J Korean Med Sci 2007; 22: 616-20
ISSN 1011-8934

Sulfonylurea Therapy in Two Korean Patients with Insulin-treated Neonatal Diabetes due to Heterozygous Mutations of the *KCNJ11* Gene Encoding Kir6.2

Permanent neonatal diabetes (PND) is a rare form of diabetes characterized by insulin-requiring hyperglycemia diagnosed within the first three months of life. In most cases, the causes are not known. Recently, mutations in the *KCNJ11* gene encoding the Kir6.2 subunit of the ATP-sensitive K^+ channel have been described in patients with PND. We report the first two Korean cases with PND due to a lysine-to-arginine substitution at position 170 (K179R) and a valine-to-methionine substitution at position 59 (V59M) mutations of *KCNJ11* encoding Kir6.2, respectively. After several years of insulin therapy, these patients were managed by oral glibenclamide therapy at a daily dose of 0.8-0.9 mg/kg. Their basal c-peptide levels increased after one week of glibenclamide therapy, and one month later, the insulin and c-peptide levels were in the normal ranges without any episodes of hyper- or hypoglycemia. These cases demonstrate that oral sulfonylurea may be the treatment of choice in PND patients with *KCNJ11* mutations even at a young age.

Key Words : *Neonatal Diabetes; Permanent; Korean; Sulfonylurea; Kir6.2 Channel; Mutation*

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Received : 5 December 2006
Accepted : 12 January 2007

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INTRODUCTION

Neonatal diabetes, defined as insulin-requiring hyperglycemia within the first three months of life, is a rare disease entity, with an estimated incidence of 1 in 400,000 neonates (1). Transient neonatal diabetes usually resolves by a median of 12 weeks and is generally associated with an abnormality of the imprinted region of 6q24 (2). By contrast, permanent neonatal diabetes (PND) requires insulin treatment for life, and until recently the genetic etiology was largely unknown. Recently, Gloyn et al. (3) reported that heterozygous activating mutations in the *KCNJ11* gene are a common cause of PND. *KCNJ11* encodes Kir6.2, the pore-forming subunit of the ATP-sensitive K^+ channel (KATP channel) (4). KATP channels play a central role in glucose-stimulated insulin secretion from pancreatic β-cells (5).

Sulfonylurea stimulates insulin secretion by binding to the β-cell's high-affinity sulfonylurea receptor and closing the ATP-sensitive K+ channels by an ATP-independent mechanism (6). Sagen et al. (7) recently reported that oral sulfonylurea should be considered for treatment of patients with Kir6.2 mutations.

We report the first two Korean cases of PND with mutations in the *KCNJ11* gene encoding Kir6.2. In addition, we also show that oral sulfonylurea therapy is as good as or better for glycemic control, compared with insulin therapy.

MATERIALS AND METHODS

Two Korean children with permanent neonatal diabetes were evaluated. Neither the parents nor their siblings had diabetes. The mothers did not have gestational diabetes. Written informed consent was obtained from the parents for study participation and publication.

Case 1

Case 1, born at 40 weeks gestation, was well at birth but had intrauterine growth retardation with a birth weight of 2,300 g (<3 percentile). She was the first child of two siblings. At six weeks of age, diabetes was diagnosed based on hyperglycemia (1,460 mg/dL) and glucosuria (3+) with ketoacidosis. The serum C-peptide was 0.4 ng/mL (normal range: 1.0-3.5) and HbA_{1C} was 6.9% (normal range, 2.1-7.7). Autoantibodies associated with type 1 diabetes and pancreatic

ultrasonography were negative. The patient did not have dysmorphic features. Insulin therapy was initiated and insulin doses of 0.6-0.8 units/kg/day were required. There was marked catch-up growth after birth, and the weight and height were normally distributed on follow-up after 6.5 yr.

Psychomotor development and muscle strength were within normal limits. We recently introduced oral sulfonylurea to the patient. We started with glibenclamide at a dose of 0.2 mg/kg/day, consisting of four doses a day and increased the doses up to 0.9 mg/kg/day over eight days. One month later, the glibenclamide dose was 7.5 mg three times a day.

Case 2

This patient was born after 37 weeks gestation by cesarean section delivery with a birth weight of 3,000 g (10-25 percentile). He was the second child of two siblings. At six weeks of age, diabetes was diagnosed based on hyperglycemia (>500 mg/dL) and glucosuria (3+) with ketoacidosis. Autoantibodies associated with type 1 diabetes were not present, and the pancreatic ultrasonography was negative. Insulin therapy was initiated and the average daily insulin dose before sulfonylurea therapy was 0.6 units per kg.

Table 1. Sequences of primers for PCR reaction

	Sense (5′-to-3′)	Antisense (5′-to-3′)
Exon 1	gtctggtggggagttatctcag	gtgaagatgagcaatgtgtgtg
Exon 2	tttgtgtccaagaaaggcaact	gatgttctgcacgatgaggat
Exon 3	ttttctccattgaggtccaagt	cgttctccatggggatgtc
Exon 4	catgatcatcagcgccac	agtccacagagtaacgtccgtc
Exon 5	acctcctacctggccgatga	ctggcccagcctcacaccag

PCR, polymerase chain reaction.

The patient is now 3.8 yr of age, and his weight is 13.5 kg (3-10 percentile), and height is 100.0 cm (50-75 percentile), and the head circumference is 51.0 cm (50-75 percentile). The serum C-peptide was 0.08 ng/mL, and HbA_{1C} was 8.7%. There were no dysmorphic features. However, he had moderate motor and mental developmental delay. He also had muscle weakness in both extremities. He did not have epilepsy, and the electroencephalogram was normal limits. At the age of 3.8 yr, he was admitted for a trial of glibenclamide therapy. The insulin therapy was discontinued over two days, and glinbenclamide was given with an initial dose of 0.05 mg/kg four times a day, and the dose was increased up to 0.9 mg/kg/day.

Molecular genetic analysis

Genomic DNA was isolated from peripheral blood using a PUREGENE DNA isolation kit (Gentra, Minneapolis, MN, U.S.A.). Five exons of the *KCNJ11* gene and their intronic flanking sequences were amplified by polymerase chain reaction (PCR) with five sets of primers (Table 1). Electrophoresis and analysis of the reaction mixtures were performed on the ABI 3100 Genetic analyzer (Applied Biosystems, Foster city, CA, U.S.A.).

Continuous glucose monitoring system

Continuous glucose monitoring was performed using on the continuous glucose monitoring system (CGMS, Medtronic MiniMed, Sylmar, CA, U.S.A.) three times in case 1: at the initiation of glibenclamide therapy, one week and one month later after glinbenclamide therapy. CGMS is a Holter-style sensor that monitors glucose values continuously in sub-

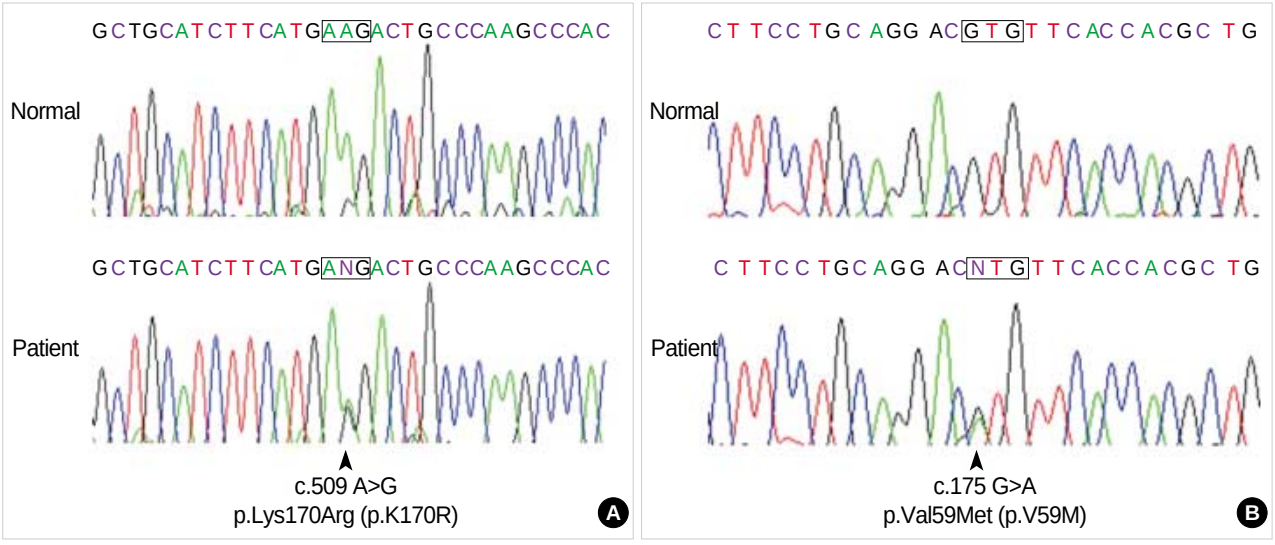

Fig. 1. Direct DNA sequence analysis of two patients with permanent neonatal diabetes revealed mutations of the *KCNJ11* gene encoding Kir6.2. Arrows indicate the nucleotide position affected by the mutation. Case 1 (A) had a nucleotide substitution with AAG to AGG at position 509 in the exon 3. Case 2 (B) had a nucleotide substitution with GTG to ATG at position 175 in the exon 2.

cutaneous tissue fluid within a range of 40-400 mg/dL. The monitor records glucose levels every five minutes for 72 hr.

RESULTS

We identified two previously reported heterozygous mutations (a a-to-g change at nucleotide 509, which resulted in a lysine-to-arginine substitution at position 170 of the gene encoding Kir6.2, K170R, in case 1, and a g-to-a change at nucleotide 175, which resulted in a valine-to-methionine substitution at position 59 in the gene encoding Kir6.2, V59M, in case 2, Fig. 1).

In order to assess the pancreatic β-cell function, C-peptide levels were measured in the patients with PND. On admission for glibenclamide therapy, C-peptide levels were 0.1 in case 1 and 0.1 ng/mL in case 2 with corresponding blood glucose levels of 119 and 400 mg/dL, respectively. The basal levels of C-peptide levels, after one week of glibenclamide treatment, were increased to more than 18 times higher than the levels obtained during insulin therapy in case 1 and case 2 (Table 2).

In order to compare the clinical response to glibenclamide treatment with the response to the insulin therapy, frequent capillary glucose measurement and CGMS were performed. After seven days of glibenclamide treatment, no hyper- or hypoglycemic episode was observed in these patients. Fig. 2 shows three representative 24-hr glucose profiles obtained by the CGMS in case 1. These patients remain well and off insulin at 6 and 15 months of follow-up.

Table 2. Serum insulin, C-peptide, and HbA_{1C} levels after glibenclamide therapy

Days after sulfonylurea	Day 0 (Case 1/2)	Day 7 (Case 1/2)	Day 30 (Case 1/2)	Day 150 (Case 1/2)	1 yr (Case 1/2)
Insulin (μIU/mL)	NA/15.4	11.5/9.47	13.4/NA	10.4/15.8	NA/12.0
C-peptide (ng/mL)	0.1/0.1	2.2/1.8	3.3/3.2	2.9/3.0	NA/2.0
Glucose (mg/dL)	119/400	294/NA	150/NA	135/NA	NA/NA
HbA_{1C}	7.4/8.7	NA/NA	6.9/7.2	6.4/5.9	NA/5.8

NA, not available.

DISCUSSION

Recently, it has been shown that PND can result from mutations in Kir6.2 that reduce the ability for ATP to close the ATP-sensitive K+ channel. We report here two different heterozygous activating mutations of the *KCNJ11* gene in Korean children with PND.

PND is defined as hyperglycemia diagnosed within the first three months of life and requires insulin therapy for life (1). Although PND is a rare entity and the genetic etiology is unknown in about half of affected patients, Kir6.2 muta-

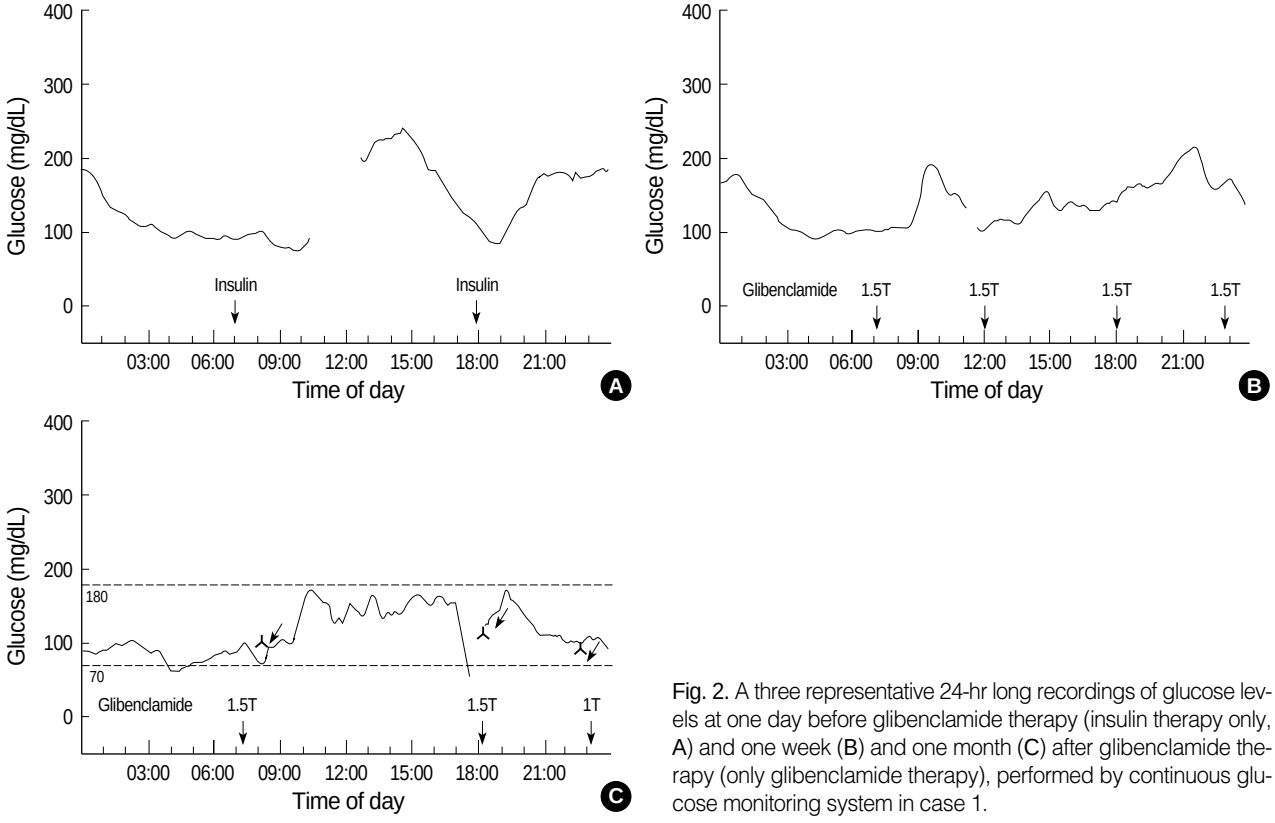

Fig. 2. A three representative 24-hr long recordings of glucose levels at one day before glibenclamide therapy (insulin therapy only, A) and one week (B) and one month (C) after glibenclamide therapy (only glibenclamide therapy), performed by continuous glucose monitoring system in case 1.

tions are a common cause of PND. To date, at least 63 patients with activating mutations in Kir6.2 have been described from Europe, the Middle East, Australia, and North and South America, comprising 21 different mutations in 49 families (8, 9). The majority of cases have been Caucasian, but mutations have been found in other ethnic groups. However, there has been only one report from Japan in Asia with PND caused by a Kir6.2 mutation (10). The cases reported here are the first cases of PND with Kir6.2 mutations identified in Korea.

We found two different mutations (V59M and K170R) in the Korean patients with PND. The V59M mutation is one of the most common mutations identified to date (8). However, the K170R mutation is rare, and only one case has been reported. There is emerging evidence for a clear genotype-phenotype relationship for Kir6.2 mutations. Most patients with a mutation at R201, the most common mutation, have nonremitting neonatal diabetes without neurological features. However, patients with the V59M mutation have developmental delay and features consistent with intermediate DEND (developmental delay, epilepsy, and neonatal diabetes) syndrome (8). The phenotypic expressions of the two patients presented here are similar to those reported in cases previously (8, 9). Our patients did not have type 1 associated antibodies and had low levels of C-peptide. One of two patients had low birth weight. Moreover, this patient was small for gestational age. Both exhibited severe hyperglycemia and the need for subsequent exogenous insulin within three months of life. The patient with the K170R mutation had only PND. By contrast, patients with the V59M mutation have neurological features (developmental delay and muscles weakness) in addition to diabetes.

All mutations studied to date produce a marked decrease in the ability of ATP to block the KATP channels when expressed in the heterologous systems (11-13). This reduction in ATP sensitivity causes the channel to open more fully at physiologically relevant concentrations of ATP, leading to an increase in the KATP current. In pancreatic β-cells, an increase in KATP current will hyperpolarize the membrane, suppressing electrical activity, Ca^{2+} influx, and insulin secretion, and thereby causes diabetes (14). KATP channels that are insensitive to ATP as a consequence of Kir6.2 mutations can still be closed by sulfonylurea that bind to the SUR subunit and directly close the channel (6). A number of patients with Kir6.2 mutations have been able to discontinue their insulin injections completely and obtain as good as, or better glycemic control, using oral sulfonylurea (7, 15-17). In our cases, we discontinued insulin injection and introduced high dose oral glibenclamide therapy (0.8-0.9 mg/kg/day). The continuous glucose monitoring systems were comparable during and after insulin treatment, and the HbA_{1C} levels declined after insulin had been discontinued. However, these patients still required high doses of sulfonylurea, compared with adults with type 2 diabetes, as has been reported in other studies (15-17). Mutations that affect the probability of opening the channel are less sensitive to inhibition by sulfonylurea in vitro (13, 18). Therefore, even higher drug doses may be necessary in patients with PND. To date, the longest duration recorded in a patient has remained off insulin has been about two years (8). Therefore, long-term follow-up studies are now required to assess whether the response to sulfonylurea is maintained, and whether sulfonylurea therapy is better than insulin therapy.

In conclusion, mutations in the gene *KCNJ11* encoding Kir6.2 can cause PND in Korean children, and oral sulfonylurea therapy is as good as, or better than glycemic control, obtained with insulin therapy. All patients with PND should be tested for *KCNJ11* mutations for appropriate management and follow-up.

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