

# HARVARD NEUROMUSCULAR DISEASE PROJECT NEWSLETTER

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## 20 Years of Muscular Dystrophy

By Louis Kunkel, Ph.D.

It has been 20 years since the publication that described the cloning of the dystrophin gene. This manuscript has served as a model for all subsequent human gene-cloning strategies. Today, with the human genome completely sequenced, what took years to complete in the late 1980's now takes just months. We know that when mutated, the dystrophin gene causes the form of muscular dystrophy originally described by Guillaume Duchenne in the 1860's. This gene is the largest known mammalian gene and encodes a protein called dystrophin. Much has been learned about the function of dystrophin at the muscle cell membrane. Many of the proteins that interact with dystrophin have been shown to cause other forms of muscular dystrophy when mutated. These studies have improved diagnosis, unmasked the pathogenesis of the disease and opened a number of therapeutic options that are being studied by research groups around the world. I would like to briefly review these therapeutic approaches, along with their strengths and weaknesses.

*Continued on Page 3*

### Kunkel lab ( Left-Right/Back-Front):

*Chris Feener, Eric Luth, Peter Kang, Tim Pusack,  
Mike Galdzicki, Adam Darity, Dick Bennett  
Kalliopi Liadaki, Julie Goswami, Alal Eran,  
Melanie Howell, Hal Schneider, Marielle Thorne  
Elicia Estrella, Louis Kunkel*

*Missing: Jeff Guyon, Hart Lidov, Leta Steffen,  
Steve Boyden, Iris Eisenberg*

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## Kunkel Lab Mission

Dr. Kunkel and his laboratory at Children's Hospital Boston have been hard at work discovering and understanding the many types of muscular dystrophy. The Kunkel lab uses fish, mice and human models to study and learn about the effects of MD. The laboratory is currently studying several types of muscular dystrophy:

**Limb Girdle Muscular Dystrophy (LGMD)**  
**Myoshi Myopathy (MM)**  
**Duchenne/Beckers Muscular Dystrophy (DMD/BMD)**  
**Facioscapulohumeral Muscular Dystrophy (FSHD)**  
**Myotonic Muscular Dystrophy (MMD)**

Dr. Kunkel uses his expertise and resources to continue his goal of finding new therapies and ultimately a cure for the many people suffering from this family of muscle diseases.



# Genetics 101

By Elicia Estrella, MS, CGC

People, animals and many other living organisms are made of cells. All cells within an organism contain DNA (**Deoxyribonucleic Acid**). DNA is the molecule that encodes our genetic information carrying all the instructions for making a person. DNA is made up of two twisted strands of chemical building blocks called bases. There are 4 bases that make up the language of our genes: **Adenosine**, **Thymine**, **Guanine**, and **Cytosine**. Certain areas of our DNA contain our genes. These genes are considered our genetic blueprint or instructions. Our genes determine all of our features like height and eye color. This is why we have similarities to our parents and grandparents. Our DNA strands are coiled up and tightly packaged into structures called **chromosomes**. We have 46 chromosomes in total. They come in pairs (23 pairs), with one chromosome from each pair inherited from your mother and the other from your father. This is how our parents and grandparents have passed their genes down to the next generation. How our genes are expressed in our family is called the **inheritance pattern**.

Most types of muscular dystrophy are inherited in one of three ways;

## 1. AUTOSOMAL DOMINANT:

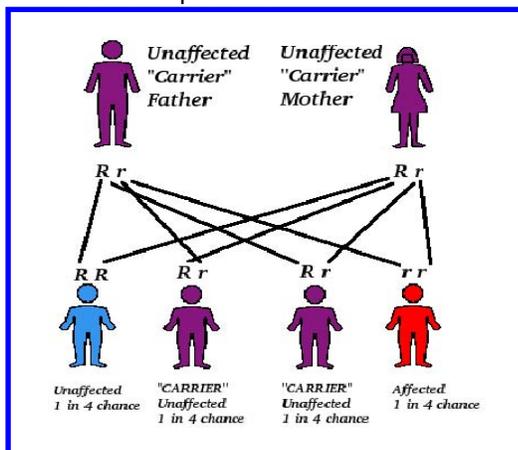
- Autosome - gene located on chromosome pairs 1-22
- Dominant
  - 1 copy of gene is mutated (altered)
  - Trait is expressed

## 2. AUTOSOMAL RECESSIVE:

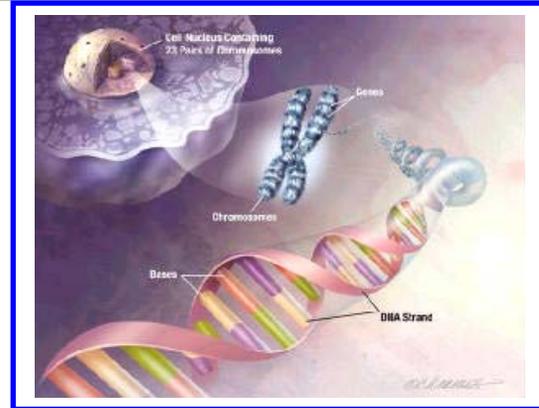
- Autosome - gene located on chromosome pairs 1-22
- Recessive
  - Both copies of a gene are mutated (altered)
  - Trait is expressed

## 3. X-LINKED RECESSIVE:

- X-linked - gene located on the X chromosome
  - 23<sup>rd</sup> pair of chromosomes are the sex chromosomes
  - XY (male) or XX (female)
- Recessive
  - Both copies of a gene are mutated
  - Trait is expressed



Autosomal recessive inheritance pedigree -  
Key  $R$ =unaltered gene /  $r$ =altered gene



Structure of DNA within the Cell

The inheritance of specific traits can be determined by reviewing the family history, drawing a pedigree and looking at the pattern of family members with a specific trait (i.e.: muscular dystrophy). Inheritance can also be determined by comparing a person's **genotype** (set of genes) to their **phenotype** (expression of a person's genes). Since we inherited 2 copies of all of our genes, one from each of our parents, subtle differences in the 2 copies can exist. How these differences, sometimes called **mutations**, are expressed also defines the inheritance pattern.

It is believed that each person contains 10-15 mutations within their genome. This is part of the reason no two people are alike. The differences we see in our gene copies (mutations) come in many types;

## 1. DELETIONS:

- a. large chunk of a gene is cut out
- b. like tearing out a chapter of a book
- c. 60% of DMD/BMD cases have deletions

## 2. DUPLICATIONS:

- a. adding extra genetic material
- b. like having 2 chapter 5's in a book
- c. 5% of DMD/BMD cases have duplications

## 3. POINT MUTATIONS:

- a. Like typos in a book
- b. typos can cause word/meaning changes
- c. 35% of DMD/BMD cases have point mutations

## 4. REPEATS: triplet repeat most common

- a. 3 bases are repeated over and over (ATG,ATG,ATG etc...)
- b. In a book having the word "THE" repeated for several pages would be confusing to the reader
- c. Repeats cause Myotonic Dystrophy
- d. The more repeats the more severe the disease

*"It is believed that every person has 10-15 mutations within their genome."*

## 20 Years of MD Cont. from pg1

**Exon Skipping:** The dystrophin gene is encoded by 79 pieces, called exons that are arranged in a certain order. Many patients with DMD or BMD lack some of these pieces. The missing pieces can affect the resulting protein, (DMD- no protein/ BMD-reduced protein). The exon skipping approach blocks exons converting someone with DMD to the more mild disease of BMD. This was a very inefficient process and the molecules that block exons were quickly degraded by the body. Recently, new molecules have been developed which are much more stable and more has been learned about how to block certain exons. Exon skipping is being safety tested in the Netherlands at this time. The exon skipping approach shows a lot of promise for a subset of DMD patients.

**Stop codon read-through:** About 25% of DMD patients have a mutation called a stop or nonsense mutation. The stop results in no dystrophin protein production. Read-through uses a chemical that fools the cell's stop codon detection system to continue, allowing protein production. PTC pharmaceutical has a chemical called PTC 124, which has shown read through in mouse models of human dystrophy. The molecule is now in human trials (see pg 5). This method will not be useful for most patients. It is unclear how it will work over the lifetime of a patient.

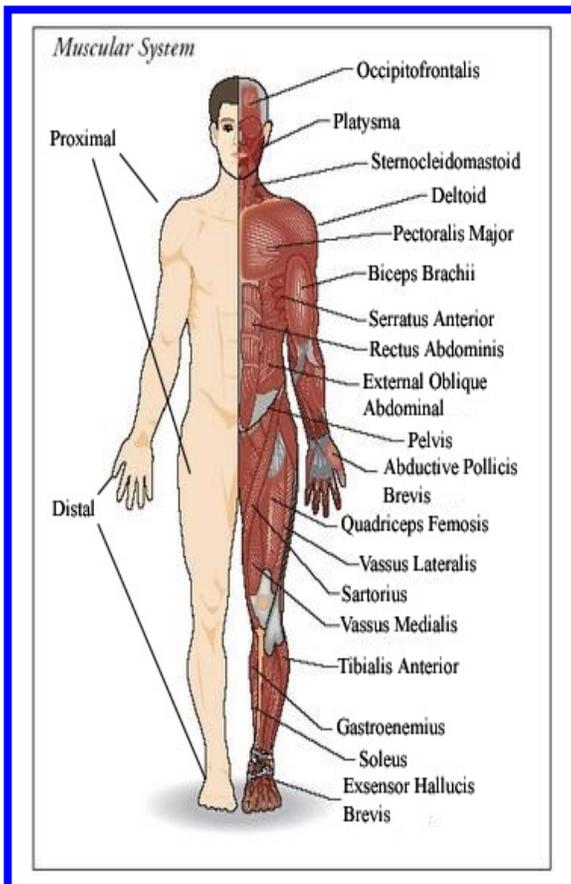
**Myostatin Repression:** A major modulator of muscle size is the growth factor myostatin. When it is decreased in amount, muscle gets larger and stronger. Studies in mice missing dystrophin have shown that blocking myostatin action can result in the correction of muscle disease. Human trials are currently in progress in BMD, LGMD and FSHMD. The results of these trials should be out soon. This promising approach may alleviate some symptoms and slow the progression of the disease process.

**Gene Therapy:** The biggest hurdles involved with this approach have been delivering the new gene to the right cells and then turning on the gene to the right amount. The current method uses the infectious nature of viruses. Our immune system recognizes these viral cells and destroys them. Currently there are types of virus that will target muscle and introduce absent muscle genes. This is being safety tested in animals and may soon be brought to human studies. The two biggest problems with gene therapy are keeping the transplanted gene turned on long-term and fooling the immune system. These are not insurmountable hurdles, but they will delay the use of this therapy in large-scale studies.

**Stem Cell Therapy:** The first use of stem cells to treat muscle disease in dystrophin-deficient mice seemed quite successful. However, early human trials were not at all successful. Better cells have since been identified, as well as more effective methods for growing and introducing these cells to muscle. Also, specialized signals that recruit stem cells into the muscle have been identified. The expansion of cells in tissue culture is being studied in anticipation of human experiments, as well as immunosuppressive agents that might allow easier transplantation of the stem cells into muscle.

**Less Developed Approaches:** Dystrophin has a closely related protein, utrophin, which can substitute for dystrophin. Numerous attempts have been made to increase utrophin expression in skeletal muscle. There are also attempts underway to repair the mutation in the dystrophin gene. These approaches have not been very successful, but may be something on the horizon. Overall there are quite a few approaches currently being tested. It is likely that one or more of methods will be an effective treatment for the many types of muscular dystrophy.

## MD Types of Interest in the Kunkel Research Laboratory



### Limb Girdle Muscular Dystrophy - LGMD

- ◆ Group of 13+ disorders
- ◆ Inheritance - Autosomal dominant or recessive
- ◆ Affect mainly voluntary muscles of shoulder & hip
- ◆ Begins in proximal (close to trunk) muscles -progressive

### Duchene/Becker Muscular Dystrophy - DMD/BMD

- ◆ Inheritance - X-linked recessive -affects mostly boys
- ◆ Affects voluntary muscles in arms, legs, & trunk
- ◆ Progressive - boys will use a wheelchair
- ◆ Toe walking, weakness, & large calves are signs
- ◆ Heart & breathing muscles may be affected
- ◆ BMD is a more mild version of DMD

### Facioscapulohumeral Muscular Dystrophy (FSHD)

- ◆ Inheritance - Autosomal Dominant
- ◆ Affects mainly face, shoulder & upper arm muscles (but not only these muscles)
- ◆ Usually slowly progressive
- ◆ Muscle weakness asymmetric - right vs. left
- ◆ Sleeping with eyes open, inability to whistle or use a straw are common features

### Myotonic Muscular Dystrophy (MMD)

- ◆ Inheritance - Autosomal dominant
- ◆ Myotonia - inability to relax muscle at will - is seen
- ◆ Affects distal (close to hands/feet) muscles first
- ◆ Heart, eyes, breathing, hearing & many other organ systems can be affected
- ◆ Several forms; mild, classic, congenital- increase in severity with increase repeat number

## Current Research News

By Tim Pusack

### Genetics:

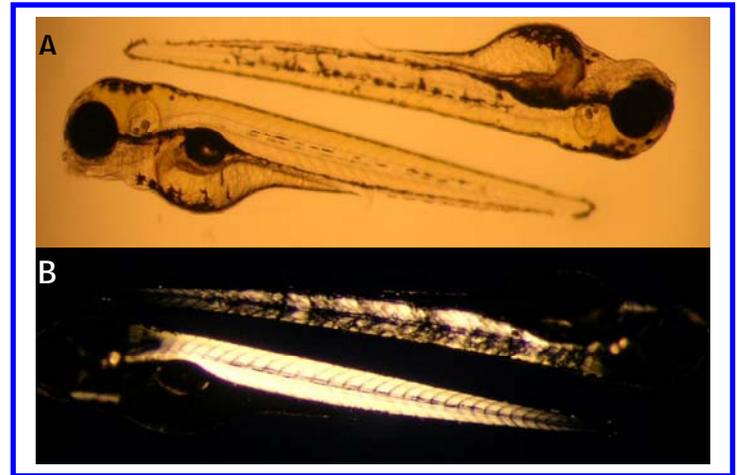
Through research we are continually learning new information about Muscular Dystrophy (MD). New studies have shown that in 30% of LGMD cases the gene responsible is still unknown. We are collecting families with LGMD to find these unknown genes. In the past year, Dr. Peter Kang has completed a project comparing gene function in human muscle vs. mouse muscle. The goal of the study was to determine how alike are mouse and human muscle. He found that only certain mouse muscle is similar to human muscle. This is very important as we frequently use the mouse as a model system to try therapies. Dr. Kang's studies tell us which mouse muscle is most directly applicable to the human muscle.

A large part of our genetics work uses zebrafish as a model for MD. At this time, we have 13 fish lines with MD. We are currently working to characterize all 13 types of MD in our fish lines (see figure - example of Fish Muscular Dystrophy). Two of the 13 fish lines with MD have already been shown to have mutations in dystrophin, the gene associated with DMD/BMD. Another fish line is believed to have a mutation in the gene titin which is associated with LGMD2J. We also have found two fish lines with mutations in a new gene and are working to determine if it is truly a new cause of MD. This work partnered with our family studies will allow us to find new genes for MD.

## New Therapeutic Frontiers

By Tim Pusack

We are testing two types of therapies in the effort to treat and to cure MD: cell-based therapies and chemical-based therapies. The cell-based therapy is a technique of transplanting healthy cells to re-grow muscle in individuals with muscular dystrophy. We are currently using the zebrafish and mouse as models for this technique. Cells taken from the muscles of healthy mice and zebrafish are injected into DMD mice or injured fish, respectively, to see which cells are best at repairing the muscle. Once we find which cell types perform well, we examine them more closely to see what specific genes they express. These genes can be used to determine what proteins are best at helping cells re-grow healthy muscle. In both models, we have been able to transplant cells from healthy mice or fish into dystrophic or injured animals and see some new muscle cells. We are also researching to see if bone marrow cells can be made into muscle cells when put into a muscle environment. This would allow us to use bone marrow transplantation as a potential therapy for MD.



(A) normal light (B) polarized light. In each picture the top fish has DMD, the bottom fish is unaffected. (B) White muscle is healthy muscle, dark patches are damaged muscle. The fish are 7 days old and still in the larval form.

### Clinical:

Other patient related studies in our lab include improving DNA testing for MD. Molecular diagnosis of the specific type of MD can be very important for family planning, at risk family members, and also for access and acceptance into clinical trials. Two of our lab members, Dick Bennett and Hal Schneider are currently developing new genetic tests for many types of MD. They are attempting to automate the process using robotics. We believe that this will improve genetic testing by making the test quick, reliable and more affordable. We hope that this would increase testing availability to all patients with MD allowing a definitive diagnosis to be made for each family.

We also would like to test the ability of different chemicals to repair damaged muscle. This work is being done in the zebrafish and is headed by Dr. Jeffery Guyon. We will screen a chemical library of 2,640 bio-reactive chemicals in the DMD fish looking for those chemicals that have a positive affect on the MD symptoms in the zebrafish. We believe that identifying reactive chemicals will yield new information about the pathogenesis of MD. This may help lead us to new treatments for many types of MD.

Many of the chemicals are already FDA approved, which would decrease the time needed to get to humans trials.

*“Improve genetic testing by making it quick, reliable and more affordable”*

# Clinical Update

By Erica Sanborn, MS, CGC

The Muscular Dystrophy Association (MDA) clinic at Children’s Hospital Boston is a multi-disciplinary clinic involving neurology, orthopedics, physical therapy, genetics, and social work. The clinic has been very busy over the last year with upward of 386 patient visits during 2006, a 16% increase from 2005. While the MDA clinic follows children with a variety of neuromuscular diagnoses, Duchenne Muscular Dystrophy (DMD) and Becker Muscular Dystrophy (BMD) collectively are the biggest subset of our population. While significant research is being done in the field of muscular dystrophies at Children’s Hospital Boston at this time, we do not have any therapeutic clinical trials for DMD or BMD. Our clinic staff does investigate new therapeutic studies worldwide.

Currently, we would like to make you aware of a new study at Children’s Hospital of Philadelphia (CHOP). CHOP is currently looking for boys with DMD for a clinical trial, studying the effects of a drug called PTC124. PTC124 has been shown to partially restore dystrophin production in animals with DMD with a specific type of point mutation called a nonsense mutation (or premature stop codon). The main purpose of this study is to see if PTC124 can safely increase working dystrophin protein in the muscles of boys with DMD caused by a nonsense mutation. Investigators are looking for boys over age 5 with confirmed DNA testing of a point mutation, who are willing to comply with scheduled



*Children's Hospital Boston MDA Clinic team: From L-R Dr. Shapiro (orthopedics), Elicia Estrella (research GC), Dr. Darras (neurology), Erica Sanborn (clinical GC), Janet Quigley (PT), missing Dr. Peter Kang and Brenda (MDA rep)*

visits, drug administration plan, laboratory tests, study restrictions, and study procedures (including muscle biopsies, myometry, and PK sampling).

If you are interested in more information about this study please contact Michele Toms, Study Coordinator at 215-590-7727 or toms@email.chop.edu. If you do not know if you have had DNA testing or what type of mutation you have feel free to contact us to discuss. (Elicia Estrella 617-919-4552 or Erica Sanborn 617-355-2752)

## What Do You Think of the Newsletter?

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3. Update us on your contact information (phone/email/address)
4. Let us know how you are doing
5. Take me off your mailing list
6. Request a copy of the newsletter to be sent to someone not on our mailing list (family member, new study participant, your doctor, etc.)

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#### Other Websites of Interest:

- [www.hhmi.org](http://www.hhmi.org)
- [www.clinicaltrials.gov](http://www.clinicaltrials.gov)
- [www.mda.org](http://www.mda.org)

## Other Useful Information

### Would you like to join our study?

- Clinical and/or pathological diagnosis of
  - o Duchenne/Becker Muscular Dystrophy (DMD/BMD)
  - o Limb-Girdle Muscular Dystrophy (LGMD)
  - o Facioscapulohumeral Dystrophy (FSHMD)
  - o Myotonic Dystrophy (DM)
  - o Miyoshi Myopathy (MM)
- Previous muscle biopsy
- Gene testing results *unless the diagnosis is LGMD (no gene testing required)*
- Relative of someone with the diagnosis of dystrophy

### New Publications from our Research:

Guyon JR, Steffen LS, Howell MH, Pusack TJ, Lawrence C, Kunkel LM.

Modeling human muscle disease in zebrafish. *Biochim Biophys Acta*. 2006 Jul 20;

Dalkilic I, Schiend J, Thompson TG, Kunkel LM. Loss of FilaminC (FLNc) results in severe defects in myogenesis & myotube structure. *Mol Cell Biol*. 2006 Sep;26(17):6522.

Duncan DR, Kang PB, Rabbat JC, Briggs CE, Lidov HG, Darras BT, Kunkel LM. A novel mutation in two families with limb-girdle muscular dystrophy type 2C. *Neurology*. 2006 Jul 11;67(1):167.

Bachrach E, Perez AL, Choi YH, Illigens BM, Jun SJ, del Nido P, McGowan FX, Li S, Flint A, Chamberlain J, Kunkel LM. Muscle engraftment of myogenic progenitor cells following intraarterial transplantation. *Muscle Nerve*. 2006 Jul;34(1):44.

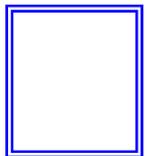
Kunkel LM, Bachrach E, Bennett RR, Guyon J, Steffen L. Diagnosis and cell-based therapy for Duchenne muscular dystrophy in humans, mice, and zebrafish. *J Hum Genet*. 2006;51(5):397.

Kho AT, Kang PB, Kohane IS, Kunkel LM. Transcriptome-scale similarities between mouse and human skeletal muscles with normal and myopathic phenotypes. *BMC Musculoskelet Disord*. 2006 Mar 7;7:23.

Kunkel LM. 2004 William Allan Award address. Cloning of the DMD gene.

*Am J Hum Genet*. 2005 Feb;76(2):205.

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