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ORIGINAL ARTICLE

Activating Mutations in the *ABCC8* Gene in Neonatal Diabetes Mellitus

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ABSTRACT

BACKGROUND

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The ATP-sensitive potassium (K_{ATP}) channel, composed of the beta-cell proteins sulfonylurea receptor (SUR1) and inward-rectifying potassium channel subunit Kir6.2, is a key regulator of insulin release. It is inhibited by the binding of adenine nucleotides to subunit Kir6.2, which closes the channel, and activated by nucleotide binding or hydrolysis on SUR1, which opens the channel. The balance of these opposing actions determines the low open-channel probability, P_o , which controls the excitability of pancreatic beta cells. We hypothesized that activating mutations in *ABCC8*, which encodes SUR1, cause neonatal diabetes.

METHODS

We screened the 39 exons of *ABCC8* in 34 patients with permanent or transient neonatal diabetes of unknown origin. We assayed the electrophysiologic activity of mutant and wild-type K_{ATP} channels.

RESULTS

We identified seven missense mutations in nine patients. Four mutations were familial and showed vertical transmission with neonatal and adult-onset diabetes; the remaining mutations were not transmitted and not found in more than 300 patients without diabetes or with early-onset diabetes of similar genetic background. Mutant channels in intact cells and in physiologic concentrations of magnesium ATP had a markedly higher P_o than did wild-type channels. These overactive channels remained sensitive to sulfonylurea, and treatment with sulfonylureas resulted in euglycemia.

CONCLUSIONS

Dominant mutations in *ABCC8* accounted for 12 percent of cases of neonatal diabetes in the study group. Diabetes results from a newly discovered mechanism whereby the basal magnesium-nucleotide-dependent stimulatory action of SUR1 on the Kir pore is elevated and blockade by sulfonylureas is preserved.

NEONATAL DIABETES IS A FORM OF diabetes mellitus defined by the onset of mild-to-severe hyperglycemia within the first months of life. Permanent neonatal diabetes requires lifelong therapy; transient neonatal diabetes remits early, with a possible relapse during adolescence. More than half of cases of transient neonatal diabetes are associated with abnormalities of an imprinted region on chromosome 6q24.^{1,2} Some cases of permanent neonatal diabetes and rare cases of transient neonatal diabetes are caused by mutations in the *KCNJ11* gene encoding the inwardly rectifying potassium-channel subunit (Kir6.2) of the ATP-sensitive potassium (K_{ATP}) channel expressed at the surface of the pancreatic beta cell.^{3,4} A few cases of permanent neonatal diabetes are attributed to mutations in the genes that encode glucokinase,⁵ insulin promoter factor 1,⁶ pancreas transcription factor 1 α ,⁷ *FOXP3*,^{8,9} or the eukaryotic translation initiation factor 2-alpha kinase 3.¹⁰

K_{ATP} channels of the pancreatic beta cell regulate insulin release. A small number of overactive mutant channels can hyperpolarize the beta cell, reduce calcium influx through voltage-gated calcium channels, and decrease insulin secretion. The beta-cell K_{ATP} channel is a hetero-octamer¹¹⁻¹³ assembled from Kir6.2¹⁴ and the high-affinity beta-cell sulfonylurea receptor (SUR1, encoded by *ABCC8*).¹⁵ K_{ATP} channels link nutrient metabolism with membrane electrical activity by sensing adenine nucleotides. Nucleotide binding to the tetrameric Kir6.2 pore reduces the mean open-channel probability (P_o),^{16,17} whereas magnesium-nucleotide binding or hydrolysis (or both) on SUR1 counterbalances this inhibition to increase the P_o .¹⁸⁻²⁰ Mutations affecting either subunit could alter this balance. Consistent with this mechanism are the findings that mutant Kir6.2 with reduced sensitivity to inhibitory ATP causes neonatal diabetes in mice²¹ and humans,^{3,22} whereas mutations in *ABCC8* that compromise the stimulatory effect of magnesium-nucleotide binding cause persistent hyperinsulinemic hypoglycemia.^{23,24} Since there are numerous cases of neonatal diabetes of unknown cause, we screened patients with neonatal diabetes for mutations in *ABCC8*.

METHODS

Between 1995 and 2005, we studied 73 patients from the French Network for the Study of Neonatal Diabetes Mellitus: 44 had received a diagnosis

of transient neonatal diabetes, whereas 29 had received a diagnosis of permanent neonatal diabetes, 3 of whom had associated pancreatic aplasia or hypoplasia.² We screened the patients for abnormalities in chromosome 6q24 and mutations in the *KCNJ11* gene; glucokinase sequencing was restricted to patients with permanent neonatal diabetes. Alterations in chromosome 6q were found in 25 patients with transient neonatal diabetes (34 percent of the total study group), and mutations in the *KCNJ11* gene were found in 13 patients (18 percent of the total study group; 12 with permanent neonatal diabetes and 1 with transient neonatal diabetes). A glucokinase mutation was identified in one patient.^{2,4} We screened the 34 remaining patients (16 with permanent neonatal diabetes and 18 with transient neonatal diabetes) for mutations in the *ABCC8* gene using sequence analysis, as described in the Supplementary Appendix (available with the full text of this article at www.nejm.org). We genotyped the parents of each proband with mutated *ABCC8*, and we confirmed family relationships by genotyping the DNA of probands and their parents with six microsatellite markers. Other members of Family 16 and Family 17, both of which had a history of transient neonatal diabetes, were genotyped to determine whether the mutant alleles segregated with diabetes. The study was approved by the local ethics committees, and all participating patients gave written informed consent. Parental consent was given on behalf of patients younger than 18 years of age; the study was explained to children capable of giving assent, and they provided oral assent.

CLINICAL STUDIES

Probands with mutations of the *ABCC8* gene had a thorough clinical examination, and their medical records were reviewed. The two patients with permanent neonatal diabetes underwent a glucagon stimulation test (1 mg was given intravenously, with C-peptide measured 5 and 0 minutes before glucagon administration and 5, 10, and 15 minutes afterward) and glyburide (also known as glibenclamide) stimulation test (0.2 mg per kilogram of body weight). Treatment with sulfonylureas was indicated on the basis of the mutant SUR1-channel response to tolbutamide. The patients with permanent neonatal diabetes and the father of the proband in Family 13 with transient neonatal diabetes received oral glyburide, starting at a dose of 0.2 mg per kilogram per day and gradually increasing the dose during a one-week

period. Glucose levels were monitored and insulin was discontinued when satisfactory metabolic control was achieved. Therapy for members with recurrent diabetes in Family 28 and Family 19 was similarly switched to glipizide and glyburide, respectively.

We compared the clinical features of the patients with transient neonatal diabetes carrying mutations of *ABCC8* and *KCNJ11*⁴ in our case series and 25 persons with transient neonatal diabetes linked to anomalies of chromosome 6 (see the Supplementary Appendix).² The Wilcoxon rank-sum test was used to evaluate differences in quantitative variables, and the chi-square and Fisher's exact tests were used for qualitative data. All tests were two-sided. Differences with a P value of less than 0.05 were considered to indicate statistical significance.

MOLECULAR BIOLOGY AND ELECTROPHYSIOLOGICAL ANALYSIS

The observed mutations were introduced separately into hamster *ABCC8* complementary DNA (cDNA)¹⁵ with the use of standard methods as described in the Supplementary Appendix. Wild-type or mutant *SUR1* cDNA, or both, were coexpressed with human Kir6.2 and green fluorescent protein (to mark transfection) in COSm6 cells.²⁵ Patch-clamp recordings and analyses of the K_{ATP} -channel current were carried out as described previously.^{18,25} To compare the responses of the different channels to physiologic nucleotide conditions, the channels in the same patch were recorded in intact cells and inside-out mode immediately after patch isolation and the values were normalized relative to their maximum ligand-independent response and expressed as the fraction of maximal activity (see the Supplementary Appendix).

A homology model²⁶ of the human *SUR1* core was used to map the mutant residues.²⁷

RESULTS

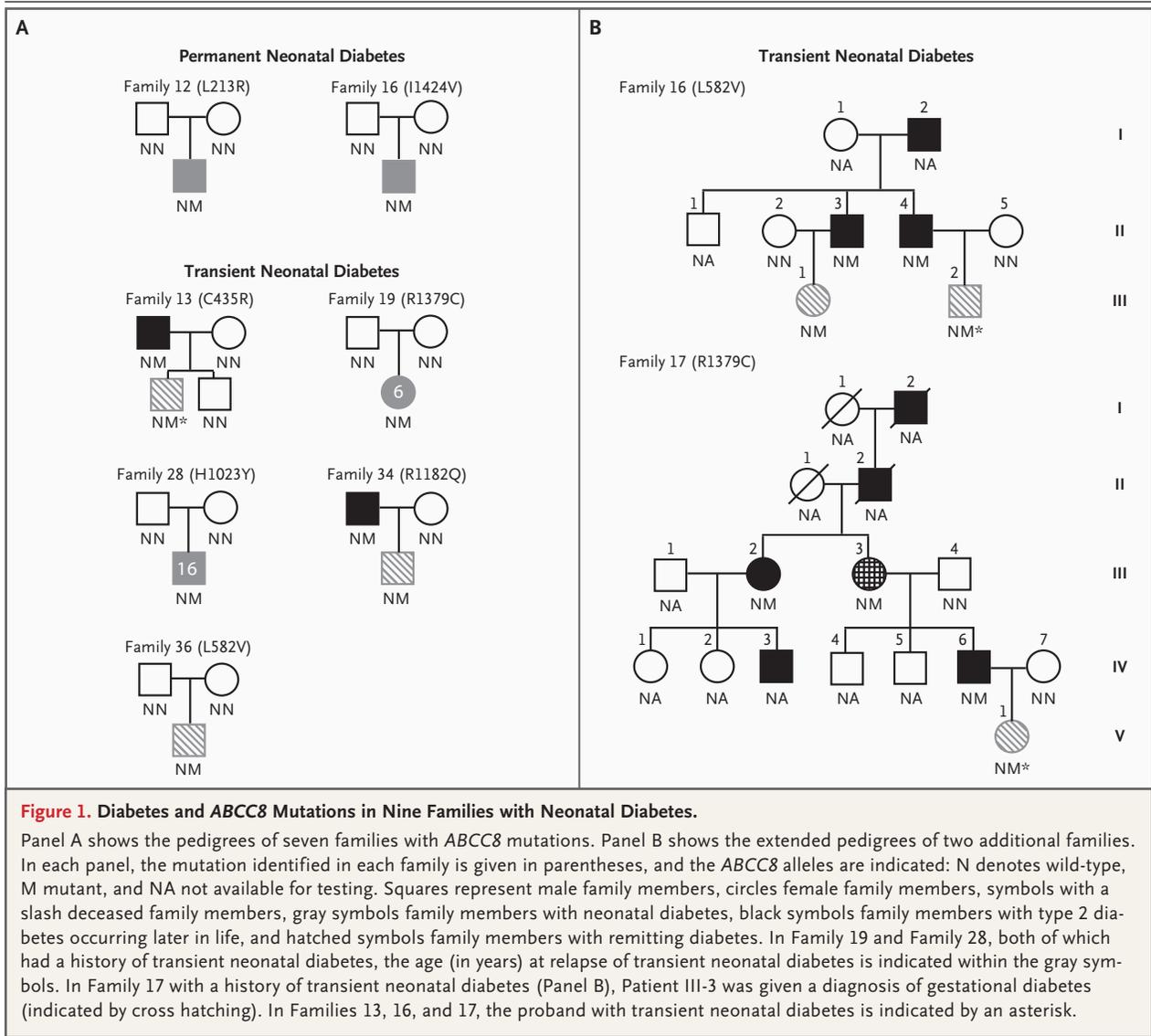
ABCC8 MUTATIONS IN PATIENTS WITH PERMANENT OR TRANSIENT NEONATAL DIABETES

We identified seven heterozygous *ABCC8* mutations in 9 of 34 patients with neonatal diabetes: L213R and I1424V in 2 with permanent neonatal diabetes and C435R, L582V, H1023Y, R1182Q, and R1379C in patients with transient neonatal diabetes. The affected amino acids are conserved in the rat, mouse, chicken, and Japanese Takifugu fish;

this suggests that they are critical for channel function. We did not observe these mutations on sequencing the relevant exons of 180 patients with diabetes and 140 unrelated white persons of French origin without diabetes. Furthermore, we detected no additional nonsynonymous changes in the *ABCC8* exons that were unaffected by mutations in a subgroup of 110 patients with diabetes, including 24 probands with maturity-onset diabetes of the young (MODY) from families without known MODY-associated mutations.

The partial pedigrees of families carrying mutations of *ABCC8* are shown in Figure 1. The L213R, H1023Y, and I1424V were noninherited mutations, as were the L582V and R1379C mutations in one family each. The L582V and R1397C mutations were also inherited in one family each, as were the C435R and R1182Q mutations. In these four families, the father of each proband was heterozygous for the mutation and the mutant alleles cosegregated with diabetes. The father of the proband with a C435R mutation in Family 13 was given a diagnosis of diabetes mellitus at 13 years of age; after he was found to have the C435R mutation, he discontinued insulin (after 24 years of treatment) after a successful response to glyburide (10 mg per day). An oral glucose-tolerance test showed that the father of the proband with an R1182Q mutation in Family 34 had diabetes; he is currently being treated with diet alone. In Family 16 with a history of transient neonatal diabetes, family members II-3 and II-4 were given a diagnosis of diabetes after 30 years of age on the basis of the results of an oral glucose-tolerance test and are currently being treated with diet alone. Diabetes developed in the father of the proband (with an R1379C mutation) in Family 17 when he was 32 years old, and he is receiving glyburide. The R1379C allele was also identified in the proband's paternal grandmother who had had gestational diabetes and is currently being treated with diet and in a paternal great-aunt who was given a diagnosis of diabetes at 44 years of age and is currently being treated with sulfonylureas.

We found mutations of *ABCC8* in 9 of the 34 patients with neonatal diabetes in our case series in whom no genetic defect had previously been identified. These 9 patients account for 12 percent of the 73 patients with neonatal diabetes included in the study. Of the 29 patients with permanent neonatal diabetes in the study, 12 had *KCNJ11* mutations (41 percent), 2 had *ABCC8* mutations



(7 percent), and 15 had no apparent mutations (52 percent). Fifty-seven percent of the 44 cases of transient neonatal diabetes were attributable to anomalies of chromosome 6q, 2 percent to mutations of *KCNJ11*, and 15 percent to mutations of *ABCC8*. The cause of 11 cases of transient neonatal diabetes remains to be determined.

DIABETES

Table 1 summarizes the clinical characteristics of probands with mutant *SUR1*. Diabetes mellitus was diagnosed in patients at a median of 32 days of age (range, 3 to 125) with hyperglycemia leading to polyuria and polydipsia in five patients and ketoacidosis in two patients. Probands in Family

17 and Family 34 had low birth weights and hyperglycemia. There were no detectable anti-islet antibodies, and ultrasonography revealed no pancreatic abnormalities. Initial insulin treatment was required for 1, 2.5, 3, 4, 4, 8.5, and 10 months in probands with transient neonatal diabetes in Families 16, 34, 17, 19, 13, 36, and 28, respectively. The last documented dose of insulin varied from 0.12 to 1.2 U per kilogram per day, with a mean of 0.67 U per kilogram per day. After identification of the mutations in the patients with permanent neonatal diabetes, glyburide therapy was initiated and found to be successful and insulin was discontinued after 2 days in the proband from Family 12 and after 15 days in the proband from

Table 1. Clinical Characteristics of Probands with Neonatal Diabetes with Mutant SUR1.*

Family No.	Mutation	Sex	Wk of Gestation	Birth Weight g (percentile)	At Diagnosis		At Metabolic Testing			Current Treatment			
					Age days	Weight g	Presentation	Glucose mmol/liter	Age yr		Height cm (SD)†	Weight kg (percentile)	Insulin U/kg/day
Permanent neonatal diabetes													
12	L213R	Male	41	3065 (22)	125	5320	Polyuria, polydipsia	28.6	4.75	107.5 (0)	17 (50)	0.12	Glb, 10 mg/day
16	I1424V	Male	40	3080 (25)	33	3360	Ketoacidosis	66	16.5	178 (+0.9)	69 (85)	0.88	Glb, 15 mg/day
Transient neonatal diabetes													
13	C435R	Male	40	3040 (25)	32	3575	Polyuria, polydipsia	44.5	4.75	108.8 (+0.5)	17.5 (75)		
16	L582V	Male	40	3350 (50)	15	3210	Polyuria, polydipsia	51.4	5.25	117 (+1.9)	18.4 (50)		
17	R1379C	Female	40	2050 (<3)	3	2100	Hyperglycemia	6.9	5.25	114.5 (+1.6)	19.5 (82)		
19	R1379C	Female	40	2330 (<3)	60	4900	Polyuria, polydipsia	22	15.7	158 (-0.8)	54 (70)	1.2	Glb, 10 mg/day
28	H1023Y	Male	40	3400 (55)	21	NA	Ketoacidosis	37.8	16	180 (+1.2)	59.5 (60)	0.5	Glp, 10 mg/day
34	R1182Q	Male	34	1830 (8)	4	1680	Hyperglycemia	13.6	2	82 (-1.5)	10.3 (8)		
36	L582V	Male	40	3570 (67)	74	6100	Polyuria, polydipsia	34	1.8	92 (+2)	14 (90)		

* Glb denotes glyburide, NA not available, and Glp glipizide. To convert values for glucose to milligrams per deciliter, divide by 0.05551.

† Values in parentheses are the standard deviation from the norm.

Family 16. The current doses of glyburide are 0.59 and 0.22 mg per kilogram per day, respectively.

Two of the patients with transient neonatal diabetes required insulin again later in life. Hyperglycemia recurred in the proband from Family 28 at 16 years of age; this patient was treated with insulin and then was given glipizide (0.16 mg per kilogram per day). The proband from Family 19 required insulin at 11 years of age, and when he was 16 years of age, his treatment was switched to glyburide (0.28 mg per kilogram per day). These doses are at the high end of, or exceed, doses of glipizide and glyburide currently recommended by the Food and Drug Administration for treating type 2 diabetes in adults.

NEUROLOGIC FEATURES

The proband with permanent neonatal diabetes from Family 12 presented with developmental delay, but in contrast to some persons carrying a mutation of *KCNJ11*, did not have seizures or muscle weakness. His parents reported that he had motor and developmental delays, which were subsequently documented to include dyspraxia. The proband with transient neonatal diabetes from Family 17 presented with minor dystonia. The proband with transient neonatal diabetes from Family 16 showed slow ideation, and the proband with transient neonatal diabetes from Family 13 had minor visual and spatial dyspraxia. None of the probands had the facial features associated with some mutations of *KCNJ11*.³ None of the other index patients had abnormal cognitive function or development.

METABOLIC TESTS

The baseline fasting levels of C-peptide in the proband with permanent neonatal diabetes from Family 12 and Family 16 were low (0.24 and 0.63 nM, respectively) but increased by 358 percent to 1.1 nM and by 222 percent to 1.4 nM, respectively, two hours after treatment with oral glyburide. Consistent with the presence of beta-cell dysfunction, stimulation by glucagon was impaired, with increments of 79 percent (0.19 nM) and 106 percent (0.67 nM), respectively, over baseline levels. (A normal response is an increment of at least 150 percent.)

Clinical Features of Neonatal Diabetes According to Genetic Cause

The birth weights of 25 patients with transient neonatal diabetes linked to anomalies of chromosome

6 were low, with the birth weight of 20 of 25 in the lowest 3 percent of the population, as compared with 3 of 7 patients with transient neonatal diabetes caused by mutant SUR1 ($P=0.01$) (Table 1, and the Supplementary Appendix). Macroglossia was present in 4 of the 25 probands with transient neonatal diabetes related to an anomaly in chromosome 6q24 region, but not in the patients with transient neonatal diabetes associated with a SUR1 mutation. Diabetes was diagnosed earlier in patients in the former group than in the latter group (mean of 4.0 vs. 29.9 days, $P<0.05$), which probably reflects, at least in part, the lower birth weights of the patients in the former group (20 vs. 2 with low birth weights, $P=0.02$), since patients with a low birth weight are more likely to have systematic glucose monitoring. Other clinical features, including developmental delay and the frequency and time of recurrence of diabetes, did not differ significantly between the groups. Anomalies of chromosome 6 were not associated with permanent neonatal diabetes.

As compared with 9 patients with neonatal diabetes caused by mutant SUR1, 13 patients with neonatal diabetes caused by mutant Kir6.2 had a similar frequency of low birth weight, similar distribution of age at diagnosis, and similar glucose levels at presentation. Ketoacidosis was more frequently associated with diabetes caused by mutant Kir6.2 than with diabetes caused by mutant SUR1 (9 of 13 patients vs. 2 of 9), but this difference was not significant ($P=0.09$), possibly because of the small number of patients in each group. The prevalence of developmental delay was not significantly different (3 of 13 patients vs. 1 of 9) and epilepsy was diagnosed in 1 of 13 patients with neonatal diabetes caused by mutant Kir6.2, but in none of the patients with neonatal diabetes caused by mutant SUR1. Dyspraxia was observed in two patients with neonatal diabetes caused by mutant Kir6.2 and in one patient with neonatal diabetes caused by mutant SUR1. In our case series, mutations of *KCNJ11* were mainly associated with permanent neonatal diabetes (12 of 13), whereas most mutations of *ABCC8* (7 of 9) were linked to transient neonatal diabetes.

EFFECT OF *ABCC8* MUTATIONS ON K_{ATP} CHANNEL ACTIVITY

The amino acids of SUR1 affected by mutation are at positions consistent with the mutations that affect protein function (see the Supplementary Ap-

pendix). To determine whether mutations in the *ABCC8* gene changed the P_o through a change in the intrinsic effect of SUR1 on channel activity or by amplifying the stimulatory effect of magnesium ATP, we compared the activities of mutant and wild-type channels in intact mammalian cells and under controlled nucleotide conditions. Measurements were made on the same patch in the intact cells and in the excised, inside-out configuration at a quasi-physiologic concentration of ATP in the presence and absence of physiologic concentrations of magnesium (free magnesium concentration, 0.7 mM) (examples of currents are provided in Fig. S2 of the Supplementary Appendix). Figure 2 shows that the normalized activities of mutant channels (containing the I1424V or H1023Y variant) in intact cells and in 1 mM magnesium ATP are nearly four and seven times as great, respectively, as those of wild-type channels under similar nucleotide conditions. A similar concentration of submembrane nucleotides (1 mM) in simian kidney cells has been gauged by others.²⁸ In the absence of magnesium, and in the presence of 1 mM ATP, the P_o of the mutant and wild-type channels did not differ significantly.

To test the effect of heterozygosity, we mixed both wild-type and mutant SUR1 with Kir6.2 and found that the average mean activities (in intact cells and in the presence of 1 mM magnesium ATP) were significantly greater than those of wild-type channels ($P<0.001$), although somewhat lower than those of "homozygous" mutant channels. In addition, the channels associated with neonatal diabetes were more active than wild-type channels at other concentrations of magnesium ATP ≥ 0.1 mM (see Fig. S3 of the Supplementary Appendix). These findings suggest that the heterozygous mutations of *ABCC8* overactivate beta-cell K_{ATP} channels by overstimulating the pore. To exclude the possibility that overactivity of the mutant I1424V and H1023Y channels is caused by either a gain in the intrinsic, ligand-independent, activity or by attenuation of the inhibitory action of ATP on Kir6.2, we measured the mean ligand-independent P_o values and steady-state ATP-inhibitory curves (i.e., without magnesium) (Fig. 3). The maximal P_o ($P_{o_{MAX}}$) values and the ATP-inhibitory curves for both mutant channels overlapped those of wild-type channels. We conclude that mutant I1424V and H1023Y channels overactivate beta-cell K_{ATP} channels under physiologic magnesium-nucleotide conditions by increasing the magne-

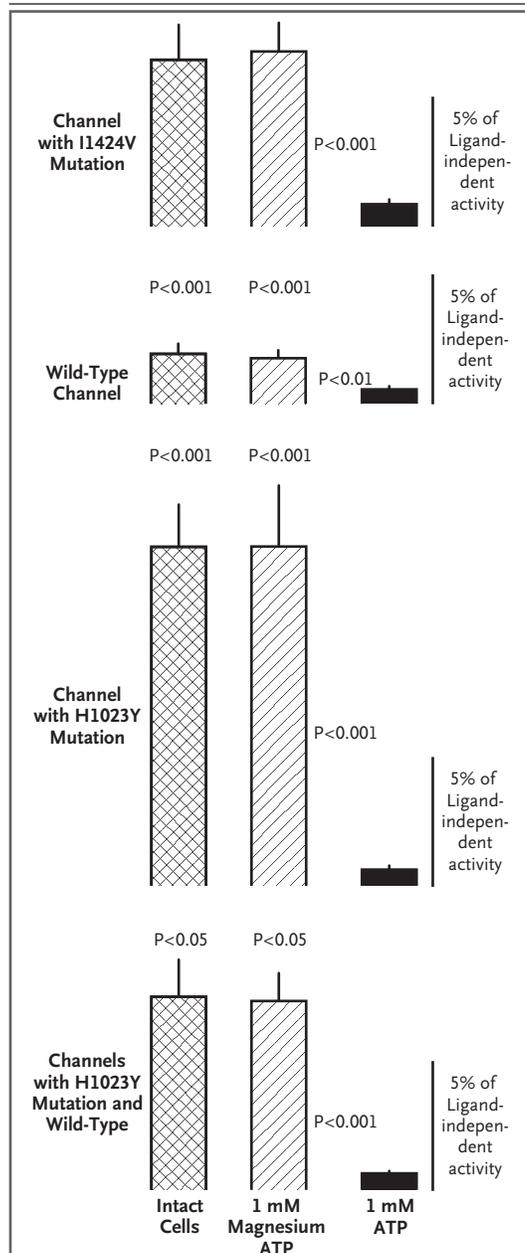


Figure 2. Activities of Mutant and Wild-Type K_{ATP} Channels in Intact Cells and in Physiologic Concentrations of ATP in the Presence or Absence of Magnesium.

The mean (\pm SE) proportion of maximal channel activity for each type of channel is shown. The results were from 10 cells in five independent transfections of Kir6.2 plus the mutant or wild-type SUR1, or both, as indicated on the left. (See the Methods section, and Fig. S2 of the Supplementary Appendix, for details of the electrophysiologic analysis.) The P values between rows of bars are for the comparison between different types of channels, and the P values to the right of the bars are for the comparison of channels in the presence and absence of magnesium.

sium-nucleotide-dependent stimulatory action of SUR1 on the pore.

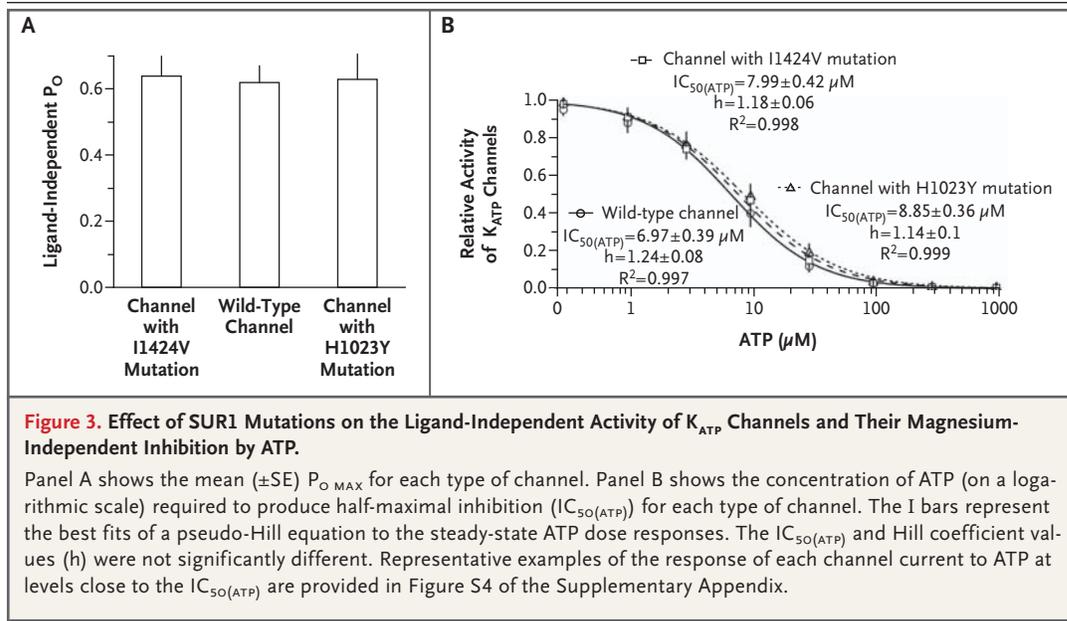
INHIBITION OF MUTANT CHANNELS BY SULFONYLUREAS

A key issue in the treatment of neonatal diabetes is whether mutant channels are inhibited by sulfonylureas; if they are, they could be used in place of insulin.²⁹ We used tolbutamide (which binds SUR1 specifically and is more rapidly reversible than glyburide and glipizide) to assess the sensitivity of the mutant recombinant channels (Fig. 4A and 4B). A concentration of 200 μ M tolbutamide, which saturates the high-affinity binding site of wild-type SUR1,²⁵ inhibited wild-type and mutant channels (containing the I1424V or H1023Y variant) to a similar degree in the absence of magnesium nucleotides (Fig. 4A). This inhibition indicated that tolbutamide binding to SUR1 and its functional coupling to the Kir6.2 pore were not altered by the I1424V or H1023Y mutations.

To determine whether the increased stimulatory activity of the mutant receptor compromised the ability of a pharmacologic concentration of sulfonylureas to abolish the stimulatory action of magnesium nucleotides,^{25,30} we assessed the effect of 50 μ M tolbutamide under magnesium-nucleotide conditions that maintain substantial steady-state K_{ATP} currents in inside-out patches, where accurate detection of zero current level is possible (Fig. 4B). We observed the normal, enhanced inhibition of both permanent neonatal diabetes and mutant channels of transient neonatal diabetes in the presence of magnesium nucleotides. On the basis of these results, treatment with sulfonylureas, glyburide, and glipizide, respectively, was initiated in patients with permanent neonatal diabetes and transient neonatal diabetes and has proved effective.

DISCUSSION

Our results indicate that heterozygous activating mutations in *ABCC8*, encoding the SUR1 regulatory subunit of the ATP-sensitive potassium channels found in beta cells, cause both permanent and transient neonatal diabetes. Although the molecular mechanisms of the *ABCC8* and *KCNJ11* mutations are distinct, the cellular mechanism reducing insulin release is common to both (Fig. 4C, 4D, 4E, and 4F). Our results are consistent with a report that neonatal diabetes develops in transgenic mice expressing a mutant Kir6.2 subunit with



reduced sensitivity to inhibitory ATP and that some cases of permanent neonatal diabetes and transient neonatal diabetes are caused by mutations in *KCNJ11*. These studies underscore the key role of K_{ATP} channels in coupling beta-cell membrane potential (and thus the requirement for calcium to release insulin-containing granules) with nutrient metabolism. The common pathway accounts for the direct overlap of the clinical features related to abnormal insulin release secondary to mutations in *ABCC8* and *KCNJ11*. As compared with patients with neonatal diabetes related to a SUR1 mutation, patients with neonatal diabetes related to a Kir6.2 mutation revealed no significant differences in the prevalence of low birth weight, age at diagnosis, or severity of hyperglycemia or accompanying ketoacidosis. Mutations of *KCNJ11* are typically associated with permanent neonatal diabetes, whereas most mutations of *ABCC8* are associated with transient neonatal diabetes, perhaps reflecting a less severe form of diabetes.

Previous reports^{3,4} have highlighted a heterogeneity of symptoms associated with neonatal diabetes caused by mutant Kir6.2, which may reflect the sharing of the Kir6.2 pore by both SUR1–Kir6.2 neuroendocrine channels and sarcolemmal SUR2A–Kir6.2 channels. The neurologic features of several persons with neonatal diabetes related to a mutant SUR1 channel imply that SUR1-containing K_{ATP} channels can control the membrane potential of neuronal cells, such as inhibitory motor neurons.

Our data show that the net inhibitory action of ATP on two types of neonatal diabetes caused by a mutant SUR1 channel was unchanged (as compared with wild-type SUR1) and that the increased activity of the channels, under physiologic conditions, was caused by an increase in the magnesium-dependent stimulatory action of SUR1 on the pore. The position of the mutations that we have described, together with further investigations, should provide insight into the stimulatory mechanism. This finding is at variance with that of a recent study³¹ showing an alteration in the inhibitory action of ATP on a mutant SUR1 channel.

Experiments designed to approximate the heterozygous condition by expression of 1:1 mixtures of mutant with wild-type subunits resulted in average mean activities, under physiologic magnesium-nucleotide conditions, intermediate to those of wild-type and mutant channels. The maximal activity of mixed K_{ATP} channels declines exponentially as the number of wild-type subunits (with the less stable active conformation)¹⁸ increases in the complex. Therefore, the overactivating effect of a mutation is expected to drop off rapidly with the inclusion of wild-type subunits. The results imply that a small subpopulation of pure mutant channels can make a considerable contribution to the hyperpolarization of beta cells and perhaps of some neurons with electrically tight membranes whose potential is dominated by a small number of channels.

In clinical practice, there is no way to distin-

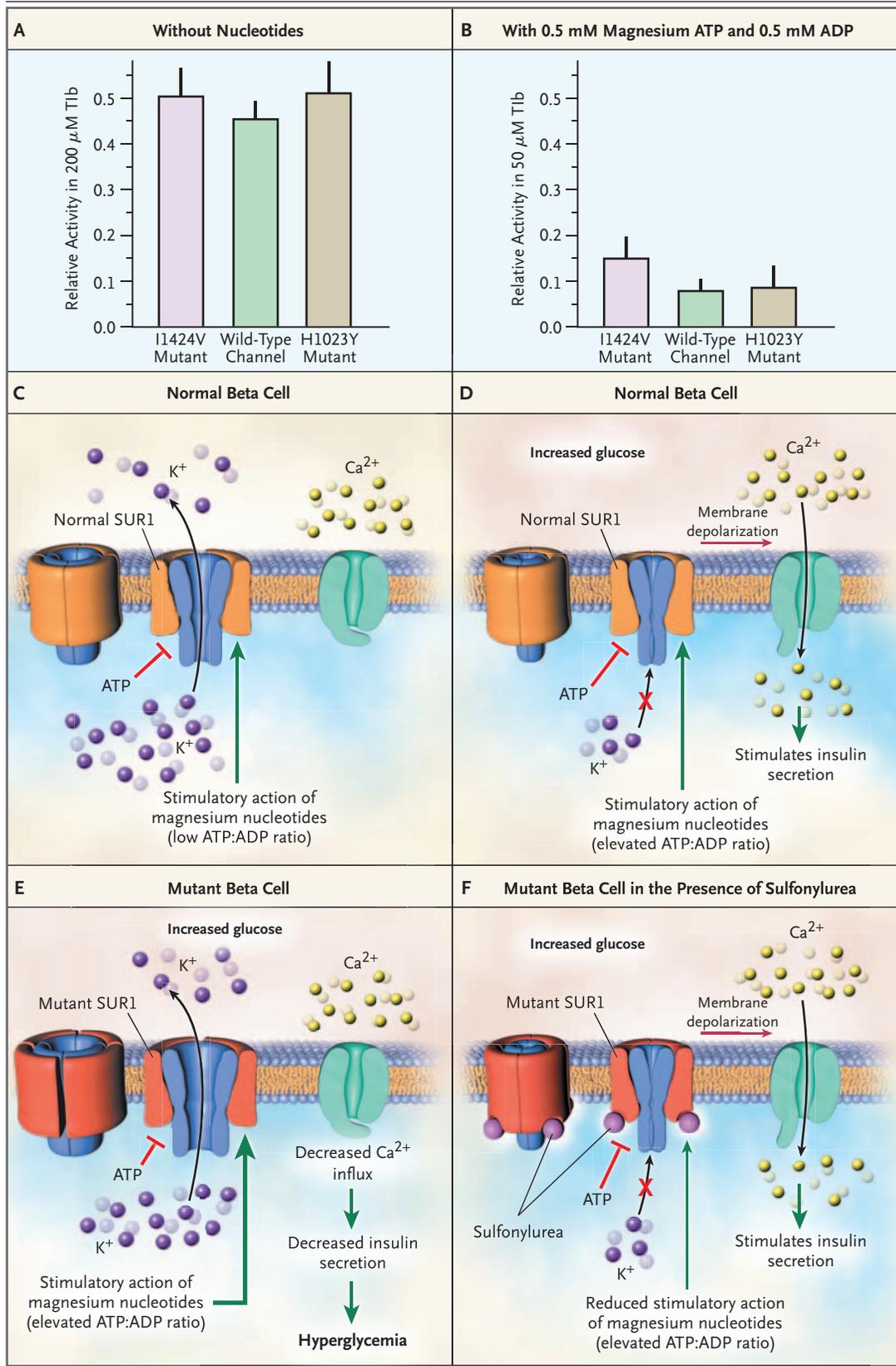


Figure 4 (facing page). Effect of SUR1 Mutations on Response to Tolbutamide (Tlb).

The mean (\pm SE) activity of each type of channel is shown in response to 200 μ M tolbutamide in magnesium-free conditions (Panel A) and to 50 μ M tolbutamide in the presence of 0.5 mM magnesium ATP and 0.5 mM magnesium ADP (Panel B). The data were derived from six experiments for each condition and type of channel. Representative current records are provided in Figure S5 of the Supplementary Appendix. Panel C shows how ATP inhibits beta-cell K_{ATP} channels through its interaction with Kir6.2 in a control subject. When glucose levels are low, this inhibitory action is balanced by the stimulatory action of magnesium nucleotides on SUR1 (indicated by the large green arrow), which increases channel activity. Membrane depolarization activates voltage-dependent calcium channels, and calcium influx stimulates insulin secretion. An increase in glucose metabolism increases the ratio of ATP to ADP and thereby reduces the stimulatory action of SUR1 (Panel D). In a patient with neonatal diabetes, the enhanced stimulatory action of the mutant receptor is sufficient to keep K_{ATP} channels open even at an elevated ratio of ATP to ADP, thus attenuating insulin release and producing hyperglycemia (Panels E and F). The data presented in Panels A and B, however, suggest that a sulfonylurea (tolbutamide) counters the stimulatory effect of the mutation to stimulate insulin release. This result is confirmed by the response of patients with permanent neonatal diabetes and transient neonatal diabetes to glyburide and glipizide.

guish patients with *ABCC8* or *KCNJ11* mutations from those with abnormalities in chromosome 6q24. Gene sequencing is required, and a useful strategy is to screen chromosome 6 and the short, one-exon *KCNJ11* gene first, unless the patients present with hyperglycemia after an overnight fast, in which case *GCK* is analyzed. If no mutations are identified, *ABCC8* is analyzed. Genetic testing clearly has profound implications for counseling and therapy for patients with neonatal diabetes. In the absence of the discovery of mutations in *ABCC8* that compromise inhibition by sul-

fonylureas, oral sulfonylurea therapy should be effective for most patients with neonatal diabetes caused by mutant SUR1. The efficacy of such therapy was also demonstrated for patients with Kir6.2 mutations in a study by Pearson et al.,³² which appears elsewhere in this issue of the *Journal*.

The diagnosis of diabetes in fathers with *ABCC8* mutations is consistent with adult-onset type 2 diabetes mellitus or a mild form of transient neonatal diabetes. Systematic blood screening in French newborns makes the latter unlikely, and we propose that mutations of the *ABCC8* gene may give rise to a monogenic form of type 2 diabetes with variable expression and age at onset. The potential contribution of overactive *ABCC8* mutations to familial early-onset type 2 diabetes remains to be evaluated, but our findings emphasize how molecular understanding of a rare pediatric form of diabetes may illuminate the more common form of the disease.

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