




# Widening the phenotypic spectrum caused by pathogenic *PDX1* variants in individuals with neonatal diabetes

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## ABSTRACT

**Introduction** Biallelic *PDX1* variants are a rare cause of isolated pancreatic agenesis and neonatal diabetes (NDM) without exocrine pancreatic insufficiency, with 17 cases reported in the literature.

**Research design and methods** To determine the phenotypic variability caused by this rare genetic aetiology, we investigated 19 individuals with NDM resulting from biallelic disease-causing *PDX1* variants.

**Results** Of the 19 individuals, 8 (42%) were confirmed to have exocrine insufficiency requiring replacement therapy. Twelve individuals (63.2%) had extrapancreatic features, including 8 (42%) with conditions affecting the duodenum and/or hepatobiliary tract. Defects in duodenum development are consistent with previous *Pdx1* ablation studies in mice which showed abnormal rostral duodenum development.

**Conclusions** Our findings show that recessive *PDX1* variants can cause a syndromic form of NDM, highlighting the need for clinical assessment of extrapancreatic features in individuals with NDM caused by *PDX1* variants.

## INTRODUCTION

Diabetes diagnosed before the age of 6 months (neonatal diabetes, NDM) is a genetically and clinically heterogeneous condition, with disease-causing variants in 1 of >35 known causative genes identified in >85% of cases.<sup>1</sup> Pathogenic variants in >25 genes cause syndromic forms of NDM, each associated with specific extrapancreatic features affecting a range of organs.<sup>2</sup> Clinical management of these individuals is often challenging due to the rarity of the conditions, heterogeneity of clinical features, disease severity and lack of specific treatments. The identification and assessment of individuals with syndromic NDM is essential to define the phenotype, guide clinical management, and inform on prognosis and recurrence risk within families.

Biallelic *PDX1* variants are a rare genetic cause of NDM, with 17 cases reported worldwide.<sup>3–12</sup> Initially, recessive loss-of-function

## WHAT IS ALREADY KNOWN ON THIS TOPIC

- ⇒ Biallelic *PDX1* variants are a rare genetic cause of diabetes diagnosed in the first 6 months of life (neonatal diabetes, NDM).
- ⇒ Initially, recessive loss-of-function *PDX1* variants were identified as a cause of pancreatic agenesis; later studies expanded the pancreatic phenotype to include NDM with or without subclinical exocrine insufficiency.
- ⇒ Recent reports have described extrapancreatic features in three unrelated cases with *PDX1*-NDM variants, highlighting the need to comprehensively assess the clinical phenotype caused by these variants.

## WHAT THIS STUDY ADDS

- ⇒ In our cohort of 19 individuals with *PDX1*-NDM, extrapancreatic features were common (detected in 63.2% of patients), with defects affecting the duodenum and/or hepatobiliary tract being present in 8 individuals (42% of the cohort).

## HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- ⇒ Our findings highlight the need for clinical assessment of extrapancreatic features in individuals with *PDX1*-NDM.

*PDX1* variants were identified as a cause of pancreatic agenesis without extrapancreatic features in three unrelated families.<sup>8–10</sup> Later studies expanded the pancreatic phenotype to include NDM with or without subclinical exocrine insufficiency.<sup>3,6</sup> Recent reports have described extrapancreatic features in three unrelated cases with homozygous *PDX1* variants,<sup>4,5,7</sup> highlighting the need to comprehensively assess the clinical phenotype caused by these variants.

In this study we assessed the genetic and clinical features of 19 individuals with biallelic *PDX1* variants to define the pancreatic and extrapancreatic features of this NDM subtype.

## RESEARCH DESIGN AND METHODS

## Subjects and genetic analysis

The study complied with the Declaration of Helsinki. Patients' parents or guardians gave their informed written consent.

Individuals with biallelic *PDX1* variants classified as pathogenic or likely pathogenic according to the American College of Medical Genetics and Genomics (ACMG) guidelines<sup>13</sup> (n=19 from 17 families) were selected from an international cohort of 2601 individuals referred for NDM testing to the Exeter Genomics Laboratory. Genetic analysis of the *PDX1* gene (NM\_000209.3) was performed by Sanger sequencing or targeted next-generation sequencing as previously described.<sup>14</sup> Variant testing for family members was performed by Sanger sequencing. No functional assessment of the variants was performed as part of this study.

Consanguinity had been reported in 13/17 families. Eight patients had been included in previous publications, including the three reports describing individuals with extrapancreatic features.<sup>3-5 7 15</sup>

Haplotype analysis was performed using off-target reads from next-generation sequencing data using a software developed inhouse (<https://github.com/rdemolgen/SavvySuite>) in individuals I-4, I-5, and I-6 (who were homozygous for the p.(Ala152Gly) variant).

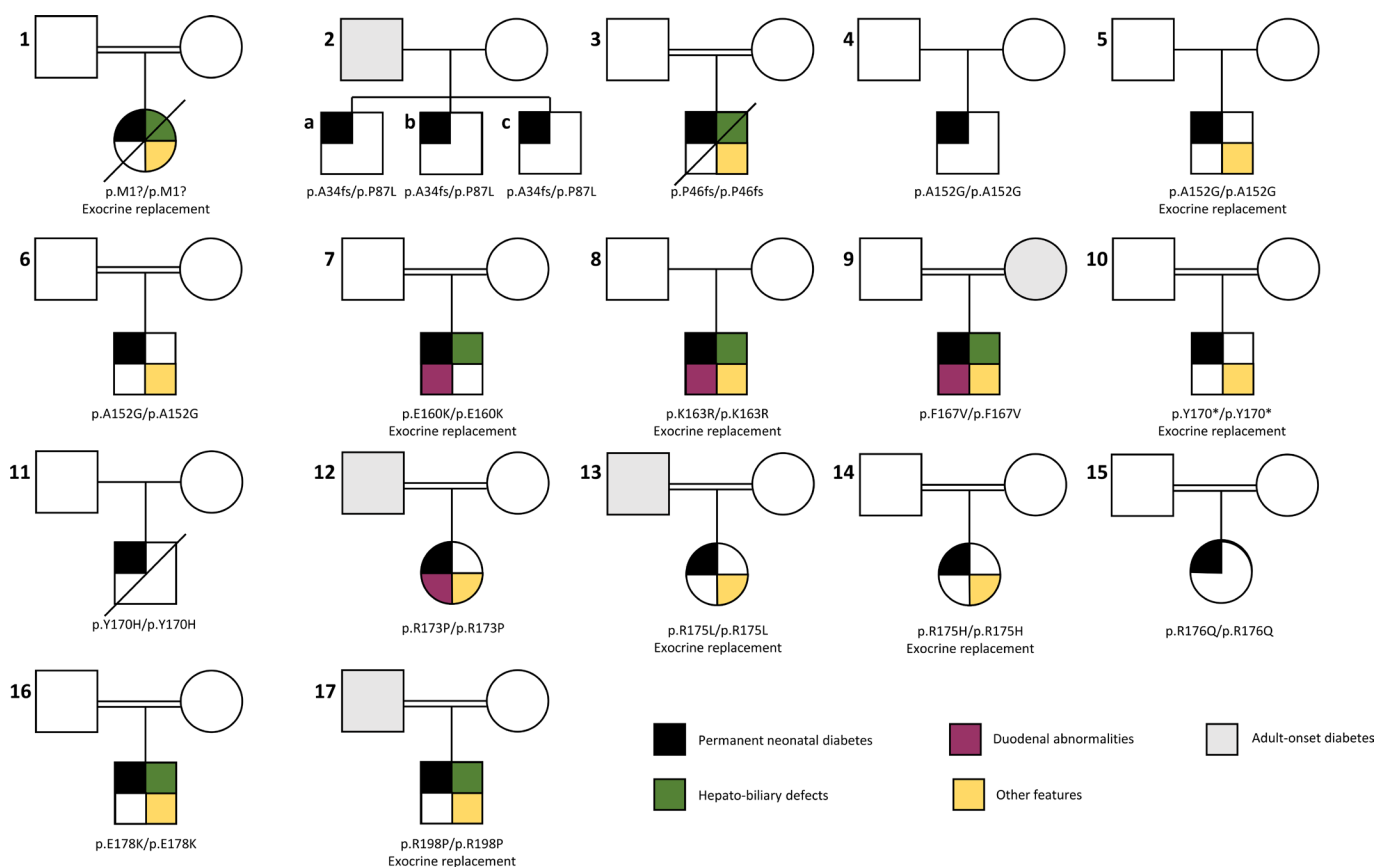
## Assessment of clinical phenotype

Clinical details at diagnosis were collected through a standardised NDM referral form with follow-up information collected for 14 individuals following email contact with the clinicians. The average age at last assessment was 5.3 years (range 2 months to 16 years). Given the difficulties in obtaining abdominal imaging in this international cohort, we clinically defined pancreatic agenesis/hypoplasia as insulin-treated NDM and exocrine insufficiency requiring replacement therapy.<sup>16</sup>

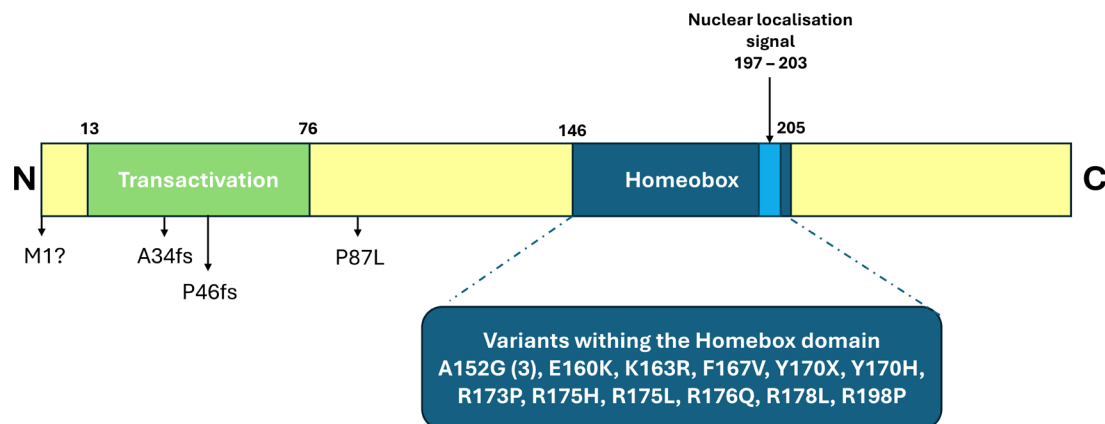
The  $\chi^2$  test was used to assess associations between extrapancreatic features and variant type (missense vs stop gain/start loss/frameshift variants). Three individuals from family 2 who were compound heterozygotes for a missense and a frameshift protein-truncating variant were excluded from this analysis since they harboured both types of variants.

## RESULTS

We identified 16 different *PDX1* variants, including 2 frameshifts, 1 start-loss, 1 stop-gain, and 12 missense, in 19 individuals with NDM (16 singletons and 3 affected siblings) (figures 1 and 2, online supplemental tables 1 and 2).



**Figure 1** Partial pedigrees of the 17 families reported in this study with biallelic *PDX1* pathogenic variants. The variant is shown underneath each pedigree, along with information on whether the patient was on exocrine replacement therapy, consistent with a clinical diagnosis of pancreatic agenesis/hypoplasia. Key shown at the bottom right of the figure.



**Figure 2** Variant location within the PDX1 protein. This schematic shows the location of each variant mapped to the PDX1 protein. The location of the protein domains is shown as reported by UniProt (<https://www.uniprot.org/>). The transactivation domain is essential for transactivation of promoters by PDX1, including the *INS* gene promoter in beta cells.<sup>24</sup> The highly conserved homeobox domain mediates DNA binding and contains the nuclear localisation signal. Only one of the missense variants identified in our study affects a residue within the Homeobox domain which is also part of the nuclear localisation signal (p.R198P).

All but one of the missense variants, p.(Pro87Leu), affected a residue within the homeobox domain of the protein. The variants were homozygous in all cases except for family 2, where the three affected siblings were compound heterozygotes for a missense and a frameshift variant (p.(Ala34fs)/p.(Pro87Leu)).

Patients I-4, I-5, and I-6 were homozygous for the same missense variant, p.(Ala152Gly), despite no known family relationship. Haplotype analysis detected a shared ~4 Mb haplotype on chromosome 13 including *PDX1*, consistent with a common distant ancestor.

### Pancreatic phenotype

All 17 individuals for whom birth weight data were available were born small for gestational age (median birth weight z-score -3.09; range: -4.51 to -1.65). The diabetes was permanent in all cases, with a median age at diagnosis of 20 days (range: 1–181 days, data available for 19/19 individuals). All patients were insulin-treated (median dose 0.93, range 0.2–2.5 U/kg/day at last assessment, data available for 16 individuals).

In eight individuals, NDM and exocrine pancreatic insufficiency requiring replacement therapy was consistent with a clinical diagnosis of pancreatic agenesis/hypoplasia. Fecal elastase measurement was normal (>200 µg) for two of the three affected siblings from family 2 (I-2a and I-2b), suggesting normal exocrine function. Exocrine pancreatic insufficiency was not biochemically investigated in the remaining nine individuals (including the third affected sibling of family 2, I-2c). Clinical symptoms of exocrine insufficiency were reported in only one of these individuals, I-9, who had vitamin D malabsorption and a BMI less than the first centile. Abdominal imaging in this individual showed an annular pancreas.

### Extrapancreatic features

Twelve patients had at least one extrapancreatic feature (63.2% of the cohort at last assessment), (online

supplemental table 1). Developmental abnormalities affecting the duodenum, gallbladder, and hepato-biliary system were most common (8/19, 42%) either in combination or individually. Rarer extrapancreatic features included anemia (n=4), developmental delay (n=4), dysmorphic features (n=3), heart defects (n=2), structural kidney defects (n=2), and epilepsy (n=2).

Three individuals died before 1 year of age. I-1 and I-3, who were homozygous for variants predicted to result in complete loss of PDX1, died of sepsis following multi-organ failure and worsening liver function, respectively. The cause of death was not known in I-11 who was homozygous for a missense variant affecting a residue within the homeobox domain.

### Genotype-phenotype relationship

No association was observed between pancreatic exocrine insufficiency, duodenal/hepatobiliary features, or all extrapancreatic features, and variant type (frameshift/stop-gain/stop-loss vs missense, p=1 for all three conditions).

### DISCUSSION

Our study of 19 individuals with *PDX1*-NDM broadens the phenotype associated with this condition, with 42% of cases having extrapancreatic features affecting the duodenum, gallbladder, and/or hepato-biliary system.

Our results confirm that variability in the pancreatic phenotype exists between individuals with biallelic *PDX1* variants. These variants were originally reported to cause pancreatic agenesis, with subsequent studies identifying patients diagnosed with NDM with either subclinical or no exocrine pancreatic insufficiency.<sup>3 6 8–10</sup> In our cohort, 8/19 patients had NDM and biochemically confirmed exocrine pancreatic insufficiency consistent with pancreatic agenesis/hypoplasia, while 2 individuals (siblings) had no biochemical or clinical evidence of exocrine

pancreatic insufficiency (previously reported<sup>3</sup>). For the nine remaining patients, exocrine pancreatic function was not assessed. While exocrine insufficiency was not clinically suspected in eight of these individuals, in the absence of biochemical tests we cannot exclude the possibility that some had subclinical exocrine insufficiency. Confirming pancreatic exocrine insufficiency in these patients is important because, if untreated, it can lead to impaired growth.

Developmental abnormalities of the duodenum, gallbladder, and hepatobiliary system were identified in 8/19 (42%) individuals in our cohort. These features are consistent with mouse models where *Pdx1* loss results in abnormal development of the pancreas and rostral duodenum.<sup>17</sup> In humans, a recent study showed strong reduction of enteroendocrine cells in intestinal organoids differentiated from induced pluripotent stem cells collected from a patient with pancreatic agenesis and chronic diarrhoea caused by a homozygous p.(Pro63fs) *PDX1* variant.<sup>18</sup> The observation of duodenum and hepatobiliary developmental abnormalities in almost half of our patients, together with the evidence provided by in vivo and in vitro studies, confirm the critical role of *PDX1* in development of the pancreas, duodenum, and hepatobiliary tract.

The phenotypic spectrum observed in our patients with *PDX1*-NDM overlaps with features seen in Mitchell-Riley syndrome caused by recessive *RFX6* variants (OMIM:615710). In vitro studies in human embryonic stem cells have shown that *PDX1* and *RFX6* regulate each other's expression during pancreatic development. *RFX6* binds to an enhancer 10 kb upstream of the *PDX1* transcription start site at the pancreatic gut tube differentiation stage<sup>19</sup> and *PDX1* binds to a putative pancreatic progenitor enhancer ~1 kb downstream of *RFX6*.<sup>20</sup> The overlapping phenotype observed in *PDX1*-NDM and *RFX6*-NDM suggests that these two transcription factors share important regulatory roles in the development of other endodermal organs, in addition to the pancreas.

Genotype-phenotype relationships have been suggested for some *PDX1* variants. Heterozygous variants are associated with type 2 diabetes,<sup>21</sup> the dominant negative p.(Pro63fs) variant was identified in a large pedigree with Maturity-onset diabetes of the young (MODY),<sup>22 23</sup> and recessive variants cause pancreatic agenesis<sup>8-10</sup> or NDM.<sup>3 6</sup> The p.(Pro87Leu) variant identified in family 2 in this study has been suggested to be a hypomorphic variant.<sup>3</sup> Consistent with this, individuals I-2a and I-2b were the only individuals in our cohort with normal fecal elastase measurement and none of the three siblings in family 2 have extrapancreatic features. Furthermore, I-4, I-5, and I-6, who shared the same homozygous variant, did not have hepatobiliary or duodenal defects which were otherwise common in the cohort, suggesting the possibility that the genotype affects the manifestation of these extrapancreatic features. Despite this, we did not find a significant association between the type of variant (missense vs stop-gain/start-loss/frameshift) and the

presence of extrapancreatic features or exocrine insufficiency when assessing the variants as a group. However, our power to detect an association was limited by the size of our cohort.

In conclusion, our findings show that *PDX1* recessive variants can result in syndromic NDM, providing further insights into the role of this transcription factor in human development. Understanding the broader phenotypic spectrum caused by *PDX1* recessive variants is essential for managing patients with this condition.

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**Contributors** NJ researched the follow-up clinical information, analyzed the data, and wrote the first draft of the manuscript. EDF, OAN, SEF, and JALH analyzed the genetic data. MNW performed the bioinformatic analysis on next-generation sequencing data. EG, KAP, ATH and the clinicians of the *PDX1*-NDM consortium analyzed the clinical data. EDF analyzed the genetic data, directed the research, contributed to the discussion, and wrote the manuscript. All authors critically revised the manuscript. The International NDM-*PDX1* Consortium authors identified the patients and reviewed the manuscript. EDF is the guarantor of this work and had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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**Patient consent for publication** Not applicable.

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#### REFERENCES

- De Franco E, Flanagan SE, Houghton JAL, *et al*. The effect of early, comprehensive genomic testing on clinical care in neonatal diabetes: an international cohort study. *Lancet* 2015;386:957–63.
- De Franco E, Johnson MB. Classification of neonatal diabetes. In: Rabbone I, Iafusco D, eds. *Neonatal and Early Onset Diabetes Mellitus: From Pathogenesis to Novelty in Treatment*. Cham: Springer International Publishing, 2023: 39–61.
- De Franco E, Shaw-Smith C, Flanagan SE, *et al*. Biallelic PDX1 (insulin promoter factor 1) mutations causing neonatal diabetes without exocrine pancreatic insufficiency. *Diabet Med* 2013;30:e197–200.
- Globa E, Zelinska N, Johnson MB, *et al*. Neonatal and early-onset diabetes in Ukraine: Atypical features and mortality. *Diabet Med* 2023;40:e15013.
- Kulkarni A, Sharma VK, Nabi F. PDX1 Gene Mutation with Permanent Neonatal Diabetes Mellitus with Annular Pancreas, Duodenal Atresia, Hypoplastic Gall Bladder and Exocrine Pancreatic Insufficiency. *Indian Pediatr* 2017;54:1052–3.
- Nicolino M, Claiborn KC, Senée V, *et al*. A novel hypomorphic PDX1 mutation responsible for permanent neonatal diabetes with subclinical exocrine deficiency. *Diabetes* 2010;59:733–40.
- Sahebi L, Niknafs N, Dalili H, *et al*. Iranian neonatal diabetes mellitus due to mutation in PDX1 gene: a case report. *J Med Case Rep* 2019;13:258.
- Schwitzgebel VM, Mamin A, Brun T, *et al*. Agenesis of human pancreas due to decreased half-life of insulin promoter factor 1. *J Clin Endocrinol Metab* 2003;88:4398–406.
- Stoffers DA, Zinkin NT, Stanojevic V, *et al*. Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. *Nat Genet* 1997;15:106–10.
- Thomas IH, Saini NK, Adhikari A, *et al*. Neonatal diabetes mellitus with pancreatic agenesis in an infant with homozygous IPF-1 Pro63fsX60 mutation. *Pediatr Diabetes* 2009;10:492–6.
- Korula S, Ravichandran L, Paul PG, *et al*. Genetic Heterogeneity and Challenges in the Management of Permanent Neonatal Diabetes Mellitus: A Single-Centre Study from South India. *Indian J Endocrinol Metab* 2022;26:79–86.
- Rapini N, Patera PI, Schiaffini R, *et al*. Monogenic diabetes clinic (MDC): 3-year experience. *Acta Diabetol* 2023;60:61–70.
- Richards S, Aziz N, Bale S, *et al*. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17:405–24.
- Ellard S, Lango Allen H, De Franco E, *et al*. Improved genetic testing for monogenic diabetes using targeted next-generation sequencing. *Diabetologia* 2013;56:1958–63.
- Flanagan SE, De Franco E, Lango Allen H, *et al*. Analysis of transcription factors key for mouse pancreatic development establishes NKX2-2 and MNX1 mutations as causes of neonatal diabetes in man. *Cell Metab* 2014;19:146–54.
- Allen HL, Flanagan SE, Shaw-Smith C, *et al*. GATA6 haploinsufficiency causes pancreatic agenesis in humans. *Nat Genet* 2011;44:20–2.
- Offield MF, Jetton TL, Labosky PA, *et al*. PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. *Development* 1996;122:983–95.
- Krishnamurthy M, Kechele DO, Broda T, *et al*. Using Human Induced Pluripotent Stem Cell-Derived Organoids to Identify New Pathologies in Patients With PDX1 Mutations. *Gastroenterology* 2022;163:1053–63.
- Nakamura T, Fujikura J, Ito R, *et al*. Human RFX6 regulates endoderm patterning at the primitive gut tube stage. *PNAS Nexus* 2024;3:pgae001.
- Wang X, Sterr M, Burtscher I, *et al*. Genome-wide analysis of PDX1 target genes in human pancreatic progenitors. *Mol Metab* 2018;9:57–68.
- Macfarlane WM, Frayling TM, Ellard S, *et al*. Missense mutations in the insulin promoter factor-1 gene predispose to type 2 diabetes. *J Clin Invest* 1999;104:R33–9.
- Stoffers DA, Ferrer J, Clarke WL, *et al*. Early-onset type-II diabetes mellitus (MODY4) linked to IPF1. *Nat Genet* 1997;17:138–9.
- Stoffers DA, Stanojevic V, Habener JF. Insulin promoter factor-1 gene mutation linked to early-onset type 2 diabetes mellitus directs expression of a dominant negative isoprotein. *J Clin Invest* 1998;102:232–41.
- Peshavaria M, Cissell MA, Henderson E, *et al*. The PDX-1 activation domain provides specific functions necessary for transcriptional stimulation in pancreatic beta-cells. *Mol Endocrinol* 2000;14:1907–17.