Brief Genetics Report

Kir6.2 Mutations Are a Common Cause of Permanent Neonatal Diabetes in a Large Cohort of French Patients

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Permanent neonatal diabetes (PND), requiring insulin within the first months of life, is unexplained at the molecular level in most cases. It has very recently been shown that heterozygous activating mutations in the *KCNJ11* gene, encoding the Kir6.2 subunit of the pancreatic ATP-sensitive K^+ channel involved in the regulation of insulin secretion, cause PND. In the present study, we screened the *KCNJ11* gene for mutations in French patients with PND. Patients were recruited through the French network for the study of neonatal diabetes. Seventeen at-term babies with a median age at diagnosis of diabetes of 64 days (range 1–260) were included. We identified in nine patients seven heterozygous nonsynonymous mutations: three of them (V59M, R201C, and R201H) were already described, and the four novel mutations resulted in an amino acid change of Kir6.2 at positions F35L, G53N, E322K, and Y330C. More patients with a Kir6.2 mutation (six of nine) were reported to have a smaller birth weight than those without mutation (two of eight). Although Kir6.2 mutation carriers do not represent a phenotypically specific form of PND, an impaired function of Kir6.2 is associated with in utero insulin secretory insufficiency and growth retardation. In conclusion, we confirmed that Kir6.2 mutations are a common cause (53%) of PND in Caucasians. *Diabetes* 53:2719–2722, 2004

Neonatal diabetes, defined as insulin-requiring hyperglycemia within the first months of life, is a rare entity, with an estimated incidence of 1 in 400,000 neonates (1). In about one-half of the neonates, diabetes is transient (transient neonatal diabetes [TND]), with remission within the first 6 months of life, whereas the rest of the patients have a permanent form of diabetes. In many cases of TND, abnormalities of chromosome 6 have raised the possibility that an imprinted, paternally expressed gene is involved in its pathogenesis (1,2).

By contrast, very few cases of permanent neonatal diabetes (PND) have been elucidated at the molecular level. Complete deficiency of the glycolytic enzyme glucokinase (*GCK*) caused by homozygous or compound heterozygous mutations was found in five case subjects (3,4). Other rare cases were also described (1), with mutations in the genes coding for insulin promoter factor-1 (*IPF-1*), forkhead box P3 (*FOXP3*), and the eukaryotic translation initiation factor 2α kinase 3 (*EIF2AK3*). Recently, Gloyn et al. (5) reported in an ethnically diverse patient cohort that six heterozygous activating mutations in the *KCNJ11* gene, encoding the Kir6.2 subunit of the pancreatic β-cell ATP-sensitive K^+ channel, caused PND in 10 probands and that some of these mutations are also associated with developmental delay, muscle weakness, and epilepsy. Homozygous inactivating mutations in *KCNJ11* encoding Kir6.2 have also been shown (6) to cause familial persistent hyperinsulinemic hypoglycemia of infancy.

Here we present the screening for mutations of the coding sequence of *KCNJ11* in 17 case subjects presenting with PND and having no *GCK* mutation and 7 case subjects with TND with no abnormalities of the chromosome 6q24 region. The patients were recruited through the French Network for the Study of Neonatal Diabetes (2). The 17 PND patients had a mean birth weight of 2,817 g (range 1,890–3,600) (Tables 1 and 2). None of the patients were born prematurely. Median age at diagnosis of diabetes was 60 days (range 1–260). At last follow-up visit, they were all on insulin, at a mean dose of 0.7 units · kg^{-1} · day^{-1} (range 0.18–1.2). The coding exon of the *KCNJ11* gene was screened for mutations by direct sequencing of genomic DNA. No mutations were found in the patients with TND. On the contrary, in nine patients with PND (Table 1), we identified seven heterozygous missense mutations. Three of them (V59M, R201C, and R201H) have been previously described (5), and the four novel mutations resulted in amino acid substitutions at the following positions: F35L, G53N, E322K, and Y330C (Table 1). No Kir6.2 mutation or familial transmission was observed after testing both parents of each the nine patients, suggesting that they are carriers of de novo Kir6.2 mutations. In addition, six microsatellite markers from the

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PND, permanent neonatal diabetes; TND, transient neonatal diabetes.

TABLE 1
Clinical features of the PND patients with Kir6.2 mutations

	DPN-2	DPN-3	DPN-4	DPN-7
Mutation	R201C	R201H	V59M	E322K
Sex (M/F)	M	M	M	F
At birth				
Weight (g/percentile)	2,610/<5	2,780/<5	2,590/<5	2,110/<5
Gestation week	40	41	39	38
At presentation				
Age (days)	37	17	127	3
Weight (g)	3,820	2,900	4,700	2,110
Presentation	Hyperglycemia	Polyuria/polydipsia	Ketoacidosis	Hyperglycemia
Glucose at presentation (mmol/l)	25	22.4	46	19
Autoantibodies	0	NA	0	0
Pancreas ultrasonography	Normal	Normal	Normal	Normal
Current status				
Age (year and month)	10 years	4 years	9 years, 3 months	10 years, 10 months
Height (cm/SD)	147/+1.9	101.5/0	NA	139.8/0
Weight (kg/SD)	18.5/−0.6	16.5/+0.3	29/0	27.7/−1
Insulin dose (units^{-1} · kg^{-1} · day^{-1})	0.79	0.70	0.88	0.62
Neurological features	No	No	Leucodystrophy/ developmental delay	No
Dysmorphic features	No	No	No	No
Other features	None	None	Severe oesophagia	None

*Median, rather than mean. NA, not applicable.

chromosome 6q region have been analyzed in all nine of these families, allowing us to confirm paternity (7). None of the mutations were present in 90 nondiabetic unrelated French Caucasian subjects. The R201H mutation was found in three unrelated probands and occurred at a highly conserved codon, supporting a critical role for residue 201 in the channel function and ATP binding (5,8). All of the other mutated residues are highly conserved between mammals (man, rat, and mouse) and the Japanese Fugu fish (data not shown).

Therefore, *KCNJ11* mutations are a common cause (53%) of PND in our French cohort, which allows us to look for genotype/phenotype correlations. No statistically significant phenotypic differences were noted between patients with or without a *KCNJ11* mutation (Tables 1 and 2). Nevertheless, more patients with a Kir6.2 mutation were reported to have intrauterine growth retardation (<5th percentile for gestational age) compared with those without a mutation (6 of 9 vs. 2 of 8; $P = 0.15$); the statistically nonsignificant P value is most likely due to a

TABLE 2
Clinical features of the PND patients with no Kir6.2 mutation

	DPN-5	DPN-6	DPN-8
Sex (M/F)	F	M	F
At birth			
Weight (g/percentile)	3,600	2,900/10	3,150
Gestation week	Term	40	Term
At presentation			
Age (days)	90	60	89
Weight (g)	5,700	NA	5,400
Presentation	Ketoacidosis	Ketoacidosis	Ketoacidosis
Glucose at presentation (mmol/l)	17.3	NA	>26
Autoantibodies	NA	NA	NA
Pancreas ultrasonography	Normal	NA	NA
Current status			
Age (year and month)	3 years	2 years, 5 months	11 years, 6 months
Height (cm/SD)	11/−1.88	96/+2	145.1/+1.44
Weight (kg/SD)	87/−1.73	14.5/+1	39.1/+1.6
Insulin dose (units^{-1} · kg^{-1} · day^{-1})	0.73	0.27	0.90
Neurological features	Seizure at 2 months	No	No
Dysmorphic features	No	No	No
Other features	Deceased in acute renal failure and lactic acidosis	No	No

*Median, rather than mean. NA, not applicable.

TABLE 1
Continued

DPN-9	DPN-10	DPN-13	ND-J	ND-C	Mean (range)
G53N	R201H	Y330C	R201H	F35L	—
M	F	F	M	M	—
3,000/30	2,710/<5	2,400/<5	3,150/18	3,260/60	2,734 (2,110–3,260)
38.5	40	41	41	39	39.7 (38–41)
95	73	37	68	42	42 (3–127)*
5,040	4,300	2,700	4,670	3,360	—
Ketoacidosis	Ketoacidosis	Ketoacidosis	Hyperglycemia	Ketoacidosis	—
55	22.7	70	37.8	38	37.3 (19–70)
0	NA	NA	NA	0	—
Normal	NA	NA	NA	Normal	—
4 years, 8 months	5 months	2 years, 8 months	3 months	2 years, 4 months	—
100.6/0	63/+2	88/−0.8	61/+0.5	NA	—
16/0	7.05/0	12.3/−0.6	6/+0.4	13.5/+0.5	—
0.40	0.68	0.77	1.20	0.70	0.75 (0.40–1.20)
No	No	Hemiplegia	No	No	—
No	No	No	No	No	—
None	None	None	None	None	—

lack of power because of the small number of case subjects. This suggests that mutations in *KCNJ11* are associated with an in utero insulin secretory insufficiency, as previously suggested (5). However, two patients (DPN-9 and ND-C, carriers of a novel mutation, which were G53N and F35L, respectively) have a normal birth weight (Table 1). DPN-9 showed a normal weight gain in the few first postnatal weeks, suggesting that insulin was secreted in an adequate amount before an abrupt secretory failure. The patient ND-C also had a normal birth weight but was unable to gain weight in the neonatal period, suggesting inadequate insulin secretion of very early postnatal onset. Therefore the correlations between the type of mutation and the insulin secretory capacity both in utero and in the postnatal period as well as the effect of sulfonylureas need to be studied.

Two patients with a *KCNJ11* mutation had diabetes with developmental or neurological features: one presenting with a severe developmental delay with leucodystrophy and no seizures and another has a history of hemiplegia. The patient with severe developmental delay had the V59M mutation, which has previously been reported (5) in an individual with developmental delay and muscle weakness. On the other hand, one subject with PND with no

TABLE 2
Continued

DPN-11	DPN-12	DPN-16	DPN-17	DPN-18	Mean (range)
F	M	M	M	M	—
1,890/<5	3,065/10	3,080/12	2,240/<5	3,370/52	2,912 (1,890–3,600)
37.5	41	40	41	39	39.7 (37.5–41)
11	125	33	1	260	74.5 (1–260)*
1,700	5,320	3,360	2,240	7,580	4,267 (1,700–7,580)
Polyuria polydipsia	Hyperglycemia	Ketoacidosis	Hyperglycemia	Ketoacidosis	—
28.8	28.6	66.6	10.7	33.5	30.2 (10.7–66.6)
Negative	NA	Negative	NA	Negative	—
Normal	Normal	NA	Normal	Normal	—
2 years, 9 months	9 months	15 years, 1 month	2 years, 11 months	2 years	—
87/−1	74.5/+1.61	172.5/+ 0.75	83.5/−3	85.5/0	—
12.3/−1	9.45/+0.61	59.9/0	12.3/−1	11.17/−0.8	—
0.78	0.18	1	0.65	0.40	0.62 (0.18–1)
No	No	No	No	No	—
No	No	No	No	No	—
No	Renal hypertrophy	No	Exocrine pancreatic insufficiency	No	—

mutation in Kir6.2 had partial epilepsy too, which suggests that an unknown gene involved in the same pathway may be responsible for a similar but possibly milder phenotype than PND due to a Kir6.2 defect.

In this regard, although screening for *KCNJ11* mutations should elucidate >50% of all PND cases in Caucasians, it does not identify a phenotypically specific form of disease. In addition, in our cohort, Kir6.2 deficiency is not involved in TND, which should be considered an etiologically different disease from PND.

RESEARCH DESIGN AND METHODS

The patients were recruited through the French network of physicians or pediatricians for the study of neonatal diabetes (2). The physicians were asked to complete a questionnaire containing items about family history of diabetes, the pregnancy, birth characteristics, the circumstances of the diagnosis of diabetes in the neonate, the initial treatment, biochemical and immunological test results, and long-term outcome. Written informed consent was obtained from all parents, and the local ethics committee approved the study.

Mutation identification. Since the human *KCNJ11* gene is intronless, we amplified six overlapping fragments of the coding exon (1,173 bp) using previously described primers (5) from patients' genomic DNA. All PCR products were directly sequenced on both strands by standard protocols (Applied Biosystems, Foster City, CA). We confirmed the identified mutations by resequencing the original genomic DNA of each patient.

Statistical analysis. Clinical parameters are presented as individual data and mean or median with range or SD. Differences between the two groups of patients were determined using a χ^2 analysis for categorical data and an unpaired t test for quantitative variables.

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