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CASE REPORT

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Absence of a PDX-1 mutation and normal gastroduodenal immunohistology in a child with pancreatic agenesis

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Abstract Pancreatic agenesis is a rare condition, of which only a limited number of cases have been described. One recent paper reported a homozygous mutation in the pancreatic duodenal homeobox gene 1 (PDX-1) in a child with pancreatic agenesis. We report a 6-year-old boy with pancreatic agenesis, treated medically, without abnormalities in the PDX-1 gene coding sequence and with normal gastroduodenal endocrine cell distribution. Genes other than PDX-1 also appear to be involved in human pancreatic agenesis.

Keywords PDX-1 · Pancreas · Agenesis · Immunohistochemistry · Mutation analysis

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Introduction

Pancreatic agenesis is a rare condition, of which only a limited number of cases have been described in the literature so far [1, 3, 6, 7, 8, 9, 11]. Recently, in one of these patients, a homozygous frameshift mutation in the pancreatic duodenal homeobox gene 1 (PDX-1) was described, leading to a 16-kDa truncated PDX-1 protein [9]. Both parents were heterozygous for the mutation. Interestingly, there was a strong family history of non-insulin dependent diabetes mellitus (NIDDM), with the father satisfying the criteria for maturity-onset diabetes of the young (MODY) [10].

The PDX-1 gene has been shown to be of critical importance for pancreatic development [2]. Mice with a homozygous PDX-1 deletion present with pancreatic agenesis and abnormalities of gastric and duodenal endocrine cells [5]. In man, no data are available about these endocrine cells in pancreatic agenesis. The PDX-1 gene is localized on chromosome 13q12.1, consists of two exons spanning more than 5 kb of genomic DNA, and encodes 283 amino acids [4]. The protein is believed to function as a key transcription factor in pancreatic development, binding to regulatory DNA sequences [2].

In this report, we describe a 6-year-old boy with pancreatic agenesis, originally presenting with hyperglycemia and cholestasis. We analyzed the patient's genomic DNA for PDX-1 mutations, performed immunohistochemistry on gastric and duodenal biopsies from the patient, and then compared them with those of normal healthy age-matched controls.

Clinical history

A 6-year-old boy with a normal karyotype was born at term in September 1993 by Cesarean section. The pregnancy was complicated with intrauterine growth retardation without evidence of infections or placental dysfunction. He is the only child of healthy non-consanguineous parents with unremarkable obstetric and fam-

ily histories. Notably, there were no individuals with type-1 or type-2 diabetes mellitus or unexpected infantile deaths in either family. At birth, the patient was small for gestational age (SGA), with a weight of 1500 g [below the third percentile (P3)] and a head circumference of 32.5 cm (P25), but he had a good start. At 10 days of age, he was transferred to the Sophia Childrens' Hospital/University Hospital Rotterdam because of hyperglycemia and cholestasis. Echographic and computer tomographic analysis of the abdomen suggested absence of the pancreas (Fig. 1). In addition, extrahepatic biliary atresia was suspected because of the persistently elevated bilirubin and moderate liver insufficiency. Therefore, percutaneous cholangiography was performed, showing a choledochal duct but no cystic duct or gallbladder (Fig. 2). A small atrial septal defect (type II) and a small ventricular septal defect were detected by means of echocardiography. An expectative management was instituted for both.

Neither a pancreas nor a gallbladder was detected by means of explorative laparotomy. A choledochotomy was performed and a transhepatic drain was inserted because of a distal stenosis of the choledochal duct. This drain stayed in situ for 10 days, during and after which all liver function tests normalized and the jaundice resided. During this period, blood glucose concentrations were repeatedly above 15 mM, while at the same time insulin and C-peptide concentrations were below 2 mU and 0.05 nM, respectively, which were the lower limits of detection. Treatment was started with intravenous short-acting insulin preparations, later followed by long-actin insulin preparations. It was difficult to obtain normal glucose levels in the patient's first year of life but, after the first year, the situation improved. Presently, there is adequate control of glycemia. In general, his insulin requirements are comparable with any other infant or child with early onset of type-1 diabetes mellitus. During the first year of life, some time after the introduction of insulin therapy, serum was taken for antibody detection. There were antibodies to insulin, but no islet cell antibodies (ICA) or antibodies to glutamic acid decarboxylase (GAD) were detectable. The exocrine pancreatic insufficiency was treated with pancreatin and supplements of vitamin A, vitamin D, vitamin E, and vitamin K. Presently, the patient is a healthy child with adequate growth, psychological and motor development, and adequate control of his exocrine and endocrine pancreatic insufficiency.

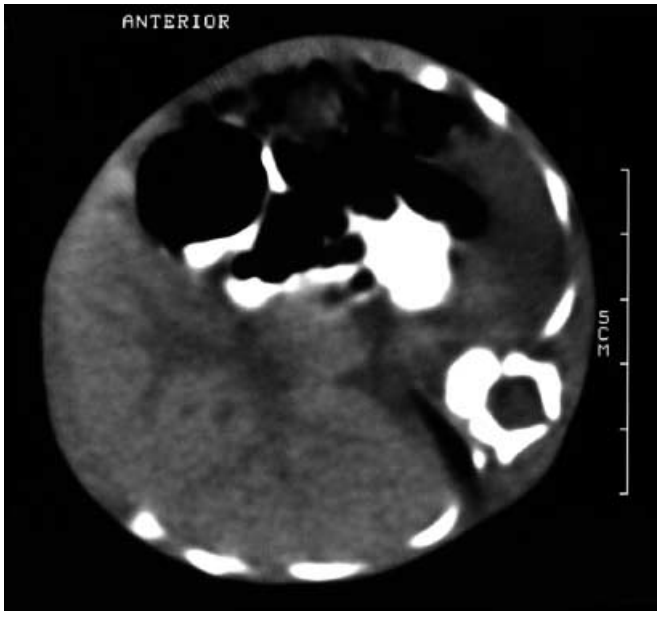

Fig. 1 Computerized tomography scan after oral contrast, which shows a normal position of the liver and spleen but no pancreas configuration and no gallbladder

Materials and methods

Histology and immunohistochemistry

Sections (5-μm thick) of paraffin-embedded gastric and duodenal biopsies of the index patient and three age-matched controls were deparaffinized. Hematoxylin and eosin staining was performed according to standard methods. For immunohistochemistry, sections were incubated for 30 min at room temperature with antibodies to insulin, glucagon, somatostatin, synaptophysin (1:500, 1:40, 1:2000, and 1:100, respectively; Dako, Glostrup, Denmark), chromogranin-A, vasoactive intestinal polypeptide (VIP; 1:150 and 1:100, respectively; Klinipath Biogenex, Uden, The Netherlands), pancreatic polypeptide (PP), serotonin (undiluted and 1:100, respectively; Eurodiagnostica, Apeldoorn, The Netherlands), PGP9.5 (1:1000; Biogenesis, Poole, UK), PDX-1 (1:400; a gift from Dr. H. Edlund,

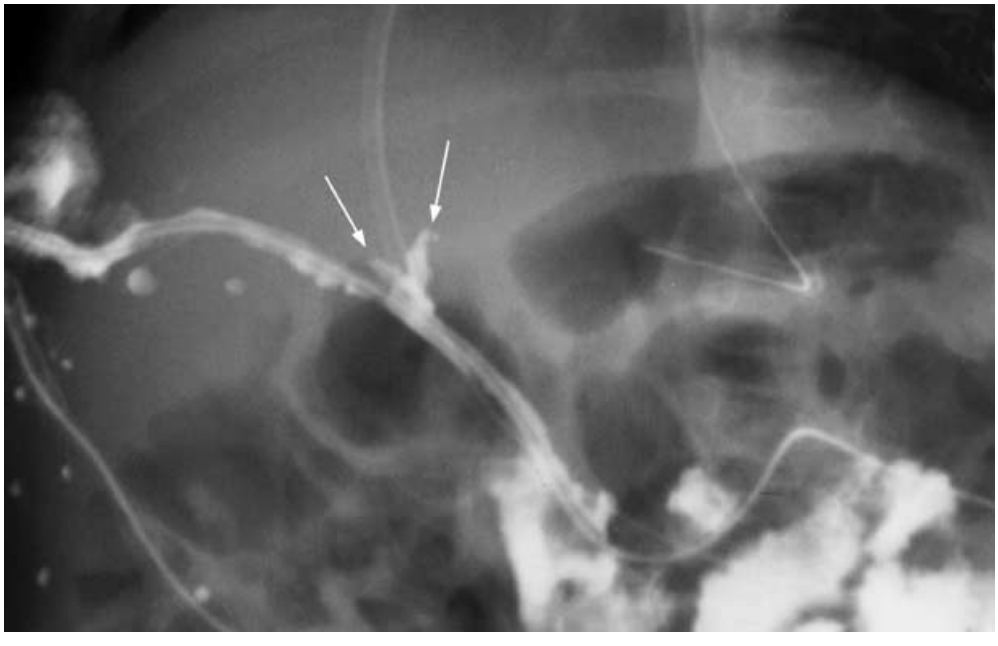

Fig. 2 Percutaneous cholangiography showing a normal caliber and position of the choledochal duct and sparse filling of the intrahepatic bile ducts (*arrows*) but no filling of a cystic duct or gallbladder

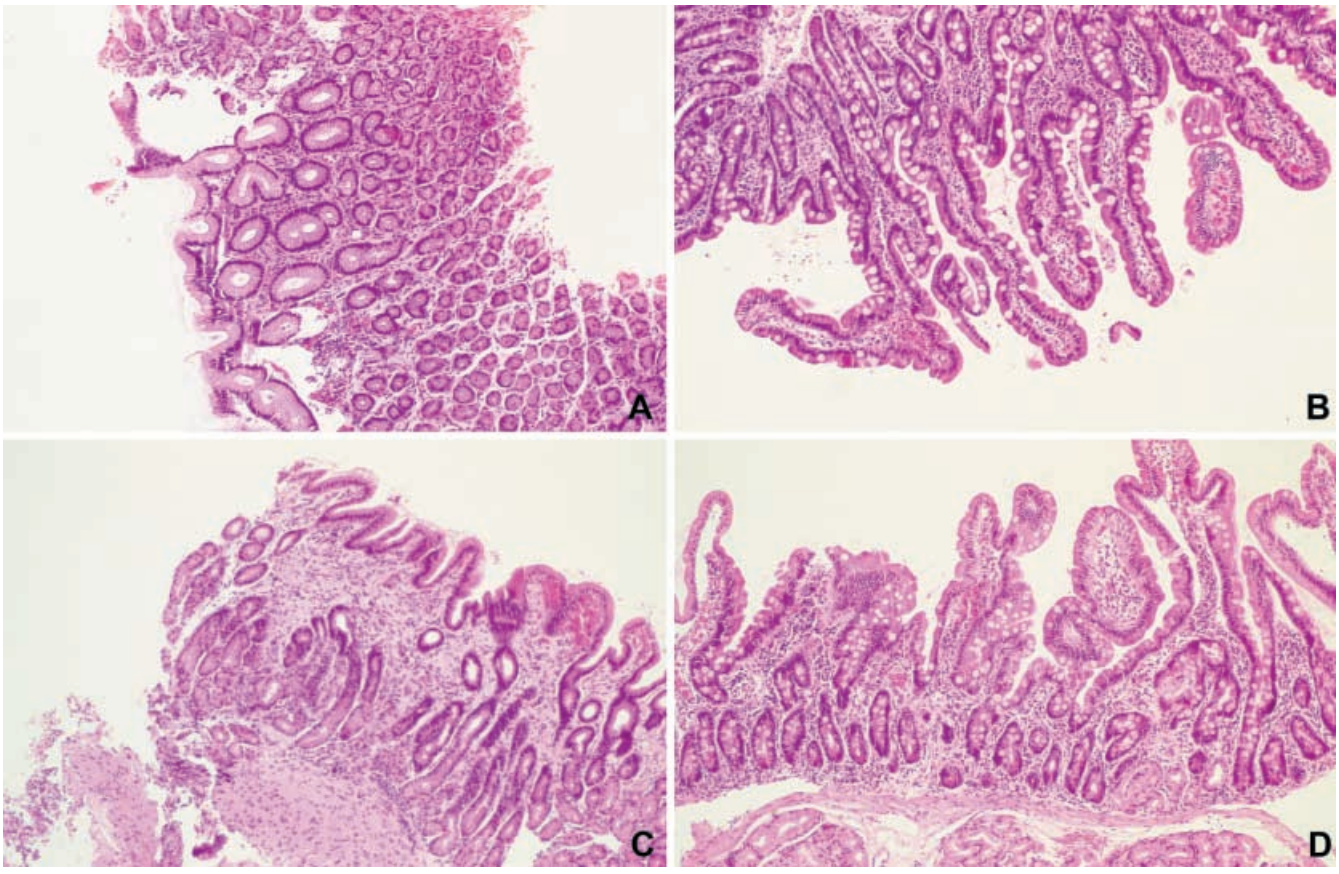

Fig. 3 Comparison of gastric (**A**, **C**) and duodenal (**B**, **D**) histology on routine hematoxylin and eosin-stained sections of the index patient (**A**, **B**) and an age-matched control patient (**C**, **D**). No mucosal differences are apparent

Umeå, Sweden), and gastrin (1:8000; a gift from Prof. J.F. Rehfeld, Copenhagen, Denmark). For synaptophysin only, a 15-min microwave antigen retrieval method in citrate buffer (pH 6.0) was used.

Sections were then washed and a biotinylated goat-anti-multilink (1:50; Klinipath Biogenex) with 2% normal human serum and 2% normal goat serum (Dako) was added for 30 min, followed by the avidin-biotin complex (1:50; Klinipath Biogenex) for 30 min. Sections were developed with diamino-benzidine tetrahydrochlorate (Fluka, Neu-Ulm, Germany) with 0.3% H_2O_2 for 7 min, counterstained, dehydrated, and mounted. For PDX-1 detection, sections were microwaved twice, 5 min each time, in citrate buffer (pH 6.0) and then incubated overnight. Subsequently, a biotinylated anti-rabbit immunoglobulin (Ig)G was applied, followed by alkaline phosphatase-conjugated streptavidin (Dako). Alkaline phosphatase activity was revealed by incubation in bromo-chloro-indolyl phosphate-nitroblue tetrazolium medium [5]. Negative control slides were prepared by omitting the primary antiserum or replacing it by a pre-immune serum. No immunoreactivity was observed in the control sections.

PDX-1 gene analysis

Genomic DNA was prepared from patient and control peripheral blood samples according to standard methods. For the polymerase chain reaction (PCR), two pairs of intron primers were designed according to the available PDX-1 gene sequence information (Genbank accession number U35632) to amplify the two exons and part of the flanking intron sequences. The primers exon1f (AACGCCACACAGTGCCAAAT), exon1r (AGGCTTACCTGCCCACTG), exon 2f (GCCCTGTGTCGCCCGCAGG), and exon 2r (CGCCTACGCTGCGGAGC) were used, yielding PCR products of 480 bp and 500 bp, respectively. PCR reactions for both exons were performed with 2.5 U *Taq* DNA polymerase (AmpliTaq GoldTM, Perkin Elmer, Norwalk, Conn.) and consisted of 35 cycles (94°C for 1 min, 55°C for 1 min, 72°C for 2.5 min) on a PTC100 Thermocycler (MJ Research, Waltham, Mass.), followed by a final extension step at 72°C for 10 min. PCR products were analyzed on a 1.5% agarose gel.

Sequencing of PCR products was done with the Big Dye Terminator system (Perkin Elmer) on an automatic sequence analyzer (ABI377, Applied Biosystems, Foster City, Calif.) after purification on a column (Qiagen, Valencia, Calif.). The PCR reaction consisted of 15 cycles (96°C for 10 s, 55°C for 5 s, and 70°C for 4 min) followed by another 15 cycles (96°C for 10 s and 70°C for 60 s). The primers used were the same as those used for the PCR reactions.

Results

Histology and immunohistochemistry

The gastric biopsy from the index patient was taken from the gastric corpus and had a normal histological architecture (Fig. 3A, C). In comparison with biopsies from the same gastric area from control patients, there was a normal frequency and distribution of endocrine cells, evidenced by positive immunoreactivity with chromogranin-A (Fig. 4A, C) and synaptophysin. A major proportion of these cells also showed immunoreactivity with antibodies to serotonin (Fig. 4B, D). None of the other markers was detected.

In the duodenal biopsy from the index patient, the histological picture was also normal and similar to that of

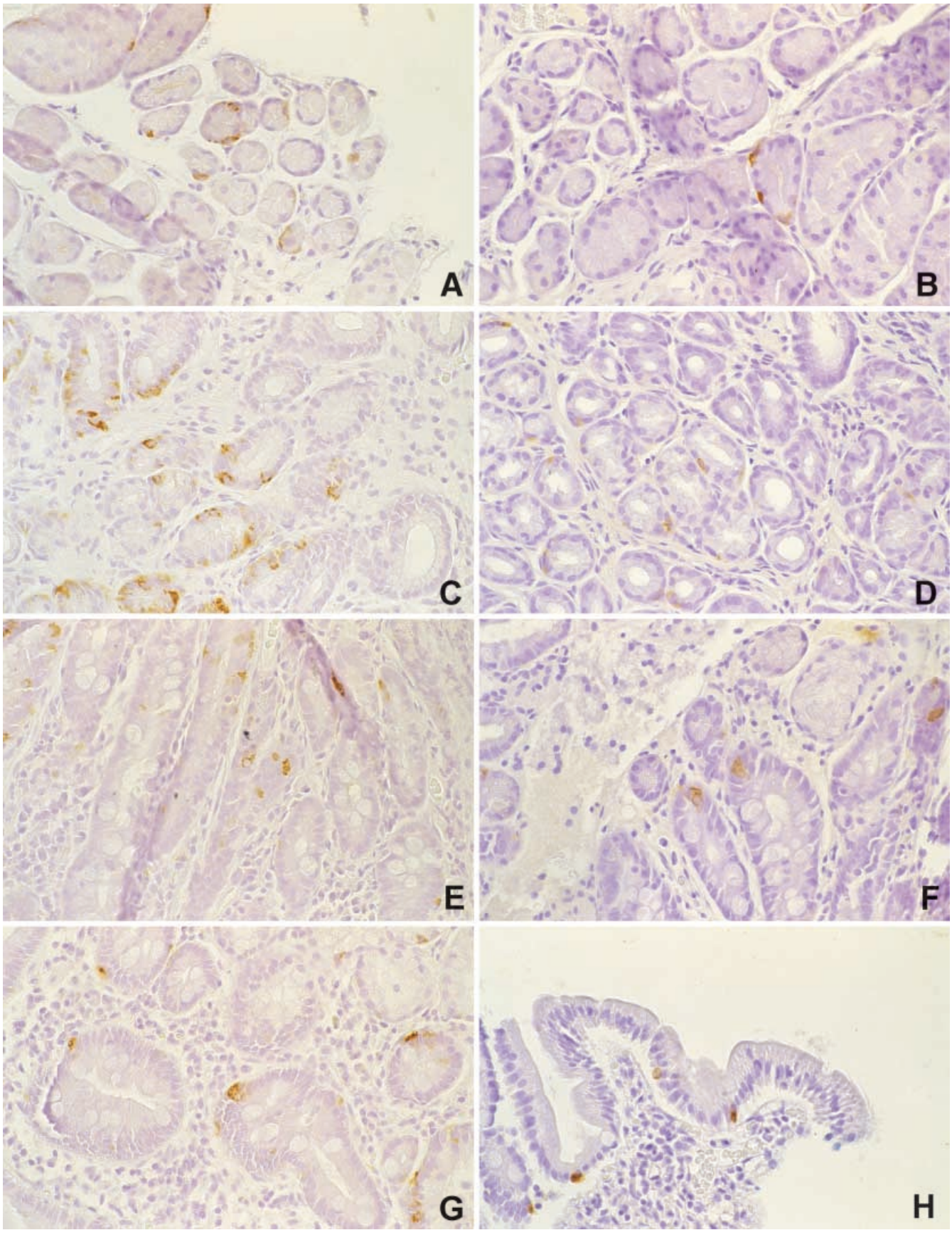

Fig. 4 Legend see page 684

the control patients, with villi of normal length (Fig. 3B, D). Also, immunohistochemistry did not yield quantitative or qualitative differences in the staining pattern of the endocrine cells, which were highlighted with chromogranin-A and synaptophysin immunoreactivity (Fig. 4E, G). Many of these cells also showed reactivity with gastrin and serotonin antibodies, with a predominance of serotonin-reactive cells (Fig. 4F, H). In addition, few somatostatin-reactive cells were detected. There was a weak but clear nuclear reactivity with the PDX-1 antibody in a subpopulation of duodenal epithelial cells in both index patient and normal controls. All other markers tested were negative. In both tissues, PGP9.5 showed reactivity to nerve fibers but not to epithelial mucosal cells.

PDX-1 gene analysis

Compared with the above-mentioned genome database, no mutations were found in the coding and noncoding regions of both amplified exons of the PDX-1 gene.

Discussion

In this case report, we presented a patient with pancreatic agenesis in whom no PDX-1 mutation could be detected. The absence of the pancreas in our patient was suspected because of the echography and further substantiated by laparotomy and biochemical analyses, in which no insulin or C-peptide levels could be detected in the absence of auto-antibodies (ICA, GAD). So far, only a limited number of patients with complete pancreatic agenesis has been published in the international literature; most of these patients died shortly after birth [1, 3, 6, 7, 8, 9, 11]. In our patient, there was a concomitant absence of the gallbladder, which has been reported in four of eight patients with pancreatic agenesis. In addition, one patient had hypoplasia of the gallbladder. Also, abnormalities of the bile duct system, which were present in our patient in the form of a choledochal duct stenosis, have been found in two cases in the literature. An explanation for the frequent concurrence of disturbed extrahepatic bile duct development and pancreatic agenesis may be the common embryological origin of the pancreas and bile duct system from the distal foregut. In some of the reported patients, additional congenital anomalies were found, but there was no consistent pattern resulting in a syndromic diagnosis. To our knowledge, there is also no known syndrome that comprises the anomalies that were found in our patient. Fetal vascular incidents or abnormalities are unlikely to account for the foregut anomalies, because the vascular supply for the (different parts of the) pancreas and the gallbladder and the choledochal duct comes from different arterial branches.

Because a PDX-1 mutation was recently described in a patient with pancreatic agenesis, and null mice lacking the PDX-1 protein also demonstrate pancreatic agenesis, we hypothesized that our patient could have a similar defect. Despite repeated sequence analysis, no mutation in the PDX-1 gene could be detected. Our PDX-1 analysis comprised the entire coding region, including the intron–exon boundaries. We cannot entirely exclude abnormalities in the PDX-1 regulatory regions, which are not known in detail at present. However, the normal histology and immunohistochemistry in the stomach and duodenum and the presence of nuclear PDX-1 immunoreactivity in the duodenum, appear to support the absence of regulatory PDX-1 gene mutations. Abnormalities in another gene or combination of genes may be responsible for pancreatic agenesis in this patient, but no other candidate genes are known. Our findings indicate that the gene(s) responsible for pancreatic agenesis in this patient has an effect on pancreas (and possibly gallbladder) development but not on that of the gastroduodenal endocrine cells, as in the case of PDX-1 null mice.

◀ **Fig. 4** Comparison of gastric (**A**–**D**) and duodenal (**E**–**H**) immunohistochemistry with antibodies to chromogranin-A (**A**, **C**, **E**, **G**) and serotonin (**B**, **D**, **F**, **H**). The pattern is essentially similar in sections from the index patient (**A**, **B**, **E**, **F**) and from an age-matched control patient (**C**, **D**, **G**, **H**)

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