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Role of genetics and the environment in the etiology of congenital diaphragmatic hernia

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ABSTRACT

Congenital diaphragmatic hernia (CDH) is a congenital malformation characterized by failure of diaphragm closure during embryonic development, leading to pulmonary hypoplasia and pulmonary hypertension, which contribute significantly to morbidity and mortality. The occurrence of CDH and pulmonary hypoplasia is theorized to result from both abnormalities in signaling pathways of smooth muscle cells in pleuroperitoneal folds and mechanical compression by abdominal organs within the chest cavity on the developing lungs. Although, the precise etiology of diaphragm maldevelopment in CDH is not fully understood, it is believed that interplay between genes and the environment contributes to its onset. Approximately 30% of patients with CDH possess chromosomal or single gene defects and these patients tend to have inferior outcomes compared with those without genetic associations. At present, approximately 150 gene variants have been linked to the occurrence of CDH. The variable expression of the CDH phenotype in the presence of a recognized genetic predisposition can be explained by an environmental effect on gene penetrance and expression. The retinoic acid pathway is thought to play an essential role in the interactions of genes and environment in CDH. However, apart from the gradually maturing retinol hypothesis, there is limited evidence implicating other environmental factors in CDH occurrence. This review aims to describe the pathogenesis of CDH by summarizing the genetic defects and potential environmental influences on CDH development.

INTRODUCTION

Birth defects are defined as structural or functional anomalies that impact physical, intellectual, or social well-being. The prevalence of birth defects is approximately 3%-6%, affecting 8 million newborns globally each year.[1] According to the 2019 Global Burden of Diseases statistics,[1] a total of 549 305 (7.1%) children died due to birth defects. Although progress is being made in understanding birth defect causation, up to 80% of cases still lack a known cause.[2] Genetic factors contribute to approximately 25% of birth defects including monogenic defects such as achondroplasia[3] and chromosomal defects such as Down syndrome.[4] Environmental factors account for about 10%[5] of birth defect causes including teratogenic exposures such as thalidomide,[6] alcohol,[7] and certain drugs during pregnancy. In addition, the majority of birth defects including congenital heart defects and congenital diaphragmatic hernia (CDH)[8] are felt to result from interactions between genes and environment. In this review, we summarize the genetic and environmental factors thought to play contributing roles in the development of CDH.

OVERVIEW ON CONGENITAL DIAPHRAGMATIC HERNIA

CDH is a rare structural-developmental anomaly affecting approximately 2.3 per 10 000 live births.[9] It is characterized by failure of closure of the diaphragm during embryonic development resulting in herniation of abdominal organs into the chest cavity. Despite improvements in prenatal diagnosis, surgical treatment, and postoperative monitoring and management of CDH, infant mortality is still in the range of 30%–50%.[10] Pulmonary hypoplasia and pulmonary hypertension are the main causes of death in CDH patients, while long-term morbidity[11] may be a significant concern in survivors.

Anatomic classification of CDH

CDH can be categorized into three anatomic types. The developing diaphragm contains a vulnerable region known as the lumbar rib triangle, situated on its posterior lateral side. Diaphragmatic hernias in this location are called Bochdalek or thoracoabdominal hiatus hernias. Bochdalek hernias account for approximately 70% of all CDH cases[12] with 85% of Bochdalek hernias being left-sided.[13] The second most common (27%) type of CDH occurs ventrally, and is known as a Morgagni or posterior sternal hernia.[12] The third type is a centrally located hernia, also known as a septum transversum hernia, which occurs in 2%–3% of patients with CDH.[12]

Associated malformations

The clinical manifestations of CDH are closely tied to its type, the presence and severity of lung and pulmonary vascular maldevelopment, and the coexistence of associated malformations which occur in approximately 40% of patients.[14] These concurrent malformations range from clinically insignificant to lethal. Cardiovascular and urogenital anomalies are the most commonly associated malformations,[15] while others congenital eye,[16] spinal,[17] and limb reduction defects[18] are infrequently observed. CDH can also be syndromic with recognized malformation patterns (e.g., Fryns, Simpson-Golabi-Behmel syndromes). Recognizing and describing these associations with CDH is useful for both hypothesizing disease pathogenesis, prognosticating outcomes and establishing appropriate trajectories of care for families, especially when the diagnoses are made prenatally.

The role of genes and the environment in CDH

It is likely that both genes and the environment contribute to CDH occurrence. At present, more than 40 genetic knockout mouse models associated with CDH have been successfully constructed, with most of these mice exhibiting a diaphragmatic defect accompanied by a pulmonary hypoplasia phenotype.[19] This not only demonstrates the direct connection between genes and CDH occurrence, but also highlights the substantial genetic heterogeneity of CDH. In humans, nearly 30% of CDH cases can be attributed to a variety of genetic factors, including chromosomal anomalies (10%) such as aneuploidies, structural rearrangements, and copy number variations (CNVs),[20] and individual gene anomalies (10%–22%) such as de novo damaging variants including single nucleotide variants and small insertions or deletions (indels).[21] Included among the approximately 150 gene variants identified in association with CDH,[22] more than 70 are single gene syndromes.[14] Although there is no direct evidence of environmental causation in human CDH, several (but not all) population-based correlative studies suggest maternal exposure risk factors for CDH including maternal age,[9] alcohol,[23] pregestational diabetes,[24] smoking,[25] and agricultural pesticide exposure.[26–28]

EMBRYOLOGY OF HUMAN DIAPHRAGMATIC DEVELOPMENT

The diaphragm is derived from mesenchyme and is composed of muscle, connective tissue (tendon), nerve, and blood vessels. From the embryologic perspective, the diaphragm muscle originates from the paraxial mesoderm, while the central tendon originates from the transverse septum of the splanchnic mesoderm.[29] Normal development of the diaphragm involves four phases: Formation of the pleuroperitoneal folds (PPFs), formation of the postnatal mesenchymal plate, formation of the diaphragm, and closure of the pleuroperitoneal canal[29] (figure 1). The development of the diaphragm occurs between the 4th and 12th weeks of development.[22] By the 5th week, the PPFs that separate the thoracic and abdominal cavities begin to extend into the surrounding regions, eventually merging with the diaphragm and mesentery of the esophagus. By the 8th week of embryonic development, the PPFs, postnatal mesenchymal plate, and diaphragms have all fully matured, and the pleuroperitoneal canals have closed leading to the complete separation of the thoracic and abdominal cavities.[8 30] Failure of closure of the pleuroperitoneal canal by week 10 when reduction of physiological midgut herniation occurs results in visceral herniation through the Bochdalek foramen into the chest, usually on the left side.

During embryonic development, muscle progenitor cells (myoblasts) originating from the somites migrate and integrate into the developing PPFs. Coincident with myoblast migration, the phrenic nerves exit the cervical spinal cord following the septum transversum and migrating myoblasts, leading to diaphragmatic innervation. The migration, proliferation, differentiation, and

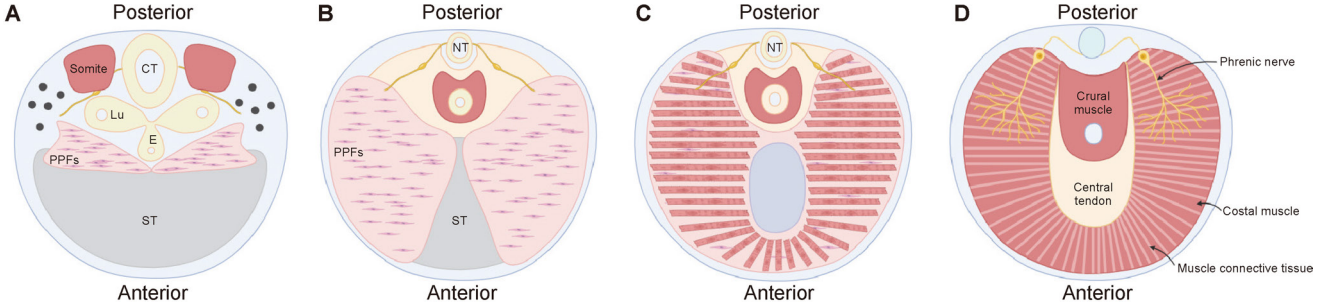

Figure 1 The normal embryological development of the diaphragm. (A) By the 5th week of human embryonic development, PPFs separating the chest and abdominal cavity begins to form and extends horizontally to the surrounding area. (B) By the 6th week, PPFs further expand and gradually fuse with the diaphragm and mesentery of the esophagus. The postnatal mesenchymal plate begins to form and develop. (C) By the 7th week, PPFs are basically mature, and the postnatal mesenchymal plate continues to develop. The diaphragm and other muscle connective tissue begin to form. (D) By the 8th week, the PPFs, postnatal mesenchymal plate, and diaphragm have fully matured, the pleuroperitoneal canals have closed, and the chest and abdominal cavity have also been completely separated by the diaphragm. CT, central tendon; E, esophagus; Lu, lung; NT, neural tube; PPFs, pleuroperitoneal folds; ST, septum transversum.

functional impact of these muscle progenitor cells are regulated by myogenic regulatory transcription factors, such as Myf5, MyoD, and myogenin.[8] When the signaling pathways of muscle progenitor cells within the PPF are disrupted, muscular development is affected contributing to the occurrence of CDH.

PULMONARY HYPOPLASIA IN CDH

Pulmonary hypoplasia is a critical determinant of mortality in patients with CDH, yet its exact etiology is unknown. It is theorized that both the mechanical compression of the developing lungs by abdominal organs within the chest cavity and an abnormal signaling pathway of smooth muscle cells in PPFs, which dysregulates migration of progenitor smooth muscle airway cells, lead to the simultaneous occurrence of CDH and pulmonary hypoplasia.[29] Accordingly, two explanatory hypotheses have been proposed.

Mechanical compression paradigm

While evidence exists that mechanical compression of the developing lung leads to a reduction of ipsilateral lung growth,[31] the etiology of pulmonary hypoplasia, which is known to affect both lungs in human CDH, is likely more complex. In the nitrofen rat model (described in a future section), pulmonary hypoplasia is evident prior to the development of the mediastinum and diaphragm.[32] In addition, other human pathologies that cause ipsilateral and contralateral (through mediastinal displacement) lung compression, such as large, space-occupying fetal lung lesions are not associated with the development of pulmonary hypoplasia observed in CDH.[33] These observations suggest that pulmonary hypoplasia in CDH does not arise solely from mechanical compression, but is likely also influenced by factors that initiate pulmonary hypoplasia earlier in embryonic development prior to the onset of mechanical compression.

Dual-hit hypothesis

Given the limitations of the mechanical compression theory, attention has shifted towards understanding the developmental role of the embryonic mediastinum in human CDH, specifically the role of pulmonary mesothelial cells and their ability to undergo epithelial–mesenchymal transition and cellular differentiation.[34] The "smooth muscle hypothesis" suggests that mesenchymally-derived progenitor cells of airway smooth muscle produce FGF-10. FGF-10 plays a crucial role in promoting the growth of pulmonary branches and facilitating the differentiation and coordination of airway smooth muscle contraction during the embryonic stage ensuring normal lung development.[35] In the presence of abnormal epithelial–mesenchymal transition, there is dysfunction of airway smooth muscle cells responsible for producing FGF-10, which likely contributes to the development of pulmonary hypoplasia.[36] In addition, mesenchymal cell maldevelopment can also lead to dysregulation of pulmonary vascular smooth muscle, further contributing to the characteristic features of pulmonary arterial hypertension.[37] Moreover, studies have shown that in CDH models with pulmonary hypoplasia, treatment with amniotic fluid stem cell-derived extracellular vesicles (AFSC-EVs) can lead to phenotypes of alveolarization and branching in the previously underdeveloped lungs. AFSC-EV is a type of nanoparticle that combines AFSCs with a biofilm, which enhances the function and activity of mesenchymal cells indicating the important role of mesenchymal action in the embryonic development of the diaphragm and lungs.[38]

These data suggest plausibility for a dual-hit mechanism[39] as being responsible for pulmonary hypoplasia in human CDH: The initial hit involves aberrant smooth muscle cell signaling within PPFs during early embryonic development. This disruption triggers abnormal epithelial–mesenchymal transition leading to primary pulmonary hypoplasia. The second hit is the mechanical compression of the developing lungs caused by abdominal organs entering the chest, further exacerbating the severity of pulmonary hypoplasia.

ANIMAL MODELS OF CDH

Researchers have successfully established animal models of CDH by interfering with normal diaphragm development in the embryo using three methods: Surgical creation of a diaphragmatic hernia (sheep, rabbits), pharmacological interference (nitrofen rat model), and gene knockout techniques in mice. This discussion will focus on rodent models of CDH.

The most frequently used animal model for studying CDH involves the use of the herbicide nitrofen (2,4-di chlorophenyl-p-nitrophenyl ether).[12] Administering nitrofen to pregnant rats on the 9th day of their 21-day gestation results in abnormal development of the heart, lungs, diaphragm, and embryonic skeleton in newborn pups.[39] Characteristics of nitrofen-induced teratogenesis in the rat closely resemble the findings of human CDH making it a valuable tool for investigating the underlying mechanisms of CDH.[40] Importantly, in the rat embryo, lung development begins on the 11th day of pregnancy. If the dams are exposed to nitrofen on the 9th day of pregnancy, approximately 70% of newborn pups develop CDH, and almost all of them exhibit pulmonary hypoplasia.[40]

With advancements in gene knockout technology, more than 40 gene knockout mouse models, including *Gata4*[8 41] and *COUP-TFII*,[42–44] have been developed for functional investigation of signaling pathways on the diaphragm and fetal lung development (table 1). The *Gata4* heterozygous mouse model is one of the most commonly used animal models for studying birth defects. A notable proportion of *Gata4* heterozygous mice die within 1 day of birth, exhibiting one or multiple developmental defects such as midline diaphragmatic hernias, dilated distal airways, and cardiac malformations.[41]

Table 1 Genetic mice models of CDH with lung abnormalities

Gene	Full gene name	Model	Diaphragmatic defects	Lung abnormalities	PMID
CTNNB1	Catenin (cadherin associated protein), b1	*Ctnnb1*$^{fl2\text{-}6/fl2\text{-}6}$	Diaphragmatic hernia	Absent lung buds	26278035
EYA1, *EYA2*	EYA transcriptional co-activator and phosphatase 1 and 2	*Eya1*$^{-/-}$, *Eya2*$^{-/+}$	Amuscular diaphragm	Pulmonary hypoplasia, abnormal epithelium morphologic features	24528972
FGFRL1	Fibroblast Growth Factor Receptor Like 1	*Fgfrl1*$^{del1\text{-}2/del1\text{-}2}$ or *Fgfrl1*$^{del3\text{-}7/del3\text{-}7}$	Posterior diaphragm muscle	–	19383940,17986259
FRAS1	Fraser extracellular matrix complex, subunit 1	*Fras1*$^{Q1263*/Q1263*}$	Retrosternal hernia (with sac)	–	29618029
FREM1	Fras1-related extracellular matrix 1	*Frem1*$^{eyes2/eyes2}$	Retrosternal hernia (with sac)	Long lobulation defects, fused pulmonary lobes	23221805
FREM2	Fras1-related extracellular matrix 2	*Frem2*$^{ne/ne}$	Retrosternal hernia (with sac)	Fused pulmonary lobes	29618029
GAB1	GRB2 Associated Binding Protein 1	*Gab1*$^{LacZ/LacZ}$	Amuscular diaphragm	–	10995442
GATA4	GATA-binging protein 4	*Gata4*$^{del2/+}$; *Gata4*$^{del3\text{-}5/fl3\text{-}5}$	Retrosternal hernia (with sac)	Dilated distal airways, increased saccule size, thickened mesenchyme, abnormal vasculature	25807280,17069789
GLI2, GLI3	GLI familiy zinc finger 2 and 3	*Gli2*$^{-/-}$;*Gli3*$^{-/-}$ or *Gli3*$^{+/-}$	Posterior hernia	Hypoplasia, absent right accessory lobe, thickened mesenchyme	9731531
HLX	H2.0-like homeobox	*Hlx*$^{neo/neo}$	Muscular hypoplasia with unspecified hernia	Enlarged lungs with normal structure	8557196
KIF7	Kinesin family number 7	*Kif7*$^{dd/dd}$ or *Kif7*^{maki}	Posterior hernia	Hypoplasia, reduced alveolar epithelial cell differentiation	23650387
LOX	*Lysyl oxidasec*	*Lox*$^{neo/neo}$	Central hernia with rupture	Hypoplasia, abnormal acini, abnormal elastic fibers	12473682
MET	Mesenchymal-epithelial transition factor	*Met*$^{neo/neo}$	Diaphragmatic hernia, thin diaphragm muscle	Abnormal saccule morphologic features	12388344
MYOD1	Myogenic differentiation 1	*Myod1*$^{neo/neo}$	Thin diaphragm muscle	Pulmonary hypoplasia	14499644
NDST1	N-deacetylase-N-sulfotransferase 1	*Ndst1*$^{fl2/fl2}$	Central hernia	Thick interalveolar septa	24355925
NR2F2	Nuclear receptor subfamily 2, group F, number 2	*Nr2f2*$^{fl1\text{-}3/fl1\text{-}3}$	Posterolateral hernia	Hypoplasia	16251273
PAX3	Paired Box 3	*Pax3*$^{Sp/Sp}$	Amuscular diaphragm	–	10226008
PBX1	Pre-B-cell leukemia transcription factor 1	*Pbx1*$^{neo/neo}$	Muscularization and tissue patterning defect	Hypoplasia, alveolar simplification	22315423
PDGFRα	Platelet-derived growth factor receptor, α	*Pdgfrα*$^{del2\text{-}4/del2\text{-}4}$	Posterolateral hernia	Hypoplasia, failure of alveogenesis	17568391
PLS3	*Plastin 3*	*Pls3*^{W499C} ; *Pls3*^{14bpdel}	Posterolateral and anterior muscular thinning, hernia	–	37751738

Continued

Table 1 Continued

Gene	Full gene name	Model	Diaphragmatic defects	Lung abnormalities	PMID
RARα, RARβ	Retinoic acid receptor α and β	$Rar\alpha^{neo/neo}$, $Rar\beta^{neo/neo}$	Posterior hernia	Hypoplasia, abnormal alveoli, lung agenesis	7607068
SIN3A	SIN3 transcription regulator family member A	$Sin3a^{CKO}$	Left-sided diaphragm malformation	Pulmonary hypoplasia, pulmonary hypertension	38295182
SIX1	Six homeobox 1	$Six1^{-/-}$	Absent diaphragm	Hypoplasia, reduced branching morphogenesis, narrow bronchi, arrested expansion of epithelial tubules, dense mesenchymal cellularity, failure of lung maturation	24528972
SLIT3	Slit guidance ligand 3	$Slit3^{lacZ/lacZ}$	Central hernia (with sac)	–	12702769
SOX7	Sex determining region Y-box 7	$Sox7^{del2/+}$	Retrosternal hernia (with sac)	–	22723016
WT1	Wilms tumor 1	$Wt1^{del1/del1}$; $Wt1^{fl1/fl1}$	Posterolateral hernia	Hypoplasia, abnormally fused and malformed lung lobes, collapsed distal air spaces	27642710,17071579

Furthermore, all *Gata4* conditional knockout mice exhibit maldeveloped diaphragms with nearly 70% of pups possessing Bochdalek hernias.[8] *COUP-TFII* is an important gene in mammalian embryonic development and is highly expressed in the mesenchymal components of different organs. Mice with homozygous deletion of *COUP-TFII* generally die within 10 days after birth due to growth restriction and severe bleeding caused by defects in angiogenesis and vascular remodeling.[42] Compared with wild-type mice, *COUP-TFII* heterozygotes have smaller body sizes, various cardiovascular developmental abnormalities, and embryonic developmental issues, including those affecting the diaphragm.[43 44]

GENETIC IMPLICATIONS IN HUMAN CDH

As noted previously, approximately 30% of patients with CDH have disease-causing genetic aberrations with chromosomal imbalance or single genetic de novo or inherited variants,[45 46] and these patients typically experience inferior outcomes compared with patients with CDH without genetic aberrations.[47] Syndromic CDH, which accounts for approximately 8% of all cases, is a recognizable constellation of anomalies that include CDH.[48] It includes monogenic syndromes (3.2%), syndromes with chromosomal anomalies or microdeletions/duplications (3.1%), and other syndromes with unknown genetic causes (1.9%). When compared with non-syndromic CDH, patients with syndromic CDH demonstrate lower birth weight, increased rates of prematurity, higher rate of bilateral CDH, lower survival rates, and higher rates of non-repair.[48]

Monogenic syndromes with CDH

The monogenic syndromes associated with CDH can be classified according to their inheritance patterns: Autosomal recessive, autosomal dominant, and X-linked (table 2). The following are representative syndromes that co-occur with CDH.

Autosomal recessive

Fryns syndrome (OMIM: 229850) is an autosomal recessive multiple anomaly syndrome that includes CDH, craniofacial anomalies, distal limb hypoplasia, cardiac anomalies, and internal malformations. It is the most common syndrome associated with CDH (>90%), accounting for 1.3%–10% of all cases.[48 49] Biallelic *PIGN* mutations have been described in Fryns syndrome.[50] However, not all patients meeting the diagnostic criteria for Fryns syndrome carry *PIGN* mutations, suggesting genetic heterogeneity.

Donnai-Barrow syndrome (DBS; OMIM: 222448) is a rare autosomal recessive genetic disorder characterized by craniofacial anomalies, high myopia, sensorineural hearing loss, and low molecular weight proteinuria. Mutations in the LDL receptor-related protein 2 (*LRP2*) gene can result in DBS.[51] Approximately 43% of cases of DBS have an associated CDH phenotype.[52]

Table 2 Monogenic syndromes related to CDH

Inheritance model	Syndrome	Gene	Chromosome	Phenotype	% with CDH	PMID
Autosomal recessive	Donnai–Barrow syndrome	*LRP2*	2q31.1	Agenesis of the corpus callosum, facial dysmorphology, ocular anomalies, sensorineural hearing loss and developmental delay	43%	25682901
Autosomal recessive	Fryns syndrome	*PIGN*	18q21.3	CDH, craniofacial anomalies, distal limb hypoplasia, Cardiac anomalies and internal malformations	10%	37418285,12483630
Autosomal recessive	Cutis laxa	*LTBP4*	19q13.2	Loose and/or wrinkled skin, pulmonary emphysema and/or vascular complications.	56%	19836010,22829427
Autosomal recessive	Pulmonary hypoplasia, Diaphragmatic anomalies, microphthalmia, and Cardiac defects (PDAC) syndrome	*STRA6* or *RARB*	15q24.1 or 3p24	Small or absent eyes, heart defects, CDH, pulmonary hypoplasia and renal malformations	Rare	22686418,24075189
Autosomal dominant	Denys–Drash syndrome	*WT1*	11p13	Male pseudohermaphroditism, GU anomalies, renal anomalies, nephrotic syndrome. Wilms tumor predisposition	Rare	7645607
Autosomal dominant	Meacham syndrome	*WT1*	11p13	Male pseudohermaphroditism, GU anomalies, renal anomalies, nephrotic syndrome, CHD, Wilms tumor predisposition	Rare	16932893
Autosomal dominant	Frasier syndrome	*WT1*	11p13	Male pseudohermaphroditism, GU anomalies, renal anomalies, nephrotic syndrome	Rare	10792605
Autosomal dominant	MYRF-related cardiac urogenital syndrome	*MYRF*	11q12.2	Urogenital defects, congenital heart defects and eye anomalies	62.5%	31069960
Autosomal dominant	Cornelia (Brachmann) de Lange syndrome	*NIPBL*	5p13.2	Limb defects, dysmorphic features: synophrys, brachyrrhinia, long philtrum, thin lip,	5~20%	32762940
X-linked	Simpson–Golabi–Behmel syndrome	*GPC3*	Xq26.2	Prenatal/ postnatal overgrowth, coarse facies, bifid uvula, macroglossia, macrognathia, CHD, rib anomalies, renal anomalies, nephromegaly, Wilms tumor predisposition	24%	20950395
X-linked	Craniofrontonasal syndrome	*EFNB1*	Xq13.1	Coronal synostosis, hypertelorism, facial asymmetry, bifid nasal tip and skeletal anomalies	Rare	16639408,32022998

Pulmonary hypoplasia, diaphragmatic anomalies, microphthalmia, and cardiac defects (PDAC) syndrome is a rare monogenic disease with a complex clinical phenotype including small or absent eyes, heart defects (55%), congenital diaphragmatic hernia (29%), pulmonary hypoplasia (39%), and renal malformations.[53] It is caused by the recessive mutations in the retinol-binding protein gene *STRA6* or recessive and dominant mutations in the retinoic acid receptor beta (*RARB*) gene.[54]

Cutis laxa is a group of rare connective tissue disorders characterized by loose and/or wrinkled skin. Mutations in the gene coding the latent transforming growth factor beta binding protein 4 (*LTBP4*) will cause cutis laxa and other physical system problems including pulmonary, gastrointestinal, genitourinary, and musculoskeletal malformations. Approximately 56% of patients with *LTBP4* recessive mutations will have CDH.[55 56]

Autosomal dominant

Denys-Drash syndrome (DDS; OMIM 194080), Meacham syndrome (MS; OMIM 608978), and Frasier syndrome (FS; OMIM 136680) are all genetic diseases caused by dominant mutations in the Wilms tumor suppressor gene *WT1* that are rarely associated with CDH.[57–59] DDS and FS are both early renal developmental disorders, and their clinical features include male pseudohermaphroditism and progressive glomerular disease.[57 60] DDS can also lead to the development of Wilms tumor, and patients may experience end-stage renal failure earlier.[61] MS is an autosomal dominant congenital connective tissue disease characterized by developmental abnormalities in the skeletal and cardiovascular systems.[58] Early onset Marfan syndrome (EOMS) is the most severe type of MS and approximately 20% of EOMS patients exhibit a phenotype of diaphragmatic eventration.[62] How the loss of *WT1* function alone might directly induce diaphragmatic defects, or whether other genetic or environmental conditions are also necessary for this to occur is unknown.

MYRF-related cardiac urogenital syndrome (MYRF-CUGS) is primarily characterized by urogenital defects, congenital heart defects, and eye anomalies. Other features of the condition include a broad range of developmental delay/intellectual disability, pulmonary hypoplasia and CDH. It is the result of de novo dominant mutations in a myelin regulatory factor (*MYRF*) gene.[63] *MYRF* is a membrane-related transcription factor. It is highly expressed during the embryonic development of the heart and diaphragm.[64 65] Approximately 60% of MYRF-CUGS will present with CDH.[66]

Cornelia de Lange syndrome 1 (CDLS1; OMIM 122470) is a rare genetic syndrome caused by mutations of NIPBL cohesin loading factor (*NIPBL*). It is characterized by proportional short stature and characteristic facial features accompanied by heart defects, and genital abnormalities. CDH occurs in 5%–20% of patients with CDLS and has a reported mortality of approximately 80%.[67]

X-linked syndromes

Simpson Golabi Behmel syndrome (OMIM 312870) is an X-linked recessive disorder caused by mutations in the *GPC3* gene, which is highly expressed in embryonic mesoderm and is involved in local growth regulation. It is characterized by excessive fetal and neonatal growth, dysmorphic features and multiple congenital anomalies, including CDH in approximately 24% of cases.[68]

Craniofrontonasal syndrome (CFNS; OMIM 304110) is an X-linked dominant condition caused by mutations in Ephrin B1 (*EFNB1*) gene[69] that is characterized by coronal synostosis, hypertelorism, facial asymmetry, bifid nasal tip, and skeletal anomalies. Women with CFNS are more severely affected than men.[70] CDH is reported in both affected men and women with CFNS.[71]

Chromosomal anomalies in CDH

Chromosomal anomalies, especially de novo events spanning multiple genes including complete or partial aneuploidy, cytogenetic rearrangements, and CNVs are present in approximately 10% of patients with CDH.[20] Aneuploidies associated with CDH mainly include trisomy 18 (Edwards syndrome), trisomy 21, trisomy 13, Turner syndrome (45, X), Trisomy X (46, XXX), and Pallister-Killian syndrome (PKS).[14] PKS is a rare chromosomal disease caused by short-arm tetrasomic chimerism on chromosome 12 and the occurrence of CDH in combination with PKS is in the range of 5%–27%.[72]

The CNVs associated with the occurrence of CDH include 8p23.1 deletion, 15q26.1 deletion, 1q41-42 deletion, 8q23.1 deletion, 4p16 deletion, 3q22 deletion, 4p16 deletion, 6p25 deletion, 11p13 deletion, 11q23.2 duplications, 1q25q31.2 duplications, 8p21-p23.1 duplications, and unbalanced translocation of der[22] t(11:22)(q23:q11).[14 20] The majority of CNVs are deleted copy numbers of candidate genes associated with CDH occurrence. Notably, deletion of 8p23.1 is the most common CNV associated with CDH accounting for 3%–5% of patients with CDH.[20 73] The deleted 8p23.1 region precisely contains the GATA binding protein 4 (*GATA4*) and SRY-box transcription factor 7 (*SOX7*) genes, which are important transcription factors in the development of the heart and diaphragm and have been confirmed to be closely related to the occurrence of CDH.[73] Similarly, the deleted region of 15q26.1 contains the protein-encoding gene nuclear receptor subfamily 2 group F member 2 (*NR2F2*, also known as *COUP-TFII*),[43] accounting for approximately 1.5% of patients with CDH[14]; 1q41-42 deletion contains the H2.0 like homeobox (*HLX*) and dispatched RND transporter family member 1 (*DISP1*) genes; CNVs in these regions have been associated with Fryns syndrome with CDH, dysmorphic features, and other multiple congenital anomalies.

The 4p16 deletion contains fibroblast growth factor receiver like 1 (*FGFRL1*); 11p13 deletion contains Wilms tumor 1 (*WT1*) gene; 8q23 deletion includes zinc finger protein family member 2 (*ZFPM2*); 3q12 deletion contains the candidate genes for cellular retinol-binding

Table 3 Overview of candidate genes for human CDH

Gene	GATA4	GATA6	ZFPM2	NR2F2	LONP1
Gene Locus	8q23.1	18q11.2	8q23.1	15q26.2	19q13.3
Expression	Lung mesenchyme, diaphragm, liver	Lung mesenchyme, diaphragm	Lung mesenchyme, diaphragm	Lung mesenchyme	Lung, heart, skeletal muscle
Function	Transcription factor	Transcription factor	Transcription factor	Transcription factor	Mitochondrial protease
Protein Activity	Zinc finger transcription	Zinc finger transcription	Zinc finger protein Regulating the expression of GATA	Nuclear receptor subfamily two group F	Bind to mtDNA and degrade damaged proteins
Anomalies Associated	Congenital Heart Disease, Atrial Septal Defect,Testicular Anomalies	Diaphragmatic Hernia, Congenital Heart Defects, Pancreatic Agenesis	Diaphragmatic Hernia, 46Xy Sex Reversal, Tetralogy Of Fallot	Congenital Heart Defects, Xx Sex Reversal	Codas (Cerebral, Ocular, Dental, Auricular And Skeletal) Syndrome

proton 1 (*RBP1*) and cellular retinol-binding proton 2 (*RBP2*).[14 74]

Genes identified in non-syndromic CDH

The use of next-generation sequencing techniques has proven invaluable in identifying candidate genes in patients with CDH. There are more than 150 gene variants identified in patients with CDH.[22] Notably, genes such as *GATA4*, the GATA binding protein 6 (*GATA6*), zinc finger protein, FOG family member 2 (*FOG2*), and Lon peptidase 1 (*LONP1*) (table 3) are suspected to be involved in diaphragm development.[75]

GATA genes

GATA4 is located at 8q23.1. GATA transcription factors are a family of transcription factors with zinc finger domains, composed of six subtypes. GATA1, GATA2, and GATA3 are hematopoietic expression factors, while GATA4, GATA5, and GATA6 (and especially GATA4) play important roles in heart and endoderm development.[76] The *GATA4* gene is highly expressed in cells of PPFs during the embryonic stage. De novo *GATA4* mutations have been identified in patients with CDH.[46 77] *Gata4*$^{-/-}$ mice experience embryonic lethality; but heterozygotes develop midline diaphragm defects in approximately 15% of offspring.[41] Moreover, conditional deletion of *Gata4* in the PPF caused diaphragm defects in all mutant mice.[8]

Approximately 18% of *GATA6* mutants cause diaphragmatic hernia.[78] *GATA6* is only expressed in the mesenchymal region of PPFs, but plays a pivotal role in the regulation of mammalian hematopoietic and cardiac development.[76] In the nitrofen rat model, the expression of *Gata6* was significantly reduced in the PPFs and lung interstitium.[79] In *GATA6* gene knockouts, mRNA expression of Clara cell 10 kDa proteins (*CC10*) and surfactant protein C (*SPC*) markers for proximal and distal lung epithelial cells differentiation is significantly reduced,[80] suggesting the impact of *GATA6* gene on pulmonary growth and maturation in CDH. Furthermore, deletion of the *GATA6* gene leads to the activation of the Wnt/-catenin signaling in the lungs, influencing the growth and differentiation of developing lung epithelial cells, ultimately resulting in decreased lung tissue branching.[81]

FOG2

FOG2 (also known as *ZFPM2*), located at 8q23.1, encodes a transcription factor that regulates the expression activities of *GATA4* and *GATA6*.[82] Studies have shown that mice with *FOG2* gene deletion exhibit significantly reduced hepatocyte growth factor/scattering factor (*HGF*) expression and a higher incidence of CDH.[82] The deletion of *FOG2* is believed to impact the HGF/c-MET signaling pathway and the migration of muscle progenitor cells to PPFs leading to diaphragm and lung maldevelopment.[22] Genetic analysis of a multigenerational family identified a heritable intragenic *FOG2* deletion with an estimated penetrance of 37.5%.[83]

Other implicated genes

The *COUP-TFII* gene, also known as the *NR2F2* gene, is located at 15q26.2 of the common CNVs associated with CDH.[84] Conditional deletion of *COUP-TFII* in the PPF induces Bochdalek CDH in ~50% of offspring.[44] At least 10 loss of function variants in *NR2F2* have been identified in patients with CDH.[43] LONP1 is a nuclear-encoded mitochondrial protease with ATP-dependent protease activity, crucial for degrading misfolded or damaged proteins. Analysis of de novo and inherited rare genetic variants highlight *LONP1* as a potential risk gene for CDH. Notably, patients with *LONP1* variants not only had CDH, but also had cardiac structural abnormalities or skeletal abnormalities. In addition, the loss of LONP1 in embryonic lung epithelium of mice led to abnormal lung development and a 100% mortality rate at birth.[85]

MATERNAL AND ENVIRONMENTAL RISK FACTORS IN HUMAN CDH

No teratogens have been causally linked to human CDH. Maternal risk factors implicated from population-based case-control studies include maternal age (risk ratio (RR) =1.69, 95% confidence interval (CI) 1.26 to 2.25),[9] pregestational diabetes (OR=12.53, 95% CI 2.40 to 65.43),[24] smoking (OR=1.30, 95% CI 1.10 to 1.50),[25] alcohol (OR=1.64, 95% CI 1.10 to 2.44),[86] OR=2.9, 95% CI 1.6 to 5.2),[23] and maternal periconceptional use of hairspray (OR=2.07, 95% CI 1.33 to 3.23).[86]

Several case-control studies have looked at preconceptual or periconceptual maternal dietary intake in relation to CDH occurrence in offspring. In one large study of nearly 5500 cases and controls from the National Birth Defects Prevention Study (NBDPS), ORs of 1.4 or higher were observed with lower (≤10th percentile) intake of lutein, selenium, and vitamin A. Conversely, ORs were decreased (0.7 or less) for≥90th percentile intakes of folate, iron, thiamin (B1), vitamin B6, and zinc.[87] Other case-control studies have looked specifically at vitamin A intake and CDH occurrence. A Dutch study showed that daily vitamin A intake below recommended levels in normal-weight mothers was significantly associated with lower serum retinol levels and an increased CDH risk (OR=7.20, 95% CI 1.50 to 34.40).[88] Conversely, a reduced risk of CDH was observed in similar normal-weight Japanese mothers with high maternal vitamin A intake (OR=0.50, 95% CI 0.20 to 1.00).[89] Furthermore, clinical and experimental evidence suggest that maternal alcohol use and cigarette smoking modifies vitamin A metabolism,[90 91] suggesting a possible mechanistic role for vitamin A in smoking or alcohol-related CDH occurrence.

It is important to acknowledge that the body of evidence describing environmental associations of CDH is inconsistent with some studies reporting no associations between CDH and smoking,[27] alcohol consumption,[25] or vitamin A intake.[92] This suggests that the role of environmental exposure in the development of CDH is likely that of a modifier of genetic phenotype rather than as a direct teratogen.

ENVIRONMENTAL AND GENETIC INTERACTIONS IN CDH: ROLE OF THE RETINOIC ACID PATHWAY

An attractive hypothesis to explain the variable expression of a CDH phenotype in the presence of known genetic predisposition is sensitization by environmental conditions (maternal and indirectly, environmental) on gene penetrance and expression. The retinoic acid (RA) pathway may play such a role in CDH.[93 94] RA is a metabolite of dietary vitamin A. Vitamin A, sourced as β-carotene from plants and as retinol from animal-derived retinoids,[95] is oxidized and metabolized in the liver to produce retinol. Retinol binds to RBP and transthyroxine and the RBP-retinol complex is actively internalized into cells through STRA6 to initiate intracellular signaling. Inside the cell, retinol binds to cytoplasmic retinol-binding protein-1 where it is oxidized to retinal by retinol dehydrogenase and then irreversibly dehydrogenated to the active molecule RA by cytosolic retinal dehydrogenases (RALDH). RA enters the cell nucleus and binds to intracellular RA receptors or retinol-like X receptors to form transcription factors. These factors bind to the retinoic acid responsive elements on the target gene, initiating the transcription of the target gene, thereby regulating gene transcription and expression[22] (figure 2).

As noted previously, maternal vitamin A deficiency is associated with human CDH,[95] and a number of mechanistic explanations are plausible. Low dietary vitamin A intake increases the incidence of teratogen-induced CDH in mice[96] through inhibited RA synthesis via RALDH2.[97] In addition, evidence from mice and humans suggest that genes involved in the RA signaling pathway can cause diaphragm defects. Mutations of the retinol-binding protein gene *STRA6* and RA receptor beta (*RARB*) gene can cause PDAC syndrome which has a CDH/eventration phenotype in 29% of patients.[53] *RALDH2* is expressed in the developing diaphragm[64] and mutations have been discovered in patients with a variety of different CDH phenotypes.[98 99] Other genes such as *RBP1* and *RBP2*, located in the recurrent 3q12 deletion region associated with CDH, could also be linked to CDH, although no specific gene mutations have been identified.[100]

RA target genes or genes that are involved in the interaction with RA receptor (RAR) are also implicated in CDH. As discussed above, genes of *WT1*, *LRAT*, *COUP-TFII*, *FOG2*, and *GATA4* that interact with RAR in CDH have been observed in both animal models and human gene mutations associated with CDH. WT1 regulates the RA signaling by activating the *RALDH2* gene.[101] Interestingly, the diaphragmatic defect can be partially rescued by RA dietary supplement in conditional *Wt1* deletion CDH mice.[102] LRAT can esterify retinol to retinol esters, preventing the oxidation of retinol in cells and preventing its further role in the RA signaling pathway.[93] COUP-TFII heterodimerizes with the RXR family to regulate gene expression.[103] *FOG2* interacts with *GATA4*[104] and both are target genes of RA.[105] It has been suggested that retinoids can regulate downstream gene expression through direct interaction with GATA4 and FOG2.[106] In addition, TBX1 can interact with the transcription factor GATA4 through the GATA-TBX1 regulatory axis, promoting the expression of GATA4.[99] Prenatal treatment with RA in the nitrofen model of CDH results in upregulated expression of COUP-TFII, FOG2, and GATA4.[105]

CONCLUSION

Although its precise origin remains uncertain, there is abundant evidence from mouse and human studies that genetics plays a key role in CDH development with more than 150 genetic variants implicated.

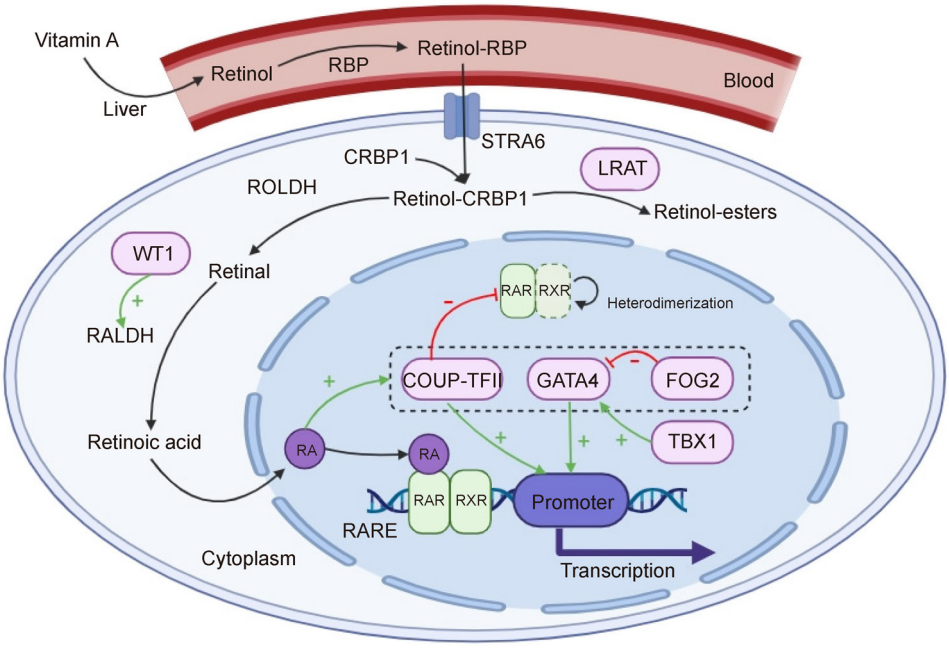

Figure 2 Environmental and genetic interactions in CDH. Dietary vitamin A undergoes oxidative metabolism in the liver, producing retinol. Retinol then binds to retinol binding protein (RBP) for transport in the blood. Retinol-RBP complex enters into specific cells, such as hepatic stellate cells or placental trophoblast cells, through transmembrane receptor STRA6. Inside the cell, retinol binds to retinol binding protein-1 (CRBP-1) and forms retinal under the action of retinol dehydrogenase (ROLDH), which further metabolizes retinoic acid (RA) under the action of retinal dehydrogenase (RALDH). RA enters the nucleus and binds to intracellular RA receptors (RARs) or retinol like X receptors (RXRs), while promoting the expression of *GATA4*, *FOG2*, *WT1*, and *COUP-TFII* genes. WT1 can activate the *RALDH* gene, thereby promoting RA signaling. COUP-TFII chelates RAR, inhibits RAR/RXR heterodimerization. GATA4 directly regulates the expression of the *RARE* gene promoter. FOG2 can bind to GATA4 and inhibit its expression. The interaction between the retinol signaling pathway and genes jointly regulates the transcription and expression of the *RARE* gene, maintaining the normal development of the diaphragm.

Environmental/maternal factors such as vitamin A/retinoic acid likely interact with genes to contribute the phenotype of CDH. Enhanced understanding of how aberrant RA signaling can lead to abnormal diaphragm development should clarify insights into CDH pathogenesis. Because CDH is usually detected antenatally; there is an increasing need for rapid, accurate, and predictive genetic diagnostics to support prenatal decision-making. From the perspective of the parents of a child with CDH, it is important to provide accurate genetic forecasting of the severity of CDH-specific disease as well as the impact of associated anomalies on both survival and quality of life as well as the risk to subsequent children. Families must be offered comprehensive genetic counseling to make decisions based on a thorough understanding of the potential benefits and risks.

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