

Pancreatic Agenesis due to Compound Heterozygosity for a Novel Enhancer and Truncating Mutation in the *PTF1A* Gene

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What is already known on this topic?

Homozygous truncating mutations in *PTF1A* have been reported in patients with pancreatic and cerebellar agenesis, while recessive mutations located in a distal *PTF1A* enhancer cause isolated pancreatic agenesis.

What this study adds?

This is the first report of a patient with isolated pancreatic agenesis resulting from compound heterozygosity for truncating and enhancer mutations in the *PTF1A* gene. This study broadens the spectrum of mutations causing pancreatic agenesis and the phenotypic variability of this condition.

Abstract

Neonatal diabetes, defined as the onset of diabetes within the first six months of life, is very rarely caused by pancreatic agenesis. Homozygous truncating mutations in the *PTF1A* gene, which encodes a transcriptional factor, have been reported in patients with pancreatic and cerebellar agenesis, whilst mutations located in a distal pancreatic-specific enhancer cause isolated pancreatic agenesis. We report an infant, born to healthy non-consanguineous parents, with neonatal diabetes due to pancreatic agenesis. Initial genetic investigation included sequencing of *KCNJ11*, *ABCC8* and *INS* genes, but no mutations were found. Following this, 22 neonatal diabetes associated genes were analyzed by a next generation sequencing assay. We found compound heterozygous mutations in the *PTF1A* gene: A frameshift mutation in exon 1 (c.437_462 del, p.Ala146Glyfs*116) and a mutation affecting a highly conserved nucleotide within the distal pancreatic enhancer (g.23508442A > G). Both mutations were confirmed by Sanger sequencing. Isolated pancreatic agenesis resulting from compound heterozygosity for truncating and enhancer mutations in the *PTF1A* gene has not been previously reported. This report broadens the spectrum of mutations causing pancreatic agenesis.

Keywords: Pancreatic agenesis, neonatal diabetes, *PTF1A* gene

Introduction

Neonatal diabetes, defined as onset of diabetes within the first 6 months of life, is a genetically heterogeneous condition with 22 known genetic causes (1,2,3). Its causal genes are involved in the development of the pancreas or islets; beta cell apoptosis or destruction; or beta-cell function (1). The *PTF1A* gene on chromosome 10 encodes a transcription factor with a key role in early pancreas development and cerebellar neurogenesis (4,5). Homozygous truncating

mutations in *PTF1A* have been reported in patients with pancreatic and cerebellar agenesis, whilst mutations located in a distal pancreatic-specific enhancer cause isolated pancreatic agenesis (5,6,7,8).

Here, we report a patient with isolated pancreatic agenesis due to compound heterozygous mutations in *PTF1A*: A coding frameshift mutation (p.Ala146Glyfs*116) and a novel regulatory mutation located in the distal enhancer, 25 kb downstream of this gene.



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Case Report

The index patient is a 1-year and 9-month-old boy, the second child of healthy, Caucasian, non-consanguineous parents. He was born with a birth weight of 1,935 g (below the third percentile), length of 43 cm (below the third percentile), and head circumference of 32 cm (third percentile). Intrauterine growth retardation was noted at 34 weeks of gestation, and the delivery was by cesarean section at 37 weeks of gestation. Physical examination revealed no dysmorphic features. During the first week of life, hyperglycemia (blood glucose = 250 mg/dL) without ketoacidosis was observed, and treatment with subcutaneous NPH insulin was initiated on the 7th day of life, followed by continuous subcutaneous insulin infusion. Despite intensive insulin therapy, the infant did not show satisfactory weight gain and at 4 months of age, his weight was 3,670 g (still below the third percentile). Abdominal distension and fatty stools were noted. Abdominal ultrasound was performed at that time and the pancreas could not be identified. No other abnormality was found. Pancreatic enzyme replacement was begun at the age of 4 months and a catch-up growth occurred (Figure 1). Over the subsequent months, his growth was satisfactory

and his developmental milestones were all reached at an appropriate age.

Genetic Analysis

Initial Sanger sequencing of the coding and flanking intronic regions of the *KCNJ11* (NM_000525), *ABCC8* (NM_000352), and *INS* (NM_000207) genes was undertaken as previously described (3), but no mutations were found. Following this, a next-generation sequencing assay was performed to analyze the coding regions and conserved splice sites of the 22 neonatal diabetes genes: *KCNJ11*, *ABCC8*, *INS*, *EIF2AK3*, *FOXP3*, *GATA4*, *GATA6*, *GCK*, *GLIS3*, *GNAS1*, *GNAS2*, *GNAS3*, *GNAS4*, *GNAS5*, *GNAS6*, *GNAS7*, *GNAS8*, *GNAS9*, *GNAS10*, *GNAS11*, *GNAS12*, *GNAS13*, *GNAS14*, *GNAS15*, *GNAS16*, *GNAS17*, *GNAS18*, *GNAS19*, *GNAS20*, *GNAS21*, and *ZFP57* (Agilent custom capture v5.1/Illumina HiSeq) at the Molecular Genetics Laboratory, University of Exeter Medical School, UK (9). A compound heterozygous mutations in the *PTF1A* gene was found: a frameshift mutation in exon 1 (c.437_462 del, p.Ala146Glyfs*116) and a mutation affecting a highly conserved nucleotide within the distal pancreatic enhancer (g.23508442A > G) (Figure 2). The two mutations were predicted to be pathogenic and likely to be pathogenic, respectively, according to the American College of Medical Genetics and Genomics variant interpretation guidelines (10). Both mutations were confirmed by Sanger sequencing. Family member testing showed that the proband's mother is a heterozygous carrier of the distal enhancer mutation and the father is a heterozygous carrier of the frameshift mutation.

Discussion

Pancreatic agenesis characterized by exocrine pancreatic insufficiency and permanent neonatal diabetes (11) is a rare condition. An international cohort study of patients with diabetes diagnosed before 6 months of age found that only 4.9% of them had pancreatic agenesis (12). Mutations in genes that encode transcription factors with a key role in pancreatic development, such as *PDX1*, *GATA6*, *GATA4*, and *PTF1A*, have been reported as genetic causes of congenital absence of the pancreas (5,6,7,8,12,13,14). However, the origin of this disorder remains unknown in ~ 15% of patients (De Franco E, unpublished data). Here, we report a patient with neonatal diabetes and exocrine pancreas insufficiency resulting from compound heterozygous mutations in the *PTF1A* gene. *PTF1A*, a 48kDa binding subunit of the pancreatic transcription factor 1 (PTF1), is required for normal pancreas development (4). This transcription factor is also expressed in embryonic neural tissues and plays a role in cerebellar neurogenesis. *Ptf1a*^{-/-} mice show a size reduction in the cerebellar primordium in embryos, resulting in cerebellar agenesis at birth (5). Consistently with this,

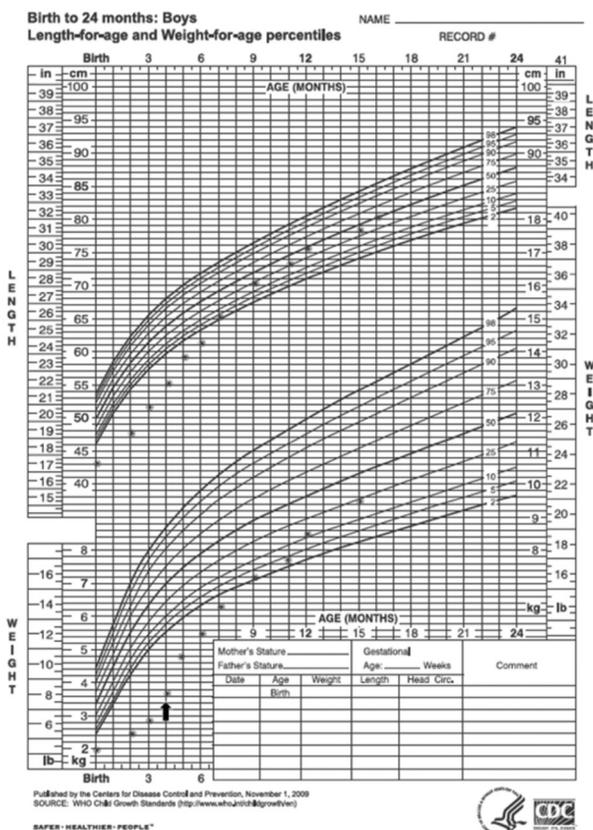


Figure 1. Growth of the patient plotted on the World Health Organization growth chart. Arrow denotes the start of pancreatic enzyme replacement

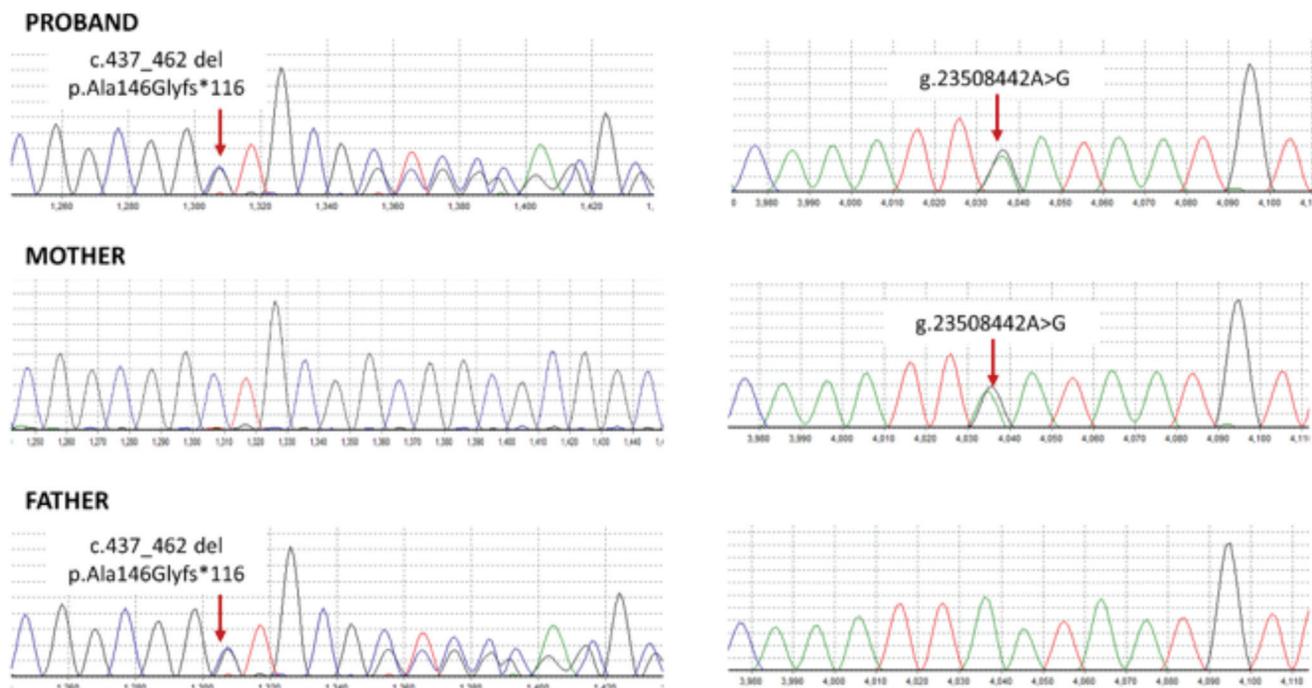


Figure 2. Sequence chromatograms showing the *PTF1A* mutations identified in the proband and his parents

recessive loss of function mutations in the *PTF1A* gene have been previously reported to cause agenesis of the pancreas and the cerebellum with additional dysmorphic features (5,6,15). A homozygous missense mutation, p.Pro191Thr, resulting in a protein with a 75% reduced transactivation activity, has been recently reported in patients with isolated pancreatic aplasia/hypoplasia, indicating a correlation between coding mutation severity and phenotype (16). Furthermore, mutations in the enhancer region located 25 kb downstream from the coding region of the *PTF1A* gene, which acts as a developmental enhancer of this gene, have been found to cause isolated pancreatic hypoplasia/agenesis, sparing the cerebellum (7,8).

To the best of our knowledge, this is the first report of a patient with isolated pancreatic agenesis resulting from compound heterozygosity for truncating and enhancer mutations in the *PTF1A* gene. Regarding the age of onset of diabetes, previous reports of patients with *PTF1A* truncating mutations showed that they had diabetes in the first month of life (5,6,15). However, patients with *PTF1A* enhancer mutations had phenotypic variability: The majority of cases are diagnosed in the first month of life, but diabetes at later ages was also observed (8). The patient we report had diabetes diagnosed in the first week of life and his neurological development has been normal, indicating no associated anomaly in the cerebellum. Interestingly, the lack of a severe neurological phenotype in patients with homozygous/compound heterozygous regulatory

mutations has been recently reported for another congenital disease, polycystic kidney disease with hyperinsulinemic hypoglycemia (HIPKD) (17). A specific promoter mutation in the *PMM2* gene, either homozygous or in trans with a coding *PMM2* mutation, was reported to cause HIPKD in 11 families. Homozygous coding mutations in *PMM2* have been previously reported to cause a congenital disorder of glycosylation type 1a, a severe multisystem disease with prominent neurologic features which were not observed in patients with the promoter mutation. The phenotype-genotype relationship observed in patients with coding versus non-coding mutations in *PTF1A* and *PMM2* highlights the fundamental role of non-coding sequences in development of specific organs.

In summary, we report the case of a patient with isolated pancreatic agenesis due to compound heterozygosity for a truncating and novel enhancer mutation in *PTF1A*, broadening the spectrum of mutations causing pancreatic agenesis and phenotypic variability of this condition.

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Ethics

Informed Consent: Written consent was obtained from the patient's parents.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Monica Gabbay, Sian Ellard, Elisa De Franco, Regina S. Moisés, Data Collection or Processing: Monica Gabbay, Sian Ellard, Elisa De Franco, Regina S. Moisés, Analysis or Interpretation: Monica Gabbay, Sian Ellard, Elisa De Franco, Regina S. Moisés, Literature Search: Monica Gabbay, Sian Ellard, Elisa De Franco, Regina S. Moisés, Writing: Monica Gabbay, Sian Ellard, Elisa De Franco, Regina S. Moisés.

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