May 2010, Volume 4

Kunkel Laboratory Newsletter

Children's Hospital, Boston

Screening for New Drugs to Treat Duchenne/Becker Muscular Dystrophy (DMD/BMD) using Zebrafish as an Animal Model

Special points of interest:

- Genetics 101!
- Meet the Kunkel Lab!
- Clinical Trial Update!
- We are online! www.chb-genomics.org

Chemical compounds or drugs can bind to specific proteins, altering their functions and resulting in changes in a disease state. We were able to gain access to a chemical library (group of chemical compounds) containing 1120 chemical compounds. These compounds were chosen because they had been shown to be safe in humans.

Zebrafish are an excellent animal model for studying many genetic diseases (see Genetics 101 pp.2). For example, there are currently zebrafish containing mutations in the DMD gene causing the zebrafish to have muscular dystrophy.

Zebrafish with DMD are easily identified using a non-invasive test called birefringence. The birefringence test uses polarized light to reflect off the muscle in a sleeping fish. Zebrafish with DMD/BMD have a patchy, unorganized appearance to their muscle (see Figure 1).

Zebrafish embryos are grown in small pools containing different chemical compounds.

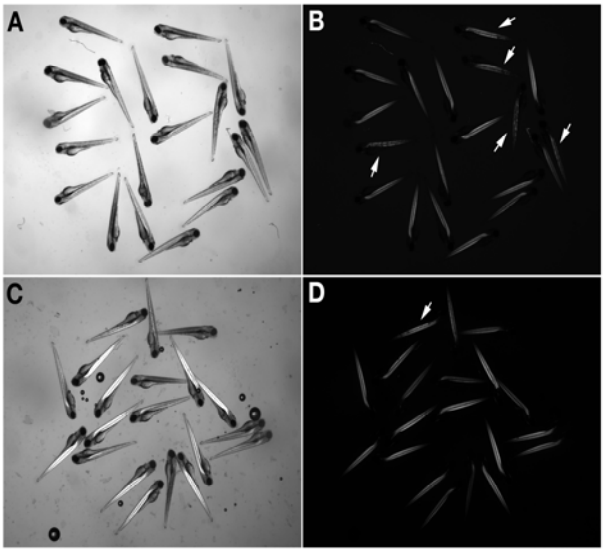

Figure 1: Zebrafish Embryos (4 days post fertilization) Panel A , B— Untreated Fish & Panel C, D—Treated Fish Panel B & D show the Birefringence assay with DMD fish near white arrows. Notice in panel D the chemicals in the water of the Treated Fish group have decreased the number of DMD Fish

On day 4 post fertilization (4 dpf), the birefringence of each zebrafish embryo is analyzed. Each experimental group has a matching control group of embryos raised in pools without any chemical additives (non-treated). The birefringence of the chemical treated fish (experimental group) was compared to the non-treated fish (control group). The main study goal is to perform a chemical screen in these zebrafish to identify compounds or drugs which might correct the muscle pathology detected by birefringence. Currently, we are investigating several promising chemical compounds identified by our zebrafish screen. We hope this will lead us to new drugs eligible for trials in humans.

Harvard Neuromuscular Disease Project
Harvard Medical School • Boston Children's Hospital

Genetics 101: Zebrafish As Model Organism for Studying The Muscular Dystrophies

Top Panel: Adult Zebrafish
Bottom Panel: Zebrafish Embryo

Humans & Zebrafish have the same Genes!

Recently, researchers have begun to use the zebrafish (*Danio rerio*), a small freshwater fish as a model organism for studying human disease. Zebrafish are easy to maintain and breed, with each female producing 100–200 eggs per mating, providing large numbers of animals for use in studies. This is exceedingly helpful when doing genetic studies, as the number of offspring can be important in observing disease state.

Due to their small size, zebrafish embryos and early larvae can be raised in as little as three ounces of water. Additionally, zebrafish embryos are transparent and develop externally from the mothers, allowing easy observation of experimental effects on internal organs and tissues while the fish grows and develops (See bottom Panel).

The zebrafish has most of the same organs found in mammals and humans making it a valuable and cost effective model organism, especially when compared with mice, rats or rabbits. Most human genes have a zebrafish counterpart, and the proteins coded by zebrafish genes function like human proteins. This makes zebrafish an excellent model for studying gene function and drug effects in humans. The zebrafish is the only vertebrate species for which large-scale genetic screens have been carried out. There are now fish with symptoms of human disorders, including cardiovascular disease, muscular dystrophy, and several types of cancer.

Meet The Kunkel Laboratory Members!

Laboratory Members: (left-Right & Front-Back) Elicia Estrella, Jennifer Meyers, Louis Kunkel, Jeremy Karpf, Jessica Egan, Marie Torres, Tran Tram, Christin Collins, Marielle Thorne, Gendi Kawahara , Patricia Arashiro, Peter Kang, Anna Duncan, Hal Schneider, Kristin Cabral, Richard Bennet, Matt Alexander, norio Motohasi, Juan-Carlos Casar
Missing From Photo: Steven Boyden, Hart Lidov, Fedik Rahimov, Mei Han

Clinical Update:

PTC124–at Children's Hospital , Boston

The Muscular Dystrophy Association (MDA) clinic at Children's Hospital Boston participated in clinical trials using the drug PTC124 also known as Ataluren. The trial called Study 007 was designed to test the safety and efficacy of 48 weeks of Ataluren therapy in patients with DMD/BMD. The study enrolled 174 participants at 37 sites in North America, Europe, Australia, and Israel. The primary outcome measure was the total distance walked during a 6-minute walk test, a commonly used test for walking. Patients completing Study 007 were eligible to receive up to 96 weeks of open-label, high-dose Ataluren in an extension study, Study 007e. In addition, Study 008 was a safety study of Ataluren in non-ambulatory boys with DMD/BMD.

Available analyses of the study results indicate that the primary endpoint of Study 007, change in 6-minute walk distance over 48 weeks, was not statistically different between the Ataluren-treated and placebo-treated groups. Safety results indicate that both low-dose and high-dose Ataluren were well tolerated. No cumulative toxicity was evident and no clinical trial patients discontinued treatment due to an adverse event.

An independent Data Monitoring Committee (DMC) has reviewed the available efficacy and safety results from Study 007. Based upon the recommendation of the DMC, PTC Therapeutics intends to discontinue ongoing studies in DMD/BMD (Studies 007e and 008).

If at any time, you would like to discuss the results of Study 007, 007e or 008 and what they mean, please feel free to contact Elicia Estrella, genetic counselor at 617-919-4552. Also, the PTC Therapeutics Patient Advocacy department is available to speak with families concerning this matter.

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Recent Publications:

1. Zebrafish models for human FKRP muscular dystrophies. Kawahara G, Guyon JR, Nakamura Y, Kunkel LM. *Hum Mol Genet.* 2010 Feb 15;19(4):623.

2. Automated DNA mutation detection using universal conditions direct sequencing: application to ten muscular dystrophy genes. Bennett RR, Schneider HE, Estrella E, Burgess S, Cheng AS, Barrett C, Lip V, Lai PS, Shen Y, Wu BL, Darras BT, Beggs AH, Kunkel LM.*BMC Genet.* 2009 Oct 18;10:66.

3. CXCR4 enhances engraftment of muscle progenitor cells. Perez AL, Bachrach E, Illigens BM, Jun SJ, Bagden E, Steffen L, Flint A, McGowan FX, Del Nido P, Montecino-Rodriguez E, Tidball JG, Kunkel LM. *Muscle Nerve.* 2009 Oct;40(4):562.

4. A role for nephrin, a renal protein, in vertebrate skeletal muscle cell fusion. Sohn RL, Huang P, Kawahara G, Mitchell M, Guyon J, Kalluri R, Kunkel LM, Gussoni E. *Proc Natl Acad Sci U S A.* 2009 Jun 9;106(23):9274.

5. Transcriptional regulation differs in affected facioscapulohumeral muscular dystrophy patients compared to asymptomatic related carriers. Arashiro P, Eisenberg I, Kho AT, Cerqueira AM, Canovas M, Silva HC, Pavanello RC, Verjovski-Almeida S, Kunkel LM, Zatz M. *Proc Natl Acad Sci U S A.* 2009 Apr 14;106(15):6220.

6. miRNAS in normal and diseased skeletal muscle. Eisenberg I, Alexander MS, Kunkel LM. *J Cell Mol Med.* 2009 Jan;13(1):2-11. Review.

7.Genetic isolation & characterization of a splicing mutant of zebrafish dystrophin. Guyon JR, Goswami J, Jun SJ, Thorne M, Howell M, Pusack T, Kawahara G, Steffen LS, Galdzicki M, Kunkel LM. *Hum Mol Genet.* 2009 Jan 1;18(1):202.

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Our offices have moved to a new location…

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We'd love to hear from you!