Neonatal diabetes; two cases with isolated pancreas agenesis due to homozygous PTF1A enhancer mutations and one with DEND syndrome due to KCNJ11 mutation

What is already known on this topic

Neonatal diabetes is a rare form of monogenic diabetes. Permanent neonatal diabetes can be due to either disruption of pancreas development or insulin secretion. PTF1A (pancreas-specific transcription factor 1a) is a transcription factor which is required for normal development of pancreas. Mutations at this site cause pancreas agenesis. Closure of ATP sensitive KATP channels depolarize cell membrane, and subsequently open voltage-dependent Ca channels that trigger release of stored insulin granules. KCNJ11 activating mutations result in the ATP sensitive K channel remain open and disrupt insulin secretion.

What this study adds

In two patients with neonatal diabetes and exocrine pancreas insufficiency homozygous g. 23508363 > G and g.23508437A > G mutations in the distal
PTF1A enhancer were identified. Interestingly father of the second patient had the same mutation with his daughter who was currently healthy. Patients were started insulin treatments. A previously reported heterozygous KCNJ11 missense mutation, p.C166Y, was identified in the third patient who had DEND syndrome. Patient was started oral sulfonylurea treatment. In the patients with neonatal diabetes genetic causes should be investigated not just for finding the underlying cause but also for planning treatment.

Abstract

Neonatal diabetes mellitus is a rare form of monogenic diabetes which is diagnosed in the first 6 months of life. Here we report three patients with neonatal diabetes; two with isolated pancreas agenesis due to mutations in the PTF1A enhancer and one with DEND syndrome (developmental delay, epilepsy, and neonatal diabetes) due to a KCNJ11 mutation.

The two cases with mutations in the distal enhancer of PTF1A had a homozygous g.23508363A>G and a homozygous g.23508437A>G mutation respectively. Previous functional analysis showed that these mutations can decrease expression of PTF1A which is involved in pancreas development. Both patients were born SGA to consanguineous parents. Both were treated with insulin and pancreatic enzymes. One of these patients’ father was also homozygous for the PTF1A mutation whilst his partner and the parents of the other patient were heterozygous carriers.

In the case with DEND syndrome, a previously reported heterozygous KCNJ11 mutation, p. Cys166Tyr (c.497G>A) was identified. This patient was born AGA to nonconsanguineous parents. The majority of neonatal diabetes patients with KCNJ11 mutations are known to respond to sulphonylurea treatment. Therefore Glibenclamide, an oral antidiabetic belonging to the sulphonylurea group, was started. This treatment regimen relatively improved blood glucose levels and neurological symptoms in the short term. Because we could not follow the patient in the long term we can not draw conclusions about the treatment.

Although neonatal diabetes mellitus can be diagnosed clinically, genetic analysis is important since it allows treatment and prognosis to be determined.

Keywords: neonatal diabetes, PTF1A, pancreas agenesis, KCNJ11
Introduction

Neonatal diabetes mellitus (NDM) is a rare form of monogenic diabetes that can be caused by mutations in different genes and presents in the first 6 months of life [1]. There are two main clinical groups; transient neonatal diabetes mellitus (TNDM) and permanent neonatal diabetes mellitus (PNDM). Transient neonatal diabetes mellitus is a developmental insulin production disorder that resolves spontaneously postnatally. Permanent neonatal diabetes mellitus does not go into remission. The underlying genetic defect can be found in most of the patients with TNDM. The majority of cases with TNDM are due to methylation defects in the imprinted region on chromosome 6q24; these can be either paternal uniparental disomy, paternal duplication, or defective methylation of the maternal allele [2].

Permanent neonatal diabetes mellitus is a genetically heterogeneous disorder due to mutations in 23 different genes described to date: KCNJ11, ABCC8, FOXP3, GCK, PDX1, PTF1A, EIF2AK3, SLC2A2, GATA6, GATA4, SLC19A2, WFS1, NEUROD1, NEUROG3, RFX6, LRBA, NKX2-2, MNX1, IER3IP1, INS, STAT3, GLIS3 and HNF1B [3-9]. These mutations can either compromise insulin secretion, disturb pancreas or islet cell development or result in autoimmune destruction of the beta cells. Genes associated with pancreatic agenesis are PDX1, PTF1A, RFX6, HNF1B and GATA6 [5, 6]. Disruption of pancreas development leads to exocrine as well as endocrine pancreatic insufficiency. Mutations in the genes encoding the ATP-sensitivite potassium channel (K\textsubscript{ATP}) subunits, KCNJ11 (Kir6.2), ABCC8 (SUR1) and INS (insulin) compromise insulin secretion by affecting the mechanisms involved in insulin secretion [10-14].

In this report we describe three patients with neonatal diabetes, two of them have isolated pancreatic agenesis due to mutations in a distal enhancer of the PTF1A gene. The third patient has normal pancreatic development and additional neurological symptoms due to a mutation in the KCNJ11 gene.

Case 1:

The first case was a female infant born to consanguineous parents (first cousins). She was born at 37 weeks gestation by normal vaginal delivery with a birth weight of 1900 gr. After birth she was followed in the neonatal intensive unit for respiratory distress and hyperglycemia. She was treated with subcutaneous regular insulin. She has 2 healthy siblings. There was no family history of diabetes.
At the age of 1 month she was referred to our clinic because of uncontrolled high blood glucose levels. On admission her body weight was 2330gr (-3.05 SDS), height was 47 cm (-2.67 SDS), head circumference was 35 cm (-1.72 SDS). Her physical examination was normal except hip dysplasia.

Laboratory tests revealed a venous glucose of 354 mg/dl with glycosuria. She did not have ketonuria or acidosis. Serum C-peptide level was 0.01ng/ml (normal range: 0.9-4.3 ng/ml), hemoglobin A1c was 7.3% (normal range: 4.8-6%) and diabetes autoantibodies tests (antiGAD, ICA, IA2) were negative. Hb level was 9.5mgr/dl, and mean corpuscular volume MCV was 85.1 fl (normal range: 81-99 fl). The peripheral blood smear showed no signs of megaloblastic anemia. Serum folic acid, thiamine and vitamin B12 levels were normal. Serum thyroid hormones were within normal limits (TSH: 1.75 mU/L, fT4: 1.06 ng/dl). Renal and hepatic function tests were all within normal ranges. The patient was diagnosed with neonatal diabetes and insulin regimen was changed to subcutaneous (SC) NPH insulin with which blood glucose levels could not be stabilized. Thus the insulin regimen was changed to detemir insulin, with rapid acting insulin adjustment when needed. But this regimen was also not successful in controlling the blood glucose levels. Finally detemir insulin was replaced with glargine insulin (1U/day) that achieved more stable blood glucose levels. Humalog insulin (0.5 U/dose) was added when needed. Insulin doses were adjusted according to blood glucose levels. She also had significant diarrhea episodes and stool tests revealed malabsorption. Abdominal ultrasonography revealed normal liver and kidneys but the pancreas could not be visualized. Her echocardiography was normal. For exocrine pancreas insufficiency, enzyme replacement therapy was added to her treatment to which she responded well (Table). At her last visit she was 3.5 years old, her body weight was 13.9 kg (-0.64 SDS), height was 92.3 cm (-1.57 SDS), head circumference was 48 cm (-1.08 SDS) with normal mental and motor development. Her glucose regulation was in acceptable ranges with HbA1c level of 7.3 %.

DNA sequencing and genetic analysis

A homozygous g.23508363A > G mutation affecting a highly conserved nucleotide within a previously identified distal enhancer of PTF1A was identified (Fig 1). Previous functional analysis showed that this mutation disrupts enhancer activity and is likely to result in decreased PTF1A expression during pancreatic development [15]. This result confirms a diagnosis of neonatal diabetes and exocrine pancreatic insufficiency due to a recessive PTF1A mutation. Her mother and sister were heterozygous for the same
mutation. Her brother did not carry the mutation. Father’s sample was not available for testing.

Case 2:

The second case was a female infant born to consanguineous (first cousins) parents. She was born at 37 weeks of gestation by C section delivery due to oligohydramnios and IUGR with a birth weight of 1520 gr. She was followed in the neonatal intensive unit for hyperglycemia and was treated with subcutaneous regular insulin. She was the first child with no siblings. There was no family history of diabetes.

She was referred to our clinic for glucose regulation when she was 44 days old. Her body weight was 1980 gr (-3.93 SDS), height was 43 cm (-5.06), head circumference was 32 cm (-4.75). Her physical examination was normal.

In the laboratory tests; venous glucose was 300 mg/dl accompanied with glycosuria. She did not have ketonuria or acidosis. Her serum C-peptide level was 0.01 ng/ml (normal range: 0.9-4.3 ng/ml), hemoglobin A1c was 7.4 % (normal range: 4.8-6 %) and diabetes autoantibodies tests (antiGAD, ICA,IA2) were negative. Hb level was 8 mgr/dl, and mean corpuscular volume MCV was 85 fl (normal range: 81-99 fl). The peripheral blood smear showed no signs of megaloblastic anemia. Serum folic acid, thiamine and vitamin B12 levels were normal. Thyroid functions were normal (TSH:5.8 mIU/L, fT4: 0.9 ng/dl). Renal and hepatic function tests were all within normal ranges. The patient was diagnosed with neonatal diabetes. We started glargine insulin (1U/day). Humalog insulin (0.5U /dose) was added when needed. Her mother was eager to use insulin pump therapy. Thus she was trained for it and a better glycemic control was acheived by continous subcutaneous insulin pump treatment. She was fed breast milk, thus her baseline insulin dose was 0.125 U/hour which was modified according to her blood glucose levels. Her bolus insulin dose was 0,5U which was again modified according to her blood glucose levels.

Abdominal ultrasonography and MRI images failed to visualize the pancreas whilst liver and kidneys appeared normal. Her stool tests were significant for malabsorbtion. Her echocardiography revealed patent foramen ovale, thin patent ductus arteriosus and peripheral pulmonary stenosis. Enzyme replacement treatment for pancreatic insufficiency was added to her treatment regimen and she responded well (Table).

At her last visit she was 4 months old, her body weight was 4500 gr (-2.64 SDS), height was 53 cm (-3.92), head circumference was 38 cm (-2.68 SDS) with normal mental and motor development. Her family and she was adapted to
insulin pump. Her blood glucose levels were in appreciable levels with a HbA1c level of 7.1%.

**DNA sequencing and genetic analysis**

A homozygous g.23508437A > G mutation was identified within the distal enhancer of the *PTF1A* gene which is known to affect a highly conserved nucleotide (Fig 2). Previous functional analysis had shown that this mutation disrupts enhancer activity and is likely to result in decreased PTF1A expression during pancreatic development [15]. This result confirms a diagnosis of neonatal diabetes and exocrine pancreatic insufficiency due to a recessive *PTF1A* mutation. Her mother was heterozygous for the mutation whilst the unaffected father was also homozygous. One patient with a homozygous g.23508437A>G mutation who developed diabetes in adulthood has been previously reported [15]. Her father is therefore at increased risk of developing diabetes and annual monitoring of his HbA1c is recommended. The risk that this couple’s next pregnancy will be affected with neonatal diabetes is 1 in 2.

**Case 3:**

The third case was a male infant born to non-consanguineous parents. He was born at 35 weeks gestation by spontaneous vaginal delivery with a birth weight of 3400 gr. His seizures as arm movements started when he was 1 month old which then progressed as tonic clonic convulsions. He was the first and only child of his family and there was no family history of diabetes.

High blood glucose levels and failure to thrive were the referral reasons to our clinic at the age of 3 months. On admission his body weight was 4460 gr (-2.24 SDS), height was 62.5 cm (0.44), head circumference was 40 cm (-0.81 SDS). His physical examination revealed hypotonia and decreased muscle strength of all 4 extremities. He wasn’t following with his eyes.

Laboratory measurements of venous glucose level was 600 mg/dl with glycosuria, which was not accompanied with ketonuria or acidosis. Serum C-peptide level was 0.72 ng/ml (normal range: 0.9-4.3 ng/ml), hemoglobin A1c was 11.4% (normal range: 4.8-6 %) and diabetes autoantibodies tests (antiGAD, ÎÇA,Î²A) were negative. Hb level was 11.4 mgr/dl, and mean corpuscular volume MCV was 84 fl (normal range: 81-99 fl). The peripheral blood smear showed no signs of megaloblastic anemia. Serum folic acid, thiamine and vitamin B12 levels were normal. Serum thyroid hormone measurements were within normal limits (TSH: 1.75 mIU/L, FT4: 1.06 ng/dl). Renal and hepatic function tests were all within normal ranges. According to our previous experience we started glargine insulin. Humalog insulin was added when needed according to his blood glucose levels. His tonic clonic convulsions continued and were unrelated
to blood sugar levels. An EEG was performed and phenobarbital was started. Developmental delay, epilepsy, and neonatal diabetes suggested DEND syndrome. Genetic testing detected a heterozygous missense mutation, c.497G>A p.C166Y, in KCNJ11 which had been previously reported.

Glibenclamide, an oral antidiabetic belonging to the sulfonylurea group, was started according to the protocol provided by the Exeter team (available at http://www.diabetesgenes.org/content/genetic-testing-neonatal-diabetes) (Table). Glibenclamide dose was gradually increased while insulin dose was decreased. With this treatment regimen his blood sugar levels were well controlled and a relative improvement (normal muscle tone, eye contact) in his neurological status was observed at 7 months follow-up. At his last visit he was 10 months old, his body weight was 6190 gr (-3.39 SDS), height was 74 cm (-0.29 SDS), head circumference was 44 cm(-1.65 SDS). He was on glibenclamide and insulin treatment at doses of 10mg/day and 4U/day (2U glargine and 2 U Humalog insulins) respectively.

**DNA sequencing and genetic analysis**

The patient was heterozygous for a previously reported KCNJ11 missense mutation, p.C166Y (Fig 3). The p.C166Y mutation has been reported previously in patients with developmental delay, epilepsy and neonatal diabetes (DEND) syndrome. This mutation is predicted to be pathogenic and the result confirmed a diagnosis of neonatal diabetes, epilepsy and developmental delay due to a mutation in the Kir6.2 subunit of the KATP channel [16].

**Discussion**

Here we report three cases with neonatal diabetes caused by three different mutations, two homozygous mutations in the PTF1A enhancer and one heterozygous mutation in the KCNJ11 gene. Their common finding was hyperglycemia before 6 months of age. Cases 1 and 3 were born SGA and had exocrine pancreatic insufficiency. Case 2 was born AGA and had neurological symptoms. Although all were diagnosed with neonatal diabetes, their clinical findings suggested different mode of disease development.

Heterozygous activating mutations in KCNJ11, encoding the Kir6.2 subunit of the ATP sensitive potassium channel, are common cause of neonatal diabetes. It has been reported as being the reason in 30-58 % of the neonatal diabetes cases [12, 16-19]. However in populations with high incidence of consanguineous marriages homozygous mutations causing neonatal diabetes seem more common [20, 21].
Exocrine pancreas insufficiency in the first 2 cases suggested pancreas agenesis. Thus, homozygous g. 23508363 >G and g.23508437A >G mutations in the distal PTF1A enhancer were identified. Spatiotemporally regulated expression of transcription factors is important for cell fate specification and organogenesis [22]. Ptf1a (pancreas-specific transcription factor 1a) is a transcription factor that is required for the formation of the exocrine pancreas and the correct spatial organization of the endocrine pancreas of mice [23]. In mice models, Ptf1a inactivation reverted pancreatic cells to intestinal cells, suggesting its function as a switch between pancreatic and intestinal cell fates [24] and Ptf1a dose reduction resulted in pancreatic hypoplasia and insufficient insulin secretion in a dosage dependent manner [22]. Furthermore in humans coding mutation in PTF1A were shown to cause neonatal diabetes due to pancreas agenesis [25]. Weedon et al have identified six different recessive mutations in a downstream enhancer of PTF1A in 10 families with isolated pancreas agenesis [15]. This region acts as a developmental enhancer of PTF1A and the mutations abolish enhancer activity. It was interesting to find the same homozygous mutation in the distal PTF1A enhancer in the healthy father of the second case. However a patient who developed diabetes in adulthood with a homozygous g.23508437A>G mutation has been previously reported [15].

Neurological symptoms in case 3 suggested DEND syndrome and a previously reported heterozygous KCNJ11 missense mutation, p.C166Y, was identified. ATP sensitive KATP channels couple cell metabolism to membrane excitability in various cell types, including neurons, pancreatic beta cells, endocrine and muscle cells. The archetypal KATP channel is an octameric complex of Kir6.2 and either SUR1 or SUR2 subunits. Pancreatic beta cells and many neurons involve SUR1, muscle cells involve SUR2. Four Kir6.2 subunits form the channel pore, and each is associated with a SUR subunit that regulates channel gating [26]. In pancreatic beta cells, ATP-potassium channels regulate glucose-induced insulin secretion. In the unstimulated state, the beta cell ATP-sensitive K channels are open. Following the uptake and metabolism of glucose, intracellular ATP/ADP ratio increases which results in closure of ATP sensitive K channels, depolarization of the cell membrane, and subsequent opening of voltage-dependent Ca channels. Increase in cytosolic Ca concentration triggers the release of stored insulin granules. KCNJ11 activating mutations result in the ATP sensitive K channel to remain open and insulin secretion is therefore disrupted. Pancreas development is normal and diabetes is due to impaired insulin secretion. This channel is important in numerous sites such as neurological cells. Which is why mutations can result not only in diabetes but also neurological disorders.
The KCNJ11 gene mutation can be treated with an oral antidiabetic agent, sulfonylurea, which can close the K\textsubscript{ATP} channel and induce insulin secretion [27]. This treatment has been shown to also improve neurological symptoms [28-33]. However, in patients with mutations resulting in severe DEND syndrome, sulfonylurea treatment is often ineffective [10, 16, 27, 34]. Although in a Brazilian patient having the same mutation as our second patient (p.C166Y mutation in the KCNJ11 gene) sulfonylurea treatment was unsuccessful in controlling blood glucose levels and neurological symptoms [35], we observed relative improvements in blood glucose levels and neurological symptoms in short term. Nevertheless, we can not comment on treatment success because we couldn’t follow up this patient in the long term.

Although neonatal diabetes is a rare disorder, it should be promptly evaluated for additional clinical findings. Clinical findings can be clue for choosing the genetic tests. Genetic testing is important because they not only reveal the underlying mechanism for the disorder but also guide treatment and follow up of the patients.

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References:

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Table: Clinical features, genetic defects and treatments of the patients.
Normal sequence

Patient: Homozygous PTF1A g.23508363A>G

Mother: Heterozygous PTF1A g.23508363A>G

Sister: Heterozygous PTF1A g.23508363A>G
Figure 1. A homozygous g.23508363A > G mutation within distal enhancer of *PTF1* was identified in the first case. Mother and sister were heterozygous for the same mutation.
Normal sequence

Patient: Homozygous PTF1A g.23508437A>G

Mother: Heterozygous PTF1A g.23508437A>G

Father: Homozygous PTF1A g.23508437A>G
Figure 2. A homozygous g.23508437A > G mutation was identified within the distal enhancer of the PTF1A gene in the second case. For the same mutation mother was heterozygous, father was also homozygous.
Normal sequence

Patient: Heterozygous KCNJ11 p.(Cys166Tyr)
Figure 3. A heterozygous missense mutation, p.C166Y within KCNJ11 gene was identified in the third case.