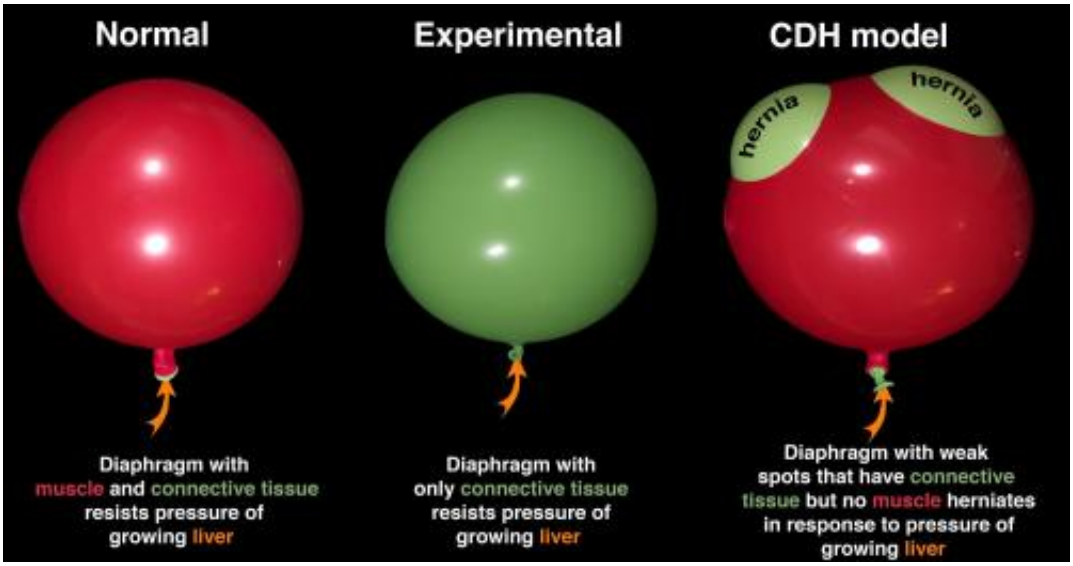
New insights into little known but common birth defect: Congenital diaphragmatic hernia

March 25 2015



CDH is a common defect that occurs in 1 in 3,000 births, and 50 percent patients die from related complications. Research from the University of Utah provides new information on how CDH arises, opening avenues for researching therapeutic interventions. LEFT - The diaphragm acts as a barrier that separates the liver and guts in the abdominal cavity from the heart and lungs in the chest cavity. A normal, healthy diaphragm is comprised of a dome-shaped layer of muscle (red) that overlies a layer of connective tissue (green, not visible on left). CENTER - Experiments in mice show that diaphragms missing muscle do not develop hernias. RIGHT -- Research suggests that CDH originates from regions with connective tissue but no diaphragm muscle. Internal pressure from growing organs in the abdominal cavity causes them to protrude through the weak spots and into the chest cavity. Credit: Gabrielle Kardon

Congenital diaphragmatic hernia (CDH) is not as well known as muscular dystrophy and cystic fibrosis, but like them it is a life-threatening birth defect, and is just as common. Occurring in one in 3,000 births, CDH causes the guts and liver to protrude through a defective diaphragm and into the chest cavity, where they interfere with the lungs.

Although many genetic mutations have been linked to CDH, a new study from the University of Utah School of Medicine is the first to demonstrate a linkage between genetic variation and a physiological mechanism that gives rise to defects in the diaphragm. The research points to a crucial role for connective tissue in CDH, and in guiding normal development of the diaphragm. These findings will be published March 25, 2015, in *Nature Genetics*.

Research like this could tell physicians when and how CDH occurs in fetal development, opening the door to preventative treatments. "We learned that these defects happen really early - much earlier than previously thought," says Gabrielle Kardon, Ph.D., associate professor of human genetics and principal investigator on the study. She stressed there are currently no therapeutic interventions to prevent CDH, and even with surgical patching of the diaphragms CDH is "silently killing half of the affected babies" - referring to the 50 percent mortality rate with this condition. Her lab is poised to test whether drugs can prevent the birth defect in animal models.

The study began with a developmental question: how is the diaphragm built as the embryo grows? The diaphragm is the only essential skeletal muscle. It is necessary for breathing and acts as a barrier that separates the liver and guts from the heart and lungs. While it's known that the mature diaphragm is composed of a domed muscle surrounded by connective tissue that attaches to tendons and bones, how the diaphragm develops has been a mystery. The researchers genetically tagged

different cell populations in mice, a technique used to visualize how the cells come together to make the diaphragm. Surprisingly, they found that the connective tissue cells are key: they send molecular signals that tell the muscle cells how to assemble properly.

If connective tissue is important for the normal development of the diaphragm, it might also be involved in CDH. "We have lots of data suggesting that CDH is due to genetic mutations," says Kardon. "But there's no line connecting the genetic mutation to the diaphragm defect." The team saw that in mice, the diaphragm's connective tissue cells expressed a gene, Gata4. Mutations in this same gene have been strongly correlated with CDH in humans. This suggested that genetic defects within connective tissue might be a cause of CDH.

They tested their hypothesis by silencing the Gata4 gene in the connective tissue cells and observing how this affected development. In every case in which the Gata4 gene was "knocked out," the mouse developed a hernia in its diaphragm.

But how does the hernia form? Surprisingly, the hernias did not develop from a hole in the diaphragm, as was assumed. Without Gata4 in the connective tissue, muscle develops incompletely - creating a localized region made entirely of connective tissue. As the growing liver presses on this weak spot, the diaphragm eventually gives way and the liver bulges through.

Their genetic studies, aided by computer models made in collaboration with bioengineers, show that CDH only develops when a weaker region of connective tissue is surrounded by stronger muscular tissue; counterintuitively, diaphragms made entirely of connective tissue did not develop hernias. These results show that small defects in muscle development lead to hernias, potentially making fixing the underlying problem more approachable. "Using our animal model of CDH, we are

now in an excellent position to test therapies and provide hope for CDH patients," says Kardon.

More information: Muscle connective tissue controls development of the diaphragm and is a source of congenital diaphragmatic hernias. A. Merrell, B. Ellis, Z. Fox, J. Lawson, J. Weiss, G. Kardon, *Nature Genetics* March 25, 2015. DOI: 10.1038/ng.3250

Provided by University of Utah Health Sciences