Genomic Alterations that Contribute to the Development of Isolated and Non-Isolated Congenital Diaphragmatic Hernia

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Abstract

Background—Congenital diaphragmatic hernia (CDH) is a life-threatening birth defect. Most of the genetic factors that contribute to the development of CDH remain unidentified.

Objective—Identify genomic alterations that contribute to the development of diaphragmatic defects.

Methods—A cohort of 45 unrelated patients with CDH or diaphragmatic eventrations were screened for genomic alterations by array comparative genomic hybridization (aCGH) or SNP-based copy number analysis.

Results—Genomic alterations that were likely to have contributed to the development of CDH were identified in eight patients. Inherited deletions of *ZFPM2* were identified in two patients with isolated diaphragmatic defects and a large de novo 8q deletion overlapping the same gene was found in a patient with non-isolated CDH. A de novo microdeletion of chromosome 1q41q42 and

two de novo microdeletions on chromosome 16p11.2 were identified in patients with non-isolated CDH. Duplications of distal 11q and proximal 13q were found in a patient with non-isolated CDH and a de novo single gene deletion of *FZD2* was also identified in a patient with a partial pentalogy of Cantrell phenotype.

Conclusions—Haploinsufficiency of *ZFPM2* can cause dominantly inherited isolated diaphragmatic defects with incomplete penetrance. Our data define a new minimal deleted region for CDH on 1q41q42, provide evidence for the existence of CDH-related genes on chromosomes 16p11.2, 11q23-24 and 13q12 and suggest a possible role for *FZD2* and Wnt signaling in pentalogy of Cantrell phenotypes. These results demonstrate the clinical utility of screening for genomic alterations in individuals with both isolated and non-isolated diaphragmatic defects.

Keywords

Diaphragmatic hernia; ZFPM2; microdeletion 1q41q42; microdeletion 16p11.2; *FZD2*

INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a life-threatening birth defect that occurs in approximately 1/4000 live births and is defined as a protrusion of abdominal viscera into the thorax through an abnormal opening or defect in that is present at birth [1,2] In some cases the herniated viscera are covered by a membranous sac and can be difficult to distinguish from a diaphragmatic eventration in which there is extreme elevation of a part of the diaphragm that is often atrophic and abnormally thin. The majority of CDH cases are sporadic with the recurrence risk for isolated CDH typically quoted as <2% based on a multifactorial inheritance.[3] However, there is a growing body of evidence that small chromosomal anomalies can predispose to the development of CDH.[4–10]

The identification of a predisposing genomic change greatly enhances the ability of physicians to provide individualized genetic counseling and to formulate optimal treatment and surveillance plans. This would suggest that screening for genomic changes by array comparative genomic hybridization (aCGH) or SNP-based copy number analysis should be performed on all patients with diaphragmatic defects.[10,11] However, this practice has not been universally applied.

Efforts to map the locations of all reported chromosomal anomalies associated with CDH have revealed many regions of the genome that are recurrently deleted, duplicated or translocated in individuals with CDH.[12, 13] Each of these genomic regions is likely to harbor one or more CDH-related genes. For some of theses regions, a specific gene has been implicated in the development of diaphragmatic defects. Chromosome 8q22q23, for example, is recurrently deleted and translocated in individuals with CDH. This region harbors the zinc finger protein, multitype 2 (*ZFPM2*) gene that has been implicated in the development of diaphragmatic defects based the identification of a de novo truncating mutation in a child with a severe diaphragmatic eventration and the development of similar diaphragmatic defects in mice that are homozygous for a hypomorphic allele of *Zfpm2*.[14] In contrast, chromosome 1q41q42—which is also recurrently deleted in individuals with CDH—harbors a number of genes, none of which have been clearly shown to cause diaphragmatic defects in humans or animal models.[15]

In this report we screened a cohort of 45 individuals with diaphragmatic defects for genomic alterations by high-resolution aCGH or SNP-based copy number analysis. Chromosomal anomalies that are likely to have caused or contributed to the development of diaphragmatic defects were identified in eight patients. These findings allow us to define the inheritance pattern of diaphragmatic defects associated with haploinsufficiency of *ZFPM2* and delineate

a new minimal deleted region for CDH on 1q41q42. They also provide evidence for the existence of CDH-related genes on chromosomes 16p11.2, 11q23-24 and 13q12 and suggest a possible role for *FZD2* and Wnt signaling in the development of various phenotypes seen in pentalogy of Cantrell.[16]

METHODOLOGY

Patient accrual

For array studies, informed consent was obtained from a convenience sample of 45 patients with CDH or diaphragmatic eventrations and, when possible, their parents in accordance with IRB-approved protocols. None of these patients have been previously published with the exception of Patient 5 and Patient 7 whose clinical findings were summarized by Shinawi et al. and Fruhman et al. respectively.[17,18]

For sequence analysis of *ZFPM2*, a cohort of 52 patients with CDH and 10 patients with diaphragmatic eventrations were screened for deleterious changes. This cohort consisted of a subset of patients from the array cohort and similarly consented individuals. *ZFPM2* sequence analyses for these individuals have not been previously published.

Array comparative genomic hybridization and SNP-based copy number analyses

In most cases, chromosomal deletions and duplications were identified or confirmed by array comparative genomic hybridization on a research basis using Human Genome CGH 244K or SurePrint G3 Human CGH 1M Oligo MicroarrayKits (G4411B, G4447; Agilent Technologies, Santa Clara, CA) prepared according to the manufacturer's protocols and analyzed as previously described using individual sex matched controls with no personal or family history of CDH.[7] Putative copy number changes were defined by twoor more adjacent probes at 244K resolution or three or more adjacent probes at 1M resolution with $\log_2$ ratios suggestive of a deletion or duplication when compared to those of adjacent probes.

In two cases—Patient 2 and Patient 6—causative chromosomal deletions were identified prior to accrual and further aCGH testing was unwarranted based on the molecular data already available. The deletion in Patient 2 was identified using an Illumina CytoSNP bead version 12.2 (Illumina, Inc., San Diego, CA, USA) and the deletion in Patient 6 was identified and defined on a clinical basis using a 105K Combimatrix Molecular Diagnostics array (Combimatrix Molecular Diagnostics, Irvine, CA, USA) hybridized, extracted, and evaluated according to manufacturers' instructions.

Identification of previously reported copy number variants

To determine if putative changes identified by aCGH or SNP-based copy number analysis had been described previously in normal controls, we searched for similar deletions or duplications in the Database of Genomic Variants (http://projects.tcag.ca/variation/).

Confirmation of Genomic Changes

Changes that were not identified in the Database of Genomic Variants were confirmed by real-time quantitative PCR with the exception of causative changes identified in Patients 3 and 7 which were confirmed by chromosome analysis and Patients 4 and 6 which were confirmed by FISH analysis.

Quantitative real-time PCR

Quantitative real-time PCR (qPCR) analysis experiments were designed and carried out as previously described.[7] For quantitative real-time PCR analysis within the *ZFPM2* gene experiments were designed in a manner similar to the standard curve method described by Boehm et al. with a region of the c14orf145 gene serving as a control locus.[19]

Chromosome Analyses and FISH Studies

Chromosome analyses were performed for Patients 3 and 7 on a clinical basis by the Molecular Genetics Laboratory at Baylor College of Medicine. FISH analyses were performed for Patient 4 by the Cytogenetic Laboratory at Texas Tech University Health Sciences Center School of Medicine and for Patient 6 by Combimatrix Molecular Diagnostics.

Long-range PCR amplification and sequencing

Long amplification PCR was carried out using the TaKaRa long range PCR system (TaKaRa Bio, Otsu, Shiga, Japan) according to manufacturer's instructions. PCR products were gel-purified, sequenced, and analyzed using Sequencher 4.7 software (Gene Codes Corporation, Ann Arbor, Michigan, USA).

Identification of *ZFPM2* sequence changes

Primers were designed to amplify the coding sequence and the intron-exon boundaries of *ZFPM2* and used to amplify patient DNA. Sequence changes in PCR amplified products identified by comparison with control DNA sequences using Sequencher 4.7 software (Gene Codes Corporation, Ann Arbor, Michigan, USA).

Hispanic control samples were obtained from the Baylor Polymorphism Resource, a collection of approximately 600 anonymous control samples from major ethnic and racial backgrounds.

RESULTS

Identification of genomic changes in patients with CDH or diaphragmatic eventrations

Forty-five subjects with congenital diaphragmatic hernias or diaphragmatic eventrations of varying severity were screened for chromosomal anomalies by aCGH or SNP-based copy number analysis. Eight subjects carried genomic changes that likely contributed to the development of their diaphragmatic defects. Clinical and molecular data from these subjects are summarized in Table 1.

Patient 1 is a male of mixed ancestry who was diagnosed at 9 days of age with intestinal malrotation and a large, left-sided diaphragmatic eventration (Figure 1). Later in life, he was diagnosed with left-sided radioulnar synostosis. Despite normal motor development, at 24 months of age he had only two words. However, he made rapid progress with speech therapy and by 31 months of age he was speaking in 4-word sentences.

aCGH analysis revealed an ~1 Mb deletion that included only the *ZFPM2* gene on chromosome 8q22.3-23.1 (Figure 1). Quantitative and long-range PCR analysis revealed that the patient's father carried the same deletion (data not shown). A review of his father's medical records did not reveal evidence of a diaphragmatic anomaly despite an extensive evaluation after a severe automobile accident which included an abdominal CT scan. We screened the coding sequence and associated intron exon boundaries of Patient 1's remaining *ZFPM2* allele looking for changes that might account for the difference in phenotype between him and his father but no sequence changes were identified.

Patient 2 is a Caucasian male that was diagnosed prenatally with a left-sided CDH. His brother was similarly affected with CDH and died of complications related to his hernia. Except for a single umbilical artery, no additional anomalies were identified after birth. He died at 2 day of age from respiratory insufficiency and pulmonary hypertension. SNP-based copy number analysis revealed a deletion on chromosome 8q23.1 that included a portion of the 3′ coding region of *ZFPM2* and the first coding exon of oxidation resistance gene 1 (*OXR1*) based on mRNA transcript variant 1. Quantitative PCR analysis revealed that exons 7 and 8 of *ZFPM2* were included in the deletion (Figure 1). No sequence changes were identified in the coding sequence and intron/exon boundaries of the remaining allele.

Patient 3 had a de novo ~32 Mb deletion from 8q22.3 to 8q24.23 that overlapped the *ZFPM2* deletions seen in Patient 1 and 2 and also included the *EXT1* and *TRPS1* genes that are associated with Langer-Giedion syndrome (OMIM #150230). This Hispanic female was diagnosed prenatally with a large left-sided posterolateral CDH. Fetal MRI at 34 5/7 weeks also revealed an omphalocele and symmetrically short extremities. Due to severe pulmonary hypoplasia she was placed in neonatal hospice care immediately after birth and died shortly thereafter.

Patient 4 is a Caucasian male who had a normal prenatal course. He was born at 37 weeks gestation and his perinatal course was complicated by persistent hypoglycaemia. A diaphragmatic hernia was identified incidentally on a chest x-ray at 11 months of age during an investigation for failure to thrive. His motor and language development were delayed and he developed seizures at 20 months of age. CNS studies revealed right hemisphere cerebral volume loss with ex vacuo dilatation of the right lateral ventricle with diffuse thin cortical mantle, atrophy in the right hippocampus and a thin corpus callosum. aCGH analysis revealed a de novo 2.2 Mb interstitial deletion of 1q41–1q42.12 (Figure 2).

Patients 5 and Patient 6 were found to have de novo interstitial deletions of 16p11.2—a recurrent microdeletion region flanked by low-copy repeats. Patient 5 is a two-year old male of mixed ancestry who had a severe right-sided posterolateral CDH, micrognathia, a U-shaped palatal cleft, paternally inherited autosomal dominant polydactyly, and dysmorphic features consistent with 16p11.2 which were previously described.[17] He continues to need dietary supplementation via G-tube and supplemental oxygen when ill.

Patient 6 was a male infant conceived by intrauterine insemination with non-consanguineous Caucasian parents as donors. A left-sided diaphragmatic hernia was diagnosed prenatally. After birth, both of his thumbs were found to be proximally placed, hypoplastic and non-articulating with the left thumb having a pedunculated appearance. Additional findings included an extra thoracic vertebra and 13 pairs of ribs. He required extra corporal membrane oxygenation within the first day of life, developed severe diffuse edema, and died on the 17th day of life as a result of his severe respiratory insufficiency and pulmonary hypertension.

Patient 7 was born at 37 6/7 weeks to non-consanguineous Hispanic parents. She had persistent respiratory distress at birth and intubation was complicated by micrognathia and a tongue anomaly. Further evaluation revealed a left-sided CDH, a ventricular septal defect (VSD), and atrial septal defect (ASD), partial cleft palate, small anteriorly placed anus, and dysmorphic features. A marker chromosome was identified by G-banded chromosome analysis and was shown be the result of an unbalanced maternal translocation between chromosome 13q and 11q resulting in a 47,XX,+der(13)t(11;13)(q23;q12.3) chromosome complement.[18] Her prognosis was deemed poor in light of her multiple anomalies, and she died shortly after support was withdrawn on day of life 8. Additional findings at autopsy included abnormal lung fissures and coronary artery anomalies.

Patient 8 is a two-year old Caucasian male who was diagnosed prenatally with a left-sided anterior CDH, and a large omphalocele. Postnatal cardiac evaluation revealed a large perimembranous VSD and a patent foramen ovale (PFO) versus ASD. Additional anomalies included bilateral inguinal hernias, left-sided cryptorchism and premature fusion of the coronal sutures evident on head CT performed at 7 and 12 months of age. An MRI obtained at 11 months of age revealed diminished bifrontal and bitemporal parenchyma white matter associated with thinning of the corpus callosum, delayed myelination and generous extra-axial CSF spaces.

aCGH analysis revealed a de novo 17.4 kb deletion that involved only the frizzled homolog 2 gene (*FZD2*) on chromosome 17q12.2. Amplification of the breakpoint by long-range PCR followed by sequencing revealed that the deletion had occurred between two Alu repeats; an Alu-Sb proximally and an Alu-Sq distally. Matsuzaki et al. has reported deletions of this gene in 3/90 normal Yoruban individuals from Ibadan, Nigeria.[20] However, these changes were not identified by McCarroll et al. who analyzed the same cohort.[21]

To determine if the mutations in Patient 8's remaining *FZD2* allele may have contributed to his phenotype, we sequenced the coding region of his remaining *FZD2* allele but did not identify any sequence changes.

Twenty changes not reported in the Database of Genomic Variants were identified in this cohort by aCGH and confirmed by either qPCR and/or FISH analysis (Table 2). In two cases the extent of the copy number variation was larger than that seen in the parents. Patient TX-28 appeared to have four copies of a region on 11q13.5 while her parents each had 3 copies compared to control. Patient TX-50 had no copies of a region on 17q25.1 while his parents each had one copy compared to control. TX-50 also had two copies of a region on Xp27.1, similar to his mother, which could represent a de novo event.

Identifying *ZFPM2* sequence changes in patients with CDH and diaphragmatic eventrations

To determine if detrimental sequence changes in *ZFPM2* were a common cause of CDH or diaphragmatic eventrations, we screened the coding region and intron-exon boundaries of this gene in 52 patients with CDH (Table 3) and 10 patients with diaphragmatic eventrations (Table 4). Three non-synonymous changes were identified which had not been previously described in the 1000 Genomes project or dbSNP. A D98N change was identified in a male with an isolated posterior left-sided hernia with a large sac. His mother did not carry a D98N allele but the father was unavailable for analysis. This change was predicted to be possibly damaging by PolyPhen (http://genetics.bwh.harvard.edu/pph/) but non-deleterious by SNPs3D (http://www.snps3d.org/).[22, 23] The same change was reported by Bleyl et al. in an individual with bilateral CDH.[24] However, they concluded that the change was likely to be a benign SNP since it was also identified in control samples.

A Q889E change was identified in a Hispanic female with a right-sided CDH, ambiguous genetalia and a double vagina. Her mother did not carry the change but the father was unavailable for analysis. This change was predicted to be benign by PolyPhen, non-deleterious by SNPs3D, and was subsequently identified in 5 of 152 (3.3%) Hispanic control chromosomes.

A paternally inherited S210T mutation in an African American male with a left-sided CDH and dysmorphic features. This change was predicted to be benign by PolyPhen and non-deleterious by SNPs3D.

DISCUSSION

aCGH and SNP-based copy number analyses are commonly used as a primary screening tool to identify genomic aetiologies in patients with congenital anomalies. Identification of these changes can provide information that physicians can use to hone the prognostic and recurrence risk information given to families and to create individualized therapeutic and surveillance plans for patients.

Haploinsufficiency of *ZFPM2* causes autosomal dominant isolated diaphragmatic defects with incomplete penetrance

ZFPM2 is a transcriptional co-factor that forms heterodimers with GATA transcription factors to regulate gene expression during development.[25] The role of *ZFPM2* in development of diaphragmatic defects was first demonstrated by Ackerman et al. who showed that mice homozygous for a hypomorphic mutation in *Zfpm2* develop diaphragmatic eventrations.[14] In the same publication, they reported a de novo heterozygous R112X mutation in an infant with severe diaphragmatic eventrations and pulmonary hypoplasia who died shortly after birth.[14]

Patient 1 is the second child in which haploinsufficiency for *ZFPM2* has been seen in association with a severe diaphragmatic eventration. Although Patient 2's deletion affects both the 3′ end of *ZFPM2* and the first coding exon of *OXR1*, it is likely that disruption of *ZFPM2* underlies the development of CDH in this patient since OXR1 appears to be involved in the prevention of oxidative damage—a process that has not been implicated in the development of diaphragmatic defects.[26] Although DNA is not available on Patient 2's brother, it is likely that he inherited the same deletion from his mother which contributed to his CDH. Alterations in *ZFPM2* activity may also be the major, if not the sole, genetic factor that contributed to the development of CDH in Patient 3 and previously reported individuals with CDH with deletions and translocation involving 8q22q23 (Supplemental Table 2).[13] Since both Patient 1 and Patient 2 inherited their deletions from unaffected parents we conclude that loss-of-function alleles of *ZFPM2* are associated with autosomal dominant diaphragmatic defects—CDH or diaphragmatic eventrations—with incomplete penetrance.

The identification of the causative genomic changes in Patient 1 and 2, both of whom had only minor anomalies—radioulnar synostosis diagnosed at 2 and half years of age and a single umbilical artery, respectively—provide further evidence that such studies should be considered in individuals who appear to have isolated diaphragmatic defects.

Alterations in *ZFPM2* underlie a small fraction of congenital diaphragmatic defects

Screening of the *ZFPM2* gene in 52 patients with CDH and 10 patients with diaphragmatic eventrations failed to identify any clearly deleterious changes in the *ZFPM2* gene. Similarly, Ackerman et al. did not find deleterious *ZFPM2* sequence changes in 29 additional post mortem samples from children with diaphragmatic defects.[14] However, Bleyl et al., screened 96 CDH patients—53 with isolated CDH, 36 with CDH and additional anomalies, and 7 with CDH and known chromosomal anomalies—and identified a rare amino acid change, M703L, in a patient with isolated CDH that is predicted to be possibly damaging by PolyPhen and deleterious by SNPs3D (Supplemental Table 3).[24] These data suggests that severe, deleterious alterations in *ZFPM2* account for only a small portion of diaphragmatic defects. It is unclear, however, whether individual changes, like M703L, increase the risk for CDH and, if so, to what degree.

Delineation of new minimal deleted region for CDH on 1q41q42

Chromosome 1q41q42 has been shown to be recurrently deleted in individuals with CDH. The 1q41q42 minimal deleted region for CDH was previously defined by the maximal region of overlap between a patient reported by Kantarci et al. and a patient described by Van Hove et al. who were subsequently referred to as Patient 7 and Patient 4, respectively, by Shaffer et al.[6, 15, 27] This interval spanned ~6.3 Mb (220,120,384–226,397,002 hg19) and contained 48 RefSeq genes.

The maximal proximal and distal breakpoints of our Patient 4's deletion are located inside the CDH region defined by Shaffer et al.[15] These breakpoints can be used to define a new minimal deleted region for CDH on chromosome 1q41q42 which spans ~2.2 Mb (223,073,839–225,318,623 hg19) and contains only 15 RefSeq genes (Figure 2).

This refined minimal deleted region for CDH does not contain the H2.0-like homeobox (*HLX*) gene which has been considered a candidate gene for CDH based on its expression in the murine diaphragm and the diaphragmatic defects identified in $Hlx^{-/-}$ mice.[28–30]. Although it is possible that haploinsufficiency of *HLX* can contribute to the formation of diaphragmatic defects in humans, its exclusion from the minimal deleted region for CDH suggests that alterations in *HLX* function are not required for the development of CDH in association with 1q41q42 deletions.

The dispatched homolog 1 (*DISP1)* gene has also been considered a candidate gene for CDH and is located within the new minimal deleted interval for CDH. DISP1 is a membrane spanning protein that plays an essential role in SHH signaling.[31] The potential role of *DISP1* in the formation of CDH is supported by evidence that murine *Disp1* is expressed in the pleuroperitoneal fold (PPF), a structure that is often considered to be the primordial mouse diaphragm and the identification of a somatic Ala1471Gly mutation—predicted to be benign by PolyPhen but "not tolerated" by SIFT (http://blocks.fhcrc.org/sift/SIFT.html)—in a patient with CDH and other anomalies.[32] However, abnormalities in other SHH signaling proteins have yet to be clearly shown to cause CDH. Truncating mutations (W475X and Y734X) in *DISP1* have been found in two individuals with microforms of holoprosencephaly and their unaffected mothers—consistent with *DISP1*'s role in SHH signaling—but not in individuals with diaphragmatic defects.[32, 33] This suggests that consideration should be given to the possible role of other 1q41q42 genes in the development of CDH. The tumor protein p53 binding protein, 2 (*TP53BP2)* gene could play a role in CDH development by modulating apoptosis and cell proliferation through interactions with other regulatory molecules and has been shown to play a critical role in heart and CNS development in mice.[34, 35] The WD repeat domain 26 (*WDR26*) gene is another candidate gene that is widely expressed and could play a role in the development of CDH by regulating cell signaling pathways and modulating cell proliferation.[36, 37]

CDH is recurrently seen in 16p11.2 microdeletion syndrome

Recurrent 16p11.2 microdeletions result in a constellation of defects including developmental delay, cognitive impairment, behavioral problems, seizures and congenital anomalies.[17] Both Patient 5 and Patient 6 have de novo deletions of this region suggesting that CDH should be added to the list of congenital anomalies associated with this microdeletion syndrome.

The T-box 6 gene (*TBX6*) is located on 16p11.2 and encodes a transcription factor that has been shown to play a critical role in important developmental processes including paraxial mesoderm differentiation and left right patterning.[38, 39] As a result, *TBX6* has been hypothesized to contribute to many of the congenital anomalies associated with 16p11.2.[17] It is possible that haploinsufficiency of *TBX6* also contribute to the development of CDH.

Evidence of CDH-related genes on distal chromosome 11q and proximal chromosome 13q

Patient 7 carried an extra derivative 13 chromosome making her trisomic for a portion of the chromosome 13 extending to 13q12.3 and a portion of chromosome 11 extending from 11q23 to the telomere—47,XX,+der(13)t(11;13)(q23.2;q12.3). A similar chromosomal complement 47,XY,+der(13)t(11;13)(q21;q14) was seen in a boy with CDH described by Park et al. suggesting that upregulation of genes on distal 11q and/or proximal 13q may play a role in the development of CDH.[40] In support of this hypothesis, duplications of 11q have been recurrently seen in patients with CDH with many cases being associated with an unbalanced 3:1 meiotic segregation of the common t(11;22) translocation—resulting in a 47,XX or XY,+der(22)t(11;22)(q23.3;q11.2) chromosomal complement.[13, 41] Multiple cases of CDH associated with trisomy 13 have also been described providing support for the existence of one or more CDH-related genes on chromosome 13.[13]

The role of *FZD2* and Wnt signaling in the development of CDH and defects associated with pentalogy of Cantrell

The cardiac, diaphragmatic and anterior wall defects seen in Patient 8, are similar to those described in pentalogy of Cantrell—a constellation of defects described by Cantrell et al. that includes, a supraumbilical omphalocele, a lower sternal cleft, a defect in the central tendon of the diaphragm, a defect in the pericardium, and an intracardiac anomaly.[16] A de novo deletion of *FZD2* was identified in Patient 8 but similar deletions have been reported in normal individuals from Ibadan, Nigeria.[20] Although this suggests that haploinsufficiency of *FZD2* is unlikely to be the sole cause of this patient's phenotype, several lines of evidence suggest that decreased *FZD2* expression may have contributed to this child's phenotype.

Frizzled genes encode a family of seven transmembrane domain proteins that act as receptors for Wnt signaling proteins.[42] *FZD2* is expressed in a variety of organs during development (Supplemental Table 1) and its protein product has been shown to interact with WNT3A and WNT5A and to signal through both the canonical (Wnt/beta-catenin) and non-canonical (Wnt/Ca++ and cyclic GMP) Wnt pathways.[43] In chick embryos, Wnt3a and Wnt5a modulate the migration of mesodermal precursors and Wnt3a has been shown to control the movement patterns of cardiac progenitor cells.[44, 45] Wnt signaling has also been shown to play a role in the development of CDH and omphalocele in humans. X-linked dominant mutations in *PORCN*, a gene that encodes a protein that modifies Wnt proteins—including WNT3A—for membrane targeting and secretion, have been shown to cause CDH and omphalocele as part of focal dermal hypoplasia (Goltz syndrome; OMIM #305600).[46, 47]

It is possible that deletion of *FZD2*, in combination with other genetic and/or environmental factors, caused dysregulation of these Wnt signaling pathways, leading to the development of the cardiac defects, CDH and omphalocele seen in Patient 8. The potential role of frizzled signaling in developmental processes that require directional tissue movements followed by tissue fusion is supported by the work of Yu et al. who recently showed that mouse frizzled 1 (*Fzd1*) and frizzled 2 (*Fzd2*) genes play an essential and partially redundant role in the correct positioning of the cardiac outflow tract and closure of the palate and ventricular septum.[48] Genetic changes that disrupt these processes may ultimately be found to contribute to other cases of partial or complete pentalogy of Cantrell.

Genomic changes of unknown significance

The majority of the rare genomic changes catalogued in Table 2 are not located within genomic regions that have been shown to be recurrently deleted or duplicated in individuals with CDH and do not affect genes/pathways that have been clearly implicated in the development of diaphragmatic defects. Although the significance of these changes is

presently unknown, it is likely that future studies, in humans and animal models, will help to clarify whether these changes increase the risk of developing diaphragm defects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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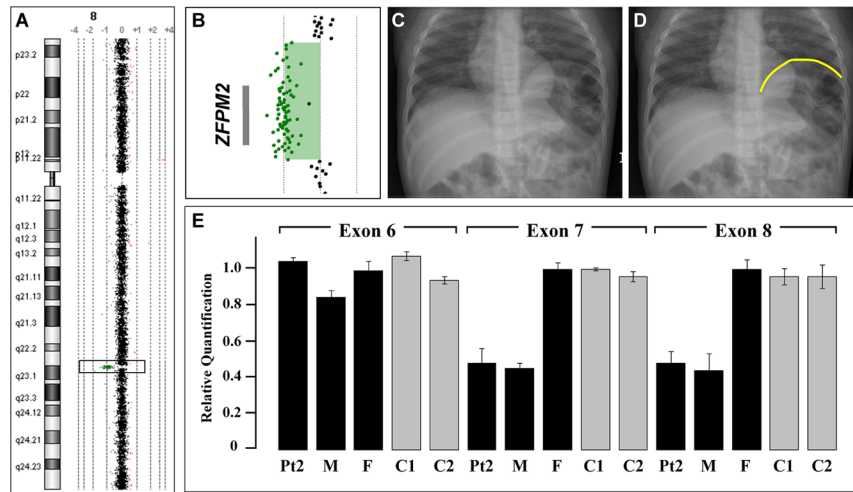
Figure 1.
A) Array comparative genomic hybridization data from Patient 1 showing an 8q22.3q23.1 single gene deletion of *ZFPM2*. B) The relative location of the *ZFPM2* gene in relation to aCGH data from the deletion region in Patient 1 is represented by a gray bar. C) A chest radiograph of Patient 1 demonstrating a severe diaphragmatic eventration containing loops of bowel. D) The same radiograph shown in panel C with the limits of the diaphragmatic eventration outlined in yellow. E) Quantitative PCR analysis demonstrates a normal copy number for *ZFPM2* Exon 6 but a reduced copy number for Exons 7 and 8 in DNA from Patient 2 (Pt2) and his mother (M). Normal copy number values are seen in DNA from Patient 2's father (F) and DNA from two unrelated controls (C1, C2).
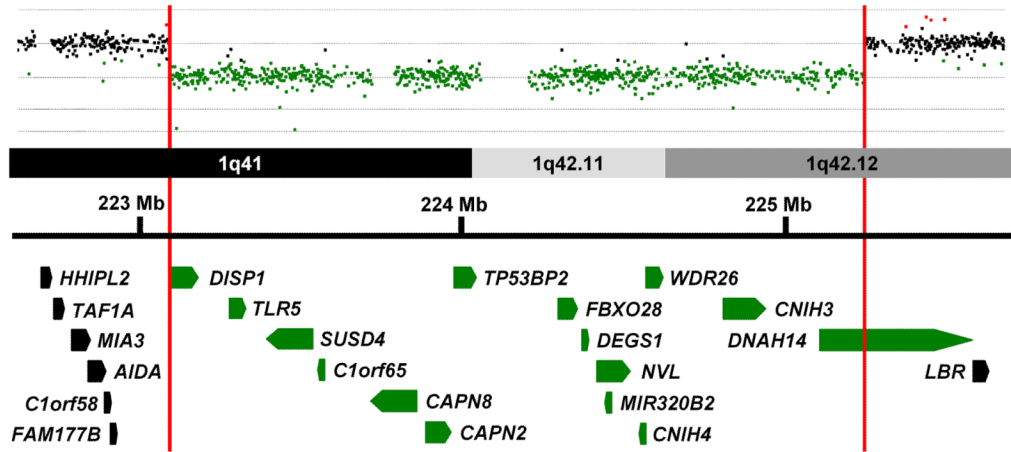
Figure 2.
Array comparative genomic hybridization data from Patient 4 is shown above a schematic representation of a portion of the 1q41–1q42 region. Genes in this region are represented by block arrows. Red vertical bars mark the limits of the deletion in Patient 4 and delineate the minimal deleted region for congenital diaphragmatic hernia (CDH) in this region of the genome. Genes with all or a portion of their coding sequence inside the minimal deleted region are shown in green.

Table 1

Summary of data from subjects with chromosomal changes likely to have contributed to diaphragm defects

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Final Karyotype	46,XY,del(8)(q2 2.3q23.1)	46,XY,del(8)(q2 3.1q23.1)	46,XX,del(8)(q2 2.3q24.23)	46,XY,del(1)(q4 1q42.12)	46,XY,del(16)(p 11.2p11.2)	46,XY,del(16)(p 11.2p11.2)	47,XX+der(13)t(11 ;13)(q23.2;q12.3)	46,XY,del(17)(q 12.2-q12.3)
Minimal/maximal affected region (hg19)	Chromosome 8 deletion 105,914,640–106,907,764 (~993 kb)/105,880,441–106,922,626 (~1.04 Mb)	Chromosome 8 deletion 106,812,277–107,420,029 (~608 kb)/106,800,200–107,511,467 (~711 kb)	Chromosome 8 deletion 104,516,737–136,746,871 (~32.2 Mb)/104,508,082–136,809,150 (~32.3 Mb)	Chromosome 1 deletion 223,076,895–225,311,293 (2.2 Mb)/223,073,839–225,318,623 (2.2 Mb)	Chromosome 16 deletion 29,652,999–30,199,351 (~554 kb)/29,350,831–30,332,522 (~982 kb)	Chromosome 16 deletion 29,502,653–30,274,073 (~771 kb) defined clinically with no minimum or maximum region specified.	Chromosome 11 duplication 112,355,952–134,927,114 (26.6 Mb)/112,347,267–134,927,114 (26.6 Mb) Chromosome 13 duplication 19,296,527–32,250,725 (12.9 Mb)/19,168,012–32,271,202 (13.1 Mb)	Chromosome 17 deletion 42,633,066–42,650,463 (~17.4 kb)/42,622,151–42,657,504 (~35.4 kb)
Confirmation Method	Real-time qPCR	Real-time qPCR	Chromosome analysis	FISH	Real-time qPCR	FISH	Chromosome analysis	Real-time qPCR
Inheritance	Paternal	Maternal	De novo	De novo	De novo	De novo	Maternal balanced translocation	De novo
Age	4 year-old	2 day-old	1 day-old	2 year-old	2 year-old	17 day-old	8 day-old	2 year-old
Gender	Male	Male	Female	Male	Male	Male	Female	Male
Ethnicity	Mixed	Caucasian	Hispanic	Caucasian	Mixed	Caucasian	Hispanic	Caucasian
Prenatally identified abnormalities, exposures, and prenatal karyotype	No abnormalities identified	CDH, intrauterine growth retardation, single umbilical artery, 46,XY	CDH, short extremities, edema/ascites, polyhydramnios, 46,XX,del(8)(q22q24)	No abnormalities identified on two prenatal ultrasounds	CDH, Maternal smoking in first trimester	CDH, normal chromosomes 46,XY	CDH, polyhydramnios and intrauterine growth retardation	CDH and omphalocele detected, normal chromosomes 46,XY
Birth history	Vaginal delivery at term, vomiting 2 days after birth	Caesarean section at 36 3/7 weeks for abnormal fetal heart rate and decreased movement	Spontaneous vaginal delivery at 34 5/7 wks	Spontaneous vaginal delivery at 37 weeks	Delivery at 37 weeks, after birth he decompensated and required 20 minutes of CPR	Repeat caesarean section at 39 6/7 weeks	Spontaneous vaginal delivery at 37 3/7 weeks	Scheduled caesarean section at 37 1/7 weeks
Birth weight, length and OFC	Weight 3.8 kg	Weight: 1.72 kg; length 42 cm; OFC 29.5 cm	No information available	Weight 3.2 kg; length 49.5 cm	Weight estimated at 2.5 kg; length 49 cm; OFC 34 cm.	Weight ~3.5 kg; length 50 cm; OFC 35.5 cm.	No information available	Weight 2.2 kg; length 45 cm, OFC 33 cm
Diaphragm	Diaphragmatic eventration	Left-sided CDH	Large left-sided CDH	CDH identified at 11 months	Right-sided CDH	Left-sided CDH	Large left-sided CDH	Anterior medial CDH
Cardiac	No known abnormalities	No known abnormalities	No known abnormalities	No known abnormalities	No structural abnormalities noted on ECHO	No structural abnormalities noted on ECHO	Small perimembranous VSD, secundum ASD	Large perimembranous VSD, PFO verses ASD
Additional anomalies	Intestinal malrotation, radioulnar synostosis	No known abnormalities	All extremities were symmetrically short with fixed flexion of the distal upper extremities	Right cerebral volume loss with ex vacuo dilatation of the right lateral ventricle, diffuse thin cortical mantle, atrophy of right hippocampus, this corpus callosum, left-sided	Micro-retrognathia, cleft palate, right inguinal hernia and bilateral post axial polydactyly (paternal)	Hypoplastic, non-articulating thumbs, extra thoracic vertebra, 13 pairs of ribs	Clouded corneas, cupped optic nerves, cleft palate, small anterior anus, prominent forehead, large anterior fontanel posteriorly-rotated low-set ears, Micro-retrognathia wide-spaced hypoplastic nipples,	Coronal craniosynostosis, diminished CNS white matter with thinning of the corpus callosum omphalocele, bilateral

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Final Karyotype	46,XY,del(8)(q22.3q23.1)	46,XY,del(8)(q23.1q23.1)	46,XX,del(8)(q22.3q24.23)	46,XY,del(1)(q41q42.12)	46,XY,del(16)(p11.2p11.2)	46,XY,del(16)(p11.2p11.2)	47,XX+der(13)t(11;13)(q23.2;q12.3)	46,XY,del(17)(q12.2q12.2)
				cryptorchidism with non-attachment to the epididymis			right single palmar crease, tapering fingers, prominent heels.	inguinal hernias, left-sided cryptorchidism.
Clinical course/development	Only two words at age two. Responded well to speech therapy. Now normal	Died on the 2nd day of life with respiratory insufficiency and pulmonary hypertension	Died shortly after delivery	Persistent hypoglycaemia after birth, gross motor, fine motor and language delay, seizures starting at 20 months	No oxygen requirement at 24 months but still receiving supplemental nutrition via g-tube	Died on the 17th day of life with severe respiratory insufficiency and pulmonary hypertension	Support was withdrawn at 8 days of age. Findings at autopsy included abnormal lung fissures and coronary artery anomalies	At 28 months could walk with a walker, nutritional support via g-tube, ventilator support with acute illness

CDH = congenital diaphragmatic hernia, OFC = occipital frontal circumference, ASD = atrial septal defect, VSD = ventricular septal defect, ECHO = echocardiogram, CPR = cardiopulmonary resuscitation, CNS = central nervous system, PFO =patent foramen ovale.

Table 2

Rare inherited changes identified in patients with CDH

Patient Number	Copy Number Change	Inheritance Pattern	Chromosome	Genes Involved	Minimum/Maximum hg19	Clinical Synopsis
TX-02	Increase	Paternal	15q24.2	COMMD4, NEIL1	75,605,889–75,644,263 75,600,108–75,648,132	Female, left-sided CDH, large bronchopulmonary sequestration
TX-04	Increase	Paternal & maternal duplication	3q26.33	SOX2, SOX2OT	181,430,003–181,431,902 181,425,656–181,432,270	Male, isolated right-sided CDH
	Increase	Maternal	5q11.2	ARL15, HSPB3	53,450,417–53,759,016 53,443,541–53,765,448	
	Increase	Paternal & maternal duplication	18p11.21	GNAL, MPPE1*	11,872,270–11,878,083 11,863,962–11,884,082	
TX-12	Decrease	Paternal	7q32.1	AHCYL2	128,988,273–128,996,545 128,978,921–129,002,744	Male, isolated left-sided CDH
TX-14	Increase	Paternal	13q21.33	KLHL1, SCA8, ATXN8OS	70,673,470–71,577,278 70,666,491–71,594,524	Male, isolated left-sided CDH
TX-23	Increase	Maternal	1p31.3	CACHD1, RAVER2	65,144,120–65,210,203 65,137,345–65,218,480	Female, right-sided CDH, ASD, misshaped kidney
TX-27	Decrease	Parents not available	14q21.1	FBXO33	39,884,781–40,262,115 39,875,206–40,301,762	Female, isolated left-sided
TX-28	Increase	Patient n=4 Mother and Father n=3	11q13.5	AQP11, CLNS1A, RSF1	77,297,657–77,424,591 77,270,540–77,431,584	Female, left-sided CDH, double outlet right ventricle, VSD
TX-36	Decrease	Not maternal, father not available	1p12	HSD3B2	119,962,240–119,983,891 119,957,963–119,994,447	Male, isolated left-sided CDH
TX-39	Decrease	Maternal	16p13.3	DNASE1, TRAP1	3,684,132–3,716,095 3,672,598–3,726,606	Female, left-sided posterior CDH, bicommissural aortic valve
TX-42	Increase	Paternal & maternal Increased copy number	1q24.3	DNM3	171,944,462–171,958,347 171,936,289–171,970,634	Male, isolated left-sided posterior CDH
	Decrease	Paternal	15q21.2	USP8*, USP50	50,802,672–50,822,065 50,791,758–50,833,347	
TX-50	Decrease	Patient n=0 Parents n=1	17q25.1	TRIM4*	73,875,459–73,878,627 73,870,461–73,882,792	Male, isolated left-sided CDH
	Increase	Maternal n=2	Xq27.1	F9, MCF2, ATP11C, MIR505, CXorf66	138,594,571–139,089,290 138,556,308–139,102,322	
TX-52	Decrease	Paternal	15q23	AAGAB*, IQCH, C15orf61, MAP2K5	67,548,229–68,040,596 67,540,042–68,049,656	Female, right-sided CDH, ASD
TX-56	Increase	Maternal	Xp11.1	ZXDA	57,704,902–57,987,522 57,673,005–58,051,706	Male, right-sided CDH, left-sided inguinal hernia

Patient Number	Copy Number Change	Inheritance Pattern	Chromosome	Genes Involved	Minimum/Maximum hg19	Clinical Synopsis
TX-65	Decrease	Maternal	7q31.1	*DOCK4*	111,668,580–111,784,369 111,653,357–111,789,489	Male, left-sided CDH, extra-lobar pulmonary sequestration,
TX-72	Increase	Paternal & maternal Increased copy number	3q13.31	*GRAMD1C* , ZDHHC23*	113,668,345–113,672,579 113,664,314–113,679,817	Male, left-sided posterior CDH,
TX-73	Increase	Paternal	20p12.2	*SNAP25, MKKS, C20orf94, JAG1*	10,223,539–10,902,949 10,213,446–10,937,659	Male, left-sided CDH, ASD: sibling died shortly after birth with CDH

*
genes located outside the minimal deleted region but are located, at least partially, inside the maximal deleted region; CDH = congenital diaphragmatic hernia, ASD = atrial septal defect, VSD = ventricular septal defect

Table 3

ZFPM2 sequence changes in patients with CDH

Observed change	Number of Patients (Inheritance Pattern)	Exon	1000 Genomes[*]/dbSNP (Heterozygosity)	Predicted effect (PolyPhen/SNPs3D)
Non-synonymous changes				
D98N	1 (Non-maternal, father not available)	3	No/No	Possibly damaging/Non-Deleterious
S210T	1 (Paternal)	6	No/No	Benign/Non-Deleterious
A403G	11	8	Yes/Yes (0.341)	Benign/Non-Deleterious
E782D	10	8	Yes/Yes (0.078)	Benign/Non-Deleterious
Q889E	1 (Non-maternal father not available)	8	No/No—3.3% Hispanic control chromosomes	Benign/Non-Deleterious
A1055V	1 (Non-maternal, father not available)	8	Yes/Yes (0.146)	Benign/Non-Deleterious
Synonymous changes				
P454P	4	8	Yes/Yes (0.234)	--
P592P	3	8	Yes/Yes (0.101)	--
V795V	2	8	Yes/Yes (0.120)	--
Y992Y	3	8	Yes/Yes (0.104)	--
H1069H	4	8	Yes/Yes (0.234)	--
L1123L	3	8	Yes/Yes (0.156)	--

[*]
Data accessed on 3/8/2011

Table 4

ZFPM2 sequence changes in patients with diaphragmatic eventrations

Observed change	Number of Patients	Exon	1000 Genomes[*]/dbSNP (Heterozygosity)	Predicted effect (PolyPhen)
Non-synonymous changes				
A403G	1	8	Yes/Yes (0.341)	Benign/Non-Deleterious
E782D	2	8	Yes/Yes (0.078)	Benign/Non-Deleterious
Synonymous changes				
P454P	1	8	Yes/Yes (0.234)	--
P592P	1	8	Yes/Yes (0.101)	--
V795V	2[**]	8	Yes/Yes (0.120)	--
H1069H	1	8	Yes/Yes (0.234)	--

[*]
Data accessed on 3/8/2011

[**]
Homozygous in one of the patients