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Stem-Cell Defect Underlies Common Genetic Disorder

Howard Hughes Medical Institute researchers have found that Hirschsprung disease, one of the most common genetic disorders, is caused by a defect that blocks neural stem cells from forming nerves that control the lower intestine.

Hirschsprung disease occurs in one in 5,000 live births and causes a potentially fatal disorder that prevents the proper transport of food through the gut. The new findings suggest that it might one day be possible to correct the disease by transplanting neural stem cells from a different part of the gut.

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Neural crest stem cells (NCSCs) are cells that mature into neurons and supporting neural cells found in the gut. The studies provide important general insight into how stem cells — the immature cells that can develop into mature nerve and other cells — are controlled. While the properties of stem cells have been widely studied, relatively little is known about how they are regulated during development.

The researchers, led by Howard Hughes Medical Institute (HHMI) investigator Sean J. Morrison, HHMI associate Toshihide Iwashita, and graduate student Eve Kruger at the University of Michigan, published their findings in the August 15, 2003, issue of *Science*.

"Some of the genetic mutations that cause Hirschsprung have been identified, but they explain only about half the cases," said Morrison. "Our work identifies new genes whose mutations might underlie the disease. We've found the mechanism by which one type of mutation impairs the function of the neural crest stem cells that give rise to the enteric nervous system."

The researchers began by conducting a global comparison of genes expressed in whole mouse fetuses with those genes expressed only in the fetal gut NCSCs. To make this comparison, they applied RNA extracts from the two

sources to microarrays, or "gene chips," which are arrays of thousands of gene probes that can signal the activity of specific genes. Using this process, the researchers found that the ten genes that were most highly expressed in the gut NCSCs relative to the whole fetus, included four that had already been linked to Hirschsprung disease in humans.

"This finding was exciting because if four of our top ten genes have already been implicated in Hirschsprung disease, it's an attractive hypothesis that some of the other genes we found upregulated could also cause the disorder when mutated," said Morrison.

Subsequent studies by Morrison and his colleagues focused on understanding the function of one of the identified genes, called *Ret*. They chose *Ret* because it is known to code for a receptor protein that enables stem cells to respond to a neuronal guidance protein called GDNF (glial-derived neurotrophic factor). Mutations in either *Ret* or *GDNF* genes had already been shown to cause Hirschsprung disease in both humans and mice, said Morrison.

Using antibody markers and NCSC cultures, the researchers confirmed that *Ret* proteins were expressed on the surface of stem cells and that the *Ret* receptor was required for the migration of the stem cells in response to GDNF in culture.

To test whether the loss of *Ret* prevented normal NCSC migration in the gut, the researchers examined the behavior of the NCSCs in the guts of *Ret*-deficient mice. These experiments revealed a dramatic decrease in the migration of NCSCs in the animals' guts.

"Until this work, what was missing was whether these molecular pathways act within neural crest stem cells to promote migration," said Morrison. "Our finding that these pathways are all expressed in neural crest stem cells and that they regulate the function of the cells, provides a cellular locus for people to study directly how those pathways interact."

Morrison also speculated that the research could have implications for correcting the genetic defect underlying Hirschsprung disease. "Our findings suggest that in people with mutations in *Ret*, the primary reason the enteric nervous system doesn't form in the hindgut is because neural crest stem cells just never migrate into the hindgut. Perhaps we can bypass that migratory defect by taking stem cells from the foregut, expanding them in culture, and then transplanting them into the hindgut."

Morrison emphasized that the findings demonstrate the value of a relatively new approach that uses microarrays for identifying activated genes and then knocking out those activated genes in mice to determine how those genes regulate stem cell function. "We think that this represents a powerful combination for getting important insights into the causes of other types of birth defects or other types of diseases," he said.