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Elisabeth Martin, Marie Vanier, Manuela Tavian, Eric Guerin, Claire Domon-Dell, et al.. CDX2 in Congenital Gut Gastric-Type Heteroplasia and Intestinal-Type Meckel Diverticula. *Pediatrics*, 2010, 126 (3), pp.e723-e727. 10.1542/peds.2009-3512 . hal-03789463

HAL Id: hal-03789463

<https://hal.science/hal-03789463v1>

Submitted on 7 Oct 2022

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CDX2 in Congenital Gastric-Type Heteroplasia and
Intestinal-Type Meckel Diverticula

Journal:	<i>Pediatrics</i>
Manuscript ID:	2009-3512.R1
Article Type:	Case Report
Date Submitted by the Author:	30-Apr-2010
Complete List of Authors:	Martin, Elisabeth; INSERM, U682 Vanier, Marie; INSERM, U682 Tavian, Manuela; INSERM, U682 Guérin, Eric; CHU Strasbourg-Hautepierre, Service de Biochimie et Biologie Moléculaire Domon-Dell, Claire; INSERM, U682 Duluc, Isabelle; INSERM, U682 Gross, Isabelle; INSERM, U682 Rowland, Jon; Children's Hospital and Research Center, Department of Pathology Kim, Sunghoon; Children's Hospital and Research Center, Department of Pediatric Surgery Freund, Jean-Noel; INSERM, U682
Keyword/Category:	Developmental



CDX2 in congenital Gut Gastric-Type Heteroplasia and Intestinal- Type Meckel Diverticula

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Short title: Gastric heteroplasia in the ventral midline of midjejunum

Keywords: gastric heteroplasia, Meckel's diverticulum, endoderm, CDX2, homeobox gene

We declare no financial relationships relevant to this article.

Abstract

The mechanisms determining organ identity along the digestive tract are poorly understood in humans. Here we describe a rare case of a young patient, who presented with congenital gastric-type heteroplasia in the midjejunum. The lesions, located along the antimesenteric midline of the gut, are made of histologically and functionally normal gastric epithelium without inflammation or in situ / invasive carcinoma. They resemble the anatomy of the lesions developing in the mouse gut as a result of haploinsufficiency of the *Cdx2* homeobox gene. The lesions were devoid of CDX2, yet without mutation in the coding sequence or in a cis-regulatory element required for intestine-specific expression. Combining these data with the CDX2 expression pattern established in human embryos and in cases of Meckel's diverticula, we propose a scenario for this patient's presentation, in which CDX2 was missing in the site of ventral closure during gut morphogenesis, with subsequent default differentiation into gastric instead of intestinal tissue. Altogether, these observations argue in favour of a pivotal role played by *CDX2* in determining intestinal identity during human embryonic development, as previously shown experimentally in mice.

Introduction

Gastric metaplasia in the gut is a relatively frequent finding in adults, associated with inflammatory process and clinically linked to diarrhea, ulceration, obstruction or gastrointestinal bleeding. Unlike metaplasia in adults, congenital gastric-type heteroplasia in the small intestine or colon is extremely rare (1), except in cases of Meckel's diverticulum or intestinal duplications. In mice, intestinal heteroplasia made of gastric and esophageal tissue is the major phenotype exhibited by animals heterozygous for the gene encoding the homeotic transcription factor *Cdx2*, and they result from the sporadic loss of function of the second wild type allele during gut morphogenesis (2). Moreover, targeted disruption of *Cdx2* in the endoderm earlier at gastrulation converts the presumptive intestinal epithelium into esophagus-like epithelium (3). Even if these experimental data point to the crucial role played by *Cdx2* for intestinal fate determination in mice, evidence is still missing in human. Here we report the case of a 9-year old patient who had gastric heteroplasia along the ventral midline of the midjejunum, and we show that the lesions are devoid of CDX2 expression. We discuss a possible scenario for the development of these lesions. Analyzing CDX2 expression during human embryonic development and in remnants of the vitelline duct in Meckel's diverticulum corroborates the model.

Patient presentation

The patient, a young boy, was first hospitalized at the age of 8 years for an episode of syncope. He was found to have significant anemia and diagnosed with hereditary elliptocytosis with iron deficiency anemia. There was no family history of anemia and he was

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3 on iron therapy since his diagnosis, but antenatal history was not available. By the age of 9
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5 years, he had again significant bleeding episode requiring ICU admission. He underwent
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7 upper and lower endoscopies with biopsies which did not reveal any abnormality. Tc-99m
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9 pertechnetate scans performed at the time of hospitalization and again two weeks later were
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11 read as normal. Without performing small bowel follow through, MRI or capsule endoscopy,
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13 a decision was then made to perform intraoperative laparoscopic examination of the small
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15 intestine which revealed an elongated thickened area of nodularity on the antimesenteric side
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17 of midjejunum. The procedure was converted to an open operation and about 50 cm of
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19 jejunum containing the palpable nodularity and a normal appearing appendix were resected.
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22 The patient recovered from the operation without complications. Retrospective analysis of
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24 the pertechnetate scans showed a very faint elongated uptake in the left upper abdomen
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26 corresponding to the jejunal lesion which was missed on initial analyses. Patient has done
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28 well subsequently to the operation without gastrointestinal bleeding in the following three
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30 years.
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36 Pathologic analysis showed a non-duplicated jejunum containing numerous foci of
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38 gastric-type heterotopias along the antimesenteric line of the intestine (Fig 1a,b). Histological
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40 examination revealed that the unremarkable jejunal mucosa merged into well developed
41
42 gastric-like mucosa with numerous chief and parietal cells (Fig 1c). There was a band of
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44 atypical epithelium in the heterotopic mucosa but no in-situ or invasive carcinoma. The
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46 atypical glands showed mitotic activity with hyperchromatic nuclei situated basally, and
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48 maturation at the surface. A few foci of inflammation were noted in the heterotopic mucosa.
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50 Bland appearing glands extended below the muscularis mucosa in numerous areas. No
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52 vascular abnormalities were noted. The appendix was normal.
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58 The gastric-type epithelium was functional, including H/K-ATPase expression in
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60 parietal cells, Muc6 in fundic glands and in bland glands underneath the muscularis mucosa,

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3 Muc5AC in foveolar and mucous neck cells (Fig 1d,e,f), as well as gastrin and chromogranin
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6 A in endocrine cells, and lysozyme in fundic glands (not shown). Conversely, the atypical
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8 epithelium failed to express intestinal differentiation markers like Muc2 and Alkaline
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10 Phosphatase characterizing goblet and absorptive cells respectively (Fig 1g,i), in contrast to
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12 the normal adjacent intestinal epithelium (Fig 1h,j). Thus, the lesions are made of ectopic but
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14 cytologically normal gastric mucosa.
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18 Since gastric-type heteroplasia in the intestine is a hallmark of *Cdx2*^{+/-} mice upon total
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20 loss of expression of *Cdx2* (2), we examined CDX2 expression in the ectopic gastric tissue. A
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22 complete loss of expression was found in the lesions unlike the normal adjacent intestinal
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24 epithelium (Fig 2a,b). However, genomic DNA sequencing failed to reveal any mutation in
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26 the three exons and in the exon/intron boundaries of the *CDX2* gene of the patient. Single
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28 nucleotide polymorphism was found at two positions in the 3'-untranslated gene region (T>G
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30 and C>T respectively 10 and 18 nucleotides downstream of the translation stop codon), and at
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32 two positions within the coding sequence. One is a C>T transition in codon 293, which
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34 changes the amino acid sequence from Ser to Pro, and corresponds to a polymorphism already
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36 reported (SNP rs1805107) without association with gut malformation. The second is a G>T
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38 transversion that changes amino acid 149 from Ala to Ser and creates a putative
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40 phosphorylation site. Thus, we constructed three expression plasmids encoding respectively
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42 wild-type CDX2, the mutated form containing Ser instead of Ala at position 149 and a
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44 mutated form containing Gln at position 149 to mimic constitutive phosphorylation. Reporter
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46 luciferase assays with a plasmid containing a typical target of the CDX2 protein, the sucrase-
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48 isomaltase promoter, showed no difference in transcriptional activation by the wild-type and
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50 the two mutated CDX2 forms, suggesting that changing the amino acid 149 has no deleterious
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52 effect on protein activity. Recently, an upstream regulatory region required for intestine-
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54 specific expression of *Cdx2* has been described in mice (4). Sequencing the corresponding
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3 region in the patient (between -10186 to -9646 upstream of the translation start site) and the
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5 highly conserved proximal promoter and 5'untranslated region of the *CDX2* gene (from -
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7 1058 to -1) also failed to detect any nucleotide change, indicating no alteration of these cis-
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9 regulatory elements in the patient.
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13 Although the cause of *CDX2* deficiency in the lesions remains elusive, we propose a
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15 scenario based on the fact that the lesions are distributed along the ventral antimesenteric
16
17 midline of the gut. During gastrulation, cells of the definitive endoderm intercalate and
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19 displace the visceral endoderm that later forms the yolk sac endoderm; then, by lateral flexure
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21 of the embryonic disc, the definitive endoderm folds and its lateral margins meet ventrally to
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23 close the digestive tube that remains eventually connected to the yolk sac by the constricted
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25 vitelline duct. In the patient, we propose that *CDX2* expression was missing from a portion of
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27 the lateral margins of the definitive endoderm, so that the site of ventral closure became
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29 devoid of *CDX2* and subsequently adopted a gastric-type identity. To challenge this scenario,
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31 we analyzed *CDX2* expression in 25-day human embryos, which corresponds to a gut
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33 morphogenesis stage where the tube is still largely open to the yolk sac at the midgut level but
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35 already closed in the hindgut (Fig 2c) (5). Midgut sections showed *CDX2* protein in the
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37 dorsal and lateral sides of the open gut tube, with a decreasing gradient towards the yolk sac
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39 endoderm (Fig 2d). Interestingly, sections at the hindgut level revealed that *CDX2* is present
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41 not only in the entire epithelial layer lining the closed gut tube, but also in the dorsal-most
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43 part of the yolk sac (Fig 2e). This indicates that the territory of *CDX2* expression extends
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45 beyond the gut endoderm into the yolk sac endoderm to ensure intestinal fate when closure
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47 occurs in the ventral midline. Inversely, the scenario proposed for the patient suggests that the
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49 ventral limit of the *CDX2* territory failed to reach the site of closure, leaving a ventral area
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51 devoid of *CDX2* which then differentiated as gastric epithelium.
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3 The vitelline duct connecting the yolk sac to the gut tube is resorbed after gestation
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5 week eight. However, resorption is incomplete in 2% of the human population (Fig 2f) and
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7 remnants, known as Meckel's diverticula, differentiate into several types of digestive
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9 structures. Since CDX2 expression extends into a portion of the yolk sac endoderm in
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11 embryos, we anticipated that areas of the vitelline duct remnants which exhibit an intestinal-
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13 type differentiation may express CDX2, whereas those differentiated into a gastric or
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15 pancreatic fate may be free of CDX2. This was corroborated by immunohistochemistry on a
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17 collection of Meckel's diverticula, which showed a strict correlation between the areas
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19 exhibiting an intestinal-type differentiation and those expressing CDX2 (Fig 2g).
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27 Discussion

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29 The congenital lesions of this young patient share properties with the gastric-type
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31 heteroplasia reported in mice upon loss of function of the homeobox gene *Cdx2* in the
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33 developing gut endoderm. Despite the lack of inactivating mutation in the *CDX2* gene, the
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35 remarkable location of the lesions along the antimesenteric midline suggests a scenario in
36
37 which CDX2 was missing ventrally in the closure site of the gut. The profound morphological
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39 changes associated here with the defect of *CDX2* in the gut were accompanied by an increase
40
41 in Shh expression and a loss of α -smooth-muscle-actin-positive myofibroblasts (not show),
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43 which correlates with the inverse effects observed in the intestine-type metaplasia that
44
45 develop in transgenic mice upon ectopic expression of *Cdx2* in the gastric mucosa (6-8). The
46
47 CDX2 pattern shown here in the developing gut and vitelline endoderm, together with the
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49 close relationship between the presence or absence of CDX2 respectively in intestinal-type
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51 Meckel's diverticula and in congenital gastric heteroplasia strongly support the notion that
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53 *CDX2* plays a primary role in determining intestinal identity during human gut development,
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55 as demonstrated experimentally in mice.
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8 **Acknowledgements**
9

10 We thank Dr Isabelle Van Seuningen (Inserm U837, Lille, France) for the anti-mucins
11 antibodies.
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Review Copy

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LEGENDS OF THE FIGURES

Figure 1

Morphology at low (a) and high (b) magnification, and histology (c) of the ectopic lesions located at the antimesenteric side of the midjejunum. Immunohistochemistry shows gastric markers, H/K ATPase (d), Muc6 (e) and Muc5AC (f) in the lesions, but loss of expression of intestinal markers, Muc2 (g) and Alkaline Phosphatase (i), in contrast to the Muc2 (h) and Alkaline Phosphatase expression (j) in the normal adjacent intestinal mucosa. Bar is 100 μm in c-j.

Figure 2

Lesions are devoid of CDX2 protein (a) in contrast to the normal adjacent intestinal epithelium (b). (c) Schematic drawing of a 25d human embryo showing the midgut (mg), hindgut (hg) and yolk sac (ys); sections at the level of the midgut and hindgut are indicated. (d) and (e), CDX2 immunostaining at the level of the midgut and hindgut, respectively. (f) Schematic representation of the abdomen showing Meckel's diverticulum corresponding to remnants (rmn) of the vitelline duct (dotted) that connects the ileum (il) to the umbilicus (umb). (g) CDX2 immunodetection in Meckel's diverticula shows restricted staining in intestinal-type area (right) and absence of CDX2 in gastric-type area (left). Bar is 100 μm in a,b,d,e and 200 μm in g.

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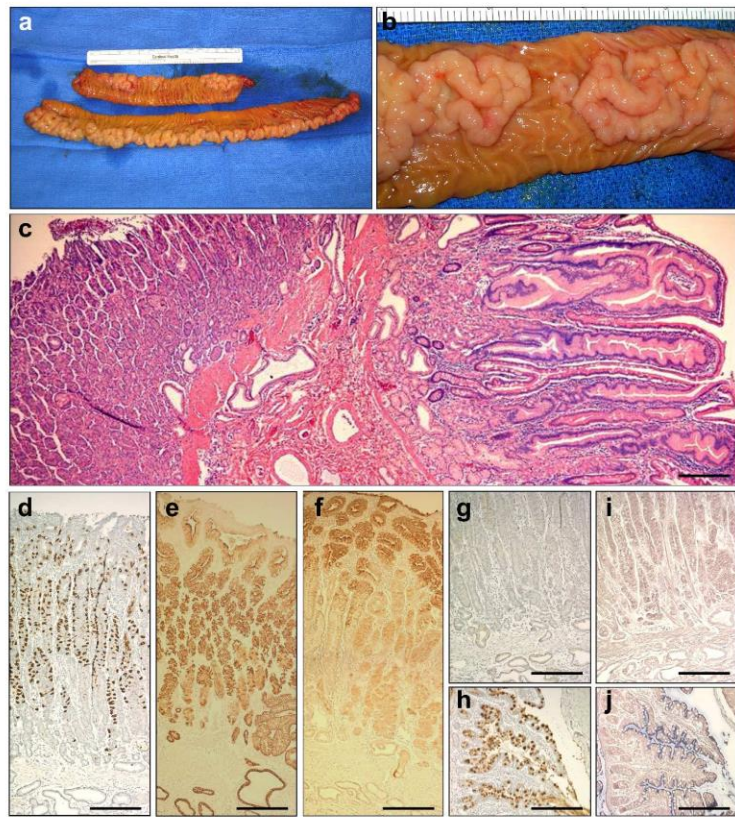


Figure 1

210x297mm (600 x 600 DPI)

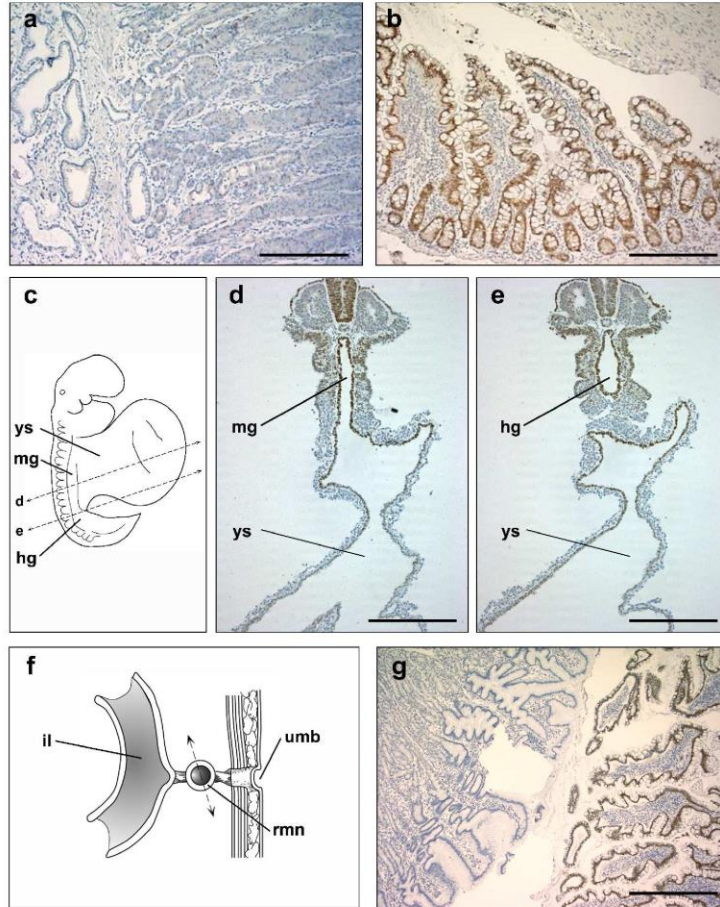


Figure 2

210x297mm (600 x 600 DPI)