New insight into most common forebrain malformation

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St. Jude Children's Research Hospital scientists have identified one of the molecular mechanisms underlying the genetic brain malformation called holoprosencephaly (HPE). The findings not only yield insights into the most common developmental malformation of the anterior brain and face in newborns, but also help in understanding the intricate process by which the brain forms in the developing fetus.

Led by St. Jude geneticist Guillermo Oliver, Ph.D., the researchers published their findings in the August 11, 2008, issue of the journal *Developmental Cell*.

"These findings are important, because, while genes that cause HPE have been identified, the interactions among those that produce HPE are not only complex, but poorly understood," Oliver said. "This represents a first step in understanding the mechanism of that interaction."

HPE occurs in about one in every 250 fetuses, frequently causing miscarriage or stillbirth. HPE is present in varying degrees in one in every 16,000 newborns. The disorder is characterized by a failure of the developing brain to separate into right and left hemispheres. Besides brain abnormalities, HPE is characterized by facial malformations such as cleft lip and cleft palate. HPE has been traced to mutations in any of nine genes, although researchers believe the disorder is also influenced by environmental factors such as maternal diabetes, infections during pregnancy or drugs.

In their studies, the researchers sought to understand the role in HPE of a gene that codes for a protein called Six3. In previous studies, Oliver and his colleagues had identified the Six3 protein as critical to fetal brain formation. Also, other studies had implicated defect-causing mutations in Six3 as involved in causing HPE, but they did not know the molecular mechanism by which Six3 promotes HPE.

One clue to Six-3's role in HPE arose from the fact that most of the other HPE-causing genes produce malfunctions in an important piece of molecular machinery, the Sonic Hedgehog pathway, a major regulator of fetal brain development.

Thus, the researchers theorized that Six3 might also trigger HPE by compromising the function of the Sonic Hedgehog pathway. For their first experiments, they turned to zebrafish, a widely used animal model for studying genetics and development. Co-authors of the paper from Vanderbilt University used the zebrafish to analyze some of the same defective mutant Six3 genes known to be associated with HPE in humans. They found that the mutations caused partial loss of function of the Six3 gene, an important clue to how the mutations work to cause HPE.

In studies with mice, Oliver and his colleagues found that both Six3 and the Sonic Hedgehog pathway were active at the same time and place in the brain, important evidence that they could work together.

The researchers also developed evidence that the two genes cooperate to cause HPE. Although engineered mice carrying a mutant Six3 gene known to be associated with HPE in humans exhibited only occasional symptoms of HPE, when crossed with mice that also have defects in Sonic Hedgehog, all the animals developed severe HPE.

"This is good evidence for what has been called the 'multi-hit' model of

HPE," Oliver said. "In this model it takes mutations in both Six3 and other genes in the Sonic Hedgehog pathway to produce the severe pathology of HPE."

Furthermore, the researchers' molecular studies showed that Six3 and Sonic Hedgehog are intimately involved in regulating normal brain development. Six3 is a significant controller of Sonic Hedgehog; Sonic Hedgehog in turn influences the function of Six3—the two working together in a regulatory loop.

The researchers findings about Six3's role offer key insights into HPE, Oliver said. "Sonic Hedgehog is a major player in embryonic development, in the brain as well as elsewhere in the body," Oliver said. "So, this new understanding of its regulation in this very limited time and place in the brain helps us understand at a molecular level some of its overall role in brain formation."

In further studies, the researchers plan to use their understanding of the roles of Six3 and Sonic Hedgehog in HPE to explore why various multi-hit combinations of gene mutations may produce a spectrum of disease from mild to severe.

Source: St. Jude Children's Research Hospital