

2017

The evolution and treatment of congenital diaphragmatic hernias in neonates

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BOSTON UNIVERSITY
SCHOOL OF MEDICINE

Thesis

**THE EVOLUTION AND TREATMENT OF
CONGENITAL DIAPHRAGMATIC HERNIAS IN NEONATES**

by

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B.A., Boston University, 2015

Submitted in partial fulfillment of the
requirements for the degree of
Master of Science

2017

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ACKNOWLEDGMENTS

Special thanks to my thesis readers, the Boston University School of Medicine, as well as family and friends for their continued support in my pursuit of medicine.

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ABSTRACT

Congenital diaphragmatic hernia (CDH) is a potentially fatal condition found in neonates where embryological defects in the diaphragm negatively impact fetal maturation and growth. The defect allows contents below the diaphragm to potentially migrate into the thoracic cavity during development, which could lead to secondary complication including pulmonary hypertension and left ventricular hypoplasia. CDH tends to have a high neonate mortality rate in congruence with the severity of the condition. Several risk factors for CDH include accompanying chromosomal abnormalities and the anatomical positions of organs in the fetus. Diagnosis is typically found with an ultrasound (US) in utero. There have been several studies in order to better understand the pathology of the disease and new techniques to try and alleviate the cases prenatally, however the risks involved with these procedures may outweigh the benefits. The standard practice for neonates that qualify for postnatal treatment is the use of extracorporeal membrane oxygenation (ECMO) postnatally, to facilitate oxygenated blood to the fetus via a bio-mechanical device. Recent treatment techniques that have revolutionized care for CDH include a delayed surgical intervention in order to reduce the risk of developing a pulmonary ailment such as pulmonary hypertension and/or lung hypoplasia. Interventions with inhaled nitric oxide have also been shown to relegate a

similar outcome to those with ECMO intervention. Despite the advancements in knowledge, treatment, and technology, the mortality rate for CDH still hovers around 50% on average, yet that percentage can increase or decrease depending on the severity of the condition and any genetic abnormalities associated with it. Overall, while there have been great strides in treatment and understanding of CDH, additional research is necessary in order to provide the utmost care for future generations of CDH patients.

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LIST OF ABBREVIATIONS

AD.....	Alveolar Duct
ALV.....	Alveolus
CDH.....	Congenital Diaphragmatic Hernia
CdLS.....	Cornelia de Land Syndrome
CGH.....	Comparative Genomic Hybridization
DBP.....	Vitamin D Binding Protein
DBS.....	Donnai-Barrow Syndrome
ECMO.....	Extracorporeal Membrane Oxygenation
FBN1.....	Fibrillin 1
FETO.....	Fetal Endoscopic Tracheal Occlusion
FISH.....	Fluorescence In-Situ Hybridization
FLV.....	Fetal Lung Volume
FOAR.....	Facio-Oculo-Acoustico-Renal
FOXP2.....	Forkhead Box Protein P2
LHR.....	Lung Volume to Head Circumference Ratio
LRP2.....	Lipoprotein-Related Protein 2
LV.....	Left Ventricular
MGI.....	McGoon Index
MM HG.....	Millimeters of Mercury
MRI.....	Magnetic Resonance Imaging
MWS.....	Matthew-Wood Syndrome

NICU.....	Neonatal Intensive Care Unit
PKS.....	Pallister-Killian Syndrome
PPFs.....	Pleuroperitoneal Folds
RB.....	Respiratory Bronchiole
RBP1.....	Retinol binding Protein 1
RBP2.....	Retinol Binding Protein 2
SHH.....	Sonic Hedgehog
SVD.....	Spontaneous Vaginal Delivery
TB.....	Terminal Bronchiole
TGF- β	Transforming Growth Factor Beta
US.....	Ultrasound
WHS.....	Wolf-Hirschorn Syndrome
WNT.....	Wingless Type
WT1.....	Wilms Tumor 1
ZFPM2.....	Zinc Finger Protein, Multitype 2

INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a developmental birth defect resulting in discontinuity of the diaphragm, attributed to several embryological factors. Foremost is the incomplete formation of the diaphragm which results in a diaphragmatic deficiency (Pober et al., 2010). The lack of closure in the diaphragm facilitates a herniation of the abdominal viscera into the thoracic cavity (Kosiński & Wielgoś, 2017). This herniation is caused from incomplete muscularization of the diaphragm, occurring on the posterolateral aspect of the diaphragm, known as a Bochdalek hernia, or on the anterior aspect of the diaphragm, known as a Morgagni hernia. Most commonly found on the left side of the diaphragm (in 75-90% of cases), CDH can also occur on the right-side (10-15%). Additionally, bi-lateral cases have been reported (1-2% incidence). A majority of cases involve isolated CDH, meaning that the diaphragmatic defect occurred spontaneously, as opposed to complex or non-isolated CDH, which is attributed to a combination of chromosomal abnormalities and genetic complications (Pober et al., 2010).

CDH is present in approximately 1 in 3000 live births and has the potential for high neonatal morbidity (approximately 50% of cases), in particular with complex CDH, where genetic abnormalities can negatively affect the survival rate (Kosiński & Wielgoś, 2017; Kumar, 2015). The defect is correlated with other complications including pulmonary hypertension and severe pulmonary hypoplasia, all of which demand a therapeutic approach that has shifted the treatment of CDH from one of immediate

surgical repair, to the progressive management of these associated complications (Kosiński & Wielgoś, 2017). Over the last 30 years, medical advances have led to new discoveries in diagnosis, intervention, and clinical management to better treat patients affected by CDH. An understanding of the embryologic and pathologic progression of CDH can evolve into a pursuit for answers to questions of why CDH occurs, and why it has high mortality rates in neonates (Gallindo et al., 2015).

Embryology and Development

The inception of the evolutionary journey of CDH begins with embryology and development. The diaphragm is a structure in the human body that is a physical barrier between the abdominal and thoracic cavity. It maintains pressure differences between these two cavities, which makes its role vitally important to fetal development. The development of the diaphragm occurs within the fourth and twelfth weeks of gestation (Rohana et al., 2008).

The diaphragm's formation and closure is essential to a normal functioning infant. During normal organogenesis, the diaphragm consists of a variety of differentiating tissue types. The central and anterior regions of the diaphragm are derived from the septum transversum, which is fused to the liver during the early stages of embryonic development, before becoming the non-muscular central tendon of the diaphragm (Poerber et al., 2010). A defect in the embryonic process of development in the diaphragm correlated with CDH is the lack of a fully formed diaphragmatic mesothelium. The mesothelium is an epithelial-like, single cell layer which lines the pleurae, peritoneum, and pericardium, and is important for the lubrication of internal organs through the

release of surfactant. The mesothelium also has an important developmental role, contributing to growth of mesoderm-derived organs such as the heart, lungs, and liver (Merrell et al., 2015). Mesothelial cells are able to differentiate, coat highly significant internal organs such as the heart, liver, and lungs, promote signaling that is critical for the development of the central tendon of the diaphragm, and are important for the diaphragm's tensile strength. Mesothelial cells are found with varied gene expressions at several points in development, located mostly on the thoracic surface of the diaphragm during the early stages, (approximately week 4 of development), while the mesothelium in the abdomen matures and grows at later stages of development, (approximately week 10 of development gestation) (You et al., 2005).

Furthermore, the diaphragm has embryonic connections to the pleuroperitoneal folds (PPFs), dorsal and esophageal mesenteries, body wall, and pre-muscle massing on the fourth cervical segment of the embryo. In particular, mutations in PPFs are vital to the development of the diaphragmatic defect. PPFs are temporary embryonic structures with the initial function of regulating the diaphragm's muscle and connective tissue development. PPFs can be attributed to the sheet of connective tissue that encompasses the diaphragm. By day 22 of development, the septum transversum is at the cervical level, and under normal conditions, the septum transversum fuses with the PPFs and the esophageal mesentery. The muscles of the body wall invade the PPFs, which then form the muscles of the diaphragm (Merrell et al., 2015). In a typical embryonic trajectory, by week 6 of development, the fused PPFs become pleuroperitoneal membranes, which separate the pleural and peritoneal cavities, with the free ends of the membranes

projecting into the pericardioperitoneal canals. If the PPFs do not fuse with the septum transversum and the mesentery of the esophagus, then the developmental path of the diaphragm is disrupted. Any incomplete fusion of the PPFs may produce a variety of effects, ranging from a pleuroperitoneal defect to a hiatal hernia, all depicted in **Figure 1**. (Kumar, 2015).

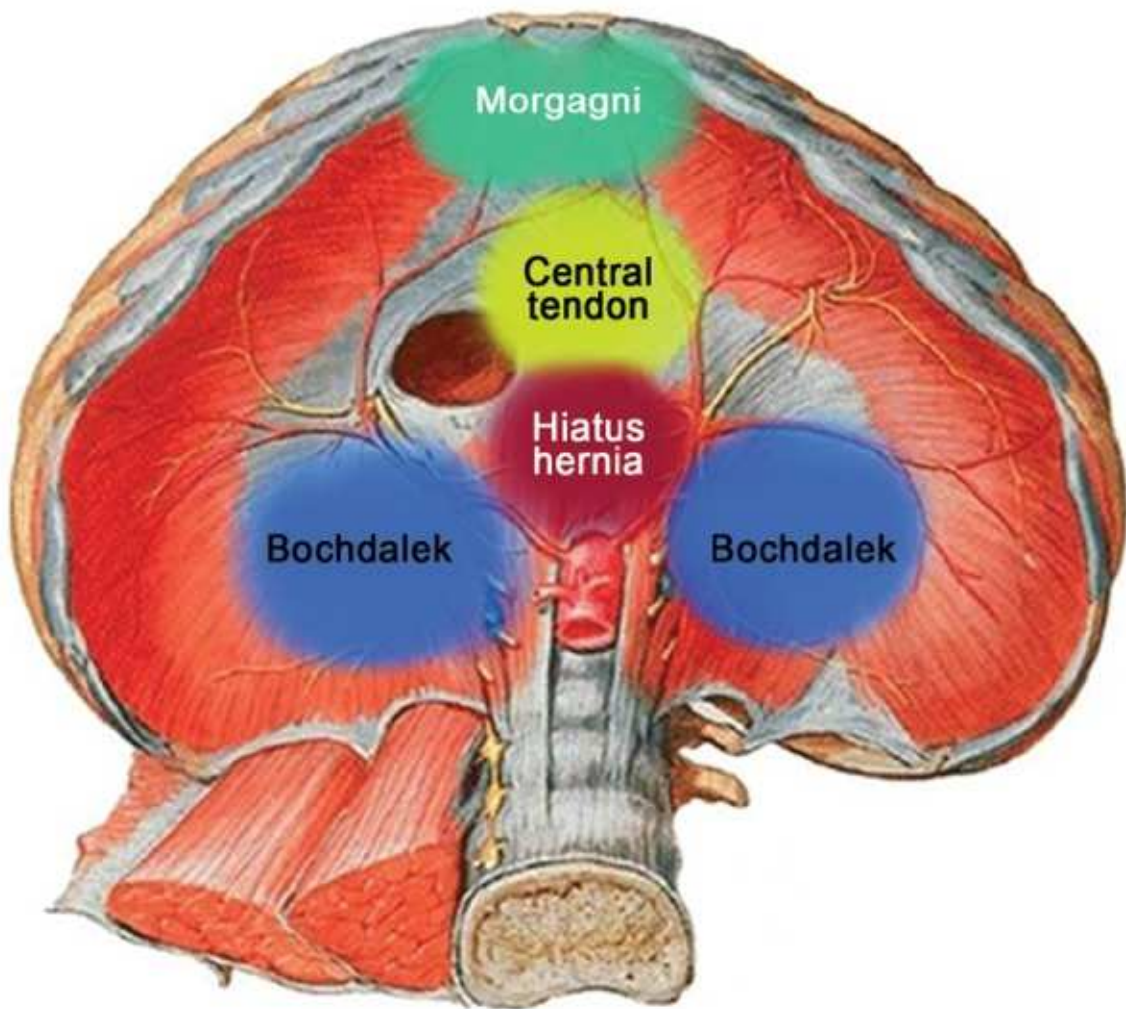


Figure 1. Schematic Representation of Diaphragmatic Hernias

This figure illustrates the variety of locations within a diaphragm where a diaphragmatic hernia can occur. Looking at the transverse section of the diaphragm, the vertebral column is in the most posterior part of the body. Traveling anteriorly to the front of the

diaphragm, on both the left and right posterolateral sides of the diaphragm are Bockdalek hernias. A CDH hernia is known as a hiatus hernia, and continuing to the most anterior part of the diaphragm, is known as a Morgagni hernia (Marlow & Thomas, 2013).

The most common diaphragmatic defect occurs on the posterolateral diaphragm, known as a Bockdalek hernia (Kays et al., 2015; Kays et al., 2013). This type of hernia is thought to develop from mutations in PPFs. Bockdalek hernias are often associated by herniation of the stomach, liver, intestines, and potentially the spleen into the thoracic cavity. A large Bockdalek hernia, seen as an absence of the hemidiaphragm (half of the diaphragm), is known as agenesis of the diaphragm, and is the more severe spectrum of Bockdalek hernias (Poerber et al., 2010).

On the anterior portion of the diaphragm, the hernias are less frequent, and known as Morgagni hernias (Kumar, 2015). The sternocostal hiatus is a triangular space between the xiphisternum and the muscular components of costal margin fibers associated with the central tendon of the hemidiaphragm. A Morgagni hernia is correlated with a hernia of the right sternocostal hiatus and a Morgagni-Larrey hernia takes place through the left sternocostal hiatus, both of which can result in a herniation of the liver and intestines into the thoracic cavity. These hernias are generally accompanied by a hernia sac and typically do not necessarily show signs and symptoms in the newborn period. However, during childhood, these hernias may elicit respiratory distress. Other rare types of Morgagni hernias are associated with the Pentology of Cantrell, involving defects of the supraumbilical midline abdominal wall, as well as the diaphragmatic pericardium, heart, and lower sternum (Poerber et al., 2010). While the exact embryological development of Morgagni hernias is unclear, it has been suggested that spontaneous or prolonged intra-

abdominal pressure may cause the abdominal organs to migrate into the thoracic cavity (Pattnaik et al., 2016).

By week 9 of development, the development of the diaphragm should be complete. If a pleuroperitoneal defect continues after week 10 of development, further complications can unfold (Kumar, 2015). For example, the intestines may enter the thoracic cavity typically on the left side, as a result of the pleuroperitoneal defect. The absence of the proper diaphragmatic continuity is associated with abnormal position of adjacent organs in the abdominal cavity. In left-sided diaphragmatic hernias, the stomach is typically an abdominal organ that enters the thoracic cavity, whereas movement of the liver is mostly found in right sided hernias. Nevertheless, there have been some cases where the liver has entered the thoracic cavity in left sided CDH as well. A bilateral CDH, an uncommon phenomenon, would see the most paramount abdominal displacement, establishing it as one of the most severe types of CDH (Kumar, 2015).

Pathology

The pathogenesis of CDH continues into development at weeks 14-16 of development, which is most frequently associated with an increased risk for lung hypoplasia and pulmonary hypertension as depicted in **Figure 2**. The correlation arises from competition for thoracic space with abdominal organs occupying the available space in the thoracic cavity. One of the most significant coexisting issues is the development of abnormal pulmonary vasculature. The pulmonary vasculature bed size is decreased in a hypoplasia lung, which can lead to lung deficiencies as well as thickening of arterial wall. (Poerber et al., 2010). The vasoconstriction caused by impeding abdominal organs

facilitates deprived blood circulation and flow throughout the fetus. These deficits of the pulmonary circulation elicit vascular resistance, which halts the capabilities for gas exchange in the lungs (Kosiński & Wielgoś, 2017). Dilemmas manifesting in lung growth could lead to pulmonary hypoplasia and a lower amount of bronchiolar branching, which could lead to dysfunctional surfactant levels and further the risk factors for poor lung development (Zalla et al., 2015). The underdevelopment of the lung and arterial thickening of the lung can be tracked to the compression of the lung due to the herniated diaphragm. Lung weight is diminished and the number of alveoli and alveolar branches are reduced significantly. Pulmonary arteries and veins typically present with muscularized walls and increased thickness at the expense of the media and adventitial layers.

Moreover, left ventricular (LV) hypoplasia is a congenital heart abnormality that is associated with the pathophysiological development CDH. LV hypoplasia is an obstruction in LV outflow. The severity of the LV obstruction is often correlated with the severity of the hypoplasia. Only in rare cases where there is an alternative pathway for LV blood to exist, such as a ventricular septal defect, would the severity of the obstruction not be correlated with the severity of the LV hypoplasia. The term hypoplastic left heart syndrome is used to describe severe cases of LV hypoplasia. If severe, the left ventricle cannot support systemic circulation, and a neonatal cardiac transplantation or complex open heart surgeries leading to a univentricular circulation. In this situation, the patient would be a candidate for a neonatal cardiac transplant or require a series of complex open-heart surgeries in order to establish univentricular circulation.

Univentricular circulation enables the right ventricle to support the systemic circulation, while pulmonary blood flow becomes a passive process. However, in mild cases of LV hypoplasia, the left ventricle is capable of supporting systemic circulation once the obstruction of the blood flow is fixed via biventricular repair (Hickey et al., 2011).

Often times with CDH, neonates present with severe respiratory distress, with breath and lung sounds diminished ipsilateral to the hernia's position due to the hypoplastic lung. While acute respiratory distress can be an onset symptom, 5-10% of CDH cases show no respiratory distress symptoms, even as far as little to low grade abdominal pain (Kumar, 2015). These resistance factors contribute to the evolution of lung hypoplasia in CDH patients due to the decreased airway pressure in the embryological state (Tovar, 2012).

Pharmacologic and teratogenic models of CDH have been critical to the discoveries of other pathogenic mechanisms behind the disease. Another pathogenesis track of CDH has been seen in fetal rats, where nitrofen-induced CDH has been correlated with the pulmonary issues seen in isolated and/or non-isolated CDH (Allan & Greer, 1997). Prenatal administration of herbicide nitrofen can inhibit in vitro retinol-dehydrogenase-2, and these rats have a striking resemblance to the diaphragmatic defects of CDH, and can be used to better understand the pathophysiological progression of the disease (Tovar, 2012). In 25-57% of CDH cases, structural defects are associated with CDH, including heart, brain, renal, and gastrointestinal deficits. These structural findings are organized and displayed in **Table 1** (Kumar, 2015).

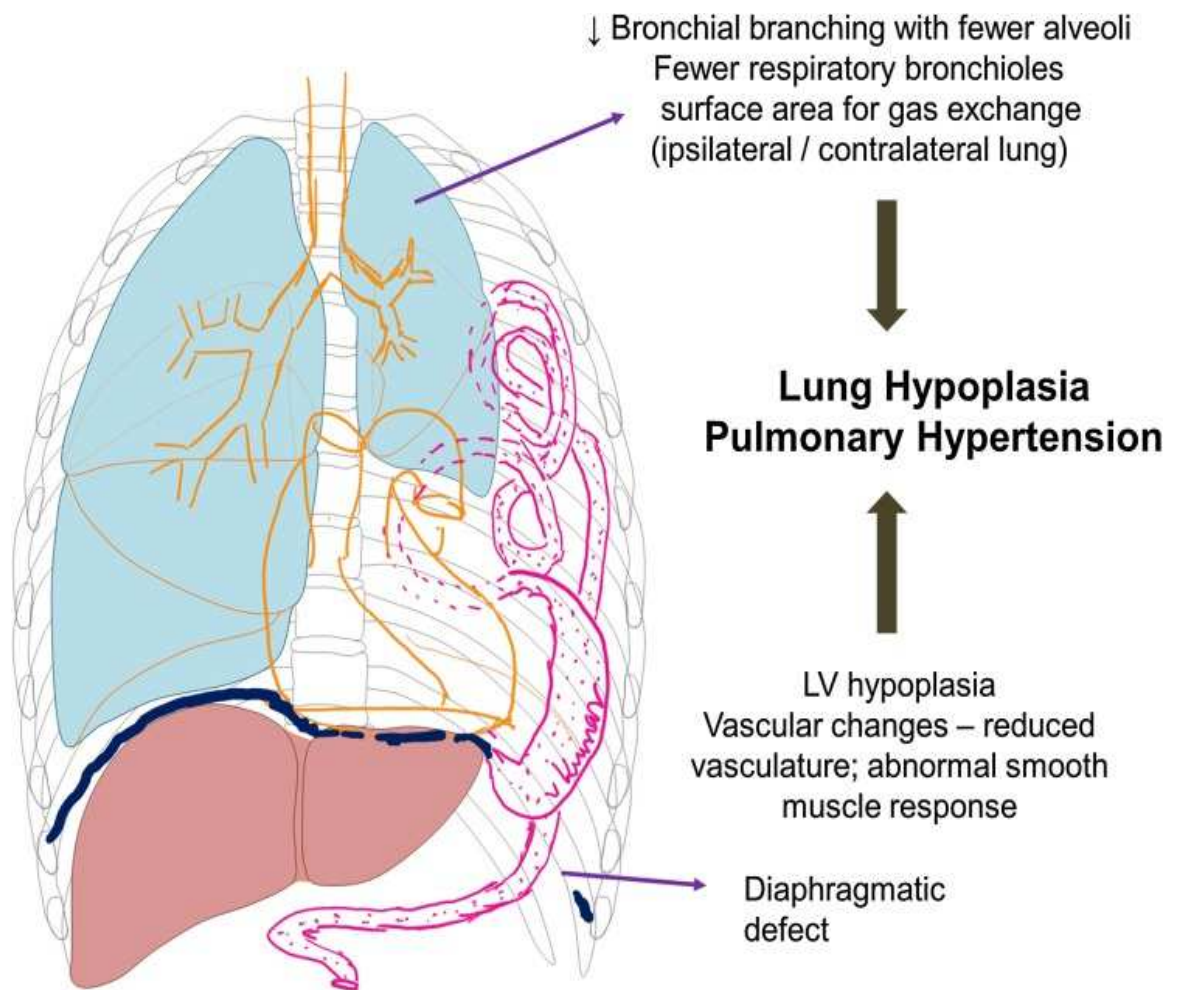


Figure 2. Lung Hypoplasia

This figure illustrates a schematic diagram of CDH with lung hypoplasia, LV hypoplasia, and abdominal contents in the left thorax due to the diaphragmatic defect in the left side (Kumar, 2015).

Body system involved	Type of defects	Estimated frequency
Cardiovascular	Ventricular septal defect	6%
	Atrial septal defect	3%
	Coarctation of aorta	2%
	Hypoplastic left heart syndrome	2%
	Dextrocardia	1%
	Tetralogy of Fallot	1%
	Transposition of the great vessels	1%
	Single ventricle	1%
	Tricuspid atresia	1%
	Pulmonary stenosis	1%
Gastrointestinal	Malrotation	4%
	Imperforate anus	3%
	Absent gallbladder	1%
	Accessory spleen	1%
Urogenital	Renal agenesis	3%
	Cystic kidney	1%
	Absent testes	1%
	Bicornuate uterus	1%
Musculoskeletal	Limb deficiency	5%
	Club foot	4%
	Omphalocele	3%
	Vertebral anomalies	2%
	Arthrogryposis	2%
	Sternal defect	2%
	Abdominal wall defect	1%
	Rib anomalies	1%
	Hip dislocation	1%
	Ectopia cordis	1%
Respiratory	Pulmonary sequestration	1%
	Tracheoeseophageal fistula	1%
Central nervous system	Neural tube defects	3%
	Hydrocephalus	3%
	Ocular hypoplasia	1%
Craniofacial	Cleft lip and/or palate	2%
	Cleft palate	2%

Table 1. Structural Defects Associated with CDH

This table depicts the wide-range of structural abnormalities that are associated with CDH. The left column denotes the body system affected, including the cardiovascular, gastrointestinal, urogenital, musculoskeletal, respiratory, central nervous system, and craniofacial systems, with the middle column specifying the region, and the right column including the frequency of those specific regions (Kumar, 2015).

Genetic Factors

While a majority of CDH cases appear to be isolated-CDH, approximately 10-30% of cases are related to some form of chromosomal defects and genetic syndromes (Arrington et al., 2012; Kumar, 2015). A complex, or non-isolated CDH, is a more complex congenital abnormality, meaning that there are other factors involved in the diaphragmatic defect. Some non-isolated CDH cases have genetic patterns of development that suggest a syndromic diagnosis and inheritance probability of CDH (Holder et al., 2007). CDH can appear with an isolated structural defect, or it could be involved with several other additional abnormal growth factors attributed to these genetic influences (Wat et al., 2012). These include but are not limited to lung hypoplasia, positional abnormalities of the chest, malrotation of the intestines, and a patent ductus arteriosus (Wat et al., 2011). These are referred to as secondary effects of CDH. Through application of karyotype methods over the last few decades of discoveries, CDH regions of the human genome have been uncovered for non-isolated CDH cases (Clugston et al., 2008). A few selected genetic syndromes have been identified and described in **Table 2**, including rare genetic disorders such as Pallister-Killian syndrome, Beckwith-Wiedemann syndrome, and Denys-Drash syndrome (Holder et al., 2007).

While the large majority of CDH cases are sporadic, some cases of autosomal dominant, autosomal recessive, and X-lined patterns of inheritance have been recorded. While the etiology remains unknown for the sporadic CDH cases, genetic factors have been found to attribute to these cases, and de novo mutations of the diaphragm development illustrate this inheritance factor surrounding some CDH reports (Kosiński &

Wielgoś, 2017). The existence of genetic syndromes in conjunction with CDH illustrates the evident connection between genetic components and the development of CDH in some cases (Holder et al., 2007).

Syndrome	Gene/locus	Phenotype features
Pallister-Killian syndrome	Tetrasomy 12p	CDH, developmental disability, epilepsy, hypotonia, epicanthal folds, flat nose, vision and hearing impairments, congenital heart defects, gastroesophageal reflux, cataracts
Fryns syndrome	Unknown (autosomal recessive inheritance is suggested)	CDH, pulmonary hypoplasia, hypoplasia of the distal phalanges and nails, flat nasal bridge, dysplastic ears, micrognathia, orofacial clefts
Gershoni-Baruch syndrome	Unknown (autosomal recessive inheritance is suggested)	CDH, omphalocele, radial ray malformations
Simpson-Golabi-Behmel	GPC3; Xq26	CDH, macrosomia (prenatal and postnatal), polydactyly, hypoplastic nails, developmental delay
Beckwith-Wiedemann syndrome	IGF2/H19/p57KIP; 11p15.5	CDH, macrosomia, omphalocele, macroglossia, neonatal hypoglycemia
Microphthalmia with linear skin defects	HCCS	CDH, cardiomyopathy, microphthalmia, dermal aplasia
Goltz syndrome	PORCN; Xp22	CDH, focal dermal hypoplasia, dental hypoplasia, syndactyly
Craniofrontonasal syndrome	EFNB1; Xp22	CDH, coronal synostosis, hypertelorism, digital anomalies
PAGOD syndrome	Unknown	CDH, omphalocele, dextrocardia, pulmonary artery hypoplasia
Denys-Drash syndrome	WT1; 11p13	CDH, glomerulopathy, male pseudohermaphroditism

Table 2. Selected Genetic Syndromes Associated with CDH

This table illustrates the eclectic variety of genetic influences to CDH. Several of these genetic factors listed are rare conditions seen in 5% or less of CDH cases. Most CDH cases remain sporadic, but while these non-isolated cases are the minority, they are still critical to discovering the design of the condition (Kumar, 2015).

Etiology of non-isolated CDH chromosomal abnormalities have been identified through advanced genomic technologies such as fluorescence in-situ hybridization (FISH) and array-based comparative genomic hybridization (array CGH). **Figure 3** below depicts the genomic variations that are associated with CDH formation, including partial and full deletions and duplications of human genes (Holder et al., 2007).

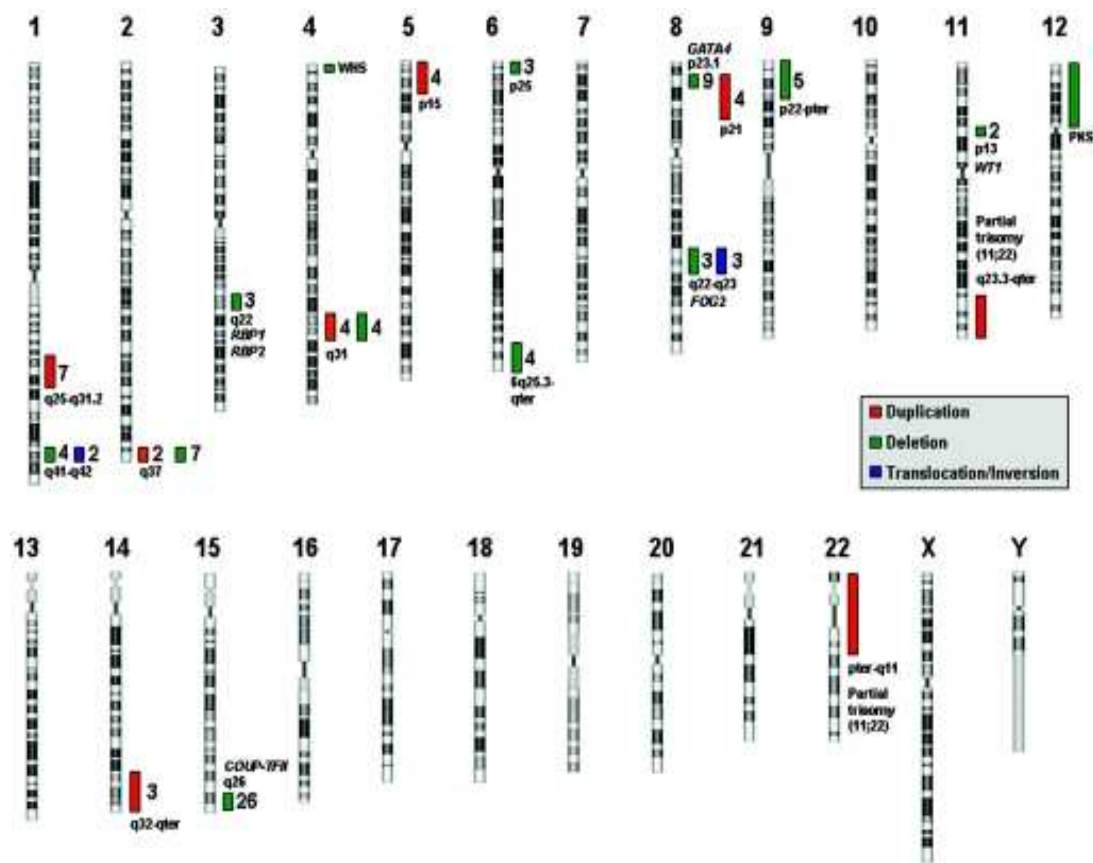


Figure 3. Chromosomal Regions and Selected Genes for CDH

This figure depicts the specific genes and chromosomal regions for CDH. Recurrent chromosomal abnormalities associated with patients with CDH are represented by the colored bars. For each region, the number of patients described with that duplication (red bar), deletion (green bar), or translocation/inversion (blue bar) is given. The genes and genetic syndromes are included beside their respective regions. PKS = Pallister-Killian syndrome; WHS = Wolf-Hirschhorn syndrome (Holder et al., 2007).

Through karyotyping and genomic hybridization over time, several prominent studies that link chromosomal abnormalities with cases of CDH, and these abnormalities have been documented over time (Holder et al., 2007). Duplications of chromosome 1q25q31.2 have been seen in seven patients with CDH. In addition, at least three of those cases were associated with cleft palate as well (Clark & Fenner-Gonzales, 1989). Deletion of chromosome 1q41-q42 have been seen in four cases of CDH (Kantarci et al., 2006). Moving on to chromosome 2, duplication or deletion of chromosome 2q37 has been noted. Seven patients with CDH have been found to involve deletions of 2q37 and two patients with 2q37 duplications (Tonks et al., 2004; Holder et al., 2007). The deletion of chromosome 3q22 has been discovered in three CDH patients, two of which had blepharophimosis and dysmorphic facial expressions, attributed to deletions of FOXL2 (Wolstenholme et al., 1994; Wynn et al., 2014). The diminished or deleted FOXL2 is known to cause certain blepharophimosis, ptosis, and epicanthus inversus syndrome. While FOXL2 genes in these regions have certain effects on the prognosis of CDH, the Chromosome 3q22 region is also home to the genes for cellular retinol binding protein 1 (RBP1) and cellular retinol binding protein 2 (RBP2) which are both part of the retinol signaling pathway associated in Vitamin A effectiveness and lung growth factors. However, no mutations in RBP1 or RBP2 have been found in CDH patients (Holder et al., 2007).

Chromosome 4 in the p16 region has been correlated with Wolf-Hirschhorn syndrome, noted by a facial appearance that models a “Greek helmet”, with associated mental retardation and cardiac abnormalities. CDH has been associated with a minimum

of 14 cases of chromosome 4p16 deletion (Slavotinek et al., 2006). Further down chromosome 4 in the q31 region, duplication or deletion of this region has been described in eight CDH cases, four in regards to duplication of chromosome 4q31 and four with deletion of chromosome 4q31 (van Dooren, 2004). Duplications of chromosome 5p15 have been elicited in four patents with CDH, and all of these conditions were correlated with chromosomal abnormalities in other areas of the genome such as a partial deletion of chromosome 9p22 (Aviram-Goldring et al., 2000). On chromosome 6, a deletion of 6p25 has been documented with three cases of CDH, in which all have additional chromosomal abnormalities. In addition, further down chromosome 6 in the q25.3-qter region, four cases of CDH have been attributed to it through the use of array-CGH (Bender et al., 1969). Chromosome 8 deletions in the 8p23.1 region have been included in nine cases of CDH (Slavontinek et al., 2006; Holder et al., 2007). Notably, GATA4 resides in this chromosomal region. The loss of function in GATA4 binding protein has been attributed to cardiac abnormalities, and a majority of CDH cases involving the deletion of 8p23.1 are associated with atrial, ventricular cardiac defects. GATA4, in conjunction with FOG2, a transcription factor, both act to regulate mesenchymal cell function in the developing diaphragm. (Xiao et al., 2015).

A mutation in both the GATA4 binding protein and/or the FOG2 transcription factor is correlated with the prevalence of CDH and the discontinuity of the developing diaphragm (Jay et al., 2007; Yu et al., 2013). Furthermore, the 8q22q23 chromosomal region facilitates another gene, zinc finger protein, multitype 2 (ZFPM2) which has been associated with CDH formation based on its mutation in children with diaphragmatic

hernias, which is further illustrated by **Figure 4**, documenting the evidence of deletion in ZFPM2 with CDH (Vuckovic et al., 2016). Consequently, duplication of this same region on chromosome 8 has also been identified in four cases of CDH (Holder et al., 2007; Klassens et al., 2006).

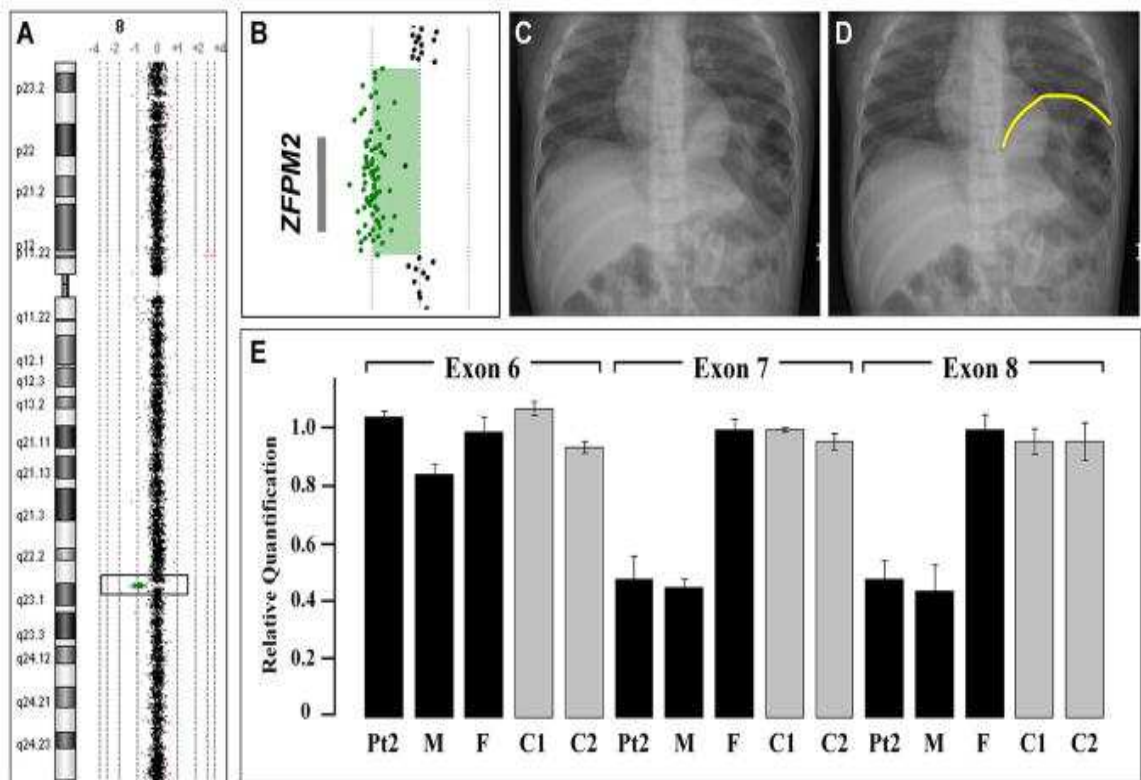


Figure 4. ZFPM2 Deletion on Chromosome 8q22.3q23.1

This figure is a representation of ZFPM2 deletion in from a CDH patients. In A) there is an array comparative genomic hybridization data showing an 8q22.3q23.1 single gene deletion of ZFPM2. In B), the approximate location of the ZFPM2 gene in relation to aCGH data from the deletion region from the patient is represented by a gray bar. C) A chest radiograph demonstrating a severe diaphragmatic eventration with loops of the patient’s bowel. D) The same radiograph in C) with the diaphragmatic eventration limits shown in yellow. E) Quantitative analysis demonstrating a normal copy number for ZFPM2 Exon 6 but a reduced copy number for Exons 7 and 8 in DNA from Patient 2 and his mother. Normal copy number values are seen in DNA from Patient 2’s father and DNA from two unrelated controls, C1 and C2 (Longoni et al., 2015, Vuckovic et al., 2016).

Several other copy number variants are prevalent with genetic syndromes correlated to CDH. Tetrasomy 12p, also known as Pallister-Killian Syndrome (PKS), is one of the more well-known chromosomal abnormalities associated with CDH. The cause of PKS is a mosaic isochromosome 12p, and up to 50% of PKS cases are associated with CDH (Longoni et al., 2015). Features of this syndrome include but are not limited to short limb and cognitive impairments. For autosomal recessive chromosomal abnormalities, Donnai-Barrow syndrome (DBS)/facio-oculo-acoustico-renal (FOAR) are found in over 50% of CDH cases. DBS/FOAR is linked with increased retinol binding protein 1 (RBP1) and a gene called lipoprotein-related protein 2 (LRP2) which is mutated and interacts with sonic hedgehog (SHH) pathways for signaling. Defects found in this syndrome cause developmental defects and attribute to the physical deficits of CDH. Matthew-Wood Syndrome (MWS) is another autosomal recessive chromosomal defect that typically causes a diaphragmatic agenesis. Mutations in genes caused by MWS are correlated with retinoid signaling pathways which lead to the diaphragm defects in CDH (Russell et al., 2012; West et al., 2012).

In mouse genome studies for autosomal dominant chromosomal abnormalities, several genes have been associated with human CDH, in particular Wilms Tumor 1 (WT1), a gene necessary for urogenital development. WT1 is a zinc finger transcription factor as well as a tumor suppressor important for organogenesis, and is often found on the mesothelial cells of the body wall, heart, and diaphragm (Carmona et al., 2016). Deficiencies in WT1 have been linked to diseases such as Denys-Drash and Fraiser Syndromes, which has a direct correlation to CDH in certain conditions (Paris et al.,

2015). WT1 deficiencies, due to loss of signaling of PFFs in early development, lead to defects in the posterolateral region of the diaphragm, where Bockdalek hernias originate (Clugston et al., 2008). Additionally, not only do WT1 deficiencies have a correlation to the formation of CDH, but WT1 signaling pathway connections to wingless type (WNT) signaling pathways in the diaphragm in early organogenesis appear to play an indirect role in the development of CDH as well. WT1 and WNT pathways seem to act upstream of β -catenin to facilitate epithelial mesenchymal transition (EMT). Loss of WT1 in these cases would produce a reduced β -catenin and thus a lack of EMT, resulting in an embryonic diaphragmatic deficit, as depicted in **Figure 5** (Paris et al., 2015).

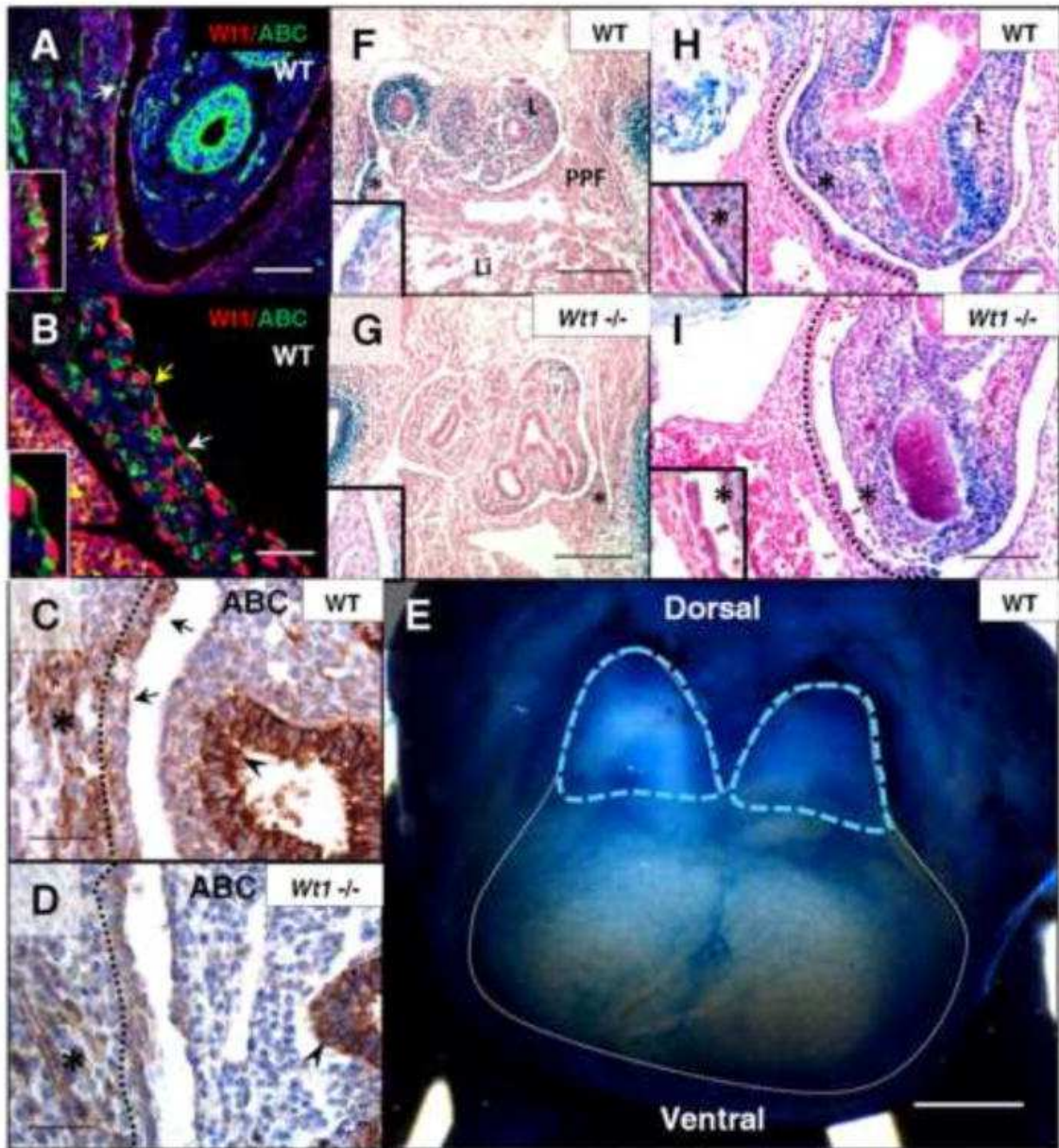


Figure 5. Loss of *Wt1* results in reduced β -catenin Expression

WT1/Active β -catenin co-immunofluorescence was performed on a sagittal section of wildtype embryos in (A) and (B). WT1 nuclear protein correlates to regions of active β -catenin staining in the mesothelial cell cytoplasm, illustrated by the arrows. Insets are in the cropped images with yellow arrows pointing to their regions. Active β -Catenin immunohistochemistry (C, D) of WT1 wildtype and mutant WT1 diaphragms labels regions of mesothelium (the dotted line), indicated by arrows, as well as diaphragm muscle (asterisks) and lung epithelium (arrowheads). E12.5 whole mount X-gal stained Axin2 wildtype embryonic diaphragm (E) has been outlined in white, with blue dotted lines marking late PPFs. Axin2 and WT1 embryos were harvested at (F–I) and sectioned

in a transverse field of view (F, G) and sagittal view (H, I) following whole mount X-gal staining. Insets are high magnification of region marked by asterisks. Abbreviations: ABC, Active β -catenin; L, lung; Li, liver; PPF, pleuroperitoneal fold. Scale bars: A, B, H and I, 100 μ m; C and D, 50 μ m; F and G, 200 μ m; E, 0.5 mm (Paris et al., 2015).

Besides the previously mentioned autosomal dominant chromosomal abnormalities associated with CDH, there are two other relevant syndromes that plague the diagnosis. Cornelia de Lang syndrome (CdLS) involves facial dysmorphia and is commonly correlated with CDH (West et al., 1992; You et al., 2005). In severe cases, CdLS causes genetic mutations that break during the G2 mitosis phase. Secondly, Marfan Syndrome is a connective tissue disorder associated with a Fibrillin 1 (FBN1) mutation. In rare but severe cases of Marfan Syndrome, CDH and paraoesophageal hernias corroborate with the FBN1 mutation (Beck et al., 2015). Lastly, an increase in TGF- β has been linked to tracheal occlusions and an increased risk for CDH (Vuckovic et al., 2016). Overall, while there are a variety of genetic causalities to CDH, the percentage of cases as a result of genetic factors remains relatively low in comparison to sporadic CDH cases (Holder et al., 2007; van Dooren, 2004).

Diagnosis

The prenatal diagnosis of CDH patients is typically based on an ultrasound (US) scan, which has become the standard mode of detection of congenital abnormalities as a whole. For the majority of cases, CDH is detected during routine scan, with an average diagnosis at approximately 24-weeks gestation (Kumar, 2015; Lusk et al., 2015). CDH may be diagnosed during a first trimester scan, however, often they are too difficult to decipher due to the visual feedback of the diaphragm (Kosiński & Wielgoś. 2017). CDH

detections in the first trimester are typically when the diaphragmatic defect is extremely large which often leads to a poor prognosis of survival. With increased gestational age, the detection rate increases as the fetus becomes larger, making the scan of micromanipulations in the diaphragm easier to see. Despite the benefits of US, the use of fetal magnetic resonance imaging (MRI) has become more prevalent to diagnosis similar cases of CDH, and further utilized to predict a prognosis of survival (Le et al., 2012). Fetal MRI does not have the limitations that US does in regards to maternal obesity or oligohydramnios. In addition, it contains a better soft tissue contrast when compared to US. Some studies have suggested that fetal MRI with a lung volume assessment test could be a better diagnostic tool for the diagnosis of CDH (Kumar, 2015). A diagnostic parameter for predicting the severity of the lung condition is by measuring an element known as fetal lung volume (FLV). This prognosis marker is done at 34-weeks gestation and uses a fetal MRI. The higher the FLV, the greater prognostic indicator for survival.

CDH diagnosis is made through a variety of appearances and factors. First, the presence of a mediastinal shift is evidence of a diaphragmatic defect, in addition to a fluid-filled stomach in the thoracic cavity, either in front of, or behind, the heart. The appearance of a liver herniation in the thoracic cavity is also a diagnostic marker of CDH (Kumar, 2015). In some cases where the liver is in the thoracic cavity, it will appear as a homogeneous mass located near the heart. In comparison to left and right sided CDH, right-sided CDH is more difficult to diagnose via US since the fetal liver appears comparable to the fetal lungs upon examination (Kays et al., 2015; Khemakhem et al., 2012). Therefore, color doppler US is a useful diagnostic tool in finding the fetal liver

because it allows the ductus venous as well as the intrahepatic vessels to be more distinguishable in comparison to the surrounding organs. In addition, polyhydramnios may be present in a CDH pregnancy due to esophageal and arterial compression, noted in **Figure 6**. If the pulmonary arteries are hypertrophied with thickening in the tunica media and adventitial layers it increases the risk for premature delivery due to the protruded lung vascularization. In severe cases of CDH, hydrops fetalis has been shown to occur in relation to the compression of vessels in the thoracic cavity as well as a mediastinal shift in the fetal stage (Kosiński & Wielgoś, 2017). Hydrops fetalis is a fetal condition defined by an abnormal fluid accumulation in two or more fetal compartments, typically localized to the abdomen, pleura, and pericardium. To analyze and diagnosis the severity of the pulmonary compressions, the McGoon index (MGI) on US combined with the modified McGoon index on MRI are used as calculations of the sum of the right and left pulmonary artery diameters. Both of these indices are helpful for predicting infant survival and the possible severity of postnatal pulmonary hypertension (Lusk et al., 2015). For diagnostic confirmation of pulmonary hypertension, a 2D echocardiography within the first 24 hours should enable the best real time performance in pulmonary arterial pressure and cardiac function in CDH patients (Kumar, 2015).

The differential diagnosis for CDH includes a variety of abdominal-related defects, but is rarely necessary because CDH is often detected before birth. Diaphragmatic eventrations involve a stable but thin diaphragm that is elevated from its proper anatomical position. Eventrations of the diaphragm are defined as abnormal elevations in one area of an otherwise intact diaphragms, which are the precursors to the

development of diaphragmatic hernias. Due to the thin nature of the diaphragm, eventrations have a tendency to bulge and lead to the abdominal cavity organs traveling into the thoracic cavity (Kays et al., 2013; Marshall & Sumner, 1982). While some large eventrations are difficult to diagnose as CDH in prenatal images, diaphragmatic agenesis, thought to be more extreme form of CDH, has eventrations that are identifiable via prenatal screenings. In addition to eventrations, thoracic lesions noted in the differential diagnosis of CDH that should be ruled out are congenital cystic adenomatoid malformation (CCAM), bronchopulmonary sequestration, teratomas, and enteric cysts. Furthermore, through US scans, diaphragmatic hernias can be indicators for other diseases to be ruled out in the differential diagnosis, including Apert syndrome, which is a genetic disorder defined by the premature fusion of cranial bones (Kosiński et al., 2016). A final diagnostic determining factor of CDH is the discovery of intestinal peristalsis inside the fetal thorax during scanning (Kosiński & Wielgoś, 2017).

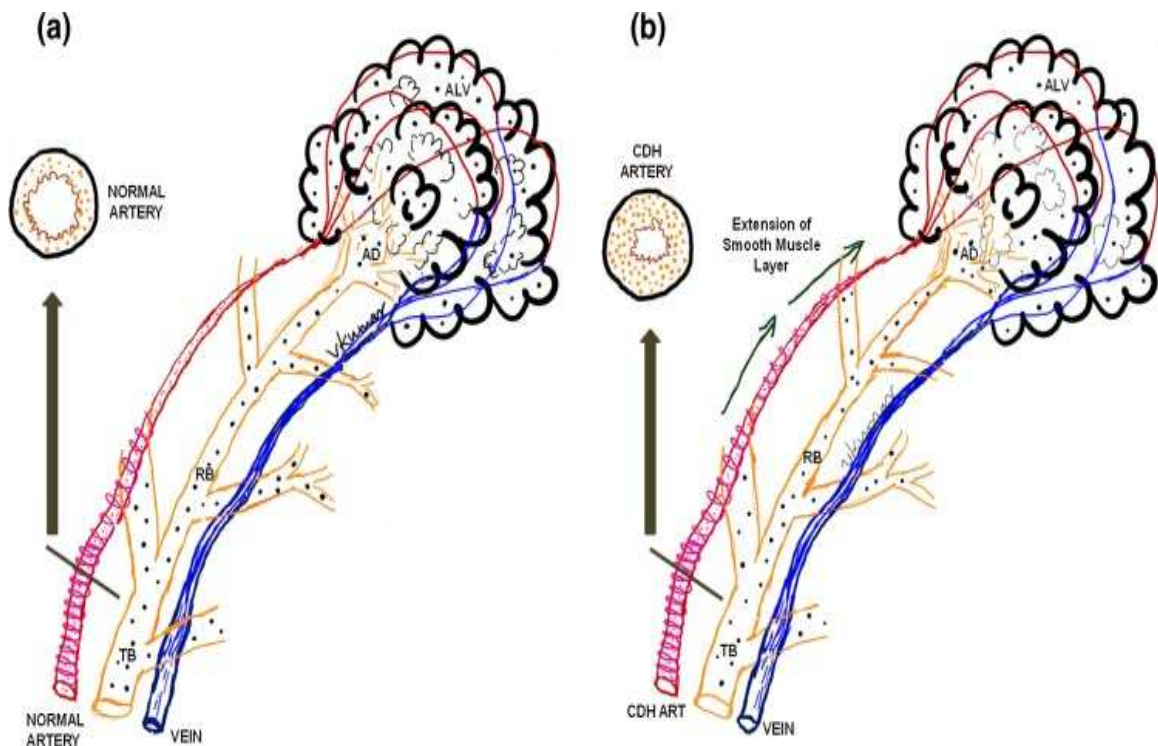


Figure 6. Pulmonary Vasculature with and without CDH

This figure illustrates the anatomy of pulmonary vasculature in normal (a) and CDH (b) infants. In (a), arterioles in the pulmonary vasculature made up of smooth muscles in the medial layer, enables a moderately large lumen. Moreover, the vascular smooth muscles do not extend beyond the terminal bronchioles in the normal, control infants. In (b) the vascular restructuring seen in CDH infants results in two major changes. The first change is that the vascular smooth muscle layer is hypertrophied. Secondly, the smooth muscle layer extends into the vasculature beyond the terminal bronchioles, into the respiratory bronchioles, and towards the alveolar duct. TB = terminal bronchiole, RB = respiratory bronchiole, AD = alveolar duct, ALV = alveolus (Kumar, 2015).

Prognosis

The survival prognosis for CDH depends on many factors ranging from chromosomal defects, types of CDH, structural placements, as well as lung maturation. In regards to chromosomal abnormalities, if CDH is correlated with a chromosomal defect, the prognosis relies on the type of genetic abnormality and any other defects associated with the condition (Cordier et al., 2015). In addition, an early diagnosis of CDH is

paramount, so an expedited examination of the associated abnormalities may proceed (Kumar, 2015; Mesas Burgos et al., 2015). One of the most accepted and profound measures of prognosis in CDH is the lung area to head circumference ratio (LHR). To correct and account for the different developmental growth ranges of fetal lungs and head, the LHR is represented as a percentage of observed and expected LHR. This tool is one of the most valid measurements for the prediction of fetal lung size calculated by the US screening (Kosiński & Wielgoś, 2017; Merrell et al., 2015).

Moreover, a poor prognosis is the presence of the liver in the chest cavity as a result of herniation. On average, infants with a right-sided CDH have a worse prognosis of survival when compared to left-sided CDH. This is due to the abdominal contents, such as the liver, that travel into the thoracic cavity. The total amount of the liver herniated into the chest can be measured by MRI and calculated as herniated-liver-to-thoracic-volume-ratio, and the larger amount of liver in the thoracic cavity, the poorer the attached prognosis for survival (Marshall & Sumner, 1982). Low LHR totals mixed with the herniation of the liver are consequently poor prognostications for infant survival with CDH (Perez-Egido et al., 2015). In addition, with the US test, the fetal stomach position is a determining factor in the prognosis of survival. Fetal stomach position is associated with a prognosis of survival in CDH. In the thoracic cavity, the infant has a poorer prognosis for survival, whereas if the fetal stomach is found within the intra-abdominal cavity, there is a more favorable prognosis of survival (Kosiński & Wielgoś, 2017). Finally, intragastric pressure monitoring in some cases has been shown to be a very

useful indicator of CDH and facilitate an early prognosis of survival (Mandal et al., 2016).

Prenatal Treatment

Once the discovery and diagnosis of CDH is confirmed, prenatal steps are then taken to appropriately treat the diaphragmatic hernia. For severe diaphragmatic hernias that have poor prognoses, the infant could be offered a fetal endoscopic tracheal occlusion (FETO) (Gallindo et al., 2015; Rohana et al., 2008). The main objective of FETO is to reduce the mortality rate and minimize the impact and risk of pulmonary hypoplasia. Since its clinical inception, FETO has been performed for severe cases at 26–28 weeks and for moderate cases at 30–32 weeks of gestation. FETO is a specialized undertaking, where the trachea is occluded with a balloon for approximately 6 weeks. This tracheal occlusion leads to the accumulation of the lung fluid, which in turn causes increased lung tissue stretch and accelerated growth of the lung itself. In response, FETO reduces the number of type II pneumocytes and surfactant in the pleural space for the period after the treatment is given. Administration of antenatal glucocorticoids may be considered as a prenatal treatment. If there is a risk of preterm delivery, antenatal steroids may decrease morbidity resulting from preterm delivery. From animal studies, it has been confirmed that with improved gas exchange and lung volume, there is a higher prognosis of survival even with severe cases of CDH. Nevertheless, following delivery, the surviving neonates still require a diaphragm surgery, often including a prosthetic patch repair (Kumar, 2015).

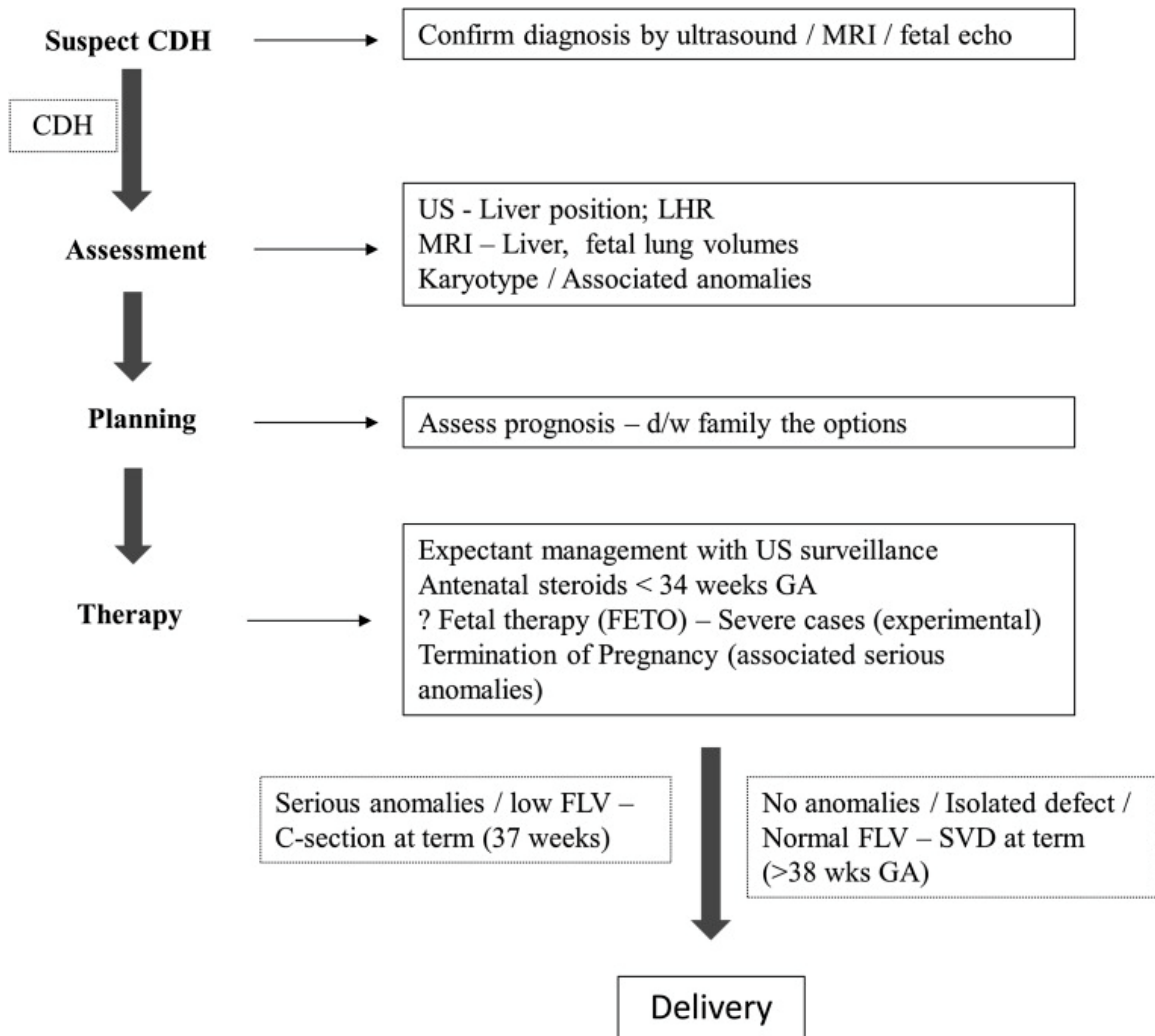


Figure 7. Prenatal Management of CDH

This figure illustrates an algorithm of prenatal management of CDH. US ultrasound, LHR lung area-to-head circumference ratio, FLV fetal lung volume, SVD spontaneous vaginal delivery, FETO fetal endoscopic tracheal occlusion (Kumar, 2015).

Postnatal Treatment

The postnatal management of CDH involves a variety of guidelines aimed and tested to reduce the morbidity of CDH cases. The postnatal management begins immediately after birth, in the delivery room. Once the infant is born, the infant's heart rate as well as blood pressure need to be monitored vigorously in the postnatal time period. Other important monitors are pre-and postductal oxygen saturations. Preductal refers to the area of the aorta proximal to the aortic opening of the ductus arteriosus. This pulse oximeter is located on the right hand. Postductal is the area distal to the aortic opening of the ductus arteriosus, and is located on the foot (Dalton et al., 2015). In regards to oxygen saturation, the infant would ideally be kept at levels ranging from 80-95% for an acceptable range of preductal saturations. The avoidance of high airway pressures and high oxygen concentration in the delivery room itself will minimize ventilation induced lung injury in the infant.

The infant needs to be intubated for ventilation without a bag and mask. The bag and mask technique could result in distention of the stomach or even worse, could exacerbate a complication with hypoplastic lungs and limit their expansion capabilities. Moreover, there would be an instantaneous placement upon delivery of an oro- or nasogastric tube with suction, at either continuous or intermittent levels. This suction will help decompress the bowel and thus allow lung expansion to further emanate in the thoracic cavity. With this, a central venous line is inserted for fluids and inotropes, as well as an arterial line for blood pressure and blood sampling once the infant is stabilized and conditions warrant the innervation for the samples. If any complications arise, such

as arterial blood pressure falling below its threshold for gestational age of 30 mm Hg, saline solutions would be administered to the infant in addition to the consideration of inotropes (Kumar, 2015).

For adequate ventilation in the neonatal intensive care unit (NICU) after delivery, a preductal saturation of 85-95% is acceptable, with a postductal aim of over 70% for saturation. It is important to wean oxygen off the infant to maintain saturation levels in order to prevent or minimize the possibility for ventilator-induced lung injury. Through permissive hypercapnia and gentle ventilation, this targeted maintenance can be achieved. Furthermore, hemodynamic monitoring is relegated to focus on organ perfusion, through the collection of patient data such as heart rate, capillary refill, urine output, and lactate levels in the infant. Inadequate perfusion or any faults in these factors would result in the need to maintain systemic blood pressure for the infant. The need to maintain systemic blood pressure is imperative due to the risk of hypoxemia, and is accomplished by shunting across the ductus arteriosus due to pulmonary hypertension. If the patient does not respond to these methods, certain inotropes including dopamine and epinephrine would be used to increase the infant's systemic blood pressure and negate the effects of the ductal shunts (Kumar, 2015).

Pulmonary hypertension is exacerbated by the increased resistance at the vascular level after birth due to the diaphragmatic hernia. While a large gap between the pre- and postductal saturation percentages is an indication of pulmonary hypertension, an absence of this gap does not completely eliminate the possibility of pulmonary hypertension in the infant. The treatment of pulmonary hypertension in CDH is a difficult undertaking, and

there have been several indications of possible nuanced techniques for care including inhaled nitric oxide, which is the immediate remedy for tertiary pediatric hospitals, however its success rate is up for debate. There have been counter-balanced cases of positive effects on survival, and no effect at all in regards to CDH infant mortality rate when inhaled nitric oxide is used. As described in **Figure 8**, milrinone is an inotrope and pulmonary vasodilator. Its use in cases of CDH improved ventricular systolic and diastolic function in association with low-cardiac output syndromes. Milrinone improved systolic and diastolic pressures in the right ventricle. However, milrinone has been used with caution in infants due to the risk of systemic hypotension and cardiac arrhythmias. In addition, sildenafil has been shown to increase oxygenation and cardiac function with CDH patients with pulmonary hypertension.

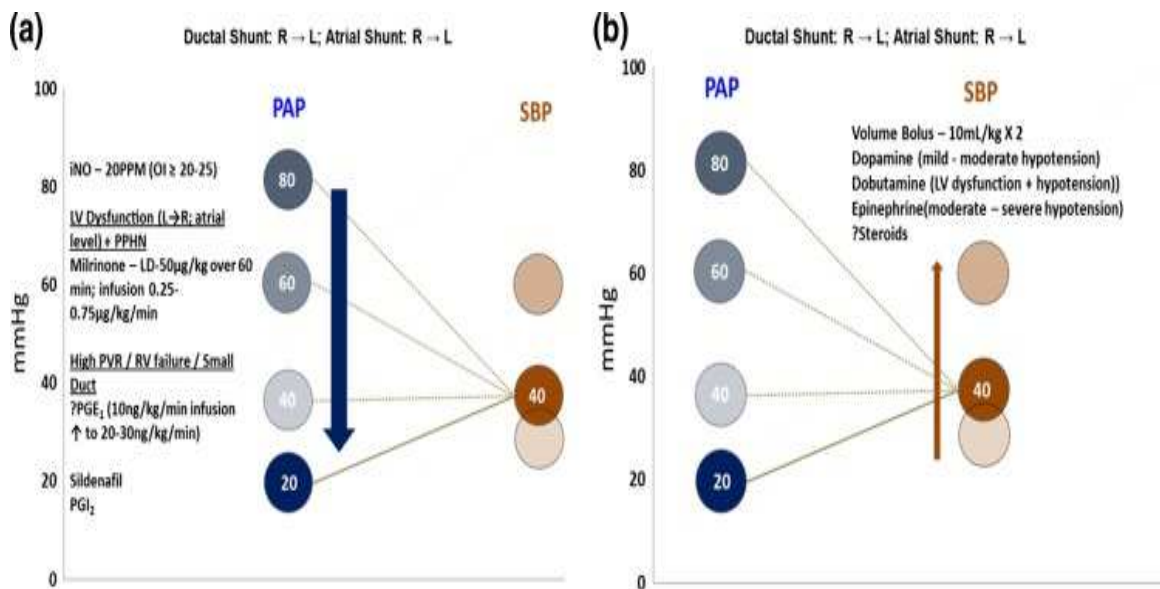


Figure 8. Management of Systemic Blood Pressure in CDH

This figure represents a schematic diagram of management with regard to pulmonary arterial pressure in (a) and systemic blood pressure in (b) in infants with CDH.

Decreasing right to left shunting at the ductal level often includes raising systemic blood

pressure, managing systemic hypotension, or decreasing pulmonary hypertension. The lines shown illustrate how changes in pulmonary arterial pressure and systemic blood pressure could alter hemodynamics and oxygenation. The therapies to increase systemic blood pressure and to decrease pulmonary arterial pressure are described above (Kumar, 2015).

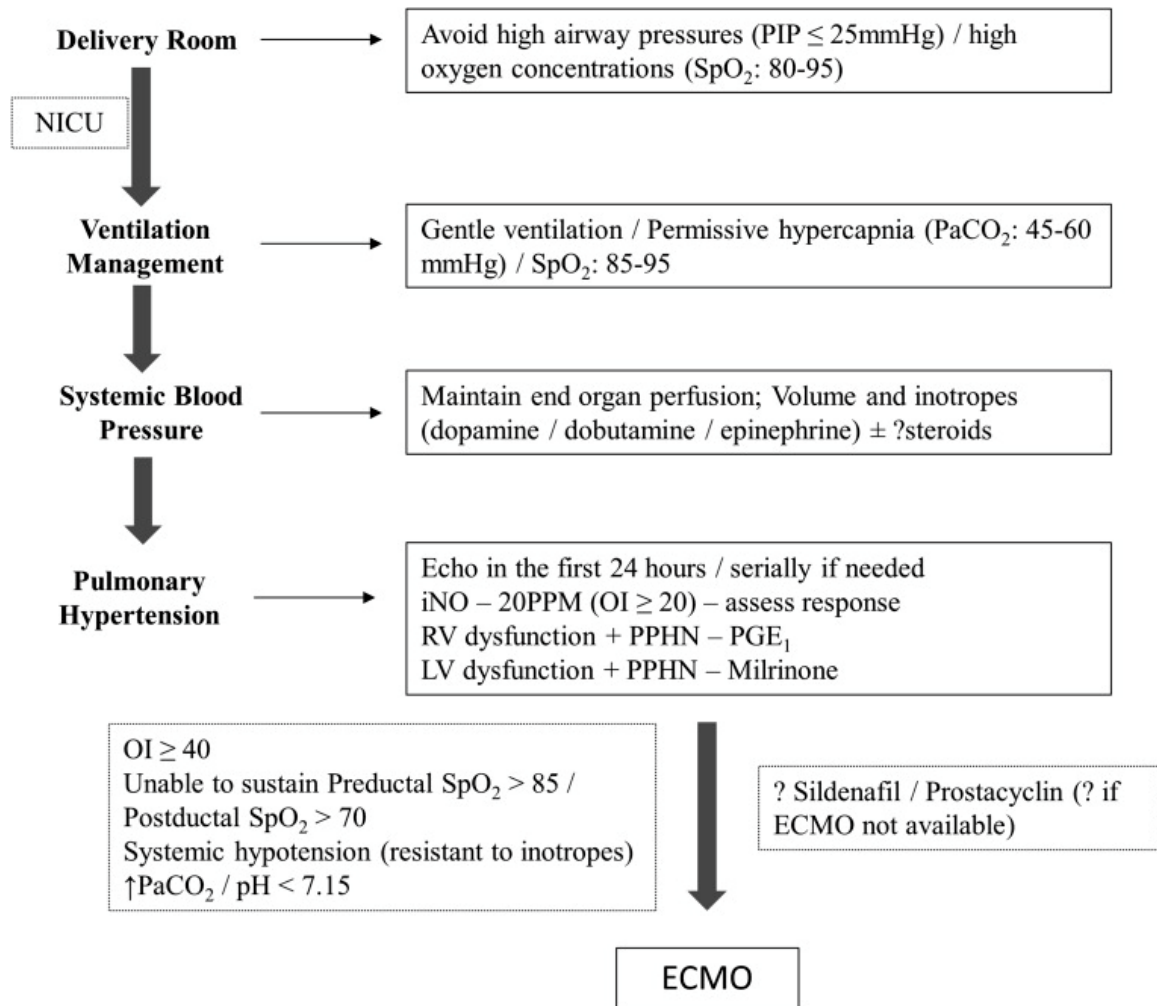


Figure 9. Postnatal Management of CDH

This figure shows an algorithm of postnatal management of CDH., While the algorithm is not a complete representation of postnatal management of CDH,it represents a general consensus based on the evolving data surrounding postnatal CDH treatment (Kumar, 2015).

Extracorporeal membrane oxygenation (ECMO) has also been used as a therapy for patients with CDH. ECMO is a collection of bio-medical devices that support important functions in a CDH infant, such as the heart, lungs, kidneys, and nutrition filtration systems. The goal of the ECMO machine is to provide oxygenated blood to the infant with CDH and remove the carbon dioxide from the blood for a period of time until the infant's own lungs can maintain their normal physiological function. In infants with severe lung and oxygenation difficulties in the blood, ECMO is comparable to a heart-lung bypass machine used in open heart surgery (Ruano et al., 2015). When an infant is placed on ECMO, oxygen is added to the blood, but it is done outside the body by the ECMO machine. In order to prepare for placement on ECMO, infants are fitted with two cannulae in the right side of the neck, with one tube placed in the jugular vein and the other in the carotid artery (Tsao et al., 2010). Those tubes will connect with the ECMO system, which drains the unoxygenated blood from the jugular vein, into an artificial lung, which oxygenates the blood, and sends it back via the carotid artery. There is also a veno-venous approach, in which the cannula is placed in the jugular vein only, but has two channels, one for the unoxygenated blood and another for the oxygenated blood respectfully. The concentration of oxygen in this system is reduced to allow the lungs to rest and heal.

While on ECMO, blood is thinned with heparin, an anti-coagulant, which helps prevent blood clots from forming in the ECMO circuit. The length of stay on ECMO is determined by three major factors; the severity of the lung disease, lung damage, and complications during the ECMO course. While a typical course on ECMO lasts about a

week, there is no standard criteria to define a date and time of physiological stabilization. The complication factors of lung disease and damage can reduce or increase the amount of time an infant is on ECMO before the period of stabilization prior to surgery (Tudorache et al., 2013). CDH candidates that qualify for the use of ECMO include those that do not have any congenital or medical management anomalies after birth. To be placed on ECMO, infants must show an inability to maintain at least 85% preductal saturations or 70% postductal oxygenation. Poor perfusion is another indicator for the use of ECMO with systemic hypotension which is resistant to fluid and inotropic treatment. In **Figure 9** above, the algorithm for determining the use of ECMO, as well as a synopsis of the postnatal management steps is outlined (Kumar, 2015).

The standard surgical approach for CDH is to repair the diaphragmatic defect. This approach consists of a subcostal incision with the removal of the abdominal contents from the thoracic region back to the abdominal cavity, and a complete closure of the defective area. For diaphragmatic defects that are too large to be closed by primary repair, prosthetic alternatives can be used to patch the region and close the gap in the diaphragm. A synthetic non-absorbent material called Gore-Tex is typically used in this event, or a natural absorbable patch called Surgisis. Due to the invasive nature of the surgery, minimally invasive surgical techniques for CDH repair have been gaining popularity, but with a downside and high risk of recurring herniation. Lastly, CDH repair after ECMO treatment has been associated with an increased survival rate compared to repair on ECMO, given that all CDH severity factors are controlled (Danzer & Kim, 2014; Kumar, 2015).

PUBLISHED STUDIES

Throughout the last few decades, several published studies in regards to CDH have been chronicled, and the discoveries over time have led to the impactful techniques and decisions of today. Several of the more novel discoveries in recent years are noted below, with particular consideration for nuanced methods of treatment of CDH. In Steurer et al., a newfound prognostic marker called B-type natriuretic peptide (BNP) was correlated to the severity of pulmonary hypertension and long-term outcomes of CDH infants. High BNP levels within 24 hours of delivery were associated with poorer clinical outcomes. In healthy infants, the BNP levels decreased after the first 24-48 hours of life, whereas steady higher levels of BNP after the first few days of life were associated with a poor prognostic indicator for long-term survival. These patients continued to receive supplemental oxygen and mechanical ventilations. The consistently high BNP levels were found to be an indicator of persistence pulmonary hypertension in infants via echocardiograph studies (Steurer et al., 2014). Findings such as Steurer et al., show the significant diversity and complexity of CDH. Prenatal and postnatal care has come a long way over the last few years, but there are still prevalent severe cases of CDH which still are being researched, in order to potentially find prenatal treatments and care for the diaphragmatic defect earlier in utero (Xiao et al., 2015).

Subsequently, one of the main prenatal management techniques for severe CDH cases was designed by Harrison et al., who were the first to introduce deflation of a previously inflated intra-thoracic balloon in animal subjects. Harrison hypothesized that

if hypoplasia is truly a developmental consequence of compression by a herniated diaphragm, then decompression in the prenatal state could allow further lung and pulmonary development, which would increase the chance of survival during the delivery. However, with these more invasive, in utero techniques, the risks elevate. The need for a tracheal occlusion is correlated with a poor prognosis, and most often involves a tracheomegaly. A tracheomegaly is an abnormally dilated trachea, which may result from prolonged positive pressure ventilation (Harrison et al., 1980).

In severe cases of CDH, Jani et al., demonstrated that the FETO procedure increased the rate of survival in patients from 24.1% to 49.1%. This study also reinforced the prognostic risk factors of CDH, specifically demonstrating that in isolated CDH, the size of the contralateral lung is a significant predictor of survival, but also the need for a prosthetic patch of diaphragmatic repair (Jani et al., 2009; Deprest et al., 2004). Consequently, the need for patch repair is an inherent measure of the severity of diaphragmatic hernia and of the CDH infant's long-term morbidity. The study also noted the relationship being a higher LHR and survival based on a sample size of 127 cases of isolated diaphragmatic hernia (Jani et al., 2009). In Basta et al., the study was undertaken to uncover the connection between the degree of stomach herniation and neonatal outcomes with left-sided CDH patients. It was hypothesized that the stomach position in the fetal cavities would have a significant impact on the rate of survival depending on its position in either the abdominal cavity or the thorax. There was a strong correlation between fetal stomach position when the stomach herniates from the abdominal cavity to the anterior left chest, and eventually into a retrocardiac position on the right side of the

thoracic cavity. Stomach position is a prognostic tool based on studies such as this one to predict the survival rates of neonates (Basta et al., 2015).

In Babiuk et al., animal models were used to test two hypotheses regarding the pathogenesis of CDH, and the results revealed findings consistent with prior studies. The hypothesis stated that the malformation of an amuscular mesenchymal aspect of the primitive diaphragm was responsible for the myogenic process, and secondly, that the defect in the primitive diaphragm influencing the diagnosis of CDH is not secondary to defects in developing lungs. The idea that lung hypoplasia is in fact secondary to the diaphragmatic defect is supported by data in this study. Taken from a sample of a surgically induced sheep model of CDH, the model depicts that a hole in the posterolateral diaphragm, a symptom of CDH, results in an underdeveloped lung on that side. The underdevelopment of the lung is due to the evasion of abdominal organs into the thoracic cavity via the diaphragmatic hernia (Babiuk et al., 2002).

Following the postnatal transition, there have been a variety of studies that attempt to corroborate certain interventions and surgical timing techniques in hopeful concordance with increased survival rates in CDH infants. One study performed by Campbell et al., examined the utilization of inhaled nitric oxide with CDH patients postnatally. The study concluded that in several tertiary hospitals without the use of ECMO, inhaled nitric oxide was an effective in reducing the mortality rate in the postnatal CDH patients. Ultimately, the study showed that there is widespread use of inhaled nitric oxide across the United States without a decrease or drop off in ECMO use, yet no net variation between inhaled nitric oxide and ECMO in mortality rates. Inhaled

nitric oxide has been found to be an effective measure to treat persistent pulmonary hypertension in CDH patients shown in **Figure 10** (Cambell et al., 2014). Moreover, the findings reveal that while previous cohorts of studies regarding more effective treatments of CDH have been published over the years, there are still hurdles to overcome regarding the treatment of CDH in the most effective way possible.

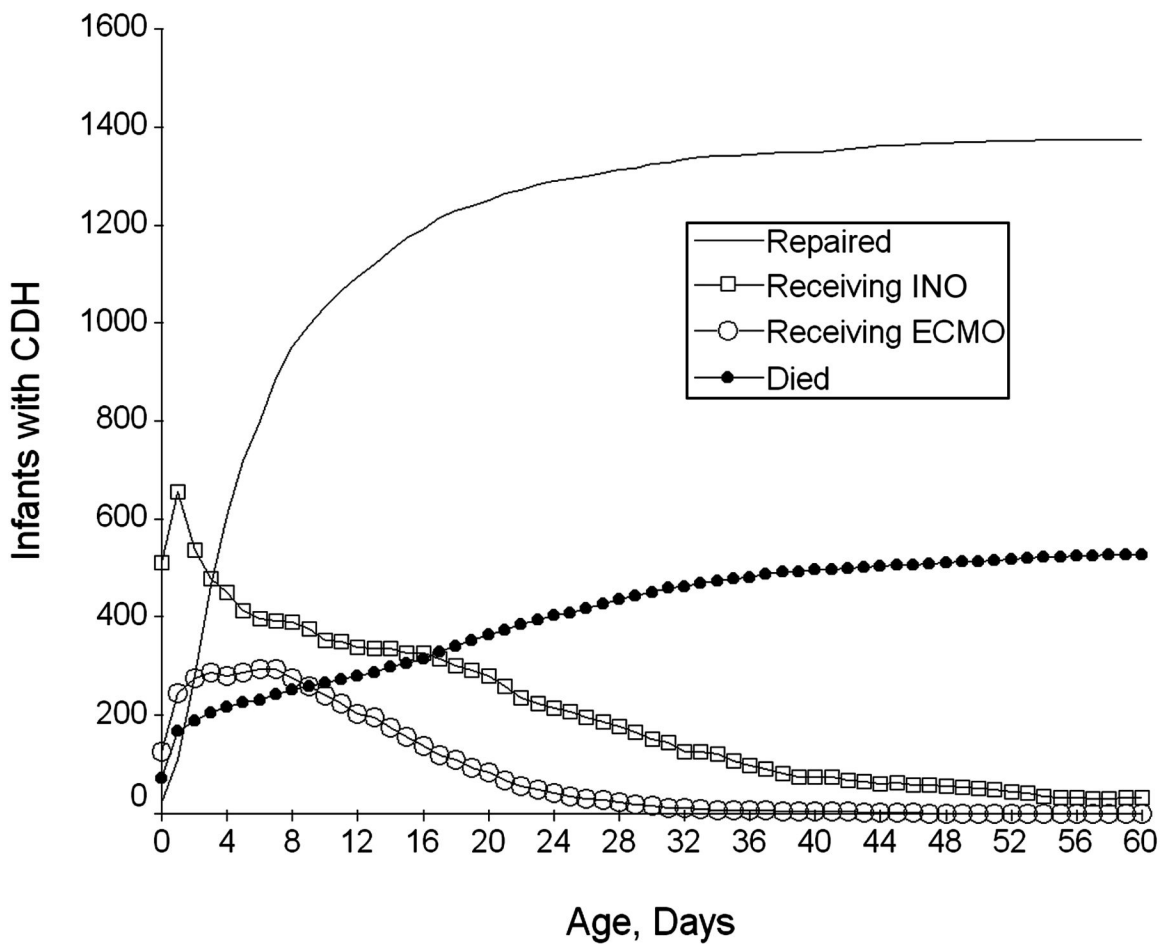


Figure 10. Inhaled Nitric Oxide Therapy in CDH
 Timing of Inhaled Nitric Oxide therapy and ECMO correlated with cumulative repair and mortality among 1,713 infants with CDH at 33 hospitals, from 2003 to 2011 (Campbell et al., 2014).

DISCUSSION AND CONCLUSIONS

Congenital diaphragmatic hernia is an abnormality riddled in complexities and evolving treatment techniques. Despite decades of research, mortality rates remain high. These high rates are in spite of the immense amount of nuanced intensive care (Kumar, 2015). While there are still impactful areas of CDH research that could be studied with future developments, one of the most vital treatment tactic involves the timing of surgery in CDH. In a study by Charlton et al., 86 patients with CDH presented to a neonatal surgical unit were analyzed for factors involving surgical timelines. CDH patients with delayed surgery showed significant survival rate improvement over those who were rushed into surgery.

This discovery altered the strategy of CDH postnatal treatment, and with the combination of ECMO, has shaped the contemporary form of treatment for infants with CDH. Delay in surgery intervention until the infant is at a sustainable lung volume is supported by this literature review, rather than rushing to surgery and risking complications such as pulmonary hypertension (Charlton et al., 1991). These notions are reinforced through the continued use of ECMO and a delayed surgical repair, which have improved survival rates in CDH patients (Chatziioannidis et al., 2014; Wat et al., 2012). Nevertheless, even with such strong corroborations such as the timing of surgical intervention, there are still post-surgical and long-term conditions that have been shown to be involved with CDH infants (Rygl et al., 2015; West et al., 1992). For instance, the rate of readmission to a hospital or clinic with regard to wheezing is significantly high in infants who had CDH. These children are in a high-risk category for a respiratory ailment

and morbidity long term after the initial extended hospital stay after birth. Abnormal lung development is one of the leading prognostic factors in wheezing for infants who had CDH (Benoist et al., 2016). Severity of pulmonary abnormalities such as hypoplasia are correlated with increased risk of long-term dilemmas after departure from the hospital, particularly on the side of the complication. The severity of left heart hypoplasia is directly associated with the severity of the left-sided CDH, and thus the severity of the long-term prognosis (Byrne et al., 2015). Another long-term factor with CDH is the post-surgical scarring, which is more of a superficial factor, but a factor in leaning towards minimally invasive surgical techniques. However, minimally invasive surgeries, while showing a lower infant mortality rate at the post-operative level, are also correlated with a higher risk of reoccurrence of CDH (Scott et al., 2005; van Dooren, 2004). In typical cases, the larger the patch repair, the greater the risk for post-discharge and long-term complications there will be (Shah et al., 2014). Liver herniation is also a major factor in long-term morbidity (Tsao et al., 2010).

Even in the most common of cases, there could be long-term prognostic indications for morbidity. Follow up visits for neurological, cardiac, and respiratory systems are recommended for patients varying in any and all forms of CDH. To improve long-term prognosis, some institutions have developed protocols for gentle ventilation to improve the survival in patients with CDH, in particular during the preoperative stage in the management process (Cartlidge et al., 1986). These protocols have universally been associated with increased survival rates because gentle ventilation allows the lung to grow and form on its own, while limiting the risks for further pulmonary complications

(Cauley et al., 2015). The protective ventilation has been depicted in several cases to see an increase in the survival rate of a majority of CDH patients (Bojanić et al., 2015). Overall, congenital diaphragmatic hernias are a significant area of pediatric research and research into the evolution and treatment of the condition are of vital importance to those who are affected. As can be seen through the process, CDH begins at the embryologic period, with a diaphragmatic defect, leading to several pathological events that set off a chain reaction where contents of the abdominal cavity can migrate up to the thoracic cavity, causing pulmonary circulation to be diminished (Olson et al., 2015). Genetic abnormalities increases the mortality rate and severity of the CDH condition. Therefore, several prenatal and postnatal treatment modifications are utilized to help the infant's fetal lungs modulate on their own with hopefully a positive long-term prognosis for survival (Bairdain et al., 2015).

APPENDIX



Left-Sided CDH depicting the stomach and liver within the four-chamber view, and a distinct mediastinal shift of the heart to the right side (Marlow & Thomas, 2013).

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CURRICULUM VITAE

