

Congenital Diaphragmatic Hernia Overview -- Animal Models

The use of surgical, teratogenic, nutritional, and genetic animal models of CDH have provided further insight into the pathogenesis of congenital diaphragmatic defects and associated pulmonary hypoplasia.

Surgical Models

The first animal models widely used were surgical models of CDH. In these models, hernias were surgically induced in the diaphragms of developing fetuses (most commonly sheep followed by dogs and rabbits) [Bütter et al 2005, Sandoval et al 2006, Roubliova et al 2009]. Although these surgical models do not allow for investigation of the etiology of CDH, they have been useful for evaluating the pulmonary consequences of CDH such as the effect of compression and decreased diaphragmatic activity on lung development. These models have also provided insight into prediction algorithms of lung tissue mechanics and treatment strategies for pulmonary hypertension and pulmonary hypoplasia [Jani et al 2009, Flemmer et al 2007]. As tracheal occlusion is evolving as a treatment for the fetus with high risk CDH, the need for good animal models is growing. Tracheal occlusion may be performed in both surgically induced CDH models as well as in the nitrofen rat model described below [Mayer et al 2008].

Nitrofen and Vitamin A Metabolism

Administration of the herbicide nitrofen to pregnant rats reliably produces Bochdalek diaphragmatic hernia and bilateral pulmonary hypoplasia. Nitrofen (2,4-dichlorophenyl 4-nitrophenyl ether) is a polyhalogenated aromatic compound with structural similarity to thyroid hormone [Kling et al 2007]. Although its mechanism of teratogenesis is unknown, evidence suggests that nitrofen acts through the retinoic acid signaling pathway [Clugston et al 2010]. The phenotype produced in rats closely resembles that found in humans in diaphragm type, pulmonary hypoplasia, and associated cardiovascular anomalies, though it is not known whether the mechanisms of pathogenesis are the same. Nitrofen is no longer commercially available as an herbicide for agricultural applications in the US.

In several species, dietary deficiency of vitamin A (retinol) leads to a spectrum of malformations, often including congenital diaphragmatic hernia. In the nitrofen model, administration of vitamin A ameliorates its teratogenic effect, reducing the frequency and severity of CDH in exposed rat fetuses [Babiuk et al 2004]. Studies have shown that nitrofen inhibits RALDH2, an important enzyme involved in the conversion of retinal to retinoic acid [Noble et al 2007]. Additional evidence that retinoid signaling plays an important role in diaphragm development includes the presence of diaphragmatic defects in *RAR α* and *RAR β 2* retinoic acid receptor knock-out mice [Mendelsohn et al 1994].

There is increasing evidence that abnormalities in retinoic acid/vitamin A metabolism play a role in human diaphragmatic development. This is discussed in the Environmental Causes section.

Genetic Models

Mouse genetic models are useful in unraveling the etiology and pathogenesis of human CDH. A number of gene mutations which result in an array of diaphragmatic defects have been created in mice. A small number of these malformations have been linked to human defects or malformation syndromes.

The gene *Fog2* (*Friend of Gata2*, officially *Zfpm2*) is necessary for independent development of the lung and the diaphragm in mice. It is also required for cardiac and gonadal development. In humans, translocations or deletions of the FOG2 locus are associated with CDH, and detrimental coding mutations have been identified in humans with nonsyndromic CDH [Ackerman et al 2005, Bleyl et al 2007]. Although coding mutations appear to be a rare cause of CDH in humans, the FOG2 genetic pathway likely plays an important role in human lung and diaphragm development warranting further investigation. The *Fog2* cofactor, *Gata4* is also a CDH candidate gene, as *Gata4* mutant mice have diaphragmatic defects [Jay et al 2007] and *Gata4* sits in a CDH cytogenetic hotspot region on human chromosome 8p [Slavotinek et al 2006]. Both *Fog2* and *Gata4* are expressed in the lung and are necessary for lung development [Jay et al 2007, Ackerman et al 2007] strengthening the hypothesis that some cases of CDH are associated with primary pulmonary developmental defects.

Mutations in the Wilms tumor suppressor gene, *Wt1*, are also associated with lung and diaphragm defects. In mice, *Wt1* mutations are associated with posterior diaphragmatic defects and structural abnormalities within the primordial diaphragm's pleuripertoneal folds [Clugston et al 2006]. Although mutations in human WT1 were not found in a small series of nonsyndromic CDH cases, a mutation in WT1 has been associated with CDH in a few cases of Denys-Drash syndrome [Antonius et al 2008] and one case of Meacham syndrome [Suri et al 2007]. It is interesting to note that the candidate genes *Wilm's tumor 1*, *Fog2*, and *Gata4* are all necessary for both diaphragmatic and genitourinary development in mice [Ackerman et al 2005, Manuylov et al 2008, Miyamoto et al 2008, Scholz & Kirschner 2005, Jay et al 2007].

A conditional deletion of the gene, *Coup-TFII*, a transcription factor in the steroid/thyroid hormone receptor super family, suggests an important role in posterior diaphragm and lung development [You et al 2005]. Although there are no reports of COUP-TFII causative sequence variations within several CDH study cohorts, the COUP-TFII gene maps to a commonly deleted CDH hotspot region on chromosome 15q26 [Klaassens et al 2005]. Among the few genes contained within this critical region, COUP-TFII is considered an extremely strong candidate for CDH.

The homeobox transcription factor, *Hlx*, may also contribute to CDH. Mutations in the gene cause abnormal diaphragmatic development in mice [Bates et al 2006], although the phenotype has not been well characterized. One study found *Hlx* sequence variants in 4 isolated CDH patients, though it is unknown whether these variants were *de novo* [Slavotinek et al 2009].

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