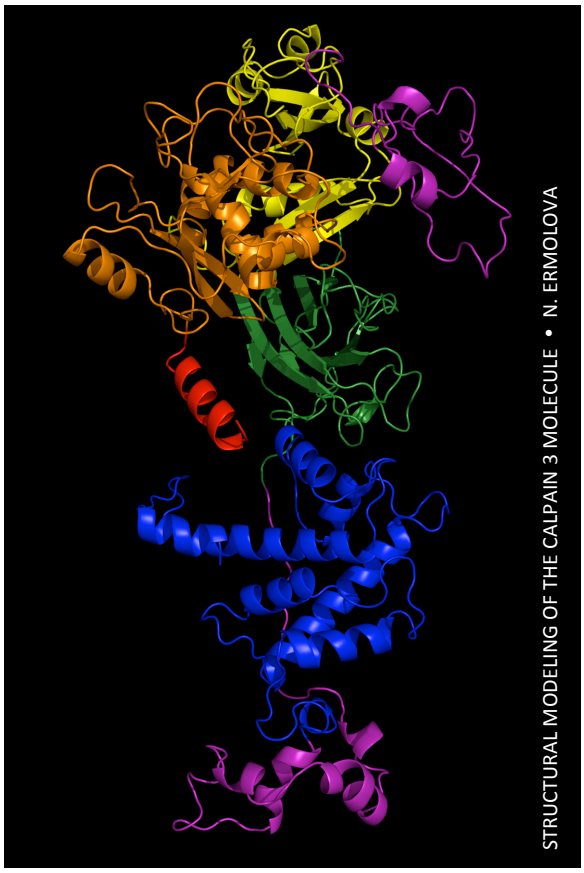
CLINICAL CHARACTERISTICS AND PATHOGENIC MECHANISMS OF LGMD2A/CALPAINOPATHY



OCTOBER 27, 2011
SANTA MONICA, CALIFORNIA

SPONSORED BY

COALITION TO CURE CALPAIN 3
OVERCOMING WEAKNESS WITH STRENGTH

COALITION TO CURE CALPAIN 3
OVERCOMING WEAKNESS WITH STRENGTH

Dear Scientists, Physicians and Guests,

Limb-girdle muscular dystrophy type 2A (LGMD2A)/calpainopathy is not completely understood, there is no cure or even a treatment, and it is under-researched and underfunded. Until today, there had never been a workshop held in the U.S. focused solely on LGMD2A; there was no nationally – much less globally – recognized patient registry and therefore, no list of people for researchers to contact about clinical trials of promising therapies; there is no way to diagnose the disease that is both reliable and affordable; there is little awareness of LGMD2A among the general public.

This was the sad state of affairs when we met in the summer of 2010. We both had been diagnosed with the disease and were frustrated by the lack of progress. While we wanted to help out, we believed that alone we would probably be unable to make a difference. We knew that it would take a community working together to overcome this disease. After meeting, we knew almost instantly that together, we had the strength, the knowhow, and the passion to create a foundation that could address this list of glaring unmet needs. We founded Coalition to Cure Calpain 3 (C3) in October of 2010.

Since that time, we have completed our business plan; began populating the Board of Directors that includes the two of us along with Dr. Lee Wrubel and Jordan Boslego; established a Scientific Advisory Board that includes Drs. Kevin Campbell, Eric Hoffman, and Melissa Spencer; filed the 501(c)(3) application for tax exempt status for which approval was granted on May 19, 2011; organized and sponsored this first ever U.S. workshop focused solely on LGMD2A, and in partnership with Dr. Melissa Spencer, applied for and were awarded an MDA grant to help fund the conference; created the first global patient registry for LGMD2A which has been sanctioned by TREAT-NMD; began to build awareness of LGMD2A and unite the community by designing and launching our website (www.curecalpain3.org) and by entering into and becoming active on the social networking sites of Facebook and Twitter as well as the online media-based fundraising programs of Network For Good and Crowdrise. Through these efforts, the increased awareness of our mission has resulted in an impressive financial advance despite the effects of our weakened economy.

Our priorities for the coming year are to continue to build the C3 infrastructure and financial base and to evaluate and choose the most promising research project(s) to support. It will be another busy year!

We want to thank you for your participation in this workshop and for your efforts on behalf of all those living with LGMD2A. We are certain that our combined efforts will make an important difference as we work together toward overcoming weakness with strength.

Warm regards,

Kris Kurnit and Michele Wrubel
Co-founders

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ACKNOWLEDGMENTS

The Board of Directors of Coalition to Cure Calpain 3 gratefully acknowledges ***Dr. Melissa Spencer*** for organizing today's meeting and thanks The UCLA Department of Neurology and The Center for Duchenne Muscular Dystrophy at UCLA.

This workshop was made possible with additional generous support from:

C3 BOARD OF DIRECTORS

Kristine Kurnit, *Co-Founder and President*

- Co-founded SensiGen, a biotechnology company, with her husband, Dr. David Kurnit
- As Chief Executive Officer at SensiGen, Kris's work included: strategic and tactical business planning for the development and commercialization of two gene-based medical technologies; relationship building with strategic partners; and general management of the company
- Prior to SensiGen, Kris worked as a clinical psychologist in private practice for 25 years. She simultaneously co-founded a medically based eating disorders clinic that was eventually acquired by Beaumont Hospital. She was diagnosed with LGMD2A in 1995
- B.A. from the University of Michigan with high distinction; M.A. from Oakland University in Clinical Psychology

Michele Wrubel, *Co-Founder and Senior Vice President*

- A decade of non-profit experience and numerous leadership roles on the executive committee and general boards of The Conservative Synagogue and for the public school system of her community in Westport, Connecticut
- Launched her advertising career at HBM/Creamer in Boston (later Della Femina, McNamee WCRS). After moving to New York, she became a media supervisor at Ammirati & Puris/Lintas putting her strategic planning skills to work on national accounts for Johnson & Johnson (Vistakon and McNeil divisions) and Sara Lee Corporation
- A 1985 *summa cum laude* graduate of the University of Rhode Island, Michele was awarded a full scholarship to the Tufts University Graduate School of Drama before learning at the age of 25 that she had muscular dystrophy

Dr. Lee Wrubel, *Treasurer*

- Co-founder and General Partner of Foundation Medical Partners, a venture capital firm
- Fifteen years of venture capital experience including association with many private and now-public companies
- Previously an investment professional with Canaan Partners and Highland Capital Partners, where he specialized in biotechnology and medical device investments
- Board of Directors member of EndoGastric Solutions, Inc., CardioMEMS, Inc., IlluminOss and Circulite, Inc.
- Serves on the Translational Research Advisory Committee of the Muscular Dystrophy Association
- A.B. from Lafayette College, MD and MPH from Tufts University, and MBA from Columbia University; pediatric internship at Mount Sinai Medical Center in New York

Jordan Boslego, Member

- Spearheads the C3 patient registry effort, including site development and coordination with various partners to maximize the patient reach, standardization and utility to researchers of the registry
- Vice President and Head of Portfolio Risk at Global Thematic Partners, an independent asset management firm headquartered in New York City
- Previously a member of the natural resources investment banking group at J.P. Morgan
- Graduated from Harvard College with an A.B. in Economics and Statistics, *cum laude* with High Honors
- Diagnosed with LGMD2A in 2005

C3 SCIENTIFIC ADVISORY BOARD

Melissa Spencer, PhD, *Chair*
University of California, Los Angeles

- Professor of Neurology at the University of California, Los Angeles
- Co-Director of the Center for Duchenne Muscular Dystrophy at UCLA
- Recipient of a PECASE, the nation's highest honor for professionals at the outset of independent research careers (2001)
- Has spent the past two decades studying calpain-3 and LGMD2A

Kevin Campbell, PhD, *Member*
University of Iowa

- Roy J. Carver Biomedical Research Chair in Molecular Physiology and Biophysics
- Investigator with the Howard Hughes Medical Institute
- Director of the Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center
- Internationally recognized for fundamental contributions to muscular dystrophy research
- March of Dimes Prize in Developmental Biology for pioneering research on cell mechanisms involved in MD (2009)

Eric Hoffman, PhD, *Member*
George Washington University

- A. James Clark Chair in Molecular Genetics and Director of the Center for Genetic Medicine Research, Children's National Medical Center
- Chair, Department of Integrative Systems Biology, George Washington University
- Over 400 publications; laboratory increasingly focused on novel drug development programs
- Emphasis on gene identification, pathophysiological studies, molecular diagnostics and therapeutics

C3 GRANT REVIEW PROCESS

C3 will solicit grant proposals from scientists or companies that could further the scientific goals of C3 and will require written grant proposals that specify:

- Project Title
- Principal Investigator/Project Director and Contact Information
- Proposed Period of Support
- Total Amount of Funding Requested
- Type of Research (Basic, Translational, Clinical)
- Overview of Proposed Project
- Identification of Specific Aims
- Background and Significance
- Experimental Methods, Design, Technologies and Techniques
- Timeline

Our Scientific Advisory Board (SAB) will review each application and provide a recommendation on the relevance of the project to the goals of C3, its scientific soundness, and an assessment of the appropriateness of the level of funding requested relative to the project's design. If, at any time, the SAB is considering a proposal by a member of the SAB, or a related institution, such member will not participate in the review and recommendation process for such proposal.

Upon recommendation of a project by the SAB, the Board of Directors will review and evaluate such project and will have final authority in the decision to grant funds. Before any funds are disbursed, C3 will work with the grantee organization to specify the responsibilities of both parties and obligate the grantee to use the funds only for specific purposes. Records will be kept at each stage of the approval process and continue with periodic reports from the grantee organization, to be reviewed by both the Board and SAB. A final report for each project will be required of the grantee.

C3 plans to fund promising research in line with its scientific goals wherever that research is taking place. Currently, C3 has no connections with any potential foreign grantee organizations, but hopes that interest in LGMD2A is not limited to the United States. C3 plans to use the same control and oversight mechanisms with foreign grantee organizations that it will with domestic organizations, noting that, in all cases, the SAB will evaluate the potential grantee to determine their ability to accomplish the purposes for which the resources would be provided and require oversight authority to ensure that the funds are used to further the tax-exempt purposes of C3, including both periodic and final reporting. Should any further measures be deemed advisable to ensure compliance in a specific case, the Board has the authority to condition the granting of funds on such measures.

AGENDA

7:30 am – 8:15 am	***Breakfast*** *– Check In – Espada Foyer*
8:30 am – 8:40 am	***Welcome*** – Sponsor, Kris Kurnit (President, C3) and Organizer, Dr. Melissa Spencer
	Overview
8:45 am – 9:00 am	Dr. Jerry Mendell: Overview of LGMD2A, Clinical
9:05 am – 9:15 am	Dr. Melissa Spencer: Overview of Calpain 3, Structure, Activation Mechanism
9:20 am – 9:40 am	Dr. Melissa Spencer: Overview of Calpain 3 Physiological Roles
	Mechanisms of LGMD2A and Preclinical Studies – Dr. Jacques Beckmann, Moderator
9:45 am – 10:00 am	Dr. Mayana Zatz: Preclinical Studies with Adult Stem Cells in Animal Models for Muscular Dystrophy
10:05 am – 10:20 am	Dr. Hiroyuki Sorimachi: Studies of Titin Signaling and Calpain 3
10:25 am – 10:40 am	Dr. Silvère van der Maarel: Identification of a Calpain 3 Consensus Cleavage Motif Facilitates Bioinformatic Reconstruction of LGMD2A Pathogenesis
10:45 am – 11:00 am	*Break*
11:05 am – 11:20 am	Dr. Isabelle Richard: Calpain 3 Gene Transfer
11:25 am – 11:40 am	Dr. Kevin Flanigan: Planning for Preclinical Gene Therapy Studies in Calpain 3 Knock Out Mice
11:45 am – 12:00 pm	Dr. Carrie Miceli: Use of Patient Derived Cells For Drug Screening for Muscular Dystrophy Treatments
	Calpain 3 and Membrane Repair – Dr. Elizabeth McNally, Moderator
12:05 pm – 12:20 pm	Dr. Kevin Campbell: Dysferlin and Muscle Membrane Repair
12:25 pm – 12:40 pm	Dr. Ronald Mellgren: Calpain 3 Is Not Required for Plasma Membrane Repair
12:45 pm – 1:45 pm	***Lunch*** *– Alaria Room*
	Calpain 3 and Other Pathways that Might Be Impacted by Calpain – Dr. Hiroyuki Sorimachi, Moderator
1:50 pm – 2:05 pm	Dr. Kanneboyina Nagaraju: Comprehensive Phenotyping of Calpain 3 Knock Out Mice
2:10 pm – 2:25 pm	Dr. Irina Kramerova: Impaired Ca2+ Mediated Signaling and Muscle Adaptation Response in the Absence of Calpain 3
2:30 pm – 2:45 pm	Dr. Mathias Gautel: Links from the Sarcomere to Mechanical Strain Sensing and Muscle Protein Turnover Control
2:50 pm – 3:05 pm	Dr. Denis Guttridge: NF-κB Signaling in Muscular Dystrophies
3:10 pm – 3:25 pm	Dr. Siegfried Labeit: Towards Selective Perturbation of Titin Based Signaling by Small Molecules to Identify Novel Tools for Muscle Research and for Testing Therapeutic Approaches
3:30 pm – 3:45 pm	*Break*
	Group Discussion Regarding Prioritization of Therapeutic and Diagnostic Strategies and Natural History Studies
3:50 pm – 4:00 pm	Dr. Lee Wrubel (Foundation Medical Partners, C3), Introduction
4:00 pm – 5:30 pm	Dr. Robert Griggs, Moderator
Additional Participants	Joana Capote, Dr. Valerie Cwik, Dr. Natalia Ermolova, Leonel Martinez, Dr. Ekaterina Mohkanova, Dr. Yasuko Ono, Dr. Susan Sparks, Dr. Nicolas Wein
6:00 pm	***Dinner*** *– Alaria Room*

NOTES

OVERVIEW OF CALPAIN 3: STRUCTURE & ACTIVATION MECHANISM •

DR. MELISSA SPENCER

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SESSION I AGENDA: MECHANISMS OF LGMD2A AND PRECLINICAL STUDIES •
DR. JACQUES BECKMANN, MODERATOR

9:45 am – 10:00 am	Dr. Mayana Zatz: Preclinical Studies with Adult Stem Cells in Animal Models for Muscular Dystrophy
10:05 am – 10:20 am	Dr. Hiroyuki Sorimachi: Studies of Titin Signaling and Calpain 3
10:25 am – 10:40 am	Dr. Silvère van der Maarel: Identification of a Calpain 3 Consensus Cleavage Motif Facilitates Bioinformatic Reconstruction of LGMD2A Pathogenesis of Substrates
10:45 am – 11:00 am	*Break* *Beverages will be available just outside the entrance to the Espada Conference Room*
11:05 am – 11:20 am	Dr. Isabelle Richard: Calpain 3 Gene Transfer
11:25 am – 11:40 am	Dr. Kevin Flanigan: Planning for Preclinical Gene Therapy Studies in Calpain 3 Knock Out Mice
11:45 am – 12:00 pm	Dr. Carrie Miceli: Use of Patient Derived Cells For Drug Screening for Muscular Dystrophy Treatments

PRECLINICAL STUDIES WITH ADULT STEM CELLS IN ANIMAL MODELS FOR MUSCULAR DYSTROPHY • DR. MAYANA ZATZ

Vieira NM, Secco M, Zucconi E, Valadares M, Bueno Junior CR, Brandalise V, Landini V, Andrade T, Vainzof M, Zatz M

Human Genome Research Center, Biosciences Institute, University of São Paulo, São Paulo, Brazil

Before testing stem-cells as a potential therapy for progressive muscular dystrophies many questions need to be addressed. What is the best way of delivery? What are the best cells to be injected? One or multiple injections? Is immunosupression required? What directs homing? In order to address these questions we have injected human mesenchymal stem-cells(hMSCS) in 3 animal models: SJL mice, LAMA2dy/2j mice and GRMD dogs without immunosuppression. Systemic hMSCs injections from both adipose tissue (AT) and umbilical cord tissue (UCT) into the SJL mice and from UCT in LAMA2dy/2j mice showed that cells from both sources were able to reach the recipient muscle and had a beneficial effect in all injected animals. In order to assess if the dystrophic process interfere in the process of homing we repeated the same experiment with AT-hMSCs in normal mice. No cells were found after two months which suggests that factors released during muscle degeneration direct homing. Systemic injections of AT-hMSCs into GRMD dogs using the same protocol showed comparable results. The expression of human muscle dystrophin was observed in muscle biopsies of injected dogs, through western blot up to 6 months after the last injections. Most importantly, the injected dogs, that are currently 3-years old did not develop any tumor and their condition seems to have stabilized. These results suggest that stem-cell therapy might have a clinical application for different forms of muscular dystrophy regardless of specific mutations.

NOTES

STUDIES OF TITIN SIGNALING AND CALPAIN 3 • DR. HIROYUKI SORIMACHI

Hiroyuki Sorimachi, Koichi Ojima, and Yasuko Ono

Calpain Project, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan

CAPN3 (previously called calpain-3/p94) is a skeletal-muscle-specific member of calpains (H.Sorimachi *et al.* (1989) *J.Biol.Chem.*, 264:20106-11), Ca^{2+}-requiring Cys-protease family encoded by 15 genes in humans (H.Sorimachi *et al.* (2011) *J.Biochem.*, 150:23-37; H.Sorimachi *et al.* (2011) *Proc.Jpn.Acad.Ser.B*, 87:287-327). Importance of CAPN3 protease activity for muscle functions was established by the fact that defects in CAPN3 activity originating from its gene mutations cause LGMD2A, also called "calpainopathy". CAPN3 is a unique protease in that it site-specifically interacts with connectin/titin, a gigantic elastic muscle protein (H.Sorimachi *et al.* (1995) *J.Biol.Chem.*, 270:31158-62), that it auto-degrades itself very quickly ($t_{1/2}$ < 10 min) *in vitro* (H.Sorimachi *et al.* (1993) *J.Biol.Chem.*, 268:10593-605), and that this autolytic activity is regulated by connectin/titin-binding (Y.Ono *et al.* (2006) *J.Biol.Chem.*, 281:18519-31) and dependent both on Ca^{2+} and Na^{+} (Y.Ono *et al.* (2010) *J.Biol.Chem.*, 285:22986-98). Using “knock-in” (*Capn3*$^{CS/CS}$) mice, in which an inactive mutant CAPN3:C129S is expressed in place of the endogenous wild-type CAPN3, we demonstrated that a loss of CAPN3 protease activity caused muscular dystrophy (K.Ojima *et al.* (2010) *J.Clin.Invest.*, 120:2672–83). Surprisingly, CAPN3 dynamically translocates to various sites of muscle cells, and CAPN3’s proteolytic activity is involved in its mobility. Moreover, the activity loss resulted in deficient upregulation of heat-shock proteins upon exercise. On another front, compared to *Capn3* null (*Capn3*$^{-/-}$) mice, *Capn3*$^{CS/CS}$ mice showed less severe dystrophic symptoms, suggesting that CAPN3 has a non-proteolytic function, *i.e.*, that CAPN3 is a component of the sarcoplasmic reticulum (SR), in which the non-proteolytic role of CAPN3 is required for proper Ca^{2+} efflux from SR to cytosol (K.Ojima *et al.* (2011) *J.Mol.Biol.*, 407:439-49). Based on these data, pathogenic mechanisms of calpainopathy will be discussed.

NOTES

IDENTIFICATION OF A CALPAIN 3 CONSENSUS CLEAVAGE MOTIF FACILITATES BIOINFORMATIC RECONSTRUCTION OF LGMD2A PATHOGENESIS •

DR. SILVÈRE VAN DER MAAREL

Silvère van der Maarel

Department of Human Genetics, Leiden University Medical Center, The Netherlands

Limb-Girdle Muscular Dystrophy 2A (LGMD2A) is caused by mutations in Calpain 3 (CAPN3). Due the rapid autolysis of CAPN3 little is known of its substrates, and consequently the pathomechanism of LGMD2A has largely remained illusive. By combining bio-informatics with biochemistry and molecular biology we identified a primary amino acid sequence motif underlying CAPN3 substrate cleavage. This motif is common to all known CAPN3 substrates, can transform a non-substrate into a substrate, and accurately identifies >300 new substrates. Among the new CAPN3 target proteins we identified the Protein Inhibitors of Activated Stats (PIAS) family of E3 SUMO ligases as substrates for CAPN3. CAPN3 can negatively regulate PIAS3 activity in vivo and subsequent analysis of LGMD2A patient tissue showed SUMO2 to be deregulated. Further bioinformatic analysis of our predicted substrates revealed CAPN3's involvement in apoptosis and calcium signaling and suggests that CAPN3 functions as a self-limiting orchestrator of rapid, local changes in cyto-architecture.

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CALPAIN 3 GENE TRANSFER •
DR. ISABELLE RICHARD

Isabelle Richard

Genethon, Evry, France

Genetic defects in Calpain 3 leads to Limb-Girdle Muscular Dystrophy type 2A, a disease of the skeletal muscle that affects predominantly the proximal limb muscles. There is no treatment for this disease to date. In a attempt to define a therapeutic strategy, we evaluated the potential of recombinant adeno-associated virus (rAAV) vectors for gene therapy in a murine model for LGMD2A.. Efficient and stable transgene expression was obtained in the skeletal muscle after intramuscular and loco-regional administration. Moreover, its presence resulted in improvement of the histological features and in therapeutic efficacy at the physiological levels, including correction of atrophy and full rescue of the contractile force deficits. Experiments are on-going for evaluation of AAV-mediated transfer of calpain 3 transfer by systemic injections in mice.

NOTES

PLANNING FOR PRECLINICAL GENE THERAPY STUDIES IN CALPAIN 3 KNOCK OUT MICE •
DR. KEVIN FLANIGAN

Kevin M. Flanigan, MD[1,2,3]; Nicolas Wein, PhD[1]; Louise Rodino-Klapac, PhD[1,2]

[1]Center for Gene Therapy, Nationwide Children's Hospital
Departments of Pediatrics[2] and Neurology[3], The Ohio State University, Columbus, Ohio

LGMD2A is due a wide spectrum of mutations in CAPN3, leading to a loss of the calpain 3 (Capn3) protein or its proteolytic function. No curative treatment is available, although two therapeutic approaches have shown promise. Delivery of a rAAV1.CAPN3 vector to a Capn3 deficient mouse results in exogenous Capn3 protein expression after intramuscular injection, albeit with only little improvement in muscle strength. In the same mouse model, inhibition of myostatin by AAV-mediated expression of a mutated propeptide results in improved muscle strength, strongly suggesting that blockade of myostatin signaling pathways may be a therapeutic approach in LGMD2A patients.

We propose to evaluate the benefit of full-length CAPN3 gene transfer into the C3KO model (a gift from M. Spencer) using a recombinant adeno-associated virus vector that is currently entering clinical trials at Nationwide Children's Hospital (NCH). This vector (rh74) shares near identity to AAV8 (and is hereafter termed AAV8), and has undergone extensive preclinical testing that forms the basis of a current IND application for gene transfer of SGCA (alpha-sarcoglyan).

Our goal is to move an LGMD2A gene therapy toward the clinic by making use of the extensive pre-clinical experience available at the NCH Center for Gene Therapy. We will begin by comparing efficacy of CAPN3 expression from several promoters, including the tMCK promoter. Preclinical studies using other AAV8.tMCK encapsidated transgenes performed in the Center provide comparative data. Studies will make use of intramuscular injection, as well as isolated limb perfusion methods through the femoral artery developed in the Center for Gene Therapy (slated for clinical trial in LGMD2D). Presuming that we achieve success in achieving calpain 3 expression in skeletal muscle, we plan on assessing the effect of AAV8.CAPN3 delivery along with an AAV8.follistatin vector, as well as the effect of AAV8.follistatin alone in the C3KO mouse. Potential therapeutic benefit in all studies will include testing of force generation, and assessment of protection from eccentric contraction injury.

The NCH Center for Gene Therapy is equipped to carry successful preclinical results forward, with the demonstrated capability of production of clinical trial quality (cGMP) vector in our in-house Clinical Manufacturing Facility; for performance of IND-enabling preclinical safety and efficacy evaluations in our rodent and non-human primate facilities; and satisfactory preparation of regulatory documentation required for IND submission and approval.

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SESSION II AGENDA: CALPAIN 3 AND MEMBRANE REPAIR • DR. ELIZABETH McNALLY, MODERATOR

12:05 pm – 12:20 pm	Dr. Kevin Campbell: Dysferlin and Muscle Membrane Repair
12:25 pm – 12:40 pm	Dr. Ronald Mellgren: Calpain 3 Is Not Required for Plasma Membrane Repair
12:45 pm – 1:45 pm	*Lunch – Lunch will be served in the Alaria Room*

DYSFERLIN AND MUSCLE MEMBRANE REPAIR • DR. KEVIN CAMPBELL

Kevin P. Campbell

Howard Hughes Medical Institute, Departments of Molecular Physiology and Biophysics, Neurology, and Internal Medicine, University of Iowa Carver College of Medicine, Iowa City, Iowa, USA

Muscular dystrophy includes a diverse group of inherited muscle diseases characterized by skeletal muscle wasting and weakness. Mutations in dysferlin are linked to two clinically distinct muscle diseases, limb-girdle muscular dystrophy type 2B and Miyoshi myopathy. We previously showed that dysferlin-null mice maintain a functional dystrophin-glycoprotein complex nevertheless develop a progressive muscular dystrophy. In normal muscle, membrane patches enriched in dysferlin can be detected in response to sarcolemma injuries. In contrast, there are sub-sarcolemmal accumulations of vesicles in dysferlin-null mice. Membrane repair assays using a 2-photon laser-scanning microscope demonstrated that wild type muscle fibers efficiently reseal their sarcolemma in the presence of Ca2+ but dysferlin-deficient muscle fibers are defective in Ca2+ dependent sarcolemma resealing. We hypothesized that the combined deficiency of dystrophin and dysferlin would cause a more severe muscle pathology due to a structural defect in the sarcolemma caused by the dystrophin deficiency and a membrane repair defect caused by the dysferlin deficiency. To test this we generated dysferlin/dystrophin double-null mice. Double-null mice developed more severe muscle pathology than either one of the single-null mice, which is reflected by the higher number of regenerated muscle fibers, serum creatine kinase levels, and significantly more Evans blue dye uptake in their muscle. Thus, confirming that dysferlin and dystrophin work on two different physiological pathways required for normal muscle function. Further analysis revealed that double-null mice develop cardiomyopathy as early as 7-weeks of age suggesting a possible role for dysferlin in the membrane repair process of cardiac muscle similar to skeletal muscle. This study reveals a novel mechanism of muscular dystrophy in which the membrane repair machinery of muscle is defective as opposed to several other types of muscular dystrophy where the primary defect lies in the sarcolemma stability.

NOTES

CALPAIN 3 IS NOT REQUIRED FOR PLASMA MEMBRANE REPAIR • DR. RONALD MELLGREN

Ronald L. Mellgren

University of Toledo College of Medicine, Toledo, Ohio, USA

Mechanical damage to the plasma membrane appears to be an ongoing phenomenon in many tissues, including striated muscle. Repair is mediated by an organized sequence of events that requires the participation of several proteins, as well as internal membrane vesicles and high concentrations of calcium ion. Recently, several studies have presented evidence that the ubiquitous, conventional calpains, m- and μ-calpain, are needed for calcium-dependent plasma membrane repair, and probably function by remodeling cortical cytoskeleton. These observations prompted an investigation of the possible role of calpain-3 in muscle plasma membrane (sarcolemma) repair. Initial studies employing myotubes or muscle fibers derived from Capn3-null mice indicated that membrane repair after a single mechanical insult was not compromised. In other ongoing studies, the intermediate filament protein vimentin was found not to be required for repair of initial damage to cultured fibroblasts. Instead it was found to be important for facilitated repair that follows repetitive damage, probably via previously described p38 MAP kinase or JNK signal transduction pathway(s). These observations led to investigation of the possible role of calpain-3 in facilitated muscle membrane repair. Myotubes derived from the C2C12 mouse cell line were pre-damaged with small glass beads and subjected to laser injury 20 hours later. Membrane resealing rates were increased compared with non-injured control myotubes. Bead pre-injury of myotubes derived from Capn3-null myoblasts resulted in facilitated repair as well. Overall, these studies indicate that calpain-3 does not have a major role in repairing initial membrane damage, or in facilitated membrane repair.

NOTES

SESSION III AGENDA: CALPAIN 3 AND OTHER PATHWAYS THAT MIGHT BE IMPACTED BY CALPAIN •
DR. HIROYUKI SORIMACHI, MODERATOR

1:50 pm – 2:05 pm	Dr. Kanneboyina Nagaraju: Comprehensive Phenotyping of Calpain 3 Knock Out Mice
2:10 pm – 2:25 pm	Dr. Irina Kramerova: Impaired Ca2+ Mediated Signaling and Muscle Adaptation Response in the Absence of Calpain 3
2:30 pm – 2:45 pm	Dr. Mathias Gautel: Links from the Sarcomere to Mechanical Strain Sensing and Muscle Protein Turnover Control
2:50 pm – 3:05 pm	Dr. Denis Guttridge: NF-κB Signaling in Muscular Dystrophies
3:10 pm – 3:25 pm	Dr. Siegfried Labeit: Towards Selective Perturbation of Titin Based Signaling by Small Molecules to Identify Novel Tools for Muscle Research and for Testing Therapeutic Approaches
3:30 pm – 3:45 pm	*Break* *Beverages will be available just outside the entrance to the Espada Conference Room*

COMPREHENSIVE PHENOTYPING OF CALPAIN 3 KNOCK OUT MICE • DR. KANNEBOYINA NAGARJU

Jahnke VE, VanderMeulen JH, Phadke A, Nagaraju K

Research Center for Genetic Medicine, Children's National Medical Center, Washington DC, USA

Systematic phenotyping of mouse models of human diseases is critical not only to understand the disease pathogenesis but also to screen various therapeutic modalities before planning human clinical trials. The preclinical phenotyping and drug testing facility at CNMC is a state-of-the art facility equipped to assess functional, behavioural, imaging, biochemical and histological features of neuromuscular disease mouse models. Calpain-3 knockout (CKO) mice that were generated by Dr. Spencer were used to understand the role of calpain-3 in this muscle disease.

We have performed systematic phenotyping of these mice at different age groups (3, 5, 9 and 15 month age) using behavioural (Grip strength, Rotarod, Open field Digiscan scan), functional (in vitro force measurement on EDL), histological (H&E), and biochemical (serum CK) measures. We found that CKO mice have a lower body weight, loss of the forelimb grip strength, and reduced motor coordination at 3 months age. We did not detect any changes in Hindlimb grip strength. In contrast their behavioural activity level (vertical activity, horizontal activity and movement number) as well as specific force of EDL was higher than WT. Analysis of the same parameters at 15 months indicated lower body weight, lower specific force, and high serum CK levels. To unmask the mild disease phenotype we have subjected the mice for downhill treadmill exercise and found that forelimb grip strength, motor coordination, maximal force of EDL are significantly reduced. We also found increased serum CK levels and inflammation in skeletal muscle suggesting that exercise exacerbates the disease phenotype. Exercise model may be useful to test therapeutic interventions in CKO mice.

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LINKS FROM THE SARCOMERE TO MECHANICAL STRAIN SENSING AND MUSCLE PROTEIN TURNOVER CONTROL • DR. MATHIAS GAUTEL

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In striated muscle sarcomeres, mechanics and signalling appear tightly interdependent, but the exact contributions of the various sarcomeric cytoskeleton proteins to strain handling or signalling are only just emerging. Recent work has led to insight into the interactions, structure, and mechanical stability of sarcomeric protein complexes that fulfil both structural and signalling roles in the adaptation of muscle to mechanical strain. In particular, the Z-disk and M-band are emerging as local hubs for the integration of mechanical signals with pathways controlling muscle protein turnover and muscle gene expression. The M-band emerges as a yet enigmatic integrator of mechanical, protein kinase and GTPase signals via the giant proteins titin and obscurin that seem to control the activity of the ubiquitin-proteasomal (UPS), autophagy-lysosomal and calpain protease degradation pathways. Our current work is beginning to shed light on the contribution of the adaptor and scaffold proteins SQSTM1 and nbr1 in mechanically modulated remodelling of the sarcomere, where these three pathways cooperate in the turnover of M-band titin and associated proteins. Disruption of M-band mechanics is likely to play a major role in several myopathies by disrupting both the mechanical stability of the sarcomere, as well as the mechanosignalling pathways involved in sarcomere remodelling. Activation of complementary pathways might be a possible therapeutic approach to compensate the failure of a specific turnover mechanism, but these mechanisms will need to be much better understood for their ultimate implementation.

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NF-κB SIGNALING IN MUSCULAR DYSTROPHIES • DR. DENIS GUTTRIDGE

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The classical NF-κB signaling pathway is commonly associated with skeletal muscle disorders affecting myofiber atrophy and degeneration. Of the muscular dystrophies, most of what we know about NF-κB derives from studies in the *mdx* mouse model of Duchenne muscular dystrophy. In that model, NF-κB activity is chronically elevated in both innate immune cells and in regenerative myofibers. The activity in macrophages functions to regulate expression of inflammatory cytokines and chemokines to promote muscle damage, while persistent NF-κB in myofibers inhibits muscle regeneration by suppressing satellite cell activation. Inhibition of NF-κB through pharmacological means provides both a histological and functional improvement of limb and diaphragm muscles, and recent evidence demonstrates that NF-κB blockade also rescues cardiomyopathy in double mutant dystrophin/ utrophin mice. Thus, classical NF-κB signaling pathway has been considered a potential therapeutic target for DMD treatment, and similar strategies are currently being discussed for treatment of laminin-α2/merosin congenital muscular dystrophy. In contrast, the role of NF-κB signaling in limb-girdle muscular dystrophy Type 2A (LGMD2A) appears unique in comparisons to the other muscle disorders. Here, calpain 3 mutations lead to the inability to cleave and proteolyze the calpain substrate, IκBα, which serves as the inhibitor protein of NF-κB. As a result of calpain 3 deficiency, NF-κB is not activated and is unable to contribute its pro-survival function to dying fibers. More recent substrates of calpain 3, which also impinge on NF-κB activity have been described. Thus for LGMD2A, therapeutic strategies may entail boosting NF-κB transcriptional activity to protect myofiber integrity. These and other topics relating to NF-κB signaling in muscular dystrophy will be discussed.

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TOWARDS SELECTIVE PERTURBATION OF TITN BASED SIGNALING BY SMALL MOLECULES TO IDENTIFY NOVEL TOOLS FOR MUSCLE RESEARCH AND FOR TESTING THERAPEUTIC APPROACHES • DR. SIEGFRIED LABEIT

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The giant elastic protein titin provides a complex array of binding sites for signaling proteins, including proteases such as C3, ubiquitin E3 ligases that regulate access of sarcomeric proteins to the UPS system, co-transcriptional regulators, and cytokine-like stress-induced factors.

The complex set of titin binding proteins with regulatory roles is arranged mainly four clusters along the titin filament. Their arrangement in cluster raises the possibility of their cooperativity within their shared complexes. Understanding how signals are generated and transmitted within these signalosomes by individual components and how such signals are possibly regulated by strain is a key challenge in titin physiology.

To address this issue, we have expressed titin fragments from its Z-disk, I-band and M-line regions where binding sites for signaling molecules cluster. These fragments have been used in high through-put ALPHA screens in an attempt to identify small molecules that disrupt the interaction of titin fragments with its specific binding partners, because this strategy could generally useful to dissect the function of individual components in titin-based signalosomes.

Here, we present current data on small molecules that disrupt either the interaction of titin with the ubiquitin E3 ligase MuRF1, or with the cytokine-like molecule CARP: Because CARP targets together with C3 the titin N2A segment within the I-band region, both CARP and C3 may cooperate in titin based stress signaling. We discuss preliminary data on the in vitro and in vivo effects of lead compounds under study with a focus on their potential as tools to study titin based signaling and stress adaptation.

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GROUP DISCUSSION REGARDING PRIORITIZATION OF THERAPEUTIC AND DIAGNOSTIC STRATEGIES AND NATURAL HISTORY STUDIES •

DR. ROBERT GRIGGS, MODERATOR

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