Neonate with permanent neonatal diabetes mellitus; a very rare homozygous missense mutation in the glucokinase gene

Abstract
Permanent neonatal diabetes mellitus (PNDM) appears within the first six months of life. It represents 50% of cases of neonatal diabetes. Affected neonates present with hyperglycemia, intrauterine growth retardation, polyuria, dehydration, and failure to thrive.

PNDM requires lifelong insulin treatment leading to catch-up growth. The course varies according to the underlying genetic cause. GCK mutations cause 4% of PNDM that present from the first day of life. Homozygous mutations in the glucokinase gene (GCK) causes PNDM while heterozygous GCK mutations cause maturity-onset diabetes of the young (MODY).

We report a male neonate of Arab ancestry who was delivered by cesarean section at 36 weeks gestation. He had intrauterine growth retardation with birth weight 1.720kg.

He presented in the first few days of life with hyperglycemia (Serum glucose > 250 mg/dl (13.8 mmol/l)). On 10th day of life insulin treatment was started. The initial insulin requirement ranged from 0.05-0.3 units per Kg body weight per day. Blood glucose control was difficult initially to be achieved with difficulties in preparing such small doses of insulin, and large variations in blood glucose concentrations but no ketosis.

His investigations revealed low serum insulin and C-peptide. The glycated hemoglobin (HBA1c) after birth was 4.8% which raised up to 6.1% at age of 2 months. Whole exome sequence revealed a very rare novel homozygous missense pathogenic variant c.667G>A (p.Gly223Ser) in the glucokinase gene. His parents are first cousins. His father has impaired fasting hyperglycemia that was treated with diet control. His mother has systemic lupus, primary hypothyroidism, protein S and anti-thrombin deficiency. She had gestational diabetes in all her pregnancies. Very strong family history of type 2 diabetes mellitus and MODY in his first-degree relatives.

To date, this missense p.Gly223Ser mutation has only been identified in heterozygous state in patients with GCK-MODY and type 2 diabetes, this is the first study to identify the homozygous form of this novel mutation in PNDM.

Abbreviations: MCT, medium chain triglyceride; IUGR, intrauterine growth restriction; USS, Ultrasound; SLE, systemic lupus erythematosus; SC, subcutaneous; NDM, neonatal diabetes mellitus; GCK, glucokinase

Introduction
While the early onset of NDM in the first six months of life rarely occurs as a result of autoimmune disorder, genetic mutations and chromosomal abnormalities are recognizable causes of NDM.1-7

Mutations in KCNJ11 or ABCC8 gene, that encode for subunits of the ATP sensitive potassium channel, are the most commonly encountered mutations in permanent NDM.3 Previous reports showed that permanent NDM may result from mutations in insulin or glucokinase (GCK) genes as well.8,9 Homozygous GCK-related permanent NDM is associated with complete GCK deficiency and severe hyperglycemia.10,11

In this report, we presented a case of permanent NDM due to a very rare, novel homozygous missense mutation in GCK gene.

Case report
A male baby born to a second-degree consanguineous parent at 36 weeks gestation with a low birth weight of 1.720 kg and APGAR score of 9 and 10 at 1 and 5 min respectively. Antenatal ultrasound (USS), showed intrauterine growth restriction (IUGR). Mother was 30 years old, P3+, with multiple autoimmune disorders including hypothyroidism due to autoimmune thyroiditis, systemic lupus erythematosus (SLE), protein S deficiency, and anti-thrombin deficiency. In her previous pregnancies, she was diagnosed with gestational diabetes then she developed a permanent type 2 diabetes mellitus two months before this pregnancy. His father has mild fasting hyperglycemia controlled by diet. Strong family history of type II diabetes mellitus in first degree relatives (one maternal aunt, five maternal uncles, both maternal grandparents, paternal grandfather and four of father’s paternal aunts). His male maternal cousin, who is currently 25 years old, had early onset diabetes during first month of life, controlled by insulin, but no confirmatory genetic test was done.

The baby’s initial examination was normal apart from low birth weight. He underwent a routine blood glucose monitoring for hypoglycemia for the first 24 hours which showed mild hyperglycemia. From day 3 of life, the blood glucose level started rising more and ranged from 180-250 mg/dl (10-13.8 mmol/l). This persistent hyperglycemia raised our index of suspicion of the possibility of neonatal diabetes especially after exclusion of other...
causes of hyperglycemia such as (sepsis and stress) especially in view of the family history. On day 9 of life, baby’s investigations revealed glucosuria without ketonuria. Serum insulin 2.8 UI/ml (4.0-16.0), C-peptide 0.4 ng/ml (1.8-4.7) when serum glucose was 218mg/dl (12mmol/l). The HbA1c was 4.8% on day 5, which raised up 6.1% at age of three months. The diagnosis of NDM (Neonatal Diabetes Mellitus) was raised by day 10 of life and the insulin was started in the form of intermediate acting insulin (Isophan insulin) with initial dose of 0.1 units subcutaneous (SC) daily (0.05iu/kg/day). Whole exome sequence test was sent for the baby and his parents to delineate the underlying genetic cause.

On day 15 of life, blood glucose readings were still high; therefore, the Isophan insulin (0.1 units SC) was given twice daily (0.1iu/kg/day). Later, on day 30 of life, baby was started on multiple daily injections (MDI) by adding short acting insulin (Aspart) 0.1 units with 4th hourly feeds. Insulin doses were frequently increased and adjusted according to blood sugar readings and feeds. Baby was reviewed regularly by dietician to optimize calories intake and ensure normal growth. It was a challenge to achieve the optimum growth with minimizing the hyperglycemia. Baby was started on high caloric feedings with low glucose content, that lead us to use of EBM (expressed mother milk) plus HMF (Human milk fortifiers) or preterm formula. Later, to achieve the high caloric requirements while maintaining the low glucose content, Medium Chain Triglyceride (MCT) oil was introduced with term formula or the breast milk aiming to maintain normal growth.

On day 74 of life, in view of hypoglycemia at the time of Isophane insulin peak action, we changed basal insulin to insulin Glargine 1 unit daily, with Aspart insulin 4th hourly with feeding according to blood glucose level. He was discharged from the hospital at age of 3 months in good general condition, body weight of 4 kg and during follow up in pediatric diabetes clinic, insulin Aspart was changed to Lispro.

Whole exome sequence revealed a very rare homozygous missense pathogenic mutation c.667G>A (p.Gly223Ser) in the GCK (glucokinase) gene. Both parents carried the same mutation in heterozygous state. Currently the baby is twelve months old, on multiple daily injections of SC insulin of Glargine and Lispro. He is thriving well with good blood sugar control and occasional hypoglycemia. His last HbA1c at age of twelve months was 7.1%. He is on continuous glucose monitoring by Dexcom G5 device and planned for insulin pump.

**Discussion**

In this report, we describe a male baby with permanent NDM due to a very rare homozygous missense pathogenic mutation c.667G>A (p.Gly223Ser) in the GCK gene because of heterozygous mutations in both parents. Glycemic control was challenging, patient was treated with multiple daily injections of SC insulin and eventually performed well.

The role of genetic mutations in the development of permanent NDM is well established. GCK is a member of the hexokinase family which produce glucose-6-phosphate. Alteration in GCK level or function is associated with abnormalities in glucose metabolism and insulin secretion. The current published literature showed few permanent NDM cases, with homozygous or compound heterozygous mutations, with decreased GCK activity, however, no common mutations have been identified yet. Bennett and colleagues reported four cases of permanent NDM due to novel homozygous nonsense GCK mutation in exon 3 (Q98X) or missense GCK mutation in exon 7 (G261R), which were previously found in heterozygous state in GCK-MODY and type 2 diabetes only.

Gly223 residue is located in the β-sheet of the large domain hydrophobic core, the missense mutation in the present case resulted in the substitution of glycine with serine which may lead to defect in the GCK structure and activity. Given the homozygous form of the mutation in the reported case, the baby presented with severe hyperglycemia in the first few days of life, which required prompt insulin therapy, and gradual titration of its dose. The baby is currently treated with multiple daily injections of SC insulin. Homozygous GCK mutations were reported to be associated with severe hyperglycemia, and sometimes ketoadiposis, due to complete GCK deficiency, while heterozygous mutations were linked to mild form that rarely require treatment. Interestingly, Borowiec and colleagues reported a permanent NDM case presented with ketoacidosis in the first weeks of life due to heterozygous p.Gly223ser mutation in GCK. The authors attributed this severe form to the coexistence of potential additional genetic mutation or an interaction between different genetic factors.

As mentioned, heterozygous mutations in GCK gene was linked to the development of MODY2, Capuano and colleagues reported 19 heterozygous GCK mutations in 28 children with suspected MODY2. In the present report, both parents carried the same mutation in heterozygous state, and the mother has established type 2 diabetes on insulin and metformin.

We faced many obstacles in order to achieve the glycemic control in our case. Being IUGR neonate with lack of subcutaneous fat, unpredictible insulin subcutaneous absorption with the rapid development of hyperglypemia due to the coexistence of potential additional genetic mutation or an interaction between different genetic factors.

**Acknowledgments**

None.

**Conflict of interest**

The author declares no conflict of interest.

**References**


Neonate with permanent neonatal diabetes mellitus; a very rare homozygous missense mutation in the glucokinase gene