HHS Public Access

Author manuscript

J Med Genet. Author manuscript; available in PMC 2023 March 01.

Published in final edited form as:

J Med Genet. 2022 March ; 59(3): 270–278. doi:10.1136/jmedgenet-2020-107317.

Clinical exome sequencing data reveals high diagnostic yields for congenital diaphragmatic hernia plus (CDH+) and new phenotypic expansions involving CDH

Tiana M. Scott[1,2], **Ian M. Campbell**[3], **Andres Hernandez-Garcia**[4], **Seema R. Lalani**[2,4], **Pengfei Liu**[4,5], **Chad A. Shaw**[4], **Jill A. Rosenfeld**[4], **Daryl A. Scott**[2,4,6,*]

[1)]Department of Microbiology and Molecular Biology, College of Life Sciences, Brigham Young University, Provo, UT, 84602, USA

[2)]Texas Children's Hospital, Houston, TX, 77030, USA

[3)]Division of Human Genetics, Children's Hospital of Philadelphia, Philadelphia, PA, 19104, USA

[4)]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, 77030, USA

[5)]Baylor Genetics, Houston, TX, 77021, USA

[6)]Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX, 77030, USA

Abstract

Background: Congenital diaphragmatic hernia (CDH) is a life-threatening birth defect that often co-occurs with non-hernia-related anomalies (CDH+). While copy number variant (CNV) analysis is often employed as a diagnostic test for CDH+, clinical exome sequencing (ES) has not been universally adopted.

Methods: We analyzed a clinical database of ~12,000 test results to determine the diagnostic yields of exome sequencing in CDH+ and to identify new phenotypic expansions.

Results: Among the 76 cases with an indication of CDH+, a molecular diagnosis was made in 28 cases for a diagnostic yield of 37% (28/76). A provisional diagnosis was made in seven other cases (9%; 7/76). Four individuals had a diagnosis of Kabuki syndrome caused by frameshift variants in *KMT2D*. Putatively deleterious variants in *ALG12* and *EP300* were each found in two individuals, supporting their role in CDH development. We also identified individuals with de novo pathogenic variants in *FOXP1* and *SMARCA4*, and compound heterozygous pathogenic variants in *BRCA2*. The role of these genes in CDH development is supported by the expression of their mouse homologs in the developing diaphragm, their high CDH-specific pathogenicity

[*]Correspondence Daryl A. Scott, R813, One Baylor Plaza. BCM225, Houston, TX 77030, USA, dscott@bcm.edu, Phone: +1 713-203-7242.

COMPETING INTERESTS

The Department of Molecular and Human Genetics at Baylor College of Medicine derives revenue from the clinical ES performed at Baylor Genetics.

scores generated using a previously validated algorithm for genome-scale knowledge synthesis, and previously published case reports.

Conclusion: We conclude that ES should be ordered in cases of CDH+ when a specific diagnosis is not suspected and CNV analyses are negative. Our results also provide evidence in favor of phenotypic expansions involving CDH for genes associated with *ALG12*-congenital disorder of glycosylation, Rubinstein-Taybi syndrome, Fanconi anemia, Coffin-Siris syndrome and *FOXP1*-related disorders.

Keywords

Congenital diaphragmatic hernia; exome sequencing; diagnostic yield; EP300; Rubinstein-Taybi syndrome; ALG12-congenital disorder of glycosylation; Fanconi anemia; Coffin-Siris syndrome; FOXP1; Kabuki syndrome

INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a life-threatening birth defect that affects approximately 1 in 4,000 newborns [1]. In approximately 40-60% of cases, CDH occurs in conjunction with other non-hernia-related anomalies (CDH+) [2]. The mortality rate for newborns treated in surgical centers is approximately 30%, but this may be a significant underestimate due to pregnancy termination and postnatal mortality prior to transfer to a surgical center [3]. The mortality rate is particularly high in the 25-40% of cases in which CDH co-occurs with congenital heart defects (CDH+/CHD) [4]. Among CDH survivors, chronic oxygen dependency, gastroesophageal reflux, poor growth and cognitive delay are common [5].

A variety of chromosomal, genomic, and single gene disorders are associated with an increased risk for developing CDH [6]. In some of these disorders, CDH is a major clinical feature and is found in a high percentage of affected individuals. Examples of such disorders include Donnai-Barrow syndrome (MIM #222448, *LRP2*), microphthalmia, syndromic 9 (MIM #601186, *STRA6*) and cardiac-urogenital syndrome (MIM #618280, *MYRF*), also known as cardiac-urogenital-diaphragm-lung (CUDL) syndrome [7-10]. In other disorders, CDH is not a major clinical feature but is found at a frequency that is higher than what would be expected by random chance. Examples of such disorders include Simpson-Golabi-Behmel syndrome, type 1 (MIM #312870, *GPC3*), focal dermal hypoplasia (MIM #305600, *PORCN*), also known as Goltz-Gorlin syndrome, and Marfan syndrome (MIM #154700, *FBN1*) [11-13]. Despite advances in our understanding of the genes that contribute to the development of CDH, the genetic variants that contribute to most cases of isolated CDH and CDH+ have not been identified [14].

Array-based copy number variant (CNV) analysis and exome sequencing (ES) studies are routinely used to identify genetic alterations in individuals with more than one congenital anomaly, particularly in cases where a specific diagnosis is not clinically apparent. The diagnostic and clinical utility of array-based CNV analyses has been well established, and is clearly indicated, in individuals with CDH+ [6]. Although studies have shown that ES can be

used to identify a molecular diagnosis in individuals with CDH+ [7 14 15], ES has not been universally adopted as a diagnostic test for this disorder.

Here we analyze a clinical database of ~12,000 test results to determine the diagnostic yield of ES in individuals with CDH+ and identify new phenotypic expansions involving CDH.

METHODS

Database analysis and clinical review

We searched coded information from a clinical database of ~12,000 individuals who were referred to Baylor Genetics for ES (accessed May, 2019) for individuals with CDH or CDH+ based on phenotypes included in their test indications. Individuals with an indication of diaphragmatic eventration or an abnormality of the diaphragm, and fetuses/newborns with an indication of a suspected CDH, diaphragmatic eventration or diaphragm abnormality, were also included. In this manuscript, we refer to all of these individuals as having CDH. Individuals whose diaphragmatic defect was described only as paralysis, or atrophy in the setting of systemic muscular atrophy, or for whom a diagnosis was made using array-based CNV detection assays, were not included in this study.

Each reported variant was classified as pathogenic, likely pathogenic, a variant of unknown significance (VUS), likely benign, or benign based on American College of Medical Genetics and Genomics (ACMG) standards for the interpretation of sequence variants and using the most current information available publicly and in local databases (Nov. 2020) [16]. The ES results of individuals with CDH had also been previously classified by clinical laboratory geneticists as solved, probably solved, possibly solved, partially solved, or unsolved. All cases that were not considered unsolved were then independently reviewed by a clinical geneticist to determine if a definitive, probable, or provisional diagnosis could be made based on molecular findings and phenotypic information contained in the test identification. The designation “partially solved” was retained as an indication that the diagnosis could only explain a distinct subset of the phenotypes included in the test indication.

A definitive diagnosis was made if the individual carried pathogenic variant(s) in a gene whose inheritance was consistent with a disorder associated with that gene, and the phenotypic data provided in the indication were suggestive of the disorder. A definitive diagnosis was also made if the case was previously published regardless of the variant designation made by the laboratory.

A probable diagnosis was made if one of the following criteria were met: 1) the individual carried a likely pathogenic variant in a gene associated with an autosomal dominant or x-linked gene whose inheritance was consistent with a disorder associated with that gene, and the phenotypic data provided in the indication were suggestive of the disorder, 2) the individual carried a pathogenic variant and a likely pathogenic variant or VUS in trans, or two likely pathogenic variants in trans, in a gene associated with an autosomal recessive disorder, and the phenotypic data provided in the indication were suggestive of the disorder, or 3) the individual was mosaic for a pathogenic or likely pathogenic variant in an autosomal

dominant gene, or an X-linked gene in a male, and the phenotypic data provided in the indication were suggestive of the disorder.

A provisional diagnosis was made if one of the following criteria were met: 1) the individual carried only a single pathogenic variant in a gene associated with an autosomal recessive disease, and the phenotypic data provided in the indication were suggestive of the disorder, 2) the individual carried two VUSs in trans, or a likely pathogenic variant and a VUS in trans, in a gene associated with an autosomal recessive disease, and the phenotypic data provided in the indication were suggestive of the disorder, 3) the individual was a female who carried a heterozygous pathogenic variant in a gene associated with an X-linked disorder, and the phenotypic data provided in the indication were suggestive of the disorder, or 4) the individual carried a VUS(s) in a gene whose inheritance was consistent with a disorder associated with that gene, and the phenotypic data provided in the indication were suggestive of the disorder.

The number of cases with a definitive or probable molecular diagnosis was then divided by the total number of CDH+ cases to determine the diagnostic yield. This process was then repeated for isolated CDH cases, and CDH+ cases in which CHD was reported in the test indication with the exception of: 1) isolated patent foramen ovale and/or patent ductus arteriosus which may persist in the presence of pulmonary hypertension, 2) dextroposition which, in the setting of CDH, is most likely a secondary finding due to the intrusion of the abdominal viscera into the thorax, 3) dilated aorta in an individual with a definitive, probable, or provisional diagnosis of a connective tissue disorder associated with aortic dilation, 4) isolated functional anomalies such as valve regurgitation, 5) isolated ventricular hypertrophy, or 6) cardiomyopathy.

Calculation of CDH frequency

For genes that were recurrently implicated in the development of CDH in our cohort, we searched coded information from a clinical ES database at Baylor Genetics to determine the frequency of CDH among all individuals in which the gene was suspected to contribute to phenotypes listed in the testing indication. Individuals in the database for whom no phenotypes were provided in the test indication were not included since it could not be determined whether they had CDH.

Human subjects research

This work was approved by the institutional review board of Baylor College of Medicine (protocol H-47546) and was conducted in accordance with the ethical standards of this institution’s committee on human research and international standards.

Literature and database searches

We searched for reports in which CDH candidate genes and/or their associated genetic disorders were mentioned in conjunction with key words such as diaphragm, diaphragmatic hernia, CDH, or diaphragmatic eventration.

Genomic knowledge fusion

We have previously developed and validated a computational algorithm whose core function is to integrate large-scale genomic knowledge from various sources—Gene Ontology (GO), Mouse Genome Informatics (MGI) phenotype annotations, the Protein Interaction Network Analysis (PINA), the Kyoto Encyclopedia of Genes and Genomics (KEGG) molecular interaction network data and micro RNA (miRNA) targeting data, the GeneAtlas expression distribution, and transcription factor binding and epigenetic histone modification data from the NIH Roadmap Epigenomics Mapping Consortium—to develop a phenotype-specific score that can be used to rank genes based on their similarity to a set of training genes known to cause that phenotype in humans and/or mice [17]. We have previously used this algorithm to generate CDH-specific pathogenicity scores for all RefSeq genes [18]. The CDH pathogenicity scores reported here represent the centile rank of each gene as compared to all other RefSeq genes.

Statistical analyses

For comparisons of the diagnostic yield between groups, we performed two-tailed Fisher's exact tests were performed using a 2 X 2 contingency table calculator available through GraphPad QuickCalcs (https://www.graphpad.com/quickcalcs/contingency1/). When defining the penetrance of CDH associated with genetic disorders, 95% confidence intervals were calculated using a binomial "exact" calculator available through the UCSF Clinical and Translational Science Institute (http://www.sample-size.net/confidence-interval-proportion/).

RESULTS

Diagnostic yield of clinical ES

In a clinical database of approximately 12,000 individuals who were referred for ES, we identified 76 individuals with CDH+ based on phenotypes included in their test indications (Table 1, Table S1). ES provided a definitive (n = 19; 25%) or a probable (n = 9; 12%) diagnosis in 28 individuals for a diagnostic yield of 37% (28/76). In addition, 7 provisional diagnoses were made. If these were included, the diagnostic yield would be 46% (35/76).

In the subset of 37 individuals with CDH+/CHD, a definitive (n = 9; 24%) or probable (n = 3; 8%) diagnosis was made in 12 individuals for a diagnostic yield of 32% (12/37). In addition, 3 provisional diagnoses were made. If these were included, the diagnostic yield would be 41% (15/37).

In the same clinical database, we identified 6 patients with what appeared to be isolated CDH based on their test indications. Among these individuals, ES provided a diagnosis in only one individual for a diagnostic yield of 16.7% (1/6). This individual, Subject 36, carried a maternally inherited c.1396_1399dup, p.(Y467fs) pathogenic variant in *ZFPM2*, the gene associated with autosomal dominant diaphragmatic hernia 3 (MIM #610187). CDH associated with *ZFPM2* haploinsufficiency is known to be incompletely penetrant [22 23], which provides an explanation of why the mother in this family was unaffected, and yet this individual's brother and sister both had CDH.

The diagnostic yields of ES were not statistically different between individuals with CDH+ (28/76) and the subset of individuals with CDH+/CHD (12/37)(P = 0.68). The same is true between the diagnostic yields of individuals with CDH+ with (12/37) or without CHD (16/39)(P = 0.48). Although the diagnostic yield of ES in CDH+ and CDH+/CHD cases were higher than that seen associated with isolated CDH (1/6), the isolated CDH sample size was too low to reach statistical significance (P = 0.42 and P = 0.65, respectively).

Recurrently altered genes

Variants in six genes were identified in more than one individual with CDH or CDH+ in our cohort. We identified four individuals, Subjects 18-21 (5.3%; 4/76), who carried frameshift variants in *KMT2D*, the gene associated with Kabuki syndrome 1 (MIM #147920). Subsequent to our initial analysis, a male with CDH, hypoplastic left heart, and posteriorly rotated ears was referred for ES and was found to carry a de novo c.7223dupT, p.(S2409Lfs*25) [NM_003482.3] pathogenic variant in *KMT2D*. We then searched the database to determine the frequency of CDH among all individuals in which *KMT2D* was suspected to contribute to phenotypes listed in the testing indication. We found the CDH penetrance to be 5/56 (8.9%; 95% confidence interval (CI) = 3% - 19.6%).

Three individuals, Subjects 1-3 (3/76; 3.9%), carried de novo pathogenic variants in *ABL1*, the gene associated with congenital heart defects and skeletal malformations syndrome (MIM #617602). Two of these individuals have been published previously [19]. We found a total of 9 individuals in the clinical database who carried *ABL1* variants that were thought to contribute to phenotypes listed in the testing indication. This suggests that CDH is a relatively common finding in individuals with congenital heart defects and skeletal malformations syndrome (33%; 3/9), but this small number of cases is insufficient to generate a clear estimate of penetrance (CI = 7.5% to 70.1%).

Similarly, two individuals, Subjects 25 and 26 (2/76; 2.6%), whose cases were previously published, carried pathogenic variants in *MYRF*, the gene associated with cardiac-urogenital syndrome (MIM #618280) [7 8]. Subsequent to our initial analysis, a male with CDH, dextrocardia, hypoplastic left heart syndrome, pulmonary vein stenosis, a bifid scrotum, cryptorchidism, and hypospadias was identified who carried a non-maternally inherited c.2057G>A, p.(R686H) [NM_013279.4] VUS in *MYRF*. Including this individual, 75% (3/4) of individuals in our database who carried putatively causative *MYRF* variants had CDH. This suggests that CDH is commonly seen in individuals with cardiac-urogenital syndrome, but this small number of cases is insufficient to generate a clear estimate of penetrance (95% CI = 19.4% to 99.4%)

As previously mentioned, Subject 36 was found to carry a maternally inherited pathogenic variant in *ZFPM2*. A second individual, Subject 35, with both CDH and a sinus venosus atrial septal defect (CDH+/CHD), was also found to carry a heterozygous pathogenic variant in *ZFPM2*, consistent with this gene's known function in cardiac development [24 25]. In our database, we found one other individual with tetralogy of Fallot who carried a pathogenic variant in *ZFPM2*. Identification of CDH in 66% (2/3) of individuals in our database is consistent with a relatively high CDH penetrance, but this small number of cases

is insufficient to generate a clear estimate of penetrance (95% CI = 9.4% to 99.2%). We also note that *ZFMP2*-related disorders are associated with incomplete penetrance.

Two individuals, Subjects 11 and 12 (2/77; 2.6%), were found to carry heterozygous variants in *EP300*, the gene associated with Rubinstein-Taybi syndrome 2 (MIM #613684). The first carried a de novo, mosaic (12%) pathogenic *EP300* variant. The second was a female who carried a c.3592T>C, p.(Y1198H) VUS in *EP300*, and also carried a c.3298G>T, p.(D1100Y) VUS in *SOS1*, the gene associated with autosomal dominant, Noonan syndrome 4 (MIM #610733), and a heterozygous, pathogenic c.−142C>G, p.? variant in *DKC1*, the gene associated with dyskeratosis congenita, X-linked (MIM #305000). The inheritance pattern of these variants could not be determined due to a lack of parental samples, and the phenotypes provided in the indication are not sufficient to clearly delineate which of these variants, alone or in combination, are contributing to her symptoms. A total of 20 individuals in the clinical database carried *EP300* variants that were thought to contribute to phenotypes listed in the testing indication. This suggests that CDH occurs in a low percent of individuals with Rubinstein-Taybi syndrome 2 (10%; 95% CI = 1.2% to 31.7%).

Two individuals, Subjects 5 and 6 (2/76; 2.6%), were found to carry compound heterozygous pathogenic variants in *ALG12*, the gene associated with *ALG12*-congenital disorder of glycosylation (*ALG12*-CDG, formerly CDG Ig; MIM #607143) [26]. These individuals were the only ones in the clinical database (2/2, 100%) in which *ALG12* variants in trans were thought to contribute to phenotypes listed in the testing indication.

Other novel candidate genes for CDH

Among the 76 individuals with CDH+, several carried putatively deleterious variants in genes that are clearly associated with an increased risk of developing CDH including *ABL1, B3GALT6, FBN1, HCCS, KMT2D, LRP2, MRYF, POGZ, SLC2A10* and *ZFPM2* [7 19]. The remainder carried putatively deleterious variants in genes whose association with CDH has not been clearly established (Table S1). To determine if deleterious variants in these genes were likely to be associated with the development of CDH, we determined whether each gene is expressed in the developing mouse diaphragm at embryonic day (E)11.5, E12.5 and E16.5 based on whole-transcriptome expression profiles published by Russell et al [27]. We then determined if these genes were associated with high CDH-specific pathogenicity scores (≥85% based on all RefSeq genes), previously generated using a validated genome-scale knowledge fusion algorithm [17 18]. We also performed a literature review to identify reports in which these genes, or their corresponding syndromes, have been previously linked to CDH. Based on these data we determined which candidate genes, or their associated syndromes, had sufficient evidence to support a phenotypic expansion including CDH (Table 2) and which, currently, did not (Table 3).

DISCUSSION

The use of clinical ES has been shown to change medical management and genetic counseling regardless of testing outcome [35 36]. The diagnosis of a specific genetic syndrome may reveal the need to address medical problems or risks that may not have been recognized prior to the diagnosis and can help to reduce medical waste by minimizing

the use of tests, imaging studies and medical interventions that are unlikely to provide significant benefit. ES also has the potential to identify novel genes that cause medically important phenotypes including CDH [7 14 15]. Despite these benefits, ES has not been universally adopted as a diagnostic test in individuals with CDH+. This may be due to uncertainty regarding the diagnostic yield of clinical ES in these cases.

The high diagnostic yield of clinical ES in CDH+

Using information from a clinical database, we estimated the diagnostic yield of ES in individuals with CDH+. Among the 76 individuals with CDH+ in the database, ES was able to identify a diagnosis in 28 individuals for a diagnostic yield of 37% (28/76). In the subset of 37 individuals with CDH+/CHD, a diagnosis was made in 12 individuals for a diagnostic yield of 32% (12/37). This suggests that clinical ES can be used to identify a molecular diagnosis in a significant percentage of individuals with CDH+.

Although the diagnostic yield of clinical ES in CDH+ cases was not statistically different than the 16.7% (1/6) diagnostic yield seen in individuals with isolated CDH ($P = 0.42$), we note that the number of isolated CDH cases in the database was too low to allow us to confidently estimate the diagnostic yield in this population (CI = 0.4% - 64.1%). We also note that Subject 36, the only individual with isolated CDH who was positive on ES, has a positive family history of CDH in a brother and sisters.

CDH is a relatively common phenotype associated with Kabuki syndrome 1

Four individuals, Subjects 18-21, carried frameshift variants in *KMT2D*, the gene associated with Kabuki syndrome 1 (MIM #147920). This represented 5% (4/76) of our CDH+ cohort and 14% (4/28) of the subset of individuals with a molecular diagnosis. After completing our initial analysis, we identified a fifth patient with CDH+ who carried a *KMT2D* frameshift variant. By reviewing all cases in our clinical database, we estimated the CDH penetrance in Kabuki syndrome 1 to be 8.9% (5/56; CI = 3% - 19.6%). In contrast, no individuals with CDH+ were found to carry putatively causative variants in *KDM6A*, the gene associated with Kabuki syndrome 2 (MIM #300867).

Phenotypic expansions involving CDH

Phenotypes with relatively high penetrance levels are easily to identify using small cohorts of affected individuals. As penetrance drops, larger cohorts are needed to conclude that a phenotype is associated with a disorder based on clinical descriptions alone. Here we seek to identify known human disease genes that contribute to the development of CDH but have previously escaped detection due to their low levels of CDH penetrance. In attempting to do so, we recognize that CDH is a relatively common birth defect with an incidence of approximately 1 in 4,000 newborns [1]. As a result, the CDH seen in some of the individuals in this cohort may not be associated with the variants we identified by clinical ES. Instead, such cases could represent single or dual diagnoses in which the causative pathogenic variant was not discovered by ES, or cases in which CDH was associated with a multifactorial inheritance pattern in which a variety of genetic, environmental, and stochastic factors lead to abnormal diaphragm development.

To distinguish which genes harboring putatively pathogenic variants were more likely to be associated with the development of CDH, we considered how frequently variants in these genes were seen in our cohort, and we determined if the mouse homologs of these genes have been shown to be expressed in the developing mouse diaphragm [27], if these genes had high CDH-specific pathogenicity scores (≥85%) [17 18], and whether there was additional evidence in the literature to support the association of these genes—or their corresponding genetic disorders—with CDH. Evidence in favor of a phenotypic expansion involving CDH was highest for: *ALG12, EP300, BRCA2, FOXP1*, and *SMARCA4* (Table 1).

We identified two fetuses, Subjects 5 and 6, which had CDH+ associated with diagnoses of *ALG12*-CDG (MIM #607143). This disorder is characterized by neurodevelopmental phenotypes and a variety of structural birth defects involving the brain, heart, genitourinary system, and skeleton and is commonly lethal in the newborn period [26]. Although *ALG12* has a relatively low CDH-specific pathogenicity score (48.2%), its mouse homolog is expressed in the developing mouse diaphragm. Based on these findings, we conclude that CDH is a feature of *ALG12*-CDG.

Rubinstein-Taybi syndrome can be caused by autosomal dominant, pathogenic variants in *CREBBP* (type 1; MIM #180849) or *EP300* (type 2; MIM #613684) and is characterized by neurodevelopmental phenotypes, distinct facial features, broad thumbs and first toes, short stature and a high rate of CHD and renal anomalies [37]. Benjamin et al. reported a possible association between CDH and Rubinstein-Taybi syndrome prior to the discovery of the genes that cause this disorder [29]. We identified two individuals, Subjects 11 and 12, with variants in *EP300* but none in *CREBBP*. Subject 12 also carried a pathogenic variant in *DKC1*, the gene associated with dyskeratosis congenita, X-linked (MIM #305000), and a variant of unknown significance in *SOS1*, the gene associated with Noonan syndrome 4 (MIM #610733). It is possible that these variants contributed to the development of CDH in this individual, particularly since CDH has been previously documented in individuals with Noonan syndrome [7 33 34]. We note that the mouse homologs of *EP300* and *CREBBP* are expressed in the developing mouse diaphragm [27] and have high CDH-specific pathogenicity scores (94.7% and 91.6% respectively). This leads us to conclude that CDH is a phenotype that can be associated with Rubinstein-Taybi syndrome, particularly Rubinstein-Taybi syndrome 2, which is caused by pathogenic variants in *EP300*.

Fanconi anemia is a genetically heterogeneous bone marrow failure syndrome associated with an increased risk for cancer and a variety of structural birth defects [38]. We identified two individuals, Subjects 9 and 13, with CDH+ who carried changes in Fanconi anemia genes. Subject 9 had a definitive diagnosis of Fanconi anemia, complementation group D1 (MIM #605724) caused by compound heterozygous, pathogenic variants in *BRCA2*. The family history of this individual was positive for a paternal aunt with breast cancer and maternal great-grandmother with pancreatic cancer. This is consistent with transmission of heterozygous, pathogenic *BRCA2* variants, and a diagnosis of autosomal dominant *BRCA2*-associated hereditary breast and ovarian cancer syndrome (MIM #612555, #613347) on both sides of the family. Subject 13 had a provisional diagnosis of Fanconi anemia,

complementation group I (MIM #609053) and phenotypes suggestive of this disorder. However, only a single pathogenic variant in *FANCI* was identified.

The mouse homologs of *BRCA2* and *FANCI* are expressed in the developing mouse diaphragm [27], and CDH has been reported in at least one other individual who was clinically diagnosed with Fanconi anemia [28]. *BRCA2* had a high CDH-specific pathogenicity score of 88.8%, while *FANCI*'s was much lower at 25%. These results leads us to conclude that CDH is a phenotype that can be associated with Fanconi anemia, particularly Fanconi anemia, complementation group D1, which is caused by biallelic pathogenic variants in *BRCA2*. In contrast, our results do not provide any evidence that individuals who carry a single pathogenic variant in *BRCA2*—or other genes that cause autosomal recessive forms of Fanconi anemia—have an increased risk of developing CDH.

Coffin-Siris syndrome is a genetically heterogeneous disorder characterized by neurodevelopmental phenotypes, distinctive facial features, hypoplasia of the distal phalanx or nail of the fifth digits, and congenital anomalies involving the central nervous, cardiac, gastrointestinal and genitourinary systems [39]. Subject 31 had a definitive diagnosis of Coffin-Siris syndrome 4 (MIM #614609) caused by a pathogenic variant in *SMARCA4*. Subject 32 had a provisional diagnosis of Coffin-Siris syndrome 8 (MIM #618362) and carried a splice junction variant in *SMARCC2*. Previous reports have suggested that Coffin-Siris syndrome is associated with an increased risk of CDH [30-32]. However, a specific association between CDH and *SMARCA4* and *SMARCC2* has not been suggested previously. The homologs of these genes are expressed in the developing mouse diaphragm and both have high CDH-specific pathogenicity scores of 93.7% and 89.9% respectively. This leads us to conclude that CDH is a phenotype that can be associated with Coffin-Siris syndrome, particularly Coffin-Siris syndrome 4 caused by pathogenic variants in *SMARCA4*.

Pathogenic variants in *FOXP1* have been shown to cause mental retardation with language impairment and with or without autistic features (MIM #613670), a genetic disorder characterized by neurodevelopmental phenotypes, dysmorphic features, and congenital anomalies of the kidney and urinary tract [40 41]. Subject 16 had a definitive diagnosis of mental retardation with language impairment and with or without autistic features. At least one other individual with CDH associated with a *FOXP1* variant has been published previously [7]. The mouse homolog of *FOXP1* is expressed in the developing diaphragm, and *FOXP1* has a CDH-specific pathogenicity score of 96.9%. This leads us to conclude that CDH is a phenotype that can be associated with mental retardation with language impairment and with or without autistic features.

Putatively pathogenic variants in *ANKRD11, FOXC2, MED12, MCPH1, RASA1*, and *TCF12* were identified in one individual with CDH+. The mouse homologs of these genes are expressed in the developing diaphragm and all have CDH-specific pathogenicity scores ≥80%. Although these findings provide some evidence that deleterious variants in these genes may predispose to the development of CDH, we feel that the data at hand are insufficient to allow us to conclude that CDH is a phenotype that can be associated with their respective genetic disorders. Additional evidence for an association may came from the

publication of more human cases or evidence of diaphragmatic defects in transgenic mouse models. Experience suggests that dedicated diaphragm studies may be needed to identify CDH in existing mouse models due to early lethality and incomplete penetrance which can vary based on genetic background [42-45].

Study limitations

This study was based on coded data available from a clinical database. As such, we recognize several limitations. First, this is a retrospective study on a subset of patients with CDH+ referred for clinical ES. As a result, it is possible that our cohort may represent a selected population of individuals with CDH+ in which there was a higher likelihood of an underlying genetic diagnosis. This type of selection bias would tend to inflate the diagnostic yield. At the same time, individuals with CDH+ for whom a genetic diagnosis could have been made by clinical ES may not have been referred since a molecular diagnosis was made using single gene or panel testing. The exclusion of these individuals from our cohort would tend to lower the diagnostic yield. With this in mind, we would suggest that future studies aimed at confirming the results obtained here be conducted in a prospective manner in which clinical ES is ordered on all individuals presenting with CDH+, or on all individuals with CDH+ for whom array-based CNV analysis was negative.

The second limitation of this study is our inability to access additional clinical and molecular information on individuals in our cohort. As a result, we cannot indicate what percentage of individuals in our cohort underwent a chromosome analysis, array-based CNV analysis, or targeted molecular testing. We are also unable to access information that might have allowed us to confirm or refute a particular diagnosis. This lack of information is unlikely to be of great importance with regards to individuals who received a definitive diagnosis based on their ES results but may have allowed us to reclassify individuals who received a probable or provisional diagnosis. This limitation could be minimized in a prospective study in which researchers have full access to the medical record.

Recommendations for clinical practice

Given the high diagnostic yield of clinical ES we identified in our cohort of individuals with CDH+ (37%; 28/76), we conclude that ES should be ordered in cases of CDH+ when a specific diagnosis is not suspected and CNV analyses are negative. Physicians and surgeons should also consider ordering clinical ES in cases of isolated CDH in which there is a positive family history. We also conclude that CDH is a relatively common phenotype associated with Kabuki syndrome 1, and that CDH may be a phenotype associated with *ALG12*-congenital disorder of glycosylation, Rubinstein-Taybi syndrome, Fanconi anemia, Coffin-Siris syndrome and *FOXP1*-related disorders. With this in mind, physicians should not discount the possibility that a patient has one of these diagnoses based on their also having CDH.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

FUNDING

This research was funded in part by NIH/NICHD grant HD098458 to D.A.S.

REFERENCES

1. Langham MR Jr., Kays DW, Ledbetter DJ, Frentzen B, Sanford LL, Richards DS. Congenital diaphragmatic hernia. Epidemiology and outcome. Clin Perinatol 1996;23(4):671–88. [PubMed: 8982563]
2. Pober BR. Genetic aspects of human congenital diaphragmatic hernia. Clin Genet 2008;74(1):1–15 doi: 10.1111/j.1399-0004.2008.01031.x. [PubMed: 18510546]
3. Brownlee EM, Howatson AG, Davis CF, Sabharwal AJ. The hidden mortality of congenital diaphragmatic hernia: a 20-year review. J Pediatr Surg 2009;44(2):317–20 doi: 10.1016/j.jpedsurg.2008.10.076. [PubMed: 19231525]
4. Gray BW, Fifer CG, Hirsch JC, Tochman SW, Drongowski RA, Mychaliska GB, Kunisaki SM. Contemporary outcomes in infants with congenital heart disease and bochdalek diaphragmatic hernia. Ann Thorac Surg 2013;95(3):929–34 doi: 10.1016/j.athoracsur.2012.07.010. [PubMed: 22939449]
5. Juretschke LJ. Congenital diaphragmatic hernia: update and review. J Obstet Gynecol Neonata Nurs 2001;30(3):259–68.
6. Longoni M, Pober BR, High FA. Congenital Diaphragmatic Hernia Overview. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, Amemiya A, editors. GeneReviews® Seattle (WA), 1993.
7. Qi H, Yu L, Zhou X, Wynn J, Zhao H, Guo Y, Zhu N, Kitaygorodsky A, Hernan R, Aspelund G, Lim FY, Crombleholme T, Cusick R, Azarow K, Danko ME, Chung D, Warner BW, Mychaliska GB, Potoka D, Wagner AJ, ElFiky M, Wilson JM, Nickerson D, Bamshad M, High FA, Longoni M, Donahoe PK, Chung WK, Shen Y. De novo variants in congenital diaphragmatic hernia identify MYRF as a new syndrome and reveal genetic overlaps with other developmental disorders. PLoS Genet 2018;14(12):e1007822 doi: 10.1371/journal.pgen.1007822. [PubMed: 30532227]
8. Rossetti LZ, Glinton K, Yuan B, Liu P, Pillai N, Mizerik E, Magoulas P, Rosenfeld JA, Karaviti L, Sutton VR, Lalani SR, Scott DA. Review of the phenotypic spectrum associated with haploinsufficiency of MYRF. Am J Med Genet A 2019;179(7):1376–82 doi: 10.1002/ajmg.a.61182. [PubMed: 31069960]
9. Kantarci S, Al-Gazali L, Hill RS, Donnai D, Black GC, Bieth E, Chassaing N, Lacombe D, Devriendt K, Teebi A, Loscertales M, Robson C, Liu T, MacLaughlin DT, Noonan KM, Russell MK, Walsh CA, Donahoe PK, Pober BR. Mutations in LRP2, which encodes the multiligand receptor megalin, cause Donnai-Barrow and facio-oculo-acoustico-renal syndromes. Nat Genet 2007;39(8):957–9 doi: 10.1038/ng2063. [PubMed: 17632512]
10. Pasutto F, Sticht H, Hammersen G, Gillessen-Kaesbach G, Fitzpatrick DR, Nurnberg G, Brasch F, Schirmer-Zimmermann H, Tolmie JL, Chitayat D, Houge G, Fernandez-Martinez L, Keating S, Mortier G, Hennekam RC, von der Wense A, Slavotinek A, Meinecke P, Bitoun P, Becker C, Nurnberg P, Reis A, Rauch A. 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(3):550–60 doi: 10.1086/512203. [PubMed: 17273977]
11. Beck TF, Campeau PM, Jhangiani SN, Gambin T, Li AH, Abo-Zahrah R, Jordan VK, Hernandez-Garcia A, Wiszniewski WK, Muzny D, Gibbs RA, Boerwinkle E, Lupski JR, Lee B, Reardon W, Scott DA. FBN1 contributing to familial congenital diaphragmatic hernia. Am J Med Genet A 2015;167A(4):831–6 doi: 10.1002/ajmg.a.36960[. [PubMed: 25736269]
12. Kunze J, Heyne K, Wiedemann HR. Diaphragmatic hernia in a female newborn with focal dermal hypoplasia and marked asymmetric malformations (Goltz-Gorlin syndrome). Eur J Pediatr 1979;131(3):213–8. [PubMed: 477680]

13. Chen E, Johnson JP, Cox VA, Golabi M. Simpson-Golabi-Behmel syndrome: congenital diaphragmatic hernia and radiologic findings in two patients and follow-up of a previously reported case. Am J Med Genet 1993;46(5):574–8 doi: 10.1002/ajmg.1320460523. [PubMed: 8322824]

14. Yu L, Hernan RR, Wynn J, Chung WK. The influence of genetics in congenital diaphragmatic hernia. Semin Perinatol 2019:151169 doi: 10.1053/j.semperi.2019.07.008. [PubMed: 31443905]

15. Longoni M, High FA, Russell MK, Kashani A, Tracy AA, Coletti CM, Hila R, Shamia A, Wells J, Ackerman KG, Wilson JM, Bult CJ, Lee C, Lage K, Pober BR, Donahoe PK. Molecular pathogenesis of congenital diaphragmatic hernia revealed by exome sequencing, developmental data, and bioinformatics. Proc Natl Acad Sci U S A 2014;111(34):12450–5 doi: 10.1073/pnas.1412509111. [PubMed: 25107291]

16. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL, Committee ALQA. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17(5):405–24 doi: 10.1038/gim.2015.30. [PubMed: 25741868]

17. Campbell IM, Rao M, Arredondo SD, Lalani SR, Xia Z, Kang SH, Bi W, Breman AM, Smith JL, Bacino CA, Beaudet AL, Patel A, Cheung SW, Lupski JR, Stankiewicz P, Ramocki MB, Shaw CA. Fusion of large-scale genomic knowledge and frequency data computationally prioritizes variants in epilepsy. PLoS Genet 2013;9(9):e1003797 doi: 10.1371/journal.pgen.1003797. [PubMed: 24086149]

18. Callaway DA, Campbell IM, Stover SR, Hernandez-Garcia A, Jhangiani SN, Punetha J, Paine IS, Posey JE, Muzny D, Lally KP, Lupski JR, Shaw CA, Fernandes CJ, Scott DA. Prioritization of Candidate Genes for Congenital Diaphragmatic Hernia in a Critical Region on Chromosome 4p16 using a Machine-Learning Algorithm. J Pediatr Genet 2018;7(4):164–73 doi: 10.1055/s-0038-1655755. [PubMed: 30430034]

19. Wang X, Charng W-L, Chen C-A, Rosenfeld JA, Al Shamsi A, Al-Gazali L, McGuire M, Mew NA, Arnold GL, Qu C, Ding Y, Muzny DM, Gibbs RA, Eng CM, Walkiewicz M, Xia F, Plon SE, Lupski JR, Schaaf CP, Yang Y. Germline mutations in ABL1 cause an autosomal dominant syndrome characterized by congenital heart defects and skeletal malformations. Nat Genet 2017;49(4):613–17 doi: 10.1038/ng.3815. [PubMed: 28288113]

20. Thomas E, Lewis AM, Yang Y, Chanprasert S, Potocki L, Scott DA. Novel Missense Variants in ADAT3 as a Cause of Syndromic Intellectual Disability. J Pediatr Genet 2019;8(4):244–51 doi: 10.1055/s-0039-1693151. [PubMed: 31687266]

21. Zastrow DB, Zornio PA, Dries A, Kohler J, Fernandez L, Waggott D, Walkiewicz M, Eng CM, Manning MA, Farrelly E, Undiagnosed Diseases N, Fisher PG, Ashley EA, Bernstein JA, Wheeler MT. Exome sequencing identifies de novo pathogenic variants in FBN1 and TRPS1 in a patient with a complex connective tissue phenotype. Cold Spring Harb Mol Case Stud 2017;3(1):a001388 doi: 10.1101/mcs.a001388. [PubMed: 28050602]

22. Wat MJ, Veenma D, Hogue J, Holder AM, Yu Z, Wat JJ, Hanchard N, Shchelochkov OA, Fernandes CJ, Johnson A, Lally KP, Slavotinek A, Danhaive O, Schaible T, Cheung SW, Rauen KA, Tonk VS, Tibboel D, de Klein A, Scott DA. Genomic alterations that contribute to the development of isolated and non-isolated congenital diaphragmatic hernia. J Med Genet 2011;48(5):299–307 doi: 10.1136/jmg.2011.089680. [PubMed: 21525063]

23. Longoni M, Russell MK, High FA, Darvishi K, Maalouf FI, Kashani A, Tracy AA, Coletti CM, Loscertales M, Lage K, Ackerman KG, Woods SA, Ward-Melver C, Andrews D, Lee C, Pober BR, Donahoe PK. Prevalence and penetrance of ZFPM2 mutations and deletions causing congenital diaphragmatic hernia. Clin Genet 2015;87(4):362–7 doi: 10.1111/cge.12395. [PubMed: 24702427]

24. Crispino JD, Lodish MB, Thurberg BL, Litovsky SH, Collins T, Molkentin JD, Orkin SH. Proper coronary vascular development and heart morphogenesis depend on interaction of GATA-4 with FOG cofactors. Genes Dev 2001;15(7):839–44 doi: 10.1101/gad.875201. [PubMed: 11297508]

25. Pizzuti A, Sarkozy A, Newton AL, Conti E, Flex E, Digilio MC, Amati F, Gianni D, Tandoi C, Marino B, Crossley M, Dallapiccola B. Mutations of ZFPM2/FOG2 gene in sporadic cases of tetralogy of Fallot. Hum Mutat 2003;22(5):372–7 doi: 10.1002/humu.10261. [PubMed: 14517948]

26. Chantret I, Dupre T, Delenda C, Bucher S, Dancourt J, Barnier A, Charollais A, Heron D, Bader-Meunier B, Danos O, Seta N, Durand G, Oriol R, Codogno P, Moore SE. Congenital disorders of glycosylation type Ig is defined by a deficiency in dolichyl-P-mannose:Man7GlcNAc2-PP-dolichyl mannosyltransferase. J Biol Chem 2002;277(28):25815–22 doi: 10.1074/jbc.M203285200. [PubMed: 11983712]
27. Russell MK, Longoni M, Wells J, Maalouf FI, Tracy AA, Loscertales M, Ackerman KG, Pober BR, Lage K, Bult CJ, Donahoe PK. Congenital diaphragmatic hernia candidate genes derived from embryonic transcriptomes. Proc Natl Acad Sci U S A 2012;109(8):2978–83 doi: 10.1073/pnas.1121621109. [PubMed: 22315423]
28. Unal S, Ozbek N, Kara A, Alikasifoglu M, Gumruk F. Five Fanconi anemia patients with unusual organ pathologies. Am J Hematol 2004;77(1):50–4 doi: 10.1002/ajh.20130. [PubMed: 15307106]
29. Benjamin DR, Juul S, Siebert JR. Congenital posterolateral diaphragmatic hernia: associated malformations. J Pediatr Surg 1988;23(10):899–903 doi: 10.1016/s0022-3468(88)80380-4. [PubMed: 3069995]
30. Delvaux V, Moerman P, Fryns JP. Diaphragmatic hernia in the Coffin-Siris syndrome. Genet Couns 1998;9(1):45–50. [PubMed: 9555587]
31. Sweeney NM, Nahas SA, Chowdhury S, Campo MD, Jones MC, Dimmock DP, Kingsmore SF, RCIGM Investigators. The case for early use of rapid whole-genome sequencing in management of critically ill infants: late diagnosis of Coffin-Siris syndrome in an infant with left congenital diaphragmatic hernia, congenital heart disease, and recurrent infections. Cold Spring Harb Mol Case Stud 2018;4(3) doi: 10.1101/mcs.a002469.
32. Fleck BJ, Pandya A, Vanner L, Kerkering K, Bodurtha J. Coffin-Siris syndrome: review and presentation of new cases from a questionnaire study. Am J Med Genet 2001;99(1):1–7 doi: 10.1002/1096-8628(20010215)99:1<1::aid-ajmg1127>3.0.co;2-a. [PubMed: 11170086]
33. Stoll C, Alembik Y, Dott B, Roth MP. Associated malformations in cases with congenital diaphragmatic hernia. Genet Couns 2008;19(3):331–9. [PubMed: 18990989]
34. Bojanic K, Pritisanac E, Luetic T, Vukovic J, Sprung J, Weingarten TN, Schroeder DR, Grizelj R. Malformations associated with congenital diaphragmatic hernia: Impact on survival. J Pediatr Surg 2015;50(11):1817–22 doi: 10.1016/j.jpedsurg.2015.07.004. [PubMed: 26259558]
35. Niguidula N, Alamillo C, Shahmirzadi Mowlavi L, Powis Z, Cohen JS, Farwell Hagman KD. Clinical whole-exome sequencing results impact medical management. Mol Genet Genomic Med 2018;6(6):1068–78 doi: 10.1002/mgg3.484. [PubMed: 30318729]
36. Matias M, Wusik K, Neilson D, Zhang X, Valencia CA, Collins K. Comparison of medical management and genetic counseling options pre- and post-whole exome sequencing for patients with positive and negative results. J Genet Couns 2019;28(2):182–93 doi: 10.1002/jgc4.1054. [PubMed: 30648779]
37. Milani D, Manzoni FM, Pezzani L, Ajmone P, Gervasini C, Menni F, Esposito S. Rubinstein-Taybi syndrome: clinical features, genetic basis, diagnosis, and management. Ital J Pediatr 2015;41:4 doi: 10.1186/s13052-015-0110-1. [PubMed: 25599811]
38. Mehta PA, Tolar J. Fanconi Anemia. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, Amemiya A, editors. GeneReviews®. Seattle (WA), 1993.
39. Schrier Vergano S, Santen G, Wieczorek D, Wollnik B, Matsumoto N, Deardorff MA. Coffin-Siris Syndrome. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, Amemiya A, editors. GeneReviews®. Seattle (WA), 1993.
40. Bekheirnia MR, Bekheirnia N, Bainbridge MN, Gu S, Coban Akdemir ZH, Gambin T, Janzen NK, Jhangiani SN, Muzny DM, Michael M, Brewer ED, Elenberg E, Kale AS, Riley AA, Swartz SJ, Scott DA, Yang Y, Srivaths PR, Wenderfer SE, Bodurtha J, Applegate CD, Velinov M, Myers A, Borovik L, Craigen WJ, Hanchard NA, Rosenfeld JA, Lewis RA, Gonzales ET, Gibbs RA, Belmont JW, Roth DR, Eng C, Braun MC, Lupski JR, Lamb DJ. Whole-exome sequencing in the molecular diagnosis of individuals with congenital anomalies of the kidney and urinary tract and identification of a new causative gene. Genet Med 2017;19(4):412–20 doi: 10.1038/gim.2016.131. [PubMed: 27657687]
41. Hamdan FF, Daoud H, Rochefort D, Piton A, Gauthier J, Langlois M, Foomani G, Dobrzeniecka S, Krebs MO, Joober R, Lafreniere RG, Lacaille JC, Mottron L, Drapeau P, Beauchamp MH, Phillips MS, Fombonne E, Rouleau GA, Michaud JL. De novo mutations in FOXP1 in cases with

intellectual disability, autism, and language impairment. Am J Hum Genet 2010;87(5):671–8 doi: 10.1016/j.ajhg.2010.09.017. [PubMed: 20950788]

42. Jordan VK, Beck TF, Hernandez-Garcia A, Kundert PN, Kim BJ, Jhangiani SN, Gambin T, Starkovich M, Punetha J, Paine IS, Posey JE, Li AH, Muzny D, Hsu CW, Lashua AJ, Sun X, Fernandes CJ, Dickinson ME, Lally KP, Gibbs RA, Boerwinkle E, Lupski JR, Scott DA. The role of FREM2 and FRAS1 in the development of congenital diaphragmatic hernia. Hum Mol Genet 2018;27(12):2064–75 doi: 10.1093/hmg/ddy110. [PubMed: 29618029]
43. Beck TF, Veenma D, Shchelochkov OA, Yu Z, Kim BJ, Zaveri HP, van Bever Y, Choi S, Douben H, Bertin TK, Patel PI, Lee B, Tibboel D, de Klein A, Stockton DW, Justice MJ, Scott DA. Deficiency of FRAS1-related extracellular matrix 1 (FREM1) causes congenital diaphragmatic hernia in humans and mice. Hum Mol Genet 2013;22(5):1026–38 doi: 10.1093/hmg/dds507. [PubMed: 23221805]
44. Jay PY, Bielinska M, Erlich JM, Mannisto S, Pu WT, Heikinheimo M, Wilson DB. Impaired Mesenchymal Cell Function in Gata4 Mutant Mice Leads to Diaphragmatic Hernias and Primary Lung Defects. Dev Biol 2007;301(2):602–14 doi: 10.1016/j.ydbio.2006.09.050. [PubMed: 17069789]
45. Wat MJ, Beck TF, Hernandez-Garcia A, Yu Z, Veenma D, Garcia M, Holder AM, Wat JJ, Chen Y, Mohila CA, Lally KP, Dickinson M, Tibboel D, de Klein A, Lee B, Scott DA. Mouse model reveals the role of SOX7 in the development of congenital diaphragmatic hernia associated with recurrent deletions of 8p23.1. Hum Mol Genet 2012;21(18):4115–25 doi: 10.1093/hmg/dds241 [PubMed: 22723016]

Table 1.

Individuals with CDH+ for whom a definitive, probable or provisional diagnosis was made by exome sequencing.

Gene†	Variant(s)	Inheritance	Variant Type	Diagnosis
Definitive 19/76 = 25%				
ABL1 *[19]	c.1066G>A, p.(A356T)	De Novo	Pathogenic	Congenital heart defects and skeletal malformations syndrome (MIM #617602)
ABL1 *[19]	c.734A>G, p.(Y245C)	De Novo	Pathogenic	”
*ADAT3** [20]	c.[586_587delinsTT];[587C>T], p.[(A196L)];[(A196V)]	Inherited in Trans	VUS, VUS	Mental retardation, autosomal recessive 36; (MIM #615286)
ALG12	c.[165C>A];[437G>A], p.[(Y55*)];[(R146Q)]	Inherited in Trans	Pathogenic, Pathogenic	*ALG12*-CDG (MIM #607143)
ANKRD11	c.1372C>T, p.(R458*)	Paternal	Pathogenic	KBG syndrome (MIM #148050)
BRCA2	c.[4965C>G];[7007G>C], p.[(Y1655*)];[(R2336P)]	Inherited in Trans	Pathogenic, Pathogenic	Fanconi anemia, complementation group D1 (MIM #605724)
FBN1 *[21] *TRPS1*	c.4786C>T, p.(R1596*); c.1630C>T, p.(R544*)	De Novo De Novo	Pathogenic Pathogenic	Marfan syndrome (MIM #154700); Trichorhinophalangeal syndrome type I (MIM #190350)
FOXC2 §	c.563_573del, p.(P188fs)	De Novo	Pathogenic	Lymphedema-distichiasis syndrome (MIM #153400)
FOXP1	c.1718_1722+8del, p.?	De Novo	Pathogenic	Mental retardation with language impairment and with or without autistic features (MIM #613670)
KMT2D	c.10258dupA, p.(I3420fs)	De Novo	Pathogenic	Kabuki syndrome 1 (MIM #147920)
KMT2D	c.13543dupA, p.(R4515fs)	De Novo	Pathogenic	“
KMT2D	c.7613dupT, p.(Q2540Sfs)	De Novo	Pathogenic	”
MCPH1	c.[586delC];[586delC], p.[(Q196fs)];[(Q196fs)]	Inherited in Trans	Pathogenic, Pathogenic	Microcephaly 1, primary, autosomal recessive (MIM #251200)
MYRF *[8]	c.3239dupA, p.(E1081Gfs)	De Novo	Likely Pathogenic	Cardiac-urogenital syndrome (MIM #618280)
MYRF *[8]	c.350_366delinsT, p.(G117Vfs)	Not Maternal	Likely Pathogenic	”
PDHA1	c.1035_1036dupGA, p.(I346Rfs) (Het in female)	Not Maternal	Pathogenic	Pyruvate dehydrogenase E1-alpha deficiency (MIM #312170)
RASA1	c.1103-1G>T, p.?	Paternal	Pathogenic	Capillary malformation-arteriovenous malformation 1(MIM #608354); Partially solved
SMARCA4	c.2936G>A, p.(R979Q)	De Novo	Pathogenic	Coffin-Siris syndrome 4 (MIM #614609)
ZFPM2	c.757_761dup, p.(C255fs)	?	Pathogenic	Diaphragmatic hernia 3 (MIM #610187)
Probable 9/76 = 12%				
ABL1	c.352T>C, p.(W118R)	De Novo	Likely Pathogenic	Congenital heart defects and skeletal malformations syndrome (MIM #617602)
ALG12	c.[437G>A];[930_931delAC], p.[(R146Q)];[(R311fs)]	Inherited in Trans	Pathogenic, Likely Pathogenic	*ALG12*-congenital disorder of glycosylation (MIM #607143)
DDX3X	c.1304T>C, p.(L435P) (Sex not indicated);	De Novo	Likely Pathognic	Intellectual developmental disorder, X-linked, syndrome, Snijders Blok type (MIM #300958)

Gene†	Variant(s)	Inheritance	Variant Type	Diagnosis
EP300	c.2660C>T Mosaic 12%, p.(T887I) Mosaic	De Novo	Likely Pathogenic	Rubinstein-Taybi syndrome 2 (MIM #613684)
HCCS	c.308_309insAGT, p.(V103dup) (Sex not indicated)	De Novo	Likely Pathogenic	Linear skin defects with multiple congenital anomalies 1 (MIM #309801)
KMT2D	c.1967delT, p.(L656fs)	?	Likely Pathogenic	Kabuki syndrome 1 (MIM #147920)
POGZ	c.2849dupC, p.(V951Sfs)	?	Likely Pathogenic	White-Sutton syndrome (MIM #616364)
TCF12	c.1808G>A, p.(R603Q)	De Novo	Likely Pathogenic	Craniosynostosis 3 (MIM #615314)
TRAF7	c.1886G>A, p.(S629N)	De Novo	Likely Pathogenic	Cardiac, facial, and digital anomalies with developmental delay (MIM #618164)
Provisional 7/76 = 9%				
B3GALT6 *IDS*	c.[929A>G];[795A>C], p.[(Y310C)];[(E265D)] c.1144G>C, p.(D382H) (Hemi in male)	Inherited in Trans Maternal	VUS, VUS VUS	Ehlers-Danlos syndrome, spondylodysplastic type, 2 (MIM #615349) Mucopolysaccharidosis II (MIM #309900)
EP300 *DKC1* *SOS1*	c.3592T>C, p.(Y1198H) c.−142C>G, p.? (Het in female) c.3298G>T, p.(D1100Y)	? ? ?	VUS Pathogenic VUS	Rubinstein-Taybi syndrome 2 (MIM #613684) Dyskeratosis congenita, X-linked (MIM #305000) Noonan syndrome 4 (MIM #610733)
FANCI	c.2422A>T, p.(K808*)	Maternal	Pathogenic	Fanconi anemia, complementation group I (MIM #609053)
LRP2	c.[3667+1G>A];[5390A>G], p.[?];[(N1797S)]	Inherited in Trans	Likely Pathogenic, VUS	Donnai-Barrow syndrome (MIM #222448)
MED12	c.5691_5692delGT, p.(Y1898fs) (Het in female)	De Novo	Pathogenic	MED12-related disorder (MIM #300895, #305450, #309520)
SLC2A10	c.[67G>A];[67G>A], p.[(G23S)];[(G23S)]	?	VUS, VUS	Arterial tortuosity syndrome (MIM #208050); Partially solved
SMARCC2 *LMX1B*	c.1651-2A>G, p.? c.904C>T, p.(Q302*)	Not Maternal Not Maternal	VUS VUS	Coffin-Siris syndrome 8 (MIM #618362) Nail-patella syndrome (MIM #161200)

†Genes clearly associated with an increased risk of developing CDH are shown in bold.

‡Originally reported as VUS/VUS prior to publication.

*Subject was previously published.

§Molecular diagnosis made by exome sequencing on identical twin. “ = same as above. ? = unknown. LP = Likely Pathogenic, P = Pathogenic, VUS = variant of unknown significance.

Table 2.

Candidate genes for which there is sufficient evidence to support a phenotypic expansion involving CDH.

Gene	Disorder[†]	Expressed in the developing diaphragm?[‡]	CDH-Pathogenicity Score	Number of individuals in our CDH+ cohort with changes in this gene and level of certainty	Other cases of CDH reported for this gene/disorder in humans?
ALG12	*ALG12*-congenital disorder of glycosylation, (MIM #607143)	YES	48.2%	2; Definitive, Probable	No/No
BRCA2	Fanconi anemia, complementation group D1 (MIM #605724)	YES	88.8%	1; Definitive	No/Yes [28]
EP300	Rubinstein-Taybi syndrome 2 (MIM #613684)	YES	94.7%	2; Probable, Provisional	No/Yes [29]
FOXP1	Mental retardation with language impairment and with or without autistic features (MIM #613670)	YES	96.9%	1; Definitive	Yes [7]/Yes [7]
SMARCA4	Coffin-Siris syndrome 4 (MIM #614609)	YES	93.7%	1; Definitive	No/Yes [30-32]

[†]Disorder associated with the gene of interest that most closely fits the phenotype descriptions of the individuals in which CDH+ was identified.

[‡]Is the mouse orthologue expressed in the developing diaphragm at embryonic day (E)11.5, E12.5 and E16.5 based on whole-transcriptome expression profiles published by Russell et al [27].

[*]Subject was previously published. N/D = Not done or not reported.

Table 3

Candidate genes for which there is currently insufficient evidence to support a phenotypic expansion involving CDH.

Gene	Disorder†	Level of diagnostic certainty	Expressed in the developing diaphragm?‡	CDH-Pathogenicity Score	Other cases of CDH reported for this gene/disorder in humans?
*ADAT3** [20]	Mental retardation, autosomal recessive 36 (OMIM #615286)	Definitive	N/D	48.5%	No/No
ANKRD11	KBG syndrome (OMIM #148050)	Definitive	YES	99.0%	No/No
DDX3X	Mental retardation, X-linked 102 (OMIM #300958)	Provisional	YES	28.2%	No/No
DKC1	Dyskeratosis congenita, X-linked (OMIM #305000)	Provisional	YES	51.3%	No/No
FANCI	Fanconi anemia, complementation group I (OMIM #609053)	Provisional	YES	25.0%	No/Yes [28]
FOXC2	Lymphedema-distichiasis syndrome (OMIM #153400)	Definitive	YES	83.3%	No/No
IDS	Mucopolysaccharidosis II (OMIM #309900)	Provisional	YES	79.0%	No/No
LMX1B	Nail-patella syndrome (OMIM #161200)	Provisional	YES	98.6%	No/No
MCPH1	Microcephaly 1, primary, autosomal recessive (OMIM #251200)	Definitive	YES	79.7%	No/No
MED12	MED12-related disorder (OMIM #300895, #305450, #309520)	Provisional	YES	81.7%	No/No
PDHA1	Pyruvate dehydrogenase E1-alpha deficiency (OMIM #312170)	Definitive	YES	73.2%	No/No
RASA1	Capillary malformation-arteriovenous malformation 1 (OMIM #608354)	Definitive (Partial)	YES	91.35	No/No
SMARCC2	Coffin-Siris syndrome 8 (OMIM #618362)	Provisional	YES	89.9%	No/Yes [30-32]
SOS1	Noonan syndrome 4 (OMIM #610733)	Provisional	YES	74.3%	No/Yes [7 33 34]
TCF12	Craniosynostosis 3 (OMIM #615314)	Probable	YES	84.9%	No/No
TRAF7	Cardiac, facial, and digital anomalies with developmental delay (OMIM #618164)	Probable	YES	71.2%	No/No
*TRPS1** [21]	Trichorhinophalangeal syndrome, type I (OMIM #190350)	Definitive	YES	84.6%	No/No

† Disorder associated with the gene of interest that most closely fits the phenotype descriptions of the individuals in which CDH+ was identified.

‡ Is the mouse orthologue expressed in the developing diaphragm at embryonic day (E)11.5, E12.5 and E16.5 based on whole-transcriptome expression profiles published by Russell et al [27].

* Subject was previously published. N/D = Not done or not reported.