Abstract #: 2025PA-0000000022

Presenter: Shrey Dalwadi

Title: Cancer Prevalence in Primary Mitochondrial Disease Patients and their Families

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Abstract:

The diverse roles of mitochondria in energy production, reactive species generation, and cell death are increasingly recognized as significant contributors underlying a wide spectrum of disease pathophysiology. While hijacking mitochondrial pathways to optimize their metabolism and prevent apoptosis can allow neoplasms to flourish, dysfunctional mitochondrial enzymes themselves may contribute to cancer formation or proliferation. Primary mitochondrial disease (PMD) patient cells are often highly glycolytic, analogous to the Warburg effect of cancer metabolism, as a physiologic adaptation to meet their physiologic needs in the face of defective oxidative phosphorylation (oxphos) capacity. Nonetheless, individuals with PMD have not been observed or widely reported to develop cancer. To further investigate the potential role of PMD genetic disorders or carrier status in increasing or mitigating cancer prevalence, we conducted a survey through the Mitochondrial Medicine Frontier Program (MMFP) at Children's Hospital of Philadelphia (CHOP) using a previous database of PMD patients. Results were recorded via REDCap and processed in MATLAB and GraphPad Prism for statistical analyses. Here, we report survey results among 727 individuals from 97 unrelated PMD kindreds, including 100 individuals affected with PMD. Overall, the onset of cancer in this study population was generally reported to occur in the later phases of life, aligning with the national median diagnosis age of 67 years old according to NCI SEER statistics. However, the five genetically confirmed PMD cases with cancer (two with basal cell/squamous cell carcinoma and one each with breast, testicular, or colorectal cancer) had an earlier age of diagnosis (mean 33.67 years). Interestingly, the overall cancer prevalence within PMD families across the entire cohort (including PMD patients, asymptomatic carriers, and non-PMD first- and second-degree relatives) was more than twice the national average after adjusting for any age difference. A statistically significant increased cancer prevalence (Odds ratio [OR] 6.12, $P < 0.0001$) was seen for older individuals (> age 50), regardless of PMD status. Even so, having a genetically confirmed PMD, being an asymptomatic carrier for a pathogenic variant in a PMD disease gene, or being a first or second degree relative of a PMD proband did not increase the likelihood of having cancer; rather, a

non-significant OR of 0.57 was seen, suggesting a trend that PMD may be protective of cancer. Notably among PMD patients, those with *POLG* pathogenic variants that cause mitochondrial depletion syndrome (MDS) had a 9-fold greater likelihood to develop cancer compared to those with any other kind of PMD. Shortcomings in this study include limited cohort size, explicit interpretation required from survey responses, and lack of confirmatory genetic testing for PMD in earlier generations. These findings highlight the need for further multi-site prospective studies of cancer incidence in a larger cohort of PMD patients and family members, as well as careful investigation of cellular mechanisms that may further elucidate mitochondrial dependencies, and therefore therapeutic opportunities, in cancer.

Abstract #: 2025PA-0000000036

Presenter: Elizabeth M. McCormick, MS, LCGC

Title: Expert Panel Curation of Mitochondrial DNA Variants According to the Existing Interpretation Specification Guidelines: Conclusions and Plans for Optimization

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Abstract:

Special consideration is required when classifying the pathogenicity of mitochondrial DNA (mtDNA) variants owing to unique features of this genome, which include its composition and structure, evolutionarily conserved haplogroups and phylogeny, maternal inheritance, heteroplasmy, specific databases and computational algorithms, and unique functional analyses. The Clinical Genome Resource (ClinGen) Mitochondrial Disease Variant Curation Expert Panel (Mito-VCEP) has engaged over 50 international experts for a highly productive effort over 9 years (https://clinicalgenome.org/affiliation/50027). Funded by the National Institutes of Health (NIH) first in 2016 by the National Human Genome Research Institute (NHGRI) via ClinGen to establish the Mito-VCEP, grant funding was obtained in 2017 through the National Institute of Child Health and Human Development (NICHD) U24 grant program to curate variant pathogenicity in association with Leigh syndrome spectrum. This initiative was then co-funded in 2021 by the NICHD and National Institute of Neurological Disorders and Stroke (NINDS) to classify mtDNA variants in the context of Primary

Mitochondrial Disease (PMD). Through this work, we developed and published further specifications and guidance for mtDNA variant classification to the Richards et al. 2015 American College of Medical Genetics (ACMG) and Association of Molecular Pathology (AMP) standards and guidelines, which has now become widely used for clinical interpretation of DNA sequence variants (McCormick et al., 2020). The Mito-VCEP has now completed mtDNA variant expert curation according to the specifications published by this group for prioritized mtDNA variants as follows: (1) "Confirmed" in MITOMAP as of 2022 (n=97); (2) conflicting pathogenicity assertions in ClinVar (n=36); (3) entered as pathogenic (P) in ClinVar (n=103); (4) entered as likely pathogenic (LP) in ClinVar (n=49); and (5) requested by Mito-VCEP members (n=5). Curation and expert panel consensus have been reached to date for all of these 290 mtDNA variants. Among these, 23 variants reached a P classification, including 4 that initially met criteria for LP but were reclassified as P by the Mito-VCEP; 85 reached a classification of LP, including 17 that required Mito-VCEP modification; 172 were classified as VUS, including 1 that required Mito-VCEP modification; 5 were classified as likely benign (LB), including 1 that required Mito-VCEP modification, and 5 were classified as benign. Importantly, the 23 variants that did not reach the appropriate classification upon Mito-VCEP consensus review highlight the limitations to the current ACMG/AMP classification system specified for mtDNA variants. To further optimize mtDNA variant classification and interpretation guidelines, the Mito-VCEP is developing a second specification version which will bolster mtDNA variant classification guidelines around functional studies, *in silico* predictor informatics tools, proband counting, and beyond. In addition, MITOMAP now includes the Mito-VCEP's assessed P and LP variants as "Confirmed," where a total of 130 mtDNA variants now have this categorization.

Abstract #: **2025PA-0000000038**

Presenter: Elizabeth M. McCormick, MS, LCGC

Title: Standardized Assessment of the Relationship Between Select Nuclear Genes and Primary Mitochondrial Disease using the ClinGen Clinical Validity Framework.

Authors: McCormick EM1, Peterson JT1, Taylor JP2, Ertmanska I3, Bluske K3, Clause AR4, Chandrasekhar A3, Lowry J3, Coffey AJ3, Gai X5,6, Falk MJ1,7, Zolkipli-Cunningham Z1,7, Rahman S8

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Abstract:

Primary mitochondrial diseases (PMD) are caused by pathogenic variants in either mitochondrial DNA (mtDNA) or more than 350 genes in nuclear DNA (nDNA). The ClinGen Mitochondrial Diseases Gene Curation Expert Panel (Mito-GCEP, https://clinicalgenome.org/affiliation/40027/), funded since 2017 through the National Institutes of Health (NIH) National Institute of Child Health and Human Development (NICHD) U24-HD093483 grant program (Falk and Gai, Multi-PIs) and re-funded in 2021 jointly by NICHD and the National Institute of Neurological Disorders and Stroke (NINDS), has been a highly productive effort engaging more than 50 international PMD experts. The first 3-year project period focused on systematic expert panel evaluation of the strength of evidence between select nuclear and mtDNA genes and Leigh syndrome spectrum (LSS; McCormick et al., 2023). The current project period has expanded to perform rigorous expert panel curation of all genes with published associations with PMD. After completing curation of the 37 mtDNA genes for their association with PMD (October 2022 – March 2024), nDNA genes were prioritized for curation based on disease mechanism. Although the ClinGen Gene-Disease Validity Curation Process was developed for curation of nuclear genes, there is an opportunity to optimize the process for characteristics of specific classes of disorders. Specific curation approaches for nDNA genes associated with PMD were then established, including defining baseline inclusion criteria for scoring variants in reported cases and a unified approach to scoring variants with varying levels of functional validation published (e.g., biochemical evidence, mtDNA depletion, and founder variants). This new standardized framework allowed for systematic and rigorous review of published literature by biocurators to reach Mito-GCEP consensus on the strength of the relationship between each nDNA gene and PMD. Genes were prioritized for curation based on known mechanisms that include roles in specific mitochondrial processes, including OXPHOS subunits and assembly factors, mtDNA gene expression, mtDNA maintenance, mitochondrial protein import and processing, mitochondrial dynamics, iron-sulfur cluster biogenesis, metabolite transport, mitochondrial toxicity, and cofactor biosynthesis. To date, the Mito-GCEP has completed curation of 184 nDNA genes in association with PMD. Among these curations, 111/184 (60%) reached a “Definitive” classification, 3/184 (2%) reached a “Strong” classification, 33/184 (18%) reached a “Moderate” classification, 33/184 (18%) reached a “Limited” classification, 2/184 (1%) were classified as “Disputed,” and 2/184 (1%) had no known human disease relationship. Overall, this Mito-GCEP curation work has rigorously evaluated the varied strength of relationship of many nDNA genes to PMD based on standardized review of evidence available in published literature. Collaborative international effort to reach consensus on gene-disease relationships for PMD is critical for accurate variant interpretation and confirmation of genetic diagnoses necessary in individual cases, to optimize medication management, tailored multi-system organ screening, accurate recurrence risk counseling and prevention, and clinical trial inclusion.

Abstract #: 2025PA-0000000049

Presenter: Melis Kose

Title: Cockayne Syndrome Human Cell and *C. elegans* Models Exhibit Pronounced Mitochondrial Dysfunction, Oxidative Stress Sensitivity, and Developmental Defects Rescued by Antioxidant Therapies

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Abstract:

Cockayne syndrome (CS) is a rare autosomal recessive genetic disorder caused by pathogenic variants in *ERCC6* (*CSB*), leading to transcription-coupled DNA repair defects and progressive neurodegeneration1. Given the overlap in clinical features with primary mitochondrial disease (PMD), accumulating evidence suggests mitochondrial dysfunction plays a key role in CS pathophysiology2. However, no mitochondrial-targeted therapies are available. Here, we report characterization of mitochondrial physiology in *CSB*$^{-/-}$ human patient fibroblasts and *C. elegans* models and the effects of potential therapeutic interventions.

Patient-derived *CSB*$^{-/-}$ human fibroblast cell lines from two affected siblings carrying a compound heterozygous mutation in *ERCC6* (c.1526+1G>T and c.2800C>A, p.P934T) exhibited significant mitochondrial dysregulation, including altered oxidative phosphorylation (OXPHOS) capacity. Basal respiration and extracellular acidification rates were increased in *CSB*$^{-/-}$ cells compared to controls, while maximal respiration was significantly reduced, indicative of mitochondrial inefficiency. *CSB*$^{-/-}$ fibroblasts displayed a marked reduction in mitochondrial DNA content, retaining only 47% of control levels ($p<0.05$). Patient platelet enzymatic analyses revealed reduced complex I activity and increased citrate synthase levels, suggesting altered mitochondrial biogenesis. Cell survival analysis was performed under metabolic stress conditions in glucose-free media containing galactose (10 mM), glutamine (0.5 mM), and L-buthionine (S, R)-sulfoximine (BSO, 50 µM) to acutely induce oxidative stress by depleting glutathione. *CSB*$^{-/-}$ fibroblasts were highly sensitive to oxidative stress, displaying severe viability loss (10-15% of control mean). Coenzyme Q10 (CoQ10, 50 µM) supplementation partially rescued cell survival, increasing viability by 45% to approximately 50-55% of control level. Taurine treatment (1 mM and 2 mM) effectively rescued *CSB*$^{-/-}$ cell survival under oxidative stress conditions, while N-acetylcysteine (NAC, 5 mM) also provided significant protection from cell death, together highlighting the therapeutic potential of antioxidant strategies in CS.

To explore mitochondrial dysfunction in a whole-organism model, we utilized the *C. elegans ok2335* strain, which carries a 1.6 kb deletion in *csb-1*. c*sb-1-/-* worms exhibited developmental delay and growth deficiency, with 46% shorter worm length at the L4 larval stage than wild-type worms ($p<0.05$). Progeny count was also significantly reduced, indicating a defect in reproductive fitness. Furthermore, mitochondrial DNA (mtDNA) copy number was reduced by 62% ($p<0.05$) and mitochondrial unfolded protein response (UPRmt) induction was increased in c*sb-1-/-* worms, supporting the direct role of mitochondrial dysfunction in CS pathogenesis. While neuromuscular thrashing assays at the L4 stage showed no statistically significant difference between c*sb-1-/- a*nd wild-type worms, it remains possible this would develop with age and/or be influenced by their smaller body size on the microscopy analysis performed.

Collectively, these preclinical modeling findings demonstrate that *CSB* deficiency leads to profound mitochondrial dysfunction across evolutionary distinct species, with mtDNA depletion, impaired oxidative phosphorylation complex I capacity, mitochondrial unfolded protein response stress, developmental delay, and growth deficiency in worms, recapitulating key aspects of human CS phenotypes. The significant rescue effects of CoQ10, NAC, and taurine support the pursuit of rigorous clinical trials to evaluate mitochondrial-targeted therapies for CS.

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Abstract #: 2025PA-0000000054

Presenter: Donna M. Iadarola

Title: Characterizing Intermediary Metabolism in Complex I and Complex IV Deficient Zebrafish Models of Leigh syndrome Spectrum

Authors: Donna M. Iadarola1, Vernon E. Anderson1, Eiko Nakamaru-Ogiso1,2, Marc Yudkoff1, and Marni J. Falk*1,2

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Abstract:

Leigh syndrome spectrum (LSS) are genetically heterogenous disorders that share defective mitochondrial energy generation, where individual gene disorders yield a distinct array of clinical and biochemical phenotypes. To characterize the metabolism of major biochemical classes of LSS, zebrafish models of respiratory chain complex I (CI) deficiency and complex IV (CIV) deficiency were generated with CRISPR/Cas9 technology to generate stable knockout mutants for a subunit of complex I (Ndufs2) and an assembly factor for complex IV (Surf1), respectively. Pharmacologic inhibitor-based LSS models of CI and CIV deficiency using mitochondrial toxins for CI (rotenone) and CIV (azide) were also studied in wild-type (WT, AB) zebrafish to determine if metabolic phenotypes of acute pharmacological respiratory

chain inhibition mimics chronic disease in genetic models. Metabolomic profiling was performed by liquid-liquid extraction of organic acids on flash frozen 7 days post-fertilization (dpf) zebrafish larvae (n=100 larvae/sample), followed by gas chromatography/ mass spectrometry (GC/MS) to quantify levels of intermediary metabolites such as tricarboxylic acid (TCA) cycle intermediates and amino acids relative to an internal standard. *ndufs2* and *surf1* knockout zebrafish were studied relative to AB fish at baseline on 7 dpf, while pharmacological inhibitor-based AB models were studied at baseline relative to exposure to either 50 nM rotenone (at 4, 8, or 24 h) or to 40 μM azide (at 8 or 24 h) for analysis on 7 dpf**.** Distinct metabolic differences were identified between wild-type (WT), CI-deficient, and CIV-deficient zebrafish larvae, where principal component analysis (PCA) effectively separated discrete populations by genetic and/or pharmacologic condition. CI-deficient and CIV-deficient larvae shared a similar profile of increased lactate, butyric acids, branched chain amino acids, and malate. Both genetic and azide-inhibited CIV-deficient larvae models further uniquely had significantly increased levels of succinate and glycine, revealing that CI- and CIV-deficiency can fact be discriminated by GC/MS profiling. Interestingly, increased threonine levels distinguished CI- and CIV-deficient larvae genetic from pharmacologic models. As elevated lactate, butyric acids, and branched chain amino acids are established hallmarks of primary mitochondrial disease (PMD), these GC/MS data demonstrate that zebrafish models reliably reflect the metabolic phenotype of human LSS patients. Statistical analyses such as Spearman's rank correlation were used for biomarker identification, with elevated valine and malate identified to be reliable biomarkers of PMD in all conditions. Ongoing analyses include hierarchical clustering to study acute effects of toxin exposure time and concentration on intermediary metabolism, as well as stable isotopic metabolic flux measurements to further investigate the distinct metabolic adaptations between CI- and CIV-deficient zebrafish larvae. Overall, GC/MS analyses in zebrafish models recapitulate metabolic hallmarks of PMD in major biochemical classes of CI- and CIV-deficiency and provides novel insights that enable biomarker identification. In addition, GC/MS analysis discriminates acute pharmacologic and chronic genetic oxidative phosphorylation inhibition on metabolic profiles of distinct LSS disorders.

Abstract #: 2025PA-0000000059

Presenter: Sonal Sharma, MD **Title:** Unraveling Infantile Epileptic Spasms Syndrome (IESS) in the Context of Primary Mitochondrial Disease **Authors**: Sharma S1-3, Catenaccio E 1,2, Ganetzky R2,3, Goldstein A1-3 **Institution**: *1Division of Neurology, Department of Pediatrics, Children's Hospital of Philadelphia, PA, USA 2Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA, 3Mitochondrial Medicine Frontier Program, Division of Genetics, Children's Hospital of Philadelphia, PA, USA*

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Abstract:

Epilepsy is a common manifestation in primary mitochondrial disease (PMD), with a prevalence of approximately 23% in adults and 40-60% in pediatric cohorts. In this study, we reviewed 17 patients with

genetically confirmed mitochondrial disease who developed infantile epileptic spasms syndrome (IESS). In a cohort of 17 patients, 10 (59%) were male. A few recurrent genetic causes were seen: *MT-ATP6* in 6, *PDHA1* and *NDUFAF8* in 2 patients, with single cases of *FBXL4, MT-ND5, NDUFS8, ATP5O, RARS2, FARS2* and *POLG* variants. PMD was caused by pathogenic variants in nuclear DNA genes in 10 (59%) patients. Age of onset of spasms ranged from 2 months to 2 years, with a median of 7 months. Age of diagnosis of PMD ranged from 2 months to 34 yrs with a median of 14 months. Epileptic spasms were the initial type of seizure in all patients except one, a patient with *PDHA1*-related disease who presented with focal seizures at 5 weeks and developed spasms at 3 months. EEG findings showed hypsarrhythmia in 9 (53%) and modified hypsarrhythmia in 2 (12%). MRI brain around age of spasm onset was normal in 2 (12%) and abnormal in 11 (65%). Findings included bilateral basal ganglia and brainstem T2/FLAIR hyperintensities (typical of Leigh syndrome), agenesis or thinning of corpus callosum, volume loss and ventriculomegaly. Polymicrogyria was noted in 1 patient with *NDUFAF8*- related disease. First medication to treat spasms was ACTH in 9 (53%), oral steroids in 3 (18%) and vigabatrin in 3 (18%) patients. Four (23.5%) patients required one, 2 (12%) required two and 11 (65%) required three or more medications for treatment of spasms. Therapies resulted in resolution of spasms in 9 patients (53%) within 1 to 24 months. Spasms remained uncontrolled in 8 (47%) patients. Nine (53%) patients eventually developed different types of seizures with 3 meeting criteria for Lennox-Gastaut syndrome. All 9 patients who received ACTH responded well except one who developed worsening acidosis. Oral steroids were the second medication of choice in one, Vigabatrin in 3 and Topamax in 2 of these 9 patients as spasms returned after ACTH was weaned. 7 out of these 9 patients eventually had resolution of spasms. Ketogenic diet was used in 5 (29%) including the two *PDHA1* patients. Vigabatrin toxicity was reported in one patient with *MT-ATP6* related disease, this was identified as restricted diffusion in bilateral basal ganglia and brainstem. However, this impression was prior to establishing the genetic diagnosis. IESS can be the presenting seizure type in patients with PMD, highlighting the need for comprehensive metabolic and genetic evaluation in cryptogenic cases. It is possible for PMD to present with epileptic spasms with normal neuroimaging. We did not identify any significant side effects with ACTH, oral steroids or vigabatrin except one patient who developed worsening acidosis. We believe ACTH or oral steroids are a safe treatment option. Majority of patients who experience resolution of spasms go on to develop other types of seizures including generalized, focal, atonic or myoclonic seizures and therefore need ongoing surveillance.

Abstract #: 2025PA-0000000063

Presenter: Cristina Remes

Title: Phenotypic Characterization of 19 ARS2 Disorders in C. Elegans Recapitulates Major Aspects of Human Disease and Demonstrates Therapeutic Benefit of Cognate Amino Acids on Growth, Neuromuscular Activity, Fecundity, and Mitochondrial Physiology

Authors: Cristina Remes1, Neal D. Matthew1, Maia Perrault1, Shannon Schrope1, Eiko Nakamaru-Ogiso1,2, Marni J. Falk1,2

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Abstract:

Defects in mitochondrial translation cause severe impaired respiratory chain deficiencies, variably affecting the brain, liver, skeletal muscle, heart, and other organs. Mitochondrial aminoacyl-tRNA synthetases (mtARS, generally encoded by ARS2 genes) are enzymes that charge mitochondrial tRNAs with their cognate amino acid. Although mtARS share a common biochemical function, patients with mtARS disorders develop variable, severe, neurological dysfunction and multi-system problems. Here, we report characterization of mtARS deficiency in *C. elegans* by (1) using feeding RNAi interference to individually knock down expression of the full set of 19 conserved mtARS genes; and (2) using CRISPR/Cas9 technology to generate 4 stable worms strains harboring patient specific pathogenic variants in: *aars-2*-/-, *dars-2*-/-, *ears*-2-/-, and *vars*-2-/-. In addition, cognate amino acids were modeled as a possible therapy for ARS2 deficiencies. Individual *ars-2* gene expression was knocked down by feeding RNAi clones to wild-type (N2 Bristol) worms for one or two generations. Four CRISPR stable mutant strains were generated by InVivo Biosystems. Worm linear growth was quantified in larval stage L4+1 Day adults. To determine *in vivo* mitochondrial stress (UPRmt) induction, RNAi for each ARS2 gene was performed in a. *C. elegans* transgenic strain carrying both *hsp-6p*::*GFP* and *myo-2p::mCherry* reporters. Worm neuromuscular activity in liquid media was quantified by thrashing assay. *C. elegans* lifespan was analyzed with a semi-automated image acquisition system (WormScan). Worm fecundity was analyzed by progeny count assay. Egg hatch rate and larval development were also studied by microscopy. Steady-state OXPHOS subunit levels were quantified by western immunoblot analysis. Cognate amino acid treatment specifically for each ARS2 gene was performed on solid media from the embryo phase, with analyses performed in stage L4 + 1 Day adult worms.*ars-2* knockdown in *C. elegans* was associated with significant decrease in worm length in all strains, and with variable reduction of neuromuscular activity, variable impact on lifespan, and increased mitochondrial stress relative to wild-type worms. First generation RNAi knockdown of *cars-2, fars-2*, *hars-1*, *kars-1,and mars-2* resulted in a near-complete sterile phenotype in *C. elegans*. The 4 *C. elegans ars-2* stable mutant strains each had significantly decreased worm linear growth and neuromuscular activity in liquid media. A consistent developmental defect was present, with *dars-2*-/- mutant worms showing the most severe developmental delay relative to wild-type worms. Fecundity analysis showed *aars-2-/-* and *dars-2-/-* mutants had significant egg hatching defects, while *aars-2-/-, ears-2-/-* and *vars-2-/-* mutants had significantly reduced numbers of progeny. *aars-2-/-*, *dars-2-/-* and ears-2-/- mutants each had significantly increased mitochondrial stress induction. Treatment of the first- and/or second-generation RNAi knockdown worms with their cognate amino acid significantly increased worm length and neuromuscular activity and reduced mitochondrial stress in a dose-dependent fashion. Additionally, fecundity of *hars-1* and *fars-2* knockdown strains were rescued with 100 µM treatment of histidine and phenylalanine, respectively. Steady-state OXPHOS protein levels in *dars-2* RNAi knockdown worms showed decrease complex I and III levels, which were significantly increased upon treatment with 100 µM aspartate. Similarly, 100 µM aspartate treatment significantly increased worm linear growth and neuromuscular activity and significantly decreased mitochondrial stress in *dars-2-/-* mutant worms. A

comprehensive study of *ars-2* gene inhibition was performed by RNAi knock-down in *C. elegans* of all 19 conserved human ARS2 genes that replicated the major but variable neurologic, survival, growth, and mitochondrial phenotypes of human mtARS deficiencies, with subsequent CRISPR/Cas9 creation of 4 stable genetic lines harboring ARS2 patient mutations. Importantly, cognate amino acids significantly rescued multi-dimensional disease phenotypes in the mtARS deficiency knockdown models and in the *dars-2-/-* stable genetic mutation worms model. These preclinical studies provide compelling evidence that treatment with cognate amino acids could be considered a potential therapy to be studied in rigorous clinical trials for human ARS2 deficiencies.

Abstract #: 2025PA-0000000072

Presenter: Jean Flickinger, PT,PCS

Title: UMDF Study of Digital Gait and Balance Sensors Characterize Fatigue and Enhance MM-COAST Data in Primary Mitochondrial Disease

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Abstract:

We utilized the ZenoTM Walkway Gait Analysis System1 (Zeno walkway) and the mSwayTM inertial sensor2 (mSway) to characterize gait and balance in genetically confirmed and verified subjects with primary mitochondrial disease (PMD) and individuals with self-reported PMD (SR-PMD), compared to healthy volunteers at the 2023 and 2024 United Mitochondrial Disease Foundation (UMDF) Symposium Clinical Research Pavilion. To demonstrate clinical meaning, digital wearables results were correlated to Mitochondrial Myopathy Composite Assessment Tool (MM-COAST) assessments3 previously validated in Mitochondrial Myopathy patients. Subjects completed a six-minute walk test (6MWT) incorporating the Zeno walkway, and MM-COAST static balance assessments of single leg stance with eyes closed (SLEC), and tandem stance with eyes closed (TSEC) and eyes open (TSEO) incorporating mSway sensors. Gait assessments were performed in 51 PMD (mean age ± SD, 28.3 ± 15.4 years, 49.0% female), 14 SR-PMD

(45.2 ± 17.9 years, 92.8% female), and 35 controls (42.3 ± 15.9 years, 65.7% female). Balance assessments were completed in 18 PMD (22.3 ± 10.8 years, 77.8% female), 17 SR-PMD (37.1 ± 16.4 years, 82.4% female), and 14 controls (34.6 ± 10.9 years, 78.6% female). Subjects were asked to hold a stance for as long as possible (maximum duration of 20 seconds) with an mSway sensor secured to their lower back and right lower extremity. Gait characteristics, including stride width (cm), length (cm), velocity (cm/sec), cadence (steps/min), stance percentage (%), single support percentage (SSP%), and single support center of pressure distance percentage1 (SSCOPD%), were significantly different between PMD (n=51) and controls (n=35), $p<0.05$. Longitudinal analysis by linear mixed-effects models (LMMs) for repeated measures revealed significant decrease over 6 minutes (slope) in PMD subjects in stride velocity (-1.99 cm/s/min, $p<0.001$), cadence (-1.29 steps/min2, $p<0.001$), and SSCOPD% (-0.54 %/min, $p<0.001$), that demonstrate worsening fatigue and imbalance from the 1st to 6th minute, compared to controls with no significant change, $p>0.05$. The SR-PMD group also demonstrated significant decline in stride velocity (-1.44 cm/s/min, $p<0.001$), cadence (-0.99 steps/min2, $p<0.001$), and SSCOPD% (-0.3 %/min, $p<0.001$) compared to controls. For PMD, we identified significant correlations between all gait parameters with MM-COAST measures including composite score ([min, max]), r= [-0.68,0.52]) and SLEC z-score (r=[-0.33,0.5]), $p<0.05$. For PMD (n=18), analysis of mSway balance parameters including change in mean sway velocity in the vertical (V), anterior-posterior (AP), and medial-lateral (ML) direction (m/s2) and normalized vertical jerk index4 were observed to be significantly different compared to controls (n=14), $p<0.05$, demonstrating higher and more variable sway while standing tandem stance or on one leg. Longitudinal analysis by LMM revealed a significant decrease over 20s (slope) for TSEO sway (-22.4 mm2/s2, $p<0.001$), demonstrating a larger range of movement in PMD compared to controls, that did not change over time (-7.36 mm2/s2, $p=0.3$). Our results demonstrate the utility of digital assessments in PMD for enhancing MM-COAST clinical assessments through quantification of specific gait impairments that could be measured in future natural history studies and clinical trials.

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Abstract #: 2025PA-0000000076

Presenter: Kaushal Prajapati

Title: Determining Meaningful Change of the Mitochondrial Myopathy-Composite Assessment Tool Informed by a Mitochondrial Myopathy-Specific Global Impression of Change (MM-GIC) Scale

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Abstract:

Our Mitochondrial Myopathy-Specific Global Impression Change (MM-GIC) scale, modified from Duong et al.1, assesses change in the Mitochondrial Myopathy-Composite Assessment Tool (MM-COAST)2. The MM-GIC scale facilitates an *anchor-based approach* to determine the Minimally Clinically Important Difference (MCID) of the MM-COAST. This seven-point global scale integrates the patient/caregiver perspective on i) magnitude of perceived change and ii) whether this change is considered meaningful. Results will be compared to the *distribution-based statistical equation estimates* of MCID. In our MM natural history study (NIAMS 1R01AR083552-01A1), we obtained data from 35 paired patient visits in a subset of MM patients, including MM-GIC and MM-COAST assessments. Subjects rated their progression on a 7-point scale ("very much improved" to "very much worse") alongside whether the change was meaningful compared to the previous visit, with interim periods of 14.7 ± 9.7 months. We collapsed GIC scores into "improved" (scores 1-3), "no change" (score 4) and "worse" (scores 5-7) categories. Caregivers completed 8/35 (23%) MM-GIC assessments. Least squares (LS) mean change in MM-COAST scores were estimated using linear regression. The MM-COAST includes 30-second sit-to-stand (30s STS), assessing exercise intolerance and 9-hole peg test (9HPT), assessing dexterity, used in recent mitochondrial disease trials3,4,5,6, and considered 'gold standard'. Analyses across our natural history MM cohort showed that MM-COAST correlated with both 30sSTSs ($r = -0.56$, $p<0.0001$, $n=97$) and 9HPT ($r = 0.63$, $p<0.0001$, $n=134$). Deming regression further demonstrated narrow confidence intervals (CI) to support 30s STS (slope -0.092; 95% CI: -0.12, -0.07; n=97) and 9HPT (slope 0.027; 95% CI: 0.021, 0.033; n=134) being significantly predictive of MM-COAST scores, confirming MM-COAST clinical relevance. We then compared change in the MM-GIC to corresponding MM-COAST scores across 2 visits in 15 pediatric (13.5 ± 2.6 years; 66.7% male) and 18 adult (40.4 ± 13.5 years; 44.4% male) subjects. Across the MM-GIC

cohort (n=33), mean patient-reported score was 3.44 (SD = 2.5), falling between “little improvement” and “no change”, and reported as meaningful. A significant positive correlation (r = +0.54, p = 0.04, n=14) between MM-GIC scores and change (?) in MM-COAST scores was observed, demonstrating subject perception of worsening symptoms with higher MM-COAST scores that indicate greater disease severity2. Subjects reporting "improved" show no significant change in MM-COAST score (-0.004, p = 0.97, n = 9). However, subjects who reported "no change" and "worse" showed significant change (+0.62, p = 0.012, n=2; +0.90, p = 0.001, n=3) respectively. This preliminary data suggests that a LS mean change of +0.90 (raw score change of +0.5) in MM-COAST scores is considered clinically meaningful. *Distribution-based* MCID estimates were variable depending on the equation, including Standard Error of Mean (SEM = +0.2) and Minimal Detectable Change with 95% CI (MDC95 = +0.57). Further study is needed in a larger MM cohort to identify the MCID of MM-COAST using *anchor-based* MM-GIC. Despite preliminary results showing MM-GIC estimates are concordant with *statistically-derived distribution-based* estimates, there remains a critical need for MCID qualitative interviews in addition to GIC assessment to ensure that MM-COAST MCID estimates truly represent the patient voice.

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Abstract #: 2025PA-0000000078

Presenter: Kelsey Keith

Title: Broad Immunophenotyping Panel with Supervised Immune Cell Classification Reveals Age-Dependent Differences Between Mitochondrial Disease Subjects and Healthy Controls

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Abstract:

Primary mitochondrial diseases (PMDs) are a highly heterogenous group of genetic disorders, with multi-system symptoms ranging in severity and timing of clinical progression. Immune system dysfunction is common in PMD, where patients are often vulnerable to disease progression at the time of viral infections and may not completely respond to vaccination. PMD patients with pathogenic mtDNA variants often show evidence of purifying selection against the variant in blood cells, indicative of mitochondrial dysfunction directly harming immune function. Immune system dysfunction characterization in PMD patients has been limited, with existing work largely limited to case reports or examination of a single cell type in a single PMD disorder.

To systematically characterize the immune system in diverse PMD patients, deep cell immunophenotyping of peripheral blood mononuclear cells (PBMCs) was performed on a spectrally enhanced BD Symphony X50 instrument using a customized 41 parameter flow cytometry panel designed for simultaneous broad typing of lymphocytes and monocytes, with particular focus on T cell activation, memory, and senescence markers. After data acquisition, analysis was performed with an automated computational pipeline, classifying cell types in a hierarchical fashion using multinomial logistic regression to assign each cell as debris or to one of the 8 major cell types. Major cell types were then divided into predetermined phenotypes using relevant markers, ending with 56 immune sub-features, represented as proportions of the major cell populations characterized.

Testing for association between PMD and immune features in pediatric subjects, defined as less than 18 years old (27 cases, 16 controls), and adult subjects (18 cases, 14 controls) were tested separately due to immune system maturation in adults. In the pediatric cohort, 19 of 56 features were significantly different in PMD patients, with differential features associated with more mature or more exhausted T cells (fewer Naïve and CD28+ T cells, more EMRA, CD57+, and KLRG1+ T cells). PMD patients also had more NK cells (CD57+). No significant differences were found in PMD adults, suggestive of age-

dependent immune effects in PMD. Principal component analysis (PCA) also revealed an immune maturity axis, progressing from pediatric controls to pediatric cases, then to all adults irrespective of disease status.

This study delineates a unique immune signature in pediatric PMD, characterized by heightened immune cell maturity and senescence, which may contribute to immune dysregulation and increased susceptibility to infections in affected individuals. Furthermore, the novel 41-parameter immunophenotyping panel and computational methodology established in this work offer broad applicability to human immunology research.

Abstract #: 2025PA-0000000083

Title: A Virtual Registry of 9,300 Primary Mitochondrial Disease Cases Constructed Through Semi-Automated Literature Mining and Expert Curation

Presenter: Xiaowu Gai, PhD

Authors: Lishuang Shen1, Marie T. Lott2, Elizabeth M. McCormick3, Colleen C. Muraresku3, Kierstin Keller2, ClinGen Mitochondrial Diseases Gene Curation Expert Panel, Douglas C. Wallace 2,3,4, Zarazuela Zolkipli-Cunningham2,3,4, Shamima Rahman5, Marni J. Falk2,3,4, Xiaowu Gai1,6

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Abstract:

Introduction: The aggregated minimal prevalence of primary mitochondrial disease (PMD) is 1 in 4,300 people, and 1 in 34,000 for the most common pediatric subtype - Leigh Syndrome Spectrum (LSS). Creation of a PMD disease case registry requires intensive collaborative effort, which is a challenge that has limited the cohort sizes to limited regional or ethnic sources in most *ad-hoc* patient registries worldwide.

Methods: The Mitochondrial Disease Sequence Data Resource (MSeqDR) Consortium has strived to enhance knowledge of PMD by building a comprehensive virtual registry of case-level data from published cases in the literature, using bioinformatics tools that leverage the latest Large Language Models (LLMs). We developed a semi-automated platform (https://mseqdr.org/virtualregistry.php) to capture and standardize case-level data along with metadata extracted from publications. In parallel, the NIH-funded ClinGen Mitochondrial Disease Gene Curation Expert Panel manually curated 594 deeply-

phenotyped LSS cases associated with 113 causative genes from literature. These efforts were further enhanced by 3,600 virtual cases shared by MitoPhen.org.

Results: The MSeqDR Primary Mitochondrial Disease (PMD) Virtual Registry, as of March 2025, aggregates 9,300 de-identified pseudo-cases from over 20 ethnic backgrounds and countries, representing more than 130 distinct PMD diseases. Notable cohorts include 2,190 LSS, 174 mitochondrial encephalomyopathy and lactic acidosis syndrome (MELAS,) 300 chronic progressive external ophthalmoplegia (CPEO), and 1,263 Leber's Hereditary Optic Neuropathy (LHON) cases. To standardize the highly heterogeneous data, 872 clinical and demographic terms were mapped to 99 standardized terms, including Human Phenotype Ontology (HPO) and OMIM diseases. Inferring inheritance mode was possible for nearly 6,000 cases, which revealed a predominance of mitochondrial inheritance (3,699 cases), followed by autosomal recessive (498), autosomal dominant (314), and X-linked (104) inheritance modes.

Key clinical trends were identified upon analysis of this large PMD virtual cohort. 45% of cases manifest within the first 5 years of life, including 26.7% in the 1st year of life. LSS displayed a significantly earlier onset than other PMDs (HP:0003593 infantile onset: 30.1% vs. 5.55%) and higher mortality by childhood (95% vs. 76%). The most common phenotypes included skeletal muscle atrophy (HP:0003202), increased serum lactate (HP:0002151), global developmental delay (HP:0001263), and intellectual disability (HP:0001249), Pathogenicity assessments for 6,100 cases linked clinical features to variants in 638 genes, including 3,349 cases carrying mtDNA variants, expanding the list of potential disease-causing candidates.

The Web-based PMD Case Browser supports Ajax/jQuery empowered Google-style fuzzy searches. Additionally, search by single-feature or multi-feature composite filters offers sophisticated but precise search capabilities across 30+ clinical features. Search results link to individual case report pages, which present case-level data in both standardized and original terms and further allow authorized users to collaboratively contribute crowdsource curations. A private in-house AI server prototype, built on open-source LLM models, can generate private knowledge base and clinical reports, as well as treatment or medicine recommendations, enhancing its utility as a community research resource.

Conclusions: The MSeqDR's PMD Virtual Registry of 9,300 de-identified cases was created to facilitate ClinGen Mitochondrial Disease variant and gene expert curation per ACMG guidelines. The virtual registry platform can also support new virtual registry creation from case-level data, Future works include the expansion of clinical phenotype data standardization into disease and phenotype dictionaries for better interoperability, and the adaptation of AL tools which have shown promise in accelerating heterogeneous clinical data capture, transformation, and reporting.

Abstract #: 2025PA-0000000097

Presenter: Amy Goldstein

Title: Retrospective Natural History Study of *POLG* Disease in a Mitochondrial Medicine Clinical Research Center

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Abstract:

Polymerase gamma (*POLG*) is the most common nuclear gene cause of primary mitochondrial disease. Clinical symptoms may range broadly and affect all ages, with higher morbidity and mortality in younger patients due to refractory epilepsy and fulminant liver failure. Once thought to cause clinically distinct phenotype syndromes, *POLG*-related mitochondrial disease has been reclassified, where age of symptom debut and epilepsy status are recognized as the two sentinel features defining clinical outcome. Here, we report a semi-automated retrospective data query performed using MMFP-Tableau under CHOP IRB 08-6177, with support of a POLG Foundation sponsored research award, of the Children's Hospital of Philadelphia (CHOP) Mitochondrial Medicine Frontier Program (MMFP) *POLG* clinical cohort since 2003, describing the age of onset, debut symptom, genotype, epilepsy status, electrophysiology results, neuroimaging findings, biomarker data, medications, and survival outcomes. Retrospective data query identified 40 CHOP patients with molecularly confirmed *POLG* disease; 95% (38/40) had autosomal recessive disease. 70% (28/40) had epilepsy, of whom 82% (23/28) were under age 12 years at symptom debut and 52% (12/23) are now deceased; of the18% (5/28) of epilepsy patients who had symptom debut between ages 12 and 40 years, none are deceased; all are female. 25% (10/40) autosomal recessive *POLG* patients never had epilepsy, of whom 60% (6/10) had symptom debut under age 12 years (33% (2/6) are now deceased) and 40% (4/10) had symptom debut between ages 12 and 40 years. The remaining 5% (2/40) *POLG* patients had autosomal dominant disease with symptom debut between ages 12 and 40 years and no history of epilepsy. Debut symptoms in epilepsy patients presenting under age 12 years included 2 with febrile status epilepticus, 2 with unprovoked status epilepticus, 10 with global developmental delay, 3 with unprovoked seizures, 4 with refractory focal motor status and 1 with feeding intolerance and hepatopathy. In all 5 female patients who presented with epilepsy between ages 12 and 40 years, the debut symptom was status epilepticus with cortical focal lesions on brain MRI. Among patients without epilepsy, debut symptom in those who presented under age 12 years were motor delay in 67% (4/6) and those who presented between ages 12 and 40 years had neuropathy in 100% (6/6 patients, including 2 with autosomal dominant *POLG*). No single biomarker was diagnostic or predictive of *POLG* disease severity or prognosis. The observed 52% mortality rate in the younger epilepsy cohort was consistent with other reported cohorts. Notably, early mortality (between ages 1.2 and 11 years, median 4.3 years) was 100% in *POLG* premature truncation and missense variant compound heterozygotes (n=5/5), all of whom had epilepsy. The underlying cause leading up to death was variable, including respiratory failure (n=5), status epilepticus (n=2), liver failure (n=2), and sepsis (n=2), although compassionate withdrawal was the most common cause. This single-site retrospective natural history highlights important clinical insights into *POLG* disease, which will guide prospective

natural history study design and serve as a baseline by which to compare future interventional clinical trials designed to reduce the pronounced morbidity and mortality of *POLG* disease.

Abstract #: 2025PA-0000000100

Presenter: Katelynn Stanley

Title: Development and Implementation of a Patient-Driven Patient Reported Outcomes System for Mitochondrial Disease

Authors: Stanley, KD1, MacMullen, LD1, George-Sankoh, I1, Tormey, C1, Goldstein, AC1,2, Ganetzky, R 1,2, Muraresku, C1, Demczko, M1,2, Zolkipli-Cunningham, Z1,2, Falk, MJ1,2

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Abstract:

Primary Mitochondrial Diseases (PMD) are a heterogenous collection of individually rare genetic disorders that present across the lifespan with a wide range of often progressive symptoms and severity. Patient Reported Outcomes (PROs) surveys are useful instruments for patient-centered clinical care, as they help clinicians objectively understand and track specific aspects of the patient experience. At the Mitochondrial Medicine Frontier Program (MMFP) at the Children's Hospital of Philadelphia (CHOP), we have been collecting PROs since 2019 via an electronic capture and analysis system that integrates data on Quality of Life, Fatigue, and Functional Status, which are commonly impaired in many different PMDs, with other clinical care metrics1. One noted limitation in the original PRO system was lack of granularity in capturing the multi-system variety of PMD symptoms. As a result, we have redesigned and expanded the system to include additional PROs in PMD-relevant domains, including Mood, Pain, Gastrointestinal Symptom Burden, Sleep, Headache, Review of Systems, and Caregiver Burden. This new system also utilizes a more patient-centric design that allows patients or caregivers to self-select up to 5 domains that are most relevant to their disease manifestations. By allowing patients to self-select domains of interest, we ensure that longitudinal data collection is capturing symptoms considered most meaningful to patients and be sensitive to change due to relevance of symptoms to perceived disease progression or as a result of therapeutic interventions. The flexible functionality of the PRO system also allows for clinicians to select domains of particular concern for a patient, for example, if they are interested in tracking the impact of sleep disturbance on symptom manifestations or have particular concerns about anxiety or depression. The automated REDCap system in which the surveys are administered automatically flags any extreme results in e.g. depression, anxiety, or stress and alerts the clinical team for immediate follow-up. The PROs are administered clinically to intermittently track symptom progression, identify gaps in patient care, and track outcomes for precision therapeutic trials. Electronic survey administration is scheduled at intervals determined by the clinical team for each patient. Survey results are also analyzed on a research basis for any patients enrolled in CHOP MMFP IRB study #08-

006177 and #16-013364. Survey data are visualized for real-time evaluation using the integrated data system MMFP-Tableau2. We will present data on the first year of implementation of optimized system, including metrics on the most frequently selected domains among CHOP MMFP patients as well as preliminary survey data results. Recognition of how crucial the patient voice and active participation in their care is essential to optimized mitochondrial disease patient management and advance clinical research, allowing researchers to understand natural histories on multiple aspects of mitochondrial disease in a wide variety of individual genetic etiologies and to develop therapies and clinical trials that target outcomes that matter most to patients and their daily quality of life. Longitudinally tracking additional disease-relevant domains and allowing patients to drive the direction of long-term symptom tracking will help researchers and clinicians better understand the unique patient experiences of PMD patients.

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Abstract #: 2025PA-0000000105

Presenter: Daniel E. McGinn

Title: The Utility of Passive Exercise Strategies in Primary Mitochondrial Disease

Authors: McGinn DE1*, Flickinger J1, Sultan L2, Morgan T2, Gendler L2, Rahaman I1, Nguyen S1, Ogiso E1, Morisue J1, Serai S3,4, Nguyen J5, Senthil K6, George-Sankoh I1, Chinwalla A7, Xiao R8,9, Anderson VE1, Ischiropoulos H10, Nadkarni VM6,12, Falk MJ1,12, Adams JA11, Zolkipli-Cunningham Z1,12

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Abstract:

Primary mitochondrial disease (PMD) affects high-energy-demanding organs, leading to exercise intolerance, fatigue, and muscle weakness1, often resulting in a sedentary lifestyle. Periodic acceleration (pGz) passive exercises, involving head-to-footward motion of a supine body using a specialized bed (pGz-BED) and passive jogging using a specialized rhythmic-device (Gentle Jogger [GJ]), have demonstrated cardiovascular benefits in other patient populations by inducing pulsatile shear stress, which upregulates endothelial nitric oxide synthase and nitric oxide2,3. This study evaluates the feasibility and physiological impact of pGz-BED and GJ passive exercise strategies compared to conventional active cardiopulmonary exercise testing (CPET) in PMD. Older children and adults (10-60 years) with PMD and healthy controls completed three separate exercise interventions: (i) conventional CPET, (ii) pGz-BED passive exercise, and (iii) GJ passive exercise. Participants avoided strenuous activity 48 hours prior to each study visit. The primary outcome was maximal oxygen consumption (VO_2max), a key measure of mitochondrial aerobic capacity, assessed before and after intervention. Secondary outcomes included the pulse waveform a/b ratio (a biomarker of nitric oxide-mediated vasodilation4), contrast-enhanced ultrasound (CEUS) imaging for muscular flow assessment, and serum lactate/pyruvate, glutathione, ketone bodies panel, and nitric oxide metabolites. Outcome measures were compared between the intervention types and between PMD and healthy participants. 18 subjects have enrolled to date, including 8 PMD and 10 control subjects. Following conventional exercise, VO_2max increased as expected in both PMD (change (□),17.9 ± 6.55, n=2) and control (26.2 ± 4.81, n=3) subjects. However, following pGz-exercise (pGz-BED and GJ), VO_2 levels showed only a modest increase in PMD (0.17 ± 0.11, n=5) subjects and did not increase for controls (0.06 ± 0.05, n=4). An increase in the a/b ratio was observed in PMD subjects after both active (2.4 ± 1.4) and pGz-exercise (0.39 ± 0.23). Notably, following pGz-exercise, a/b ratio was higher in PMD subjects compared to controls (0.14 ± 0.10) trending towards significance (p=0.08). CEUS imaging demonstrated a significant increase in perfusion index (PI) in PMD subjects (7.35 ± 1.1, n=3) compared to controls (0.02 ± 0.37, n=2) following GJ intervention (p=0.03). PMD and control subjects had similar increase in PI following active exercise. Metabolic markers revealed no change in lactate levels following passive exercise, whereas plasma lactate markedly increased [~5 fold] following CPET for all subjects. However, the Beta-Hydroxybutyrate(BHB)/Acetoacetate(AcAc) ratio, a measure of NADH/NAD^+ redox balance, was elevated (2.94, normal 0.48-2.56) in PMD subjects following pGz-exercise compared to controls (2.49), suggesting altered mitochondrial redox status. PMD and healthy controls had similarly elevated BHB/AcAc ratios following active exercise. Preliminary findings suggest that pGz-exercise induces a vasodilatory response in PMD patients, as evidenced by increased a/b ratio and PI on CEUS imaging, which may be linked to underlying systemic endothelial dysfunction not previously recognized in PMD. Furthermore, results demonstrate PMD patients had similar, though less pronounced, physiologic responses to pGz-exercise compared to active exercise. These findings highlight passive exercise as a feasible intervention to

quantify dysfunction in PMD. It may also merit further investigation into its long-term cardiovascular and metabolic benefits as a treatment intervention in PMD.

References:

We will be conducting Gentle Jogger assessments in the Clinical Research Pavilion at the 2025 annual conference.

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Abstract #: 2025PA-0000000107

Presenter: Daniel E. McGinn

Title: Next-Generation Sequencing Genomic Data Analysis, Utility, and Diagnostic Rate for a Patient Advocacy Group-Led, No-Cost, Genetic Testing Program for Primary Mitochondrial Diseases

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Abstract:

Primary mitochondrial diseases (PMD) are caused by pathogenic variants in more than 400 genes encoded by both nuclear (nDNA) and mitochondrial DNA (mtDNA) genomes. Still, many individuals with

medical concerns for suspected PMD lack a confirmed genetic etiology, often without prior comprehensive genetic diagnostic testing. The lack of a confirmed genetic etiology is a significant hindrance, decreasing treatment and research opportunities. To address this, the United Mitochondrial Disease Foundation (UMDF), in collaboration with Medical Neurogenetics (MNG) Laboratories, launched a no-cost pilot genetic testing program in 2022. The aim was to aid clinicians in obtaining genetic diagnoses for patients with suspected PMD, where participating clinicians could order next-generation sequencing (NGS) testing of 320 PMD nDNA genes on an exome backbone and the mtDNA genome (Comprehensive Cellular Energetics Defects, NGS301). For patient privacy, the program-wide diagnostic yield was not disclosed by MNG Laboratories, requiring secondary dataset reanalysis. Proband-only NGS sequencing, raw genomic sequencing data quality control, and variant calling were conducted by MNG Laboratories for 344 participants without prior genetic testing. Genetic diagnostic testing reports were shared with ordering providers by MNG Laboratories, limiting understanding of the overall impact of the program. To facilitate cohort analysis, de-identified Variant Call Format (VCF) files were securely transferred from MNG Laboratories to UMDF. De-identified VCF files were then transferred to the Children's Hospital of Philadelphia's Mitochondrial Medicine Frontier Program (MMFP) research group for systematic reanalysis using the Mitochondrial Disease Sequence Data Resource Quick-Mitome Web resource1, followed by MMFP Genetic Counselor expert review. A genetic diagnosis was made in 34 individuals, as evidenced by pathogenic and/or likely pathogenic variants found in genes associated with their phenotype. A mtDNA etiology was found in 26 individuals, including 13 with m.3243A>G, 5 with m.11778G>A, 4 with m.8344A>G, and 4 with other mtDNA variants. Five had pathogenic variants confirming an autosomal recessive (AR) condition (2 *SURF1*-related PMD, 1 *POLG*-related PMD, 1 *PYGM*-related Glycogen Storage Disorder V, and 1 *SLC22A5*-related Carnitine Deficiency). One had an X-linked condition (*PHKA1*-related Glycogen Storage Disorder IX). Two had autosomal dominant conditions (*OPA1*-related Optic Atrophy, *CPT2*-related Carnitine Deficiency). Ten individuals had compelling variants but lacked information, such as segregation, age and tissue type tested, necessary to confirm whether these variants were causal of symptoms. 19 individuals had one pathogenic variant for an AR disease gene, interpreted as being asymptomatic carriers. An additional 202 participants had variant(s) of uncertain significance, which did not appear to be associated with their reported phenotype. Significant barriers to accessing genetic diagnostic testing exist in individuals with clinical features highly concerning for PMD. Secondary data analysis confirmed at least a 10% diagnostic yield of this industry sponsored, no-cost pilot genetic testing program, demonstrating the utility of providing patients and clinicians with direct access to cutting-edge NGS genetic testing. Confirming a precise genetic etiology is critical for optimizing medication management, tailoring multi-system organ screening, providing accurate recurrence risk counseling and prevention, and enabling clinical trial inclusion for PMD. Data from this pilot program will inform future UMDF strategies for genetic diagnostic testing initiatives.

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Abstract #: 2025PA-0000000112

Presenter: Elizabeth McCormick

Title: Facilitating Community-Based Genomic Data Analysis in Primary Mitochondrial Disease: mitoSHARE Patient Registry and Mitochondrial Disease Sequence Data Resource (MSeqDR) Collaboration to Accelerate Genomic Data Discoveries

Authors: Bogush E.1, Wilson N.2, McCormick E.M.1, Qunell E.1, Yeske P.2, Falk M.J.1,3

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Abstract:

Over 400 genes from both nuclear and mitochondrial genomes have been associated to primary mitochondrial disease (PMD), yet many individuals with a clinical presentation highly concerning for PMD do not have a confirmed genetic etiology. While clinical diagnostic genomic testing for PMD has become widely utilized, genomic data analysis is often restricted to clinical diagnostic laboratories, which typically report only variants in known disease-related genes. This presents an opportunity for novel gene discovery and/or more complex genomic analyses of existing genomic data from PMD subjects. We describe here a collaborative, community-driven approach to facilitate such analyses, involving the United Mitochondrial Disease Foundation (UMDF) worldwide patient registry, mitoSHARE, and the Mitochondrial Disease Sequence Data Resource (MSeqDR) Consortium. Participants enrolled in mitoSHARE are encouraged to share their diagnostic status during enrollment, enabling the identification of individuals who have undergone genetic testing. If a participant indicates interest in sharing their genomic data, they receive information on how to participate in the MSeqDR genomic data repository study, which is approved by the Children's Hospital of Philadelphia (CHOP) Institutional Review Board (IRB). Once consented and enrolled, participants' genomic data is transferred from clinical diagnostic testing laboratories to a secure cloud-based server. This data may include exome, genome, and RNA-Seq transcriptome data to allow for a more extensive data analysis. Participants are assigned a global universal identifier (GUID), which they can then share with a medical or scientific professional of their choice to serve as a proxy. The proxy can then access the data through a secure, web-based user-friendly data query platform called Genesis, allowing for proxy's direct analysis of the full dataset without needing bioinformatics expertise. 488 mitoSHARE participants have reported previous genetic testing, however, only 333 of these participants have indicated having a genetic diagnosis. To date, 83 participants have completed the informed consent process, and 25 genomic datasets have been transferred. The GUID-encoded datasets are made accessible to proxies within Genesis. Video tutorials created by the MSeqDR study team are available to

proxies to learn how to self-navigate Genesis' platform. Enrollment and genomic data collection continue, with opportunities for both those with known and unknown genetic causes of PMD to participate in research. This collaborative effort between mitoSHARE and MSeqDR offers PMD patients a chance to engage in clinical research, contributing to a more robust genomic analysis pipeline for mitochondrial disease research. Importantly, this project allows individuals and families with PMD, whether diagnosed or undiagnosed, to choose specific medical professionals to access and meaningfully analyze their complex genomic data.

Abstract #: 2025PA-0000000113

Presenter: Leonard Burg, PhD

Title: Characterization of a Zebrafish *surf1-/-* Model of Leigh Syndrome.

Authors: Burg L1, O'Hara T1, Haroon S1, Reesey Gretzmacher E1, Magnitsky S2, Seiler C3, Nakamaru-Ogiso E1, Falk MJ1,4

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Abstract:

Introduction: Mitochondrial respiratory chain defects caused by pathogenic variants in both nuclear and mitochondrial DNA genes often result in impaired exercise capacity and reduced oxygen utilization by muscles. Zebrafish (*Danio rerio*) offer an increasingly robust translational model to investigate human mitochondrial respiratory chain disease pathophysiology, owing to an array of tractable forward and reverse genetic approaches and their relative ease of use for large-scale pharmacologic treatment screens. We have used CRISRP/Cas9 technology to generate genetic knockout zebrafish with *surf1-/-* deficiency as a model of Leigh Syndrome spectrum1 (LSS), which have reduced whole body oxygen consumption and swimming speed as adults. Development of zebrafish MRI methods further identified growth abnormalities in these animals and their organs over time2. Here, we describe ongoing investigations in this model including (1) brain cell death mechanistic studies to understand the pathophysiology of metabolic stroke in LSS, (2) high-throughput drug library screening efforts to identify therapeutics that will rescue the neurologic manifestations of SURF1 disease, and (3) novel live-animal metabolic imaging techniques we have developed to advance understanding of organ-specific metabolic adaptations that occur in LSS disorders.

Methods: (1) Using our *surf1-/-* zebrafish model, we have evaluated whether specific cell death pathway inhibitors prevent brain cell death upon exposure to low-dose sodium azide in this stressor-hypersensitive LSS model. (2) We are pursuing a high-throughput screen to identify candidate therapies from a 2,600 FDA approved drug compound and natural products library in a larval *surf1-/-* zebrafish

swimming-based neuromuscular activity assay. (3) We have developed a novel live-animal imaging protocol to analyze adult zebrafish by PET/CT to assess their *in vivo* glycolytic activity, as well as a protocol for MRI/NMR to evaluate multiple metabolites in distinct organs of adult *surf1-/-* and wild-type zebrafish.

Results: Interestingly, pharmacologic inhibitors of 5 major cell death pathways in *surf1-/-* zebrafish larvae exposed to low dose sodium azide exposure have failed to prevent acute brain cell death. Reduced swimming activity at 7 days post fertilization in *surf1-/-* zebrafish larvae exposed to low-dose azide can be prevented by pretreatment with *N*-acetylcysteine (NAC), which is being used as a positive control to complete a high-throughput screen to identify additional therapeutic leads that improve health and survival in SURF1 disease. A novel protocol has been effectively established to perform PET/CT scans using 18-FDG tracer in living adult zebrafish, which we have applied to identify *surf1-/-* adult zebrafish having increased uptake of 18-FDG in the brain relative to wild-type animals, suggestive of 2-fold higher glycolytic activity in *surf1-/-* disease. A novel MRI/ NMR protocol has been developed in live adult zebrafish, with ongoing work to characterize their organ-specific metabolic adaptations at baseline or upon candidate treatments in living s *surf1-/- adult zebrafish*.

Conclusion: *surf1-/-* zebrafish offer an important animal model that faithfully replicates key aspects, and thereby facilitates the preclinical translational research study, of human mitochondrial respiratory chain disease including neurologic acute metabolic stroke and exercise intolerance manifestations commonly seen in LSS. **References:** 1Haroon et al, 2023 PMID: 36795052. 2Sharm et al, 2024 PMID: 37603286

Abstract #: 2025PA-0000000122

Presenter: Marni Falk

Title: A Retrospective Study to Characterize the Natural History of Cardiac Findings in CHOP Mitochondrial Medicine Patients with Primary Mitochondrial Disease

Authors: MacMullen, LE1*, Weis, M, George-Sankoh, I1, Reesey Gretzmacher, E1, Anderson, VE1, Xiao, R2, Owens, A3, Banerjee, A4, Campbell, J5, Carr, J5, Zolkipli-Cunningham, Z1,6, Goldstein, AC1,6, Falk, MJ1,6

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Abstract:

Primary mitochondrial diseases (PMD) are inherited genetic disorders characterized by impaired mitochondrial oxidative phosphorylation , leading to various end organ manifestations that may include the heart, which can lead to heart failure. The aim of this retrospective study was to investigate the prevalence of cardiac manifestations in PMD patients seen in the Children's Hospital of Philadelphia Mitochondrial Medicine Frontier Program (CHOP MMFP), and define the natural history of cardiomyopathy, heart failure, and/or cardiogenic shock in PMD patients by semi-automated analysis of historical medical and research records. This collaborative research was performed with iterative data queries and analyses with Stealth BioTherapeutics (SBT) and CHOP/Penn multi-disciplinary team members. The dataset included retrospective clinical information pulled for research purposes for human subjects enrolled under research protocol #08-01677, approved by the CHOP Institutional Review Board. Cohort selection and data extraction were completed using MMFP-Tableau, an integrated data system that extracts patient data from the back end of the Electronic Medical Record (EMR, EPIC) system, integrates it with research data sets, cleans and processes data (Alteryx) to prepare it for visualization (Tableau) and analysis1. Subjects identified from 3 iterative data queries resulted in a total list of n = 243 genetically confirmed PMD subjects having either confirmed, suspected, or at risk for cardiac involvement. This list was then manually vetted via individual chart review by clinicians with expertise in PMD-related cardiac involvement, who classified each subject as having (1) PMD-related cardiac involvement (n = 67), (2) Non PMD-related cardiac involvement (n = 20), or (3) no cardiac involvement (n = 156). The final cohort of 67 subjects with definitive PMD-related cardiac involvement was used for all subsequent analyses. Of these 67 individuals, n = 36 were female (54%). A total of 41, subjects had a pathogenic variant or deletion in mitochondrial DNA (mtDNA, 62%), and 26 had pathogenic variants in a nuclear DNA gene (nDNA, 38%). A total of 20 subjects from the PMD-related cardiac involvement cohort are deceased (30%). Age of death ranges from 20 days – 65 years (mean = 17.76 years, median = 16 years). The most common genetic etiology among the deceased cohort was SLSMDS (40%). Characterization and categorization of cardiac manifestations in these 67 individuals were manually curated by a team of expert cardiologists and mitochondrial medicine clinicians. Each subject's medical history was reviewed for (1) presence or history of a cardiomyopathy, (2) presence or history of arrhythmia, and (3) presence or history of a conduction abnormality. Cardiac phenotypes were defined by the presence of these abnormalities, and each abnormality was sub-characterized by type. Data will be presented on the prevalence of each cardiac manifestation by age, genetic etiology, phenotypic presentation, and survival status, as well as longitudinal data characterizing the progression of cardiac presentations. Collectively, this extensive characterization of a large, heterogeneous PMD cohort highlights the prevalence and nature of serious cardiac findings in PMD subjects, which will be useful to inform design of future cardiac-focused therapeutic intervention studies.

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Abstract #: 2025PA-0000000129

Presenter: Suraiya Haroon

Title: Therapy Development for OPA1 Disease Using Worm Models, With Validation in Zebrafish and Patient Fibroblast Models

Authors: O'Hara TG1, Lu A1, Tara Z1, Haus E1, Campbell C1, Wei S1, Mendel R1, Mathew N1, Keith K2 Seiler C3, Chen S4, Nakamaru-Ogiso E1, Falk MJ1,5, and Haroon S1,5

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Abstract:

Background: Pathogenic *OPA1* variants lead to progressive vision loss, with 20% of OPA1+/- patients developing additional symptoms such as motor and/or sensory neuropathy, ataxia, myopathy, and sensorineural hearing loss. OPA1 is a mitochondrial GTPase, where pathogenic OPA1 variants induce fragmented mitochondria, mitochondrial dysfunction, and mtDNA depletion that lead to bioenergetic dysfunction and subsequent mitochondrial degradation via mitophagy. One approach to prevent the continual cycling of mitochondrial degradation and biogenesis is to modulate mitophagy, which we postulate may reduce ATP consumption, preserve mitochondrial mass, and stabilize mtDNA content. Our long-term goal is to utilize mitophagy modulator screening approaches to develop OPA1+/- disease therapies. Here, we report the development of a humanized worm model for OPA1+/- disease in which to screen candidate therapies, with lead candidates then validated across 3 evolutionarily distinct species, including 2 worms, a zebrafish, and various patient cell line models.

Methods: Using CRISPR/Cas9, we generated the R289Q mutant worm strain of *eat-3*, which is orthologous to the pathogenic R345Q (previously R290Q) OPA1 variant and obtained a V328I missense mutant *eat-3* strain. Both mutant worm strains showed defects in mtDNA content, mitochondrial respiration, fecundity, animal development, and neuromuscular function. Substantially increased mitochondrial unfolded protein stress response (UPRmt) induction in R289Q worms was used to screen (i) potential therapies identified for complex I disease worm models, (ii) 62 mitophagy modulating drugs, and (iii) a library of 2,560 FDA-approved and natural product compounds.

Results: Using the UPRmt fluorescence screen, Thiamine, Lipoic Acid, Celastrol and Hemin were identified from the first two compound sets, and the library screen identified 16 compounds as potential therapeutic candidates. Among these, Thiamine, Celastrol, and Bromindione reproducibly reduced UPRmt, increased neuromuscular activity, and improved development in the OPA1 mutant (*eat-3*) worm strains. *OPA1-/-* zebrafish larvae have been found to have quantified impaired visual function by optokinetic assay, which will be used to screen candidate therapies identified in the worm models on

visual function. In *OPA1-/-* R345Q patient fibroblast cells, Thiamine, Celastrol, and Bromindione rescued cell death (CellTox) to healthy patient cell levels. Additional validation studies are underway on multiple fitness outcomes in *eat-3* mutant worms and in human fibroblast cell lines (R345Q+/- and I458T+/-) on cell death (CellTox) and mitochondrial physiology. We have also derived R345Q+/- induced-Pluripotent Stem Cells (iPSC) from *OPA1-/-* patient peripheral blood mononuclear cells and are using CRISPR-Cas9 technology to generate the revertant wild-type cells. iPSC-derived retinal ganglion cells (RGC) are being generated to better characterize *OPA1-/-* disease and validate lead therapies. Work is ongoing to evaluate whether Red Light Therapy shows preclinical efficacy in these translational models of *OPA1-/-* disease.

Conclusion: Overall, we describe several novel OPA1 disease models across multiple species that manifest key aspects of human disease. Candidate therapy screens have identified 20+ possible therapeutic leads, with validation studies showing preclinical efficacy of Thiamine, Celastrol, and Bromindione on multiple outcomes in OPA1 worm models and a human patient fibroblast cell line.

Abstract #: **2025PA-0000000133**

Presenter: Suraiya Haroon

Title: Identification of FDA-Approved Compounds That Rescue Mitochondrial Stress in a Heteroplasmic Single Large-Scale mtDNA Deletion (SLSMD) C. Elegans Animal Model

Authors: Mendel R1, Cheng Y1, Tara Z1, Schrope S1, Keith K3, Matsuno S1, O'Hara TO1, Mathew N1, Nakamaru-Ogiso E1, Falk MJ1,2, and Haroon S1,2

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Abstract:

Introduction: Kearns-Sayre syndrome (KSS) and Pearson Syndrome (PS) are primary mitochondrial diseases caused by single large-scale mitochondrial DNA (mtDNA) deletions (SLSMD). SLSMD syndromes, as with most mitochondrial diseases, have no effective treatments or FDA-approved therapies. One major roadblock to therapeutic development is the lack of tools to genetically engineer models harboring mtDNA deletions that are stable and transgenerational. As such, studying naturally occurring animal models helps to advance understanding of disease progression and enable therapeutic development. Specifically, the *uaDf5 C. elegans* model harbors a heteroplasmic 3.1 kilobase mtDNA deletion of 11 genes that encode 7 MT-tRNAs and 4 mitochondrial proteins.

Methods: Here, we use a mitochondrial stress phenotype in the *uaDf5* animals to screen for potential therapeutic leads from two libraries, consisting of 62 mitophagy modulators and 2,560 FDA-approved and natural compounds. To conduct the screen, *uaDf5* animals were crossed with the *myo2::mcherry* reporter, a red fluorescent pharyngeal bulb marker for automated animal count, and an *hsp6p::GFP*

reporter that fluoresces green upon mitochondrial stress induction. These triple transgenic animals were used to screen potential therapies using the CX5 high content imager (Thermo Fisher). We use mtDNA heteroplasmy analysis (qPCR), mitochondrial fitness (mitochondrial stress reporter), fecundity (hatch rate and progeny count), and organismal fitness (development) in worms, and cell survival (Cell Tox) in patient fibroblast cells to assess disease progression and therapeutic treatment efficacy.

Results: Two drugs, Thiamine and Lipoic Acid, were identified as positive controls for the library screen based on their ability to rescue mitochondrial stress in *uaDf5* animals. Thiamine reduced mtDNA heteroplasmy in *uaDf5* animals and increased fitness their fitness. Thimaine also increase survival in patient cells. Hemin and Celastrol were identified in the mitophagy modulator screen to rescue mitochondrial stress in *uaDf5* mutant animals. Furthermore, Celastrol reduced mtDNA heteroplasmy and improved organismal fitness in *uaDf5* animals. Celastrol also improved patient cell survival. Finally, multiple repeat experiments have validated 4 new potential therapeutic candidates that were identified from a screen of 2,560 FDA-approved and natural compounds in the *uaDf5* animals.

Conclusions: The *uaDf5* worm model of SLSMD Syndromes enable mechanistic and therapeutic modeling insights, as well as high throughput screening of drug libraries to identify novel therapeutic leads for SLSMD diseases. Therapies identified to ameliorate disease in *uaDf5* worm model can also rescue patient cell death, showing remarkable cross-species conservation of therapy efficacy. We are currently developing patient-derived neuronal cells and zebrafish as additional models for better understanding of disease progression in SLSMD Syndromes and validating therapies identified in the *uaDf5* worms.

Abstract #: 2025PA-0000000134

Presenter: Jean Flickinger

Title: Mitochondrial Mobility Performance Levels (IMPROVE) to Facilitate Enrollment of a Homogenous Clinical Trial Cohort

Authors: Flickinger, J.1,2, Rahaman, I.1, Prajapati, K.1, Santos, J.D.1, Ly, A.1, Martin, I1, Ballance, E.1,2, O'Leary, S. 1,2, Fogliano, J.1,2, Peterson, J.1, Stanley, K.1, MacMullen, L.1, Hill, D3., Chinwalla, A3., Victoria Lazariu4, Xiao, R.5,6, Zolkipli-Cunningham, Z.1,5*

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Abstract:

A major challenge to studying Mitochondrial Myopathy (MM) cohorts is the broad genetic and clinical heterogeneity1-2. We developed the Mitochondrial Mobility Performance Levels (IMPROVE) that stratifies MM subjects by mobility levels to discriminate by clinical phenotype in order to attenuate heterogeneity and thus facilitate targeted enrollment for future clinical trials. We enrolled 131 pediatric (mean age ± SD, 11.5 ± 3.2 years, 44.8% males) and adult (mean age ± SD, 36.9 ± 13.32 years, 26.6% males) MM subjects who completed MM-COAST at their baseline clinic visit as part of a MM natural history study (NIAMS 1R01AR083552-01A1). Cohort MM-COAST informed development of clinically relevant IMPROVE levels. Given the broad variability in cohort MM-COAST scores with outliers, we considered subjects with MM-COAST scores between the 25th and 75th percentiles as the majority (63/121,52%) of the ambulatory cohort scores were within this range. We designed the IMPROVE classification based on subjects with scores within this range (Levels 3-5, ambulant with severe, moderate and mild fatigue, respectively) and outside of the range (Level 2, non-ambulatory and Level 6 ambulant without fatigue). Level 1 subjects (n=22) were unable to complete MM-COAST assessments due to severe phenotype therefore were not included in analyses; however, this level was incorporated into IMPROVE to be fully inclusive of MM disease spectrum. Analysis of baseline MM subject MM-COAST scores identified a composite score of 0.43 (25th percentile) and 1.80 (75th percentile), while mean ± SEM MM-COAST scores in healthy volunteers was -0.47 ± 0.27, n=65, with total possible scores from -1.0 to +3.0 and higher composite scores indicating greater disease severity3. Level 2 (non-ambulatory subjects requiring physical assistance) have corresponding higher mean composite scores (2.47 ± 0.40, n=11) while milder Level 6 subjects (community-level ambulators without fatigue) have lower mean composite scores (0.03 ± 0.58, n=7) with both levels falling well outside the 25th- 75th percentile ranges. Ambulatory MM subjects were classified as Levels 3-5 with mean composite score ± SEM for Level 3 (1.53 ± 0.65, n=25), Level 4 (1.19 ± 0.80, n=50), and Level 5 (0.70 ± 0.72, n=38), within the 25th-75th percentile range. Thus, each IMPROVE level successfully defined distinct subsets of mobility phenotypes that aligned with MM-COAST scores. The goal of the IMPROVE is to attenuate heterogeneity and therefore reduce variability. For example, the standard deviation (SD) for six-minute walk test (6MWT) in Level 3 individuals was 297.8 ± 128.3 (n=17), mean meters ± SD. Combined Levels 4-5 was 443.6 ± 99.1 (n=82), compared to the total MM cohort of 425.0 ± 129.1, n=108,($p < 0.01$, Kruskal-Wallis), confirming decreased 6MWT variability in the Level 4/5 cohort. Additionally, longitudinal MM-COAST analysis revealed distinct disease trajectories. Level 3 subjects with severe fatigue revealed a faster rate of change in MM-COAST scores of 0.13/year ($p<0.01$, n=17) while Level 4 and 5 subjects revealed slower rates of change (0.04/year, $p=0.02$, n=50), supporting implementation of IMPROVE levels for targeted enrollment. We propose the IMPROVE classification to enhance patient enrollment strategies for future clinical trials by diminishing broad heterogeneity in MM clinical phenotype.

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Abstract #: 2025PA-0000000136

Presenter: Elizabeth M. McCormick, MS, LCGC

Title: Expanding Reproductive Options for mtDNA Disorders in the United States

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Abstract:

Primary mitochondrial disorders (PMD), caused by pathogenic variants in genes located in nuclear (nDNA) or mitochondrial DNA (mtDNA) genomes, are often progressive, have limited treatment options, and may be present early in life. Reproductive options for families wishing to have a genetic relationship to their child depend on the molecular etiology of PMD in their family. Those with an nDNA disorder have the same options as other single gene disorders, namely preimplantation genetic testing for monogenic disorders (PGT-M), in the setting of in vitro fertilization (IVF). In contrast, PGT for mtDNA disorders is complicated by unique biologic features of mtDNA however has now been successfully performed by several centers globally. Despite these advances, PGT for mtDNA disorders is not offered in the United States (US) due to inexperience with specialized techniques for accurately assessing mtDNA heteroplasmy in embryo

biopsies. To address this gap in options for US families with mtDNA disorders and the cost of traveling overseas, we, by collaboration between the Mitochondrial Medicine Frontier Program at the Children's Hospital of Philadelphia (CHOP), Penn Fertility Care at the Hospital of the University of Pennsylvania (Penn), and the Newcastle Rare Mitochondrial Disorders Service (Newcastle, UK), established a process to facilitate PGT for mtDNA disorders for families living in the US. The PGT for mtDNA disorders process

for US patients includes (1) patient and family counseling, clinical evaluation, and necessary specialist referrals at CHOP; (2) baseline fertility evaluation, IVF cycle planning and monitoring, oocyte retrieval, fertilization, and day 3 single cell biopsy at Penn, followed by biopsy shipment from Penn to Newcastle for mtDNA PGT; (3) fitness for fertility assessment, blastomere heteroplasmy analysis, and telehealth consultation for embryo biopsy heteroplasmy analysis and discussion of PGT outcomes by the Newcastle team; (4) selected embryo transfer based on embryo biopsy mtDNA heteroplasmy analysis at Penn; with (5) follow-up of resultant children born after successful life birth at CHOP. A female patient aged 29 years with m.3243A>G (5-11% heteroplasmy in blood, buccal, and urine) pursued IVF and PGT for the m.3243A>G variant at Penn Fertility Care, with consultation at CHOP, IVF cycle planning and care at Penn, and telehealth consultation and samples sent to Newcastle for mtDNA variant heteroplasmy determination. One IVF cycle yielding 13 oocytes and biopsy of 7 embryos was completed. Embryos had low to undetectable heteroplasmy, and one with undetectable heteroplasmy was transferred resulting in a successful live birth. Post-natal follow-up at age 20 months has confirmed the female child's normal health and development, with post-natal heteroplasmy level testing actively being coordinated. Families with mtDNA disorders living in the US have had limited family planning options given the unique nature of mtDNA variants, despite the potentially devastating nature of these conditions. Families carrying a known pathogenic mtDNA disorder with a desire for a genetic relationship to their child can now remain in the US while undergoing IVF and PGT for mtDNA disorders. Formal establishment of this process was completed to help other individuals with mtDNA disorders achieve their family planning goals.

Abstract #: 2025PA-0000000137

Presenter: Jean Flickinger

Title: Mitochondrial Myopathy Composite Assessment Tool is a Relevant Outcome Measure in Leigh Syndrome Spectrum and Other Primary Mitochondrial Disorders

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Abstract:

The Mitochondrial Myopathy Composite Assessment Tool (MM-COAST) was developed and validated as an objective outcome measure to quantify key symptoms of Mitochondrial Myopathy (MM)1 including muscle weakness, muscle fatigue, exercise intolerance, imbalance and poor dexterity. In addition to MM, we proceeded to administer MM-COAST assessments to individuals with Leigh syndrome spectrum (LSS) and other phenotypes to show its broader relevance, as imbalance and impaired dexterity are common in LSS2. As part of a MM natural history study funded by NIAMS (1R01AR083552-01A1), we assessed 131 PMD subjects aged ≥ 5 years, including MM subjects (n=100), LSS (n=20), and 'Other Primary Mitochondrial disease (PMD) phenotypes', including *OPA1*-related PMD and Leber's Hereditary Optic Neuropathy (LHON, n=14), in comparison to healthy controls (n=65). The LSS cohort consists of 14 pediatric (mean age ± SD,12.9 ±3.9 years, 50% male) and 6 adult (30.8 ±12.7 years, 66.7% male) subjects with either nuclear (n=11, 55%) or mtDNA (n=9, 45%) genetic etiologies. Raw data for all domains were transformed into z-scores for adult and pediatric comparisons with z-scores < -2 S.D. (standard deviations) considered abnormal. In the pediatric cohort, MM-COAST composite (mean score ± SEM) was -0.52 ± 0.24 in pediatric controls (n=28), 0.14 ± 0.40 in pediatric 'Other PMD' (n=4), 0.85 ± 0.73 in pediatric MM (n=51), and 1.39 ± 0.65 in pediatric LSS (n=14) subjects, p<0.001, ANOVA. Pediatric LSS MM-COAST scores were significantly higher than MM subjects (p<0.01, Kruskal Wallis), indicating higher disease severity1. In the adult cohort, mean composite score was -0.43 ± 0.29 in controls (n=37), 0.98 ± 0.99 in 'Other PMD' (n=7), 0.99 ± 0.85 in MM (n=49), and 1.52 ± 0.37 in LSS (n=6) subjects, p< 0.001, ANOVA. Adult LSS MM-COAST scores were significantly higher than adult MM subjects (one-way ANOVA, p<0.001). Dexterity results showed a mean functional dexterity test (FDT) z-score ± SD of 0.42 ± 0.99 in pediatric healthy controls (n=28), -0.28 ± 0.19 in pediatric 'Other PMD (n=4), -3.56 ± 3.22 in pediatric MM (n=51), and –6.58 ± 4.60 in pediatric LSS (n=11) subjects, p<0.001, ANOVA. Mean FDT z-score was significantly lower in pediatric LSS compared to pediatric MM (p<0.01, Kruskal Wallis), indicating greater dexterity impairment. Adult FDT mean z-score was 0.19 ± 0.83 in healthy controls (n=36), -3.61 ± 4.13 in adult 'Other PMD" (n=7), -4.35 ± 5.14 in adult MM (n=46), and –8.63 ± 5.97 in adult LSS (n=5), p<0.001, ANOVA with mean FDT z-score significantly lower in adult LSS compared to MM, (p<0.01, Kruskal Wallis). Balance testing revealed single-leg stance with eyes closed (SLEC) mean z-score was significantly lower in pediatric LSS (-3.05 ± 1.11) compared to MM (-1.53 ± 1.54, p<0.01, Kruskal Wallis) indicating more severe balance impairment. In contrast, SLEC mean z-score was not significantly lower in adult LSS (-1.67 ± 0.24) compared to adult MM subjects (-1.23 ± 1.05) (p=0.44, Kruskal Wallis). Results demonstrate the ability of the MM-COAST to quantify clinically relevant impairments in LSS and other PMD phenotypes and indicate greater disease severity in LSS compared to MM.

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Abstract #: 2025PA-0000000143

Presenter: Tyler O'Hara

Title: Development of Osteosarcoma Xenografts in Zebrafish Models of Mitochondrial Disease

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Abstract:

While primary mitochondrial diseases (PMD) and osteosarcoma are both diseases common in early development, it is remarkably rare to find a clinical presentation of cancer in patients with PMD. Both disease classes favor the less energy-efficient but carbon-sparing glycolytic pathway over the respiratory chain for ATP generation, known as the "Warburg effect". To understand further connections between PMD and cancer, we have developed osteosarcoma xenografts in established zebrafish PMD models. Human osteosarcoma cell lines 143B, MG-63, and U-2 OS were transduced via lentivirus to express dual reporters, green fluorescent protein (GFP) and luciferase. Orthotopic bone xenografting is not possible at early developmental timepoints, as zebrafish ossification occurs at 10-14 dpf. In lieu of this, three candidate injection sites were tested (Duct of Cuvier, yolk sac, and hindbrain), where results indicated the greatest metastatic potential when injecting at the Duct of Cuvier. Therefore, to assess the implication of perturbed host metabolism on *in vivo* tumor growth, dual-labeled osteosarcoma cells were harvested and transplanted into 2-day post-fertilization (dpf) wild-type, transgenic and pharmacological zebrafish models of Complex I and Complex IV deficiency at the duct of Cuvier. Zebrafish were studied for tumor cell migration and tumor growth 1-hour post-injection (hpi) and again at 96 hpi. Tumorigenicity of these human osteosarcoma lines was observed to decrease in the order 143B > MG-63 > U2-OS, consistent with published results in mouse xenografts, which solidified 143B for future zebrafish xenograft studies. Xenografted zebrafish harboring *ndufs2* or *surf1* deletions exhibited different degrees of increased 143B cell fluorescence from 2-dpf to 6-dpf. Complex I deficiency, achieved either by genetic (*ndufs2cri4/cri4*) and pharmaceutical (rotenone) means, resulted in increased fluorescence in a stepwise dose-dependent manner, indicative of a more favorable environment for tumor development. However, neither the genetic (*surfcri1/cri1* or *surf1cri2/cri2*) nor pharmaceutical (sodium azide) models of complex

IV deficiency resulted in increased fluorescence of xenografted 143B cells relative to wild-type zebrafish. To further assess changes in tumorigenicity of injected cells due to PMD, a cybrid cassette of 143B with variable mitochondrial DNA (mtDNA) genomes was engrafted into wild-type zebrafish. Injections of 143B, ρ0 (143B with fully depleted mtDNA), Q1875 (143B with m.8993 T>G in *MT-ATP6*), and Q1875p1 (143B with mtDNA from healthy fibroblasts) yielded mean fluorescence intensities that did not significantly differ; however, distributions of individual fish fluorescence values over the four-day growth period were narrower in cybrid xenografts with depleted or mutated mtDNA than in those with wild-type mtDNA, indicative of reduced likelihood per fish to have increased tumor growth. Overall, we have established novel zebrafish xenograft models that inform that relative interaction between pediatric osteosarcoma proliferation capacity and PMD, demonstrating clear dependencies of cancer growth on host mitochondrial complex I function.

Abstract #: 2025PA-0000000144

Presenter: Kelsey Keith

Title: *ndufs2-/-* Zebrafish Have Impaired Survival, Neuromuscular Activity, Morphology, and One-Carbon Metabolism Treatable with Folic Acid

Authors: Dana V. Mitchell1*, Donna M. Iadarola2, Neal D. Mathew2, Kelsey Keith1, Christoph Seiler3, Sanghyeon Yu4, Man S. Kim4, Niki Woodard2, Vernon E. Anderson2, Eiko Nakamaru-Ogiso2,5, Deanne M. Taylor1,5, and Marni J. Falk2,5

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Abstract:

Mitochondrial complex I deficiency manifests with complex multi-system dysfunction, commonly in the Leigh syndrome spectrum (LSS), with early-onset metabolic strokes, lactic acidemia, and early mortality. To facilitate pre-clinical screening of novel therapeutic candidates for complex I diseases including LSS disorder, we developed a stable genetic knockout zebrafish animal model for a highly conserved nuclear-encoded complex I subunit, Ndufs2. CRISPR/Cas9 technology was used to generate a 16 base pair deletion in *ndufs2,* which are maintained as heterozygotes and in-crossed to generate homozygous *ndufs2-/-* mutants and phenotypically wild-type (WT), *ndufs2+/+* and *ndufs2+/-* siblings for larval stage analyses. Larvae were phenotypically characterized for survival, neuromuscular swimming activity, and

gross morphologic defects. RNA-Seq transcriptome profiling was performed to evaluate pathway adaptations in *ndufs2-/-* larvae, utilizing traditional enrichment analysis as well as a novel metabolic modeling approach that simulates potential metabolic flux capacity through a genome-scale metabolic model reconstructed from biochemical reactions comprising cellular processes of interest. RNA-Seq zebrafish results were compared to previous transcriptome profiling datasets from *ndufs2-/-* missense mutant *C. elegans* (*gas-1(fc21)*) and complex I disease patient fibroblasts. Therapies previously identified in the *C. elegans ndufs2-/-* missense model were screened for their ability to rescue morphological malformations in the zebrafish *ndufs2-/-* knockout model. *ndufs2-/-* zebrafish had severely reduced survival to a median of 11 days post fertilization (dpf) relative to wild-type (WT) animals that live greater than 2 years. *ndufs2-/-* zebrafish had marked neuromuscular dysfunction with ~50% decreased swimming activity and showed 80% reduced CI enzyme activity. Morphological analysis showed an uninflated swim bladder, decreased yolk absorption, an enlarged and dark liver phenotype, and small eyes. Transcriptome profiling of *ndufs2-/-* larvae revealed dysregulation of the electron transport chain, TCA cycle, fatty acid beta-oxidation, and one-carbon metabolism. Interestingly, pathways associated with eye development were downregulated in mutant larvae. Similar transcriptomic profiles were observed in *ndufs2-/-* missense mutant *C. elegans* (*gas-1(fc21)*) and two human CI-disease fibroblast cell lines stressed in galactose media. Metabolic modeling revealed specific dysregulation of pentose phosphate pathway reactions. One-carbon metabolism associated pathway alterations appeared to contribute to CI disease pathophysiology, as folic acid treatment rescued the growth defect and hepatomegaly in *ndufs2-/-* larvae. We have established and validated a novel vertebrate animal model of severe complex I deficiency using CRISPR/Cas9 technology to knock-out *ndufs2* in zebrafish. This preclinical model recapitulated many phenotypes associated with mitochondrial disease and LSS, provides novel insight into the unique gene expression profiles of LSS, and objectively demonstrates the therapeutic value of folic acid to replete one carbon metabolism in mitochondrial complex I disease. This translational model of PMD will enable future investigations of organ specific mechanisms of disease and high-throughput treatment studies.

Abstract #: 2025PA-0000000145

Presenter: Kelsey Keith

Title: Unmasking Metabolic Dysregulation in Primary Mitochondrial Disease using Galactose-Based Culture and Metabolic Modeling

Authors: Mitchell DV1*, Zhang Y1, Keith K1, McCormick EM1,2, George-Sankoh I1, Kim MS3, Zhang Z1, Falk MJ2,4, Taylor D1,4

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Abstract:

Primary mitochondrial disease (PMD) cellular and animal models with impaired aerobic energy production have improved health and viability when given glucose, exploiting their upregulated glycolytic anaerobic energy production capacity. However, our previous preclinical modeling studies showed that glucose administration masks mechanistic investigation into metabolic stress adaptations that occur in PMD cells. We hypothesized that culturing PMD fibroblast cells in galactose medium would unmask underlying cellular adaptations, enabling evaluation of mitochondrial disorders' effects on intermediary metabolic processes and physiologic functions. 12 human fibroblast cell lines molecularly confirmed (n=2) or clinically suspected (n=10) PMD patients, and 2 healthy controls were cultured at ~80% confluence in glucose (10 mM) or galactose (10 mM) media for 24 hours, after which RNA-Seq based transcriptome profiling was performed. Pairwise comparisons identified differentially expressed genes and enriched pathways. In addition, a novel metabolic modeling approach was performed to discern variations in metabolic potential between healthy and diseased cells. This systems biology focused method uses genome-scale metabolic models reconstructed from biochemical reactions comprising cellular processes of interest, which, when constrained by the RNA-seq data, simulate metabolic capacity and potential metabolic flux alterations. Having established the utility of galactose media to elucidate cellular metabolic adaptations in mitochondrial disease cells, our methodology and analysis was validated in a larger dataset of 92 additional samples comprising 12 healthy controls and 45 definite PMD patients cohorted by molecularly-related etiologies, including 6 complex I deficiency, 2 complex III deficiency, 5 complex IV or V deficiency, 2 fission/fusion defects, 9 mtDNA maintenance disorders, 1 single large-scale mtDNA deletion (SLSMD), 2 nDNA gene disorders, 5 pyruvate dehydrogenase complex disorders, and 13 mitochondrial translation disorders. This expanded investigation sought to elucidate discrepancies in the potential activity of biochemical reactions between healthy controls and PMD patients with varying subgroups of genetic etiologies. Significant differences were identified in gene expression, pathway enrichment, and biochemical regulation between PMD cells grown in galactose versus glucose media. For multiple patients, significant downregulation in galactose media were observed of pathways associated with cell cycle activities, chromosomal organization, and translational regulation. By contrast, few differences were identified in glucose vs galactose exposed healthy control cells. Across the larger cohort, metabolic flux capacity modeling unveiled significant differences between PMD patients and healthy controls in a number of biochemical subsystems (including glycolysis and the TCA cycle), after only subtle differences in transcriptomic profiles could be elucidated through differential expression and pathway enrichment analyses. In vitro transcriptome analyses in PMD demonstrated that significant differences occurred in gene expression of cell growth related pathways and potential metabolic flux capacity across numerous metabolic reactions in PMD patient cells grown in galactose compared to glucose media. This study underscores the critical importance of carefully controlling for cellular growth conditions when studying PMD and utilizing transcriptome profiling to investigate cellular mechanisms underlying complex metabolic disorders. Further, metabolic flux modeling offers a novel approach to unveil previously unrecognized systemic biochemical disparities

that occur among different classes of mitochondrial pathology that may be overlooked with canonical RNA-seq analyses.