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CASE REPORT

# Permanent Neonatal Diabetes Mellitus Due to a C96Y Heterozygous Mutation in the Insulin Gene. A Case Report

**Anish Ahamed[1], Ambika Gopalakrishnan Unnikrishnan[1], Sanket Sharad Pendsey[1], Sheela Nampoothiri[2], Nisha Bhavani[1], Valliyaparambil Pavithran Praveen[1], Harish Kumar[1], Rohinivilasam Vasukutty Jayakumar[1], Vasantha Nair[1], Sian Ellard[3], Emma L Edghill[3]**

Departments of [1]Endocrinology and [2]Pediatric Genetics, Amrita Institute of Medical Sciences. Cochin, India. [3]Institute of Biomedical and Clinical Science, Peninsula Medical School. Exeter, United Kingdom

## ABSTRACT

**Context** Neonatal diabetes is a rare disorder with an incidence of 1 in 215,000-500,000 live births with 50% of them having permanent neonatal diabetes mellitus.

**Case report** We present a case of permanent neonatal diabetes mellitus due to a C96Y (c.287G>A; p.Cys96Tyr) heterozygous mutation in the insulin (*INS*) gene. Both the patient and his father (who had childhood-onset insulin-requiring diabetes) were found to be carriers of a heterozygous missense mutation C96Y in exon 3 of the *INS* gene. It has been hypothesized that these mutations disrupt the folding of the proinsulin molecule and result in a misfolded protein or retention of the protein in the endoplasmic reticulum, resulting in endoplasmic reticulum stress and beta cell apoptosis. Subjects with this form of diabetes will need lifelong insulin therapy.

**Conclusion** Insulin gene mutations appear to be an important cause of neonatal diabetes worldwide. This is the first report of a case from the Indian subcontinent. It is important to carry out genetic tests for mutations linked to pancreatic beta cell dysfunction in all patients with persistent neonatal diabetes mellitus in order to decide on therapy.

## INTRODUCTION

Neonatal diabetes mellitus is defined as hyperglycemia lasting for more than 2 weeks and occurring within the first month of life [1]. Subjects diagnosed with diabetes in the first 6 months of life are more likely to have monogenic neonatal diabetes rather than type 1 diabetes. A rare entity affecting approximately 1 in 500,000 births, it is categorized into permanent neonatal diabetes mellitus and transient neonatal diabetes mellitus. A definitive diagnosis of transient neonatal diabetes mellitus is only possible retrospectively [2]. At the molecular level, abnormalities of the imprinted region on chromosome 6q24 constitute the most frequent cause of transient neonatal diabetes mellitus. Heterozygous activating mutations in the potassium inwardly-rectifying channel, subfamily J, member 11 (*KCNJ11*) and ATP-binding cassette, sub-family C (CFTR/MRP), member 8 (*ABCC8*) genes which encode the Kir6.2 and SUR1 subunits of the ATP-sensitive potassium ($K_{ATP}$) channels controlling insulin secretion are the commonest causes of permanent neonatal diabetes mellitus and a rarer cause of transient neonatal diabetes mellitus [3, 4]. Apart from these mutations, the other rare genetic causes of transient neonatal diabetes mellitus or

permanent neonatal diabetes mellitus include mutations in HNF1 homeobox B (*HNF1B*), pancreatic and duodenal homeobox 1 (*PDX1*, also known as *IPF1*), pancreas-specific transcription 2 factor, 1a (*PTF1A*), forkhead box P3 (*FOXP3*), eukaryotic translation initiation factor 2-alpha kinase 3 (*EIF2AK3*), GLIS family zinc finger 3 (*GLIS3*) and the glucokinase (hexokinase 4) (*GCK*) genes [2, 4, 5]. Recently, missense mutations in the genes encoding insulin and its precursors, preproinsulin and proinsulin, have also been reported as causes of permanent neonatal diabetes mellitus [6, 7, 8].

We report the case of a 4-month-old boy who was referred to us as having neonatal diabetes mellitus and who was subsequently diagnosed as having permanent neonatal diabetes mellitus due to an insulin gene mutation.

## CASE REPORT

A 4-month-old boy, born to non-consanguineous parents, was evaluated for hyperglycemia. The antenatal period was normal and there was no history of gestational diabetes. He was delivered at term by a normal vaginal delivery, the birth weight being 2.6 kg. The postnatal period was uneventful with no history of neonatal jaundice, seizures or renal failure. The family history was significant; his father had been diabetic since the age of 3 and he was on insulin. At 18 years of age, insulin was substituted with 5 mg of glibenclamide. However, as the hyperglycemia was not controlled with this, insulin was restarted after 1 month. The mother and two elder sisters of the baby were normoglycemic.

The general condition of the baby was unremarkable. He was playful and active. His weight was 7.6 kg (greater than the 95$^{th}$ percentile) and length 55 cm (less than 5$^{th}$ percentile). The rest of the examination, including the nervous system and fundus, was normal. The complete blood count was normal and there was no ketonuria. The fasting blood glucose was 177 mg/dL (reference range: 70-110 mg/dL), the 2-hour postprandial blood glucose was 210 mg/dL (reference range: 100-140 mg/dL) and HbA1c was 8.2% (reference range: 4.0-5.9%). Thyroid function tests and the fasting lipid profile were normal. Cushing syndrome was ruled out by an overnight dexamethasone suppression test. The structural diseases of the pancreas were excluded by ultrasonography of the abdomen. Glutamic acid decarboxylase-65 antibody (GAD-65) and insulinoma antigen-2 (IA-2) were negative. A fasting C-peptide level for the assessment of the beta cell reserve was only 0.6 ng/mL (reference range: 1-3 ng/mL).

Genetic tests for mutations in the *KCNJ11* and *ABCC8* gene subunits of the ATP-sensitive potassium channel, which are commonly associated with neonatal diabetes mellitus, were negative. Both the baby and father were found to be carriers of a heterozygous missense mutation c.287G>A in exon 3 of the *INS* gene. The mother was negative for this mutation. A mutation study was not carried out on the siblings. This mutation results in the substitution of tyrosine for cysteine at codon 96 (p.Cys96Tyr) in exon 3 of the insulin gene which confirms the diagnosis of permanent neonatal diabetes due to an insulin gene mutation. The child was started on 2 units of neutral protamine Hagedorn (NPH) insulin and, at present, he is on 2 units twice daily. He is being regularly followed up with satisfactory glycemic control.

## DISCUSSION

Neonatal diabetes mellitus due to an insulin gene mutation is quite rare. Several studies using linkage and candidate gene-based approaches have reported heterozygous missense mutations in the insulin gene causing permanent neonatal diabetes mellitus [6, 7, 8, 9].

It has been postulated that these mutations in the insulin gene result in the formation of abnormally folded proinsulin molecules which are consequently degraded in the endoplasmic reticulum. This leads to severe endoplasmic reticulum stress followed by beta cell death from apoptosis. Disulfide bonding seems to be crucial for proinsulin folding in the endoplasmic reticulum, and about 60% of

the insulin gene mutations disrupt disulfide bridge formation within this protein either by substitution of a cysteine residue or by the creation of an additional cysteine [8].

In the diagnosis of neonatal diabetes, it is vital to diagnose the channel mutations involving damage to the potassium channels which control insulin secretion. These patients do not require insulin and can respond to sulfonylureas [10, 11]. The sulfonylureas bind to the SUR1 subunit (coded by *ABCC8* gene) of the ATP-sensitive potassium channel and inhibit its function, leading to beta cell depolarization - this causes calcium influx and insulin release [10, 11]. It is also important to test for islet cell antibodies in order to diagnose autoimmune type 1 diabetes in this setting. In our patient, both GAD-65 and IA-2 antibodies were negative.

Clinically, patients with an insulin gene mutation have severe hyperglycemia, ketoacidosis and an elevated birth weight [8, 9, 12]. However, presentation is variable and, of these patients, only 40% had marked hyperglycemia and about 59% had ketoacidosis [8]. The present patient also had only moderate hyperglycemia and no diabetic ketoacidosis. The birth weight was normal. In general, more than 80% of insulin gene mutations are sporadic cases which result from a *de novo* mutation. However, our case is suggestive of a dominant inheritance as both the father and the son were affected, and both were heterozygous for the mutation.

In summary, insulin gene mutations appear to be an important cause of permanent neonatal diabetes mellitus. To the best of our knowledge, this is the first case of an insulin gene mutation from the Indian subcontinent. We recommend that genetic testing for both the insulin gene as well as the channel mutations be carried out in all cases of neonatal diabetes, as this will help in deciding on the appropriate therapy.

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**Abbreviations** *ABCC8*: ATP-binding cassette, sub-family C (CFTR/MRP), member 8; GAD-65: glutamic acid decarboxylase-65 antibody; IA-2: insulinoma antigen-2; *KCNJ11*: potassium inwardly-rectifying channel, subfamily J, member 11

**Conflict of interest** The authors have no potential conflicts of interest

**Correspondence**
Ambika Gopalakrishnan Unnikrishnan
Department of Endocrinology and Diabetes
Amrita Institute of Medical Sciences
Elamakkara PO
Cochin-682026
India
Phone: +91-484.280.1234-ext-1099; +91-484.220.5343
Fax: +91-484-280-2131
E-mail: unnikrishnanag@aims.amrita.edu

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