Congenital anomalies of the tubular gastrointestinal tract

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Summary

Congenital anomalies of the tubular gastrointestinal tract are an important cause of morbidity not only in infants, but also in children and adults.

The gastrointestinal (GI) tract, composed of all three primitive germ layers, develops early during embryogenesis. Two major steps in its development are the formation of the gut tube (giving rise to the foregut, the midgut and the hindgut), and the formation of individual organs with specialized cell types.

Formation of an intact and functioning GI tract is under strict control from various molecular pathways. Disruption of any of these crucial mechanisms involved in the cell-fate decision along the dorsoventral, anteroposterior, left-right and radial axes, can lead to numerous congenital anomalies, most of which occur and present in infancy. However, they may run undetected during childhood.

Therapy is surgical, which in some cases must be performed urgently, and prognosis depends on early diagnosis and suitable treatment.

A precise pathologic macroscopic or microscopic diagnosis is important, not only for the immediate treatment and management of affected individuals, but also for future counselling of the affected individual and their family. This is even more true in cases of multiple anomalies or syndromic patterns.

We discuss some of the more frequent or clinically important congenital anomalies of the tubular GI, including atresia's, duplications, intestinal malrotation, Meckel's diverticulum and Hirschsprung's Disease.

Key words: gastrointestinal tract, gastrointestinal malformations, intestinal atresia, intestinal duplication, Hirschsprung Disease

Introduction

The gastrointestinal (GI) tract, which emerges from the endoderm during gastrulation, initially extends from the buccopharyngeal membrane to the cloacal membrane. During these early embryological weeks, the development of the gut tube occurs simultaneously to the turning and folding of the embryo, consequently forming three structures in the sagittal plane: the foregut (located cephalically in the head fold), the hindgut (located caudally with its allantoid outgrowth), and the midgut (located in between). Subsequent endodermal invaginations in the cranial and caudal regions form the anterior intestinal and caudal intestinal portals, which gradually extend towards each other. Ventral folding of the lateral midgut endoderm leads to formation of a sealed gut tube, around which sub-adjacent splanchnic mesenchyme differentiates into smooth

muscle. By the end of the fourth week of gestation, foregut, midgut and hindgut are craniocaudally discernible, and later on they will be distinguishable by their different arterial supplies [1].

The epithelial cells of the GI tract proliferate, and by the sixth week of gestation completely obliterate the lumen of the gut tube. Degeneration of the centrally located cells at eight weeks leads to a once again patent lumen. Formation of an intact and functioning GI tract during embryologic development can be derailed through numerous factors, causing disruption of GI tract morphogenesis due to specification and maintenance problems [2].

Some of the congenital developmental anomalies involving the GI tract discussed in this article are atresia, stenosis, duplication, intestinal malrotation, Meckel's diverticulum, lymphangiectasia and Hirschsprung's Disease.

Esophagus

Congenital foregut malformations present as a wide variety of surgically important entities. Of these, developmental disorders of the esophagus are relatively common, occurring in approximately 1 in 3000 live births and are particularly relevant to pediatric surgical practice.

The separation of the primitive foregut tube into tracheobronchial tree and the esophagus occurs between the fourth and fifth week of development [3]. Aberrations in normal development at this time can cause esophageal atresia, esophageal stenosis, and esophageal duplications cysts.
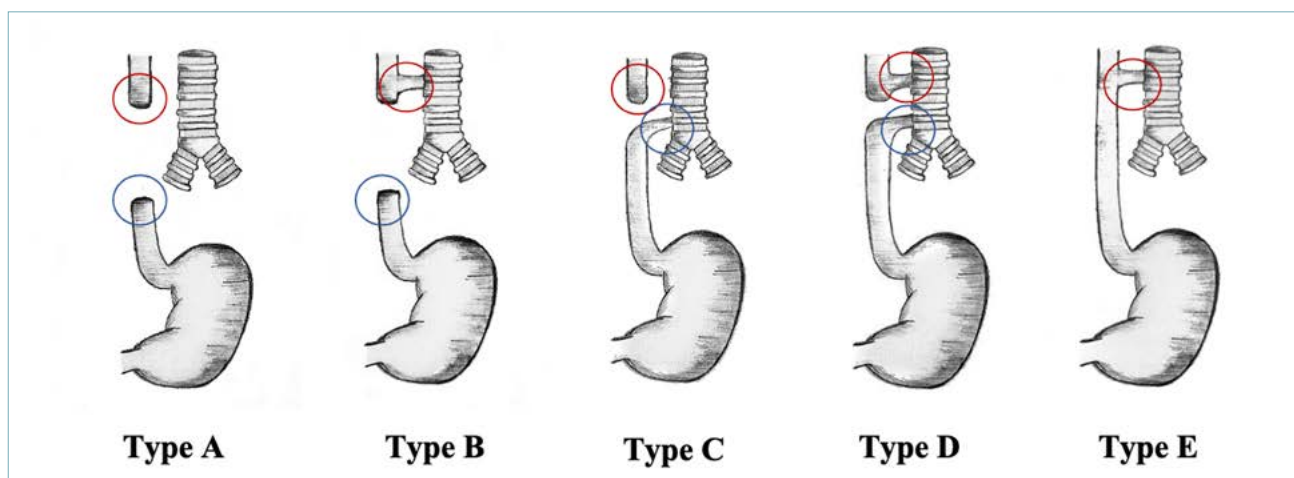
ESOPHAGEAL ATRESIA AND TRACHEOESOPHAGEAL FISTULA

Esophageal atresia (EA) is a congenital malformation characterized by an interruption of the continuity of the esophagus with or without a persistent communication with the trachea (tracheo-esophageal fistula – TEF). EA is relatively common, occurring in 1/2500-3000 live births.

The etiology of EA, with or without TEF, is still not completely understood and is likely to be multifactorial. Various hypotheses have been proposed, in particular the occurrence of esophageal vascular/ischemic events or failure of those processes that allow the tracheo-esophageal separation, with subsequent incomplete development of the foregut into tracheal and esophageal channels during embryonal development [4-6].

There is emerging evidence of an important role for genetic factors. In fact, additional congenital anomalies (usually midline) are present in over 50% of cases. Cardiovascular anomalies occur in 35% of cases, genitourinary (GU) in 20% of cases, and associated GI anomalies occur in approximately 20% of cases. Approximately 10% of EA patients have a nonrandom VACTERL association (Vertebrae, Anal, Cardiac defects, Tracheo-Esophageal, Renal, and Limb anomalies); other associations include the CHARGE syndrome (Coloboma, Heart defects, Atresia of choanae, developmental Retardation, Genital hypoplasia and Ear deformities), the congenital pulmonary airway malformations (CPAMs) [7,8], and chromosomal anomalies, such as trisomy 18 (Edwards syndrome) and trisomy 21 (Down syndrome).

The original classification by Vogt in 1929, modified by Ladd and Gross in 1953, recognizes 5 types of EA (Fig. 1) [4,7,9-11].

Figure 1. Types of esophageal atresia (EA) and tracheoesophageal fistula (TEF) according to the Gross classification: (A) EA without TEF (8%); (B) EA with proximal TEF (2%); (C) EA with distal TEF (85%); (D) EA with distal and proximal TEF (1%), and (E) TEF in the absence of EA (H-type TEF; 4%).

Improvements in fetal imaging have led to increased prenatal diagnoses of this condition, which may be difficult to recognize. Maternal polyhydramnios is present in a significant number of cases [12], and a small or absent stomach bubble can be recognized after the 18th week of gestation.

Neonates are unable to swallow oral and pharyngeal secretions and are known to have excessive salivation requiring repeated suctioning. If the atresia is not immediately recognized, babies cough, choke and may become cyanotic, especially during first attempts at feeding. The diagnosis is confirmed by the inability of nasogastric tube to pass beyond 10-11 cm from the lips. Cases with Type E are normally diagnosed late during infancy due to recurrent episodes of aspiration pneumonia.

Chest radiography is mandatory and should be performed as soon as possible. It demonstrates an air-filled proximal esophageal pouch dilated with the tip of the catheter coiled in the esophagus, arrested in the superior mediastinum (T2-4). The presence or absence of gastrointestinal air below the diaphragm is an important finding: the presence of gas denotes the presence of a TEF, while the complete absence denotes the absence of the fistula.

There are no pathognomonic histologic features features in EA. Diagnosis is based on the gross anatomy of the lesions, often observed during fetal autopsies. However, histology of TEF often reveals foci of squamous metaplasia, primarily along the posterior wall of the trachea, but frequently extending around the entire internal surface of the trachea and into the bronchi. The esophageal segment may show primitive ciliated columnar epithelium, respiratory glands, and even cartilage [13].

The indications and timing of surgical repair may be determined by using the Waterston and Spitz prognostic classification system, which assigns risk of mortality based on the baby's size, presence of respiratory distress and associated anomalies (Tab. I).

Usually, EA is surgically corrected in the first few days of life, after stabilization of the babies and a proper study of all possible associated anomalies. Survival rates currently exceed 90% due to improved advances in surgical techniques and preoperative and postoperative care.

In most cases, infant present with Type C EA. In these cases, a direct anastomosis of the proximal and distal esophageal pouches, after division of TEF and closure of the trachea, is possible. This operation may be performed according to surgeons' preferences (open procedure or thoracoscopy). Babies with EA without fistula represent a challenging subgroup. Everything must be done to obtain a primary repair, and many esophageal growth procedures have been utilized. However, a substitution is still sometimes necessary (gastric tube, gastric transposition, esophago-colonplasty, jejunal vascularized graft segments).

Although long-term survival and outcome for infants with EA are very good, several complications can occur post-operatively after repair. Early and late complications include anastomotic leaks, postoperative stricture formation at the side of anastomosis, recurrent TEF, tracheomalacia, peptic esophagitis and/or eosinophilic esophagitis (secondary to an associated esophageal motor disorder) [4].

ESOPHAGEAL STENOSIS

Congenital esophageal stenosis (CES), defined by Nihoul-Fekete as "an intrinsic stenosis of the esophagus present although not necessarily symptomatic at birth which is caused by congenital malformation of the esophageal wall architecture" [14], has an estimated incidence of 1 in 25,000 to 50,000 live births in the general population, while its incidence in patients with EA is significantly higher (between 3% and 47% of patients). CES can be isolated, or associated with other malformations, such as cardiac anomalies, intestinal atresia, duodenal duplication, anorectal malformations, celiac disease, TE, and chromosomal anomalies [15].

Table I. Waterston and Spitz Classification System.

Class	Description	Survival (%)
Waterston Classification		
A	Birth weight > 2500 g and otherwise well and free from complications	100
B	Birth weight 2000-2500 g and well, or higher weight with moderate associated anomalies	100
C	Birth weight < 2000 g and well, or higher weight with sever associated anomalies	80
Spitz Classification		
I	Birth weight > 1500 g without major cardiac anomalies	99
II	Birth weight < 1500 g or major cardiac anomalies	84
III	Birth weight < 1500 g with major cardiac anomalies	43

CES can be subdivided into three types: segmental fibromuscular hypertrophy of the muscle and submucosal layers (FMS; 54%), ectopic tracheobronchial remnants in the esophageal wall (TBR; 30%), and a membranous webbing or esophageal membrane (EM; 16%) [16,17]. Microscopic examination is not routinely performed; however, histologic alterations have been described in all three entities.

FMS, which is mainly localized in the middle or lower third of the esophagus, is histopathologically characterized by a circumferential proliferation of smooth muscle fibers and fibromuscular thickening of the wall. TBR mainly involves the lower third of the esophagus (within 1 cm of the gastroesophageal junction) [18] and may contain mature and immature cartilage, respiratory epithelium or seromucous glands. The presence of ectopic respiratory epithelium in deep structures of the esophageal wall is mandatory and, frequently, non-inflammatory lymphoid tissue is found ("lymphoepithelial bronchogenic tissue") [15,19,20].

EM, which mainly involves the upper or middle third of the esophagus, is histologically composed of a fold of normal esophageal epithelium with underlying loose connective tissue, while the muscular layers of the esophagus are not involved in esophageal webs [16]. Webs of the cervical esophagus have been associated with the Plummer-Vinson (Paterson-Kelly) syndrome, and biopsies can show reactive epithelial changes with basal cell proliferation, parakeratosis, chronic inflammation, and fibrosis. Squamous dysplasia is occasionally found. However, most upper esophageal webs are not associated with Plummer-Vinson syndrome and are apparently of no clinical consequence, unless they are associated with idiopathic or allergic eosinophilic esophagitis.

Clinical symptoms of CES include dysphagia, vomiting, feeding difficulties, recurrent aspiration pneumonia and respiratory symptoms. Esophagogram identifies CES in the majority of patients and esophagoscopy is also an important diagnostic tool to confirm diagnosis and to rule out additional pathologies [21]. Options for treatment of congenital esophageal stenosis include dilation or surgery, depending on the pathologic features of the stenosis, its pathohistologic type and severity of the CES. Surgical complications reported include, but are not limited to, anastomotic leakage (treated operatively and nonoperatively), anastomotic stenosis, hiatal hernia, and reflux esophagitis [22].

ESOPHAGEAL DUPLICATION CYSTS

Esophageal duplications are rare lesions that are predominantly congenital in nature and represent the second most common duplication of the alimentary tract, after the ileum. Most alimentary tract duplications are cystic. Duplication cysts may be asymptomatic and found incidentally; however, when located in the proximal or distal esophagus they can present with respiratory symptoms or dysphagia, respectively. They occur most frequently within the thoracic cavity, in proximity of the distal two-thirds of the esophagus. Endoscopic ultrasound has been widely used as a modality for the evaluation and diagnosis of duplication cysts [19].

When the esophageal mucosa is normal, biopsy and aspiration should be avoided, as they can lead to infection of the cyst and obliteration of the surgical planes due to scarring.

Duplication cysts are usually small and round, but they may also be long tubular structures adjacent to the esophagus. Histologically, the mucosa may contain stratified squamous or columnar epithelium. The differentiation between an esophageal duplication cyst and a bronchogenic cyst can be challenging, as both can show similar epithelial linings and occur in the same location.

To be diagnosed as an esophageal duplication, the cyst must have two layers of muscularis externa and be associated with the esophageal wall.

The treatment of choice of an esophageal duplication cyst is complete surgical resection. The surgical approach depends upon the location of the cyst and surgeon's preference. Thoracoscopy provides excellent exposure and is the preferred approach for cysts located above the lower third of the esophagus. Laparoscopy plays a role when the cyst is located in the distal esophagus, particularly when abnormal reflux is present, because a fundoplication can be added [23].

Stomach

INFANTILE HYPERTOPHIC PYLORIC STENOSIS

Infantile hypertrophic pyloric stenosis (IHPS) is a complex disorder characterized by gastric outlet obstruction due to an abnormal thickening of the pyloric muscle. IHPS has an overall incidence of 4 to 5 per 1000 livebirths, being more common in the Caucasian population [24]. It is known to have a male predilection, is more frequent in first-born infants and frequently recurs in siblings [25, 26].

The precise etiology of IHPS in unknown, although an association with genetic and environmental factors, such as exposure to erythromycin in the first weeks of life, cow's milk protein allergy, maternal smoking and young age, preterm birth and cesarean section, has been reported [26].

The marked hypertrophy of the circular and, to a lesser degree, longitudinal muscular layers of the pyloric muscle leads to a narrowing/obstruction of the gastric outlet lumen and an increase of width and length of the pylorus. Subsequently there is an edematous thickening of the antral mucosa, often associated with a mild inflammatory infiltrate, and secondary dilatation of the stomach. Histologically there is an increase in the amount of pyloric muscle fibers, which however are structurally normal.

IHPS classically presents around the fourth to sixth week of age with a characteristic projectile, non-bilious vomiting. Prolonged delay in diagnosis can lead to dehydration, poor weight gain, malnutrition, metabolic alterations, and lethargy.

At physical examination of the abdomen, it is difficult to locate the olive-sized mass in the right upper abdominal quadrant, and diagnosis is confirmed by ultrasound, which needs to evaluate the pyloric muscle thickness and length: muscle wall thickness of 3 mm (or greater) and length of 14 mm (or greater) are considered abnormal.

Treatment is surgical and consists of pyloromyotomy (according to Ramstedt), performed with an open procedure or with mini-invasive technique.

A milder form of congenital pyloric stenosis has occasionally been reported to present later in adult life [27]. As in its infantile counterpart, the adult form affects mainly males and co-occurrence of both forms (infantile and adult) have been reported within the same family [27,28].

Small and large intestines

### CONGENITAL STENOSIS AND ATRESIA

Atresia is a loss of continuity of the bowel lumen, either by complete absence of a variably long segment, or occlusion of the lumen, and is accompanied by intestinal obstruction, while stenosis is a localized luminal narrowing that may produce a partial obstruction to passage of intestinal contents.

All parts of the intestinal tract can be affected. Based on anatomical characteristics four types of intestinal atresia have been described by Grosfeld and colleagues in 1979 (Fig. 2) [29].

Duodenal Atresia

Duodenal atresia occurs in approximately 1 in 6,000-10,000 live births [30]. Unlike most of the other intestinal atresias, duodenal atresia is frequently associated with other congenital anomalies. The majority of cases (25-40%) are associated with Down's syndrome, while less frequently related anomalies are malrotation, biliary tract and cardiac malformations and the VATER association (vertebral defects, anal anomalies, tracheo-esophageal atresia, renal anomalies) [30,31]. An annular pancreas, which surrounds the entire circumference of the duodenum, is frequently observed, and it is likely due to failure of duodenal development rather than abnormal growth of the pancreatic buds.

The etiology is unknown, although various hypotheses exist, which include failure of recanalization of the intestinal lumen following florid epithelial proliferation around the 8th week of gestation [32], and vascular acci-
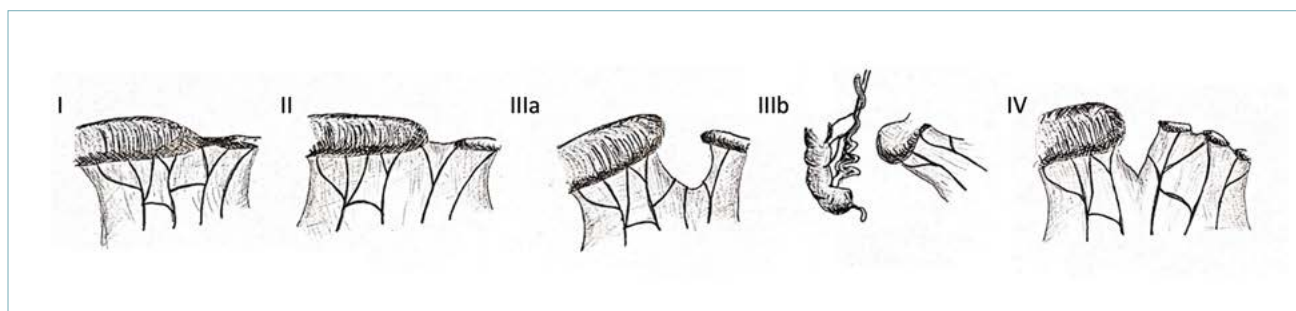
Figure 2. Types of intestinal atresia according to the Grosfeld classification: Type I atresia is characterized by an intact bowel wall and a transluminal septum which causes proximal dilatation and collapse of the distal intestinal segment. In type II atresia the two blind-ending pouches are connected by a fibrous band, located along the edge of the intact mesentery. Type IIIa is similar to type II but is accompanied by a small mesenteric defect. Type IIIb is characterized by proximal jejunal atresia, an extensive mesenteric defect with loss of the normal blood supply and subsequent coiling of the ileum around the ileocolic artery. Type IV is defined as the presence of multiple atresias (types I, II, and III)("string of sausages").

Figure 3. Plain abdominal X-ray displaying the classic double-bubble sign in a patient with duodenal atresia.

dent resulting in necrosis of the affected segment with subsequent resorption [33].

Duodenal atresia is accompanied by polyhydramnios in up to 80% of cases, often leading to premature labor, and, unlike the more distal intestinal atresia, appears to result from defective embryogenesis [30]. Prenatal ultrasound shows a characteristic "double-bubble" sign, which is also visible at abdominal X-ray, performed soon after birth (Fig. 3) [34].

Newborns present non-bilious vomit, if atresia is proximal to the ampulla of Vater, or bilious, if the atresia is distal. Dehydration, weight loss and electrolyte imbalance may follow unless fluids and electrolyte losses are adequately replaced.

Treatment is surgical and consists of a side-to-side, or, preferably, proximal transverse-to-distal longitudinal (Diamond-shape) anastomosis.

Jejunal and Ileal Atresia

Jejunoileal atresia (JIA) is the commonest type of in-

testinal atresia and accounts for approximately 1 to 3 in 5,000-10,000 live births [35]. A localized intrauterine vascular event with necrosis of the bowel and subsequent reabsorption of the affected segment is the best accepted etiologic theory. JIA associated with in-utero intussusception, perforation, volvulus or others may confirm the occurrence of the vascular accident. Over one-third of affected children are born prematurely [30]. The presence of fetal squamae and lanugo hairs in the lumen distal to the atresia provides highlight that the atresia develops after patency of the bowel lumen and peristalsis have become established [36].

In contrast to duodenal atresia there is no sex predilection, and Down's syndrome is uncommon, as well as other congenital anomalies. Cystic fibrosis, malrotation and gastroschisis are the most common associations, being found in around 10% of cases [37].

The bowel proximal to the atresia is always bulbous and its vascularity may be seriously impaired; perforation is a recognized complication. The distal bowel is contracted with a narrow lumen (Fig. 4). Type IIIb atresia is a rare variant, so called "Christmas tree," "Maypole," or "Apple peel" deformity (Fig. 5). In this type, the two intestinal segments are separated as in Type IIIa atresia, and the mesenteric defect is wide. The proximal atretic segment is high, in proximity of ligament of Treitz. The superior mesenteric artery distal to the middle colic branch is absent, and the distal small bowel is considerably reduced in length and twisted around a marginal artery. This variety of intestinal atresia has been reported in families [38]. A familial variant of multiple intestinal atresias [hereditary multiple intestinal atresia (HMIA)] with autosomal recessive inheritance, due to mutations in the tetratricopeptide repeat domaine-7A (*TTC7A*) gene, has been described in French Canadians [39].

The prenatal ultrasound shows polyhydramnios and distension of bowel loops, but the differential diagnosis may include other anomalies, such as meconium ileus or Hirschsprung disease.

Newborns usually present with abdominal distension, bilious vomit and failure to pass meconium in the first 24-48 hours. Signs of hypovolemia occur over time if dehydration and electrolyte imbalance are not adequately treated. Imaging is characteristic with dilated bowel loops with air-fluid levels, and gasless distal intestine.

Treatment is surgical with resection of the atretic segments and reconstruction of the continuity of the intestine.

Colonic Atresia

As isolated entity, without imperforated anus or cloacal extrophy, colonic atresia and stenosis are very ra-
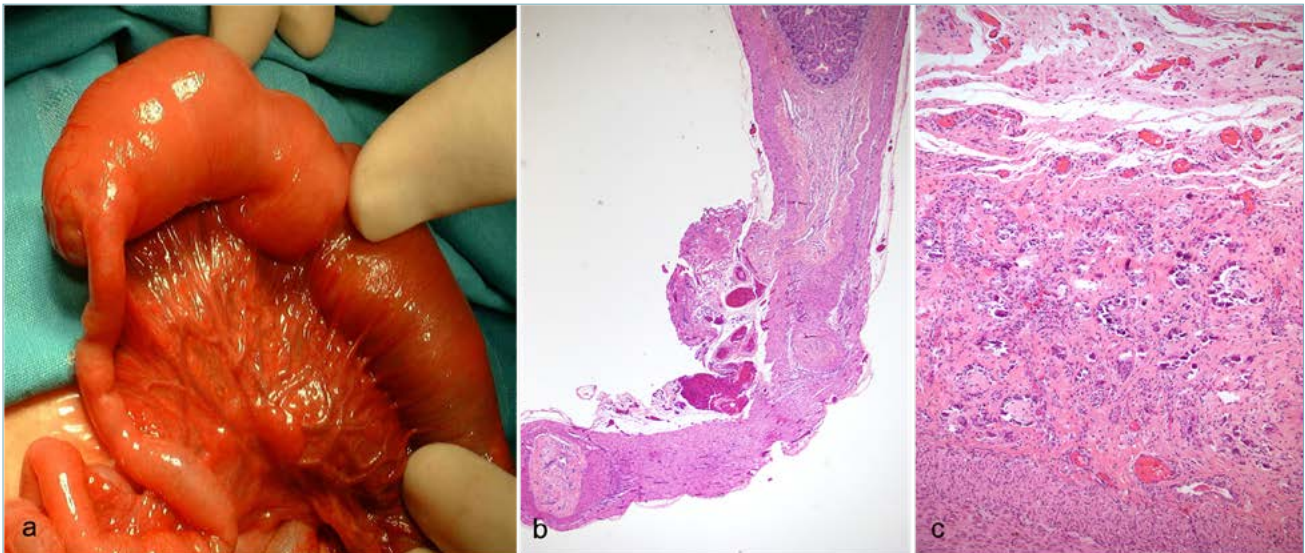
Figure 4. Type I and II atresia's according the Grosfeld classification: (a) Intraoperative image of ileal type I atresia according to the Grosfeld classification displaying dilatation of the proximal intestinal tract and a collapsed distal intestinal segment; (b) Histology of a fibrous band in type II atresia reveals a partially intact muscularis propria with fibrous obliteration of the lumen (H&E, x 50); (c) Higher magnification of serial sections revealed calcification and granulation tissue, suggestive of intrauterine ischemia and healing (H&E, x 200).

Figure 5. Intraoperative image of the "apple peel deformity" or atresia type IIIb according to the Grosfeld classification.

re, occurring in 4/10,000 live births [40]. They are mainly localized in the ascending and transverse colon. There is a slight predominance of male versus female patients and in a small number of cases association with Hirschsprung disease has been reported [41].

Patients generally present, within the first two days of life, with symptoms of distal intestinal obstruction and imaging reveals an important dilatation of the proximal intestinal tract. Delayed diagnosis is believed to increase mortality significantly [42].

Surgical correction is the mainstay of treatment and generally a primary resection and anastomosis is the recommended procedure.

CONGENITAL DUPLICATIONS (ENTERIC CYSTS)

Duplications of the gastrointestinal tract are rare congenital anomalies that may occur throughout the whole intestinal tract (Fig. 6), with an overall reported incidence of 1 in 4,500 live births [43]. They are usually diagnosed within the first two years of life. However, cases presenting with abdominal pain and obstruction in adults have been described as well as cases of multiple intestinal duplications [44].

Overall, males are more frequently affected (especially when occurring in the small bowel) and around one third of cases have been described to have associated congenital anomalies. The etiology of intestinal duplications is still to be elucidated. Different theories include vascular insults, persistence of embryologic structures, abnormal recanalization and others [30].

The vast majority of cases are located in the midgut, especially in the ileum (more than 60% of cases), fol-
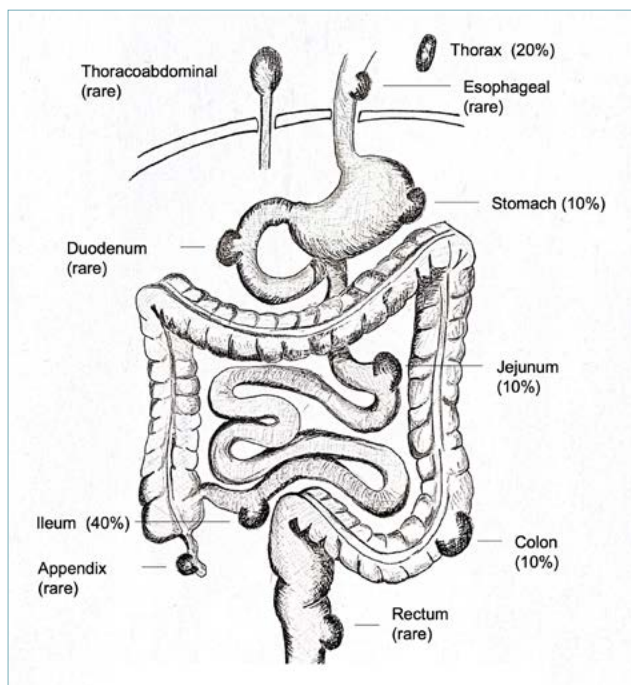
Figure 6. Location and incidence of duplications along the tubular gastrointestinal tract.

lowed by esophagus, rectum and colon (Fig. 6). They are either part of, or located directly adjacent to, the intestinal wall. Duplications are found on the mesenteric side (mesenteric cysts) and vary in shape and size. In 80% of cases the duplication is cystic, while in the remaining "20% is tubular" [44]. Macroscopically they are most often single structures, ranging in size from 2 to 7 cm, variably displaying communications with the normal bowel.

Histologically, duplications mimic the native gastrointestinal tract, with enteric mucosa, submucosa, muscolaris propria and a myenteric plexus. Intramural duplications usually share their muscular layer with the adjacent native intestine. The mucosal layer is usually simplified, difficult to categorize, consisting of a simple columnar epithelium resembling intestinal surface epithelium with or without cilia (as in embryonic intestinal epithelium).

Many cases are incidentally diagnosed by antenatal ultrasound. Clinical symptoms depend on size and location of the duplication and include pain, obstructive symptoms due to direct effect of compression of adjacent organs. Hemorrhage may also be observed in case of cysts lined with ectopic gastric mucosa due to ulceration and erosion [43,45]. Complete surgical removal of the duplication is required.

Yolk sac or Omphalomesenteric duct remnants

The omphalomesenteric duct (OMD) is an embryonic structure, which connects the yolk sac to the midgut, and it is the last point to close after the separation of the intestine from the yolk sac during embryonic development. The communication reduces gradually during the first weeks and closure is completed by the 10th weeks of embryonic life. After closure, a fine fibrous cord remains to connect the two structures. The point of closure is about 8-10 cm above the ileocecal valve in the term neonate. The failure of its resorption results in various anomalies including Meckel's diverticulum, the most common congenital anomaly of the gastrointestinal tract, which can be found in about 2% of the general population with a male to female ratio ranging between 2:1 to 4:1 [30].

Meckel's diverticulum, typically located 40 to 70 cm proximal to the ileocecal valve, presents as an out pouching of the anti-mesenteric side of the intestine and is a true diverticulum, characterized by the presence of all three layers of the native bowel (Fig. 7). Ectopic mucosa is frequently encountered during histologic examination, with gastric mucosa being identified in 50-60% of the cases. Less frequently, Meckel's diverticulum contains pancreatic, hepatobiliary, duodenal, or colonic mucosa [46].

Meckel's diverticulum can remain asymptomatic for many years, having been identified in 0.14% to 4.5% of autopsy series, and lifetime risk for related complications has been estimated in 4% [47-49].

However, the presence of ectopic acid secreting gastric mucosa, may cause ulceration and erosion of the diverticular mucosa, with important rectal bleeding. Less frequent complications are intestinal obstruction and peritonitis, all of which require prompt diagnosis and surgical intervention. Persistence of part of the vitelline duct at the umbilicus may manifest as an umbilical sinus, which presents with mucous discharge from the umbilicus (intestinal contents). It must be distinguished histologically from urachal remnants, which are also characterized by mucous discharge, but not from the intestine.

Malrotation

Malrotation refers to a group of congenital abnormalities of the intestinal position, resulting from non-rotation, incomplete rotation, or abnormal fixation of the embryonic gut. As an anatomic entity it can be identified in 0.2-1% of the normal population. The incidence is estimated to be around 1 in 6000 live births. The majority of anomalies remain asymptomatic, so the true incidence is actually unknown [50].

Intestinal rotation occurs during the fourth through twelfth week of gestation. During the first two weeks of
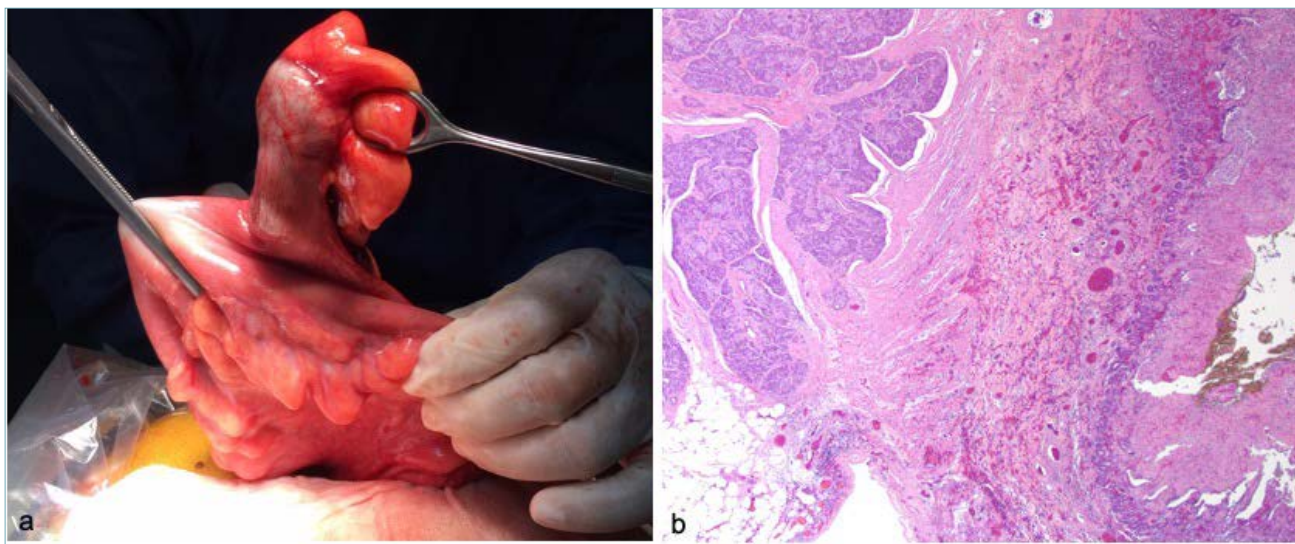
Figure 7. Meckel diverticulum: (a) Intraoperative image of ileal Meckel diverticulum; (b) Histology of Meckel diverticulum with extopic endocrine and exocrine pancreatic tissue within the muscular wall.

this period the straight tube of primitive intestine elongates more rapidly than the abdominal cavity, causing the midgut (duodenum, jejunum, ileum, ascending and transverse colon) to protrude into the base of the umbilical cord (physiologic herniation). During the tenth week of gestation, after having completed a series of rotations, the intestines return into the abdomen [42,51].

Formation of intestinal laterality was thought to occur via rotation. Recently, a hierarchical looping model has been proposed, based on the identification of four stereotypical loops forming in a predictable and hierarchical order along the cranio-caudal axis of the midgut, analogous to their order of re-entry into the body cavity [51,52]. After re-entering the abdomen, the terminal ileum, and the entire colon, located distally to the yolk sac attachment, lie in the left side of the abdomen, while the rest of the small bowel lies to the right. Subsequently the ileum and the cecum slide dorso-caudally into their final position, where the latter, together with the ascending colon, becomes fixed by fusion of the mesentery with the posterior abdominal wall. The small bowel below the duodenum remains free to move because of its wide mesentery.

Rotation and movement of the intestine may end prematurely at any stage before the caecum has descended and becomes fixed to the right iliac fossa.

In the absence of rotation, the cecum does not undergo proper fixation and subsequently the entire colon remains on the left side of the abdomen, with the small bowel lying on the right and the duodenum descending directly along the course of the superior mesenteric artery. Due to its wide-based mesentery, patients are not at risk of developing midgut volvulus. Non-rotation is usually associated with diaphragmatic hernia and exomphalos, but may be an incidental finding at necropsy.

Incomplete intestinal rotation (classic malrotation) generally results in a narrowed mesenteric base, predisposing the affected children to midgut volvulus. Malrotation most frequently is characterized by localization of the duodenal-jejunal junction in the right upper quadrant and the cecum in the middle/upper abdomen. The latter is fixed by adhesive bands (Ladd bands) to the duodenum, gallbladder, and right sided abdominal wall. In the remaining number of cases the intestine rotates clockwise, thus positioning the duodenum anteriorly to the superior mesenteric artery and the colon posteriorly, creating a retro-arterial tunnel with possible obstruction of venous, lymphatic or arterial mesenteric obstruction.

In approximately 30-60% of cases, associated congenital malformations can be identified, including cardiac, anorectal and other [53]. Around 13% of cases of jejunoileal atresia are associated with malrotation, as are trisomy of chromosomes 9, 13, 18, 21 and triploidy, in varying percentage of cases [42,53]. A number of genes involved in the molecular events guiding these early steps of gut development and "rotation" have been identified, and those implicated in the "Left-Right" patterning have been shown to be the most conserved among all species [1,52,54]. Molecules crucial

to intestinal left-right patterning are Sonic hedgehog (Shh), activin, nodal, forkhead box transcription factor (Foxf-1), paired-like homeodomain transcription factor (Ptix)-2, bagpipe homeobox homolog (BAPX)-1 and fibroblast growth factor (FGF)-8 [1,52]. Defects in genes involved in left-right patterning can lead to disorders such as situs inversus viscerum and heterotaxy syndrome [1].

Most patients with malrotation are asymptomatic, and this anomaly may not be disclosed during life. Malrotation can be characterized by an acute or chronic process. The most severe acute presentation is the mid-gut volvulus, and onset of symptoms may be insidious or sudden. The mesentery and the intestinal loops are twisted, resulting in intestinal occlusion and bowel ischemia due to the interrupted blood supply. Symptoms include abdominal distension, pain, vomiting, constipation and bloody stools. Over the hours, hemodynamic instability from hypovolemia and shock, as well as peritonitis occur, and prompt diagnosis and treatment are necessary to avoid necrosis of the bowel [50]. In older children and adults, the presentation is more variable and may be mixed with symptoms of intermittent/chronic abdominal pain.

Syndromic intestinal malrotations, non-syndromic familial malrotation with autosomal dominant transmission and malrotation in alveolar capillary dysplasia with misalignment of pulmonary veins, a mostly sporadic and fatal disease due to mutation in *Foxf1,* have been described as well [55]. Recent studies suggest the role of differentially expressed plasma miRNAs as biomarkers of malrotation, thus improving early diagnosis [56].

Hirschsprung Disease

Hirschsprung disease (HSCR), also known as congenital aganglionic megacolon or congenital colonic aganglionosis, is a complex multigenetic congenital anomaly characterized by the absence of ganglion cells in a portion of the intestinal tract, usually the distal colon, due to a disruption of the normal neural crest cell migration, proliferation, differentiation, survival and/or apoptosis [57].

The aganglionic segment, which starts at the internal anal sphincter and extends proximally for a variable length, in around 80% of affected patients, is limited to the recto-sigmoid (short-segment HSCR), while in 3-10% of patients the entire colon is aganglionic (total colonic aganglionosis; TCA) [58]. A very short-segment HSCR is defined as an aganglionic segment involving less than 2 cm of the distal rectum [59]. In a very small percentage of cases the aganglionosis involves the distal small intestine.

According to various studies, the incidence ranges from 1 in 5000 to 1 in 10,000 live births and varies among different ethnic groups [58,60,61].

A skewed male-to-female ratio of 3:1 to 4:1 has been reported especially for short-segment HSCR [60].

In most cases, HSCR is not an inherited disease, even if it has long been appreciated that genetic alterations may play a pivotal role in the etiology. Moreover, familial recurrence has been reported, and some studies have suggested that the longer the aganglionic segment, the higher is the rate of familial recurrence [62]. Genetic associations have been shown to be complex, including incomplete penetrance, alterations in non-coding and coding DNA segments. Alterations in more than 30 different genes have been reported so far with frequent combinatorial effects, often times impairing signal transduction by the RET receptor tyrosine kinase [63]. In approximately 30% of affected children an associated chromosomal anomaly or congenital malformation can be identified. HSCR has been shown to have an incidence of 2.6% in patients affected by Trisomy 21 [64].

Genetic syndromes associated with HSCR include Ondine syndrome, Waardenburg-Shah syndrome, Mowat Wilson syndrome, multiple endocrine neoplasia type 2A, and others, while associated malformations frequently involve the cardiac, gastrointestinal, genitourinary and central nervous system [58,60].

The ability of GI tract in activating peristalsis, controlling blood flow and secretions depends on the enteric nervous system (ENS), which represents the largest part of the peripheral nervous system and functions almost independently of the central nervous system. ENS neurons and glia are organized into ganglia. The enteric ganglia are interconnected to form the outer myenteric plexus (Auerbach), involved in motility, and inner submucosal plexus (Meissner), which regulates motility, blood flow and transport of ions across the intestinal epithelium.

Aganglionosis is thought to be due to arrested migration of parasympathetic ganglion cells, which normally occurs between the second and third month of fetal life [57,65].

The earlier the arrest of cell migration occurs, the more extensive will the affected intestinal tract be, resulting in a poorer clinical outcome and more severe and disabling symptoms.

Once thought to be a disease of the neonatal period, today we know that only about 6.5% of patients present within the first week of life, and roughly 40% within the first 6 months [58, 66].

Neonates with megacolon are often full-term infants, of normal weight and without other malformations, whose initial symptom is a delay in the emission of meconium within the first 48 hours. The newborn may

also present with acute symptoms, consistent with distal occlusions, characterized by abdominal distension and bilious/fecaloid vomiting. In the most fulminant picture, infants present with overwhelming sepsis, characterized by poor general conditions, fever, distended abdomen and emission, under stimulation, of very smelly stools, usually gray in color (such as fine sand), soft or liquid [58,67].

In older children, the disease is discovered during the evaluation for chronic constipation. Progressive abdominal distension with malnutrition and failure to thrive is characteristic. Although they exist, cases of HSCR presenting after childhood are rare [68].

In cases suspicious for HSCR, there are two available screening tests: contrast enema and anorectal manometry. Manometry, however, is not feasible in small babies.

The most sensitive and specific examination to obtain the diagnosis is rectal suction biopsy. It is a minimally invasive procedure performed at the patient's bed which consists in the removal of fragments of the mucosa and submucosa.

Biopsies should ideally be obtained at 1, 2, and 3 cm above the dentate line. Labeled regarding their position (distance from the dentate line and circumferential location), they should be submitted separately. An additional unfixed biopsy should is performed and submitted to the laboratory for frozen sections if acetylcholinesterase histochemistry be needed.

Adequacy evaluation of the biopsies is of uttermost importance, as the absence of ganglion cells from biopsies obtained too close to the dentate line might be the consequence of physiological paucity in this area, rather than pathology.

The pathology report for diagnosis or exclusion of HSCR on rectal suction biopsies should contain information regarding the adequacy of the tissue sample (mucosa and submucosa), presence/absence of ganglion cells and/or submucosal nerve hypertrophy and the used of immunohistochemical and histochemical techniques [69].

An adequate biopsy should be lined by colonic mucosa (mucosal segments lined by either transitional or anal transitional mucosa, and in the absence of ganglion cells, should be regarded as indagate) and the submucosa should constitute approximately 50% or more of the tissue sample [58]. Processing of an adequate biopsy usually includes serial sections of the tissue fragment, sometimes exhausting the block, and careful histologic examination of a large number of slides.

Ganglion cells, characterized by the presence of abundant cytoplasm and eccentrically placed round nuclei, conspicuous nucleoli, a perinuclear halo, and peripheral chromatin (Fig. 8), can be diagnosed by the experienced pathologist on H&E-stained sections. Immature ganglion cells in very young children, characterized by less cytoplasm and an indistinct nucleolus with stippled chromatin, can be more difficult to identify and in some instances, immunohistochemistry may be helpful. The identification of an unequivocal ganglion cell usually excludes the diagnosis of HSCR. However, in the presence of features suggestive of a transition zone biopsy very short-segment HSCR must be excluded. Cytomegalovirus (CMV) infection can sometimes produce cytologic alterations reminiscent of mature ganglion cells, and that should be considered [58].

Submucosal nerve hypertrophy can usually be identified in HSCR specimens and consists of the presence of numerous large nerves with variable diameters in the submucosa (in the normal neonate nerve diameters rarely exceed 40 μm) [70]. Immunohistochemical stains (glucose transporter-1, glial fibrillary acidic pro-
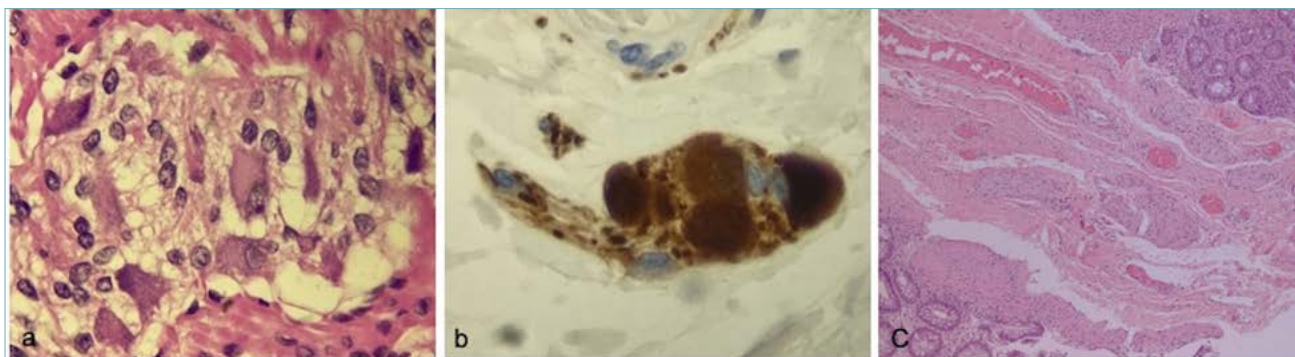
Figure 8. (a) Ganglion cells of the myenteric plexus from a normally innervated colon biopsy (H&E, x 400); (b) Calretinin immunohistochemistry displaying ganglion cells with nuclear and cytoplasmic positivity from a normally innervated tissue sample (x 400); (c) Hypertrophic submucosal nerves in a patient with HSCR (H&E, x 200).

tein, and others) can be used to highlight the presence of the hypertrophic nerve fibers. However, a higher sensitivity and specificity can be achieved by acetylcholinesterase (AChE) stains. AChE histochemistry highlights the cholinergic nerve fibers within the lamina propria, which appear abnormally coarse and dense.

Although a reliable diagnostic feature in most HSCR patients, submucosal nerve hypertrophy has been found to be strangely absent in some patients with TCA [71].

Ancillary techniques, namely immunohistochemical and histochemical stains, can be useful to facilitate the diagnosis, and most centers apply these techniques routinely. The most widely used methods are AChE histochemistry and Calretinin immunohistochemistry [58]. However, numerous other, mainly immunohistochemical markers, have been described in HSCR.

In cases of ambiguous histology on rectal suction biopsy and, depending on clinical practice in older children and adults, full-thickness biopsies should be obtained.

Differential diagnosis includes conditions such as intestinal neuronal dysplasia and colon hypoganglionosis, in which there is a generalized hypoplasia of the nervous structures of the intestinal wall.

After a proper diagnosis, the treatment is surgical and consists in most of cases in the resection of the aganglionic tract with further anastomosis of the distal rectum to normal colon, in various manners (pull-through). The treatment for long segment aganglionosis is very challenging, but refinements in surgical techniques and pre-and post-operative care have reduced morbidity and mortality and improved significantly the long-term outcome.

ANORECTAL MALFORMATIONS

Congenital malformations of the anorectum (ARMs) form a broad and complicated spectrum of anal anomalies and associated regional defects, ranging from mild phenotypes w to extensive anomalies of the hindgut and urogenital organs, which require individualized treatments for newborn, sophisticated approaches for reconstruction and management of long term outcomes.

During embryogenesis, the urorectal septum grows caudally and fuses with the cloacal membrane to divide the urogenital sinus and the anal and perianal openings. Anorectal malformations occur if there is abnormal migration and/or fusion of the urorectal septum or if the cloacal membrane fails to rupture. It has been suggested that a defect in the dorsal cloacal membrane interferes with its migration toward the tail groove, and that different types of anomalies are produced, depending on the form and size of the defect. Thus, superficial defects cause low malformations such as stenotic anus and ectopic anus, while deeper defects cause anal and anorectal agenesis, with and without fistulas. Abnormalities may be categorized generally as high, intermediate, or low, relative to the level of the puborectalis sling of the elevator ani. All types may be associated with fistulas, which may open into the bowel, perineum, vagina, urethra, or, rarely, the bladder, depending on the sex and the level of the lesion [72]. Numerous complex classification schemes, based mainly on clinical features, have been proposed [73-77]. The most widely accepted classification is the Krickenbeck classification, which is based on prognostic and therapeutic implications (Tab. II) [75].

Table II. Anorectal malformations according to the Krickenbeck Classification.

Major Clinical Groups	Rare/Regional Variants
Perineal (cutaneous) fistula	Pouch colon
Rectourethral fistula	Rectal atresia/stenosis
Prostatic	Rectovaginal fistula
Bulbar	H fistula
Rectovesical fistula	Others
Vestibular fistula	
Cloaca	
No fistula	
Anal stenosis	

The prevalence of ARMS has been estimated in 3 per 10,000 live births in Europe [78]. Males are more frequently affected than females and there is a recurrence risk of 1% for families with a previous case of ARM [79,80]. Associated congenital abnormalities are more common with high malformations, frequently involving the genitourinary system, heart and skeleton [78, 81]. Abnormalities of the lumbosacral spine are found in over 50% of the "high anomaly" group and include sacral agenesis or hemi-sacral anomalies. In all infants with ARM, a possible diagnosis of VACTERL association and caudal dysplasia syndrome must be taken into consideration. An imperforate anus occurs regularly in sirenomelia [82]. The overall incidence of associated anomalies in ARMs is approximately 45-65% [83,84].

Despite advances in basic embryology, animal models and genetics, the etiopathogenesis of the majority of ARMs remains mainly unclear and supposedly multifactorial [72, 85-87].

Non-genetic factors found to be associated with ARM are assisted reproductive techniques, multiple pregnancy, preterm delivery, low birth weight, maternal overweight or obesity, and preexisting diabetes [88,89].

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