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The influence of genetics in congenital diaphragmatic hernia

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Abstract

Congenital diaphragmatic hernia (CDH) is a common birth defect that is associated with significant morbidity and mortality, especially when associated with additional congenital anomalies. Both environmental and genetic factors are thought to contribute to CDH. The genetic contributions to CDH are highly heterogeneous and incompletely defined. No one genetic cause accounts for more than 1–2% of CDH cases. In this review, we summarize the known genetic causes of CDH from chromosomal anomalies to individual genes. Both *de novo* and inherited variants contribute to CDH. Genes causing CDH are increasingly identified from animal models and from genomic strategies including exome and genome sequencing in humans. CDH genes are often transcription factors, genes involved in cell migration or the components of extracellular matrix. We provide clinical genetic testing strategies in the clinical evaluation that can identify a genetic cause in up to ~30% of patients with non-isolated CDH and can be useful to refine prognosis, identify associated medical and neurodevelopmental issues to address, and inform family planning options.

Introduction

Congenital diaphragmatic hernia (CDH) is a common and severe birth defect that affects around 1 in 3,000 live births¹. It is characterized by incomplete formation of the diaphragm, leading to the herniation of the abdominal viscera into the chest cavity. CDH can occur as an isolated defect but ~40% of cases are non-isolated and associated with additional anomalies in other systems, most commonly including heart, brain, renal and genitourinary malformations^{1,2}. Newborns with CDH often have severe respiratory distress resulting from pulmonary hypoplasia and pulmonary hypertension, which are the most significant causes of morbidity and mortality of CDH patients. Today, over 70% of cases can be prenatally diagnosed based on the imaging techniques including ultrasonography and fetal

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DISCLOSURE

There is no conflict of interest for all authors

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magnetic resonance imaging (MRI) at a mean gestational age of 24 weeks^{3,4}. Prenatal diagnosis has altered the neonatal outcomes and significant progress has been made in the postnatal care of patients with CDH, with mortality rates reduced to ~30%⁵. However, the long-term morbidity and the medical cost for caring for CDH survivors remains a significant burden. Understanding the etiology of CDH is essential to discovery of new therapies to improve outcomes.

Both environmental and genetic factors contribute CDH. It has been known that nitrofen and manipulating retinoids cause CDH in animal models^{6,7}. Most human cases are unexplained by known environmental factors. Evidence from genetic studies in patients with CDH and animal models provides important insight into the molecular mechanisms of diaphragm development. The genetic etiologies of CDH are highly heterogeneous. Chromosomal anomalies of aneuploidies, structural rearrangements, copy number variants (CNVs) and single-gene mutations all contribute to CDH. Identifying a genetic etiology for CDH in patients is increasingly clinically relevant to provide accurate prognostic information and guide medical management. Studies indicate that chromosomal anomalies detectable by karyotype are observed in 10% of CDH cases^{8,9} and 3–10% of CDH are associated with a syndromic diagnosis^{8,10}. Recent massively parallel sequencing techniques demonstrate that 10–22% of CDH patients have *de novo* damaging variants that are associated with a high relative risk of developmental disorders^{11–13}. In this review, we summarize the current knowledge of CDH genetics.

Chromosomal anomalies associated with CDH

Chromosomal anomalies have been reported in about 10% of all individuals with CDH¹ and include complete or mosaic aneuploidies, cytogenetic rearrangements detectable by karyotype, and copy number variants (CNVs) identified by chromosome microarrays or other methods. Structural anomalies involve almost all chromosomes^{10,14–16}, and an updated list of CDH associated chromosomal anomalies (Supplementary Table 1) and recurrent CNVs are provided (Figure 1).

Aneuploidies

Aneuploidy is an abnormal number of chromosomes. CDH is observed infrequently with many aneuploidies including trisomies 13, 18 and 21 and rarely with Turner syndrome (45,X) and trisomy X^{17,18}. Pallister-Killian syndrome (PKS) is a multisystem developmental disorder caused by tissue limited mosaic tetrasomy of chromosome 12p (isochromosome 12p) and is associated with CDH in 5%–29% of prenatal cases^{19,20}.

CNVs

CNVs that range in size from 50 bp to large segmental deletions or duplications of several megabases have been implicated in human disorders that include CDH^{9,14,21}. Frequently reported CNVs in CDH include 8p23.1 deletion, 15q26 deletion, 1q41–42 deletion, 4p16 deletion, unbalanced translocation of der(22)t(11:22)(q23;q11)¹⁸. CNVs that encompass multiple genes can have many associated features because of both the pleiotropic effects of single genes on multiple phenotypes and/or the additive impact of multiple individual genes

on phenotypes. Analysis of these CNVs have identified candidate genes for CDH (Figure 1 and Supplementary Table 1). Defining the critical regions by comparing CNVs across CDH patients can assist in identifying relevant CDH genes. Several of the CNVs are not consistently associated with CDH and suggest that the CDH aspect may depend on additional interacting genetic or non-genetic factors.

Deletion CNVs

Deletion of 15q26 is the most frequent CNV associated with CDH, observed in 1.5% of CDH cases^{22, 23}. Patients with a 15q26 deletion have intra-uterine growth restriction, cardiovascular malformations, CDH and characteristic facial features associated with Fryns syndrome^{22–24}. The critical region is 1.7 Mb spanning chr15:96420020–98119250 (hg19)^{25, 26} and contains the protein-coding gene: *NR2F2* (nuclear receptor subfamily 2 group F member 2, also known as *COUP-TFII*). *NR2F2* is a transcription factor in the steroid/thyroid hormone receptor superfamily and conditional *Nr2f2* knockout mice develop CDH²⁷. *NR2F2* interacts with ZFPs²⁸, and is regulated by the retinoid signaling pathway²⁹. Pathogenic sequence variants in *NR2F2* have been described in humans with isolated CDH or patients with an atrial septal defect³⁰. These genetic data suggest that *NR2F2* is a critical gene in the development of CDH in patients with 15q26 deletions.

Deletions of 8p23.1 of variable size are common in CDH (OMIM 222400).³¹ and are frequently associated with cardiac anomalies, neurodevelopmental disabilities (NDD), and facial dysmorphisms^{32, 33}. The CDH-critical region at 8p23.1 is a 3.7 Mb region between 8079861 and 11860569 (hg19)³¹. *GATA4* and *SOX7* in this critical interval have been implicated in CDH in mice^{34–36}. Chromosome 8q22–q23 is recurrently deleted and translocated in individuals with CDH^{15, 37, 38} and is associated with dysmorphic features^{15, 38}. The minimum deletion is ~1Mb and includes *ZFPM2*³⁷ which has been associated with diaphragmatic defects in mice.³⁹ Inherited or *de novo* damaging *ZFPM2* sequence variants are observed in up to 5% of CDH in a cohort of 275 patients, but the penetrance is reduced in families with *ZFPM2* variants, and the predominant phenotype is usually isolated⁴⁰.

Deletions of 1q41–42 are consistently associated with seizures, NDD, dysmorphic features, and less commonly with a range of birth defects including cleft palate, clubfeet, and 18% with CDH^{41, 42}. Some patients have a clinical diagnosis of Fryns syndrome including CDH, pulmonary hypoplasia, cleft palate, hypoplastic nails, contractures of the digits, clubfeet, and dysmorphic features^{41, 43}. The critical region for CDH is 219,914,853 to 224,637,114 (hg19)^{41, 43, 44}. Genes *HLX* (H2.0 like homeobox) and *DISP1* (dispatched RND transporter family member 1) have been implicated in CDH. *HLX* is a homeobox transcription factor, and the diaphragms of *hlx* null mice were herniated⁴⁵. Heterozygous and homozygous missense variants in *HLX* have been described in CDH patients^{44, 46}. *DISP1* is highly expressed in the pleuroperitoneal folds (PPF)⁴⁷, and a mosaic *de novo* missense variant of *DISP1* in a CDH patient with heart abnormalities and bilateral cleft palate has been identified although the role of *Disp1* during diaphragm development is unknown⁴⁷.

The 4p16 deletion causes Wolf–Hirschhorn syndrome (WHS), characterized by typical facial features, skeletal anomalies, intellectual disability, and growth problems. WHS is

rarely associated with CDH^{48, 49}. A 2.3 Mb critical region (chr4:1–2,336,628)⁵⁰ contains *FGFRL1* (fibroblast growth factor receptor like 1) and may contribute to CDH^{50, 51}.

CDH has been reported occasionally in 22q11.2 deletion syndrome (22q11.2DS)^{52–54}. The 22q11.2 deletion involves microdeletions of varying sizes (0.7–3 Mb)⁵⁵. A retrospective study of a cohort of patients with 22q11.2 DS revealed that the prevalence of CDH is 0.8%¹⁷. The hotspot for CDH in chromosome 22 was estimated between chr22:18640,000–20,680,000 (hg19)¹⁷.

Microdeletion of 17q12 is associated with maturity-onset of diabetes of the young type 5, macrocephaly, developmental delay and renal disease^{56, 57}. There are six patients with CDH with 17q12 deletion and the minimal region for CDH is a 1.2 Mb deletion from 34,984,100 to 36,207,688 (hg19)^{9, 56, 58, 59}.

Duplication CNVs

The most frequently implicated duplication in CDH is the 11q23 duplication⁶⁰ which usually results from the unbalanced translocations 11;22(q23;q11)^{9, 61}, 11;12(q23;q24)⁶¹, or 11;13(q23;q12.3)^{37, 62}. Ten cases with CDH have been reported to be associated duplications of chromosome 1q⁶³. Other duplications include duplications of 8p21–p23.1, 4q31, and 14q32¹⁵.

Monogenic Syndromes associated with CDH

CDH can be isolated but more commonly is an inconsistent feature of a syndrome with other developmental anomalies or neurodevelopmental disabilities. There are more than 70 well characterized syndromes associated with CDH^{18, 64, 65} and over 20 of these syndromes have established genetic causes^{18, 65}. It is estimated that 3–10% of CDH is associated with an underlying syndromic diagnosis^{8, 10} which has important implications for prognosis and management. The syndromes can be autosomal recessive, dominant or X-linked⁶⁴. Genes associated with these syndromes are summarized in Figure 1 and in Supplementary Table 2.

X-linked Syndromes

CDH has been reported in 18% of individuals with Simpson-Golabi-Behmel Syndrome [SGBS; OMIM 312870]⁶⁶ which is a X linked recessive disorder characterized by prenatal and postnatal macrosomia, hypertelorism, polydactyly and syndactyly, congenital heart disease (CHD), renal anomalies, intellectual disability, and a predisposition to the development of embryonal tumors⁶⁶. SGBS is caused by microdeletions or missense mutations in *GPC3* (glypican-3)⁶⁷. CDH has also been associated with other X-linked recessive disorders, including myotubular myopathy 1 [OMIM: 310400], which is due to loss of function mutations in the *MTM1* (myotubularin 1) at Xq28⁶⁸; Opitz GBBB syndrome [OMIM:300000] caused by mutations of gene *MID1*⁶⁹ and Lowe syndrome [oculocerebrorenal syndrome; OMIM:309000] caused by mutations in *OCRL* (OCRL inositol polyphosphate-5-phosphatase)⁷⁰.

Craniofrontonasal syndrome [CFNS; OMIM 304110] is an X-linked dominant condition characterized by coronal synostosis with hypertelorism, facial asymmetry, a broad and bifid

nasal tip and skeletal anomalies. Family studies have shown that the phenotype in males with CFNS is milder than for females with CFNS^{71–73}. CDH has been described in both males and females with CFNS⁷³. Mutations in *EFNB1* (Ephrin B1) cause CFNS⁷². CDH also has been reported in focal dermal hypoplasia/Goltz Syndrome [305600]^{74, 75}, a X-linked dominant condition with linear pigmentation of the skin, fat herniation, microphthalmia and iris defects, hypoplastic teeth, digital anomalies and bone striation. The syndrome is caused by heterozygous *PORCN* (Porcupine O-Acyltransferase) mutations⁷⁶.

Autosomal Dominant Syndromes

Cornelia De Lange Syndrome 1 [CDLS1; OMIM 122470] is a rare and clinically variable disorder affecting multiple organs. It is characterized by distinctive facial dysmorphisms, intellectual disability, and prenatal and postnatal growth restriction. Congenital anomalies include malformations of the upper limbs, gastrointestinal malformation/rotation, pyloric stenosis, CDH, heart defects and genitourinary malformations. A retrospective review of causes of death with CDLS identified CDH as the cause in 10% of patients⁷⁷. Heterozygous mutations in *NIPBL* are the cause of CDLS with CDH^{78, 79}. Denys Drash syndrome [DDS; OMIM 194080] is characterized by urogenital abnormalities, congenital nephropathy, progressive glomerulopathy and the development of Wilms tumors in early childhood. CDH in DDS is rare. Only three cases with *WT1* (Wilms tumor 1) mutations have been described^{80–82}. CDH can be seen with other conditions associated with *WT1* mutations, including Frasier syndrome [FS; OMIM 136680]⁸³ and Meacham syndrome [OMIM: 608978]⁸⁴. Marfan syndrome [MFS; OMIM:154700] is a genetic disorder of the connective tissue, with a wide spectrum of clinical variability with skeletal, cardiovascular, pulmonary, and ocular involvement. MFS is caused by mutations of *FBNI* (fibrillin-1) that alter the homeostasis of elastic fibers⁸⁵. Early onset Marfan syndrome (EOMS) is the most severe form of MFS, and approximately 20% of patients with EOMS have a diaphragmatic eventration⁸⁶.

Autosomal Recessive Syndromes

Donnai–Barrow syndrome [DBS; OMIM: 222448] is associated with CDH in >50% of patients⁸⁷. It is an autosomal recessive disorder characterized by CDH, exomphalos, absent corpus callosum, myopia, and sensorineural deafness⁸⁸. Biallelic pathogenic variants in *LRP2* cause DBS⁸⁹. *LRP2* encodes the multiligand receptor megalin. Megalin ligand vitamin A or retinol⁹⁰ is critical for diaphragm development⁹¹. Microphthalmic syndrome 9 [MCOPS9, 601186] also known as Matthew-Wood syndrome or Spear Syndrome is another autosomal recessive condition which is caused by biallelic mutations in *STRA6*^{92, 93}. The encoded protein acts as a receptor for retinol/retinol binding protein complexes⁹⁴. Another retinoic signaling pathway related gene *RARB* (retinoic acid receptor beta) causes syndromic Microphthalmia [MCOPS12; OMIM: 615524]⁹⁵. Both syndromes share similar features of bilateral clinical anophthalmia, pulmonary hypoplasia/aplasia, cardiac malformations, and diaphragmatic hernia/eventration⁹⁵.

CDH genes identified by animal model and humans

Recently, next-generation sequencing technology including whole exome sequencing (WES) and whole genome sequencing (WGS) facilitated the identification of novel single nucleotide variants and genes in sporadic and familial CDH patients^{12, 96–98}. The genes identified so far are mainly in two broad groups: transcription factors, and molecules involved in cellular migration, or the components of extracellular matrix (ECM)⁹⁹ (Table 1). Genetically manipulated mouse models of many of these genes are associated with diaphragm anomalies, further supporting their role in CDH (Supplementary Table 3, Figure 1).

Transcription factors

Transcription factors involved in retinoic acid (RA) signaling and sonic hedgehog (Shh) signaling pathways implicated in CDH include *WT1*, *ZFPM2*, *GATA4*, *SOX7*, *GATA6*, *NR2F2*, *MYRF*, *KIF7*, *GLI3*, and *PBX1*. *WT1* is involved in RA signaling pathway and is expressed in PPF⁷. Heterozygous mutations in *WT1* cause a series of overlapping clinical syndromes that can include CDH. *Wt1* null mice exhibit diaphragmatic hernia^{100, 101}.

ZFPM2 (also called *FOG-2*, friend of GATA-2) is located in a CNV recurrently associated with CDH. *Fog2*^{-/-} mice generated through chemical mutagenesis have been shown to have diaphragmatic eventration and pulmonary hypoplasia³⁹. In humans, inherited and *de novo* variants in *ZFPM2* gene were identified predominantly in isolated CDH patients^{39, 40, 102}. *GATA4* and *SOX7* are both located on chromosome 8p23.1. *GATA4* interacts with *ZFPM2*¹⁰³ and is an important component of the retinoic signaling pathway¹⁰⁴. *Gata4* mice have cardiac, lung or diaphragm defects³⁶. In humans, mutations in *GATA4* have been associated with congenital heart defects^{105, 106} and CDH^{107, 108}. *Sox7* is another transcription factor. Haploinsufficiency of *Sox7* can produce anterior CDH in mice¹⁰⁹. No mutations in human CDH patients have yet been identified in *SOX7*.

Several lines of evidence suggest that haploinsufficiency of *NR2F2* causes human CDH. *NR2F2* is expressed in diaphragm, lungs and heart during embryonic development²⁷, and expression is regulated by the retinoid signaling pathway¹¹⁰. Homozygous tissue-specific ablation of *NR2F2* in mice cause posterolateral CDH²⁷. Furthermore, *NR2F2* physically interacts with *ZFPM2*, suggesting these two factors together with *GATA4* may cooperate in diaphragm development²⁸.

Several *de novo* and inherited damaging variants in *GATA6* have been identified in CDH patients,¹¹¹ many with additional cardiac or pancreatic malformations¹¹². In mice, *GATA6* is expressed in the septum transversum mesenchyme and the lateral PPFs^{113, 114}. *Gata6* expression was significantly decreased in PPF2 and in muscularized diaphragms in the nitrofen-induced CDH model¹¹⁵. Decreased *Gata6* expression results in the reduction of proliferating mesenchymal cells in nitrofen-exposed fetuses, causing malformed PPFs and consequently leading to diaphragmatic defects in the nitrofen-induced CDH model¹¹⁵.

De novo variants in Myelin regulatory factor (*MYRF*) recently have been reported in patients with phenotypes including CDH, CHD and urogenital anomalies^{12, 116, 117},

suggesting a new syndrome caused by *MYRF* mutations. MYRF is a membrane associated transcriptional factor which was originally identified to be essential for the development and maintenance of central nervous system myelination ¹¹⁸. It is highly expressed in the developing diaphragm ¹¹³. Analyzing the transcriptome of patient-derived diaphragm fibroblast cells suggest that disease associated variants are due to loss of function ¹².

The sonic hedgehog (Shh) signaling pathway has been shown to be involved in diaphragm development ¹¹³. The Shh transcription factors *GLI2* and *GLI3* knockout mice have CDH ¹¹⁹. *KIF7* (kinesin family member 7) and PBX1 (PBX Homeobox 1) are regulators of Gli3, a transcription factor in the Shh signaling pathway ¹²⁰. Homozygous *Kif7* mice ¹²¹ and *Pbx1* knockout mice have diaphragmatic defects ¹¹³. Haploinsufficiency of *PBX1* has recently been associated with multiple developmental defects including CDH ¹²². Two rare and predicted pathogenic variants in *KIF7* gene were identified in human CDH patients ⁹⁸.

Cellular migration, or the components of extracellular matrix (ECM)

Genes involved in cell migration, myogenesis or extracellular matrix formation are critical to normal diaphragm development ¹¹³. Mutations in genes such as *SLIT3*, *ROBO1*, *GPC3*, *NDST1*, *FREM1*, *FRAS1*, and *FREM2* are associated with CDH. *SLIT3* (slit guidance ligand 3) is one of the three human homologs of the *Drosophila Slit* gene. The slit proteins are secreted molecules with multiple roles in cell migration and adhesion through interacting with roundabout (Robo) homolog receptors to affect cell migration ^{123, 124}. *SLIT* genes are expressed in the central nervous system during development, but *SLIT3* is also expressed in the mesothelium of the diaphragm during embryonic development ¹²³. The majority of *Slit3* deficient mice developed central-type diaphragmatic hernias ^{123, 125}. No *SLIT3* mutations have been reported in humans with CDH to date. *ROBO1* encodes a surface membrane protein that functions in axon guidance and neuronal precursor cell migration and cardiac development ^{126, 127}. Most *Robo1* homozygous mice died at birth of respiratory failure because of delayed lung maturation, and ~6% of homozygous mice also exhibited CDH ¹²⁸. Recent studies identified a second Slit receptor heparin sulfate as an integral component of Slit-Robo signaling complex which stabilized the Slit-Robo-interaction ¹²⁹. Mutations in a heparin sulfate proteoglycan, *GPC3*, cause the CDH associated syndrome SGBS (discussed above). Loss of another heparin sulfate biosynthetic enzyme *NDST1* in mice also show defective diaphragm development ¹³⁰.

The FRAS/FREM extracellular matrix (ECM) proteins (*FRAS1*, *FREM1* and *FREM2*) form a complex and mediate cell adhesion and intercellular signaling during embryonic development ¹³¹. The FRAS1 related extracellular matrix1 (*FREM1*) gene encodes an extracellular matrix protein that plays a critical role in the development of multiple organs including diaphragm ¹³². Recessive loss-of-function mutations affecting *FREM1* have been shown to cause isolated CDH in humans and up to 47% of *Frem1* homozygous mice have CDH ¹³³. Biallelic variants were identified in a CDH cohort ¹³⁴. Diaphragmatic hernia was seen in 8% of *Frem2*-deficient mice and 1% of *Fras1*-deficient mice ¹³⁴. Loss of the *FREM1/FREM2/FRAS1* complex is thought to result in anterior sac CDH through its effects on mesothelial fold progression ¹³⁴.

Other genes identified by sequencing

Due to the historically high mortality with CDH, few familial studies of CDH have been conducted because of decreased reproductive fitness. The low recurrence rates¹³⁵ among siblings and the lack of family history in the majority of CDH patients with CDH support the model of *de novo* variants causing a substantial fraction of CDH, especially syndromic cases with low reproductive fitness. Exome/genome sequencing has supported the identification of new genes for CDH, often due to *de novo* mutations^{11–13}. Recent studies identified *de novo* and inherited variants in *GATA4*⁹⁷, *GATA6*⁹⁶, *ZFPM2*⁴⁰, and *MYH10*¹³⁶ for CDH (Figure 1). In the case series to date, few genes had more than two independent deleterious variants, suggesting that there is significant heterogeneity in genetic etiology, similar to congenital heart disease and neurodevelopmental disorders¹². Prioritizing among identified *de novo* or inherited rare variants is a challenge for the genes that are not recurrent. Studies across diseases may be helpful since many genes identified with deleterious *de novo* variants are highly enriched at the top quartile of expression in developing diaphragm, heart and brain^{11–13}, suggesting pleiotropy in complex cases. Since CHD and NDD are the most common syndromic features in CDH, evidence from genes involved across these conditions may help to prioritize risk genes in CDH. For example *STAG2*, which encodes a subunit of the cohesin complex and has been associated with intellectual disability and behavioral problems¹³⁷.

In our prior studies, 21% of complex cases and 12% of isolated CDH cases are attributable to *de novo* likely gene-disrupting or deleterious missense variants¹². Inherited coding variants, *de novo* noncoding variants, and somatic variants may also contribute to CDH and are currently being assessed⁶⁴ with genome sequencing and RNA sequencing.

Genetic counseling

Prenatal ultrasound screening enables early CDH diagnosis. Assessment of the family history should include prenatal or neonatal deaths of uncertain cause, congenital anomalies and NDD in family members. Diaphragm defects in other family members can be subtle, and MRI imaging in parents can identify small hernias or eventrations that are clinically asymptomatic but that suggest a higher risk of recurrence due to incomplete penetrance^{40, 96, 97}. Perinatal genetic data from chromosome microarray and fetal exome/genome sequencing may enable diagnosis of a genetic syndrome and refine prognosis and inform clinical management. But even with a comprehensive genetic evaluation, a definitive genetic cause may not be identified in the majority of CDH cases. In approximately 30% of non-isolated CDH cases a genetic etiology can be identified with a combination of karyotype, chromosome microarray and trio exome sequencing^{8–13}. Most of large chromosome anomalies are diagnosable with a conventional karyotype. Chromosome microarray detects small genomic deletions and duplications and offers incremental yield over karyotyping and is recommended in clinical evaluation. Chromosome anomalies are detected in ~10% of patients with CDH with standard clinical karyotype and chromosome microarray⁸. Exome sequencing including both parents is recommended, especially in patients with multiple anomalies or NDD. Approximately 10–22% of CDH patients have a *de novo* sequence variant that is a major contributing factor to CDH^{11–13, 98}. Because we do not yet know all

the genes causing CDH, reanalysis of the sequence data in the future may be helpful even if a diagnosis is not established. As *de novo* deleterious variant are identified in novel genes not previously associated with human disease, an immediate question is whether the identified genetic variant is expected to be associated with isolated CDH or to have pleiotropic effects on other organs such as the lungs, heart and brain. We use data from mice with comparable mutations and gene expression data across tissues to predict outcome based upon the type of gene implicated and phenotypes associated with mutations in other similar genes; however, we cannot always accurately predict the prognosis.

Identifying an established genetic etiology clarifies the recurrence risk for the family. The recurrence risk for parents to have another affected children after having one child with CDH is about 2% in families without a known family history or known genetic etiology¹²². A *de novo* genetic cause has a 1% recurrence risk for the parents but up to a 50% recurrence risk for the individual carrying the genetic mutation.

Conclusion

With the availability of new molecular genetic techniques to facilitate gene discoveries for CDH, our understanding of the role of genetics in the pathogenesis of CDH may make it possible to use preventative strategies and therapeutic interventions to partially alleviate the pulmonary disease in patients with CDH. Because CDH is so genetically heterogeneous, further studies need to include analysis of larger numbers of CDH patients using exome/genome sequencing, single cell sequencing or RNA sequencing of the developing PPF, bioinformatics analysis, and animal models to identify additional pathogenic genes and variants and determine the associated clinical features and outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Chromosomal anomalies and genes associated with CDH from mouse models and humans.

Duplicated (blue) and deleted (red) copy number variants identified in patients with CDH. Genes implicated by mouse models (black), monogenetic syndromes (green) and/or patients with isolated CDH or with multiple malformations (purple). Aneuploidies are not shown.

Table 1.

Transcription factors, cell migration and extracellular matrix genes associated with congenital diaphragmatic hernia

Gene	Gene name	Function	Chromosome location	genetic variant type in humans	CDH in mice model reference
<i>GATA4</i>	GATA binding protein 4	transcription factor	8p23.1	heterozygous missense ^{97, 108}	36
<i>WT1</i>	Wilms Tumor 1	transcription factor	11p13	heterozygous missense ⁸⁰	100, 101, 138
<i>ZFPM2</i>	Zinc Finger Protein, FOG Family Member 2	transcription factor	8q23	heterozygous missense/LGD ^{40, 102, 108, 139}	39
<i>SOX7</i>	SRY-Box 7	transcription factor	8p23.1	N/A	109
<i>GATA6</i>	GATA Binding Protein 6	transcription factor	18q11.2	heterozygous missense/LGD ^{96, 140, 141}	N/A
<i>MYRF</i>	Myelin Regulatory Factor	transcription factor	11q12.2	heterozygous missense/LGD ^{12, 116}	N/A
<i>NR2F2</i>	Nuclear Receptor Subfamily 2 Group F Member 2	transcription factor	15q26.2	heterozygous LGD ¹⁰⁸	27
<i>KIF7</i>	Kinesin Family Member 7	transcription factor	15q26.1	heterozygous missense ⁹⁸	142
<i>GLI3</i>	GLI Family Zinc Finger 3	transcription factor	7p14.1	N/A	119
<i>PBX1</i>	PBX Homeobox 1	transcription factor	1q23.3	heterozygous missense ^{108, 122}	113
<i>SLIT3</i>	Slit Guidance Ligand 3	cell migration	5q34-q35.1	N/A	123, 125
<i>ROBO1</i>	Roundabout Guidance Receptor 1	cell migration	3p12.3	heterozygous LGD ¹⁴³	128
<i>GPC3</i>	Glypican 3	cell migration	Xq26.2	heterozygous LGD ¹⁴⁴	N/A
<i>NDST1</i>	N-Deacetylase And N-Sulfotransferase 1	cell migration	5q33.1	N/A	130
<i>FREM1</i>	FRAS1 Related Extracellular Matrix 1	extracellular matrix	9p22.3	biallelic ¹³³	133
<i>FRAS1</i>	Fraser Extracellular Matrix Complex Subunit 1	extracellular matrix	4q21.21	N/A	134
<i>FREM2</i>	FRAS1 Related Extracellular Matrix Protein 2	extracellular matrix	13q13.3	N/A	134

CDH, congenital diaphragmatic hernia; LGD, likely gene disrupting, N/A, not available