UNITED MITOCHONDRIAL DISEASE FOUNDATION®

HOPE. ENERGY. LIFE.

The United Mitochondrial Disease Foundation is proud to present...

MITOCHONDRIAL MEDICINE 2018

Mitochondrial Chemical Biology

Sheraton Music City
Nashville, TN

Scientific Meetings: June 27 - 30, 2018

2018 Course Chair: Vamsi K. Mootha, MD

2018 CME Chair: Bruce H. Cohen, MD

A special thanks to those organizations serving on the Planning Commitee:
Akron Children's Hospital, the Mitochondrial Medicine Society,
and the Mitochondria Research Society

Mitochondrial Medicine 2018: Nashville

Scientific Program
June 27 - 30, 2018

Mitochondrial Medicine 2018: Nashville

2018 Course Description

The United Mitochondrial Disease Foundation and Children's Hospital Medical Center of Akron (CHMCA) have joined efforts to sponsor and organize a CME-accredited national symposium. Mitochondrial diseases are more common than previously recognized and mitochondrial pathophysiology is now a recognized part of many disease processes, including heart disease, cancer, AIDS and diabetes. There have been significant advances in the molecular genetics, proteomics, epidemiology and clinical aspects of mitochondrial pathophysiology.

This conference is directed toward the scientist and clinician interested in all aspects of mitochondrial science. The content of this educational program was determined by rigorous assessment of educational needs and includes surveys, program feedback, expert faculty assessment, literature review, medical practice, chart review and new medical knowledge. The format will include didactic lectures from invited experts intermixed with peer-reviewed platform presentations. There will be ample time for professional discussion both in and out of the meeting room, and peer-reviewed poster presentations will be given throughout the meeting. This will be a four-day scientific meeting aimed at those with scientific and clinical interests.

Learning Objectives

At the end of the scientific program, attendees will:

- Learn about newly characterized mitochondrial enzymes.
- Become familiar with new insights into mitochondrial redox biochemistry and how this may be applied to developing new therapies for mitochondrial diseases.
- Learn how mitochondrial dysfunction affects the metabolic profile.
- Discover how imagining mass spectrometry can be used to understand mitochondrial function.
- Summarize how drugs can be developed that target them to the mitochondria.
- Describe the efforts of drug repurposing and how old medications may result in mitochondrial therapeutics.
- List the new developments in clinical trials in mitochondrial disease and distinguish the differences in their mechanisms of action.
- Demonstrate how the mitochondria can serve as an undesired target of pharmaceuticals and environmental exposures.

Mitochondrial Medicine 2018: Nashville

Credits

The Children's Hospital Medical Center of Akron designates this live activity for a maximum of 19.75 *AMA PRA Category I Credits™*. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

Accreditation Statement

This activity has been planned and implemented in accordance with the accreditation requirements and policies of the Ohio State Medical Association (OSMA) through the joint providership of Children's Hospital Medical Center of Akron and The United Mitochondrial Disease Foundation. The Children's Hospital Medical Center of Akron is accredited by the OSMA to provide continuing medical education for physicians.

Special Announcements

Name Badges

All attendees must wear a name badge to all course functions.

Scientific Sessions

All scientific sessions will be held in Hermitage ABC.

Refreshment Breaks

Exhibits will be open in the Hermitage Lobby during all breaks and lunches. Posters will be in Two Rivers/ Kingsley Rooms. All posters are assigned numbers in the back of this syllabus. Presenters will station themselves at their poster to field questions according to those numbers as follows: even numbers on Wednesday from 5:30 pm to 7:30 pm and odd numbers on Thursday from 5:45 pm to 7:45 pm.

Meals

Continental Breakfasts and Lunches will be held in Hermitage DE.

Friday Night Banquet and Awards Ceremony

Scientific and family program attendees are invited to attend the Friday Night Banquet and Award Ceremony in the Heritage Ballroom.

CME Verification Form

Physicians: All physicians were given a 3-part CME Verification Form at Registration. Fully complete, sign, and turn in the carbon copy of the form to the registration desk at the end of the meeting (or upon your departure if you will not be staying for the duration of the meeting). Keep the WHITE copy for your records.

Non-Physicians: All non-physicians were given a 3-part Verification Form at registration. Fully complete, sign, and turn in the carbon copies of the form at the end of the meeting (or upon your departure if you will not be staying for the duration of the meeting). Keep the WHITE copy for your records.

Mitochondrial Medicine 2018: Nashville

2018 Scientific Planning Committee

- **Vamsi K. Mootha, MD,** 2018 Course Chair
  Harvard Medical School, Boston, MA
- **Bruce H. Cohen, MD**, 2018 CME Chair
  Akron Children's Hospital, Akron, OH
- **William Copeland, PhD**
  NIEHS, Research Triangle Park, NC
- **Marni Falk, MD**
  Children's Hospital of Philadelphia, Philadelphia, PA
- **Amy Goldstein, MD**
  Children's Hospital of Pittsburgh, Pittsburgh, PA
- **Larry Grossman, PhD**
  Wayne State University School of Medicine, Detroit, MI
- **Adam Hartman, MD**
  NINDS/NIH, Rockville, MD
- **Amel Karaa, MD**
  Massachusetts General Hospital, Boston, MA
- **Carla Koehler, PhD**
  University of California Los Angeles, Los Angeles, CA
- **Giovanni Manfredi, MD, PhD**
  Weill Cornell Medicine, New York, NY
- **Robert K. Naviaux, MD, PhD**
  University of California San Diego, LaJolla, CA
- **Sumit Parikh, MD**
  The Cleveland Clinic, Cleveland, OH
- **Russell Saneto, DO, PhD**
  Seattle Children's Research Institute, Seattle, WA
- **Peter Stacpoole, PhD, MD**
  University of Florida, Gainesville, FL
- **Keshav Singh, PhD**
  University of Alabama at Birmingham, AL
- **Philip E. Yeske, PhD**
  UMDF Science & Alliance Officer, Pittsburgh, PA
- **Kara Strittmatter, CMM, MA**
  UMDF Meeting Event Director, Columbus, OH

2018 Abstract Presenters

- **Rachael Baker, PhD**
  Calvin College, Grand Rapids, MI
- **Emanuele Barca, MD, PhD**
  Columbia University Medical Center, New York, NY
- **William Copeland, PhD**
  2017 Course Co-Chair, NIEHS, Research Triangle Park, NC
- **Marilena D'Aurelio, PhD**
  Weill Cornell Medicine, New York, NY
- **Larry Grossman, PhD**
  Wayne State University School of Medicine, Detroit, MI
- **Sujay Guha, PhD**
  Children's Hospital of Philadelphia, Philadelphia, PA
- **Nahid Khan, PhD**
  University of Helsinki, Helsinki, Finland
- **Shannon Kruk RN, BS**
  NIH Bethesda, MD
- **Giovanni Manfredi, MD, PhD**
  Weill Cornell Medicine, New York, NY
- **Edward McKee, PhD**
  College of Medicine, Central Michigan University, Mt Pleasant, MI
- **Meagan McManus**
  Children's Hospital of Philadelphia, Philadelphia, PA
- **Bryce Mendelsohn MD, PhD**
  UCSF, San Francisco, CA
- **Carlos Moraes, PhD**
  University of Miami, Miami, FL
- **Raphael Morscher, MD, PhD**
  University Children's Hospital Zürich, Zürich, Switzerland
- **Tomas Mracek, PhD**
  Institute of Physiology CAS, Prague, Czech Republic
- **Derek Narendra, MD, PhD**
  NINDS/NIH, Bethesda, MD
- **Martin Picard, PhD**
  Columbia University Medical Center, New York, NY
- **Rocio Rius, MD, MPH, MCRI**
  Melbourne, Australia
- **Hongying Shen, PhD**
  Massachusetts General Hospital, Boston, MA
- **Keshav Singh, PhD**
  University of Alabama at Birmingham, AL
- **Marketa Tesarova, PhD**
  Department of Pediatrics and Adolescent Medicine, Charles University, First Faculty of Medicine, and General University Hospital in Prague, Prague, Czech Republic
- **Atif Towheed, PhD**
  Children's Hospital of Philadelphia, Philadelphia, PA
- **Jesse Wilson, PhD**
  Colorado State University, Fort Collins, CO
- **Linlin Zhao**
  Central Michigan University, Mount Pleasant, MI
- **Zarazuela Zolkipli-Cunningham**
  Children's Hospital of Philadelphia, PA
- **Olga Zurita Rendon, PhD**
  University of Utah, Salt Lake City, UT

Mitochondrial Medicine 2018: Nashville

2018 Scientific Meeting Faculty

- **Vamsi K. Mootha, MD, 2018 Course Chair**
  Harvard Medical School, Boston, MA
- **Richard Caprioli, PhD**
  Vanderbilt University, Nashville, TN
- **Christopher J. Chang, PhD**
  UC Berkeley, CA
- **Sarah Elsea, PhD**
  Baylor College of Medicine, Houston, TX
- **Marni Falk, MD**
  Children's Hospital of Philadelphia, PA
- **Grainne Gorman, PhD**
  Wellcome Trust Centre for Mitochondrial Research, UK
- **Michio Hirano, MD**
  Columbia University, New York, NY
- **Dean P. Jones, PhD**
  Emory University, Atlanta, GA
- **Shana Kelley, PhD**
  University of Toronto, Canada
- **Kiyoshi Kita, PhD**
  Nagasaki University, Japan
- **Carla Koehler, PhD**
  University of California, Los Angeles, CA
- **Daria Mochly-Rosen, PhD**
  Stanford University, Stanford, CA
- **Anne N. Murphy, PhD**
  University of California San Diego, La Jolla, CA
- **Mike Murphy, PhD**
  MRC Mitochondrial Biology Unit, University of Cambridge, Cambridge, UK
- **Jane Newman, MCSP, PhD**
  Wellcome Centre for Mitochondrial Research, UK
- **David J. Pagliarini, PhD**
  Morgridge Institute for Research at UW-Madison, WI
- **Sumit Parikh, MD**
  The Cleveland Clinic, Cleveland, OH
- **Ethan Perlstein, PhD**
  CEO, Perlara, San Francisco, CA
- **Pere Puigserver, PhD**
  Dana-Farber Cancer Institute, Boston, MA
- **Jared Rutter, PhD**
  University of Utah, Salt Lake City, UT
- **Rohit Sharma, MD, PhD**
  Massachusetts General Hospital, Boston, MA
- **Gerald I. Shulman, MD, PhD**
  Yale University School of Medicine, New Haven, CT
- **Jan Smeitink, MD, PhD**
  Radboud Center for Mitochondrial Medicine, Radboudumc, Nijmegen, The Netherlands
- **Anu Suomalainen, MD, PhD**
  University of Helsinki, Finland
- **Tanja Taivassalo, PhD**
  University of Florida College of Medicine, Gainesville, FL
- **Mark Tarnopolsky, MD, PhD**
  FRCP(C) McMaster University Medical Center, Ontario, Canada
- **Alice Ting, PhD**
  Stanford University, Stanford, CA
- **Kendall B. Wallace, PhD**
  University of Minnesota, Duluth, MN
- **Yvonne Will, PhD**
  Pfizer, Groton, CT
- **Philip Yeske, PhD**
  UMDF Science & Alliance Officer, Pittsburgh, PA

Mitochondrial Medicine 2018: Nashville

Disclosure of Relevant Financial Relationships

The potential for a conflict of interest may be considered to exist if a faculty member has any relevant interest or other relationship with the manufacturer of any commercial product discussed in her/his presentation.

As required by the Accreditation Council for Continuing Medical Education (ACCME), each speaker is required to complete a Speaker Disclosure Form which outlines any relationship the speaker has with any commercial company whose products he/she will be discussing. The prospective audience must be made aware of the affiliation/financial interest by an acknowledgment in the program or syllabus or in the faculty listing.

The intent of this policy is not to prevent a speaker from making a presentation. It is intended only that any potential conflict of interest should be identified openly so that the listeners may form their own judgments about the presentation with the full disclosure of the facts. The following presenters have a financial arrangement or affiliation with an organization or company:

Bruce Cohen, MD
Grant/Research Support: BioElectron, Reata, Stealth Biotherapeutics, Horizon; Consultant: Stealth Biotherapeutics

Marni Falk, MD
Grant/Research Support: Stealth BioTherapeutics, Neurovive, Mitobridge, Raptor Pharmaceuticals; Consultant: Fortress Biotech, Neurovive, Stealth, Mitobridge; Speaker's Bureau: UMDF; Stock Shareholder: Perlstein Labs

Amy Goldstein, MD
Grant/Research Support: NAMDC; Consultant: Biomarin, Stealth BioTherapeutics; Speaker's Bureau: UMDF

Adam L. Hartman, MD
Consultant: Best Doctors, Inc.

Amel Karaa, MD
Grant/Research Support: NIH, Genzyme Sanofi, Stealth BioTherapeutics, Shire, Protalix; Consultant: Genzyme Sanofi, Steatlh BioTherapeutics, MitoBridge, Akros, Vasuda, Homology

Daria Mochly-Rosen, PhD
Consultant: Mitoconix Biosciences, Foresee; Stock Shareholder: Mitoconix Biosciences

Vamsi Mootha, MD
Consultant: Raze Therapeutics, Janssen Pharmaceuticals

Bryce Mendelsohn MD
Grant/Research Support: Pediatric Scientist Development Program (NIH); Consultant: Genome Medical; Speaker's Bureau: Medscape; Stock Shareholder: Hygea Precision Medicine, Clear Genetics; Other Financial or Material Support: McGraw Hill Education

Carlos Moraes, PhD
Other financial or material support: Precision Biosciences

Anne N. Murphy, PhD
Consultant: Agilent Technologies, Inc.

Mike Murphy, PhD
Antipodean Pharmaceuticals; Takeda Pharmaceuticals, Novintum Biosciences; Stock Shareholder: Antipodean Pharmaceuticals

Derek Narendra, MD
Grant/Research Support: NINDS Intramural Program

Robert K. Naviaux, MD, PhD
Consultant: Stealth BioTherapeutics

Ethan Perlstein, PhD
Grant/Research Support: NIH subaward; Stock Shareholder: Perlara PBC

Olga Zurita Rendon, PhD
Grant/Research Support: American Heart Association Postdoctoral Fellowship

Jared Rutter, PhD
Grant/Research Support: Calico Life Sciences; Consultant: Centaurus Therapeutics, Merck; Stock Shareholder: Centaurus Therapeutics, BioEnergenix, Vetttore

Russell Saneto, DO, PhD
Grant/Research Support: Stealth BioTherapeutics

Gerald I. Shulman, MD, PhD
Grant/Research Support: Gilead Sciences, Celgene; Consultant: SAB for Astra-Zeneca, Merck, Janssen, Novo Nordisk

Jan Smeitink, MD, PhD
Stock Shareholder: Khondrion B.V.

Peter Stacpoole, PhD, MD
Other Financial or Material Support: Medosome Biotech, LLC

Mark Tarnopolsky, MD, PhD
Speaker's Bureau: Speaker Honarario – Sanofi/Genzyme; Stock Shareholder: Exerkine Corporation – Nutritional Supplements for aging

Hilary Vernon, MD, PhD
Consultant: Stealth Biotherapeutics, Auburndale, MA

The following CME Committee/Planning Committee members have the following commercial/financial relationships to disclose:

Rajeev Bhatia, MD
Consultant: Merck Medical Manuals (Chapter update)

Todd Ritzman, MD
Grant/Research Support: NIH STTR Grant; Consultant: OrthoPediatrics Medical Advisory Board ;Speaker's Bureau: OrthoPediatrics; Stock Shareholder: Apto Orthopedics

Mitochondrial Medicine 2018: Nashville

CME Information/ CEU Information

CME Evaluations

TO COMPLETE EVALUATIONS and receive CME credit GO TO:
www.akronchildrens.org/cme and click on "Complete a CME Evaluation" link
The CME Activity Code is: 5708
Please be aware that the evaluation period will remain open for 30 days post activity date
as implemented by the Continuing Medical Educations subcommittee.

Attention Genetic Counselors

The National Society of Genetic Counselors (NSGC) has authorized the United Mitochondrial Disease Foundation to offer up to 1.625 CEUs or 16.25 contact hours (Category 1) for the event UMDF Mitochondrial Medicine 2018. The American Board of Genetic Counseling (ABGC) will accept CEUs earned at this program for the purposes of certification and recertification.

GCs must submit this $25 Category 1 CEU Filing Fee to claim credit for participation in this event. Final CEU certificates will be issued by NSGC after the conclusion of the event to individuals that have submitted the filing fee AND completed all of the *required paperwork on site.

**Required paperwork includes signing in daily, completing speaker evaluations and completing the Self Reporting Form provided at registration. YOU MUST also complete the online evaluation form using the information below. Please turn in the Self Reporting Form and Evaluation Form at registration by 1pm on Saturday.*

www.akronchildrens.org/cme and click on "Complete a CME Evaluation" link

The CME Activity Code is: 5708
Please be aware that the evaluation period will remain open for 30 days post activity date
as implemented by the Continuing Medical Educations subcommittee.

Save the Date!

Mitochondrial Medicine 2019: Washington DC
Scientific Program: June 26 - 29, 2019

Hilton Alexandria Mark Center
Alexandria, VA

Mitochondrial Medicine 2018: Nashville

Exhibitors

Thank you to the following exhibitors for attending:

Agilent Technologies

Akron Children's Hospital

Baylor Genetics

ChemRx

Epic4Health/Tishcon Corp

GeneDx

Iliad Neurosciences

Mitochondrial Research Guild

MNG Laboratories

NAMDC

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Exhibitors

PerkinElmer Genomics

Permobil

Quten Research

Reata Pharmaceuticals

Santhera Pharmaceuticals

Solace Nutrition

Stealth BioTherapeutics

Summit Health Pharmacy

Undiagnosed Diseases Network

UT Mitochondrial Center of Excellence

Thank you also to any exhibitors who have been added since the printing of this syllabus.

SCHEDULE

Mitochondrial Medicine 2018: Nashville

Scientific Program Schedule

Day 1: Wednesday, June 27, 2018

Morning Platform Session (1) ***Hermitage ABC***
Advances in Mitochondrial Biochemistry and Metabolism

8:00 am **Welcome**
Vamsi K. Mootha, MD, Course Chair, Harvard Medical School, Boston, MA

8:15 am **Mitochondria, Metabolism and Cellular Coordination**
Jared Rutter, PhD, University of Utah, Salt Lake City, UT

8:45 am **Defining Mitochondrial Protein Function through Systems Biochemistry**
David J. Pagliarini, PhD, Morgridge Institute for Research at UW-Madison, WI

9:15 am **Abstract Presentations (2)**

9:45 am Break - *Hermitage Foyer*

10:15 am **Bioenergetic Rescue of Human Mitochondrial Mutations**
Pere Puigserver, PhD, Dana-Farber Cancer Institute, Boston, MA

10:45 am **Abstract Presentations (6)**

12:15 pm **North American Mitochondrial Disease Consortium (NAMDC) Update** *(Non-CME)*
Michio Hirano, MD, Columbia University, New York, NY

12:30 pm Lunch - *Hermitage DE*

Afternoon Platform Session (2) ***Hermitage ABC***
New Chemical Tools for Investigating Mitochondria

2:00 pm **Chemo-Genetic Tools for Probing Mitochondrial Proteomes and Transcriptome**
Alice Ting, PhD, Stanford University, Stanford, CA

2:30 pm **Activity-Based Sensing Approaches to Studying Metal and Redox Biology**
Christopher J. Chang, PhD, University of California, Berkeley, CA

NOTE: Abstract times follow this schedule and a five minute question and answer period is included in each of the speaker's allotted time.

3:00 pm **Abstract Presentations (2)**

3:30 pm Break - *Hermitage Foyer*

4:00 pm **Integration of High-Resolution Metabolomics, Redox Proteomics, Transcriptomics and Respiratory Activities to Understand Complexities of Mitochondria-Cell Signaling**
Dean P. Jones, PhD, Emory University, Atlanta, GA

4:30 pm **Abstract Presentations (4)**

5:30 pm Break & Poster Reception and Cash Bar *(Non-CME) - Two Rivers/Kingsley*
Even Numbers Stay at Posters

7:30 pm Adjourn

Day 2: Thursday, June 28, 2018

Morning Platform Session (3) ***Hermitage ABC***
Mitochondrial Metabolomics: from Basic Mechanisms to Biomarkers

8:00 am **Plasma Metabolic Profiles of Mitochondrial Disease**
Rohit Sharma, MD, PhD, Massachusetts General Hospital, Boston, MA

8:30 am **Broad Scale Untargeted Metabolomics Provides Functional Evidence for Diagnosis of Inborn Errors of Metabolism**
Sarah Elsea, PhD, Baylor College of Medicine, Houston, TX

9:00 am **Imaging Mass Spectrometry**
Richard Caprioli, PhD, Vanderbilt University, Nashville, TN

9:30 am **Abstract Presentations (2)**

10:00 am Break - *Hermitage Foyer*

10:30 am **Targeting Mitochondria**
Shana Kelley, PhD, University of Toronto, Canada

SCHEDULE

Clinical Platform Session Track ***(organized by MMS)*** ***Hermitage D***

Management of Fatigue and Exercise Intolerance in Mitochondrial Disease

11:00am	MMS Business Updates
11:15am - 11:45am	Mitochondrial Care Network *Sumit Parikh, MD; Amel Karaa, MD; and Amy Goldstein, MD*
11:50am - 12:30pm	Risks and Benefits of Exercise in Mitochondrial Disease Patients *Mark Tarnopolsky, MD, PhD* *FRCP(C) McMaster University Medical Center, Ontario, Canada*
12:30pm - 1pm	Lunch Served
1:00pm - 1:40pm	Exercise Testing for Mitochondrial Disease Patients *Tania Taivassalo, PhD, University of Florida College of Medicine, Gainesville, FL*
1:40pm - 2:20pm	Fatigue Characteristic and Fatigue Outcome Measures in Mitochondrial Disease *Grainne Gorman, MD and Jane Newman, MCSP, PhD* *Wellcome Trust Centre for Mitochondrial Research, UK*
2:20pm - 2:30pm	Closing Remarks

11:00 am **Abstract Presentations (6)**

12:30 pm Lunch - *Hermitage E*

Afternoon Platform Session (4) ***Hermitage ABC***

Drug Discovery: From Structure Based Approaches to High Throughput Screening

2:30 pm **Mitochondrial Drug Development: a Multi-Partner Endeavor**
Jan Smeitink, MD, PhD, Radboud Center for Mitochondrial Medicine, Radboudumc, Nijmegen, The Netherlands

3:00 pm **Small Molecule Inhibitors in Mitochondrial Protein Import**
Carla Koehler, PhD, University of California, Los Angeles, CA

3:30 pm Break - *Hermitage Foyer*

4:00 pm **Model Organisms for N=1 Drug Discovery**
Ethan Perlstein, PhD, CEO, Perlara, South San Francisco, CA

4:30 pm **Therapeutic Cross-training: High-throughput Screening Across Evolutionary-Distinct Genetic Models to Optimize Precision Mitochondrial Disease Therapies**
Marni Falk, MD, Children's Hospital of Philadelphia, PA

5:00 pm **Abstract Presentations (3)**

5:45 pm Break & Posters Reception Odd Numbers Stay at Posters *(Non-CME)*

Day 3: Friday, June 29, 2018

Morning Platform Session (5) *(All Talks are Non-CME)* ***Hermitage ABC***
Clinical Trials Updates and Challenges
Moving Drugs from Concept to Clinic

8:00am **Welcome and Clinical Trials Session**
(Combined with Scientific, Clinician, Patient, Family and LHON Attendees)
Brent Fields, Chuck Mohan, Philip Yeske, PhD

8:10am **History of Clinical Trials in Mitochondrial Disease**
Bruce H. Cohen, MD, Akron Children's Hospital, Akron, OH

8:30am **Planning an Externally-led Patient-Focused Drug Development Meeting**
- EL-PFDD Meeting Background (James Valentine)
- EL-PFDD Mitochondrial Disease Plan (Philip Yeske, PhD)
- EL-PFDD Meeting Timeline and Q&A

9:00am **Clinical Trial Updates Session 1: Small Molecule Approaches**
- Mitochondria Disease Program Update
  Reenie McCarthy, Chief Executive Officer, Stealth BioTherapeutics
- Safety, Efficacy, and Pharmacodynamics of Omaveloxolone in Mitochondrial Myopathy Patients (Reata MOTOR Trial): Part 1 Results
  Karen Lindhardt Madsen, PhD, University of Copenhagen
- NeuroVive - KL1333 - Mitochondrial Disease Treatment Opportunity by NAD+ Modulation and Mitochondrial Biogenesis
  Magnus Hansson, PhD, Chief Medical Officer, NeuroVive Pharmaceuticals

10:00am Break - *Hermitage Foyer*

10:30am **Clinical Trial Updates Session 2: Vision Disorders**
Moderators: Lissa Poincenot and Nancy Newman, MD
- Overview of Mitochondrial Vision Disorders
  Nancy Newman, MD
- GenSight: What we have Done, What we are Doing
  Barrett Katz, MD, MBA, Chief Medical Officer, GenSight Biologics
- Santhera's Real World Experience with Idebenone in LHON. Update on Programs
  Xavier Lloria, Medical Affairs Director, Santhera Pharmaceuticals
- LHON Development Update
  Jim Carr, PharmD, Chief Clinical Development Officer, Stealth BioTherapeutics
- Vision Disorder Panel Q&A

11:55am Scientific/Clinical Adjourn
(LHON Attendees stay in Hermitage ABC for their closing)

SCHEDULE

12:00pm **LHON Scientific Q&A**
Moderator: Lissa Poincenot - Speaker: Nancy Newman, MD
LHON Community Update, Group Photo, and Closing Remarks

12:30 pm Lunch - *Hermitage DEF*

Afternoon Platform Session (6) *Hermitage ABC*

Targeting Mitochondria in Common Diseases

2:00 pm **Small Molecular Activators of Aldehyde Dehydrogenases - a New Treatment for Mitochondrial Dysfunction and Mitopathies?**
Daria Mochly-Rosen, PhD, Stanford University, Stanford, CA

2:30 pm **Mitochondria and Diabetes**
Gerald I. Shulman, MD, PhD, Yale University School of Medicine, New Haven, CT

3:00 pm **Neuroprotective Effects of Inhibition of the Mitochondrial Pyruvate Carrier**
Anne N. Murphy, PhD, University of California San Diego, La Jolla, CA

3:30 pm Break - *Hermitage Foyer*

3:45 pm **Medicines for Malaria and African Sleeping Sickness**
Kiyoshi Kita, PhD, Nagasaki University, Japan

4:15 pm **UMDF Funded Projects**
Philip Yeske, PhD, UMDF Science & Alliance Officer, Pittsburgh, PA

4:45 pm Adjourn

6:00 pm Reception - *Hermitage Foyer*

7:00 pm Friday Night Banquet and Awards Ceremony - *Hermitage Ballroom*

Day 4: Saturday, June 30, 2018

Morning Platform Session (7) ***Hermitage ABC***
Platform*: *Mitochondrial Stress Responses

8:00 am **Mitochondrial Disease Sequence Data Resource (MSeqDR)**
Marni Falk, MD, Children's Hospital of Philadelphia, Philadelphia, PA
and Colleen Clarke Muraresku, CGC

9:00 am **Mitochondria Drug Toxicity and the Pharmaceutical Industry**
Yvonne Will, PhD, Pfizer R&D, Groton, CT

9:30 am **Compensatory Responses to Mitochondrial Toxicity**
Kendall B. Wallace, PhD, University of Minnesota Medical School, Duluth, MN

10:00 am Break - *Hermitage Foyer*

10:30 am **Manipulating Mitochondrial ROS and Oxidative Damage as Therapeutic Strategies**
Mike Murphy, PhD, MRC Mitochondrial Biology Unit, University of Cambridge, UK

11:00 am **Stress Responses in Mitochondrial Disease**
Anu Suomalainen, MD, PhD, University of Helsinki, Finland

11:30 am **Closing**
Vamsi K. Mootha, MD

ABSTRACTS

Mitochondrial Medicine 2018: Nashville

Abstract: Presentation Schedule

Day 1: Wednesday, June 27, 2018

Platform Session 1: Abstracts

#	Time	Presenter	Title
0444	9:15am	Hongying Shen	De-orphaning the enzyme activity of CLYBL reveals a new mechanism of mitochondrial vitamin B12 regulation
0417	9:30am	Tomas Mracek	Knockout of DAPIT protein disrupts ATP synthase oligomerisation and has a profound role in regulation of glucose homeostasis
0384	10:45am	Keshav Singh	Reversing Wrinkled Skin and Lost Hair in Mice by Restoring Mitochondrial Function
0374	11:00am	Raphael Morscher	Mitochondrial translation requires folate dependent tRNA methylation
0404	11:15am	Martin Picard	Mitochondrial 3D Network Organization and Nanotunnels in Mitochondrial Disease
0415	11:30am	Lawrence Grossman	MNRR1 (CHCHD2) and mitochondrial UPR: a novel nexus
0383	11:45am	Olga Zurita Rendon	The stress-responsive mitochondrial protein, Vms1, is a release factor for the Ribosome Quality control Complex
0492	12:00pm	Emanuele Barca	USMG5 Ashkenazi Jewish founder mutation impairs mitochondrial complex V dimerization and ATP synthesis

Platform Session 2: Abstracts

#	Time	Presenter	Title
0458	3:00pm	Linlin Zhao	Mitochondrial Transcription Factor A Induces DNA-Protein Cross-Links and DNA Strand Breaks at Abasic DNA Lesions

All Abstracts are listed in the back of this syllabus.

0488	3:15pm	Carlos Moraes	MitoTALEN reduces mutant mtDNA load and restores tRNA alanine levels in a mouse model of heteroplasmic mtDNA mutation
0432	4:30pm	Bill Copeland	Ultrasensitive detection of mtDNA deletions in POLG patients elucidates the mechanism of mtDNA replication
0493	4:45pm	Meagan McManus	Superoxide sensitive PET radiotracer as a mitochondrial biomarker for neurodegenerative disease
0482	5:00pm	Giovanni Manfredi	New mouse models of mutant CHCHD10 mitochondrial disease
0414	5:15pm	Derek Narendra	Parkinson-related CHCHD2 is strictly necessary for oligomerization of ALS/FTD-related CHCHD10

Day 2: Thursday, June 28, 2018

Platform Session 3: Abstracts

#	Time	Presenter	Title
0489	9:30am	Jesse Wilson	Picosecond spectroscopy of respiratory hemes: towards non-invasive optical biopsy of mitochondrial function
0418	9:45am	Marketa Tesarova	Impact of DNM1L mutation in GTPase domain on mitochondrial network
0380	11:00am	Marilena D'Aurelio	Glutamate anaplerosis as a mechanism of metabolic adaptation in mitochondrial diseases
0421	11:15am	Edward McKee	Mitochondrial DNA depletion diseases and compartmentalization of the salvage pathway for TTP synthesis in isolated mitochondria from rat tissues.
0416	11:30am	Rachael Baker	Higher Order Structural Analysis to Elucidate Genotype-Phenotype Relationships in BCS1L-Related Rare Diseases

All Abstracts are listed in the back of this syllabus.

ABSTRACTS

ABSTRACTS

0490	11:45am	Emanuele Barca	The challenge of genetic diagnoses in patients with mitochondrial disease: data from the North American Mitochondrial Disease Consortium (NAMDC)
0393	12:00pm	Rocio Rius	The epidemiology and natural history of pediatric mitochondrial diseases – a population-based study.
0433	12:15pm	Shannon Kruk	Measles, Mumps, Rubella And Varicella Titers In Patients With Mitochondrial Disease

Platform Session 4: Abstracts

#	Time	Presenter	Title
0442	5:00pm	Bryce Mendelsohn	A high throughput screen of real-time ATP levels in individual cells reveals mechanisms of energy failure
0375	5:15pm	Nahid Khan	Pharmacological Inhibition of Poly(ADP-Ribose) in mouse model of mitochondrial myopathy
0468	5:30pm	Sujay Guha	"Mitochondrial cocktail" combinatorial compound screening in Caenorhabditis elegans and zebrafish models of mitochondrial complex I disease

Day 3: Friday, June 29, 2018

Platform Session 6: Abstracts

#	Time	Presenter	Title
0509	4:15pm	Atif Towheed	Allotopically Expressed RNA Mediated Genetic Complementation of a Mitochondrial-encoded ND6 Frameshift Mutant
0496	4:30pm	Zarazuela Zolkipli-Cunningham	Development of a Mitochondrial Myopathy Rating Scale

***All Abstracts are listed in the back of this syllabus.**

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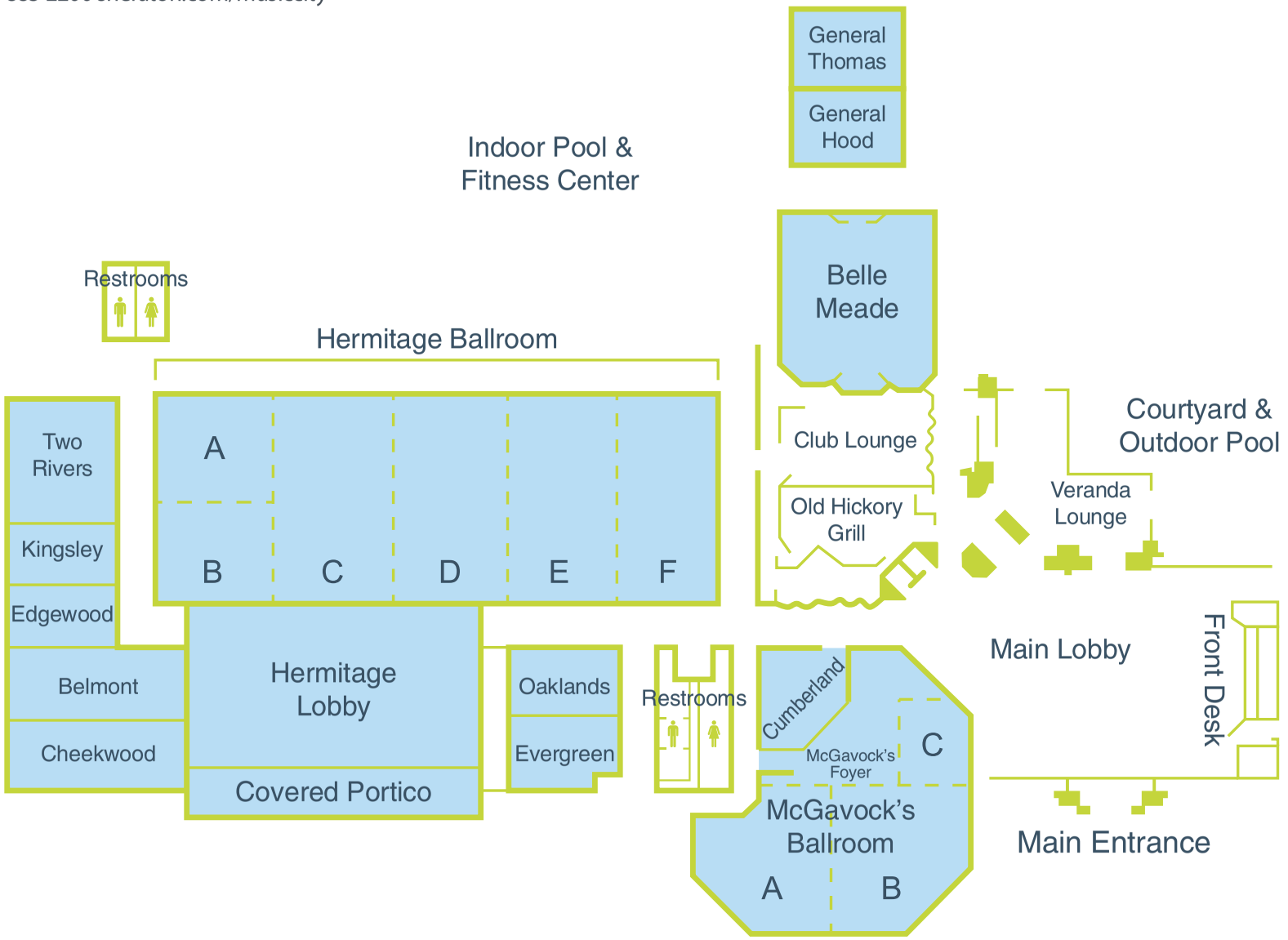

Mitochondrial Medicine 2018: Nashville

Sheraton Music City

Mitochondrial Medicine 2018: Nashville

Wednesday, June 27, 2018

Notes

Morning Session

Advances in Mitochondrial Biochemistry and Metabolism

Welcome

Vamsi K. Mootha, MD, Course Chair

Notes

Morning Session

Advances in Mitochondrial Biochemistry and Metabolism

Mitochondria, Metabolism and Cellular Coordination

Jared Rutter, PhD

Notes

Functionalizing the Unannotated Mitochondrial Proteome

Jared Rutter

Howard Hughes Medical Institute and Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, UT, USA

Email: rutter@biochem.utah.edu

Mitochondria are dynamic and complex organelles that play a central role in all aspects of biology, including energy production, intermediary metabolism, and apoptosis. These broad cellular functions also place mitochondria as a central player in human health. Mitochondrial dysfunction is associated with a wide range of diseases, including cancer, type 2 diabetes, and most neurodegenerative disorders. As a result of these wide-ranging critical activities, many efforts have focused on identifying and characterizing the mitochondrial proteome, with over 1,000 proteins identified to date in mammals. Remarkably, however, roughly one-quarter of these proteins remain essentially uncharacterized. These include many proteins that are highly conserved throughout eukarya, a strong indication that they perform a fundamentally important function. The overall goal in this research is to provide a new understanding of the biochemical and cellular function of each conserved uncharacterized mitochondrial protein, determine how they contribute to normal mitochondrial activity and human disease. Our studies of a handful of these uncharacterized mitochondrial conserved proteins have revealed new roles for these proteins in critical aspects of mitochondrial function, including mitochondrial protein quality control, lipid synthesis and mitochondrial ETC complex and supercomplex assembly.

Notes

Afternoon Session

Advances in Mitochondrial Biochemistry and Metabolism

Defining Mitochondrial Protein Function through Systems Biochemistry

David J. Pagliarini, PhD

Notes

Defining mitochondrial protein function through systems biochemistry

David J. Pagliarini[1,2]

[1]Morgridge Institute for Research, Madison, WI 53715, USA

[2]Department of Biochemistry, University of Wisconsin–Madison, Madison, WI 53706, USA

Despite their position as the iconic powerhouses of cellular biology, many aspects of mitochondria remain remarkably obscure—a fact that contributes to our poor ability to address mitochondrial dysfunction therapeutically. Such dysfunction contributes to a vast array of human diseases through distinct means. For instance, aberrant mitochondrial biogenesis can fail to properly set cellular mitochondrial content; dysregulated signaling processes can fail to calibrate mitochondrial activity to changing cellular needs; and malfunctioning proteins can render core bioenergetic processes ineffectual. A major bottleneck to understanding—and ultimately addressing—these processes is that the proteins driving them are often undefined. Concurrently, the functions of hundreds of mitochondrial proteins that may fulfill these roles are not known, or at best are poorly understood. Thus, the high-level goal of my research program is to help achieve a more complete, systems-level understanding of mitochondrial biology by systematically establishing the functions of orphan mitochondrial proteins and their roles within disease-related processes. We do so by first devising multi-dimensional analyses designed to make new connections between these proteins and established pathways and processes. We then employ mechanistic and structural approaches to define the functions of select proteins at biochemical depth. This ‘systems biochemistry’ strategy is helping us address three outstanding biological questions: Which orphan mitochondrial proteins fulfill the missing steps in classic mitochondrial processes, including the biosynthesis of coenzyme Q and other aspects of respiratory chain function? What proteins assist in the orchestrated assembly of lipids, metabolites, and proteins (from two genomes) to ensure proper mitochondrial biogenesis? And, which resident signaling proteins direct the post-translational regulation of mitochondrial activities? In answering these questions, we aim to help transform the mitochondrial proteome from a component list into a metabolic circuitry of connected functions, and to elucidate the biochemical underpinnings of mitochondrial dysfunction in human disease.

Notes

Morning Session

Advances in Mitochondrial Biochemistry and Metabolism

Abstract Presentations

Platform Session 1: Abstracts

#	Time	Presenter	Title
0444	9:15am	Hongying Shen	De-orphaning the enzyme activity of CLYBL reveals a new mechanism of mitochondrial vitamin B12 regulation
0417	9:30am	Tomas Mracek	Knockout of DAPIT protein disrupts ATP synthase oligomerisation and has a profound role in regulation of glucose homeostasis

Notes

Morning Session

Advances in Mitochondrial Biochemistry and Metabolism

Bioenergetic Rescue of Human Mitochondrial Mutations

Pere Puigserver, PhD

Notes

Presenter: Puigserver, Pere, PhD

Authors: Eduardo Balsa, Meghan S. Soustek, Joeva Barrow, Pere Puigserver

Institution: Dana-Farber Cancer Institute, Department of Cancer Biology and Harvard Medical School, Department of Cell Biology, Boston, MA 02115

Title: Bioenergetic Rescue of Human Mitochondrial Mutations

Body of Abstract: Mitochondrial diseases comprise a heterogenous group of genetically inherited disorders that cause failures in energetic and metabolic function. Bioenergetic failures are the initial cause of the different pathologies observed in mitochondrial disease patients. In these diseases, mutations in nuclear or mitochondrial encoded genes cause bioenergetic defects associated with failures to use the electron transfer chain and oxidize substrates. These defects are exacerbated under energetic or metabolic stress conditions and cause cell dysfunction and death. Cellular strategies that rescue these mitochondrial stress failures and rescue cell dysfunction and death have important implications in potential therapies for mitochondrial diseases. For example, boosting residual oxidative phosphorylation (OXPHOS) activity can partially correct these bioenergetic failures. The addition of galactose, instead of glucose, to experimentally force mitochondrial respiration, causes a metabolic and energetic vulnerability of cells carrying human mitochondrial disease mutations. We have designed and performed a series of high-throughput chemical and CRISPR screens using human complex I mutations under conditions of energetic stress. Two main sets of positive hits are identified using these experimental platforms, 1) chemical compounds and genes that rescue oxygen consumption rates and OXPHOS, among them are bromodomain inhibitors that are able to bypass complex I defects through increases of complex II-dependent respiration and 2) chemical compounds and genes that suppress mitochondrial-dependent cell death pathways, for example, sulfonylureas inhibit the ER stress inflammatory pathway (IRE1alpha and stress-activated MAP kinases) and maintain survival of human mitochondrial complex I mutant cells. In this presentation, we will show the results of these screens and discuss the potential therapeutic implication of the different cellular strategies that rescue mitochondrial mutations derived from mitochondrial disease patients.

Notes

Morning Session

Advances in Mitochondrial Biochemistry and Metabolism

Abstract Presentations

Platform Session 1: Abstracts

#	Time	Presenter	Title
0384	10:45am	Keshav Singh	Reversing Wrinkled Skin and Lost Hair in Mice by Restoring Mitochondrial Function
0374	11:00am	Raphael Morscher	Mitochondrial translation requires folate dependent tRNA methylation
0404	11:15am	Martin Picard	Mitochondrial 3D Network Organization and Nanotunnels in Mitochondrial Disease
0415	11:30am	Lawrence Grossman	MNRR1 (CHCHD2) and mitochondrial UPR: a novel nexus
0383	11:45am	Olga Zurita Rendon	The stress-responsive mitochondrial protein, Vms1, is a release factor for the Ribosome Quality control Complex
0492	12:00pm	Emanuele Barca	USMG5 Ashkenazi Jewish founder mutation impairs mitochondrial complex V dimerization

Notes

Morning Session

Advances in Mitochondrial Biochemistry and Metabolism

North American Mitochondrial Disease Consortium (NAMDC) Update

Michio Hirano, MD

Notes

Notes

Afternoon Session

New Chemical Tools for Investigating Mitochondria

Chemo-Genetic Tools for Probing Mitochondrial Proteomes and Transcriptome

Alice Ting, PhD

Notes

Notes

Afternoon Session

New Chemical Tools for Investigating Mitochondria

Activity-Based Sensing Approaches to Studying Metal and Redox Biology

Christopher J. Chang, PhD

Notes

Presenter: Christopher J. Chang, PhD

Authors: Christopher J. Chang[1,2,3]

Institution: [1]Department of Chemistry, [2]Department of Molecular and Cell Biology, and [3]Howard Hughes Medical Institute, University of California, Berkeley, CA 94720

Title: Activity-Based Sensing Approaches to Studying Metal and Redox Biology

Body of Abstract: Our laboratory advanced the concept of activity-based sensing (ABS), which uses dynamic chemical reactivity, rather than static lock-and-key binding, to build highly selective and sensitive molecular detection systems in the same spirit as binding-based sensors have revolutionized the study of calcium and other important biological signaling messengers. This presentation will describe the development and application of ABS chemical reagents as imaging and proteomics probes to identify and decipher new principles of metal and redox biology, with a focus on reactive oxygen and carbonyl species.

Notes

Afternoon Session

New Chemical Tools for Investigating Mitochondria

Abstract Presentations

Platform Session 2: Abstracts

#	Time	Presenter	Title
0458	3:00pm	Linlin Zhao	Mitochondrial Transcription Factor A Induces DNA-Protein Cross-Links and DNA Strand Breaks at Abasic DNA Lesions
0488	3:15pm	Carlos Moraes	MitoTALEN reduces mutant mtDNA load and restores tRNA alanine levels in a mouse model of heteroplasmic mtDNA mutation

Notes

Afternoon Session

New Chemical Tools for Investigating Mitochondria

Integration of High-Resolution Metabolomics, Redox Proteomics, Transcriptomics and Respiratory Activities to Understand Complexities of Mitochondria-Cell Signaling

Dean P. Jones, PhD

Notes

Notes

Afternoon Session

New Chemical Tools for Investigating Mitochondria

Abstract Presentations

Platform Session 3: Abstracts

#	Time	Presenter	Title
0432	4:30pm	Bill Copeland	Ultrasensitive detection of mtDNA deletions in POLG patients elucidates the mechanism of mtDNA replication
0493	4:45pm	Meagan McManus	Superoxide sensitive PET radiotracer as a mitochondrial biomarker for neurodegenerative disease
0482	5:00pm	Giovanni Manfredi	New mouse models of mutant CHCHD10 mitochondrial disease
0414	5:15pm	Derek Narendra	Parkinson-related CHCHD2 is strictly necessary for oligomerization of ALS/FTD-related CHCHD10

Notes

Mitochondrial Medicine 2018: Nashville

Thursday, June 28, 2018

Notes

Morning Session

Mitochondrial Metabolomics: from Basic Mechanisms to Biomarkers

Plasma Metabolic Profiles of Mitochondrial Disease

Rohit Sharma, MD, PhD

Notes

Presenter: Rohit Sharma, MD, PhD

Authors: Rohit Sharma[1], Bryn Reinstadler[1], Kris Engelstad[2], Erin Stackowitz[2], Michio Hirano[2], Darryl De Vivo[2], Devin Oglesbee[3], Vamsi K. Mootha[1]

Institution: [1]Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114. [2]Columbia University Medical Center, New York, NY 10032. [3]Mayo Clinic, Rochester, MN 55905.

Title: Identification of Biochemical Biomarkers for Mitochondrial Diseases

Abstract: The clinical management of patients with inherited mitochondrial disease is extremely challenging. These disorders display significant heterogeneity at the genetic, biochemical, and clinical levels, and we have no proven therapies to offer patients. While next-generation sequencing has transformed our diagnostic approach, we still lack robust biomarkers. Facile, mechanistically sound biomarkers are urgently needed as they could potentially help to classify mitochondrial disorders, aid as a companion in their diagnosis, enable tracking of disease progression, and provide a quantitative readout of treatment response. We have focused on applying liquid chromatography-mass spectrometry (LC-MS) based metabolomics to discover plasma and urine biomarkers in cohorts of patients with genetically defined mitochondrial disease. In my talk, I will present our latest work aimed at identifying biochemical markers emerging from metabolomics studies of individuals with m.3243A>G mutation, the most frequent cause of mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), and compare these results to previously published metabolic profiles of Mendelian forms of mitochondrial disease. The suite of metabolites emerging from these studies can mechanistically be traced back to impairments in the electron transport chain. Our long-term vision is to define a collection of metabolites that reliably report mitochondrial dysfunction and to understand their biochemical provenance.

Support: This work was funded by grants from the Marriott Mitochondrial Disorders Collaborative Network (MMDCRN), the Howard Hughes Medical Institute, and the Eunice Kennedy Shriver National Institute of Child Health & Human Development (NICHD).

Notes

Morning Session

Mitochondrial Metabolomics: from Basic Mechanisms to Biomarkers

Broad Scale Untargeted Metabolomics Provides Functional Evidence for Diagnosis of Inborn Errors of Metabolism

Sarah Elsea, PhD

Notes

Presenter: Sarah H. Elsea, PhD

Authors: Sarah H. Elsea, Ning Liu, Jing Xiao Michael Wangler, Lisa Emrick, William E Craigen, Joseph P. Alaimo, Yaping Yang, Fernando Scaglia, Qin Sun, and V. Reid Sutton

Dept. of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX USA

Title: A multi-omics precision medicine approach to diagnosis of inborn errors of metabolism

Body of Abstract: Metabolomics is the study of the distinctive chemical fingerprint produced by specific cellular processes. Untargeted mass spectrometry-based metabolomic profiling for small molecules in body fluids is an emerging technique used to produce and analyze this chemical fingerprint. This technology holds the promise of providing new insights into human disease states and serving as a primary diagnostic tool for novel and previously characterized inborn errors of metabolism (IEM), as well as for the identification of biomarkers of disease and treatment. Clinical metabolomic profiling allows for parallel screening of hundreds of metabolites in a single biological specimen. On average, ~900 small molecules are detected in a given plasma sample with a core group of ~350 analytes found in all specimens tested to date. The analytes detected encompass numerous classes of small molecule biomarkers including acylcarnitines, amino acids, bile acids, carbohydrates, lipids, neurotransmitters, organic acids, and nucleotides. In addition, metabolomic data in many cases affords a much richer view of a patient's metabolic disturbance by identifying: (1) elevated metabolites located far upstream of or consequential to the genetic defect, (2) treatment related compounds, including commonly tested therapeutic drug monitoring analytes, (3) spectrally unique analytes that are not yet associated with a biochemical phenotype, and (4) identify metabolites across multiple pathways and from multiple classes of molecules in a single analysis. While the potential for untargeted metabolomics in biomarker discovery and diagnosis of ultra-rare diseases is significant, the untargeted and semi-quantitative approach does require additional follow-up for confirmation of findings. Coupling these studies with whole genome approaches, however, enhances the power of metabolomics. In our clinical experience, the integration of whole exome sequencing data with the metabolomics profile has improved the interpretation of genetic variants in 38% of cases, including ruling out the diagnosis of IEMs, as well as supporting a specific diagnosis, and for the identification of new disease and/or treatment biomarkers. For undifferentiated clinical phenotypes such as intellectual disability, hypotonia, autism, or seizures, many different tests involving different sample types are often needed for diagnosis. This can lead to prohibitive costs and ongoing diagnostic odysseys. Data will be presented on genomic and metabolomic profiling of previously undiagnosed cases which resulted in diagnosis of a variety of disorders including transketolase deficiency, aromatic amino acid decarboxylase deficiency, GABA transaminase deficiency, adenylosuccinate lyase deficiency, and peroxisome biogenesis disorders, among others, illustrating the powerful synergy of genomic and metabolomic analysis. We will also share data related to monitoring of patients during treatment or post-transplant, including ethylmalonic encephalopathy. Ultimately, a clinical systems biology approach to the integration clinical data with genomic, transcriptomic, epigenomic, proteomic, and metabolomic data will provide a comprehensive precision medicine approach to improve understanding of natural biological variation and to improve diagnosis and management of genetic disease.

Notes

Morning Session

Mitochondrial Metabolomics: from Basic Mechanisms to Biomarkers

Imaging Mass Spectrometry

Richard Caprioli, PhD

Notes

Presenter: Richard Caprioli

Authors: Richard Caprioli, Jeremy Norris

Institution: Departments of Biochemistry and the Mass Spectrometry Research Center, Vanderbilt University School of Medicine, Nashville, TN, U.S.A.

Title: Imaging Mass Spectrometry: Molecular Microscopy for Biology and Medicine

MALDI Imaging Mass Spectrometry (IMS) produces molecular maps of peptides, proteins, lipids and metabolites present in intact tissue sections. It employs desorption of molecules by direct laser irradiation to map the location of specific molecules from fresh frozen and formalin fixed tissue sections without the need of target specific reagents such as antibodies. Molecular images of this nature are produced in specific m/z (mass-to-charge) values, or ranges of values. Each imaged specimen gives rise to many hundreds of specific molecular images from a single raster of the tissue. In a complementary approach where only discrete areas within the tissue are of interest, we have developed a histology-directed approach that integrates mass spectrometry and microscopy.

We have employed IMS in studies of a variety of biologically and medically relevant research projects, several of which will be presented including studies in diabetic nephropathy involving both proteins and lipids and the differentiation of benign skin lesions from melanomas. In addition, IMS has been applied to drug targeting and metabolic studies both in specific organs and also in intact whole animal sections following drug administration. Recent work with the image analysis of single cells, although just beginning, will be discussed along with the pros and cons of such research analyses.

This presentation describes recent technological advances both in sample preparation and instrumental performance to achieve images at high spatial resolution (1-10 microns) and at high speeds so that a typical sample tissue once prepared can be imaged in minutes. Instrumentation used in these studies includes both MALDI FTICR MS and MALDI TOF mass spectrometers. Applications utilize MS/MS, ultra-high mass resolution, and ion accumulation devices for IMS studies. PIMS (Pathology Interface for Mass Spectrometry) software will be described that allows investigators worldwide to access the imaging technologies over the internet. Finally, new biocomputational approaches will be discussed that deals with the high data dimensionality of IMS and our implementation of ‘image fusion’ in terms of predictive integration of MS images with microscopy and other imaging modalities.

Notes

Morning Session

Mitochondrial Metabolomics: from Basic Mechanisms to Biomarkers

Abstract Presentations

Platform Session

#	Time	Presenter	Title
0489	9:30am	Jesse Wilson	Picosecond spectroscopy of respiratory hemes: towards non-invasive optical biopsy of mitochondrial function
0418	9:45am	Marketa Tesarova	Impact of DNM1L mutation in GTPase domain on mitochondrial network

Notes

Afternoon Session

New Chemical Tools for Investigating Mitochondria

Targeting Mitochondria

Shana Kelley, PhD

Notes

Presenter: Shana O. Kelley, PhD

Authors: Shana O. Kelley, Sae Rin Jean, Eric Lei, Tanja Sack, Simon Wisnovsky

Institution: Department of Pharmaceutical Sciences, University of Toronto

Title: Molecular Delivery to Mitochondria

The mitochondrion is the powerhouse of the cell and also plays a key role in regulating programmed cell death. In particular, the elucidation of the mitochondrion's function in various human diseases has generated an appreciable amount of interest in exploring this organelle as a potential drug target. Delivering molecular agents to mitochondria is challenging, however, due to the difficulty of penetrating the hydrophobic inner mitochondrial membrane. We have developed a set of peptide-based agents that are able to carry drug molecules into mitochondria, and have studied the effects of delivering clinically used therapeutics to mitochondrial targets. We have also mitochondrially-targeted drug conjugates to discover new proteins with previously undetected activities in this cellular subcompartment. Our work on using this approach to i) deliver new active molecular cargo into mitochondria, iii) define structure-function relationships controlling mitochondrial transport and iii) perform high-throughput screens for previously unidentified mitochondrial proteins will be presented.

Notes

Clinical Platform Session Track

Management of Fatigue and Exercise Intolerance in Mitochondrial Disease

11:00am	MMS Business Updates
11:15am - 11:45am	Mitochondrial Care Network *Sumit Parikh, MD; Amel Karaa, MD; and Amy Goldstein, MD*
11:50am - 12:30pm	Risks and Benefits of Exercise in Mitochondrial Disease Patients *Mark Tarnopolsky, MD, PhD*
12:30pm - 1:00pm	Lunch
1:00pm - 1:40pm	Exercise Testing for Mitochondrial Disease Patients *Tania Taivassalo, PhD*
1:40pm - 2:20pm	Fatigue Characteristic and Fatigue Outcome Measures in Mitochondrial Disease *Grainne Gorman, MD*
2:20pm - 2:30pm	Closing Remarks

Notes

Morning Session

Mitochondrial Metabolomics: from Basic Mechanisms to Biomarkers

Abstract Presentations

Platform Session

#	Time	Presenter	Title
0380	11:00am	Marilena D'Aurelio	Glutamate anaplerosis as a mechanism of metabolic adaptation in mitochondrial diseases
0421	11:15am	Edward McKee	Mitochondrial DNA depletion diseases and compartmentalization of the salvage pathway for TTP synthesis in isolated mitochondria from rat tissues.
0416	11:30am	Rachael Baker	Higher Order Structural Analysis to Elucidate Genotype-Phenotype Relationships in BCS1L-Related Rare Diseases
0490	11:45am	Emanuele Barca	The challenge of genetic diagnoses in patients with mitochondrial disease: data from the North American Mitochondrial Disease Consortium (NAMDC)
0393	12:00pm	Rocio Rius	The epidemiology and natural history of pediatric mitochondrial diseases – a population-based study.
0433	12:15pm	Shannon Kruk	Measles, Mumps, Rubella And Varicella Titers In Patients With Mitochondrial Disease

Notes

Afternoon Session

Drug Discovery: From Structure Based Approaches to High Throughput Screening

Mitochondrial Drug Development: a Multi-Partner Endeavor

Jan Smeitink, MD, PhD

Notes

Presenter: Jan Smeitink[1,2]

Authors: Jan Smeitink[1,2], Werner Koopman[1], Peter Willems[1], Ria de Haas[1], Frans Russel[1], Tom Schirris[1], Lisanne van Oppen[1], Richard Rodenburg[1], Sarah Foriel[1,2], Eligio Iannetti[2], Saskia Koene[1], Mirian Jansssen[1], Julien Beyrath[2]

[1]Institution/[2]Company: [1]Radboud Center for Mitochondrial Medicine (www.rcmm.info), Geert Grooteplein Zuid 10, 6500 HB, Nijmegen, The Netherlands [2]Khondrion BV (www.khondrion.com), Philips van Leydenlaan 15, 6525EX Nijmegen, The Netherlands,

Title: Mitochondrial Drug Development: A Multi-Partner Endeavor

Mitochondrial diseases caused by defects in the oxidative phosphorylation system (OXPHOS) are devastating multi-system disorders with a high unmet medical need for treatment development. Detailed understanding of the OXPHOS in health and disease at all levels of complexity, validating every result obtained with alternative approaches, is a fundamental principle for success. Parallel strategies and contingency plans and timely translation of knowledge from academia to industry via public-private partnerships are crucial as are ongoing dialogues with individual patients, patient organizations and key-opinion leaders. Whatever development strategy applied the important questions to be solved should always relate to the burden mitochondrial disease patients suffer from taken transparent expectation management into account. This presentation will illustrate examples of preclinical and clinical academic and industrial research which has served or may serve as starting points for the development of new treatment strategies for mitochondrial disease.

Notes

Afternoon Session

Drug Discovery: From Structure Based Approaches to High Throughput Screening

Small Molecule Inhibitors in Mitochondrial Protein Import

Carla Koehler, PhD

Notes

Small Molecule Inhibitors in Mitochondrial Protein Import

Carla Koehler

Department of Chemistry and Biochemistry, UCLA, Los Angeles, CA

Mitochondrial dysfunction is a contributing factor in degenerative diseases. Modulation of the mitochondrial protein import pathways can have regulatory effects on mitochondrial function. Studying these pathways by conventional methods such as RNAi in mammalian cells can be difficult because it takes several days to knock-down proteins coupled with an overall loss of mitochondrial function. Therefore, we have developed several approaches to develop small molecule modulators for mitochondrial protein translocation. To date, we have conducted screens to identify modulators for the TOM-TIM23, TIM22, and MIA protein import pathways. Our efforts now focus on identifying the specific target of the small molecules in mitochondria using structural and genetic approaches and then using the modulators in model systems to understand how defects in mitochondrial protein translocation impact the rest of the cell. In some cases, we find that specific stress pathways, including mitophagy and apoptosis pathways, are activated. In addition, we also show that these probes are a valuable platform for therapeutic strategies, because the small molecules can modulate mitochondrial stress pathways that have been implicated in degenerative diseases. Our latest advances show that the small molecules can differentially regulate Pink1/Parkin-dependent and independent mitophagy pathways. Therefore, our small molecule screening strategy has been useful in generating a toolbox of small molecule modulators for mitochondrial translocation that can be used in a variety of experimental systems and for regulating mitochondrial stress pathways.

Notes

Afternoon Session

Drug Discovery: From Structure Based Approaches to High Throughput Screening

Model Organisms for N=1 Drug Discovery

Ethan Perlstein, PhD

Notes

Presenter: Ethan Perlstein, PhD

Authors: Ethan Perlstein, Nina DiPrimio, Sangeetha Iyer, Joshua Mast, Jessica Lao, Julide Bilen, Tamy Portillo Rodriguez, Feba Sam, Hillary Tsang, Kausalya Murthy, Gabriela Colmenares, Aras Rezvanian, Madeleine Prangley, Zach Parton, Jay Patel

Institution: Perlara PBC, 6000 Shoreline Court, Suite 204, South San Francisco, CA 94080

Title: Model Organisms for N=1 Drug Discovery

Body of Abstract: Conventional drug discovery involves human cell-based phenotypic screens or human protein target-based in vitro (or in silico) screens followed ultimately by validation in mice. While model organisms routinely identify and elucidate fundamental principles and mechanisms in biology, well studied simple animals remain largely absent from or under-utilized by academic and biopharma drug discovery efforts, especially for rare monogenic diseases. Mitochondrial diseases are well-suited to invertebrate models because causal genes, underlying biochemical defects and pathophysiology are evolutionarily conserved. Here we describe results from natural history studies, drug repurposing screens and drug discovery screens using *Saccharomyces cerevisiae* (yeast), *Caenorhabditis elegans* (nematode), and *Drosophila melanogaster* (fly) patient avatars of rare and ultra-rare genetic diseases, including lysosomal diseases and congenital disorders of glycosylation and deglycosylation. Yeast models of Phosphomannomutase 2 Deficiency (PMM2-CDG) exhibit a conserved genotype-phenotype relationship that can be exploited in a simple growth/no growth multi-well plate assay. A nematode model of Niemann-Pick Type C (NPC) was used to identify a novel chemical entity with an unexpected bypass mechanism that is orally bioavailable, brain-penetrant and well-tolerated, and is bioactive in a mouse model of NPC without the need for lead optimization. Fly and worm models of N-glycanase 1 (NGLY1) Deficiency were used in combination with the proteasome inhibitor bortezomib to identify small-molecule modifiers. This body of work suggests that modeling patient-derived mutations causing mitochondrial diseases in invertebrates will create new avenues for drug discovery as well as gene modifier and biomarker discovery.

Notes

Afternoon Session

Drug Discovery: From Structure Based Approaches to High Throughput Screening

Therapeutic Cross-training: High-throughput Screening Across Evolutionary-Distinct Genetic Models to Optimize Precision Mitochondrial Disease Therapies

Marni Falk, MD

Notes

Therapeutic Cross-Training:
High-throughput across evolutionary-distinct genetic models to optimize precision mitochondrial disease therapies

Marni J. Falk, MD

Executive Director, Mitochondrial Medicine Frontier Program, Division of Human Genetics, Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA 19104; Associate Professor of Pediatrics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104 USA. E-mail: falkm@email.chop.edu

Mitochondria have extensive evolutionary conservation in all living species. This allows robust insights to be gained into mitochondrial dysfunction, and potential therapeutic approaches for mitochondrial disease, that can be pre-clinically tested in a range of *in vitro* cellular and *in vivo* animal models. Dramatic advances in genetic technologies, such as CRISPR/Cas9, has enabled the ready establishment of mitochondrial disease models for a plethora of individual nuclear gene disorders, complemented by pharmacologic inhibition stressor models to characterize mitochondrial dysfunction in distinct respiratory chain complexes or pathways. Our Mitochondrial Medicine research group has developed a series of *C. elegans* (invertebrate, worm), *D. rerio* (vertebrate, zebrafish), *M. musculus* (mammal, mouse), and human patient cell models of primary mitochondrial disease in which we "cross-train", identifying candidate therapies that reliably demonstrate optimal efficacy and minimal toxicity in distinct mitochondrial diseases. The conservation of therapeutic effect across species is remarkable, where molar concentrations found toxic or efficacious in one species are typically highly conserved in the other mitochondrial disease models. Phenotypic outcomes at the level of survival, function, and feeling are prioritized that reflect the ultimate goal of ultimate human therapies for mitochondrial disease, with lead compounds more deeply characterized to decipher their cellular mechanism(s) and biochemical effects. Novel high-throughput technologic advances will be discussed that now enable us to efficiently screen in parallel a large number of compound concentrations, drug libraries, and combinatorial therapies in a wide range of mitochondrial disease disorders and subclasses. Overall, this "therapeutic cross-training" approach has proven invaluable to identify compounds having the highest potency and safety profile in different mitochondrial disease subtypes to prioritize compounds, concentrations, and disease phenotypes for clinical research trial design in human subjects with mitochondrial disease.

Notes

Afternoon Session

Drug Discovery: From Structure Based Approaches to High Throughput Screening

Abstract Presentations

Platform Session

#	Time	Presenter	Title
0442	5:00pm	Bryce Mendelsohn	A high throughput screen of real-time ATP levels in individual cells reveals mechanisms of energy failure
0375	5:15pm	Nahid Khan	Pharmacological Inhibition of Poly(ADP-Ribose) in mouse model of mitochondrial myopathy
0468	5:30pm	Sujay Guha	"Mitochondrial cocktail" combinatorial compound screening in Caenorhabditis elegans and zebrafish models of mitochondrial complex I disease

Notes

Mitochondrial Medicine 2018: Nashville

Friday, June 29, 2018

Notes

Morning Session

Clinical Trials Updates and Challenges
Moving Drugs from Concept to Clinic

Welcome and Clinical Trials Session

Brent Fields, Chuck Mohan, Philip Yeske, PhD

UNITED MITOCHONDRIAL DISEASE FOUNDATION®

HOPE. ENERGY. LIFE.

Mitochondrial Disease-Focused Industry-Sponsored U.S. Clinical Trials Update

June 2018

Here is a brief summary of industry-sponsored, US-based mitochondrial disease-focused clinical trials as of June 2018:

Pediatric Mitochondrial Disorders Clinical Trials

BioElectron Technology Corporation, formerly Edison Pharmaceuticals (BioE-743, formerly EPI-743)- BioE-743 is administered orally, passes into the brain, and works by regulating key enzymes involved in the synthesis and regulation of energy metabolism. A pediatric-focused Phase 2b trial of BioE-743 was completed at the end of 2014 and BioElectron continues to analyze trial data, meet with regulatory authorities worldwide and prepare for a next set of clinical trials across a number of mitochondrial disorders. https://clinicaltrials.gov/ct2/show/NCT01721733 (not actively recruiting)

Adult Mitochondrial Disorders Clinical Trials

Reata Pharmaceuticals (RTA 408, omavaloxolone)- In preclinical studies RTA 408 was shown to target inflammatory, metabolic, and mitochondrial pathways by inducing Nrf2 and suppressing NF-kB. Unlike other drugs in development for the treatment of mitochondrial disease, RTA 408 directly affects mitochondrial function and energy production in muscle cells. In 2015, Reata launched a Phase 2 clinical trial (the MOTOR study) to evaluate the efficacy, safety, and pharmacodynamics of RTA 408 in the treatment of adult patients with mitochondrial myopathy. In an analysis of top-line data, Reata reported in March 2018 that omaveloxolone failed to sufficiently separate from placebo in the MOTOR study, with patients in the treatment group not performing better than those treated with placebo during exercise testing (peak work), the primary endpoint, or in the 6-minute walk test, the secondary endpoint. No safety concerns were identified and the highest dose tested of omaveloxolone (160 mg) reduced heart rate and blood lactate levels in the submaximal exercise test, which are indicative of improved mitochondrial function. No additional trials for mitochondrial disease are planned by Reata at this time. https://clinicaltrials.gov/ct2/show/NCT02255422 (not actively recruiting)

Stealth BioTheapeutics (MTP-131, elamipretide)- MTP-131 is a small molecule peptide designed to penetrate the cellular and outer mitochondrial membrane and target cardiolipin, which is found exclusively in the inner mitochondrial membrane. MTP-131 has been shown to positively impact dysfunctional mitochondria in nonclinical studies, with no effect in healthy mitochondria. In June 2016 Stealth reported positive preliminary findings on topline data from a Phase 1/2 study to evaluate safety, tolerability and efficacy of MTP-131 to treat mitochondrial myopathy in adult patients with genetically-confirmed mitochondrial disease (MMPOWER study). A follow-on Phase 2 trial (MMPOWER-2) focused on subcutaneous efficacy launched returned positive data in 2017. In anticipation of a Phase 3 trial in 2018, Stealth has launched a 1-year observational study (RePOWER) from which Phase 3 participants will be recruited. That Phase 3 trial (MMPOWER-3) is now underway at multiple clinical sites with a goal of enrolling over 200 participants. Top-line data from MMPOWER-3 are anticipated in Q1 2019. https://clinicaltrials.gov/ct2/show/NCT03323749 (enrolling by invitation)

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Mitochondrial Disease-Focused Industry-Sponsored U.S. Clinical Trials Update

June 2018

Ophthalmic Mitochondrial Disorders Clinical Trials

Santhera Pharmaceuticals (Raxone®, Idebenone)- Raxone is an approved drug in Europe for the treatment of Leber's Hereditary Optic Neuropathy (LHON). While Raxone is not approved for treatment in the US, Santhera is conducting a Phase IV study (LEROS) as an open-label interventional study of the use of idebenone for patients with LHON up to 5 years after clinical onset. https://clinicaltrials.gov/ct2/show/NCT02774005 (actively recruiting)

GenSight Biologics (GS010)- GS010 is an investigational gene therapy being evaluated in multiple clinical trials for the treatment of Leber's Hereditary Optic Neuropathy (LHON). In the REVERSE Phase 3 clinical trial, GS010 was evaluated the safety and efficacy of a single intravitreal injection of GS010 (rAAV2/2-ND4) in 37 subjects whose visual loss due to the 11778-ND4 mutation commenced between 6 and 12 months prior to study treatment. The primary endpoint was the ETDRS visual acuity (quantitative score) at Week 48 after intravitreal injection. GenSight reported in April 2018 a clinically meaningful improvement of +11 ETDRS letters in both eyes- an unexpected improvement in the untreated eye suggests a bilateral treatment effect that needs to be further investigated. GS010 is currently being investigated in two additional ongoing Phase 3 trials, RESCUE and REFLECT, while patients in REVERSE continue to be followed for another 4 years. RESCUE is a randomized, double-masked, sham-controlled Phase III trial designed to evaluate the safety and efficacy of a single intravitreal injection of GS010 in subjects affected by LHON with < 6 months of onset of vision loss. GenSight expects to report topline data for RESCUE in the third quarter of 2018. REFLECT is a randomized, double-masked, placebo-controlled Phase III trial evaluating the safety and efficacy of bilateral injections of GS010 in patients with < 1 year of onset of vision loss in LHON. The first patient in REFLECT was treated in March 2018.

REVERSE: https://clinicaltrials.gov/ct2/show/NCT02652767 (not actively recruiting)
REFLECT: https://clinicaltrials.gov/ct2/show/NCT03293524 (actively recruiting)
RESCUE: https://clinicaltrials.gov/ct2/show/NCT02652767 (not actively recruiting)

Stealth BioTheapeutics (MTP-131, elamipretide)-Stealth launched in June 2016 a Phase 2 trial focused on front-of-the-eye delivery of MTP-131 for Leber's Hereditary Optic Neuropathy (LHON), with topline data expected in late 2018. https://clinicaltrials.gov/ct2/show/NCT02693119 (not actively recruiting)

Among the most important things that patients and family members can do to contribute to the development of treatments and cures is to sign up for the Mitochondrial Disease Community Registry (MDCR), a central repository for patient-derived data that provides registrants with full control of who can see and analyze their de-identified data as well as who can make contact with them about relevant research studies.

Philip E. Yeske, Ph.D.
UMDF Science & Alliance Officer
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Notes

Morning Session

Clinical Trials Updates and Challenges
Moving Drugs from Concept to Clinic

History of Clinical Trials in Mitochondrial Disease

Bruce H. Cohen, MD

Notes

Morning Session

Clinical Trials Updates and Challenges
Moving Drugs from Concept to Clinic

Clinical Trial Updates Session 1: Small Molecule Approaches

Moderator: Bruce Cohen, MD

- *8:40-9:00*
  Mitochondria Disease Program Update
  Reenie McCarthy, Chief Executive Officer, Stealth BioTherapeutics

- *9:00-9:20*
  Safety, Efficacy, and Pharmacodynamics of Omaveloxolone in Mitochondrial Myopathy Patients (MOTOR Trial): Part 1 Results
  Karen Lindhardt Madsen, PhD, University of Copenhagen

- *9:20-9:40*
  The KHENERGY study: an exploratory, double-blind, randomized, placebo-controlled, two-way cross-over phase II trial in m.3243A>G patients
  Jan Smeitink, CEO, Khondrion

Notes

Morning Session

Clinical Trials Updates and Challenges
Moving Drugs from Concept to Clinic

Clinical Trial Updates Session 2: Vision Disorders

Moderators: Lissa Poincenot and Nancy Newman, MD

- *10:15-10:30*
  Overview of Mitochondrial Vision Disorders
  Nancy Newman, MD

- *10:30-10:50*
  GenSight: What we have Done, What we are Doing
  Barrett Katz, MD, MBA, Chief Medical Officer, GenSight Biologics

- *10:50-11:10*
  Santhera's Real World Experience with Idebenone in LHON. Update on Programs
  Xavier Lloria, Medical Affairs Director, Santhera Pharmaceuticals

- *11:10-11:30*
  LHON Development Update
  Jim Carr, PharmD, Chief Clinical Development Officer, Stealth BioTherapeutics

- *11:30-11:40*
  Vision Disorder Panel Q&A

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Morning Session

Clinical Trials Updates and Challenges
Moving Drugs from Concept to Clinic

Pre-Clinical Data Updates

Philip Yeske, PhD

- *11:45-12:00*
  KL1333 - Mitochondrial Disease Treatment Opportunity by NAD+ Modulation and Mitochondrial Biogenesis
  Magnus Hansson, PhD, Chief Medical Officer, NeuroVive Pharmaceuticals

Notes

Morning Session

Clinical Trials Updates and Challenges
Moving Drugs from Concept to Clinic

Planning an Externally-led Patient-Focused Drug Development Meeting

- EL-PFDD Meeting Background (James Valentine)
- EL-PFDD Mitochondrial Disease Plan (Philip Yeske, PhD)
- EL-PFDD Meeting Timeline and Q&A

Notes

Afternoon Session

Targeting Mitochondria in Common Diseases

Small Molecular Activators of Aldehyde Dehydrogenases - a New Treatment for Mitochondrial Dysfunction and Mitopathies?

Daria Mochly-Rosen, PhD

Notes

Title: Small Molecular Activators of Aldehyde Dehydrogenases – a New Treatment for Mitochondrial Dysfunction and Mitopathies?

Presenter: Daria Mochly-Rosen, PhD

Authors: Daria Mochly-Rosen[1], Katia Gomes[2], Julio C. B. Ferreira[2]

Institutions: [1]Stanford University School of Medicine, Stamford CA 94305-5174; [2]Biomedical Sciences Institute-University of Sao Paulo, Brazil

Abstract:

A significant amount of evidence has recently emerged to indicate a greater role of endogenous and exogenous aldehydes in the establishment and progression of acute and chronic diseases. We have identified a class of novel molecules called Aldas, for aldehyde dehydrogenase (ALDH) activators, using a fluorescence-based high-throughput screening. A family of ALDH2-specific agonists were found to selectively increase the catalytic activity of ALDH2 in vitro and in vivo. ALDH2 is a mitochondrial enzyme that provides a critical shield from damaging aldehydes that arise under oxidative stress. ALDH2 activation using Alda-1 protects against aldehydic stress when tested in cultured cells, in intact organs and in whole organisms. As an example, I will discuss our work on activation of ALDH2 using Alda-1 in protecting against cardiac diseases, by increasing the clearance of stress-generated toxic aldehydes, including the lipid peroxidation by-product 4-hydroxynonenal. Sustained ALDH2 activation improves cardiomyocyte shortening, cardiac function, left ventricular compliance and diastolic function under basal conditions, and after isoproterenol stimulation. Importantly, sustained Alda-1 treatment promotes a cardiac anti-remodeling effect and malfunction by suppressing myocardial hypertrophy and fibrosis. Alda-1 reverses the accumulation of 4-hydroxynonenal (4-HNE)-protein adducts and protein carbonylation in hearts subjected to myocardial infarction. This benefit was associated with improved mitochondrial function, including elevated mitochondrial respiratory control ratios and reduced H2O2 release. This selective ALDH2 activation decreased mitochondrial Ca2+-induced permeability transition opening and cytochrome c release in failing hearts. Further supporting a mitochondrial mechanism for ALDH2 activation, Alda-1 treatment preserved mitochondrial function following in vitro increase in aldehydic load. Notably, sustained activation of ALDH2 using Alda-1 improved the clinical outcome following myocardial infarction and heart failure in rodents, through decreasing cardiac reactive aldehydes and improving mitochondrial bioenergetics. Finally, Alda-1 treatment in vitro reversed ALDH2 inactivation in ventricular specimens of patients with end-stage heart failure and protected human induced pluripotent stem cell-derived cardiomyocytes against ischemic damage. Together, these data show that increased aldehydic load causes mitopathy and drugs that reduce aldehyde toxicity and ameliorate mitochondrial dysfunction by targeting mitochondrial ALDH2 may prevent or reduce the progression of a number of pathologies associated with mitopathy, such as heart failure.

Notes

Afternoon Session

Targeting Mitochondria in Common Diseases

Mitochondria and Diabetes

Gerald I. Shulman, MD, PhD

Notes

Mitochondria, NAFLD, NASH and Type 2 Diabetes

Gerald I. Shulman, M.D., Ph.D.
Departments of Medicine and Cellular & Molecular Physiology
Howard Hughes Medical Institute
Yale University School of Medicine

Nonalcoholic fatty liver disease (NAFLD) is a major factor in the pathogenesis of type 2 diabetes (T2D) and nonalcoholic steatohepatitis (NASH). In this talk I will discuss: 1) the cellular and molecular mechanisms for lipid-induced liver and muscle insulin resistance, 2) how alterations in hepatic mitochondrial activity, due to ablation of N-acetyltransferase 1, can cause NAFLD and hepatic insulin resistance, 3) a novel positional isotopomer NMR tracer analysis (PINTA) method that can noninvasively assess hepatic mitochondrial function in awake rodents and humans and 4) a novel liver-targeted mitochondrial uncoupling approach that we are actively pursuing to treat NAFLD, NASH and T2D.

References

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Notes

Afternoon Session

Targeting Mitochondria in Common Diseases

Neuroprotective Effects of Inhibition of the Mitochondrial Pyruvate Carrier

Anne N. Murphy, PhD

Notes

Presenter: Anne N. Murphy, PhD

Authors: Ajit S. Divakaruni[1a], Lynn A. Raymond[2],Ian J. Reynolds[3], Brenda Bloodgood[4], Geoffrey Chang[1], Christian M. Metallo[5], and Anne N. Murphy[1]

Institution: [1]Pharmacology, [4]Neurobiology, [5]Bioengineering, University of California, San Diego, La Jolla, CA, 92093. [2]Department of Psychiatry, University of British Columbia, Vancouver, BC, V6T 1Z3, [3]Discovery Research, Teva Pharmaceutical Industries Ltd., West Chester, PA, 19380.. [a]Current address: Molecular and Medical Pharmacology, David Geffen School of Medicine, Los Angeles, CA 90095

Title: Neuroprotective Effects of Inhibition of the Mitochondrial Pyruvate Carrier

Glutamate is the dominant excitatory neurotransmitter in the brain. Under conditions of metabolic stress it can accumulate in the synapse to levels that compromise neuronal function and viability by excitotoxic stress. Specifically, excitatory amino acid receptor-mediated accumulation of Na+ and Ca2+ levels within the cytoplasm triggers damage to mitochondria and a bioenergetic crisis, and can ultimately result in neuronal death. Excitotoxicity is a hallmark of diseases and conditions including epilepsy, traumatic brain injury, stroke, and progression of Alzheimer's Disease. Although pharmacologic modulation of excitatory amino acid receptors is well studied, minimal consideration has been given to targeting mitochondrial glutamate metabolism to control neurotransmitter levels. This may in part be due to conceptualization of glutamate handling within the glutamine/glutamate cycle, whereby glutamate packaged within neurotransmitter vesicles in a presynaptic neuron is released to the synapse, where it binds to ionotropic and metabotropic receptors on postsynaptic neurons to trigger activity. It is then rapidly cleared by high affinity transporters particularly on astrocytes, where it is converted to glutamine, transported back to neurons, deamidated to glutamate and repackaged into vesicles, allowing a continuous cycle of neurotransmitter activity.

For an energy source, the brain is known to be highly reliant on glucose as fuel. This is in contrast to other tissues such as skeletal muscle, that demonstrate significant metabolic flexibility, or switching to alternative substrates as availability and hormonal stimulation dictate. The brain is described to make use of ketone bodies as an alternative substrate, particularly during periods of starvation or intermittent fasting. Heavy reliance on glucose is a liability, as multiple forms of neurodegenerative disease are associated with energetic and metabolic deficits. In fact, the loss of metabolic flexibility and insulin resistance associated with type 2 diabetes is a significant risk factor for Alzheimer's disease. Further, hypometabolism of glucose in the brain can manifest long before clinical symptoms in Alzheimer's Disease. The liability created by a lack of metabolic flexibility in the brain is further underscored by evidence that oxidation of non-glucose substrates may be beneficial in certain forms of neurodegeneration. For instance, low carbohydrate diets are known to confer dramatic benefits to some forms of medically refractory seizure disorders. In fact, this promise has triggered the clinical evaluation of ketogenic diets for several forms of acute and chronic neurodegenerative disease, although a mechanistic understanding of the potential benefits remains unresolved.

To reconcile the potential benefits from oxidation of non-glucose substrates with the reliance of neuronal metabolism on glucose, we mapped the pattern of cellular metabolism in response to reduced mitochondrial pyruvate carrier (MPC) activity using the tool compound UK5099. The MPC is an inner membrane transporter that facilitates pyruvate uptake from the cytoplasm into mitochondria. It is a central regulator of mitochondrial substrate utilization, and restrictions in mitochondrial pyruvate uptake can potentiate the use of fatty acids and a range of amino acids to fuel cellular energetics and biosynthesis in non-neuronal cells. Mapping the pattern of neuronal metabolism upon MPC inhibition allowed us to determine the extent of metabolic flexibility in neurons, and determine whether adjustments in neuronal substrate oxidation could affect susceptibility to injury. We report that metabolism of cortical neurons in culture and hippocampal slices is characterized by metabolic plasticity, and oxidative metabolism can be maintained in spite of large reductions in mitochondrial pyruvate uptake because of a selective increase in glutamate oxidation. Thus, perhaps counterintuitively, reductions in mitochondrial pyruvate metabolism can be neuroprotective: increased glutamate oxidation decreases the glutamate available for synaptic release and, in turn, minimizes the positive feedback cascade of excitotoxic injury. Our findings link mitochondrial pyruvate metabolism to glutamatergic neurotransmission and establishes the MPC as a therapeutic target to treat neurodegenerative diseases characterized by excitotoxic stress.

Notes

Afternoon Session

Targeting Mitochondria in Common Diseases

Medicines for Malaria and African Sleeping Sickness

Kiyoshi Kita, PhD

Notes

Presenter: Kiyoshi Kita, PhD

Institution: School of Tropical Medicine and Global Health & Institute of Tropical Medicine, Nagasaki University 1-12-4, Sakamoto, Nagasaki 852-8523, Japan

Title: Medicines for Malaria and African Sleeping Sickness – Mitochondria as a drug target

Parasites have developed a variety of physiological functions necessary for their survival within the specialized environment of the host. Using metabolic systems that are very different from those of the host, they can adapt to low oxygen tension present within the host animals. Most parasites do not use the oxygen available within the host to generate ATP even they reside oxygen rich circumstance such as blood, but rather employ systems anaerobic metabolic pathways. In addition, all parasites have a life cycle. In many cases, the parasite employs aerobic metabolism during their free-living stage outside the host. In such systems, parasite mitochondria play diverse roles. In particular, marked changes in the morphology and components of the mitochondria during the life cycle are very interesting elements of biological processes such as developmental control and environmental adaptation. As mitochondrial function is essential for the survival of the parasites, it should be promising target of chemotherapy.

Malaria

One good example is atovaquone, which is widely used electron transport inhibitor and a major component of Malarone. By using mutant strains and biochemical study on the mutant respiratory chain, we clearly showed that atovaquone binds Qo site of cytochrome *b* in *Plasmodium* mitochondrial respiratory chain. Furthermore, we showed that malaria parasites with mutations in the mitochondrial encoded cytochrome *b* gene *(cytB)*, which are resistant to atovaquone are essentially unable to transmit resistance to new hosts. This means that atovaquone resistance would not spread in the endemic region (Goodman et al., Science, 2016).

Recently, we found a new anti-malarial drug candidate “5-Aminolevulinic Acid”, which is 1st step intermediate of heme biosynthesis of the parasite (Suzuki et al., Antimicrob. Agents and Chemother., 2015). It should be noted that the cured mice were protected from homologous rechallenge, even when reinfection was attempted more than 230 days after the initial recovery, indicating long-lasting resistance to reinfection with the same parasite. Interestingly, parasite-specific antibodies against typical vaccine candidate antigens were detected and persistently maintained in the sera of the cured mice.

African Sleeping Sickness

Cyanide-insensitive respiration in plants has long been recognized since 1920s. Intensive biochemical studies revealed that the mitochondrial membrane enzyme, alternative oxidase (AOX), is responsible for the cyanide-insensitive respiration. AOX, which is cyanide-insensitive and salicyl hydroxamic acid (SHAM)-sensitive, is a non-proton-pumping ubiquinol oxidase catalyzing the 4-electron reduction of dioxygen to water. AOX has been found in higher plants, algae, yeast, slime molds, free-living amoebae, eubacteria and nematodes, as well as protozoan including trypanosomes.

T. brucei, which causes African sleeping sickness in human and Nagana in livestock, those are serious health and economic problem in sub-Saharan Africa had been known to show the cyanide-insensitive respiration. For this parasite cyanide-insensitive respiration, the trypanosome alternative oxidase (TAO) functions in the African trypanosomes as a cytochrome-independent terminal oxidase, which is essential for their survival in the mammalian host. TAO has been thought as a good target for theanti-trypanosomal drugs because mammalian hosts do not possess this protein.

We found that the ascofuranone, isolated from pathogenic fungus, specifically inhibits the quinol oxidase activity of TAO and rapidly kills the parasites. In addition, we have confirmed the chemotherapeutic efficacy of ascofuranone *in vivo*. Structure activity relationship analysis revealed the essential structure in the ascofuranone for its potent inhibition of quinol oxidase activity. Furthermore, our first 3D structure study provided a conclusive information of inhibitor-binding mechanism. The result mentioned here leads to a rational design of more potent and safe anti-trypanosomal drugs. In fact, at least, 3 completely synthetic derivatives of ascofuranone cured the infected mice.

Notes

Afternoon Session

Targeting Mitochondria in Common Diseases

UMDF Funded Projects

Philip Yeske, PhD

Notes

Afternoon Session

Targeting Mitochondria in Common Diseases

Abstract Presentations

Platform Session 6: Abstracts

#	Time	Presenter	Title
0509	4:15pm	Atif Towheed	Allotopically Expressed RNA Mediated Genetic Complementation of a Mitochondrial-encoded ND6 Frameshift Mutant
0496	4:30pm	Zarazuela Zolkipli-Cunningham	Development of a Mitochondrial Myopathy Rating Scale

Notes

Mitochondrial Medicine 2018: Nashville

Saturday, June 30, 2018

Notes

Morning Session

Mitochondrial Stress Responses

Mitochondrial Disease Sequence Data Resource (MSeqDR)

Marni Falk, MD and Colleen Clarke Muraresku, CGC

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Morning Session

Mitochondrial Stress Responses

Mitochondria Drug Toxicity and the Pharmaceutical Industry

Yvonne Will, PhD

Notes

Presenter: Yvonne Will, PhD

Affiliation: Pfizer Inc., Groton CT

Presentation Title: Mitochondrial Toxicity and the Pharmaceutical Industry

Presentation Description: Mitochondrial toxicity can lead to late stage attrition of drugs, mostly due to hepatotoxicity. Many drugs carrying black box warnings (especially cardiac and hepatic toxicity) also have been shown to have mitochondrial liabilities. Over the past decade, we have made great progress in building high-throughput applicable assays that can help screen out drug induced mitochondrial toxicity early in the drug discovery process. In this lecture I will introduce currently available technologies that can help to elucidate potential mitochondrial toxicity using examples of a variety of drug classes such as glitazones, statins, NSAIDS, antidepressants, antibiotics etc. This includes a high throughput applicable screens using isolated mitochondria and soluble oxygen sensors and a cell viability assays that truly reveals mitochondrial toxicity, by growing them in galactose containing media and an imaging based assay to detect adverse effects on mitochondrial replication/translation. I will discuss the strength and limitations for the various assays.

Notes

Morning Session

Mitochondrial Stress Responses

Compensatory Responses to Mitochondrial Toxicity

Kendall B. Wallace, PhD

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Kendall B. Wallace, PhD
University of Minnesota Medical School
Duluth, MN 55812

Mitochondrial stress may be either the result of direct interference with mitochondrial bioenergetics, i.e. genetic or acquired mitochondrial dysfunction, or secondary to an “external”metabolic challenge, such as exercise or hyperglycemia. Regardless, and apparently indiscriminately, the cell responds by attempting to restore bioenergetic homeostasis by way of two major compensatory pathways; Transcriptionally reprogramming intermediary metabolism in favor of glucose oxidation and stimulating mitochondrial biogenesis. If successful, the fully compensated bioenergetic state is, for practical purposes, “clinically silent”. It is only when this compensatory response is insufficient to restore cellular bioenergetics in response to severe mitochondrial stress that a clinical phenotype is manifest. The current presentation will describe the two compensatory pathways including key cell signaling and intermediary steps, and give examples for both genetic and acquired disorders. An attempt will be made during the discussion to define critical crossover points in the manifestation of a clinically diagnosable disorder. The presentation will conclude with the proposition that these compesatory pathways may provide sensitive and robust bioindicators of early stage clinically silent mitochondrial disease.

Notes

Morning Session

Mitochondrial Stress Responses

Manipulating Mitochondrial ROS and Oxidative Damage as Therapeutic Strategies

Mike Murphy, PhD

Notes

Presenter: Mike Murphy

Authors: Mike Murphy

Institution: MRC Mitochondrial Biology Unit, University of Cambridge, Hills Road, Cambridge, CB2 0XY, UK

Title: Manipulating mitochondrial ROS and oxidative damage as therapeutic strategies

Mitochondrial redox metabolism is central to the life and death of the cell. For example, mitochondrial production of free radicals and subsequent oxidative damage has long been known to contribute to damage in conditions such as ischaemia-reperfusion (IR) injury in stroke and heart attack. More recently mitochondrial redox changes have also been implicated in redox signalling. Over the past years we have developed a series of mitochondria-targeted compounds designed to ameliorate or determine how these changes occur. I will outline some of this work, which suggested that ROS production in IR injury during stroke was mainly coming from complex I. This led us to investigate the mechanism of the ROS production and using a metabolomic approach we found that the ROS production in IR injury came from the accumulation of succinate during ischaemia that then drove mitochondrial ROS production by reverse electron transport at complex I during reperfusion. This surprising mechanism led up to develop further new therapeutic approaches to impact on the damage that mitochondrial ROS do in pathology and also to explore how mitochondrial ROS can act as redox signals. I will discuss how these unexpected mechanisms may lead to redox and metabolic signals from mitochondria in a range of conditions under both healthy and pathological conditions.

Notes

Morning Session

Mitochondrial Stress Responses

Stress Responses in Mitochondrial Disease

Anu Suomalainen, MD, PhD

Notes

Presenter: Anu Suomalainen, MD PhD

Authors: Christopher Jackson[1], Eija Pirinen[1], Saara Forsström[1], Joni Nikkanen[1], Nahid Khan[1], Jana Buzkova[1], Rebecca Kohnz[2], Daniel Nomura[2], Liya Wang[3], Anne Roivainen[4,] Vidya Velagapudi[5] & Anu Suomalainen[1]

Institutions: [1] Biomedicum-Helsinki, Molecular Neurology Research Program, University of Helsinki, 00610 Helsinki, Finland; [2]Departments of Chemistry and Nutritional Sciences and Toxicology, University of California, Berkeley, Berkeley, CA 94720, USA; [3]Department of Anatomy, Physiology, and Biochemistry, Swedish University of Agricultural Sciences, 75007 Uppsala, Sweden; [4] Turku PET Centre, University of Turku, 20520 Turku, Finland; [5] Metabolomics Unit, Institute for Molecular Medicine Finland, University of Helsinki, 00290 Helsinki, Finland Institute of Molecular Medicine Finland, FIMM, 00610 Helsinki, Finland.

Title: Stress Responses in Mitochondrial Disease

Body of Abstract

Mitochondrial diseases manifest with an unprecedented clinical variability, affecting children and adults, and involving different organ systems. However, knowledge of the molecular mechanistic basis of such variability is lacking. To elucidate the tissue-specificity, we have created mouse models and stem cell-derived neural cultures for mtDNA replication defects and characterized tissue-specific metabolic consequences in the primary affected tissues and the whole organism. Using these disease models, we characterized an ”integrated mitochondrial stress response” (ISRmt), which has tissue-specific characteristics and involves transcriptional components, remodels one-carbon cycle and induces secretion of metabolic cytokines. This response is turned on in mtDNA expression disorders (mitotranslation, mtDNA deletions), but not in structural defects of the respiratory chain, indicating that the type of mitochondrial dysfunction determines the local stress responses. We present the orderly and sequential induction of ISRmt, with feed-back loops and redox-regulation in mammalian tissues and cells. The metabolic component of ISRmt involves anabolic cytoplasmic biosynthesis reactions and activates mTORC1 in the skeletal muscle and the heart. Our results suggest that mitochondrial replisome and translation are part of a complex signaling system linking nutritional intake to energy metabolism, cellular biosynthetic reactions and organellar turnover. Furthermore, we propose that mitochondrial dysfunction modifies this signaling, which has consequences to disease progression. We show that the core components of the mouse response is conserved in human patient muscle. Also, we show that these pathways offer multiple strategies for intervention, demonstrated in our first human pilot trial with NAD+ precursor intervention for mitochondrial myopathy patients.

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Morning Session

Mitochondrial Stress Responses

Closing

Vamsi K. Mootha, MD

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UMDF Funded Grant Projects

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2017 UMDF Grant Recipients

Chairman's Award
Prashant Mishra, MD, PhD
University of Texas Southwestern Medical Center
Children's Research Institute
$100,000

"Identification of SLC Family Members as Predictive Biomarkers for Mitochondrial Disease"
The development and validation of blood based biomarkers for mitochondrial disease remains a top priority in the research community. This project investigates the potential use of specific solute carrier (SLC) family members in blood serum as novel predictive biomarkers of mitochondrial disease. Preliminary data generated in Dr. Mishra's lab indicates that SLC family members are not only altered in the disease setting, but could also serve as therapeutic development targets.

New and Early Principal Investigator Award
Rustum Karanjia, MD, PhD
Doheny Eye Center
UCLA
University of Ottowa
$100,000

"Photopic Negative Response as an Objective Biomarker in Mitochondrial Disease"
Many forms of mitochondrial disease have eye-related symptoms, including LHON, DOA, Leigh Syndrome and MELAS. There is a significant and need for better measurements of eye function as it relates to disease progression. Dr. Karanjia intends to measure eye cell electrical activity over 18 months in affected individuals utilizing Photopic Negative Response (PhNR). This data will help to validate PhNR as a potential biomarker for optic nerve function in mitochondrial disease patients.

Postdoctoral Fellowship Award
Melissa Anne Walker, MD, PhD
Massachusetts General Hospital
$100,000

"A Single Blood Draw Test of Mitochondrial Disease"
here are currently no consensus biomedical tests for mitochondrial disease due to its complex and diverse nature, a situation which makes effective diagnosis and treatment extremely challenging. The goal of Dr. Walker, working in the lab of Dr. Vamsi Mootha, is to develop a rapid, facile blood test for mitochondrial activity based on a single blood draw. Preliminary data points to a simple, inexpensive test that could be applied in any standard clinical laboratory. The funds provided by the UMDF will allow for continued development and validation of this assay in both healthy controls as well as affected individuals with a genetically confirmed diagnosis of mitochondrial disease.

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2016 UMDF Grant Recipients

Short Summaries by Phil Yeske, PhD

Chairman's Award

Nicola Brunetti-Pierri, MD, FACMG, Associate Investigator, Telethon Institute of Genetics and Medicine (Italy), "*Phenylbutyrate Therapy for Pyruvate Dehydrogenase Deficiency, Small Clinical Study Award*" - 1 year/$25,000

This grant, winner of the 2016 Chairman's Award for highest rated research proposal after peer review, is a clinical study of a new potential therapy for pyruvate dehydrogenase complex (PDHC) deficiency by lowering lactate levels. This project comes 5 years after Dr. Brunetti-Pierri received a UMDF grant to first test phenylbutarate on patient cells. Subsequent animal model studies confirmed the promising in vitro data that resulted from the first grant, and now a pilot clinical trial will be carried out across multiple centers in Italy. Positive results from the pilot study would lead to a larger study directed toward PDHC deficiency patients.

Brendan J. Battersby, Ph.D., Research Director, Biomedicum Helsinki, Research Programs Unit-Molecular Neurology, University of Helsinki (Finland), "*Investigating the Pathogenesis of C12orf65 Deficiency in Mitochondrial Translation and Mitochondrial Disease*" - Principal Investigator Award, 2 years/$70,000

The goal of this research project led by Dr. Battersby is to address a significant gap in mechanistic knowledge within the mitochondrial field- ribosome function and translation. The outcome of this work could provide unique insights into the broad range of mitochondrial disease symptoms that result from mutations in the C12orf65 gene.

Alessandro Bitto, Ph.D., Department of Pathology, University of Washington Medical Center (USA), "*Molecular Mechanisms for Suppression of Mitochondrial Disease by Acarbose, Postdoctoral Fellowship Award*" - 2 years/$70,000

Dr. Bitto, under the mentorship of Dr. Matt Kaeberlein, will evaluate an FDA-approved drug called acarbose for efficacy in a translational mouse model of Leigh Syndrome. The drug impacts mTOR signaling, an important mitochondrial function pathway whose understanding could open up a broad therapeutic strategy for mitochondrial disease.

Adam Hughes, Ph.D., Assistant Professor of Biochemistry, University of Utah School of Medicine (USA), "*Quality Control of Unimported Mitochondrial Precursor Proteins, Principal Investigator Award*" - 2 years/$100,000

Utilizing yeast models, Dr. Hughes intends to explore the link between loss of mitochondrial membrane potential and mis-targeted mitochondrial proteins. That the accumulation of such proteins and their associated "waste disposal" is a source of mitochondrial pathology is a novel and intriguing premise that could open up many new avenues in future research.

Leo Nijtmans, Ph.D., Radboud University Medical Centre, Nijmegen (Netherlands), "*Mitochondrial Complexome Profiling Provides a Novel Tool to Diagnose and Understand Complex I Deficiency*" - Principal Investigator Award, 1 year/$40,000

Complex I disorders are some of the most common types of mitochondrial disease. Dr. Nijtmans will utilize a profiling technique to study protein interactions within Complex I using patient cell lines. The results will provide insight into Complex I assembly and function, and could ultimately lead to new therapeutic targets for investigation.

George A. Porter, Jr., MD, Ph.D., Assistant Professor, Department of Pediatrics, Division of Cardiology, University of Rochester Medical Center (USA), "*Manipulating the Permeability Transition Pore to Ameliorate Neonatal Heart Failure*" - Principal Investigator Award, 2 years/$100,000

Many types of mitochondrial disease have associated cardiomyopathies. In this translational research project Dr. Porter will test potential therapies for cardiomyopathies in a mouse model. Success in this project would initially open the possibility for treating neonates with bioenergetics disorders, and eventually have potential for more broadly treating mitochondrial disease patients with Complex I disorders.

Eric A. Shoubridge, Ph.D., Professor and Chair, Department of Human Genetics, Montreal Neurological Institute, McGill University (Canada), "*Interrogating the Mitochondrial Interactome Using BioID, Principal Investigator Award*" - 2 years/$75,000

Dr. Shoubridge's project will identify functional networks within the mitochondria based on the analysis of protein-protein interactions. In addition to the potential for revealing new insights into mitochondrial disease, the availability of a mitochondrial protein interactome will be a generally useful resource for addressing basic questions regarding mitochondrial structure and function in both a normal and diseased state.

Zarazuela Zolkipli Cunningham, MBChB MRCP, Division of Neurology,The Children's Hospital of Philadelphia (USA), "*Development and Validation of a New Outcome Measure in Mitochondrial Disease*" - Small Clinical Study Award, 1 year/$25,000

Dr. Zolkipli Cunningham and collaborators aim to develop a new outcome measure for mitochondrial myopathy that is specifically designed for use in Phase II/III clinical trials. The patient perspective will be critical to the project, helping to ensure that meaningful measures are developed over the full range of disease state- from early ambulatory to late non-ambulatory. Recognizing the urgent need for improved clinical trial endpoints, the development of this scale will build upon existing scales and tools.

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2015 UMDF Grant Recipients

Short Summaries by Phil Yeske, PhD

Chairman's Award

John Christodoulou, PhD, Children's Hospital at Westmead, New South Wales, Australia.
"Utility of FGF21 and GDF15 as Diagnostic and Prognostic Biomarkers of Mitochondrial Respiratory Chain Disorders." - $100,000 over 2 years

Dr. Christodoulou will validate optimal methodology in a clinical diagnostic laboratory setting to determine the utility of measuring FGF-21 and GDF-15 as biomarkers of pediatric mitochondrial disease. This has become a major question in the field, as to how potentially useful in terms of sensitivity and specificity these biomarkers are for mitochondrial disease.

Daniel F. Bogenhagen, MD, Department of Pharmacological Sciences, Stony Brook University.
"Kinetics of Mitochondrial Complex Assembly." - $100,000 over 2 years

Dr. Bogenhagen is utilizing mass spectrometry techniques to study the assembly map of the mitoribosome as well as the mitochondrial respiratory complexes. The improved understanding of both of these mitochondrial construction projects will enhance the diagnosis and future therapy of mitochondrial disorders.

Peter W. Stacpoole, PhD, MD, Department of Medicine, University of Florida.
"Validation of an Observer Reported Outcome (ObsRO) Measure of Home Functionality in Children with Pyruvate Dehydrogenase Complex Deficiency (PDCD)." - $24,898 for 1 year

Dr. Stacpoole, in response to a specific request from the FDA for information from the patient or patient family to aid in regulatory decisions, has developed an innovative computer tool to track home functionality of pediatric PDCD patients. The pilot study in 10 PDCD families will test the feasibility of the survey instrument and refine it as needed for its eventual use in a planned Phase III trial of dichloroacetate (DCA). If the trial shows DCA is found safe and effective, it could lead to this drug being designated as the first FDA-approved therapy for PDCD.

Dr. Atif Towheed, PhD, Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia.
"Allotopic RNA Rescue of LHON Mouse Model." - $70,000 over 2 years

The goal of Dr. Towheed's work is to develop a novel gene therapy strategy for the treatment of Leber hereditary optic neuropathy (LHON) utilizing a mouse model developed in the labs of Dr. Douglas C. Wallace. If this therapeutic approach is successful it could inhibit the onset of the optic nerve pathology.

Sara M. Nowinski, PhD, Department of Biochemistry, University of Utah.
"Characterizing the Function of the Atypical Mitochondrial Kinase ADCK3." - $70,000 over 2 years

The studies in Dr. Nowinski's grant will improve the understanding of ADCK3 function in the synthesis of coenzyme Q and cerebellar ataxia. Additionally, better treatment strategies for mitochondrial disease could be developed in the future if new roles for ADCK3 are identified.

Anu Suomalainen Wartiovaara, MD, PhD, University of Helsinki, Finland.
"Vitamins B as Therapy for Disorders with mtDNA Instability." - $100,000 over 2 years

Dr. Suomalainen Wartiovaara will utilize a mouse model to build upon preliminary results indicating that vitamins B, especially B3 (Niacin) play key metabolism regulatory roles in patients with mitochondrial myopathies. Pre-clinical data generated in mice will inform the creation of a follow-up human clinical trial on the impact of Niacin supplementation for the alleviation of symptoms due to mitochondrial disease.

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2014 UMDF Grant Recipients

Short Summaries by Steven G. Bassett, PhD

Chairman's Award

Hubert Smeets, PhD, Department of Genetics and Cell Biology, Maastricht University, The Netherlands - $25,000 for 1 year
"Development of an autologous myogenic stem cell therapy for carriers of a heteroplasmic mtDNA mutation, a proof of principle study in m.3243A>G mutation carriers."
Dr. Smeets has developed a process using transplantation of a patient's own muscle stem cells that have been freed of mitochondrial DNA mutations. The resulting formation of normal muscle fibers promises to set the stage for significant new therapies for mitochondrial disease.

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Carlos Moraes, PhD, Department of Neurology, University of Miami Miller School of Medicine - $120,000 over 2 years
"Developing specific mitochondrial nucleases to eliminate mutant mtDNA."
Dr. Moraes has developed a process for removing disease-causing mitochondrial DNA mutations from affected mitochondria. Extension of this research seems likely to lead to the development of gene therapies for human mitochondrial disease.

……..

Michael James Bell, MD, University of Washington DC - $25,000 for 1 year
"Improving CNS delivery of brain antioxidants after acute metabolic decompensation in mitochondrial disease."
Dr. Bell will investigate a combination of two FDA-approved drugs for their effectiveness in treating children and young adults with Leigh's Syndrome. This work has the potential to improve brain function in patients with a mitochondrial disease for which there are currently no proven treatments.

……..

Francisca Diaz, PhD, Department of Neurology, University of Miami Miller School of Medicine - $80,000 over 2 years
"Modulation of GSK3 activity and enhancement of glycolysis to maintain neuronal survival in Complex IV deficient mice."
Dr. Diaz is using a much studied mouse model in which a mitochondrial respiratory enzyme has been deactivated in nerve cells. She will study the effectiveness of modulating glucose metabolism as a treatment for these mice, with the potential for extending this therapy to human mitochondrial disease patients.

……..

Scot Leary, PhD, Department of Biochemistry, University of Saskatchewan - $120,000 over 2 years
"Targeted delivery of copper to mitochondria: investigating its therapeutic potential for the effective treatment of patients with mutations in SCO1 and SCO2."
Dr. Leary is investigating therapies for copper delivery to mitochondria in patients with impaired ability to synthesize a vital mitochondrial respiratory enzyme that requires copper as a building block. This research could lead to the development of early intervention therapies for mitochondrial disease.

……..

Erin Seifert, PhD, Department of Pathology, Thomas Jefferson University, Philadelphia - $120,000 over 2 years
"Pathogenesis of myopathies caused by mitochondrial phosphate carrier mutations."
Dr. Seifert is studying mutations that severely affect the delivery of phosphate for ATP synthesis in the mitochondria of skeletal muscle and the heart. This foundational research will provide new insights into important mechanisms responsible for mitochondrial disease.

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2013 UMDF Grant Recipients

Short Summaries by Steven G. Bassett, PhD

Chairman's Award
James Stewart, PhD, Max Planck Institute for the Biology of Ageing, Cologne, Germany - $90,000 over 2 years
"Using mtDNA mutator mouse-derived lineages to generate mouse models of human mitochondrial diseases."
By working with mice that are prone to mitochondrial mutations, Dr. Stewart will develop new genetic models of human disease. Once established, these mouse models can be used for the development of new drug therapies.

Alberto Sanz-Monterro, PhD, University of Tampere, Tampere, Finland - $100,000 over 2 years
"A Genome-wide RNAi Screening to Identify New Genes Involved in Mitochondrial Diseases."
Dr. Sanz-Monterro will use a well-understood fruit-fly model to discover previously unknown genetic defects that can cause mitochondrial disease. Many mitochondrial disease patients have not had a specific genetic mutation linked with their disease, and this research will help to fill that gap.

Rajesh Ambasudhan, PhD, Sanford-Burnham Medical Research Institute, La Jolla, California - $84,000 over 2 years
"A Human Reprogrammed-Cell Model of MELAS."
Dr. Ambasudhan will obtain skin cells from MELAS patients and reprogram them as nerve cells to be grown in culture. This "disease-in-a-dish" model will be used to gain insights into mitochondrial dysfunction in MELAS and other mitochondrial diseases.

Natalie Niemi, PhD, University of Wisconsin, Madison, Wisconsin - $75,000 over 2 years
"Utilizing dynamically regulated phosphorylation as a means to modulate mitochondrial metabolism."
Dr. Niemi will study mechanisms that activate enzymes in the mitochondria, with the goal of understanding how this regulation is impaired in mitochondrial disease. This could lead to new therapeutic options for mitochondrial disease patients.

Gerald Shadel, PhD, Yale University Medical School. - $120,000 over 2 years (co-funded by UMDF and MitoCon)
"Characterization of disease-specific mitochondrial stress-signaling pathways in vivo as potential therapeutic targets for mitochondrial diseases."
Dr. Shadel will use a mouse model to identify important stress signaling pathways than can then be related to mitochondrial dysfunction in humans. This could enable repurposing of existing therapeutics as well as development of new therapies.

2013 Clinical Fellowship Training Award

Amel Karaa, MD, Harvard Medical School and Massachusetts General Hospital, Boston MA - $70,000 for 1 year

"Hypogonadotropic hypogonadism in mitochondrial disease: prevalence, phenotypic heterogeneity and hormonal spectrum variations in a tertiary hospital cohort."

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2012 UMDF Grant Recipients

Short Summaries provided by Steven G. Bassett, PhD

Chairman's Award

Carla Giordano, MD, PhD, University of Rome - $108,000 over 2 years (co-funded by UMDF and MitoCon)

"Estrogen mediated regulation of mitochondrial biogenesis and functions: possible therapeutic implications for Leber's hereditary optic neuropathy."

Dr. Giordano will use phytoestrogens, plant compounds with estrogenic properties, to enhance mitochondrial energy metabolism. This research could result in effective treatments for a progressive mitochondrial disease that severely impairs vision.

William James Craigen, MD, PhD, Baylor College of Medicine - $100,000 over 2 years

"Testing Gene Therapy in an Animal Model of Mitochondrial Respiratory Chain Disorders."

Dr. Craigen's lab is developing procedures using viruses to deliver the correct genetic information to mice that have defective mitochondria, in an attempt to greatly improve their energy metabolism. This could lead to the development of an effective gene therapy for young mitochondrial disease patients.

Mariana G. Rosca, MD, Case Western Reserve University - $100,000 over 2 years

"Rescuing complex I defective mitochondria and target organs with methylene blue."

Dr. Rosca is developing a treatment that could bypass a defective mitochondrial enzyme, enhancing energy metabolism. Improving the performance of mitochondria in this way could address a defect that is responsible for a third of all mitochondrial disease cases.

Javier Torres-Torronteras, PhD,Vall d'Hebron Research Institute, Barcelona, Spain - $93,000 over 2 years

"Preclinical studies for the gene therapy of mitochondrial neurogastrointestinal encephalomyopathy (MNGIE). Long-term follow-up and use of adeno-associated viral vectors."

Dr. Torres-Torronteras will investigate the effectiveness of a gene therapy that he developed for use in mice with mitochondrial disease. This research will aid in determining the best approaches for treating human mitochondrial disease with gene therapy.

David A. Sinclair, PhD, Harvard Medical School - $80,000 over 2 years

"Ultra-high-throughput screening for mitochondrial enhancers as novel targets for treating mitochondrial diseases."

Dr. Sinclair will screen a large number of genes for their ability to enhance mitochondrial energy metabolism. Applying his findings to cell culture models of various mitochondrial diseases could lead to the discovery of new treatments.

Nuno Raimundo, PhD, Yale University School of Medicine - $50,000 over 1 year

"Mechanisms and treatment of mitochondrial deafness."

Dr. Raimundo has developed an animal model for the study of hearing loss due to a mutation in mitochondrial DNA. The insights gained from this research could lead to the prevention and treatment of a type of deafness that is linked to mitochondrial malfunction.

2012 Clinical Fellowship Training Award

Lisa Emrick, MD, Baylor College of Medicine, Houston, TX - $60,000 over 1 year

"Characterization of Neurologic Deficits and Response to Treatment in Patients with MELAS and other Mitochondrial Disorders."

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2011 UMDF Grant Recipients

Short Summaries provided by Steven G. Bassett, PhD

Chairman's Award
Brett A. Kaufman, PhD, Department of Animal Biology, University of Pennsylvania - $120,000 for 2 years
"Regulatory mechanisms governing TFAM-mediated mtDNA copy number control."
Dr. Kaufman research could lead to therapies that would increase the number of copies of normal mitochondrial DNA in patients with specific types of mitochondrial disease.

Chairman's Award
Nicola Brunetti-Pierri, MD, Telethon Institute of Genetics and Medicine, Fondazione Telethon, Rome, Italy - $120,000 for 2 years
"Therapeutic Interventions for Pyruvate Dehydrogenase Deficiency."
Dr. Brunetti-Pierri is seeking safe and effective treatments for a genetic condition that causes deficiencies of an important enzyme in the energy pathway of mitochondria.

Miguel Garcia-Diaz, PhD, Department of Pharmacological Sciences, Stony Book University, New York - $100,000 for 2 years
"Deficiencies of tRNA maturation and the pathogenesis of mitochondrial diseases."
Dr. Garcia-Diaz will study the regulation of mitochondrial transfer RNAs, molecules that are intrinsic to synthesis of the energy metabolism enzymes in mitochondria.

Ying Dai, MD, PhD, Department of Neurology, Beth Israel Deaconess Medical Center, Boston - $80,000 for 2 years
"Driving Selection Against Heteroplasmic Mitochondrial DNA Mutations by Enhancing Mitophagy."
Dr. Dai hopes to develop a mechanism whereby mitochondria with abnormal mutated mitochondrial DNA will be eliminated from cells, with the goal of restoring normal function.

2011 Clinical Fellowship Training Award

Anna-Kaisa Niemi, MD, PhD, Stanford University Medical Center, Stanford, CA - $75,000 for one year
"Redox imbalance in methylmalonic acidemia mut0 subtype"

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2010 UMDF Grant Recipients

Cornelius Boerkoel, MD, PhD, University of British Columbia, Vancouver - $130,348
Spinocerebellar ataxia with axonal neuropathy: defining the mitochondrial component

Robert Jensen, PhD, Johns Hopkins School of Medicine, Baltimore, MD - $110,000
DCMA and Barth syndromes—similar diseases caused by defects in mitochondrial protein import?

Ingrid Tein, MD, Hospital for Sick Children, Toronto, Canada - $75,000
Pilot study to investigate the efficacy of L-arginine therapy on endothelium-dependent vasodilation & mitochondrial metabolism in MELAS syndrome

2009 UMDF Grant Recipients

Christoph Handschin, PhD, Institute of Physiology, University of Zurich - $130,000
Mitochondrial dysfunction, exercise intolerance and myopathy in skeletal muscle-specific PGC-1a-deficient mice

Michael Murphy, PhD, Medical Research Council Dunn Human Nutrition Unit, Cambridge, UK - $110,000
Development of a Novel Mass Spectrometric Approach to Measure Mitochondrial Oxidative Damage In Vivo

Sarika Srivastava, PhD, Harvard Medical School - $90,804
Investigating the Rescue of Mitochondrial Dysfunction by SIRT1 and Calorie Restriction

Patrick O'Farrell, PhD, Biochemistry & Biophysics, University of California, San Francisco - $81,857
Selecting for Transformation with Mitochondrial

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UMDF Grant Recipients 1998-2008

2008

$126,563	Stuart Smith, PhD, DSc	*Children's Hospital & Research Center at Oakland*
$116,428	Bridget Elizabeth Bax, PhD	*St. George's University of London*
$100,469	Beverly A. Rzigalinski, PhD	*Virginia College of Osteopathic Medicine*
$100,000	Deepa Vinay Dabir, PhD	*University of Helsinki*
$99,998	Timothy E. Shutt, PhD	*University of California - Los Angeles*
$99,998	Sion L. Williams, PhD	*University of Miami*
$99,990	Rebeca Acin-Perex, PhD	*Weill Medical College, Cornell University*
$98,300	Elizabeth Ann Amiott, PhD	*University of Utah*
$83,334	Leo Joseph Pallanck, PhD	*University of Washington*

2007

$157,450	Mindong Ren, PhD	*New York University School of Medicine*
$118,648	Michael King, PhD	*Thomas Jefferson University*
$114,189	Patrice Hamel, PhD	*Ohio State University*
$111,779	Brett H. Graham, MD, PhD	*Baylor College of Medicine*
$110,000	Konstantin Khrapko, PhD	*Beth Israel Deaconess Medical Center/Harvard Medical School*
$100,000	Ludivine Walter, PhD	*Cornell University*
$99,000	Paul Cobine, PhD	*University of Utah*
$98,340	Ann Saada (Reisch), PhD	*Hadassah Hebrew University Medical Center*
$94,481	Tina Wenz, PhD	*University of Miami*
$86,250	Paolo Pinton, PhD	*University of Ferrara*
$60,500	Orly Elpeleg, MD	*Hadassah Hebrew University Medical Center*

2006

$125,000	Brian H. Robinson, PhD	*Hospital for Sick Children, Toronto, Canada*
$125,000	Thomas W. O'Brein, PhD	*University of Florida*
$1122,720	Håkan Westerblad, MD, PhD	*Karolinska Institute, Sweden*
$115,000	Haya Loberboum-Galski, PhD	Hebrew University of Jerusalem
$110,000	Zaza Khuchua, PhD	*Vanderbilt University Medical Center*
$98,500	Stephane Chiron, PhD	*University of California - San Diego*
$98,457	Michael J. Palladino, PhD	*University of Washington DC*
$98,000	Doron Rapaport, PhD	*Institute for Physiological Chemistry, Germany*
$88,850	Vishal Gohil, PhD	*Massachusetts General Hospital*
$43,494	John Gordon Lindsay, PhD	*University of Glasgow*

2005

$ 110,980	Linda Spremulli, PhD	*University of North Carolina, Chapel Hill*
$ 86,455	Jan-Willem Taanman, PhD	*University College London, UK*
$162,878	Patrick Chinnery, PhD	*University of Newcastle upon Tyne*
$126,500	Elena Rugarli, MD	*Telethon Institute of Genetics and Medicine, Naples, Italy*
$94,000	Luca Scorrano, MD, PhD	*Dulbecco-Telethon Institute, Padova, Italy*
$141,027	Michael Frohman, MD, PhD	*Stony Brook University, Stony Brook, NY*
$70,525	Tal Mia Lewin, PhD	*University of North Carolina at Chapel Hill*
$116,133	Mair Churchill, PhD	*University of Colorado Health Sciences Center*
$109,991	Richard Haas, MB, B.Chir.	*University of CA San Diego*

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UMDF Grant Recipients 1998-2008

2004

$128,000	David Chan, MD, PhD	*California Institute of Technology, Pasadena, CA*
$108,305	Miriam Meisler, PhD	*University of Michigan, Ann Arbor, MI*
$99,360	Volkmar Weissig, PhD, ScD	*Northeastern University, Boston, MA*
$90,200	Vamsi Mootha, MD	*Massachusetts Institute of Technology*
$88,852	Joseph Garcia, MD, PhD	*University of Texas SW Medical Center at Dallas,*
$88,000	Stefan Strack, PhD	*University of Iowa, Carver College of Medicine*
$44,000	Brian Robinson, PhD	*Hospital for Sick Children, Toronto, Canada*
$34,179	Gregory Enns, MB, ChB	*Stanford University, Stanford, CA*
$33,776	Ramon Marti, PhD	*Fundacio Institut Hospital Universitari Vall d'Hebron, Barcelona, Spain*

2003

$100,000	Immo Scheffler, PhD	*University of California, San Diego, La Jolla, CA*
$100,000	Mikhail Alexeyev, PhD	*University of South Alabama, Mobile, AL*
$90,000	Matthew Freeman, PhD	*Laboratory of Molecular Biology – M.R.C., UK*
$83,400	Koji Okamoto, PhD	*University of Utah, Salt Lake City, UT*
$76,780	Bernard Lemier, PhD	*University of Alberta, Edmonton, Alberta, Canada*
$50,000	Giovanni Manfredi, MD, PhD	*Weill Medical College of Cornell University*

2002

$81,574	Philip Schwartz, PhD	*Children's Hospital of Orange County*
$66,000	Yidong Bai, PhD	*University of Texas Health Science Center*
$61,389	Tanja Taivassalo, PhD	*Institute for Exercise & Environmental Medicine*
$41,085	Jose Hernandez-Yago, PhD	*Institute for Cell Research, Valencia, Spain*

2001

$33,000	Min-XIn Guan, PhD	*Cincinnati Children's Hospital Medical Center*
$15,000	Brian Robinson, PhD	*The Hospital for Sick Children, Toronto, Canada*
$12,000	Edwin Kirk, MD	*Sydney Children's Hospital, Randwick, New South Wales, Australia*

2000

$36,719	Dikoma Shungu, PhD	*Columbia University*
$18,281	George Perry, PhD	*Case Western Reserve University*

1999

$31,137	Cecilia Giulivi, PhD	*University of Minnesota*
$30,000	John Shoffner, MD	*Horizon Molecular Laboratory*
$8,863	Brian Robinson, PhD	*The Hospital for Sick Children, Toronto, Canada*

1998

$5,000	Carolyn Bay, MD	*Children's Hospital of Washington DC*
$30,000	Richard Boles, MD	*Children's Hospital of Los Angeles*

Notes

Mitochondrial Medicine 2018: Nashville

Abstracts

Notes

Mitochondrial Medicine 2018: Nashville

Abstracts

Abstract #: 2018 PA-0372

Presenter: Yanyan Peng

Authors: Yanyan Peng[1], Deepali N. Shinde[2], Alex C. Valencia[1], Jun-Song Mo[1], Jill Rosenfeld[3], Megan Truitt Cho[4], Adam Chamberlin[2], Zhuo Li[1], Jie Liu[1], Baoheng Gui[1], Rachel Brockhage[1], Alice Basinger[5], Brenda Alvarez-Leon[5], Peter Heydemann[6], Pilar L. Magoulas[3], Andrea M. Lewis[3], Fernando Scaglia[3], Solange Gril[7], Shuk Ching Chong[8], Matthew Bower[9], Kristin G. Monaghan[4], Rebecca Willaert[4], Maria-Renee Plona[10], Rich Dineen[10], Francisca Milan[4], George Hoganson[10], Zoe Powis[2], Katherine L. Helbig[2], Jennifer Keller-Ramey[4], Belinda Harris[11], Laura C. Anderson[11], Torrian Green[11], Stacey J. Sukoff Rizzo[11], Julie Kaylor[12], Jiani Chen[13], Min-Xin Guan[14], Elizabeth Sellars[12], Steven P. Sparagana[15], James B. Gibson[16], Laura G. Reinholdt [11], Sha Tang[2], Taosheng Huang[1]

Institution: [1]Division of Human Genetics, Cincinnati Children's Hospital, Cincinnati, Ohio, USA.
[2]Clinical Genomics, Ambry Genetics, Aliso Viejo, California, USA.
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[4]GeneDx Inc. 207 Perry Pkwy, Gaithersburg, MD, USA.
[5]Department of Metabolic Genetics, Cook Children's Physician Network, Fort Worth, Texas, USA.
[6]Section of Pediatric Neurology, Rush University Medical Center, Chicago, Illinois, USA.
[7]Neuropediatric Department, Raul Carrea Institute for Neurological Research -FLENI Montañeses 2325 (C1428AQK), Argentina.
[8]Department of Pediatrics and Department of Obstetrics & Gynecology, the Chinese University of Hong Kong, Hong Kong, China.
[9]University of Minnesota Medical Center, Fairview Molecular Diagnostics Laboratory Neurology clinic, 420 Delaware Street SE, Minneapolis USA.
[10]University of Illinois at Chicago, Pediatric Genetics, 840 S. Wood St. Chicago, IL USA
[11]The Jackson Laboratory, Bar Harbor, Maine, USA.
[12]Arkansas Children's Hospital, 1 Children's Way, Little Rock, AR USA.
[13]University of Oklahoma Health Sciences Center, 1200 Children's Ave, Oklahoma City, OK USA.
[14]Institute of Genetics, Zhejiang University, Hangzhou, China.
[15]Pediatric Neurology, Texas Scottish Rite Hospital for Children, Dallas, Texas, USA.
[16]Dell Children's Medical Center, 1301 Barbara Jordan Boulevard, Austin, TX, USA.

Title: Biallelic mutations in the ferredoxin reductase gene cause novel mitochondriopathy with optic atrophy

Body of Abstract: Iron-sulfur (Fe-S) clusters are ubiquitous cofactors essential to various cellular processes, including mitochondrial respiration, DNA repair, and iron homeostasis. A steadily increasing number of disorders are being associated with disrupted biogenesis of Fe-S clusters. Here, we conducted whole-exome sequencing of patients with optic atrophy and other neurological signs of mitochondriopathy and identified 17 individuals from 13 unrelated families with recessive mutations in FDXR, encoding the mitochondrial membrane-associated flavoprotein ferrodoxin reductase required for electron transport from NADPH to cytochrome P450. In vitro enzymatic assays in patient fibroblast cells showed deficient ferredoxin NADP reductase activity and mitochondrial dysfunction evidenced by low oxygen consumption rates (OCRs), complex activities, ATP production and increased reactive oxygen species (ROS). Such defects were rescued by overexpression of wild-type FDXR. We also found that the evolutionarily conserved FDXR mutations caused iron overload, dissipation of mitochondrial membrane potential and inhibition of cell proliferation. Moreover, we found that mice carrying a spontaneous mutation allelic to the most common mutation found in patients displayed progressive gait abnormalities and vision loss, in addition to biochemical defects consistent with the major clinical features of the disease. Compound muscle action potentials in peripheral nerves showed peripheral neuropathy associated with degeneration and demyelination in axons. Taken together, these data provide the first demonstration that germline, hypomorphic mutations in FDXR cause a novel mitochondriopathy and optic atrophy in humans via iron homeostasis.

Mitochondrial Medicine 2018: Nashville

Abstracts

Abstract #: 2018 PA-0374

Presenter: Raphael J. Morscher

Authors: Raphael J. Morscher[1,2,3], Gregory S. Ducker[1,2], Sophia Hsin-Jung Li[4], Johannes A. Mayr[5], Zemer Gitai[4], Wolfgang Sperl[5] & Joshua D. Rabinowitz[1,2]

Institution: [1]Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, New Jersey 08544, USA; [2]Department of Chemistry, Princeton University, Princeton, New Jersey 08544, USA; [3] Current address: University Children´s Hospital Zürich Zurich – Eleonore Foundation, Zürich 8032, Switzerland; [4]Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544, USA; [5]Department of Pediatrics, Salzburger Landeskliniken and Paracelsus Medical University, Salzburg 5020, Austria.

Title: Mitochondrial translation requires folate-dependent tRNA methylation

Body of Abstract: Mitochondrial co-factor utilization and protein expression have recently developed into highly dynamic fields within the study of inborn errors of mitochondrial metabolism. Here we present an unexpected link between the two fields, identifying mitochondrial folate one-carbon (1C) activation as being essential for mitochondrial translation.

The essential vitamin folate is well known for its role in the activation and transfer of 1C units for the biosynthesis of purines, thymidine and methionine. Studies on mitochondrial folate enzymes have thus focused on their support of anabolic metabolism in the cytosol of proliferating lymphocytes and human cancers. The full range of uses of folate-bound one-carbon units in the mitochondrial compartment itself, however, has not been explored. When characterizing a set of human CRISPR-deletion cell lines lacking folate 1C enzymes, we serendipitously discovered that loss of catalytic activity of the mitochondrial folate enzyme serine hydroxymethyltransferase 2 (SHMT2), but not of other folate enzymes, leads to a combined respiratory chain deficiency phenotype. We find that SHMT2, by generating mitochondrial 5,10-methylenetetrahydrofolate, provides methyl donors to produce the taurinomethyluridine base at the wobble position of select mitochondrial tRNAs. Mitochondrial ribosome profiling in SHMT2-knockout cells reveals that the lack of this modified base causes defective translation, with preferential mitochondrial ribosome stalling at certain lysine (AAG) and leucine (UUG) codons. This results in the impaired expression of respiratory chain subunits. Stalling at these specific codons also occurs in certain inborn errors of mitochondrial metabolism, either directly affecting enzymes catalyzing the modification reaction (MTO1) or mt-DNA mutations precluding tRNA modification (MT-TL1/MELAS). Disruption of whole-cell folate metabolism, by either folate deficiency or antifolate treatment, also impairs the respiratory chain.

In summary, mammalian mitochondria use folate-bound one-carbon units to methylate tRNA, and this modification is required for mitochondrial translation and thus oxidative phosphorylation.

Abstract #: 2018PA-0375

Presenter: Nahid A. Khan

Authors: Nahid A Khan[1], Eija Pirinen[1], Ilse Paetau[1], Riikka Kivelä[2], Vidya Velagapudi[3], Johan Auwerx[4] and Anu Suomalainen[1,5]

Institution : [1]Research Programs Unit, Molecular Neurology, University of Helsinki, 00290 Helsinki, Finland, [2]Research Programs Unit, Translational Cancer Biology, University of Helsinki, 00290 Helsinki, Finland, [3]Metabolomics Unit, Institute for Molecular Medicine Finland, University of Helsinki, 00290 Helsinki, Finland, [4]Laboratory of Integrative Systems Physiology, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland,[5]Department of Neurology, Helsinki University Hospital, 00290 Helsinki, Finland.

Title: Pharmacological Inhibition of Poly(ADP-Ribose) in a mouse model of mitochondrial myopathy

Body of Abstract: Mitochondrial disorders are the most common group of inherited neurometabolic diseases. These disorders manifest with respiratory chain deficiency (RCD), and lead to a multitude of clinical manifestations. Despite their progressive and often fatal outcome, no curative treatment is available. Therapy trials have been hampered by the absence of patient groups with homogenous genetic and clinical

presentations. We have previously generated Deletor mice, with adult-onset mitochondrial myopathy (MM). These mice carry a dominant patient mutation in the mitochondrial replicative helicase, Twinkle, resulting in progressive MM after 12 months of age. They accumulate multiple mtDNA deletions in skeletal muscle and brain, leading to subtle progressive respiratory chain deficiency (RCD). The histological findings and physiological responses in Deletors mimic closely those of patients with the same mutation, making these mice a valuable model for therapy trials. We have shown that vitamin B3, which acts as NAD+ booster, can efficiently delay the progression of mitochondrial myopathy in Deletor mice and human patients. As a second strategy, inhibition of poly ADP ribose polymerases (PARP), DNA repair enzymes that utilize NAD+ pools, have also been shown to increase NAD+ levels and delay disease progression in structural defects of the respiratory chain. We found PARPs to be increased in Deletor muscle, and asked whether inhibiting PARPs that have an important role in DNA damage detection and repair is safe and beneficial also in diseases with mtDNA instability. We applied PARP inhibitor to Deletor mice, and tested the treatment effect. Here we report the consequences of this strategy in a disorder mitochondrial myopathy and mtDNA instability.

Abstract #: 2018 PA-0377

Presenter: Shereen Ghosh

Authors: Shereen Ghosh

Institution: UC San Diego, California

Title: Molecular mechanisms of the ADPRHL2-mediated pediatric neurodegenerative disease

Body of Abstract: ADP-ribosylation is a posttranslational modification of proteins characterized by the addition of poly-ADP-ribose (PAR) in response to cellular stressors, such as excitotoxicity or oxidative stress. The PAR Polymerase (PARP) gene family catalyzes this reaction, which is reversed by two known genes, Poly-ADP Ribose Glycohydrolase (PARG) and ADP-ribosylhydrolase-like protein 2 (ADPRHL2, aka ARH3). Using genome-wide linkage analysis and exome sequencing, inactivating mutations in ADPRHL2 have been identified in several families. The patients exhibit a pediatric onset neurodegenerative disorder with brain atrophy and sudden death from epilepsy. Previous studies have identified ADPRHL2 as the only active glycohydrolase present in mitochondria; however, its importance and role in the mitochondria remain understudied. Until now, ADPRHL2 has not been implicated in any disease. The identification of homozygous loss-of-function mutations indicates a requirement for its function in neuronal homeostasis, specifically as it relates to mitochondrial function. The generation of iPSCs from patient fibroblasts, which can be further reprogrammed into a neural differentiation lineage, offers an exciting and relevant tool to test our model. Patient cells lack ADPRHL2 protein, display increased basal and stress-associated PAR levels, and enhanced susceptibility to cell death following insult. PARP inhibitors, already in clinical trials, rescued lethality, suggesting that this class of drugs may be used to treat this lethal disorder. The goal is to describe this clinical condition as a new syndrome cause of neurodegeneration and study oxidative-stress-induced mechanisms by which loss of ADPRHL2 promotes cell death both in vitro and in vivo. The outcome of this work will not only provide novel insight into mitochondrial function, but will also identify potential treatments for a new early-onset neurodegenerative disease.

Abstract #: 2018 PA-0378

Presenter: Min Liang

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310058, China.

Title: Biochemical evidence for a mitochondrial genetic modifier in the phenotypic manifestation of Leber's hereditary optic neuropathy-associated mitochondrial DNA mutation

Body of Abstract: Leber's hereditary optic neuropathy (LHON) is the most common mitochondrial disease. Mitochondrial modifiers are proposed to modify the phenotypic expression of primary LHON-associated mitochondrial DNA (mtDNA) mutations. In this study, we demonstrated that the LHON susceptibility allele (m.14502T>C, p. 58I>V) in the ND6 gene modulated the phenotypic expression of primary LHON-associated m.11778G>A mutation. Twenty-two Han Chinese pedigrees carrying m.14502T>C and m.11778G>A mutations exhibited significantly higher penetrance of optic neuropathy than those carrying only m.11778G>A mutation. We performed functional assays using the cybrid cell models, generated by fusing mtDNA-less ρo cells with enucleated cells from LHON patients carrying both m.11778G>A and m.14502T>C mutations, only m.14502T>C or m.11778G>A mutation and a control belonging to the same mtDNA haplogroup. These cybrids cell lines bearing m.14502T>C mutation exhibited mild effects on mitochondrial functions compared with those carrying only m.11778G>A mutation. However, more severe mitochondrial dysfunctions were observed in cell lines bearing both m.14502T>C and m.11778G>A mutations than those carrying only m.11778G>A or m.14502T>C mutation. In particular, the m.14502T>C mutation altered assemble of complex I, thereby aggravating the respiratory phenotypes associated with m.11778G>A mutation, resulted in a more defective complex I. Furthermore, more reductions in the levels of mitochondrial ATP and increasing production of reactive oxygen species were also observed in mutant cells bearing both m.14502T>C and m.11778G>A mutation than those carrying only 11778G>A mutation. Our findings provided new insights into the pathophysiology of LHON that were manifested by interaction between primary and secondary mtDNA mutations.

Abstract #: 2018 PA-0379

Presenter: Juanjuan Zhang[1]

Authors: Juanjuan Zhang[1,2,3,4], Yanchun Ji[1,2], , Yuanyuan Lu[2,3,4], Runing Fu[3,4], Man Xu[3,4], Xiaoling Liu[3,4] and Min-Xin Guan[1,2, 3,4,*]

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Title: Leber's hereditary optic neuropathy (LHON)-associated ND5 12338T>C mutation altered the assembly and function of complex I, apoptosis and mitophagy

Body of Abstract: Mutations in mitochondrial DNA (mtDNA) have been associated with Leber's hereditary optic neuropathy (LHON) and their pathophysiology remains poorly understood. In this study, we demonstrated that a missense mutation (m.12338T>C, p.1M>T) in the ND5 gene contributed to the pathogenesis of LHON. The m.12338T>C mutation affected the first methionine (Met1) with a threonine and shortened two amino acids of ND5. We therefore hypothesized that the mutated ND5 perturbed the structure and function of complex I. Using the cybrid cell models, generated by fusing mtDNA-less (ρo) cells with enucleated cells from LHON patients carrying the m.12338T>C mutation and a control subject belonging to the same mtDNA haplogroup, we demonstrated that the m.12338T>C mutation caused the reduction of ND5 polypeptide, perturbed assemble and activity of complex I. Furthermore, the m.12338T>C mutation caused respiratory deficiency, diminished mitochondrial ATP levels and membrane potential, and increased the production of reactive oxygen species. The m.12338T>C mutation promoted apoptosis, evidenced by elevated release of cytochrome c into cytosol and increased levels of apoptosis-activated proteins: caspases 9, 3, 7 and PARP

in the cybrids carrying the m.12338T>C mutation, as compared to control cybrids. Moreover, we also document the involvement of m.12338T>C mutation in decreased mitophagy, as showed by reduced levels of autophagy protein LC3 and accumulation of autophagic substrate p62 in the in mutant cybrids as compared with control cybrids. These data demonstrated the direct link between mitochondrial dysfunction caused by complex I mutation and apoptosis or mitophagy. Our findings may provide new insights into the pathophysiology of LHON.

Abstract #: 2018 PA-0380

Presenter: Marilena D'Aurelio

Authors: Qiuying Chen[2], Steven S. Gross[2], Giovanni Manfredi[1], Marilena D'Aurelio[1].

Institution: Weill Cornell Medicine, New York, NY 10065, [1]Feil Family Brain and Mind Research Institute; [2]Department of Pharmacology.

Title: Glutamate anaplerosis as a mechanism of metabolic adaptation in mitochondrial diseases

Body of Abstract: Mitochondrial diseases are heterogeneous genetic disorders caused by impaired oxidative phosphorylation (OXPHOS). Although the genetic and bioenergetic defects are known, many aspects of mitochondrial disease pathogenesis are yet to be elucidated. Currently, there are no effective treatments for mitochondrial diseases, due to the limited understanding of the metabolic consequences of OXPHOS defects and the lack of defined targets.

Using untargeted stable isotope tracing approaches, we identified an increased glutamine-derived alpha-ketoglutarate (alpha-KG) anaplerotic flux in mitochondrial DNA (mtDNA) mutant cells that harbor human disease-associated OXPHOS defects. We determined that glutamine-glutamate anaplerosis provides an alternative energy-generating mechanism for mutant cells that can be metabolically stimulated to rescue energy stores and cell survival in otherwise lethal oxidative conditions. We demonstrated that this energy generating alpha-KG oxidative flux prevails over the anabolic reductive carboxylation flux when the residual mitochondrial respiration in mitochondrial DNA mutant cells exceeds 45% of control levels.

Increased glutamate anaplerosis was also identified in vivo, in a mouse model of OXPHOS dysfunction and mitochondrial myopathy, the muscle-specific COX10 KO mouse. In this mouse, increased oxidative alpha-KG flux in muscle arises from enhanced alanine synthesis and release into blood, concomitant with accelerated amino acid catabolism from protein breakdown. Interestingly, the resulting intramuscular amino acid imbalance was normalized by supplementation of dimethyl alpha-ketoglutarate a membrane permeable analog of alpha-KG.

Similarly, in muscle of patients with severe mitochondrial myopathy associated with Myoclonus Epilepsy and Ragged Red Fibers (MERRF) we found increased glutamate anaplerosis and increased alanine synthesis and release. Our hypothesis is that in mitochondrial disorders, increased glutamate flux into the TCA cycle is a metabolic adaptation that affects the amino acid metabolism and alters pH and ammonia homeostasis, contributing to disease pathogenesis. Our work provides novel mechanistic links between bioenergetic defects and dysmetabolism and supports the rationale for dietary supplementation with glutamate derivatives in mitochondrial diseases.

Abstract #: 2018 PA-0382

Presenter: Yudong Wang, PhD

Authors: Yudong Wang[1], Johan Palmfeldt[2], Neils Gregersen[2], Alexander Makhov[3], James F. Conway[3], Meicheng Wang[4], Stephen P. McCalley[5], Shrabani Basu[5], Hana Alharbi[1], Claudette St. Croix[6], Mike Calderon[6] Xuemei Zeng[6], Simon Watkins[6], Nathan Yates[6], and Jerry Vockley[1,5,7]

Mitochondrial Medicine 2018: Nashville

Abstracts

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Title: Characterizing the Molecular Architecture of Mitochondrial Energy Metabolism

Body of Abstract: In mitochondrial energetics, the electron transfer equivalents generated from fatty acid oxidation (FAO), QH2 and NADH, are subsequently transferred as substrates to the electron transport chain (ETC). Supercomplexes (SC), separated from mammalian cells, contain complex I, III, and IV. SC shows the importance in stabilizing individual complexes and enhances the electron transfer efficiency functions. We have recently shown that many of the functions of fatty acid oxidation are also contained in SC. Also, we shown SC display electron transfer start from oxidation of long chain CoA and to reduce cytochrome c, the electron acceptor of ETC. The activity of oxidation of C16 CoA is about 2.6 times higher by SC than pure VLCAD. Currently we provide more evidence to show the physical interaction between FAO and ETC. Cross link plus Co-IP shown two physical associations point between FAO and ETC. (a) ETFDH physically interacts with com III at Q reduction side. By means of a connecting com III ETFDH indirectly binds to com I in SC through com III. (b) Trifunctional protein (TFP) associates with NADH biding domain of com I. The reducing equivalents, QH2 and NADH, from FAO enter ETC at the level of complexes III and I respectively. The physical interactions would prevent the QH2 and NADH from be oxidized during transportation. Funding the interaction between FAO and ETC reveal (a) individual energy metabolism pathways physically linked to each other for the electron transfer substrate and product transportation to meet the needs of the most efficient metabolism. (b) The abnormal of the enzyme in one pathway would affect the other as the existing linkages among pathways.

Patients with deficiencies of either FAO or OXPHOS often show clinical and/or biochemical findings indicative of a disorder of the other pathway. Genetic disorders of FAO and OXPHOS are among the most frequent inborn errors of metabolism. Patients with deficiencies of either FAO or OXPHOS often show clinical and/or biochemical findings indicative of a disorder of the other pathway.

Abstract #: 2018 PA-0383

Presenter: Olga Zurita Rendón[1]

Authors: Olga Zurita Rendón[1,2*], Eric Fredrickson[2*], Conor Howard[3,5,6*], Christopher Hill[2], Adam Frost[2,3,6], Jared Rutter[1,2]

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Title: The stress-responsive mitochondrial protein, Vms1, is a release factor for the Ribosome Quality control Complex

Body of Abstract: The majority of the nuclear-encoded mitochondrial genome is imported into mitochondria in a co-translational fashion, where cytosolic ribosomes associate with the Translocase of the Outer mitochondrial Membrane (TOM) complex. Therefore, understanding the mechanisms that secure mitochondrial translation fidelity is key for the maintenance of mitochondria biogenesis and health.

Defects that impair the proper read-out of an mRNA during translation can cause ribosomes to stall. To prevent the accumulation of potentially deleterious nascent chains (NC) and to maintain ribosome homeostasis, eukaryotic cells employ surveillance and clearance mechanisms, including the Ribosome Quality control (RQC) Complex. In yeast, the RQC is comprised of the E3 ubiquitin ligase Ltn1, the ATPase Cdc48 and the novel proteins Rqc1 and Rqc2—whose homologs are Listerin, VCP/p97, TCF25, and NEMF, respectively in humans. The RQC assembles on 60S ribosomal subunits containing incomplete polypeptides linked to a tRNA. Rqc2 non-canonically synthesizes the

C-term addition of poly-Ala and Thr extensions (CAT-tails) to the NC. A primary function of CAT-tailing is to expose from the exit tunnel Lys residues in the NC allowing ubiquitination by Ltn1, which stimulates Cdc48-dependent degradation. If the proteasome system is overwhelmed, CAT-tails mediate the formation of aggregates. A primary unanswered question concerns the identity of the hydrolase that liberates ubiquitinated and CAT-tailed NCs from the tRNA for degradation.

In this work, we show that the mitochondrial stress-responsive protein, Vms1, genetically interacts with all RQC components and the mRNA degradation protein, Ski7. Vms1 physically associates with Rqc2 and the 60S and prevents the accumulation/aggregation of cytosolic and mitochondrial reporters engineered to stall translation. Finally, our high-resolution structural data reveals that Vms1 harbors an eRF1 release factor-like domain that is required for both the genetic functions in living cells and release factor enzymatic activity in vitro. Our data demonstrate that Vms1 plays an essential role in the RQC pathway by catalyzing the hydrolysis of stalled peptidyl-tRNA species.

Abstract #: 2018 PA-0384

Presenter: Keshav K. Singh

Authors: Bhupendra Singh[1], Trenton R. Schoeb[1], Prachi Bajpai[1], Andrzej Slominski[2], Keshav K. Singh[1]*

Institution: Departments of Genetics[1] and Dermatology[2], School of Medicine, University of Alabama at Birmingham, Kaul Genetics Building, Suite 620, 720 20th St. South, Birmingham, AL, USA 35294,

Title: Reversing Wrinkled Skin and Lost Hair in Mice by Restoring Mitochondrial Function

Mitochondrial DNA (mtDNA) depletion impairs mitochondrial function that leads to mtDNA depletion syndrome (MDS). The MDSs are a heterogeneous group of disorders, characterized by low mtDNA levels in specific tissues. In different target organs, mtDNA depletion presents specific phenotype. Furthermore, mtDNA copy number declines with age, and such changes increase the risk for age-associated diseases. The causative association of the decline in mtDNA copy number in aging and aging-related diseases, however, has not been addressed.

To evaluate the consequences of depletion of mtDNA in the whole animal, we created an inducible mouse (mtDNA-depleter) expressing, in the polymerase domain of POLG1, a dominant-negative mutation to induce depletion of mtDNA in different tissues. These mice showed reduced mtDNA content, changes in mitochondrial protein expression and reduced stability of mitochondrial oxidative phosphorylation complexes. We demonstrate that ubiquitous depletion of mtDNA in mice has profound and predominant effects on the skin resulting in wrinkles and hair loss. Development of skin wrinkles was associated with the hyperproliferation of epidermis, increased expression of MMPs and decreased expression of TIMP1. We also found increased inflammation that may be an underlying contributing factor in phenotypic changes in the skin. Histopathologic analyses revealed dysfunctional hair follicles. The mice also showed changes in expression of aging-associated markers including IGF1R, KLOTHO, VEGF, and MRPS5. The rescue experiment revealed that, by turning off the mutant POLG1 transgene expression and restoring the mtDNA content to the wild-type level in the whole animal (mtDNA-repleter) the skin and hair phenotypes revert to normal. These studies present first *in vivo* evidence that the skin wrinkles and loss of hair can be reversed by restoring mitochondrial function.

Together, we have developed a mtDNA-depleter and repleter mouse model. This mouse will provide an unprecedented opportunity to achieve tissue-specific modulation of mitochondrial functions to determine, for various tissues and organs, the role of mitochondria in vivo and expand our knowledge of how mitochondria contribute to the pathogenesis of MDS and other human diseases beyond their well-established roles in metabolism and cell death. The development of mtDNA-depleter and repletor mouse would also provide an impetus to the research about the development of preventative and therapeutic strategies to augment the mitochondrial functions for the treatment of mitochondrial and mitochondria related diseases.

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Abstract #: 2018-PA-0385

Presenter: Anne Chiaramello

Authors: Martine Uittenbogaard[1], Lee-Jun Wong[2], Andrea Gropman[3], Anne Chiaramello[1]

Institution: [1]George Washington University School of Medicine and Health Sciences, Department of Anatomy and Regenerative Biology, Washington, D.C. 20037,[2]Baylor College of Medicine, Department of Molecular and Human Genetics, Houston, TX, 77030, [3]Children's National Medical Center, Division of Neurogenetics and Developmental Pediatrics, Washington, D.C. 20010

Title: Dysregulation of Energy Reprogramming by Mitochondrial Pathogenic Variants

Body of Abstract: The cardinal feature of mitochondrial respiratory disorders is chronic energy deficit due to mutations in the nuclear and/or mitochondrial genome affecting the oxidative phosphorylation (OXPHOS) responsible for ATP synthesis. Mitochondrial DNA mutations only affect a subset of the multi-copy mitochondrial genome, causing heteroplasmy that in part dictates the clinical phenotype, the disease severity and the age of onset. Currently, no therapeutic options are available to these patients and palliative therapies fail to prevent progression of these diseases. Therefore, understanding the metabolic consequences due to pathogenic mitochondrial DNA variants may improve the design of therapeutic strategies. We investigated the comprehensive metabolic signature of three pathogenic mitochondrial variants targeting different OXPHOS complexes. The m.3243 A>G variant causing MELAS (Mitochondrial Encephalopathy with Lactic Acidosis and Stroke-like episodes) affects both the first and third OXPHOS complexes, with a bigger impact on complex I due to its high leucine content, with complex I being one of the two points of entry for electrons to create the electrochemical proton gradient necessary for ATP synthesis by complex V. The m.11778 A>G variant linked to LHON-MS (Leber's Hereditary Optic Neuropathy with Multiple Sclerosis-like) affects the ND4 subunit of complex I. The m.8993 T>G variant responsible for MILS (Maternally Inherited Leigh Syndrome) affects the subunit 6 of complex V, the last OXPHOS complex, which is essential for linking ATP synthesis to the electrochemical proton gradient. We used patient-derived fibroblasts to investigate their metabolic consequences by performing three distinct, but complementary Seahorse-based bioenergetics assays: Mitochondrial Stress Test, Glycolysis Stress Test, and Glycolytic Rate Test. All tested patients harboring one of the three pathogenic variants exhibited clinical symptoms compatible with their respective disease. Heteroplasmy levels were accurately quantified by the long-range PCR-based next generation sequencing method. Even though all three pathogenic variants impaired the mitochondrial OXPHOS pathway, they each lessened the maximal rate of oxidative phosphorylation and the spare respiratory capacity to varying degrees when compared to a healthy subject. Surprisingly, all three variants altered the glycolytic pathway by curtailing the glycolytic capacity and reserve. Moreover, they not only reduced the basal glycolytic rate, but also stunted the compensatory glycolysis response. Thus, proband's fibroblasts harboring one of the three mtDNA variants exhibit a defective metabolic switch from OXPHOS to glycolysis under conditions simulating an acute ATP crisis. In conclusion, our study provides a novel metabolic perspective of these three pathogenic mtDNA variants vis-à-vis dysregulation of energy reprogramming as a result of a defective interplay between OXPHOS and glycolysis.

Abstract #: 2018-PA-0386

Presenter: Andrea Gropman

Authors: Anne Chiaramello[1], Martine Uittenbogaard[1], Andrea Gropman[2]

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Title: Novel Metabolic Signatures of Szt2 Variants of Uncertain Clinical Significance and its role in mitochondrial homeostasis in a Patient with Epileptic Encephalopathy

Body of Abstract: With the advent of whole exome sequencing, variants of uncertain clinical significance are more frequently discovered in undiagnosed patients with a suspected mitochondrial disease. Our study pertains to a four-year-old patient with severe developmental

delay, hypotonia, and cognitive deficiencies who suffered a hypoxic-ischemic event accompanied by seizures one hour after birth. An electroencephalogram (EEG) indicated excessive left and right frontal sharp wave discharges, while brain MRI showed increased T1 signal in the basal ganglia with possible involvement of the globus pallidum. At the age of three, his encephalopathy has worsened, resulting in physical and cognitive regression. The patient, has not gained weight for the last two years even though he is G-tube fed. In the absence of a definite clinical diagnosis, the proband was genetically tested by whole exome sequencing (WES) and next-generation sequencing (NGS) of the mitochondrial genome. While no mutations, deletions and duplications were detected in his mitochondrial genome, WES revealed two compound recessive nuclear mutations mapping in the Szt2 (Seizure Threshold 2) gene associated with epileptic encephalopathy. The c.5174 C>T (p.A1725V) is maternally inherited, while the c.5949_5951del TGT (p.V1984del) is paternally inherited. Both are variants of uncertain clinical significance that map in a non-conserved domain of the SZT2 protein. The maternally inherited substitution variant maps in exon 34, while the paternally inherited in-frame three-base-pair-deletion variant maps in exon 37. Despite being highly conserved in evolution, the function of the SZT2 protein has remained elusive until recently. The SZT2 protein is a component of the KICSTOR complex involved in modulating the activity of the mechanistic target of rapamycin complex I (mTORC1), known to regulate mitochondrial biogenesis and respiration. Thus, we initially performed a mitochondrial morphometric analysis by transmission electron microscopy, which revealed ultrastructural alterations of mitochondria. The proband's fibroblasts exhibited an exaggerated mitochondrial elongation, suggestive of dysregulated mitochondrial dynamics. Furthermore, mitochondria displayed a weak electron density in their mitochondrial matrix, indicative of impaired mitochondrial metabolic functions. Using a Seahorse-based Mitochondrial Stress Test assay, we found that these two Szt2 variants significantly repressed several bioenergetics OXPHOS parameters, such as basal and ATP-linked respiration. More critically, they exhausted the spare respiratory capacity, rendering the proband's fibroblasts nearly incapable of generating ATP during an acute energy crisis. Using the Glycolytic Rate Test, we investigated whether the proband's fibroblasts could meet the energy demand via glycolysis. We detected a decrease in basal glycolysis and compensatory glycolysis response, highlighting a decreased ability to readily switch from OXPHOS to glycolysis. In conclusion, our mitochondrial morphometric and metabolic analyses reveal the first evidence of pathological consequences from these two Szt2 variants, until now characterized as variants of uncertain clinical significance.

Abstract #: 2018 PA-0388

Presenter: Robert L. Elliott

Authors: Robert L. Elliott, Xian-Peng Jiang, Catherine C. Baucom

Institution: Sallie A. Burdine Breast Foundation, Baton Rouge, Louisiana 70806, USA

Title: Antibiotic abuse causing modern plagues: "mitochondrial dysfunction promoting neurodegeneration and tumorigenesis"

Body of Abstract: According to the endosymbiotic theory, mitochondria originated from free-living, aerobic bacteria. It is inevitable that antibiotics target mitochondria and mitochondrial components in mammalian cells. Azithromycin and doxycycline target bacterial ribosomes, while ciprofloxacin suppresses DNA gyrases. In this study, we examined the effect of these three antibiotics on mitochondrial function and metabolism of human normal mammary epithelial MCF-12A and mouse motor neuronal NSC-34 cells. Mitochondrial morphology was examined by electron and fluorescent microscopy. Mitochondrial membrane potential gradient and reactive oxygen species (ROS) were studied by fluorescent probe staining with fluorescent microscopy. Cell metabolism gene expression was measured by quantitative real time PCR (polymerase chain reaction). Antibiotics inhibited the proliferation and mitochondrial membrane potential (JC-1 aggregates with bright red fluorescence) of MCF-12A and NSC-34 cells. Ultrastructural microscopy revealed that antibiotics cause vacuolated, swollen mitochondria with disrupted cristae. MCF-12A cells were treated with IC_{50} concentration of antibiotics for 3 hours. The antibiotics significantly or completely suppressed mitochondrial membrane potential. However, surviving cells recover from antibiotic inhibition and regain red JC-1 fluorescence at 48 hours and 7 days of antibiotic incubation. All three antibiotics increased mitochondrial ROS production (staining with MitoSOX Red) during both mitochondrial suppression and activity. Addition of human superoxide dismutase to the antibiotic culture compartment significantly decrease the MitoSOX red staining of MCF-12A cells. Gene expression of cell metabolism confirmed the microscopy findings. Three hours of antibiotic treatment caused decreased gene expression of mitochondrial oxidative phosphorylation (OXPHOS) enzymes, MT-ATP6 (mitochondrially encoded ATP synthase 6), MT-CYB (mitochondrially encoded cytochrome b), MT-CO1 (mitochondrially encoded

cytochrome C oxidase 1) and MT-ND1 (mitochondrially encoded NADH dehydrogenase 1). After the initial stage of mitochondrial inhibition, MCF-12A cells gradually upregulate gene expression of HIF1-a (hypoxia inducible factor 1- alpha), MT-ATP6, MT-CYB, MT-CO1, MT-ND1, and mitochondrial biogenesis transcription factors of PGC1a (peroxisome proliferator activated receptor gamma coactivator-1-alpha), NRF1 and NRF2 (nuclear respiratory factor 1 and 2) and Tfam (transcription factor A, mitochondrial). The expression of glycolysis related genes such as HK2 (hexokinase), PFKM (phosphofructokinase 1), PKM2 (pyruvate kinase) and LDHA (lactate dehydrogenase A) are also increased. Gene expression levels reach to peak levels at 7 days of antibiotic treatment. Antibiotics induced aerobic glycolysis and increased glucose transporter gene expression. In response to ROS overproduction, MCF-12A cells upregulated gene expression of several antioxidants, catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPX). However, the response was much slower than ROS production induced by antibiotics. Antibiotics directly suppress mitochondria and cause ROS-associated damage of genomic and mitochondrial DNA and cell components. Glycolysis and DNA damage caused by antibiotics may contribute to neurodegeneration, tumorigenesis, and aggravate existing mitochondrial diseases.

Abstract #: 2018 PA-0390

Presenter: Maxim Jestin

Authors: Maxim Jestin[1], Senta Kapnick[1], Tatyana Tarasenko[1], Patricia Zerfas[2], Francisca Diaz[3], Peter J. McGuire[1]

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Title: TNFα mediates hepatic metabolic decompensation due to influenza in a mouse model of mitochondrial hepatopathy

Body of Abstract: Mitochondrial hepatopathy is estimated to occur in up to 20% of patients with mitochondrial disease and can present as hepatic steatosis, cholestasis, or even neonatal liver failure. Patients with mitochondrial hepatopathy are susceptible to potentially fatal episodes of metabolic decompensation which can manifest as hypoglycemia, lactic acidosis or organ failure as a result of an underlying precipitant. Although infection is known to be a common trigger of metabolic decompensation, the mechanisms underlying the disruption of metabolic equilibrium during infection is not clear. Previously, our group has shown that the immune system modulates hepatic metabolism during systemic immune activation as a result of viral infection. To examine the role of infection-induced immune activation in precipitating metabolic decompensation in mitochondrial hepatopathy, we infected a mouse model of mitochondrial hepatopathy (LivCox10$^{-/-}$) with mouse-adapted influenza (H1N1 PR8). In response to infection, LivCox10$^{-/-}$ mice developed elevated blood transaminases and lactate, grossly-enlarged and abnormally shaped mitochondria in hepatocytes, and hepatic steatosis. Serum cytokine profiling of influenza-induced immune response revealed significant elevations of TNFα. Given the role of TNFα in modulating hepatic metabolism, we targeted this pro-inflammatory cytokine in vivo using etanercept, a TNFα antagonist, during infection. In response, LivCox10$^{-/-}$ mice demonstrated an improvement in hepatic phenotype indicated by a reduction in lipid accumulation and restoration of mitochondrial morphology. Our findings suggest that during influenza infection, TNFα released during immune activation exacerbates underlying metabolic dysfunction in mitochondrial hepatopathy. The resolution of metabolic perturbations by etanercept suggests that targeting the immune system may be a viable strategy for alleviating the complications associated with metabolic decompensation.

Abstract #: 2018 PA-0391

Presenter: Sumit Parikh

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Title: Advances in Development of a Mitochondrial Disease Care Network (MCN) in the US

Body: The Mitochondrial Medicine Society and US-based Patient Advocacy Groups are collaborating to form a clinical care network to formally unify US-based clinicians who provide medical care to individuals with mitochondrial disease; to define, design and implement best practices in mitochondrial medicine building on current consensus guidelines for mitochondrial diagnosis and care; and to optimize management and care to improve patient outcomes.

The model for these centers is based on other previously created expert rare disease centers. While clinical research is a crucial part of the endeavor, the primary goal will be optimizing mitochondrial disease clinical care including proper evaluation and diagnosis and comprehensive multi-disciplinary care.

We previously highlighted the current clinical landscape and physician practice patterns of mitochondrial medicine in the US, the MMS's attempt at developing consensus criteria for diagnosis and care and the patient's need for improved coordinated care.

Here we review the steps taken after the initial review of MCN goals and expectations by stake-holders and showcase advances made in defining the MCN over the past year with development of a Request-For-Applications, review of initial applications and expected launch of a network in 2018.

Abstract #: 2018 PA-0393

Presenter: Rocio Rius

Authors: Rocio Rius[1,2], Alison G. Compton[1,2], Hayley S. Mountford[1,2,3], S Thirukeswaran[1,2], Nicole J. Lake[1,2], Sarah E. Calvo[4,5,6], Vamsi K. Mootha[4,5,6], John Christodoulou[1,2,7], David R. Thorburn[1,2,7]

Institution: [1]Murdoch Children's Research Institute, Melbourne, Australia, [2]Department of Paediatrics, University of Melbourne, Melbourne, Australia, [3]Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, UK [4]Howard Hughes Medical Institute and Department of Molecular Biology, Massachusetts General Hospital, Boston, USA , [5]Department of Systems Biology, Harvard Medical School, Boston, USA, [6]Broad Institute, Cambridge, USA, [7]Victorian Clinical Genetics Services, Royal Children's Hospital, Melbourne, Australia.

Title: The epidemiology and natural history of pediatric mitochondrial diseases – a population-based study.

Body of Abstract: The epidemiological impact of mitochondrial diseases in childhood is currently not well understood. Given that population-based studies are limited the estimated prevalence has been an evolving number.

Aims: (1) To describe the clinical and molecular diagnoses of patients with mitochondrial diseases in childhood and to identify potential factors influencing the natural history. (2) To estimate the minimum birth prevalence (MBP) of pediatric mitochondrial disorders and of the different subgroups.

Methods: We retrospectively analyzed demographic, clinical, biochemical, and molecular information on known pediatric mitochondrial disease cases in south-eastern Australia between 1987 and 1996.

Results: During the 10-year study period, there were 111 patients with a definite diagnosis of mitochondrial disease using the Bernier criteria with disease onset by age 16 years.

The MBP was estimated as 6.50 in 100,000 (95% CI 5.40-7.83). A molecular diagnosis was achieved for 83% (N=92/111) of the patient cohort, with 68% of the pathogenic in the nuclear genome (N=63), giving an estimated MBP of 3.69 (95% CI [2.89, 4.72]), whilst 32% were in the mitochondrial genome (N=29), giving an estimated MBP of 1.70 (95% CI [1.18, 2.44]).

The most common causative gene was POLG (11.7%; N=13/111) associated with Childhood myocerebrohepatopathy spectrum (MCHS) including Alpers-Huttenlocher Syndrome.

The most prevalent syndrome was Leigh (-like) Syndrome (N=39) with a MBP of 2.28 (95% CI, 1.67-2.28), this group was the most molecularly heterogeneous with 15 different genes, the most common being SURF1 (n=6).

The overall estimated median age of onset was 4 months (range 0-187 months) and only 12% (n=13) of the patients presented after 4 years. The estimated median survival was 66 months; 60% of patients (N=67) are deceased (median 5 months, range 0-322), 18% (N=20) are alive (median 26 years, range 21-29), and 22% (N=24) have an unknown status (median age at last follow-up 6 years, range 8 months-25 years).

The estimated median age at presentation was older for patients in the mtDNA-related disease group than the rest of the cohort (23 vs. 3.35 months, p<0.0001). Within the mtDNA cohort, those with variants in protein-encoding genes (N=15) presented earlier than with tRNA genes (N=7) or rearrangements (N=7) (7.9, 131.7, 82.6 months respectively p<0001).

The estimated median survival was also longer for mtDNA than the rest of the cohort (322 vs. 41 months p=0.002). The estimated survival for patients with variants in mtDNA protein-encoding genes was not significantly different than those with nuclear mutations (45 vs 41 months p=0.77). In contrast, all patients with rearrangements (n=7) were alive at age 14 years, with a median age of last follow up of 23.5 years (range 14.3 to 28 years). The median age of last follow up for patients with pathogenic variants in the mt-tRNA gene MTTL1 (N=7) was 15 years (range 7.1 to 27.8 years).

Conclusion: This well-defined pediatric population-based study has the highest diagnosis rate in mitochondrial diseases reported to date. Therefore, we were able to analyze the natural history in different subgroups and provide prevalence estimates that could be translated into patient care, surveillance, and identification of research priorities.

Abstract #: 2018 PA-0394

Presenter: E. J. Neren

Authors: E. J. Neren

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Title: Mitochondrial Reactive Oxygen (ROS) Species as a Non-Toxic Adjuvant Integrative Anticancer Therapy Option for Adult Stage IV Solid Tumor Patients (Brain, Lung, Breast, and Prostate). When Traditional Therapy Options Have Been Exhausted: Palladium/Lipoic Acid Complex and Coenzyme Q10 Impacting the ROS Production and Apoptosis

Body of Abstract:

Background: Metal compounds (Platinum, etc.) have been investigated as potential cancer therapies; however, patient toxicity resulted. ROS (superoxide, hydrogen peroxide, hydroxyl ions) has also been investigated. Dr. Merrill Garnett synthesizing organometallic compounds (1960-1990) encapsulated palladium in alpha lipoic acid (non-toxic in treating mice with Ehrlich carcinoma). Cat/dog tumors were also treated. Rudy Falk, MD. (1992 University of Toronto) determined human safety, found patient improvement/remissions in gravely advanced cancer cases. Since then, 200+ U.S. physicians have used the Palladium/ Lipoic Acid Complex (PdLAC=Poly-MVA) and Coenzyme Q10 (COQ10), as an integrative late stage cancer therapy.

Objective: To provide a non-toxic integrative therapy option and mechanism for late stage cancer patients/physicians justifying physician calls to physicians with clinical experience, and determine if the PdLAC/CoQ10 is appropriate for the given patient.

Methodology: Cell line studies (NCI protocol apoptosis/48 hours: Brain [Glioblastoma/35.8%, Astrocytoma/38.3%], Lung [Non-Small Cell Carcinoma/37.5%], Breast [Adenocarcinoma/41.4%],

Prostate [DU-145/22.7%]) were conducted by Calvert Laboratories. The PdLAC/CoQ10 is administered orally as nutritionals, and tumor marker/CoQ10 levels are determined. PdLAC (water/Fat soluble) impacts both cancer and normal cells. CoQ10 Ubiquinol) dose is 4-100mg softgels/day. PdLAC dose is 8-12 teaspoons (in juice) 4-times/day based on patient body weight (1-teaspoon /30 pounds). Positive clinical response (tumor growth [slowed/ stopped/reduced] and improved patient energy/quality of life) is expected within 3-months. Patient progress is monitored with traditional clinical chemistries/tumor markers, and imaging. This therapy seeks a balance between therapy/nutrition/detoxification and energy enhancement.

Mechanism: The PdLAC enters cancer/normal cells and the mitochondrial outer membrane at the voltage dependent anion channel, and then through the inner membrane at the Complex 1. In cancer cells, the oxidative phosphorylation channel (OXPHOSC) produces low levels of adenosine triphosphate (ATP) due to deficient CoQ10. The deficient CoQ10 results in the PdLAC donating electrons generating excessive ROS. In normal cells, PdLAC (acting as an electrical shunt) donates electrons to the OXPHOSC producing more ATP/patient energy. In the cancer cell (damaged OXPHOSC); excessive ROS builds up between the outer and inner mitochondrial membranes. When the outer membrane ruptures, ROS, Cytochrome C, and the Procapases 2, 3, and 9 enter the cancer cell anaerobic cytoplasm and apoptosis occurs.

Results: James Forsythe, MD/HMD conducted clinical outcome studies (500 Stage IV patients/5-year survival 33% 2004-2012). He reported improvement in quality of life issues directly proportional to overall response rate and that stable disease can be tolerated//transformed into a chronic livable condition.

Conclusion: Scientific//clinical documentation, from several public/professional sources, provides a non-toxic adjuvant integrative nutritional therapy option for advanced (Brain/Lung/Breast/Prostate) cancer patients/physicians, when traditional therapies are exhausted. Physician calls are justified to physicians with PdLAC/CoQ10 clinical experience to determine if this therapy/monitoring is appropriate for a given patient. This therapy is not intended to circumvent traditional therapies, is administered orally as a nutritional (not a “drug or cure”), prescription is not required, 3-month cost $3000, positive results within 3-months, is within good medical practice/medical ethics/FDA guidelines, and should meet physician/hospital/hospice legal obligations. The PdLAC/CoQ10 proposed mechanism focuses on the creation of excessive ROS acting as a natural chemotherapy/anticancer agent when entering the cancer cell anaerobic cytoplasm. PdLAC/CoQ10 represents an application of mitochondrial medicine, with the potential of a patient tolerated stable/chronic livable disease condition. A PdLAC cell line study correlation with adult cell line studies warrants a protocol development adapting the adult therapy dosage to children’s solid tumors (relapsed neuroblastoma/ carcinomas/sarcomas/glioblastomas).

Abstract #: 2018 PA-0395

Presenter: E.J. Neren

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Title: Hypothesis: Mitochondrial Reactive Oxygen Species (ROS) as an Anticancer Agent Impacting Stage IV Children’s Relapsed Neuroblastoma With a Palladium/Lipoic Acid Complex and Coenzyme Q10 as a Non-Toxic Adjunct Integrative Therapy Option Monitored With Clinical Chemistries and Imaging When Traditional Therapies Have Been Exhausted

Body of Abstract:

Background: Neuroblastoma (NB) is the most common children’s extracranial solid tumor and accounts for more than 15% of all pediatric cancer-related deaths. Virtually no options exist for relapsed NB patients except palliative care, when traditional therapies have been exhausted. A Palladium/Lipoic Acid Complex (PdLAC=Poly-MVA)/Coenzyme Q10 (CoQ10) therapy has been utilized for adult late stage solid tumor adult patients (Brain/Lung/Breast/Prostate) as a non-toxic adjunct integrative anticancer option (supported with cell line studies and clinical outcome data), when traditional therapies have been exhausted.

Objective: To provide a hypothesis and cell line study data justifying a PdLAC/CoQ10 adult adapted protocol development for children’s

relapsed NB patients that have exhausted traditional therapies.

Mechanism: The PdLAC enters normal/cancer cells and the mitochondrial outer membrane at the voltage dependent anion channel, then through the inner membrane at Complex 1. In the cancer cell, the oxidative phosphorylation channel (OXPHOSC) produces low levels of adenosine triphosphate (ATP/energy) due to deficient CoQ10. The deficient CoQ10 results in the PdLAC donating electrons producing excessive cancer cell ROS (super oxide//hydrogen peroxide/hydroxyl ions). The excessive ROS builds up between the outer and inner mitochondrial membranes. When the outer membrane ruptures, the ROS, Cytochrome C, and the Procapases 2, 3, and 9 enters the anaerobic cytoplasm of the cancer cell and apoptosis occurs. In the normal cell, the PdLAC (acting as an electrical shunt) donates electrons to the OXPHOSC producing more ATP/patient energy.

Methodology: J.W. Forsythe, MD/HMD (outcome study of 500 Stage IV adult patients 2004-2012), established the PdLAC to be non-toxic and efficacy; however, children were not included. A PdLAC NB

SH-SY5 cell line study (NCI protocol/apoptosis in 48 hours) was performed resulting in 37% apoptosis.

The PdLAC NB SH-SY5 apoptosis coincides with adult cell line studies (Brain [Glioblastoma /35.8%, Astrocytoma /38.3%], Lung {Non-Small Cell Carcinoma/37.5%), and Breast [Adenocarcinoma/41.4%]). PdLAC (water/fat soluble) impacts both cancer and normal cells. Positive clinical response/ improved patient energy/quality of life are expected within 3-months. The PdLAC (adult dose 4- teaspoons 3-times/day, 1-teaspoon/30 pounds of body weight) could be protocol adjusted to the NB patient utilizing Clark's Rule (Adult Dose X (Weight ÷ 150) = Childs Dose) or Young's Rule (Adult Dose X (Age ÷ (Age ÷ 12)) = Childs Dose). Progress would be physician monitored (clinical chemistries/ tumor markers, and imaging). Coenzyme Q10/Vitamin D/Zinc/ Magnesium levels would also be deficiency monitored.

Conclusion: PdLAC/NB cell line study positive data and related positive adult cell line study data suggests a non-toxic adjuvant integrative PdLAC/CoQ10 therapy option for relapsed NB patients and warrants protocol adaptation and investigation. The utilization of Clark's Rule / Young's Rule allows the adult PdLAC/CoQ10 therapy adaptation to relapsed NB children. The PdLAC/CoQ10 therapy (taken orally as a nutritional not as a "drug or cure") may be a nontoxic/integrative means of managing the disease (slowing/stopping/reducing tumor growth within 3-months and with improved patient energy/ quality of life. The PdLAC/CoQ10 option should be a potential application of mitochondrial medicine and within good medical practice/palliative care/medical ethics/FDA guidelines and legal obligations of a physician/hospital/hospice. The PdLAC/CoQ10 therapy ROS mechanism/protocol should also apply to other children's solid tumors (carcinomas/sarcomas/glioblastomas/ astrocytomas).

Abstract #: 2018 PA-0396

Presenter: Romain Cartoni

Authors: Romain Carton[1,3], Michael W. Norsworthy[1], Fengfeng Bei[1], Chen Wang[1], Siwei Li[1], Yiling Zhang[1], Christopher V. Gabel[2], Thomas L. Schwarz[1] and Zhigang He[1].

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Title: THE MAMMALIAN SPECIFIC PROTEIN ARMCX1 REGULATES MITOCHONDRIAL TRANSPORT DURING AXON REGENERATION

Body of Abstract: Mitochondrial transport is crucial for neuronal and axonal physiology. However, whether and how it impacts neuronal injury responses, such as neuronal survival and axon regeneration, remain largely unknown. Recent genetic manipulations have revealed several pathways that regulate intrinsic regenerative ability[1,2,3,4]. Because most of these treatments involve oncogenes, there is a special

interest to identify more specific and safer targets. In an established mouse model with robust optic nerve regeneration[3], we show that Armcx1, a mammalian-specific gene encoding a mitochondrially-localized protein, is up-regulated in Retinal Ganglion Cells (RGCs) with high regenerative capacities after axotomy induced by optic nerve crush. Armcx1 overexpression enhances mitochondrial transport in RGCs axons by mobilizing stationary mitochondria. Importantly, Armcx1 also promotes both neuronal survival and axon regeneration after injury in vivo, and these effects depend on its mitochondrial localization. Specifically, Armcx1 overexpression promotes axonal regeneration of non-alpha RGCs a subtype that has been shown to be refractory to other genetic manipulations[5]. Furthermore, Armcx1 knockdown undermines both neuronal survival and axon regeneration in the high regenerative capacity model, further supporting a key role of Armcx1 in regulating neuronal injury responses in the adult central nervous system (CNS). Our findings suggest that Armcx1 controls mitochondrial transport during neuronal repair and provide a new fascinating approach to develop novel therapies for mitochondria related CNS injury and diseases[6].

Abstract #: 2018 PA-0398

Presenter: T. Yardeni[1]

Authors: T. Yardeni[1], C. Tanes[2], K. Bittinger[2], Lisa M. Mattei[2], DG. Murdock[1], DC. Wallace[1,3]

Institution: [1.] Center for Mitochondrial and Epigenomic Medicine, Children's Hospital of Philadelphia,
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[3.] Department of Pathology and Laboratory Medicine, University of Pennsylvania

Title: Mitochondrial Influence on the Microbiome: A Role for Reactive Oxygen Species (ROS)

Body of Abstract:

The human gut contains trillions of microorganisms that have a significant effect on individual health, interestingly, changes in the gut microbiome or the mitochondrial genotype have both been correlated with a variety of clinical phenotypes including diabetes mellitus, autism, and Parkinson disease. What then causes these diseases, the microbiome or the mitochondrial genotype? Since the microbiome cannot change the mitochondrial genotype, we hypothesized that perhaps the mitochondrial genotype controls both the phenotype and the microbiome.

To address this hypothesis, we analyzed the gut microbiota in mouse models harboring mtDNA and/or nDNA variants in mitochondrial genes. Our data revealed that mice with different mitochondrial genotypes developed characteristic mitochondrial genotype-associated microbiomes. For example, a pathogenic mutation in the mtDNA (ND6 P25L) is associated with significantly different microbiota compare to control. Additionally, we found that miss much nuclear/ mitochondria mice which having the same inbred nucleus (C57BL/6J) but different mouse mtDNAs [129 (from 129 mouse model), NZB (from NZB mouse model)] and heteroplasmic 129 + NZB revealed an incremental difference in the ratio of Bacteroidetes to Firmicutes for mice homoplasmic for C57BL/6J mtDNA, to those homoplasmic for 129 mtDNA, to those heteroplasmic for a mixture of 129 and NZB mtDNAs, to those homoplasmic for NZB mtDNA. These results show that the ratio of Bacteroidetes to Firmicutes is effected by the percentage of mt-NZB, this ratio has a linear correlation with the increasing of the percentage of mt-NZB. In addition, by Shannon diversity of the bacterial richness and evenness a linear decrease was also observed with increasing NZB mtDNA levels.

Importantly, mitochondrial physiology was linked to microbiota composition by studying mice that differed in the expression of the mitochondrial antioxidant gene for the nicotinamide nucleotide transhydrogenase (NNT). The $Nnt^{+/+}$ and $Nnt^{-/-}$ mice had markedly different gut microbiomes. To confirm that the Nnt genotype causes these differences, we fostered newborn pups with the $Nnt^{+/+}$ and $Nnt^{-/-}$ genotypes on mothers with the reciprocal Nnt genotype. Within 2 months, the Nnt genotype of the pups restructured the microbiota from that of the nursing mother back toward the characteristics of the pup's Nnt genotype. When the $Nnt^{-/-}$ mutation was combined with the mitochondrially-targeted human catalase (mCAT), the microbiome of the $Nnt^{-/-}$ mice with the mCAT was markedly different from cohoused $Nnt^{-/-}$ littermates without mCAT. These data indicate that the mitochondrial genotype can control both the mammalian clinical phenotype and the gut microbiome. Moreover, mitochondrially-generated ROS may be one of the factors that modulates the gut microbiome.

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Abstracts

Abstract #: 2018 PA-0399

Presenter: Russell L. D'Souza

Authors: Russell L. D'Souza[1], Yvonne Latour[2], and Peter J. McGuire[1]

Institution: [1]Metabolism, Infection and Immunity Section, [2]Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD

Title: Development of iPS-derived cerebral organoids for studying mitochondrial disease

Abstract: Mitochondria are multifaceted organelles that are ubiquitously present and partake in a number of metabolic processes such as fatty acid oxidation, urea production, gluconeogenesis etc. Mitochondrial diseases are a type of in-born errors of metabolism characterized by dysfunctional mitochondria resulting in metabolic decompensation. They are clinically heterogeneous, and patients can manifest a number of symptoms. The disease is multisystemic but affects organs that are highly dependent on aerobic metabolism the most. Because of this clinical heterogeneity, disease diagnosis relies mostly on genetic testing. Therefore, there is a need of a model system that closely resembles the affected tissue type. We evaluated a 13 – year old boy with a diagnosis of a mitochondrial disease via our clinical protocol. Prior to generating iPS cells from our patient, the possibility of a mitochondrial disease was established when decreased Complex III activities was reported in muscle biopsies. Furthermore, we also observed lower oxygen consumption rates when compared to healthy volunteers. Mitochondrial gene expression arrays revealed a downregulation of BCS1L, a chaperone that aids in the assembly of Complex III. This observation was corroborated by probing for antibodies against BCS1L in mitochondria isolated from patient lymphoblast cells. A whole genome sequencing is in progress to evaluate the type of mutation in BCS1L. PBMCs isolated from our patient were reprogrammed to form inducible pluripotent stem (iPS) cells via the NHLBI iPS Core. Using an established protocol, patient derived iPS cells will be used to grow cerebral organoids that produce encephalopathy. Beginning with healthy iPS cells, we set out to establish the growth and development parameters of cerebral organoids. Using specialized media, iPS cells were observed to form embryoid bodies at 2 weeks with neuronal epithelium. The embryoid bodies further developed into cerebral organoids at 9 weeks (~300μm) in tissue culture bioreactors containing differentiation medium. The organizational structure of the organoids was observed by IHC and imaging analysis. Antibodies against MAP2 show presence of terminally differentiated neurons throughout the tissue section. The ventricular folds were observed using DAPI. Together, these data demonstrate that cerebral organoids demonstrate structures and topography analogous to human brain tissue. Following reprogramming of patient PBMCs into iPS cells, cellular growth was restricted when compared to healthy iPS cells. This phenotype suggests that the BCS1L mutation in our patient affects mitochondrial function leading to impaired growth. Organoid generation and evaluation from the patient's iPS cells is in progress.

Abstract #: 2018 PA-0400

Presenter: Shanmughapriya Santhanam

Authors: Shanmughapriya Santhanam[1,2], Katherine J. Slovik[3], Neeharika Nemani[1,2], Natarajaseenivasan Kalimuthusamy[1,2], Zhiwei Dong[1,2], Christy Lu[1,2], Edmund Carvalho[1,2], Sudarsan Rajan[2], Ying Tian[2], Wenli Yang[3], Madesh Muniswamy[1,2]

Institution: [1]Temple University, Department of Medical Genetics and Molecular Biochemistry, Lewis Katz School of Medicine, Philadelphia, PA 19140, [2]Temple University, Center for Translational Medicine, Lewis Katz School of Medicine, Philadelphia, PA 19140, [3]University of Pennsylvania, Institute for Regenerative Medicine, Philadelphia, PA 19104

Title: Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes with Remodeled Mitochondrial Ca2+ Transients Develop Adult Cardiac

Phenotype

Body of Abstract: Introduction: Effective differentiation of embryonic or induced pluripotent stem cells (ESCs/iPSCs) into cardiomyocytes remain a promising approach for cardiac regeneration. Beyond the clear novelty, iPSCs-derived cardiomyocytes (iPSCs-CMs) exhibit a functionally immature, disorganized, fetal-like phenotype that is not equivalent to adult CM. Indeed, transplantation of immature CMs could result in both poor graft-host integration and lethal arrhythmia. The present work is corroborated on elucidating how the fetal microenvironment drives myocyte maturation by modulating mitochondrial Ca^{2+} channels, leading to varying $_{c}Ca^{2+}$ transients. Hypothesis: The fine regulation of $_{c}Ca^{2+}$ transient by mitochondria is a key regulator of fetal myocyte maturation. **Results:** We employed a tissue-wide developmental array screening before settling on a cell-based functional approach to find modulators of mitochondrial Ca^{2+}-dependent myocyte maturation. As shown by the tissue array and protein profiling, the auxiliary protein of the uniporter complex, MICU1 was differentially regulated with no observable changes in the pore component. To our surprise, iPSCs-CMs had significantly lower MICU1 and exhibited immature spontaneous $_{c}Ca^{2+}$ transients resembling fetal myocytes (from E13.5 to P0). We identified in either glycolytic or hypoxic environment MICU1 is under the repression of Foxd1 transcription factor. Reconstitution of MICU1 either by Foxd1 knock-down or ectopic expression modulated mitochondria's ability to maintain $_{c}Ca^{2+}$ transients, facilitated a metabolic switch to β-oxidation, and ultimate electrical and mechanical maturation. **Conclusion:** The goal of myocyte maturation by modulating mitochondria's ability to maintain $_{c}Ca^{2+}$ transients will layout a roadmap for future experimental confirmation to molecularly define the mechanism of fetal myocyte maturation and cardiac regeneration. Upon the validation of a pathway central to myocyte maturation, future efforts will be aimed to elucidate the in vivo therapeutic potential. With the birth and expansion of iPSCs-CMs coupled with modulated mitochondrial function, we see our approach as the future of scientific discovery and a powerful tool to open the door for future therapies.

Abstract #: 2018 PA-0401

Presenter: Adam P. Fischer

Authors: Zhihong Li[1], Adam P. Fischer[2], Ke Zhang[1], Jia Jun Guan[1], Jie Zhang[2], Hugo J. Bellen[1], and Brett H. Graham[2]

Institution: [1]Baylor College of Medicine, Department of Molecular and Human Genetics, Houston, TX 77030; [2]Indiana University School of Medicine, Department of Medical and Molecular Genetics, Indianapolis, IN 46202

Title: The Generation of Drosophila melanogaster lacking NDUFS3 as a model of Complex I deficiency for High Throughput screens

Body of Abstract: Medical conditions caused by mitochondrial dysfunction are broadly defined as mitochondrial diseases. Epidemiological evidence suggests as many as 1 in 5,000 individuals may be affected by these disorders. The majority of mitochondrial diseases are attributed to mutations or deletions to nuclear or mitochondrial encoded genes that are necessary for efficient ATP production via oxidative phosphorylation (OXPHOS). Among OXPHOS defects known to cause disease phenotypes, isolated complex I (NADH:ubiquinone oxidoreductase) deficiency is the most commonly observed respiratory chain defect in patients. Clinical phenotypes of complex I deficiency are diverse and are accompanied by a vast array of symptoms that typically manifest neonatally or later during childhood, however encephalomyopathies usually predominate. Among these illnesses, Leigh syndrome is the most common clinical diagnose for patients suffering from complex I deficiency. Despite complex I deficiency being the most prevalent cause of mitochondrial disease, few treatment options are available for these patients, causing most to succumb to this condition within a few years after diagnosis. One limitation for the development of innovative new therapies for mitochondrial disease is the paucity of diverse, relevant, and reliable in vivo disease models. While murine models of mitochondrial disease and complex I deficiency exist and are providing important insights, they are not ideal for high throughput screens often necessary for drug discovery. Alternatively, in vivo Drosophila models have been extensively used to study mechanisms of neurologic dysfunction and drug intervention. An estimated 75 % of human disease-causing genes are thought to have a homolog in Drosophila, making them an invaluable tool for investigating intervention strategies for mitochondrial diseases. To gain further insight into the pathophysiological mechanisms that facilitates the acquisition of mitochondrial diseases and to aid in novel drug discovery, our laboratory has generated and characterized fruit flies lacking CG12079, the Drosophila melanogaster ortholog of the known nuclear encoded complex I subunit, NADH Dehydrogenase (Ubiquinone) Fe-S Protein 3 (NDUFS3). NDUFS3 mutant Drosophila are characterized as exhibiting inhibited complex I activity, elevated oxidative stress, and significant retinal degeneration consistent with clinical phenotypes observed in patients. Furthermore, a

preliminary drug screen of established therapeutic agents using our Drosophila model identified a cohort of drugs capable of suppressing the mutant phenotype, suggesting known drug candidates may be repurposed to treat mitochondrial disease and warrant further investigation.

Abstract #: 2018 PA-0403

Presenter: Sarika Srivastava

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Title: Loss of CASK Function Causes Mitochondrial Metabolic and Neurodevelopmental Defects in Mice

Body of Abstract: CASK is an X-linked gene that encodes for a calcium/calmodulin-dependent serine protein kinase. Constitutive CASK deletion is lethal in mammals indicating its essential role for survival. Genetic mutations in human CASK associate with a wide spectrum of clinical phenotypes including intellectual disability, autism spectrum disorders, microcephaly with pontine and cerebellar hypoplasia (MICPCH), optic nerve hypoplasia, epileptic encephalopathy, myoclonic epilepsy, infantile spasms, growth retardation, sensorineural hearing loss, hypotonia and scoliosis. To better understand the molecular function of CASK and underlying pathogenic mechanisms, we generated the $CASK^{(+/-)}$ heterozygous knockout and neuronal-specific CASK knockout ($CASK^{NKO}$) mice utilizing the Cre-LoxP mediated conditional gene knockout strategy. We found that the $CASK^{(+/-)}$ heterozygous knockout mice recapitulate human clinical phenotypes with high fidelity. Specifically, the $CASK^{(+/-)}$ knockout mice exhibited postnatal microcephaly, cerebellar hypoplasia, optic nerve hypoplasia, growth retardation, ataxia and scoliosis compared to the age- and sex-matched $CASK^{(+/+)}$ wild-type control mice. Using functional assays, we demonstrate that brain mitochondrial respiration and glucose oxidation rates were significantly lowered in $CASK^{(+/-)}$ knockout mice compared to the $CASK^{(+/+)}$ wild-type littermate controls. Furthermore, $CASK^{(+/-)}$ knockout mice displayed abnormal metabolic cage activity and a significant reduction in skeletal muscle glucose and fatty acid oxidation rates compared to the $CASK^{(+/+)}$ littermate controls. A significant increase in glutamate and GABA (γ-amino butyric acid) neurotransmitter levels were also observed in the brain of $CASK^{(+/-)}$ knockout mice compared to the $CASK^{(+/+)}$ littermate controls indicating abnormal neurotransmission activity. Interestingly, the neuronal-specific CASK deletion in mice caused severe growth retardation as well as recurrent tonic spasms and myoclonic epilepsy beginning postnatal day 17 (P17) that culminated in death on or before P25. A significant decrease in brain mitochondrial respiration was observed both before and after the onset of myoclonic epilepsy in $CASK^{NKO}$ mice compared to the age- and sex- matched $CASK^{floxed}$ control mice. Moreover, electron microscopy analysis revealed reduced number of mitochondria in the cortex of $CASK^{NKO}$ mice compared to the $CASK^{floxed}$ control mice. Our findings suggest that CASK is essential for mammalian neurodevelopment and plays a critical role in regulating mitochondrial metabolic function.

Abstract #: 2018 PA-0404

Presenter: Martin Picard

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Title: Mitochondrial 3D Network Organization and Nanotunnels in Mitochondrial Disease

Body of Abstract: Background Mitochondrial DNA (mtDNA) defects are a major cause of disease, but the consequence of genetic and biochemical defects on mitochondrial morphology in human tissues is not fully understood. Here we provide the first quantitative three-dimensional (3D) assessment of the human skeletal muscle mitochondrial network.

Methods In tissues from six patients with rare mtDNA diseases and eight healthy individuals, we develop a novel electron microscopy approach to quantify the morphological complexity of individual mitochondria and extract intuitive metrics of mitochondrial population distributions in each individual.

Results The resulting mitochondrial complexity index (MCI) was validated in mice, and a quantitative cross-species comparison of the mitochondrial network demonstrates significantly more morphological complexity in mouse than human muscle. Using high-resolution 3D imaging, we find that the prevalence of mitochondrial nanotunnels – ~100nm-wide tubular protrusions of outer and inner mitochondrial membranes connecting the matrix space of non-adjacent mitochondria – increases with age and is 6-fold higher in patients with disease than healthy controls. Furthermore, analyses from three genetically-related family members with increasing mtDNA mutation load (m.8344A>G) suggests a bi-phasic relationship between heteroplasmy and morphology. Finally, we use machine learning to isolate key morphological features and combination of network characteristics that differentiate mtDNA disease from healthy individuals. Combined, elevated proportions of i) small mitochondria and ii) nanotunnels represents a potential signature of mtDNA disease.

Conclusions Overall, these results define the nature of the mitochondrial network in human skeletal muscle, demonstrate systematic differences with mice, and identifies new morphological features of mitochondrial disease, including nanotunnels, in human tissues.

Abstract #: 2018 PA-0405

Presenter: Liming Pei

Authors: Juanjuan Zhao[1,2], Katherine Lupino[1,2], Benjamin J. Wilkins[2,3], Chengxiang Qiu[4], Jian Liu[1,2], Yasuhiro Omura[5], Amanda L. Allred[5], Caitlin McDonald[1,2], Katalin Susztak[4], Grant D. Barish[5], Liming Pei[1,2,3*]

Institution: [1]Center for Mitochondrial and Epigenomic Medicine; [2]Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, Philadelphia, PA 19104; [3]Department of Pathology and Laboratory Medicine; [4]Renal Electrolyte and Hypertension Division, Department of Medicine; Department of Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104 ; [5]Department of Medicine, Division of Endocrinology, Metabolism and Molecular Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL 60611

Title: Regulation of mitochondrial and cell type-specific functions by nuclear receptor ERRγ

Body of Abstract: Maintaining optimal mitochondrial function is central to health. Mutations of mitochondrial DNA and proteins directly cause mitochondrial disease with severe defects often observed in organs of high mitochondrial content and energetic demand including the brain, heart and kidney. Most cells do not have an extensive capacity to fully store mitochondrial-generated energy, therefore cellular energy production and consumption must be well coordinated. It remains poorly understood how mitochondrial metabolism is coordinated with cell type-specific cellular functions that depend on mitochondrial-generated ATP (i.e. muscle contraction in the heart or renal reabsorption in the kidney). Recent work has revealed nuclear receptor ERRγ as a critical transcriptional regulator of mitochondrial oxidative phosphorylation (OxPhos) and fatty acid oxidation (FAO). We performed ChIP-Seq to map and compare the genome-wide binding patterns of ERRγ in the brain, heart and kidney. The results reveal that in addition to binding to hundreds of mitochondrial OxPhos and FAO genes in all three tissues, ERRγ also binds to and activates cell type-specific energy-consumption genes, i.e. muscle contraction and conduction in the heart and renal

reabsorption in the kidney. We show that ERRγ regulates these genes through functional cooperation with cell-type specific transcription factors. In the kidney, ERRγ directly regulates mitochondrial metabolism but cooperatively controls renal reabsorption via convergent binding with HNF1β. Deletion of ERRγ in renal epithelial cells (RECs), in which it is highly and specifically expressed, results in severe renal energetic and reabsorptive dysfunction and progressive renal failure that recapitulates phenotypes of animals and patients with HNF1β loss-of-function gene mutations. Together with our previous studies in the brain and heart, we demonstrate that ERRγ is a central transcriptional coordinator of cell-type specific energy production and utilization.

Abstract #: 2018 PA-0406

Presenter: Eija Pirinen

Authors: Eija Pirinen[1], Mari Auranen[1,2], Nahid A. Khan[1], Niina Urho[2], Antti Hakkarainen[3], Juho Kuula[3], Ulla Heinonen[2], Mark Schmidt[4], Kimmo Haimilahti[1], Sari Räsänen[5], Päivi Piirilä[6], Nina Lundbom[3], Marja-Riitta Taskinen[5], Charles Brenner[4], Vidya Velagapudi[7], Kirsi H. Pietiläinen[5] and Anu Suomalainen[1]

Institution: [1]Research Programs Unit, Molecular Neurology, University of Helsinki, FIN-00290 Helsinki, Finland, [2]Clinical Neurosciences, Neurology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland, [3]Department of Radiology, University of Helsinki and Helsinki University Hospital Radiology, Helsinki, Finland, [4]Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, Iowa 52242, USA, [5]Diabetes and Obesity Research Unit, Research Programs Unit, University of Helsinki, FIN-00290 Helsinki, Finland, [6]Department of Clinical Physiology and Nuclear Medicine, Laboratory of Clinical Physiology, Helsinki University Hospital, Helsinki, Finland, [7]Metabolomics Unit, Institute for Molecular Medicine Finland (FIMM), FIN-00290 Helsinki, Finland.

Title: Niacin supplementation alleviates disease symptoms in patients with mitochondrial myopathy

Body of Abstract: Mitochondrial disorders are characterized by mitochondrial respiratory chain defects which has been suggested to result in impaired NADH utilization in electron transport chain and subsequently decreased energy production and NAD^+/NADH ratio. The most frequent form of adult-onset mitochondrial disorders is mitochondrial myopathy, often manifesting with progressive external ophthalmoplegia (PEO), progressive muscle weakness and exercise intolerance. Currently, no curative treatment exists for this disease. In mice, supplementation with a NAD^+ precursor vitamin B3, nicotinamide riboside, prevented and delayed disease symptoms by increasing mitochondrial biogenesis in two mouse models for mitochondrial myopathy. Here, we tested NAD^+ boosting therapy for patients with mitochondrial myopathy.

We recruited five PEO patients either with sporadic single mtDNA deletions or a mutation in a mtDNA maintenance gene Twinkle. For every patient, two gender- and age-matched healthy controls were recruited. All manifested with only myopathic symptoms. Study subjects were supplemented with a 1 g/day dose of niacin, which is a NAD^+ precursor vitamin B3 form with a proven safety record in humans. Clinical examinations and collection of blood samples and muscle biopsies were performed in patients at the time points 0, 4 and 10 months and in controls at 0 and 4 months.

Before the niacin supplementation, PEO patients showed significantly lower whole blood and muscle NAD^+ levels compared to controls at the baseline. Niacin increased blood NAD^+ levels 8-fold already after 4 months, and the level stayed the same after 10 months of supplementation. In the muscle, niacin elevated NAD^+ content to near control levels in 4 months. The NAD^+ repletion improved findings in muscle histology and elevated mitochondrial amount in vastus lateralis muscle of PEO patients after 10 months. Niacin also enhanced exercise capacity by 3% in PEO patients in the six-minute walking test after 10 months. All patients demonstrated a significant increase in muscle strength of core muscles (back and abdominal muscles) by 90% and 995%, respectively, and upper extremities by 150% after 10 months of niacin supplementation. Spiroergometry indicated improved post-exercise lactate clearance in niacin-treated patients. Eye muscle symptoms did not, however, improve in any patient. Analysis of muscle metabolome revealed that niacin supplementation clearly shifted the global metabolite profile of PEO patients towards controls after 10 months niacin supplementation.

In summary, niacin, a vitamin B3 form, mitigated symptoms of mitochondrial myopathy in our patients irrespective of patients' age. These results

highlight the potential of niacin to restore whole blood and muscle NAD^+ levels and improve muscle mitochondrial biogenesis in humans. Our pilot study indicates the potential of micronutrients as metabolic modifiers, and NAD^+ boosters, including niacin, in treatment of diseases with global NAD^+ deficiency, i.e. PEO and adult-onset mitochondrial myopathy caused by mtDNA deletions. Our study did not, however, provide any evidence for efficacy or exclude harmful effects in other mitochondrial diseases

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Abstract #: 2018 PA-0407

Presenter: Senta M Kapnick

Authors: Senta M Kapnick, Peter McGuire

Institution: National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, 20892

Title: Exploring the impact of mtDNA mutations on T lymphocyte metabolism and function in transmitochondrial cytoplasmic hybrids

Body of Abstract: Mitochondria arose over 2 billion years ago as endosymbionts and owe their persistence to their remarkable ability to convert organic molecules from the environment into energy through mitochondrial respiration. Although many genes required for mitochondrial maintenance and function were transferred to the nucleus during evolution, mitochondria maintain multiple copies of maternally-inherited mitochondrial DNA (mtDNA). Approximately 17kb in length, mtDNA contains 37 genes: 22 transfer RNAs, 2 ribosomal RNAs, and 13 genes encoding protein subunits of the respiratory chain necessary for efficient mitochondrial metabolism. Mitochondrial disease (MD) is a group of clinically heterogeneous disorders that result from mutations in either nuclear or mitochondrial genes involved in mitochondrial function. Although MDs due to mtDNA mutations manifest most severely in the nervous system, patient reports reveal an emerging phenotype of immune deficiency; notably, infections not commonly seen in immunocompetent populations. During an immune response, T lymphocytes undergo significant metabolic changes that determine their ability to perform the effector functions vital for protection against pathogenic organisms and tumors; thus, there exists an intimate link between lymphocyte function and mitochondrial metabolism that is vital for mounting effective immune responses. Despite these recent contributions to the MD patient literature and heightened interest in the central role of cellular metabolism in regulating immune cell function, how deleterious mutations in mtDNA impact T cell bioenergetics and T cell-mediated immunity is not known. Using transmitochondrial cytoplasmic hybrids (“cybrids”), we are currently evaluating how specific, patient-derived mtDNA mutations impact the ability of T cells to meet the bioenergetic demands required for proper effector function. Rho0 T lymphocyte cell lines were produced using the viral nuclease isoform, UL12.5 (pMZS3F UL12.5-SPA, gift from J. Smiley), shown to exclusively trigger mtDNA depletion in the absence of other viral gene products. To generate cybrid lines, we perform polyethylene glycol (PEG)-mediated fusion of Rho0 lymphocytes with platelets isolated from blood collected from patients with MD. By studying perturbed T cell metabolism due to mtDNA mutations, we expect to contribute to our current knowledge of the link between T cell function and mitochondrial-mediated metabolism. This work has the potential to aid in the development of improved strategies for evaluating and treating immune dysfunction in patients with MD due to mutations in mtDNA.

Abstract #: 2018 PA-0409

Presenter: Matthew Thompson

Authors: Matthew Thompson[1], Di Hu[1], Anniefer Magpusao[1], Xin Qi[1], Drew Adams[1]

Institution: [1]Case Western Reserve University School of Medicine, Case Western Reserve University, Cleveland, OH, 44106

Title: A Kinase Inhibitor That Reverses Huntington’s Disease Phenotypes

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Body of Abstract: Huntington's Disease (HD) is a fatal genetic disorder characterized by an expanded polyglutamine tract in the first exon of the huntingtin (Htt) gene. Accumulation of mutant Htt (mtHtt) leads to the death of medium spiny neurons (MSNs) in the basal ganglia, and as a result, HD patients suffer progressive motor and cognitive deficiencies, vision and speech loss, and an elevated suicide risk. Recent evidence suggests that mtHtt causes mitochondrial defects within affected neurons, causing decreased mitochondrial membrane potential (MMP) and oxygen consumption, and increased neuronal death. Despite our understanding of HD's genetic and cellular mechanisms, current therapies merely alleviate HD symptoms but cannot halt or even slow disease progression. Our recent success in reversing HD-induced mitochondrial deficits via a peptide inhibitor led us to conduct a high-throughput screen to discover small molecules capable of mitochondrial rescue. Inhibitor 1 (which remains undisclosed for the purposes of future patent application), a Kinase X inhibitor, emerged as a leading candidate capable of restoring MMP in HD striatal neurons. Follow-up studies showed Inhibitor 1 enhanced mitochondrial oxygen consumption and neuronal viability in vitro. In the R6/2 HD mouse model, Inhibitor 1 increased the density of MSNs, decreased mtHtt aggregation, and prolonged survival compared to a vehicle control. The failure of other Kinase X inhibitors to reproduce the same cellular phenotypes produced by Inhibitor 1 suggests that Inhibitor 1 likely does not modulate HD pathology through inhibition of its canonical target. Furthermore, preliminary siRNA knockdown experiments have confirmed our hypothesis that Kinase X is not the mechanistic target of Inhibitor 1. Instead, genetic knockdown of Kinase Y, a validated off-target enzyme of Inhibitor 1, confers increases in MMP and cell viability analogous to those observed with Inhibitor 1 treatment. Ultimately, further optimization of Inhibitor 1 as an HD therapeutic and elucidation of its mechanism could open new avenues for HD drug development and provide much needed therapies capable of reversing disease progression.

Abstract #: 2018 PA-0410

Presenter: Masako Ueda

Authors: Masako Ueda[1], Jesus A. Tintos-Hernandez[1], Atif Towheed[1], Meagan McManus[1], Xilma Ortiz- Gonzalez[1], Deborah G. Murdock[1], Daniel J. Rader[2], Douglas C. Wallace[1].

Institution: [1]The Children's Hospital of Philadelphia, Philadelphia, PA, [2]the University of Pennsylvania, Philadelphia, PA 19104.

Title: T10I LMNA Mutation Alters Mitochondrial Quantity in Patients with Lipodystrophy

Body of Abstract: Lipodystrophies are heterogeneous disorders of adiposity associated with the phenotypes similar to the severe metabolic syndrome. Generalized lipodystrophy with virtual absence of adipose tissues, and partial lipodystrophy with a selective lack and/or excess of adiposity as well as inherited and acquired are known subtypes. Mitochondrial (mt) dysfunction has been well- characterized in acquired lipodystrophy which is seen in patients with human immunodeficiency virus (HIV) infection treated with an antiretroviral therapy, such as a nucleoside reverse transcriptase inhibitor (NRTI) or a protease inhibitor (PI). Mitochondrial dysfunction is reported to be the result of NRTI's inhibition of mitochondrial DNA (mtDNA) polymerase γ that facilitates mtDNA replication, and PI's inhibition of zinc metalloproteinase ste24 (ZMSTE24) that is required for endoproteolytic processing of the lamin A/C (LMNA). Altered mitochondrial quantities (mtDNA to nuclear DNA ratios (mtDNA:nDNA)) have been reported in patients with HIV who received NRTI and developed lipodystrophy. Therefore, it is plausible that mitochondrial abnormality and dysfunction may exist in inherited lipodystrophy. In fact, abnormal oxidative phosphorylation has been reported in congenital lipodystrophy.

LMNA gene is a causal gene of inherited lipodystrophy. As part of a larger lipodystrophy project planned, quantitative mitochondrial analyses in a proband-parent pair with markedly distinct phenotypes with a LMNA mutation (p.T10I) were performed by quantitative polymerase chain reaction (qPCR), targeting five mitochondrial genes (NADH dehydrogenase 1, mt leucine tRNA, mt ribosomal protein 12S, cytochrome B, and NADH dehydrogenase 4) that were normalized to several nuclear housekeeping genes. DNA was collected from peripheral blood leukocytes (PBL), saliva, oral mucosa, and hair follicles.

Compared to the mtDNA:nDNA ratio of 21.57 found in the control DNA (haplogroup H1a), increased ratios were observed in both the proband (haplogroup D1) with 24.65 (+14%), and the parent (haplogroup U562a1b) with 28.30 (+31%). The proband's PBL DNA obtained post-cardiac transplantation revealed a tremendous increase to 96.91 (~4-fold increase), compared to the pre- transplant level. This mt proliferation may be a compensatory response to the effect of steroid immunosuppressive therapy, which is known to affect oxidative phosphorylation and mt-

dependent apoptosis. Higher mtDNA:nDNA ratios of saliva and buccal cells were found in the father (~2X) than the proband: (17.24 vs 9.10) and (250.42 vs 142.57), respectively. These trends were similar to the PBL ratio prior to the proband's transplant. However, these were not aligned with the quantitative mt decline often seen in aging and the predicted mt proliferation with leptin therapy (the proband). A higher ratio was identified in the proband's hair follicles than the father, which may be due to a larger stem cell reservoir in younger individuals, and to an aging process.

There results may represent effects of the LMNA mutation on mitochondria, and may underlie their phenotypic differences. The findings may also signify tissue-specific adaptive or compensatory responses. Additional studies with age-gender, and possibly haplogroup-matched controls may enable elucidating the role of the LMNA mutation. Additional qualitative and functional mt studies in their explanted heart tissues and in iPSC may further clarify these findings and to characterize the disease process in lipodystrophy.

Abstract #: 2018 PA-0411

Presenter: Bryan L. Gitschlag

Authors: Bryan L. Gitschlag[1], Maulik R. Patel[1,2]

Institution: [1]Department of Biological Sciences, Vanderbilt University, Nashville, TN, 37232, [2]Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN, 37232

Title: Homeostatic Pathways Regulate Mutant Mitochondrial Genome Dynamics

Body of Abstract: Common sources of mitochondrial dysfunction and disease include mutations in the mitochondrial genome (mtDNA), the small circular chromosome that resides within the matrix and encodes several essential genes for aerobic metabolism. However, mutant mtDNA (ΔmtDNA) do not follow the inheritance patterns that are typical of the nuclear genome. This makes it difficult to predict the inheritance and development of ΔmtDNA-associated disease and highlights the importance of investigating the mechanisms that govern the propagation of ΔmtDNA. We have found aspects of metabolic status, namely insulin signaling and the ability to metabolize glucose, to be important regulators of ΔmtDNA proliferation in a Caenorhabditis elegans model of mitochondrial disease. Specifically, insulin signaling promotes the proliferation of ΔmtDNA, while inhibiting glucose metabolism tempers the proliferation of ΔmtDNA. Furthermore, we have found that insulin signaling promotes ΔmtDNA proliferation by suppressing the activity of the transcription factor FOXO/DAF-16. However, is the proliferation unique to the presence of this particular mutation? Although we have previously shown that this ΔmtDNA variant is able to outcompete wildtype mtDNA and become the dominant allelic variant in the organism, not all mitochondrial mutations exhibit the same proliferative dynamics. To test whether mtDNA proliferation is generalizable or unique to the presence of this mutation, we targeted insulin signaling in animals lacking this ΔmtDNA. Interestingly, these animals also exhibit reduced mtDNA copy number when insulin signaling is suppressed, suggesting that insulin signaling-dependent mitochondrial proliferation does not depend on the presence of a particular ΔmtDNA variant. We also observe that mutants defective for insulin signaling show reduced proliferation of the female germline tissue, where the majority of mtDNA replication occurs. This effect is observed in both animals harboring ΔmtDNA as well as animals with only wildtype mtDNA. Germline proliferation is restored by the loss of FOXO/DAF-16 in both mitochondrial genotypes. Taken together, we propose a model whereby insulin signaling connects nutrient status to the development of germline tissue and the propagation of mitochondria, through its regulation of FOXO/DAF-16. By promoting the wholesale proliferation of mitochondria under nutritionally permissive conditions, insulin signaling facilitates the propagation of potentially disease-causing mitochondrial mutations. These findings yield valuable insight on the risks of developing mitochondrial disorders in the context of other metabolic conditions such as obesity, insulin resistance and diabetes.

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Abstract #: 2018-PA-0412

Presenter: Joseph A. Bonanno

Authors: Diego G. Ogando[1], Moonjung Choi[1], Shimin Li[1], Edward T. Kim[1], and Joseph A. Bonanno[1*]

Institution: [1]School of Optometry, Indiana University, Bloomington, Indiana, 47405, United States

Title: The NH_3/H^+ transporter Slc4a11 Facilitates Glutamine-Dependent Mitochondrial Function and ROS Prevention by Mitochondrial Uncoupling

Body of Abstract: Mutations in SLC4A11 can cause CHED (Congenital Hereditary Endothelial Dystrophy) a disorder of the Corneal Endothelium that produces corneal edema and poor vision in early childhood and in some cases Harboyan Syndrome (CHED + hearing deficits). SLC4A11 functions as an NH_3-sensitive electrogenic H^+ transporter. Highly expressed in the corneal endothelium, it facilitates glutamine (Gln) catabolism with increased NH_3 production. SLC4A11 has been localized to the basolateral membrane and undetermined cytoplasmic locations of corneal endothelium. $Slc4a11^{-/-}$ (KO) mice recapitulate CHED and show corneal endothelial cell dysfunction and evidence of oxidative stress. Here, we ask if Gln - dependent NH_3 production is a source of oxidative stress and cell death that is ameliorated by Slc4a11 through a mitochondrial uncoupling mechanism. Indicators of oxidative stress, mitochondrial membrane potential (MMP), apoptosis and assays for ATP, oxygen consumption, and ammonia were performed over 24 hours in a minimal non-proliferative assay media using mouse corneal endothelial cell lines (MCEC) derived from $Slc4a11^{-/-}$ (KO) and $Slc4a11^{+/+}$ (WT) mice in the absence and presence of Gln. In WT MCEC, Gln increased [ATP], concomitant with mitochondrial superoxide (O_2^-) & NH_3 production, hyperpolarized MMP, and increased apoptosis. In contrast, KO MCEC show decreased [ATP] & NH_3 production, with significantly greater ROS, MMP hyperpolarization, and apoptosis that can be rescued by the mitochondrial specific anti-oxidant MitoQ, but not by the global anti-oxidant N-Acetylcysteine. NH_3 (25mM) alone increased ROS, depolarized MMP, decreased [ATP] and increased apoptosis in KO without affecting WT cells. Gln derived NH_3 toxicity was partially reduced by GLS1 inhibitors BPTES or CB839 and totally inhibited by supplementation with Dimethyl-α-Ketoglutarate. Relative to KO, WT cells had a higher oxygen consumption rate and greater proton leak in the presence of Gln. Acute application of NH_3 depolarized WT MMP, but not KO. The mitochondrial uncoupler BAM15 reduced Gln-dependent MMP hyperpolarization and mitochondrial ROS production, while increasing [ATP] and completely rescuing KO cells. This result indicates that Slc4a11 is providing NH_3 sensitive mitochondrial uncoupling that reduces Gln-dependent ROS production, consistent with the NH_3-dependent electrogenic H^+ transport properties of this protein.

Abstract #: 2018 PA-0413

Presenter: Wenyan Xu

Authors: Wenyan Xu[1], Wenxin Zhao[1], Nana L Morehouse[1], Maya O Tree[1] and Linlin Zhao[1]

Institution: [1]Department of Chemistry and Biochemistry, Central Michigan University, Mount Pleasant, Michigan, MI 48858

Title: Kinetic Basis of DNA Synthesis by Human Mitochondrial DNA Polymerase/Primase PrimPol

Body of Abstract: PrimPol is the most recently discovered human DNA polymerase/primase, which belongs to archaeo-eukaryotic primase (AEP) superfamily. In addition to its role in maintaining the nuclear genome, PrimPol is thought to be functionally important for the mitochondrial DNA replication particularly when the replication fork is stalled by DNA damage or DNA secondary structures. Such role in genomic maintenance can be attributed to PrimPol's DNA lesion bypass and de novo DNA synthesis (re-priming) activities. Despite the previous biochemical characterizations, it remains unclear concerning the kinetic basis of PrimPol-catalyzed nucleotide incorporation. Such knowledge is fundamental for further understanding the PrimPol-catalyzed DNA synthesis and for developing novel approaches to regulate the enzymatic activity of PrimPol. In the present study, we performed detailed kinetic analysis and computer simulations to better understand the mechanism by which PrimPol catalyzes the nucleotidyl-transfer reaction. Our experiments revealed that PrimPol-catalyzed nucleotide insertion

entails a rate-limiting step prior to the chemistry step. We assigned this step as the conformational change of PrimPol from a non-productive to a productive conformation for nucleotidyl transfer. The partition between these conformations is dependent on the length of single-stranded DNA of the DNA substrate, consistent with PrimPol's activities in both primer-extension and priming. The computer simulated rate of the conformational change is comparable to the experimentally measured k_{cat}, confirming that this conformational change is rate-limiting during the nucleotidyl transfer reaction. Collectively, these data provide a kinetic framework for further understanding the catalytic and regulatory mechanisms of PrimPol.

Abstract #: 2018 PA-0414

Presenter: Derek Narendra

Authors: Xiaoping Huang[1], Beverly Wu[1], Yi-Ting Liu[1], Diana Nguyen[1], Melika Marani[1], and Derek Narendra[1]

Institution: [1]Inherited Movement Disorders Unit, Neurogenetics Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892, USA.

Title: Parkinson-related CHCHD2 is strictly necessary for oligomerization of ALS/FTD-related CHCHD10

Body of Abstract: Mutations in paralogous mitochondrial proteins CHCHD2 and CHCHD10 were recently found to cause autosomal dominant Parkinson Disease (PD) and ALS/FTD, respectively. Using newly generated CHCHD2, CHCHD10, and CHCHD2/10 double knockout cell lines, we find that the proteins are partially functionally redundant, share localization throughout mitochondrial cristae, and form heterodimers. CHCHD2 is strictly required for oligomerization of CHCHD10. CHCHD2, in contrast to CHCHD10, readily forms homodimers in the absence of CHCHD10, which may account for the more severe phenotype resulting from loss of CHCHD2. We exploit the dependence of CHCHD10 oligomerization on CHCHD2 to develop a CHCHD2/CHCHD10 heterodimer incorporation assay and demonstrate that CHCHD2 and CHCHD10 with disease-causing mutations readily incorporate into heterodimers. These findings demonstrate that asymmetries in paralogous CHCHD2 and CHCHD10 mediate their heterodimerization and reveal an unanticipated link between Parkinson disease and ALS pathogenesis.

Abstract #: 2018 PA-0415

Presenter: Lawrence I. Grossman

Authors: Lawrence I. Grossman, Kezhong Zhang, Siddhesh Aras

Institution: Wayne State University, Center for Molecular Medicine and Genetics, School of Medicine, Detroit, MI 48201

Title: MNRR1 (CHCHD2) and mitochondrial UPR: a novel nexus

Body of Abstract: The mitochondrial unfolded protein response (UPR^{mt}) is a stress response that seeks to maintain metabolic homeostasis in the face of unfolded or misfolded proteins in mitochondria beyond their capacity to handle them. We previously reported that MNRR1 (CHCHD2) is a bi-organellar regulator of mitochondrial function. It directly activates cytochrome *c* oxidase in the mitochondria and functions as a transcription activator in the nucleus for some of the electron transport chain subunit genes and mitochondrial coactivators like PGC-1α. Our further characterization has identified a broader role for MNRR1. We now report that MNRR1 is a mediator of the UPR^{mt}. MNRR1-knockout (MNRR1-KO) cells are unable to induce key UPR^{mt} markers either in response to direct induction (in the mitochondria) or indirect induction via activation of the endoplasmic reticulum stress response (ER stress). The activation of UPR^{mt} by ER stress may arise in two ways. One is that a key ER-stress responsive transcription factor, CREBH, is a direct transcriptional activator for MNRR1. Furthermore, markers of UPR^{mt} are induced in wild type MNRR1 cells in the absence of applied stress when they are expressing a constitutively active version of CREBH (CREBH-CA). Secondly, known small molecule inducers of ER stress activate the MNRR1 gene promoter. The mechanism by which MNRR1

facilitates UPR^{mt} activation is not yet clear. ATF-5, a transcription factor similar to ATFS-1 in worms, has been identified previously as a mediator of UPR^{mt}. MNRR1-KO cells display an ~40% reduction in the protein levels of ATF5, suggesting that MNRR1 plays an important role upstream of the known mediators of UPR^{mt}. Applied stress causes MNRR1 to display an altered localization profile between the mitochondria and the nucleus. In addition to the trans-activation activity in the nucleus under ER stress, MNRR1 is also involved in the UPR^{mt} process in the mitochondria, where it is turned over rapidly by YME1L1, a key protease induced in response to UPR^{mt}. This response is absent in the MNRR-KO cells, uncovering a critical role of MNRR1 in UPR^{mt}. We discuss our findings with respect to exploiting this novel role of MNRR1 as a regulator of metabolic homeostasis.

Abstract #: 2018 PA-0416

Presenter: Rachael A. Baker and Amy M. Wilstermann

Authors: Rachael A. Baker[1], Amy M. Wilstermann[1]

Institution: [1]Calvin College, Grand Rapids, MI 49506

Title: Higher Order Structural Analysis to Elucidate Genotype-Phenotype Relationships in BCS1L-Related Rare Diseases

Body of Abstract: BCS1L is a nucleus-encoded mitochondrial member of the AAA+ (ATPases associated with various cellular activities) family of proteins. It functions as a translocase in the integration of Rieske Fe/S protein during respiratory chain complex III assembly. More than 40 BSC1L mutations have been identified, making them the most frequent cause of isolated complex III deficits. Despite their location within the same gene, BCS1L mutations are associated with a wide variety of phenotypes. These can be as mild as Björnstad syndrome, characterized by pili torti and sensorineural hearing loss, or as severe as GRACILE syndrome, characterized by growth restriction, aminoaciduria, cholestasis, iron overload, lactic acidosis, and early death. BCS1L mutations are also linked to undefined complex III deficiency, an intermediate heterogenous condition generally involving low birth weight, renal and hepatic pathologies, hypotonia, and developmental delay. However, some patients with phenotypes more severe than Björnstad and less severe than GRACILE have no demonstrable reduction in complex III activity or quantity, confounding characterization of BCS1L mutation phenotypes. While BCS1L is known to be an ATPase involved in complex III assembly, the diverse patient phenotypes suggest additional roles for BCS1L in the mitochondria. To date, the identification of genotype-phenotype relationships in BCS1L-related diseases has been unsuccessful. Here, we present a higher order structural analysis that shows connections between disease-causing BCS1L mutations and phenotypic outcomes. Using our knowledge of protein structures and drawing upon what is currently known about proteins homologous to BCS1L, we identify patterns between structural protein changes and phenotypic impacts. We focus on the three domains within BCS1L and the key secondary and tertiary structural features found within these domains that are affected by disease-causing mutations. Our observations include: 1) mutations in the mitochondrial targeting domain that are predicted to impede BCS1L trafficking amplify the effect of a second BCS1L mutation, 2) the location of BCS1L domain mutations in three-dimensional space is a better predictor of phenotype than location of the mutation in the primary sequence, 3) mutations in the ATPase domain linked to Björnstad syndrome localize to the interface between monomers while complex III deficiency-causing mutations localize to regions conserved in the ATPase family of proteins. This work suggests that structural analysis can be used to understand phenotypes of patients with compound heterozygous BCS1L mutations and may be used to aid in prognosis determination for patients with novel BCS1L mutations.

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Abstract #: 2018 PA-0417

Presenter: Tomas Mracek

Authors: Petr Pecina[1], Hana Nuskova[1], Jana Kovalcikova[1], Alena Pecinova[1], Vaclav Zidek[1], Vladimir Landa[1], Vilma Kaplanova[1], Frantisek Kolar[1], Frantisek Papousek[1], David Habart[2], Ludmila Kazdova[2], Kristyna Bardova[1], Katerina Tauchmannova[1], Zdenek Drahota[1], Jan Kopecky[1], Michal Pravenec[1], Josef Houstek[1], Tomas Mracek[1]

Institution: [1]Institute of Physiology, Czech Academy of Sciences; [2]Institute of Clinical and Experimental Medicine, Prague, Czech Republic

Title: Knockout of DAPIT protein disrupts ATP synthase oligomerisation and has a profound role in regulation of glucose homeostasis

Body of Abstract: F_oF_1-ATP synthase is the key enzyme of mitochondrial energy provision, responsible for production of most of the cellular ATP. Recently, small 7 kDa proteolipid DAPIT, originally recognised as “diabetes associated protein in insulin sensitive tissues” (also termed Usmg5), has been found to be loosely attached to the enzyme, but its biological role is largely enigmatic. To elucidate the importance of this novel protein we produced zinc-finger rat knockout model of DAPIT deficiency on unique SHR background.

DAPIT$^{-/-}$ animals were fully viable and contrary to previous data on cell lines, we observed normal levels of fully assembled ATP synthase, however, it was predominantly present in the monomeric form. Contrary to proposed role of ATP synthase dimers in mitochondrial cristae formation, we observed only minor changes in cristae morphology in heart of DAPIT$^{-/-}$ animals. We observed analogous phenotype of ATP synthase dimers absence and almost normal cristae morphology in HEK293 DAPIT knockdown model, further verifying our animal observations. From the biochemical standpoint, we observed mild isolated ATP synthase deficiency in DAPIT$^{-/-}$ animals. Both ADP phosphorylating and ATP hydrolyzing activities were reduced by circa 10% in studied tissues, i.e. liver and heart.

DAPIT$^{-/-}$ animals had 20-30% lower body weight and pronounced decrease in total adiposity (by 40%). Based on indirect calorimetry, DAPIT$^{-/-}$ animals preferred utilisation of glucose to other substrates. This was also replicated at the tissue level, with higher glucose oxidation in DAPIT$^{-/-}$ skeletal muscle. Serum levels of glucose were unchanged in both fed and fasted state, but DAPIT$^{-/-}$ animals were significantly more insulin sensitive with decreased levels of serum insulin as well as area under curve in OGTT test. This is due to the improved peripheral insulin sensitivity, as glucose-stimulated insulin secretion from pancreatic islets was normal in DAPIT$^{-/-}$ animals. High fat diet led to further dissociation of phenotype between control and knockout animals.

In conclusion, absence of DAPIT protein leads towards preferential oxidation of glucose, increases insulin sensitivity and decreases total adiposity in rat. In addition, it implicates for the first time that mitochondrial ATP synthase can be directly involved in regulation of glucose homeostasis.

Supported by Czech Science Foundation grant 16-01813S.

Abstract #: 2018 PA-0418

Presenter: Markéta Tesařová

Authors: Nikol Volfová[1], Lukáš Alán[2], Tereza Daňhelovská[1], Marie Rodinová[1], Jana Sládková[1], Jana Křížová[1], Hana Hansíková[1], Jiří Zeman[1], Markéta Tesařová[1]

Institution: [1]Charles University and General University Hospital in Prague, Department of Pediatrics and Adolescent Medicine, Prague, Czech Republic, 12800, [2]Czech Academy of Sciences, Institute of Physiology, Czech Republic, 14220.

Title: Impact of DNM1L mutation in GTPase domain on mitochondrial network

Body of Abstract: DNM1L gene encodes the dynamin 1-like protein (Drp1), which is crucial in the mitochondrial and peroxisomes fission

process. Several mutations in DNM1L gene were described. Most of them were de novo missense mutations in middle domain, which is important for the self-assembly of the protein and its oligomerization and few, cases were in GTPase domain. We identified novel de novo mutation c.176C>T (p.Thr59Ile) in DNM1L gene, that affects highly conserved Thr59 in the GTPase domain of the protein. Thr59 has been previously shown to be indispensable for GTPase reaction, since it is involved in the positioning of the catalytic water molecule and Mg^{2+} coordination (Wenger et al 2013). In patient cells, regular occurrence of “mega-mitochondria” along elongated mitochondrial network was found in cultured myoblasts and fibroblasts confirming impaired mitochondrial dynamic. An immunocytochemistry staining with catalase antibody revealed disturbances of peroxisomal fission. The dominant-negative effect of the mutation on DMN1L function was confirmed in cultured skin fibroblasts, where protein analysis revealed decreased amount of some complex IV subunits, decreased amount of Mitofilin protein and different distribution of OPA1 isoforms compared to control cells. Furthermore, we compared impact of DNM1L mutations localized in GTPase domain (p.Thr59Ile) and middle domain (p.Gly362Ser) on mitochondrial network and ultrastructure. Supported by research projects AZV 17-30965A, GAČR 14-36804G, RVO-VFN64165/2012 and Progres Q26/LF1.

Abstract #: 2018 PA-0419

Presenter: Alena Pecinova

Authors: Alena Pecinova[1], Andrea Brazdova[1], Jana Kovalcikova[1], Petr Pecina[1], Lukas Alan[1], Michal Zima[1], Zdenek Drahota[1], Josef Houstek[1], Tomas Mracek[1]

Institution: [1]Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic

Title: Targeting mitochondrial metabolic pathways in anti-cancer therapies

Body of Abstract: Targeted therapies are currently in the primary focus of anti-cancer drug development. In response to fluctuating microenvironmental conditions, mitochondrial metabolism is essential for tumorigenesis – it serves as a prerequisite to malignant cell proliferation, allowing metabolic adaptations of cellular metabolism comprising high rates of glucose utilization and subsequent NADH reoxidation. In order to sustain high glycolytic rate, many cancer cells depend on functional mitochondrial respiration (aerobic glycolysis). Glycerophosphate-(GP)-shuttle represents one of the key cellular pathways for regeneration of cytosolic NAD^+. Rate limiting component of GP shuttle is mitochondrial FAD-dependent glycerol-3-phosphate dehydrogenase (mGPDH) - inducible and highly tissue/cell specific enzyme, typically active in glycolytic cells.

In this work, we tested whether the proliferation of selected cancer cells is affected by mGPDH inhibitors (metformin-MF, α-tocopheryl succinate-TOS and benzimidazole-phenyl-succinamides-iGP) with the aim to verify mGPDH as a druggable target. TOS and iGP appeared to be potent inhibitors of mGPDH activity, yet poor inhibitors of cell proliferation. MF, the drug already repurposed for cancer therapy, had only a minor effect on enzyme activity. However, it represented the only mGPDH inhibitor which affected cancer cell proliferation, albeit still at suprapharmacological concentrations. We hypothesized, that pharmacologically relevant concentrations of MF confer its anti-neoplastic effect through subtler mechanism than direct inhibition of cell proliferation. We therefore examined 2 approaches of MF actions: (1) how mitochondrial substrate utilization underlies metformin sensitivity, and (2) its direct effect on immune system in hallmarks of cancer. Interestingly, the response to physiological concentrations of MF can be modulated by nutrient-restricted environment. Concerning MF action on immune system, isolated circulating monocytes showed higher potential for differentiation into immature dendritic cells. Despite MF associated inhibitory effect on mitochondrial respiratory chain, particularly on complex I, the overall effect did not appear to be toxic. Altogether, our results might provide new insights into cancer progression and related therapies.

Supported by the Grant Agency of the Czech Republic (16-12726S).

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Abstract #: 2018 PA-0420

Presenter: Akihiko Miyauchi

Authors: Akihiko Miyauchi [1], Takeshi Kouga [1], Eriko Jimbo [1], Tetsuro Matsuhashi [2], Takaaki Abe [2], Takanori Yamagata [1], Hitoshi Osaka [1]

Institution: [1]Department of Pediatrics, Jichi Medical University, Tochigi, Japan, [2]Department of Clinical Biology and Hormonal Regulation, Tohoku University Graduate School of Medicine, Sendai, Japan

Title: Drug screening for mitochondrial disease using fibroblasts from patients with mitochondrial disease

Body of Abstract: Introduction: Among the specific mitochondrial diseases, Leigh syndrome (LS) and MELAS (myopathy encephalopathy lactic acidosis and stroke-like episodes) account for approximately 60% of pediatric mitochondrial disease. However, the treatment options for these diseases are very limited and effective, approved treatments are highly expected. We examined an existing chemical library using fibroblasts from patients with mitochondrial disease to search for potential therapeutic drugs for mitochondrial disease.

Results: Our study was carried out in four fibroblast cell lines from LS and MELAS patients after obtaining their informed consent. We performed a cell viability assay under increased oxidative stress by adding L-butionine (S, R)-sulfoximine (BSO), a glutathione synthesis inhibitor. Idebenone, an analogue of coenzyme Q10, was used as a positive control. Under oxidative stress, the fibroblasts from the LS and MELAS patients showed enhanced cell death. We found the 6 chemicals that had a greater effect than idebenone. Among them, MA-43 was the most effective in preventing cell death. Subsequently, we measured the oxygen consumption rate (OCR) after 24 hours of MA-43 treatment using Seahorse XF 96 as a mitochondrial bioenergetic analysis. MA-43, significantly improved the mitochondrial bioenergetic functions of basal respiration, ATP production, and maximal respiration. In addition, to investigate the mechanism of action, we performed a microarray analysis of ~63,000 genes in MA-43-treated fibroblasts from a patient with Leigh syndrome.

The microarray analysis revealed 475 MA-43-responsive genes. Among these, 13 genes in WNT-mTOR signaling pathway were found to act in a direction that inhibited the WNT-mTOR signaling pathway. Moreover, a number of inflammatory chemokines and cytokines were decreased.

Conclusion: We identified that MA-43 rescued fibroblasts from cell death under oxidative stress. MA-43 also improved the mitochondrial respiratory activity. The results of the microarray analysis and Western blotting MA-43 suppressed the mTOR activity. As rapamycin, a specific inhibitor of the mTOR signaling pathway, was reported to improve survival and alleviate disease progression in a mouse model of Leigh syndrome. MA-43 appears have a potential application in the treatment of patients with mitochondrial disease due to its anti-cell death effect and its promotion of increased mitochondrial respiratory activity.

Abstract #: 2018 PA-0421

Presenter: Edward E. McKee

Authors: Chia-Heng Hsiung[1,2], Vasudeva G Kamath[1,3], Avery S Ward[1], Alexander G Gillish[1] and Edward E McKee[1]

Institution: [1]Department of Foundational Sciences, College of Medicine, Central Michigan University, Mount Pleasant, Michigan, United States [2]Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, Pennsylvania, United States [3]Touro College of Osteopathic Medicine, Middletown, New York, United States

Title: Mitochondrial DNA depletion disease and compartmentalization of the salvage pathway for TTP synthesis in isolated mitochondria from rat tissues.

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Body of Abstract: Thymidine triphosphate (TTP), along with other deoxynucleoside triphosphates (dNTPs) are required for the replication and repair of nuclear and mitochondrial DNA (mtDNA). TTP can be synthesized in cells via de novo synthesis of UDP and CDP via an energy consuming series of reactions employing smaller biomolecules, followed by their reduction to dUDP and dCDP with conversion of dUMP to TMP followed by final phosphorylation. Alternatively, the salvage pathway can synthesize TTP directly by phosphorylation of thymidine, or indirectly by phosphorylation of deoxyuridine and conversion of dUMP to TMP. Thymidine and deoxyuridine are found in the serum and may be released from cells with a de novo pathway, or released from cells via turnover of DNA. Dividing cells have a high demand for TTP and the other dNTPs, and the de novo enzymes that synthesize dNTPs are strongly expressed as well as the cytosolic thymidine salvage enzyme, thymidine kinase 1 (TK1). Conversely, differentiated non-dividing tissues have far less TTP and dNTPs, as they are needed only for mtDNA replication and nuclear DNA repair, and the de novo and cytosolic salvage pathways for TTP are poorly expressed, if at all. Hence, TTP synthesis in non-dividing tissues must rely on the mitochondrial thymidine salvage pathway via thymidine kinase 2 (TK2). Work from our laboratory in perfused rat hearts indicated that TTP could only be synthesized by phosphorylation of thymidine. Further, our laboratory has shown that the synthesis of TTP in isolated heart mitochondria prefers to start with thymidine even when thymidylate (TMP) is provided, as the TMP must be dephosphorylated to thymidine prior to entering a compartmentalized salvage pathway to produce TTP. In this study, we extend our observations in heart mitochondrial to mitochondria from rat liver, kidney, and brain. Liver mitochondrial metabolism of thymidine and TMP (7.7±0.9, and 3.1±1.5 pmol/mg protein respectively) was more rapid but otherwise similar to that of heart mitochondria (6.9 ± 1.0 and 1.8±0.4 pmol/mg protein). As with heart, the amount of TTP synthesized from thymidine was higher than TTP formed from the same amount of TMP. In contrast, TTP formed from thymidine or TMP was nearly identical and much more rapid in the kidney and brain, (~11.6 +/- 2.2 and ~18.1 +/- 1.2 pmol / mg protein, respectively) for either thymidine or TMP as precursor. However, using azidothymidine (AZT) to block the activity of mitochondrial TK2 in liver, brain and kidney, we demonstrate that TMP cannot serve as a precursor for TTP synthesis in isolated mitochondria from any of these tissues unless it dephosphorylates to thymidine first. This remains true even in mitochondria that have lost inner-membrane integrity and so is not a function of TMP transport. We show that the dephosphorylation of TMP in brain and kidney is more rapid than in heart and liver, which allows TMP to be as good a precursor for TTP synthesis as thymidine. This has important ramifications for individuals with mitochondrial DNA depletion disease caused by inherited defects in TK2, as provision of TMP is unlikely to correct the defect in many tissues.

Abstract #: 2018-PA-0422

Presenter: Rebecca D. Ganetzky

Authors: Rebecca D. Ganetzky[1,2], Amy Goldstein[1,2], Zarazuela Zolkipli-Cunningham[1], Elizabeth M. McCormick[1], Colleen Muraresku[1], Marni J. Falk[1,2]

Institution: [1]Mitochondrial Medicine Frontier Program, Division of Human Genetics, Children's Hospital of Phiadelphia, Philadelphia, PA 19104 USA; [2]Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104 USA.

Title: The Future is Now: Leveraging Electronic Health Records to Standardize Mitochondrial Care

Background: Health care for patients with primary mitochondrial disease (MD) is complicated by the large clinical diversity of patient symptoms. Patients with primary MD are highly complex, with multi-system involvement and an average of 16 symptoms per patient. Most patients see multiple subspecialty providers to manage their myriad symptoms. Providing comprehensive, efficient, and standardized care to this broad patient population that presents for care across potentially every medical discipline is a challenge. Modern electronic health record (EHR) systems can be effectively utilized to improve integrated care for MD patients.

Methods: We have devised a new system comprised of multiple EHR tools to increase the efficacy, accuracy, and standardization of care provided to patients cared for by the Children's Hospital of Philadelphia (CHOP) Mitochondrial Medicine Frontier Program. These tools include a custom electronic pre-visit survey that captures directly-entered patient information and automatically integrates responses into the body of an EHR clinical note; a standardized inpatient note template that captures physical examination findings as discrete data elements, and allows for the personalized selection of standard phrases applicable to each patient, as well as an ordering smart-set that standardizes dosing practices, lab methodologies, and ICD10 coding selection across providers. Development is underway to establish an inpatient decision support tool that consolidates recommendations for best practices in several possible mitochondrial emergency situations (e.g. lactic acidosis,

stroke-like episode) as EHR orderable items, providing evidence to support complex decision making in a centralized location for rapid access.

Results: By combining these tools to efficiently streamline several different types and sources of EHR data throughout diverse access points and utilization needs within our health system, we are able to collect consistent, reliable medical data that is rapidly accessible to all healthcare providers caring for a MD patient. We are also able to provide consistent care in both the outpatient and inpatient setting from a centralized location. Beyond the obvious clinical advantages, these tools pave the way for the use of wide-scale data science in mitochondrial medicine because of the consistency of coding and centralized electronic implementation that allows for practice modifications to be applied simultaneously across an institution. For instance, diagnostic coding consistency allows for fine mapping of data onto other research systems, such as Human Phenotype Ontology (HPO) terminology, to enable big-data mining strategies that will correlate phenotypic features with MD patient prognosis and therapeutic response. Consistent ordering practices that allow for real-time change to keep up with guidelines will enable better evaluation of practice care management results. As drug trials in mitochondrial medicine begin, these standardizations will be invaluable for selection of appropriate individualized endpoints to be used in clinical trials. Electronic tools will also enable large-scale collaboration beyond individual health systems as well as multi-center natural history studies and clinical trials, since they can be implemented in any Epic instance and readily shared between centers through Epic Community Library.

Conclusion: Collaborating to develop consistent and standardized EHR tools that support the effective practice of mitochondrial medicine within and across health centers will enable the mitochondrial disease community to provide the best clinical care and research collaborations for these patients.

Abstract #: 2018 PA-0424

Presenter: Rahmat Adejumo, MBBS, MPH

Authors: Rahmat Adejumo[1], Parisa Mehrzad [1], Mary Kay Koenig[1]

Institution: [1]University of Texas McGovern Medical School, Houston, TX

Title: Update on The International Leigh syndrome Registry

Introduction: Leigh Syndrome (LS) is defined by the Online Mendelian Inheritance in Man (OMIM) as an early-onset progressive neurodegenerative disorder with a characteristic neuropathology consisting of focal, bilateral lesions in one or more areas of the central nervous system, including the brainstem, thalamus, basal ganglia, cerebellum, and spinal cord. The lesions are areas of demyelination, gliosis, necrosis, spongiosis, or capillary proliferation. The most common underlying cause is a defect in oxidative phosphorylation. The outcome of Leigh syndrome remains grave and its natural history is poorly understood.

In an effort to improve the understanding of natural history of LS, The International Leigh Syndrome Registry was created at the University of Texas Mitochondrial Center of Excellence, in collaboration with People Against Leighs (PALS) and the UMDF. This registry is an electronic database for people with LS with self-directed questionnaires. It is designed to collect demographic, clinical, and quality of life information on people with LS. The first phase, launched in 2015, enrolled eighty-five participants and collected demographic information. The second phase, launched in 2017, collects information on medical and family history, quality of life, and research participation. As of January 31, 2018, 20 participants have completed Phase II. Of these, one participant restricted access (a feature of the registry giving participants control of their data).

Results: The registry has enrolled participants from Asia, Australia, Europe and North America. Ages range from 1-53 years with a preponderance of females (68%). Of the 19 unrestricted participants completing Phase II, the age at onset ranged from birth to 18 years, while clinical diagnosis ranged from birth to 22 years, with a median diagnostic age of 1 year, and a majority (50%) being a year old or less. A majority (95%) had had genetic testing with 61% having mutations in the mtDNA, 22% in the nDNA, and no mutation found in 6%. The other 11% were unaware of their test results. Presenting symptom was musculoskeletal in 56% and neurological in 33%. Other common presenting symptoms included respiratory (28%), gastrointestinal (22%), ocular (22%), ENT (17%), and sleep (11%). Over the course of their illness, the

majority of participants experienced neurological (81%), musculoskeletal (75%) and gastrointestinal (69%) symptoms. Of the participants with neurological symptoms, 75% reported movement disorders (ataxia, chorea, fasciculation, hypertonia, hypotonia, motor tics and myoclonus), and 69% reported developmental delay. 23% experienced at least one acute regressive episode.

Discussion: Leigh syndrome is a heterogeneous disease with multi-system involvement producing significant socioeconomic burden on the patients, their families, and the healthcare system. A primary goal of The International Leigh Syndrome Registry is to collect data for utilization by the medical community to bridge the gaps in our knowledge of LS. An understanding of the natural history will inform improvements in medical management as well as provide outcome measures for clinical trials.

Abstract #: 2018 PA-0425

Presenter: Dr. Barrett Katz[1], MD, MBA

Authors: The LHON Study Group

Institution: [1]GenSight Biologics, Paris, France

Title: Efficacy and Safety of Bilateral Intravitreal Injection of GS010: A Randomized, Double-Masked, Placebo-Controlled Trial in Subjects Affected with G11778A ND4 LHON

Body of Abstract:

Leber Hereditary Optic Neuropathy (LHON), the most common inherited mitochondrial disease, leads to sequential bilateral vision loss mostly in young adults. G11778A LHON is caused by a mutation in the ND4 gene - a subunit of Complex I in the respiratory chain - that induces apoptosis of ganglion cells on the surface of the retina.

rAAV2/2-ND4 is an investigational gene therapy enabling allotopic expression of mitochondrial transgene ND4 via a Mitochondrial Targeting Sequence. This technology actively shuttles the nuclear mRNA to mitochondrial associated ribosomes and translocates the synthesized ND4 protein into the mitochondrial matrix.

Following two ongoing Phase III studies in which subjects with LHON received a unilateral intravitreal injection of rAAV2/2-ND4, we are instituting an interventional randomized placebo-controlled double-masked trial to assess the safety and efficacy of bilateral intravitreal injections of rAAV2/2-ND4 in subjects with LHON [the REFLECT Study - ClinicalTrials.gov Identifier: NCT03293524]. Subjects in treatment arm 1 will receive intravitreal GS010 in both eyes. Subjects in treatment arm 2 will receive GS010 in the first-affected eye and placebo intravitreal injection in the second-affected eye. Subjects will be randomized in a 1:1 allocation. GS010 will be administrated via intravitreal injection of 9E10 viral genomes in 90µL balanced salt solution (BSS) as a single baseline intravitreal injection.

The primary endpoint will be the Best-Corrected Visual Acuity (BCVA) reported with logMAR at 1-year post-treatment. LogMAR BCVA will be used for statistical purposes. Secondary outcome measures will include responder analyses, Spectral Domain OCT parameters, Humphrey Visual Field metrics, Pelli Robson low vision contrast sensitivity, quality of life visual function questionnaire-25, and quality of life 36-item Short Form Health Survey. Safety and tolerability will be monitored during the study period with physical exams, EKGs, laboratory results of blood collections, and evaluations of immune response.

The study started in March 2018. Main enrollment criteria include age 15 years or older, clinically manifested vision loss due to ND4 LHON in at least one eye, and vision loss duration of ≤ 1 year. The REFLECT study will be conducted worldwide with sites in the USA to include Nashville, Denver, Atlanta, Philadelphia, Boston, NYC, and Los Angeles.

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Abstract #: 2018 PA-0426

Presenter: Peter J. McGuire

Authors: Tatiana N. Tarasenko[1], Jessica Sudderth[2], Ralph J. DeBerardinis[2], and Peter J. McGuire[1]

Institution: [1] Metabolism Infection and Immunity Section, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; [2] Children's Medical Center Research Institute, University of Texas Southwestern Medical Center, Dallas, TX; [3] Department of Pediatrics, University of California at San Diego, San Diego, CA.

Title: Pyruvate dehydrogenase deficiency reveals metabolic flexibility in T-cells

Body of Abstract: T-cells undergo metabolic reprogramming with major changes in cellular energy metabolism during activation. Given the importance of acetyl-CoA and the TCA cycle for bioenergetics and biosynthesis, we created a model of T-cell pyruvate dehydrogenase deficiency using a cre-recombinase system ($TPdh^{-/-}$). In T-cells Pdha1 mRNA was greatly reduced and PDHA was not detected by immunoblot. Stable isotope tracing with [U-^{13}C] glucose in $TPdh^{-/-}$ T-cells revealed intact glycolysis and decreased citrate, suggesting that transition from pyruvate to acetyl-CoA was impaired. Probing the TCA cycle with [U-^{13}C] glutamine, $TPdh^{-/-}$ T-cells showed increased anaplerosis with severely depressed cycling beyond the first turn of the TCA. Extracellular flux analysis of $CD4^+$ and $CD8^+$ T-cells was consistent with depressed glycolysis and oxidative phosphorylation in activated $TPdh^{-/-}$ T-cells. Despite these perturbations in bioenergetics, surprisingly, T-cell function was spared. Cell proliferation was relatively spared, lagging behind by one cycle, possibly compensated by aspartate generation from TCA anaplerosis. Immunization with T-dependent antigens elicited an antibody response in vivo, and T-cell subset differentiation in vitro (TH1, TH2, TH9, TH17, Treg) and memory responses to influenza in vivo were also intact. Overall, our results indicate that the metabolic link between glycolysis and the TCA cycle is not necessary for T-cell function. In addition, we propose that T-cells are metabolically flexible, employing ancillary pathways to circumvent a block in metabolism, a subject for continuing investigations.

Abstract #: 2018 PA-0427

Presenter: Megan L. Rasmussen

Authors: Megan L. Rasmussen[1], Alejandra I. Romero-Morales[1], Natalya A. Ortolano[1], Leigh A. Kline[1], Kathryn E. Beckermann[3], Jeffrey C. Rathmell[3], Vivian Gama[1,2]

Institution: [1]Vanderbilt University, Department of Cell and Developmental Biology, Nashville, TN 37232, [2]Vanderbilt University, Vanderbilt Center for Stem Cell Biology, Nashville, TN 37232, [3]Vanderbilt University Medical Center, Department of Pathology, Microbiology, and Immunology, Nashville, TN 37232

Title: MCL-1 maintains stem cell pluripotency through its regulation of mitochondrial dynamics and metabolism

Body of Abstract: Mitochondrial dynamics, which are maintained by the balance of mitochondrial fission and fusion, affect not only cellular metabolic profiles, but also proliferation and apoptosis. Preserving this balance is essential to maintain mitochondrial genome integrity, efficient ATP generation, and control ROS levels. Emerging studies show that the mitochondrial network in stem cells is shifted to promote a highly fragmented state. However, the mechanisms by which mitochondrial dynamics are maintained and regulated in pluripotent stem cells remain unknown. Data from our laboratory demonstrate that Myeloid Cell Leukemia-1 (MCL-1), an anti-apoptotic protein belonging to the BCL-2 family, is a fundamental regulator of mitochondrial dynamics in human pluripotent stem cells. My studies demonstrate that MCL-1 not only inhibits cell death, but also has additional roles in the regulation of stem cell fate through the modulation of mitochondrial fragmentation and metabolism. MCL-1 is induced upon reprogramming, and MCL-1 inhibition or knockdown induces dramatic changes to the mitochondrial network, as well as the loss of the key pluripotency transcription factors, NANOG and OCT-4. Aside from localizing at the outer mitochondrial membrane, as with other BCL-2 family members, MCL-1 is unique in that it also resides at the mitochondrial matrix in pluripotent stem cells. Mechanistically, my studies show that MCL-1 interacts with DRP-1 and OPA1, two GTPases responsible for remodeling of the mitochondrial

network. We were able to disrupt these interactions using a recently published small molecule inhibitor of MCL-1, which specifically binds with high affinity to MCL-1's BH3-binding groove. To further decipher this mechanism for MCL-1 regulation of DRP-1 and OPA1, we will perform structure-function analysis coupled with high resolution imaging to examine the effects of disrupting these protein interactions. Our experimental system is ideal for elucidating how MCL-1 can regulate both cell death and mitochondrial dynamics in stem cells. In addition, our preliminary data indicate that MCL-1 may also support stem cell metabolism through its interaction with OPA1 at the matrix. Upon treatment with the MCL-1 inhibitor, pluripotent stem cells showed reduced levels of basal respiration, ATP production, and respiration capacity by Seahorse analysis. These findings suggest that MCL-1 mediates a potential link between mitochondrial dynamics and metabolism, and uncovers an unexpected, non-apoptotic function for MCL-1 in stem cell fate. Future studies using cellular reprogramming will examine the requirement of this non-canonical function of MCL-1 for cell fate conversion.

Abstract #: 2018 PA-0429

Presenter: Danica (Donna) Novacic, MD

Author: Danica Novacic[1], Lynne Wolfe[1], William Gahl[1]

Institution: [1]National Institutes of Health, National Human Genome Research Institute, Undiagnosed Diseases Network, Bethesda, MD 20892

Title: Synergy Amongst Variants in Complex One? – An unsolved case report.

Body of Abstract: A 37-year-old female presented to the Undiagnosed Diseases Network with 4 years of curious symptoms including recurrent monthly episodes of tachycardia, nausea, abdominal pain and malaise. Events occur suddenly and require hospitalization, each time finding a metabolic acidosis from lactic acidosis with properly drawn lactate levels of 9-10mmol/L, hyperglycemia to the 400s but no diabetic keto-acidosis. She does develop increases in multiple urine organic acids. She responds to fluids, insulin and beta blockers. The initial events led to cardiac evaluation labeling the arrhythmia as "inappropriate sinus tachycardia" which led to sinus ablations four times. Regarding her diabetes, a timed glucose tolerance test with insulin levels reveals a type one diabetes pattern with lack of appropriate insulin production. After an otherwise unrevealing work up, her providers considered mitochondrial disease perhaps affecting the pancreas and heart. A nuclear mito gene panel showed heterozygous variants of undetermined significance (VUS) in NUBPL c.815-27T>C and NDUFS2 c.1212G>A. An exome revealed a heterozygous VUS in GPAM c.2132C>G. All of these changes are not expected to be pathologic alone but do all affect complex one of the electron transport chain. In particular the NUBPL plays a role in assembly of complex one by transferring an iron sulfur moiety to the NDUFS2, a subunit of complex one to which the iron sulfur moiety is attached. Diabetes type 1, cardiac conduction abnormalities and lactic acidosis are potential symptoms of complex one disease. The patient was evaluated at NIH with muscle biopsy and lumbar puncture to compete her mitochondrial disease work up. CNS work up did not show any evidence of mitochondrial disease. A muscle biopsy showed some subtle findings most notably with electron microscopy showing an increase in enlarged lipid droplets between myofibrils and subsarcolemmal areas which can be associated with mitochondrial disease. Electron transport chain enzymology on muscle however was normal with complex one at 88% of control. Mito DNA genome on muscle was normal. Complex one appears to be present in the tissue sampled however, it's not clear if it is functioning properly during times of high ATP demand. Perhaps the iron-sulfur moiety is not properly oriented or attached impairing optimal functioning in a state of high demand for electron transport. Pancreatic beta cells may be exquisitely sensitive to such a configuration rendering them unable to secrete insulin in a high demand state. An oxidative stress experiment is now underway on the patient's fibroblast using Agilent Seahorse XF Cell Mito Stress Test. Results are pending. We are postulating that hypomorphic mutations and synergy amongst them acting on a single complex protein could lead to deficiencies which become apparent in a high energy demand state. Additional discussion and suggestions for further work up are welcome.

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Abstracts

Abstract #: 2018 PA-0430

Presenter: David C. Samuels

Authors: David C. Samuels

Institution: Vanderbilt University, Vanderbilt University School of Medicine, Vanderbilt Genetics Institute, Department of Molecular Physiology and Biophysics, Nashville, TN, 37212

Title: Likely unrecognized mitochondrial disease patients identified in an Electronic Medical Record by a mitochondrial disease risk score and confirmed by genetics.

Abstract:

From 20,065 adult Caucasian patients in the Vanderbilt biobank, BioVU, we identified patients with discussion of mitochondrial disease in their electronic medical record (EMR). After manual review, 50 patients were assigned as potential mitochondrial disease patients. We then defined sets of ICD codes (International Classification of Disease) with similar ICD codes binned into phenotype classes following the PheWAS method. Within the 20,065 person cohort, we found 16 phenotype classes significantly associated with the potential mitochondrial disease patient subset (each with p<1E-4, adjusting for sex and age). These included phenotypes that fit the expectation of mitochondrial disease, including myopathy, peripheral neuropathy, lack of coordination and visual disturbances. These 16 phenotypes were combined into a weighted score to define a Mitochondrial Disease Risk Score (MDRS), which was then assessed for the full patient cohort. The suspected mitochondrial disease patients were concentrated in the patients with score >5 standard deviations above the mean (MDRS>5). However, only 15% of the patients with this extreme high MDRS had mention in their record of consideration of mitochondrial disease. The other 85% of these high risk patients are possible unrecognized mitochondrial disease patients.

This cohort was genotyped on the Illumina Exome chip, which focuses on rare coding variants. To test for unrecognized mitochondrial disease, we considered the most severe variants, premature stop variants in the first half of a protein. 8 genotyped SNPs fit this definition in mitochondrial proteins (defined by MitoCarta2.0). 24% of the cohort were carriers of these premature stop variants, while 0.66% were homozygous, in NDUFV3, PRODH, COQ2, or PCK2. These homozygous patients were highly more likely to occur in the MDRS>5 group compared to those with lower scores (OR [95% CI] = 13 [4-33], p=7E-5 by Fisher's test). The extremely high OR is indicative of a Mendelian disease, as expected for a homozygous premature stop within the first half of a protein. The results remained significant (OR = 12 [3-34], p=4E-4) when the 50 identified possible mitochondrial disease cases were excluded. Carriers of heterozygous stop variants were actually slightly depleted in the high MDRS group (OR=0.46 [0.19-0.98], p=0.039).

We next tested rare nonsynonymous variants in a known mitochondrial disease gene, POLG. 23 of these SNPs were genotyped on the Exome Chip. Patients with 2 or more of these rare nonsynonymous POLG variants were also concentrated in the MDRS>5 group (OR=6.9 [1.4-21.4], p=0.011). These patients were mainly carriers of the T251I and the P587L POLG mutations, which have been identified in cis in several families with PEO and MNGIE.

These results show that unrecognized mitochondrial disease can be identified in an EMR by a phenotype risk score using data generally available in any EMR. This risk score could be implemented automatically to identify patients who should be considered for investigation of mitochondrial disease. In our cohort, the patients with high mitochondrial risk scores were highly enriched for solid organ transplants (heart, p<2E-16; kidney, p<2E-16; liver, p=4E-8; lung, p=2E-12), indicating the high medical burden of these patients.

Mitochondrial Medicine 2018: Nashville

Abstracts

Abstract #: 2018 PA-0431

Presenter: Siu-Hin Wan

Authors: Siu-Hin Wan[1], Teresa M. Kruisselbrink[2], Ralitza H. Gavrilova[3], Margaret A. Lloyd[1]

Institution:
[1]Department of Cardiovascular Diseases, Mayo Clinic, Rochester MN, 55905
[2]Laboratory Medicine and Pathology, Mayo Clinic, Rochester MN, 55905
[3]Medical Genetics and Neurology, Mayo Clinic, Rochester MN, 55905

Title: SEPN1 Selenoprotein-Related Myopathy, Mitochondrial Dysfunction, and Risk of Sudden Cardiac Death

Body of Abstract:

Background: The risk of sudden cardiac death in patients with selenoprotein N1 gene mutations is unknown.

Description: A 44-year-old woman with progressive symmetric proximal muscle weakness and elevated creatine kinase was referred to cardiology clinic due to an abnormal EKG. Whole exome sequencing was performed but did not identify a specific etiology for the patient's myopathy. A comprehensive neuromuscular disorders panel demonstrated three variants in the selenoprotein N1 gene, which is associated with autosomal recessive congenital myopathy with fiber type disproportion. A muscle biopsy showed small type 1 muscle fibers. While dilated cardiomyopathy has previously been reported with SEPN1 mutations, given the rarity of the disease, there are no evidence based guidelines regarding risk stratification for sudden cardiac death in these patients. The patient was found to have nonspecific intraventricular conduction delay on EKG and the patient's echocardiogram demonstrated mild to moderate generalized left ventricular hypokinesis with ejection fraction of 45%. A 24 hour Holter monitor demonstrated frequent PVCs and a three-beat run of ventricular tachycardia. Upon patient-centered discussion of the risks and benefits, the patient elected to proceed with an implantable cardioverter-defibrillator implantation. The patient's first degree relatives were recommended to have an echocardiogram and EKG for cardiomyopathy and arrhythmia screening.

Discussion: SEPN1 mutations and selenoprotein-related myopathies are rare neuromuscular conditions with potential association with cardiomyopathy and arrhythmias. SEPN1 is found at the mitochondria-associated endoplasmic reticulum membranes, and plays an essential role in calcium transfer from the endoplasmic reticulum to the mitochondria. Mutation leads to dysregulation in mitochondrial levels of calcium in skeletal muscle. As part of the family of multiminicore diseases, SEPN1 mutations lead to muscle sarcomeric disorganization and depletion of mitochondria. While the pathophysiology and prognoses of these patients remain incompletely defined, the intraventricular conduction delay, reduced systolic function, and frequent ventricular ectopy suggests that this patient may be at higher risk of lethal arrhythmias and sudden cardiac death.

Conclusion: SEPN1 selenoprotein-related myopathy is a rare neuromuscular condition that may have cardiac involvement. Structural and arrhythmogenic abnormalities from calcium dysregulation and mitochondrial dysfunction may increase sudden cardiac death risk and a patient-centered discussion of the risks and benefits of an implanted defibrillator is useful.

Abstract #: 2018 PA-0432

Presenter: William C Copeland

Authors: Scott A Lujan[2], Margaret H Humble[1], Christopher A Lavender[3], Matthew J Longley[1], Adam B. Burkholder[3], Thomas A Kunkel[2], Robert W Taylor[4] and William C Copeland[1],

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DNA Replication Fidelity Group, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC 27709, USA; [3] Integrative Bioinformatics, Division of Intramural Research, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC 27709, USA; [4] Mitochondrial Research Group, School of Neurology, Neurobiology and Psychiatry, The Medical School, University of Newcastle upon Tyne, NE2 4HH, United Kingdom.

Title: Ultrasensitive detection of mtDNA deletions in POLG patients elucidates the mechanism of mtDNA replication

Body of Abstract: The human mitochondrial genome (mtDNA) encodes 37 genes, including 13 essential subunits of the oxidative phosphorylation system, the indispensable primary metabolic pathway for cellular ATP production. Each human cell can have over a thousand copies of circular 16.5 kb mtDNA (approximately 0.2% of all DNA in a cell), and the term heteroplasmy refers to the relative fraction of wild-type and variant sequences. Mitochondrial dysfunction is a characteristic of both natural aging and mitochondrial disease, and alterations in the mitochondrial genome are symptoms and drivers for both processes. The heteroplasmic mixture of mtDNAs containing wild-type sequence, single point mutations, rearrangements, insertions, and deletions places stringent requirements for sensitivity and low-artifact requirements on mutation detection and quantitation. Previous ultrasensitive point mutation assays suggest little role for substitution mutations in driving the aging process. In contrast, large mtDNA deletion mutations have been linked with aging and are common in late onset mitochondrial disorders. The underlying mechanisms responsible for generating large scale mtDNA deletions are somewhat uncertain and competing hypotheses exist for both the generation of large scale mtDNA deletions and the mechanism of mtDNA replication. Deletions may result from replication errors with slipped-strand mispairing between direct repeats, from processing single- or double-strand DNA breaks, or by ectopic priming events driven by micro-homology in regions of single-stranded mtDNA. Using techniques that should be applicable to ultrasensitive deletion detection in any population of circular dsDNA molecules, we report deletion maps of skeletal muscle mtDNA from 41 individuals: 19 from individuals without mitochondrial diseases (17 to 93 years of age) and 22 from mitochondrial disease patients (aged 17 to 80). The patients bear known mutations in POLG, the nuclear gene that encodes the catalytic subunit of the mitochondrial replicative polymerase, DNA Polymerase γ (Pol γ). Age- and disease-correlated patterns among the over 35 million deletions detected (~470,000 unique deletion spans detected) implicate Pol γ in the formation of mtDNA deletions during both processes and are consistent with the strand displacement model of mtDNA replication. Our new methodology applies to all mitochondrial diseases and can be adapted to any tissue sample. By extending the scope and sensitivity of conventional methodologies, our approach offers a more extensive look at the entire mitochondrial genome and helps to define the role of DNA Polymerase γ in the formation of mtDNA deletions.

ABSTRACT #: 2018 PA-0433

PRESENTER: SHANNON KRUK

AUTHORS: Shannon Kruk[1], Tatiana N. Tarasenko[1], Susan Pacheco[2], Mary Kay Koenig[2], and Peter J. McGuire[1]

INSTITUTION: [1]Metabolism, Infection and Immunity Section, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; [2]Department of Pediatrics, The University of Texas Health Science Center, Houston, Texas, USA.

TITLE: MEASLES, MUMPS, RUBELLA AND VARICELLA TITERS IN PATIENTS WITH MITOCHONDRIAL DISEASE

BODY OF ABSTRACT:

Background: Infection can be life threatening for patients with mitochondrial disease (MD). The toll infection takes on patients with MD is well recognized by clinicians: up to 50% of cases can be life-threatening or result in significant neurodegenerative sequelae. Previous work by our group has shown that patients with mitochondrial disease can have clinical markers of immunodeficiency including increased rates of infection, memory T-cell defects, and hypogammaglobulinemia. Given the recent outbreaks of vaccine preventable diseases (e.g. measles, varicella) due to depressed herd immunity, we examined the titer status for measles, mumps, rubella and varicella in patients with MD.

Methods: Patients with MD were phenotyped for immune function via a longitudinal natural history protocol known as the NIH MINI Study: Metabolism, Infection and Immunity in IEM (NCT01780168). Titers for measles, mumps, rubella and varicella were determined through

the Department of Laboratory Medicine at the NIH Clinical Center. Individuals who were either equivocal or negative for serum titers were considered unprotected (i.e. seronegative) in accordance to clinical guidelines.

Results: We evaluated 22 pediatric patients (8 female, 14 male, mean age = 10.6 years) with “Definite” or “Probable” MD as defined by modified Walker Criteria. All patients were up to date on their pediatric vaccinations as confirmed by record review. Enzymology for diagnosis of MD showed a variety of deficiencies in individual and combined respiratory chain complexes. Serum IgG levels were greater than 500 mg/dL for all patients. The seropositive rate for measles (N=22), mumps (N=22), and rubella (N=22) were 72%, 86%, and 100% respectively. These results are consistent with reported seropositive rates in historical cohorts (>90%). In contrast, the seropositive rate for varicella (N=22) was only 45%, significantly below historical controls (>90%). Up to 23% of patients with MD were seronegative for 2 or more MMRV titers.

Conclusions: Immunity to varicella is complex and involves multiple arms of the immune system. The current definition of immune protection relies on serum varicella titers. Immunocompromised individuals who contract varicella are at risk of developing visceral dissemination leading to pneumonia, hepatitis, encephalitis, and disseminated intravascular coagulopathy. The immune phenotype of patients with MD is evolving and is suggestive of immune dysfunction. Since metabolic decompensation due to infection can lead to devastating results, and rates of herd immunity are decreased due to reduced adherence to vaccination recommendations in the general population, understanding the vaccination status of patients with mitochondrial disease is an important part of maintaining health in this vulnerable population.

Abstract #: 2018 PA-0435

Presenter: Eiko Nakamaru-Ogiso[1]

Authors: Eiko Nakamaru-Ogiso[1], Julian Ostrovsky[1], Heeyong Yoon[1], Min Peng[1], Chigoziro Konkwo[1], James Byrnes[1], Rui Xiao[2], Marni J. Falk[1,3]

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Title: Pre-clinical modeling of elamipretide efficacy and toxicity in human cell and simple model animals of primary mitochondrial respiratory chain disease

Background: Elamipretide is a tetrapeptide showing encouraging results in a phase II clinical trial of human mitochondrial myopathy. It binds the phospholipid cardiolipin in the mitochondrial inner membrane and may normalize mitochondrial function under conditions of oxidative stress. We performed pre-clinical modeling of elamipretide, and an analog, in human cell and simple model animals to gain better understanding of their effects in primary mitochondrial disease.

Methods: Elamipretide and the analog SBT-100 were provided by Stealth BioTherapeutics under a researcher-initiated translational research program. Compounds were tested for toxicity and efficacy in human complex I (CI) disease fibroblasts (NDUFS8, ND5) exposed to various stressors, C. elegans genetic mutants (NDUFS2, FBXL4), and pharmacologic oxphos-inhibited zebrafish models (rotenone, azide, chloramphenicol). Outcomes studied included viability, development, integrated behaviors, and physiologic read-outs of mitochondrial function.

Results: No toxicity, developmental delay, or lethality were observed at concentrations between 1 nM and 10 uM of either elamipretide or SBT-100 in any wild-type or mitochondrial disease model tested. Human Fibroblasts. Initial studies indicated that neither compound dramatically rescued survival of CI disease fibroblasts exposed to rotenone, 2-deoxyglucose, or glucose starvation for 48-96 hours, nor demonstrated major changes in mitochondrial content, membrane potential, or matrix superoxide burden. Given these results, we decided to focus on animal models. C. elegans. Significantly improved animal lifespan occurred between 10 nM and 100 nM elamipretide in gas-1(fc21) NDUFS2 mutant worms. Trends toward improved median lifespan by 15-25% were also observed in FBXL4(tm3695) missense or FBXL4(vc3038) knockout

worms. In vivo analyses after 24 hour treatment with 100 nM of either compound showed partially improved mitochondrial membrane potential (TMRE) with no effect on mitochondrial content (mitotracker green) in gas-1(fc21) NDUFS2 mutant worms. Zebrafish. (A) CI inhibition. Improved startle and touch response occurred in two replicate studies among animals pre-treated from 5 days post fertilization (dpf) with either elamipretide or SBT-100 (100 nM > 1 uM) and then subsequently exposed to 75 nM rotenone for 5 hours on 7 dpf. While reduced swimming activity in 50-75 nM rotenone at 7 dpf was not rescued by pre-treatment from 3 dpf with 100 nM of elamipretide, mild protection from acute brain death was seen when pre-treated from 5 dpf with 10 nM of either compound. (B) CIV inhibition. Animals pre-treated with either elamipretide or SBT-100 (100 nM, 1 uM, 10 uM) from 5 dpf showed significantly improved animal survival and behavioral responses when subsequently exposed from 6-7 dpf with 75-100 uM azide. Detailed analyses of elamipretide effect following 18-24 hour exposure to 75 uM azide confirmed significantly improved survival at all concentrations between 1 nM and 10 uM, with maximal effects observed at 1 nM (n=5, $p < 0.001$). (C) Mitochondrial translation inhibition. No improvement in startle or touch response occurred with either compound in animals exposed to 2.5 mM chloramphenicol.

Conclusion: Pre-clinical evaluation of the mitochondrial-targeted molecules elamipretide and SBT-100 show promising signs of therapeutic efficacy on animal survival and stress resiliency in C. elegans and zebrafish animal models of primary complex I and IV mitochondrial dysfunction. Studies are ongoing in genetic disease model animals to gain deeper insight into potential therapeutic effects of this class of peptides in multiple mitochondrial genetic diseases.

Abstract #: 2018 PA-0436

Presenter: Elizabeth M. McCormick[1]

Authors: Elizabeth M. McCormick[1], Colleen C. Muraresku[1], Kierstin Keller[2], Marie T. Lott[2], Lishuang Shen[3], Zarazuela Zolkipli-Cunningham[1,4], Shamima Rahman[5], Matthew C. Dulik[2,6], Douglas C. Wallace[2,7], Danuta Krotoski[8], Xiaowu Gai[3,9], Marni J. Falk[1,7]

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Title: Gene-disease association and variant expert panel curation for Leigh and Leigh-like syndrome within the established ClinGen framework

Background: Mitochondrial disease is a phenotypically and genetically heterogeneous array of progressive, multi-system disorders including Leigh syndrome (LS) and Leigh-like syndromes (LLS). More than 75 nuclear and 14 mitochondrial (mtDNA) genes representing all inheritance patterns cause classical LS and LLS and dozens more cause other pediatric mitochondrial encephalopathies. Establishing an accurate nuclear or mtDNA genetic diagnosis of the diverse pediatric mitochondrial LS and LLS encephalopathy syndromes is imperative, given their increasing clinical actionability. The latter includes initiation or avoidance of specific medications, co-factors, or diets; avoiding fasting and mitochondrial-toxic medicines or anesthetics in disease subsets; improving recurrence risk counseling and prevention, and enabling targeted surveillance for reported medical complications. While establishing a definitive genetic etiology remains challenging, Mitochondrial Disease Sequence Data Resource (MSeqDR) has furthered knowledge since its inception in 2012 of mitochondrial disease genomics, phenotypes, and analysis/informatics tools. Using this foundation, MSeqDR has now begun an international collaboration funded by NICHD (U24-HD093483) to curate genes and variants relevant to the most prevalent and treatable primary mitochondrial diseases within the established framework of the NHGRI U41-HG006834 supported Clinical Genome Resource (ClinGen), with an initial focus on LS and LLS.

Results: We have successfully engaged nearly 3 dozen leading mitochondrial disease experts from 11 countries on 5 continents to assemble disease-gene and variant curation expert panel working groups. (1) Gene-disease curation: All members of the expert panel working group will participate in gene-disease curation review, an effort led by Drs. Zarazuela Zolkipli-Cunningham and Shamima Rahman. An application

for ClinGen recognition as the Mitochondrial Disease Gene Curation Expert Panel has been submitted, and we anticipate several LS and LLS genes will be curated in Spring 2018. (2) Variant curation: Members of the expert panel working group have been divided into 5 subgroups, each with a specific gene focus for variant curation. In this first year, we are focusing on LS phenotype variant curation in POLG, PDHA1, SLC19A3, ETHE1, and MT-ATP6. Each subgroup has had multiple Web meetings to review and amend current ACMG/AMP guidelines as appropriate for each gene, with variants piloted to evaluate the consistency in scoring and accuracy of pathogenicity assertions. Application for ClinGen recognition as the Mitochondrial Disease Variant Curation Expert Panel is underway, and curation will soon begin on variants in LS genes. ACMG/AMP guidelines that have been amended for each of the genes planned for LS variant curation in year 1 and will be presented, along with newly established mtDNA variant curation guidelines.

Conclusion: We have established a highly collaborative and productive international research team with demonstrated expertise and commitment to complete gene and variant expert curation for LS, LLS, and other pediatric-onset mitochondrial encephalopathies using ClinGen/ClinVar approved frameworks, processes, curation tools, and resources. Additional mitochondrial disease experts who wish to participate in this LS gene curation effort, or to participate in gene-disease curation for other mitochondrial disease phenotypes as well as variant curation in other mitochondrial disease genes using our expert panel process, are welcome and encouraged to contact mccormicke@email.chop.edu.

Abstract #: 2018 PA-0437

Presenter: Al-Walid Mohsen[1]

Authors: Al-Walid Mohsen[1], Anuradha Karunanidhi[1], Bianca Seminotti[1,2], Guilhian Leipnitz[1,2], Catherine Kochersperger[1], Mateus Grings[1,2], Areeg El-Gharbawy[1], Lina Ghaloul-Gonzalez[1,4], Peter Wipf[3], Jerry Vockley[1,4]

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Title: NOVEL DRUG THERAPIES OF FATTY ACID B-OXIDATION DISORDERS: THE FUTURE FOCUS AND HOPE

Body of Abstract:

Objective: To provide an overview of novel drug therapies of fatty acids □-oxidation disorders (FAODs) in our laboratory and discuss prospects of implementation of these therapies into clinical practice.

Background: Mitochondrial fatty acid □-oxidation (FAO) is the major source of energy for many tissues including heart and skeletal muscles and is critical during times of fasting or physiologic stress. Deficiency of enzymes of mitochondrial long-chain FAO cause serious diseases that are often lethal, if treatment is delayed. FAODs collectively represent the most frequent metabolic disorders at a worldwide frequency of ~1/10,000 of newborns. Most patients with FAODs in the US are identified through NBS. Symptoms of long-chain FAODs include fasting or stress-related hypoketotic hypoglycemia or Reye-like syndrome, cardiac conduction abnormalities, arrhythmias, cardiomyopathy, muscle weakness, and stress-induced rhabdomyolysis. Current treatment protocols are focused on alleviating acute symptoms by IV glucose infusion and L-carnitine. Long-term therapy involves replenishing carnitine stores and preventing hypoglycemia. Medium-chain triglycerides can bypass the need for the long chain FAO in some of the diseases but patients are still at risk for symptoms including late onset cardiomyopathy. Triheptanoin (UX007) is effective in treating hypoglycemia and cardiomyopathy and improves, but doesn't eliminate, muscle symptoms, and significant morbidity and mortality persist. Thus, there is continued need for therapeutic agents to treat/prevent the heterogeneous pathological symptoms caused by FAODs. Accordingly, we have been developing a series of candidate drugs that address various aspects of the cellular pathophysiology induced by various FAO enzyme deficiencies.

Results: Drugs under development in our lab are categorized under four classes:

I. Protein and lipid stabilizing chaperones: These include trimetazidine for the treatment of VLCAD, MCAD, LCHAD, and LCKAT

deficiencies, triphenylbutyrylglycerol (Ravicti), for the treatment of MCAD, and a cardiolipin binding peptide, for the treatment of LCHAD and TFP deficiencies.

II. Mitochondrial-targeted ROS electron scavengers: These include JP4-039 and XJB-5-131. FAODs increase intra-mitochondrial levels of reactive oxygen species (ROS) that is hypothesized to impair a variety of important mitochondrial functions including oxidative phosphorylation (OXPHOS) and induces inflammation. XJB-5-131 has pharmacodynamics similar to JP4-039 with both reducing ROS levels and improve OXPHOS function in cells from patients with VLCAD, LCHAD, and ACAD9 deficiencies.

III. Protein expression enhancers: Transcription activators of nuclear encoded mitochondrial genes have been demonstrated to enhance the production of defective FAO proteins and levels of activity.

IV. Anaplerotic agents: We have designed a variety of novel anaplerotic agents that function similarly to triheptanoin but enter the TCA cycle directly. Anaplerotic compounds can potentially be therapeutic not only in long chain FAODs but for methylmalonic acidemia and propionic acidemia. Their role is meant to alleviate the tertiary deficiency of specific biochemical intermediates exhausted as a result of the enzymatic block.

Conclusion: Changes in key biochemical markers suggest that some of the damaging biochemical abnormalities in FAODs can be remedied by additional therapeutic agents. Since the severity of the disease is genotype-dependent, a personalized therapeutic regime will likely be considered. Our comprehensive approach using these novel drugs with known pharmacodynamics and showing efficacy in vitro provides the impetus to bring these drugs to clinical trials soon.

Abstract #: 2018 PA-0439

Presenter: Justin B. Perry

Authors: Justin B. Perry[1], Aloka B. Bandara[1], Paloma Ruiz[2], Dain Ruiz[2], Zefeng Wang[2], David A. Brown[1], Joseph Ruiz[2]

Institution: [1]Virginia Tech, Department of Human Nutrition, Foods, and Exercise and the Virginia Tech Center for Drug Discovery, Blacksburg, VA, [2]Enzerna Biosciences, Raleigh NC

Title: Mitochondrial insights from a novel model of Leber's hereditary optic neuropathy

Body of Abstract: Leber's hereditary optic neuropathy (LHON), an ophthalmic disease affecting 1 in every 50,000 individuals, induces sudden onset vision loss in affected patients. There are few treatment options for affected patients, due in part to the difficulty in creating experimental models of LHON. Variants in several different mitochondrial proteins contribute to the LHON phenotype, notably mutations in mitochondrial electron transport system complex I. Targeted disruption of proteins encoded by mitochondrial DNA has been bolstered in recent years by several different promising approaches, yet each of these has their limitations. In this study, we utilized Artificial Site-specific RNA Endonucleases (ASREs), a novel mitochondrial RNA engineering platform, to create a cell model of LHON. By combining ASRE ablation of a target gene (phenocopy of a knockout mutation) with the expression of corresponding human disease allele integrated into the nuclear genome as a transgene, unique cell culture models of human mitochondrial diseases can be generated. In this study, human embryonic kidney (HEK293) cells and C2C12 cells were transfected with a drug-inducible ASRE designed to decrease the expression of complex I subunit ND1 (ASRE-ND1). In a separate cohort of cells, the ASRE-engineered cells were transfected with piggyBac (PB) transposon vectors that carried a cumate-inducible wild type ND1 (PB-ND1) or ND1 gene [PB-ND1(G3640A)] associated with one form of LHON. We then determined the susceptibility of each of the transfected cells to chemical injury and employed high-resolution respirometry with OROBOROS Oxygraph 2ks (OROBOROS INSTRUMENTS, Austria) to assess complex I-dependent alterations in cellular bioenergetics. ASRE-ND1 cells displayed a heightened susceptibility to mitochondrial toxicity, reflected by increased sensitivity to antimycin-A titrations. In cellular respiration studies, saponin-permeabilized cells were placed in high-resolution respirometry chambers and treated with sequential substrate-uncoupler-inhibitor titrations. Complex I-dependent respiration (glutamate/malate) decreased from 13.2 ± 0.7 pmol/sec*million cells to 11.0 ± 0.4 pmol/sec*million cells after ASRE-ND1 activation. We were able to rescue this decrement by expressing wild type PB-ND1, restoring complex I-dependent

respiration to 14.5 ± 0.4 pmol/sec*million cells. Interestingly, expression of the PB-ND1(G3649) LHON mutant in ASRE-ND1 cells did not restore complex I-dependent respiration, with these cells respiring at 12.2 ± 0.5 pmol/sec*million cells (P<0.05 significantly lower than ND1-rescue cells and no different from ASRE-ND1 cells alone). We then acutely treated cells with idebenone, a quinone analog employed in LHON clinical trials. ASRE-ND1 cells expressing PB-ND1(G3649) acutely treated with idebenone significantly improved mitochondrial respiration even when complex I was inhibited with rotenone (2.38 ±0.16 vs 4.61±0.39 pmol/sec*million cells in vehicle versus idebenone, respectively; P<0.05). These data are the first to demonstrate the feasibility of creating cell models of mitochondrial disease using the ASRE platform and the subsequent effects of knocking down ND1 expression on complex I-dependent respiration utilizing this approach. We anticipate that this technology will lead to the development of new screening platforms to identify or screen the efficacy of novel therapeutic interventions.

Abstract #: 2018 PA-0440

Presenter: Ugne Zekonyte

Authors: Ugne Zekonyte[1], Sandra R. Bacman[1], James Stewart[2], Jeff Smith[3], Derek Jantz[3], Carlos T. Moraes[1]

Institution: [1]University of Miami Miller School of Medicine, Department of Neurology, Miami, FL, USA. [2]Max Planck Institute for the Biology of Aging, Cologne, Germany. [3]Precision Biosciences, Durham, NC, USA.

Title: Using a mitochondria-targeted meganuclease to reduce mutant mtDNA load in cell and mouse models carrying a pathogenic mtDNA mutation

Body of Abstract: Mutations in mtDNA can cause a broad range of disorders. Heteroplasmy levels play an important role in the development of symptoms. Usually, a threshold of >80% mutant is required for disease phenotype manifestation. Based on this knowledge, we and others have developed approaches to selectively eliminate mutant mtDNA molecules using specific endonucleases that recognize the mutant site. After mtDNA cleavage, the linearized molecule is degraded and the mitochondria repopulate the cell with residual mtDNA, increasing the wild-type mtDNA levels. Shifting mutant mtDNA levels to below the threshold for disease could rescue the disease phenotype. In collaboration with Precision Biosciences, we have designed a meganuclease that has a mitochondrial targeting sequence, and recognizes and cleaves a mouse mtDNA region harboring the C5024T mtDNA mutation. This mutation, in the $tRNA^{Ala}$ gene causes a decrease in mitochondrial $tRNA^{Ala}$ levels. Using embryonic fibroblasts from this mouse model we observed a reduction in the relative levels of mutant mtDNA 24 hours after transfection with the mitochondrial meganuclease. This change was sustained over time and was associated with an increase in cellular respiration. We also transduced mice carrying this mutation with a recombinant AAV-9 expressing the mitochondrial-targeted meganuclease. AAV9 has tropism to skeletal and cardiac muscle but can also transduce liver. Six weeks after injections we observed shifts in heteroplasmy towards wild-type in heart, skeletal muscle, and liver. We are currently testing whether there is a concomitant increase in $tRNA^{Ala}$ levels, and a corresponding increase in OXPHOS function in vivo.

Abstract #: 2018 PA-0441

Presenter: Manuela Lavorato, PhD

Authors: Manuela Lavorato[1], Neal Mathew[1], Julian Ostrovsky[1], Eiko Nakamaru-Ogiso[1], Marni J. Falk[1,2]

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Mitochondrial Medicine 2018: Nashville

Abstracts

Title: Dichloroacetate treatment improves survival, health, and mitochondrial morphology in FBXL4 disease human fibroblasts and C. elegans models

Body of Abstract:

Background: FBXL4-related encephalomyopathic mitochondrial DNA depletion syndrome is a severe, multi-systemic mitochondrial disease caused by 47 pathogenic mutations reported to date. While FBXL4 function is not well understood, recessive mutations in FBXL4 lead to mitochondrial depletion and multiple respiratory chain complex deficiencies with pronounced lactic academia. Here, we report the physiologic effects of FBXL4 genetic mutations in C. elegans and human fibroblasts, and stressor screens used to identify effective treatment strategies.

Methods: Animal brood size, egg hatching rate, larval development, body length, neuromuscular activity, and lifespan were quantified in the FBXL4 (vc3038) C. elegans strain, which harbors a homozygous FBXL4 700 basepair deletion (ok3741). A semi-automated screening method (Mathew et al, 2016) was used to test drug treatment effects on an integrated C. elegans health endpoint of fecundity, brood size, and behavior. Human fibroblasts were studied from a subject harboring a 1067del (p.Gly356Alafs*15) nonsense mutation in the maternal FBXL4 allele and a c.1790A>C (p.GLn597Pro) missense mutation in the paternal FBXL4 allele (Gai et al, 2013). Light, fluorescence (mitotracker green), and transmission electron microscopy (TEM, Lavorato et al, 2017) methods were used to analyze proband fibroblasts and mitochondrial morphology at baseline and following metabolic stress induced by incubating cells for 48 hours in glucose/uridine-free media.

Results: C. elegans. FBXL4 (vc3038) knockout worms at 20°C had significantly reduced survival (87% and 73% of wild-type N2 Bristol median and maximal lifespan respectively, $p<0.0001$) and brood size (17% of controls, $p<0.0001$). Their neuromuscular function was impaired, with significantly reduced motility (17% body bends/min relative to N2, $p<0.001$). Treating FBXL4 mutant young adult worms with 25 mM dichloroacetate (DCA), a pyruvate dehydrogenase kinase inhibitor that activates the pyruvate dehydrogenase complex, significantly improved brood size by day 3 (from 15% to 85%, $p<0.0001$). Human fibroblasts. FBXL4 proband fibroblasts' morphology and ultrastructure were comparable at baseline to healthy controls except for increased lysosomes on TEM. When subjected to metabolic stressors (such as nutrient-deplete media) for 48 hrs, however, these cells developed very abnormal mitochondrial morphology and ultrastructure, including increased mitochondrial fragmentation on mitotracker green analysis and substantially increased lysosome content with damaged mitochondria ultrastructure on TEM analysis. Treating FBXL4 proband fibroblasts with 20 mM DCA during exposure to the metabolic stressor rescued their cell morphology and mitochondrial structure.

Conclusions: FBXL4 knockout C. elegans have significantly reduced survival, fecundity, development, and neuromuscular function. Remarkably, 25 mM DCA significantly rescued their fecundity, with study ongoing of additional treatment effects. FBXL4 disease fibroblasts similarly showed reduced survival and abnormal mitochondrial morphology in metabolic stressors, with increased lysosome number suggestive of increased autophagic-lysosomal activity, severe phenotypes that were rescued with 20 mM DCA treatment. These results are suggestive that DCA is a therapeutic candidate for FBXL4 disease that should be evaluated in robust clinical trials, and further highlight the utility of simple cell and animal model systems to identify therapeutic leads for complex mitochondrial diseases.

Abstract #: 2018 PA-0442

Presenter: Bryce A. Mendelsohn

Authors: Bryce A. Mendelsohn[1,2], Maxwell Darch1, Neal K. Bennett[1], Katharine Yu[1], Daniela Pucciarelli[3], Max Horlbeck[4], Luke Gilbert[4], William Hyun[5], Martin Kampmann[6,7], Jean L. Nakamura[3] and Ken Nakamura[1,8,9]

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Title: A high throughput screen of real-time ATP levels in individual cells reveals mechanisms of energy failure

Abstract: ATP is the key energy-carrying molecule in all cells, and failure to maintain adequate ATP levels may be critical in many diseases, ranging from mitochondrial disorders to cancer and neurodegeneration. Despite the importance of ATP, the genes and pathways that regulate and maintain ATP levels are incompletely understood yet may be therapeutic targets for diseases of energy failure or candidates for novel inborn errors of metabolism. Genome-scale analysis of ATP regulators has been limited by a lack of high-throughput tools; existing studies have relied on luciferase-based methods that require lysis of pooled cells, or surrogates for ATP such as cell growth or survival.

Genetically encoded fluorescent biosensors specifically report the level of their target metabolite in living cells and can be used to detect ATP. Measuring ATP in individual cells instead of cell lysates has produced important observations of the temporal, spatial, and sub-cellular regulation of cellular metabolism. However, high-throughput technologies, such as flow cytometry, have yet to be combined with fluorescent biosensors to detect metabolites in screens and other large studies. Here we report a novel screening paradigm to identify genes that maintain cellular ATP levels that combines fluorescence resonance energy transfer (FRET) and fluorescence activated cell sorting (FACS) to measure ATP in individual living cells transduced with a pooled CRISPRi library at high-throughput.

In the screen, approximately 2500 genes were knocked down—one gene per cell—with a mitochondrial gene-enriched CRISPRi library. Prior to sorting, cells were acutely exposed to drug/substrate combinations that forced ATP to derive from glycolysis or oxidative phosphorylation. Millions of cells/hour were then separated by FACS based on cellular ATP levels as determined by the FRET signal. Cells with increased or decreased ATP relative to the population were collected to quantify the abundance of each knocked-down gene in the high- or low-ATP fractions. We used a mutant ATP sensor with poor ATP binding to eliminate ATP-independent FRET artifacts.

With this approach, we identified genes not known to be involved in energy metabolism. Most mitochondrial ribosomal proteins are essential in maintaining ATP levels specifically under respiratory conditions, and impaired mitochondrial function predicted poor growth. We also identified genes for which CoQ10 supplementation rescued ATP deficits caused by knockdown, including a subset of CoQ10 biosynthetic genes associated with human disease and at least one gene not linked to CoQ10 biosynthesis.

This screening paradigm shows that FRET and FACS can be used to screen a metabolite—ATP—based on its real-time levels in living cells, and reveals novel candidates for mitochondrial diseases, mechanisms of metabolic control, and genetic defects responsive to energy-based therapies.

Abstract #: 2018 PA-0443

Presenter: Peter J. Oates

Authors: Peter J. Oates[1], James Carr[1]

Institution: [1]Stealth Biotherapeutics Inc., 275 Grove Street, Newton, MA 02466

Title: Urinary Metabolite Changes and Correlations with Six Minute Walk Test Distance in Elamipretide-treated Subjects with Mitochondrial Disease in the MMPOWER Trial

Body of Abstract:

Introduction: SPIMM-201 was a multi-center, randomized, double-blind, three-dose-ascending, placebo (PCBO)-controlled study of once-a-day elamipretide (ELAM) for 5 days in 36 subjects with genetically confirmed primary mitochondrial myopathy (PMM). The study demonstrated a dose-dependent (P=0.014) increase in six-minute-walk distance (6MWD), P=0.0297. Exploratory data on selected urinary metabolites from SPIMM-201, their correlations and some possible implications for PMM are now presented. **Methods:** Three first-morning-void (FMV)

urines were collected: day 1 (baseline; first 6MWD prior to drug), day 5 (prior to last ELAM dose, second 6MWD) and day 7. The UCSD Biochemical Genetics and Metabolomics Laboratory analyzed 294 metabolites in 106 of 108 intended urine samples. Metabolites were normalized to creatinine and internal standards. Data were log-transformed, filtered using interquartile ranges and analyzed by traditional univariate approaches and by nonparametric methods with non-log-transformed data. **Results:** After 4 days of ELAM, urinary creatinine, pyruvate, lactate and Krebs cycle intermediates were unchanged from baselines. ELAM dose-dependently lowered the aggregate Day 5-Day 1 change in 20 amino acids (AA) from +22% in PCBO to -12% in the high dose ELAM group (HD) (P=0.0003, ANOVA). ELAM treatment showed dose-dependent lowering of isoleucine from +34% rise in PCBO to -14% in HD (P=0.0001 ANOVA); of leucine from +32% rise in PCBO to -17% in HD (P = 0.0023); of urea cycle intermediates ornithine from +56% in PCBO to -19% in HD (P=0.0041) and arginine from +157% in PCBO to -27% in HD (P=0.012), findings all consistent with a reduction of proteolysis. The HD group had ~30-55% decreases in C_6-C_9 dicarboxylic acids (DCA), e.g., adipic acid -37% (P=0.023 vs. 0 (0=no change)), suggesting reduced microsomal fatty acid ω-oxidation and peroxisomal β-oxidation. These observations were reinforced by correlation analyses: changes in HD group ornithine (r_S=0.883, P=0.0031 (uncorrected for multiple comparisons)) and adipic acid (r_S=0.767, P=0.021) correlated more strongly with changes in 6MWD than other AAs or DCAs examined. Finally, in the HD group, on a per subject basis, decreases in ornithine (urea cycle catalyst) and in adipic acid (product of peroxisomal DCA β-oxidation) strongly correlated (r_S=0.933, P=0.0007) with one another, a finding suggesting a quantitatively coordinate downshift in resting proteolysis and fatty acid oxidation after 4 days of ELAM. CONCLUSIONS: These exploratory findings are consistent with the hypothesis that in PMM although high intra-mitochondrial NADH typically impedes Krebs cycle metabolism and mitochondrial β-oxidation of fatty acids, energetic impairment can be partially compensated by 1) robust glycolytic metabolism largely disconnected from mitochondrial metabolism, 2) proteolysis with muscle metabolism of isoleucine and related AAs to succinyl-CoA (“bypassing” dysfunctional Complex I / elevated mitochondrial NADH), and 3) ω-oxidation of medium chain fatty acids in the endoplasmic reticulum to DCAs, followed by peroxisomal β-oxidation of longer DCAs to adipic and succinic acids to support mitochondrial post-Complex I electron transfer. These exploratory data further suggest a working hypothesis that after 4-5 days of HD ELAM PMM subjects had improved mitochondrial bioenergetic efficiency as evidenced not only by increased 6MWD, but also by coordinated decreases in resting rates of proteolysis and microsomal/peroxisomal fatty acid oxidation.

Abstract #: 2018 PA-0444

Presenter: Hongying Shen

Authors: Hongying Shen[1,2,3], Gregory C. Campanello[4], Daniel Flicker[1,2,3], Zenon Grabarek[1,2,3], Junchi Hu[5], Cheng Luo[5], Ruma Banerjee[4], and Vamsi K. Mootha[1,2,3]

Institution:
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Title: De-orphaning the enzyme activity of CLYBL reveals a new mechanism of mitochondrial vitamin B_{12} regulation

Body of Abstract: CLYBL encodes a ubiquitously expressed mitochondrial metabolic enzyme that is conserved across all vertebrates. However, its enzyme activity and pathway assignment are unknown. Curiously, approximately 3-5% of human CLYBL alleles are premature stop variants, and homozygous loss of CLYBL is evidently well tolerated with subclinical vitamin B_{12} deficiency being the only phenotype.

Here, by combining enzymology, structural biology, and activity-based metabolomics, we report that CLYBL functions as a citramalyl-CoA lyase. Cells lacking CLYBL accumulate citramalyl-CoA, an intermediate in the C5-dicarboxylate metabolic pathway that also includes itaconate, a recently identified human anti-microbial metabolite and immunomodulator. We show that CLYBL loss leads to a cell-autonomous defect in

the mitochondrial B_{12} metabolism. Further cellular and in vitro study reveals the underlying mechanism. A metabolite related to the CLYBL's substrate, the CoA ester of itaconate (itaconyl-CoA), is a cofactor-inactivating, substrate-analog inhibitor of the mitochondrial B_{12}-dependent methylmalonyl-CoA mutase (MUT).

Our work de-orphans the function of human enzyme CLYBL, and provides the mechanism underlying the association between CLYBL loss and low B_{12} levels in humans. Our study also reveals that an unexpected consequence of exposure to itaconate is B_{12} inactivation, which may have important roles in inflammation and immunity.

Abstract #: 2018 PA-0447

Presenter: Justina Šileikytė

Authors: Justina Šileikytė[1,2], Marco Schiavone[2], Paolo Bernardi[2] and Michael Forte[1]

Institution: [1]Vollum Institute, Oregon Health & Science University, Portland, Oregon 97239, USA, [2]Department of Biomedical Sciences, University of Padova, Padova, I-35131 Italy.

Title: On the Road to Fixing Mitochondria: Discovery of Small Molecule Inhibitors of the Mitochondrial Permeability Transition Pore

Body of Abstract: Impaired mitochondrial fitness that leads to inappropriate cell death is well recognized as playing a pivotal role in a wide variety of human diseases. Studies have demonstrated that pathogenesis of ischemia-reperfusion injury, muscular dystrophies and neurodegenerative diseases share a common element – a lowered threshold for activation of the mitochondrial permeability transition pore (PTP). PTP, a high conductance channel in the inner mitochondrial membranes, opens in response to Ca^{2+} and reactive oxygen species. It results in mitochondrial depolarization, burst in oxidative stress, impaired cellular Ca^{2+} homeostasis, diminished ATP generation and release of pro-apoptotic factors into the cytosol and ultimately leading to cell death. In spite of the importance of the PTP, its potential as a drug target is currently not fully exploited. Indeed, at present our ability to treat diseases characterized by inappropriate PTP opening is restricted to the use of cyclosporine A (CsA) and its analogs, which desensitize the PTP indirectly by acting on the PTP regulator cyclophilin D, not the pore itself, and thus afford limited efficacy. Here we report the screening of NIH MLPCN ~360,000-compound chemical library with an aim to identify novel small molecules that would serve as direct and specific inhibitors of the PTP. We describe the screening strategy, the identification of hits belonging to isoxazole and benzamide scaffolds and their medicinal chemistry optimization leding to several very potent analogues. In isolated mouse liver mitochondria matrix swelling due to Ca^{2+} overload was inhibited with EC_{50} as low as 7.6 pM (1,000-fold lower than classical PTP inhibitor CsA) and the Ca^{2+} retention capacity was increased up to 15-fold (3-fold higher than CsA). Moreover, isoxazoles proved beneficial in zebrafish models of muscular dystrophies and protective in ischemia-reperfusion injury. Compared to prior art, these compounds are the best-in-class inhibitors of the PTP and a promising basis for the development of novel therapeutic agents for some of the most challenging human diseases featuring mitochondrial dysfunction. Supported by funds from NIH and Leducq fondation.

Abstract #: 2018 PA-0449

Presenter: Katy E Beckermann

Authors: Katy E. Beckermann[1], Kirsten Young[1], Peter Siska[2], Frank Mason[1], Katie Carbonell[2], Gabriella Andrejeva[2], W. Kimryn Rathmell[1], and Jeffrey C. Rathmell[2]

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2. Department of Pathology, Microbiology, and Immunology., Vanderbilt University Medical Center, 648 PRB 2220 Pierce Avenue, Nashville, TN 37232.

Title: Targeting Metabolic Dysregulation of T cells in Kidney Cancer

Body of Abstract: Cancer cells can inhibit effector T cells through both immunomodulatory receptors and alteration of the tumor microenvironment because of cancer metabolism. Models of chronically stimulated T cells suggest that dependence on glycolysis and glucose uptake is limited by the microenvironment and an inability to switch to oxidative phosphorylation leads to disabled effector function. The extent to which metabolic conditions within the tumor impede T cell activation and anti-tumor effector function in renal cell carcinoma (RCC) are under heavy investigation.

Through work with Rag deficient mice lacking functional B and T cells, we have established that RenCa tumor growth is regulated in a T cell dependent manner as evidenced by earlier formation and faster tumor growth. In a syngeneic mouse model of RCC (RenCa), we find that inhibition of PD-1 delays tumor growth and size. Tumor infiltrating lymphocytes (TIL) isolated from xenograft exhibit markers of activation and chronic stimulation with high PD-1. Ex vivo analysis of CD8 TIL suggested differences in metabolic dependency compared to control CD8 from splenocytes. Patient samples analyzed were found to be phenotypically distinct and impaired both functionally and metabolically from healthy surveilling CD8 control. At a global level, based on RNA-seq data, CD8 from TIL rely on distinct metabolic pathways compared to control. Instead of efficient use of aerobic glycolysis, TILs fail to increase glucose metabolism, and instead display increased reactive oxygen species (ROS) and mitochondrial dysfunction. CD8 effector cells found in tumors have notable differences in mitochondrial morphology compared to healthy control CD8 T cells by electron microscopy and immunofluorescence where CD8 TIL are punctate and dispersed throughout the cell while healthy control CD8 mitochondria are fused in networks. CD8 TIL have decreased glucose transport and glycolysis. By circumventing this metabolic deficiency through supplementation with sodium pyruvate, the end product of glycolysis, CD8 TIL in vitro show increases in markers of activation and effector function. Targeting alternative metabolic pathways using the glutaminase inhibitor CB-839 shows in vitro improvement of surface markers of activation and CD8 TIL effector function. Healthy donor CD8 cells from peripheral blood when stimulated under these conditions show increases in mitochondrial mass and changes in mitochondrial respiration and glycolysis.

Bypassing metabolic defects restore markers of TIL activation and effector function. Preclinical data suggests that improved understanding of metabolic dysfunction in TIL of RCC may allow for combined therapies to improve response rates of checkpoint inhibition in this disease.

Abstract #: 2018 PA-0450

Presenter: Yi Guo

Authors: Yi Guo, Siqi Hong, Li Jiang

Institution: Department of Neurology, Children's Hospital of Chongqing Medical University, Chongqing, China

Title: Clinical features of Mitochondrial Disease in children: a monocenter retrospective study of 45 patients in southwestern China

Body of Abstract:

Objective. The aim of this study was to review the clinical manifestations and laboratory data of children with mitochondrial disease from southwestern China. and ultimately improve the diagnostic rate and treatment options for these patients.

Methods. The clinical features, laboratory data, gene mutations,neuroimaging, myopathological features, and immunohistochemical findings of 45 genetically-confirmed patients evaluated at the Children's Hospital of Chongqing Medical University from January 2012 to December 2017 were retrospectively analyzed.

Results. Among the 45 patients with mitochondrial disease enrolled in this study, 34 patients had pathogenic mtDNA mutations and 11

had nDNA disorders. We detected 16 different gene disorders, of which 8 were novel and 8 were previously reported pathogenic mutations in known disease genes. MELAS appears to be the most common clinical mitochondrial disease syndrome, with a common m.3243A>G hot-spot mutation(64.4%) seen in this population. The most common presenting features included seizure (95.6%), lactic acidosis(95.6%), hypertrichosis (88.8%),short stature (84.4%), fatigue (80%) , hypotonia (77.8%), abnormal tendon reflexes (64%), dystonia (62%), developmental regression (56%), ptosis (51%), headache (48%), visual abnormalities(42%),ataxia(40%), dysarthria (35.6%), hearing loss (22%),and paroxysmal hemiparalysis (20%),hypertrophic cardiomyopathy(17%),Hypoparathyroidism(7%) diabetes(2%).Neuroimaging showed typical abnormality in 40 patients .Muscle biopsy showed red ragged fiber in 3 patients.FGF-21 showed a markedly higher diagnostic odds ratio than lactate,creatine kinase and L/P ratio(42.8, $p<0.05$). Compared with nDNA disorder patients, serum FGF-21 concentrations were significantly increased in patients with pathogenic mtDNA mutation ($p<0.01$).

Conclusions. Mitochondrial Disease in Southwest China is likely underdiagnosed, but patients recognized with both mtDNA and nuclear gene etiologies for their complex phenotypes. FGF-21 appears to be a potentially sensitive biomarker to raise suspicion for a mtDNA-based mtDNA disease.

Abstract #: 2018 PA-0451

Presenter: Melissa A. Walker MD, PhD

Authors: Melissa A. Walker MD, PhD (1), Stephanie Libzon MScPT (2) , Keren Yosovich (3), Dorit Lev MD (3), Tally Lerman-Sagie MD (3), Kathryn J Swoboda MD (1), Lubov Blumkin MD (2)

Institution: (1) Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA (2) Department of Pediatric Neurology, Sackler School of Medicine, Tel- Aviv University, Israel (3) Rina Mor Institute of Medical Genetics Sackler School of Medicine, Tel- Aviv University, Israel

Title: THG1L/IGH1 p.Val55Ala Mutations in 2 Unrelated Families Support a Novel, Mitochondrial Autosomal Recessive Ataxia Syndrome

Body of Abstract:

THG1L (tRNA-histidine guanylyltransferase 1 like) catalyzes the 3'-5' addition of guanine to the 5'-end of tRNA-histidine. First identified in yeast, it was subsequently found to localize to the mitochondrion in HeLa cells. Expression of THG1L—also known as Induced in High Glucose-1 (IHG1)—is transcriptionally upregulated in cultured renal mesangial cells exposed to high glucose and in patients with diabetic nephropathy (Murphy et al., 2008). THG1L shRNAi knock down cells show decreased NRF-1, cyto B, and Atp6 expression, as well as decreased Tfam activity (Hickey et al. 2011). Renal derived HK2 cells expressing doxycycline-inducible IGH1 shRNAi constructs show diminished baseline oxygen consumption rates and reserve capacity; and exogenous overexpression THG1L results in increased numbers of fused mitochondria in HeLa cells (Hickey et al. 2014).

Recently, 3 siblings from a single Ashkenazi Jewish family were reported to carry homozygous V55A mutations in THG1L segregating within the family in an autosomal recessive pattern. All three individuals displayed cerebellar ataxia, dysarthria, developmental delay, pyramidal signs, and cerebellar hypoplasia versus atrophy on MRI brain. The authors of that work demonstrated growth defect in yeast expressing V55A mutant THG1L as well as abnormal mitochondrial networks in patient fibroblasts subjected to obligatory oxidative phosphorylation, though enzyme activity was not affected in vitro (Edvardson et al. 2016).

Here we report nearly identical phenotypes in 2 patients from unrelated families each carrying homozygous V55A mutations. In both cases, the variants were inherited from unaffected, heterozygous parents and segregated in an autosomal recessive pattern among other family members. We present morphologic EM studies in patient cells, as well as bioninformatics analyses of the V55A mutation demonstrating high conservation in metazoan species. While mitochondrial number, size, and morphology in peripheral blood mononuclear cells (PBMC) were normal, and all clinical laboratory metabolic assays negative for evidence of mitochondrial dysfunction. Edvardson and colleagues have found a carrier rate of 0.8% but no THG1L V55A homozygotes in a cohort of 3232 unrelated Ashkenazi Jewish individuals. This variant is reported with an allelic

frequency of 0.02% in Exac, also with no homozygotes and is not listed in gnomAD.

The finding of THG1L V55A homozygous individuals with strikingly similar presentations in 3 unrelated families in combination in the absence of unaffected homozygotes within these families or published databases constitutes moderate evidence of pathogenicity per 2015 ACMG criteria. The experimental evidence of pathogenicity presented by Edvardson et al. could be considered sufficient to support a designation of strong evidence. Ultimately, however, further research will be required to prove pathogenicity of the variant.

Though precise counts vary based on clinical definitions, roughly 40 distinct genes have been linked to autosomal recessive ataxia syndromes (Beaudin et al., 2017). Of these, at least 10 encode gene products localizing to the mitochondrion. In this abstract we review the diverse molecular etiologies and clinical phenotypes of these syndromes, highlighting common themes in mechanism and clinical presentation.

Abstract #: 2018 PA-0452

Presenter: Anil Shanker, PhD

Authors: Roman V Uzhachenko[1], Michel Buferne[2], Claude Boyer[2], J. Shawn Goodwin[1], Lee Leserman[2], Anne-Marie Schmitt-Verhulst[2], Alla V Ivanova[3], Anil Shanker[1,4,5]

Institution: [1]Department of Biochemistry, Cancer Biology, Neuroscience and Pharmacology, Meharry Medical College School of Medicine, Nashville, TN; [2]Centre d'Immunologie de Marseille-Luminy, Aix Marseille Université UM2, Institut National de la Santé et de la Recherche Médicale U1104, Centre National de la Recherche Scientifique UMR7280, Marseille, France; [3]Department of Surgery, Section of Otolaryngology, Yale University School of Medicine, New Haven, CT; [4]Host–Tumor Interactions Research Program, Vanderbilt-Ingram Comprehensive Cancer Center, Vanderbilt University School of Medicine, Nashville, TN; [5]Vanderbilt Institute for Infection, Immunology and Inflammation, Vanderbilt University School of Medicine, Nashville, TN.

Title: Mitochondrial Ca^{2+} transport-guided intermembranous crosstalk between $CD8^+$T and NK cells provides immunosurveillance against tumor escape

Body of Abstract: The mammalian immune system is unique in its dynamic interplay of cells with often highly specialized functions that are cross-regulated by feedback loops. Thus, unraveling the complex nature and dynamics of immune networks are key to understanding lymphocyte functions and improving cancer immunotherapy. We explored the functional dynamics between T and NK cells in various adoptive T cell transfer protocols in lymphocyte deficient $RAG^{-/-}$, $RAG^{-/-}$gamma $chain^{-/-}$ and $RAG^{-/-}$GzmB-Tom mice established with solid tumors expressing an unmutated tumor-specific self-antigen P1A encoded by a cancer-germline X-linked gene Trap1a. Data show that activated tumor-infiltrating $CD8^+$ T cells augmented NK cell effector function by mechanisms involving physical interaction between activated $CD8^+$ T and NK cells through intermembranous pseudopodia-like outgrowths and exchange. This interaction forged a functional teamwork necessary for efficient immunosurveillance against the development of tumors including escaping antigen-loss tumor variants. We also found that the intercellular interaction between activated ($CD69^{high}CD25^{high}$) $CD8^+$ T cells and NK cells cross-regulated mitochondria-related parameters, increasing mitochondrial Ca^{2+} (mitoCa^{2+}) transport in both cell types. This crosstalk resulted in NK cell differentiation into effectors and $CD8^+$T cell polarization towards a central memory phenotype. Intracellularly, $CD8^+$ T cells increased JAK1, JAK3, TYK2, STAT2 and STAT6 phosphorylation and oxidative signaling in NK cells, whereas NK cells restrained IL-2 signaling in $CD8^+$ T cells by dampening activation-induced STAT5-dependent signaling. Blocking lymphocyte mitoCa^{2+} uptake abrogated the differentiation of these lymphocyte changes. Moreover, mice deficient in mitoCa^{2+} handling-regulatory gene Fus1 showed increased incidence of a range of spontaneous sarcomas, lymphomas and leukemia. These data suggest that mitoCa^{2+} transport-guided intercellular crosstalk between $CD8^+$ T and NK cells is critical for efficient immunosurveillance against tumor. The findings provide a rationale for the development of novel cancer immunotherapy approaches based on mitochondrial Ca^{2+} transport-guided intermembranous crosstalk between antitumor $CD8^+$ T and NK cells that could provide strong immunosurveillance capable of preventing tumor development and escape.

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Abstracts

Abstract #: 2018 PA-0453

Presenter: Claus Desler

Authors: Claus Desler[1], Jon Ambæk Durhuus[1], Maria Angleys[1], Jens R. Bundgaard[4], Flemming Dela[2], Ian David Hickson[3], Lene Juel Rasmussen[1]

Institution: [1]University of Copenhagen, Center for Healthy Aging, Department of Cellular and Molecular Medicine, Copenhagen, Denmark

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[4.] University of Copenhagen, Department of Clinical Biochemistry, Copenhagen, Denmark

Title: Mitochondrial function is a potential link between statin usage and risk of diabetes type II and myalgia

Body of Abstract:

Body of Abstract: Statins are HMG-CoA reductase inhibitors that effectively reduce hypercholesterolemia and thereby prevents atherosclerosis that over time can result in cardiovascular diseases such as heart attack and stroke. Because of the effectiveness of statins, they are some of the most commonly prescribed drugs worldwide with 15 million users in the US. Statin usage increases the risk of diabetes type II as well as myalgia and to a low degree rhabdomyolysis. All side-effects that have a major impact on life-quality and where the molecular mechanism responsible is unexplained. In this study we have measured the effect on statin usage on mitochondrial bioenergetics, mitochondrial production of reactive oxygen species (ROS) and other markers of mitochondrial fitness and related metabolism. Peripheral blood mononuclear cells (PBMCs) and platelets where extracted from blood samples from 61 simvastatin treated users and 16 well-matched control subjects. We found that statin usage was correlated with significant changes of mitochondrial bioenergetics and mitochondrial produced ROS. As mitochondrial function has been linked to both risk of diabetes type 2 and myalgia, we hypothesize that statin induced impact on mitochondrial function is an important mediator of the side effects of statin-usage. A better elucidation of this mechanism will allow statin usage without increased risk of mitochondrial related side-effects

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Abstract #: 2018 PA-0454

Presenter: Sunil Kumar Sain

Authors: Sunil Kumar Saini, Ponnusamy Kalaiarasan

Institution: School of Biotechnology, Jawaharlal Nehru University, New Delhi, Delhi 110067, India

Title: MicroRNA (hsa-miR-19b-2-5p) targets key mitochondrial biogenesis genes and regulates its biology.

Body of Abstract:

MicroRNAs have been shown to be involved in a diverse range of biological processes. We here report the potential role of a miRNA, hsa-miR-19b-2-5p, in regulating mitochondrial biogenesis. The microRNA's were explored to target mitochondrial biogenesis genes that were epigenetically upregulated on the exogenous expression of DNMT1-isoform3 and were predicted using four different target prediction programs. A directed network was constructed and analyzed where these microRNAs were further refined for the potential candidate

microRNAs targeting all of these genes. The results provided only one such candidate microRNA, hsa-miR-19b-2-5p, which acted as a hub and could target all those genes involved in regulating nuclear-coded mitochondrial biogenesis related genes. The miRNA has shown to be involved in critical biological processes of cellular metabolic, macromolecule biosynthetic processes and gene expression pathways. Also, this miRNA targeted a total of 112 mitochondria-related genes, establishing further the crucial role of the candidate miRNA in mitochondrial biology.

Abstract #: 2018 PA 0455

Presenter: Kostas Tokatlidis

Authors: Mauricio Cardenas-Rodriguez[1], Ruairidh Edwards[1], Allan Dale[1] and Kostas Tokatlidis[1,2]

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[2] **corresponding Author:** Kostas.tokatlidis@glasgow.ac.uk

Title: Rescuing mitochondria in peril during oxidative stress by unconventional translocation of cytosolic antioxidant proteins

Maintaining healthy mitochondria requires a finely tuned redox balance in the organelle. Alterations in redox balance affect the flux of several redox pathways and are related to ageing, neurodegenerative disorders and cancer. We have recently discovered that under severe redox stress mitochondria are able to import protective antioxidant proteins that can promote reactions to counteract this stress. We describe here how critical antioxidant proteins that normally reside in the cytoplasm are redirected into redox stressed mitochondria and how they discharge their antioxidant function to ameliorate this stress. Protein import into mitochondria is essential for their construction. Most mitochondrial proteins (~10-15% of the human proteome) are synthesized in the cytosol and so must be imported following their synthesis. Under healthy conditions the import of most of these proteins is powered by the mitochondrial transmembrane energy gradient. Remarkably, the antioxidant proteins are imported by a system that does not require this energy gradient, which is just as well since that gradient is compromised under these severe redox stress conditions. Our findings offer the first opportunity to explore the mechanism of this entirely new import pathway that is critical to defend cells against deleterious oxidative stress. This analysis should further our understanding of mitochondrial protein import and cell stress mechanisms and it is likely to provide a novel paradigm for understanding the coordination of oxidative stress signalling in eukaryotic cells. Understanding mechanisms that can potentially rescue mitochondria from irreversible damage are clearly very important.

Abstract #: 2018 PA-0456

Presenter: Zarazuela Zolkipli-Cunningham

Authors: Zarazuela Zolkipli Cunningham[1,2], Didi She[3,7], Katherine Mitchell[4], Douglas C. Wallace[4,5], Mark Allen[3,7], M J. Falk[2,6,8]

Institution: [1]Division of Neurology, [2]Mitochondrial Medicine Frontier Program, [3]Krishna P. Singh Center for Nanotechnology, [4]Center for Mitochondrial and Epigenomic Medicine (CMEM), [5]Department of Pathology and Laboratory Medicine [6]Division of Human Genetics, Department of Pediatrics, The Children's Hospital of Philadelphia, [7]School of Engineering, [8]University of Pennsylvania, Philadelphia, PA 19104.

Title: Development of a minimally invasive muscle O_2 nanosensor in Mitochondrial Myopathy

Background: While Mitochondrial Myopathy (MM) treatment intervention trials are increasingly being conducted, a current major challenge

is the absence of quantitative outcome measures that reliably reflect and predict disease severity, progression, and response to therapy. Historically, diagnostic open needle biopsies have been the gold standard approach to obtain direct, ex vivo, objective measurements of muscle oxidative phosphorylation (OXPHOS) capacity. The opportunity for in vivo intramuscular measurements of oxygen (O_2) in MM patients has not previously been feasible. In MM, whole body and tissue O_2 levels are elevated due to impaired muscle O_2 extraction efficiency when mitochondrial OXPHOS function is compromised. Development of a Clark-type O_2 nanosensor would transform clinical practice where decision-making is based on historical results, and clinical trials where repeatable, objective outcome measures in MM are lacking. Here, we report results of a pre-clinical study to test the safety and efficacy of a prototype O_2 sensor to measure in vivo muscle O_2 in mouse models having the biochemical and physiological characteristics of MM.

Methods: Our collaborators at UPenn Singh Center for Nanotechnology have custom designed, fabricated and characterized a prototype Clark-type O_2 nanosensor. The working principle is that O_2 diffuses through the O_2-permeable membrane into the inner electrolyte cell, producing a current that is proportional to the amount of reduced O_2 when a negative potential is applied between the working and reference electrodes. The nanosensor was manually inserted in gluteus muscle under isoflurane (1.5%) anesthesia within 90 seconds of completing treadmill-exercise in MM mice harboring the homogenous mt-ND6 mutation and in transgenic MCAT mice at baseline and after 20 minutes of standard protocol treadmill exercise.

Results: The partial pressure of muscle O_2 in control (C57BL/6J) mice was reported as 50 Torr (Reinke, 1985). Before and after in vivo measurements in gluteal muscle, room air nanosensor measurement was found to be ~ 160 Torr. MCAT transgenic mice that over-express mitochondrial catalase had measured muscle O_2 of 53.4 ± 3.58 Torr (n=2), similar to historic controls. mt-ND6 mutant mice (n=2) had elevated muscle O_2 of 60.5 Torr when sedentary (n=1), and 72.2 Torr following treadmill exercise (n=1).

Conclusions: O_2 nanosensor analysis demonstrated that mt-ND6 mutant mice, but not long-lived MCAT transgenic mice, had elevated muscle O_2 relative to control mice when sedentary that further increased after moderate exercise. These preliminary results indicate the feasibility and efficacy of our prototype O_2 nanosensor to accurately quantify in vivo muscle O_2., and are the first to confirm in a MM mouse model that tissue O_2 is elevated. These results support similar observations of elevated muscle O_2 previously made by other techniques in human MM subjects (Chance, 2003). These data highlight the possibility of developing minimally-invasive, quantitative, bedside assessments of muscle O_2 as a biomarker of in vivo mitochondrial OXPHOS capacity in MM, a capability that may change our understanding of MM and shift the treatment assessment paradigm.

Reference: Reinke et al, (1985) Journal of Applied Physiology.

Abstract #: PA-0457

Presenter: Kalyn S. Specht

Authors: Kalyn S. Specht[1], Abinash Padhi[2], Amrinder S. Nain[2], David A. Brown[1]

Institution: [1]Virginia Tech, Department of Human Nutrition, Foods, and Exercise and the Virginia Tech Center for Drug Discovery, Blacksburg, VA, 24060, [2]Virginia Tech, Department of Mechanical Engineering, Blacksburg, VA, 24060

Title: Energetics of Myoblasts Sensing and Crawling on Aligned Fibers

Body of Abstract: Cell migration is pivotal in many physiological processes such as morphogenesis and wound healing, as well as in disease states such as cancer metastasis. Native cellular environments are composed of fibrous proteins of varying geometric features (diameter, orientation, spacing and hierarchical assembly). Cellular migration starts with cells sensing their environment through formation of finger-like protrusions which, depending upon favorable conditions, leads to whole cell-body migration. The bioenergetic pathways responsible for migratory behavior remains poorly understood, in part due to lack of appropriate in vitro assays capable of mimicking extracellular matrices. In this study, we engineered fiber networks of controlled diameter (curvature) and alignment, and interrogated the bioenergetic pathways utilized in single cell C_2C_{12} myoblast migration. The fibers were coated with fibronectin, and cell migration was studied using aligned fiber networks. To investigate the bioenergetic contribution of different metabolic pathways in these migrating cells, we introduced mitochondrial inhibitors,

including antimycin-a (AMA) [2μM], oligomycin [2.5μM] and a glycolysis inhibitor, 2-deoxy-d-glucose (2-DG) [12mM]. Despite clear effects on mitochondrial respiration, cell speed was not markedly altered after treatment with AMA (34.5±2.3 μm/hr and 30.1±2.5 μm/hr for pre- and post-AMA, respectively, n=21; P = NS). Rates of cell migration remained unchanged for the next six hours. Next, we treated cells with oligomycin, and again found cell speed to remain unaffected (pre: 30.1±3.8 μm/hr and post: 28.72.8 μm/hr (n=11); P = NS). Unexpectedly, inhibition of glycolytic metabolism with 2-DG significantly decreased cell migration velocity from 32.9±3.8 μm/hr to 23.9 ±1.4 μm/hr (P<0.05, (n=25)) within one hour and remained decreased for the subsequent 6-hour treatment. Interestingly, the successive addition of 2DG and AMA further decreased cell velocity from 35.4 ±2.1 μm/hr to 14.9±1.3 μm/hr (P<0.05, (n=10)). Altogether our data suggest that myoblast migration is heavily reliant on glycolysis and contrary to current understanding suggests a diminished role of mitochondrial bioenergetics in cell-ECM interactions during development, repair, and pathology.

Abstract #: 2018 PA-0458

Presenter: Linlin Zhao

Authors: Riley Boyd, Wenyan Xu, and Linlin Zhao

Institution: Department of Chemistry and Biochemistry, Central Michigan University

Title: Mitochondrial Transcription Factor A Induces DNA-Protein Cross-Links and DNA Strand Breaks at Abasic DNA Lesions

Body of Abstract: Mitochondrial DNA (mtDNA) is packaged by mitochondrial transcription factor A (TFAM) protein into nucleoid structures. MtDNA encodes important protein components for cellular bioenergetics, and therefore the integrity of mtDNA is critical for mitochondrial and cellular functions. MtDNA is susceptible to damage by endogenous and foreign chemicals; mtDNA damage has been implicated in human diseases and aging. Abasic lesion is a ubiquitous DNA damage produced by hydrolysis of the glycosidic bonds of nucleotide building blocks of DNA. The hydrolysis is accelerated when certain nucleobases are chemically modified. In addition, abasic lesion is an important intermediate during base excision DNA repair. The steady-state level of mitochondrial AP lesions is estimated at hundreds per cell, which makes abasic lesion one of the most abundant endogenous mtDNA lesions. Importantly, abasic lesions are chemically reactive and form secondary DNA damage, such as DNA interstrand cross-links, DNA-protein cross-links, and strand breaks. This chemical reactivity of abasic lesion has been shown with histone and several nuclear DNA repair proteins. Both abasic lesions and their derivatives are highly mutagenic. In this work, we discovered that TFAM causes the formation of TFAM-DNA cross-links and DNA strand breaks when abasic lesions are formed in TFAM-DNA complexes in vitro. The half-life of abasic lesions within a TFAM-bound DNA is approximately 50-fold shorter than in naked DNA. Several lysine residues of TFAM catalyze the formation of TFAM-DNA cross-links and DNA strand breaks. Our research has revealed a potential role of TFAM in facilitating the degradation of damaged mtDNA and pave the way to further validating this new function of TFAM in the cellular environment.

Abstract #: 2018 PA-0460

Presenter: Pankaj Prasun

Authors: Pankaj Prasun[1] Cassie Mintz[1] Sajel Lala[1] Bryn Webb[1] George Diaz[1]

Institution: [1]Icahn School of Medicine at Mount Sinai, New York, NY 10029

Title: Unusual presentation of EARS2 associated mitochondrial disease

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Body of Abstract: Introduction: EARS2 is associated with leukoencephalopathy with thalamus and brain stem involvement and high lactate (LTBL). Presentation in all cases described so far is neurological with onset in infancy and characteristic MRI findings of diffuse white matter changes and symmetrical signal abnormalities in thalamus and brain stem. The presentation falls into two group- severe and mild. The patients in severe group present before 6 months of age with marked neuro-regression and then clinical stagnation. Patients in mild group present later in infancy with neuro-regression but they partially recover and regain some milestones. Here we describe a patient with atypical presentation.

Case report: Patient is a 6 month old girl who presented at 3 months of age with generalized tonic seizures. Her growth and development was appropriate. She had hepatomegaly, abnormal liver function tests (AST 237, ALT 146, GGT 356), and high lactate in blood (9.8), and CSF (9.4).Abdominal ultrasound showed diffusely echogenic enlarged liver and MRI brain showed restricted diffusion in white matter and corpus callosum. Basal ganglia and brain stem structures were normal. Two mutations in the EARS2, c.322C>T (p.R108W) and c.328 G>A (p.G110S) were found. She was started on riboflavin, thiamine, coenzyme q 10, and L-carnitine. At 6 months, her growth was normal. Mild motor delay but normal language and social development were noted. Both lactate and liver enzymes were trending down.

Discussion: EARS2 associated mitochondrial disease is characterized by neurological symptoms and characteristic MRI finding. Our patient does not fall into the typical severe or mild presentations. Although, onset of symptoms is before 6 months, the characteristic neuro-regression is lacking. In addition, brain MRI is not typical of LTBL. Hepatomegaly and elevated liver enzymes were the main concerns at presentation. Although liver involvement is described in few cases before, a primarily liver presentation without typical neuroimaging finding has not been described.

Conclusion: The clinical spectrum of EARS2 associated mitochondrial disease is likely broader than known at present and it should be considered in a patient with high lactate and neurological findings even in absence of characteristic MRI findings. Spectrum of EARS2 related conditions includes liver abnormalities and it should be considered in the differential diagnosis of unexplained hepatopathy with high lactate.

Abstract #: 2018 PA-0462

Presenter: Rafael Toro

Authors: Rafael Toro[1], Paul van Ginkel[2], Tomas Prolla[2], Nuray Ugras[3], Blake Hill[1]

Institution: [1]Medical College of Wisconsin, Milwaukee WI 53226, [2]University of Wisconsin, Madison WI 53706, [3]CyteGen Corp, Wauwatosa WI 53226

Title: Rapid determination of protein expression levels by gel-free immunodetection to evaluate mitochondrial homeostasis

Mitochondrial homeostasis involves the growth of new mitochondria (mitochondrial biogenesis) and removal of damaged mitochondria (mitophagy). Improper mitochondrial homeostasis causes or contributes to a wide variety of diseases including many neuromuscular disorders. To monitor mitochondrial homeostasis, it is common to measure gene expression at the mRNA level using RNA-SEQ and gene microarray. These are powerful tools but correlate with protein expression levels poorly (typically less than 40%). Thus, direct measurement of protein levels via immunodetection can provide a more accurate reflection of mitochondrial homeostasis. The ability to monitor changes in protein levels involved in mitochondrial biogenesis and mitophagy may have many potential applications, including use as a diagnostic tool and in drug discovery. In addition, changes in levels of proteins that reflect and/or and regulate mitochondrial homeostasis can be used elucidate mitochondrial response mechanisms due to cellular stresses or disease. Here we apply a commercial adaption of traditional Western blotting techniques that take advantage of microfluidic technology and robotics to rapidly generate a qualitative and quantitative profile for a panel of proteins involved in mitochondrial homeostasis. A panel of antibodies for proteins involved in mitochondrial biogenesis and mitophagy was optimized for use. This optimization process involved determining a saturating dilution of antibody for accurate quantification, determining an appropriate lysate concentration for optimal signal detection, and the testing of cell lysates cultured under conditions that should alter the balance of mitochondrial biogenesis and mitophagy (peroxide treatment, increased mutational load, impairment of fusion/fission machinery). The result is a core panel of antibodies that allows for the rapid determination of up or down regulation of proteins involved in mitochondrial homeostasis.

Abstract #: 2018 PA-0463

Presenter: Laura Stanley

Authors: Laura Stanley[1], Kira Mann[2], Tracy Wall[3], Michael DiMatteo[3]

Institution: [1]Foundation for Mitochondrial Medicine, Atlanta, GA 30327, [2]MitoAction, Boston, MA 02109, [3]Stealth BioTherapeutics, Newton, MA 02466

Title: Primary Mitochondrial Myopathy Research: Patient Perspectives on Their Journey Through the Healthcare System

Background: Primary mitochondrial myopathies (PMM) are genetic disorders that impair normal mitochondrial function, ultimately affecting neurologic (central & peripheral), musculoskeletal, gastrointestinal, and cardiovascular function. PMM patients tolerate physical exercise less because of skeletal muscle respiratory chain dysfunction that leads to debilitating muscle weakness, muscle atrophy, limited exercise capacity, and symptoms of fatigue. PMM severity is variable, but disease progression significantly compromises daily activity performance.

Objectives: We sought to better understand how to positively impact the lives of patients with PMM by improving their access to timely and appropriate care and management.

Methods: Perspectives from PMM patients and/or their caregivers (N=19) were gained through a three-part study: Symptom-form completion, one-on-one interview, and completion of a 7-day journal. All respondents had a diagnosis of PMM, with or without genetic confirmation.

Results: PMM patients ≥22 years of age (n=9) experienced the longest time from initial symptom-presentation to diagnosis. Typically, younger children had a shorter time to diagnosis; those with more overt symptoms (ie, failure to thrive or reach expected milestones) were diagnosed even more rapidly. Fatigue and muscle weakness were the most frequently experienced symptoms in respondents aged 3+ years; the number of different symptoms experienced by patients increased with age. In patients <3 years of age, parents noted delays in achieving expected developmental milestones. All respondents were routinely seeing 4-10 physicians (generalists and various specialists), and received a variety of diagnoses and medical/cognitive tests before a definitive diagnosis of PMM was obtained from a neurologist or neuro-geneticist. Prior to diagnosis, pediatricians used the 'wait and see' approach. All respondents reported being on a variety of supplements and prescription treatments throughout the course of their disease. As expected, the social and emotional impact of PMM is huge, affecting rational, emotional, physical, and social parameters; the older the patient, the greater the impact on day-to-day functioning. Physician education was identified as a primary need by adult patients. Supportive needs include education, insurance, finance and transportation; education requires better understanding and services for school age students and young adults. Support and charitable organizations were deemed to be therapeutic.

Conclusions: New patient-journey insights reinforced the need for a more timely diagnosis in PMM. Specialists in neurogenetics, geneticists and some neurologists are most adept at achieving a diagnosis; generalist physicians and specialists wait too long to refer patients to other specialists. PMM patients also see numerous other specialists over the span of many years, especially older adult patients, without obtaining a definitive diagnosis. Generally, the younger the patient, the faster the PMM diagnosis. Geography also remains an ongoing diagnostic barrier. Younger patients tend to have a brighter outlook on their future, while there is a sense of resignation and depression among older PMM patients.

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Abstract #: 2018 PA-0464

Presenter: Chynna N Broxton[1]

Authors: Chynna N Broxton[1], Manuela Lavorato[1], Sujay Guha[1], Tara Gallagher[2], Christoph Seiler[2], Eiko Nakamaru-Ogiso[1], Marni J Falk[1,3]

Institution: [1]Mitochondrial Medicine Frontier Program, Division of Human Genetics, Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA 19104; [2]Aquatics Core Facility, The Children's Hospital of Philadelphia Research Institute, Philadelphia, PA 19104; [3]Department of Pediatrics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104

Title: Development of robust animal models to investigate mitochondrial Dihydrolipoamide dehydrogenase (DLD) deficiency and therapeutic efficacy.

Body of Abstract:

BACKGROUND: Dihydrolipoamide dehydrogenase (DLD) is a key enzyme in mitochondrial metabolism that serves as the E3 subunit of three mitochondrial matrix enzymes involved in glucose metabolism (PDH), the tricarboxylic acid cycle (AKGDH), and amino acid catabolism (BCAADH). Autosomal recessive DLD mutations result in a rare mitochondrial disease that manifests as developmental delay, lactic acidosis, liver dysfunction, neurological problems, and/or failure to thrive. Although generally attributed to PDH deficiency, full understanding of the biochemical basis for the complex disease phenotype remains largely unknown. As a result, current therapies are non-specific and generally ineffective. Here, we explored the molecular and biochemical mechanisms underlying DLD mutation phenotypes in novel zebrafish (D. rerio, vertebrate) and nematode (C. elegans, invertebrate) genetic model animals of primary DLD deficiency.

METHODS: Brood size, egg hatching rate, lifespan, and DLD-1 protein expression were studied in DLD-1(tm4879) homozygous mutant C. elegans. DLDH deletion zebrafish mutants were generated using CRISPR/Cas9 gene editing via microinjection at the 1-cell stage. Animal viability, organ development, swim behavior, DLD-1 protein expression, and electron transport chain enzyme activity analyses were evaluated in DLDH mutant relative to AB (wild-type) zebrafish. Liver physiology was studied by Oil Red O staining and transmission electron microscopy.

RESULTS: C. elegans: DLD-1(tm4879) worms had 50% decreased DLD1 protein expression, 75% reduced brood size, 23% egg hatching rate, and 30% median lifespan relative to N2 Bristol wild-type worms at 20°C ($p < 0.0001$). Zebrafish: DLDH mutant fish had a grossly enlarged liver and deflated swim bladder visible by 5 days post fertilization (dpf). Abnormal swimming behavior was observed at 7 dpf, with 100% mortality occurring by 11 dpf. Transmission electron microscopy of DLDH mutant liver showed pronounced mitochondrial degeneration and a high accumulation of lipid droplets, which was further confirmed by Oil Red O staining. Electron transport chain enzyme activity analysis surprisingly revealed DLDH mutant zebrafish had decreased complex IV activity by 20% at 6 dpf and by 40% at 7 dpf relative to wild-type AB controls.

CONCLUSION: Two novel, recessive DLD mutant animal models in C. elegans and zebrafish display the classical hallmarks of mitochondrial and liver dysfunction that occur in human DLD disease. Further studies are ongoing in these models to investigate the impact of DLD mutations on E3-containing enzyme activities, mitochondrial physiology, and intermediary metabolite profiles. In addition, drug screens are underway to evaluate the ability of current standard-of-care and emerging therapies to rescue the severe phenotypes seen in DLD disease model animals, as well as identify novel treatment leads to test in human DLD subject clinical trials.

Abstract #: 2018 PA-0465

Presenter: Kira Mann

Authors: Kira Mann[1], Laura Stanley[2], Tracy Wall[3], Michael DiMatteo[3]

Institution: [1]MitoAction, Boston, MA 02109, [2]Foundation for Mitochondrial Medicine, Atlanta, GA 30327, [3]Stealth BioTherapeutics, Newton, MA 02466

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Title: Primary Mitochondrial Myopathy Research: Neurologists Understanding of Primary Mitochondrial Myopathies in Their Patients

INTRODUCTION: Patients with primary mitochondrial myopathies (PMMs) are not diagnosed in a timely manner and have difficulty reaching the appropriate specialist to manage their neuromuscular symptoms.

OBJECTIVE: We sought to understand how the lives of PMM patients with neuromuscular manifestations can be positively impacted by improvements in their management and journey through the healthcare system. Goals included obtaining an understanding of the neurologist's practice type/focus, and experience with PMM patients.

Methods: Qualitative research including one-on-one in-person exploratory interviews (8 neurologists, varied areas of training/specialization) and web-assisted telephone interviews (11 neurologists, 8 specializing in neuromuscular disorders, 3 neurology generalists) were conducted.

Results: Neurologists are generally aware of primary mitochondrial disease (PMD) and it may be more common in their practice than originally thought. Some of those interviewed reported having at least one patient diagnosed with a specific form of PMD (ie, LHONs, MELAS, Kearns-Sayre syndrome). Several neurologists indicated that they had seen a case of PMD as a resident; some thought it was only a pediatric condition. Because the term PMM was not recognized (neurologists are more familiar with specific types of PMD), the neurologists' primer that detailed PMM symptoms and its impact on organ systems, along with prevalence data was well received. As a result, neurologists felt that there were probably undiagnosed patients exhibiting symptoms of PMM in their practice since they don't immediately consider PMM as a diagnosis. Currently, neurologists are rarely ordering muscle biopsy and genetic testing, but did express an increased interest after reviewing educational materials that indicated such diagnostic testing is available. General neurologists and subspecialists indicated that neuromuscular subspecialists would be the primary specialty to manage adult PMM patients.

Conclusion: Neuromuscular neurologists are clearly the best poised medical professionals to provide a diagnosis in adults who present with signs and symptoms of PMM; however, they still require additional educational materials to directly support the identification of symptoms possibly indicative of PMM in adults. The results indicate a call to action for educating adult neurologists on the epidemiology, clinical presentation and diagnostic approach for adults with PMM.

Abstract #: 2018 PA-0466

Presenter: Mitchell E. Allen

Authors: Alexander H. Thomson[1], Mitchell E. Allen[1], Justin B. Perry[1], Andrew J. Nichols[2], John F. Reilly[2], Pradeep Bista[2], Diana Lee[2], Chi B. Vu[2], David A. Brown[1]

Institution: [1]Virginia Tech, Department of Human Nutrition, Foods, and Exercise and the Virginia Tech Center for Drug Discovery, Blacksburg, VA, [2]Catabasis Pharmaceuticals, One Kendall Square, Suite B14202, Cambridge, MA

Title: Protection of Mitochondrial Bioenergetics in Injured Myoblasts with CAT-4001

Body of Abstract: Mitochondrial dysfunction is noted across disease states and is a primary target for emerging treatments. In these studies, we determined the bioenergetic efficacy of a novel cell-permeable compound, CAT-4001. CAT-4001 is a conjugate of monomethyl fumarate (activates Nrf2) and docosahexaenoic acid (inhibits NF-kB). Once inside cells, the conjugate was hydrolyzed by intracellular enzymes to synergistically decrease inflammation (by inhibiting NF-kB) and stimulate cellular resistance to oxidative stress (by activating Nrf2). Oxygen consumption rates (OCR) were assessed in undifferentiated C2C12 myoblasts exposed to a 500μM hydrogen peroxide insult. The effects of CAT-4001 were compared to positive controls N-acetylcysteine (NAC) and catalase, using a 24-hour treatment paradigm. Peroxide treatment significantly decreased maximal OCR (90±19 pmol/min) compared to untreated control (184±34 pmol/min, $P<0.05$). Treatment with 1μM CAT-4001 significantly improved maximal OCR (206±19 pmol/min) to levels comparable to positive controls (192±25 pmol/min for 5mM NAC and 194±19 pmol/min for catalase). 10μM of CAT-4001 resulted in substantially higher maximal OCR (348±20 pmol/min). ATP-dependent respiration was also significantly reduced after peroxide insult, decreasing ATP-dependent OCR from 51±8 pmol/min in untreated cells to 32±5

pmol/min after hydrogen peroxide. NAC and catalase treatment led to modest improvements in ATP-dependent respiration that did not reach statistical significance (47±5 pmol/min and 49±4 pmol/min, respectively). Both 1μM and 10μM CAT-4001 evoked significantly higher ATP-dependent respiration (69±5 pmol/min and 113±5 pmol/min, respectively; P<0.05 compared to all other peroxide-treated groups). These data highlight the efficacy of CAT-4001 as a treatment to improve mitochondrial function by improving cellular tolerance to oxidative insults.

Abstract #: 2018 PA-0467

Presenter: Palmiro Cantatore

Authors: Giordano L[1], d'Adamo P[2], Cappellari M[1], Carelli V[3], Roberti M, Loguercio Polosa P[1], Cantatore P[1,]

Institution: [1]Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Bari, Italy; [2]IRCCS-Burlo Garofolo Children Hospital, Trieste, Italy; [3]Department of Biomedical and NeuroMotor Sciences (DIBINEM), Neurology Unit, University of Bologna, Bologna, Italy

Title: Effect of cigarette smoking on the penetrance of Leber's hereditary optic neuropathy

Leber's hereditary optic neuropathy (LHON), the most frequent mitochondrial disease, is associated with mitochondrial DNA point mutations affecting Complex I subunits, usually homoplasmic. This blinding disorder is characterized by incomplete penetrance, possibly related to several modifying factors. Increased mitochondrial biogenesis in unaffected mutation carriers is a compensatory mechanism, which reduces penetrance. Among environmental factors cigarette smoking has been implicated as disease triggers. To investigate this issue, we investigated the relationship between cigarette smoke and mtDNA copy number in blood cells from large cohorts of LHON families and found that affected smokers had a lowest mtDNA copy number. To get insights on the mechanism of tobacco toxicity in LHON, we treated fibroblasts from affected individuals, unaffected mutation carriers and controls with cigarette smoke condensate (CSC). We found that CSC caused a decrease of mtDNA content in all cells. Moreover mutated cells including carriers exhibited a significant reduction of ATP level. This result indicated that smoke derivatives hampered the bioenergetic compensation in carriers. We also found that in untreated cells the content of of carbonylated proteins was highest in affected individuals, whereas the level of several detoxifying enzymes, was highest in carriers; this shows that carriers were particularly successful in reactive oxygen species (ROS) scavenging capacity. In the presence of CSC the detoxifying enzymes were increased in all cells, while carbonylated proteins increased only in LHON mutant cells, mostly from affected individuals. Overall these data indicate that exposure to smoke derivatives has a more deleterious effect in affected individuals and that carriers are more efficient in mitigating ROS rather than recovering bioenergetics. Therefore, the identification of genetic modifiers that modulate LHON penetrance must take into account also the exposure to environmental triggers such as tobacco smoke.

Abstract #: 2018 PA-0468

Presenter: Sujay Guha[1]

Authors: Sujay Guha[1], Erzsebet Polyak[1], Julian Ostrovsky[1], Young Joon Kwon[1,] Christoph Seiler[2], Rui Xiao[3], Zhe Zhang[4], Eiko Nakamaru-Ogiso[1], Marni J Falk[1, 5]

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"Mitochondrial cocktail" combinatorial compound screening in Caenorhabditis elegans and zebrafish models of mitochondrial complex I disease

Background: Numerous vitamin and cofactor supplement therapies are combined in 'mitochondrial cocktails' to empirically treat mitochondrial disease patients. Pre-clinical studies in primary respiratory chain (RC) disease cell and animal models have been suggestive that some of these compounds may have desirable physiological effects (Kuszak A, 2017; Polyak E, 2018). Here, we investigated whether combining the individually most potent lead compounds that have different underlying cellular mechanisms would have synergistic or toxic effects in Caenorhabditis elegans (C. elegans) and zebrafish models of primary RC complex I (CI) disease.

Methods: Validated gas-1(fc21) NDUFS2 CI mutant C. elegans were used to systematically screen and identify effects of combined drug combinations that improve mitochondrial dysfunction related phenotypes. Validation of lead treatment combinations was performed in a pharmacologic CI inhibition (rotenone) zebrafish model (Byrnes J et al, 2017). 13 triplicate combinations were evaluated based on combining compounds from 3 major categories, namely antioxidants, signaling modifiers, and intermediary metabolic modifiers, previously shown to have individual efficacy in improving gas-1(fc21) worm lifespan. Outcomes evaluated included animal lifespan, metabolic and genomic profiling, and mitochondrial physiology in C. elegans, as well as animal survival, brain death, neurologic response, and swim bladder development in zebrafish.

Results: C. elegans. (1) Lifespan. Synergistic lifespan rescue of gas-1(fc21) beyond that of healthy wild-type N2 controls, and also beyond the effects of any single component of the triplicate was observed for 1 of 13 triplicate drug combinations for which the treatment was started at early development (L1) stage: glucose, nicotinic acid, N-acetyl cysteine (Glu+NA+NAC). Subsequent pair-wise component lifespan evaluation revealed maximal synergy was obtained with Glu+NAC. (2) In vivo mitochondrial physiology. The Glu+NA+NAC triplicate 24 hour treatment in young adult gas-1(fc21) worms significantly improved their reduced in vivo mitochondrial membrane potential, but did not rescue their reduced mitochondrial content. Interestingly, Glu+NAC 24 hour treatment significantly rescued their reduced in vivo mitochondrial membrane potential but not mitochondrial content, while Glu+NA 24 hour treatment had the opposite effects. NA+NAC did not affect the altered mitochondrial physiology of gas-1(fc21) worms. (3) Intermediary metabolism. The broadly altered amino acid profile of gas-1(fc21) worms were not rescued by any pairwise or triplicate Glu+NA+NAC treatment. Studies are ongoing to identify the central signaling mechanisms underlying the observed treatment synergy. Zebrafish. Glu+NA+NAC combinatorial evaluation demonstrated that treatment of zebrafish larvae from 3 days post-fertilization (3 dpf) effectively rescued brain death ('gray brain') that otherwise developed when subsequently exposed to rotenone at 7 dpf.

Conclusion: Among 13 combinatorial "mitochondrial treatment cocktails" of antioxidants, signaling modifiers, and intermediary metabolic modifiers that have individual benefit in a C. elegans model of CI dysfunction, only Glu+NA+NAC resulted in synergistic lifespan rescue. Mechanistic evaluations revealed this combination significantly rescued in vivo mitochondrial membrane potential, and prevented brain death in a zebrafish CI disease model. Demonstrating similar dosing efficacy of a lead combinatorial treatment in invertebrate and vertebrate models highlights the conserved pathophysiological mechanisms and therapeutic targets in primary RC disease.

Abstract #: 2018 PA-0469

Presenter: Emmanuele Valentina

Authors: Emmanuele Valentina, MD, PhD[1]; Jaya Ganesh, MD[2]; Georgirene Vladutiu, PhD[3]; Richard Haas, MD[4]; Charles Hoppel, MD[5]; Douglas Kerr, MD, PhD[6]; Russell Saneto, DO, PhD[7]; Bruce Cohen, MD[8]; Johan Van Hove, MD, PhD[9]; Fernando Scaglia, MD[10]; Xiomara Q. Rosales, MD[1]; Emanuele Barca, MD, PhD[1]; Richard Buchsbaum[11]; John L. Thompson, PhD[1,11]; Salvatore DiMauro, MD[1]; Michio Hirano, MD[1]; and the North American Mitochondrial Disease Consortium (NAMDC).

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Title: Development of diagnostic criteria to facilitate research in mitochondrial disorders: a proposal from the North American Mitochondrial Disease Consortium (NAMDC).

Body of Abstract:

Objective: To develop updated research diagnostic criteria for primary mitochondrial disorders.

Background: Mitochondrial diseases are clinically, biochemically, and genetically heterogeneous and therefore challenging to classify and diagnose. Since the publication of the original mitochondrial disease diagnostic criteria more than 10 years ago, there has been an explosion of phenotypic and genotypic data including identification of numerous novel molecular genetic defects. To incorporate this new information, and to harmonize diagnostic criteria and terminology, we propose revised diagnostic criteria for mitochondrial disorders.

Methods: The North American Mitochondrial Disease Consortium (NAMDC) established a Diagnostic Criteria Committee. Comprised of members with diverse expertise, the panel included: clinicians, researchers, diagnostic laboratory directors, statisticians and data managers. The committee conducted a comprehensive review of literature, evaluation of current clinical practices, diagnostic modalities, surveys, and teleconferences to reach a consensus in order to establish and validate diagnostic criteria for mitochondrial disease. Greater emphasis was placed on the molecular genetic criteria for the diagnosis of mitochondrial diseases. Refinement of the existing diagnostic criteria was achieved after manual application of the criteria to patients enrolled in the NAMDC registry.

Results: The Diagnostic Criteria Committee generated consensus criteria for the clinical definition of canonical and non-canonical mitochondrial syndromes and for achieving the level of certainty (definite, suspected, and unlikely) in the diagnosis of mitochondrial diseases.

Conclusions: Building upon existing diagnostic criteria and integrating them with the expanding knowledge in the field, NAMDC has generated updated research diagnostic criteria for mitochondrial diseases. The NAMDC Research Diagnostic criteria will be very helpful to confirm or exclude mitochondrial disease diagnoses, and improve enrollment in future natural history studies and clinical trials.

Abstract #: 2018 PA-0470

Presenter: Larisa Emelyanova

Authors: Larisa Emelyanova[1], Sean Ryan[1], Catherine Warner[1], Farhan Rizvi[1], Gracious Ross[1], Susan Olet[2], David Kress[3], Daniel O'Hair[3], Francis Downey[3], Arshad Jahangir[3]

Institution: [1]Center for Integrative Research on Cardiovascular Aging, Aurora Research Institute, Milwaukee, WI 53233, [2]Investigator Initiated

Research, Aurora Sinai/Aurora St. Luke's Medical Centers, Milwaukee, WI 53219, [3]Aurora Cardiovascular Services, Aurora St. Luke's Medical Center, Aurora Health Care, Milwaukee, WI 53219

Title: Mitochondrial OXPHOS Dysfunction and Oxidative Stress Contribute to the Development and Progression of Atrial Fibrillation in Human

Introduction: Although, electrical and structural remodeling of the atria has been well characterized in atrial fibrillation (AF), metabolic basis for such alterations in human atria has not been fully defined. Since mitochondria are critical for maintaining cardiac function, a deficit in mitochondrial energetics and oxidative stress can lead to the development of the substrate that promotes AF and its progression.

Objective: The aim was to assess AF-related changes in expression of genes involved in mitochondrial respiration and OXPHOS along with functional activity and expression levels of OXPHOS proteins and oxidative stress in AF and non-AF patients.

Methods: Right atrial appendage tissues (RAA) were obtained from 72 patients with well-preserved left ventricular function who were undergoing open heart surgery. Gene expression profiling was performed using Human Mitochondrial Energy Metabolism RT^2 Profiler PCR Array. Activity of OXPHOS complexes I-V (CI-V) was measured spectrophotometrically. Protein expression level of OXPHOS protein subunits was determined by Western blot. Level of 4-hydroxynonenal (4-HNE) was measured using OxiSelect HNE adduct competitive ELISA. The level of superoxide production in myofibers was determined as a change in fluorescence intensity of MitoSOX Red in response to antimycin A. Comparison between groups was done applying the two sample t- and Wilcoxon rank sum tests with 5% level of significance.

Results: Out of 84 genes, the expression of 14 genes was significantly reduced in AF patients. These genes included 4 genes coding for CI (NDUFB8, p=0.03; NDUFC2, p<0.01; NDUFS7, p=0.02; NDUFS8, p=0.03), 1 gene for CII (SDHD, p=0.04), 2 genes for CIII (UQCRC1, p=0.01; UQCRFS1, p=0.02), 4 genes for CIV (COX5A, p<0.05; COX6A2, p=0.04; COX8A, p<0.05; CYC1, p=0.04), and 3 genes for CV (ATP5J2, p=0.03; ATP5L, p=0.04; ATP6V1C2, p=0.04). There was a significant reduction in OXPHOS CI (p=0.02) and CII (p<0.01) activities in AF patients. The activity of CIII and CIV was not significantly different between the two groups. However, CV activity was increased in AF group (p<0.01). At the protein level, there was a significant reduction in expression of CI in the AF group compared to non-AF group (p=0.02) without difference in the expression of the remaining four OXPHOS complexes. The level of 4-HNE protein adducts was significantly increased in AF compared to non-AF patients (p<0.05). The mitochondrial superoxide production was more than 6 fold higher in AF compared to non-AF patients (p=0.03).

Conclusion: AF is associated with energetic remodeling characterized by downregulation of genes encoding for OXPHOS, reduced OXPHOS functional activity and increased oxidative stress that can contribute to the progression of the substrate for AF.

Abstract #: 2018 PA-0471

Presenter: Alvar Grönberg[1]

Authors: Alvar Grönberg[1], Eleonor Åsander-Frostner[1, 2], Steven Moss[3], Sarah Piel[1, 2], Imen Chamkha[1, 2], Lee Webster[3], Sonia Simon Serrano[1, 2], Michael Karlsson[1, 2], Johannes Ehinger[1, 2], Matt Gregory[3], Eskil Elmér[1, 2], Magnus J. Hansson[1, 2]

Institution: [1]NeuroVive Pharmaceutical AB, Lund, Sweden; [2]Mitochondrial Medicine, Lund University, Lund, Sweden; [3]Isomerase Therapeutics Ltd., Chesterford Research Park, Cambridge, UK

Title: A novel brain penetrant, orally bioavailable prodrug of succinate

Body of Abstract:

Background: Reduced activity or stability of complex I is a common cause of respiratory chain dysfunctions with clinical manifestations that are often severe. We have previously shown that cell-permeable prodrugs of succinate, which acts as a substrate for complex II, provide efficient restoration of oxygen consumption in cells from patients with complex I dysfunction and mitigate rotenone-induced lactate production

(Ehinger et al, Nat Commun. 2016;7:12317). We have now developed a new generation of succinate prodrugs represented by the compound NV354, which has properties making it suitable for animal efficacy studies in models of complex I disease and is a potential candidate for drug development.

Methods: NV354 and its 13C-labeled analogue NV865 were synthesized and tested in vitro and in vivo. Oxygen consumption was studied in freshly isolated human platelets and cell lines. In vitro Caco-2 cell permeability was used to estimate oral bioavailability and an in vitro rat brain endothelial cell assay was used to estimate penetration of the blood-brain barrier. Prodrug metabolism was studied in vitro in plasma, blood and isolated microsomes. Pharmacokinetic studies with unlabelled and 13C-labeled compound were performed in mice.

Results: NV865 was used to demonstrate release of drug-derived 13C-succinate in vitro and in vivo. NV354 was able to increase oxygen consumption in human platelets, fibroblasts and liver cells. Caco-2 permeability of NV354 was high with no signs of efflux and the compound could penetrate brain endothelial cells. Excellent oral bioavailability and good brain penetration was confirmed in vivo by measuring plasma, blood and organ concentrations of drug-derived 13C-labelled succinate after oral and intraperitoneal administration of NV865. Compared to an equimolar dose of native 13C-labelled succinate, NV865 gave rise to several-fold higher concentrations of 13C-labelled succinate in all investigated organs, including the brain, after oral, intraperitoneal and intravenous administration. NV354 showed good solubility and stability in water and could be administered in drinking water at high doses.

Conclusion: NV354 is a prodrug of succinate with drug-like properties suitable for investigating the impact of efficient tissue succinate delivery in models of complex I dysfunction.

Abstract #: 2018 PA-0472

Presenter: Claudia V. Pereira

Authors: Claudia V. Pereira[1], Susana Peralta[1], Sandra R. Bacman[1], Tania Arguello[1], Paula Lima and Carlos T. Moraes[1]

Institution: [1]Department of Neurology, University of Miami Miller School of Medicine, Miami, USA

Title: rAAV9-mediated Ndufs3 replacement reverts the myopathy phenotype in pre- and post-symptomatic muscle Ndufs3 KO mice

Body of Abstract: Mitochondrial disorders can be caused by mutations in nuclear genes, such as Ndufs3. This gene encodes one of the iron-sulfur protein components of complex I and is believed to be required for catalysis. We have created and characterized a conditional muscle Ndufs3-KO model which presents a severe myopathy at 2-3 months and shortened life span (7-9 months). We treated these mice by delivering rAAV9-Ndufs3 systemically, via retro-orbital injections. rAAV9-Ndufs3 injections in P15-18 mice effectively restored NDUFS3 levels which was accompanied by a block in the development of the myopathy phenotype, assessed by treadmill and wire hanging time tests. Quadriceps of 6 months old mice showed normalized weight and oxidative phosphorylation protein levels. In-gel activity of Complexes I and IV showed a complete reversion of the KO mice biochemical phenotype in rAAV-Ndufs3 injected mice. Complex I activity in muscle homogenates was similar to control animals, as was serum lactate levels. Furthermore, we tested whether it was possible to treat post-symptomatic mice (2 months). The results showed an overall significant improvement of the mitochondrial myopathy phenotype, 4 months after the injection. Our results showed a wide gene therapy window for replacing missing genes in mitochondrial myopathies.

Mitochondrial Medicine 2018: Nashville

Abstracts

Abstract #: 2018-PA-0474

Presenter: Rebecca D. Ganetzky

Authors: Rebecca D. Ganetzky[1], Amy Goldstein[1], Zarazuela Zolkipli-Cunningham[1], Elizabeth M. McCormick[1], Colleen Muraresku[1], Marni J. Falk[1]

Institution: [1]Mitochondrial Medicine Frontier Program, Division of Human Genetics, Children's Hospital of Philadelphia, Philadelphia, PA, 19104 USA; [2]Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104 USA.

Title: GDF15 clinical performance in the practice of mitochondrial medicine

Background: Growth differentiation factor 15 (GDF15) is a cytokine whose levels increase in response to various stressors. While GDF15 level has been proposed as a candidate biomarker for primary mitochondrial respiratory chain (RC) disease, its clinical utility is still unknown.

Methods: We measured GDF15 levels on a large, diverse cohort of patients evaluated in the Mitochondrial Medicine Frontier Program at the Children's Hospital of Philadelphia (n=207). Plasma GDF15 levels were measured on a clinical basis (Mayo laboratory) and reported in mg/dL. Levels above the limits of assay detection were rounded to 6000, the largest definite value reported. GDF15 levels were compared between the subset of patients who were ultimately diagnosed with molecularly-confirmed primary mitochondrial disease (24%; n=49) and patients who did not have a final molecular diagnosis (n=149). Five patients who had a molecularly-confirmed pyruvate metabolism defect were also included. Three patients who ultimately were diagnosed with a Mendelian disease that was not a primary RC disorder were excluded from further analysis. One patient with severe mitochondrial dysfunction on muscle biopsy without molecular confirmation was also excluded. Statistical analyses were performed in R studio with the pROC package.

Results: GDF15 levels were significantly higher in the cohort of patients with confirmed RC disease (mean 1400 pg/mL) relative to patients without a molecular diagnosis (mean 576 pg/mL, p=0.0004.) None of the 5 patients with a pyruvate metabolism defect had elevated GDF15 (mean 386 pg/mL). However, a significantly overlapping range was seen between the primary RC disease cohort and the molecular unknown patient cohort. The area under the receiver-operator curve was 0.6637, suggesting overall poor ability to differentiate primary RC disease patients from other diagnostic entities. For example, a GDF15 cutoff of 750 had 78% a sensitivity and 45% specificity. On subgroup analysis, GDF15 was reliably elevated in 6 subjects with single mtDNA deletions (5 Kearns Sayre Syndrome; 1 Pearson syndrome), where the average GDF15 level was 4,231 pg/mL and the minimum level was 1,887 pg/mL (normal < 750 pg/mL). GDF15 levels also tended to be higher in patients with mitochondrial depletion and translation defects, as compared to patients with single RC complex deficiencies. Further subgroup analysis is ongoing to inform the utility of GDF15 in different patient populations, and provide insight as to whether GDF15 can meaningfully inform an individual patient's prognosis, disease progression, and/or response to therapies.

Conclusion: Overall, our Mitochondrial Medicine Programs' clinical experience has shown that GDF15 is a biomarker with overall limited sensitivity and specificity in the diverse clinical setting. However, it may have particular utility in mtDNA deletion patients where it was uniformly elevated, as well as in patients with mitochondrial depletion or translation defects.

Abstract #: 2018 PA-0475

Presenter: Divakar S. Mithal

Authors: Gregory S. McElroy[1], Colleen R. Reczek[1], Joshua S. Stoolman[1], Divakar S. Mithal[1,2], Navdeep S. Chandel[1]

Institution: [1]Division of General Internal Medicine, Department of Pulmonary Critical Care, Northwestern Feinberg School of Medicine, Chicago IL, 60611; [2]Department of Pediatrics, Section of Neurology, Ann and Robert H. Lurie Children's Hospital of Chicago, Chicago, IL 60611

Mitochondrial Medicine 2018: Nashville

Abstracts

Title: NDI1 extends lifespan in a mouse model of Leigh syndrome

Body of Abstract: Mitochondrial disorders are clinically heterogeneous, but Leigh Syndrome (LS) is the single most common diagnosis[1]. LS is characterized by symmetric basal ganglia and cerebellar degeneration with ataxia, seizures, and rapid progression to death by two years of age for most children. The pathophysiology remains unclear with care focused on symptomatic and supportive treatments.

In mammalian mitochondria, complex I of the ETC is necessary for NADH oxidation to regenerate NAD+. Complex I is necessary for proton pumping across the inner mitochondrial membrane to produce both ATP and reactive oxygen species (ROS). Complex I is composed of 45 subunits encoded by both the nuclear and mitochondrial genomes and one gene with a large number of known LS causing mutations is NDUFS4[2]. Mice lacking NDUFS4 either globally or selectively in neural tissue, recapitulate many features of LS, including rapid progression to death by day 60[3,4].

NDI1 is a single yeast protein that converts NADH to NAD in S. Cerevisiae. It can be stably expressed in mammalian cells to rescue NADH to NAD conversion in mitochondria[5]. NDI1 conducts NADH oxidation without translocating protons, however, which does not contribute to generation of ATP or ROS. This allows for potential rescue of functionally impaired complex I.

Here we use a cre-lox system to introduce NDI1 in a model of LS where nestin-lineage central nervous system cells simultaneously lack NDUFS4 and express NDI1. We demonstrate that this conditional expression of NDI1 is sufficient to both prevent growth regression and extend lifespan in the LS mice. By contrast, NDI1 expression does not prevent the severely ataxic phenotype of the LS mice. Further studies are required to identify the mechanism whereby NDI1 prolongs survival.

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Abstract #: 2018 PA-0476

Presenter: Larisa Emelyanova

Authors: Larisa Emelyanova[1], Steven Komas[2], Susan Olet[3], Sean Ryan[1], Catherine Warner[1], Farhan Rizvi[1], Gracious R Ross[1], David Kress[4], Daniel O'Hair[4], Francis Downey[4], Arshad Jahangir[4]

Institution: [1]Center for Integrative Research on Cardiovascular Aging Center, Aurora Research Institute, Aurora Health Care, Milwaukee, WI 53233, [2]Department of Biophysics, Medical College of Wisconsin, Milwaukee, WI 53226, [3]Investigator Initiated Research, Aurora Sinai/Aurora St. Luke's Medical Centers, Aurora Research Institute, Milwaukee, WI 53219, [4]Aurora Cardiovascular Services, Aurora St. Luke's/Aurora Sinai Medical Center, Milwaukee, WI 53219

Title: Differences in Atrial-Fibrillation-associated Energetic Remodeling between the Right and Left Atria in Human

Mitochondrial Medicine 2018: Nashville

Abstracts

Introduction: Right and left atria have different susceptibilities toward developing atrial fibrillation (AF). The molecular bases of these differences are not well characterized. Given the complexity of AF development and progression, understanding AF-associated changes in myocardial energetics between the atria will help improve mechanistic insights and therapeutics for better clinical management of AF. The aim was to compare changes in mitochondrial oxidative phosphorylation system (OXPHOS), glycolysis and Krebs cycle metabolites in right atrial (RAA) and left atrial (LAA) appendage tissue from patients with (AF) and without (non-AF) AF.

Methods: RAA and LAA from well-matched AF (n=54) and non-AF (n=58) patients undergoing elective open heart surgery were collected. Functional activity of OXPHOS complexes I-V was measured spectrophotometrically. Protein expression level of OXPHOS complexes was determined by Western blot. OXPHOS gene expression level was performed using RT-PCR. Metabolites were profiled using highperformance liquid chromatography coupled to tandem mass spectrometry. Comparison between groups was done applying the 2 sample t- and Wilcoxon rank sum tests with 5% level of significance.

Results: The most significant AF-associated alterations in myocardial energetics were observed in RAA. In AF patients, out of 84 OXPHOS genes, expression of 14 genes was significantly reduced in RAA ($p<0.05$) and 2 genes was reduced in LAA ($p<0.05$). There was an AF-associated reduction in complex I ($p=0.01$) and IV ($p=0.04$) protein expression in RAA without changes in LAA. Unlike LAA, mitochondria from RAA revealed a decline in complex I ($p=0.01$) and II ($p=0.03$) activity in AF compared to non-AF patients. In AF patients, glycolysis metabolites level of glucose-6-phosphate and phosphoenolpyruvate was reduced in RAA ($p=0.03$), whereas 2-phosphoglycerate was reduced in LAA ($p=0.02$). AF was related with decrease in NAD^+ ($p=0.03$), GDP ($p=0.05$), citrate ($p=0.03$), total pool of adenine nucleotides ($p=0.02$) and glutathione ($p=0.03$) level in RAA without changes in LAA.

Conclusion: AF is associated with different energetic remodeling in right and left atria, suggesting that dissimilar mechanisms may contribute to development and progression of AF.

Abstract #: 2018 PA-0478

Presenter: Amel Karaa

Authors: Amel Karaa on behalf of MMPower Investigators

Institution: Massaschusetts General Hospital, Boston, MA

Title: RePOWER: a Global Prospective Observational Study of Patients with Primary Mitochondrial Myopathy

Body of Abstract:

Background: Primary mitochondrial myopathies (PMMs) are genetic disorders of the mitochondrial respiratory chain that predominantly affect skeletal muscle, thereby leading to fatigue, exercise intolerance, and muscle weakness, which adversely affect physical functioning and quality-of-life (QoL). The investigational agent, elamipretide (ELAM), localizes to the inner mitochondrial membrane, associating with cardiolipin and improving ATP production, resulting in improved exercise performance, as demonstrated in multiple preclinical models and clinical trials. ELAM treatment of patients with genetically-confirmed PMM produced favorable results on Six Minute Walk Test (6MWT) and PMM Symptom Assessment (PMMSA) Total Fatigue and Total Fatigue During Activities scores in MMPOWER-1 and MMPOWER-2. These results supported the development of the RePOWER Registry and pivotal phase 3 trial, MMPOWER-3.

Objectives: The primary objectives of RePOWER (global prospective, non-interventional study) was to assess the genotype-phenotype relationship in patients with PMM and current clinical practice/standard of care.

Methods: RePOWER enrolled patients (29 sites; N=409) with a known or suspected diagnosis of PMM based on available genetic data and signs/symptoms. Ambulatory patients, aged ≥16 and ≤80 years, were required to attempt functional assessments and complete questionnaires about their current symptoms and QoL. Patient demographics, and genetic testing results were also captured.

Results: Preliminary analyses of RePOWER data demonstrated the subject average age to be 43.6 years (SD±14.8 years), a predominance of females (60.1%), and a majority classified as white (92.4%). At the time of analysis, 243/409 patients were sub-classified by genetic mutation according to DiMauro (2003). A majority (>95%) of patients have been genetically classified into "disorders involving mtDNA mutations that impair mitochondrial protein synthesis in toto" (75.3%) and "disorders involving nDNA mutations causing defects of intergenomic signaling" (20.6%). Of those that attempted the 6MWT (n=405), the average distance walked was 367.4m (SD±113.6m; range 3m–661m) with most (78.8%) between 100m-450m. Of those that attempted the 5X Sit-to-Stand Test (5XSST) (n=376), the average time-to-completion was 20.0s (SD±17.7s; range 5s–182s). Of those that completed the Triple-Timed Up and Go (3TUG) Test (n=399), the average time-to-completion was 42.5s (SD±27.4s; range 14s–366s). Enrolled RePOWER patients are eligible for recruitment into MMPOWER-3, an ongoing 24-week, randomized, double-blind, placebo-controlled, parallel-group trial examining the efficacy and safety of ELAM treatment of patients with PMM, followed by an open-label extension of up to 144 weeks, with results expected in 2019.

Conclusions: Patients with PMM suffer from symptoms that adversely affect physical functioning and QoL. The PMM population captured in the RePOWER Registry demonstrated a significant level of disease burden. From a qualitative perspective, the RePOWER results parallel those previously reported in patients with mitochondrial disease. The RePOWER results support the substantial disease burden of PMM and the make clear the need for investigations for effective therapies. The MMPOWER-3 clinical trial will investigate the therapeutic potential of ELAM for patients with PMM.

Abstract #: 2018 PA-0479

Presenter: Sujay Guha[1]

Authors: Sujay Guha[1], Corey Burrough[1], Julian Ostrovsky[1], Chigoziri Konkwo[1], Eiko Nakamaru-Ogiso[1], Marni J. Falk[1, 2]

Institution: [1]Mitochondrial Medicine Frontier Program, Division of Human Genetics, Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA 19104; [2]Department of Pediatrics, University of Pennsylvania Perelman School of medicine, Philadelphia, PA 19104.

Title: Pre-clinical in vivo analysis of novel cell-permeable succinate prodrugs tolerability, dosing, and efficacy in a Caenorhabditis elegans model of mitochondrial complex I disease

Background: Complex I (CI) disease is the most common biochemical defect in human mitochondrial respiratory chain disease, a heterogeneous collection of genetic disorders caused by mutations in more than 300 genes. No FDA-approved drug therapies currently exist for mitochondrial disease. Identifying pharmacologic candidates that show efficacy and lack of toxicity, and understanding dose-range effects in simple model animals will facilitate pre-clinical development of rational therapies for the currently highly morbid and heterogeneous class of human mitochondrial disease. Here, we evaluated the effects of a novel cell-permeable class of succinate pro-drugs that bypass defective CI by providing a complex II (CII) substrate, succinate, in a Caenorhabditis elegans (C. elegans) animal model of primary CI dysfunction.

Methods: Four cell-permeable succinate pro-drugs (NV099, NV185, NV294, NV302) were provided by NeuroVive Pharmaceutical AB under a researcher-initiated translational research program. These compounds were initially tested for toxicity and developmental effects on wild-type (N2 Bristol) worms using the highest soluble concentration (500 uM) of each drug in DMSO. Drugs were then analyzed at different dose ranges (2.5 uM, 25 uM, 50 uM, 100 uM) on the well-validated C. elegans genetic NDUFS2 mutant gas-1(fc21) model of CI dysfunction to evaluate their ability to rescue the animals' short lifespan. In vivo mitochondrial functional effects were also assessed using a high-throughput Biosorter method we recently developed (Kwon et al, Mitochondrion, 2017).

Results: Developmental death curve analysis in wild-type N2 worms showed no toxicity on animal growth or development from any pro-drug tested at doses up to 500 uM. Lifespan analyses showed that compounds NV099, NV302 and NV294 at 100 uM final concentration significantly rescued the short lifespan of gas-1(fc21) NDUFS2 mutant worms. In addition, NV294 at 50 uM final concentration significantly rescued these CI mutant worms' short lifespan. No lifespan effect was observed of any pro-drug at 2.5 uM or 25 uM, suggesting these

drugs work in a dose-dependent manner. In vivo mitochondrial physiology Biosorter analyses in young adult worms exposed to each drug 24 hours revealed that NV294 and NV302 significantly improved the reduced mitochondrial membrane potential (TMRE fluorescence) of gas-1(fc21) NDUFS2 mutant worms at 50 uM final concentration, with no alteration of their reduced mitochondrial content (Mitotracker green fluorescence).

Conclusion: Promising therapeutic efficacy with cell-permeable succinate pro-drugs in the 50-100 uM range, with no signs of toxicity, were seen in the well-established gas-1(fc21) NDUFS2 C. elegans animal model of primary mitochondrial CI disease. Studies are ongoing in additional animal models to further characterize the in vivo efficacy and toxicity of similar class compounds that have greater bioavailability. These pre-clinical data highlight the utility of simple animal model studies to efficiently gain robust insight into novel compounds' tolerability and efficacy at various concentrations in specific subclasses of mitochondrial disease.

Abstract #: 2018 PA-0480

Presenter: Chigoziri Konkwo[1]

Authors: Chigoziri Konkwo[1], Marni J. Falk[1, 2], Eiko Nakamaru-Ogiso[1]

Institution: [1]Mitochondrial Medicine Frontier Program, Division of Human Genetics, Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA 19104; [2]Department of Pediatrics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104

Title: Reduced and oxidized glutathione quantitation assay development in Caenorhabditis elegans models of mitochondrial disease

Background: A major hallmark of mitochondrial disease is increased cellular oxidative stress. Oxidants are generated in the form of harmful reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and superoxide (O_2^-). In excessive amounts, ROS oxidize and damage proteins, lipids, and nucleic acids, disrupting their functions within the cell. Glutathione (GSH), the most abundant cellular antioxidant in eukaryotes, plays a central role in cellular defense against oxidative stress and redox metabolism. GSH deficiency has been reported in a variety of disorders associated with impaired mitochondrial function, including Leigh Syndrome, Friedreich Ataxia, and neurodegenerative disorders such as Parkinson's disease. Thus, GSH levels and the GSH:GSSG ratio are a useful biometric assay to evaluate overall cellular redox balance in mitochondrial dysfunction. Here, we developed a highly sensitive and reproducible method for the quantification of GSH and GSSG levels in C. elegans models of mitochondrial disease using HPLC-ECD (electrochemical detection).

Methods: Worm populations were counted and collected using the Union Biometrica Biosorter®. Extraction buffer was added and worms were freeze-thawed twice in liquid nitrogen and room temperature water. Worms were then homogenized using a pestle and motorized homogenizer, and supernatant was then separated from worm debris by centrifugation. The supernatant was used for enzymatic analysis after it was deproteinized, and injected to our HPLC-ECD system with a gold working electrode. Results of our custom HPLC-ECD assay were compared with a commercially available spectrophotometric enzyme assay kit to measure GSH.

Results: The ratio of worm volume to total sample volume was a key factor determining overall glutathione extraction efficiency, where a 20% worm:total volume ratio was determined to be most efficient. For the HPLC mobile phase, a sodium phosphate-based buffer was much more effective than trifluoroacetic acid (TFA) for GSH separation and detection sensitivities. No difference in GSH levels was detected based on whether the deproteinizing agent used was PCA (generally used for small molecule analysis such as NAD and amino acids) and MPA (exclusively used for GSH assays published). For the enzymatic assay, populations below 2,000 worms were insufficient for accurate quantification of GSH levels in worms. Optimization of GSSG measurement by this HPLC-ECD system is underway, as well as quantitation of altered GSH, GSSG, GSH/GSSG in different C. elegans mitochondrial disease mutants.

Conclusion: We have developed a novel HPLC-ECD assay to measure absolute GSH levels in C. elegans populations that is highly reproducible and several magnitudes more sensitive than the standard commercially available enzymatic assay. This improved analytic

capability will improve our ability to interrogate oxidative stress in mitochondrial disease models at baseline, over time, and in response to treatment interventions.

Abstract #: 2018 PA-0481

Presenter: Shuo Han

Authors: Shuo Han[1], Furqan Fazal[2], Pornchai Kaewsapsak[1], Kevin Parker[2], Alistair Boettiger[3], Howard Chang[2], Alice Ting[1]

Institutions: [1]Department of Genetics, Biology and Chemistry, Stanford University, CA 94305, [2]Center for Personal Dynamic Regulomes, Stanford University School of Medicine, Stanford, CA, 94305, [3]Department of Developmental Biology, Stanford University, CA 94305

Title: Spatial transcriptome in living cells via APEX-seq

Body of Abstract: The spatial organization of RNA within cells plays a crucial role in influencing a wide range of biological functions. Where RNA is located within the cell can dictate its processing, translation, degradation, binding partners and even the fate of the protein it encodes. However, a general understanding of RNA localization has been hindered by a lack of simple, high-throughput methods for mapping the transcriptomes of subcellular compartments. Here, we develop such a method, called APEX-Seq, which combines live cell proximity biotinylation of RNAs by a genetically targeted peroxidase enzyme ("APEX") with streptavidin enrichment followed by RNA-Seq. Application of this method in mammalian cells reveals the organizing principles of RNAs targeted to the outer mitochondrial membrane and identifies specific functional classes of mRNAs that are enriched near the mitochondrion.

Abstract #: **2018 PA-0482**

Presenter: Giovanni Manfredi

Authors: [1]Corey Anderson, [1]Kirsten Bredvik, [1]Samantha Meadows, [1]Anna Stepanova, [1]Suzanne Burstein, [2]Cathleen Lutz, [1]Giovanni Manfredi

Institutions: [1]Weill Cornell Medicine, New York, NY; [2]The Jackson Laboratories, Bar Harbor, ME

Title: **New mouse models of mutant CHCHD10 mitochondrial disease**

Mutations in the coiled-coil-helix-coiled-coil-helix domain containing 10 (CHCHD10), a mitochondrial protein with unknown function, cause autosomal dominant forms of familial amyotrophic lateral sclerosis (ALS), frontotemporal dementia, Parkinson disease, and myopathy. To investigate the pathogenic role of CHCHD10 *in vivo*, we have generated CHCHD10 knock out (KO) mice and mutant (S55L) CHCHD10 knock in (KI) mice. CHCHD10 KO lack behavioral abnormalities and have a normal lifespan, suggesting that mice can adapt to the loss of CHCHD10. Instead, mutant S55L KI mice show progressive body weight loss, motor deficits, and die prematurely. Homozygote mice show earlier disease onset and fatal outcome than heterozygote mice, suggesting a gene dosage effect. Interestingly, young S55L females die during pregnancy due to a fatal dilative cardiomyopathy. Gross pathology reveals significant reductions in skeletal muscle and cardiac mass. We characterized the pathology of S55L KI mice in the CNS, skeletal muscle, and heart, which are the most affected tissues in CHCHD10 mutant patients and found strong vacuolization in the muscle and heart, while in the brain, where CHCHD10 is expressed mostly in dopaminergic neurons, we found altered protein localization in the cytosol. By western blot analyses, we measured CHCHD10 protein levels in both mitochondrial enriched and cytoplasmic fractions of brain and heart. We found that mutant S55L mice have altered CHCHD10 levels in mitochondria enriched fractions, which is accompanied by altered mitochondrial function with decreased respiration and ATP synthesis.

Importantly, transcriptomic analyses of the heart of S55L KI mice suggest that the mutant protein causes a profound integrated mitochondrial stress response with elevation of enzymes of one carbon metabolism, and stress-related proteins, such as ATF4 and ATF5. Taken together, these results support the hypothesis that pathogenic mutations in CHCHD10 alter the normal functions of the protein and cause a toxic gain of function that damages mitochondria.

Abstract #: 2018 PA-0483

Presenter: Iyar Mazar

Authors: Iyar Mazar

Institution: Boston College, Sociology Department, McGuinn Hall 426, Boston College, 140 Commonwealth Avenue, Chestnut Hill, MA 02467-3807

Title: Legitimization of chronic illness at the intersection of severity, visibility, and control over symptoms: A case study of Barth Syndrome

Body of Abstract: Individuals with unapparent, severe symptoms that cannot be treated or controlled may struggle to gain legitimacy for their health condition. These individuals must make their health status known to others to mitigate others' doubts and negative perceptions (e.g., that one is lazy or demotivated) and to obtain the necessary institutional support to manage the impacts of their condition. Research thus far has primarily focused on commonly experienced, less severe pediatric or adult-onset illnesses, as opposed to life-limiting and life-threatening early-onset, rare health conditions. Studies report that males with chronic illnesses tend to disassociate from their condition, aiming to appear healthy, rather than seeking to openly manage their illness. This research evaluated how individuals with Barth Syndrome (BTHS) experience and aim to legitimize their condition. BTHS is a rare and severe condition in males characterized by largely unapparent symptoms including cardiomyopathy, fatigue, muscle weakness, neutropenia, and growth delay. The risk of mortality in BTHS remains high, however, overall survival with the condition has improved due to advancements in its diagnosis and management. Therefore, a growing number of individuals with BTHS will require institutional support as their health outcomes improve. Thirty-three open-ended interviews were conducted face-to-face with an international sample of individuals with BTHS and/or their caregivers. Each 60-minute interview was audio-recorded, transcribed, and anonymized. Transcripts were analyzed using a qualitative data analysis program. The analytic approach was deductive (i.e., informed by prior theory regarding the role of symptom severity, visibility, and control in the social and institutional legitimization of illness) as well as inductive (i.e., informed by the responses elicited from participants in this sample). It was hypothesized that individuals with BTHS would publicly manage and make their condition known to others to mitigate negative perceptions and promote the legitimization of BTHS. Interviews were conducted with participants ≤15 years of age (n=18, mean age=8.6 years, SD=±3.9, range 2.5-15.0) and/or their caregivers, and with individuals ≥16 years of age (n=15, mean age=22.9 years, SD±5.8, range 16.0-34.0). Almost all participants reported being Caucasian. Social legitimization of BTHS was informed by the severity, visibility, and uncontrollability of the condition. The symptoms of BTHS are severely limiting on a daily basis and life-threatening, requiring preventive management (e.g., guarding against infection and heart complications). Symptom severity made it unrealistic and dangerous for individuals to pass as healthy (e.g., participation in physical activities such as contact sports was not possible or too risky). The public management of BTHS-related physical impacts (e.g., the use of assistive devices to facilitate walking) made the otherwise unapparent symptoms of weakness and fatigue visible to others. This confirmed one's status as being sick rather than lazy or disinterested. Individuals with BTHS and their caregivers attempted to make others aware of the uncontrollable, severe nature of the condition to promote understanding of and support for BTHS. The process by which individuals aim to legitimize unapparent chronic health conditions is informed by the intersecting experience and levels of disease severity, visibility, and control as is evidenced by the case of BTHS.

Mitochondrial Medicine 2018: Nashville

Abstracts

Abstract #: 2018 PA-0484

Presenter: Neal D. Mathew[1]

Authors: Neal D. Mathew[1], Tara L. Gallagher[2], Christoph Seiler[2], Eiko Nakamaru-Ogiso[1] and Marni J. Falk [1,3]

Institution: [1]Mitochondrial Medicine Frontier Program, Division of Human Genetics, Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA 19104; [2]Aquatics Core Facility, The Children's Hospital of Philadelphia, Philadelphia, PA 19104; [3]Department of Pediatrics, University of Pennsylvania Perelman School of medicine, Philadelphia, PA 19104

Title: Optimising precision therapy for NUBPL disease using C. elegans and Zebrafish models

Background: Complex I (CI) dysfunction impairs neurodevelopment and survival due to a range of gene disorders that critically alter cellular metabolism, energy homeostasis, and reactive oxygen species production. Pathogenic mutations in NUBPL, an assembly factor for the CI iron-sulfur cluster within CI, strongly decrease CI activity and lead to multi-system mitochondrial disease with predominant cerebellar dysfunction. Despite recognition of the importance of NUBPL to CI function, its precise function in CI iron-sulfur complex assembly remains unclear. Here, we report the phenotypic and biochemical characterization of C. elegans deletion mutants and zebrafish CRISPR/Cas9 genetic deletion models of NUBPL disease, as well as a staged approach to high-throughput treatment discovery.

Methods: A genetic NUBPL(tm3754) knock-out worm strain was obtained from the National Bioresource Project in Japan. Animals are preliminarily screened with a high-throughput imaging technique, WormScan (Mathew et al. 2016), for integrated analysis of motility, mortality, brood size, and growth. Hits from the preliminary screen will be then be evaluated for detailed lifespan effect using WorMotel (Churgin et al. 2017), with detailed Biosorter screening to assess effects on mitochondrial membrane potential and oxidative stress response. An initial screen is underway with 17 lead compounds previously identified in the Falk laboratory in another CI disease model due to a structural CI subunit deficiency (gas-1(fc21)), with future expansion to treatment screening in an FDA-approved drug library. In addition, we used CRISPR-Cas9 technology to generate two NUBPL knockout zebrafish lines, as well as missense mutations found in a NUBPL patient.

Results: NUBPL knockout zebrafish lines have been established and undergoing phenotype characterization in the CHOP Aquatics Core Facility. C. elegans NUBPL knock-out mutant worms are being characterized. A high throughput automated robotic worm lifespan system is under construction that will 10-fold increase our capacity for high-throughput drug screen. Therapies will be prioritized that improve animal development, stress resistance, mobility, and survival.

Conclusion: We have established stable genetic models of NUBPL deficiency in which to characterize disease phenotypes at the whole animal survival, behaviour, and organ-specific functional level. These animals. These models will facilitate a precision medicine approach, which can be readily extended to other gene disorders, to characterize optimal therapeutic leads in both candidate screens and larger scale compound libraries in an effort to efficiently develop safe and effective therapies for mitochondrial disease.

Abstract #: 2018 PA-0485

Presenter: Alessia Angelin

Authors: Alessia Angelin[1], Luis Gil-de-Gómez[2], Satinder Dahiya[3], Jing Jiao[4], Lili Guo[2], Matthew H. Levine[5], Zhonglin Wang[5], William Quin[6], Piotr K. Kopinski[1], Liqing Wang[3], Joseph A. Baur[6], Ian A. Blair[2], Wayne W. Hancock[3], Douglas C. Wallace[1,7] and Ulf H. Beier[4]

Institution: [1]Center for Mitochondrial and Epigenomic Medicine, Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA; [2]Penn SRP Center, University of Pennsylvania, Philadelphia, PA 19104, USA; [3]Division of Transplant Immunology, Children's Hospital of Philadelphia, University of Pennsylvania; [4]Division of Nephrology, Department of Pediatrics, Children's Hospital of Philadelphia, University of Pennsylvania; [5]Department of Surgery, University of Pennsylvania, Children's Hospital of Philadelphia; [6]Department of Physiology and Institute of Diabetes, Obesity, and Metabolism, University of Pennsylvania; [7]Department of Pathology and Laboratory Medicine, University of Pennsylvania

Mitochondrial Medicine 2018: Nashville

Abstracts

Title: Foxp3 increases oxidative phosphorylation and NAD oxidation, adapting regulatory T cells to low glucose high lactate environments

Body of Abstract: T-regulatory (Treg) cells maintain peripheral tolerance and prevent autoimmunity, but their immune suppressive properties can also work to a disadvantage in the tumor microenvironment. Solid tumors can be depleted of glucose and enriched with lactic acid. This can weaken anti-tumor immunity, as cytotoxic and effector T cells require glycolysis to proliferate and produce cytokines. We observed that Treg cells reprogram their metabolism through the transcription factor Foxp3, which suppresses Myc gene expression and glycolysis, enhances oxidative phosphorylation (OXPHOS), and increases NAD oxidation. These metabolic adaptations allow Treg cells to maintain function, while effector T cells lose the ability to recycle NAD through reversal of the lactate dehydrogenase reaction in lactate rich environments. We also investigated the role of different mitochondrial respiration complexes in Treg cells using two mouse models: one carries a mutation in the ND6 subunit of complex I which maintains normal ATP production, but has impaired NAD oxidation; while the second has a mutation on COI subunit of complex IV causing an impairment of ATP production, but leaves NAD oxidation intact. While T cell development was not altered in either model, we found that Treg cells isolated from ND6 mice, but not CO1 mice, have reduced suppressive function suggesting that complex I function may be particularly important in Treg cells activity. Our findings may explain how Treg cells adapt to function in low glucose high lactate environments promoting peripheral immune tolerance during tissue injury, but also weaken anti-cancer immunity in the tumor microenvironment. Understanding Treg cells metabolism may therefore lead to novel approaches for selective immune modulation in cancer and autoimmune diseases, but also in therapeutic immunosuppression during transplant.

Abstract #: 2018 PA-0486

Presenter: Nadee Nissanka

Authors: Nadee Nissanka[1], Sandra R. Bacman[2], Melanie J. Plastini[1], Carlos T. Moraes[1,2]

Institution: [1]Neuroscience Graduate Program, University of Miami Miller School of Medicine, Miami, FL 33136, [2]Department of Neurology, University of Miami Miller School of Medicine, Miami, FL 33136.

Title: Polymerase gamma exonuclease activity degrades linear mtDNA following double-strand breaks

Body of Abstract: Double-strand breaks (DSBs) in the mitochondrial DNA (mtDNA) result in the formation of linear fragments which are rapidly degraded. However, the identity of the nuclease(s) which perform this function are unknown. To explore this phenomenon, we used a mouse model in which the mtDNA polymerase gamma (Polg) is deficient in the 3'-5' exonuclease activity (known as the mutator mouse). We created one, two, or three DSBs in mtDNA using viruses encoding different mitochondrially-targeted restriction endonucleases. We visualized a prolonged presence of the linear mtDNA fragments, accompanied by a partial degradation of the mtDNA in the mutator models via Southern blot and qPCR. In contrast, the wild-type mouse showed rapid mtDNA degradation. Different linear fragments were degraded at a similar rate, suggesting that Polg was recruited to the linearized mtDNA fragments in an origin of replication independent manner. Additionally, the prolonged existence of the linear fragments in the mutator models following the DSBs was associated with increased levels of mtDNA rearrangements. These large rearrangements have previously been identified in the mutator mouse model, patients with Polg mutations, and normal aging.

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Abstracts

Abstract #: 2018 PA-0487

Presenter: Joel H. Wheeler

Authors: Joel H. Wheeler, Matthew J. Young Ph.D.

Institution: Southern Illinois University School of Medicine, Carbondale IL 62901

Title: Analysis of mitochondrial DNA maintenance in a human hepatoma cell line, HepaRG

Body of Abstract: One goal of our laboratory is to investigate mechanisms of drug- and toxicant-induced mitochondrial injury. In vitro biochemistry has demonstrated that certain pharmaceuticals, such as nucleoside reverse transcriptase inhibitors (NRTIs), block human DNA polymerases that have been established to localize to mitochondria. Also, when human cell lines are grown and exposed to NRTIs in tissue culture mitochondrial DNA (mtDNA) depletion has been observed. For these reasons, we wanted to develop a tool to estimate the ratio of mtDNA to nuclear DNA (nDNA) in human cells for future exposure studies. HepaRG is a proliferative human hepatoma-derived cell line that can be differentiated into hepatocyte-like and biliary-like cells. HepaRG cells maintain key hepatic functions including drug transporters and xenobiotic-metabolizing enzymes at levels comparable to primary hepatocytes. To determine the effectiveness of utilizing HepaRG cells as a model to study mtDNA maintenance Southern blot analysis was employed. Non-radioactive probes specific to mtDNA and to nDNA were developed. PCR amplified fragments of human mtDNA and nDNA were separately cloned into the pCR 2.1-TOPO vector. The fragments were removed from plasmids by restriction endonuclease digestion and agarose gel electrophoresis followed by gel extraction. Next, the mtDNA and nDNA fragments served as DNA templates for the in vitro synthesis of digoxigenin-labeled probes. Samples of whole cell HepaRG DNA were subjected to BamHI restriction digestion and the resulting reactions were run on agarose gels followed by Southern blotting. Lambda HindIII fragments and the exACTGene 1 kb Plus DNA ladder were run alongside the reactions and served as linear double-stranded DNA standards to determine molecular weights of whole cell mtDNA and nDNA restriction fragments. The calculated molecular weights of mtDNA and nDNA bands observed on various Southern blots are consistent with expected values. These results indicate that our probes are highly specific to their mtDNA and nDNA target sequences. This new tool provides the means to investigate mtDNA maintenance in human cells treated with pharmaceuticals, exposed to mitochondrial toxicants, or harboring mitochondrial disease mutations.

Abstract #: 2018 PA-0488

Presenter: Carlos T. Moraes

Authors: Sandra R. Bacman[1], Johanna H. K. Kauppila[2], Claudia V. Pereira[1], Maria Miranda[2], Sion L. Williams[1], Nadee Nissanka[1], Nils- Göran Larsson[2], James B. Stewart[2] and Carlos T. Moraes[1]

Institution: [1]Department of Neurology, University of Miami Miller School of Medicine, Miami, USA, [2]Department of Mitochondrial Biology, Max Planck Institute for Biology and Ageing, Cologne, Germany.

Title: MitoTALEN reduces mutant mtDNA load and restores tRNA alanine levels in a mouse model of heteroplasmic mtDNA mutation

Body of Abstract: Mutations in the mitochondrial DNA (mtDNA) are responsible for several disorders, commonly involving muscle and the CNS. Because of its important role to oxidative phosphorylation function, most pathogenic mtDNA mutations are heteroplasmic, co-existing with wild-type molecules. Using a mouse model with a heteroplasmic mtDNA mutation in the tRNA alanine gene, we have tested whether mitochondrial-targeted TALENs (mitoTALENs) could reduce the mutant mtDNA load in muscle and heart. AAV9-mitoTALEN was administered via intramuscular, intravenous and intraperitoneal injections. Muscle and heart were efficiently transduced and showed a robust reduction in mutant mtDNA, which was stable over time. The molecular defect, namely a decrease in tRNA alanine levels, was restored by the treatment. These results showed that mitoTALENs, when expressed in affected tissues, decrease the mutant mtDNA load and normalize the molecular defect responsible for impaired mitochondrial function.

Mitochondrial Medicine 2018: Nashville

Abstracts

Abstract #: 2018 PA-0489

Presenter: Jesse W. Wilson

Authors: Erkang Wang[1], Randy A. Bartels[1,2], Adam J. Chicco[3], Jesse W. Wilson[1,2]

Institution: Colorado State University, [1]Department of Electrical and Computer Engineering, [2]School of Biomedical Engineering, [3]Department of Biomedical Sciences, Fort Collins, CO 80523.

Title: Picosecond spectroscopy of respiratory hemes: towards non-invasive optical biopsy of mitochondrial function.

Body of Abstract: While genetic testing has advanced by leaps and bounds over the past decades, functional testing of the respiratory chain remains costly, time consuming, and invasive. Non-invasive 'virtual biopsy' of tissue and cell morphology is now possible thanks to recent advances in laser-scanning in vivo microscopy, but respiratory chain composition and redox remains inaccessible to current techniques. We are developing a new minimally invasive, label free, optical imaging technique for observing respiratory chain composition and redox by measuring the picosecond optical responses of heme porphyrins.

Electron transfer along the respiratory chain can be monitored through optical spectroscopy, allowing defects to be pinpointed. The molecules that feed electrons into the chain are fluorescent, making them easy to measure. Unfortunately, the electron transfer molecules within the chain itself are dark. These can only be measured through absorption spectroscopy, which is notoriously difficult in live tissue, due to optical scattering and an overwhelming background from hemoglobin.

In our approach, tissue is illuminated with a focused laser beam containing a pump-probe pulse pair. The pump prepares target molecules in an excited state, which induces subtle changes in absorption of the probe pulse. Essentially, pump-probe combines absorption spectroscopy with picosecond-timescale dynamics to provide strong contrast between non-fluorescent molecules, such as the electron transport porphyrins along the respiratory chain. This picosecond contrast is robust to optical scattering, and can be localized to sub-cellular volumes. Pump-probe microscopy can be performed in live tissue, and provides clear separation between hemoglobin-containing vasculature and the tissue of interest

We have found the pump-probe response of unstained, cryosectioned tissue to be sensitive to heme composition, permitting classification of brown adipose versus myocardial tissue. In addition, we have found the pump-probe response of cytochrome c to be sensitive to redox state. We will present these results and discuss progress towards live cell and in vivo mitochondrial microscopy for diagnosis, monitoring of progression, and quantitative assessment of therapies.

Abstract #: 2018 PA-0490

Presenter: Emanuele Barca

Authors: Barca E MD[1], Cooley V BS[2], Schoenaker RMD BS[3], Emmanuele V MD[1], PhD, DiMauro S MD[1], Cohen BH MD[4], Karaa A MD[5], Vladutiu GD PhD, Haas R MD[6], BChir, Van Hove JLK MD[7], PhD, Scaglia F, MD PhD[8], Parikh S, MD[9], Bedoyan JK, MD[10], PhD, DeBrosse SD[10], MD, Gavrilova R MD[11], Saneto RP DO PhD[12], Enns GM, MD[13], Stacpoole PW, MD, PhD[14], Ganesh J, MD[15], Larson, A, MD[7], Zolkipli-Cunningham Z, MD[16], Falk MJ, MD[17], Goldstein AC, MD[18], Tarnopolsky M, MD, PhD[19], Camp K[20], Krotoski D, PhD[21], Engelstad K, MS[1], Rosales

Mitochondrial Medicine 2018: Nashville

Abstracts

XQ, MD[1], Kriger JF, BA[2], Grier J[2], Buchsbaum R[2], Thompson JLP, PhD[2], Hirano M, MD[1]

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Title: The challenge of genetic diagnoses in patients with mitochondrial disease: data from the North American Mitochondrial Disease Consortium (NAMDC).

Background: Mitochondrial disorders comprise a group of highly heterogeneous diseases. Although rare if considered as single disease entities, as a group they represent one of the most common inherited metabolic disorders. Mitochondrial function depend upon the control of two genomes, nuclear DNA and mitochondrial DNA. Mitochondrial diseases can be due to variants in either genome. Due to the high genetic complexity, mitochondrial diseases present challenges for diagnosis, clinical management, and therapeutic research.

Methods: Since 2011, more than 1,200 patients with mitochondrial disease have been enrolled in the NAMDC Registry. Subjects have been enrolled based on their diagnoses (clinical and biochemical). A large collection of information about disease manifestations, biochemical/histological findings, and molecular genetic defects has been recorded through a secure web-based entry system from 16 sites in the United States and Canada. We compared demographic, clinical and laboratory features of the patients with genetic diagnosis versus patients without genetic diagnosis.

Results: As of May 2017, 999 patients had sufficient data for the analysis. Age of disease onset showed a biphasic distribution with two peaks, one below age of 2 years and one in adult age (>18 years). The most frequent canonical clinical syndromes were: Leigh Syndrome, Chronic Progressive External Ophthalmoplegia (CPEO), CPEO-plus and mitochondrial encephalo-myopathy, lactic acidosis, and stroke-like episodes (MELAS); however, we enrolled a large number of patients with non-specific syndromes, including "multisystemic" (200 patients), and "other clinical syndromes" (150 patients). Genetic diagnosis was available in 589 out of 996 patients (59%). We analyzed the characteristics of patients without genetic diagnosis and compared with the genetically defined group. We observed a higher genetic diagnostic rate in patients who fulfilled the criteria for a canonical clinical syndrome. The lowest frequency of genetic diagnoses was observed in patients with non-canonical syndromes, in particular patients with myopathy and encephalomyopathy. We also noted a low percentage of genetic diagnoses in patients with complex syndromes such as multisystemic syndrome. No differences were observed in molecular diagnosis among the different groups stratified by age-at-onset.

Conclusion: Achieving a molecular diagnosis in mitochondrial patients is often problematic, costly, and time consuming with a high proportion

of undiagnosed patients even after applying new sequencing techniques. Registry data reflect this problem, with a genetic diagnostic rate of 59%, which is similar to other published cohort. Analysis of characteristics of patients without a genetic diagnosis will facilitate recognition of difficult to-to-diagnose syndromes and focus genetic analysis.

Abstract #: 2018 PA-0491

Presenter: Neil Otto

Authors: Neil Otto[1] Leah Hogdal[1], Sandeep Kumar[1], Travis Cordie[1], Beau Webber[1], Jarryd Campbell[2], Stephen Ekker[2], David Largaespada[1], Branden Moriarity[1]

Institution: [1]B-MoGen Biotechnologies, Minneapolis MN 55413, [2]Mayo Clinic Rochester MN 55905

Title: Generation of de-novo site specific mitochondrial gene deletions in mammalian cells

Mitochondria are involved in numerous critical cellular processes ranging from energy production and metabolism, to control of cell signaling, differentiation, and death. Considering their importance, functional genomics research would be greatly advanced by tools to generate targeted mutations in the mitochondrial genome. This is critical considering the vital roles that mitochondria play in human biology and disease states including cancer, cardiovascular disease and autoimmunity. To address this, our team has developed a novel method to introduce targeted deletions in the mitochondrial genome. Our strategy employs paired mito-TAL nickases to 'seed' targeted deletions within individual mitochondrial genomes, which are subsequently enriched via heteroplasmic shift using mito-TAL nucleases that selectively deplete remaining wild-type mitochondrial genomes. Our technology represents the first reliable method to introduce site specific deletions in the mitochondrial genome, and promises to bring several revolutions to mitochondrial biology and genomics.

Abstract #: 2018 PA-0492

Presenter: Emanuele Barca

Authors: Emanuele Barca,[1‡] Rebecca D. Ganetzky[2, 3‡] Prosanth Potlouri[4], Marti Juanola Falgarona,[1] Xiaowu Gai,[5,6] Dong Li,[7] Chaim Jalas,[8] Yoel Hirsch, Valentina Emmanuele,[1] Saba Tadesse,[1] Marcello Ziosi,[1] Hasan O. Akman,[1] Wendy K. Chung,[9] Yoel Hirsch[10] Kurenai Tanji,[11] Elizabeth McCormick,[2] Emily Place, [6] Mark Consugar,[6] Eric A. Pierce, 6 Hakon Hakonarson,[2,3,7] Douglas C. Wallace,[4,12] Michio Hirano[*1] Marni J. Falk[*2,3]

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Mitochondrial Medicine 2018: Nashville

Abstracts

Title: USMG5 Ashkenazi Jewish founder mutation impairs mitochondrial complex V dimerization and ATP synthesis

Body of the abstract: Leigh syndrome is a frequent, heterogeneous pediatric presentation of mitochondrial oxidative phosphorylation (OXPHOS) disease, manifesting with psychomotor retardation and necrotizing lesions in brain deep gray matter. OXPHOS occurs at the inner mitochondrial membrane through the integrated activity of 5 protein complexes, of which complex V (CV) functions in a dimeric form to directly generate adenosine triphosphate (ATP). Mutations in several different structural CV subunits cause Leigh syndrome; however, dimerization defects have not been associated with human disease. We report four Leigh syndrome subjects from three unrelated Ashkenazi-Jewish families harboring a homozygous splice-site mutation (c.87+1G>C) in a novel CV subunit disease gene, USMG5. The Ashkenazi population allele frequency is 0.57%. This mutation produces two USMG5 transcripts the wild-type and one lacking exon 3. Fibroblasts from two Leigh syndrome probands had reduced wild-type USMG5 mRNA expression and undetectable protein. The mutation did not alter monomeric CV expression, but reduced both CV dimer expression and ATP synthesis rate. Rescue with wild-type USMG5 cDNA in proband fibroblasts restored USMG5 protein, increased CV dimerization and enhanced ATP production rate. These data demonstrate that a recurrent USMG5 splice-site founder mutation in the Ashkenazi Jewish population causes autosomal recessive Leigh syndrome by reduction of CV dimerization and ATP synthesis.

Abstract: 2018 PA-0493

Presenter: Meagan J. McManus, PhD

Authors: Meagan J McManus, PhD[1], Robert Doot, PhD[2], Robert Mach, PhD[2], Douglas C Wallace[1], PhD

Institution: [1]Center for Mitochondrial and Epigenomic Medicine, Children's Hospital of Philadelphia, Philadelphia, PA; [2]Department of Radiochemistry, University of Pennsylvania, Philadelphia, PA

Title: Validation of Novel Neuroimaging PET Probe as a Predictive Mitochondrial Biomarker for Neurodegenerative Disease

Mitochondrial dysfunction and oxidative stress are not only responsible for primary mitochondrial diseases, but also prominent early features in complex, age-related pathologies, such as Alzheimer's, Parkinson's, cancer, and cardiomyopathy. Defects in the mitochondrial DNA (mtDNA), nuclear DNA encoding mitochondrial proteins, and environmental insults all impinge on mitochondrial function, often causing toxic levels of reactive oxygen species (ROS) production. Due to their transient nature, it is currently difficult to directly measure ROS in vivo, and therefore analysis of ROS levels is largely dependent on the molecular footprints left in their wake of destruction. Among all organs of the body, the brain is perhaps most vulnerable to oxidative damage. As such, elevation of virtually every established marker of oxidative damage has been documented in brain tissue from the most prevalent neurodegenerative diseases. However, the lack of a noninvasive method to detect mitochondrial dysfunction and oxidative stress in the living brain has led many to believe that these early changes are simply epiphenomenon. To address this issue, we designed the first superoxide-sensitive, radioactive tracer, ROStrace, for positron emission tomography (PET). Here we provide evidence that ROStrace crosses the blood brain barrier and detects increased mitochondrial oxidative stress in the brain and eyes of mice harboring the mtDNA ND6P25L mutation, which causes Leber's hereditary optic neuropathy (LHON) in humans. In mice, the mtDNA ND6P25L also causes a progressive neurodegenerative phenotype that closely recapitulates that of Parkinson's disease (PD). Prior to the onset of PD-related motor symptoms in ND6P25L mice, ROStrace retention increased 33% over that of age-matched WT controls, whereas there was no change in presymptomatic alpha-synuclein A53T PD mice. We then measured ROStrace retention over time and found that it correlated with age and progression of neurodegenerative disease in ND6P25L mice. Just as patients with primary mitochondrial disease are often hypersensitive to immune challenge, the mtDNA ND6P25Lmice were also more sensitive to inflammatory activation by lipopolysaccharide (LPS; 5mg/ml). Neural ROStrace retention correlated neurological demise and increased mortality in the ND6P25L + LPS mice. In summary, our results establish the first PET probe for ROS detection in the living brain, which promises to resolve the etiological significance of aberrant ROS production in complex diseases involving mitochondrial dysfunction. This probe may also be used to enhance clinical trial design by identifying patients in the prodromal phase of neurodegenerative disease that would benefit most from preventative mitochondrial therapeutics.

Mitochondrial Medicine 2018: Nashville

Abstracts

Abstract: 2018 PA-0494

Presenter: Zarazuela Zolkipli-Cunningham

Authors: Zarazuela Zolkipli-Cunningham[1,2], Allan Glanzman[3], Jean Flickinger[3], Eileen Barr[2], Colleen Muraresku[2,4], Rui Xiao[5,6], Marni J. Falk[2,4,6]

Institution: [1]Division of Neurology, [2]Mitochondrial Medicine Frontier Program, [3]Department of Physical Therapy, [4]Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia PA 19104 USA; [5]Department of Biostatistics and [6]Department of Pediatrics, University of Pennsylvania Perelman School of Medicine, Philadelphia PA 19104 USA.

Title: Modified Analyses of the Six Minute Walk Test in Mitochondrial Myopathy

Background: The Six Minute Walk Test (6MWT) is a validated measure of exercise capacity and motor function that has been widely applied in the study of several neuromuscular diseases. The ability to quantify exercise capacity and the sensitivity to change makes the 6MWT an attractive clinical outcome measure and is accepted by the Food and Drug Administration as a primary endpoint in late-phase clinical treatment trials. Previous evaluations of the 6MWT in patients harboring the m.3243 mutation compared to control individuals (n=10-18) found the 6MWT to be highly sensitive and correlated with proximal muscle strength measurements (Newman et al., 2015). Whereas Duchenne Muscular Dystrophy (DMD) and healthy individuals maintain consistent walking speed throughout the 6MWT, this was shown not to be true of Spinal Muscular Atrophy patients (Montes et al., 2010). We hypothesized that MM fatigue patients would show similar results, with inability to maintain consistent walking speed throughout the 6MWT.

Here, we investigated whether alternative data analyses of 6MWT may effectively quantify fatigue in Mitochondrial Myopathy (MM). Specifically, we evaluated whether change in walking speed, rather than the standard assessments of absolute distance walked and deviations from normative values during a 6MWT, might offer a more reliable measure of fatigue in clinical trials of MM.

Methods: Thirty-three subjects with genetically and/or biochemically confirmed MM enrolled in Children's Hospital of Philadelphia IRB study protocol (IRB #16-013364) underwent 6MWT evaluations following standard protocol. Documentation of the distance walked at each minute was obtained, in addition to total distance walked during the full 6 minute period. Paired t-test and linear mixed-effects model were performed to analyze the data.

Results: Preliminary data (n=33) demonstrate that the mean distance walked during the 6MWT at minute 1 (77.7 ± 20.5 meters) was significantly higher than at minute 6 (70.3 ± 22.4 meters, p = 0.0039). This suggests MM subjects have a ~10% decrement in performance over time, which is consistent with their self-reported fatigue.

Conclusion: We report here a novel analytic approach that compares walking distance in the last minute relative to the initial minute (rather than cumulative time) of the 6MWT, which may provide a more sensitive outcome measure of fatigue in MM trials.

Abstract #: 2018 PA-0495

Title: Validation of Novel Neuroimaging PET Probe as a Predictive Mitochondrial Biomarker for Neurodegenerative Disease

Authors: Meagan J McManus, PhD[1], Robert Doot, PhD[2], Robert Mach, PhD[2], Douglas C Wallace[1], PhD

[1]Center for Mitochondrial and Epigenomic Medicine, Children's Hospital of Philadelphia, Philadelphia, PA; [2]Department of Radiochemistry, University of Pennsylvania, Philadelphia, PA

Mitochondrial dysfunction and oxidative stress are not only responsible for primary mitochondrial diseases, but also prominent early features in complex, age-related pathologies, such as Alzheimer's, Parkinson's, cancer, and cardiomyopathy. Defects in the mitochondrial DNA (mtDNA),

nuclear DNA encoding mitochondrial proteins, and environmental insults all impinge on mitochondrial function, often causing toxic levels of reactive oxygen species (ROS) production. Due to their transient nature, it is currently difficult to directly measure ROS in vivo, and therefore analysis of ROS levels is largely dependent on the molecular footprints left in their wake of destruction. Among all organs of the body, the brain is perhaps most vulnerable to oxidative damage. As such, elevation of virtually every established marker of oxidative damage has been documented in brain tissue from the most prevalent neurodegenerative diseases. However, the lack of a noninvasive method to detect mitochondrial dysfunction and oxidative stress in the living brain has led many to believe that these early changes are simply epiphenomenon. To address this issue, we designed the first superoxide-sensitive, radioactive tracer, ROStrace, for positron emission tomography (PET). Here we provide evidence that ROStrace crosses the blood brain barrier and detects increased mitochondrial oxidative stress in the brain and eyes of mice harboring the mtDNA ND6P25L mutation, which causes Leber's hereditary optic neuropathy (LHON) in humans. In mice, the mtDNA ND6P25L also causes a progressive neurodegenerative phenotype that closely recapitulates that of Parkinson's disease (PD). Prior to the onset of PD-related motor symptoms in ND6P25L mice, ROStrace retention increased 33% over that of age-matched WT controls, whereas there was no change in presymptomatic alpha-synuclein A53T PD mice. We then measured ROStrace retention over time and found that it correlated with age and progression of neurodegenerative disease in ND6P25L mice. Just as patients with primary mitochondrial disease are often hypersensitive to immune challenge, the mtDNA ND6P25Lmice were also more sensitive to inflammatory activation by lipopolysaccharide (LPS; 5mg/ml). Neural ROStrace retention correlated neurological demise and increased mortality in the ND6P25L + LPS mice. In summary, our results establish the first PET probe for ROS detection in the living brain, which promises to resolve the etiological significance of aberrant ROS production in complex diseases involving mitochondrial dysfunction. This probe may also be used to enhance clinical trial design by identifying patients in the prodromal phase of neurodegenerative disease that would benefit most from preventative mitochondrial therapeutics.

Abstract #: 2018 PA-0496

Presenter: Zarazuela Zolkipli-Cunningham

Authors: Allan Glanzman[1], Jean Flickinger[1], Natalie Burrill[1], Eileen Barr[1], Colleen Muraresku[1], Patrick Chinnery[2], Rui Xiao[3], Richard Haas[4], Zarazuela Zolkipli-Cunningham[1].

Institution: 1-Mitochondrial Medicine Frontier Program, The Children's Hospital of Philadelphia, 2- Mitochondrial Biology Unit, University of Cambridge, 3- Department of Biostatistics and Epidemiology, University of Pennsylvania, UK, 4- Metabolic and Mitochondrial Disease Center, University of California, San Diego.

Title: Development of a Mitochondrial Myopathy Rating Scale

Background: The increasing pursuit of Mitochondrial Myopathy (MM) treatment trials has created a pressing need for quantitative outcome measures that reliably reflect MM disease severity, progression, and therapeutic response. Since effective therapies may incrementally slow disease progression, quantitative outcome measures are needed that are specific to MM and sensitive to change. Supported by the United Mitochondrial Disease Foundation, this abstract serves to provide an update on the development of the Mitochondrial Myopathy Rating Scale (MMRS) as a phase II/III clinical trial outcome measure. We prioritized the integration of MM patient perspectives and included patient-reported (PROM), functional/quality of life (QoL), examination and composite measure domains in the design.

Methods: CHOP IRB study protocol was established, and involved two study sites (CHOP, UCSD). A cohort of ~ 60 adult and pediatric MM individuals, having genetically and/or biochemically-confirmed mitochondrial disease with predominant symptoms of myopathy are enrolled thus far. We conducted a systematic review of the literature to gather existing, validated scales of motor performance in other diseases and evaluated MM patient perception of these measures using a RedCap-administered survey. We conducted a qualitative study to evaluate the impact of MM symptoms on daily life to provide content validity for the MMRS. We then systematically evaluated existing exam-based motor performance measures validated in other neuromuscular disorders (Mancuso et al., 2017) in MM subjects to assess how well these measures characterized the diverse MM clinical domains of strength, exercise intolerance and fatigue. Statistical analyses using standard descriptive statistics and Rasch were conducted in R. The qualitative study was analyzed in NVivo.

Results: MM patient-reported preferred questions and results of the Rasch analysis were used to guide selection of items to assess their most frequent symptoms of exercise intolerance, fatigue, muscle strength, imbalance and neuropathy in the PROM and QoL domains. Notably, MM subjects relayed the importance of including QoL questions in the MMRS. We will present results of the qualitative study which provides insight into impacted daily activities in MM subjects with either 1 or a combination of the symptoms listed above. In the evaluation of existing motor performance measures, we concluded that all were not applicable to MM unless modified. We have adapted an exam-based measure from the North Star Ambulatory Assessment in Duchenne Muscular Dystrophy and are currently validating new fatigue measures. For feasibility of standardized assessments across an age spectrum, we have limited the utility of the MMRS to ambulatory individuals and > 7 years of age. Finally, administration of the MMRS in its entirety to the MM cohort is ongoing and these results will be presented.

Conclusion: We have been methodical in our approach to the development of a patient- centered MMRS and have sought FDA guidance through this project period. Our efforts have focused on incorporating MM patient perception, the systematic evaluation of existing motor performance measures for modification in MM and the development of muscle fatigue measures. Further work will be needed to assess the sensitivity, reproducibility, reliability and clinical meaningfulness of the MMRS.

Abstract #: 2018 PA-0497

Presenter: Cait S. Kirby

Authors: Cait S. Kirby, Maulik R. Patel

Institution: Vanderbilt University, Department of Biological Sciences, Nashville, TN

Title: Mitochondrial DNA copy number in C. elegans is regulated by a functional output of the electron transport chain

Body of Abstract: Mitochondria contain their own genomes (mtDNA), which encodes essential components of the mitochondrial electron transport chain that produces ATP via oxidative phosphorylation. This circular 16.5kb chromosome can exist in hundreds to thousands of copies per cell. mtDNA copy number varies across different cell and tissue types, with insufficient mtDNA copy number resulting in debilitating diseases, called “mtDNA depletion syndromes.” These data suggest tightly controlled regulation of mtDNA copy number. However, the mechanisms of mtDNA copy number regulation are poorly understood. A major challenge has been the lack of a tractable system which exhibits robust and active mtDNA copy number control.

My preliminary data suggest the existence of an active homeostatic control mechanism that regulates mtDNA copy number in the Caenorhabditis elegans germline, providing a genetically tractable system in which to study mtDNA copy number control. I have utilized a collection of heteroplasmic C. elegans strains, which contain mtDNA molecules with two distinct genetic sequences: mutant and wild-type. For each strain, the mutant molecules contain a single mtDNA deletion. Together, these deletions span nearly the entire mitochondrial genome. These strains provide a powerful tool to interrogate which regions of mtDNA are necessary for mtDNA copy number regulation. In many of these heteroplasmic strains, the number of wild-type copies is maintained at a stable amount, even as the frequency of mutant copies increases. These data suggest the presence of a homeostatic mechanism that maintains wild-type copy number, but not mutant copy number. My analysis suggests that deletions which affect certain complexes of the electron transport chain result in mutant molecules escaping copy number regulation. Based on these data, it appears that an overall functional output of the electron transport chain is being used in a cellular sensing mechanism to maintain wild-type copy number. My work provides insights into potential generation of therapeutic medical intervention for patients with mtDNA diseases such as disorders of mtDNA depletion.

Mitochondrial Medicine 2018: Nashville

Abstracts

Abstract #: 2018 PA-0498

Presenter: Johan L.K. Van Hove

Authors: Johan L.K. Van Hove[1], Marisa W. Friederich[1], Kaz Knight[1], Rudy Van Coster[2], Joel Smet[2], Elise Vantroys[2], Michio Hirano[3], Amy Goldstein[4]

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4 Department of Pediatrics, Pittsburgh, Pittsburgh, PA, USA

Title: Clinical utility of established and new mitochondrial diagnostic testing in fibroblasts, a minimally invasive tissue, identifies sensitive functional testing methods for complex I deficient mitochondrial disorders.

Background: Primary mitochondrial disorders are clinically, biochemically, and genetically heterogenous. Next-generation DNA sequencing has become a primary means of diagnosis, but does not always identify the genetic cause, and often requires functional validation for variants of unknown significance or when only a single mutation in a recessive gene was identified. Diagnosis by functional testing often required invasive biopsies of muscle or liver because respiratory chain enzyme activities in material from minimally invasive tissue such as fibroblasts have low sensitivity (abnormal in <50% of patients). New methods are needed to expand the functional testing capability using minimally invasive tissues.

Methods: Respiratory chain enzyme activities were measured spectrophotometrically and complexes were assessed by blue native PAGE with in-gel activity staining (BNP). Complex I assembly was assessed using a solubilized mitochondrial inner membrane preparation separated on a gradient native gel followed by western blotting and probing with an antibody against a subunit that integrates at the earliest stage of complex I assembly. High resolution respirometry using a substrate-uncoupler-inhibitor titration (SUIT) protocol specific for fibroblasts was performed on an Oroboros Oxygraph 2k instrument. To evaluate the clinical utility of these assays, we evaluated a large set of cells from controls and from patients with known primary mitochondrial genetic defects.

Results: In 42 controls, fully assembled complex I was detected at 1000 kDa, with an additional faint band at 230 kDa observed in 60% of samples at 7.15±3.50% of the main band. In isolated complex I deficiency, respirometry was specific using a combination of three parameters, but at low sensitivity of 38%. Blue native PAGE was abnormal in 50% of cases and enzyme activity assay in 70%, but the assembly analysis had high sensitivity at 92%. In isolated complex V cases, blue native gel analysis had the highest sensitivity. Surprisingly, complex I assembly was sometimes abnormal in isolated complex V defects, the mechanism of which is still under study. In combined deficiencies, enzyme activities were not sensitive (30%), but complex I assembly and BNP had better results at 60 and 66% sensitivity. Particularly, disorders of aminoacyl-transferase genes (_ARS2) often showed normal results under routine culture conditions, but abnormal if the cognate amino acid concentration was lowered in the culture media.

Conclusions: These preliminary data highlight the sensitivity and specificity of new and established assays in detection of respiratory chain complex deficiency, and illustrate the potential to develop a panel of functional tests in fibroblasts for diagnosis using a tissue requiring a minimally invasive procedure.

Abstract #: 2018 PA-0499

Presenter: Amy C. Goldstein

Authors: Amy Goldstein, Zarazuela Zolkipli-Cunningham, Rebecca Ganetzky, Shana McCormack, Marni J. Falk

Institution: Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA

Mitochondrial Medicine 2018: Nashville

Abstracts

Title: Opportunities and Challenges to Measuring Fatigue in Primary Mitochondrial Disease

Introduction: Despite fatigue being one of the most common symptoms of primary mitochondrial disease, there is a lack of quantitative, objective and validated fatigue measures for clinical trials. Ongoing clinical trials for mitochondrial myopathy do not effectively measure fatigue. As clinical trials continue, validated outcome measures are needed to reliably measure fatigue in primary mitochondrial disease. This requires comparative analysis of available fatigue scales to assess their relative merits and drawbacks.

Methods: Fatigue outcome measure instruments were reviewed that were either used in active or completed mitochondrial disease clinical trials, listed in the NINDS Common Data Elements for mitochondrial disease, or discussed in a recent mitochondrial myopathy outcome measures workshop held in Rome, Italy. The Rome workshop instruments reviewed included Checklist individual strength (CIS), Fatigue Severity Scale (FSS) and Multidimensional Fatigue Inventory (MFI). Additional fatigue instruments reviewed include Fatigue Severity Scale, Modified Fatigue Impact Scale (FIS), PROMIS-Fatigue, and FACIT (Functional assessment of chronic illness therapy).

Results: Multiple different instruments exist for fatigue in different disorders, none of which has been validated in mitochondrial disease. Currently a lack of correlation exists between subjective complaints and these existing scales in mitochondrial disease patients. While there have been recommendations on instruments to measure fatigue in mitochondrial disease based on expert consensus, none have been validated in mitochondrial disease and the study design of various clinical trials in mitochondrial disease have not implemented the same outcome measures, hence limiting inter-study comparison. A comparative analysis of components in different fatigue scales highlights the diverse aspects of fatigue and need for greater understanding of the underlying mechanism of fatigue in mitochondrial disease and development of validated outcome measures to promote targeted treatments. The Multidimensional Fatigue Inventory (MFI) might best capture the multifaceted aspects of fatigue typical of mitochondrial disease, as it assesses general, physical, mental and/or cognitive fatigue in addition to reduced motivation and reduced activity.

Conclusions: Validation and harmonization of outcome measures for mitochondrial across clinical trials will be essential to quantify and promote development of effective treatments for fatigue.

Abstract #: 2018 PA-0500

Presenter: Amy Goldstein

Authors: Amy Goldstein, Zarazuela Zolkipli-Cunningham, Rebecca Ganetzky, Colleen Muraresku, Elizabeth McCormick and Marni Falk

Institution: Children's Hospital of Philadelphia, Division of Human Genetics, Philadelphia, PA

Title: Expanding the phenotype of m.10191T>C (p.Ser45Pro) recurrent pathogenic variant in MT-ND3

Body of Abstract: The m.10191T>C was first described in an adult with a progressive history including stroke-like episodes, progressive myoclonic epilepsy, ataxia, bilateral optic atrophy, and cognitive decline, and was also the first pathogenic mitochondrial DNA mutation in the ND3 gene to be reported (Taylor 2001). Brain MRI was asymmetric and abnormal for increased signal within the midbrain involving the right superior colliculus and the posterior aspect of the right lentiform nucleus.

Pathogenicity was subsequently established via in vitro biochemical assays of patient-derived fibroblasts and lymphoblasts, in addition to transmitochondrial cybrid analysis, showing complex I deficiency. The serine to proline change at position 45 occurs within a highly conserved region of ND3 protein that could affect folding and interaction with other complex I subunits (Bugiani 2004). In silico tools predict pathogenicity and it is not seen in healthy controls and is therefore classified as pathogenic.

Subsequently, this pathogenic variant was reported in multiple heteroplasmic patients with mitochondrial complex I deficiency either de novo

or from asymptomatic mothers with much lower heteroplasmy levels. The spectrum of clinical phenotypes has ranged from infant lethality to adult onset Leigh syndrome (Leshinsky-Silver E 2005, Taylor 2001). Nearly all patients have abnormal neuro-imaging and elevated plasma and CSF lactate, meeting criteria for Leigh syndrome. Most patients have developmental delay, regression, hypotonia, dystonia, spasticity, and epilepsy. Ataxia, myoclonus and optic atrophy were also reported in some patients. Neither the degree of heteroplasmy nor the mode of inheritance has correlated with symptom severity (Nesbitt 2012; Levy 2014) and it has been suggested that the survival rate for patients harboring this mutation may be higher than other complex I deficiencies and/or Leigh syndrome (McFarland 2004; Levy 2014). A recent review by Nesbitt et al 2012 describes 16 patients with m.10191T>C (p.Ser45Pro) MT-ND3 causing isolated complex I deficiency with variable prognosis; most having central nervous system involvement evident on neuro-imaging (when available); mainly bilateral putamen and caudate, rarely brainstem, midbrain and medulla, but all typical of Leigh syndrome. 2 adults reported have cognitive impairment and optic atrophy, not observed in pediatric patients. In another recent update citing 22 patients published in the literature to date, including updates on those previously published, Levy et al (2014) reported that of 17 available histories, 9/17 patients had died (6 prior to age 10, 3 after age 10), 8/17 remaining alive were older than age 10 and only 1 patient had adult onset disease. Therefore only 35% of these patients died prior to age 10, with the majority surviving beyond childhood; compared to Leigh syndrome overall the m.10191T>C has a higher survival rate than others where death prior to age 10 is seen in 75-85% (Levy 2014).

We have 2 additional cases for which our patients had a clinical course and neuro-imaging typical of Leigh syndrome and then progressed to a MELAS-like phenotype. We would like to suggest that the pathogenic variant m.10191T>C causes a LS/MELAS overlap syndrome for which, once a patient survives earlier childhood, a MELAS-like course develops and could be amenable to further treatment and prevention.

Abstract #: 2018 PA-0501

Presenter: Emine C. Koc

Authors: Emine C. Koc[1], Funda Kartal[1], Maria Tirona[2], and Hasan Koc[3]

Institution: Marshall University Joan C. Edwards School of Medicine, Departments of Biological Sciences[1], and Edwards Cancer Center[2], and Marshall University School of Pharmacy, Department of Pharmaceutical Research and Science[3]

Title: Regulation of Mitochondrial Biogenesis and Translation in ER/PR Positive Breast Cancers

Body of Abstract: Remodeling of energy metabolism is described as one of the major hallmarks of cancer and contributes to cancer heterogeneity and survival in a dynamic environment with reduced nutrient and oxygen levels. Defects in oxidative phosphorylation (OXPHOS) can also cause a switch in energy metabolism from oxidative to aerobic glycolysis, also known as the Warburg effect in cancer. Mitochondrial translation plays a crucial role in the biogenesis of OXPHOS complexes by synthesizing 13 mitochondrially-encoded subunits of complexes I, III, IV, and V (ATP synthase). Interestingly, the changes in expression of mitochondrial translation components and single nucleotide polymorphisms (SNPs) of their genes have been associated with breast cancer. In this study, we investigated the expression of OXPHOS components in 34 ER/PR (+) breast tumors. Our findings suggest that the changes in mitochondrial ribosomal protein (MRP) expression contribute to the remodeling of oxidative energy metabolism and deregulation of apoptosis in breast cancer. Understanding the role of MRPs in the remodeling of energy metabolism and apoptosis will be essential in characterization of heterogeneity in breast tumors at molecular levels.

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Abstract #: 2018 PA-0502

Presenter: Bruce Cohen

Authors: BH Cohen[1], Cristy Balcells,[2] B Hotchkiss[3], Karaa, Amel[4]

Institution: 1. Akron Children's Hospital, Akron OH 2. RN, MSN 3. Stealth BioTherapeutics, Newton MA 4. Massachusetts General Hospital, Boston, MA, USA.

Title: Retrospective, Comparative, Cohort Analysis of Health Care Utilization for Neurological Disorders in Patients with Mitochondrial Disease, Multiple Sclerosis, and Amyotrophic Lateral Sclerosis

Background: Primary Mitochondrial disease (PMD) is a heterogeneous group of disorders characterized by impaired energy production caused by abnormal oxidative phosphorylation, affecting several systems including the neurologic system. This retrospective patient-claims analysis compared the financial impact (healthcare utilization and cost) of PMD to those observed for patients with multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS).

Methods: The analysis used the Truven Marketscan database and Milliman's Consolidated Health Cost Guidelines Sources Database (CHSD) for the calendar year 2015. Patients of any age with an ICD9/10) for PMD between 2008 and 2015 were included. Specifically, ICD9 277.87 disorders of mitochondrial metabolism and ICD10 E88.40, E88.41, E88.42 and E88.49 mitochondrial metabolism disorders were used to identify PMD-related claims.

Results: Mean age of PMD patients was 26.1 years (46.3%, 0 to 15 years [pediatric]) and 53.7%, ≥16 years [adult]). Males represented 46.8% (N=3,832) and females represented 53.2% (N=4,348) of PMD patients. A larger proportion of female PMD patients (63.1%) were adults. A larger proportion of males (57%) were pediatrics. Health care resource utilization and cost for adult PMD patients were compared to those to patient members diagnosed with MS (N=125,434) and ALS (N= 4,579) . Resource utilization per 1000 for PMD was 95,404, which was lower than ALS (107,133) but greater than MS (71,859). Overall PMD allowed per member per month (PMPM) costs were comparable to MS and ALS, which are all significantyl higher than the baseline member population.

Conclusions: ALS and MS, used for comparison to PMD, are two neurodegenerative disorders that carry a significant morbidity and mortality. We demonstrated that MD imposes a cost burden comparable to the more widely recognized neuromuscular disorders, MS and ALS, making it important for health plans to ensure that patients with PMD receive the most appropriate care across multiple health disciplines. The management of neurological comorbid conditions in pediatric and adult patients incurs significant costs and burden of care.

This study was funded by Stealth BioTherapeutics.

Abstract #: 2018 PA-0504

Presenter: Ajibola B. Bakare

Authors: Ajibola B. Bakare[1],Olivia Kolenc[2], Isaac Vargas[2],Joshua Stabach[2],Abigail Harris[1], Julienne Daniel[1], Kyle Quinn[2] and Shilpa Iyer[1]

Institution: [1]University of Arkansas, Biological Sciences, J. William Fulbright College of Arts & Sciences, Fayetteville, AR 72701.[2]University of Arkansas, Biomedical Engineering, College of Engineering, Fayetteville, AR 72701.

Title: Comparative Analysis of Mitochondrial Bioenergetics and Dynamics in Leigh's disease

Body of Abstract:

Introduction: Leigh's Syndrome (LS) is a classic mitochondrial disease and currently there are neither effective treatments nor adequate

model systems for understanding the rapid fatality associated with the disease. Fatality with LS results from excess accumulation of mutant mtDNA molecules or failure of mitochondrial bioenergetics. Other symptoms of LS usually include developmental, neural, cardiac and muscle impairments resulting in a chronic lack of energy in these patients.

Results: In this study we characterized five LS patient-derived fibroblast cells, carrying mutations in Complex I and Complex V of the mitochondrial genome. Three of the patients were diagnosed with mutations in the (m.8993T>G and m.9185T>C) ATP6 gene; while the other two mutations (m.10158T>C) and (m.12706T>C) were present in ND3 and ND5 genes respectively. We carried out a comparative analysis of mitochondrial redox potential, mitochondrial bioenergetics and mitochondrial morphology between the five Leigh's patient fibroblast cells. Label-free multiphoton microscopy was used to non-invasively evaluate redox potential in each cell line based on an optical redox ratio of FAD and NADH auto-fluorescence. A higher optical redox ratio was detected in cells with a Complex V mutation ($p=0.0249$) relative to normal fibroblasts, indicating an increase in ETC activity. Then we measured basal oxygen consumption rate (OCR mitochondrial respiration) and extracellular acidification rate (ECAR which measures glycolysis) using the extracellular flux analyzer. Complex V mutants showed a significantly higher maximal respiration rate ($p=0.0027$) and spare respiratory capacity rate ($p=0.0036$) compared to control fibroblast cells. In addition, we also observed significantly higher non-mitochondrial oxygen consumption rate (<0.0001) and higher ($p=0.0036$) compensatory Glycolytic rate relative to normal fibroblast cells. These results indicated that the cells were consuming oxygen at a faster rate to compensate for the defect in ATP synthase. Results indicated no significant change in Basal respiration or ATP production in the Complex V mutant cells relative to normal fibroblast control, supporting our observation of higher maximal respiration and compensatory glycolytic rate. Surprisingly, we did not observe significant differences in any of the OCR or ECAR measurements in Complex I mutant cells relative to the control fibroblast cell. We observed differences in cell growth and morphology between the five mutant cell lines. We hypothesized that the morphological differences could be attributed to altered mitochondrial dynamics, respiration, and redox potential between mutant and control cell lines. Cells were stained with Mitotracker Red CMH2X RoS (stains actively respiring mitochondria in live cells) and Mitotracker Green (stains all mitochondria in live cells). Fluorescence images of live cells were obtained using EVOSFL imaging system. The cells were analyzed by Fractal Analysis and ImageJ macro tools. Preliminary data indicated that Complex I mutant cells demonstrated mitochondrial organization with a significantly higher fractal dimension ($p\leq0.0438$), suggesting less clustering.

Conclusion: We are currently in the process of generating isogenic induced pluripotent stem cell lines from Leigh's patient fibroblast cells to further understand the role of mitochondrial dysfunctions in neuronal development and differentiation. Future work will also explore how imaging techniques can be used as a non-invasive diagnostic tool for Leigh's and other mitochondrial disorders.

Abstract #: 2018 PA-0505

Prenenter: Aurora Gomez-Duran

Authors: Aurora Gomez-Duran[1,2], Zoe Golder[1,2], Claudia Calabrese*[1,2], Yaobo Xu*[3], Gavin Hudson[3], Mauro Santibanez-Koref[3], Eduardo Ruiz-Pesini[4], Patrick F. Chinnery[1,2]¶

Institution: [1]Department of Clinical Neurosciences, School of Clinical Medicine, University of Cambridge, CB2 0QQ, UK.
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Title: Cytochrome b regulates nuclear-mitochondrial homeostasis through mTORC1 and HIF1α

Body of Abstract: Maternally inherited polymorphic variants of mitochondrial DNA (mtDNA) alter the risk of developing common late-onset human diseases, but the underlying mechanisms are not understood. Here we show that mTORC1 is a key mediator between

the mitochondrion and cell nucleus under homeostatic conditions, and without activating the integrated mitochondrial stress response mechanisms. mTORC1 inhibition exposes major differences in respiratory capacity between common mtDNA population haplogroups through compromised mitochondrial biogenesis. We show that mTORC1 is a rheostat regulating glycolysis through HIF1α in response to subtle differences in complex III due to the mtDNA sequence. Although critical for maintaining ATP levels, this has an impact on cell proliferation and survival. Thus, our findings provide a new mechanism linking mtDNA variation with cancer and neurodegenerative disease that does not directly involve oxidative phosphorylation. Although manipulating mTORC1 may have therapeutic benefits, it will disrupt mitochondrial homeostasis in genetically defined groups, with potentially adverse effects.

Abstract #: 2018 PA-0506

Presenter: Ralitza H. Gavrilova

Authors: Ralitza H. Gavrilova, [2,3] Fernando C. Fervenza, [1] Samih H. Nasr, [4] Maria V. Irazabal, [1] Karl A. Nath [1]

Institution: Mayo Clinic College of Medicine, Rochester, Minnesota
[1]Division of Nephrology and Hypertension, [2]Department of Clinical Genomics, [3]Department of Neurology, [4]Department of Laboratory Medicine and Pathology,

Title: Chronic Renal Disease and Rhabdomyolysis Due to a Novel Mitochondrial DNA Mutation

Mitochondrial myopathies may present isolated or as a component of multi-system disorder. Kidney disease is recognized in 5% of mitochondrial cytopathies but the extent to which mitochondrial pathology contributes to renal disease is uncertain. The prevalence of kidney disease in mitochondrial cytopathies may be underestimated. Here we present a case of rhabdomyolysis and chronic renal disease due to novel mtDNA mutation. Kidney biopsy unrevealed underlying mitochondrial pathology as the cause for his kidney dysfunction which otherwise may be presumed as secondary to rhabdomyolysis. 42 yo man was evaluated at Mayo Clinic for elevated serum creatinine after episode of rhabdomyolysis. He had no prior history or family history of muscle disease. Evaluation revealed elevations in CK (>20,000U/L), creatinine (1.6 mg/dL), plasma lactic acid and pyruvate (4.7 mmol/L and 3.0 mg/dL). The patient was hospitalized and treated symptomatically. Kidney biopsy revealed globally sclerotic glomeruli minimal tubular atrophy and interstitial fibrosis 10% of cortex. Rare collecting ducts showed granular swollen epithelial cells, distinct morphologic feature of mitochondrial nephropathy. Electron microscopy revealed distal tubular cells with abnormal mitochondria, enlarged and rounded, with internal rounded or linear osmiophilic inclusions and dense parallel cristae. EMG revealed mild diffuse myopathy. Muscle biopsy showed many COX negative fibers, few had subsarcolemmal increase in SDH reactivity, and a single ragged-blue fiber. MtDNA massively parallel sequencing from skeletal muscle revealed novel pathogenic heteroplasmic 61.7% mitochondrial DNA variant 6145G>A in the MTCO1 gene (W81*). First, created stop codon and premature termination of subunit I COX protein, COX deficiency was confirmed on muscle biopsy. Second, mitochondrial abnormalities were detected in both muscle and kidney. Third, was heteroplasmic, feature commonly associated with pathogenic mtDNA mutations. Finally, it was not reported as polymorphism or with other pathogenic mtDNA mutations. This mutation adversely affected skeletal muscle and kidney. In muscle, the deficiency of the COX protein, critical component of ETC, would predictably impair ATP generation; diverting electrons into generating partially reduced ROS. ATP maintains homeostasis of the skeletal muscle, especially under exertional stress and impaired ATP generation in muscle predisposes to rhabdomyolysis. In the kidney, impaired ATP production compromises tubular function, and with enhanced generation of ROS, predisposes to tubular interstitial disease (TID). Thus, this mtDNA mutation can cause, independently rhabdomyolysis and TID. Additionally, chronic TID may reflect the interaction of abnormalities in skeletal muscle and kidney. In experimental model repetitive muscle injury resulted in chronic TID because of proinflammatory and profibrogenic effects of heme proteins, enhanced when there is increased renal ROS generation. It is thus possible that the renal mitochondrial complex IV defect in this patient renders the kidney sensitive to the nephropathic effects of myoglobin during rhabdomyolysis. The patient exhibited mitochondrial abnormalities in muscle and kidney, chronic tubulointerstitial changes, and recurrent episodes of rhabdomyolysis. We outline mechanisms that may underlie the occurrence of chronic kidney disease in the setting of this novel mtDNA mutation. We also underscore the need to consider in relevant kidney diseases the presence of an underlying mitochondrial cytopathy, as the latter more commonly exists than is generally recognized.

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Abstracts

Abstract #: 2018 PA-0507

Presenter: Jomênica de Bortoli Livramento

Authors: Jomênica B Livramento[1]; Thiago C. Araújo[1]; Gabriela S. Rodrigues[1]; Camila D. S. Barros[1]; Beatriz H. Kiyomoto[1]; Beny Schmidt[2]; Acary S. B. Oliveira[1]; Célia H. Tengan[1]

Institution: [1] Departments of Neurology & Neurosurgery and Pathology[2], Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo/Brazil

Title: Nitrative stress in skeletal muscle of patients with mitochondrial DNA mutations

Protein nitration is a modification related to nitrative stress, which is caused by the presence of reactive nitrogen species. Tyrosine nitration is an indicator of protein nitration and has been used as a biomarker of nitrative stress in different conditions including inflammatory, autoimmune and degenerative diseases. Considering that mitochondrial diseases have a higher generation of oxygen radicals (ROS) and that there is NO production in the skeletal muscle, the presence of nitrative stress is likely and may be an additional factor that may be considered in the pathogenesis of these diseases. The aim of this study was to evaluate the presence of nitrated proteins in muscle from patients with mitochondrial disease. We studied muscle biopsy specimens of patients with mitochondrial DNA (mtDNA) mutations (multiple deletions, N=3 single deletions, N=2; mutations in MT-TL1, N=3) and a control muscle sample with no abnormalities. Nitrated proteins were detected by immunofluorescence with the anti-3-nitrotyrosine antibody and quantified using ImageJ software. We found immunoreactivity in both sarcolemma and sarcoplasm but with different levels of intensity and proportions of affected muscle fibers. Most of the patients (87%) had immunoreactivity affecting more than 25 % of the sarcolemmal membrane. Muscle samples with sarcolemmal immunoreactivity had a mean of 54% affected muscle fibers, but the proportions varied from 3% to 100%. Two patients presented sarcolemmal immunostaining in the majority of muscle fibers, one with multiple deletions due to a TK2 gene mutation and the other with the m.3243A>G. Nitrotyrosine was detected in the sarcoplasm in 50% of patients with proportions of affected fibers that varied from 14% to 53%. Ragged red fibers (RRF) had a significant increase in the intensity of sarcoplasmic immunoreactivity (72,43±16,82 %, n=9) when compared to normal fibers (50.59±13,67 %, n=8). No correlation was detected between positive immunoreactivity and the genotype, however, these are preliminary results and a larger study group is necessary. Our findings show the presence of nitrative stress in muscle samples with mtDNA mutations, especially in the sarcolemmal membrane and sarcoplasm of RRF. These results suggest that nitrative stress can induce modifications that can be relevant to the pathogenesis of mitochondrial diseases.

Financial support: CAPES, CNPq and FAPESP.

Abstract #: 2018 PA-0509

Presenter: Atif Towheed

Authors: Atif Towheed[1], Jesus Tintos[1], Piotrek Kopinski[1], Prasanth Potluri[1], Santhanam Shanmughapriya[6], Howard Gamper[5], Deborah Murdock[1], Ya-Ming Hou[5], Madesh Muniswamy[6,7], Michael J. Palladino[3,4], Douglas C. Wallace[1,2]

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Pennsylvania 15261, USA. 4) Pittsburgh Institute for Neurodegenerative Diseases (PIND), University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261, USA. 5) Thomas Jefferson University, Department of Biochemistry and Molecular Biology, 233 South 10th Street, BLSB 220, Philadelphia, PA, United States. 6) Department of Medical Genetics and Molecular Biochemistry, Lewis Katz School of Medicine, Temple University, Philadelphia, PA 19140, USA. 7) Center for Translational Medicine, Lewis Katz School of Medicine, Temple University, Philadelphia, PA 19140, USA.

Title: Allotopically expressed RNA mediated genetic complementation of a mitochondrial-encoded ND6 frameshift mutant

Body of Abstract: mtDNA mutations occur with an incidence of 1 in 4000. Diagnosis and treatment of disorders caused by mtDNA mutations are relatively challenging due to their complex genetics and tissue specificity. Mutations in the mtDNA encoded ND6 subunit of Complex I is known to cause ~15% of LHON in human population. Due to challenges in the treatment of complex mitochondrial disorders, gene-therapy has been proposed as a viable option. However, gene-therapy using protein targeting to the mitochondria has its own sets of limitations such as aggregation due to hydrophobicity of mtDNA-encoded proteins. An alternate strategy is to target RNA instead of the protein. Using mtND6 null mutant cell cybrid model, we expressed mitochondrial encoded ND6 gene in the nucleus and targeted it into the mitochondria using RNA sequences that are naturally imported into the mitochondria as targeting signals. The targeting signals allow localization of the allotopically expressed full length recoded mtND6 mRNA into the mitochondria. Once localized into the mitochondria, these mRNA have the potential to be translated using the endogenous mitochondrial translational machinery. Our results indicate that using RNA allotopic expression of the normal copy of ND6 gene, the mtND6 null mutant can be rescued. This is evident by decrease in extracellular acidity, NAD:NADH ratio and ROS, increase in mitochondrial respiration, partial restoration Complex I protein (NDUFB8), calcium kinetics as well as changes in mitochondrial content. We thus successfully engineered and evaluated a vector to express a normal copy of a mitochondrial gene in the nucleus, target it into the mitochondria and complement the defect due to a frameshift mutation in mtND6 gene. This tool has the potential to be used as a gene therapy for mitochondrial DNA mutation diorders.

Abstract #: 2018 PA-0510

Presenter: Pushpa Sharma, PhD

Authors: Pushpa Sharma, Ph.D. and Brandi Benford MS

Institution: Department of Anesthesiology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814

Title: DIPSTICK TEST FOR ELECTRON TRANSPORT CHAIN DAMAGE IN RATS FOLLOWING MILD TRAUMATIC BRAIN INJURY

Mild traumatic brain injury (mTBI) has been recognized as major cause of death and disabilities in younger and older population. Bioenergetics failure due to dysfunctional mitochondria is increasingly recognized as a key component in the progression of neuronal cell death in TBI (1) The ineffective treatments for TBI is mainly due to 1) lack of early blood- based biomarkers of metabolic failure and mitochondrial damage following brain injury, and 2) unsuccessful mitochondrial targeted treatment strategies. Objectives: 1) develop blood based mitochondrial biomarkers of brain- injury severity, and 2) develop mitochondrial targeted therapeutic strategies. Method: We will use blood -based dipstick test (2) to determine the activity of electron transport chain complexes I and IV and PDH (pyruvate dehydrogenase complex) in rats with mild TBI (fluid percussion) and treated with/out mitochondrial substrate sodium pyruvate (1g/kg in d water) orally every 24 h for 7 days). **Results:** Complex I activity was significantly lower in plasma and hippocampus of TBI animals than controls at day 7, and pyruvate improved the complex I activities and GFAP staining following TBI. **Conclusion:** There was a direct correlation between serum complex I in blood and hippocampus suggesting the use of dipstick test as biomarker for TBI.

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Abstracts

Abstract #: 2018 PA-0514

Presenter: Suraiya Haroon

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Title: A POLG model identifies regulators of mitochondrial disease and generates new mitochondrial DNA mutants.

Abstract: Energy is a necessity for all biological processes and most of this energy is made by the mitochondria. In order to facilitate with the energy production, the mitochondria carry their own genome, the mitochondrial DNA (mtDNA). Mutations in the mtDNA can disrupt energy production and is detrimental to high-energy consuming cells like neurons and muscle fibers. These pathogenic mtDNA mutations can accumulate during natural aging or be inherited by children and they lead to diseases characterized by neuromuscular dysfunction. Currently, there are no cures for mtDNA disease. Two important impediments in developing new treatments are (i) a lack of animal models available for study and (ii) a lack of cost-effective ways to identify new therapeutic targets. To address these concerns, we have developed a new worm model for mtDNA disease that carries an error-prone allele of polg-1, the polymerase that replicates the mitochondrial genome. First, the polg-1 strain is a new model to study mtDNA diseases and they can be used to generate new mtDNA mutant models. Secondly, since worms are amenable to genetic and chemical manipulations, we can cost-effectively screen the animals to identify novel targets for therapy.

Remarkably, the polg-1 mutants exhibit hallmark features of mtDNA disease in humans, which include mtDNA instability, mitochondrial dysfunction, loss of neuromuscular function and a shortened lifespan. The mimicry of mammalian disease progression in the polg-1 mutant worms make them ideal for discovery experiments. With a small, targeted RNAi screen of 130 genes, the knockdown of 22 genes were identified to rescue mtDNA disease. These genes belong to different biological processes, including the IGF/Insulin signaling (IIS) pathway, mitochondrial unfolded protein response (UPR^{mt}), autophagy and apoptosis. Studies with genetic mutants verified that reducing IGF/Insulin signaling (IIS) and mitophagy improves the health of the polg-1 worms by improving mtDNA copy number, mitochondrial function and tissue function. Furthermore, constitutively activating the UPR^{mt} also improves these phenotypes in the polg-1 mutants. Currently, we are studying the role of reducing insulin signaling in the $POLG^{D207A}$ mouse model.

Fortuitously, even though manipulating mtDNA directly is prohibitive, the polg-1 mtDNA mutator strain allows us to develop mtDNA mutant lines. Once the polg-1 mutants have accumulated mtDNA mutations to detectable levels, we breed out the polg-1 mutant allele to create stable lines with mtDNA mutations. We have already established 7 separates mtDNA mutant strain that consist of ranging complexity in the mutation profile of the mtDNA. We are currently in the process of developing more mtDNA mutant models and we plan to characterize the effects of these mutations on organismal health.

Abstract #: 2018 PA-0515

Presenter: Brian Cunniff

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Title: Miro1-mediated mitochondrial positioning shapes intracellular energy gradients required for cell migration

It has long been postulated that mitochondria are positioned in the cytoplasm to meet local requirements for energy. Here we show that positioning of mitochondria in mouse embryonic fibroblasts (MEFs) determines the shape of energy gradients in living cells. Specifically, the ratio of ATP to ADP was highest at perinuclear areas of dense mitochondria and gradually declined approaching peripheral sites. Furthermore, the majority of mitochondria were positioned at the ventral surface of the cell, correlating with high ATP:ADP ratios at the ventral membrane that rapidly decreased towards the dorsal surface. We used cells deficient for Miro1, an essential mediator of microtubule-based mitochondrial motility, to study how changes in mitochondrial positioning affect energy distribution and cell migration. The mitochondrial network in Miro1$^{-/-}$

MEFs was restricted to the perinuclear area, with few mitochondria at the cell periphery. This change in mitochondrial distribution dramatically reduced the ratio of ATP to ADP at the cell cortex and disrupted events essential for cell movement (focal adhesion dynamics and actin based membrane reorganization). Consequently, Miro1$^{-/-}$ MEFs migrated slower than control cells during both collective and single cell migration. These data establish that Miro1-mediated mitochondrial positioning at the leading edge provides localized energy production that promotes cell migration by supporting membrane protrusion and focal adhesion stability. These results provide context for ongoing work in our laboratory investigating specific signaling pathways influenced by local and temporal production of mitochondrial metabolites.

Abstract #: 2018 PA-0516

Presenter: Liam Coyne

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Title: Mitochondrial proteostatic stress induces cytosolic aggresome formation.

Body of Abstract: Mitochondrial proteostatic stress is causally implicated in many human diseases. Proteostatic stress in mitochondria has multiple causes including protein misfolding, defects in mitochondrial protein quality control, and an over-abundance of protein in mitochondrial sub-compartment. How mitochondrial proteostatic stress kills cells and causes disease is poorly understood, at least in part due to the multifunctional nature of mitochondria, which engenders complex cellular consequences. We recently showed that multiple mitochondrial proteostatic insults can kill yeast cells by reducing protein import efficiency and thereby inducing mitochondrial Precursor Overaccumulation Stress (mPOS). mPOS is characterized by the toxic accumulation of unimported mitochondrial precursors in the cytosol. Here, we show that mPOS can be directly visualized in human cells challenged by two different mitochondrial proteostatic stress mechanisms: inner mitochondrial membrane (IMM) protein overexpression and protein misfolding on the IMM. Upon overexpression of mitochondrial carrier proteins, such as Ant1, we observe the formation of large membrane-bound aggresomes in the cytosol. Aggresomes are cytosolic sequestrations of misfolded proteins that form when the cell's protein degradation machinery, such as the proteasome, is overwhelmed. These mitochondria-induced aggresomes are also observed upon expression of pathogenic Ant1 variants that misfold on the IMM. Surprisingly, despite this drastic cytosolic response, we do not observe IMM uncoupling or severe bioenergetic deficiency. The experiments therefore captured a profound effect of IMM stress on cytosolic proteostasis and an important role of aggresome formation in handling the proteostatic burden. These findings could have broad implications for numerous degenerative diseases with defective IMM protein quality control, and also for our general understanding of how mitochondrial deterioration affects cell viability.