

FEBRUARY 27, 2005

Tailing the Cause of a Rare Heart Disease

Using genetic analyses and the translucent tail of a fish, researchers have pinpointed the underlying cause of a rare, mysterious heart disease that is preceded by hearing loss. Discovering the genetic cause of this disease provides researchers with a wealth of new ideas about the molecules involved in building the developing heart, as well as how diseases weaken heart muscle.

In an advance online publication on February 27, 2005, in the journal *Nature Genetics*, Howard Hughes Medical Institute investigators Christine E. Seidman and Jonathan G. Seidman and their colleagues identified the mutation that causes the disorder, dilated cardiomyopathy preceded by sensorineural hearing loss. The Seidmans and their colleagues at Harvard Medical School collaborated with researchers at University Hospital Würzburg in Germany, Massachusetts General Hospital, Children's Hospital Boston and The Wellcome Trust Sanger Institute in Britain.

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— Christine E. Seidman

In dilated cardiomyopathy, muscle weakness causes the left ventricle to stretch. As a result, the heart becomes enlarged to the point where it can no longer pump blood efficiently. In earlier studies of patients with cardiomyopathy preceded by hearing loss, the Seidmans and their colleagues identified a region of chromosome 6 as the location of the culprit gene.

In the latest study, they sought to pinpoint the gene and identify the mutation that was responsible. A search of the human genome database identified candidate genes in the region, and subsequent tissue analysis revealed that one gene, called *EYA4*, was expressed in both the heart and the cochlea of the ear. The researchers confirmed that affected people possess a characteristic mutation of the *EYA4* gene, a finding which offered surprises,

said Christine Seidman.

"The *EYA4* gene had been implicated in hearing loss before, but in none of the patients where mutations had been characterized had there ever been an abnormality of the heart reported," she said. The Seidmans also found that the gene was expressed in adult heart tissues, which is unusual because other *EYA* gene family members are thought to function primarily during development.

Curiously, the gene does not code for a structural protein involved in known myocyte functions such as contraction, as is the case with other mutations that cause cardiomyopathies. Rather, *EYA4* codes for a protein involved in activating other genes—controlling the copying, or transcription, of genetic information to messenger RNA that carries that genetic blueprint to the cell's protein-making machinery.

To confirm that the mutant *EYA4* does indeed cause cardiomyopathy, the researchers turned to the zebrafish, whose genetic machinery for cardiovascular development closely resembles that of mammals. In their experiments with the fish, they used antisense genetic techniques to reduce the fish's production of *eya4* protein and measured the resulting changes to the heart. The treated fish developed swelling of the heart ventricle that suggested cardiac dysfunction. High-speed video of the fish's beating heart—visible because the fish are translucent—indicated that the pumping function of the heart was dramatically reduced.

However, the researchers turned to the fish's translucent tail to confirm independently that blood flow was, indeed, reduced in the treated fish. "Since the cardiac imaging of zebrafish remains technically challenging," said Seidman, "we devised a means of tracking the movement of a single red blood corpuscle through the tail to assess flow. That analysis revealed a dramatically lower pumping velocity in the treated fish and provided a functional readout of heart performance," she said.

To understand the molecular mechanism by which the mutant *eya4* protein might compromise cardiac function in humans, the researchers compared its function with that of other mutant forms that only caused hearing loss. They found that the mutant protein that caused both cardiomyopathy and hearing loss lacks a region necessary for the protein to attach to other proteins that help it enter the cell nucleus. Only if the *eya4* protein enters the nucleus can it play the appropriate role in gene regulation, said Seidman.

"That finding suggests that this mutation causes a dramatic reduction in the amount of *eya4* within the nucleus, and we presume that is what accounts for heart disease," said Seidman. "The implication of this finding is that the function of *eya4* in the heart may be different than its functions in other tissues, such as the ear."

Although the researchers' findings will not aid treatment of the cardiomyopathy immediately, said Seidman, they do offer the potential for important basic insights into the mechanisms of cardiomyopathies.

"Whenever a new disorder affecting the human population is identified, it adds new basic knowledge to the field of medicine," she said. "And sometimes rare disorders such as this one can provide very powerful insights into the biology of more common problems. In this case, the finding that transcriptional regulation goes awry in this disorder gives us a top-down look at the molecules that must be appropriately expressed and regulated for normal function of the heart throughout life."