TITLE PAGE

PDX1-MODY and dorsal pancreatic agenesis: new phenotype of a rare disease

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ABSTRACT

Maturity-Onset Diabetes of the Young (MODY) type 4 or *PDX1*-MODY is a rare form of monogenic diabetes caused by heterozygous variants in *PDX1*. Pancreatic developmental anomalies related to *PDX1* are reported only in neonatal diabetes cases. Here, we describe dorsal pancreatic agenesis in two patients with *PDX1*-MODY. The proband presented with diabetes since 14 years of age and maintained regular glycemic control with low doses of basal insulin and detectable C-peptide levels after 30 years with diabetes. A diagnosis of MODY was suspected. Targeted next-generation sequencing identified a heterozygous variant in *PDX1*: c.188delC/p.Pro63Argfs*60. Computed tomography revealed caudal pancreatic agenesis. Low fecal elastase indicated exocrine insufficiency. His son had impaired glucose tolerance, presented similar pancreatic agenesis, and harbored the same allelic variant. The unusual presentation in this Brazilian family enabled expansion upon a rare disease phenotype, demonstrating the possibility of detecting pancreatic malformation even in cases of *PDX1*-related diabetes diagnosed after the first year of life. This finding can improve the management of MODY4 patients, leading to precocious investigation of pancreatic dysgenesis and exocrine dysfunction.

Key words: MODY; *PDX1;* heterozygous variant; dorsal pancreatic agenesis

INTRODUCTION

PDX1-MODY (MODY4) is a rare subtype of monogenic diabetes caused by heterozygous variants in *PDX1* (OMIM *600733), which encodes a transcription factor crucial for regulating pancreatic function and development (1).

Pancreatic agenesis related to *PDX1* is reported only in cases of neonatal diabetes mellitus (NDM), caused by homozygous or heterozygous compound variants (2-6). To date, there is no description of this pancreatic developmental anomaly associated with heterozygous *PDX1* variants (MODY4) (3).

Here, we describe the unprecedented association between *PDX1*-MODY and dorsal pancreatic agenesis (DPA) in two cases from a Brazilian family.

RESEARCH DESIGN AND METHODS

The proband was selected for molecular analysis of MODY since he met the clinical criteria (age of diabetes onset < 35 years, family history of diabetes, absence of obesity, negative pancreatic antibodies and detectable C-peptide levels 3-5 years after diabetes diagnosis) (7).

We designed a customized next-generation sequencing (NGS) panel (SureSelect assay, Agilent Technologies, Santa Clara, CA, USA), which included 51 nuclear genes and the mitochondrial genome, all associated with different types of monogenic diabetes (see Supplemental Methods). Sequencing was performed on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA). The pathogenicity of variants was evaluated according to American College of Medical Genetics and Genomics (ACMG) guidelines (8). Sanger sequencing was performed to confirm the most likely causal variant and also to analyze

available individuals of the family. Informed consent was obtained from the patient and his family.

RESULTS

Patient and Family description

The proband, a 52-year-old male, had a diagnosis of diabetes at 14 years of age. The presumed hypothesis at the time was Type 1 Diabetes and he started insulin treatment. During 38 years of follow-up, he only used NPH insulin at low doses (average 0.4 IU/Kg/d), maintaining regular glycemic control (glycated hemoglobin range: 6.8–9.2%) with no episode of diabetic ketoacidosis. After this long duration of diabetes, he had a detectable C-peptide level of 0.47 nmol/L, fasting glucose 24.5 mmol/L, A1c 7.4%, and normal renal function. A diagnosis of MODY was suspected and sulfonylurea treatment was initiated with subsequent reduction of the insulin dose (NPH 0.2 IU/Kg/d). His 5-year-old son had a normal body mass index and impaired glucose tolerance. The proband's mother (deceased) and two siblings also had diabetes. The latter two were overweight and were diagnosed with late onset hyperglycemia, in addition to hypertension and dyslipidemia, resembling Type 2 Diabetes.

Genetic analysis

Targeted-NGS sequence analysis (see Fig. S1) identified a heterozygous variant in *PDX1* (NM_000209), a deletion of a cytosine in codon 63 of exon 1 resulting in a frameshift: c.188delC/p.Pro63Argfs*60 (Fig. 1a). The variant found in *PDX1* has been previously described in cases of both types of monogenic diabetes, MODY and NDM (1, 2, 5, 9), and is considered pathogenic according to the ACMG criteria (8). Familial co-segregation analysis

(Fig. 1b) showed the same variant in his son with prediabetes. Five relatives were available for testing, of which 2 had late onset diabetes and 3 had normal glycemia; none harbored this variant in *PDX1*.

Imaging investigation and assessment of exocrine deficiency

Since variants in *PDX1* related to NDM are associated with pancreatic dysgenesis, we performed a pancreatic morphological evaluation. Abdominal computed tomography (CT) scan of these two patients harboring the heterozygous *PDX1* variant showed agenesis of the caudal pancreas and no sign of the main pancreatic duct of this segment. The proband's brother had a normal pancreas on CT scan (Fig. 1c).

The proband had reduced fecal elastase (< 100 mcg/g), which characterizes exocrine pancreatic insufficiency. Despite the absence of abdominal pain or weight loss, he presented diarrhea after a high fat meal, but did not require exocrine supplementation. His son had normal fecal elastase (> 200 mcg/g).

The clinical, laboratory, imaging, and genetic data of this Brazilian family are presented in Table 1.

DISCUSSION

Until 2010, only 3 families with heterozygous *PDX1* variants related to MODY have been reported, and two of them were possibly related (1, 9, 10). In 2015, 3 new patients with MODY were described harboring variants in *PDX1* (11-13). Two of these recent cases were detected by Targeted-NGS, demonstrating the importance of this new sequencing approach to diagnose rare MODY subtypes. In these previous studies, pancreatic morphology and exocrine function were not reported. The phenotype of described *PDX1*-MODY cases was variable: the age of diabetes onset occurred later than that of other forms of MODY, affecting both obese and nonobese patients, and there was a wide variety of therapeutic responses (some patients were treated only with diet, others with oral antidiabetics and/or insulin). All

cases with reported segregation had a prominent pattern of autosomal dominant inheritance (1, 9, 10, 12, 13).

Previously reported cases of diabetes and pancreatic agenesis related to *PDX1* were verified in probands with NDM (homozygous/compound heterozygous variants) (1, 2, 4-6, 9). In our cases, a heterozygous loss-of-function variant was identified in a father and his son with MODY and dorsal pancreatic malformation. The two proband's siblings, who had phenotype compatible with type 2 Diabetes, were homozygous for *PDX1* wild type allele. This phenomenon of coexistence between other type of diabetes and MODY in the same family has already been reported by other groups (1, 9, 10).

The present NGS panel allowed the analysis of all MODY genes and also of six known genes previously associated with pancreatic agenesis (*PDX1, HNF1B*, *PTF1A*, *RFX6*, *EIF2AK3,* and *GATA6*) (3, 14). We have ruled out any pathogenic variant in those other genes, except for the one in *PDX1* (c.188delC/p.Pro63Argfs*60). This variant was previously described in families with concomitance of NDM and MODY. Relatives with MODY who harbored the same *PDX1* variant, in heterozygosis, had no pancreatic evaluation reports (1, 9).

In a recent review, there was no description of pancreatic malformation imaging related to *PDX1*-MODY (3). CT scan of our patients with *PDX1*-MODY revealed agenesis of the pancreatic tail. The pancreatic parenchyma in the head, uncinate process and body were preserved intact and the patients had no documented history of pancreatitis, discarding the possibility of pancreatic involution of the tail by chronic pancreatitis (pseudo-agenesis).

Partial anomalies of pancreatic formation can be associated with subclinical exocrine deficiency (6). In our cases, the proband had exocrine pancreatic insufficiency and his son

had normal fecal elastase with no clinical manifestation of exocrine dysfunction. As the son is still a child, it is possible that he may develop pancreatic failure in the future.

Previous functional studies corroborate the association of heterozygous *PDX1* variants with MODY (15, 16). Haploinsufficient mice experiments showed depletion of ² -cell function, while not impairing pancreas formation (17). However, there are limitations of animal models for the functional validation of allelic variants. Discrepant phenotypes between mice and humans with MODY have already been described, as seen in heterozygous variants in the transcription factor gene *HNF1B* (14, 18).

In NDM cases, severe pancreatic malformation was detected – complete agenesis or complete DPA (2, 4-6). Since our MODY subjects presented partial DPA, we hypothesized that heterozygous null variants in *PDX1* could lead to a milder phenotype of pancreatic dysgenesis, secondary to haploinsufficiency mechanism.

Although we did not study the effects of this heterozygous *PDX1* variant on the genesis of pancreas, several lines of evidence suggest that this variant is likely to have caused the disorder. First, there is strong biological plausibility. *PDX1* is one of the most critical genes for pancreas development (4). Further, homozygous variants in *PDX1* had already been reported to cause pancreatic agenesis and diabetes. Second, this loss-of-function variant, an early-frameshift deletion, is located in a highly conserved region of a known haploinsufficient gene, hence strongly predicts to affect protein function. Third, the variant cosegregates with the phenotype (MODY/DPA) in the reported family. We cannot exclude the additional influence of unevaluated genes on pancreatic agenesis. Nevertheless, we believe that the identified heterozygous *PDX1* variant has a causal relationship with the

clinical phenotype. The lack of report on DPA in previous heterozygous *PDX1* cases may be due to a subclinical condition underdiagnosed.

CONCLUSIONS

We report DPA in two cases from a Brazilian family with *PDX1*-MODY, representing a new spectrum of heterozygous *PDX1* variants. Increasing awareness of rare MODY subtypes is crucial since few cases are reported and disease characterization is not fully elucidated. This new finding should be confirmed for unrelated subjects with *PDX1*-MODY and also heterozygous *PDX1* variants should be tested for patients with DPA. Expansion of the described phenotype of *PDX1*-MODY can improve the management of such patients, leading to precocious investigation of pancreatic dysgenesis and exocrine dysfunction.

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Legends

Table 1 - Clinical and laboratory characterization of the Brazilian family with *PDX1*-MODY

Figure 1a – Heterozygous deletion (c.188delC / p.Pro63Argfs*60) in exon 1 of *PDX1* identified by Targeted-NGS and visualized using the Integrative Genomics Viewer software: IGV 2.3 - Broad Institute/MIT/Harvard (top). Confirmation of the NGS-detected variant by Sanger sequencing (bottom).

Figure 1b – Pedigree of the Brazilian *PDX1*-MODY family

Arrow indicates the proband. Squares represent male patients and circles females. Lozenges symbolize siblings of unknown sex (not available for testing). Subjects with diabetes are indicated by black symbols. Gray symbol indicates impaired glucose tolerance. Red symbol designates dorsal pancreatic agenesis. Nondiabetic individuals are represented by open symbols. Question mark indicates unknown glycemic state. Genotype of family members is indicated by: N, normal sequence allele (wild allele) and M, mutant allele (p.Pro63Argfs*60). Age at diagnosis of diabetes is specified below the genotype.

Figure 1c – Arterial phase contrast-enhanced computed tomography in (A) the proband, (B) his son, and (C) his brother. The red arrows indicate the pancreatic tail limit. Images A and B depict a short pancreatic tail which edge lies far from the splenic hilum. There is a lack of pancreatic parenchyma anterior to the splenic vein. Image C shows a pancreatic tail with normal length extending up to the splenic hilum.

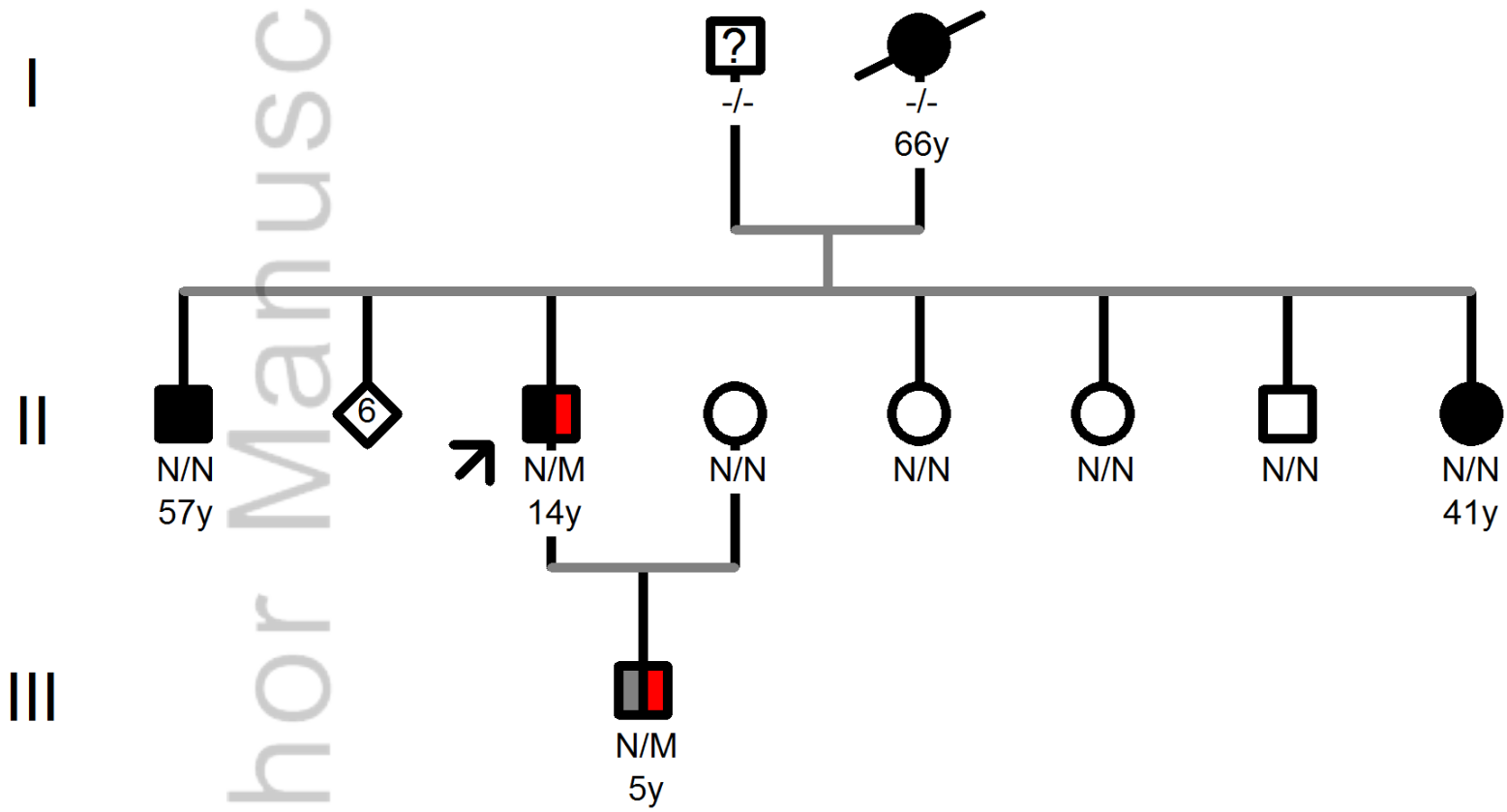


Figure1b.tiff

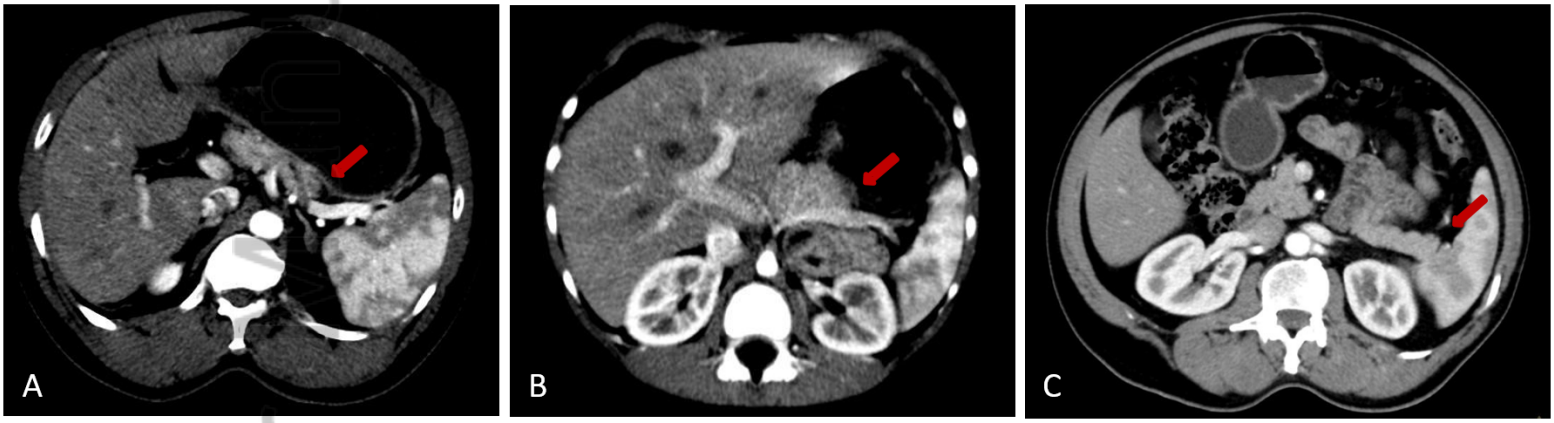
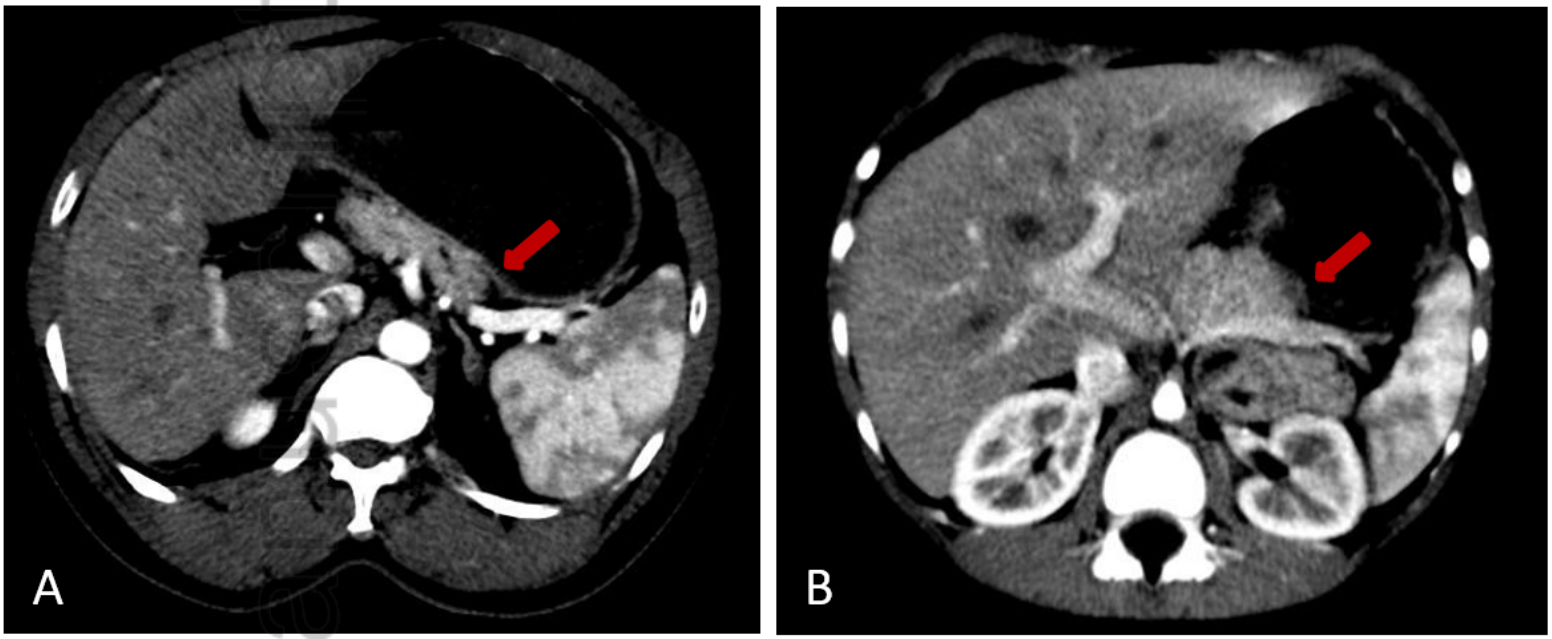


Figure1c.tiff

PDX1-MODY and dorsal pancreatic agenesis: new phenotype of a rare disease



- PDX1-MODY (MODY4): rare form of monogenic diabetes caused by heterozygous variants in PDX1

- Pancreatic agenesis related to PDX1: described only in neonatal diabetes cases (homozygous or compound heterozygous variants)

- We report two related cases with PDX1-MODY and dorsal pancreatic agenesis

- Proband (A) presented diabetes since 14 yo and his son (B) had impaired glucose tolerance detected at 5 yo

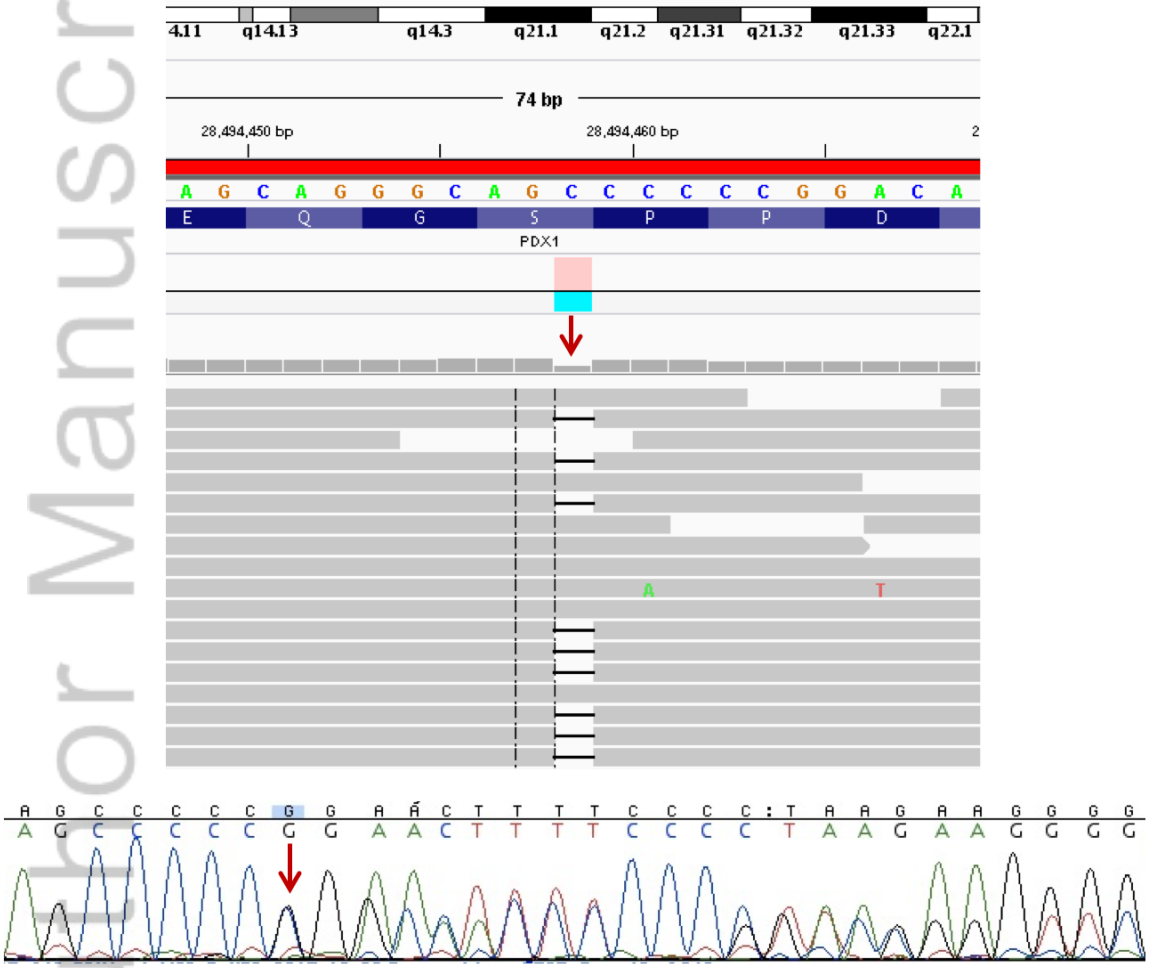
- Targeted-NGS: heterozygous variant in PDX1: c.188delC/p.Pro63Argfs*60

- Computed tomography (similar in both patients): caudal pancreatic agenesis (red arrows)

- Proband had exocrine pancreatic insufficiency and his son had normal fecal elastase

- Dorsal pancreatic agenesis: new spectrum of heterozygous PDX1 variants

Graphical Abstract.tiff



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