Switching from Insulin to Oral Sulfonylureas in Patients with Diabetes Due to Kir6.2 Mutations

Ewan R. Pearson, M.R.C.P., Ph.D., Isabelle Flechtner, M.D., Pål R. Njølstad, M.D., Ph.D., Maciej T. Malecki, M.D., Ph.D., Sarah E. Flanagan, B.Sc., Brian Larkin, Ph.D., Frances M. Ashcroft, D.Sc., Ph.D., Iwar Klimes, M.D., D.Sc., Ethel Codner, M.D., Violeta Iotova, M.D., Annabelle S. Slingerland, M.D., Julian Shield, M.B.Ch.B., M.D., Jean-Jacques Robert, M.D., Ph.D., Jens J. Holst, M.D., D.Med.Sc., Penny M. Clark, F.R.C.Path., Ph.D., Sian Ellard, Ph.D., M.R.C.Path., Oddmund Søvik, M.D., Ph.D., Michel Polak, M.D., Ph.D., and Andrew T. Hattersley, F.R.C.P., D.M., for the Neonatal Diabetes International Collaborative Group*

ABSTRACT

BACKGROUND

Heterozygous activating mutations in *KCNJ11*, encoding the Kir6.2 subunit of the ATP-sensitive potassium (K_{ATP}) channel, cause 30 to 58 percent of cases of diabetes diagnosed in patients under six months of age. Patients present with ketoacidosis or severe hyperglycemia and are treated with insulin. Diabetes results from impaired insulin secretion caused by a failure of the beta-cell K_{ATP} channel to close in response to increased intracellular ATP. Sulfonylureas close the K_{ATP} channel by an ATP-independent route.

METHODS

We assessed glycemic control in 49 consecutive patients with Kir6.2 mutations who received appropriate doses of sulfonylureas and, in smaller subgroups, investigated the insulin secretory responses to intravenous and oral glucose, a mixed meal, and glucagon. The response of mutant K_{ATP} channels to the sulfonylurea tolbutamide was assayed in xenopus oocytes.

RESULTS

A total of 44 patients (90 percent) successfully discontinued insulin after receiving sulfonylureas. The extent of the tolbutamide blockade of K_{ATP} channels in vitro reflected the response seen in patients. Glycated hemoglobin levels improved in all patients who switched to sulfonylurea therapy (from 8.1 percent before treatment to 6.4 percent after 12 weeks of treatment, $P<0.001$). Improved glycemic control was sustained at one year. Sulfonylurea treatment increased insulin secretion, which was more highly stimulated by oral glucose or a mixed meal than by intravenous glucose. Exogenous glucagon increased insulin secretion only in the presence of sulfonylureas.

CONCLUSIONS

Sulfonylurea therapy is safe in the short term for patients with diabetes caused by *KCNJ11* mutations and is probably more effective than insulin therapy. This pharmacogenetic response to sulfonylureas may result from the closing of mutant K_{ATP} channels, thereby increasing insulin secretion in response to incretins and glucose metabolism. (ClinicalTrials.gov number, NCT00334711.)

From the Institute of Biomedical and Clinical Sciences, Peninsula Medical School, Exeter (E.R.P., S.E.F., A.S.S, S.E., A.T.H.); the Division of Medicine and Therapeutics, University of Dundee, Dundee (E.R.P.); University Laboratory of Physiology, Oxford, (B.L., F.M.A.); the Department of Child Health, University of Bristol, Bristol (J.S.); and University Hospital Birmingham, Birmingham (P.M.C.) — all in the United Kingdom; the Faculty of Medicine, René Descartes University, Pediatric Endocrinology and Diabetology, Necker Enfants Malades Hospital, Paris (I.F., J-J.R., M.P.); the Department of Clinical Medicine, University of Bergen (P.R.N., O.S.), and the Department of Pediatrics, Haukeland University Hospital (P.R.N.) — both in Bergen, Norway; the Department of Metabolic Diseases, Jagiellonian University, Medical College, Krakow, Poland (M.T.M.); Diabgene, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic (I.K.); Institute of Maternal and Child Research, School of Medicine, University of Chile, Santiago (E.C.); Medical University, Varna, Bulgaria (V.I.); the Department of Cardiology, Leiden University Medical Center, Leiden, the Netherlands (A.S.S.); and the Department of Medical Physiology, the Panum Institute, University of Copenhagen, Copenhagen (J.J.H.). Address reprint requests to Dr. Hattersley at the Peninsula Medical School, Barrack Rd., Exeter EX2 5DW, United Kingdom, or at andrew.hattersley@pms.ac.uk.

Drs. Pearson, Flechtner, and Njølstad contributed equally to this article.

*Other members of the Neonatal Diabetes International Collaborative Group are listed in the Appendix.

The pancreatic ATP-sensitive potassium (K_{ATP}) channel is a critical regulator of beta-cell insulin secretion. Heterozygous activating mutations in the *KCNJ11* gene, which encodes the Kir6.2 subunit of the K_{ATP} channel, have recently been found to cause diabetes in the neonatal period or early infancy.[1] Such mutations account for 30 to 58 percent of cases of permanent diabetes diagnosed in patients under six months of age.[1-7] Less commonly, *KCNJ11* mutations are associated with transient neonatal diabetes[8] or with neonatal diabetes accompanied by neurologic features.[6]

In the pancreatic beta cell, glucose metabolism results in increased intracellular levels of ATP and reduced levels of ADP. These changes in adenine nucleotides lead to the closure of K_{ATP} channels and, ultimately, to insulin secretion. Patients with diabetes caused by *KCNJ11* mutations have K_{ATP} channels with decreased sensitivity to ATP inhibition.[8-11] Consequently, their channels remain open in the presence of glucose, thereby reducing insulin secretion.[1,6] These patients present with diabetic ketoacidosis or marked hyperglycemia with low levels of circulating endogenous insulin[1] and are therefore treated with insulin. Sulfonylureas, a class of drugs used to treat type 2 diabetes mellitus, close K_{ATP} channels by an ATP-independent route, thereby causing insulin secretion. This fact suggests that sulfonylureas may represent a suitable therapy for patients with *KCNJ11* mutations.

Previous reports have described six patients with neonatal diabetes whose treatment was switched from insulin to oral sulfonylurea with short-term follow-up.[2,4,12,13] We investigated the initial response and the sustained response to sulfonylureas in a large consecutive cohort of patients with diabetes caused by *KCNJ11* mutations. We also examined the mechanism of insulin independence in patients who were able to switch from insulin to oral sulfonylurea therapy.

Methods

Patients

A total of 49 consecutive patients from 40 families were identified as having diabetes caused by a heterozygous *KCNJ11* mutation through sequencing performed in molecular genetics laboratories in Exeter, United Kingdom (34 patients), Paris (5 patients), and Bergen, Norway (10 patients). All 49 patients either switched from insulin to sulfonylurea therapy or were unable to switch but received an adequate dose of sulfonylureas before October 2005. No other selection criteria were applied. An adequate dose of sulfonylureas was defined as a dose of glyburide (also known as glibenclamide) of at least 0.8 mg per kilogram of body weight per day of glyburide, since this high sulfonylurea dose was required in two of the published cases.[4,12] Five patients whose initial treatment was previously reported[2,12,13] were included in this series.

Switch to Sulfonylureas

An oral sulfonylurea was introduced, and the insulin dose was reduced on the basis of frequent measurements of capillary blood glucose. Glyburide was administered to 43 of the patients and was introduced according to two standardized protocols. Glyburide was chosen because it blocks K_{ATP} channels containing both the beta-cell (SUR1) and muscle (SUR2) types of sulfonylurea receptors and because of previous experience with its use in children. To switch treatment rapidly in hospital inpatients, glyburide was started at a dose of 0.1 mg per kilogram twice daily and was increased daily by 0.2 mg per kilogram per day. To switch treatment more slowly in outpatients, glyburide was introduced at a dose of 0.1 mg per kilogram per day and was increased by 0.1 mg per kilogram per day once a week. The dose of glyburide was increased until insulin independence was achieved or the dose was at least 0.8 mg per kilogram per day. For young children unable to take tablet medication, oral glyburide suspension was produced by a local pharmacy. (Details about the switching protocol and sulfonylurea formulations appear in the Supplementary Appendix, available with the full text of this article at www.nejm.org.)

The change to sulfonylureas was considered to be successful if a patient was able to stop insulin treatment completely at any dose of glyburide and was deemed to be unsuccessful if insulin was still required with a dose of glyburide of at least 0.8 mg per kilogram per day. Owing to preferences of clinicians, two patients were treated with glipizide gastrointestinal therapeutic system (Pfizer), two patients were treated with gliclazide, one patient with tolbutamide, and one patient with glimepiride. To allow for inclusion of these data, the sulfonylurea dose was expressed as a percentage of the maximum recommended dose (according to the British National Formulary) and converted to an equivalent dose of glyburide.

Measurements of glycated hemoglobin levels, which are closely linked to the mean glucose values during the previous four to six weeks,[14] were available before and after treatment in all patients who switched to sulfonylureas, except in the case of one patient in whom the presence of fetal hemoglobin could affect the assay and in another five patients who switched to sulfonylureas less than six weeks before the end of the study.

To assess the frequency of hypoglycemia, we continuously monitored glucose levels (CGMS, Medtronic) in eight patients for 48 hours while they were receiving insulin and at least one month after treatment was successfully changed to sulfonylureas. Hypoglycemia was assessed as the percentage of time the capillary glucose level was below 3.3 mmol per liter in a 48-hour period.

PHYSIOLOGICAL STUDIES

The following studies were carried out before sulfonylureas were administered and again after treatment was successfully switched to sulfonylureas: an intravenous glucose-tolerance test to assess early insulin response (in 16 patients); an oral glucose-tolerance test with an assay of glucose, insulin, and the incretins glucagon-like peptide 1 (GLP1) and gastrointestinal peptide (GIP) (in 5 patients); and insulin secretion in response to intravenous glucagon (in 5 patients). In addition, to test the response of patients to various stimuli, seven patients receiving sulfonylureas were given a mixed-meal test and oral and intravenous glucose-tolerance tests. (Details of the protocols of all physiological studies are given in the Supplementary Appendix.)

FUNCTIONAL STUDIES

Heterozygous K_{ATP} channels (composed of Kir6.2 and SUR1) containing Kir6.2 mutations that corresponded to the mutations found in the study patients were expressed in xenopus oocytes, as previously described.[10,11] We tested the ability of tolbutamide (at a dose of 0.5 mmol per liter, a saturating concentration for wild-type channels) to block whole-cell K_{ATP} currents activated by 3 mmol of azide per liter.

LABORATORY ASSAYS

Venous blood was collected in lithium heparin for a centralized insulin assay. Insulin was measured by an immunoenzymometric assay (Insulin EASIA, Biosource-Invitrogen) with no detectable cross-reactivity with intact proinsulin and 32-33 split proinsulin. In cases in which the insulin concentration was below the lower limit of the assay (<10 pmol per liter) a value of 10 pmol per liter was used in calculations. Venous blood was collected in EDTA with 50 μmol of aprotinin (Trasylol, Bayer Pharmaceuticals) per milliliter of blood for assays of total GLP1 and GIP, which were carried out as previously described.[15-17] Assays for glycated hemoglobin (glycated hemoglobin with values aligned with those in the Diabetes Control and Complications Trial[18]), glucose, and C-peptide were performed by local laboratories according to their standard procedures.

STATISTICAL ANALYSIS

Changes in glycated hemoglobin levels were assessed by the paired t-test, and data are expressed as means with 95 percent confidence intervals. In cases in which data were not normally distributed (i.e., for insulin, C-peptide, glucose, and hypoglycemia data from continuous glucose monitoring), the Wilcoxon signed-rank test and Friedman's test were used for related sample analyses and the Mann–Whitney test (for GLP1 and GIP) for independent sample analyses; data are expressed as medians with interquartile ranges.

All patients or their parents gave written informed consent. The studies were approved by the ethics committees of the institutions in Exeter, United Kingdom; Paris; and Bergen, Norway.

RESULTS

TREATMENT WITH SULFONYLUREAS

Successful Switching

Of the 49 patients who were treated with an adequate dose of sulfonylureas, 44 were able to stop insulin treatment; their genetic and clinical characteristics are shown in Table 1. The switch to sulfonylureas was successful regardless of the type of sulfonylurea used, suggesting a class effect. C-peptide was undetectable (<165 pmol per liter) before sulfonylurea therapy in 83 percent of the patients whose treatment was successfully switched from insulin, which indicated that they had not secreted a significant amount of endogenous insulin in the absence of sulfonylurea. The youngest patient to switch treatment was 3 months of age, and the oldest was 36 years of age. The median equivalent dose of glyburide that was initially required for insulin independence was 0.45 mg per kilogram per day (range, 0.05 to 1.5 mg per kilo-

Table 1. Baseline Characteristics of the Patients, According to the Success of the Switch to Sulfonylurea Therapy.*

Characteristic	All Patients (N=49)	Patients with Successful Switch (N=44)	Patients with Unsuccessful Switch (N=5)	P Value†
Mutation	NA	F35V, H46Y, R50Q, G53N, G53R, V59M (6 patients), K170T, R201C (5 patients), R201H (23 patients), R201L, E322K, Y330S, and F333I	Q52R, G53R, L164P, R201C, and I296L	NA
Neurologic features — no. (%)	NA	6 (14), all with intermediate DEND (1 with G53N and 5 with V59M)	4 (80), including 2 with DEND (with Q52R and I296L) and 2 with intermediate DEND (with G53R and R201C)	0.004
Male sex — %	51	55	20	0.39
Birth weight — g				0.08
Median	2710	2740	2550	
Interquartile range	2405 to 3090	2420 to 3115	2075 to 2685	
Birth weight — SD score				
Median	–1.0	–1.0	–2.0	0.18
Interquartile range	–2.2 to –0.5	–2.2 to –0.5	–2.7 to –1.1	
Age at diagnosis — mo				
Median	1.5	1.5	1.2	0.76
Interquartile range	0.5 to 3.0	0.6 to 3.0	0.4 to 4.0	
Ketoacidosis at diagnosis — %	33	34	20	0.66
Age at initiation of sulfonylurea treatment — yr				
Median	7	6	18	0.04
Interquartile range	3 to 14	3 to 12	6 to 35	
Weight at time of switch to sulfonylurea treatment — SD score				
Median	0.05	0.12	–0.41	0.38
Interquartile range	–0.69 to 0.90	–0.63 to 0.98	–2.63 to 1.13	
C-peptide levels undetectable — no./total no. (%)‡	24/28 (86)	20/24 (83)	4/4 (100)	0.11
Insulin dose — U/kg/day				
Median	0.7	0.7	0.4	0.07
Interquartile range	0.6 to 0.9	0.6 to 0.9	0.4 to 0.7	
Glycated hemoglobin — %				
Median	8.0	8.1	7.4	0.65
Interquartile range	7.1 to 9.5	7.1 to 9.4	5.3 to 9.5	
Equivalent dose of glyburide — mg/kg/day				
Median	NA	0.45	1.0	NA
Range		0.05 to 1.50	0.80 to 2.28	
Interquartile range		0.25 to 0.82	0.90 to 1.74	

* DEND denotes developmental delay, epilepsy, and neonatal diabetes; and NA, not applicable.
† P values are for the comparison of the patients with a successful switch with patients with an unsuccessful switch and were calculated by the Mann–Whitney test or Fisher's exact test for categorical data.
‡ C-peptide levels were considered undetectable if they were below 165 pmol per liter.

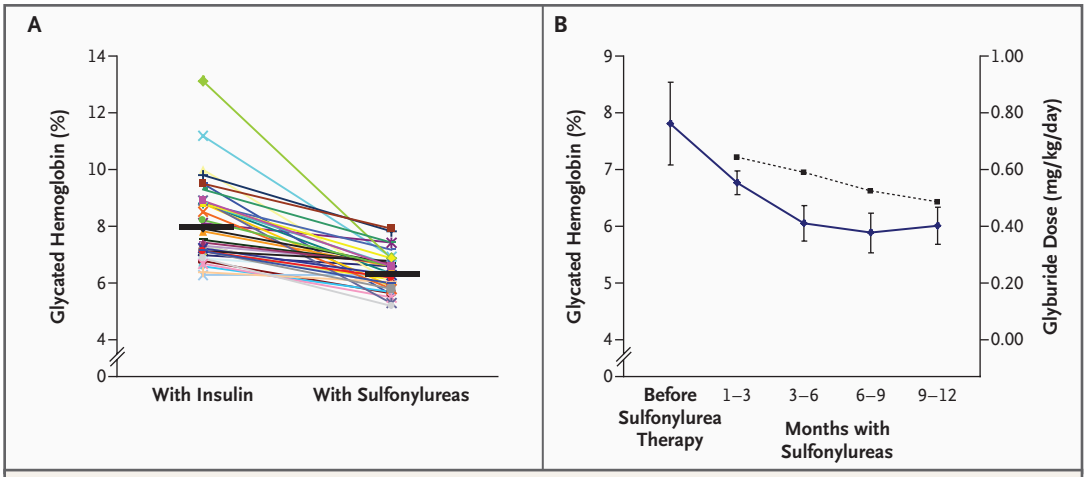

Figure 1. Reduction in Glycated Hemoglobin Levels Associated with Switching from Insulin to Sulfonylurea Therapy.
Panel A shows glycated hemoglobin levels for 38 patients (for whom data were available) recorded when they were taking insulin alone; the mean values measured between 4 and 26 weeks after the cessation of insulin and the switch to sulfonylurea therapy. Data are included for patients whose response to initial sulfonylurea treatment was previously reported. Panel B shows mean glycated hemoglobin levels and sulfonylurea doses for 12 patients who had switched from insulin to sulfonylurea therapy at least 12 months previously. The dashed line represents the mean equivalent dose of glyburide. I bars denote 95 percent confidence intervals.

gram per day), which is higher than the maximum recommended dose for the treatment of type 2 diabetes in an adult weighing 60 kg (0.25 mg per kilogram per day). Glycemic control was improved in all 38 patients tested (Fig. 1A). The mean glycated hemoglobin level before treatment with sulfonylurea fell from 8.1 percent (95 percent confidence interval, 7.7 to 8.6 percent) to 6.4 percent (95 percent confidence interval, 6.2 to 6.6 percent), with an absolute reduction in glycated hemoglobin of 1.7 percentage points (95 percent confidence interval, 1.3 to 2.1) ($P<0.001$) at a mean of 12 weeks after the cessation of insulin. The initial improvement in glycated hemoglobin levels was sustained in the 12 patients who were insulin-independent for more than one year (Fig. 1B), despite a reduction in the dose of sulfonylureas. Four patients were insulin-independent for more than 15 months, with a mean glycated hemoglobin level of 6.0 percent, and the longest duration was 2.0 years, with a glycated hemoglobin level of 5.7 percent.

Unsuccessful Switching

Five patients (10 percent) were unable to stop receiving insulin despite receiving glyburide at a dose of at least 0.8 mg per kilogram per day (Table 1). Four of these patients (80 percent) had neurologic features, including two patients (whose *KCNJ11* mutations were Q52R and I296L) who had severe developmental delay, epilepsy, and neonatal diabetes, known as the DEND syndrome.[6] In contrast, only six patients (14 percent) whose treatment was successfully switched to sulfonylureas had neurologic features ($P=0.004$).

In two families, the mothers (who were 43 and 27 years of age) were unable to switch to treatment with sulfonylureas, even though their affected children (11 and 3 months of age, respectively) were able to do so. This fact suggests that the lack of response to sulfonylurea therapy in these parents was not a specific characteristic of the mutation.

In Vitro Block of K_{ATP} Channel Current

The response of wild-type and mutant K_{ATP} channels expressed in xenopus oocytes to the sulfonylurea tolbutamide is shown in Figure 2. The use of tolbutamide blocked more than 75 percent of the K_{ATP} current through all the channels carrying *KCNJ11* mutations found in patients who had a clinical response to sulfonylureas. In contrast, the tolbutamide block was less than 65 percent for the three mutations (Q52R, I296L, and L164P) in which there was no response to sulfonylureas in patients. Thus, the functional properties of the channel predict the clinical response seen in patients.

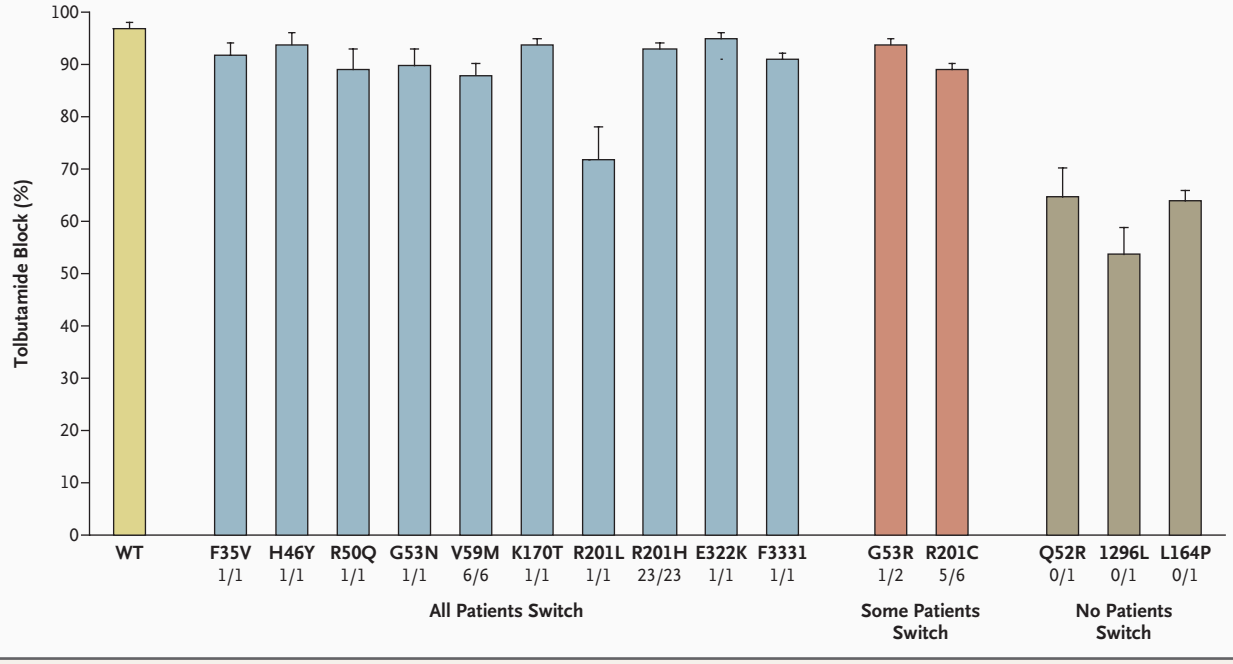

Figure 2. Correlation between the Sensitivity of K_{ATP} Channels to Sulfonylurea and Clinical Response in Patients with Kir6.2 Mutations.

The chart shows the mean percentage of inhibition of K_{ATP} currents through wild-type (WT) or mutant K_{ATP} channels by 0.5 mM of tolbutamide. The Kir6.2 mutation is indicated below the bar. The fractions below the bars represent the number of patients with the mutation who had a response to treatment, divided by the total number of patients with that mutation. Currents were measured in the presence of 3 mM of azide. Data indicate the mean of 5 to 13 oocytes. The T bars denote SE. The extent of the block was greatest for Kir6.2 mutations carrried by patients who had a response to sulfonylureas (indicated in blue) and smallest for mutations carried by patients who did not have a clinical response to sulfonylureas (indicated in brown).

Side Effects

Five patients had transitory diarrhea (lasting less than four days) without pyrexia, which was associated with abdominal pain in two patients. No dose reduction was necessary, and after the resolution of diarrhea, the sulfonylurea dose was increased. No other side effects of sulfonylureas were reported. In 12 children between the ages of 1.6 and 12.4 years who had received sulfonylurea treatment for more than one year, such treatment had no detrimental effect on growth, as compared with that in an age-matched population. Among these children, the mean (±SE) standard-deviation score for weight was −0.30±0.26 before treatment was switched and −0.32±0.26 after treatment was switched (P=0.86) and for height, −0.16±0.36 and −0.01±0.33, respectively (P=0.11). Despite improved glycated hemoglobin levels, no patients reported having severe hypoglycemia (grade 3 in the 2000 consensus guidelines of the International Society for Pediatric and Adolescent Diabetes). Continuous glucose monitoring showed no significant change in the frequency of hypoglycemic episodes when patients were receiving sulfonylureas, despite improved glycemic control. The median percentages of measurements that were below 3.3 mmol per liter were 2 percent (interquartile range, 0.3 to 15.6) in patients receiving insulin and 5 percent (interquartile range, 0.3 to 17.0) in patients who received four to eight weeks of sulfonylurea therapy (P=0.80).

PHYSIOLOGICAL STUDIES

The near normalization of glycated hemoglobin levels without significant hypoglycemia in patients receiving sulfonylurea treatment suggests that insulin secretion was well regulated. To determine whether this was the case, we examined basal C-peptide levels and the insulin secretion in response to intravenous glucose, oral glucose, a mixed-meal test, and intravenous glucagon. Samples from subgroups of a varying number of patients were used in these assays (Table 1 of the Supplementary Appendix).

In 19 patients, fasting levels of C-peptide increased by a median of 75 pmol per liter (interquartile range, 4 to 194; P=0.001) after treatment was switched to sulfonylureas. In 16 patients who had successfully transferred to sulfonylureas, the maximum insulin increment in response to intravenous glucose increased from 1.9 pmol per liter to 20.4 pmol per liter (P=0.01) (Fig. 3A). In five patients, the maximum insulin increment in response to oral glucose increased from 7.1 pmol per liter to 53.6 pmol per liter (P=0.04) (Fig. 3A).

To investigate further the difference between intravenous and oral stimuli, seven patients who had successfully switched to sulfonylurea treatment underwent intravenous and oral glucose-tolerance tests and a mixed-meal test. We observed a greater maximum insulin secretory response to oral glucose (132.0 pmol per liter, P=0.03) or a mixed meal (143.6 pmol per liter, P=0.02) than intravenous glucose (26 pmol per liter), despite the smaller increment in plasma glucose concentration effected by oral administration (Fig. 3B).

GLP1 concentrations in four patients before and after treatment was switched to sulfonylureas were similar before the intake of oral glucose (P=0.28) and after the intake of oral glucose (P=0.20) (Fig. 3C). Similar results were seen for GIP (data not shown). This finding suggests that the improved insulin secretory response of the beta cell to oral glucose or a mixed meal in patients receiving sulfonylurea therapy does not reflect an increased secretion of incretins but, rather, an improved response of the beta cell to incretins. Basal and stimulated GLP1 levels were similar to those of healthy control subjects.[19,20]

Because glucagon, like GLP1, has an effect on insulin secretion through the elevation of beta-cell cyclic AMP (cAMP), we assayed insulin secretion in response to exogenous glucagon. Glucagon had very little effect on insulin secretion in patients before treatment was switched to sulfonylureas (an increment of 1.6 pmol per liter), but it stimulated insulin secretion in patients whose treatment was successfully switched to sulfonylureas (an increment of 57.7 pmol per liter, P=0.04) (Fig. 3D).

DISCUSSION

We found that most patients with diabetes caused by *KCNJ11* mutations can successfully switch from treatment by insulin injection to oral sulfonylurea therapy. This finding is in contrast to that for most patients with diabetes and undetectable insulin secretion, who need lifelong insulin therapy. This is an example of pharmacogenetics, since the genetic cause of the insulin deficiency determines the response to treatment.

The success of sulfonylurea therapy in patients with Kir6.2 mutations reflects the in vitro response of the K_{ATP} channel with the same mutation to tolbutamide. The blockade of the mutated K_{ATP} channel by sulfonylureas is related to the molecular mechanism of the mutation on channel function.[10,11,21] K_{ATP} channels with the Q52R, I296L, and L164P mutations, which affect the channel kinetics,[10,11] showed only a small response to tolbutamide. Conversely, K_{ATP} channels with Kir6.2 mutations that affect ATP sensitivity independently of the channel kinetics (such as mutations at residue 201) had a greater response to tolbutamide.[10,21]

Despite the relatively high doses used in this study, sulfonylurea treatment appears to be safe, with the only reported side effect being transitory diarrhea. Doses were reduced at the discretion of the clinician, usually in response to measurements of capillary blood glucose. Continued follow-up will be required to determine the long-term outcome of sulfonylurea therapy in this group of patients.

A mean glycated hemoglobin level of 6.4 percent in patients receiving sulfonylureas compares favorably with levels in the Diabetes Control and Complications Study, in which patients undergoing intensive insulin treatment and education had a mean glycated hemoglobin level of 7.1 percent.[18] It is particularly encouraging that in our study, this level was achieved without any severe hypoglycemia and no increase in mild-to-moderate hypoglycemia, although further long-term study is needed to assess this finding fully. The near normalization of glycated hemoglobin levels with minimal hypoglycemia reflects regulated insulin secretion, as demonstrated by continuous glucose monitoring.[4,13]

Patients with Kir6.2 mutations who were receiving sulfonylurea therapy had marked insulin secretion in response to both oral glucose and a mixed meal but only moderate first-phase insulin secretion in response to intravenous glucose (6 percent of that seen in controls[22]). This finding was consistent with the hypothesis that improved glycemic control is largely mediated by incretins, such as GLP1. Our data suggest that

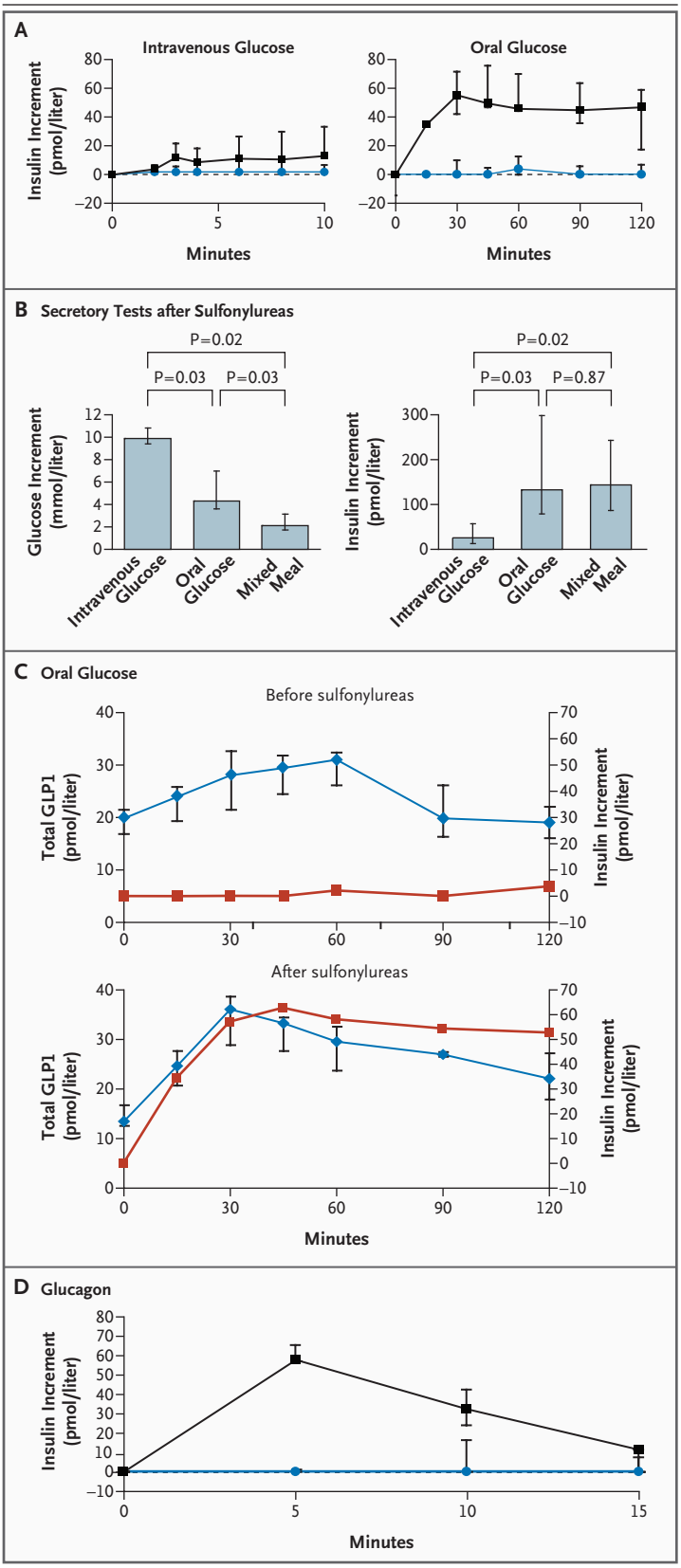

Figure 3. Physiological Studies of the Effect and Associated Mechanisms of Sulfonylurea Treatment on Insulin Secretion.

Panel A shows the median incremental increase in insulin concentration above baseline in an intravenous glucose-tolerance test (16 patients) and an oral glucose-tolerance test (5 patients) before treatment was switched from insulin to sulfonylureas (blue lines) and after treatment (black lines). Panel B shows the median incremental increase in insulin and glucose concentration from baseline in response to intravenous glucose, oral glucose, and a mixed meal in seven patients whose treatment was successfully switched from insulin to sulfonylurea. Panel C shows median concentration of total glucagon-like peptide 1 (GLP1) (blue lines) and the median incremental increase in insulin concentration above baseline (red lines) in response to an oral glucose-tolerance test in four patients before and after treatment was switched to sulfonylurea. Panel D shows the median incremental increase in insulin concentration above baseline after glucagon stimulation in five patients before sulfonylurea therapy (blue line) and after sulfonylurea therapy (black line). The I bars in all panels denote interquartile ranges.

sulfonylureas do not increase GLP1 secretion by gastrointestinal L cells (Fig. 3C) but change the ability of beta cells to secrete insulin in response to GLP1. In keeping with this explanation, glucagon (which, like GLP1, acts through the action of cAMP) does not stimulate insulin secretion unless sulfonylureas are present (Fig. 3D). We propose that in the absence of sulfonylureas, the beta-cell membrane is hyperpolarized, which prevents the beta cell from responding to incretins or other stimuli (Fig. 4A).[23-26] Sulfonylureas close the K_{ATP} channel and depolarize the membrane potential enough that beta cells become able to respond to GLP1 and other secretagogues (Fig. 4B).

Our study has several limitations. First, since the study was multicentered and multinational, investigators chose to use various sulfonylureas and approaches for introducing them. We included data on all consecutive patients tested with sulfonylurea therapy. The success with various sulfonylureas suggests this is a class effect and not specific to a single drug. Second, assays of glycated hemoglobin, glucose, and C-peptide were performed by local laboratories. However, since paired statistical analyses were applied to these data, differences between laboratory assays should not alter the results. A third limitation is the small number of patients in some of the physiological studies. A large number of patients were infants or young children, so repeated studies were difficult be-

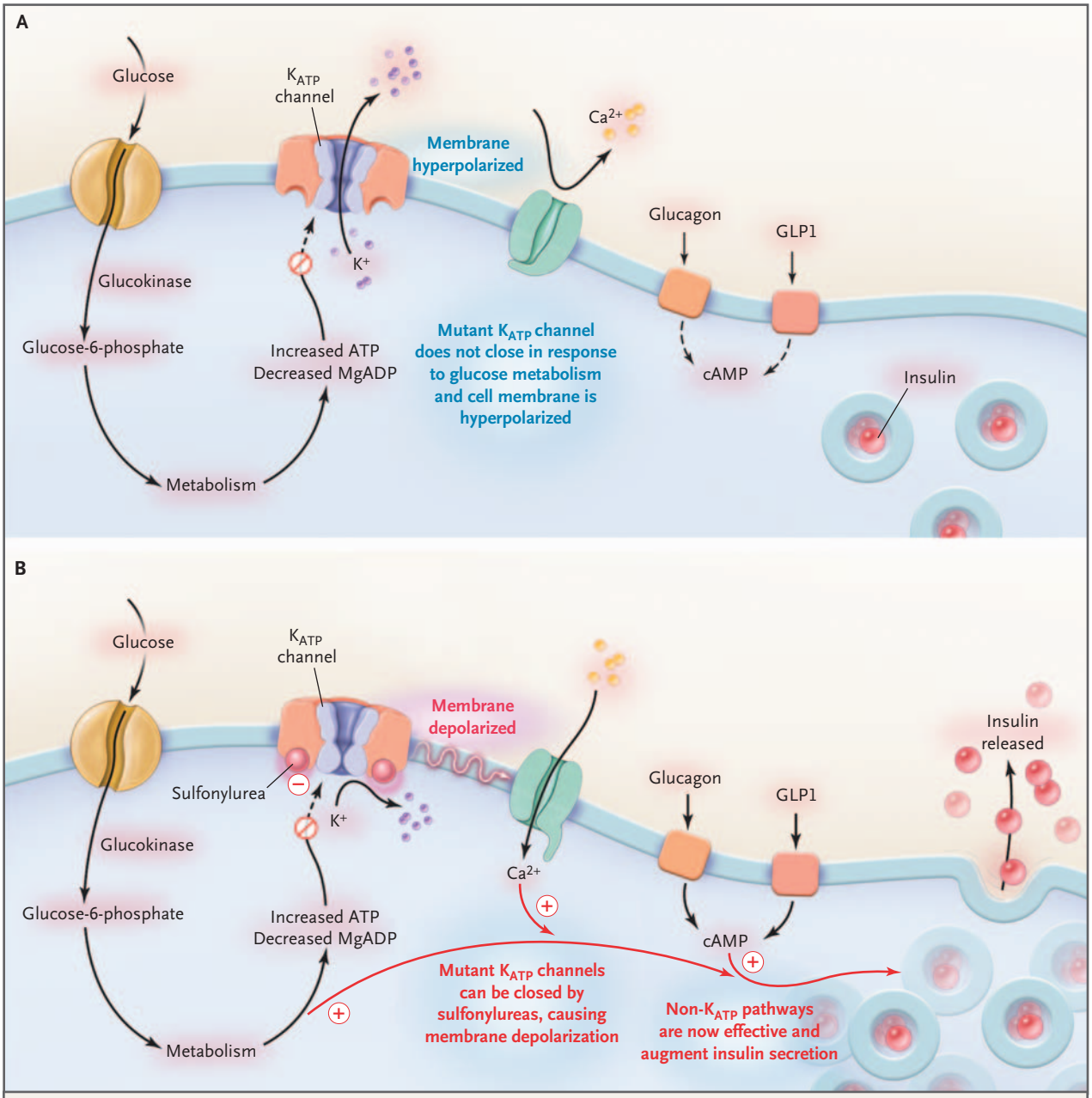

Figure 4. Proposed Model of the Action of Sulfonylurea on Beta Cells Expressing Mutations in the Kir6.2 Subunit of the K_{ATP} Channel.

In Panel A, glucose enters the beta cell and is metabolized, which leads to an increase in intracellular ATP and a decrease in magnesium ADP (MgADP).[23-25] Since mutations in the Kir6.2 subunit of the K_{ATP} channel are less sensitive to ATP inhibition, K_{ATP} channels remain open in the presence of glucose, which keeps the plasma membrane hyperpolarized. This hyperpolarization keeps voltage-gated calcium channels closed, preventing calcium influx and insulin release. Other stimuli, such as GLP1, are ineffective because the membrane is hyperpolarized and cytosolic calcium levels remain low. In Panel B, sulfonylureas bind to the SUR1 subunit of the K_{ATP} channel, closing mutant K_{ATP} channels, which results in membrane depolarization. This process triggers the opening of voltage-gated calcium channels, causing calcium influx and a small increase in insulin release. The rise in the level of intracellular calcium renders potentiators of insulin secretion, such as incretins (e.g., GLP1), capable of augmenting insulin secretion. It is also possible that the sulfonylurea dose may not be sufficient to depolarize the membrane completely but that GLP1, for example, can produce an additional small depolarization that facilitates calcium influx if most K_{ATP} channels are shut.[26]

cause the amount of blood that could be drawn was limited and patients were very reluctant to stop sulfonylurea therapy once oral treatment was established. However, we were able to do multiple physiological studies in various groups of patients, and in most cases, we presented paired data before and after sulfonylurea therapy. A final issue is that we did not carry out a randomized trial of sulfonylureas as compared with intensive insulin treatment, so caution is necessary before stating the superiority of sulfonylurea therapy over insulin therapy in this group of patients, even though glycated hemoglobin levels improved in 100 percent of patients who switched to sulfonylureas.

Our study has shown that most patients with Kir6.2 mutations can be switched from insulin injections to oral sulfonylurea therapy and that such treatment is both safe and highly effective in the short term. Recently, mutations in the gene encoding SUR1, the other component of the K_{ATP} channel, have been shown to cause neonatal diabetes, as reported by Proks et al.[27] and by Babenko et al.[28] (The latter article appears elsewhere in this issue of the *Journal*.) The fact that four patients with SUR1 mutations were able to switch from insulin to sulfonylurea therapy suggests that many of these patients may also be successfully treated with sulfonylureas.[28] However, not all forms of neonatal diabetes have been shown to respond to sulfonylurea treatment. Response to a sulfonylurea was not seen in patients with homozygous mutations in the glucokinase gene (unpublished data) and is not expected in patients with mutations in *FOXP3* (which cause the immunodysregulation, polyendocrinopathy, enteropathy, and X-linked [IPEX] syndrome)[29] or *IPF1* (which cause pancreatic agenesis).[30] Therefore, a molecular diagnosis is required before the use of sulfonylurea therapy in neonatal diabetes is considered. We recommend early molecular genetic diagnosis in all patients with diabetes whose disease was diagnosed before the age of six months,[7] whatever their current age, since the identification of patients with Kir6.2 mutations has important therapeutic implications.

Supported by grants (067463, to Dr. Hattersley; 076436, to Dr. Ashcroft; and 065686, to Dr. Pearson) from the Wellcome Trust, a grant (to Dr. Pearson) from the National Health Service Education for Scotland, a grant (2P0E-13629, to Dr. Malecki) from the Ministry of Scientific Research and Information Technology, a grant (to Dr. Njølstad) from the University of Bergen and Haukeland University Hospital, a grant (to Dr. Holst) from the Danish Medical Research Council and European Federation for the Study of Diabetes, a grant (LSHM-CT-2006-518153, to Drs. Hattersley and Ashcroft) from the European Union, a grant (to Dr. Polak) from the Aide aux Jeunes Diabétiques, a grant (to Dr. Slingerland) from the Child Health and Wellbeing Fund, and a grant (51-014205, to Dr. Klimes) from the Slovakian Research and Development Support Agency. No other potential conflict of interest relevant to this article was reported.

We are indebted to Helene Cave of the Robert Debré Hospital in Paris for her collaboration.

APPENDIX

The following investigators also participated in the Neonatal Diabetes International Collaborative Group study: *Hackensack University Medical Center, Hackensack, N.J.* — J. Aisenberg; *Comenius University Pediatric Hospital, Bratislava, Slovak Republic* — L. Barak, J. Stanik; *Birmingham Children's Hospital, Birmingham, United Kingdom* — T. Barrett, N. Shaw; *Hôpital Saint-Jacques, Besancon, France* — A. Bertrand; *Motol University Hospital, Prague, Czech Republic* — O. Cinek, Z. Sumnik; *Stollery Children's Hospital, University of Alberta, Edmonton, Alta., Canada* — B. Couch; *Centre Hospitalier, Saint-Germaine-en-Laye, France* — H. Crosnier; *Dalhousie University, Halifax, N.S., Canada* — E. Cummings; *Diabgene, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic* — D. Gasperikova; *University Laboratory of Physiology, Oxford, United Kingdom* — H. de Wet, C. Girard; *Women's and Children's Hospital, Adelaide, Australia* — J. Fairchild; *Centro Endocrinologico Clinica Santa Maria, Santiago, Chile* — H. Garcia; *Bradford Hospitals, Bradford, United Kingdom* — S. Gorman; *Children's Hospital "Lindenhof," Berlin* — H. Haberland; *Barnet General Hospital, Barnet, United Kingdom* — V. Hakeem; *Loma Linda University, Loma Linda, Calif.* — E. Hathout; *Children's Hospital at Westmead, Sydney* — N. Howard, S. Srinivasan; *Wishaw General Hospital, Wishaw, United Kingdom* — I. Hunter; *Innlandet Hospital, Lillehammer, Norway* — A.K. Høgåsen, H. Baevre; *Edinburgh Royal Infirmary, Edinburgh* — A. Jaap; *Derbyshire Royal Infirmary, Derby, United Kingdom* — P. King; *Jagiellonian University Medical College, Krakow, Poland* — T. Klupa, J. Nazim, J. Sieradzki; *Louisiana State University Health Sciences Center, Shreveport* — R. McVie; *Rikshospitalet University Hospital, Oslo* — A.G. Myhre; *Royal Edinburgh Hospital for Sick Children, Edinburgh* — K. Noyes; *Meander Medical Center, Amersfoort, the Netherlands* — R. Nuboer; *Safarik University, Kosice, Slovak Republic* — M. Paskova; *University Hospital, Rovaniema, Finland* — S. Pontynen; *Center for Research of Diabetes, Metabolism and Nutrition, Charles University, Prague, Czech Republic* — S. Pruhova; *University of Tennessee College of Medicine, Chattanooga* — M.-L. Rincon; *Carmarthen Hospitals, Carmarthen, United Kingdom* — V. Saxena; *Rambam Medical Center, Haifa, Israel* — N. Shehadeh; *Piaui State University Medical School, Teresina, Piaui, Brazil* — J.M. Silva; *Hôpital Jeanne de Flandre, Lille, France* — C. Stuckens; *Medical University, Varna, Bulgaria* — V. Tzaneva; *Derbyshire Children's Hospital, Derby, United Kingdom* — T. Tinklin; *University Clinic, Luebeck, Germany* — V. Wagner; *Sheffield Children's Hospital, Sheffield, United Kingdom* — J. Wales; *Sydney Children's Hospital, Sydney* — J. Walker, H. Woodhead; *Norwegian University of Science and Technology, Trondheim, Norway* — R. Ødegård; and Sophia Children's Hospital, Rotterdam, the Netherlands — G. Bruining.

REFERENCES

1. Gloyn AL, Pearson ER, Antcliff JF, et al. Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. N Engl J Med 2004;350:1838-49. [Erratum, N Engl J Med 2004;351:1470.]
2. Sagen JV, Raeder H, Hathout E, et al. Permanent neonatal diabetes due to mutations in KCNJ11 encoding Kir6.2: patient characteristics and initial response to sulfonylurea therapy. Diabetes 2004;53:2713-8.

3. Vaxillaire M, Populaire C, Busiah K, et al. Kir6.2 mutations are a common cause of permanent neonatal diabetes in a large cohort of French patients. Diabetes 2004; 53:2719-22.
4. Zung A, Glaser B, Nimri R, Zadik Z. Glibenclamide treatment in permanent neonatal diabetes mellitus due to an activating mutation in Kir6.2. J Clin Endocrinol Metab 2004;89:5504-7.
5. Massa O, Iafusco D, D'Amato E, et al. KCNJ11 activating mutations in Italian patients with permanent neonatal diabetes. Hum Mutat 2005;25:22-7.
6. Hattersley AT, Ashcroft FM. Activating mutations in Kir6.2 and neonatal diabetes: new clinical syndromes, new scientific insights, and new therapy. Diabetes 2005;54:2503-13.
7. Flanagan SE, Edghill EL, Gloyn AL, Ellard S, Hattersley AT. Mutations in KCNJ11, which encodes Kir6.2, are a common cause of diabetes diagnosed in the first 6 months of life, with the phenotype determined by genotype. Diabetologia 2006;49:1190-7.
8. Gloyn AL, Reimann F, Girard C, et al. Relapsing diabetes can result from moderately activating mutations in KCNJ11. Hum Mol Genet 2005;14:925-34.
9. Gloyn AL, Cummings EA, Edghill EL, et al. Permanent neonatal diabetes due to paternal germline mosaicism for an activating mutation of the KCNJ11 gene encoding the Kir6.2 subunit of the beta-cell potassium adenosine triphosphate channel.
J Clin Endocrinol Metab 2004;89:3932-5.
10. Proks P, Antcliff JF, Lippiat J, Gloyn AL, Hattersley AT, Ashcroft FM. Molecular basis of Kir6.2 mutations associated with neonatal diabetes or neonatal diabetes plus neurological features. Proc Natl Acad Sci U S A 2004;101:17539-44.
11. Proks P, Girard C, Haider S, et al. A gating mutation at the internal mouth of the Kir6.2 pore is associated with DEND syndrome. EMBO Rep 2005;6:470-5.
12. Codner E, Flanagan S, Ellard S, Garcia H, Hattersley AT. High-dose glibenclamide can replace insulin therapy despite transitory diarrhea in early-onset diabetes caused by a novel R201L Kir6.2 mutation. Diabetes Care 2005;28:758-9.
13. Klupa T, Edghill EL, Nazim J, et al. 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. Diabetologia 2005; 48:1029-31.
14. Rohlfing CL, Wiedmeyer HM, Little RR, England JD, Tennill A, Goldstein DE. Defining the relationship between plasma glucose and HbA(1c): analysis of glucose profiles and HbA(1c) in the Diabetes Control and Complications Trial. Diabetes Care 2002;25:275-8.
15. Krarup T, Madsbad S, Moody AJ, et al. Diminished immunoreactive gastric inhibitory polypeptide response to a meal in newly diagnosed type I (insulin-dependent) diabetics. J Clin Endocrinol Metab 1983;56:1306-12.
16. Holst JJ. Evidence that enteroglucagon (II) is identical with the C-terminal sequence (residues 33-69) of glicentin. Biochem J 1982;207:381-8.
17. Orskov C, Rabenhoj L, Wettergren A, Kofod H, Holst JJ. Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. Diabetes 1994;43:535-9.
18. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977-86.
19. Nauck MA, El-Ouaghlidi A, Gabrys B, et al. Secretion of incretin hormones (GIP and GLP-1) and incretin effect after oral glucose in first-degree relatives of patients with type 2 diabetes. Regul Pept 2004;122: 209-17.
20. Muscelli E, Mari A, Natali A, et al. Impact of incretin hormones on {beta}-cell function in subjects with normal or impaired glucose tolerance. Am J Physiol Endocrinol Metab (in press).
21. Koster JC, Remedi MS, Dao C, Nichols CG. ATP and sulfonylurea sensitivity of mutant ATP-sensitive K+ channels in neonatal diabetes: implications for pharmacogenomic therapy. Diabetes 2005;54:2645-54.
22. Sagen JV, Pearson ER, Johansen A, et al. Preserved insulin response to tolbutamide in hepatocyte nuclear factor-1alpha mutation carriers. Diabet Med 2005;22: 406-9.
23. Kennedy HJ, Pouli AE, Ainscow EK, Jouaville LS, Rizzuto R, Rutter GA. Glucose generates sub-plasma membrane ATP microdomains in single islet beta-cells: potential role for strategically located mitochondria. J Biol Chem 1999;274:13281-91.
24. Maechler P, Wang H, Wollheim CB. Continuous monitoring of ATP levels in living insulin secreting cells expressing cytosolic firefly luciferase. FEBS Lett 1998; 422:328-32.
25. Freeman H, Shimomura K, Horner E, Cox RD, Ashcroft FM. Nicotinamide nucleotide transhydrogenase: a key role in insulin secretion. Cell Metab 2006;3:35-45.
26. Ashcroft F, Rorsman P. Type 2 diabetes mellitus: not quite exciting enough? Hum Mol Genet 2004;13:Spec No. 1:R21-R31.
27. Proks P, Arnold AL, Bruining J, et al. A heterozygous activating mutation in the sulphonylurea receptor SUR1 (ABCC8) causes neonatal diabetes. Hum Mol Genet 2006;15:1793-800.
28. Babenko AP, Polak M, Cavé H, et al. Activating mutations in the *ABCC8* gene in neonatal diabetes mellitus. N Engl J Med 2006;355:456-66.
29. Slingerland AS, Hattersley AT. Mutations in the Kir6.2 subunit of the KATP channel and permanent neonatal diabetes: new insights and new treatment. Ann Med 2005;37:186-95.
30. Stoffers DA, Zinkin NT, Stanojevic V, Clarke WL, Habener JF. Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. Nat Genet 1997;15:106-10.