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Evidence that *FGFRL1* contributes to congenital diaphragmatic hernia development in humans

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Abstract

Fibroblast growth factor receptor-like 1 (*FGFRL1*) encodes a transmembrane protein that is related to fibroblast growth factor receptors but lacks an intercellular tyrosine kinase domain. in vitro studies suggest that FGFRL1 inhibits cell proliferation and promotes cell differentiation and cell adhesion. Mice that lack FGFRL1 die shortly after birth from respiratory distress and have abnormally thin diaphragms whose muscular hypoplasia allows the liver to protrude into the thoracic cavity. Haploinsufficiency of *FGFRL1* has been hypothesized to contribute to the development of congenital diaphragmatic hernia (CDH) associated with Wolf-Hirschhorn syndrome. However, data from both humans and mice suggest that disruption of one copy of *FGFRL1* alone is insufficient to cause diaphragm defects. Here we report a female fetus with CDH whose 4p16.3 deletion allows us to refine the Wolf-Hirschhorn syndrome CDH critical region to an approximately 1.9 Mb region that contains *FGFRL1*. We also report a male infant with isolated left-sided diaphragm agenesis who carried compound heterozygous missense variants in *FGFRL1*. These cases provide additional evidence that deleterious *FGFRL1* variants may contribute to the development of CDH in humans.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The FGFRL1 variants seen in Subject 2 have been submitted to the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/).

Keywords

4p16.3; congenital diaphragmatic hernia; critical region; FGFRL1; Wolf-Hirschhorn syndrome

1 | INTRODUCTION

The fibroblast growth factors (FGFs) regulate a variety of cellular functions including cell proliferation, differentiation, migration, and apoptosis and play a critical role in embryonic development (Belov & Mohammadi, 2013). FGFs act by binding to and activating members of the fibroblast growth factor receptors (FGFR) subfamily of tyrosine kinases. Fibroblast growth factor receptor-like 1 (*FGFRL1*; MIM# 605830) encodes a transmembrane protein that is related to FGFRs but lacks an intercellular tyrosine kinase domain (Wiedemann & Trueb, 2000). in vitro studies suggest that FGFRL1 inhibits cell proliferation (Trueb, Zhuang, Taeschler, & Wiedemann, 2003), and promotes cell differentiation (Baertschi, Zhuang, & Trueb, 2007) and cell adhesion (Rieckmann, Kotevic, & Trueb, 2008).

In mice, *Fgfrl1* is expressed at a relatively high level in the developing diaphragm (Trueb & Taeschler, 2006). *Fgfrl1*$^{-/-}$ mice die shortly after birth due to respiratory distress caused by generalized hypotrophy of the diaphragm muscle (Baertschi et al., 2007). In some regions, the muscular diaphragm is replaced by connective tissue, and in some cases the liver protrudes into the thoracic cavity (Gerber, Steinberg, Beyeler, Villiger, & Trueb, 2009). Decreased expression of FGFRL1 may also contribute to the development of congenital diaphragmatic hernia (CDH) in mice exposed to nitrofen in utero (Dingemann, Doi, Ruttenstock, & Puri, 2011).

In humans, *FGFRL1* is located in an ~2.3 Mb CDH critical region on 4p16.3 defined by a patient described by Casaccia, Mobili, Braguglia, Santoro, and Bagolan (2006). Deletions of 4p16.3 cause Wolf–Hirschhorn syndrome (WHS; MIM# 194190). Deletion of *FGFRL1* has been previously hypothesized to contribute to the development of CDH associated with WHS (Callaway et al., 2018; LopezJimenez et al., 2010). However, the low loss-of-function intolerance of *FGFRL1* in the Genome Aggregation Database (gnomAD ver2.1.1; https://gnomad.broadinstitute.org/; pLI = 0.01; e/o ratio = 0.37) suggests that haploinsufficiency of *FGFRL1* alone is unlikely to be sufficient to cause CDH (Karczewski et al., 2020).

Here we refine the CDH critical region associated with WHS to an ~1.9 Mb region of 4p16.3 based on a deletion identified in a female fetus with CDH. We also report a male infant with isolated left-sided diaphragm agenesis who was compound heterozygous for missense variants in *FGFRL1*. These cases provide additional evidence that deleterious *FGFRL1* variants may contribute to the development of CDH in humans.

2 | MATERIALS AND METHODS

2.1 | Editorial policies and ethical considerations

Subjects 1 and 2 were enrolled in research studies in accordance with protocols approved by local institutional review boards. The procedures followed were in accordance with the

ethical standards of Baylor College of Medicine's committee on human research and were in keeping with international standards.

2.2 | Molecular testing

The 4p16.3 deletion in Subject 1 was detected by array-comparative genomic hybridization (a-CGH) performed according to manufacturer's recommendations using a 60 K Agilent array. Genetic testing for Subject 2 was performed on a clinical basis at Baylor Genetics using a cord blood sample and included a chromosome analysis with 550-band resolution, array-based copy number variant (CNV) analysis (CMA-HR + SNP version 11.2) and critical trio whole exome sequencing (Meng et al., 2017). Tests for maternal blood contamination were negative.

2.3 | In silico variant analyses

In silico analyses of *FGFRL1* sequence variants were performed using SIFT (https://sift.bii.a-star.edu.sg/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), MutationTaster (http://www.mutationtaster.org/), and Combined Annotation Dependent Depletion (CADD; https://cadd.gs.washington.edu/).

3 | RESULTS

3.1 | Clinical presentations and molecular studies

3.1.1 | Subject 1—Subject 1 (DECIPHER ID: 339928) was a Caucasian, 21-week gestation female fetus with left-sided CDH identified by prenatal ultrasound. The left lung was hypoplastic and the heart was displaced to the right. The stomach was in the thoracic cavity, but the remaining abdominal organs had normal conformations and positions. The brain was of normal size and conformation for the gestational age. Parents chose to terminate the pregnancy. Postmortem biometric values were consistent with a fetus of 21 weeks gestational age with a weight of 480 g (82nd centile), length of 28.5 cm (94th centile), and occipitofrontal circumference of 21.3 cm (99th centile, Z-score = 2.59). The fetus was found to harbor a de novo 4p16.3 deletion (minimum deletion chr4:71,552–1,800,425, maximum deletion chr4:1–1,875,255; hg19) by array-comparative genomic hybridization (Figure 1).

3.1.2 | Subject 2—Subject 2 was a Hispanic male of Mexican descent who was conceived by healthy, nonconsanguineous parents with the aid of oral medications for ovulation induction. His parents had one spontaneous abortion at 8 weeks gestation and have a 5-year-old son who is in good health. There was no family history of functional or structural birth defects. The pregnancy was complicated by diet-controlled gestational diabetes.

At 31 weeks gestation, an ultrasound examination showed a left-sided CDH. A fetal MRI at 38 weeks gestation confirmed a left-sided CDH with herniation of the stomach, spleen, and a portion of the left lobe of the liver, with no evidence of a hernia sac. The total fetal lung volume was 16.8 ml, with a right lung volume of 15.6 ml, and a left lung volume of 1.2 ml. The observed/expected total fetal lung volume was 15.4% based on the formula proposed by

Rypens et al. and 19% based on mean values reported by the same group (Rypens et al., 2001). The lung area to head circumference ratio was 1.0, the observed/expected lung to head ratio was 30%, and 24% of the liver was herniated.

Subject 2 was born at 39 3/7 weeks gestation via an induced vaginal delivery. Apgar scores were 8 and 9. The patient had a birth weight of 3.06 kg (27th centile), a birth length of 50.8 cm (69th centile), and a head circumference of 35 cm (66th centile). He was intubated and sedated immediately after delivery. There were no dysmorphic features or other congenital anomalies. A head ultrasound, performed on the first day of life, was normal. An echocardiogram on the sixth day of life showed severe pulmonary hypertension, severe tricuspid regurgitation, a severely dilated right ventricle with qualitatively moderately to severely depressed systolic function, and decreased left ventricle cavity size with hyperdynamic systolic function. There was a small patent ductus arteriosus and a patent foramen ovale/small atrial septal defect, both with right to left shunting.

On day of life 11, he was put on veno-arterial extracorporeal membrane oxygenation (VA-ECMO) due to respiratory failure and cardiac dysfunction. He underwent a CDH repair surgery on day of life 12. The left-sided diaphragmatic defect was estimated as 95%, with no diaphragm along the left lateral aspect and the left anterolateral and posterolateral aspect of the chest. There was a rim of diaphragm medially that was about 5 mm in its the largest dimension anteriorly, and a small crural remnant posteromedially. The abdominal contents were returned to the abdomen and diaphragmatic defect was closed with a GORE-TEX Dual Mesh patch.

On day of life 24, he had a successful trial off VA-ECMO; however, he suffered an unexplained decompensation shortly thereafter. Despite maximal support, he died on day of life 25. Postmortem CT and MRI evaluations did not identify additional birth defects.

A chromosome analysis showed a 46,XY chromosomal complement. Array-based CNV analysis did not identify variants that were associated with known microdeletion or microduplication syndromes, deletions of the mitochondrial genome, or increased blocks of absence of heterozygosity (AOH). Trio exome sequencing performed on a clinical basis did not reveal any pathogenic variants, likely pathogenic variants, or variants of uncertain significance in known CDH genes. However, the same test revealed that Subject 2 was compound heterozygous for two *FGFRL1* missense variants; a paternally inherited c.886A>G, p.(I296V) variant located in a region that codes for an Ig-like C2-type 3 domain (amino acids 246–354; UniProt https://www.uniprot.org/uniprot/Q8N441), and a maternally inherited c.1328G>C, p.(G443A) variant. All variants described in this article are based on transcript NM_001004356.2. In silico analyses of these variants are summarized in Table 1.

4 | DISCUSSION

Despite advances in diagnostic techniques, the molecular etiology of the majority of CDH cases remains undetermined (Yu, Hernan, Wynn, & Chung, 2019). This is due, in part, to an incomplete understanding of the genes that contribute to the development of CDH. Often the first indication that a gene plays a role in diaphragm development comes from mouse

models with evidence of a role in human diaphragm development slowly accumulating over time.

The first indication that FGFRL1 deficiency could cause CDH came from mouse models. *Fgfrl1*$^{-/-}$ mice die shortly after birth from respiratory distress and have abnormally thin diaphragms whose muscular hypoplasia allows the liver to protrude into the thoracic cavity (Baertschi et al., 2007; Gerber et al., 2009). Decreased expression of FGFRL1 during the late stages of gestation has also been hypothesized to contribute to the development of CDH in mice exposed to nitrofen in utero (Dingemann et al., 2011).

In humans, *FGFRL1* is located in the previously defined ~2.3 Mb CDH critical region on chromosome 4p16.3 (Casaccia et al., 2006), and haploinsufficiency of *FGFRL1* has been hypothesized to contribute to the development of CDH associated with WHS for over a decade (Callaway et al., 2018; LopezJimenez et al., 2010). Among the ~61 RefSeq genes located in this region, *FGFRL1* was identified using a machine learning algorithm to be the second most similar to a group of training genes previously shown to cause CDH (Callaway et al., 2018). Among all RefSeq genes, *FGFRL1* was ranked at the 98.9th centile based on its similarity to genes in the CDH training set (CDH-specific pathogenicity score = 98.9%) (Callaway et al., 2018), suggesting that it represents an excellent positional candidate gene for CDH.

The deletion identified in Subject 1 allows us to refine the CDH critical region to an approximately 1.9 Mb region that still includes *FGFRL1* (Figure 1). This interval includes ~48 RefSeq genes. Since *FGFRL1* has a low loss-of-function intolerance in gnomAD (pLI = 0.01; e/o ratio = 0.37), it is likely that haploinsufficiency of *FGFRL1* combined with other epigenetic, genetic, environmental, and/or stochastic factors is responsible for the CDH seen in a subset of individuals with WHS. The genetic factors involved may include the haploinsufficiency of other protein coding genes within the new critical region that have high CDH-specific pathogenicity scores generated by Callaway et al. These genes may include *FGFR3* (99.9%; MIM# 134934; pLI = 0), *NSD2* (97.3%; MIM# 602952; pLI = 1), and *ZNF141* (97%; MIM# 194648; pLI = 0.1), *MAEA* (91%; MIM# 606801; pLI = 0.23), *CPLX1* (89%; MIM# 605032; pLI = 0.82) and/or *CTBP1* (85.6%; MIM# 602618; pLI = 0.98) (Callaway et al., 2018). Of these genes, only *CTBP1* has been clearly implicated in the development of the diaphragm with *Ctbp1*$^{-/-}$;*Ctbp2*$^{+/-}$ mouse embryos having abnormal muscle fiber formation in their diaphragms (Hildebrand & Soriano, 2002). In humans, heterozygous variants in *CTBP1* have been shown to cause hypotonia, ataxia, developmental delay, and tooth enamel defect syndrome (MIM# 617915).

The potential role of *FGFRL1* as a contributor to CDH development is also supported by Subject 2, the first child with CDH reported with biallelic variants in *FGFRL1* (c.[886A > G];[1328G > C], p. [(I296V)];[(G443A)]). As expected, based on the *Fgfrl1* mouse model, neither of Subject 2's parents, who carried only one affected *FGFRL1* allele, had CDH. Consistent with Subject 2's Hispanic (Mexican) ethnicity, these variants are seen most commonly in the Latino/Admixed American population of the gnomAD database. However, even in this population, they are rare (allele frequencies of 0.00032 and 0.00059, respectively) and have never been documented in the homozygous state. In silico evaluations

provide greater evidence for the deleterious nature of the c.886A>G variant (Table 1) that occurs in a region that codes for an Ig-like C2-type 3 domain of FGFRL1 and has a Combined Annotation Dependent Depletion (CADD; https://cadd.gs.washington.edu/) score of 23.9. In contrast, the c.1328G > C variant has a much lower CADD score of 8.5. If these variants contributed to the development of CDH in Subject 2, it is still possible that they did so in conjunction with other deleterious variants that were not identified on exome sequencing. Unidentified epigenetic, environmental and/or stochastic factors may have also played a role in Subject 2's CDH.

We conclude that the CDH critical region on 4p16.3 that is associated with WHS can be refined to an ~1.9 Mb telomeric region that contains *FGFRL1* and ~47 other RefSeq genes. We also conclude that deleterious *FGFRL1* variants may contribute to the development of CDH in humans, although definitive proof will require the identification of additional individuals with CDH that carry biallelic, pathogenic variants in *FGFRL1.*

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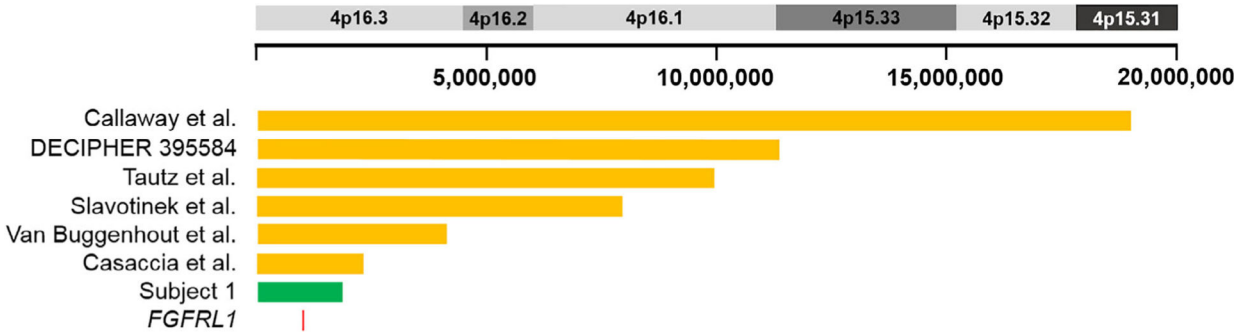

FIGURE 1.

The deletion identified in Subject 1 defines the CDH critical region on 4p16.3 that is associated with Wolf-Hirschhorn syndrome. A schematic representation of molecularly defined, isolated 4p16 deletions associated with CDH (Callaway et al., 2018; Casaccia et al., 2006; Slavotinek et al., 2006; Tautz et al., 2010; Van Buggenhout et al., 2004). In all cases, the maximal deletion is depicted. *FGFRL1* is located in the CDH critical region on 4p16.3 defined by the maximal deletion seen in Subject 1 (chr4:1–1,875,255; hg19)

TABLE 1

Results of in silico analyses performed for the *FGFRL1* variants seen in Subject 2

***FGFRL1* variant [NM_001004356.2]**	**SIFT**	**PolyPhen-2**	**MutationTaster**	**CADD**	**Allele frequency[a]**
c.886A>G, p.(I296V)	Tolerated	Probably damaging	Disease causing	23.9	11/34456 (0.00032); no homozygotes
c.1328G>C, p.(G443A)	Tolerated	Benign	Polymorphism	8.5	20/33814 (0.00059); no homozygotes

[a]Latino/Admixed American population frequencies.