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Diagnosis and treatment of neonatal diabetes: an United States experience[†]

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Abstract

Background/objective—Mutations in *KCNJ11*, *ABCC8*, or *INS* are the cause of permanent neonatal diabetes mellitus in about 50% of patients diagnosed with diabetes before 6 months of age and in a small fraction of those diagnosed between 6 and 12 months. The aim of this study was to identify the genetic cause of diabetes in 77 consecutive patients referred to the University of Chicago with diabetes diagnosed before 1 yr of age.

Methods—We used Oragene™ DNA Self-Collection kit to obtain a saliva sample for DNA. We sequenced the protein-coding regions of *KCNJ11*, *ABCC8*, and *INS* using standard methods.

Results—We enrolled 32 patients diagnosed with diabetes before 6 months of age and 45 patients diagnosed between 6 and 12 months. We identified a mutation in *KCNJ11* in 14 patients from 12 families and in *INS* in 7 patients from 4 families. Three of the patients with an *INS* mutation were diagnosed with diabetes between 6 and 12 months of age. Finally, we found that two patients had an abnormality of chromosome 6q24 associated with transient neonatal diabetes mellitus.

Conclusions—We were able to establish a genetic cause of diabetes in 63% of patients diagnosed with diabetes before 6 months of age and in 7% of patients diagnosed between 6 and 12 months. Genetic testing, which is critical for guiding appropriate management, should be considered in patients diagnosed with diabetes before 1 yr of age, especially if they are autoantibody negative, although the presence of autoantibodies does not rule out a monogenic cause.

Keywords

ABCC8; ATP-sensitive potassium channel; glyburide; insulin gene; *KCNJ11*; monogenic diabetes

Neonatal diabetes mellitus (NDM) is a rare insulin-requiring form of diabetes with an estimated prevalence of 1 in 100 000–300 000 live births (1). Patients with NDM can be grouped into

[†]For further information on the registry, see www.neonataldiabetesregistry.org.

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two well-defined subgroups, permanent and transient, each accounting for approximately 50% of patients (1). Recent studies have indicated that diabetes in the majority of patients diagnosed with permanent NDM (PNDM) before 6 months of age is likely to be non-autoimmune in nature and caused by a mutation in the heterozygous or homozygous state in one of a number of possible genes. These include mutations in the genes (*KCNJ11* and *ABCC8*) encoding the two protein subunits (Kir6.2 and SUR1, respectively) of the ATP-sensitive potassium channel and in the gene (*INS*) encoding insulin itself being the most common causes (2–13). They account for 31% (*KCNJ11*), 12% (*INS*) and 10% (*ABCC8*) of cases in the large Exeter cohort (13). The genetic basis for transient NDM (TNDM) has also been determined with abnormalities of chromosome 6q24 accounting for more than 70% of the cases and mutations in either *KCNJ11* or *ABCC8* in approximately 25% of the cases (5). The definition of the underlying genetic cause has led to improved treatment for patients with PNDM caused by a mutation in *KCNJ11* or *ABCC8* as these patients can frequently be effectively treated with orally administered sulfonylurea therapy rather than insulin, with significantly improved glycemic control and quality of life (14–17).

In this report, we studied 32 consecutive patients diagnosed with diabetes before 6 months of age and 45 patients diagnosed from 6 to 12 months with the goal of determining the underlying genetic basis for the disease. We determined the genetic cause in 23 patients and in this study present the clinical features of these patients including perinatal data, clinical presentation, family history, and management of the diabetes before genetic diagnosis. In patients with NDM caused by a mutation in *KCNJ11*, we facilitated the transition from insulin treatment to glyburide either by advising the patient's local endocrinologist or through admission at the University of Chicago Medical Center.

Methods

Subjects

The majority of the patients contacted our team directly by phone or by e-mail as a result of publicity in the *Chicago Tribune* (Monday, 11 September 2006) about the transition from insulin to glyburide in a patient with PNDM caused by a mutation in *KCNJ11*. Other patients were referred by their local pediatric or adult endocrinologist for a genetic evaluation because of a very early onset of diabetes and, in some instances, an extensive family history of diabetes. Transition from insulin to oral sulfonylurea therapy was undertaken based on a previously reported protocol (18) (and advice kindly provided by Dr A. T. Hattersley) either during inpatient admission to the General Clinical Research Center (GCRC) at the University of Chicago or through close communication with the patient's local endocrinologist who directly supervised the transition.

Genetic analysis

We sent an Oragene™ DNA Self-Collection kit (DNA Genotek Inc., Ottawa, ON, Canada) to obtain a saliva sample for DNA by mail together with a consent form and a questionnaire approved by the University of Chicago Institutional Review Board requesting details about birth data (birth weight and gestational age), glucose at presentation, results of tests if any for pancreatic islet autoantibodies, present age, present weight, present height, insulin dose at presentation and current dose, most recent hemoglobin A1c (HbA1c) and normal range, C-peptide with paired glucose if done, diabetes remission with age at remission and age at relapse, family history of diabetes including age at diagnosis and current treatment, and other medical problems associated with monogenic diabetes or autoimmune diseases in patients with true type 1 diabetes such as muscle weakness, developmental delay, and epilepsy (*KCNJ11* and *ABCC8*); macroglossia (chromosome 6q24 abnormalities); renal cysts, abnormal liver function tests, and pancreatic hypoplasia (*HNF1B/TCF2*); skeletal abnormalities and thyroid

dysfunction (*EIF2AK3*); gastrointestinal symptoms and thyroid dysfunction (*FOXP3*); thyroid dysfunction (*GLIS3*); megaloblastic anemia (*SLC19A2*); and other associations. The patients returned the consent form, questionnaire, and saliva sample by mail.

The DNA was extracted from the saliva samples as recommended by the manufacturer. *KCNJ11*, *ABCC8* and *INS* were amplified by polymerase chain reaction (PCR). Primers and PCR conditions have been described previously (6,7,19). We used the Applied Bio-systems 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) for bidirectional sequencing and Mutation Surveyor software (SoftGenetics, State College, PA, USA) for data analysis. *KCNJ11* and *INS* were sequenced in all patients, whereas *ABCC8* was sequenced only in patients who had normal *KCNJ11* and *INS* sequences and were diagnosed before 6 months of age or diagnosed from 6 to 12 months with a birth weight below 3500 g. We believe that beyond these parameters, mutations in *ABCC8* are unlikely and hence analysis is likely to be unrewarding. In patients with evidence of TNDM and in one patient with PNDM diagnosed in the first week of life who tested negative for mutations in *KCNJ11*, *INS*, *ABCC8*, and *GCK*, analysis of chromosome 6q24 was carried out to detect duplications, uniparental isodisomy, and methylation abnormalities in the University of Chicago Genetic Services Laboratories and the laboratory of Dr Deborah J. Mackay, Wessex Regional Genetics Laboratory, Salisbury District Hospital, Salisbury SP2 8BJ, UK (20–22).

Statistical analysis

Values shown are median and range unless otherwise indicated. We used the Wilcoxon signed rank sum test for paired data to compare HbA1c before and after transition from insulin to glyburide in patients with a *KCNJ11* mutation.

Informed consent and ethics committee approval

This protocol was approved by the Institutional Review Board of the University of Chicago and is in accordance with the Declaration of Helsinki. Adult and child (if able to sign their name) participants and/or parents/guardians of minors gave written informed consent.

Results

Clinical features of study population

The patients in this series were recruited for the study over an 18-month period, all from the USA, including 32 diagnosed with diabetes from birth to 6 months of age and 45 diagnosed between 6 and 12 months. Sixty-four percent of the patients were male. The majority of the patients were of European descent (85%), and those remaining were African-American (1%), Asian (1%), mixed ancestry (6.5%), Hispanic (3.9%), and unknown (2.6%). The median age at diagnosis of diabetes was 34 wk (range 0–52 wk). Median birth weight was 3359 g (range 1275–4536 g) corresponding to the 47th percentile. All the patients diagnosed after 6 months of age had PNDM, whereas two patients diagnosed before 6 months had TNDM, with two others still so young that TNDM remains a possibility (Tables 1 and 2).

Molecular genetic studies

In the 32 patients diagnosed with diabetes before 6 months of age, we identified six different mutations in *KCNJ11* in 14 patients from 12 families: H46Y, V59M, R201C, R201H, E227K, and E322K. Six patients carried the R201H mutation (two were father and son); E227K and E322K were found in one patient each; and R201C, V59M, and H46Y (the latter in a mother and her son) were found in two patients each (Fig. 1). The two patients with the V59M mutation also had developmental delay consistent with the intermediate developmental delay, epilepsy, and neonatal diabetes (DEND) syndrome. None of the 45 patients diagnosed with diabetes

from 6 to 12 months of age carried a mutation in *KCNJ11* or *ABCC8* (the latter only tested in patients with birth weight below 3500 g). Four patients diagnosed before 6 months of age (from the same family) and three patients diagnosed from 6 to 12 months were found to have a mutation in *INS*; these patients have been reported previously (7) (Table 3).

In the four patients with TNDM or possible TNDM, we were able to establish a genetic cause; two patients had a mutation in *KCNJ11* (H46Y and E227K) and two had a 6q24 abnormality. One of these patients was found to have complete loss of methylation of the maternal allele of the 6q24 locus. She was born with a birth weight below the 1st percentile and was diagnosed at birth with diabetes that required insulin treatment during the first weeks of life. The diabetes resolved within 3 months, and an oral glucose tolerance test at 1 yr of age was normal (Fig. 2). At 13 yr of age, the patient was again diagnosed with diabetes presenting with the classical symptoms of weight loss, polydipsia, and polyuria as well as recurrent cold sores and oral thrush. She has received insulin in replacement doses since then (0.8 U/kg/d) in a twice-daily regimen; her most recently available HbA1c is 6.4%. Another patient was found to have a paternal uniparental isodisomy of 6q24. He was also diagnosed at birth (birth weight also below 1st percentile) and was noted to have macroglossia. This patient was lost to follow-up, and it is unknown if his diabetes resolved.

In a patient with Down's syndrome, thyroid dysfunction, and diabetes diagnosed at 18 d of age, we identified a missense mutation in *ABCC8*, R285Q. Unlike the majority of patients with a pathogenic mutation in *ABCC8*, this patient had a normal birth weight (61st percentile). The variant was inherited from the patient's father who was diagnosed with type 2 diabetes at 40 yr of age. The mother also has type 2 diabetes diagnosed in her thirties. Because both parents have diabetes and there are no other children and the grandparents were not available for study, it is uncertain if the variant cosegregates with diabetes in this family. The inheritance of a pathogenic mutation in *ABCC8* from a healthy parent or a parent diagnosed with type 2 diabetes has previously primarily been described in patients with TNDM caused by *ABCC8* mutation (5). This patient, whose present age is 6 yr, has been treated with insulin since diagnosis and is thus classified as having PNDM. Based on these observations, we consider this to be rare variant of uncertain pathogenicity at this time.

We established a genetic diagnosis in 20 of 32 patients (63%) diagnosed with diabetes before 6 months of age. In the remaining 12 patients, we have so far been unable to establish a genetic diagnosis. However, none had extrapancreatic features consistent with a mutation in one of the genes associated with rare syndromic forms of NDM [*IPF1*, *PTF1A*, *FOXP3*, *GLIS3*, *HNF1B* (*TCF2*), and *EIF2AK3*] and these therefore were not tested.

Characteristics of patients with *KCNJ11* mutations

Fourteen patients, all diagnosed before 6 months of age, had NDM caused by a mutation in *KCNJ11*. In nine of these patients, both parents were non-diabetic, suggesting a *de novo* mutation in the proband (Fig. 1). Two patients had a parent who carried the same mutation and had a similar phenotype. The father of the patient with the E227K mutation also carried the same mutation. However, he was not diagnosed with NDM but rather had a maturity-onset diabetes of the young (MODY)-like phenotype.

The median age at diagnosis in the 14 patients was 8.5 wk (range 1–26 wk), and median birth weight was 2804 g (range 1531–4536 g) corresponding to the 5th percentile. At diagnosis, the patients had severe hyperglycemia with median blood glucose of 590 mg/dL (range 250–900 mg/dL). All patients were treated with insulin in replacement doses from the time of diagnosis (0.6 U/kg/d, range 0.13–0.8 U/kg/d). The median age at genetic testing was 5.5 yr (range 0.25–36 yr). To date, 11 of the patients have been successfully transferred from insulin to glyburide after genetic testing was completed. Two patients are in progress, and in one patient, the

diabetes is spontaneously resolving, thus there is no indication for transition (see subsequently). The patients all achieved better glycemic control after transition, as evidenced by a significant improvement in the HbA1c: median pre-glyburide HbA1c was 8.5%, while post-transition HbA1c was 6.3% ($p < 0.01$). The median dose of glyburide is 0.73 mg/kg/d (range 0.04–1.34 mg/kg/d), divided in one to five daily doses (Table 4).

As reported in other series (19,23,24), the two patients with the V59M mutation had developmental delay without epilepsy, consistent with the intermediate DEND syndrome. One patient, a male diagnosed at 25 wk of age with severe diabetic ketoacidosis (DKA) and dehydration, underwent a Bayley 3 developmental and behavioral evaluation at 2.5 yr of age, just before transition to glyburide. He was found to have a mixed developmental disorder with significant challenges in language use and fine motor skills. Overall, he was assessed to have the motor and cognitive level of an 18–21 months old. Following transfer to glyburide at age 2.5 yr, his parents reported significant improvement in previous sleep disturbances, characterized by frequent spontaneous awakenings. They also reported marked improvement in cognitive function and motor skills after the transfer to glyburide.

The other V59M patient, a 13-yr old female, also had impaired cognitive and fine motor skills. Assessment by a pediatric neurologist at age 7 yr and again at age 10 yr revealed an intelligence quotient of 44 and 55, respectively. Although her mother had not observed any changes in school performance after the transition from insulin to glyburide at 13 yr of age, her fine motor skills noticeably improved, as has been reported in another case of V59M (24).

The patient with the E227K mutation was diagnosed at 5 wk of age with hyperglycemia (646 mg/dL) and DKA and initially required insulin in replacement doses. The patient was referred to us for genetic evaluation at 3 months of age at which time she required only 0.13 U/kg/d of insulin, with diminishing demands in subsequent weeks. The patient had a family history of diabetes with autosomal dominant transmission (Fig. 1). None of the relatives had a history of NDM. All were diagnosed in their twenties and were non-obese, with some on insulin therapy, while others achieved satisfactory glycemic control on oral antidiabetic agents. Sequencing of the proband's father revealed that he was also a carrier of the E227K mutation, strongly suggesting that the familial accumulation of diabetes could be explained by this mutation.

A variable phenotype was also seen within family members carrying the H46Y mutation. The mother of the proband had TNDM, while the son had PNDM. She was diagnosed with diabetes at 12 d of age with severe hyperglycemia (900 mg/dL), but at 11 months of age, her diabetes remitted and she was taken off of insulin. At 8 yr of age, the diabetes relapsed, and she had been treated only with an uncertain oral antidiabetic agent. However, by 18 yr of age, satisfactory glycemic control could no longer be achieved on oral agents alone, so she was again started on insulin. At age 36 yr, she was successfully transitioned to glyburide, but interestingly, she only requires a very low dose (0.04 mg/kg/d). In contrast, her son was treated with insulin continuously from time of diagnosis until he was transitioned to glyburide at 16 yr of age (dose 0.44 mg/kg/d).

The proband from family BP61 was diagnosed with diabetes at 3 wk of age with hyperglycemia (571 mg/dL). He had intra-uterine growth retardation with a birth weight at the 3rd percentile. Before his genetic evaluation, his pediatric diabetologist (F. R. C.) measured an undetectable C-peptide (< 0.5 ng/mL) and a positive anti-GAD65 of 5.8 IE/mL (normal range 0.0–1.45 IE/mL). The patient was 17 yr old by the time a diagnosis of diabetes caused by the R201H mutation in *KCNJ11* was established. The patient was transferred to glyburide using an outpatient protocol. The patient responded well to the high doses of glyburide as evidenced by marked increase in C-peptide to 3.9 ng/mL (randomly obtained). However, despite receiving 1.4 mg/kg/d of glyburide, he experienced episodic hyperglycemia (300–400 mg/dL). The

presence of authentic anti-GAD65 antibodies was confirmed by a repeat measurement of 6.32 IE/mL (normal range 0.0–1.45 IE/mL), and he also had high-titer insulin autoantibodies (36 IE/mL), presumably secondary to long-standing insulin therapy. ICA were negative. Human leukocyte antigen (HLA) typing indicated neutral risk for type 1 diabetes (DQB1 *0502/0504-DQB1 *0609). Sitagliptin (100 mg/d) was added to glyburide therapy, following which he achieved good glycemic control, evidenced by improved HbA1c from a pre-glyburide value of 9.4 to 7.1% after 10 months on combined glyburide and sitagliptin treatment.

Another patient (BP0) who had been diagnosed with diabetes at 4 wk of age when glycosuria was revealed as part of a routine health exam was found to carry the R201C mutation. She successfully transitioned from insulin to glyburide when she was 6 yr old. Around this time, celiac disease was also diagnosed by positive testing for tissue transglutaminase antibodies. The patient was started on a gluten-free diet in the months following her transition to glyburide, and she remains symptom free. Before transition, her body mass index (BMI) was 17 kg/m² (81st percentile), and after 1 yr on glyburide treatment, on a strict gluten-free diet most of that time, her BMI increased modestly to 19.9 kg/m² (94th percentile), most likely reflecting less stringent carbohydrate limits in her diet combined with improved absorption. Interestingly, the parents also reported an improvement in the patient's hypoglycemia awareness after the transition to glyburide. When she had been on insulin, she would have nightmares during overnight episodes when the parents had noted hypoglycemia, whereas after the transition to glyburide, the patient would notify the parents when she correctly suspected that she was hypoglycemic.

Discussion

In this study, we describe our experience in determining the genetic basis of diabetes in 77 consecutive patients with onset of disease from birth to 12 months of age enrolled in our studies of neonatal and other monogenic forms of diabetes. We were able to establish a genetic cause in 63% of 32 patients diagnosed before 6 months of age, with mutations in *KCNJ11* (43.8%) being the most common cause and mutations in *INS* (13%) the other important etiology. The proportion of patients with diabetes caused by *KCNJ11* mutations is similar to those reported by groups in Europe (2,19,25–27). Other groups have also recently reported a similar proportion of patients with *INS* mutations causing diabetes before 6 months of age (12,13). Unexpectedly, we did not identify any patients with diabetes caused by mutations in *ABCC8* (6), likely a consequence of our sample size; in the study by Babenko et al. (6), 7% of patients with PNDM had a mutation in *ABCC8*, equal to only one to two patients in our study population. In the 45 patients diagnosed from 6 to 12 months of age, we previously reported that 3 patients had diabetes caused by a mutation in *INS* (7%), and we confirm the absence of mutations in *KCNJ11* as a cause of diabetes in this age-group and in *ABCC8* for all of those with low birth weight (1,2,28). We thus confirm in the series presented in this study that it is now possible to identify an underlying genetic defect in the majority of patients diagnosed with diabetes before 6 months of age, whereas a much smaller fraction of those diagnosed after 6 months seems to have a known monogenic cause of their diabetes, probably because of an increasing likelihood of typical autoimmune type 1 diabetes. Our results suggest that additional diabetes genes remain to be identified in the patients with onset before 6 months of age.

Eleven of the 14 patients with mutations in *KCNJ11* have been transferred successfully from insulin to glyburide. The treatment regimens for the patients have been individualized with regard to total daily dose and number of doses per day (15). All patients have improved glycemic control as evidenced by improvement in HbA1c without a concomitant increase in the frequency of hypoglycemic episodes. In most of the patients, a significant reduction in hypoglycemic events was also noted. Interestingly, the patients secrete insulin in a meal-dependent manner by uncertain mechanism(s) that may be at least partly explained by the

incretin effect on beta cells (15). Parents of both patients with the V59M mutation report subjective improvement in fine motor skills after the transition to glyburide. All patients enjoy a significant improvement in overall quality of life, without the necessary difficulties their prior intensive insulin therapy required.

Pancreatic autoantibodies

One proband with PNDM caused by the *KCNJ11* mutation R201H (BP61) had positive anti-GAD65 antibodies, confirmed by repeat testing. We considered the possibility of an underlying autoimmune attack on this patient's beta cells in addition to a *KCNJ11* mutation causing diabetes; however, true 'double diabetes' is unlikely in that he had a neutral risk HLA type, and he eventually successfully transitioned completely off insulin. Detection of pancreatic autoantibodies has been previously described in patients with long-standing diabetes caused by *KCNJ11* mutations (29). Dysfunction of beta cells possibly resulting in increased apoptosis could lead to a greater likelihood of positive autoantibodies because of increased exposure of beta-cell content to antigen-presenting cells. Alternatively, anti-GAD65 antibody titers from 5 to 10 IE/mL have been reported as an incidental finding in 1–2% of healthy controls (30). As a consequence of this finding, we recommend genetic evaluation in patients with PNDM diagnosed before 6 months of age regardless of pancreatic autoantibody status. We also recommend HLA typing of anti-GAD65-positive individuals with PNDM to identify an autoimmune cause in addition to a monogenic cause of PNDM to rule out 'double diabetes' before transition from insulin to glyburide in individuals with either *KCNJ11* or *ABCC8* mutations.

Variable phenotype

The proband with NDM caused by the *KCNJ11* mutation E227K (BP121) came from a family with an extensive history of MODY-type diabetes. The father of the proband had the same mutation. It is currently not understood why this mutation causes a variable phenotype with regard to not only the age at diagnosis of diabetes but also the severity of insulin deficiency. One possibility is that the relatives had a neonatal phase of the disease that was not recognized; however, this seems unlikely given the severe hyperglycemia and DKA exhibited by the proband at diagnosis and also considering the large number of potentially affected relatives. Other groups have reported a similar variability in the phenotype of patients carrying this mutation, and this is not readily explained by the functional studies of the mutant protein (5, 31).

We also observed a variable phenotype in the BP1 family carrying the H46Y mutation in *KCNJ11*. It remains unclear why the mother of the proband had TNDM, while her son apparently required continuous insulin therapy from the time of diagnosis until his transition to glyburide. Although variable presentation of *KCNJ11*-related TNDM has been observed (5), to our knowledge, H46Y has not previously been implicated in TNDM.

Learning disabilities

Patients with the *KCNJ11* mutation, V59M, have the intermediate DEND syndrome, which is characterized by learning difficulties and other features of developmental delay. Patients with the R201C mutation have also been reported to have a variable incidence of developmental delay and a 50% incidence of learning disabilities (2,15,32). Although not yet well characterized, one of the patients with the R201C mutation reported in this study has a learning disorder and strabismus. Until further clarified, we recommend that all patients with this mutation be considered for formal evaluation of learning disorders, such as visual-spatial processing and executive function. Patients with several other *KCNJ11* mutations (G53R, G53D, E229K, and Y330C) have also been reported to have learning disorders (5,14,33). Partial developmental improvement may follow sulfonylurea treatment, at least for the V59M

mutation (23,24). The clinical features associated with activating mutations in *KCNJ11* may partially reflect the degree of loss of ATP sensitivity as assessed *in vitro*, and we suspect that additional features of these mutations will come to light as more cases are reported (2,34).

Saliva samples for DNA

The use of the Oragene™ saliva Self-Collection kit was efficient and dependable. The sample was well preserved and gave an excellent quality and quantity of DNA for sequencing even for samples that took several days to be delivered through the mail. Parents and children prefer for obvious reasons to give a saliva sample instead of a blood draw. In the very few samples where the DNA was of poor quality, it was readily apparent by the brownish discoloration exhibited by the DNA sample upon extraction. This was likely because of coffee intake, smoking, or tobacco chewing before preparation of the saliva samples. Thus, patients need to be advised to carefully follow the instructions that accompany the kit.

Patients with unknown cause of PNDM

In 12 patients diagnosed before 6 months of age, we were unable to establish a genetic cause for their diabetes by testing *KCNJ11*, *ABCC8*, and *INS*. Six of the patients had phenotypic or biochemical features that indicated that their diabetes could be of an autoimmune nature. One patient was ICA512 positive, while another was anti-GAD65 antibody positive (both patients had normal birth weights). Three patients had other autoimmune diseases: celiac disease in one patient and thyroid dysfunction in two patients who also had Down's syndrome (one of the patients was the carrier of the *ABCC8* variant, R285Q). Lastly, a patient diagnosed at 6 months of age had a normal birth weight and a brother diagnosed with diabetes at 2 yr with positive anti-GAD65 autoantibodies. The remaining six patients had a median age at onset of 5.5 months and median birth weight at the 40th percentile. One of these patients who was born to consanguineous parents was diagnosed with diabetes at 1 wk of age. He also had developmental delay and muscle hypotonia (the glucokinase gene and the 6q24 locus were also normal in this patient). The remaining five patients did not have other health problems to suggest any of the gene-associated multisystem diseases that included NDM. The later onset and near-normal birth weight in these patients, compared with those with *KCNJ11*, *ABCC8*, or *INS* mutations, could indicate less severe *in utero* insulin deficiency and that the effects of the unknown mutant gene take more time to disrupt beta-cell function and cause diabetes. Continued studies of these patients may provide new genes that can cause NDM. The absence of a family history of diabetes suggests that the mutations may be *de novo* as for the majority of cases of PNDM caused by mutations in *KCNJ11*, *ABCC8*, and *INS*. PNDM appears to be largely a sporadic form of diabetes, which has implications for genetic counseling of parents of a child with this form of NDM.

6q24 abnormalities

Two patients had NDM because of abnormalities of chromosome 6q24. Both patients were diagnosed within the first week of life, and both were born severely growth retarded, similar to previous studies reporting earlier age of diagnosis and lower birth weight than patients with mutations in *KCNJ11* or *ABCC8* (5). One patient was noted to have macroglossia, which has been found in 30% of patients (35). The other patient with TNDM because of complete loss of methylation of the maternal allele at the 6q24 locus was healthy apart from diabetes, depression, and attention-deficit hyperactivity disorder.

Conclusions

We were able to identify an underlying genetic defect in 63% of patients diagnosed with diabetes before 6 months of age, with mutations in *KCNJ11* and *INS* being the most prominent causes. Our results demonstrate that salivary samples can be used to obtain an appropriate DNA

sample for genetic analysis even in young children. We also confirm that the majority of patients with NDM caused by a single gene defect have a low birth weight and a variable age at diagnosis of diabetes from less than 1 wk to 6 months. In contrast, monogenic diabetes is rare but possible in patients diagnosed between 6 and 12 months of age, although not because of mutations amenable to transition to glyburide therapy. Testing for underlying genetic defects in NDM is critical for guiding appropriate management, such as sulfonylurea therapy that has allowed for life-changing modification of diabetes management in those with *KCNJ11* or *ABCC8* mutations.

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Fig. 1.

Partial pedigrees of 12 families with neonatal diabetes mellitus because of a mutation in *KCNJ11*. Circles represent females and squares represent males. Black symbols represent individuals with diabetes. A slash through the symbol indicates that the family member is deceased. The genotype is indicated under the symbols. Amino acid change/N, one mutated and one normal allele; N/N, two normal alleles; NA, not available for testing. The arrow indicates the proband.

Fig. 2.

Plasma glucose values by date for patient with transient neonatal diabetes mellitus (BP90) because of a methylation defect of 6q24. The patient was discharged from hospital at 6 wk of age without insulin supplement. The values were obtained from the patient's mother who was told to monitor plasma glucose daily. This graph demonstrates the gradual normalization of glucose values as the patient enters remission. Red lines on the x -axis indicate days when the patient had a fever or other illness.

Table 1

The genetic causes of neonatal diabetes mellitus in 77 patients from the University of Chicago Neonatal Diabetes Mellitus Registry

	All (n = 77)	<i>KCNJ11</i> /6q24 (n = 16)	<i>INS</i> (n = 7)	Unknown genetic cause (n = 54)
Diagnosis ≤6 months old	32	16	4	12
Diagnosis 6–12 months old	45	0	3	42

Table 2

Clinical characteristics of 77 patients diagnosed with diabetes before 1 yr of age from The University of Chicago Neonatal Diabetes Registry

Characteristics	All (n = 77)	<i>KCNJ11/INS/6q24</i> (n = 23)	Unknown genetic cause (n = 54)
Sex (% male)	62	61	63
Age at diagnosis (wk)	34 (0–52)	11.5 (0–52)	39 (1–52)
Birth weight (g)	3359 (1275–4536)	2863 (1275–4536)	3458 (2182–4300)
Gestational age (wk)	40 (34–42)	40 (35–42)	40 (34–42)
Percentile for birth weight	47 (0–96)	23 (<1–89)	52 (1–96)

Values are median and range unless otherwise indicated.

Table 3

Clinical characteristics of 18 probands with a known genetic cause of neonatal diabetes mellitus

Characteristics	<i>KCNJ11</i> (n = 12)	<i>INS</i> (n = 4)	6q24 (n = 2)
Sex (% male)	50	75	50
Birth weight (g)	2804 (1531–3657)	3402 (2211–3402)	1786 (1275–2296)
Percentile for birth weight	6 (<1–65)	38 (5–69)	<1
Age at diagnosis (wk)	10 (1–26)	11 (9–43)	0.3 (0–0.6)
Plasma glucose at onset (mg/dL)	571 (250–834)	769 (640–898)	490 (430–550)

Values are median and range unless otherwise indicated.

Characteristics of 11 patients with permanent neonatal diabetes mellitus because of a *KCNJ11* mutation who were successfully transferred from insulin to glyburide

Table 4

Characteristics	Mutation									
	H46Y		V59M		R201C		R201H		R201H	
Patient	Son BP1		BP100		BP106		BP4		BP61	
Sex (M/F)	M		F		F		F		M	
Current age (yr)	17		14		6		6		18	
Pre-transition insulin dose (U/kg/d)	0.6		0.7		0.8		0.8		0.7	
Glyburide dose (mg/kg/d)	0.44		1.2		0.55		0.20		1.34	
Number of doses per day	2		—		2		5		3	
Hemoglobin A1c (%)	8.4		7.2		7.9		7.8		9.4	
On insulin	6.5		5.9		6.3		6.5		7.1	
On glyburide	23.1		22.9		14.8		15.1		20.5	
BMI (kg/m ²)	23.2		—		14.6		14.6		20.1	
On insulin	0.8		0.9		-0.3		-0.1		-0.3	
On glyburide	0.7		-0.1		-0.5		-0.5		-0.7	

BMI, body mass index.

* Fructosamine level is used to monitor her glycemic control because she has thalassemia. Pre-transition value was 319 μmol/L and post-transition value was 228 μmol/L (normal range 0–285 μmol/L).