

AUGUST 20, 1999

Genetic Mutation Linked to Heart Disease in Muscular Dystrophy

A team of researchers that helped pioneer new gene-based therapies for muscular dystrophy has now pinpointed a genetic defect that leads to heart damage in some of the most severe forms of muscular dystrophy. The defect, found in smooth muscle cells, causes the coronary arteries to constrict, killing the surrounding heart muscle.

But HHMI investigator Kevin Campbell and his colleagues at the University of Iowa and the Department of Veteran Affairs also found that they could prevent such damage in mice by treating the animals with a drug that relaxes smooth muscle. "With the appropriate pharmacological agent, it could be likely that we could prevent the heart damage often seen in some forms of muscular dystrophy," said Campbell.

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— **Kevin P. Campbell**

He added that this finding also means that muscular dystrophy (MD) researchers who are attempting to develop genetic therapies must target defective smooth muscle as well as the malfunctioning skeletal muscle most often associated with the crippling effects of the disease.

In addition, this study may offer new leads into the causes of coronary artery disease, a leading killer of adults in the United States. The scientists reported their work in the August 20, 1999, issue of the journal *Cell*.

Campbell and his colleagues began their work by genetically engineering knockout mice that lacked a functioning protein called δ -sarcoglycan, which is part of a larger molecular assemblage called the dystrophin-glycoprotein complex (DGC). This critical structural complex is a protective brace for muscle cells, connecting the cells' internal cytoskeleton with an external surrounding membrane to stabilize the cells and protect them from the stress of contraction. In forms of MD characterized by loss of various components

of the DGC, muscle cells die from such stresses, and the muscles degenerate.

The DGC complex includes not only the δ -sarcoglycan protein, but another protein known as α -sarcoglycan. In previous studies, Campbell's team had found that knockout mice lacking α -sarcoglycan had no heart muscle damage, even though their skeletal muscles showed the deterioration typical of MD.

"The key difference in the two kinds of knockout mice is that α -sarcoglycan is expressed in skeletal and cardiac muscle, but not in smooth muscle. However, δ -sarcoglycan is also expressed in smooth muscle, and its absence thus also affects smooth muscle function" said Campbell.

As expected, tissue studies of the δ -sarcoglycan knockout mice revealed a distinctively different pattern of muscle damage when compared to the damage the researchers found in knockout mice missing the α -sarcoglycan gene. "We saw a more severe form of the generalized muscle cell death usually seen in muscular dystrophy, but also more focal regions of cardiac muscle cell damage as if there were some type of an infarct or smooth muscle dysfunction," said Campbell. *In vivo* perfusion of the coronary arteries revealed severe constrictions in the arteries.

To explore the impact of such smooth muscle defects on cardiac function, the scientists exercised δ -sarcoglycan knockout mice on treadmills. "To our surprise, about a third of these mice died during routine exercise," said Campbell. "And when we examined the heart tissue of the mice that survived, we found that they had developed major regions of cardiac muscle tissue death."

However, treating the knockout mice with a drug that relaxed smooth muscle prevented cardiac muscle damage and death during exercise.

The scientists' discovery of the new mechanism of muscle damage will also affect development of gene therapy for MD, said Campbell. "One way of treating patients with limb-girdle muscular dystrophy might be to use a gene transfer approach to treat the muscular dystrophy, and a pharmacological approach to treat the smooth muscle dysfunction," he said. "It's much more difficult to insert genes into heart muscle, so this approach might be more effective."

"We will now have to ensure that methods that use viruses to insert corrective genes not only target skeletal muscle, but also smooth muscle," said Campbell, whose research has provided the basis for such experimental therapies.

More broadly, said Campbell, "we believe our findings open up a completely new avenue of research involving the functional role of this complex in vascular smooth muscle and its possible involvement in coronary artery disease in general."