Molecular Genetics, Clinical Characteristics, and Treatment Outcomes of $K_{ATP}$-Channel Neonatal Diabetes Mellitus in Vietnam National Children’s Hospital

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Background: Neonatal diabetes mellitus (NDM) is defined as insulin-requiring persistent hyperglycemia occurring within the first 6 months of life, which can result from mutations in at least 25 different genes. Activating heterozygous mutations in genes encoding either of the subunits of the ATP-sensitive K+ channel ($K_{ATP}$ channel; $KCNJ11$ or $ABCC8$) of the pancreatic beta cell are the most common cause of permanent NDM and the second most common cause of transient NDM. Patients with NDM caused by $K_{ATP}$ channel mutations are sensitive to sulfonylurea (SU) treatment; therefore, their clinical management can be improved by replacing insulin with oral agents.

Patients and Methods: Seventy patients were diagnosed with NDM between May 2008 and May 2021 at Vietnam National Children’s Hospital, and molecular genetic testing for all genes known to cause NDM was performed at the Exeter Genomic Laboratory, UK. Patients with $ABCC8$ or $KCNJ11$ mutations were transferred from insulin to oral SU. Clinical characteristics, molecular genetics, and annual data relating to glycemic control, SU dose, severe hypoglycemia, and side effects were collected. The main outcomes of interest were SU dose, SU failure (defined as permanent reintroduction of daily insulin), and glycemic control (HbA1c).

Results: Fifty-four of 70 patients (77%) with NDM harbored a genetic mutation and of these; 27 (50%) had activating heterozygous mutations in $ABCC8$ or $KCNJ11$. A total of 21 pathogenic mutations were identified in the 27 patients, including 13 mutations in $ABCC8$ and 8 mutations in $KCNJ11$. Overall, 51% had low birth weight (below 3rd percentile), 23 (85%) were diagnosed before 3 months of age, and 23 (85%) presented with diabetic ketoacidosis. At diagnosis, clinical and biochemical findings (mean ± SD) were pH...
INTRODUCTION

Neonatal diabetes mellitus (NDM) is defined as uncontrolled hyperglycemia with onset in the first 6 months of life. It is estimated to affect one in 90,000 newborns (1). NDM can be divided into three forms based on phenotypic characteristics; transient NDM (TNDM), permanent NDM (PNDM), and syndromic NDM (2). TNDM and PNDM account for 90% of NDM cases. NDM can result from mutations in at least 25 different genes (2). Most cases of TNDM are caused by imprinting defects on chromosome 6q24, with presentation in infancy, remission, and subsequent relapse in later life. Activating heterozygous mutations in the genes encoding either of the subunits of the ATP-sensitive K+ channel (KATP channel; KCNJ11 or ABCC8) of the pancreatic beta cell are the second most common cause of TNDM (26% of TNDM cases) and the most common causes of PNDM (44% of PNDM cases) (2). The KCNJ11 (MIM # 600937) and ABCC8 (MIM # 60059) genes are located on the short arm of chromosome 11 (11p15.1) and encode the Kir6.2 subunit and the SU receptor 1 (SUR1) regulatory subunit of the KATP channel, respectively. In the normal pancreatic beta-cell, increased glucose enters the cell via a glucose transporter and is metabolized by the enzyme glucokinase, resulting in increased production of ATP. This causes closure of the KATP channel, which, in turn, depolarizes the cell membrane, activating the influx of calcium through voltage-gated calcium channels that subsequently allows exocytosis of insulin granules. Activating KCNJ11 and ABCC8 mutations cause the KATP channels to remain inappropriately open even in the presence of hyperglycemia. Without channel closure, the cell membrane is not able to depolarize effectively; thus, insulin cannot be released from the beta-cell (3).

In the clinical setting, insulin is the immediate choice for establishing glycemic control in NDM patients because it will be effective in all cases where an insulin deficit is involved. If a diagnosis of diabetes is made before 6 months of age and genetic screening is undertaken, the identification of mutations in KCNJ11 or ABCC8 provides an alternative therapeutic strategy. This is because if NDM results from overactive KATP channels, closing these channels is a key step to suppress insulin release, and sulfonylureas (SU) are well-studied KATP channel inhibitors (4). Moreover, SU have proven to be an effective treatment for individuals with NDM resulting from KCNJ11 or ABCC8 mutations (5, 6).

In 2020, De Franco et al. (7) collected and summarized a total of 748 ABCC8 and 205 KCNJ11 pathogenic and likely pathogenic mutations associated with congenital hyperinsulinism and NDM from various countries. In the present study, we report KCNJ11/ABCC8 pathogenic mutations in Vietnamese patients with NDM diagnosed at Vietnam National Children’s Hospital between May 2008 and May 2021, and the outcomes of SU therapy transfer.

RESEARCH DESIGN AND METHODS

Study Design and Individuals

The patients were diagnosed with NDM and were admitted to Vietnam National Children’s Hospital from May 2008 to May 2021. Inclusion criteria were hyperglycemia onset before 6 months of age and fasting blood glucose ≥126 mg/dl (7.0 mmol/L). Fasting was defined as no caloric intake for at least 4 h in children aged 0–1 years or random plasma glucose concentration ≥11.1 mmol/L (200 mg/dl). Hyperglycemia lasting at least 2 weeks required insulin for treatment. All patients with KCNJ11 or ABCC8 mutations and their parents agreed to participate in the study. Exclusion criteria were hyperglycemia due to glucose infusion, infection, stress, drugs, and other factors.

Data Collection and Biochemical Analyses

Clinical phenotype and biochemical tests were performed at Vietnam National Children’s Hospital. Data included pedigree, sex, date of birth, gestational age, birth weight, date of diabetes
diagnosis, natural history, and examination at diagnosis such as weight, height, and symptoms of diabetic ketoacidosis (DKA) including tachypnea, dehydration, lethargy, coma, and other symptoms such as convulsion. Blood glucose and HbA1C were measured by the automated Beckman Coulter AU2700/AU680 system. The specimen was collected in the early morning at schedule visits. Hexokinase technology with ORS 6221 reagent of OLYMPUS and ORS6192 reagent were used for blood glucose and HbA1c testing, respectively. Insulin and C-peptide were measured using immunoassay chemiluminescent technology by automated biochemistry Hitachi 704. Arterial blood gas was measured by spectrometry, using GEM primer 300. Capillary glucose levels were measured at home by One Touch Ultrain in all patients five times/day (before breakfast, lunch, dinner, 22 h and 2 h) or whenever there was an abnormality. Continuous glucose monitoring (CGM) over 7 days was monitored by using the Medtronic Ipro™2 Professional. HbA1c was checked at 3-month intervals. Blood glucose level and HbA1c targets were determined according to the International Society of Pediatrics and Adolescent Diabetes (ISPAD) 2018 guideline (8).

**Mutation Analysis**

Mutation analysis was performed at the Exeter genomic laboratory, UK. Blood samples were taken with informed consent obtained from the patients and their parents. Genomic DNA was extracted from peripheral blood using phenol/ chloroform methods at Vietnam National Children’s Hospital.

The single exon of the KCNJ11 gene was amplified in three overlapping fragments, as previously described (9). The ABCC8 gene was analyzed at the same time as KCNJ11. The 39 exons of ABCC8 were amplified in 38 fragments using previously described primers (10). PCR products were sequenced on an ABI 3100 or ABI 3730 (Applied Biosystems, Warrington, UK). Sequences were compared with the reference sequences (KCNJ11, NM_000525.3; ABCC8, NM_001287174.1) using Staden or Mutation Survey or software version 2.61.

The identified mutations were checked in common databases such as dbSNP154 database, ClinVar database, Leiden open variation database (LOVD), human gene mutation database (HGMD), and the genome aggregation database (gnomAD). In silico analysis was performed using Alamut Visual. Pathogenic variants identified after 2018 were classified using the ACMG best practice guidelines (11). Protein visualization was generated using the Proter website (http://wlab.ethz.ch/protter/start/)

**Transfer to Sulfonylureas From Insulin**

For this study, clinicians were provided with two recommended protocols for the transfer to the SU glyburide (also known as glibenclamide) for Kir6.2 and SUR1 patients [see www. diabetesgenes.org and (12)]. One was for a rapid inpatient transfer, where the glyburide dose was increased by 0.2 mg/kg/day every day and the other for a slower outpatient transfer, where the glyburide dose was increased by 0.2 mg/kg/day every week. Both involved the gradual withdrawal of insulin, as the SU was introduced depending on blood glucose levels. These protocols were modified by the treating clinicians (5).

**Statistical Analysis**

Data analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, IL). Data are expressed as frequency (%), mean ± SD, or median and range. Continuous variables were analyzed using ANOVA or Kruskal–Wallis tests. Proportions were compared using Fisher’s exact tests. Statistical significance was defined as p < 0.05 or the corresponding Bonferroni-adjusted p value for multiple comparisons.

**RESULTS**

A total of 70 patients were diagnosed with NDM before 6 months of age. Pathogenic variants were identified in 54 (77%). The most common genetic causes were mutations in ABCC8 (26%) and KCNJ11 (24%). The current study focused on the analysis of the 27 patients with ABCC8/KCNJ11 mutations.

**Clinical Features of NDM Patients With KCNJ11/ABCC8 Mutations**

Of the 27 patients with a K<sub>ATP</sub> channel mutation, 14 had a mutation in ABCC8, and 13 had a KCNJ11 mutations. Clinical characteristics of these patients are provided in Table 1. Five of the 27 patients had TNDM, which was diagnosed before 3 months of age and onset with severe/moderate DKA. The average birth weight was 2758.3 ± 419.0 g with gestational age of 39.2 ± 1.28 weeks. Fifty-two percent (14/27) of the subjects were small for gestational age with a birth weight below the third centile. The average onset age in 27 cases was 60 ± 36.4 days, of which 85% were diagnosed before 3 months of age. At diagnosis, biochemical studies showed blood glucose, 36.2 ± 12.3 mmol/L; HbA1C, 7.16 ± 0.16% (normal range, 4 – 6.5%); HCO<sub>3</sub>, 7.9 ± 7.4 mmol/L; BE, −17.9 ± 9.1 mmol/L; and C-peptide, median of 0.09 (range, 0 – 1.61 nmol/L; normal range, 0.26 – 0.62). Twenty-three patients (85.2%) presented with DKA. Nine patients (33.3%) had a convulsion at diagnosis.

**Molecular Genetic Analysis**

A total of 21 pathogenic variants were identified, including 13 ABCC8 and 8 KCNJ11 mutations (Table 2 and Figure 1). These mutations were predicted to be disease causing by the Mutation Taster tool and evaluated as pathogenic variants in the ClinVar database. Four mutations, namely, ABCC8 (p.E747X), ABCC8 (p.R1183W), KCNJ11 (p.R201H), and KCNJ11 (p.R201H), were found in multiple patients. Except for the ABCC8 (p.E747X) mutation, which was homozygous in patient 4 (13), the remaining mutations were heterozygous.

Twelve patients inherited mutations from their parents, while in 14 patients, the mutation had arisen de novo. Patient 33 inherited the ABCC8 (p.R598Q) mutation from his mother and also had a de novo mutation ABCC8 (p.R826W). Three patients 5, 14, and 33 had compound heterozygous mutations in ABCC8. Among 21 pathogenic mutations, four were unpublished, including KCNJ11 (p.S331P), ABCC8 (p.E1141G), and ABCC8 (p.A1153G) (Table 2).
Transfer to Sulfonylureas

Of the 27 patients with mutations in KCNJ11 or ABCC8, 26 were successfully transferred from insulin to SU treatment. Patient 24 remitted after 5 months of insulin treatment and before transfer to SU. Glycemic fluctuations reduced when the patients were on SU treatment as compared with insulin treatment (Figure 2A, p = 0.000001). The mean HbA1C level dropped from 7.75% ± 3.60% on insulin treatment (13.3 ± 25 months) to 5.69% ± 1.02% on SU treatment (86.0 ± 56.9 months, p = 0.0000038) (Figure 2B). While on insulin treatment, there was one case of DKA and one case with convulsion due to severe hypoglycemia. After transfer to SU (86.0 ± 56.9 months), there were no further episodes of severe hypoglycemia or DKA. Patient 4 with a homozygous nonsense mutation in ABCC8 p.(E747X) is currently 16 years of age and can walk, speak in short sentences, and understand and answer some simple questions. When patient 4 was treated with insulin, HbA1C was 8.5% ± 0.4%, and blood glucose ranged from 4 to 20 mmol/L; however, HbA1C decreased to 5.3% ± 0.2%, and blood glucose fluctuation ranged from 5 to 10 mmol/L when he was treated with SU.

Two TNDM patients with KCNJ11 mutations (p.(R50Q) and p.(E292K)) and three TNDM patients with ABCC8 mutations (two patients with p.(R1183W) and one patient with p.(E1141G)) remitted at 6, 50, 6, 14, and 24 months of ages, respectively (Table 2).

Treatment requirements (insulin or SU dose) did not show significant differences between the patients with KCNJ11 mutations and those with ABCC8 mutations (data not shown).

Side Effects

None of the patients reported side effects during SU treatment such as diarrhea, nausea, or vomiting. Renal and liver function tests of all patients were checked every 6–12 months; all were within the normal range. No severe hypoglycemic episodes were reported while on SU treatment, and no other side effects were noted.
**TABLE 2 | Molecular analyses of 27 Vietnamese patients with NDM.**

| Gene | cDNA change | Protein change | Zygosity | Inheritance | dbSNP | ClinVar | Reference | Type (Patient No.) | Treatment |
|------|-------------|----------------|----------|-------------|-------|---------|-----------|-------------------|-----------|
| ABC08 c.382G>A | p.E128K | Het | Maternal | rs781617345 | RC0001058712 | Pathogenic (13) | PNDM (5) | Ins—SU |
| ABC08 c.1303T>C | p.C435R | Het | Paternal | rs1344172059 | VCO06523361.1 | Pathogenic (14) | PNDM (23) | Ins—SU |
| ABC08 c.1793G>A | p.R588Q | Het | Maternal | – | – | – | – | – |
| ABC08 c.2239G>T | p.E747X | Het (4) | Paternal and maternal (4) | – | – | – | – | – |
| ABC08 c.2476C>T | p.R826W | Het | De novo | – | Pathogenic (15) | PNDM (33) | Ins—SU |
| ABC08 c.2497G>A | p.G833S | Het | De novo | – | Pathogenic (16) | PNDM (40) | Ins—SU |
| ABC08 c.3403-1G>A | Splicing | Het | – | rs76684889 | VCO066370935.5 | Likely pathogenic (17) | PNDM (14) | Ins—SU |
| ABC08 c.3422A>G | p.E1141G | Het | Paternal | – | Pathogenic | unpublished | PNDM (24) | Ins—SU |
| ABC08 c.3458C>G | p.A1153G | Het | Maternal | – | Pathogenic | unpublished | TNDM (36) | Remission 24 months |
| ABC08 c.3547C>T | p.P1199L | Het | – | rs79045209 | VCO06210076.2 | Pathogenic (18) | PNDM (13) | TNNDM (24, 27) |
| ABC08 c.3596C>T | p.R1183W | Het | De novo | – | Pathogenic | unpublished | PNDM (12, 48, 49) | Ins—SU |
| ABC08 c.4139G>A | p.R1380H | Het | Maternal | – | Pathogenic (19) | PNDM (25) | Ins—SU |
| ABC08 c.4519G>C | p.E1507Q | Het | Paternal | – | Pathogenic | unpublished (20) | PNDM (32) | Ins—SU |
| KCNJ11 c.1490G>A | p.R50Q | Het | De novo | rs80356611 | VCO06638311.1 | Pathogenic (21) | PNDM (14) | Ins—SU |
| KCNJ11 c.157G>A | p.G53S | Het | De novo | rs80356613 | VCO0668861.1 | Pathogenic (22) | PNDM (30) | Remission 6 months |
| KCNJ11 c.553A>C | p.K185Q | Het | De novo | – | Pathogenic | unpublished | PNDM (23) | Ins—SU |
| KCNJ11 c.601C>T | p.R201C | Het | Maternal (2) | rs80356625 | VCO0668866.3 | Pathogenic (24) | PNDM (28) | Ins—SU |
| KCNJ11 c.602G>A | p.R201H | Het | De novo | rs80356624 | VCO0668866.4 | Pathogenic (25) | PNDM (1, 28, 37) | Ins—SU |
| KCNJ11 c.685G>A | p.E229K | Het | De novo | rs587783673 | VCO06146117 | Pathogenic | unpublished | TNNDM (16) | Ins—SU |
| KCNJ11 c.857A>G | p.E292G | Het | Maternal | – | Pathogenic | unpublished | PNDM (15) | Remission 50 months |
| KCNJ11 c.991T>C | p.S331P | Het | Paternal | – | Pathogenic | Unpublished | PNDM (35) | Ins—SU |

Het, heterozygous; Hom, homozygous; PNDM, permanent neonatal diabetes mellitus; TNDM, transient neonatal diabetes mellitus; Ins, insulin; SU, sulfonylureas.

**DISCUSSION**

In our comprehensive mutation analysis of a large cohort of 70 patients with NDM enrolled at Vietnam National Children's Hospital, we identified gene mutations in 54 cases (77%). In these 54 patients with a confirmed genetic diagnosis, mutations in the K<sub>ATP</sub> channel genes was the most common cause of NDM with a rate of 50%. Overall, 51% had low birth weight (below third percentile), 23 (85%) were diagnosed before 3 months of age, and 23 (85%) presented with DKA. Twenty-six patients were successfully transferred from insulin to SU therapy, and glycemic control subsequently improved.

The mutation rate of 77% in our study was lower than that in Ukrainian (12) and Chinese studies but similar to the University of Chicago Monogenic Diabetes Registry \(n = 88\) (27). In contrast, Russo et al. (28) found the most common genes causing NDM diagnosed during the first 6 months of life were KCNJ11 and ABCC8 (70%), but mutations in KCNJ11 were more common than ABCC8. These differences may be due to ethnicity, race, or size of the study cohort. In our study, we only investigated patients with NDM onset before 6 months of age who have mutations in the genes encoding the K<sub>ATP</sub> channel.

The rate of patients with low birth weight (under third percentile) was 52%, which is similar to the results reported by Besser et al. (29). In the study reported by Russo et al. (28), the patients diagnosed with PNDM before 6 months of age but without mutations in KCNJ11, ABCC8, or INS had higher birth weight than those with mutations in these genes.

The majority of our cohort (85%) presented in DKA. Similarly, Letourneau et al. (27) reported that 66% of patients with neonatal diabetes (and 79% of patients with KCNJ11/ABCC8 mutations) presented with DKA. While this is slightly...
less than observed in our cohort, there may have been a delay in
diagnosis in some of our cases, which is reflected in the later age
of diagnosis. This delay may be related to the challenge of
diagnosing diabetes in infants who cannot communicate
symptoms and in whom polydipsia and polyuria may not be
readily apparent—indeed, this could even be reassuring
to clinicians.

The numbers of Vietnamese patients with ABCC8 and
KCNJ11 mutations were similar (14 versus 13), which is
consistent with the findings in the Indian population (30). In
contrast, Hashimoto et al. (31) reported more patients with
KCNJ11 than ABCC8 mutations (16 versus 8). Recurrent
mutations KCNJ11 p.(R201C), KCNJ11 p.(R201H), and ABCC8
p.(R1183W) in Vietnamese patients have been reported in NDM
patients in Jordan (32), India (30), Ukraine (12), The United
States (33), Japan (34), and China (35). Therefore, these
mutations may be considered as common mutations in
different ethnic groups. The high rate of de novo mutations,
which can arise either during gametogenesis or embryogenesis in
NDM patients (15/27), identified in the current study, is
consistent with the findings of Edghill et al. (36).

None of the 12 mutations identified in ABCC8 are located in
the glibenclamide binding pocket of SUR1. However, four
mutations [ABCC8 p.(C435R) and p.(R598Q), p.(E1141G), and
p.(A1153G)] are located in the transmembrane domains TMD1
and TMD2, respectively (Figure 1A). Interestingly, p.(C435R)
was previously reported in two TNDM patients (15, 37), while
patient 23 in our study had PNDM after treatment on SU 72
months (Table 1). Three ABCC8 mutations p.(R598Q),
p.(E1141G), and p.(A1153G) are unpublished (Table 2).
Patient 33 had a compound heterozygous ABCC8 mutation,
p.(R598Q) and p.(R826W) (Tables 1, 2).

Eight KCNJ11 mutations identified in this study are located in
the N- and C-terminal regions (Figure 1B), which form the ATP
binding pocket of Kir6.2 (38). The p.(R50Q), p.(K185Q),
p.(R201C), and p.(R201H) mutations may reduce the response
of the channel to ATP, as they lie at the main binding pocket (23,
24, 39). The KCNJ11 p.R50Q mutation has been reported to

![FIGURE 1](image-url)
cause both TNDM (34) and PNDM (9); therefore, patients with this mutation can present with different phenotypes as suggested by Suzuki et al. (34). The KCNJ11 p.(K185Q) mutation was identified in PNDM patient 28 (Table 1). This mutation have been reported previously in a 3-year-old girl with PNDM, and after treatment with insulin, her HbA1C was between 6.8% and 7.8% (23). In our study, patient 28 was also treated with insulin; however, she was transferred to SU, resulting in an HbA1C of 7.6% and blood glucose level of 7.9 mmol/L. Functional studies indicated that this mutation reduced ATP binding to Kir6.2, resulting in a reduction in ATP sensitivity of the K<sub>ATP</sub> channel, leading to PNDM in the patient (23).

SU therapy is effective in the treatment of hyperglycemia in patients with NDM who have a mutation in KCNJ11 or ABCC8. Up to 90–95% of patients with NDM caused by mutations in these genes can cease insulin therapy after initiation of SU therapy (3, 5). In our study, the rate was higher, at 96.3%. The one remaining case (3.7%) had a remission of diabetes at 5 months of age before transfer to SU could be initiated. SU acts on the K<sub>ATP</sub> channel to promote closure, allowing insulin to be released from the beta cells. Since SU therapy increases insulin release, there is a risk for hypoglycemia to occur. However, there was no severe hypoglycemia reported in our study. Excellent glycemic control was maintained after commencing SU therapy (Figure 2A), which is similar to other reports (6, 40). In the study of Bowman et al. (40), there were no reports of severe hypoglycemia in 809 patient-year follow up for the whole KCNJ11 cohort, and 93% of the participants remained on SU therapy for the 10-year duration; however, a high rate (14%) of patients presented with mild and transient side effects of SU such as diarrhea, nausea, weight loss due to reduced appetite, and abdominal pain (40). Whereas, in our study, there was no side effects of SU, continued follow-up will be required to determine the long-term outcome of SU therapy in this group of patients.

Interestingly, two patients (patients 2 and 4) with DEND syndrome were successfully transferred to SU treatment, which is consistent with previous studies (41, 42).

In conclusion, we found that all patients in our cohort with ABCC8 and KCNJ11 mutations could be successfully treated with oral SU treatment even if they had previously been treated with insulin. It is essential to perform rapid genetic testing for ABCC8/KCNJ11 in any patient diagnosed with diabetes before 6 months of age, particularly given issues regarding access to and cost of insulin in some populations. On SU treatment, we observed that this therapy is safe in the short term for patients with K<sub>ATP</sub> channel NDM.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Vietnam National Children’s Hospital IRB#1, 18/879 Lathanh, Dongda, Hanoi, Vietnam. Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

CN, TD, SE, and VD conceptualized, designed the study, and wrote and reviewed the manuscript. VD, CN, BT, and NNK...
provided patients’ clinical information, and MC reviewed/edit ed the manuscript. EF, SF, JH, and NNL analyzed data and wrote and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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