



# Working on a Cure for Duchenne Muscular Dystrophy

by Kate Forster

One of the major areas in genetics research these days is using delivery shuttles derived from viruses as a way to transfer genes to tissues. For many years, Jeffrey Chamberlain, Ph.D., professor of neurology, biochemistry, and medicine and a CHDD research affiliate, has been experimenting with a viral-based delivery system, called viral vectors, as a type of gene therapy in his search for a cure for Duchenne muscular dystrophy (DMD), the most common form of muscular dystrophy, an X-chromosome-linked genetic disease affecting approximately 20,000 newborn males each year. Gene therapy is a technique for correcting or replacing defective genes responsible for the development of disease. DMD can result from a spontaneous gene mutation, or it can be inherited. It is a progressive and degenerative disease that results in muscle weakness, skeletal abnormalities, and eventual dependence on a wheelchair and other assistive devices. It takes a large emotional and economic toll on the families affected.



*Jeffrey Chamberlain is researching ways to use gene therapy to treat and cure Duchenne muscular dystrophy.*

## Promising developments with gene therapy

Because muscular dystrophy is a result of a genetic mutation, gene therapy looks to be a promising possibility as researchers work toward an effective treatment or cure. In 1986, the dystrophin gene was identified as the one responsible for causing DMD. This gene, the largest known human gene, controls the making of a protein, also called dystrophin, which is responsible for maintaining muscle structure. Without access to this protein, muscles are fragile and cannot regenerate.

Because this disease is caused by a mutation in a single gene, Chamberlain considered the possibility of creating a synthetic version of the gene and replacing the mutated version with the new one. He and his team first went about determining what was going wrong with the gene that causes DMD. After conducting a number of studies on the function and structure of the dystrophin protein, they developed a synthetic version of the dystrophin gene. They then worked on making the gene much smaller than the original. The advantage of having a very small version of the gene is that it's much easier to deliver into the bloodstream and ultimately into the muscle cells, where they do their work.

## Creating the delivery shuttle

Once Chamberlain had developed a new gene that was small enough to deliver into the muscles, he needed an effective way to deliver it into the body. Viral vectors have long been used in gene therapy as a way to deliver genetic material into cells. They are created by taking the shell of a particular virus, removing the viral DNA inside it, and replacing it with the new therapeutic gene. A large number of these are then injected into the body where the viral shell finds its target, in this case the muscle

cells, and delivers its package, in this case the synthetic dystrophin gene. Vectors derived from adeno-associated viruses (AAV) are particularly well-suited for this gene as they can efficiently target muscle cells. In addition, neither the AAV vectors nor the virus itself is harmful to the body.

### Addressing challenges

Chamberlain has had success in curing mice of muscular dystrophy using the AAV vector shuttles. However, he discovered that the delivery shuttles can cause an immune response in larger animals. The problem with injecting a viral-based shuttle is that the body can see it as a foreign invader and cause the immune system to attack it before it has the chance to deliver the synthetic gene. And the larger the dose, the more likely an immune response will result.

Chamberlain wants to make sure that immune response doesn't become a significant issue before he starts clinical trials. He and his team are currently addressing this concern, and they are being as cautious as they can. They are hoping to find the minimum number of viral vectors needed to produce a positive response in a patient. "The less we have to deliver, the smaller the chances of having a reaction against it," says Chamberlain.

### Refining the protocol

Chamberlain and his team are working on a number of refinements as they get ready to start their clinical trials. They are continuing to refine the structure of these synthetic dystrophin genes.

"By reducing the size of this gene, we can start delivering it more easily," says Chamberlain. "We're also trying to make synthetic genes that work a little better than the first ones we came up with." Chamberlain is also testing different delivery methods, including isolating a single limb and circulating the vector throughout that limb, delivering the whole vector in a single dose, and using multiple doses, work led by his colleagues Guy Odom, Ph.D., acting assistant professor of neurology, and Stephen Hauschka, Ph.D., professor of biochemistry.

Additionally, Chamberlain is trying to make the delivery system as efficient as possible. He is comparing related members of the AAV class of viruses to see which will be the least likely to activate the immune system. Chamberlain suspects that he will continue refining this protocol, making things better and safer, for a number of years. "You want to really be prepared for all possibilities," says Chamberlain, "and be able to either rapidly implement the treatment, or fix any problems that we run into along the way."

**Gene therapy for muscular dystrophy using AAV vectors**  
*A dystrophic mouse model that naturally develops a form of DMD is used to test new therapies. The AAV delivery vectors are grown in a human cell culture system by introducing genes for the AAV and the dystrophin parts of the delivery vehicle. The final AAV vector is purified from the cells and injected into the dystrophic mice. Normally, the mice do not make any dystrophin, as shown in the bottom panel. Within a few weeks of the AAV injection, all the muscles of the mice make the new microdystrophin that was introduced by the AAV vector. The microdystrophin is shown in green in the upper panel.*

CHDD is an interdisciplinary center dedicated to the prevention and amelioration of developmental disabilities through research, training, clinical service, and community outreach. CHDD includes the University Center of Excellence in Developmental Disabilities and the Eunice Kennedy Shriver Intellectual and Developmental Disabilities Research Center.

#### CHDD Outlook

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