Genetic considerations in recurrent congenital diaphragmatic hernia in two siblings

Khalid AlFaleh, Kyong-Soon Lee, Jennifer Ramsay, Margaret Nowaczyk

Congenital diaphragmatic hernia (CDH) is a congenital malformation that has a reported incidence ranging from 2.4 to 4.1 in 10,000 births.\(^1,2\) Survival of infants with CDH is dependent on the extent of lung hypoplasia and the presence of additional congenital anomalies or chromosomal abnormalities. Despite advances in neonatal care, a mortality rate of 33% is still reported with isolated CDH, predominantly due to hypoxic respiratory failure secondary to pulmonary hypoplasia.\(^3\)

Chromosomal abnormalities have been observed in 18% to 47% of cases of CDH and these patients frequently have associated malformations.\(^2,4\) The mode of inheritance of isolated non-syndromic CDH is unknown. We present two siblings with lethal CDH born within a 30-month period. The clinical course in these two infants is described and the possible mode of inheritance for CDH in this family is reviewed.

**Case 1**

The first baby was delivered at 37 weeks to a 32-year-old primigravida mother. An antenatal ultrasound at 18-weeks gestation was reported as normal. At 34 weeks a repeat ultrasound for fetal position revealed a left-sided diaphragmatic hernia. Labour was induced at 37 weeks and a baby girl weighing 2636 grams was born vaginally. Apgar scores were 6 and 9 at 1 and 5 minutes, respectively. The neonate was intubated at delivery and a left-sided CDH was confirmed on the chest x-ray film. No dysmorphic features or other congenital anomalies were apparent. She was treated with high frequency oscillatory ventilation (HFOV) due to difficulties with conventional ventilation and there was evidence of pulmonary hypertension. The echocardiogram showed mild hypoplasia of the aortic arch, but there were no other cardiac abnormalities. By day 8, the baby was weaned to conventional ventilation and underwent operative repair of her CDH two days later without complication. At surgery, the left lobe of the liver, spleen, stomach, small bowel and proximal colon were noted within the left thorax. On day 13, she had a significant deterioration with hypoxic respiratory failure requiring HFOV, paralysis, nitric oxide, and inotropic support. On day 18 she had an acute bradycardic episode requiring cardiac compressions and the following day a right pneumothorax developed. The patient continued to deteriorate with lack of response on maximal therapy. Intensive care was eventually withdrawn after discussion with parents and the baby died at 25 days of age.

A complete autopsy was performed. At postmortem examination, the left diaphragmatic patch was intact and the ipsilateral lung was small. The heart showed changes in keeping with pulmonary hypertension, but there was no aortic hypoplasia or other congenital defects. Chromosomal analysis of cultured peripheral blood lymphocytes obtained at the time of delivery showed a normal female karyotype, 46XX

**From MacMaster University, Hamilton, Ontario and the Division of Neonatology, The Hospital for Sick Children, Toronto, Ontario, Canada**

**Correspondence and reprint requests:**

Khalid AlFaleh, MBBS, FAAP, FRCPC

McMaster University Faculty of Health Sciences,
4G40 1200 Main St.
West Hamilton, ON L8N 3Z5
Canada

T: +1-905-521 2100 ext 75612
F: +1-905-521 5007
alfalek@mcmaster.ca

at 475 bands of resolution. There was no evidence of isochromosome 12p by fluorescent-in-situ hybridization (FISH) with chromosome 12 centromere-specific probes. Based on an assumed multifactorial mode of inheritance, the recurrence rate was estimated at 2%.

The second pregnancy of this couple resulted in the normal delivery of a healthy full term female infant. There was no history of consanguinity.

**Case 2**

The third child in this family was delivered 30 months after the first pregnancy. An antenatal ultrasound at 14-weeks gestation was reported as normal. However, a second ultrasound at 21 weeks revealed a left diaphragmatic hernia with an elevated stomach in the left chest. In addition, a cystic lesion was noted in the right upper quadrant near the diaphragm, which was thought to represent either the gall bladder or an intrahepatic cyst. Fetal echocardiography showed no abnormalities and amniocentesis showed a normal male karyotype. By 34 weeks, polyhydramnios had developed and labor was induced at 39 weeks. A baby boy weighing 3600 grams was delivered vaginally with Apgar scores of 8 and 5. Due to suspected CDH, the neonate was immediately intubated in the delivery room and a nasogastric tube was inserted. No dysmorphic features or other congenital anomalies were apparent. He had severe pulmonary hypertension with difficulty in oxygenation in 100% O₂ despite maximal settings on HFOV. The first chest x-ray film on admission showed opacity of lung in the right hemithorax and an elevated left hemidiaphragm (Figure 1). His clinical course was complicated by the development of pneumoperitoneum drained with an intra-peritoneal angiocatheter (Figure 2). At 5 hours of age, he had significant bradycardia unresponsive to full resuscitative measures including bilateral chest and intraperitoneal drains. He was pronounced dead at 6 hours of age.

A full autopsy was performed. The postmortem examination revealed bilateral posterior diaphragmatic hernias with herniation of the right lobe of the liver into the ipsilateral hemithorax and the transverse colon, spleen, pancreas, stomach, and left lobe.

**Figure 1.** A chest and abdomen radiograph immediately after delivery showing opacity in the lower lungs bilaterally. In the right, the opacity is the liver and in the left, the stomach, both of which have herniated through bilateral posterior congenital diaphragmatic hernias. Figure 3 shows corresponding pathologic findings at autopsy.

**Figure 2.** A chest and abdomen radiograph showing bilateral pneumothoraces and a pneumoperitoneum with a left sided abdominal drain in situ (arrow). The intra-abdominal structures that have herniated into the thoracic are clearly delineated by the presence of air.
of liver in the left hemithorax (Figure 3). A pneumomediastinum and pneumopericardium, severe pulmonary hypoplasia, malrotation of the bowel, undescended right testicle, and an inguinal left testicle were also present.

Chromosomal analysis of cultured peripheral blood lymphocytes obtained at the time of delivery showed a normal male karyotype, 46XY at 475 bands of resolution. There was no evidence of isochromosome 12p by FISH analysis with chromosome 12 centromere-specific probes.

Discussion

The mode of inheritance in CDH is heterogeneous but most cases are thought to be sporadic. Several teratogens including phenmetrazine, thalomide, quinine, and nitrofen have been reported in association with CDH as well as vitamin A deficiency in rats.5

If CDH is present in association with other major anomalies (non-isolated CDH), up to 47% have chromosomal abnormalities.2 CDH associated with abnormal numbers of chromosomes include trisomy 13, 18, and 21, Turner syndrome, partial trisomy 5, 12p tetrasomy, partial trisomy 20, and tetrasomy.5 CDH has been observed in patients with specific chromosomal abnormalities, especially different translocations such as 8q22.3.5,6

Fryns syndrome is the commonest autosomal recessive syndrome associated with CDH and is comprised of CDH, craniofacial abnormalities, digital limb hypoplasia, and internal malformations.7 Pallister-Killian syndrome (PKS), which is comprised of a combination of coarse faces, pigmentary skin anomalies, localized alopecia, profound mental retardation, seizures, and supernumerary nipples, has been frequently associated with diaphragmatic defects. PKS is caused by tetrasomy 12p5. However, the genetic mechanism causing diaphragmatic defect in PKS is unknown.

CDH has been frequently associated with recognizable syndromes. These include Cornelia de Lange syndrome (recently found to be caused by mutations in the NIPBL gene5), pentalogy of Cantrell, neonatal Marfan syndrome, Beckwith-Weidemann syndrome, Goldenhar sequence, Pierre-Robin sequence, Rubinstein-Taybi syndrome, and others,5 but the genetic mechanism has not yet been delineated for many of these syndromes. There have been several reports of either de novo deletion or unbalanced translocations involving the 15q24-q26 region, suggesting that this region is critical to normal development of the diaphragm.10,11 Recently, reports of infants with multiple congenital anomalies associated with partial trisomy of the 15q26 region have led to the discovery of submicroscopic deletions in this region in two patients with Fryns syndrome.12 The myocyte-specific enhancer factor-2 (MEF2) proteins play a critical role in the control of muscle differentiation and development. They proposed MEF2A member of the MEF2 gene family mapping to 15q26, as a candidate gene. Investigators have described central diaphragmatic hernia in mice in which a Slit3 gene was lacking.13

There have been several reports of familial CDH14 but the mode of inheritance is unknown. Multiple anomalies have been reported in about 50% of the sporadic CDH cases, but defects in familial cases tend to be isolated with a low incidence of additional malformations. When present, these additional anomalies tend to be fusion defects, i.e. neural tube defects, cleft lip and palate, and omphalocoele.

For familial isolated CDH, despite suggestions for autosomal recessive modes of inheritance, there is more support for a multifactorial mode of inheritance based on the observation of pedigree patterns suggestive of autosomally dominant and recessive as well as X-linked inheritance without a clear-cut predominance of one pattern, a lack of familial cases with greater than two siblings affected, the observation of monozygotic twins discordant for CDH, and the heterogeneity of the anatomic nature of the defect (e.g. type of hernia, side affected) and the severity of the defect. Norio et al presented 14 familial cases of five Finnish families affected by severe CDH and reviewed data of 53 previously reported familial
cases. There appeared to be a male preponderance in familial CDH with a male to female ratio of 2:1, and bilateral defects were reported in 20% of familial cases compared with 3% in non-familial cases.

With a multifactorial mode of inheritance, a recurrence risk for first-degree relatives is calculated by 1 divided by the square root of the frequency in the general population. CDH, with an estimated incidence of approximately 1 in 2200, a recurrence risk of 2% is reported, with the highest risk among close relatives of the most severely isolated cases as compared to less severe cases, or cases with other associated anomalies. After the occurrence of CDH in two siblings, a recurrence rate of 10% is suggested.

In the two cases presented here, CDH occurred in 2 of 3 children from the same parents. The first was male with a large left-sided defect, and the second was male with a large bilateral defect. Although a mildly hypoplastic aortic arch was suspected on echocardiogram in the first case, this was not confirmed at autopsy. In the second case there were minor associated abnormalities likely related to the CDH with malrotation and undescended testicles. No other congenital anomalies were found, and no other family members were affected.

Due to normal cytogenetics and the absence of major dysmorphic features, these two cases of familial CDH are likely consistent with a multifactorial mode of inheritance. No single gene mutation has been discovered for this major congenital anomaly. Further investigation into isolated and particularly familial cases may lead to the identification of genetic abnormalities detectable with FISH assay, locus-specific DNA probes, or other new techniques of analysis that target specific genetic sites, especially sites that may be important in lung development and those guiding mesenchymal growth. There remains an ongoing need for careful clinical review and blood banking of cases of CDH to allow better insight into the genetic causes of severe fetal anomalies such as CDH.

References