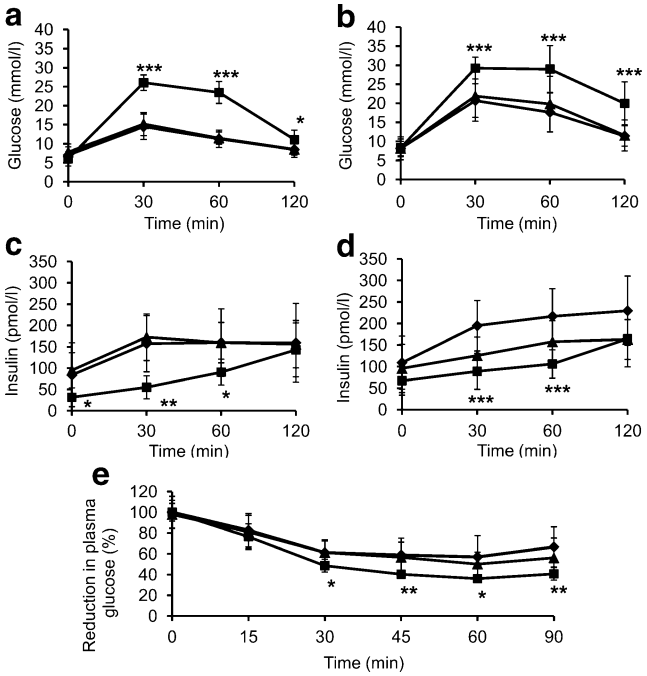

Fig. 1 Impaired glucose tolerance and increased insulin sensitivity. Glucose tolerance (**a**, **b**), insulin secretion (**c**, **d**) and insulin tolerance (**e**) in homozygous (black squares), heterozygous (black triangles) and wild-type littermate (black diamonds) backcross three *Kcnj11*^{Y12STOP} mice at 12 weeks of age. **a**, **c** Female mice and (**b**, **d**) male mice. **a**–**d** n=9, n=16 and n=7 for wild-type, heterozygous and homozygous respectively. **e** Reduction of plasma glucose in male 8-week-old mice; n=12, n=13 and n=7 for wild-type, heterozygous and homozygous respectively. Data are given as mean±SD. *p<0.05, **p<0.01 and ***p<0.001 for difference between homozygous and wild-type, Student's t test

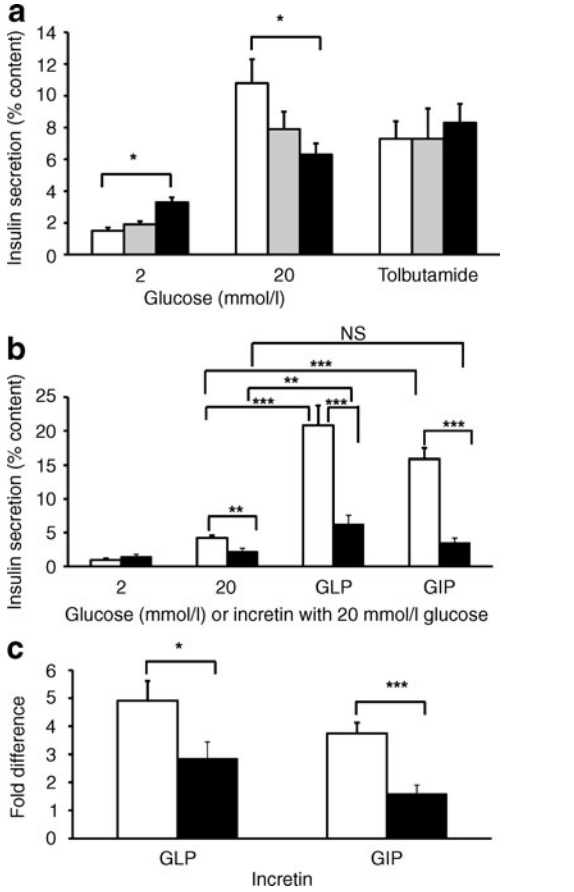

Fig. 2 Impaired insulin secretion and incretin response in islets isolated from wild-type, heterozygous or homozygous *Kcnj11*^{Y12STOP} mice. **a** Insulin secretion, expressed as percentage of insulin content, in response to 2 mmol/l glucose, 20 mmol/l glucose or 100 μmol/l tolbutamide plus 7 mmol/l glucose for islets isolated from wild-type (white bars), heterozygous (grey bars) or homozygous (black bars) littermates. Data are the mean of three separate experiments (i.e. three mice), with five replicates (each of five islets) at each concentration. **b** Insulin secretion, as percentage of content, in response to the incretins GLP-1 (5 μmol/l+20 mmol/l glucose) or GIP (10 μmol/l+20 mmol/l glucose). Data represent the mean±SEM of four independent experiments from four animals per genotype (with three to eight replicates of five islets per animal). **c** Fold increase in insulin secretion produced by 5 μmol/l GLP-1 or 10 μmol/l GIP (expressed as the amount of insulin secretion induced by 20 mmol/l glucose plus the indicated incretin, divided by that produced by 20 mmol/l glucose alone). **b**, **c** White bars, wild-type islets; black bars, homozygous islets. *p<0.05, **p<0.01 and ***p<0.001 for difference between wild-type and homozygote or between incretin +glucose response to response with 20 mmol/l glucose alone (as indicated), Student's t test

insulin (ESM Fig. 4b). However, measurement of insulin gene transcription did not reveal any differences between genotypes (ESM Fig. 4c, d). No difference in islet area, was detected between wild-type (1.02±0.08%, mean±SE, n=3 animals, eight sections each) and homozygous mice (1.06±0.07% mean±SE, n=3 animals, eight sections each) at 13 weeks of age. Similarly, immunohistochemistry on pancreas sections at 13 weeks of age showed normal distribution of insulin (beta cell) and glucagon (alpha cell) staining cells (ESM Fig. 5). Further, staining with cleaved caspase-3 showed no evidence of significant apoptosis (ESM Fig. 5; ESM Table 1).

Discussion

Like the *Kcnj11*-knockout mice [3, 9], homozygous *Kcnj11*^{Y12STOP} mice show impaired glucose tolerance in vivo, decreased insulin secretion from isolated islets and enhanced insulin sensitivity. Homozygous *Kcnj11*^{Y12STOP} mice do not recapitulate the human phenotype of hyperinsulinism although they may be useful in understanding the transition to hyperglycaemia in some patients. Similar results have been reported for homozygous *Kcnj11* and *Sur1* (also known as *Abcc8*) knockout mice [2, 10], and for inactivating *Kcnj11* mutations: they either show only neonatal hypoglycaemia or, at best, mild adult-onset hyperinsulinism, whereas total loss of functional *Kcnj11* causes hyperglycaemia.

The marked reduction in glucose tolerance in homozygous *Kcnj11*^{Y12STOP} mice, manifested by lower insulin

levels in vivo in response to a glucose challenge, occurred despite enhanced insulin sensitivity. This suggests that insulin secretion was impaired, which was confirmed by the reduced insulin secretory response in isolated islets of *Kcnj11*^{Y12STOP} mice. The lower insulin secretion results from a marked reduction in insulin content (up to 50% in 13-week-old islets) and impaired stimulus–secretion coupling.

The insulin content of *Kcnj11*-knockout islets was not significantly different from that of islets isolated from 5-month-old wild-type mice [9]. Interestingly, *Sur1*-knockout islets have only about 60% of the insulin content of wild-type [11]. The Kir6.2G132S dominant negative transgenic mouse, which develops hyperglycaemia and hypoinsulinaemia, also shows substantially reduced insulin content between 4 and 16 weeks of age, although insulin content subsequently increases leading to some improvement in glucose tolerance [12].

The reason for the reduced insulin content of *Kcnj11*^{Y12STOP} islets is unclear. As insulin mRNA levels were unchanged it cannot be the result of lower transcription. Hypersecretion under basal conditions, which could deplete insulin content, was observed in isolated islets (Fig. 2a).

Our in vitro data demonstrate a clear impairment of the coupling between glucose metabolism and insulin secretion, because insulin secretion is lower in homozygous *Kcnj11*^{Y12STOP} islets, when expressed as a percentage of insulin content. This may be related to the increased blood glucose levels or to long-term elevation of intracellular calcium.

Studies of isolated *Kcnj11*^{Y12STOP} islets showed a defective incretin response, consistent with findings of Miki et al., who showed that *Kcnj11*-knockout islets had a marked reduction in the GLP-1 response and complete unresponsiveness to GIP [13]. Similarly, knockout of *Sur1* also impaired the ability of incretins to potentiate glucose-stimulated insulin secretion [11]. It has been hypothesised that this reflects a failure of incretin-induced cAMP stimulation of insulin secretion by a mechanism independent of protein kinase A, which is likely to be mediated by *Epac* (also known as *Rapgef3*) [14].

Some HI patients have mutations (homozygous or heterozygous) that result in partially functioning channels and respond to treatment with the K_{ATP} channel opener diazoxide or with diet alone. Patients with such mutations may progress to diabetes in later life, perhaps reflecting a gradual decline of beta cell mass and insulin secretion like that seen in the AAA-TG mouse. Patients unresponsive to diazoxide are usually treated by partial pancreatectomy and consequently many develop diabetes at puberty. These individuals often have severe null mutations that are similar in type to the knockout mouse mutations. It is not known whether these patients would progress to diabetes if they did not undergo partial pancreatectomy, as is found for the transgenic mouse models and humans with less severe mutations.

Our homozygous *Kcnj11*^{Y12STOP} mouse carries the same mutation as that observed homozygously in a human patient [7]. This model may be a useful tool for studying null mutations of the *Kir6.2*/*Kcnj11* gene and the transition from HI to diabetes and its treatment. In addition, this model will be of use for investigating the function of K_{ATP} channels in other cell types and tissues.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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