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RESEARCH LETTER

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The identification of a R201H mutation in *KCNJ11*, which encodes Kir6.2, and successful transfer to sustained-release sulphonylurea therapy in a subject with neonatal diabetes: evidence for heterogeneity of beta cell function among carriers of the R201H mutation

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To the Editor

Diabetes caused by severe mutations in beta cell genes is usually diagnosed as neonatal diabetes [1] or MODY [2]. Activating mutations in *KCJN11*, the gene encoding the ATP-sensitive potassium channel subunit Kir6.2, have been described in very-early-onset diabetes (<6 months) characterised by a profound impairment of insulin secretion [3]. We screened *KCJN11* in 25 Polish patients who were diagnosed before 2 years of age and who had been on insulin from diagnosis to the time they were studied (mean duration 8.7 years). Five individuals were diagnosed during the first 6 months of life. All patients diagnosed before 6 months of age, and 15 of 20 of those diagnosed after 6 months, had at least one diabetic first- or second-degree relative. Type 1 diabetes-related antibodies were not found in the four subjects who were tested at diagnosis; the others were not tested. The intronless gene was amplified in three overlapping fragments using primers with M13 universal sequences at the 5′ end of tailed forward and reverse primers (Table 1), followed by direct sequencing. All study subjects gave informed consent. The project was approved by the local ethics committee and was conducted in accordance with the Declaration of Helsinki.

A heterozygous R201H missense mutation (c.601C>A) was found in a single patient. This child was born at 40 weeks' gestation and weighed 2,450 g. He was diagnosed with diabetes during the third week of life based on a plasma glucose of 35–50 mmol/l, without ketoacidosis, which was measured when he presented with pneumonia and bilateral acute otitis media. The patient was treated with insulin, with a dose of 0.7–1.0 U/kg used initially and subsequently decreased. At 9 years, 11 months he weighed 27.5 kg and his daily insulin requirement was 7 U (0.25 U/kg) as a single morning injection of intermediate-acting insulin. Despite little modification to his diet he rarely experienced hyperglycaemia above 11 mmol/l, and had an HbA_1c level of 6.6%. These observations suggested endogenous beta cell function, which was confirmed by a fasting plasma C-peptide of 443 pmol/l (glucose 6.7 mmol/l) and an increase in plasma insulin concentration of 70 pmol/l during an IVGTT (Fig. 1).

As patients with Kir6.2 diabetes may respond to sulphonylureas, we assessed whether this patient could successfully transfer to sulphonylurea tablets [3, 4]. Glipizide gastrointestinal therapeutic system (GITS), a controlled-release sulphonylurea, was introduced [5]. A dose of 5 mg was administered for 2 days, then 10 mg; insulin was simultaneously slowly withdrawn over a period of 5 days. As the patient experienced a few mild hypoglycaemic episodes during the first 2 days after the discontinuation of insulin the glipizide GITS dose was decreased to 5 mg. While on this dose, a day profile of his capillary glucose concentrations revealed that they were between 4 and 6 mmol/l; a result confirmed by a 72-h record obtained using a continuous glucose monitoring system (Medtronic, Northridge, CA, USA). The IVGTT was repeated 3 weeks after a stable glipizide dose was achieved. At this time the patient's fasting glucose level was 5.4 mmol/l, and his C-peptide level was 347 pmol/l.

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Table 1 The primer set used to amplify *KCNJ11*, the gene that encodes Kir6.2, with M13 tails

Fragment	5′–3′ Forward primer	5′–3′ Reverse primer
B	TGT AAA ACG ACG GCC AGT CCG AGA GGA CTC TGC AGT GA	CAG GAA ACA GCT ATG ACC TAG TCA CTT GGA CCT CAA TGG AG
C	TGT AAA ACG ACG GCC AGT CTG CTG AGC CCT GTG TCA CC	CAG GAA ACA GCT ATG ACC CAC GCC TTC CAG GAT GAC GAT
D	TGT AAA ACG ACG GCC AGT CTA CCA TGT CAT TGA TGC TGC CAA	CAG GAA ACA GCT ATG ACC CCA CAT GGT CCG TGT GTA CAC AC

An annealing temperature of 62°C was used for all three PCR protocols

The peak increase in insulin concentration during the IVGTT was 197 pmol/l—almost three times higher than that measured prior to sulphonylurea treatment (Fig. 1).

Four individuals on the maternal side of his family had diabetes.The mother developed gestational diabetes at 31 years of age, during her first pregnancy, and remained on insulin until the delivery of the proband. She was also diagnosed with diabetes during her second pregnancy, and an OGTT performed 6 weeks after delivery showed that she remained diabetic. She is treated by diet and does not have type 1 diabetes-related antibodies. Three other distant relatives had typical late-onset type 2 diabetes. These were aged >40 years at diagnosis and do not require insulin treatment. The R201H mutation was not present in the proband's diabetic mother or in three other family members examined, including one diabetic relative. The proband's father was not available; however, he is not known to have diabetes. It is therefore likely the proband has a new mutation, as is usually the case with *KCNJ11* mutations [3, 4].

Our data support earlier reports suggesting that mutations in Kir6.2 are usually found in patients diagnosed at <3 months of age [3, 4] and that these mutations are uncommon in those aged >6 months [6]. Although the proband had other relatives who had diabetes, these individuals did not have the mutation, thus demonstrating heterogeneity of aetiology in this family. A family history of later-onset diabetes should not influence the decision to test for Kir6.2 mutations. The R201H mutation found in this Polish child has previously been described in neonatal diabetes, and has been shown to reduce the response of the Kir6.2 channel to ATP in functional studies [3, 4]. Despite having a low birthweight and a similar age at diagnosis (<6 months) there are some striking differences between our patient and the previously reported cases. Our patient's insulin requirement was substantially lower than in ten previously reported R201H carriers (0.25 vs ≥0.6 U/kg) [3, 4]. In keeping with this, both his basal C-peptide concentration (443 vs <20 pmol/l) and his peak increase in insulin response during an IVGTT before treatment with sulphonylureas (70 vs <3 pmol/l) were considerably higher than those in the other R201H carriers [3, 4]. These results are consistent with our patient having greater endogenous beta cell function than others with the same mutation, supporting the presence of phenotypic heterogeneity. Its cause is not known, but it could represent polygenic or environmental factors. These might include the allele status at other *KCNJ11* polymorphic sites, since, surprisingly, the proband was a homozygote for the K allele at residue 23 of the protein, which predisposes to type 2 diabetes [7]. Interestingly, the five R201H carriers with the more severe beta cell defect carried at least one E variant [3, and E. E., S. E. and A. H. unpublished data]. Further studies are needed to

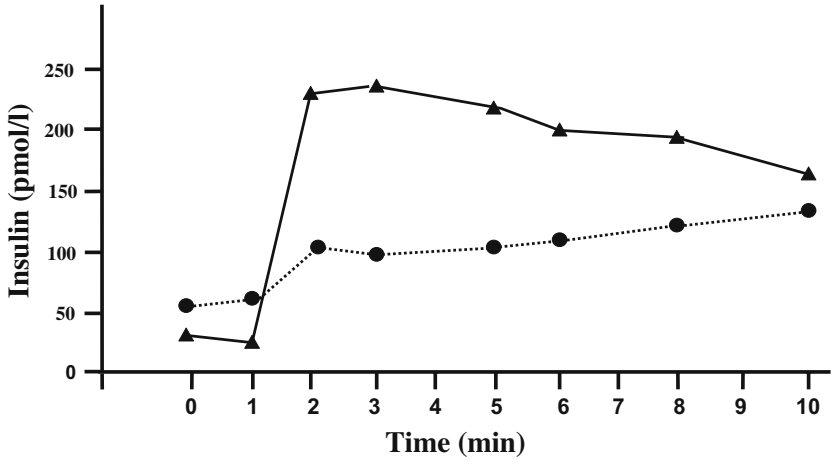

Fig. 1 The insulin response to a frequently sampled IVGTT in the R201H mutation carrier before (*filled circles*, *dotted line*) and after (*black triangles*, *solid line*) 3 weeks of 5 mg glipizide GITS therapy. The initial IVGTT was performed 24 h after the patient had received his regular insulin dose of 7 U of Insulatard HM (Novo Nordisk, Bagsvaerd, Denmark). After taking baseline samples, 0.3 g/kg of glucose was given intravenously. Blood samples were obtained at 1, 2, 3, 5, 6, 8, and 10 min and plasma insulin was assayed. The second IVGTT test was performed according to the same protocol 24 h after the last dose of sulphonylurea

determine whether the interaction between the R201H mutation and the variant at residue 23 influence the severity of beta cell dysfunction.

In summary, we have described the successful initial transfer of a patient with Kir6.2 mutation from insulin to a sustained-release sulphonylurea. The lowest adult dose of glipizide (5 mg) resulted in a markedly increased acute insulin response to an IVGTT, despite the fact that the last dose was administered 24 h before the investigation. The heterogeneity of beta cell function in association with the R201H mutation suggests that the response to sulphonylurea treatment may vary considerably between individuals even if they have the same mutation.

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