Scientists discover how an out-of-tune protein leads to muscle demise in heart failure

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A new Johns Hopkins study has unraveled the changes in a key cardiac protein that can lead to heart muscle malfunction and precipitate heart failure.

Troponin I, found exclusively in heart muscle, is already used as the gold-standard marker in blood tests to diagnose heart attacks, but the new findings reveal why and how the same protein is also altered in heart failure. Scientists have known for a while that several heart proteins—troponin I is one of them—get "out of tune" in patients with heart failure, but up until now, the precise origin of the "bad notes" remained unclear.

The discovery, published online ahead of print on Sept. 12 in the journal *Circulation*, can pave the way to new—and badly needed—diagnostic tools and therapies for heart failure, a condition marked by heart muscle enlargement and inefficient pumping, and believed to affect more than 6 million adults in the United States, the researchers say.

Troponin I acts as an on-off switch in regulating heart relaxation and contraction and, in response to, adrenaline—the "flight-fight" response. But when altered, troponin I can start acting as a dimmer switch instead, one that ever so subtly modulates cardiac muscle function and reduces the heart's ability to pump efficiently and fill with blood, the researchers found.

The Hopkins team used a novel method to pinpoint the exact sites, or epicenters, along the protein's molecule where disease-triggering changes occur. They found 14 such sites, six of them previously unknown. In revealing new details about the molecular sequence of events leading up to heart failure, the researchers said their work may spark the development of tests that better predict disease risk and monitor progression once the heart begins to fail.

"Our findings pinpoint the exact sites on troponin I's molecule where disease-causing activity occurs, and in doing so they give us new targets for treatment," says researcher Jennifer Van Eyk, Ph.D., director of the Johns Hopkins Proteomics Innovation Center in Heart Failure.

In the current study, the team analyzed tissue from the hearts of patients with end-stage heart failure and from deceased healthy heart donors. The 14 sites the researchers identified are sites where troponin I binds with phosphate, a process known as phosphorylation. Phosphate can activate or deactivate many enzymes, thus altering the function of a protein and, in the case of heart failure, ignite disease. The six newly identified sites represent new "hot spots" involved in heart contraction, the researchers say, and could be used as diagnostic markers or a target for treatment to restore function. The Hopkins researchers found that in some sections of the molecule, phosphorylation ratcheted up the dimmer switch, while ratcheting it down in other sections, but it invariably led to muscle dysfunction.

"Our goal would be to zero in on these new sites, gauge risk of heart failure and, hopefully, restore heart muscle function," Van Eyk says.

Heart failure is a complex progressive disorder, and while cardiac pacemakers can restore or "resynchronize" heart function in many people, about one-third of patients do not improve even with pacemaker therapy in addition to standard medication treatments.

"This is a devastating disorder for which we desperately need new and less invasive therapies," says senior investigator Anne Murphy, M.D., a cardiologist at Johns Hopkins Children's Center.

In their analysis, the researchers used a novel technique, called multiple-reaction monitoring (MRM), which pinpoints the exact locations along the protein's molecule where faulty signaling occurs and disrupts heart muscle function. MRM is an ultra-sensitive type of mass spectrometry that measures the exact size and chemical composition of protein fragments. Phosphorylated protein fragments have different molecular weights than non-phosphorylated ones. In this way, MRM accurately homes in on sites where phosphate is bound to troponin I to modulate heart muscle function.

The researchers found that patients with heart failure had markedly different levels of phosphorylation in certain protein segments compared with healthy heart muscle.

The advantage of MRM analysis—one of the first non-antibody based troponin I tests—is that it can measure phosphorylation levels without the need for antibodies, the traditional method currently used to monitor heart muscle function. The researchers believe that MRM can be developed as a clinical diagnostic test, and the Hopkins team is already working to develop a test that would measure phosphorlyation levels of proteins in the blood and would allow physicians to monitor the progression of the disease as well as predict which heart attack patients will progress to heart failure. About one-third of them do so.

"Right now, we don't really know which heart attacks patients will develop heart failure and which ones will maintain normal heart muscle function," Murphy says. "Monitoring specific phosphorylation sites might be one way to help us foresee and forestall this complication on an individual patient basis."

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