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REVIEW ARTICLE

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Animal models in pediatric surgery

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Introduction

The advent of animal models of pediatric surgical diseases has not only allowed us to study the etiology and pathogenesis of complex congenital anomalies but has also led to major advances in the surgical and therapeutic management of these conditions. Even though representative animal models do not exist for every human disease, the animal models available to us today are still of great importance for medical research. Animal models allow us to comprehend the molecular and biochemical basis of diseases, and possibly find new drugs and forms of therapy, both medical and surgical. Different animal models have been and may be used. Because of their easy availability, smaller animals such as rodents are often preferred as models. Mice (*Mus musculus*) offer many advantages. They are similar to humans in a biological and genetic manner, so that much, but not all, of the knowledge gained from studies in mice may be applied to humans. The genome of the mouse is similar to the human genome, with regard to the size and number of genes. Mice have a very high reproduction rate, a short life span and mature quickly. This makes it possible to easily follow the effects of changed genes over many generations. At the present time, mice have become the most important and common animal models for human diseases. For many years the mouse genome has been closely investigated, and many mutations, which have either spontaneously developed in a population or were induced through outer influences (for example chemical mutagenesis) have been demonstrated. Many international co-operations work on methods of describing the function of each single genome with different strategies. Rats (*Rattus norvegicus)* are also widely used in research, as they are particularly suitable for physiological, pharmacological and behavioral research. They have also contributed greatly to research involving surgical techniques and the study of many teratogens.

Animal models are of great importance for the analysis of genes. With their help it is possible to set up models for the examination of gene expression and gene regulation. Genes may be specifically inactivated within animals (knockout model), and the effect on the organism can be studied. By inserting an additional recombinant gene (transgenic animal model), its expression and influence on the metabolism of the organism can be examined. The setting up of expression analysis is only part of solving the functions of genes in an organism or in tissues. Since genes generally work in coordination, thus defining the characteristics of an organism, it is necessary to examine the function of the genes in connection with the organism under physiological conditions.

Broadly speaking, there are four main types of animal models in use today: naturally occurring, teratogen induced, surgically created and transgenic animals. Obviously, the animals in which diseases occur naturally are the ideal models with which to study disease pathogenesis, as there is little or no interference to the animal prior to the study. The ground-breaking development of transgenic animals has not only allowed researchers to almost mimic the natural occurrence of certain conditions but also gives insight into the specific genes involved in the regulation of the disease process and how their modification might alter the course of the disease. Teratogen-induced models, although useful, have the drawback of exposing the animal to a generalized noxious stimulus, which can result in widespread detrimental effects rather than simply targeting a specific organ system. Finally, surgical models can closely simulate surgical procedures and conditions found in hu-

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man neonates but difficulties may arise in relation to controlling variables such as anesthesia, analgesia, sepsis and feeding.

Following from these models, research has sought to develop potential therapeutic approaches, including surgical interventions, which can be employed in the management of many conditions, and as a result many different models for several important human diseases have been developed over many years of pediatric surgical research. Lesser known animal models not discussed in this review include those for spina bifida (curly tail and delayed splotch mouse models, surgical rat and lamb models), hydrocephalus (HTX rat, HSV and *L1* knockout mouse models), cloacal exstrophy (suramin chick and surgical lamb model), cryptorchidism (*Insulin-3* and *HOXA 11* knockout mice, flutamide rat, surgical rat and rabbit models), biliary atresia (rotavirus mouse model), necrotizing enterocolitis (hypoxia/lipopolysaccharide and casein piglet model, surgical rabbit model, hypoxic rat model), short bowel syndrome (surgical mouse model), vesico-ureteral reflux (surgical piglet model) and pyloric stenosis (mutant *hph-1* mouse model). Research into important and challenging neonatal conditions such as gastroschisis (GS), Hirschsprung's disease (HD), VACTERL association and congenital diaphragmatic hernia (CDH) has been greatly advanced by the ready availability of animal models and will form the basis of the discussion in this review.

Animal models of the VATER association

The vertebral, anorectal, tracheo-esophageal, renal (VATER) association is a spectrum of clinical conditions in the human neonate, which involves multiple anomalies first reported by Quan [1]. The exact incidence of VATER is difficult to quantify in view of the fact that there is such variation in clinical presentation, but it affects approximately 1 in 5,000 live births and often requires urgent surgical intervention after birth due to the foregut and hindgut anomalies. VATER has not been recognized as a specific syndrome in humans but rather represents a non-random association of congenital anomalies of poorly understood etiology and pathogenesis and its components have been variable. The most important features of the VATER association, which will be discussed in this paper from a pediatric surgical point of view, include anorectal malformations, esophageal atresia (EA), tracheoesophageal fistula (TEF), and renal anomalies. Etiologically, the VATER association may be a feature of some chromosomal anomalies [2] but the majority of cases have no recognized cause. The higher incidence of malformations occurring in association with each other than might be thought to occur randomly prompted some to suggest that the VATER association arose as a result of an insult to the developing embryo early in its development which led to abnormalities in diffuse organ systems at birth. Khoury [3] suggested that the VATER association arose as a result of defective mesodermal development during early embryogenesis, leading to abnormalities in diffuse organ systems at birth. Others have suggested that teratogens, such as the maternal use of hormones during pregnancy may have a role in bringing about the association [4].

Four types of animal model of the main surgical anomalies found in the VATER association have been developed: teratogen induced, surgically created, knockout models and naturally occurring.

Teratogen-induced models of VATER

The Adriamycin rat model (ARM) of the VATER association is a well-established model, which has provided us with a unique opportunity to investigate the mechanisms behind the origins of this congenital anomaly and its associated conditions. Similarities with the human pattern made the Adriamycin rat a useful model for pathogenetic analysis of these defects. Adriamycin (doxorubicin hydrochloride) is an anthracycline, anti-neoplastic drug that has been widely used in the treatment of many malignancies, including sarcomas, lymphomas, neuroblastoma and breast cancer [5]. One proposed mechanism of action lies in its ability to inhibit nucleic acid synthesis through intercalation with DNA [6].

The teratogenicity of Adriamycin was first established by Thompson et al. [7] in 1978, when the intraperitoneal administration of 1–2 mg/kg/day of Adriamycin to pregnant rats from gestational days (GD) 6–9 resulted in characteristic malformations, including esophageal and intestinal atresia, TEF, hypoplasia of the urinary bladder and cardiovascular malformations. The first report of the Adriamycin rat as a pediatric surgical model for anorectal malformations, EA and TEF and renal anomalies came in 1996 from Diez-Pardo et al. [8]. With the administration of 1.5–2 mg/kg/day on GD 6–9 they achieved significant rates of EA with TEF (45%), anorectal anomalies (50%) and renal anomalies (100%) and this has prompted a huge volume of research to be carried out since then.

The role of neuropeptides and innervation in the esophagus of the ARM was looked at early on as a possible cause for esophageal dysmotility. The vagus and recurrent laryngeal nerves in ARM embryos were found to have inherent abnormalities in their course and culminated in a deficient extrinsic nerve fiber plexus in the lower esophagus [9]. Elevated levels of neuropeptides of the enteric nervous system, such as S100 and galanin were found in the distal esophagus of experimental embryos compared to controls [10]. Also the density of the nerve plexus, ganglia and number of cell bodies per ganglion immunostained by neuron-specific enolase, vasoactive intestinal peptide and substance P was significantly reduced in EA–TEF fetuses [11]. These findings were all suggested as contributing factors to dysmotility in patients with EA.

The role of apoptosis has been studied as a potential mechanism for the development of tracheoesophageal abnormalities in the ARM. Initial studies demonstrated a specific and consistent pattern of apoptosis or cell death in the region of the so-called ''tracheoesophageal septum'' that might have contributed to the normal process of tracheoesophageal separation [12, 13]. Subsequent studies did not demonstrate significant apoptosis in the immediate period following Adriamycin administration, suggesting that the teratogenic effect of Adriamycin is not caused by cell death [14, 15].

The contribution of the notochord to normal embryonic development has been well established and it has been shown to play a role in the patterning of mesoderm as well as regulating endodermal development [16, 17]. The notochord has been found to be grossly abnormal in ARM embryos with foregut anomalies, such as EA and TEF [18]. The various abnormalities of the notochord include accessory ventral branching, bending, splitting and tethering to foregut structures with ventral displacement of the notochord and dorsal displacement of the foregut [19–21]. Abnormalities of Sonic hedgehog (*Shh*), an important genetic signaling molecule, have also been implicated in the pathogenesis of the anomalies seen in the rat model of VATER. Studies have shown high levels of *Shh* protein expression in the normal rat foregut during embryogenesis, which slowly declines as the embryo approaches term [22]. In contrast, Adriamycin-treated embryos demonstrated a low level of *Shh* expression throughout the same time period. In a quantitative morphological study of the notochord, Mortell et al. [23] demonstrated a significant increase in the relative notochord volume of Adriamycin-treated embryos compared to controls. Immunohistochemically, the accessory branches of notochord, which contributed to this increased volume, expressed *Shh* similar to control notochord. Interestingly, the increase in notochord volume, relative to embryo volume, was found to be maximal soon after Adriamycin administration in experimental embryos [24]. This early alteration in notochord volume, with associated *Shh* expression, during a critical phase of embryonic development was thought to contribute to the malformations found in the VATER association.

The development of anorectal malformations in the ARM has also been of great interest to pediatric surgeons engaged in research. Although less frequently seen than EA with TEF, anorectal malformations occur in 24–28% of Adriamycin-treated embryos [25, 26]. Anorectal malformations have also been produced with the application of other teratogens to either rodent or murine models. Rat embryos prenatally exposed to a single dose of oral retinoic acid on GD 10 have been shown to develop anorectal anomalies in 94% of the cases [27]. The most common defect noted was anal atresia with or without cloaca and in association with these defects, abnormalities of the tail were commonly found. Interestingly, spina bifida was also noted in 55% of the embryos studied. In pregnant mice treated with oral retinoic acid as a single dose on one of the GD 8 or 9, a large number of their offspring developed multiple anomalies [28]. These consisted of urogenital malformations, anal agenesis and rectourethral fistula. More than 95% of murine embryos treated with retinoic acid developed anorectal malformations, with rectoprostatic urethral fistula, rectocloacal fistula and a short tail being commonly found [29].

As a teratogen, ethylenethiourea (ETU) has been used to induce anorectal malformations since the early 1990s [30]. The offspring of pregnant rats, which are gavage fed 1% ETU (125 mg/kg) on GD 10, develop anal atresia with a rectourethral fistula in up to 85% of embryos [31, 32]. These abnormalities seem to be caused by aberrations in the development of several components of the hindgut and cloaca. Abnormalities of the notochord, similar to those noted in the ARM, were found in this model and suggest that abnormal notochord development may be pivotal in producing anorectal malformations [32].

Renal defects are seen in approximately 50% of patients with the VACTERL association. The most common renal defects seen in humans with VATER/VACTERL association include renal agenesis, cystic dysplasia, renal ectopia, horseshoe kidney, persistent urachus, and urethral agenesis [33]. These problems can lead to significant morbidity and progressive renal deterioration early in life and may sometimes require renal transplantation. Renal anomalies form a significant part of the spectrum of abnormalities seen in the ARM of the VATER association. These findings have allowed researchers to intensively study the pathogenesis of urinary tract anomalies, which can affect human neonates. Although bladder agenesis is rare in humans, it can be developed experimentally in up to 100% of embryos receiving 2 mg/kg/day of Adriamycin on GD 6–9. This study suggests that GD 7 is the critical embryological window for bladder development in rats [34]. In association with bladder agenesis, all embryos had significant unilateral or bilateral hydronephrosis/hydroureter, presumably as a result of distal obstruction [35]. Further work determined that primary agenesis rather than secondary resorption of the bladder occurred in this model [36]. Microscopically, affected kidneys have abnormal architecture, with an abnormal nephron configuration, decreased tubular differentiation and a thinned medulla [37]. Dilatation was noted mainly in the collecting system, ducts and tubules, with no evidence of collagen deposition or fibrosis. A further development of this animal model, using a lower dose (1.75 mg/kg/day) of Adriamycin on GD 7–9, led to the findings of bilateral hydronephroureter with preservation of the bladder and this has allowed for a more physiological study of the renal tract, as might be found in the human setting [38].

The availability of murine molecular probes and genetic strains of knockout mouse models of EA and TEF

has prompted researchers to try and develop a new murine model incorporating the known teratogenic effects of Adriamycin [39]. Although this model has not been fully developed to yield the same spectrum of anomalies seen in the ARM, early results have shown tracheoesophageal anomalies in 47% of embryos from pregnant CBA/Ca mice treated with 4 mg/kg/day of Adriamycin on GD 7.5 and 8.5. Further work demonstrated immunohistochemical evidence of maintained Nkx2.1 expression (similar to that seen in controls) in the respiratory element of Adriamycin-treated embryos despite their abnormal morphogenesis [40]. The authors concluded that the dorsal fistula retains its non-respiratory commitment, in the absence of tracheoesophageal separation, suggesting that dorso-ventral patterning of foregut development is undisturbed by Adriamycin exposure. The authors then studied the expression of Sonic hedgehog (*Shh*) and Patched (*Ptc1)* in the foregut of developing mouse embryos, which had been treated with Adriamycin [40]. *Shh* is a secreted glycoprotein, which is known to play an important patterning role in the development of many organ systems including the foregut. *Ptc1* is a transmembrane receptor, which acts as a target gene for *Shh* [41]. Their results showed a reversal in the dorso-ventral pattern of *Shh* expression during the brief period of tracheoesophageal separation, with the foregut of Adriamycin-treated embryos diffusely expressing *Shh* [40]. They suggested that a transient disturbance in *Shh* expression at this critical time might contribute to the abnormal organogenesis in this model.

Surgical models of EA and TEF

Although the chick embryo has not provided us with a reliable model of EA and TEF, its contribution to our knowledge of the normal development of the esophagus and trachea cannot be ignored. The theory of foregut hyperflexion had been proposed as a cause of EA and distal TEF as far back as 1976 [42]. However, this theory has never been proven. Kleckner et al. [43] performed hyperflexion studies on chick embryos in order to test this theory. Although one case of an ''H-type'' TEF resulted from their study, no cases of EA developed. This isolated case was felt to possibly represent a chance finding of little significance and therefore the theory was disregarded.

Embryological studies in the chick have endeavored to look at the primitive foregut during the period of differentiation, as previous work has suggested that the tracheoesophageal septum plays a significant role in the division of the foregut into trachea and esophagus. Kluth et al. [44] studied the foregut region of chick embryos from Hamburger and Hamilton [45] stages 14–26 by scanning electron microscopy and light microscopy. They found no evidence of lateral folds or fusing foregut wall components, which might have contributed to a tracheoesophageal septum. Their findings suggested that esophageal and tracheal development results from a simple reduction of the size of the primitive foregut.

Although the vast majority of animals used for research purposes today are rodents, canine models of EA have been studied as far back as the 1970s when Oh [46] described a microsurgical technique for esophageal reconstruction in puppies. Subsequently, beagles were widely used to study the post-operative results following esophageal resection or transection with subsequent re-anastomosis, utilizing an autologous jejunal mucosa transplant [47], a tubular musculopleural pedicle graft [48] or a variety of myotomies with or without delayed esophageal reconstruction [49–51]. The results from these studies were variable with no clear advantage for one particular technique. Although early contrast and manometric studies suggested a good outcome from spiral myotomy, long-term follow-up in this group demonstrated esophageal dysmotility, as observed in children following esophageal surgery. However, further studies in canine models of EA compared manometric findings following esophageal transection and re-anastomosis versus esophageal vagotomy alone [52]. Their results showed coordinated peristaltic contractions between the proximal and distal esophagus in the first group with abnormal simultaneous contractions in the vagotomized group. This suggested that post-operative dysmotility might arise from disruption of the vagus nerve as part of the congenital abnormality or secondary to surgical trauma.

An interesting paper by Carachi et al. [53] looked at the effect of an indwelling silicone transanastomotic tube (TAT) on the healing esophageal anastomosis in puppies. They found no significant difference in stenosis rates whether a TAT was used or not. Interestingly, they observed a shelf of stenotic tissue on the posterior wall of the esophagus at the site where the intraluminal silk knots had been tied, compared to a thin linear scar on the anterior wall, where knots had been tied extraluminally.

More recently, the use of a tubed latissimus dorsi musculocutaneous flap (LDMCF) for esophageal replacement has been studied in canine puppies [54, 55]. Long-term follow-up of these animals showed good survival rates with only occasional vomiting. Although contrast studies showed no evidence of stenosis, endoscopy demonstrated hair re-growth intraluminally, with no evidence of abnormal histological changes on sectioning of the LDMCF.

Most recently Tannuri et al. [56, 57] have studied the effects of circular myotomy (Livaditis) on anastomotic leak rates and anastomotic healing in dogs subjected to esophageal resection and an anastomosis under tension. They found an equal leak rate between groups with an anastomosis under tension and those with an additional circular myotomy. Morphometric and biochemical analysis showed reduced numbers of small vessels and collagen type V in the fibrous scars of the myotomy animals. The authors concluded that circular myotomy does not reduce the possibility of anastomotic leaks but

does promote deleterious changes in anastomotic healing.

Porcine (piglet) models of EA have been very useful for pediatric surgical research purposes because of their size and anatomical similarity to human neonates. Initial studies involved the evaluation of surgical techniques for esophageal replacement comparing gastric tube interposition with gastric tube in continuity and gastric transposition [58]. Gastric transposition gave very poor results with 100% mortality within 96 h. Gastric tube interposition was associated with improved growth, fewer clinical complications (such as vomiting, minor leak and stricture) and fewer histological changes (such as esophagitis or submucosal fibrosis) than either gastric tube in continuity (with or without posterior fundoplication) or gastric transposition.

The effects of a circular myotomy (to achieve esophageal elongation in long gap atresia) have sometimes been detrimental to patients, with muscular defects allowing ballooning of the esophagus post-operatively [59]. To try and prevent this adverse occurrence, Komuro et al. [60] applied collagen sponge scaffolds to the muscular defect in myotomized piglet esophagus. Although the loose connective tissue covering the defect did not contain muscle, when it was examined histologically, it did prevent distension of the esophagus at the point of myotomy.

The advent of robotic-assisted surgery has prompted a great volume of research in animal models of surgical disease. The advantages are many, but the techniques are not without their drawbacks. Although robots can filter out the fine tremor of a surgeon's hand and add an extra element of flexibility with intracorporeal instrument angulation and higher magnification, the prolonged set up time and added expense can detract from the overall usefulness of certain robots. Thoracoscopic repair of EA [61] has become a recognized method for dealing with this complex surgical problem and studies involving robotic-assisted thoracoscopic repair of EA in piglets have shown that the technique is both technically feasible and that it may facilitate a minimal approach in the small chest of a newborn [62, 63]. A recent interesting study looked at a novel approach to thoracoscopic esophageal anastomosis using a U-clip suturing technique [64]. The self-ligating metal U-clips were easily placed and appeared to be a feasible alternative to sutures but the study only involved a small number of piglets and therefore warrants further research to assess the technique more comprehensively.

A surgical rat model has recently been developed to examine the possibility of esophageal transplantation for use as an esophageal replacement. Initial studies by Yamataka et al. [65] looked at syngeneic esophageal transplantation into an omental pouch and found the transplants to be viable and well vascularized after a period of 10 days. They also found that the grafts could be easily mobilized into the thoracic cavity on an omental pedicle without tension. Following this study they employed allogeneic esophageal transplantation from newborn Brown-Norway rats into 5-week-old Lewis rats and studied the effects on the neo-esophagus with and without immunosuppression using FK-506 [66]. Syngeneic transplants were used as controls and showed no evidence of rejection with 100% graft survival. Grafts that received between 0.6 and 1.2 mg/kg of FK-506 for the duration of the study period showed minimal or no rejection. However, grafts receiving no immunosuppression, low dose FK-506 or immunosuppression for a shorter period showed definite histological evidence of rejection.

Knockout models of VATER

Faulty genetic signaling has often been implicated in the etiology of the VATER association. The hedgehog (Hh) family of signaling molecules mediates many inductive processes during invertebrate and vertebrate development. As previously mentioned, Sonic hedgehog (*Shh*) is a genetic signaling molecule crucial to normal embryonic development, which is released from the notochord and acts in a concentration dependant manner [67]. *Shh* has been shown to be involved in the control of cell fate determination in the central nervous system, dorsoventral patterning of somites, anteroposterior polarity of limbs and left–right asymmetry. In the developing embryo, the *Shh* pathway is essential for correct patterning of the various organ systems [68–70], including hindgut patterning [71] and lung growth [72], and is responsible for radial patterning of the gut wall by inducing inner components and by inhibiting outer components, such as smooth muscle and neurons [73]. In mutant mice with deletions of the *Shh* gene, VATER anomalies are commonly found [67, 74]. Homozygous *Shh*-null mutant mice demonstrate EA/stenosis, TEF and tracheal and lung abnormalities, similar to those seen in human neonates with foregut defects [75]. Anorectal malformations have also been shown in *Shh*-null mice with mutants displaying a persistant cloaca, with the distal urinary tract and anorectum sharing a common outlet [76].

Other factors implicated in *Shh*'s patterning of the embryo include the *Shh* membrane-bound protein receptors Patched (*Ptc*), Smoothened (*Smo*) and the zinc-finger transcription factors *Gli1*, *Gli2*, and *Gli3*, which are also released from the embryonic notochord and act as mediators in the nuclear transcriptional activity of *Shh* signaling [77]. These genes are known to operate in a downstream manner to *Shh* and have been shown to have an essential function in the formation of lung, trachea, esophagus and ano-rectum in mice [76].

Mutant mice lacking *Gli2* function exhibit the full spectrum of VACTERL anomalies, except renal anomalies. These include foregut defects, such as esophageal and tracheal stenosis as well as lung hypoplasia [74]. All *Gli2* null-mutants displayed an imperforate anus with recto-urethral or recto-vaginal fistula [78]. *Gli3*

null-mutants have been shown to develop vertebral, anal and limb anomalies. However, *Gli3* null-mutant mice with a 50% reduction in the gene dosage of *Gli2* developed EA and TEF with very poor lung development. Mice lacking both *Gli2* and *Gli3* did not develop esophagus, trachea or lung [76, 79].

NKX2.1 (otherwise known as *TTF-1*) is a homeodomain transcriptional factor expressed in thyroid, lungs and parts of the brain. Mouse embryos carrying a homozygous targeted disruption of the *Nkx2.1* locus demonstrate failure of septation of the anterior foregut along the dorsoventral axis, into distinct tracheal and esophageal structures [80]. This failure of septation results in a common lumen connecting the pharynx to the stomach, similar in phenotype to the human pathological condition of TEF. Further abnormalities in this knockout model include severe lung hypoplasia, with associated poor gas exchange, resulting in early postnatal death.

Naturally occurring models of anorectal malformations

Two naturally occurring animal models have long been used to study anorectal malformations, such as those seen in the VATER association. The *Sd* mouse, first bred by Danforth in 1930 [81], is a mutant of the normal house mouse and was found to develop urogenital and anorectal abnormalities [82]. Also known as "Danforth's short tail mouse", this model has a number of advantages because of the high percentage of abnormal animals per litter, the relative inexpense of a mouse model and the ease with which *Sd* mice can be bred. These mice have an autosomal-dominant trait for imperforate anus and pass on this semi-dominant trait with high penetrance, where all of the homozygotes and most of the heterozygotes are affected. Kluth et al. [83] looked at the spectrum of anorectal anomalies in this model and found that the pathological anatomical malformations in the heterozygous *Sd* mouse group were very similar to those seen in human neonates with anorectal anomalies as well as previous porcine models, which have been described. To date, the gene defective in *Sd* mice has yet to be identified.

Domestic pigs have a naturally high incidence of anorectal malformations and can be bred to generate an animal model to study the anatomy and embryology of anorectal anomalies [84]. Recent studies have developed a pig pedigree over 15 years, resulting in the development of non-syndromal anal atresia in up to 60% of newborn piglets [85]. This resource was generated by surgically treating affected piglets soon after birth and later breeding them for a number of generations. Utilizing marker analysis, the authors attempted to map susceptibility loci and found a suggestive locus on pig chromosome 15 [86]. Previous morphological studies on the internal sphincter in neonatal piglets with anorectal malformations showed that all animals had a normal internal sphincter surrounding the proximal fistula [84]. They, therefore, suggested that the fistula should be designated as an ectopic anal canal and that the incorporation of the fistula into the surgical correction of anorectal malformations may yield better results from a continence point of view.

Animal models of Hirschsprung's disease

Hirschsprung's disease (HD) is a common cause of intestinal obstruction in the newborn with an incidence estimated to be 1 in 5,000 live births. Animal models of HD have been pivotal in the understanding of the anatomy and pathophysiology of the disease and in the discovery of the genes involved in HD. Four types of HD animal models can be distinguished: natural mutants, knockout models, surgical and chemical models.

Natural mutants

Aganglionic megacolon not only occurs in humans but also affects several other species such as mice, rats and horses [87–90]. All of these mutations not only involve the intestine but another neural crest derivative, the melanocytes, since all have major areas of unpigmented skin and hair. In 1966 Lane described two strains of mice, which developed aganglionosis as an autosomal recessive condition [87]. The lethal spotting (*ls*) mice have approximately 2 mm of aganglionosis with a patched coat. Piebald lethal (s^l) mice have approximately 10 mm of aganglionosis. Later studies linked the defect to chromosome 2 and 14, respectively. In 1979 Ikadai et al. [89] described aganglionosis in rats and 80–90% of the rats had a total colonic aganglionosis. The animals had a high mortality rate and were only able to survive 3–4 weeks of age due to a severe bowel obstruction and enterocolitis. These animals showed autosomal recessive inheritance and had a white colored coat. A fourth rodent model is the dominant megacolon (*Dom*) mouse that was described by Lane and Liu in 1984. As the name suggests the inheritance is dominant [88]. These mice are characterized by distal colonic aganglionosis and a long hypoganglionic transition zone, but occasionally the entire large intestine is aganglionic. The allele for this mutation has been mapped on chromosome 15. Histological studies in all these rodent models using acetylcholinesterase whole-mounts demonstrated that they have a histological picture identical to the human. There is distal aganglionosis with increased nerve trunks, followed by a transition zone, which is often asymmetrical and variable in length and more proximal the plexus is near normal [91]. Many electrophysiological studies have been done in rodent models of aganglionosis. It has been demonstrated that there are abnormal discharges of myogenic action potentials in the aganglionic bowel associated with tonic constriction and a reduction in the luminal diameter [92] and that the aganglionic bowel has no inhibitory junction potentials,

which leads it to writhe in an uncontrolled manner tending to contraction. Therefore, the rodent models have helped us to clarify that the simple lack of nerve fibers is sufficient explanation for the functional obstruction seen in patients with HD and that there is no need to invoke selective disorders of various components of the autonomic nervous system to account for the clinical variability [91]. The rodent models of aganglionosis have also contributed to the understanding of the embryology of HD. Cass et al. [93] studied the migration of the neural crest cells to the gut in the lethal spotting mouse, the piebald mouse and the spotting lethal rat. They concluded that there was a slowing of migration in those neural crest cells derived from the vagal neural crest and that there were small numbers of enteric precursors in the aganglionic distal hindgut derived from the sacral neural crest that did not differ in number compared to normal embryos. Although they demonstrated that there is sacral neural crest contribution into the enteric nervous system, this contribution was shown to be functionally insignificant.

The mutations which give rise to these natural mutant mouse strains have now been identified as being the endothelin receptor-B gene (ENDR-B) for the piebald lethal mouse, the gene for endothelin-3 (ET-3) in the lethal spotting mouse and the Sox10 gene in the *Dom* mouse [94–97].

Knockout models for HD

Transgenic technology, particularly in the mouse, provides a powerful method with which to produce animal models to determine the function of genes associated with human inherited disease. Several genes have been disrupted in mice, producing phenotypes remarkably similar to HD in humans. In addition, three well-studied mutant mouse models of HD have been found to be due to disruptions of specific genes, as stated before. The *Ret* gene encodes a receptor tyrosine kinase, which has four ligands: glial cell line derived growth factor (*GDNF*), neurturin (*NTN*), artemin (*ATM*) and persephin (*PSP*) [98]. The complete receptor complex includes the *Ret* receptor tyrosine kinase and a glycosylphosphatidylinositol-anchored binding component (*gfrα1*, *gfrα2*, *gfrα3* or *gfrα4*). This receptor has been suggested to function as an adhesion molecule, which is required for neural crest migration and that could also play a role in either differentiation or survival of the neural crest cells which have stopped migrating [99, 100]. *Ret* (−/−) transgenic mice have a homozygous, targeted mutation of the tyrosine kinase receptor resulting in a loss of its function. These mice exhibit total intestinal aganglionosis and renal agenesis [101]. The *Ret* gene has been demonstrated to be a major gene causing HD in humans. Mutations of *Ret* account for 50% of familial and 15–20% of sporadic cases of HD [102–105]. *GDNF*, one of the *Ret* receptor ligands, stimulates the proliferation and survival of neural crest derived precursor cells in the embryonic gut [106, 107]. Mice homozygous for null mutation in *Ret*, *GDNF* and *gfrα1* have almost identical phenotypes characterized by failure of enteric nervous system development distal to the esophagus and absent kidneys [101, 108–112]. Although a causative role for *GDNF* mutations in some patients with HD has been suggested, the occurrence of such cases is uncommon and it is more likely that the *GDNF* mutations are involved via its interaction with the *Ret* receptor [113, 114]. No *gfrα1* mutations have been identified in patients with HD [115].

The endothelins are intercellular local messengers that comprise four members to date: *ET-1*, *ET-2*, *ET-3* and *VIP*. They transduce a signal via two cell surface transmembrane receptors: *ENDR-A* and *ENDR-B* [98]. In human fetuses, both *ENDR-B* and *ET-3* have been demonstrated on enteric neurones and gut mesenchyme cells, suggesting that their function may be to regulate interactions between neural crest cells and gut mesenchyme cells, necessary for normal migration [94, 95]. Both *ET-3* and *ENDR-B* genes have been disrupted and have been identified as the cause for the natural mutants lethal spotting mice and piebald lethal mice, respectively [94, 95]. Moreover, a transgenic mouse *ENDR-B* null, with a mutation targeted to the *ENDR-B* gene, has a phenotype identical to the piebald lethal mouse [116]. In the same year as the connection between mutations in the *Ret* receptor and familial HD was established, *ET-3* and *ENDR-B* mutations were also implicated in the disease [94, 95, 117]. However, these mutations have been demonstrated in less than 10% of the cases of HD in humans and particularly in Waardenburg syndrome [118–121]. Endothelins are initially produced as an inactive proendothelin that has to be activated by a specific enzyme, the endothelin-converting enzyme (*ECE*). Two *ECE* genes have been described, *ECE-1* and *ECE-2* [98]. ECE-1 knockout mice show craniofacial and cardiac abnormalities in addition to colonic aganglionosis [122]. A heterozygous *ECE1* mutation has been identified in a patient with HD who also had craniofacial and cardiac defects [123].

Sox10 is a member of the *SRY*-related family of transcription factors and is expressed by enteric nervous system precursors before and throughout colonization of the gut mesenchyme [98]. Disruption of the *Sox10* gene has been demonstrated to be the cause of the *Dom* mouse natural mutant [96, 97]. Interestingly, both homozygous and heterozygous animals produce a lethal HD-like phenotype [124]. Mutations in *Sox10* have been identified in Waardenburg syndrome associated with HD [125].

Phox2B is a transcription factor that is essential for the development of the neural crest derivates [126] as it regulates the *Ret* expression in enteric nervous system precursors [127]. Targeted *Phox2B* gene disruption leads to a complete absence of enteric nervous system in the mice, a phenotype that is very similar to that of the *Ret* knockout mouse [127]. Recently, Garcia-Barcelo et al. [128] reported that *Phox2B* deficiency might predispose

to HD in humans. *Pax3* is a member of the paired-box-containing family of nuclear transcription factors that is expressed in neural cell precursors giving rise to enteric ganglia and synergizes with *Sox10* to activate an enhancer in the *Ret* gene [129]. In the mouse, *Pax3* mutations result in a phenotype characterized by a white belly spot and deficient enteric ganglia in the heterozygous state. Homozygous deficient embryos die during midgestation with neural tube defects, cardiac defects and absence of enteric ganglia [129]. No *Pax3* mutations have been identified in patients with HD.

Surgically created HD

Most surgically generated experimental models of HD have been made in chick embryos because they are easily accessible and the development of their enteric nervous system has been well studied. Aganglionosis can be induced by surgical ablation of the premigratory neural crest [130]. Even though this procedure does not tell us about the mechanism of HD, it can be used to investigate possible treatment strategies. It has been possible to recolonize aganglionic bowel with neural crest cells by transplanting tissue obtained from the dorsal neural tube [131–133]. It has also been shown that neurons from more proximal regions of bowel, if taken at early stages after their differentiation, are capable of recolonizing distal bowel and forming enteric ganglia [133, 134].

Chemical models of HD

Sato et al. [135] first described a chemical model of HD in 1978. They created a segmental aganglionosis by topical application of benzalkonium chloride to the colon and rectum in rats. This model has also been successfully reproduced in mice and guinea pigs [136, 137] and has been tested not only in the intestine, as a model of HD, but also in the distal esophagus as a model of achalasia [138]. Benzalkonium chloride is a cationic surfactant agent that attaches to the cell membrane causing irreversible depolarization, which injures the cell membrane resulting in severe cell damage and cell death. Since neurons have a higher cell membrane negative charge, the depolarizing effect is more marked on them; thus, benzalkonium chloride induces a selective neuronal ablation in the intestinal wall [136]. This treatment eliminates almost all myenteric neurons and glia in treated segments. Although the aganglionic bowel does not show hypertrophic nerve bundles and the chemical does not affect the number of submucosal neurons, the treated part results in a narrow segment and the recto-anal reflex is abolished [135]. Compared to the other models of HD, this technique is cheap, easy to perform and the animals can survive longer. This chemical model of HD has been used to study functional and structural changes in the bowel resulting from loss of these neural elements and could be used to study the chronic changes caused by the aganglionic segment and the long-term effects of different surgical treatments [139–146].

Animal models of gastroschisis

Gastroschisis (GS) is a congenital anomaly, which occurs with an approximate incidence of 1 in 4,000 births. Based on embryologic studies, there are various theories on the pathogenesis of GS. The three main theories are somatopleure differentiation deficiency in embryonic mesenchyme [147], defects of the omphalomesenteric artery in utero [148], and premature atrophy or abnormal persistence of the right umbilical vein [149]. Although it is thought to be a rare condition, the incidence of GS appears to be increasing dramatically in recent years, possibly as a result of lower maternal age, alcohol consumption, cigarette smoking and drug abuse [150, 151]. Infants born with this condition have an abdominal wall defect, usually to the right of the umbilicus, which exposes the bowel to amniotic fluid during fetal life. Although bowel is only rarely resected in GS, histologic findings demonstrate thickened intestinal muscle layers, blunting of epithelial villi and the presence of a serosal fibrous peel [152]. Despite the fact that 90% of infants survive, children born with GS face potential prolonged difficulties with nutrient absorption and intestinal dysmotility. Although recent advances in antenatal diagnosis, surgical technique and neonatal intensive care have improved outcomes dramatically, a level of morbidity and mortality persists for neonates with GS. The etiology of intestinal damage in infants with GS is uncertain and is probably multifactorial. Studies in chick embryos, fetal mice, rats, rabbits and lambs have demonstrated similar changes in the affected intestine to those seen in human neonates with GS. These studies have suggested that both exposure to amniotic fluid and possible constriction of the bowel at the level of the abdominal defect play a role in the changes affecting the bowel.

Three types of GS animal models have been developed over the years: surgically created, teratogen induced and transgenic.

Surgically created gastroschisis models

The surgical creation of an abdominal defect in fetal rabbits and lambs in the 1970s allowed early researchers to study the pathophysiology of GS [153, 154]. Chick embryo models of GS followed in the 1980s, with the utilization of one of the most readily available and versatile animal models, which could be easily modified to avoid exposure of the bowel to fetal urine [155]. Most recently, the rat model of GS was developed to take advantage of its large litter size, short gestation and the ready availability of molecular probes and reagents [156].

With the exception of the chick embryo, the surgically created models involve a laparotomy, followed by a hysterotomy to expose the experimental fetus. This is generally performed under general anesthetic with tocolytic agents to prevent premature labor. A small fetal para-umbilical incision is then made to allow extrusion of bowel loops out of the abdomen, with care taken not to injure the umbilical vessels. The fetus is then replaced back into the uterine cavity to simulate human GS, where the bowel is bathed in amniotic fluid. Alternatively, the fetus can be partially replaced into the uterus with the bowel remaining outside the uterine cavity to prevent amniotic fluid from coming into contact with the fetal intestines.

In 1991, Philips modified the fetal rabbit model to yield higher survival rates (up to 82% in the GS group), resulting in significantly shorter and thicker bowel in experimental animals compared to controls [157]. Animals were generally operated on day 23–24 (Full term = 30–31 days) with specimens being harvested shortly before term. Further studies examining the effects of amniotic fluid on the exposed bowel were performed by creating GS either intra-amniotically or extra-amniotically [158]. This resulted in significantly shorter bowel in fetal rabbits, where the bowel was exposed to amniotic fluid compared to bowel that was maintained extra-amniotically and control bowel. However, there was no significant difference in intestinal weight or bowel wall thickness. Surprisingly, there was no evidence of serositis or adhesions in experimental bowel and all GS specimens were histologically normal, in contrast to bowel from human newborns with GS.

Nutrient uptake by GS intestine has also been studied using the rabbit model. Shaw employed an everted mucosal sleeve technique to determine the uptake of an amino acid (proline), and a sugar (glucose) in the small intestine (SI) of GS-affected fetal rabbits [159]. He found that the uptake of proline per milligram and centimeter of SI, and the uptake of glucose per milligram of SI was significantly impaired in GS fetuses compared with controls.

The role of nitric oxide (NO) in GS-induced intestinal damage has been studied in the fetal rabbit model of GS. Bealer measured NO synthase activity in the SI of full-term fetal rabbits with and without GS [160]. He found that the SI of GS fetal rabbits showed a significant increase in total NO synthase activity 2.5 times greater than that of control littermates without GS. He attributed this finding to accelerated enzyme kinetics.

The effects of prokinetic agents have also been studied to determine their potential effects on intestinal hypomotility in fetal rabbit GS [161]. Cisapride was the only agent found to improve bowel contractility in newborns with GS. Erythromycin, octreotide and metoclopramide did not improve in vitro contractility of GS bowel in fetal rabbits. Most recently, gastrointestinal motility has been determined in preterm fetal GS rabbits with the administration of intragastric fluorescein [162]. This study showed that bowel exposure to amniotic fluid reduces intestinal motility and gastric contractility in the preterm rabbit fetus. The fetal rabbit model of GS has recently been used to demonstrate the feasibility of intrauterine repair of GS for the first time [163]. This short study only assessed fetal survival for 5 h after initial GS creation and therefore has not addressed the issue of long-term survival following intrauterine GS repair or if there is any reduction in intestinal damage following the procedure.

The lamb model of GS has allowed more invasive therapeutic techniques to be studied by virtue of the size of the study animal. Fetendoscopy has been employed not only to create GS surgically but also to place an amnioinfusion catheter into the amniotic cavity of the experimental fetus [164]. This fetendoscopic technique resulted in survival rates of 46.7% in the GS group. Esophageal ligation has recently been employed in the fetal lamb model of GS in an attempt to interrupt the passage of amniotic fluid through the digestive tract [165]. However, this intervention did not have a beneficial effect on the inflammatory process affecting GS bowel in this model. In the lamb model morphometric analysis of GS bowel revealed thickening of the muscularis propria with evidence of both cellular hyperplasia and hypertrophy [166]. These changes in smooth muscle were associated with a thickened submucosa with an increase in collagen content.

The chick embryo model of GS has allowed many studies on the effects of amnio-allantoic fluid (AAF) exchange on GS bowel. The amniotic cavity of chick embryos is readily accessible once an eggshell window is created. The allantoic and amniotic membranes can then be opened to create a common cavity, which mimics the amniotic cavity in humans. The characteristic picture of GS evolved only in chick embryos where herniated bowel was exposed to urine components in the allantoic fluid [167]. The use of AAF exchange in GS chick embryos has demonstrated a reduction in macroscopic and microscopic intestinal damage [168, 169], and prevented a decrease in bowel contractility [170]. Aktug et al. [171] found that AAF exchange resulted in a reduction in the levels of gastrointestinal waste products (bile acids and bilirubin) in embryonic AAF; however, it did not alter the levels of urinary waste products, such as urea and creatinine. This dilution in the concentration of gastrointestinal waste products yielded a reduction in bowel damage seen in GS chick embryos. Studies on the effects of AAF pH on GS bowel have shown that pre-treatment with alkalinization of AAF can also reduce intestinal damage [172].

Research involving the fetal rat model of GS has centered around neuronal differentiation of the bowel, as it has been proposed that delayed maturation of intestinal smooth muscle cells might account for the dysmotility seen in human neonates with GS. Survival rates of 78–91% have been achieved in this model. In the GS rat model fetus, the myenteric plexus was noted to be disorganized with absent or scattered neuronal cells suggesting a degree of neuronal immaturity [173].

Intestinal neurofilament formation also appeared immature, demonstrating delayed cytoskeletal organization and reduced synaptic activity in the myenteric neurons in the rat model of GS [174]. Immunohistochemical analysis of GS intestine showed delayed differentiation of smooth muscle cells and interstitial cells of Cajal, with the most severely affected bowel loops demonstrating the most significant aberration in pacemaker cell morphology [175].

Although fetal urine has been implicated in the etiology of intestinal damage in GS, the role of meconium has also been investigated [176]. Correia-Pinto looked at different grades of meconium contamination in the fetal rat model of GS and found that all parameters of bowel damage were adversely affected by meconium contamination of the amniotic fluid (MCAF) [177]. Intestinal length was decreased in direct correlation with the grade of MCAF. In contrast, intestinal weight, degree of fibrous peel coverage and bowel adherence was significantly increased in experimental fetuses supporting the hypothesis that meconium is at least in part responsible for bowel damage in GS.

Transgenic gastroschisis models

Transgenic mice have played a relatively minor role in animal research of GS. The Heiligenberger (HLG) inbred mouse strain was established as an inbred strain in Freiburg (Germany) in 1939 and was subsequently colony-bred from 1975 to 1992 in Essen [178]. By 1994 more than 20 generations of inbreeding had taken place and the strain was registered (HLG/Zte). In this model GS occurs spontaneously with a frequency of about 3%. This strain demonstrates a higher incidence (up to 11%) of GS when embryos at the one-cell stage are exposed to the teratogen, X-irradiation. The pattern of inheritance in this model follows a recessive mode for the HLG susceptibility alleles. Utilizing a backcross strategy and genome-wide micro-satellite typing, this trait has been mapped chromosomally. Hillebrandt found a suggestive linkage for a locus responsible for radiation-induced GS (*Rigs1*) in a region of mouse Chromosome 7 [179]. Additional quantitative trait loci were identified on Chromosome 11 and Chromosome 13. Pils found that an increased incidence of GS (6.5 vs. 3.5% in controls) persisted in the progeny of a second generation of HLG/Zte mice even without the pregnant females receiving a dose of radiation [180]. These results support the hypothesis that genomic instability may play a role after radiation exposure of the zygote stage of HLG mice.

Teratogen-induced gastroschisis models

The investigation of teratogens in animal models has been of great benefit in determining the deleterious effects of compounds prior to their use in humans. To date there is no single teratogenic agent in use in pediatric surgical research, which yields a high incidence of GS when administered to chick embryos, mice, rats or rabbits with good reproducibility. Agents, such as doxorubicin hydrochloride [181], ethanol [182, 183], nitrous oxide [184], ethylene glycol [185], scopolamine hydrobromide [186], acetazolamide [187], and cyclooxygenase inhibitors [188, 189] have all been investigated and have resulted in embryonic malformations, including GS. However, the teratogenic rate in these studies is relatively low with GS affecting embryos in only 3.7–19.8% of cases.

The injection of doxorubicin hydrochloride/saline into the albumin of chick eggs resulted in a GS rate of 19.8% [181]. However, the development of ventral defects, such as GS, in the chick embryo seems to be a perturbation of embryogenesis at a crucial early stage rather than a direct teratogenic effect of doxorubicin itself, as controls receiving the saline vector commonly developed the malformation (15.7%).

Due to the high incidence of GS in offspring of young mothers who often smoke during pregnancy, Singh studied the effects of carbon monoxide (CO) and altered protein/zinc diet on pregnant mice of the CD-1 strain [190]. He found that the incidence of GS was 47% in the low protein/zinc deficient group, which had been exposed to CO. In contrast, no fetal mice developed GS in the other groups with either low protein/zinc diet/air or CO alone. These findings contribute to the thinking of a multifactorial etiology of GS.

Alcohol has been widely studied for its teratogenic effects and in humans results in a pattern of malformations in what is known as the fetal alcohol syndrome (FAS). The main methods of administration have included intraperitoneal injection of an alcohol solution to pregnant rats [182] and oral administration of ethanol-derived diet to pregnant C57BL/6J isogenic mice [183]. Although experimental fetuses in both rat and mouse studies developed multiple anomalies, the incidence of GS was generally low with the doses given.

An interesting chemical, which has been extensively studied in the toxicology and oncology literature, is the polar solvent N-methylformamide (NMF). This compound is a metabolite of dimethylformamide, a solvent with wide applications in the chemical industry. Its teratogenic effects were noted in rabbits as far back as 1980 when oral administration resulted in a number of developmental anomalies [191]. At this time rabbit embryos appeared to be more sensitive to these chemicals than other animals and more recent studies have demonstrated similar findings. It has also been evaluated as an antineoplastic agent because it has been shown to induce tumor inhibition in human colon carcinoma cell lines when used in combination with 5-fluorouracil [192]. In a study in 1995, Kelich [193] utilized Crl:CD (SD) COBS rats and New Zealand white rabbits [Hra:(NZW)] and administered daily NMF by gavage at doses up to 75 mg/kg from GD 6–15 in rats and 50 mg/kg from GD 6–18 in rabbits. In the rat group, only one fetus developed GS out of a total of 269 (0.4%). Interestingly, in

the rabbit group 84 out of 85 (99%) fetuses developed GS, with skull and sternal anomalies being the other major malformations. With this high incidence of fetal GS in NMF-treated rabbits the potential for future development of an animal model in pediatric surgical research is significant. Further studies of this model could yield valuable results for pediatric surgical researchers worldwide.

Animal models of congenital diaphragmatic hernia

Congenital diaphragmatic hernia is a common congenital malformation with an incidence of 1 in 3,000 births. Despite progress in neonatal and intensive care units, the outcome of these patients depends mainly on the pulmonary hypoplasia that remains the principal contributor to the high mortality of CDH. Much of the current understanding of the pathophysiology of CDH originates from experimental studies.

Four types of CDH animal models have been developed over the years: naturally occurring, surgically created, teratogen induced and transgenic.

Naturally occurring CDH

A CDH pig model was described in a herd that was originally bred to produce pigs with anorectal malformations [194]. Among 93 piglets, 13 with CDH were identified, which presented with symptoms from 1 to 4 months after birth. As with humans with CDH, herniated intra-abdominal organs, stomach and intestine were found within the thoracic cavity; but crucially, these pigs lacked the pulmonary hypoplasia that is characteristic of human CDH. This pig model represents a true natural animal model of CDH, but the main disadvantage is that it requires a large herd of animals to ensure a regular production of animals with CDH.

Surgically created CDH

De Lorimier [195] was the first to create an animal model of CDH, using third trimester fetal lambs, based upon the traditional view that lung hypoplasia in CDH results from compression by abdominal viscera. This led to hypoplastic lungs but similar pressure volume curves compared to sham-operated controls. Subsequent studies attempted to simulate CDH by placing a balloon in the left chest in fetal lambs [196, 197]. Newborns had significantly reduced lung weights and air capacities, decreased pulmonary vascular bed and decreased pulmonary compliance compared with control animals. Following this it was shown that in utero deflation of the balloon improved the pathophysiologic consequences of CDH and improved survival [197]; but none of these models accurately simulated the pulmonary vascular morphological changes seen in humans with CDH. After that, the age in which the defect was created was decreased to the second trimester in the hope that insulting the developing lung earlier in gestation would more closely simulate the human condition [198]. In this model, time-dated pregnant ewes were anesthetized and following a midline abdominal incision, the fetus was localized by palpation of the uterus. Then a hysterotomy was performed and the upper torso of the fetus delivered. Next, a posterolateral left thoracotomy was made and the diaphragm was incised widely over the superior edge of the spleen and the fundus of the stomach; the stomach, omentum and small intestine were gently pulled into the chest. The fetal thoracotomy and maternal hysterotomy were closed and pregnancy prolonged using tocolytics. This model has also been tested in rabbits [199–201]. Resulting CDH lambs have a number of similarities to the human condition: the lungs are hypoplastic and have reduced number of airway branches, diminished alveolar number, decreased size of the pulmonary vascular bed and an increase in the muscularization of the pulmonary arterial tree when compared with controls [202–204]. The prenatal repair of this surgically created diaphragmatic defect showed a reversal of pulmonary hypoplasia; however, a prospective randomized trial in humans did not show any clear benefit over standard postnatal management [205]. This redirected the efforts to approach the disease on reversing the pulmonary hypoplasia rather than focusing on the diaphragm. Extrapolating the findings from the congenital high airway obstruction syndrome in which hyperplastic lungs are found, an innovative animal model was created utilizing temporary tracheal occlusion to induce lung hyperplasia [206]. Repair of the actual diaphragmatic defect occurred postnatally. Initially, a clip was placed in the trachea through a cervical dissection. The clips, however, required surgical removal prior to birth leading to associated tracheal complications. Modification led to the current technique of endoscopic placement of a detachable balloon directly into the fetal trachea [207]. The results of this technique, in a randomized controlled trial in humans, have recently been reported [208]. The historical analysis of how pioneering fetal surgery developed for CDH reflects the importance of animal models in surgical diseases. However, since this animal CDH model is surgically created during fetal life, it is unable to yield any insights into the embryogenesis of CDH.

Teratogen-induced CDH

Although the teratological effects of the herbicide nitrofen (2,4–dichlorophenyl-*p*-nitrophenyl ether) in rodents were described a long time ago [209], they had been overlooked by pediatric surgeons until a rediscovery of this model led to an explosion in research [210–212]. Since nitrofen is administered during early organogenesis, the advantage of this model over the surgically created ones is that it gives the opportunity to examine

the embryogenesis of CDH. The exact time of administration and the right dosage of nitrofen needed to create CDH have been studied over the years. In the mouse model, time-mated CD-1 or Swiss-Webster mice have to be gavage fed with 25 mg of nitrofen dissolved in 0.5 ml of olive oil on GD 8 to produce severe pulmonary hypoplasia in the whole litter and CDH in 30% of them [213]. In the rat model, time-mated Sprague-Dawley rats are gavage fed with 100 mg of nitrofen dissolved in 1 ml of olive oil on GD 9.5 in order to obtain a 60% rate of CDH, most of them being left sided. Interestingly, if rats are given nitrofen on day 11 of gestation, they mainly develop right-sided CDH [211].

In both mouse and rat models, the CDH animals have predominantly large-sized hernial defects containing liver and stomach and a marked bilateral pulmonary hypoplasia. Surprisingly, nitrofen-treated lungs without CDH are also hypoplastic [211, 212, 214, 215]. The associated malformations found in the CDH animals have been studied and they closely mimic the incidence and spectrum of those seen in humans [216–220]. Since the defect is induced at the stage when the foregut has just separated into the esophagus and the trachea, this model of CDH has given the opportunity of carefully studying the developmental anatomy of the lungs and diaphragm in CDH [212, 221–224]. Recent studies have shown that the pulmonary hypoplasia precedes the diaphragmatic defect in this model and that there could be a common mechanism underlying the etiology of CDH or that the pulmonary hypoplasia might even be the cause of the diaphragmatic defect instead of the result [225, 226]. Although all these studies provide some information about the pathogenesis of CDH, research into the etiology of CDH had been lacking. Recently, Greer et al. [227] have provided evidence suggesting that the retinoid signaling pathway may contribute to the etiology of CDH.

Even though the nitrofen model of CDH has been widely used, it is unclear whether pulmonary hypoplasia (particularly in those nitrofen-treated animals without CDH) may arise due to a direct toxic effect of nitrofen rather than being secondary to the CDH and hence has little to tell us of hypoplastic lung development in human CDH. Moreover, since there is a liver ingrowth into the chest in most of the animals with CDH and this is rarely seen in human CDH, the nitrofen CDH model has recently been questioned as a good model of CDH [228]. Nevertheless, the nitrofen model remains one of the most popular because it is cheap, technically simple and because it provides a singular opportunity for embryological studies.

Transgenic models of CDH

Ideally, the best animal model of CDH would be a gene knockout model that could accurately reproduce the malformation seen in humans. So far, several mutant phenotypes have been described in which diaphragmatic hernia may feature but they have a range of often far more frequent and significant malformations that make them phenotypically different from human CDH. Deletion of both murine RARs is associated with a high incidence of cranial, vertebral, limb, cardiac, foregut and pulmonary malformations whilst manifesting a low frequency of CDH [229, 230]. Mice with mutations for the homeobox *Hlx* gene also have CDH; however, more remarkable in these mice are their large lungs and small liver and intestine [231]. Homozygous inactivation of *WT-1* results in CDH but they are also severely deformed and have serious defects in the urogenital system [232]. Recently, knockout mice homozygous for *Slit3* deficiency were constructed. In these mice, the most obvious phenotype is CDH, but the hernia is located on the ventral midline portion of the central tendon rather than in the posterolateral diaphragm and all hernias include the liver and the gallbladder. Moreover, 20–40% of the mice have renal and ureteral agenesis [233].

Heterogeneity between human CDH and described knockout mice restrains against the adoption of transgenic CDH models at present.

References

1. Quan L, Smith DW (1973) The VATER association. Vertebral defects, Anal atresia, T-E fistula with esophageal atresia, radial and renal dysplasia: a spectrum of associated defects. J Pediatr 82:104–107
2. Rabinowitz JGM, Mitty JE (1967) Trisomy 18, esophageal atresia, anomalies of the radius, and congenital hypoplastic thrombocytopenia. Radiology 89:488
3. Khoury MJ, Cordero JF, Greenburg F, James LM, Erickson JD (1983) A population study of the VACTERL association: evidence for its etiologic heterogeneity. Pediatrics 71:815–820
4. Nora AH (1975) A syndrome of multiple congenital anomalies associated with teratogenic exposure. Arch Environ Health 30:17
5. Young RC, Ozols RF, Myers CE (1981) The anthracycline antineoplastic drugs. N Engl J Med 305:139–153
6. Di Marco A, Arcamone F (1975) DNA complexing antibiotics: daunomycin, adriamycin and their derivatives. Arzneim Forsch 25:368–375
7. Thompson DJ, Molello JA, Strebing RJ, Dyke IL (1978) Teratogenicity of adriamycin and daunomycin in the rat and rabbit. Teratology 17:151–157
8. Diez-Pardo JA, Baoquan Q, Navarro C, Tovar JA (1996) A new rodent experimental model of esophageal atresia and tracheoesophageal fistula: preliminary report. J Pediatr Surg 31:498–502
9. Qi BQ, Merei J, Farmer P, Hasthorpe S, Myers NA, Beasley SW, Hutson JM (1997) The vagus and recurrent laryngeal nerves in the rodent experimental model of esophageal atresia. J Pediatr Surg 32:1580–1586
10. Cheng W, Bishop AE, Spitz L, Polak JM (1997) Abnormalities of neuropeptides and neural markers in the esophagus of fetal rats with adriamycin-induced esophageal atresia. J Pediatr Surg 32:1420–1423
11. Qi BQ, Uemura S, Farmer P, Myers NA, Hutson JM (1999) Intrinsic innervation of the oesophagus in fetal rats with oesophageal atresia. Pediatr Surg Int 15:2–7
12. Qi BQ, Beasley SW (1998) Preliminary evidence that cell death may contribute to separation of the trachea from the primitive foregut in the rat embryo. J Pediatr Surg 33:1660–1665

13. Zhou B, Hutson JM, Farmer PJ, Hasthorpe S, Myers NA, Liu M (1999) Apoptosis in tracheoesophageal embryogenesis in rat embryos with or without adriamycin treatment. J Pediatr Surg 34:872–875
14. Gillick J, Giles S, Bannigan J, Puri P (2002) Cell death in the early adriamycin rat model. Pediatr Surg Int 18:576–580
15. Williams AK, Qi BQ, Beasley SW (2000) Temporospatial aberrations of apoptosis in the rat embryo developing esophageal atresia. J Pediatr Surg 35:1617–1620
16. Elliott GB, Tredwell SJ, Elliott KA (1970) The notochord as an abnormal organizer in production of congenital intestinal defect. Am J Roentgenol Radium Ther Nucl Med 110:628–634
17. Cleaver O, Krieg PA (2001) Notochord patterning of the endoderm. Dev Biol 234:1–12
18. Possoegel AK, Diez-Pardo JA, Morales C, Tovar JA (1999) Notochord involvement in experimental esophageal atresia. Pediatr Surg Int 15:201–205
19. Orford J, Manglick P, Cass DT, Tam PP (2001) Mechanisms for the development of esophageal atresia. J Pediatr Surg 36:985–994
20. Gillick J, Giles S, Bannigan S, Puri P (2002) Midgut atresias result from abnormal development of the notochord in an Adriamycin rat model. J Pediatr Surg 37:719–722
21. Gillick J, Mooney E, Giles S, Bannigan J, Puri P (2003) Notochord anomalies in the adriamycin rat model: a morphologic and molecular basis for the VACTERL association. J Pediatr Surg 38:469–473
22. Arsic D, Keenan J, Quan QB, Beasley S (2003) Differences in the levels of sonic hedgehog protein during early foregut development caused by exposure to Adriamycin give clues to the role of the Shh gene in oesophageal atresia. Pediatr Surg Int 19:463–466
23. Mortell A, O'Donnell AM, Giles S, Bannigan J, Puri P (2004) Adriamycin induces notochord hypertrophy with conservation of sonic hedgehog expression in abnormal ectopic notochord in the adriamycin rat model. J Pediatr Surg 39:859–863
24. Mortell A, Gillick J, Giles S, Bannigan J, Puri P (2005) Notable sequential alterations in notochord volume during development in the Adriamycin rat model. J Pediatr Surg 40:403–406
25. Merei J, Hasthorpe S, Farmer P, Hutson JM (1999) Visceral anomalies in prenatally adriamycin-exposed rat fetuses: a model for the VATER association. Pediatr Surg Int 15:11–16
26. Merei JM (2002) Embryogenesis of adriamycin-induced hindgut atresia in rats. Pediatr Surg Int 18:36–39
27. Yu J, Gonzalez S, Martinez L, Diez-Pardo JA, Tovar JA (2003) Effects of retinoic acid on the neural crest-controlled organs of fetal rats. Pediatr Surg Int 19:355–358
28. Padmanabhan R (1998) Retinoic acid-induced caudal regression syndrome in the mouse fetus. Reprod Toxicol 12:139–151
29. Sasaki Y, Iwai N, Tsuda T, Kimura O (2004) Sonic hedgehog and bone morphogenetic protein 4 expressions in the hindgut region of murine embryos with anorectal malformations. J Pediatr Surg 39:170–173
30. Hirai Y, Kuwabara N (1990) Transplacentally induced anorectal malformations in rats. J Pediatr Surg 25:812–816
31. Qi BQ, Beasley SW, Frizelle FA (2002) Clarification of the processes that lead to anorectal malformations in the ETU-induced rat model of imperforate anus. J Pediatr Surg 37:1305–1312
32. Qi BQ, Beasley SW, Frizelle FA (2003) Evidence that the notochord may be pivotal in the development of sacral and anorectal malformations. J Pediatr Surg 38:1310–1316
33. van Heurn LW, Cheng W, de Vries B, Saing H, Jansen NJ, Kootstra G, Tam PK (2002) Anomalies associated with oesophageal atresia in Asians and Europeans. Pediatr Surg Int 18(4):241–243
34. Liu MI, Hutson JM, Zhou B (1999) Critical timing of bladder embryogenesis in an adriamycin-exposed rat fetal model: a clue to the origin of the bladder. J Pediatr Surg 34(11):1647–1651
35. Liu MI, Hutson JM (2000) Cloacal and urogenital malformations in adriamycin-exposed rat fetuses. BJU Int 86(1):107–112
36. Liu MI, Hutson JM (2001) Ontogeny of bladder agenesis in rats induced by adriamycin. BJU Int 87(6):556–561
37. Temelcos C, Hutson JM (2004) Renal abnormalities in rat fetuses exposed to doxorubicin. J Urol 171:877–881
38. Mortell A, Fourcade L, Solari V, Puri P (2005) Bilateral megaureters in the Adriamycin rat model. Pediatr Surg Int 21(3):212–216
39. Ioannides AS, Chaudhry B, Henderson DJ, Spitz L, Copp AJ (2002) Dorsoventral patterning in oesophageal atresia with tracheo-oesophageal fistula: evidence from a new mouse model. J Pediatr Surg 37:185–191
40. Ioannides AS, Henderson DJ, Spitz L, Copp AJ (2003) Role of sonic hedgehog in the development of the trachea and oesophagus. J Pediatr Surg 38:29–36
41. Hammerschmidt M, Brook A, McMahon AP (1997) The world according to hedgehog. Trends Genet 13:14–21
42. Piekarski DH, Stephens FD (1976) The association and embryogenesis of tracheo-oesophageal and anorectal anomalies. Prog Pediatr Surg 9:63–76
43. Kleckner SC, Pringle KC, Clark EB (1984) The effect of chick embryo hyperflexion on tracheoesophageal development. J Pediatr Surg 19:340–344
44. Kluth D, Steding G, Seidl W (1987) The embryology of foregut malformations. J Pediatr Surg 22:389–393
45. Hamburger V, Hamilton HL (1951) A series of normal stages in the development of the chick embryo. J Morph 88:49–92
46. Oh C, Jacobson JH 2nd (1972) Microsurgical technique for esophageal reconstruction in growing puppies. Arch Surg 104:203–205
47. Halsband H (1986) Esophagus replacement by free, autologous jejunal mucosa transplantation in long-gap esophageal atresia. Prog Pediatr Surg 19:22–36
48. Moutsouris C, Barouchas G, Karayannacos P, Dontas I, Salakos C, Skalkeas G (1987) Tubular musculopleural pedicle grafting of esophageal long gaps in dogs. J Pediatr Surg 22:117–119
49. Rossello PJ, Lebron H, Franco AA (1980) The technique of myotomy in esophageal reconstruction: an experimental study. J Pediatr Surg 15:430–432
50. Shoshany G, Kimura K, Jaume J, Sterman H, Birnbaum E, Stein T, Levine J (1988) A staged approach to long gap esophageal atresia employing a spiral myotomy and delayed reconstruction of the esophagus: an experimental study. J Pediatr Surg 23:1218–1221
51. Levine JJ, Shoshany G, Davidson M, Kimura K (1990) Manometric variations following spiral myotomy for long-gap esophageal atresia. J Pediatr Gastroenterol Nutr 10:380–384
52. Shono T, Suita S (1997) Motility studies of the esophagus in a case of esophageal atresia before primary anastomosis and in experimental models. Eur J Pediatr Surg 7:138–142
53. Carachi R, Stokes KB, Brown TC, Kent M (1984) Esophageal anastomosis—an experimental model to study the anastomotic lumen and the influence of a transanastomotic tube. J Pediatr Surg 19:90–93
54. Yamataka A, Miyano T, Segawa O, Nozawa M (1994) Tubed latissimus dorsi musculocutaneous flaps for thoracic esophageal replacement: an experimental study. J Pediatr Surg 29:590–593
55. Yamataka A, Wang K, Kobayashi H, Segawa O, Miyahara K, Sueyoshi N, Miyano T (2000) Tubed latissimus dorsi musculocutaneous flaps for esophageal replacement in puppies: long-term follow-up. J Pediatr Surg 35:1623–1625
56. Tannuri U, Tannuri AC, Fukutaki MF, de Oliveira MS, Muoio VM, Massaguer AA (1999) Effects of circular myotomy on the healing of esophageal suture anastomosis: an experimental study. Rev Hosp Clin Fac Med Sao Paulo 54:9–16

57. Tannuri U, Teodoro WR, de Santana Witzel S, Tannuri AC, Lupinacci RM, Matsunaga P, Matsumura N, Naufal RR (2003) Livaditis' circular myotomy does not decrease anastomotic leak rates and induces deleterious changes in anastomotic healing. Eur J Pediatr Surg 13:224–230
58. Samuel M, Burge DM (1999) Gastric tube interposition as an esophageal substitute: comparative evaluation with gastric tube in continuity and gastric transposition. J Pediatr Surg 34:264–269
59. Lindahl H, Louhimo I (1987) Livaditis myotomy in long-gap esophageal atresia. J Pediatr Surg 22:109–112
60. Komuro H, Nakamura T, Kaneko M, Nakanishi Y, Shimizu Y (2002) Application of collagen sponge scaffold to muscular defects of the esophagus: an experimental study in piglets. J Pediatr Surg 37:1409–1413
61. Rothenberg SS (2000) Thoracoscopic repair of a tracheoesophageal fistula in a newborn infant. Pediatr Endosurg Innov Tech 4:289–294
62. Hollands CM, Dixey LN (2002) Robotic-assisted esophagoesophagostomy. J Pediatr Surg 37:983–985
63. Lorincz A, Langenburg SE, Knight CG, Gidell K, Rabah R, Klein MD (2004) Robotically assisted esophago-esophagostomy in newborn pigs. J Pediatr Surg 39:1386–1389
64. Tirabassi MV, Banever GT, Moriarty KP, Konefal S, Reiter E, Wait R (2004) Feasibility of thoracoscopic U-clip esophageal anastomosis: an alternative for esophageal atresia reconstruction. J Pediatr Surg 39:851–854
65. Yamataka A, Kato Y, Ohshiro K, Miyazaki E, Wang K, Miyano T (1999) Fetal esophageal transplantation in rats: a treatment option for long-gap esophageal atresia. J Pediatr Surg 34:1638–1640
66. Yamataka A, Wang K, Kobayashi H, Unemoto K, Miyahara K, Sueyoshi N, Miyano T (2001) Transplantation of newborn esophagus: an experimental study. J Pediatr Surg 36:1255–1257
67. Kim JH, Kim PCW, Hui CC (2001) The VACTERL association: lessons from the sonic hedgehog pathway. Clin Genet 59:306–315
68. Ramalho-Santos M, Melton DA, McMahon AP (2000) Hedgehog signals regulate multiple aspects of gastrointestinal development. Development 127:2763–2772
69. Rennie J (1994) Super sonic. A gene named for a video game guides development. Sci Am 270:20
70. Traiffort E, Charytoniuk DA, Faure H, Ruat M (1998) Regional distribution of sonic hedgehog, patched, and smoothened mRNA in the adult rat brain. J Neurochem 70:1327–1330
71. Roberts DJ, Johnson RL, Burke AC, Nelson CE, Morgan BA, Tabin C (1995) Sonic hedgehog is an endodermal signal inducing Bmp-4 and Hox genes during induction and regionalization of the chick hindgut. Development 121:3163–3174
72. Bellusci S, Furuta Y, Rush MG, Henderson R, Winnier G, Hogan BL (1997) Involvement of sonic hedgehog (*Shh*) in mouse embryonic lung growth and morphogenesis. Development 124:53–63
73. Sukegawa A, Narita T, Kameda T, Saitoh K, Nohno T, Iba H, Yasugi S, Fukuda K (2000) The concentric structure of the developing gut is regulated by sonic hedgehog derived from endodermal epithelium. Development 127:1971–1980
74. Kim PCW, Mo R, Hui CC (2001) Murine models of VACTERL syndrome: role of sonic hedgehog signalling pathway. J Pediatr Surg 36:381–384
75. Litingtung Y, Lei L, Westphal H, Chiang C (1998) Sonic hedgehog is essential to foregut development. Nat Genet 20:58–61
76. Mo R, Kim JH, Zhang J, Chiang C, Hui CC, Kim PC (2001) Anorectal malformations caused by defects in sonic hedgehog signaling. Am J Pathol 159:765–774
77. Walterhouse DO, Yoon JW, Iannaccone PM (1999) Developmental pathways: sonic hedgehog-patched-GLI. Environ Health Perspect 107:167–171
78. Kimmel SG, Mo R, Hui CC, Kim PC (2000) New mouse models of congenital anorectal malformations. J Pediatr Surg 35:227–230
79. Motoyama J, Liu J, Mo R, Ding Q, Post M, Hui CC (1998) Essential function of Gli2 and Gli3 in the formation of lung, trachea and oesophagus. Nat Genet 20:54–57
80. Minoo P, Su G, Drum H, Bringas P, Kimura S (1999) Defects in tracheoesophageal and lung morphogenesis in Nkx2.1(−/−) mouse embryos. Dev Biol 209(1):60–71
81. Danforth CH (1930) Developmental anomalies in a special strain of mice. Amer J Anat 45:275–287
82. Gluecksohn-Schoenheimer S (1943) The morphological manifestation of a dominant mutation in mice affecting tail and urogenital system. Genetics 28:341–348
83. Kluth D, Lambrecht W, Reich P, Buhrer C (1991) SD-mice—an animal model for complex anorectal malformations. Eur J Pediatr Surg 1:183–188
84. Lambrecht W, Lierse W (1987) The internal sphincter in anorectal malformations: morphologic investigations in neonatal pigs. J Pediatr Surg 22:1160–1168
85. Ohkawa H, Matsumoto M, Hori T (1990) Establishment of a pig animal model for anorectal malformations and its application to embryological studies. Jpn J Pediatr Surg 22:499–505
86. Hori T, Giuffra E, Andersson L, Ohkawa H (2001) Mapping loci causing susceptibility to anal atresia in pigs, using a resource pedigree. J Pediatr Surg 36:1370–1374
87. Lane PW (1966) Association of megacolon with two recessive spotting genes in the mouse. J Hered 57:29–31
88. Lane PW, Liu HM (1984) Association of megacolon with a new dominant spotting gene (*Dom*) in the mouse. J Hered 75:435–439
89. Ikadai H, Fujita H, Agematsu Y et al (1979) Observation of congenital aganglionosis rat (Hirschsprung's disease) and its genetical analysis. Congen Anom 19:31–36
90. McCabeL, Griffin LD, Kinzer A et al (1990) Overo lethal white foal syndrome: equine model of aganglionic megacolon (Hirschsprung's disease). Am J Med Genet 36:336–340
91. Cass DT (2000) Animal models of aganglionosis, 2nd edn. In: Holschneider AM, Puri P (eds) Hirschsprung's disease and allied disorders. Harwood Academic Publishers
92. Wood JD (1973) Electrical activity of the intestine of mice with hereditary megacolon and absence of enteric ganglion cells. Am J Dig Dis 18:477–488
93. Cass DT, Zhang AL, Morthorpe J (1992) Aganglionosis in rodents. J Pediatr Surg 27:351–356
94. Hosoda K, Hammer RE, Richardson JA et al (1994) Targeted and natural (piebald-lethal) mutations of endothelin-B receptor gene produce megacolon associated with spotted coat colour in mice. Cell 79:1267–1276
95. Baynash AG, Hosoda K, Giaida A (1994) Interaction of endothelin 3 with the endothelin B receptor is essential for development of neural crest-derived melanocytes and enteric neurons: missense mutation in endothelin 3 gene in lethal spotting mice. Cell 79:1277–1285
96. Hersarth B, Pinganlt V, Bondurand H et al (1998) Mutation of the Sry-related SOX 10 gene in dominant megacolon, a mouse model for human Hirschsprung's disease. Proc Natl Acad Sci USA 95:5161–5168
97. Soultared-Smith EM, Kos L, Pavan WJ et al (1998) Sox 10 mutation disrupts neural crest development in Dom Hirschsprung's mouse model. Nat genet 18:60–64
98. Puri P, Shinkai T (2004) Pathogenesis of Hirschsprung's disease and its variants: recent progress. Sem Pediatr Surg 13:18–24
99. Robertson K, Mason IJ (1995) Expression of *ret* in the chicken embryo suggests roles in regionalization of the vagal neural tube and somites and in the development of multiple neural crest and placodal lineages. Mech Dev 53:329–344
100. Pouliot Y (1992) Phylogenetic analysis of the cadherin superfamily. Bioessays 14:743–748

101. Schuchardt A, D'Agati V, Larsson-Blomberg L et al (1994) Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor RET. Nature 367:380–383
102. Romeo G, Ronchetto P, Luo Y (1994) Point mutations affecting the tyrosine kinase domain of the RET proto-oncogene in Hirschsprung's disease. Nature 367:377–378
103. Edery P, Lyonnet S, Mulligan LM et al (1994) Mutations of the RET proto-oncogene in Hirschsprungs's disease. Nature 367:378–380
104. Kusafuka T, Puri P (1997) Altered RET gene mRNA expression in Hirschsprung's disease. J Pediatr Surg 32:600–604
105. Martucciello G, Checcherini I, Lerone M et al (2000) Pathogenesis of Hirschsprung's disease. J Pediatr Surg 35:1017–1025
106. Worley DS, Pisano JM, Choi ED et al (2000) Developmental regulation of GDNF response and receptor expression in the enteric nervous system. Development 127:4383–4393
107. Young HM, Hearn CJ, Farlie PG et al (2001) GDNF is a chemoattractant for enteric cells. Dev Biol 229:503–516
108. Sanchez M, Silos-Santiago I, Frisen L et al (1996) Renal agenesis and the absence of enteric neurons in mice lacking GDNF. Nature 382:70–73
109. Pichel JG, Shen L, Sheng HZ et al (1996) Defects in enteric innervation and kidney development in mice lacking GDNF. Nature 382:73–76
110. Enomoto H, Araki T, Jackman A et al (1998) GFR alpha 1-deficient mice have deficits in the enteric nervous system and kidneys. Neuron 21:317–324
111. Tomac AC, Grinberg A, Huang SP et al (2000) Glial cell line-derived neurotrophic factor receptor alpha 1 availability regulates glialcell li-derived neurotrophic factor signalling: evidence from mice carrying one or two mutated alleles. Neuroscience 95:1011–1023
112. Cacalano G, Farinas I, Wang LC et al (1998) GFR alpha 1 is an essential receptor component for GDNF in the developing nervous system and kidney. Neuron 21:53–62
113. Amiel J, Lyonnet S (2001) Hirschsprung's associated syndromes and genetics: a review. J Med Genet 38:729–739
114. Angrist M, Bolk S, Halushka M et al (1996) Germline mutations in glial cell line derived neurotrophic factor (GDNF) and RET in Hirschsprung's disease patients. Nat genet 14:341–344
115. Martucciello G, Thompson H, Mazzola C et al (1998) GDNF deficit in Hirschsprung's disease. J Pediatr Surg 33:99–102
116. Kapur RP, Yost C, Palmioter RD et al (1992) A transgenic model for studying the development of the enteric nervous system in normal and aganglionic mice. Development 116:167–175
117. Puffenberger EG, Hosoda K, Washington SS et al (1994) A missense mutation of the endothelin-B receptor gene in multigenic Hirschsprung's disease. Cell 79:1257–1266
118. Kusafuka T, Wang Y, Puri P (1996) Novel mutation of the endothelin-B receptor gene in isolated patients with Hirschsprung's disease. Hum Mol Genet 5:347–349
119. Kusafuka T, Puri P (1997) Mutations of the endothelin-B receptor and endothelin-3 genes in Hirschsprung's disease. Pediatr Surg Int 12:19–23
120. Kusafuka T, Wang Y, Puri P (1997) Mutation analysis of the RET, the endothelin-B receptor and the endothelin-3 genes in sporadic cases of Hirschsprung's disease. J Pediatr Surg 32:501–504
121. Edery P, Attie T, Amiel J et al (1996) Mutations of the endothelin-3 gene in Waardenburg–Hirschsprung's disease (Shah–Waardenburg syndrome). Nat Genet 12:442–444
122. Yanagisawa H, Yanagisawa M, Kapur RP et al (1998) Dual genetic pathways of endothelin mediated intercellular signalling revealed by targeted disruption of endothelin converting enzyme-1. Development 125:825–836
123. Hofstra RM, Valdenaire O, Asch E et al (1999) A loss of function mutation in the endothelin converting enzyme-1 (ECE-1) associated with Hirschsprung's disease, cardiac defects and autonomic dysfunction. Am J Hum Genet 64:304–308
124. Kapur RP (1999) Hirschsprung's disease and other enteric dysganglionoses. Crit Rev Clin Lab Sci 36:225–273
125. Kuhlbrodt K, Schmidt C, Sock E et al (1998) Functional analysis of Sox 10 mutatins found in human Waardenburg–Hirschsprung patients. J Biol Chem 273:23033–23038
126. Paltyn A, Morin X, Cremer H et al (1999) The homeobox gene Phox2b is essential for the development of autonomic neural crest derivates. Nature 399:366–377
127. Gariepy CE (2001) Intestinal motility disorders and development of the enteric nervous system. Pediatr Res 49:605–613
128. Garcia-Barcelo M, Sham MH, Lui VCH et al (2003) Association study of Phox2b as a candidate gene for Hirschsprung's disease Gut 52:563–567
129. Lang D, Chen F, Milewski R et al (2000) Pax3 is required for enteric ganglia formation and functions with Sox10 to modulate expression of *c-ret*. J Clin Invest 106:963–971
130. Carel Meijers JH, Tibboel D, van der Kamp A et al (1989) A model for aganglionosis in the chicken embryo. J Pediatr Surg 6:557–561
131. Payette RF, Tennyson VM, Pomeranz HD et al (1988) Accumulation of components of basal laminae: association with the failure of neural crest cells to colonise the presumptive aganglionic bowel of ls/ls mutant mice. Dev Biol 125:341–360
132. Gershon MD, Chalazonitis A, Rothman TP et al (1993) From neural crest to bowel: development of the enteric nervous system. J Neurobiol 24:199–214
133. Rothman TP, Le Douarin NM, Fontaine-Perus JC et al (1990) Developmental potential of neural crest derived cells migrating from segments of developing quail bowel back grafted into younger chick host embryos. Development 119:411–423
134. Theiry JP, Duband JL, Delovee A (1982) Pathways and mechanisms of avian trunk neural crest migration and localisation. Dev Biol 93:324–343
135. Sato A, Yamamoto M, Imamura K et al (1978) Pathophysiology of aganglionis colon and anorectum: an experimental study on aganglionosis produced by a new method in the rat. J pediatr Surg 13:399–435
136. Yoneda A, Shima H, Nemeth L et al (2002) Selective chemical ablation of the enteric plexus in mice. Pediatr Surg Int 18:234–237
137. Parr E, Sharkey K (1997) Multiple mechanisms contribute to myenteric plexus ablation induced by benzalkonium chloride in the guinea-pig ileum. Cell Tissue Res 289:253–264
138. Goto S, Grosfeld JL (1989) The effect of a neurotoxin (benzalkonium chloride) on the lower esophagus. J Surg Res 47:117–119
139. Dahl JL, Bloom DB, Epstein ML et al (1987) Effect of chemical ablation of myenteric neurons on neurotransmitter levels in the rat jejunum. Gastroenterology 92:338–344
140. Hadzijahic N, Renehan WE, Ma CK et al (1993) Myenteric plexus destruction alters morphology of rat intestine. Gastroenterology 105:1017–1028
141. Holle GE (1991) Changes in the structure and regeneration mode of the rat small intestinal mucosa following benzalkonium chloride treatment. Gastroenterology 101:1264–1273
142. Holle GE, Forth W (1990) Myoelectric activity of small intestine after chemical ablation of myenteric neurons. Am J Physiol G519–G526
143. Luck MS, Dahl JL, Boyeson MG et al (1993) Neuroplasticity in the smooth muscle of the myenterically and extrinsically denervated rat jejunum. Cell Tissue Res 271:363–374
144. See NA, Epstein ML, Schultz E et al (1988) Hyperplasia of jejunal smooth muscle in the myenterically denervated rat. Cell Tissue Res 253:609–617

145. See NA, Greenwood B, Bass P (1990) Submucosal plexus alone integrates motor activity and epithelial transport in rat jejunum. Am J Physiol 259:G593–G598
146. See NA, Epstein ML, Dahl JL et al (1990) The myenteric plexus regulates cell growth in rat jejunum. J Auton Nerv Syst 31:219–230
147. Duhamel B (1963) Embryology of exomphalos and allied malformations. Arch Dis Child 38:142–147
148. Hoyme HE, Jones MC, Jones KL (1983) Gastroschisis: abdominal wall disruption secondary to early gestational interruption of the omphalomesenteric artery. Semin Perinatol 7:294–298
149. Moore TC, Stokes GE (1953) Gastroschisis; report of two cases treated by a modification of the gross operation for omphalocele. Surgery 33:112–120
150. Stoll C, Alembik Y, Dott B, Roth MP (2001) Risk factors in congenital abdominal wall defects (omphalocele and gastroschisi): a study in a series of 265,858 consecutive births. Ann Genet 44:201–208
151. McDonnell R, Delany V, Dack P, Johnson H (2002) Changing trend in congenital abdominal wall defects in eastern region of Ireland. Ir Med J 95:236–238
152. Tibboel D, Kluck P, Molenaar JC, Gaillard JL (1987) A comparative investigation of the bowel wall in gastroschisis and omphalocele: relation to postoperative complications. Pediatr Pathol 7:277–285
153. Sherman NJ, Asch MJ, Isaacs H, Rosenkrantz JG (1973) Experimental gastroschisis in the fetal rabbit. J pediatr Surg 8:165–169
154. Haller JA, Kehrer BH, Shaker IJ, Shermeta DW, Wyllie RG (1974) Studies of the pathophysiology of gastroschisis in fetal sheep. J Pediatr Surg 9:627–632
155. Kluck P, Tibboel D, van der Kamp AW, Molenaar JC (1983) The effect of fetal urine on the development of the bowel in gastroschisis. J Pediatr Surg 18:47–50
156. Correia-Pinto J, Tavares ML, Baptista MJ, Estevao-Costa J, Flake AW, Leite-Moreira AF (2001) A new fetal rat model of gastroschisis: development and early characterization. J Pediatr Surg 36:213–216
157. Phillips JD, Kelly RE Jr, Fonkalsrud EW, Mirzayan A, Kim CS (1991) An improved model of experimental gastroschisis in fetal rabbits. J Pediatr Surg 26:784–787
158. Albert A, Julia MV, Morales L, Parri FJ (1993) Gastroschisis in the partially extraamniotic fetus: experimental study. J Pediatr Surg 28:656–659
159. Shaw K, Buchmiller TL, Curr M, Lam MM, Habib R, Chopourian HL, Diamond JM, Fonkalsrud EW (1994) Impairment of nutrient uptake in a rabbit model of gastroschisis. J Pediatr Surg 29:376–378
160. Bealer JF, Graf J, Bruch SW, Adzick NS, Harrison MR (1996) Gastroschisis increases small bowel nitric oxide synthase activity. J Pediatr Surg 31:1043–1045
161. Langer JC, Bramlett G (1997) Effect of prokinetic agents on ileal contractility in a rabbit model of gastroschisis. J Pediatr Surg 32:605–608
162. Oyachi N, Lakshmanan J, Ross MG, Atkinson JB (2004) Fetal gastrointestinal motility in a rabbit model of gastroschisis. J Pediatr Surg 39:366–370
163. Till H, Muensterer O, Mueller M, Klis V, Klotz S, Metzger R, Joppich I (2003) Intrauterine repair of gastroschisis in fetal rabbits. Fetal Diagn Ther 18:297–300
164. Guys JM, Esposito C, Simeoni J, D'Ercole C, Paut O, Bouzid A, Boubli L (2001) An experimental model of gastroschisis using fetoendoscopy: preliminary results and technical considerations. Surg Endosc 16:317–319
165. de Lagausie P, Guibourdenche J, de Buis A, Peuchmaur M, Oury JF, Aigrain Y, Sibony O, Luton D (2002) Esophageal ligature in experimental gastroschisis. J Pediatr Surg 37:1160–1164
166. Srinathan SK, Langer JC, Blennerhassett MG, Harrison MR, Pelletier GJ, Lagunoff D (1995) Etiology of intestinal damage in gastroschisis. III. Morphometric analysis of the smooth muscle and submucosa. J Pediatr Surg 30:379–383
167. Tibboel D, Kluck P, van der Kamp AW, Vermey-Keers C, Molenaar JC (1985) The development of the characteristic anomalies found in gastroschisis-experimental and clinical data. Z Kinderchir 40:355–360
168. Aktug T, Erdag G, Kargi A, Akgur FM, Tibboel D (1995) Amnio-allantoic fluid exchange for the prevention of intestinal damage in gastroschisis: an experimental study on chick embryos. J Pediatr Surg 30:384–387
169. Aktug T, Ucan B, Olguner M, Akgur FM, Ozer E (1998) Amnio-allantoic fluid exchange for prevention of intestinal damage in gastroschisis II: effects of exchange performed by using two different solutions. Eur J Pediatr Surg 8:308–311
170. Sencan A, Gumustekin M, Gelal A, Arslan O, Ozer E, Mir E (2002) Effects of amnio-allantoic fluid exchange on bowel contractility in chick embryos with gastroschisis. J Pediatr Surg 37:1589–1593
171. Aktug T, Ucan B, Olguner M, Akgur FM, Ozer E, Caliskan S, Onvural B (1998) Amnio-allantoic fluid exchange for the prevention of intestinal damage in gastroschisis. III: determination of the waste products removed by exchange. Eur J Pediatr Surg 8:326–328
172. Kanmaz T, Yagmurlu A, Aktug T, Gokcora H (2001) The effect of amnio-allantoic fluid pH on the intestines: an experimental study in the chick embryo gastroschisis model. J Pediatr Surg 36:1341–1345
173. Vannucchi MG, Midrio P, Flake AW, Faussone-Pellegrini MS (2003) Neuronal differentiation and myenteric plexus organization are delayed in gastroschisis: an immunohistochemical study in a rat model. Neurosci Lett 339:77–81
174. Vannucchi MG, Midrio P, Zardo C, Faussone-Pellegrini MS (2004) Neurofilament formation and synaptic activity are delayed in the myenteric neurons of the rat fetus with gastroschisis. Neurosci Lett 364:81–85
175. Midrio P, Faussone-Pellegrini MS, Vannucchi MG, Flake AW (2004) Gastroschisis in the rat model is associated with a delayed maturation of intestinal pacemaker cells and smooth muscle cells. J Pediatr Surg 39:1541–1547
176. Akgur FM, Ozdemir T, Olguner M, Aktug T, Ozer E (1998) An experimental study investigating the effects of intraperitoneal human neonatal urine and meconium on rat intestines. Res Exp Med (Berl) 198:207–213
177. Correia-Pinto J, Tavares ML, Baptista MJ, Henriques-Coelho T, Estevao-Costa J, Flake AW, Leite-Moreira AF (2002) Meconium dependence of bowel damage in gastroschisis. J Pediatr Surg 37:31–35
178. Hillebrandt S, Streffer C, Muller WU (1996) Genetic analysis of the cause of gastroschisis in the HLG mouse strain. Mutat Res 372:43–51
179. Hillebrandt S, Streffer C, Montagutelli X, Balling R (1998) A locus for radiation-induced gastroschisis on mouse Chromosome 7. Mamm Genome 9:995–997
180. Pils S, Muller WU, Streffer C (1999) Lethal and teratogenic effects in two successive generations of the HLG mouse strain after radiation exposure of zygotes— association with genomic instability? Mutat Res 429:85–92
181. Mortell A, Giles J, Bannigan J, Puri P (2003) Adriamycin effects on the chick embryo. Pediatr Surg Int 19:359–364
182. Beauchemin RR Jr, Gartner LP, Provenza DV (1984) Alcohol induced cardiac malformations in the rat. Anat Anz 155:17–28
183. Grinfeld H, Goldenberg S, Segre CA, Chadi G (1999) Fetal alcohol syndrome in Sao Paulo, Brazil. Paediatr Perinat Epidemiol 13:496–497
184. Lane GA, Nahrwold ML, Tait AR, Taylor-Busch M, Cohen PJ, Beaudoin AR (1980) Anesthetics as teratogens: nitrous oxide is fetotoxic, xenon is not. Science 210:899–901
185. Neeper-Bradley TL, Tyl RW, Fisher LC, Kubena MF, Vrbanic MA, Losco PE (1995) Determination of a no-observed-effect level for developmental toxicity of ethylene glycol administered by gavage to CD rats and CD-1 mice. Fundam Appl Toxicol 27:121–130

186. McBride WG, Vardy PH, French J (1982) Effects of scopolamine hydrobromide on the development of the chick and rabbit embryo. Aust J Biol Sci 35:173–178
187. Tellone CI, Baldwin JK, Sofia RD (1980) Teratogenic activity in the mouse after oral administration of acetazolamide. Drug Chem Toxicol 3:83–98
188. Cappon GD, Cook JC, Hurtt ME (2003) Relationship between cyclooxygenase 1 and 2 selective inhibitors and fetal development when administered to rats and rabbits during the sensitive periods for heart development and midline closure. Birth Defects Res Part B Dev Reprod Toxicol 68:47–56
189. Cook JC, Jacobson CF, Gao F, Tassinari MS, Hurtt ME, DeSesso JM (2003) Analysis of the nonsteroidal anti-inflammatory drug literature for potential developmental toxicity in rats and rabbits. Birth Defects Res Part B Dev Reprod Toxicol 68:5–26
190. Singh J (2003) Gastroschisis is caused by the combination of carbon monoxide and protein–zinc deficiencies in mice. Birth Defects Res Part B Dev Reprod Toxicol 68:355–362
191. Merkle J, Zeller H (1980) Studies on acetamides and formamides for embryotoxic and teratogenic activities in the rabbit (author's transl). Arzneimittelforschung 30:1557–1562
192. Arancia G, Leonetti C, Malorni W, Greco C, Formisano G, Marangolo M, Zupi G (1991) Different effects of sequential combinations of N-methylformamide with 5-fluorouracil on human colon carcinoma cells growing in nude mice. J Cancer Res Clin Oncol 117:351–358
193. Kelich SL, Mercieca MD, Pohland RC (1995) Developmental toxicity of N-methylformamide administered by gavage to CD rats and New Zealand white rabbits. Fundam Appl Toxicol 27:239–246
194. Ohkawa H, Matsumoto M, Hori T et al (1992) Familial congenital diaphragmatic hernia in the pig—studies on pathology and heredity. Eur J Pediatr Surg 3:67–71
195. De Lorimier AA, Tierney DF, Parker HR (1967) Hypoplastic lungs in fetal lambs with surgically produced congenital diaphragmatic hernia. Surgery 62:12–17
196. Haller JA Jr, Signer RD, Golladay ES et al (1976) Pulmonary and ductal hemodynamics in studies of simulated diaphragmatic hernia of fetal and newborn lambs. J Pediatr Surg 11:675–680
197. Harrison MR, Bressack MA, Churg AM et al (1980) Correction of congenital diaphragmatic hernia in utero II. Simulated correction permits lung growth with survival at birth. Surgery 88:260–268
198. Adzick NS, Harrison MR Outwater KM et al (1985) Correction of congenital diaphragmatic hernia in utero IV. An early gestational fetal lamb model for pulmonary vascular morphometric analysis. J Pediatr Surg 20:673–680
199. Fauza DO, Tannuri U, Ayoub AA et al (1994) Surgically produced congenital diaphragmatic hernia in fetal rabbits. J Pediatr Surg 29:882–886
200. Wu J, Ge X, Verbeken EK, Gratacos E et al (2002) Pulmonary effects of in utero tracheal occlusion are dependent on gestational age in a rabbit model of diaphragmatic hernia. J Pediatr Surg 37:11–17
201. Roubliova X, Verbeken E, Wu J, Yamamoto H et al (2004) Pulmonary vascular morphology in a fetal rabbit model for congenital diaphragmatic hernia. J Pediatr Surg 39: 1066–1072
202. Di Maio M, Ting A, Gil J et al (1994) A morphometric study of lung hypoplasia in a second trimester lamb model of comgenital diaphragmatic hernia. Am J Resp Crit Care Med 149:A727
203. Lipsett J, Cool JC, Runciman SI et al (2000) Morphometric analysis of preterm fetal pulmonary development in the sheep model of congenital diaphragmatic hernia. Pediatr Dev Pathol 3:17–28
204. Ting A, Glick PL, Wilcox DT et al (1998) Alveolar vascularization of the lung in a lamb model of congenital diaphragmatic hernia. Am J Resp Crit Care Med 157:31–34
205. Harrison MR, Adzick NS, Bullard KM et al (1997) Correction of congenital diaphragmatic hernia in utero VII. A prospective trial. J Pediatr Surg 32:1637–1642
206. Hedrick MH, Estes JM, Sullivan KM et al (1994) Plug the lung until it grows (PLUG): a new method to treat congenital diaphragmatic hernia in utero. J Pediatr Surg 29:612–617
207. Skarsgard ED, Meuli M, VanderWall KJ et al (1996) Fetal endoscopic tracheal occlusion ("fetendo-PLUG") for congenital diaphragmatic hernia. J Pediatr Surg 31:1335–1338
208. Harrison MR, Keller RL, Hawgood SB et al (2003) A randomised trial of fetal endoscopic tracheal occlusion for severe fetal congenital diaphragmatic hernia. N Engl J Med 349:1916–1924
209. Ambrose AM, Larson PS, Borcelleca JF et al (1971) Toxicological studies on 2,4-dichlorophenyl-*p*-nitrophenyl ether. Toxicol Appl Pharmacol 19:263–275
210. Iritani I (1984) Experimental study on embryogenesis of congenital diaphragmatic hernia. Anat Embryol 169: 133–139
211. Kluth D, Kangah R, Reich P et al (1990) Nitrofen-induced diaphragmatic hernia in rats: an animal model. J Pediatr Surg 25:850–854
212. Kluth D, Tenbrinck R, von Ekesparre M et al (1993) The natural history of congenital diaphragmatic hernia and pulmonary hypoplasia in the embryo. J Pediatr Surg 28:456–463
213. Cilley R, Zgleszewski S, Krummel T et al (1997) Nitrofen dose-dependent gestational day-specific murine lung hypoplasia and left-sided diaphragmatic hernia. Am J Physiol 272:L362–L371
214. Brandsma AE, Tenbrinck R, Ijsselstijn H et al (1995) Congenital diaphragmatic hernia: new models, new ideas. Pediatr Surg Int 10:10–15
215. Losty PD, Pacheco BA, Manganaro TF et al (1996) Prenatal hormonal therapy improves pulmonary morphology in rats with congenital diaphragmatic hernia. J Surg Res 65:42–52
216. Losty PD, Connell MG, Freese R et al (1999) Cardiovascular malformations in experimental congenital diaphragmatic hernia. J Pediatr Surg 34:1203–1207
217. Migliazza L, Otten C, Xia H et al (1999) Cardiovascular malformations in congenital diaphragmatic hernia: human and experimental studies. J Pediatr Surg 34:1352–1358
218. Migliazza L, Xia H, Alvarez JI et al (1999) Heart hypoplasia in experimental congenital diaphragmatic hernia. J Pediatr Surg 34:706–710
219. Migliazza L, Xia H, Diez-Pardo JA et al (1999) Skeletal malformations associated with congenital diaphragmatic hernia: experimental and human studies. J Pediatr Surg 34:1624–1629
220. Xia H, Migliazza L, Diez-Pardo JA et al (1999) The tracheobronchial tree is abnormal in experimental congenital diaphragmatic hernia. Pediatr Surg Int 15:184–187
221. Kluth D, Losty PD, Schnitzer JJ et al (1996) Toward understanding the developmental anatomy of congenital diaphragmatic hernia. Clin in Perinatol 23:655–669
222. Allan DW, Greer JJ (1997) Pathogenesis of nitrofen-induced congenital diaphragmatic hernia in fetal rats. J Appl Physiol 83:338–347
223. Greer JJ, Cote D, Allan DW et al (2000) Structure of the primordial diaphragm and defects associated with nitrofen-induced CDH. J Appl Physiol 89:2123–2129
224. Babiuk RP, Zhang W, Clugston R et al (2003) Embryological origins and development of the rat diaphragm. J Comp Neurol 455:477–487
225. Keijzer R, Liu J, Deimling J et al (2000) Dual-hit hypothesis explains pulmonary hypoplasia in the nitrofen model of congenital diaphragmatic hernia. Am J Pathol 156:1299–1306
226. Guilbert TW, Gebb SA, Shannon JM (2000) Lung hypoplasia in the nitrofen model of congenital diaphragmatic hernia occurs early in development. Am J Physiol Lung Cell Mol Physiol 279:L1159–L1171
227. Greer JJ, Babiuk RP, Thebaud B (2003) Etiology of congenital diaphragmatic hernia: the retinoid hypothesis. Pediatr Res 53:726–730

228. Baglaj SM, Czernick J (2004) Nitrofen-induced congenital diaphragmatic hernia in rat embryo: what model? J Pediatr Surg 39:24–30
229. Lohnes D, Mark M, Mendelsohn C et al (1994) Function of the retinoic acid receptors (RARs) during development (I). Craniofacial and skeletal abnormatilites in RAR double mutants. Development 120:2723–2748
230. Mendelsohn C, Lohnes D, Decimo D et al (1994) Function of the retinoic acid receptors (RARs) during development (II). Multiple abnormatilites at various stages of organogenesis in RAR double mutants. Development 120:2749–2771
231. Hentsch B, Lyons I, Li R et al (1996) *Hlx* homeobox gene is essential for an inductive tissue interaction that drives expansion of embryonic liver and gut. Genes Dev 10:70–79
232. Kreidberg JA, Sariola H, Loring JM et al (1993) WT-1 is required for early kidney development. Cell 74:679–691
233. Liu J, Zhang L, Wang D et al (2003) Congenital diaphragmatic hernia, kidney agenesis and cardiac defects associated with Slit3-deficiency in mice. Mech Dev 120:1059–1070