

Neonatal Diabetes in a Singapore Children's Hospital: Molecular Diagnoses of Four Cases

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Abstract

Introduction: Neonatal diabetes (ND) presents below 6 months of age, and is caused by a genetic defect in glucose homeostasis. Molecular genetic diagnosis can identify the exact molecular aetiology and guide clinical management. The objective of this study was to identify ND among children with diabetes in a major children's hospital in Singapore and to characterise their molecular and clinical features. **Materials and Methods:** The study identified all infants below 6 months of age who presented with diabetes to our centre from January 2008 to December 2010. It also reviewed diabetes database comprising 662 patients, to identify those who were diagnosed with diabetes below 6 months of age between January 1997 and December 2010. Four patients (3 females and 1 male) were identified and their molecular aetiology was investigated. **Results:** A molecular aetiology was found in each of the 4 patients identified. Two patients (Patient 1 and 2) had permanent ND (PND). Patient 1 who has *KCNJ11*/R201H mutation was successfully switched from insulin to oral glibenclamide and Patient 2 who has a novel mutation *INS*/C109Y continues to be treated with insulin. Two patients (Patient 3 and 4) had transient ND (TND) and no longer require insulin or any other intervention to maintain normoglycaemia. Patient 3 has a novel mutation *ABCC8*/F1182S and Patient 4 has a paternal duplication on chromosome 6q24. **Conclusion:** This study identified 4 cases of ND in our cohort of diabetes children and confirmed their molecular diagnosis. Molecular genetic testing for these children led to accurate diagnosis and appropriate management.

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Introduction

Neonatal diabetes (ND) is a rare form of diabetes affecting 1 in 100,000 to 300,000 live births. Its exact incidence is unclear, and it was only in recent years that ND has been recognised as an entity distinct in aetiology from type 1 diabetes mellitus (T1DM). Unlike patients with T1DM, patients who develop diabetes before 6 months of age do not have serological evidence of auto-immunity but instead have been found to carry mutations in one of a number of possible genes implicated in ND.¹⁻¹³ Known molecular aetiologies of ND now include mutations in the genes *KCNJ11* and *ABCC8* encoding the 2 protein subunits Kir6.2 and SUR1 of the ATP-sensitive potassium (K_{ATP}) channels respectively in insulin secreting beta-cells and mutations in

the insulin gene (*INS*), encoding insulin itself. Depending on the molecular aetiology, some children can be weaned off insulin therapy and achieve normal glucose control with oral sulphonylureas.¹⁴⁻¹⁶

ND may be transient (TND), resolving within a median of 3 months, or permanent (PND), in which case treatment is required for life. Each subgroup accounts for about 50% of the patients. Approximately 70% of TND is caused by the overexpression of certain genes in a region of the long (q) arm of chromosome 6 called 6q24,¹⁷ while about half of infants with PND have mutations in the *KCNJ11* gene. Infants with TND typically present in the first 4 weeks of life while those with PND develop diabetes later from 2 to 6 months of age.

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This knowledge of ND has important implications in patient management. By establishing the molecular aetiology of an infant with ND, it may be possible to predict the likelihood of insulin-dependency or of substituting insulin therapy with oral sulphonylureas. The molecular diagnosis is also important in genetic counseling. We conducted this study to identify children with ND among new and existing cases in our institution.

Materials and Methods

Patients

This study was conducted at KK Women's and Children's Hospital (KKH), an 830-bed tertiary hospital in Singapore. Only Singapore-born children were included in the study. Case-finding was conducted in the following ways:

- All children presenting with very early onset diabetes (6 months old and younger) who required insulin therapy from 1 January 2008 to 31 December 2010 were identified. Each child had the following investigations performed as part of the workup for newly diagnosed diabetes — blood gases, blood/urine for ketones, C-peptide and serum insulin levels, glutamic acid decarboxylase (GAD) and islet cell antibody (ICA) levels. Blood was sent for genetic testing.
- The hospital maintains a diabetes database which records all cases of diabetes between 0 to 16 years, with information on gender, date of birth, date of diagnosis, type of presentation and diabetes type. The database comprised 662 patients who sought treatment in our hospital between 1 January 1997 and 31 December 2010 at the time of this study. A review of the database was performed to identify those who were diagnosed with diabetes below 6 months of age. Their clinical data was extracted and parent's consent was obtained for genetic testing.

Mutational Analysis

Molecular genetic analysis was performed at the molecular genetics laboratory in Exeter, United Kingdom. Methylation-specific polymerase chain reaction (MSP) testing for chromosome 6q24-related TND was performed at the Wessex Regional Genetics Laboratory in Salisbury, United Kingdom. The methods used have been previously reported.¹³

Ethical Committee Approval

The study was approved by the hospital's Institutional Review Board. Informed consent was obtained from patients/parents for molecular testing.

Results

We identified 4 cases out of a total cohort of 662 patients over 13 years (1 January 1997 to 31 December 2010). Three patients were diagnosed over 2 years (1 January 2008 to 31 December 2009) and one was diagnosed retrospectively from the database.

Their clinical characteristics are presented in Table 1 and family pedigrees charts are shown in Figure 1.

- Patient 1 (diagnosed in June 2008), Patient 3 (diagnosed in November 2008) and Patient 4 (diagnosed in July 2009), were identified at time of diagnosis, while Patient 2 (diagnosed in 1994), was identified from the diabetes database review.
- Two of the children had PND — Patient 1 (onset 3 months old, converted to oral glibenclamide at age 6 months) and Patient 2 (onset 6 months old, still on insulin therapy 18 years on).
- The other 2 children had TND — Patient 3 (onset 3 weeks old, weaned off insulin at age 6 months) and Patient 4 (onset 3 days old, weaned off insulin at age 2 months). The 2 cases of TND presented relatively earlier within one month of life, required initial insulin treatment and later were successfully weaned off insulin.
- Two children had novel mutations — Patient 2 (C109Y in exon 3 of the *INS* gene) and Patient 3 (F1182S in exon 28 of the *ABCC8* gene). The other two children had common well-described genetic diagnoses — Patient 1 (R201H of the *KCNJ11* gene) and Patient 4 (paternal duplication of chromosome 6q24).

Patient 1

Patient 1 was 3 months old when she presented with infantile pyrexia and vomiting. Her full septic workup was negative. In view of hyperglycaemia, metabolic acidosis and ketonuria, a diagnosis of diabetic ketoacidosis (DKA) was made and intravenous insulin was started. She responded well to the treatment and her insulin was converted to subcutaneous insulatard and novorapid. In view of very early onset of presentation, a probable diagnosis of monogenic diabetes was considered and molecular genetic analysis confirmed a heterozygous missense mutation R201H in the *KCNJ11* gene. She has been successfully weaned off insulin and has excellent blood glucose control on glibenclamide twice daily.

Patient 2

Patient 2 was identified from the diabetes database. She was initially diagnosed with T1DM at 6 months of age in

Table 1. Clinical Characteristics of Singaporean Children Identified with Neonatal Diabetes

Clinical Characteristics	Patient 1	Patient 2	Patient 3	Patient 4
Sex	Female	Female	Male	Female
Race	Chinese	Chinese	Chinese	Chinese
At birth				
Weight (grams)	2145	NA	2800	2196
Gestation (weeks)	38	NA	38	37
At presentation				
Age	3 months	6 months	3 weeks	3 days
Presentation	Ketoacidosis	Ketoacidosis	Hyperglycaemia	Hyperglycaemia
Glucose (mmol/L)	20.0	NA	25.2	17.4
HbA1c (%)	14.5	NA	5	Not tested
Autoantibody	Negative	NA	Negative	Negative
C-peptide (ng/mL)	0.7	NA	0.5	0.4
Treatment initiated	Insulin injections	Insulin injections	Insulin injections	Insulin injections
Mutation	<i>KCNJ11</i> /R201H	<i>INS</i> /C109Y	<i>ABCC8</i> /F1182S	<i>Pat. Dupl. Chrom. 6q24</i>
Current status				
Age	4.5 years	18 years	3.5 years	3 years
Weight (kg)	17.4	61.1	7.7 (at 9 months)	18.6
Type of treatment	Glibenclamide	Insulin injections	Weaned off insulin	Weaned off insulin
HbA1C (%)	6.5	8.2	5.9	5.1
C-peptide (ng/mL)	2.2	0.3	0.6	Not tested
Type of ND	Permanent	Permanent	Transient	Transient

HbA1c: Glycated haemoglobin; NA: Not available; ND: Neonatal diabetes; *Pat. Dupl. Chrom. 6q24*: Paternal duplication of chromosome 6q24

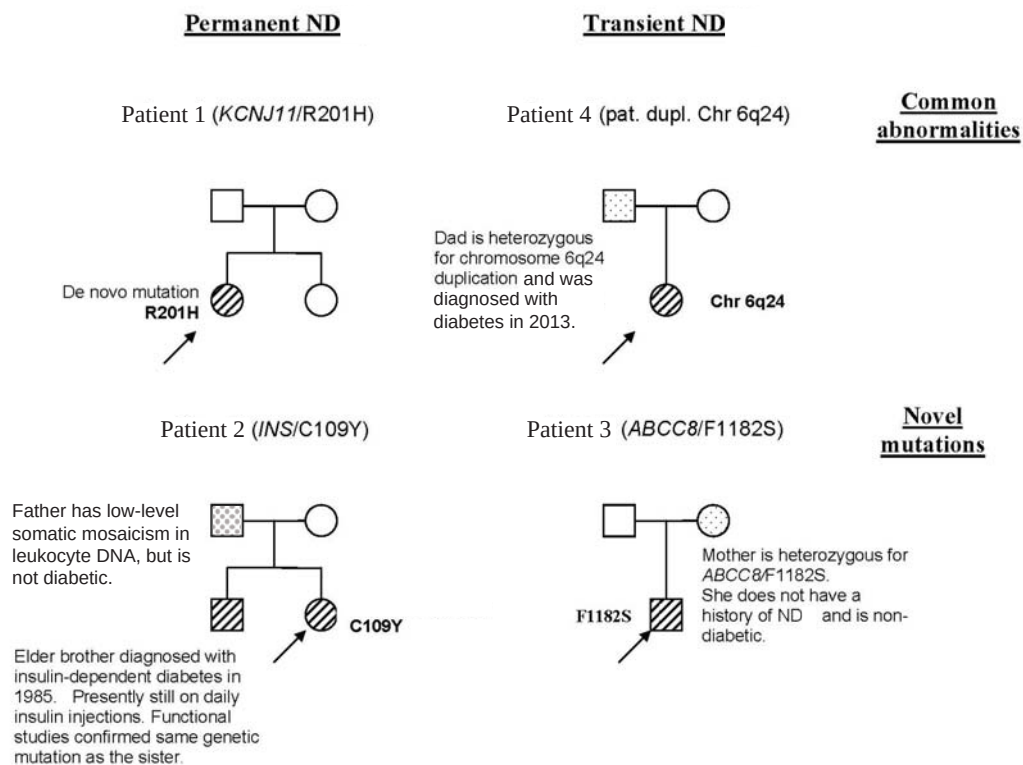


Fig. 1. Pedigree charts of Singaporean children identified with neonatal diabetes.

1994. She was found to have a novel mutation C109Y in exon 3 of the *INS* gene, which is consistent with a phenotype of PND. She has a 25-year-old brother also with insulin-dependent diabetes from infancy. Following the diagnosis of ND in Patient 2, genetic studies have been done on her brother confirming the same gene mutation. Father has low-level somatic mosaicism in leukocyte DNA, but is not diabetic.¹⁸

Patient 3

Patient 3 presented at 3 weeks of age with prolonged neonatal jaundice, and on further evaluation was found to have glycosuria and hyperglycaemia with no ketoacidosis. Molecular genetic analysis confirmed a heterozygous novel missense mutation, F1182S, in exon 28 of the *ABCC8* gene, which is consistent with a diagnosis of TND. The heterozygous mutation was inherited from his mother, who was non-diabetic and did not have a history of neonatal hyperglycaemia.

Patient 4

Patient 4 presented at 3 days of life with hyperglycaemia with no ketoacidosis. She was a term small for gestational age (SGA) baby with normal clinical examination except for macroglossia. Molecular genetic analysis confirmed a diagnosis of TND caused by paternal duplication of chromosome 6q24. Hypermethylation at this locus was present in patient's father, indicating that the father carries this duplication on his maternal chromosome.

Discussion

We report 4 cases of ND with variable clinical and molecular characteristics, identified by systematically examining new and existing patients with very early infancy-onset diabetes in our institution. Secondly, our study highlights the clinical characteristics of 2 children with novel missense mutations involving the *ABCC8*/F1182S and *INS*/C109Y genes, which expands the catalogue of known mutations that may cause TND and PND. Thirdly, our results illustrate the importance of accurate diagnosis as it has implications for treatment and prognosis.

The first case of PND (Patient 1) with missense mutation R201H in the *KCNJ11* gene represents the most common PND causing mutation. These mutations are familial, or more commonly, sporadic in nature.¹⁹ This heterozygous gain of function mutation in the *KCNJ11* gene encoding the Kir6.2 subunit results in ATP-insensitive channels that will respond to sulphonylurea with channel closure and insulin release. Clinical application of these drugs in ND with these mutations affecting the ATP sensitivity of the channel is reflected in this case. A successful introduction

of glibenclamide saw a rapid weaning in her insulin dose besides producing good glycaemic control. Recently, the efficacy of therapeutic amendment from insulin to sulphonylurea-based treatment was assessed in cases of neonates with diabetes owing to Kir6.2 mutations.¹⁵ Ninety percent of subjects had a successful therapeutic response to an oral sulphonylurea.¹⁵ Sulphonylureas were not only effective in achieving an acceptable level of glycated hemoglobin, but they also sustained the euglycemic response in patients with Kir6.2 mutations.¹⁵ Independently, other studies have also established similar success of sulphonylureas in patients with ND owing to Kir6.2 mutations.^{19,20} Oral sulphonylurea treatment thus forms an attractive alternative to lifelong exogenous injections of insulin in these patients.

The second case of PND (Patient 2) has novel mutation C109Y in exon 3 of the *INS* gene. Patient 2 was diagnosed with T1DM 18 years ago when she was 6 months old — we have reclassified her diagnosis as PND following genetic confirmation. *INS* mutations have recently been described as the second commonest cause of PND and should be assessed in neonatal and early childhood “type 1-like insulin requiring diabetes”, particularly when autoimmune markers are absent.^{7,12,13} Most mutations are located within the A or B insulin chains which disrupt the folding of the pro-insulin molecule, resulting in a misfolded protein or retention of the protein in the endoplasmic reticulum (ER), causing ER stress and beta-cell apoptosis. Disulfide bonds are crucial for pro-insulin folding in the ER and 60% of the mutations either abolish or disrupt disulfide bridge formation by substitution or addition of a cysteine residue.^{21–23} The majority of patients with *INS* gene mutations are sporadic cases that result from de novo mutations, while some cases show autosomal dominant inheritance, which was implicated in this case. The clinical characteristics of patients with *INS*, *KCNJ11*, or *ABCC8* gene mutations were compared in the large Exeter cohort. No difference in sex, birth weight, or gestational age was observed between the 3 groups, but patients with an *INS* mutation were diagnosed later (median 11 weeks) than those with K_{ATP} channel mutations. Coincidentally, this patient did present at later age among the 4 cases of ND. Also, no neuropsychological or neuromotor dysfunction were present in children with an *INS* mutation in contrast to the children with a K_{ATP} channel mutations. Our patient is now 18 years old, both she and her 25-year-old biological brother continue to remain on subcutaneous insulin therapy. In summary, these mutations are the second most common cause of PND, and *INS* gene screening should be recommended in all children diagnosed with diabetes in the first year of life.

Our first case of TND (Patient 3) has a novel mutation *ABCC8*/F1182S and has not been previously reported. The *ABCC8*/F1182S mutation may result in TND by affecting

the SUR1 subunit of the K_{ATP} channel.²⁴ Although the molecular mechanisms of *ABCC8* and *KCNJ11* mutations are distinct, the cellular mechanism reducing insulin release is common to both. We speculate that the *ABCC8*/F1182S mutation was pathogenic because the phenylalanine at position 1182 is conserved across species. A different mutation (F1182L) has been identified in another patient with transient neonatal diabetes and neither mutation has been detected in 500 patients with the opposite phenotype of hyperinsulinism.²² In addition, the adjacent residue, R1183, is frequently mutated in patients with TND. The F1182S mutation was inherited from patient's unaffected mother. This case was challenging as with a mutation possible to be TND, it was subsequently proven when the patient was successfully weaned off insulin with no other treatment. As with *KCNJ11*, activating mutations in *ABCC8* encoding the SUR1 regulatory subunit of the K_{ATP} channels cause both permanent and transient neonatal diabetes.⁵ Mutations of *KCNJ11* are typically associated with PND, whereas most mutations of *ABCC8* are associated with TND, perhaps reflecting a less severe form of diabetes.

Our second case of TND (Patient 4) has a common molecular cause — paternal duplication of chromosome 6q24. She presented at day 3 of life with hyperglycaemia and required insulin to achieve normoglycaemia. Titrating and delivering small doses of insulin with feeds was a challenging issue. She was monitored closely and the insulin was weaned off completely with good glycaemic control. Chromosome 6q24-related TND begins in the first 6 weeks of life and resolves by 18 months of age. Intermittent episodes of hyperglycaemia may occur in childhood, particularly during intercurrent illnesses. Recurrence in adolescence is more akin to type 2 DM. Women are also at risk of relapse during pregnancy and present with gestational diabetes. The cardinal features are intrauterine growth retardation, dehydration and hyperglycaemia and absence of ketoacidosis. Macroglossia and umbilical hernias are often present. These features were seen in this case which helped to clinch the diagnosis in addition to molecular testing. This form of TND is caused by overexpression of 2 genes, *PLAGL1*(ZAC) and *HYMAI*, found within an imprinted region on chromosome 6q24. Molecular diagnosis is based on identifying an additional paternal copy of *PLAGL1*(ZAC)/*HYMAI* or loss of maternal methylation within the differentially methylated region (DMR) of the promoter of *PLAGL1*(ZAC)/*HYMAI*. Three known mechanisms cause TND: paternal uniparental disomy of chromosome 6, duplication of 6q24 on the paternal allele, and a 6q24 methylation defect.²⁵ The risk to siblings and offspring of a proband of having TND or developing diabetes later in life depends upon genetic mechanism in the family. TND caused by paternal uniparental isodisomy of

chromosome 6 is typically a de novo, non-recurrent event. Duplication of 6q24 (paternal) can be de novo, inherited in an autosomal dominant manner, or inherited as a part of a complex chromosome rearrangement. It may recur in siblings and offspring of a proband if the duplication is inherited from the father. All reported instances of individuals with a methylation defect have been simplex cases (i.e. a single occurrence in the family). However, it is possible that the offspring of individual with TND caused by methylation defect may be at risk of developing TND or diabetes in later life if his/her affected parent is female. Prenatal diagnosis of TND is possible in those individuals at risk for a structural chromosome abnormality.

Conclusion

Our study is the first report in Singapore describing a series of 4 confirmed cases of neonatal diabetes representing distinct clinical and genetic variability spectrum. Two novel mutations expand the catalogue of mutations that may cause ND. Our study highlights the need for medical practitioners to consider molecular testing for all patients who present with diabetes below 6 months of age as this will facilitate accurate diagnosis and guide appropriate therapy.

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