1. Introduction

Historians, scientists, and researchers have been fascinated by the diaphragm for many centuries. Homer first described Trojan War battle wounds with reference to the diaphragm in the 9th century B.C. From 500-430 B.C., Empedocles of Agrigentum was one of the first people to study the physiology of respiration. In this early period of medical knowledge, however, the purpose of the diaphragm bewildered scientists. Hippocrates observed the diaphragm’s inherent fragility and thinness that caused it to throb at any instance of unexpected joy or sorrow. Plato hypothesized that the diaphragm was not involved with respiration but rather served as a boundary between parts of the soul. It was not until Galen in the 2nd century A.D. that the actions of the diaphragm were described as upward isovolume movements during the period of rib cage expansion in respiration (Skandalakis 2004).

In the early 17th century, the first observations of congenital diaphragmatic hernia (CDH) emerged. Through these defects, the understanding of the embryological development of the diaphragm began to form. Morgagni clearly described the hiatal hernia through the foramina of Morgagni in 1761. In 1848, Bochdalek referenced the development of the pleuroperitoneal canals while describing the hernia that formed by passing through these canals. Broman proposed the first diagram of the adult diaphragm indicating its embryonic derivatives in 1905. Wells continued the study of the diaphragm along with the pleural sacs in the mid-20th century, and Adzick diagnosed 88 of 94 infants with CDH using prenatal ultrasonography in 1985 (Skandalakis 2004). With the wide range of diaphragmatic defects that have been identified, the formation of the diaphragm still remains a topic of fascination today. However, in the last decade, a spectrum of genetic loci was identified as causative genes of this defect.

2.1 Embryology

2.1.1 Normal diaphragm development

Prior to diaphragm formation, the transverse septum, one of the four components that combine into the early diaphragm, begins its descent along the vertebral column. In the
third week of embryonic life, the transverse septum lies at the level of the third cervical vertebra. By the end of diaphragm development in week 8, the early diaphragm descends to its ultimate position at the first lumbar segment, secondary to the rapid growth of the vertebral column. Concurrently, the phrenic nerve arises from the third to fifth cervical vertebra and follows the diaphragm down to its final location (Skandalakis 2004).

Diaphragm development begins approximately at the fourth week of intrauterine life from four embryonic structures derived from the mesoderm: the transverse septum, the pleuroperitoneal membranes, the dorsal esophageal mesentery, and the musculature of the body wall (Figure 1) (Bielinska, Jay et al. 2007; Hartnett 2008). The transverse septum, an infolding of the ventral body wall, develops into the anterior central tendon, beginning the separation of the pleuro-pericardial cavity and the peritoneal cavity (Clugston and Greer 2007; Hartnett 2008; Keijzer and Puri 2010). The incomplete separation of the body cavities results in two openings adjacent to the esophagus called the pericardio-peritoneal canals (Hartnett 2008).

Another infolding of the posterolateral body wall develops into the pleuroperitoneal membranes, which originates from the caudal end of the pericardioperitoneal canals and travels medially and ventrally (Hartnett 2008; Keijzer and Puri 2010). The fusion of the transverse septum with the pleuroperitoneal membranes and structures around the esophageal mesentery begins the closure of the pleuroperitoneal canals, with the right side closing before the left (Keijzer and Puri 2010). During weeks four to six, pleuroperitoneal folds are formed as a pair of temporary pyramidal structures joining the pleuropericardial folds to the transverse septum (Clugston and Greer 2007). Closure is completed around the eighth week of gestation (Figure 2) (Clugston and Greer 2007).
After the early diaphragm is formed, distinct myogenic cells migrate from the lateral dermomyotomal lip and invade the pleuroperitoneal folds (Clugston and Greer 2007). The muscle precursor cells then proliferate and radiate out to muscularize the entire diaphragm (Clugston and Greer 2007). Simultaneously, the phrenic nerve extends to the pleuroperitoneal folds and, from there, innervates the remainder of the diaphragm (Clugston and Greer 2007). The muscularization and innervation of the diaphragm is concluded at about week ten (Clugston and Greer 2007).

2.2 Abnormal diaphragm development

Many theories have been proposed as to the cause of diaphragmatic hernias. In one theory, abdominal viscera herniate through the diaphragm to prevent closure. The presence of the viscera in the thoracic cavity then leads to pulmonary hypoplasia. In another theory, the primary insult is believed to be pulmonary hypoplasia. This is followed by the abdominal viscera migrating into the chest as a result of a lack of adequate pulmonary parenchyma. The diaphragm then fails to fuse due to the invading abdominal viscera. It has also been hypothesized in yet another theory that the same embryological accident produces pulmonary hypoplasia and the diaphragmatic defect. No theory is proven at this time. However, more support lends to the latter two theories from animal studies.

Diaphragmatic hernia can be classified under two major categories, congenital and acquired. The congenital diaphragmatic hernias are classified as 1) posterior lateral defect of the diaphragm (Bochdalek), 2) anterior defect of the diaphragm (Morgagni), 3) peritoneopericardial central diaphragmatic hernia (septum transverse type), 4)
eventration of the diaphragm, 5) hiatal hernia and paraesophageal hernia, (6) and others (Skandalakis 2004). Of the congenital hernias, Bochdalek and Morgagni represent the majority of cases of CDH (Figure 3). On the other hand, acquired diaphragmatic hernias are traumatic in nature (Skandalakis 2004).

Fig. 3. Diagram showing the site of Bochdalek and Morgagni defects

### 2.2.1 Congenital diaphragmatic hernia

#### 2.2.1.1 Posterior lateral defect of the diaphragm (Bochdalek)

This defect represents over 70% of diaphragmatic hernias. It begins above and lateral to the left lateral arcuate ligament at the vertebral costal trigone. The event occurs during the intestinal return to the abdominal cavity around the 10th week of embryonic life. At that time, the trigone is composed mainly of membranous tissue with rare muscle fibers. The increased intra-abdominal pressure causes the separation of the muscle fibers and creates the defect. The defect can be small in size or, in extreme cases, almost the entire hemidiaphragm is involved. Approximately 90% of this defect occurs on the left side while the right side represents less than 10% (Figure 4-5). In 99% of the cases it is unilateral. Herniation of the small intestines, stomach, colon, spleen, and part of the liver may occur (Figure 6-7) (Skandalakis 2004). Subsequently, pulmonary hypoplasia with mediastinal shift occurs.
Fig. 4. X-ray of left diaphragmatic hernia

Fig. 5. X-ray of right diaphragmatic hernia
2.2.1.2 Anterior defect of the diaphragm (Morgagni)

This is also known as parasternal defect of the diaphragm, which results from a small gap of the musculature on either side of the xiphoid process and the seventh costal cartilage (Skandalakis 2004). It occurs from a failure of the crural and sterna portions of the diaphragm to fuse. It is usually associated with omental herniation, hence they always contain fat (Gossios, Tatsis et al. 1991). 90% of the Morgagni type occurs on the right side, and 7% occur bilaterally.
2.2.1.3 Peritoneopericardial central diaphragmatic hernia (septum transverse type)

This rare type has been reported in newborn infants and even adults. It represents a defect in the central tendon and overlying pericardium. Incarceration of the intestines can be the early symptoms (Skandalakis 2004). The defect allows a rare occasion herniation of the liver into the pericardial sac (Davies, Oksenberg et al. 1993).

2.2.1.4 Eventration of the diaphragm

It can be classified into congenital or traumatic. It occurs when the entire leaf of the diaphragm bulges upward (Figure 8). It is more common on the left side and affects males more than females, and it is usually associated with intestinal malrotation. Additionally, sigmoid valvulus and rarely gastric valvulus can be seen with this type of herniation (Tsunoda, Shibusawa et al. 1992; McIntyre, Bensard et al. 1994). It occurs mainly due to the failure of muscularization of the diaphragm leaflets, not due to fusion of the embryonic components as in the previous three entities. In the congenital form, the phrenic nerve is intact, and the lung on the affected side is collapsed but not hypoplastic.

On the other hand, the acquired eventration is due to phrenic nerve injury with normal muscularization of the diaphragmatic leaflets (Skandalakis 2004).

Fig. 8. Diagram showing eventration of the diaphragm

2.2.1.5 Hiatal hernia

This lesion results from an enlarged hiatus and a weakened phrenoesophageal ligament. It can be classified into two subtypes, sliding hiatal hernia and paraesophageal hernia. In the sliding hiatal hernia, a part of the stomach is placed upward with the gastroesophageal junction located in the thoracic cavity. In the paraesophageal hernia, the gastroesophageal junction is normally located below the diaphragm. However, the portion of the fundis can herniate into the thoracic cavity anterior to the esophagus, and, on rare occasions, a piece of omentum can be seen in the thoracic cavity (Skandalakis 2004).
2.2.1.6 Others

**Accessory diaphragm and duplication of diaphragm**

This rare anomaly divides the corresponding hemothorax into two spaces by an accessory sheet of fibromuscular tissue. The origin of the membrane usually comes from the pericardial reflection. It can be attached to the seventh rib. However, in rare occasions, it can be attached to the apex of the pleural. The lower cavity of the hemothorax usually contains a hypoplastic portion of the lung (Skandalakis 2004).

A true duplication rarely occurs secondary to the duplication of the septum transversum (Krzyzaniak and Gray 1986).

**Diaphragmatic agenesis**

This rare entity occurs secondary to the failure of the diaphragmatic components or their failure to join properly in the early embryonic life. It usually occurs unilaterally (Skandalakis 2004). However, in rare occasions, bilateral agenesis can occur especially in association with pentalogy of Cantrell.

2.2.2 Acquired/traumatic hernia

This entity is rare and usually results from post-natal trauma. It is more common in the right side with a portion of omentum, colon, and stomach herniated into the thoracic cavity. Another rare form represents diaphragmatic rupture and rib fracture resulting from paraoxysmal coughing (Skandalakis 2004).

2.2.3 Abnormal lung development

Although repairing a diaphragmatic defect in a newborn is relatively simple via a primary closure or a patch, the major issue is that the development of the lungs is disrupted, causing pulmonary hypoplasia and constant pulmonary hypertension (van Loenhout, Tibboel et al. 2009; Keijzer and Puri 2010). Pulmonary hypoplasia, reduced airway branching, and surfactant deficiency in newborns with CDH result in respiratory failure at birth (Keijzer and Puri 2010). Children with a CDH suffer from significant levels of morbidity and mortality due to abnormal pulmonary development. Improved treatment of newborns with CDH in the neonatal intensive care unit has substantially reduced mortality rates to less than 10% to 20% in tertiary referral centers (van Loenhout, Tibboel et al. 2009; Keijzer and Puri 2010). The high morbidity rate is attributed to modern treatment methods, such as high-frequency oscillation and extracorporeal membrane oxygenation (ECMO) (van Loenhout, Tibboel et al. 2009; Keijzer and Puri 2010).

Pulmonary hypoplasia is thought to be the result of a dual-hit hypothesis. Traditionally, it was believed that a hypoplastic lung developed only with the diaphragmatic hernia. In the dual-hit hypothesis, however, the lungs suffer from two insults, the first one inherent in pulmonary development before the development of the CDH and the second insult occurring with the CDH. The lungs are inherently disturbed prior to the early formation of the diaphragm before any mechanical pressure can be applied by the CDH (van Loenhout, Tibboel et al. 2009; Keijzer and Puri 2010). A second insult to the ipsilateral lung subsequently occurs as a result of disturbance of fetal breathing movements due to the abdominal organs invading the thoracic cavity (van Loenhout, Tibboel et al. 2009; Keijzer...
and Puri 2010). Although some authors have postulated that the hypoplastic lung causes the diaphragmatic hernia, animal models of mutant mice with inactivated Fgf10, thereby preventing lung development, still had normal diaphragms, disproving that abnormal pulmonary growth creates the diaphragmatic defect(Keijzer and Puri 2010).

### 2.2.4 Associated anomalies

Many anomalies have been associated with CDH, including intestinal malrotation, congenital heart defects, pulmonary stenosis, tracheal agenesis, abdominal wall defects (omphalocele and gastroschisis), obstructive uropathy, skeletal anomalies (in particular vertebral malformation), choanal atresia, and neural tube defect(Skandalakis 2004). Additionally, the instance of CDH is seen more frequently in syndromic infants, particularly with trisomy 18, trisomy 21, and pentalogy of Cantrell (Figure 9-10).

![Fig. 9. Fetus with trisomy 18 and left diaphragmatic hernia](image_url)
3. Molecular genetics

A genetic component has been confirmed in the etiology of CDH (Bielinska, Jay et al. 2007). However, many genes have been implicated in its pathogenesis, which may be the cause of multiple developmental insults during embryological growth of the diaphragm and lung (van Loenhout, Tibboel et al. 2009). The phenotype of CDH has been observed to be significantly variable, suggesting that its etiology is most likely due to more than one single gene mutation (van Loenhout, Tibboel et al. 2009). Moreover, it has been hypothesized that CDH may also be influenced by environmental factors, increasing the complexity in
determining the specific genetic mutations that may induce diaphragmatic defects (van Loenhout, Tibboel et al. 2009).

Despite this multifactorial nature of CDH, various genes have been identified and associated in CDH. Transcriptional regulators have been hypothesized to contribute to the development of CDH (Bielinska, Jay et al. 2007). Transcription factor haploinsufficiency in humans is an accepted cause of CDH, especially when it is accompanied with other congenital defects (Bielinska, Jay et al. 2007). Transcription factors can regulate gene expression for mesenchymal cell function, and if impaired, they can cause impaired structure, apoptosis, and anomalous cell sorting (Bielinska, Jay et al. 2007).

Factors of cell migration, mesodermal patterning, or the structure of extracellular matrix have also been implicated in the morphogenesis of CDH (Bielinska, Jay et al. 2007). Mutations in genes directing cell migration and mesodermal patterning are essential for growth/guidance factors, receptors, and parts of the extracellular matrix (ECM) and have been related to CDH and extradiaphragmatic defects (Bielinska, Jay et al. 2007). The mesenchymal hit hypothesis proposes that signaling pathways common to cells in organs derived from the mesoderm are disturbed by genetic and environmental triggers in CDH (Bielinska, Jay et al. 2007). Thus, it is not surprising that diaphragmatic defects are often accompanied by abnormalities in the heart, lungs, and liver in nonisolated CDH (Bielinska, Jay et al. 2007).

In addition, the development of the diaphragm is dependent on proteins involved in the metabolism and binding of retinoids, and animal studies have shown that 25-40% of rat offspring develop a CDH when fed a vitamin A deficient diet (Goumy, Gouas et al. 2010; Keijzer and Puri 2010). In chromosome loci commonly associated with CDH, identified genes involved in the retinoic signaling pathway have been proposed as causative pathways of CDH (Goumy, Gouas et al. 2010).

3.1 Wt1

Wilms’ tumor suppressor gene, Wt1, is a zinc finger transcription factor expressed in the amuscular diaphragm, pleural/abdominal mesothelial cells, epicardium, testicular somatic cells, and kidney (Bielinska, Jay et al. 2007). Heterozygous mutations in Wt1 are known to cause syndromic CDH, such as Wilms’ tumor-Aniridia-Genitourinary anomalies-mental Retardation (WAGR), Denys-Drash, Frasier, and Meacham syndrome (Bielinska, Jay et al. 2007; van Loenhout, Tibboel et al. 2009). Symptoms of these syndromes include genitourinary, diaphragmatic, and cardiac deformations (Bielinska, Jay et al. 2007). Although no mutations in Wt1 have been linked to isolated CDH in humans, a Bochdalek hernia originating from the deformation of the pleuroperitoneal folds was observed in Wt1 null mutant mice, along with cardiac abnormalities (Clugston and Greer 2007).

3.2 Fog2

Friend of GATA-2, a multi-zinc finger transcription factor that binds to the Gata transcription factor family, is expressed in mesodermal tissue, such as the early diaphragm, lung mesenchyme, epicardium, myocardium, and testicular somatic cells (Bielinska, Jay et al. 2007; Goumy, Gouas et al. 2010). It lies on chromosome 8q23, and a nonsense mutation in a female patient with severe bilateral pulmonary hypoplasia and a posterior diaphragmatic
eventration on the left side (Bielinska, Jay et al. 2007; van Loenhout, Tibboel et al. 2009; Keijzer and Puri 2010). Rearrangements of *Fog2* in two cases of isolated CDH were found but not identified as mutations (van Loenhout, Tibboel et al. 2009). Patients with loss-of-function point mutations heterozygous for *Fog2* can develop diaphragm anomalies (eventration), pulmonary hypoplasia, and/or cardiac abnormalities (Bielinska, Jay et al. 2007). *Fog2* is the only gene thus far identified with mutations in nonsyndromic CDH patients, supporting its importance in diaphragmatic and lung development in humans.

Animal studies have displayed that a mutation in this gene causes severe pulmonary hypoplasia with a posterolateral muscularization defect instead of a developmental defect, supporting the notion that the defects of the lungs and diaphragm in CDH occur separately (van Loenhout, Tibboel et al. 2009).

### 3.3 Gata-4

The zinc finger transcription factor *Gata4*, which interacts with *Fog2*, is also expressed in mesenchymal cells of the embryological diaphragm, lungs, heart, and testicular somatic cells (Bielinska, Jay et al. 2007; van Loenhout, Tibboel et al. 2009). *Gata4* lies on chromosome 8p23.1 and is known to be involved in heart development (Wat, Shchelochkov et al. 2009). Patients heterozygous for *Gata4* loss-of-function mutations with terminal deletions extending to at least 8p23.1 developed cardiac deformations, and microdeletions on 8p23.1 have been linked to a spectrum of cardiac malformations, such as atroventricular septal defects, hypoplastic left heart, hypoplastic right ventricle, pulmonary atresia/stenosis, pulmonary valve stenosis, partial anomalous pulmonary venous return, subaortic stenosis, transposition of the great arteries, double-inlet/double-outlet right ventricle, and tetralogy of Fallot (Wat, Shchelochkov et al. 2009). Haploinsufficiency of *Sox7* with *Gata4* deletions may worsen these cardiac conditions (Wat, Shchelochkov et al. 2009).

Isolated CDH is also common in patients with *Gata4* deletions in at least nine previous cases (van Loenhout, Tibboel et al. 2009; Wat, Shchelochkov et al. 2009). CDH has occurred with 22.2% of patients with reported interstitial deletions of 8p23.1, and the majority of these patients have developed left-sided CDH (Wat, Shchelochkov et al. 2009).

In animal studies, 70% of mice heterozygous for *Gata4* knockout mutations developed heart, lung and midline diaphragmatic defects (van Loenhout, Tibboel et al. 2009). The midline diaphragm malformation was described as a ventral hernia covered by a sac that allowed the abdominal viscera to protrude and was present in about 30% of the mice (Bielinska, Jay et al. 2007; Wat, Shchelochkov et al. 2009). Moreover, it has been shown that *Gata4* is important for normal pulmonary lobar development (van Loenhout, Tibboel et al. 2009).

### 3.4 Coup-TFII

Chicken ovalbumin upstream promoter-transcription factor II (*Coup-TFII*) is a nuclear orphan receptor of *Fog2* that belongs to a steroid/thyroid hormone receptor superfamily and is expressed in a variety of tissues, including mesodermal tissues in the diaphragm, lungs, and heart (Bielinska, Jay et al. 2007; van Loenhout, Tibboel et al. 2009). The minimally deleted region on human chromosome 15q26.1-26.2 has been identified in CDH patients, and *Coup-TFII* is one of the four genes that is located in this region, suggesting its possible involvement in the CDH of patients with 15q deletions (Bielinska, Jay et al. 2007). However,
in a review of over 130 cases, no mutations in Coup-TFII were found (van Loenhout, Tibboel et al. 2009). Mutations in this gene were also absent in 73 CDH cases tested by Scott et al. and in over 100 cases reviewed by Slavotinek et al. (Goumy, Gouas et al. 2010).

Despite the lack of evidence of Coup-TFII in the implication of CDH, animal studies have proven to be more convincing. Mice with Cre-lox conditional mutagenesis of Coup-TFII in the mesenchyme and pleuroperitoneal folds developed diaphragm malformations similar to Bochdalek diaphragmatic hernias (Bielinska, Jay et al. 2007; van Loenhout, Tibboel et al. 2009). In these studies, Coup-TFII expression was shown to be significantly downregulated in the early diaphragm and pleuroperitoneal folds but only slightly reduced in the early lung (van Loenhout, Tibboel et al. 2009). Upregulation of Coup-TFII may cause hypoplastic lung in the nitrofen rat model through a negative feedback system during diaphragm development (Goumy, Gouas et al. 2010; Keijzer and Puri 2010). Coup-TFII null mice show malformations in cardiovascular development (Goumy, Gouas et al. 2010; Keijzer and Puri 2010).

3.5 Shh signaling pathway

Sonic Hedgehog (Shh) and Gli2 and Gli3 are highly conserved genes that are components of the Shh signaling pathway (van Loenhout, Tibboel et al. 2009). Shh has been shown to be a vital protein for the morphogenesis of the early respiratory system in the mouse embryo (Goumy, Gouas et al. 2010). In Shh null mutant mice, tracheo-esophageal separation is absent, and the branching of the lungs is underdeveloped, forming hypoplastic lungs (van Loenhout, Tibboel et al. 2009). In the nitrofen rat model of CDH and in the hypoplastic lungs of CDH patients, Shh has been shown to be downregulated (van Loenhout, Tibboel et al. 2009; Goumy, Gouas et al. 2010).

Similarly, animal studies have shown that Gli2 and Gli3 mutant mice have similar foregut anomalies with a more severe lack of pulmonary branching. In the original publication on the functions of Shh and Gli2 and Gli3, mutants of Gli2 and Gli3 mice demonstrated more severe failure of pulmonary branching, ranging from failure of primary branching to the agenesis of the lungs to ectopic branching and fusion of both lungs (van Loenhout, Tibboel et al. 2009). No diaphragmatic in this study was reported (van Loenhout, Tibboel et al. 2009). However, in a more recent study conducted by the same authors showed that Gli2 and Gli3 null mutant mice showed evidence of CDH (van Loenhout, Tibboel et al. 2009; Keijzer and Puri 2010). Evidence that Gli2 and Gli3 play roles in CDH in human patients has yet to be found (van Loenhout, Tibboel et al. 2009).

3.6 Slit3 and Robo1

Slit3 belongs to the Slit family proteins, which are secreted molecules involved in axon guidance through repulsion and mesodermal cell migration (Bielinska, Jay et al. 2007; van Loenhout, Tibboel et al. 2009). This gene is expressed in the embryonic diaphragm among other tissues (Bielinska, Jay et al. 2007). Seventy percent of null mutant mice have displayed central-type diaphragmatic hernias, cardiac defects, and renal deformations (Bielinska, Jay et al. 2007; van Loenhout, Tibboel et al. 2009). This diaphragmatic defect is derived from abnormal connective tissue formation in the central septum transversum, which causes liver attachment on the right side, creating a likeness to the human central CDH (van Loenhout, Tibboel et al. 2009). Moreover, these mice do not experience pulmonary hypoplasia and do
not die of respiratory failure (Keijzer and Puri 2010). One newborn with CDH was observed to have a hernia sac attached to the liver, similar to the Slit3 null mutant mice (van Loenhout, Tibboel et al. 2009).

Robo1 is a surface transmembrane protein and receptor of Slit3 found in the brain, lung, heart, liver, muscle, and kidney (Bielinska, Jay et al. 2007). Robo1−/− mice die upon birth from respiratory failure and have abnormal mesenchymal cellularity, with some displaying CDH (Bielinska, Jay et al. 2007).

No mutations of Slit3 or Robo1 have been found in patients with CDH (Bielinska, Jay et al. 2007). However, mutations in heparan sulfate, a proteoglycan possibly essential to the signaling complex of Slit3 and Robo1, have been discovered in CDH patients (Bielinska, Jay et al. 2007).

3.7 PDGFRα

Platelet-derived growth factor receptor-α (PDGFRα), known to be involved in the formation of gastrointestinal and neural tumors, was recently identified to be significant in the morphogenesis of the diaphragm and lung (van Loenhout, Tibboel et al. 2009). In PDGFRα null mice, pulmonary hypoplasia and posterolateral diaphragmatic hernias were found, similar to Fryns syndrome, a nonisolated CDH found in humans (van Loenhout, Tibboel et al. 2009; Keijzer and Puri 2010). Moreover, a genetic sequence variant of PDGFRα was found in one patient with nonisolated CDH (van Loenhout, Tibboel et al. 2009). Thus, PDGFRα is a potential candidate for syndromic CDH.

3.8 Retinoid signaling pathway

Retinoids have a major role in various biological processes, including embryogenesis and lung development (Goumy, Gouas et al. 2010). Increasing evidence in animal studies has recognized the retinoid signaling pathway as an important factor in the development of the diaphragm. For instance, 25-40% of rat offspring fed a diet deficient in vitamin A developed diaphragmatic abnormalities, and this percentage decreased upon the reintroduction of vitamin A mid-gestation (Goumy, Gouas et al. 2010). Retinoic acid receptor (RAR) double mutant mice displayed posterolateral diaphragmatic defects similar to those seen in humans (van Loenhout, Tibboel et al. 2009; Goumy, Gouas et al. 2010). In another study, RALDH2, the enzyme that converts retinal into retinoic acid, was inhibited in utero by nitrofen herbicide, which caused CDH and lung deformities in rats (Goumy, Gouas et al. 2010). Moreover, by using a pan-RAR antagonist to block RAR signaling, results showed a high level of left-sided CDH (Goumy, Gouas et al. 2010).

In humans, evidence has begun to support the role of retinoids in CDH development. Retinol and retinol-binding protein plasma levels were about 50% less in newborns with CDH than healthy newborns (Goumy, Gouas et al. 2010). Also, in a case of a pleiotrophic malformation syndrome including CDH, mutations of Stra6, a membrane receptor involved in cellular uptake of vitamin A, were found (Goumy, Gouas et al. 2010). These results support the hypothesis of the involvement of the retinoid signaling pathway in the etiology of CDH.

Many of the genes we have discussed are involved in the retinoid signaling pathway, especially genes in chromosome loci known to be associated with CDH. In chromosome 1,
deletions in the 1q41-1q42 region increases the likelihood of CDH malformation (Goumy, Gouas et al. 2010). Disp1 is a gene in this region that interacts in the Shh pathway, which targets Coup-TFII, a repressor of the retinoid pathway (van Loenhout, Tibboel et al. 2009; Goumy, Gouas et al. 2010). Thus, it may be possible that a loss of the Disp1 gene may disrupt the Shh pathway and Coup-TFII, disturbing retinoic acid signaling and leading to CDH (Goumy, Gouas et al. 2010).

Chromosome 8 is frequently involved in CDH mutations. Microdeletions on chromosome 8p23.1 are common in CDH patients, where the human Gata4 gene resides (Goumy, Gouas et al. 2010). The expression and activity of Gata4 is modulated by retinoids and may affect its function in mesodermal embryogenesis (Goumy, Gouas et al. 2010). 8q23.1, a region found to contain deletions in 6 cases, includes the Fog2 gene on its proximal end nearest 8q22.3 (Goumy, Gouas et al. 2010). Fog2 is indirectly involved in the retinoic acid pathway in various ways. It regulates target genes of Gata proteins through a heterodimer formation of the Gata family (Goumy, Gouas et al. 2010). Fog2 is also a corepressor protein for Coup-TFII and Gata4, and it has been suggested that simultaneous activity of Fog2 and Gata4 is necessary in order to direct mesenchymal cell function (Goumy, Gouas et al. 2010).

Chromosome 15 is another chromosome that has been known to have multiple deletions in CDH cases. Four patients were described with 15q24 microdeletions along with diaphragmatic hernias (Goumy, Gouas et al. 2010). Stra6, mentioned previously, encodes a receptor for RBP4-retinol on cell membranes and influences cell uptake of retinol molecules (Goumy, Gouas et al. 2010). Stra6 transcription is directly affected by retinoic acid levels (Goumy, Gouas et al. 2010). It is found to be expressed in the respiratory mesenchyme and respiratory/bronchial epithelium and is associated with severe malformations in humans, including diaphragmatic defects (Goumy, Gouas et al. 2010). 15q24 is another location on chromosome 15 that is involved in retinoic acid signaling and may be implicated in CDH etiology. Cellular retinoic acid binding protein 1 (CRABP1) is located in this region and encodes a lipid-binding protein that regulates intracellular concentration of retinoic acid by retinoic acid catabolism and transport from the cytoplasm to nuclear receptors (Goumy, Gouas et al. 2010).

The Coup-TFII gene is located on 15q26.1-q26.2 on chromosome 15, and deleted regions have been found in patients with syndromic CDH (Goumy, Gouas et al. 2010). Coup-TFII can regulate gene transcription and repress the retinoid pathway by preventing RAR heterodimer formation (Goumy, Gouas et al. 2010). Fog2, as mentioned before, is also known to interact with Coup-TFII, another gene involved in retinoid activity.

In chromosome 3, deletions have been found in 3 patients in literature in 3q21-23 (Goumy, Gouas et al. 2010). Retinol-binding protein 1 and 2 (RBP1 and RBP2) are located at the distal end of the 3q23 band adjacent to 3q22 and encode cellular RBPs (CRBPs) involved in intracellular retinol movement (Goumy, Gouas et al. 2010).

CDH has also been diagnosed in 4 cases with deletions and duplications in chromosome 4q32.1 (Goumy, Gouas et al. 2010). The LRAT gene is located in this region, and it translates into a microsomal enzyme that catalyzes the esterification of retinol for retinoid homeostasis (Goumy, Gouas et al. 2010). A downregulation of LRAT found in nitrofen models caused a shift of retinol homeostasis, disrupting the balance of the converted and stored forms (Goumy, Gouas et al. 2010). This suggested that nitrofen blocks retinoid pathways earlier than RALDH, the protein previously thought to be inhibited by nitrofen (Goumy, Gouas et al. 2010).
Other molecules involved in the retinoic acid pathway also interact with LRAT, including RARs and Gata transcription factors (Goumy, Gouas et al. 2010).

4. References


