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Sulfonylurea improves CNS function in a case of intermediate DEND syndrome caused by a mutation in *KCNJ11*

Wojciech Mlynarski, Andrei I Tarasov, Agnieszka Gach, Christophe A Girard, Iwona Pietrzak, Lejla Zubcevic, Jacek Kusmierek, Tomasz Klupa, Maciej T Malecki and Frances M Ashcroft*

SUMMARY

Background A 12-week-old female presented with neonatal diabetes. Insulin therapy alleviated the diabetes, but the patient showed marked motor and mental developmental delay. The patient underwent genetic evaluation at the age of 6 years, prompted by reports that mutations in the *KCNJ11* gene caused neonatal diabetes.

Investigations Genomic sequencing of the ATP-sensitive potassium (K_{ATP}) channel gene *KCNJ11* and *in vitro* functional analysis of the channel defect, and single-photon emission CT imaging before and after glibenclamide therapy.

Diagnosis Genetic evaluation revealed a missense mutation (His46Leu) in *KCNJ11*, which encodes the Kir6.2 subunit of the K_{ATP} channel, conferring reduced ATP sensitivity. Functional studies demonstrated that the mutant channels were strongly inhibited by the sulfonylurea tolbutamide.

Management Sulfonylurea (glibenclamide) treatment led to both improved glucose homeostasis and an increase in mental and motor function.

KEYWORDS ATP-sensitive potassium channel, *KCNJ11*, neonatal diabetes, neuropathology, sulfonylurea

CME

W Mlynarski is a Professor in the Department of Pediatrics and the Department of Molecular Genetics and Immunopathology, I Pietrzak is a Pediatrician and Diabetologist in the Department of Pediatrics, A Gach is a PhD candidate in the Department of Molecular Genetics and Immunopathology, and J Kusmierek is a Research Professor in the Department of Nuclear Medicine, at the Medical University of Lodz, Lodz, Poland. AI Tarasov and CA Girard are Postdoctoral Fellows, L Zubcevic is a Research Assistant, and FM Ashcroft is a Royal Society Research Professor, in the Department of Physiology, Anatomy and Genetics at the University of Oxford, Oxford, UK. T Klupa is a Consulting Physician, and MT Malecki is an Associate Professor, in the Department of Metabolic Diseases at the Jagiellonian University Medical College, Krakow, Poland.

Correspondence
*Department of Physiology, Anatomy and Genetics, Oxford University, Parks Road, Oxford OX1 3PT, UK
frances.ashcroft@physiol.ox.ac.uk

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THE CASE

A female infant presented with polydipsia, glucosuria, ketoacidosis (pH 7.09, −14 mmol/l base excess) and severe hyperglycemia (blood glucose 42.5 mmol/l [766 mg/dl]) 12 weeks after birth. C peptide was undetectable. Tests for islet cell autoantibodies and antibodies against glutamic acid decarboxylase 65 (GAD65), insulinoma-associated antigen 2 and insulin were negative. The patient had no family history of diabetes. She had a low birth weight (2,700 g) despite an unremarkable pregnancy and a normal gestational age. For the first 72 h after diagnosis of neonatal diabetes she was treated with intravenous insulin (2.1 U/kg, 1.5 U/kg and 1.2 U/kg body weight, for the first, second and third days, respectively). Subsequently, she received subcutaneous insulin therapy with multiple insulin injections daily (1.3 U/kg body weight daily). She had frequent episodes of hyperglycemia and hypoglycemia (blood glucose 1.1–24.8 mmol/l [19.8–446.8 mg/dl]) from the time of diabetes onset to the age of around 3 years, and experienced two severe hypoglycemic episodes (blood glucose 1.1 mmol/l [19.8 mg/dl] on each occasion), one at 2 years of age and one at 4 years of age. The first episode was associated with loss of consciousness following physical activity and the second with night-time hypoglycemia. On both occasions the patient was treated with glucagon (0.5 mg) and no hospitalization was needed. No further episodes of ketoacidosis were documented. The patient's level of hemoglobin A1c (Hb_{A1c}) averaged 6.9% between 6 months and 6 years of diabetes duration (see Supplementary Table 1 online).

The patient's motor and mental functions were normal at diabetes diagnosis, but at 12 months of age she was still unable to stand without support and her motor milestones were similar

to those of a normally developing child aged 8–10 months. No specific neurological features were found and the native electroencephalogram was normal. Importantly, developmental delay was evident before the first hypoglycemic episode. At the age of 24 months, a pediatric neurological examination revealed muscle weakness and lower limb hypotonia. The patient's reflexes and sensation were normal.

The patient underwent genetic evaluation at the age of 6 years, following reports that mutations in the *KCNJ11* gene (which encodes the ATP-sensitive potassium channel Kir6.2 subunit) caused neonatal diabetes.[1,2] At this time, the patient reportedly fell down 10–15 times per week and had difficulty running. The falls were not associated with loss of consciousness or seizures, and an electroencephalogram was normal. Muscle weakness and hypotonia of the lower extremities were still present: muscle power was graded as 3–4 on a scale of 0–5 for muscle power assessment in a cooperative child. At this time the patient also had difficulty sustaining attention. Psychological examination revealed that she fulfilled seven criteria for inattention and eight criteria for hyperactivity–impulsivity according to the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, 4th Edition) diagnostic criteria for attention deficit hyperactivity disorder (ADHD). She showed mental retardation (using the Wechsler Preschool and Primary Scale of Intelligence-revised [WPPSI-R] test), demonstrating an IQ of 57 and mental abilities similar to those of a normal child aged 42–48 months.

An MRI scan revealed no brain structural abnormalities (see Supplementary Figure 1 online). Single-photon emission computed tomography (SPECT) examination, however, showed a focal perfusion deficit in the left temporal cortex (Figure 1A). Cerebellar indices were calculated for semi-quantitative evaluation of SPECT scans (Figure 1C,D).

Sequencing of the patient's *KCNJ11* gene using genomic DNA extracted from peripheral lymphocytes identified a novel heterozygous mutation, His46Leu (c137A>T) in Kir6.2, the pore-forming subunit of the ATP-sensitive potassium (K_{ATP}) channel (Figure 2A,B).[2] Genotype analysis confirmed the family relationships, and that the His46Leu mutation was not present in either parent. *In vitro* functional studies determined that the His46Leu mutation reduced K_{ATP} channel sensitivity to ATP, and that the mutant channels were strongly inhibited by the sulfonylurea tolbutamide (Figure 2E and Supplementary Information 1 online). On the basis of the genotype and the clinical features of neonatal diabetes plus developmental delay, the patient was given a diagnosis of intermediate DEND (developmental delay, epilepsy and neonatal diabetes) syndrome.

Glibenclamide treatment was initiated because of the observed sensitivity of the mutant K_{ATP} channel to sulfonylureas in studies carried out *in vitro*. The initial dose was 0.1 mg/kg body weight daily, divided into three doses (see Supplementary Information 1 online), and this was gradually increased. The insulin dose was reduced concomitantly. During the transition period, a transitory diarrhea (lasting less than 3 days) without pyrexia was noted (as reported previously in other patients).[3] Insulin was discontinued 1 month after initiating the transition to glibenclamide and good diabetes control (Hb_{A1c} level of 5.9% 4 months after initiation of glibenclamide therapy) was obtained with a sulfonylurea dose of 0.81 mg/kg body weight daily. The dose of glibenclamide was subsequently reduced and 10 months after transition the patient required only 0.62 mg/kg body weight glibenclamide daily. No hypoglycemia has been reported in the patient since she started on glibenclamide.

Seven months after starting glibenclamide treatment a pediatric neurological examination was normal. The child no longer had running problems (she fell down infrequently less than twice a week) and showed considerable improvement in sustaining attention. She was able to draw simple pictures, write a few letters and digits and clean her teeth by herself, none of which was observed before drug therapy. She now fulfilled only three criteria for inattention and five for hyperactivity–impulsivity (DSM-IV diagnostic criteria for ADHD). Mental development assessment using the WPPSI-R test still showed mild mental retardation but her intelligence had improved (IQ 71).

A second SPECT examination performed 16 weeks after insulin was discontinued revealed that the focal perfusion deficit in the left temporal cortex observed in the initial examination was no longer present (Figure 1B). A significant decrease in the average cerebellar indices was also apparent (Figure 1C,D), indicative of an increase in cerebellar perfusion. Continuous glucose monitoring over 3 days

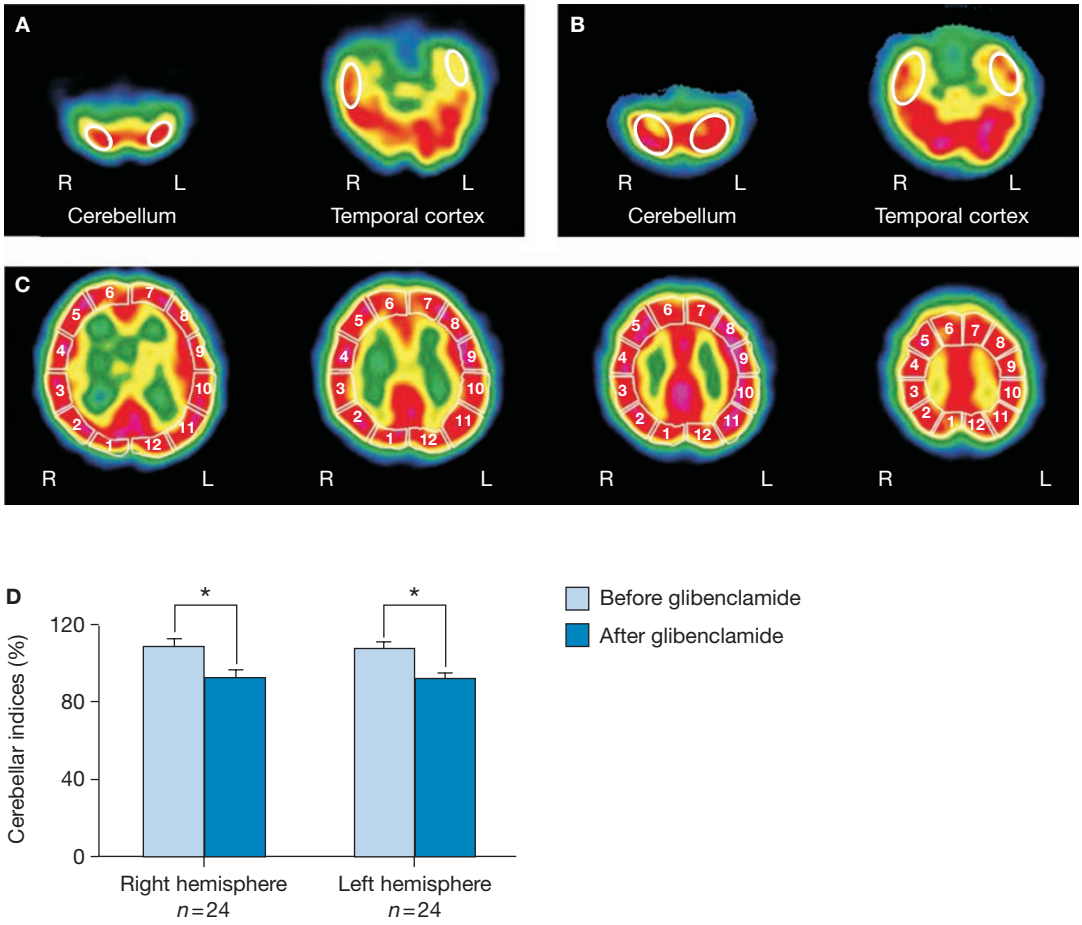

Figure 1 Single-photon emission CT imaging of a patient with neonatal diabetes caused by a missense mutation (His46Leu) in *KCNJ11* indicates an improvement in brain blood flow following glibenclamide therapy. (**A**) Single-photon emission CT scan of the patient's brain showing a focal perfusion deficit in the left temporal cortex. The white borders indicate the areas compared by two independent nuclear radiologists. (**B**) After 6 months of treatment with glibenclamide, the focal perfusion deficit is no longer present and cerebellar perfusion is enhanced. (**C**) Brain cortex areas in four consecutive transaxial slices chosen for calculation of cerebellar indices. (**D**) Mean ± SD cerebellar indices before and 6 months after initiation of glibenclamide treatment. An asterisk denotes a significant difference at the level $P<0.05$. Cerebellar indices are defined as the mean count per pixel for a specific cortical region expressed as a percentage of the average count per pixel measured across the whole cerebellum. As the average count per pixel measured across the whole cerebellum is in the denominator of the cerebellar index, a decrease in the cerebellar index indicates an increase in cerebellar perfusion.

before the second SPECT scan and during the examination did not reveal any fluctuations in blood glucose level (see Supplementary Figure 2 online).

DISCUSSION OF DIAGNOSIS

DEND syndrome is defined as developmental delay with epilepsy, muscle weakness and neonatal diabetes and is caused by mutations in the K_{ATP} channel subunits Kir6.2 (encoded by *KCNJ11)* or SUR1 (sulfonylurea receptor 1; encoded by *ABCC8*).[2] Intermediate DEND syndrome (iDEND) is a less severe condition in which neonatal diabetes is accompanied by muscle weakness and developmental delay but not epilepsy.[2] To date, two *KCNJ11* mutations have been shown to cause iDEND in some but not all patients: 4 of 5 patients with Val59Met and 2 of 7 patients with Arg201Cys had iDEND.[2,5,6] These patients were characterized by low birth weight (mean ~2.5 kg; percentile for weight 0.8–2.4%) despite normal gestational age, neonatal diabetes (mean age at diagnosis 5–6 weeks), muscle weakness, and motor and

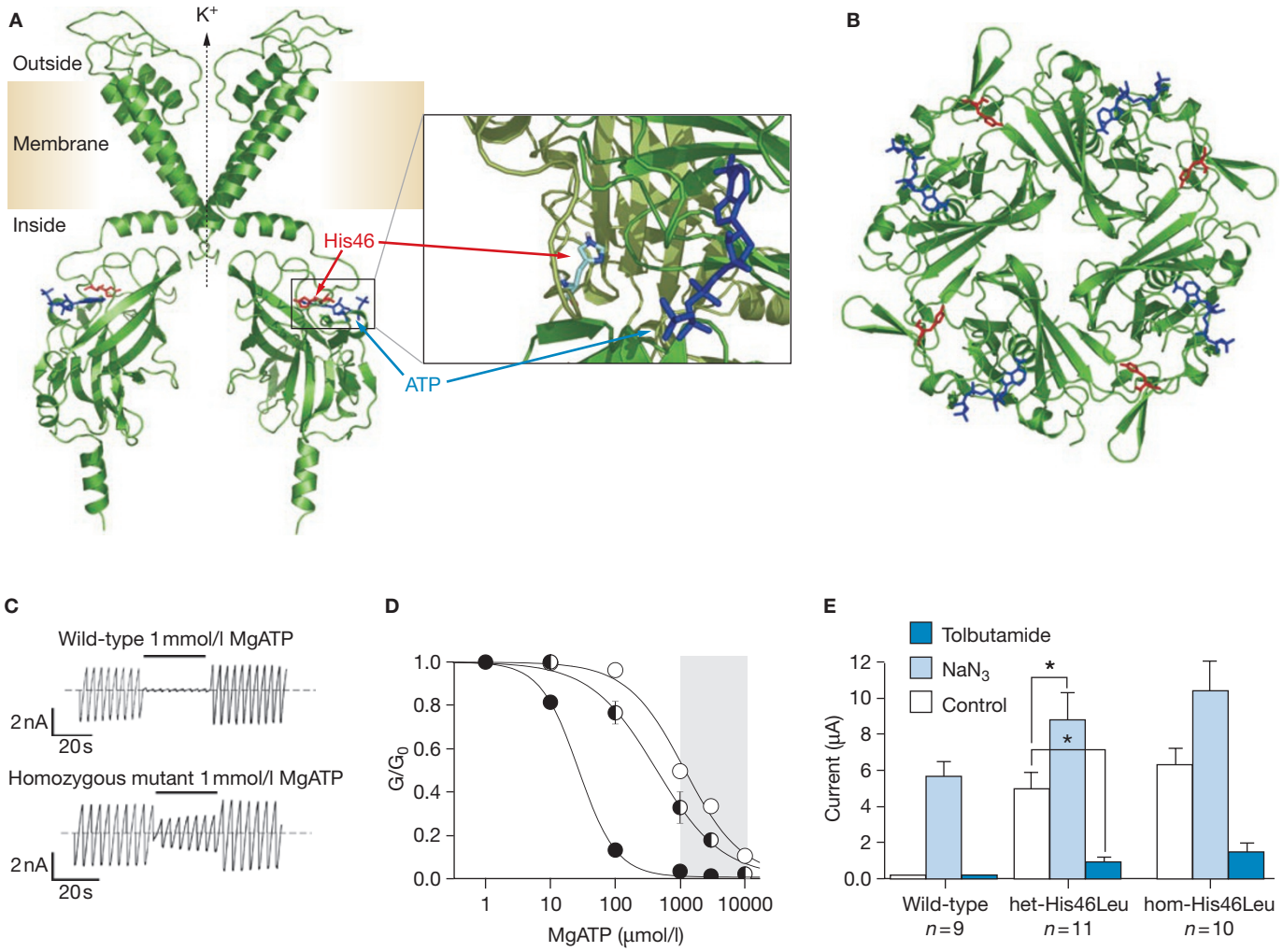

Figure 2 Functional analysis of K_{ATP} channels carrying the Kir6.2-His46Leu mutation demonstrates reduced ATP sensitivity but robust block by sulfonylureas. (**A**,**B**) Molecular model of Kir6.2,[14] viewed from the side (A) and from above (B). For clarity, in (A) only two transmembrane domains and two cytosolic domains are illustrated, and in (B) the transmembrane domains have been removed. ATP is shown in blue and His46 in red. Residue His46 sits near to the ATP-binding site, but does not form part of it. (**C**) Inhibition of mutant K_{ATP} channels by ATP was significantly impaired by the His46Leu mutation. Wild-type (Kir6.2–SUR1) and homozygous mutant (Kir6.2-His46Leu–SUR1) K_{ATP} channels were expressed in *Xenopus* oocytes by co-injection of SUR1 mRNA and either Kir6.2 or Kir6.2-His46Leu mRNA, respectively. Currents were recorded in excised membrane patches in response to successive voltage ramps from –110 mV to +100 mV. The dashed lines indicate the zero current level. MgATP, added to the intracellular side of the membrane, produced a greater block of wild-type channels than of homozygous mutant channels. (**D**) The His46Leu mutation markedly reduced K_{ATP} channel ATP sensitivity and increased the K_{ATP} current at physiological concentrations of ATP (1–10 mmol/l). The mean relationship between MgATP concentration and K_{ATP} conductance (G) is expressed relative to that in the absence of MgATP (G_0). Half-maximal block of wild-type channels (closed circles, $n=6$) was produced by 28 µmol/l ATP and half-maximal block of homozygous His46Leu channels (open circles, $n=6$) was produced by 1.17 mmol/l ATP. To simulate the heterozygous state of the patient we co-injected a 1:1 mix of wild-type and mutant mRNAs: the resulting channels were half-maximally blocked by 544 µmol/l ATP (half-open circles, $n=6$). The shaded area indicates the physiologic range of ATP concentration. See Supplementary Information 1 online for additional data. (**E**) The reduced ATP sensitivity caused by the His46Leu mutation (shown in C,D) produces a large increase in the resting whole-cell K_{ATP} current (white bars). Data for wild-type, heterozygous (het-His46Leu, as in the patient) and homomeric mutant (hom-His46Leu) K_{ATP} channels are shown. In β-cells and neurons this increased K_{ATP} current would cause membrane hyperpolarization and inhibit insulin secretion and electrical activity. Metabolic inhibition with 3 mmol/l sodium azide (NaN_3; light blue bars) lowers intracellular ATP levels and further activates all channel types. Tolbutamide (0.5 mmol/l; dark blue bars) potently blocks mutant K_{ATP} channels, suggesting that it might be effective at closing K_{ATP} channels in a patient's β-cells and neurons, thereby alleviating both their diabetes and their neurological problems. K_{ATP} currents were evoked by a voltage step from –10 mV to –30 mV. An asterisk denotes a significant difference at the level $P<0.01$ (Wilcoxon matched-pairs test). See also Supplementary Information 1 online. Abbreviations: K_{ATP} channel, ATP-sensitive potassium channel; MgATP, magnesium ATP; mRNA, messenger RNA.

mental developmental delay (see Supplementary Table 2 online).[2,6]

The symptoms of the patient discussed in this Case Study were consistent with iDEND, and a mutation in *KCNJ11* was confirmed by sequencing this gene. Mutations in *KCNJ11* that cause iDEND act by reducing the ability of cellular ATP to block to the K_{ATP} channel.[1,2,7] As a consequence, the channel remains open when blood glucose levels rise, preventing insulin secretion.[2] As the mutation (His46Leu) carried by this patient was novel, *in vitro* functional analysis was used to confirm that the mutation reduced K_{ATP} channel ATP sensitivity (Figure 2C,D and Supplementary Information 1 online).

Approximately 50% of patients with low birth weight who present with permanent neonatal diabetes within the first 6 months of life have mutations in *KCNJ11* or *ABCC8*.[2,6] Anti-islet autoantibodies are usually absent. These characteristics differentiate neonatal diabetes from type 1 diabetes, which presents later and in which autoantibodies are present. Neurological features are associated with some *KCNJ11* and *ABCC8* mutations,[2,5,6] but the extent of impairment of neurological function can vary, so patients should be assessed by a neurologist for muscle weakness and motor and mental developmental delay as well as epilepsy. These children might walk and talk later than normal.

TREATMENT AND MANAGEMENT

Recent evidence has shown that patients with gain-of-function mutations in *KCNJ11* can be successfully treated with sulfonylureas, which block open K_{ATP} channels.[8] This treatment removes the necessity for daily insulin injections and markedly enhances glycemic control.[3–5] Functional studies revealed that K_{ATP} channels heterozygous for the His46Leu mutation (as would be the case in the current patient) exhibit a marked sensitivity to inhibition by the sulfonylurea tolbutamide (Figure 2E). When tolbutamide causes a block of this magnitude, patients carrying the relevant *KCNJ11* mutations generally respond well to sulfonylurea therapy.[3] Consequently, sulfonylurea therapy was initiated in the patient presented here, resulting in improved glycemic control and a reduction in Hb_{A1c} levels.

Remediation of this patient's neurological symptoms and mental status suggests that glibenclamide blocks K_{ATP} channels in neurons enough to normalize electrical activity and transmitter release, as it presumably does in β-cells. The improvements in brain blood flow and in motor and mental function are consistent with the high density of K_{ATP} channels found in the cerebellum and cerebral cortex.[9,10] The increase in blood flow observed in the SPECT scans is most likely to reflect an increase in neuronal activity, rather than a direct effect on vascular tissue, because β-cell and neuronal (Kir6.2–SUR1) K_{ATP} channels are more sensitive to glibenclamide block than are vascular (Kir6.2–SUR2B) K_{ATP} channels.[11]

As relatively good glycemic control was achieved before both SPECT examinations, it is unlikely that variability in glucose levels had a large impact on the SPECT images. This finding is consistent with a previous SPECT study of pediatric patients with type 1 diabetes, which found that perfusion of the cerebellum and temporal lobe differed little between those patients with well-controlled and those with poorly controlled diabetes, or between patients with diabetes and healthy subjects.[12] Although we cannot exclude the possibility that the current patient's increased IQ score was attributable, at least in part, to the natural course of the disease, the magnitude of the response and the short time interval (10 months) between the two sets of WPPSI-R tests make this an unlikely scenario. It is possible that the observed increase in IQ was partly a result of the improvement in ADHD symptoms produced by glibenclamide.

A major difference between insulin therapy and sulfonylurea treatment is that the former only controls glucose homeostasis whereas sulfonylureas should block K_{ATP} channels in all tissues to which they have access, and, therefore, alleviate extrapancreatic problems. Although the extent to which sulfonylureas cross the blood–brain barrier is unknown in humans, limited penetrance has been documented in rodents.[13] The improvements in mental and motor function, and in brain blood flow, observed in the patient in this case following glibenclamide treatment suggest that the drug is able to access the brain at concentrations sufficient to influence K_{ATP} channel activity.

CONCLUSIONS

This Case Study describes a novel *KCNJ11* mutation, His46Leu, causing iDEND syndrome. Sulfonylurea (glibenclamide) therapy not only restored glucose homeostasis, but also alleviated some of the neurological symptoms associated

with the patient's impaired CNS function. Good long-term glycemic control together with an improvement in motor function following glibenclamide treatment has recently been reported in a patient with iDEND,[5] but the impact of this treatment on the CNS has not yet been documented. Here we show for the first time that CNS blood flow and function can be improved by sulfonylureas. This study is the first to report the use of SPECT to provide evidence of brain changes that correlate with mental and motor improvement following glibenclamide therapy. This finding does not, of course, prove a causal relationship between the changes in brain perfusion and psychomotor function.

This Case Study re-emphasizes the necessity of screening for K_{ATP} channel mutations in diabetes diagnosed before the age of 6 months, especially if developmental delay is also present. Furthermore, introduction of sulfonylurea treatment in patients carrying K_{ATP} channel mutations should be implemented as soon as possible, not only to improve glycemic control, but also to help alleviate neurological problems.

Supplementary Information in the form of text, tables and figures is available on the *Nature Clinical Practice Neurology* website.

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Competing interests

The authors declared no competing interests.