Published in final edited form as:
Ann Med. 2007 ; 39(4): 261–274.

Molecular genetics of congenital diaphragmatic defects

Malgorzata Bielinska¹, Patrick Y. Jay^{1,2}, Jonathan M. Erlich¹, Susanna Mannisto⁴, Zsolt Urban^{1,2}, Markku Heikinheimo^{1,4}, and David B. Wilson^{1,3}

¹*Department of Pediatrics, Washington University and St. Louis Children's Hospital, St. Louis, MO 63110 USA* ²*Department of Genetics, Washington University and St. Louis Children's Hospital, St. Louis, MO 63110 USA* ³*Department of Molecular Biology & Pharmacology, Washington University and St. Louis Children's Hospital, St. Louis, MO 63110 USA* ⁴*Program for Developmental & Reproductive Biology, Biomedicum Helsinki and Children's Hospital, University of Helsinki, 00290 Helsinki, Finland.*

Abstract

Congenital diaphragmatic hernia (CDH) is a severe birth defect that is accompanied by malformations of the lung, heart, testis, and other organs. Patients with CDH may have any combination of these extradiaphragmatic defects, suggesting that CDH is often a manifestation of a global embryopathy. This review highlights recent advances in human and mouse genetics that have led to the identification of genes involved in CDH. These include genes for transcription factors, molecules involved in cell migration, and extracellular matrix components. The expression patterns of these genes in the developing embryo suggest that mesenchymal cell function is compromised in the diaphragm and other affected organs in patients with CDH. We discuss potential mechanisms underlying the seemingly random combination of diaphragmatic, pulmonary, cardiovascular, and gonadal defects in these patients.

Keywords

birth defects; diaphragmatic eventration; embryonic development; hernia, diaphragmatic; heart defects, congenital; sex reversal, gonadal

1. Introduction

Congenital diaphragmatic hernia (CDH) is a severe developmental abnormality that affects 1 per 3000 live births (1,2). The hallmark of the disorder is a defect in the muscular or tendinous portion of the diaphragm that allows abdominal viscera to protrude into the thoracic cavity. CDH is often accompanied by malformations of the lungs, heart, and/or other organs (3). The incidence of these extradiaphragmatic birth defects ranges from 35 percent to 60 percent in newborns with CDH and may be even greater in affected stillborns (1-4). Despite advances in surgical and medical management, the neonatal mortality rate of CDH remains significant, owing in part to concomitant birth defects (3).

Correspondence to: David B. Wilson, MD PhD Box 8208, Washington University School of Medicine 660 S. Euclid Ave., St. Louis, MO 63110 USA Phone: +1.314.286.2834 FAX: +1.314.286.2892 Email: wilson_d@wustl.edu.

Key messages

- Genetic triggers of CDH can disrupt mesenchymal cell function in not only the primordial diaphragm but also the developing lungs, heart, great vessels, gastrointestinal tract, and testes.
- CDH and associated birth defects can result from mutations in genes encoding transcription factors, molecules involved in cell migration, and extracellular matrix components.
- Bimodal gene expression may underlie the ostensibly random combination of diaphragmatic, pulmonary, cardiovascular, and gonadal defects in patients with CDH.

Most cases of CDH are presumed to reflect a combination of genetic predisposition and environmental factors, but the etiopathogenesis of this disorder is poorly understood (4-6). This review focuses on recent advances in human and mouse genetics that have led to the identification of genes involved in CDH. For a discussion of potential environmental triggers of CDH, such as vitamin A deficiency or teratogens, the reader is referred to other review articles (7,8).

2. CDH as a global embryopathy

In both humans and animal models, mutation of a single gene can cause primary development defects in not only the diaphragm but also the lung, heart, testis, and other organs (Table 1) (9-11). In humans, CDH has been described as a part of known genetic syndromes (4). Thus, CDH and associated birth defects are manifestations of a global embryopathy reflecting disruption of fundamental cellular processes during organogenesis. For practical reasons, surgeons and clinical geneticists generally categorize individual cases of CDH as “isolated” or “complex.” The term “isolated”, however, tends to understate the pervasive nature of the embryopathy, because cases of “isolated” CDH are often accompanied by primary defects in lung development.

A. Diaphragmatic defects

The primordial diaphragm forms through amalgamation of four embryonic structures: the septum transversum, esophageal mesentery, pleuroperitoneal folds (PPF), and body wall (Fig 1A) (12). Each of these four tissues is derived from mesoderm, as are the mesenchymal progenitors of several other organs, including the heart, lungs, and liver [e.g., mesenchymal cells in the septum transversum contribute to not only the central tendon of the diaphragm but also liver sinusoidal cells and certain cardiac cell types (Fig 1B)]. Fusion of the PPF with adjacent structures leads to closure of the pleuroperitoneal canals at week 5–8 in humans [corresponding to embryonic day (E)12.5-E13.5 in mice]. Subsequently the PPF is invaded by muscle precursors derived from the lateral dermomyotome of cervical somites.

Several anatomical defects fall under the rubric of CDH (3). Bochdalek hernias, involving the posterolateral region of the diaphragm, account for the majority of cases (Fig 1C). This type of CDH is thought to reflect failure of fusion of the PPF with adjacent structures. Less common forms of CDH include the Morgagni hernia, which affects the retrosternal diaphragm, and the central-type hernias, involving the central tendon. Other malformations grouped with CDH include diaphragmatic agenesis and eventration (the abnormal elevation of the diaphragm due to paralysis or aberrant muscularization). Most cases of CDH, however, are presumed to result from malformation or dysfunction of the amuscular mesenchymal component of the primordial diaphragm rather than a primary defect in myogenesis (13).

B. Pulmonary defects

Lung abnormalities, such as hypoplasia, retarded acinar development, emphysematous changes, or excessive muscularization of peripheral pulmonary arteries, are evident in most patients with CDH (14-16). Traditionally it was assumed that these lung defects were solely the result of external compression by herniating abdominal viscera, but it is now apparent that the pulmonary manifestations in CDH often reflect intrinsic defects in lung development. In experimental models, pulmonary hypoplasia precedes the appearance of diaphragmatic hernias, implying that there are primary disturbances in the lung developmental program (17, 18). Postmortem analysis of the lungs of infants with unilateral CDH reveals reduced numbers of bronchi, alveoli, and arteries in both the ipsilateral and contralateral lungs (19). Finally, genes implicated in development of the primordial diaphragm are often co-expressed in mesenchymal cells of the embryonic lung (Fig 2A,B). Consequently, mutations impairing

diaphragm development may have independent effects on lung morphogenesis, a process that requires coordinated interactions between endoderm-derived airway epithelial cells and mesoderm-derived mesenchymal cells (20).

C. Cardiovascular defects

A wide variety of cardiac anomalies have been associated with CDH, including atrial septal defects (ASD), ventricular septal defects (VSD), atrioventricular canal (AV canal), tetralogy of Fallot, univentricular anatomy, truncus arteriosus, and other malformations (Fig 3A-C) (16). Some of the defects are plausibly related to a defect in the field of mesenchymal cells that contribute to the heart and diaphragm. For example, the proepicardium, which arises from the septum transversum, contributes cells to the atrioventricular cushion (21), thus suggesting a common developmental basis for endocardial cushion defects and CDH. Abnormalities of the great vessels have also been linked to CDH; these include stenosis of the aorta or pulmonary arteries, aortic aneurysm, aortic tortuosity, and anomalous pulmonary venous return (Fig 3A, 4A).

D. Other birth defects

Like the diaphragm and heart, the liver is derived in part from septum transversum mesenchyme, and hepatic lesions, such as hemangioendotheliomas and hamartomas, have been observed in patients with CDH (22,23). Interestingly, CDH has also been linked to cryptorchidism and 46XY pseudohermaphroditism (24-27), which are presumed to reflect intrinsic defects in testicular somatic cell differentiation, a process that involves mesenchymal cell migration from adjoining mesonephros (28). CDH may also be accompanied by developmental eye defects (e.g., microphthalmia) (29), spinal anomalies (30), or limb-reduction defects (31).

3. Gene mutations that impair mesenchymal cell function

In an effort to explain the association of diaphragmatic and extradiaphragmatic defects, the *mesenchymal hit hypothesis* has been proposed (17,32-34). This hypothesis posits that: 1) similar signaling pathways are involved in the differentiation of mesenchymal cells in all of the affected organs; and 2) the function of mesenchymal cells in the affected organs is disrupted by genetic or environmental triggers. Consequently, a major goal of CDH research is to characterize genes and signaling pathways critical for early mesenchymal cell function during morphogenesis of the diaphragm, lungs, cardiovascular system, and testes.

Genes involved in CDH have been identified through the analysis of recurrent chromosomal abnormalities in patients with CDH and the characterization of mutant mice. The genes identified so far encode proteins that fall into two broad groups: A) transcriptional regulators, and B) factors involved in cell migration, mesodermal patterning, or components of the extracellular matrix (ECM; Table 2).

A. Transcription factors

Retrospective surveys suggest that germline mutations in transcription factor genes account for only a small percentage of cases of CDH in humans (35,36); nevertheless, these cases highlight the pivotal role of transcriptional regulation in the pathogenesis of CDH. In mice, homozygous mutations in certain transcription factor genes elicit CDH and other anomalies that result in embryonic lethality. In humans, transcription factor haploinsufficiency is an established cause of CDH, especially in cases with concomitant extradiaphragmatic birth defects. The identified transcription factors directly or indirectly regulate expression of genes critical for mesenchymal cell function in the diaphragmatic substratum as well as the lungs,

heart, and testis. Deficiency of these transcription factors leads to impaired structural integrity, increased apoptosis, and anomalous cell sorting in the tissues.

1. *WT1*—The product of the 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 in the developing kidney (37-39). Heterozygous loss-of-function mutations in *WT1* cause a series of overlapping clinical syndromes [e.g., WAGR syndrome (Wilms tumor-Aniridia-Genitourinary anomalies-Mental Retardation), Denys-Drash syndrome, Frasier syndrome, and Meacham syndrome]. Clinical features of these syndromes include genitourinary (GU) abnormalities (e.g., male pseudohermaphroditism), diaphragmatic defects, and cardiac malformations (40-42). Mice homozygous for *Wt1* deletion mutations die between E13 and E15. In addition to major GU malformations, *Wt1*^{-/-} mice exhibit Bochdalek-type diaphragmatic hernias and cardiac anomalies (abnormally developed epicardium with perturbations of the coronary vessels) (37-39,43).

2. *FOG-2*—Friend of GATA-2 (*FOG-2*) is another zinc finger transcription factor that is expressed in embryonic diaphragm, lung mesenchyme, epicardium, myocardium, and testicular somatic cells [see Fig 2A and (44,45)]. The human *FOG2* (*ZFPM2*) gene resides on chromosome 8q23, and rearrangements of 8q22-23 have been observed in patients with CDH (46-48). Patients who are heterozygous for *FOG2* loss-of-function point mutations are predisposed to diaphragm anomalies (eventration), pulmonary hypoplasia, and/or cardiac malformations (36,49). *Fog2*^{-/-} mice, generated through either targeted or chemical mutagenesis, recapitulate this spectrum of anomalies [eventration, hypoplasia of the right middle and accessory lobes of the lung, cardiac malformations (including aberrant coronary vessels), and male-to-female sex reversal (36,50,51)].

3. *GATA-4*—This zinc finger transcription factor, which interacts with *FOG-2* during embryonic development, has also been implicated in CDH and associated anomalies. The human *GATA4* gene is found on chromosome 8p23.1, and microdeletion of this region is a recurring chromosomal abnormality in patients with CDH (52-56). Like *FOG-2* and *WT-1*, *GATA-4* is expressed in mesenchymal cells of the developing diaphragm, lungs, and heart [see Fig 2B and (57)] and in testicular somatic cells (45). *Gata4*^{-/-} mice die by E9 from defects in ventral morphogenesis and heart tube fusion, precluding studies of diaphragm development in these mice (58,59). However, C57Bl/6 mice heterozygous for a *Gata4* deletion mutation are predisposed to midline diaphragmatic hernias, dilated distal airways, thickened pulmonary mesenchyme and valvuloseptal cardiac malformations [see Fig 4B-D and (57)]. Such defects are not seen in *Gata4* heterozygotes maintained on mixed genetic backgrounds (58,59), suggesting that strain-specific modifier genes impact penetrance. Confirming the importance of *GATA4-FOG2* interactions to normal organogenesis, mice homozygous for a *Gata4* missense mutation that disrupts *GATA4-FOG2* interactions develop pulmonary hypoplasia, cardiac malformations, and male-to-female sex reversal (51,60,61). Cardiac malformations have been reported in patients heterozygous for *GATA4* loss-of-function mutations (62-64); whether point mutations or small deletions in the *GATA4* gene can cause CDH or primary pulmonary defects in humans is unknown.

4. *COUP-TFII*—Chicken ovalbumin upstream promoter-transcription factor II (*COUP-TFII*) is a nuclear orphan receptor expressed during embryonic development in a variety of tissues, including mesodermal derivatives in the diaphragm, lungs, and heart (65). In CDH patients a minimally deleted region has been identified on human chromosome 15q26.1-26.2 (65-68). *COUP-TFII* (*NR2F2*) is one of the four known genes residing within this critical region, suggesting that *COUP-TFII* deficiency contributes to the formation of CDH in individuals with 15q deletions. Supporting this premise, Cre-lox mediated mutagenesis of the *Coup-TFII* gene

in the foregut mesenchyme and PPF of mice has been shown to cause Bochdalek-type CDH (65). *Coup-TFII* null mice die by E10.5 with cardiac malformations and impaired angiogenesis (69), while *Coup-TFII*^{+/-} mice have impaired gonadal somatic cell function (70). Interestingly, COUP-TFII has been shown to physically interact with FOG-2 (71), implying that these two factors may cooperate in diaphragm morphogenesis.

5. bHLH factors—Basic helix-loop-helix (bHLH) transcription factors have been implicated in diaphragm development in mice. One of these transcription factors, capsulin (POD1, epicardin), is expressed in branchial muscle, the primordial diaphragm, and in mesenchymal cells of the heart, kidney, lung, gut, spleen, and testis (72). In myoblasts and other progenitor cells, capsulin appears to function as a negative regulator of differentiation by acting as a transcriptional repressor (73). Mice homozygous for a *capsulin* null mutation die shortly after birth due to pulmonary and cardiac defects; these animals also exhibit asplenia, male-to-female sex reversal, and renal anomalies (74,75). Interestingly, mice homozygous deficient for both capsulin and the related bHLH transcription factor, MyoR, lack facial musculature and have CDH (76). Mice homozygous deficient for another bHLH factor, MyoD, exhibit abnormal muscularization of the diaphragm on a dystrophin-deficient (*mdx*) background (77). No mutation in *Capsulin*, *MyoR*, or *MyoD* has been reported in a patient with CDH.

6. Retinoic acid receptors—Disruptions in the retinoid signaling pathway during embryogenesis may contribute to the pathogenesis of CDH (7,8). Retinoic acid receptor (RAR)- α , β 2 double mutant mice exhibit CDH and heart and lung abnormalities, a constellation of defects also seen in vitamin A deficient animals (78). Similarly, teratogens that disrupt retinoic acid production, such as nitrofen, cause CDH and primary lung defects in rodents (7,8). Mutations in *STRA6*, the membrane receptor that mediates cellular uptake of vitamin A (79), cause a spectrum of anomalies including CDH, congenital heart malformations, lung hypoplasia, and anophthalmia (80).

B. Factors involved in cell-cell signaling, mesodermal patterning, or ECM biosynthesis

Proper cell sorting and guidance is critical for normal organ development. Loss-of-function mutations in genes involved in cell migration and mesodermal patterning have been shown to cause diaphragmatic and extradiaphragmatic defects in humans and animal models. These genes encode growth/guidance factors, their cognate receptors, and components of the ECM, such as elastic fibers, which serve structural functions and bind growth/differentiation factors.

1. HGF signaling pathway—Hepatocyte growth factor (HGF) is produced in the PPF, lung mesenchyme, and other mesenchymal cell types. Its receptor tyrosine kinase, c-Met, is expressed in myoblast precursors and in certain epithelial cells. In *Hgf*^{-/-} or *cMet*^{-/-} mouse embryos the diaphragmatic substratum forms normally, but its muscularization is impaired due to a defect in myoblast migration (81,82). Similarly, mouse embryos deficient in *Grab1*, a docking protein that binds to phosphorylated c-Met and facilitates intracellular signaling, lack diaphragmatic musculature (83). HGF-signaling has also been linked to testicular differentiation in the mouse (84). In *Fog2*^{-/-} mice, there is impaired expression of HGF mRNA in the PPF, suggesting that *Hgf* may be one of the target genes for this transcription factor in the developing diaphragm (36). It is unclear whether mutations in the genes encoding HGF, c-Met, and *Grab1* cause CDH or testicular defects in humans.

2. Slit2 and Robo1—An example of a ligand-receptor pair involved in cell migration is Slit3 and its receptor, Robo1. Slit proteins are secreted molecules that serve to repel axons and direct mesodermal cell migration. Slit3 is expressed in the primordial diaphragm and other tissues, and a majority of *Slit3*^{-/-} mice develop central-type diaphragmatic hernias, cardiac defects, and renal anomalies (85,86). *Robo1* encodes a surface transmembrane protein found in

developing brain, lung, heart, liver, muscle, and kidney (87). *Robo1* transcripts are alternatively spliced in a tissue-specific fashion (87). Most *Robo1*^{-/-} mice die at birth of respiratory failure, and histological examination of newborn lung reveals abnormal mesenchymal cellularity (88). A subset of the homozygotes also exhibit CDH. Recent studies suggest that heparan sulfate is an integral component of the Slit-Robo signaling complex (89). Interestingly, mutations in a heparan sulfate proteoglycan, glypican-3, cause CDH in humans (see below). To date, neither a *SLIT3* nor *ROBO1* mutation has been detected in a patient with CDH. However, duplication of the distal region of chromosome 11q, which includes the *ROBO3* and *ROBO4* genes, is associated with CDH in humans (90).

3. Ephrin-B1—Eph receptor tyrosine kinases and their ligands, ephrins, play a critical role in cell sorting during a number of developmental processes, including diaphragm morphogenesis (91). Loss-of-function mutations in the ephrin-B1 (*EFNB1*) gene cause craniofrontonasal syndrome (CFNS), an X-linked dominant disorder in which the phenotype is paradoxically more severe in females than in males (92,93). In addition to CDH, female patients with CFNS exhibit craniosynostosis, frontonasal dysplasia, and limb anomalies (94). Such birth defects are also seen in female *Efnb1*^{+/-} mice. Due to X-inactivation, *EFNB1*^{+/-} females are mosaic for cells that express the mutant allele. The presence of pools of cells that are either Ephrin-B1-positive or Ephrin-B-negative is thought to disrupt normal tissue boundary formation and impair sorting of invading receptor-positive cells (95,96).

4. Glypican-3—CDH is one of the manifestations of Simpson-Golabi-Behmel syndrome (SGBS), which, like CFNS, is an X-linked disorder that exhibits semidominant inheritance. Along with CDH, patients with SGBS exhibit macrosomia, abnormal facial features, skeletal abnormalities, cardiac malformations, and a predisposition to Wilms tumor and other embryonal tumors of childhood (97,98). SGBS is caused by loss-of-function mutations in the *glypican-3* (*GPC3*) gene, which encodes a heparan sulfate proteoglycan that is bound to the cell surface through a glycosyl-phosphatidylinositol anchor. Glypican-3 binds and thereby regulates the activity of a wide array of growth factors (e.g., fibroblast growth factors, bone morphogenic proteins), growth factor antagonists (e.g., Noggin), and guidance molecules (e.g., Slit-Robo complex) that play a critical role in morphogenesis (89,99,100).

5. Components of the Notch signaling pathway—Diaphragmatic hernias also occur in patients with spondylocostal dysostosis (SCD), a syndrome marked by aberrant mesodermal patterning, caused by defects in the Notch signaling pathway (101-104). Notch signaling mediates short range interactions involved in cell fate decision and boundary formation. Notch proteins are cell surface receptors that function as membrane-tethered transcription factors. In response to ligand binding, Notch receptors undergo proteolytic processing, allowing the transcription factor domain to translocate to the nucleus. SCD is caused by recessive mutations in the genes for Delta like-3 (a Notch ligand), Lunatic Fringe (a glycosyltransferase that modifies and thereby regulates Notch receptors), and MESP2 (a downstream transcription factor) (105-107). Mutations in another gene implicated in the Notch signaling, *NIPBL*, cause Cornelia de Lange syndrome, a dominantly inherited developmental disorder characterized by CDH, growth and mental retardation, facial anomalies, limb defects, cardiac malformations, and GU abnormalities (108).

6. Components of the ECM—Impaired formation of the ECM, caused by disruptions in either collagen or elastic fibers, can lead to developmental defects in a wide range of organs, including the diaphragm, lungs, heart, and great vessels.

a) Elastin: Mutations in the human elastin (*ELN*) gene cause supravalvular aortic stenosis (109) or autosomal dominant cutis laxa (110), a syndrome characterized by systemic

connective-tissue abnormalities, including excess inelastic skin, vascular tortuosity, aortic aneurysm, joint laxity, CDH, and emphysematous lungs. Similarly, mice harboring homozygous mutations in the *Eln* gene develop lethal obstructive arterial disease, distal airway dilatation, and other emphysematous changes (111,112).

b) Collagen: Ehlers-Danlos syndrome (EDS) includes a group of connective tissue disorders characterized by abnormal collagen metabolism. Clinical manifestations of EDS include skin hyperextensibility, joint laxity, CDH, and other anomalies. There are several subtypes of EDS which differ in terms of the severity of skin and joint findings, involvement of other tissues, and mode of inheritance. CDH has been linked to several of these subtypes, including Type IV EDS, an autosomal dominant condition characterized by pulmonary arterial stenoses, systemic arterial tortuosity, and aneurysms (113,114). Type IV EDS is caused by mutations in *COL3A1* (type III collagen), which is expressed during embryonic development in the skin, vessel walls, heart, and other organs (113,114).

c) Crosslinking enzymes: The crosslinking of collagens and elastin, which is critical for structural integrity of the ECM, is catalyzed by lysyl oxidase (Lox), an extracellular cuproenzyme. *Lox*^{-/-} mice die perinatally of aortic aneurysms and other cardiovascular abnormalities. These animals also exhibit central-type diaphragmatic hernias (115-117). No human *LOX* mutations have been reported; however, mutation of the human *ATP7A* gene, which encodes a the α -subunit of a Cu²⁺-transporter required for biosynthesis of the Lox holoenzyme, causes Menkes disease, a lethal X-linked disorder marked by skin laxity, vascular tortuosity, aortic aneurysms, and CDH (118).

d) Fibulins: The fibulins are a family of six extracellular proteins that contain series of calcium-binding EGF-like modules and a distinctive C-terminal domain. These proteins serve to link elastic fibers to the surface of cells. Mutations in human *Fibulin-4* (*FBLN4*) cause autosomal recessive cutis laxa with vascular tortuosity, aortic aneurysm, joint laxity, CDH, and emphysematous lungs (Fig 4A) (119). *Fbln4*^{-/-} mice die as neonates and exhibit dilated distal airways and severe vascular defects, such as aortic tortuosity and aneurysm (120). Mutations in human *Fibulin-5* (*FBLN5*) also cause recessive cutis laxa syndrome. These patients develop early emphysema and supraaortic stenosis (121), and *Fbln5*^{-/-} mice have a corresponding phenotype (122).

e) Fibrillin: Another ECM protein implicated in CDH is fibrillin, a major component of microfibrils in both elastic and nonelastic connective tissue. Mutations in the *fibrillin-1* gene (*FBN1*) cause Marfan syndrome, an autosomal dominant condition characterized by aortic root dilatation, tall stature, kyphoscoliosis, ocular abnormalities, joint laxity, myxomatous mitral valve defects, and congenital diaphragmatic eventration (123,124). Congenital distal airway dilatation is seen in about 10 percent of patients with Marfan syndrome, and mice that make low amounts of fibrillin-1 develop aortic root abnormalities and have emphysema-like lung abnormalities at birth (125). In the lungs, aorta, and mitral valves of these mice there is evidence of excess accumulation of the active form of transforming growth factor- β (TGF- β). Fibrillin-1 deficiency appears to alter the matrix sequestration of the large latent form of TGF- β , and this renders the cytokine more prone to activation. Remarkably, the lung, aortic, and mitral valve defects in fibrillin-1 deficient mice can be prevented by administration of either a TGF- β neutralizing antibody or the angiotensin II type 1 receptor antagonist, losartan (126,127). This demonstrates that unraveling the molecular mechanism of a gene defect can lead to a pharmacological intervention that can, in a mouse model, prevent developmental anomalies associated with CDH, namely distal airway dilatation and valvular defects.

4. Mechanisms underlying the ostensibly random combination of diaphragmatic and extradiaphragmatic defects in patients with CDH

As noted above, CDH and related birth defects occur in random combinations, but the mechanistic basis for this phenotypic variability has remained an enigma. Recent studies suggest that bimodal gene expression, triggered by haploinsufficiency for transcription factors or signaling molecules, may underlie this phenomenon.

Transcriptional regulation is commonly viewed as a “rheostatic” process in which promoter activity increases proportionally in response to rising levels of a particular transcriptional activator (128-130). Under the rheostatic model, transcription factor haploinsufficiency leads to uniform reductions in target gene levels in all mesenchymal cells. An alternative to this model is the binary model of transcriptional activation (128-130). This model implies that genes exist in either an “on” or an “off” state and that transcription factors regulate the probability of a gene occupying either state. Accordingly, transcription factor haploinsufficiency impacts the bimodal distribution of cells that either express or do not express a target gene.

The accumulation of a separate population of “non-expressing” mesenchymal cells in sensitive areas of a particular organ could trigger a focal structural abnormality, such as a diaphragmatic hernia or valvuloseptal cardiac defect. Alternatively, the presence of expressing and non-expressing cells might impair morphogenesis by disrupting signaling molecules that influence cell migration. Indeed, the bimodal population of expressing and non-expressing cells in *EFNB1*^{+/-} females (95) is reminiscent of the situation encountered under the binary model of transcription factor haploinsufficiency. Similarly, bimodal expression of an anti-apoptotic factor could be the direct pathogenic mechanism of CDH and related defects. *Gata4* heterozygosity has been associated with a small but significant increase in the number of apoptotic cells in the stressed heart and in the developing diaphragm of the mouse (57,131). One can imagine that the increased incidence yield a higher probability of a cluster of cells in the diaphragm or heart that all die, leading to a structural defect.

Another potential cause of bimodal gene expression is somatic mutagenesis. Recently, investigators have detected somatic mutations in *GATA4* and other “cardiac” transcription factors in the hearts of patients who died of congenital heart disease (132). Thus, somatic mutations that arise during cardiogenesis may be a novel molecular cause of congenital heart disease, and it is conceivable that somatic mutations in transcription factor genes contribute to the pathogenesis of CDH.

5. Future directions

Over the past decade basic research on CDH has been dominated by a teratogen-induced model of CDH (exposure of fetal rodents to nitrofen). Although the nitrofen model has improved our understanding of the early anatomical derangements associated with CDH and highlighted the importance of retinoic acid signaling in diaphragm morphogenesis (7,8), this model has provided limited insight into the genetic basis for CDH. We are entering a new era in CDH research; the characterization of chromosomal abnormalities in patients with CDH and the analysis of mutant mice have improved our understanding of the pathophysiology of this serious embryopathy.

One challenge for the future is to elucidate the hierarchy of the genes implicated in CDH [e.g., decreased expression of HGF mRNA in *Fog2*^{-/-} mice (36)]. Another challenge is to determine how environmental triggers, such as teratogen exposure or vitamin A deficiency, interact with gene mutations to cause CDH. Based on comparisons of PPF abnormalities in nitrofen-treated

rats, vitamin A deficient rats, and *Wtl*^{-/-} mice, it has been proposed that teratogen-induced, dietary, and genetic models of congenital diaphragmatic hernia share a common mechanism of pathogenesis (43).

Once the factors and pathways involved in CDH and related anomalies are delineated, it may be possible to design rational therapeutic interventions. Recent experiments showing that developmental defects of the lung and heart in a mouse model of Marfan syndrome can be prevented with anti-TGF- β therapies offer hope that we can identify transplacental therapies to prevent (or lessen the degree of) developmental defects in the diaphragm and other organs.

Acknowledgements

We thank Dr. Debra Rita for providing the CT scan. This research was supported by NIH DK52574, Mallinckrodt Foundation, Finnish Pediatric Research Foundation, and the Juselius Foundation. PYJ is a Scholar of the Child Health Research Center of Excellence in Developmental Biology at Washington University School of Medicine (K12 HD001487).

Abbreviations

ASD, atrial septal defect
 AVC, atrioventricular canal
 bHLH, basic helix-loop-helix
 CDH, congenital diaphragmatic hernia
 CFNS, craniofrontonasal syndrome
 COUP-TFII, chicken ovalbumin upstream promoter-transcription factor II
 E, embryonic day
 ECM, extracellular matrix
 EDS, Ehlers-Danlos syndrome
 EGF, epidermal growth factor
 FBLN, fibulin
 FBN, fibrillin
 FOG, friend of GATA
 HGF, hepatocyte growth factor
 GU, genitourinary
 LOX, lysyl oxidase
 PPF, pleuroperitoneal folds
 RAR, retinoic acid receptor
 SCD, spondylocostal dysostosis
 TGF- β , transforming growth factor- β
 VSD, ventricular septal defect

Reference List

1. Colvin J, Bower C, Dickinson JE, Sokol J. Outcomes of congenital diaphragmatic hernia: a population-based study in Western Australia. *Pediatrics* 2005;116:e356–e363. [PubMed: 16140678]
2. Pober BR, Lin A, Russell M, Ackerman KG, Chakravorty S, Strauss B, et al. Infants with Bochdalek diaphragmatic hernia: sibling precurrence and monozygotic twin discordance in a hospital-based malformation surveillance program. *Am J Med Genet A* 2005;138:81–8. [PubMed: 16094667]
3. Smith NP, Jesudason EC, Featherstone NC, Corbett HJ, Losty PD. Recent advances in congenital diaphragmatic hernia. *Arch Dis Child* 2005;90:426–8. [PubMed: 15781941]
4. Slavotinek AM. The genetics of congenital diaphragmatic hernia. *Semin Perinatol* 2005;29:77–85. [PubMed: 16050525]
5. Holder AM, Klaassens M, Tibboel D, de Klein A, Lee B, Scott DA. Genetic factors in congenital diaphragmatic hernia. *Am J Hum Genet.* 2007in press

6. Donahoe PK. Genomic approaches to surgical diseases: 21st annual Samuel Jason Mixter lecture. *Arch Surg* 2006;141:409–13. [PubMed: 16618902]
7. Greer JJ, Babiuk RP, Thebaud B. Etiology of congenital diaphragmatic hernia: the retinoid hypothesis. *Pediatr Res* 2003;53:726–30. [PubMed: 12621107]
8. Gallot D, Marceau G, Coste K, Hadden H, Robert-Gnansia E, Laurichesse H, et al. Congenital diaphragmatic hernia: a retinoid-signaling pathway disruption during lung development? *Birth Defects Res A Clin Mol Teratol* 2005;73:523–31. [PubMed: 15981190]
9. Benjamin DR, Juul S, Siebert JR. Congenital posterolateral diaphragmatic hernia: associated malformations. *J Pediatr Surg* 1988;23:899–903. [PubMed: 3069995]
10. van Dooren MF, Goemaere NN, de KA, Tibboel D, de Krijger RR. Postmortem findings and clinicopathological correlation in congenital diaphragmatic hernia. *Pediatr Dev Pathol* 2004;7:459–67. [PubMed: 15547770]
11. Losty PD, Vanamo K, Rintala RJ, Donahoe PK, Schnitzer JJ, Lloyd DA. Congenital diaphragmatic hernia--does the side of the defect influence the incidence of associated malformations? *J Pediatr Surg* 1998;33:507–10. [PubMed: 9537568]
12. Greer JJ, Cote D, Allan DW, Zhang W, Babiuk RP, Ly L, et al. Structure of the primordial diaphragm and defects associated with nitrofen-induced CDH. *J Appl Physiol* 2000;89:2123–9. [PubMed: 11090558]
13. Babiuk RP, Greer JJ. Diaphragm defects occur in a CDH hernia model independently of myogenesis and lung formation. *Am J Physiol Lung Cell Mol Physiol* 2002;283:L1310–L1314. [PubMed: 12388344]
14. Migliazza L, Otten C, Xia H, Rodriguez JI, ez-Pardo JA, Tovar JA. Cardiovascular malformations in congenital diaphragmatic hernia: human and experimental studies. *J Pediatr Surg* 1999;34:1352–8. [PubMed: 10507428]
15. Losty PD, Connell MG, Freese R, Laval S, Okoye BO, Smith A, et al. Cardiovascular malformations in experimental congenital diaphragmatic hernia. *J Pediatr Surg* 1999;34:1203–7. [PubMed: 10466596]
16. Graziano JN. Cardiac anomalies in patients with congenital diaphragmatic hernia and their prognosis: a report from the Congenital Diaphragmatic Hernia Study Group. *J Pediatr Surg* 2005;40:1045–9. [PubMed: 15991194]
17. Keijzer R, Liu J, Deimling J, Tibboel D, Post M. Dual-hit hypothesis explains pulmonary hypoplasia in the nitrofen model of congenital diaphragmatic hernia. *Am J Pathol* 2000;156:1299–306. [PubMed: 10751355]
18. Jesudason EC, Connell MG, Fernig DG, Lloyd DA, Losty PD. Early lung malformations in congenital diaphragmatic hernia. *J Pediatr Surg* 2000;35:124–7. [PubMed: 10646789]
19. Hislop A, Reid L. Persistent hypoplasia of the lung after repair of congenital diaphragmatic hernia. *Thorax* 1976;31:450–5. [PubMed: 968803]
20. Shannon JM, Hyatt BA. Epithelial-mesenchymal interactions in the developing lung. *Annu Rev Physiol* 2004;66:625–45. [PubMed: 14977416]
21. Gittenberger-de Groot AC, Vrancken Peeters MP, Mentink MM, Gourdie RG, Poelmann RE. Epicardium-derived cells contribute a novel population to the myocardial wall and the atrioventricular cushions. *Circ Res* 1998;82:1043–52. [PubMed: 9622157]
22. Shah KD, Beck AR, Jhaveri MK, Keohane M, Weinberg B, Gerber MA. Infantile hemangioendothelioma of heterotopic intrathoracic liver associated with diaphragmatic hernia. *Hum Pathol* 1987;18:754–6. [PubMed: 3596593]
23. Chu DY, Olson AL, Mishalany HG. Congenital liver cyst presenting as congenital diaphragmatic hernia. *J Pediatr Surg* 1986;21:897–9. [PubMed: 3783379]
24. Meacham LR, Winn KJ, Culler FL, Parks JS. Double vagina, cardiac, pulmonary, and other genital malformations with 46,XY karyotype. *Am J Med Genet* 1991;41:478–81. [PubMed: 1844355]
25. Killeen OG, Kelehan P, Reardon W. Double vagina with sex reversal, congenital diaphragmatic hernia, pulmonary and cardiac malformations--another case of Meacham syndrome. *Clin Dysmorphol* 2002;11:25–8. [PubMed: 11822701]

26. Manouvrier-Hanu S, Besson R, Cousin L, Jeanpierre C, Kacet N, Cartigny M, et al. Sex reversal and diaphragmatic hernia in phenotypically female sibs with normal XY chromosomes. *J Med Genet* 2000;37:315–8. [PubMed: 10819644]
27. Kent A, Simpson E, Ellwood D, Silink M. 46,XY sex-reversal (Swyer syndrome) and congenital diaphragmatic hernia. *Am J Med Genet A* 2004;131:103–5. [PubMed: 15389702]
28. Brennan J, Capel B. One tissue, two fates: molecular genetic events that underlie testis versus ovary development. *Nat Rev Genet* 2004;5:509–21. [PubMed: 15211353]
29. Steiner RD, Dignan PSJ, Hopkin RJ, Kozielski R, Bove KE. Combination of diaphragmatic eventration and microphthalmia/anophthalmia is probably nonrandom. *Am J Med Genet* 2002;108:45–50. [PubMed: 11857549]
30. Grethel EJ, Farrell J, Ball R, Lee H, Nobuhara KK. Congenital diaphragmatic hernia associated with spinal anomalies. *Obstet Gynecol* 2007;109:485–8. [PubMed: 17267867]
31. van Dooren MF, Brooks AS, Tibboel D, Torfs CP. Association of congenital diaphragmatic hernia with limb-reduction defects. *Birth Defects Res A Clin Mol Teratol* 2003;67:578–84. [PubMed: 14632307]
32. Jesudason EC. Small lungs and suspect smooth muscle: congenital diaphragmatic hernia and the smooth muscle hypothesis. *J Pediatr Surg* 2006;41:431–5. [PubMed: 16481264]
33. Jesudason EC, Smith NP, Connell MG, Spiller DG, White MR, Fernig DG, et al. Peristalsis of airway smooth muscle is developmentally regulated and uncoupled from hypoplastic lung growth. *Am J Physiol Lung Cell Mol Physiol* 2006;291:L559–L565. [PubMed: 16603591]
34. Featherstone NC, Connell MG, Fernig DG, Wray S, Burdya TV, Losty PD, et al. Airway smooth muscle dysfunction precedes teratogenic congenital diaphragmatic hernia and may contribute to hypoplastic lung morphogenesis. *Am J Respir Cell Mol Biol* 2006;35:571–8. [PubMed: 16728706]
35. Nordenskjold A, Tapper-Persson M, Anvret M. No evidence of WT1 gene mutations in children with congenital diaphragmatic hernia. *J Pediatr Surg* 1996;31:925–7. [PubMed: 8811558]
36. Ackerman KG, Herron BJ, Vargas SO, Huang H, Tevosian SG, Kochilas L, et al. *Fog2* is required for normal diaphragm and lung development in mice and humans. *PLoS Genet* 2005;1:58–65. [PubMed: 16103912]
37. Kreidberg JA, Sariola H, Loring JM, Maeda M, Pelletia J, Housman J, et al. WT-1 is required for early kidney development. *Cell* 1993;74:679–91. [PubMed: 8395349]
38. Natoli TA, Alberta JA, Bortvin A, Taglienti ME, Menke DB, Loring J, et al. *Wt1* functions in the development of germ cells in addition to somatic cell lineages of the testis. *Dev Biol* 2004;268:429–40. [PubMed: 15063178]
39. Moore AW, McInnes L, Kreidberg J, Hastie ND, Schedl A. YAC complementation shows a requirement for *Wt1* in the development of epicardium, adrenal gland and throughout nephrogenesis. *Development* 1999;126:1845–57. [PubMed: 10101119]
40. Scott DA, Cooper ML, Stankiewicz P, Patel A, Potocki L, Cheung SW. Congenital diaphragmatic hernia in WAGR syndrome. *Am J Med Genet A* 2005;134:430–3. [PubMed: 15779010]
41. Devriendt K, Deloof E, Moerman P, Legius E, Vanhole C, de ZF, et al. Diaphragmatic hernia in Denys-Drash syndrome. *Am J Med Genet* 1995;57:97–101. [PubMed: 7645607]
42. Cho HY, Lee BS, Kang CH, Kim WH, Ha IS, Cheong HI, et al. Hydrothorax in a patient with Denys-Drash syndrome associated with a diaphragmatic defect. *Pediatr Nephrol*. 2006
43. Clugston RD, Klattig J, Englert C, Clagett-Dame M, Martinovic J, Benachi A, et al. Teratogen-induced, dietary and genetic models of congenital diaphragmatic hernia share a common mechanism of pathogenesis. *Am J Pathol* 2006;169:1541–9. [PubMed: 17071579]
44. Tevosian SG, Deconinck AE, Cantor AB, Rieff HI, Fujiwara Y, Corfas G, et al. FOG-2: A novel GATA-family cofactor related to multitype zinc-finger proteins friend of GATA-1 and U-shaped. *Proc Natl Acad Sci U S A* 1999;96:950–5. [PubMed: 9927674]
45. Ketola I, Anttonen M, Vaskivuo T, Tapanainen JS, Toppari J, Heikinheimo M. Developmental expression and spermatogenic stage specificity of transcription factors GATA-1 and GATA-4 and their cofactors FOG-1 and FOG-2 in the mouse testis. *Eur J Endocrinol* 2002;147:397–406. [PubMed: 12213678]
46. Temple IK, Barber JC, James RS, Burge D. Diaphragmatic herniae and translocations involving 8q22 in two patients. *J Med Genet* 1994;31:735–7. [PubMed: 7815446]

47. Wilson WG, Wyandt HE, Shah H. Interstitial deletion of 8q. Occurrence in a patient with multiple exostoses and unusual facies. *Am J Dis Child* 1983;137:444–8. [PubMed: 6601906]
48. Howe DT, Kilby MD, Sirry H, Barker GM, Roberts E, Davison EV, et al. Structural chromosome anomalies in congenital diaphragmatic hernia. *Prenat Diagn* 1996;16:1003–9. [PubMed: 8953633]
49. Pizzuti A, Sarkozy A, Newton AL, Conti E, Flex E, Digilio MC, et al. Mutations of ZFPM2/FOG2 gene in sporadic cases of tetralogy of Fallot. *Hum Mutat* 2003;22:372–7. [PubMed: 14517948]
50. Tevosian SG, Deconinck AE, Tanaka M, Schinke M, Litovsky SH, Izumo S, et al. FOG-2, a cofactor for GATA transcription factors, is essential for heart morphogenesis and development of coronary vessels from epicardium. *Cell* 2000;101:729–39. [PubMed: 10892744]
51. Tevosian SG, Albrecht KH, Crispino JD, Fujiwara Y, Eicher EM, Orkin SH. Gonadal differentiation, sex determination and normal Sry expression in mice require direct interaction between transcription partners GATA4 and FOG2. *Development* 2002;129:4627–34. [PubMed: 12223418]
52. Barber JC, Maloney V, Hollox EJ, Stuke-Sontheimer A, du BG, Daumiller E, et al. Duplications and copy number variants of 8p23.1 are cytogenetically indistinguishable but distinct at the molecular level. *Eur J Hum Genet* 2005;13:1131–6. [PubMed: 16077733]
53. Shimokawa O, Miyake N, Yoshimura T, Sosonkina N, Harada N, Mizuguchi T, et al. Molecular characterization of del(8)(p23.1p23.1) in a case of congenital diaphragmatic hernia. *Am J Med Genet A* 2005;136:49–51. [PubMed: 15937941]
54. Faivre L, Morichon-Delvallez N, Viot G, Narcy F, Loison S, Mandelbrot L, et al. Prenatal diagnosis of an 8p23.1 deletion in a fetus with a diaphragmatic hernia and review of the literature. *Prenat Diagn* 1998;18:1055–60. [PubMed: 9826897]
55. Pecile V, Petroni MG, Fertz MC, Filippi G. Deficiency of distal 8p-: report of two cases and review of the literature. *Clinical Genetics* 1990;38:271–8. [PubMed: 2190718]
56. Lurie IW. Where to look for the genes related to diaphragmatic hernia? *Genet Couns* 2003;14:75–93. [PubMed: 12725592]
57. Jay PY, Bielinska M, Erlich JM, Mannisto S, Pu WT, Heikinheimo M, et al. Impaired mesenchymal cell function in *Gata4* mutant mice leads to diaphragmatic hernias and primary lung defects. *Dev Biol* 2007;301:602–14. [PubMed: 17069789]
58. Molkentin JD, Lin Q, Duncan SA, Olson EN. Requirement of the transcription factor GATA4 for heart tube formation and ventral morphogenesis. *Genes Dev* 1997;11:1061–72. [PubMed: 9136933]
59. Kuo CT, Morrissey EE, Anadappa R, Sigris K, Lu MM, Parmacek MS, et al. GATA4 transcription factor is required for ventral morphogenesis and heart tube formation. *Genes Dev* 1997;11:1048–60. [PubMed: 9136932]
60. Crispino JD, Lodish MB, Thurberg BL, Litovsky SH, Collins T, Molkentin JD, et al. Proper coronary vascular development and heart morphogenesis depend on interaction of GATA-4 with FOG cofactors. *Genes Dev* 2001;15:839–44. [PubMed: 11297508]
61. Ackerman KG, Wang J, Luo LL, Fujiwara Y, Orkin SH, Beier DR. *Gata4* is necessary for normal pulmonary lobar development. *Am J Respir Cell Mol Biol*. 2007in press
62. Garg V, Kathiriyi IS, Barnes R, Schluterman MK, King IN, Butler CA, et al. *GATA4* mutations cause human congenital heart defects and reveal an interaction with TBX5. *Nature* 2003;424:443–7. [PubMed: 12845333]
63. Okubo A, Miyoshi O, Baba K, Takagi M, Tsukamoto K, Kinoshita A, et al. A novel GATA4 mutation completely segregated with atrial septal defect in a large Japanese family. *J Med Genet* 2004;41:e97. [PubMed: 15235040]
64. Nemer G, Fadlalah F, Usta J, Nemer M, Dbaibo G, Obeid M, et al. A novel mutation in the *GATA4* gene in patients with Tetralogy of Fallot. *Hum Mutat* 2006;27:293–4. [PubMed: 16470721]
65. You LR, Takamoto N, Yu CT, Tanaka T, Kodama T, DeMayo FJ, et al. Mouse lacking COUP-TFII as an animal model of Bochdalek-type congenital diaphragmatic hernia. *Proc Natl Acad Sci U S A* 2005;102:16351–6. [PubMed: 16251273]
66. Klaassens M, van DM, Eussen HJ, Douben H, den Dekker AT, Lee C, et al. Congenital diaphragmatic hernia and chromosome 15q26: determination of a candidate region by use of fluorescent in situ hybridization and array-based comparative genomic hybridization. *Am J Hum Genet* 2005;76:877–82. [PubMed: 15750894]

67. Castiglia L, Fichera M, Romano C, Galesi O, Grillo L, Sturnio M, et al. Narrowing the candidate region for congenital diaphragmatic hernia in chromosome 15q26: contradictory results. *Am J Hum Genet* 2005;77:892–4. [PubMed: 16252246]
68. Slavotinek A, Lee SS, Davis R, Shrit A, Leppig KA, Rhim J, et al. Fryns syndrome phenotype caused by chromosome microdeletions at 15q26.2 and 8p23.1. *J Med Genet* 2005;42:730–6. [PubMed: 16141010]
69. Pereira FA, Qiu Y, Zhou G, Tsai MJ, Tsai SY. The orphan nuclear receptor COUP-TFII is required for angiogenesis and heart development. *Genes Dev* 1999;13:1037–49. [PubMed: 10215630]
70. Takamoto N, Kurihara I, Lee K, DeMayo FJ, Tsai MJ, Tsai SY. Haploinsufficiency of chicken ovalbumin upstream promoter transcription factor II in female reproduction. *Mol Endocrinol* 2005;19:2299–308. [PubMed: 15890675]
71. Huggins GS, Bacani CJ, Boltax J, Aikawa R, Leiden JM. Friend of GATA 2 physically interacts with chicken ovalbumin upstream promoter-TF2 (COUP-TF2) and COUP-TF3 and represses COUP-TF2-dependent activation of the atrial natriuretic factor promoter. *J Biol Chem* 2001;276:28029–36. [PubMed: 11382775]
72. Lu J, Richardson JA, Olson EN. Capsulin: a novel bHLH transcription factor expressed in epicardial progenitors and mesenchyme of visceral organs. *Mech Dev* 1998;73:23–32. [PubMed: 9545521]
73. Funato N, Ohyama K, Kuroda T, Nakamura M. Basic helix-loop-helix transcription factor epicardin/capsulin/Pod-1 suppresses differentiation by negative regulation of transcription. *J Biol Chem* 2003;278:7486–93. [PubMed: 12493738]
74. Cui S, Ross A, Stallings N, Parker KL, Capel B, Quaggin SE. Disrupted gonadogenesis and male-to-female sex reversal in *Pod1* knockout mice. *Development* 2004;131:4095–105. [PubMed: 15289436]
75. Lu J, Chang P, Richardson JA, Gan L, Weiler H, Olson EN. The basic helix-loop-helix transcription factor capsulin controls spleen organogenesis. *Proc Natl Acad Sci U S A* 2000;97:9525–30. [PubMed: 10944221]
76. Lu JR, Bassel-Duby R, Hawkins A, Chang P, Valdez R, Wu H, et al. Control of facial muscle development by MyoR and capsulin. *Science* 2002;298:2378–81. [PubMed: 12493912]
77. Inanlou MR, Dhillon GS, Belliveau AC, Reid GA, Ying C, Rudnicki MA, et al. A significant reduction of the diaphragm in *mdx:MyoD*–/–(9th) embryos suggests a role for MyoD in the diaphragm development. *Dev Biol* 2003;261:324–36. [PubMed: 14499644]
78. Mendelsohn C, Lohnes D, Decimo D, Lufkin T, LeMeur M, Chambon P, et al. Function of the retinoic acid receptors (RARs) during development (II). Multiple abnormalities at various stages of organogenesis in RAR double mutants. *Development* 1994;120:2749–71. [PubMed: 7607068]
79. Kawaguchi R, Yu J, Honda J, Hu J, Whitelegge J, Ping P, et al. A membrane receptor for retinol binding protein mediates cellular uptake of vitamin A. *Science*. 2007
80. Pasutto F, Sticht H, Hammersen G, Gillesen-Kaesbach G, Fitzpatrick DR, Nurnberg G, et al. Mutations in STRA6 Cause a Broad Spectrum of Malformations Including Anophthalmia, Congenital Heart Defects, Diaphragmatic Hernia, Alveolar Capillary Dysplasia, Lung Hypoplasia, and Mental Retardation. *Am J Hum Genet* 2007;80:550–60. [PubMed: 17273977]
81. Bladt F, Riethmacher D, Isenmann S, Aguzzi A, Birchmeier C. Essential role for the c-met receptor in the migration of myogenic precursor cells into the limb bud. *Nature* 1995;376:768–71. [PubMed: 7651534]
82. Dietrich S, bou-Rebyeh F, Brohmann H, Bladt F, Sonnenberg-Riethmacher E, Yamaai T, et al. The role of SF/HGF and c-Met in the development of skeletal muscle. *Development* 1999;126:1621–9. [PubMed: 10079225]
83. Sachs M, Brohmann H, Zechner D, Muller T, Hulsken J, Walther I, et al. Essential role of Gab1 for signaling by the c-Met receptor in vivo. *J Cell Biol* 2000;150:1375–84. [PubMed: 10995442]
84. Ricci G, Catizone A, Innocenzi A, Galdieri M. Hepatocyte growth factor (HGF) receptor expression and role of HGF during embryonic mouse testis development. *Dev Biol* 1999;216:340–7. [PubMed: 10588883]
85. Liu J, Zhang L, Wang D, Shen H, Jiang M, Mei P, et al. Congenital diaphragmatic hernia, kidney agenesis and cardiac defects associated with Slit3-deficiency in mice. *Mech Dev* 2003;120:1059–70. [PubMed: 14550534]

86. Yuan W, Rao Y, Babiuk RP, Greer JJ, Wu JY, Ornitz DM. A genetic model for a central (septum transversum) congenital diaphragmatic hernia in mice lacking Slit3. *Proc Natl Acad Sci U S A* 2003;100:5217–22. [PubMed: 12702769]
87. Clark K, Hammond E, Rabbitts P. Temporal and spatial expression of two isoforms of the Dutt1/Robo1 gene in mouse development. *FEBS Lett* 2002;523:12–6. [PubMed: 12123796]
88. Xian J, Clark KJ, Fordham R, Pannell R, Rabbitts TH, Rabbitts PH. Inadequate lung development and bronchial hyperplasia in mice with a targeted deletion in the Dutt1/Robo1 gene. *Proc Natl Acad Sci U S A* 2001;98:15062–6. [PubMed: 11734623]
89. Hussain SA, Piper M, Fukuhara N, Strohlic L, Cho G, Howitt JA, et al. A molecular mechanism for the heparan sulfate dependence of slit-robo signaling. *J Biol Chem* 2006;281:39693–8. [PubMed: 17062560]
90. Klaassens M, Scott DA, van DM, Hochstenbach R, Eussen HJ, Cai WW, et al. Congenital diaphragmatic hernia associated with duplication of 11q23-qter. *Am J Med Genet A* 2006;140:1580–6. [PubMed: 16770801]
91. Davy A, Soriano P. Ephrin signaling in vivo: look both ways. *Dev Dyn* 2005;232:1–10. [PubMed: 15580616]
92. Vasudevan PC, Twigg SR, Mulliken JB, Cook JA, Quarrell OW, Wilkie AO. Expanding the phenotype of craniofrontonasal syndrome: two unrelated boys with *EFNB1* mutations and congenital diaphragmatic hernia. *Eur J Hum Genet* 2006;14:884–7. [PubMed: 16639408]
93. Morris CA, Palumbos JC, Carey JC. Delineation of the male phenotype in craniofrontonasal syndrome. *Am J Med Genet* 1987;27:623–31. [PubMed: 3631134]
94. Twigg SR, Kan R, Babbs C, Bochukova EG, Robertson SP, Wall SA, et al. Mutations of ephrin-B1 (*EFNB1*), a marker of tissue boundary formation, cause craniofrontonasal syndrome. *Proc Natl Acad Sci U S A* 2004;101:8652–7. [PubMed: 15166289]
95. Twigg SR, Matsumoto K, Kidd AM, Goriely A, Taylor IB, Fisher RB, et al. The origin of *EFNB1* mutations in craniofrontonasal syndrome: frequent somatic mosaicism and explanation of the paucity of carrier males. *Am J Hum Genet* 2006;78:999–1010. [PubMed: 16685650]
96. Compagni A, Logan M, Klein R, Adams RH. Control of skeletal patterning by ephrinB1-EphB interactions. *Dev Cell* 2003;5:217–30. [PubMed: 12919674]
97. DeBaun MR, Ess J, Saunders S. Simpson Golabi Behmel syndrome: progress toward understanding the molecular basis for overgrowth, malformation, and cancer predisposition. *Mol Genet Metab* 2001;72:279–86. [PubMed: 11286501]
98. Enns GM, Cox VA, Goldstein RB, Gibbs DL, Harrison MR, Golabi M. Congenital diaphragmatic defects and associated syndromes, malformations, and chromosome anomalies: a retrospective study of 60 patients and literature review. *Am J Med Genet* 1998;79:215–25. [PubMed: 9788565]
99. Paine-Saunders S, Viviano BL, Zupicich J, Skarnes WC, Saunders S. glypican-3 controls cellular responses to Bmp4 in limb patterning and skeletal development. *Dev Biol* 2000;225:179–87. [PubMed: 10964473]
100. Paine-Saunders S, Viviano BL, Economides AN, Saunders S. Heparan sulfate proteoglycans retain Noggin at the cell surface: a potential mechanism for shaping bone morphogenetic protein gradients. *J Biol Chem* 2002;277:2089–96. [PubMed: 11706034]
101. Sparrow DB, Clements M, Withington SL, Scott AN, Novotny J, Sillence D, et al. Diverse requirements for Notch signalling in mammals. *Int J Dev Biol* 2002;46:365–74. [PubMed: 12141422]
102. Martinez-Frias ML, Bermejo E, Paisan L, Martin M, Egues J, Lopez JA, et al. Severe spondylocostal dysostosis associated with other congenital anomalies: a clinical/epidemiologic analysis and description of ten cases from the Spanish registry. *Am J Med Genet* 1994;51:203–12. [PubMed: 8074145]
103. Lam YH, Eik-Nes SH, Tang MH, Lee CP, Nicholls JM. Prenatal sonographic features of spondylocostal dysostosis and diaphragmatic hernia in the first trimester. *Ultrasound Obstet Gynecol* 1999;13:213–5. [PubMed: 10204217]
104. Day R, Fryer A. Diaphragmatic hernia and preaxial polydactyly in spondylothoracic dysplasia. *Clin Dysmorphol* 2003;12:277–8. [PubMed: 14564219]

105. Sparrow DB, Chapman G, Wouters MA, Whittock NV, Ellard S, Fatkin D, et al. Mutation of the LUNATIC FRINGE gene in humans causes spondylocostal dysostosis with a severe vertebral phenotype. *Am J Hum Genet* 2006;78:28–37. [PubMed: 16385447]
106. Dunwoodie SL, Clements M, Sparrow DB, Sa X, Conlon RA, Beddington RS. Axial skeletal defects caused by mutation in the spondylocostal dysplasia/pudgy gene *Dll3* are associated with disruption of the segmentation clock within the presomitic mesoderm. *Development* 2002;129:1795–806. [PubMed: 11923214]
107. Whittock NV, Sparrow DB, Wouters MA, Sillence D, Ellard S, Dunwoodie SL, et al. Mutated *MESP2* causes spondylocostal dysostosis in humans. *Am J Hum Genet* 2004;74:1249–54. [PubMed: 15122512]
108. Krantz ID, McCallum J, DeScipio C, Kaur M, Gillis LA, Yaeger D, et al. Cornelia de Lange syndrome is caused by mutations in *NIPBL*, the human homolog of *Drosophila melanogaster* Nipped-B. *Nat Genet* 2004;36:631–5. [PubMed: 15146186]
109. Urban Z, Zhang J, Davis EC, Maeda GK, Kumar A, Stalker H, et al. Supravalvular aortic stenosis: genetic and molecular dissection of a complex mutation in the elastin gene. *Hum Genet* 2001;109:512–20. [PubMed: 11735026]
110. Szabo Z, Crepeau MW, Mitchell AL, Stephan MJ, Puntel RA, Yin LK, et al. Aortic aneurysmal disease and cutis laxa caused by defects in the elastin gene. *J Med Genet* 2006;43:255–8. [PubMed: 16085695]
111. Li DY, Brooke B, Davis EC, Mecham RP, Sorensen LK, Boak BB, et al. Elastin is an essential determinant of arterial morphogenesis. *Nature* 1998;393:276–80. [PubMed: 9607766]
112. Wendel DP, Taylor DG, Albertine KH, Keating MT, Li DY. Impaired distal airway development in mice lacking elastin. *Am J Respir Cell Mol Biol* 2000;23:320–6. [PubMed: 10970822]
113. Abdul WA, Janahi IA, Eltohami A, Zeid A, Ul Haque NF, Teebi AS. A new type of Ehlers-Danlos syndrome associated with tortuous systemic arteries in a large kindred from Qatar. *Acta Paediatr* 2003;92:456–62. [PubMed: 12801113]
114. Lin IC, Ko SF, Shieh CS, Huang CF, Chien SJ, Liang CD. Recurrent congenital diaphragmatic hernia in ehlers-danlos syndrome. *Cardiovasc Intervent Radiol* 2006;29:920–3. [PubMed: 16447004]
115. Hornstra IK, Birge S, Starcher B, Bailey AJ, Mecham RP, Shapiro SD. Lysyl oxidase is required for vascular and diaphragmatic development in mice. *J Biol Chem* 2003;278:14387–93. [PubMed: 12473682]
116. Mäki JM, Räsänen J, Tikkanen H, Sormunen R, Mäkilä K, Kivirikko KI, et al. Inactivation of the lysyl oxidase gene *Lox* leads to aortic aneurysms, cardiovascular dysfunction, and perinatal death in mice. *Circulation* 2002;106:2503–9. [PubMed: 12417550]
117. Mäki JM, Sormunen R, Lippo S, Kaarteenaho-Wiik R, Soininen R, Myllyharju J. Lysyl oxidase is essential for normal development and function of the respiratory system and for the integrity of elastic and collagen fibers in various tissues. *Am J Pathol* 2005;167:927–36. [PubMed: 16192629]
118. Schaefer M, Gitlin JD. Genetic disorders of membrane transport. IV. Wilson's disease and Menkes disease. *Am J Physiol* 1999;276:G311–G314. [PubMed: 9950803]
119. Huchtagowder V, Sausgruber N, Kim KH, Angle B, Marmorstein LY, Urban Z. Fibulin-4: a novel gene for an autosomal recessive cutis laxa syndrome. *Am J Hum Genet* 2006;78:1075–80. [PubMed: 16685658]
120. McLaughlin PJ, Chen Q, Horiguchi M, Starcher BC, Stanton JB, Broekelmann TJ, et al. Targeted disruption of fibulin-4 abolishes elastogenesis and causes perinatal lethality in mice. *Mol Cell Biol* 2006;26:1700–9. [PubMed: 16478991]
121. Loeys B, Van ML, Mortier G, Coucke P, Gerniers S, Naeyaert JM, et al. Homozygosity for a missense mutation in fibulin-5 (*FBLN5*) results in a severe form of cutis laxa. *Hum Mol Genet* 2002;11:2113–8. [PubMed: 12189163]
122. Yanagisawa H, Davis EC, Starcher BC, Ouchi T, Yanagisawa M, Richardson JA, et al. Fibulin-5 is an elastin-binding protein essential for elastic fibre development in vivo. *Nature* 2002;415:168–71. [PubMed: 11805834]
123. Robinson PN, rteaga-Solis E, Baldock C, Collod-Beroud G, Booms P, De PA. The molecular genetics of Marfan syndrome and related disorders. *J Med Genet*. 2006

124. Revencu N, Quenum G, Detaille T, Verellen G, De PA, Verellen-Dumoulin C. Congenital diaphragmatic eventration and bilateral uretero-hydronephrosis in a patient with neonatal Marfan syndrome caused by a mutation in exon 25 of the FBN1 gene and review of the literature. *Eur J Pediatr* 2004;163:33–7. [PubMed: 14586646]
125. Neptune ER, Frischmeyer PA, Arking DE, Myers L, Bunton TE, Gayraud B, et al. Dysregulation of TGF-beta activation contributes to pathogenesis in Marfan syndrome. *Nat Genet* 2003;33:407–11. [PubMed: 12598898]
126. Habashi JP, Judge DP, Holm TM, Cohn RD, Loeys BL, Cooper TK, et al. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. *Science* 2006;312:117–21. [PubMed: 16601194]
127. Ng CM, Cheng A, Myers LA, Martinez-Murillo F, Jie C, Bedja D, et al. TGF-beta-dependent pathogenesis of mitral valve prolapse in a mouse model of Marfan syndrome. *J Clin Invest* 2004;114:1586–92. [PubMed: 15546004]
128. Fiering S, Whitelaw E, Martin DI. To be or not to be active: the stochastic nature of enhancer action. *BioEssays* 2000;22:381–7. [PubMed: 10723035]
129. Magee JA, Abdulkadir SA, Milbrandt J. Haploinsufficiency at the Nkx3.1 locus. A paradigm for stochastic, dosage-sensitive gene regulation during tumor initiation. *Cancer Cell* 2003;3:273–83. [PubMed: 12676585]
130. Cook DL, Gerber AN, Tapscott SJ. Modeling stochastic gene expression: implications for haploinsufficiency. *Proc Natl Acad Sci U S A* 1998;95:15641–6. [PubMed: 9861023]
131. Bisping E, Ikeda S, Kong SW, Tarnavski O, Bodyak N, McMullen JR, et al. *Gata4* is required for maintenance of postnatal cardiac function and protection from pressure overload-induced heart failure. *Proc Natl Acad Sci U S A* 2006;103:14471–6. [PubMed: 16983087]
132. Reamon-Buettner SM, Spanel-Borowski K, Borlak J. Bridging the gap between anatomy and molecular genetics for an improved understanding of congenital heart disease. *Ann Anat* 2006;188:213–20. [PubMed: 16711160]

Figure 1.

A) Embryonic structures contributing to the primordial diaphragm. The diaphragm forms through amalgamation of the septum transversum, foregut mesentery, pleuroperitoneal folds, and body wall. Abbreviations: IVC (inferior vena cava), PPF (pleuroperitoneal folds). **B)** Spectrum of cell types derived from septum transversum mesenchyme. A functional defect in mesenchymal progenitors in the septum transversum could impair development of the diaphragm, heart, and/or liver. This may, in part, account for the association of CDH with malformations of the heart and liver. **C)** Chest radiograph of a newborn with a left-sided, Bochdalek-type CDH. Air-filled loops of bowel (arrows) herniate into the left chest and displace the heart into the right.

Figure 2. Genes implicated in diaphragm morphogenesis are expressed concomitantly in mesenchymal cells of the lungs and heart

Adjacent tissue sections from E11.5 wild-type mice were subjected to immunoperoxidase staining with antibodies against FOG-2 (A) or GATA-4 (B) and then counterstained with toluidine blue. Note that FOG-2 and GATA-4 are co-expressed in the central tendon of the diaphragm, lung mesenchyme, and the heart. Abbreviations: ct, central tendon; ht, heart; lm, lung mesenchyme. Bars, 75 μ m.

Figure 3.

A) Partial anomalous pulmonary venous return in a patient with CDH and a complete, unbalanced AV canal defect. In this angiogram contrast was injected into the pulmonary circulation via a Blalock-Taussig shunt. Levophase shows blood returning from the left upper pulmonary vein (LUPV) to the systemic innominate vein (IV) and superior vena cava (SVC). The left lower pulmonary veins (LLPV) drain normally to the left atrium. B, C) Echocardiography of a rare combination of heart defects in a newborn with CDH. Tricuspid atresia is shown by the thick, fibrous plate between the right atrium and ventricle (panel B). The child also had truncus arteriosus in which a single arterial valve and vessel give rise to the pulmonary artery and aorta (panel C) and an interrupted aortic arch (not shown).

Abbreviations: Ao, aorta; RA, right atrium; RV, right ventricle; LA, left atrium; LV, left ventricle; TR, truncal valve; TV, tricuspid valve, atretic.

Figure 4. Examples of extradiaphragmatic defects associated with CDH in humans and mice with known gene defects

A) Computed tomography of a girl with a homozygous E57K mutation in the *fibulin-4* gene. Note the tortuous aorta and elevated left hemidiaphragm. At 10 months of age she underwent diaphragmatic plication; surgical observations included arterial tortuosity, emphysematous lungs, and scant diaphragmatic musculature. **B)** H&E stained section of right lung from a 1-day-old C57Bl/6 mouse heterozygous for a *Gata4* deletion mutation (*Gata4*^{+/-}). Note the enlarged distal airspaces and thickened mesenchyme, presumed to represent a primary defect in lung development. Bar, 200 μ m. **C,D)** H&E stained sections of hearts from two 1-day-old C57Bl/6 *Gata4*^{+/-} mice. Panel C shows a large membranous ventricular septal defect (VSD)

with secundum atrial septal defect (ASD). Panel D shows a transitional atrioventricular canal (primum ASD, cleft mitral valve, inlet VSD). Bars, 300 μ m. Other abbreviations: Ao, aorta; AoV, aortic valve; MV, mitral valve; R, right atrium; RV, right ventricle; LA, left atrium; LV, left ventricle; TV, tricuspid valve.

Table 1

Birth defects associated with CDH

<u>Lungs and pleura</u>
Airway dilatation and alveolarization defects
Congenital cystic adenomatoid malformation
Hypoplasia (primary and secondary)
Persistent pulmonary hypertension and associated vascular changes
Pleural defects
Pulmonary sequestration
<u>Heart, pericardium, and great vessels</u>
Arterial stenosis, tortuosity, or aneurysms
ASD, VSD, and AVC defects
Hypoplastic ventricles
Pericardial defects (including agenesis)
<u>Gastrointestinal tract</u>
Aplasia of liver lobes
Accessory liver lobe
Hepatic hemangioendothelioma or hamartoma
Liver cysts
Malrotation
Tracheo-esophageal fistula or esophageal atresia
<u>GU tract</u>
Bicornuate uterus
Cryptorchidism
Hypospadias
Male-to-female sex reversal

Table 2
Genes implicated in CDH in humans or mice

<u>Transcription factors</u>
WT1
FOG2
GATA4
COUP-TFII
Capsulin + MyoR
MyoD on an <i>mdx</i> background
RAR- α + RAR- β
<u>Molecules involved in cell migration, mesodermal patterning, or ECM biosynthesis</u>
HGF, its receptor c-Met, and the docking protein Grab1
Slit3 and its receptor Robo1
Ephrin-B1
Glypican-3
Delta-like 3, Lunatic Fringe, and others factors in the Notch signaling pathway
Elastin
Type III collagen and other collagen types
Lysyl oxidase
ATP7A
Fibulin-4
Fibulin-5