A case of MODY5-like manifestations without mutations or deletions in coding and minimal promoter regions of the HNF1B gene

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Abstract. Pancreatic tail hypoplasia is a common manifestation of maturity onset diabetes of the young (MODY) 5 that can cause reno-genito-urinary malformations such as renal cysts and bicornuate uterus. A 69-year-old female was admitted to our hospital for consultation on her relatively high HbA1c value. At age 20, she was diagnosed with uterus bicornis. At age 68, she was diagnosed with pancreas tail hypoplasia, renal cysts and non-functioning pancreatic neuroendocrine tumor (NET) in addition to right hydronephrosis due to multiple ureteral bladder carcinomas. She received total right nephrectomy, ureterectomy and partial cystectomy for multiple ureteral bladder carcinomas [non-invasive papillary urothelial carcinoma, low grade (G1), pTa, LV10, u-rtx, RM0, and pN0 (0/8)]. She also received distal pancreatectomy for pancreatic NET [NET G1]. She then was referred to our department at age 69 due to increase in her HbA1c value from 6.2 to 7.2%; 75 g oral glucose tolerance test revealed impaired glucose tolerance. Her clinical characteristics (uterus bicornis, pancreas hypoplasia, and renal cysts) closely resembled the phenotype of MODY5, in which mutations in the HNF1B gene have been reported. Our genetic testing failed to detect any mutation or microdeletion in the coding or minimal promoter regions of the HNF1B gene. Although there remains a possibility that genetic mutations in introns and regulatory regions of the HNF1B gene might cause the MODY5-like manifestations in this patient, these results might suggest involvement of genes other than HNF1B in the pathogenesis of our patient’s disease.

Key words: Maturity onset diabetes of the young 5 (MODY5), Hepatocyte nuclear factor 1 beta (HNF1B), Pancreatic hypoplasia, Pancreatic neuroendocrine tumor, Pancreatic NET
with uterus bicornis, but delivered two healthy babies at age 28 and age 33. At age 50, she started olmesartan due to hypertension, and started receiving regular health checkup every year. No abnormalities in her HbA1c or plasma glucose values were noted, and she reported no history of severe abdominal or back pain. At age 68, she noticed hemosiderinuria without abdominal pain, and abdominal echosonography by her family doctor disclosed bladder tumor. She was then referred to the urology department at our hospital. Upon admission, her eGFR was 48.1 mL/min/1.76 cm². Computer tomography (CT) scan showed left renal cysts, pancreatic tumor, and pancreatic tail hypoplasia in addition to right renal hydronephrosis due to multiple ureteral bladder tumors (Fig. 2). Neither magnetic resonance cholangiopancreatography (MRCP) nor endoscopic retrograde cholangiopancreatography (ERCP), which is necessary for diagnosis of agenesis of dorsal pancreas (ADP), was performed, as physicians-in-charge prioritized surgical resection of her ureteral bladder tumors. She received total right nephrectomy, ureterectomy and partial cystectomy [non-invasive papillary urothelial carcinoma, low grade (G1), pTa, LV10, u-rtx, RM0, and pN0 (0/8)] (Fig. 3A and 3B). Papillary urothelial carcinomas originate from urothelial cells in the bladder lining. Urothelial cells also line the urethra, ureters, and other parts of the urinary tract; cancer can also originate from these areas [9]. In this patient, multiple carcinomas were seen in both bladder and ureter, but their origin was unclear. The pathological analysis of the patient’s resected right kidney also revealed multiple cysts lined with epithelial cells (Fig. 3C and 3D), while CT scan failed to detect cysts in the patient’s right kidney due to the renal hydronephrosis. Regarding her pancreatic tumor, endoscopic ultrasound-fine needle aspiration (EUS-FNA) biopsy revealed a non-functional neuroendocrine tumor (NET) G1, for which laparoscopic distal pancreatectomy was performed (Fig. 3E). Immunohistochemistry showed that the tumor was positive for CD56, chromogranin A and synaptophysin, which is consistent with the diagnosis of NET (Fig. 3F–3H), and negative for gastrin, insulin and the other hormones examined. Both the Ki-67-index (1%) and mitotic count (<2/10 HPF) were low (Fig. 3I), and the tumor was diagnosed as NET G1 according to the WHO 2019 criteria [10].

During the 6 months after pancreatectomy, her body...
weight and BMI increased from 48 kg to 53 kg and from 21.9 to 24.2, respectively. At age 69, her HbA1c value increased from 6.2 to 7.2%, and she was referred to our department. Her blood data including the concentrations of Mg$^{2+}$, K$^+$ and uric acid were almost normal except for her renal function (eGFR 43 mL/min/1.73 m$^2$) (Table 1). 75 g oral glucose tolerance test (OGTT) revealed impaired glucose tolerance: her plasma glucose levels were 0 min, 121 mg/dL; 30 min, 247 mg/dL; 60 min, 262 mg/dL; 90 min, 221 mg/dL; and 120 min, 163 mg/dL, and her serum insulin levels were 0 min, 10.7 μU/mL; 30 min, 71.6 μU/mL; 60 min, 58.8 μU/mL; 90 min, 66.8 μU/mL; and 120 min, 55.6 μU/mL. Her homeostasis model assessment (HOMA)-β, insulinogenic index, HOMA-insulin resistance (IR), and quantitative insulin sensitivity check index (QUICKI) were 66.4% (normal range; 40–60%), 0.48 (normal range; >0.4), 3.2 (normal range; 1.0–1.5), and 0.10 (normal range; 0.357–0.382), respectively; these values need to be interpreted carefully due to her moderately reduced renal function (eGFR 43 mL/min/1.73 m$^2$).

She had MODY5-like manifestations including uterus bicornis, renal cysts and pancreatic hypoplasia, and her HNF1B score was 16 (<8 predicts negative testing) (Table 2) [11], which strongly suggested that she had MODY5 caused by HNF1B gene mutation. Moreover, she had some tumors in the urogenital tract and pancreas, tissues in which HNF1B is highly expressed. We therefore searched for mutations of HNF1B and other MODY genes and microdeletions of selected MODY genes (i.e., HNF4A, GCK, HNF1A and HNF1B) after obtaining the patient’s informed consent for genetic testing. No MODY 1–14 gene mutations or microdeletions of the selected MODY genes including HNF1B were found (Fig. 4). We also failed to detect mutations in the minimal promoter region of the HNF1B gene (−580 bp relative to the initiator ATG codon). Human HNF1B is known to be expressed in pancreatic ductal epithelium cells and centroacinar cells and intercalated ductal cells with predominant nuclear and faint cytoplasmic staining [12]. Immunohistochemical analysis using anti-HNF1B antibody (Catalogue number HPA002083, Atlas antibodies) found expression of HNF1B protein in some pancreatic cells including ductal epithelium cells and possibly
centroacinar cells and intercalated ductal cells (Fig. 3J and 3K), which was similar to those previously reported (https://www.proteinatlas.org/ENSG00000275410-HNF1B/tissue/pancreas). Expression of HNF1B protein was not detected in urothelial carcinoma and pancreatic NET (Fig. 3B and 3J).

Written informed consent was obtained from the patient for publication of this report. Formal ethics approval was waived, as this is a case report.

**Genetic Analysis of MODY Genes**

MODY 1–14 gene mutations were analyzed by targeted panel sequencing using the sequence-capture

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**Fig. 3** Histological analysis of urothelial carcinoma (A and B), renal cyst (C and D) and pancreatic neuroendocrine tumor (E–K). Panels A and C–E, hematoxylin-eosin staining; panels B, J and K, anti-HNF1B staining; panel F, anti-CD56 staining; panel G, anti-chromogranin A staining; panel H, anti-synaptophysin staining; and panel I, anti-Ki67 staining. Magnification, ×10 (A–C, E and J), ×40 (D, F–I and K). Panel A shows papillary urothelial carcinoma in ureter. Multiple carcinomas are seen in both bladder and ureter. Panel C shows one of the renal cysts in the resected right kidney, which is located under the renal capsule and connected to the renal pelvis dilated due to hydronephrosis. Arrows in panel D (a magnified image of the boxed area in panel C) indicate epithelial cells lining a renal cyst. Asterisks in panels E and J indicate pancreatic endocrine tumors. Pancreatic cells including ductal epithelium cells (arrows in panel K) and possibly centroacinar cells and intercalated ductal cells were stained by anti-HNF1B antibody (Catalogue number HPA002083, Atlas antibodies) in panels J and K (a magnified image of the boxed area in panel J).
We report here a patient with MODY5-like manifestations for whom scrupulous analysis failed to detect mutations or microdeletions in coding and minimal promoter regions of the HNF1B gene. In general, the major features of MODY5 with HNF1B gene mutation include heterogeneous cystic renal disease, diabetes, pancreatic hypoplasia, liver dysfunction and genital tract anomalies [1-5]; the HNF1B score was developed previously as a simple and accurate tool to provide for a rational approach to selecting patients for HNF1B genetic testing based on the presence or absence of these features [11]. The present case demonstrated pancreatic hypoplasia, renal cysts, papillary urothelial carcinoma, and bicornuate uterus; her HNF1B score was high enough to consider genetic testing, which detected no abnormalities in coding or minimal promoter regions of the gene.

ADP results from failure or regression of the dorsal pancreatic bud to form the body and tail of the pancreas in the developing fetus [13, 14]. Gene targeting studies in mice demonstrate that several transcription factors have important roles in pancreatic development [13]. Among them, PTF1A deficiency results in a hypoplastic dorsal bud and a total lack of acinar cells [15, 16]. HNF1B is required for ventral bud development, and defect in the gene can result in pancreatic agenesis similarly to PTF1A deficiency [7, 8, 15, 16]. Pdx1 deficiency also leads to pancreatic agenesis due to failed growth of the pancreatic primordium [17]. We therefore performed a genetic test for PTF1A and PDX1, which failed to detect any abnormalities in coding regions of the genes. Thus, it is possible that abnormalities in other genes might be involved in the pathogenesis of the current case; further genetic investigation is clearly needed. In

### Table 1 Laboratory data of the patient

| Urinalysis                      | Biochemistry                 |
|---------------------------------|------------------------------|
| Specific gravity                | TP (g/dL)                    |
| Protein                         | albumin (g/dL)               |
| Glucose                         | AST (U/L)                    |
| RBC                             | ALT (U/L)                    |
| Ketone                          | LDH (U/L)                    |
| Complete Blood Count            | ALP (U/L)                    |
| WBC (×10^3/μL)                  | γGTP (U/L)                   |
| RBC (×10^3/μL)                  | ChE (U/L)                    |
| Hb (g/dL)                       | T-Bil (mg/dL)                |
| Ht (%)                          | AMY (U/L)                    |
| MCV (fL)                        | CRE (mg/dL)                  |
| MCHC (%)                        | UA (mg/dL)                   |
| Plt (×10^3/μL)                  | BUN (mg/dL)                  |
| Hb (g/dL)                       | 6.29 (3.3–8.6)               |
| RBC (×10^3/μL)                  | 4.05 (3.86–4.92)             |
| Hb (g/dL)                       | 12.8 (13.7–16.8)             |
| MCHC (%)                        | 33.3 (31.7–35.3)             |
| Plt (×10^3/μL)                  | 22.2 (15.8–34.8)             |

**AMY**, amylase; **ALP**, alkaline phosphatase; **ALT**, alanine aminotransferase; **AST**, aspartate aminotransferase; **BUN**, blood urea nitrogen; **ChE**, choline esterase; **CRE**, creatinine; **γGTP**, γ-glutamyltransferase; **Hb**, hemoglobin; **Ht**, hematocrit; **LDH**, lactate dehydrogenase; **MCHC**, mean corpuscular hemoglobin concentration; **MCV**, mean corpuscular volume; **Plt**, platelet; **RBC**, red blood cell; **T-Bil**, total bilirubin; **T-cho**, total cholesterol; **TG**, triglyceride; **TP**, total protein; **UA**, uric acid; **WBC**, white blood cell.
Table 2  HNF1B scoring of the patient

| Characteristics                                           | Points | This patient |
|-----------------------------------------------------------|--------|--------------|
| Family history                                            | 2      | 0            |
| Antenatal renal abnormalities by renal echography         | Uni/bilateral abnormality by renal echography | 2 | 0 |
| Left kidney                                               | Hyper-echogenicity          | 4 | 0 |
|                                                           | Renal cysts                 | 4 | 4 |
|                                                           | Hypoplasia                  | 2 | 0 |
|                                                           | Multi-cystic and dysplastic kidney | 2 | 0 |
|                                                           | Urinary tract malformation  | 1 | 0 |
|                                                           | Solitary kidney             | 1 | 0 |
| Right kidney                                              | Hyper-echogenicity          | 4 | 0 |
|                                                           | Renal cysts                 | 4 | 4 |
|                                                           | Hypoplasia                  | 2 | 0 |
|                                                           | Multi-cystic and dysplastic kidney | 2 | 0 |
|                                                           | Urinary tract malformation  | 1 | 0 |
|                                                           | Solitary kidney             | 1 | 0 |
| Electrolyte or uric acid disorders                        | Low serum Mg²⁺ (<0.7mmol/L) | 2 | 0 |
|                                                           | Low serum K⁺ (<3.5mmol/L)   | 1 | 0 |
|                                                           | Early-onset gout (>30 years of age) | 2 | 0 |
| Pathological findings                                     | Oligomeganephronia or glomerular cysts | 1 | 0 |
| Pancreas                                                  | MODY or hypoplasia of tail and neck of the pancreas or pancreatic exocrine insufficiency | 4 | 4 |
| Genital tract                                             | Genital tract abnormality   | 4 | 4 |
| Liver                                                     | Live test abnormalities of unknown origin | 2 | 0 |
| HNF1B score                                               | 16 (≥8)                     |   |   |

Fig. 4  Analysis for genomic DNA copy number variation of HNF1B and three other MODY genes. The copy number variation (CNV) of HNF1B and other genes (glucokinase, HNF1A, and HNF4A) was evaluated by multiplex ligation-dependent probe amplification (MLPA)-targeted gene sequencing using Salsa Multiplex Ligation-dependent Probe Amplification (MLPA) Kit P241 and 357 (MRC-Holland, Amsterdam, the Netherlands). GCK, glucokinase; hepatocyte nuclear factor 1A, HNF1A; hepatocyte nuclear factor 1B, HNF1B; hepatocyte nuclear factor 4A, HNF4A.
the current case, non-functioning pancreatic NET was seen in addition to ADP. ADP is often associated with pancreatic ductal adenocarcinoma; pancreatic NET is rarely associated with ADP. Therefore, understanding the genetic abnormalities in the current patient may shed light on the relationship between pancreatic NET and ADP [13].

The expression of HNF1B protein is known to be associated with risk of several cancers including pancreatic ductal adenocarcinoma, hepatocellular carcinoma, and renal cancer [6], and various cancers were seen in our patient’s family members (Fig. 1). While HNF1B expression was not investigated in cancer cells of the family members, no detectable expression of HNF1B protein was seen in her urothelial carcinoma as well as her pancreatic NET (Fig. 3B and 3J), suggesting that HNF1B protein played little role in the pathogenesis of her urothelial carcinoma and pancreatic NET.

The current study has several limitations. 1) We did not investigate mutations and deletions in introns and other regulatory regions of the HNF1B gene that might cause aberrant splicing variants and/or reduce expression of HNF1B mRNA. Especially, since MODY5 is a dominantly inherited disorder often caused by a loss-of-function mutation, detection of HNF1B protein in some pancreatic cells including ductal epithelium cells and possibly centroacinar cells and intercalated ductal cells in the current patient by our non-quantitative immunohistochemical study (Fig. 3K) cannot rule out half normal expression of HNF1B protein. Further RNA-sequencing analysis of the patient’s tissues may have helped ascertain the presence or absence of abnormalities in the HNF1B gene. 2) Diagnosis of ADP requires confirmation of the absence of the pancreatic body and tail using ERCP or MRCP [13]. Our patient did not receive ERCP or MRCP before dorsal pancreatectomy, as physicians-in-charge at our urology department prioritized surgical resection of her ureteral bladder tumors. However, pancreatic hypoplasia with no previous history of abdominal or back pain, chronic pancreatitis, and no fatty replacement or calcification of the pancreas on CT image does not contradict a diagnosis of ADP. 3) While we believe that HNF1B protein was expressed in pancreatic ductal epithelium cells and possibly centroacinar cells and intercalated ductal cells (Fig. 3K), we failed to determine the exact cell type of HNF1B-positive cells as we did not have antibodies that can distinguish each pancreatic cell type by immunohistochemistry.

In conclusion, this is a rare case exhibiting MODY5-like clinical manifestations without any mutations or deletion in the proximal promoter or coding regions of the HNF1B gene. Further genetic investigation may shed light on the pathogenesis of such patients.

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Authorship

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Disclosures

Katsumi Iizuka received honoraria for lectures from Novo Nordisk Pharma, Ltd and Kowa Company, Ltd. Yukio Horikawa received honoraria for lectures from MSD K.K., Kowa Company, Ltd. and Astellas Pharma Inc. Daisuke Yabe received consulting or speaker fees from MSD K.K. and Novo Nordisk Pharma Ltd. Daisuke Yabe also received clinically commissioned/joint research grants from Nippon Boehringer Ingelheim Co., Ltd., Eli Lilly and Company, Taisho Toyama Pharmaceutical Co. Ltd., MSD K.K., Ono Pharmaceutical Co. Ltd., Novo Nordisk Pharma Ltd., Arklay Co. Ltd., and Takeda Pharmaceutical Company Limited. Kenta Nonomura, Yayoi Kuwabara-Ohmura, Ken Takao, Takehiro Kato, and Yanyan Liu declare that they have no conflict of interest.

Compliance with Ethics Guidelines

All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from the patient.
References

1. Horikawa Y (2018) Maturity-onset diabetes of the young as a model for elucidating the multifactorial origin of type 2 diabetes mellitus. *J Diabetes Investig* 9: 704–712.
2. Horikawa Y, Iwasaki N, Hara M, Furuta H, Hinokio Y, et al. (1997) Mutation in hepatocyte nuclear factor-1 beta gene (TCF2) associated with MODY. *Nat Genet* 17: 384–385.
3. Clissold RL, Hamilton AJ, Hattersley AT, Ellard S, Bingham C (2015) HNF1B-associated renal and extra-renal disease—an expanding clinical spectrum. *Nat Rev Nephrol* 11: 102–112.
4. Edghill EL, Bingham C, Ellard S, Hattersley AT (2006) Mutations in hepatocyte nuclear factor-1beta and their related phenotypes. *J Med Genet* 43: 84–90.
5. Dubois-Laforgue D, Cornu E, Saint-Martin C, Coste J, Bellanné-Chantelot C, et al. (2017) Diabetes, associated clinical spectrum, long-term prognosis, and genotype/phenotype correlations in 201 adult patients with hepatocyte nuclear factor 1B (HNF1B) molecular defects. *Diabetes Care* 40: 1436–1443.
6. Yu DD, Guo SW, Jing YY, Dong YL, Wei LX (2015) A review on hepatocyte nuclear factor-1beta and tumor. *Cell Biosci* 5: 58.
7. Dassaye R, Naidoo S, Cerf ME (2016) Transcription factor regulation of pancreatic organogenesis, differentiation and maturation. *Islets* 8: 13–34.
8. Haumaitre C, Barbacci E, Jenny M, Ott MO, Gradwohl G, et al. (2005) Lack of TCF2/vHNF1 in mice leads to pancreas agenesis. *Proc Natl Acad Sci USA* 102: 1490–1495.
9. Razdan S, Kirschenbaum A (2019) Papillary urothelial carcinoma of the vagina: a case presentation and review of the literature. *Urol Case Rep* 29: 101091.
10. WHO Classification of Tumours Editorial Board (2019) Digestive System Tumours. In: WHO Classification of Tumours (5th). IARC, Lyon, France.
11. Faguer S, Chassaing N, Bandin F, Prouheze C, Garnier A, et al. (2014) The HNF1B score is a simple tool to select patients for HNF1B gene analysis. *Kidney Int* 86: 1007–1015.
12. Yang MX, Coates RF, Ambaye A, Gardner JA, Zubarick R, et al. (2018) Investigation of HNF-1B as a diagnostic biomarker for pancreatic ductal adenocarcinoma. *Biomark Res* 6: 25.
13. Jennings RE, Berry AA, Strutt JP, Gerrard DT, Hanley NA (2015) Human pancreas development. *Development* 142: 3126–3137.
14. Rittenhouse DW, Kennedy EP, Mascaro AA, Brumbaugh JL, Stein LH, et al. (2011) The novel triad of dorsal agenesis of the pancreas with concurrent pancreatic ductal adenocarcinoma and nonalcoholic chronic calcific pancreatitis: a case series and review of the literature. *J Gastrointest Surg* 15: 1643–1649.
15. Kawaguchi Y, Cooper B, Gannon M, Ray M, MacDonald RJ, et al. (2002) The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. *Nat Genet* 32: 128–134.
16. Krapp A, Knofler M, Ledermann B, Burki K, Berney C, et al. (1998) The bHLH protein PTF1-p48 is essential for the formation of the exocrine and the correct spatial organization of the endocrine pancreas. *Genes Dev* 12: 3752–3763.
17. Guz Y, Montminy MR, Stein R, Leonard J, Gamer LW, et al. (1995) Expression of murine STF-1, a putative insulin gene transcription factor, in beta cells of pancreas, duodenal epithelium and pancreatic exocrine and endocrine progenitors during ontogeny. *Development* 121: 11–18.