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Infants With Bochdalek Diaphragmatic Hernia:

Sibling Precurrence and Monozygotic Twin Discordance in a Hospital-Based Malformation Surveillance Program

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Abstract

Congenital diaphragmatic hernia (CDH) is a common and often devastating birth defect. In order to learn more about possible genetic causes, we reviewed and classified 203 cases of the Bochdalek hernia type identified through the Brigham and Women's Hospital (BWH) Active Malformation Surveillance Program over a 28-year period. Phenotypically, 55% of the cases had isolated CDH, and 45% had complex CDH defined as CDH in association with additional major malformations or as part of a syndrome. When classified according to likely etiology, 17% had a Recognized Genetic etiology for their CDH, while the remaining 83% had No Apparent Genetic etiology. Detailed analysis using this largest cohort of consecutively collected cases of CDH showed low precurrence among siblings. Additionally, there was no concordance for CDH among five monozygotic twin pairs. These findings, in conjunction with previous reports of de novo dominant mutations in patients with CDH, suggest that new mutations may be an important mechanism responsible for CDH. The twin data also raise the possibility that epigenetic abnormalities contribute to the development of CDH.

Keywords

congenital diaphragmatic hernia; discordant monozygotic twins; precurrence risk; review

INTRODUCTION

Congenital diaphragmatic hernia (CDH) refers to a group of congenital defects in the structural integrity of the diaphragm associated with often lethal pulmonary hypoplasia and pulmonary hypertension. Prevalence in liveborns ranges from 1/2,500–1/4,000 [Langham et al., 1996]. Approximately 90% of CDH cases are Bochdalek or posterolateral hernias most often occurring on the left side [Langham et al., 1996]. In most series, just over half of congenital hernias occur in isolation, while the remainder are associated with chromosome abnormalities, recognized genetic syndromes, or other major birth defects but not as part of recognized syndromes [Tibboel and Gaag, 1996]. Despite continued advances in the medical and surgical care for infants with CDH, mortality and morbidity remain high [Stege et al., 2003]. In order to improve outcomes for infants with CDH, progress is needed in understanding its causes.

The etiologies of human CDH are largely unknown. Two studies have shown that the rate of occurrence in siblings is low, in the order of 1% [David and Illingworth, 1976; Czeizel and Kovacs, 1985]. However, several observations indicate that CDH can be due to genetic abnormalities, including: (a) reports of an appreciable number families with multiple affected relatives diagnosed with CDH [Wolff, 1980; Norio et al., 1984]; (b) the identification of de novo mutations in important developmental genes in patients with CDH [Devriendt et al., 1995; Denamur et al., 2000; Reardon et al., 2004; Ackerman et al., 2005]; and (c) the many human single gene disorders and chromosome abnormalities that occur in association with CDH [Enns et al., 1998; Lurie, 2003].

To gain further insights into the issue of family clustering and possible genetic determinants of CDH, we examined our data among siblings and twins diagnosed with Bochdalek CDH.

METHODS

We evaluated infants and fetuses with CDH that were identified through the Active Malformation Surveillance Program at BWH over a 28-year period [1972 to 1974 and 1979 to 2003]. Consecutive cases of CDH among electively terminated fetuses, liveborns (<5 days of age), stillborns, and neonatal deaths were included. Additional details about the Surveillance Program have been provided elsewhere [Nelson and Holmes, 1989; McGuirk et al., 2001]. The diagnosis of CDH, as well as the presence of additional major malformations, was established from a review of prenatal medical records, postnatal medical records, and laboratory tests. Malformations were coded using the *International Classification of Diseases, Ninth Revision (ICD-9)* codes. Hernias were classified as either Bochdalek (posterolateral hernia or agenesis of the hemidiaphragm), or non-Bochdalek (Morgagni, central, or not-otherwise-specified, NOS). Cases were excluded if they had a non-Bochdalek hernia or diaphragm eventration, as the latter were not systematically ascertained. Certain malformations, such as pulmonary hypoplasia, intestinal malrotation, gastric volvulus, and patent ductus arteriosus were considered part of a diaphragm defect sequence and were not tabulated as separate malformations [Fauza and Wilson, 1994]. Importance of precise case classification [Holmes et al., 1976; Rasmussen et al., 2003] has been stressed by others and accordingly, we classified infants with CDH in two ways, by phenotype and by likely etiology (Fig. 2). For the phenotypic classification, infants with no structural anomalies other than Bochdalek hernia were classified as having isolated CDH; those with at least one additional major malformation or Bochdalek hernia occurring as part of a syndrome, were classified as having complex CDH.

Likely causes of the Bochdalek hernia were categorized as falling into either a Recognized Genetic etiology or No Apparent Genetic etiology. Specifically, CDH due to a Recognized Genetic etiology was present in the setting of a single gene disorder (such as Fryns syndrome) or a chromosome abnormality. Cases were considered as having No Apparent Genetic etiology

when the Bochdalek hernia occurred in association with twinning, a known gestational exposure such as insulin-dependent diabetes mellitus, or when an etiology could not be identified (e.g., unknown etiology).

Since this hospital is a tertiary care center, mothers were subdivided into two groups: those who planned to deliver at BWH (non-referred group) and those who changed their delivery to BWH following prenatal diagnosis of an anomaly (referred group). Information about birth status, gender, multiple gestations, and family history was obtained. Family history details were reviewed at the time of delivery of the malformed infant to identify all relatives with CDH and/or other major malformations. To calculate the precurrence risk for probands with CDH, we tallied the proband's prior siblings in whom CDH could have been recognized, including livebirths, stillbirths, fetuses >20 weeks gestation, and elective terminations for CDH. We excluded spontaneous and other elective terminations <20 weeks gestation with no known malformations, as such fetuses were not adequately studied to diagnose the presence or absence of CDH with confidence. The records of all twin sets were reviewed to establish concordance for CDH and zygosity.

The total number of births (251,400) during this 28-year study period was tabulated from all livebirths, stillbirths, and infants terminated for the presence of a major malformation at BWH. Only a single member of each twin or sibling pair was counted as an index case of CDH. Cases of conjoined twins were excluded from this data series as well as from the literature review on twins.

RESULTS

We identified a total of 226 infants with CDH who delivered at BWH. After excluding 23 patients, the final study group consisted of 203 infants with a Bochdalek hernia (Fig. 1). The birth prevalence for the total group was 8/10,000. The nonreferred birth prevalence was 1.95/10,000, since only 49 infants were born to mothers who intended to deliver at BWH.

One hundred sixty-three (80%) of the cases were liveborn. Eleven infants were stillborn and 29 pregnancies were terminated electively (21 via prostaglandin infusion and eight via D&E). One hundred thirty four (66%) of infants had a left-sided Bochdalek hernia and the male to female preponderance was 1.4 to 1.0.

As described in the Methods Section, each case was classified in two different ways (Fig. 2).

- a. Phenotypic classification: infants with isolated Bochdalek hernia comprised 55% (n = 112) of the study group. The remaining 45% of the group had complex hernia, that is a Bochdalek hernia plus additional structural birth defects, or CDH as part of a syndrome.
- b. Etiologic classification: 169 infants were classified as having CDH due No Apparent Genetic etiology. The remaining 34 cases (17%) had a Recognized Genetic etiology for their CDH, either a chromosome abnormality or a monogenic syndrome; details are listed in Table I.

Two-thirds (136/203) of the Bochdalek hernia cases had a chromosome analysis. 75% of those classified with complex hernia had a chromosome study, while 61% of those classified with isolated hernia had a chromosome study (chi-squared, $P = 0.03$). Information on the tissue source for each karyotype is not consistently available in the patient records reviewed for this study; however, the common practice at BWH is to perform blood chromosome analysis in newborns.

Occurrence in Previous Pregnancies

Among the Bochdalek hernia cases classified in the No Apparent Genetic etiology category, 89 families had a total of 149 prior siblings who could have been evaluated for the presence of CDH. Only one infant had a previous sibling also diagnosed with CDH, for a precurrence rate of 1/149 (0.7%). In that family, a female fetus had isolated left-sided CDH diagnosed on prenatal ultrasound, with a normal karyotype on amniocentesis. The pregnancy was terminated and subsequent examination of the fragmented fetus, which included inspection of all extremities, revealed no additional anomalies. The proband's mother reported that her first son, fathered by a different husband, died at several hours of life from complications secondary to CDH. We reviewed a facial photograph as well as an autopsy report on this male infant. These revealed that the baby had agenesis of the left hemidiaphragm, left pulmonary hypoplasia, but no additional anomalies or dysmorphic features. The mother subsequently had three healthy children, a girl and two boys, both of whom have mild hypospadias.

Among the 149 prior siblings, six (4%) had a major malformation other than CDH. One sibling each had: cleft lip and palate, hydronephrosis, renal agenesis/dysgenesis, and anencephaly, while the remaining two siblings had congenital heart defects.

Discordance in Twins

There were eight twin pairs in which one member had a Bochdalek hernia. Although seven of the eight twins with CDH had additional anomalies, none had either a chromosome abnormality or a recognized syndrome. All of the twin pairs were discordant for CDH, though in two cases the co-twin had other congenital malformations (Table II). Five of the eight twin pairs were monozygous based on placental pathology and/ or DNA markers, one was dizygous based on opposite genders, and two were of unknown zygosity (same gender infants with dichorionic diamniotic membranes). Although the number of twins, with one member having CDH, appears high given a total cohort of 203 cases, seven of the eight twin pairs were referred to BWH for delivery.

DISCUSSION

In terms of distribution of hernia types and percent of isolated versus complex cases, the results of this study on infants with Bochdalek hernia identified through the BWH Malformation Surveillance Program are similar to those of previous reports [Torfs et al., 1992; Robert et al., 1997; Tonks et al., 2004]. The CDH prevalence rate among infants of non-referred mothers was 1.95/10,000, a rate at the low end of the reported range. However, if we presume that Bochdalek hernias predominated among the non-classifiable CDH cases intending to deliver at BWH (Fig. 1), then the prevalence rises to 2.1/10,000, comparable to a few other series [Langham et al., 1996; Robert et al., 1997; Garne et al., 2002; Dott et al., 2003]. Additionally, we found a < 1% sibling precurrence risk, and CDH discordance among five confirmed monozygotic twin pairs.

The strengths of this study are the large number of CDH cases consecutively born at a single tertiary care hospital, the meticulous classification of the diaphragm defect as well as any associated malformations, and careful review of pedigree information.

This study also has several limitations. Since all infants are identified at birth and examined by many different physicians, the awareness of the features of specific syndromes, such as Fryns syndrome, would not be uniform. Likewise, all infants did not have chromosome analysis or skin fibroblast studies to look for the presence of 12p tetrasomy (Pallister–Killian syndrome). This cohort does not represent a discrete catchment area (e.g., is not population-based) and long-term followup of infants did not occur, so that additional syndrome diagnoses may have

been established over time. Finally, sibling precurrence is being used as a less than perfect proxy for sibling recurrence, since it is possible that family size is altered after the birth of a child with a serious congenital malformation.

The significance of the findings from this study need to be placed in the context of other studies pertaining to a genetic basis of CDH.

Literature on Sibling Precurrence and Twin Discordance in CDH

Sibling precurrence—Several series mention the birth of prior siblings affected with CDH [Torfs et al., 1992; Bollmann et al., 1995; Dillon et al., 2000; Tonks et al., 2004]. However, only two prior studies have examined occurrence in families from a systematic series of infants with CDH [David and Illingworth, 1976; Czeizel and Kovacs, 1985]. Czeizel and Kovács [1985] collected family information and reviewed selected medical records on all infants with CDH who were ascertained through the Hungarian Malformation Registry. They reported 156 cases of isolated Bochdalek hernia who collectively had a total of 231 siblings, half born before the proband and half after the proband yet before study completion. Only one infant with isolated Bochdalek hernia had a similarly affected sibling, for a sibling occurrence of 1/231 (0.43%). In addition to the 156 infants with isolated CDH, Czeizel and Kovacs identified 96 infants who had CDH along with additional defects. This group contained a mixture of monogenic and chromosome syndromes, as well as infants with multiple anomalies of unknown etiology. None of the 164 siblings of the complex cases had CDH. In the complex CDH group, the birth prevalence of non-CDH malformations was greater than expected compared to controls, with a notable increase in the frequency of neural tube defects. A second series from England [David and Illingworth, 1976] described 143 CDH cases, born between 1943 and 1974, that were identified from treatment centers or autopsy logs. Approximately half of the cases had isolated CDH. Using information available from medical records, the authors found that none (0%) of 181 siblings, the majority of whom were born before the proband, had CDH though seven siblings had a neural tube defect without CDH.

Twin discordance—Twins have been used extensively to study the heritability of traits [Boomsma et al., 2002]. A high frequency of concordance among monozygotic twins, compared to dizygotic twins, is interpreted as showing genetic contribution to the trait under study. There are no published concordance ratios between MZ and DZ twins for CDH, so we can only glean genetic information from examination of MZ twins.

Findings in the literature on Bochdalek hernia in twins, plus cases from the current study, are summarized in Table II [David and Illingworth, 1976; Eichelberger et al., 1980; Gencik et al., 1982; Watanatittan, 1983; Chu et al., 1986; Mishalany and Gordo, 1986; Torfs et al., 1992; Chao et al., 1997; Gibbs et al., 1997; Robert et al., 1997; Lucas Talan et al., 1998; Abe et al., 2001; Gallot et al., 2003; Govindaswami et al., 2004; Tonks et al., 2004]. The data show that the majority of monozygotic twin pairs described in case reports or as part of a small series are concordant for CDH. In contrast, most twin pairs reported as part of consecutive series, including the twins in our own large series, are discordant for CDH. We suggest there is over-reporting of concordantly affected twins in case reports and that furthermore, while CDH can be present in both members of a monozygous twin pair, it more frequently affects only one.

Possible Genetic Causes for Low Sibling Precurrence and MZ Twin Concordance

The findings from consecutive series of low sibling precurrence and low MZ twin concordance can be due to a number of different mechanisms, including genetic causes. Possible genetic explanations include incomplete penetrance, de novo chromosome abnormalities, and new dominant mutations; evidence in support of the latter is presented below.

De Novo Mutations Responsible for CDH

A de novo mutation in *FOG2* has recently been reported in an infant whose post-mortem examination demonstrated a congenital abnormality of the diaphragm and pulmonary hypoplasia, without any additional abnormalities [Ackerman et al., 2005]. This report extends the authors' findings that mice homozygous for a hypomorphic *Fog2* mutation have diaphragmatic abnormalities and primary pulmonary hypoplasia [Ackerman et al., 2005]. Likewise, work initially done in mice carrying *Wt1* mutations has been extended to humans. The *Wt1* mutant mice showed posterolateral diaphragm defects, resembling Bochdalek hernia, and urogenital defects [Kreidberg et al., 1993]. Several reports demonstrate similar human phenotypes caused by *WT1* mutations, namely Denys-Drash and Meacham syndromes [Devriendt et al., 1995; Denamur et al., 2000; Reardon et al., 2004]. Although the mouse phenotypes described above are autosomal recessive, the comparable human phenotypes occur in the presence of only one mutant allele, and hence are autosomal dominant. There are other examples of a normal, or mildly abnormal, heterozygous phenotype in the mouse where the homozygotes more closely resemble the dominant human disorder, such as the elastin knock-out mouse and a hypomorphic pkd mouse [Li et al., 1998; Lantinga-van Leeuwen et al., 2004].

Advanced paternal age has been convincingly associated with an increased frequency of several dominant disorders due to de novo paternal point mutations; the best studied examples include achondroplasia and Apert syndrome (as reviewed in [Crow, 2000]). If some, or many, cases of a common birth defect are caused by de novo paternal mutations, then evidence of advanced paternal age may be apparent. One study, using a population-based surveillance registry of 125 cases of CDH, has addressed this issue and found a trend suggesting an increased frequency of CDH among the offspring of older fathers [McIntosh et al., 1995]. Further study is required before this issue can be resolved.

Based on these data, we predict that new dominant (i.e., spontaneous) mutations cause many cases with CDH. The higher fatality rate of CDH in previous decades means that very few adults with repaired CDH, who could have a spontaneous autosomal dominant mutation as the underlying cause of their hernia, are in their reproductive years. A similar phenomenon was observed in the disorder, Hirschsprung disease, where familial occurrence was underappreciated until improved medical and surgical care permitted long-term survival [Bodian and Carter, 1963].

Genetic basis for discordance in MZ twins—Phenotypic discordance between monozygotic twins can arise through a number of different mechanisms, both genetic and non-genetic [Machin, 1996]. Post-zygotic de novo mutations, as well as epigenetic differences are mechanisms proven to underlie MZ twin discordance [Kondo et al., 2002; Weksberg et al., 2002]. The latter mechanism has been documented in twins with Beckwith Wiedemann syndrome (BWS). Weksberg et al. [2002] recently demonstrated an imprinting defect in *KCNQ1OT1* in a twin with BWS that was not present in her normal co-twin. Intriguingly, congenital diaphragm defects are occasionally reported in singleton patients with BWS [Irving, 1967; Thorburn et al., 1970]. These findings raise the possibility that some cases of CDH may be due to mutations that interfere with normal epigenetic modifications. This possibility had been suggested previously [Austin-Ward and Taucher, 1999].

Further Evidence That CDH has Genetic Determinants

There are additional findings to suggest that genetic abnormalities play an important role in at least some cases with CDH.

Multiplex families with CDH—Numerous multiplex families with either isolated or complex CDH have been published [Butler and Claireaux, 1962; Welch and Cooke, 1962;

Scott and Patterson, 1966; Passarge et al., 1968; ten Kate and Anders, 1970; Feingold, 1971; Harberg et al., 1976; Thomas et al., 1976; Crane, 1979; David et al., 1979; Pollack and Hall, 1979; Arad et al., 1980; Wolff, 1980; Gencik et al., 1982; Norio et al., 1984; Czeizel and Kovacs, 1985; Lipson and Williams, 1985; Toriello et al., 1985, ¹⁹⁸⁶; Bocian et al., 1986; Farag et al., 1989; Hitch et al., 1989; Carmi et al., 1990; Frey et al., 1991; Sripathi and Beasley, 1992; Narayan et al., 1993; Farag et al., 1994; Gibbs et al., 1997; Mitchell et al., 1997; Kufeji and Crabbe, 1999; Manouvrier-Hanu et al., 2000]. Several of these families are consanguineous, making it likely that a single mutant gene is responsible for their disorder [Arad et al., 1980; Norio et al., 1984; Farag et al., 1989, 1994], though multifactorial inheritance cannot be ruled out as an explanation for familial clustering. The discovery of mutations in the interferon regulatory factor 6 gene (*IRF6*) as the cause of lip pit or van der Woude syndrome could be a model for identifying multifactorial genetic changes contributing to CDH. Specifically, mutations in both the *IRF6* protein binding domain and the DNA binding domain produce van der Woude syndrome; recent studies demonstrate that a common variant in *IRF6* increases the risk for isolated cleft lip and palate [Kondo et al., 2002; Kayano et al., 2003; Zuccherro et al., 2004; Scapoli et al., 2005]. Thus, possible mutations or clusters of polymorphisms in *FOG2*, *WT1* and, other as yet unidentified major development genes or their downstream targets, could contribute to multifactorial causation of CDH.

Chromosome abnormalities and CDH—Using standard techniques chromosome abnormalities have been detected, on average, in ~10% of cases with CDH [Thorpe-Beeston et al., 1989; Philip et al., 1991; Bollmann et al., 1995; Howe et al., 1996; Faivre et al., 1998; Garne et al., 2002; Tonks et al., 2004]. Examples of commonly detected abnormalities include trisomy 18, trisomy 21, trisomy 13, tetrasomy 12p, +der 22 t(11;22), 8p-, and 4p- [Pecile et al., 1990; Howe et al., 1996; Faivre et al., 1998; Lurie, 2003; Borys and Taxy, 2004; Tonks et al., 2004; van Dooren et al., 2004]. A recent excellent review demonstrates numerous “hot spots” that may harbor genes contributing to CDH [Lurie, 2003]. The presence of two recurring cytogenetically detectable abnormalities, del 15q26 [Schlembach et al., 2001; Biggio et al., 2004; Hengstschlager et al., 2004] and del 8p23 [Faivre et al., 1998; Borys and Taxy, 2004; Shimokawa et al., 2005], indicate that hemizygosity for one or more genes located in these regions can cause CDH. Application of careful deletion mapping has recently led to the delineation of a five megabase CDH critical region in 15q26.2 with identification of four candidate genes [Klaassens et al., 2005]. New technologies, such as fluorescent in situ hybridization and comparative genomic hybridization [Knight and Flint, 2000; Shaffer and Bejjani, 2004] are likely to identify additional CDH-critical regions in the future.

CONCLUSIONS

In a systematic series of cases with CDH, we demonstrate that sibling precurrence and twin concordance for CDH are low. The recent findings of de novo dominant mutations associated with CDH suggest that a high proportion of CDH cases may be due to new dominant mutations. Additionally, the high rate of discordance in monozytic twins also suggests that epigenetic events could also be a causal factor. Based on the cumulative evidence reviewed above, the continued pursuit of the genetic basis of CDH through the use of molecular and cytogenetic strategies, the identification of candidate genes from animal models, and an assessment of human malformation syndromes with CDH, is justified to uncover genes that either cause or contribute to CDH.

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Fig. 1.
Distribution of cases with congenital diaphragmatic hernia (CDH) in this study.

Phenotypic classification

		Isolated Bochdalek Hernia (N=112)	Complex Bochdalek Hernia (N=91)	
Etiologic classification	I. No Apparent Genetic etiology (N= 169)	Ia. Unknown Etiology	111	48
		Ib. Twinning	1	7
		Ic. Maternal diabetes	0	2
	II. Recognized Genetic etiology (N=34)			
		IIa. Monogenic	0	10
		IIb. Chromosome	0	24

Fig. 2.
Classification of cases with congenital diaphragmatic hernia (CDH), of the Bochdalek type, according to phenotype and likely etiology.

TABLE I

Specific Diagnoses Among 34 CDH Cases due to a Recognized Genetic Etiology

	Diagnosis	Number of cases
Chromosome abnormalities	Trisomy 18	8
	Isochromosome 12 p	3
	Trisomy 13	2 + 1 mosaic
	Trisomy 21	2
	Tetrasomy 21	1
	Trisomy 8	1 mosaic
	Deletions:	3
	del (8)(p)	
	del (15)(q26)	
	del (4)(p16)	
	Unbalanced translocations:	3
	der(4)t(4;20)(q34.2;q13.1)pat	
	der(15)t(12;15)	
	Der (X)t(X;Y)(p22.3;q11.2) ^a	
Monogenic syndromes	Fryns	4
	Private syndrome	2
	Brachmann-de Lange	1
	Persistent Mullerian Duct syndrome	1
	Simpson-Golabi-Behmel	1
	Smith-Lemli-Opitz	1

^a Also diagnosed with Leri Weil dyschondrosteosis, but only tabulated as having a chromosome abnormality.

TABLE II

Bochdalek Hernia in Twins*

Author, Year	Gender, CDH type		Additional anomalies		CDH	Zygosity
	Twin A	Twin B	Twin A	Twin B		
Case Reports						
Eichelberger et al. [1980]	Male, L Bochdalek	Male, None	None	None	Discordant	MZ ^a
Gencik et al. [1982]	Male, L Agenesis	Male, L Agenesis	None	None	Concordant	MZ ^b
Watanaititan [1983]	Female, L Bochdalek	Female, L Bochdalek	None	None	Concordant	MZ ^c
Mishalany and Gordo [1986]	Male, L Bochdalek	Male, L Bochdalek	None	None	Concordant	MZ ^d
Chao et al. [1997]	Male, R Agenesis	Male, R Agenesis	None	None	Concordant	MZ ^d
Gibbs et al. [1997]	Male, B Agenesis	Male, B Agenesis	None	None	Concordant	MZ ^d
Lucas Talan et al. [1998]	Unknown, CDH	Unknown, CDH	None	None	Concordant	MZ ^a
Abe et al. [2001]	Female, Bochdalek	Female, Bochdalek	None	None	Concordant	MZ ^d
Gallot et al. [2003]	Unknown, CDH	Unknown, None	None reported	None reported	Discordant	MZ ^b
Govindaswami et al. [2004]	Female, L Bochdalek	Female, L Bochdalek	None	None	Concordant	MZ ^e
Consecutive Series						
Present series						
1	Male, L Bochdalek	Male, None	VSD, cystic hygroma	None	Discordant	MZ ^d
2	Female, L Bochdalek	Female, None	Enceph-alocele, Kinked spine	Anen-cephaly	Discordant: CDH (Concordant: NTD)	MZ ^e
3	Male, L Bochdalek	Male, None	Anencephaly, CLP, hypertel-orism	None	Discordant	MZ ^d
4	Female, L Bochdalek	Female, None	Aortic coarctation, IU GR, dysmor-phic	None	Discordant	MZ ^d
5	Male, L Bochdalek	Male, None	Omphal-ocle	VSD, hypospa-dias	Discordant	MZ ^d
6	Female, L Bochdalek	Male, None	Spina bifida, preaxial polydac-tyly	None	Discordant	DZ ^f
7	Male, L Bochdalek	Male, None	HLHS	None	Discordant	Unk ^g
8	Female, L Bochdalek	Female, None	None	None	Discordant	Unk ^g

Author, Year	Gender, CDH type			Additional anomalies			CDH	
	Twin A	Twin B		Twin A	Twin B		Concordance	Zygosity
David and Illingworth [1976]	Female, L Bochdalek	Male, None		None	None		Discordant	DZ ^f
	Female, L Bochdalek	Female, None		None	None		Discordant	MZ ^b
Torfs et al. [1992]	4 twin pairs	No cotwin had CDH		All had complex CDH, with additional anomalies	One w/ aortic stenosis		4/4 Discordant	2 MZ ^a ; 2 same sex ^h
Robert et al. [1997]	45 twin pairs	N/A		N/A	N/A		43/45 Discordant	28/32 known sex pairs were like-sexed ^h (c/w 75% MZ)
Tonks et al. [2004]	6 twin pairs	N/A		N/A	N/A		6/6 Discordant	Unknown zygosity ^h

Prior reports of Bochdalek hernia in twins, plus the eight cases identified in this study. Twins with a Recognized Genetic etiology for their CDH have been excluded. Although we classified diaphragm agenesis under the category of Bochdalek hernia in our series, we maintained this distinction when citing the work of others. Cases of conjoined twins have been excluded. B, bilateral; CLP, cleft lip and palate; DZ, dizygotic; F, female; HLHS, hypoplastic left heart syndrome; IUGR, intrauterine growth retardation; L, left; M, male; MZ, monozygotic; NTD, neural tube defect; R, right; Unk, unknown; VSD, ventricular septal defect. Method of determining zygosity:

^a method not stated;

^b monochorionic placenta;

^c blood groups;

^d monochorionic diamniotic placenta;

^e monochorionic monoamniotic placenta,

^f opposite genders;

^g dichorionic diamniotic placenta;

^h no details provided regarding zygosity.

* Cases with a Recognized Genetic Etiology are excluded.