Neonatal Diabetes Mellitus: Description of Two Puerto Rican Children with KCNJ11 Activating Gene Mutation

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Neonatal diabetes mellitus (NDM) is a rare disorder. A one-month-old boy presented with vomiting, hyperglycemia (968 mg/dl [53.8 mmol/L]), severe acetonemia, and metabolic acidosis (pH 6.95, HCO_3- 4.2 mmol/L). A second child (three months of age) presented with upper respiratory tract symptoms and a plasma glucose level of 835 mg/dl, without acetonemia or acidosis. Both were hospitalized and managed with intravenous fluids and then discharged on insulin. Genetic testing identified the presence of the *de novo* V59M and E322K activating mutations in the KCNJ11 gene encoding the sulphonylurea/potassium channel (Kir6.2 subunit) of the insulin β cell. Both patients were switched to glibenclamide and remain off insulin. To our knowledge, these are the first children in Puerto Rico identified with NDM secondary to a KCNJ11 activating mutation. We conclude that NDM secondary to KCNJ11/Kir6.2 activating mutations, although unusual, should be considered in similar cases since patients with these mutations could come off insulin. [*P R Health Sci J 2011;30:87-89*]

Key words: Neonatal diabetes mellitus, Potassium channel, KCNJ11 mutation, Sulphonylurea, Insulin

Neonatal diabetes mellitus (NDM) is defined as hyperglycemia lasting for more than two weeks and occurring within the first six month of life. In a normal individual, before a meal, the activity of the potassium ATP (K_{ATP}) channels of the pancreatic β-cell reduces the membrane potential below the threshold for activation of voltage-gated calcium channels, thus turning off the stimulatory action of calcium on insulin secretion. During a meal, blood glucose increases and enters β-cells via a glucose transporter. The conversion of glucose to glucose-6-phosphatase by glucokinase is the key step governing substrate input into the glycolytic pathway in β-cells. Subsequent metabolism via glycolysis and oxidative phosphorylation raises the ATP/ADP ratio. The increase in ATP/ADP ratio closes K_{ATP} channels, depolarizing the β-cell membrane to allow calcium-dependent insulin secretion via activation of voltage-sensitive calcium channels. Activating mutations of the K_{ATP} channels result in impaired binding of ATP and ADP to the inside portion of the channel, followed by lack of depolarization of β-cell membrane which results in an interrupted insulin secretion and clinically the development of diabetes (1). Subjects diagnosed with NDM are more likely to have a monogenic defect rather than type 1 diabetes mellitus (2). It is rare, affecting approximately 1 in 500,000 newborns. Also, NDM is heterogeneous and can be transient (TNDM) or permanent (PNDM).

Abnormalities of the imprinted region on chromosome 6q24 constitute the most frequent cause of TNDM (2, 3). The second most common cause of NDM is that induced by an activating mutation in the *KCNJ11* gene, which encodes the Kir6.2 subunit of the K_{ATP} channel, a critical regulator of beta-cell insulin secretion. A significant percentage of patients with the latter can be transferred to oral sulphonylurea (SU) (4, 5). Herein, we report what, to our knowledge, represents the first two cases of Puerto Rican children presenting with the KCNJ11/K_{ir}6.2 activating gene mutation.

Case Report

Case 1

A 1 month-old boy was brought for evaluation because of vomiting that had occurred during the five days prior to admission. He was a full-term newborn, adequate for gestational age (TAGA, BW 2329 g) product of an uneventful pregnancy and delivery. The parents' marriage was non-consanguineous. His family history was negative for type 1 diabetes. On the day of admission, he became hypoactive with Kussmaul-like breathing. He was dehydrated and had lost 1 kg. His laboratory results showed him to have a higher-than-normal glucose level (968 mg/dl, or 53.8 mmol/L), severe acetonemia, and metabolic acidosis (pH 6.95, HCO3- 4.2 mmol/L, PCO2 20 mmHg, base excess -26.6). His treatment included intravenous insulin and fluid administration. He was discharged home on subcutaneous

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insulin. His c-peptide serum levels were lower than 0.5 ng/ml; antibodies against islet cells (ICA), glutamic acid decarboxylase (GAD-65), and insulin were all negative.

Blood samples collected from case 1 and parents were sent to the laboratory of doctors Hattersley and Ellard in Exeter, UK. Mutation analysis of the KCNJ11 genes were undertaken on amplified genomic DNA samples using the polymerase chain reaction with previously reported primers at the Molecular Genetics Laboratory at Peninsula Medical School in Exeter, UK. The products were sequenced by standard methods on an ABI 3100 (Applied Biosystems, Warrington, UK) (6). Genetic testing identified the presence of the V59M activating mutation in the KCNJ11 gene encoding the Kir6.2 subunit. The parents tested negative for the mutation.

After one year of insulin therapy, the infant was switched to glibenclamide (GLI). He has been off insulin for 40 months. He continues to thrive (Table 1). The boy's neurological profile showed that at 11 months of age he was unable to sit up or pull himself up to stand. At 31 months of age, his speech was still delayed. At that point he underwent neurological evaluation and was found to have moderate psychomotor delay. Between his two most recent visits to the clinic, the parents increased the dose of GLI from 0.45 to 0.51 mg/kg/day, seeking neurological benefits for their child.

Case 2

The boy was brought in for evaluation at 3 months of age because of upper respiratory tract symptoms and unquantified fever for four days before admission. He had been soaking diapers and drinking lots of water for two days before diagnosis. He was the preterm AGA (adequate for gestational age; 34 weeks, BW 2215 g) newborn of a pregnancy complicated by hypertension. He was the product of a non-consanguineous union. His family history was negative for type 1 diabetes, but positive for type 2 diabetes. The day before diagnosis, he had six episodes of vomiting. His laboratory results showed him to have a higher-than-normal glucose level (835 mg/dl, or 46.4 mmol/L), traces of acetonemia, and no metabolic acidosis (pH 7.35, HCO3- 21.4 mmol/L, PCO2 39 mmHg, base excess -3.8). Urine analysis was negative for ketones. His treatment included intravenous insulin and fluid administration. He was discharged home on subcutaneous insulin. He tested negative for ICA, GAD-65, and insulin antibodies.

Genetic testing, as described above, identified the presence of the E322K activating mutation in the KCNJ11 gene encoding the Kir6.2 subunit. The parents tested negative for the mutation.

He was switched at 18 months to GLI over a 4-week period. He has been off insulin for 25 months and is being treated only with oral GLI (0.48 mg/kg/day) and continues to thrive (Table 1). At 18 months of age he was referred for speech therapy because of delayed language development.

Table 1. Characteristics of two infant boys with the KCNJ11/Kir6.2 activating gene mutation

Characteristics	Case 1	Case 2
Birth		
Weight, grams (percentile)	2329 (3rd)	2215 (50-75th)
Length, cm (percentile)	49 (25th)	46 (<3rd)
Gestational age, weeks	38	34
Findings on Presentation		
Plasma glucose, mg/dl*	968	835
Diabetic ketoacidosis at diagnosis	Yes	No
Pancreatic autoantibodies	Negative	Negative
Age at diagnosis, months	1	3
KCNJ11/K_{ir}6.2 mutation	V59M	E322K
Age stopped insulin, months	13	18
Current status		
Age, months	53	43
Weight in kilograms (percentile)	18.6 (50-75th)	20.9 (>97th)
Length/Height in cm (percentile)	105.3 (50th)	108 (>97th)
Insulin administration	0	0
Most recent HbA_{1c},%	5.8	6.2
Glibenclamide, mg/kg/day	0.51	0.48
Remarks	Psychomotor and speech delay	Speech delay

*divide by 18 to change to mmol/L

Discussion

Today, the causes of NDM are better understood, and they can be grouped as defects either secondary to impaired islet development (i.e., hepatocyte nuclear factor-1β mutations), reduced β-cell mass [i.e, reduction in pancreatic eukaryotic initiation factor-2α kinase (EIF2AK3) activity, e.g., Wolcott-Rallinson Syndrome], or β-cell dysfunction (i.e., defective glucokinase and activating mutations K/SU receptors). There is also a better understanding of the insulin secretion cascade events. Specifically, pancreatic β-cell ATP-sensitive K channels and the high-affinity SU receptor are crucial for the regulation of glucose-induced insulin secretion: insulin secretion is initiated by closure of the channels and inhibited by their opening.

To our knowledge, the cases reported herein represent the first descriptions of Puerto Rican children with *de novo* KCNJ11 receptor mutation provoking β-cell dysfunction. Genetic analyses performed on samples from the biological parents of these children demonstrated that none of the parents had a mutation in the KCNJ11 region gene. Instead, both children had *de novo* mutations resulting in a single amino acid substitution, similar to most of the cases reported until recently (7).

Different from what has been reported by Aguilar-Bryan, neither of the cases herein was demonstrated to be an SGA (small for gestational age) baby, a condition commonly seen in newborns with KCNJ11 activating mutations (8, 9). Shield reported that diabetic ketoacidosis is seen in one third of the cases with KCNJ11 activating mutations (4). Its absence did not preclude the diagnosis in our case 2. More important would

be to pursue further studies for KCNJ11 mutations in children under 6 months of age with persistent hyperglycemia even in the absence of ketosis. In fact, Hattersley and Ashcroft published the existence of a relationship between phenotype, genotype, and functional severity of Kir6.2 mutations and polymorphisms. According to them, the clinical severity of the disease is directly associated with specific mutations and the extent of reduction in the ATP-sensitive mutated K_{ATP} channel (10).

Furthermore, the cases herein are examples of translational research turning into a gratifying experience by resulting in an improvement in quality of life. The two children remain off insulin injections, and instead are on oral medication. It is unlikely that severe developmental delay seen in a subgroup of the patients with Kir6.2 mutations results from diabetes or its treatment (6). Kir6.2 is the pore-forming subunit of K_{ATP} channels in skeletal muscle and neurons throughout the brain, hence altered activity of these channels could cause developmental delay, muscle weakness, and epilepsy. Therefore, special attention should be given to the appearance of neurological symptoms in children with PNDM. Although we did not include in this report a formal neurological evaluation, we speculate on the possibility of having an instance of the intermediate DEND syndrome (developmental delay, epilepsy and neonatal diabetes) in case 1, as described by Pearson at al. (11). In fact, when switched to GLI, he showed neurological improvement. Presumably, the function of the CNS SU/K channels improved. The child still requires speech, physical, and occupational therapies, which necessity might be explained by the heterogeneity of these channels (or difference in response to SU by the CNS cells) or by SU unable to reach sufficient concentration in the brain. In fact, the parents of case 1 increased GLI hoping for better neurological outcomes for their child. It is tempting to hypothesize that higher doses of SU could possibly result in further neurological improvements. However, the risks of untoward adverse events (e.g., hypoglycemia) would have to be weighed against the benefits.

In summary, we present the cases of two Puerto Rican children, each with a *de novo* KCNJ/Kir6.2 activating mutation causing PNDM. The children were able to stop insulin therapy and continue to thrive on SU. Although NDM is an unusual disease, patients should be promptly evaluated for K channel gene mutations as such an evaluation could result in patient's being able to terminate insulin injections.

Resumen

La diabetes mellitus neonatal (DMN) es un desorden infrecuente. Se presenta un infante de un mes de edad con vómitos e hiperglucemia (968 mg/dl [53.8 mmol/L]), acetonemia severa y acidosis metabólica (pH 6.95, HCO_3- 4.2 mmol/L). Un segundo infante (de tres meses de edad) se presenta con síntomas de tracto respiratorio alto y nivel de glucosa en plasma de 835 mg/dl, sin acetonemia o acidosis. Ambos son hospitalizados y tratados con líquidos intravenosos y dados de alta en insulina. Pruebas de genética revelaron la presencia de mutaciones activadoras *de novo* V59M y E322K en el gen KCNJ11 que codifica el canal de sulfonilurea/potasio (subunidad Kir6.2) de la célula β secretora de insulina. Ambos pacientes pudieron ser cambiados a terapia con glibenclamide y permanecen sin necesitar insulina. Hasta donde se conoce, estos son los primeros niños en Puerto Rico que se identifican con DMN secundaria a una mutación activadora de KCNJ11. Concluimos que, aunque la DMN secundaria a una mutación activadora de KCNJ11/Kir6.2 es rara, ésta debe considerarse en situaciones como los casos descritos toda vez que estos pacientes pudieran detener la administración de insulina.

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