

# Kunkel Laboratory Newsletter

Children's Hospital,  
Boston

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## LGMD type 2I in Zebrafish

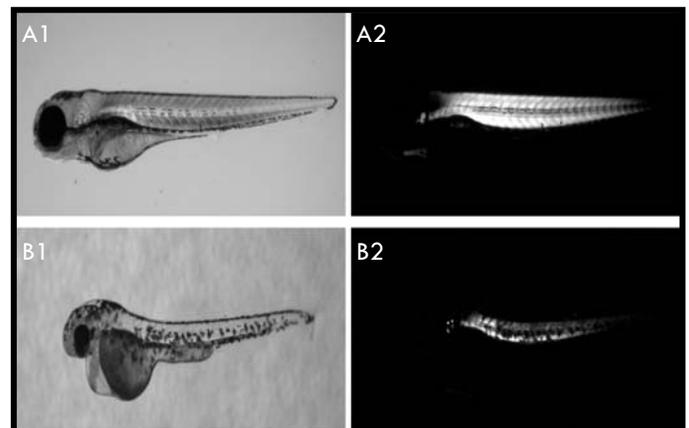
Limb Girdle Muscular Dystrophy (LGMD) is a group of muscle diseases showing a similar pattern of weakness, usually beginning with the muscles of the pelvic and shoulder areas. The cause of LGMD can be subdivided by its inheritance pattern. Type 1 LGMD is dominantly inherited while Type 2 LGMD follows a recessive inheritance pattern. Type 1 and Type 2 LGMD can be further characterized by which specific gene changes are determined to cause the muscle symptoms. LGMD type 2I is a recessively inherited muscle disease caused by changes in fukutin-related protein (FKRP) gene.

Changes in the FKRP gene also cause related forms of neuromuscular disease including; Walker-Warburg syndrome (WWS), and congenital muscular dystrophy 1C (MDC1C). Fukutin-related protein (FKRP) is a relative of the protein fukutin, the cause in Fukuyama-type Congenital Muscular Dystrophy (FCMD). The fukutin protein is thought to function as a glycosyltransferase (modifies other proteins by adding sugar groups). Recent findings show many types of muscular dystrophies are associated with defective glycosylation of  $\alpha$ -dystroglycan, an important protein at the muscle cell membrane. This problem is thought to be caused by mutations in genes encoding glycosyltransferases. However, the exact function of FKRP is currently unknown.

Zebrafish, a small, easily bred, fish could serve as an ideal animal model to help understand the mechanism of how changes in the FKRP gene cause LGMD 2I, MDC1C

and WWS. We were able to decrease FKRP gene expression in zebrafish and then show that the resulting fish embryos had symptoms similar to those observed in humans. The decreased FKRP fish showed muscle, heart and eye formation problems. In addition, the fish had curved tails and were unable to swim. They were also found to have a reduction in  $\alpha$ -dystroglycan glycosylation.

These findings suggest the decreased FKRP fish are clinically similar to findings in human patients with LGMD 2I, MDC1C, and WWS. Moreover, we were able to



**Panels A1 & A2 - Developing Zebrafish embryo -wild type**  
**Panels B1 & B2—LGMD 2I Zebrafish. Notice in B1 small eye and large heart. B2 notice unorganized muscle compared to A2.**

add back both human or fish FKRP while the embryo was growing to restore normal development. We hope to continue studying these “knockdown” or decreased FKRP fish for clues as to the function of FKRP in maintaining healthy muscle. The FKRP knockdown fish is also ideal for testing gene therapy and/or drug therapies and treatments due to their small size, short life span and similar symptoms to human muscular dystrophies.



## Special points of interest:

- ☒ LGMD 2I—Fish model
- ☒ PTC 124 Study
- ☒ New Website

## Genetics 101

Inheritance can be determined by comparing a person's **genotype** (set of genes) to their **phenotype** (expression of a person's genes). When we inherited 2 copies of all of our genes, one from each of our parents, subtle differences in the 2 copies can exist. These differences are sometimes called **mutations**.

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;

**DELETIONS:** A large chunk of a gene is cut out, like tearing out a chapter of a book  
65% of DMD/BMD cases have deletions

**DUPLICATIONS:** Adding extra genetic material or DNA, like having 2 chapter 5's in row in when reading a book. 5-10% of DMD/BMD cases have duplications

**POINT MUTATIONS:** Typos in our genes are like typos in a book, the changes can cause word/meaning differences. 25- 30% of DMD/BMD cases have point mutations

**REPEATS:** Most commonly 3 bases are repeated over & over (ATG,ATG,ATG etc...) In a book having the word "**THE**" repeated for several pages would be confusing to the reader. If this went on for many pages you would lose meaning of the story. Repeats cause Myotonic Dystrophy (DM). The more repeats the more severe the disease

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



## Meet The Team!

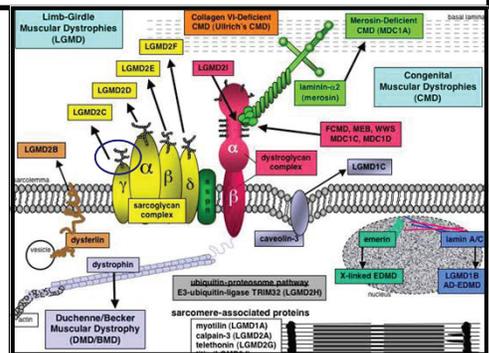
Kunkel Lab Members: Front –Back/ Right—Left

Caitlin Kreitman, Fedik Rahimov, Louis Kunkel, Marielle Thorne, Kayla Vatalaro, Jess Egan, Christine Savage, Elicia Estrella, McKensie Wessen, Kellie Haley, Hal Schneider, Josh Davis, Genri, Kawahara, Alan Eran, Jill McCarthy (hidden), Stephanie Burgess, Juan Carlos Casar, Alex White, Christin Collins, Matt Alexander, Steve Boyden, Dick Bennett

**Missing from Photo:** Peter Kang, Hart Lidov, Iris Eisenberg, Tram Tran, George Gennis

## What is Glycosylation?

All of our genes are encoded in our DNA. In order for the body to read our genes and make them into proteins for use throughout the body, our DNA is "transcribed" or re-written into RNA in a process called transcription. The RNA is then "de-coded" or "translated" to produce the correct order of amino acids in a protein. Once the protein is translated, it can still be altered by cutting it or adding things to it. These changes are called posttranslational modifications and they allow the protein to have many functions. Glycosylation is a type of posttranslational modification. Sugars are combined into poly and oligosaccharides or "glycosyl groups". These groups are then added to certain proteins. Glycosylation of certain proteins is very important to help maintain the shape of a protein, keeping it stable, and help the cells that contain glycosylated proteins interact with one another and the outside world. Many of the proteins at the neuromuscular junction are known to be glycosylated. New studies show us that glycosylation defects are frequently seen in many neuromuscular diseases.



Example of the  $\gamma$  sarcoglycan protein with a glycosyl group attached (See blue circle)  
See also  $\alpha$ ,  $\beta$ ,  $\delta$  sarcoglycan &  $\beta$ -dystroglycan

## Contact Us...We would love to hear from you!

1. *We would love feedback about the newsletter*
2. *Future topics you would like to see in the newsletter*
3. *Updated us on any changes with you*
4. *Take me off your mailing list*
5. *I know someone else who would like to get the newsletter*

### Reply Card

Name: \_\_\_\_\_

Contact Information Changes: (Please Print)

Address: \_\_\_\_\_

Phone: \_\_\_\_\_

Email: \_\_\_\_\_

Clinical Updates: \_\_\_\_\_

\_\_\_\_\_

Please check here to be taken off our mailing list

Please sign to authenticate: \_\_\_\_\_

## Clinical Update...

The Muscular Dystrophy Association (MDA) clinic at Children's Hospital Boston (CHB) is a multi-disciplinary clinic that cares for children with a variety of neuromuscular diagnoses. In July of 2008, CHB became a study site for a clinical trial of the new drug *Ataluren (PTC124™)* to treat boys with DMD/BMD, over the age of 5 with a premature stop codon (nonsense mutation) in their dystrophin gene. The study is designed to determine whether *Ataluren (PC124)* can improve walking, activity, muscle function, and strength and whether the drug can safely be given for a long period of time.

The study is a **randomized double-blind placebo controlled phase IIb trial**. Clinical trials involving new drugs are commonly classified into four phases (Phase I-IV). Phase I trials are the first stage of testing in human subjects. A small (20-80) group of healthy volunteers was selected to look at drug side effects/toxicity. Phase II studies can be divided into Phase IIA and Phase IIB. Phase IIA is specifically designed to assess dosing requirements (how much drug should be given). Phase IIB is specifically designed to study **efficacy** (how well the drug works at the prescribed dose(s)). The study is randomized and double-blinded if each study subject is randomly assigned to receive either the study treatment or a **placebo** (fake treatment) and neither the subject or researcher knows who has received treatment vs. placebo.

Currently, the study enrollment is complete, but the overall study is still ongoing. Participants have a biopsy at the beginning of the study and one at the end to compare levels of dystrophin. Muscle strength is measure many ways including a 6 minute walk test given at each study visit. Blood and urine samples are taken to observe levels of Creatine Kinase (CK) and ensure no drug toxicity. Initial data has been very promising. After completion of the study the boys involved will enroll in an open label study with *Ataluren (PTC124)* for 2 years. We hope that *Ataluren (PTC124)* will become commercially available before the end of the open label study.



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### We're on the Web!

Please check out our new website at;

**[WWW.CHB-Genomics.org](http://WWW.CHB-Genomics.org)**

**Click on Neuromuscular Disease**

Let us know what you think?

Are we missing things you would like to see?

Is it easy to find info on Muscular Dystrophy?