CHOP Poster Presentations

UMDF Symposium 2022, Phoenix, AZ

Abstract # 2022PA-0000000031

Presenters: Cassandra Pantano, CRNP, and Emily Bogush, LCGC

Title: Cardiac Arrhythmia Identified Post COVID-19 Infection in Pediatric Patient with mt- ND5 m.13042G>A Mutation

Authors: Cassandra Pantano, CRNP[1], Emily Bogush, MS, LCGC[1], Amy Goldstein, MD[1], Matt Demczko, MD[1], Marni J. Falk, MD[1,2]

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Abstract background: Numerous variants in MT-ND5 that is a mitochondrial DNA gene encoding the ND5 submit of complex I in the respiratory chain have been associated with Mitochondrial Encephalomyopathy with Lactic Acidosis and Stroke-like Episodes (MELAS), Leigh Syndrome, and Leber Hereditary Optic Neuropathy (LHON) overlap (Valentino, M., 2006). The m.13042G>A heteroplasmic mutation in MT-ND5 was initially reported as a novel heteroplasmic variant in 2005, associated with MELAS/ Myoclonic Epilepsy Ragged Red Fiber (MERRF) overlap (Naini AB, 2005). Additional m.13042G>A variant case reports expanded the phenotypic spectrum to include LHON and Leigh Syndrome. Here, we expand the phenotypic spectrum of this pathogenic variant by reporting a new m.13042G>A clinical case who developed cardiac arrhythmia post-infection with the sars-COV2 virus that causes COVID-19.

Clinical Case Description: The proband was a 27-month-old full-term Caucasian girl of Polish, Italian, and Greek ethnicity. Her past medical history was notable for developmental delay with independent walking onset at 20 months old, suspected vestibular dysfunction with severe discomfort in stroller, swings and car rides, and recurrent bilateral acute otitis media (AOM) for one year s/p bilateral myringotomy tube placement 12/2021. She had recurrent regressions secondary to infection with a significant regression secondary to sars-COV2 infection. Initial regression was 12/2020 secondary to AOM and consisted of loss of ability to pull to stand, intermittent loss of balance when not looking at the ground while ambulating and decreased from verbalizing approximately 30 words to 5 words. She began physical therapy for regression and suspected vestibular dysfunction two months later at 15 months and regained both verbal and gross motor skills by 20 months, including walking independently. Her history until 12/2021 was unremarkable except for 4 additional AOM and three sinus infections without regression. 12/2/2021 bilateral myringotomy tubes were placed under general anesthesia and 6 days later she had a viral infection with fever. At this time proband began rubbing the back of her head, verbalizing “ouch” continuously throughout the day and was coughing with liquids and solids with significantly decreased PO intake secondary to bulbar dysfunction. Exact weight loss at

that time unknown, but she had progressively decreased PO intake for 7 to 8 weeks. During this time, she had a subsequent regression in skills. She would not run, climb stairs up or down, showed imbalance when ambulating and stopped scooting. 01/13/2022 proband was found to have sars-COV2 infection with fever, nasal congestion and excessive somnolence for 3 to 4 days diagnosed via nasal swab PCR test at pediatrician. Proband did not regain skills from regression, bulbar dysfunction increased resulting in coughing with all PO intake and was noted to have left eye esotropia with intermittent beats of nystagmus. 02/11/21 brain MRI under general anesthesia for a total time of 4 hours showed symmetrical abnormal signal predominantly within the medial thalami, periaqueductal gray and within the brainstem. Upon awakening from general anesthesia left eye esotropia had progressed to more significant exotropia. She was admitted 02/10/22 for 4 days and was readmitted 02/17/22 secondary to continued choking and refusal to walk. She did not receive IV arginine during either admission. She received IVIG 1g/kg x2 secondary to suspected Multisystemic Inflammatory Syndrome in Children secondary to sars-COV2 infection. During secondary admission, 02/20/22 proband experienced agonal respirations and cyanosis secondary to respiratory failure and intubation with ventilatory support. Proband was intubated for 12 days. Wolff Parkinson White (WPW) dysrhythmia was diagnosed by EKG. Intermittent elevation in lactate were seen at this time with a maximum elevated to 6.2 mM (normal A, at 87.6% heteroplasmy). mtDNA genome sequencing was proband only at Preventions Genetics. Subsequent targeted, maternal testing for the MT-ND5 m.13042G>A variant in buccal was performed at GeneDx and not observed; targeted testing in urine is pending. Whole exome sequencing was performed as a trio at GeneDx and resulted negative. 5-year-old brother has targeted mtDNA variant testing at GeneDx pending but did have negative mtDNA sequencing at ARUP laboratories 06/25/2017 secondary to GERD and fine and gross motor delays. Discussion. Here, we report a new case of an MT-ND5 m.1304G>A pathogenic variant at high heteroplasmy in blood consistent with the known phenotypic spectrum of Leigh syndrome with early developmental delay preceding acute neurodevelopmental regression secondary to viral infections including post sars-COV2 viral infection. Additionally, this case study is the first to report WPW dysrhythmia in association with the m. 13042 G>A pathogenic variant, although it has been reported in MT-ND5 cases with the m. 13513G>A pathogenic variant (Monlleo-Neila, L., 2012). Overall, these findings suggest careful cardiac follow- up is warranted in m.13042 G>A cases, and the sars-COV2 may be another viral etiology that can precipitate neurodevelopmental regression in MT-ND5 Leigh syndrome patients.

Abstract #: 2022PA-0000000034

Presenter: Donna M. Iadarola

Title: Elucidating metabolic patterns between different zebrafish models of Leigh syndrome

Authors: Donna M. Iadarola[1], Eiko Nakamaru-Ogiso[1,2], Marc Yudkoff[1], and Marni J. Falk[1,2]

Institutions: [1]Mitochondrial Medicine Frontier Program, Division of Human Genetics, Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA; [2]Department of Pediatrics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA.
Abstract introduction: Leigh syndrome (LS) denotes a group of genetically heterogenous disorders that stem from defective mitochondrial energy generation, where each monogenic mutation yields a unique clinical and biochemical phenotype. Towards the goal of characterizing the metabolism of different genetic causes of LS, zebrafish models of respiratory chain complex I (CI) deficiency and complex IV (CIV) deficiency were constructed by using CRISPR/Cas9 technology to generate stable knockout mutants for a complex I subunit gene (Ndufs2) and a complex IV assembly factor gene (Surf1), respectively. These zebrafish models develop acute brain death with metabolic stressors, a finding that is analogous to the major phenotype of human LS. Pharmacological inhibitor-based LS models of CI and CIV deficiency using mitochondrial toxins for CI (rotenone) and CIV (azide) were also studied in a wild-type (WT, AB zebrafish) background to determine if metabolic phenotypes of acute pharmacological respiratory chain inhibition mimics genetic disease models.
Methods: Organic acid liquid-liquid extraction was performed on each sample consisting of 100 flash frozen 7 days post-fertilization (dpf) zebrafish larvae. Gas chromatography/ mass spectrometry (GC/MS) was performed on these samples to analyze organic acids such as tricarboxylic acid (TCA) cycle intermediates and amino acids (n=100 larvae/sample) in the whole larvae samples relative to an internal standard. We compared genetic lines Ndufs2 and Surf1 to AB fish at baseline on 7 dpf. For pharmacological inhibitor-based models, we treated AB zebrafish with either 70 nM rotenone for 4 h on 7 dpf or 70 uM azide for 24 h starting at 6 dpf. ANOVA and principal component analysis were performed using Graphpad Prism.
Results: Distinct metabolic differences were identified between wild-type (WT), CI-deficient, and CIV-deficient zebrafish larvae. Principal component analysis effectively separated the discrete populations by genetic and/or pharmacologic condition. CI-deficient and CIV-deficient larvae all shared a similar profile of increased lactate, butyric acids, and branched chain amino acids. Both genetic and pharmacologic inhibitor-based CI-deficient larvae models further displayed a unique profile of significantly increased levels of malate and fumarate. Interestingly, CIV-deficient larvae metabolic profiles differed between the pharmacologic and genetic models – the AB + azide inhibited animals had significantly increased levels of succinate, while Surf1-/- larvae had significantly increased malate and citrate levels.
Conclusion: We have developed novel methods to quantify intermediary metabolic profiles by GC/MS in zebrafish larvae, which we applied to a series of genetic and pharmacologic inhibitor \-based CI and CIV deficient LS models. As elevated lactate, butyric acids, and branched chain amino acids are established hallmarks of LS, our data demonstrates that zebrafish models reliably reflect the metabolic phenotype of human patients. Metabolic signatures differed between acute CIV inhibition by high-dose azide and the genetic mutant models at baseline; future studies are underway to interrogate the metabolic effects of acute pharmacologic inhibition in the Surf1-/- models, as they

have no gross phenotype until stressed with low-dose azide. Stable isotopic metabolic flux labeling is also being developed to further investigate the distinct metabolic adaptations between CI- and CIV- deficient zebrafish larvae.

Summary for relevance to mitochondrial disease community (100 words or less)
Leigh syndrome (LS) is a genetically heterogenous disease stemming from defective mitochondrial energy generation, where each monogenic mutation has a unique biochemical and clinical phenotype. We are in the process of characterizing the metabolic signatures of different zebrafish models of LS using gas chromatography/ mass spectrometry. This research will aid in identifying new therapeutic targets to treat the different monogenic causes and metabolic consequences of LS.

Abstract #: 2022PA-0000000037

Presenter: Elizabeth M. McCormick

Title: Facilitating community-based genomic data analysis in primary mitochondrial disease: Collaboration of the Mitochondrial Disease Sequence Data Resource (MSeqDR) and mitoSHARE registry to support future genomic research discoveries.

Authors: Elizabeth M. McCormick[1], Emily Bogush[1], Katelynn Stanley[1], Katarina Gray[2], Philip E. Yeske[2], Xiaowu Gai[3,4], Marni J. Falk[1,5]

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Abstract Introduction: Primary mitochondrial diseases (PMD) are a highly heterogeneous group of disorders both in terms of physical manifestations with variable involvement of many organ systems, and genetic etiologies with pathogenic variants now recognized in more than 300 genes across both the nuclear and mitochondrial genomes. Further, wide phenotypic variability exists even among individuals with the same genetic disorder. Much remains to be learned about PMD, as many individuals with medical concerns highly concerning for PMD lack a confirmed genetic etiology. While genetic diagnostic testing, including mtDNA genome, whole exome, and whole genome sequencing, has become more accessible and widely utilized, analysis of these complex data often remains limited to clinical diagnostic laboratories that typically report only on variants for already known disease genes. Limited opportunity exists for novel gene discovery, or for more complex genomic studies of existing data in PMD subjects such as those involving pharmacogenomics that might identify novel treatment responders or efforts to identify modifying factors to explain a given genetic disorders' clinical variability. Further, no community-wide mechanism has yet been established to empower PMD individuals and their families to choose who can access and meaningfully analyze their existing genomic data for reanalysis purposes. Here, we report a recently

launched new initiative to bring these capabilities to the PMD global research community.

Methods and Results: The UMDF-led mitoSHARE mitochondrial disease online patient registry was launched in March 2022. The Mitochondrial Disease Sequence Data Resource (MSeqDR) genomic data repository was granted approval by the Institutional Review Board (IRB) at Children's Hospital of Philadelphia (CHOP, Falk PI) in January 2022. Participants from around the world who have enrolled into mitoSHARE and expressed interest in sharing their existing genomic raw data files (not just their data reports) will review an electronic informed consent form. Coded genomic data will then be uploaded to a secure Cloud-based server, stripped of identifying information, and labeled with a global universal identifier (GUID). GUID-labeled genomic data will be made available within MSeqDR in a secure, Web-based, genomics data analysis environment using sophisticated data query platforms (i.e., MSeqDr-Open CGA, Genesis) made accessible through a central web portal (https://mseqdr.org) that allow for analysis of genomic data for end-users without formal training in bioinformatics. Approved medical professionals with genomic data proficiency who are approved by the mitoSHARE-MSeqDR data use committee and provided by a family with their unique GUID identifier will be able to readily analyze exome, genome, and/or mtDNA genome data from individuals and families with known or suspected PMD. Raw genomic datasets will not be permitted to be downloaded by users. However, the clinician or researcher who may know a patient or family best (and is specifically given their GUID by the family) will be empowered to directly analyze their genomic data on a research basis to attempt to find the underlying genetic etiology and potentially other research-based uses, such as for pharmacogenomic and/or modifier gene analysis. No research results will be directly provided to families by the CHOP study team that is organizing this community-wide genomic data access effort but may inform the GUID-approved clinician or researchers' interrogation of their disease etiology.

Conclusions: Success rates for genomic analyses of highly heterogeneous disorders such as PMD can be greatly improved if a large cohort of patient raw genomic data is assembled to enhance collective capabilities for accurate sequence variant annotation, analysis, and interpretation in sophisticated and researcher user friendly genomic data analysis platforms. This community research project aims to put in place a mechanism to enable individuals and families with definitive or suspected to choose specific medical professionals to meaningfully evaluate their complex genomic data.

Abstract #: 2022PA-0000000042

Presenter: Laura E. MacMullen

Title: N of 1 Trials: Elamipretide Expanded Access Program Experience in Primary Mitochondrial Disease Subjects with Nuclear Mutations and Myopathy and/or Neuropathy Phenotypes

Authors: Laura E. MacMullen[1], Ibrahim George-Sankoh[1], Jean Flickinger[1], Elizabeth Ballance[1], Marni J. Falk[1,] Amy C. Goldstein[1,2].

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

Abstract introduction: Primary mitochondrial disease (PMD) is comprised of heterogenous genetic disorders for which there are currently no FDA-approved therapies. While substantial progress has been made in the therapeutic pipeline for PMD, clinical trial availability remains limited, and trials are often inaccessible to many PMD patients due to strict eligibility criteria. Expanded Access Programs (EAPs) provide a crucial pathway for providing potentially beneficial treatments to patients and give providers a unique opportunity to evaluate patient progress using individualized treatment outcomes. Primary mitochondrial myopathy (PMM) frequently leads to skeletal muscle myopathy, with resultant fatigue, exercise intolerance, imbalance, and muscle weakness, as well as often progressive peripheral neuropathies. Elamipretide is an investigational mitochondrial-targeting agent that localizes to the inner mitochondrial membrane where it associates with cardiolipin to improve mitochondrial respiratory chain stability and function, including ATP production and reduction of damaging levels of oxidation (ROS).

Methods: Subjects (N=5) with confirmed PMD caused by nuclear mutations in either POLG or TWINKLE were enrolled into the Elamipretide EAP (sponsored by Stealth BioTherapeutics) conducted at the Children's Hospital of Philadelphia Mitochondrial Medicine Frontier Program. All subjects in this cohort presented with predominant symptoms of myopathy and/or neuropathy. Clinical and research outcomes were systematically evaluated to determine progress over the course of treatment, including objective assessments of physical function as well as patient and caregiver-reported outcomes. Objective assessments included the six-minute walk test (6MWT), balance testing, and dynamometry. Subjective assessments included the Modified Fatigue Impact Scale (MFIS), the Pediatric Quality of Life Inventory (PedsQL), the EuroQoL EQ-5D-5L, and the Lansky and Karnofsky Scales.

Results: Age at treatment start ranged from 5 years to 66 years (4 male, 1 female). Treatment duration ranged from 70 days to 368 days (mean duration 170 days). Among this cohort, one patient passed (POLG) away from her underlying illness (age 5 years, death unrelated to treatment). Repeat subjective measures were completed on four patients (age 9 years [POLG]; age 10 [POLG]; age 15 [POLG] and age 66 [TWINKLE]). Repeat objective assessments were completed on one ambulatory subject (age 66 years, TWINKLE). This subject walked 112 meters longer on the 6MWT after one year of treatment with elamipretide (490 meters) than at baseline (378 meters). This same subject also showed marked improvement in balance on the tandem stance. Preliminary analyses suggest modest improvements occurred in subjective outcomes with more marked improvements in physical function outcomes in PMD subjects with peripheral myopathy and/or neuropathy symptoms on expanded access, open label, daily subcutaneous elamipretide therapy. Detailed results analysis is pending ongoing repeat objective and subjective outcomes as well as clinical safety data.

Conclusion: Expanded access IND protocols play an important role in bringing therapeutic candidates to PMD subjects for whom no effective therapies are available. Longer treatment duration and additional follow-up assessments are needed to further evaluate treatment outcomes of elamipretide in myopathy and peripheral neuropathy, as well as objective larger cohort randomized controlled clinical trials to evaluate the safety and efficacy of elamipretide in PMD subjects with peripheral neuropathy and/or myopathy predominant disease manifestation.

Abstract #: 2022PA-0000000049

Presenter: Leonard Burg

Title: The soluble guanylate cyclase stimulator CY6463 improves neuromuscular function and swimming activity in multiple zebrafish models of mitochondrial respiratory chain disease

Authors: Leonard Burg[1], Heeyong Yoon[1], Min Peng[1], Erin Haus[1], Prabhjot Kaur[1], Bhumi Shah[1], Peter Germano[2], Eiko Nakamaru-Ogiso[1,3], Marni J. Falk[1,3]

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Abstract Introduction: Mitochondrial respiratory chain (RC) disease pathophysiology has been associated with impairment of the nitric oxide (NO)-soluble guanylate cyclase (sGC)-cyclic guanosine monophosphate (cGMP) signaling pathway. Further, central nervous system manifestations of mitochondrial RC diseases have been reported to be ameliorated by activation of the NO-sGC-cGMP pathway.

Methods: Here, we tested the sGC stimulator CY6463 in Danio rerio (zebrafish) genetic and inhibitor-based larval models of primary mitochondrial RC dysfunction: ndufs2-/- and rotenone-exposed wild-type (AB) zebrafish (complex I disease models), surf1-/- and sodium azide-exposed AB zebrafish (complex IV disease models), fbxl4-/- and c12orf65-/- (multiple respiratory chain complex disorders), and dldh-/- (pyruvate dehydrogenase complex (PDHc) deficiency). CY6463 was evaluated on larval neuromuscular dysfunction in each mitochondrial disease model at baseline and upon exposure to RC inhibition by 96-well plate format larval swim activity assay. Brain survival, touch and startle neuromuscular responses, and ability to prevent loss of heartbeat were also assessed in the RC inhibitor-based larval models. Classical mitochondrial analytes including ATP, lactate, pyruvate, and ETC enzymes were assessed to evaluate the mechanism of the CY6463 effect. BioA studies were performed to determine CY6463 concentrations achieved in larval homogenates and adult tissues. Finally, swim activity assays (Loligo) measuring maximal oxygen consumption, swim duration, and swim velocity against a defined current were performed in the surf1-/- and fbxl4-/- genetic models during adult stages.

Results: CY6463 significantly improved survival and neuromuscular touch and startle phenotypes in the complex I inhibitor and genetic disease models. In the complex IV inhibitor disease model, CY6463 improved neuromuscular phenotypes in the inhibitor model, but had no effect in the genetic (surf1-/-) model. CY6463 improved viability and

larval swim activity in the fbxl4-/- and c12orf65-/- multiple RC complex disease models but had no effect on the dldh-/- (PDHc) model. BioA analysis confirmed CY6463 exposure in the brain and muscle tissue of adult zebrafish after incubation with the compound in their water. Evaluation of CY6463 on biochemical parameters and ability to rescue swimming activity in adult animals by measuring maximal oxygen consumption, swim duration, and swim velocity in the surf1-/- and fbxl4-/- genetic models is ongoing.
Conclusion: In preclinical study of primary RC disease zebrafish larval animal models, the sGC stimulator CY6463 significantly improved animal swimming activity and neuromuscular functions, particularly in complex I and multiple RC complex disease models. Mechanistic studies remain ongoing, as well as studies of CY6463 treatment effect on exercise capacity in adult zebrafish with complex IV and multiple RC complex diseases.

Abstract #: 2022PA-0000000053

Presenter: Isaac Martin
Title: Feasible Exercise Protocols for a Primary Mitochondrial Disease Clinical Trial
Authors: Isaac T. Martin[1], Jean Flickinger[2], Elizabeth B. Ballance[2], Michael G. McBride[3], Marni J. Falk[1,4], Zarazuela Zolkipli-Cunningham[1,4]
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Abstract Introduction: Both endurance and resistance exercise training have been shown to be beneficial in Primary Mitochondrial Disease (PMD) (Taivassalo et al., 2001, Murphy et al., 2008). However, guidance is lacking on routine exercise prescription in PMD and most PMD patients are unable to exercise for 30 minutes as recommended by the American Heart Association. Here, we sought to identify newer exercise protocols, prioritizing short protocols G mutations. These patients often present with myocardial abnormalities, conduction defects and/or cardiac arrythmias. Elamipretide is an investigational mitochondrial-targeted 4 amino acid peptide that localizes to the inner mitochondrial membrane, where it associates with cardiolipin to improve membrane stability, resulting in increased ATP production and reduced toxic levels of reactive oxygen species. It has also been shown to improve mitochondrial function during heart failure and improve left ventricular ejection fraction (LVEF) in humans and animal models.
Methods: Subjects (N=3) with confirmed PMD and heart failure with reduced ejection fraction (HFrEF) were enrolled into the Elamipretide Expanded Access Program (EAP; sponsored by Stealth BioTherapeutics) conducted at the Children’s Hospital of Philadelphia. The primary outcome measure assessed in this cohort was LVEF on transthoracic echocardiogram.
Results: Age at treatment initiation in the EAP ranged from 10 years to 45 years (3 male, 0 female). Treatment duration ranged from 222 days to 229 days (mean duration 225

days). All patients showed modest to significant improvements in LVEF during the start of treatment. Subject 1 (age 10; USMG5-based complex V disease) was treated under the EAP during cardiac arrest. His reduced LVEF of 29% at baseline showed improvement to 62% after three days of treatment and was 60% on 3-month follow-up. Subject 2 (age 24; MT-TL1 m.3243A>G; heteroplasmy 44% in buccal sample) showed modest improvement in LVEF from 20% prior to treatment to 35% at 5-month follow-up, thereby delaying the need for evaluation for cardiac transplantation. In addition, he reported improved ability to gain weight as well as improved mobility and endurance. Subject 3 (age 45; MT-TL1 m.3243A>G; heteroplasmy 79% in urine) presented with 20% LVEF 6 months prior to study treatment initiation. His echocardiogram performed 3-months after starting elamipretide therapy showed stabilization of his LVEF at 23% but no improvement. Of note, subject 3's similarly affected brother had progressive LVEF decline requiring orthotopic heart transplant at age 52 years.
Conclusion: Preliminary analyses suggest that cardiac improvement and/or stabilization may occur in PMD subjects with HFrEF within days to months after treatment with elamipretide. Stable cardiac outcomes may suggest protection from disease progression that is typical of HFrEF in the absence of treatment. Longer treatment durations will provide additional information on the impact of elamipretide on cardiac outcomes, and a randomized controlled clinical trial would be valuable to objectively assess the efficacy of elamipretide in PMD subjects with HFrEF.

Abstract #: 2022PA-0000000060

Presenter: Manuela Lavorato, PhD, Research Assistant Professor
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Title: Dichloroacetate improves survival, neuromuscular function, and mitochondrial physiology and morphology in FBXL4 disease preclinical C. elegans, zebrafish, and human fibroblast models.
Background: FBXL4-based primary mitochondrial disease (PMD) is an autosomal recessive disorder that manifests with a highly variable breadth and severity of multi-system features. It has extensive allelic heterogeneity, with 48 pathogenic variants reported in 94 patients, making it one of the more common nuclear gene causes of PMD. Unfortunately, no FDA-approved, effective therapies, or cures currently exist for FBXL4 disease. Here, we report characterization of novel C. elegans and zebrafish animal models, as well as human patient fibroblast cell lines (FCL), to study FBXL4-/- disease mechanisms and identify preclinical therapeutic leads.

Methods: The efficacy of DCA was tested on a C. elegans strain, VC3038, which contains a homozygous fbxl-1(ok374) allele involving a 707 base pair deletion in the C02F5.7 (fbxl-1) gene and a zebrafish genetic model, fbxl4sa12470 (homozygous for a missense mutation in the sa12470 allele). Specifically, DCA was then extensively evaluated in both worm and zebrafish FBXL4-/- animal models on animal survival, swimming activity, gross morphological phenotypes, mitochondrial physiology, and stressor sensitivity. Validation of DCA effects on mitochondrial amount, morphology, and ultrastructure and survival was performed in primary FCLs established from two human FBXL4-/- disease subjects.
Results: fbxl-1(ok3741) C. elegans and fbxl4sa12470 zebrafish models both showed decreased neuromuscular activity. fbxl-1(ok3741) C. elegans had impaired pharyngeal pumping rate, ~70% decreased locomotor activity ($p<0.0001$), and 58% reduced fecundity relative to wild-type (WT) worms ($p<0.0001$). Seven days post-fertilization (dpf) fbxl4^{sa12470} zebrafish had 70% reduced neuromuscular response ($p<0.0001$) and increased frequency of brain death ($p<0.0001$) when exposed to a mitochondrial translation inhibitor, chloramphenicol (CAP), relative to WT controls. Electron transport chain enzyme activity analysis showed isolated reduction of complex IV activity by 60% in 7 dpf mutant zebrafish larvae ($p<0.05$). Citrate synthase (CS) enzyme activity as a marker of mitochondrial content was reduced by ~40% in both C. elegans ($p<0.05$) and human patient fibroblasts ($p<0.05$) relative to controls, but unchanged in zebrafish. Fecundity was significantly reduced in the fbxl-1 worms but rescued by 50% with 25 mM DCA treatment ($p<0.0001$), as was their pharyngeal pumping activity. 5 mM DCA prevented brain death in fbxl4^{sa12470} zebrafish larvae exposed to CAP (40% improvement, and increased their neuromuscular response (50% improvement, $p<0.001$). DCA also increased CS activity by ~50% in both the C. elegans model ($p<0.01$) and human patient FCLs ($p<0.05$), and prevented metabolic stressor-induced mitochondrial gross morphological and ultrastructural damage in FBXL4 -/- human patient fibroblasts. Finally, DCA decreased intracellular lactate levels by more than 50% in patient FCLs ($p<0.05$).
Conclusions: We have developed and extensively characterized a robust preclinical pipeline of FBXL4 disease models in which to perform disease modeling and enable therapeutic development. Detailed evaluation of a pyruvate dehydrogenase complex activator, dichloroacetate (DCA) in fbxl1(ok3741) C. elegans, fbxl4sa12470-/- zebrafish and FBXL4-/- human FCLs demonstrated its beneficial effects on survival, neuromuscular function, and mitochondrial physiology and morphology. Collectively, these data suggest that DCA holds promise as a therapeutic lead to improve neuromuscular function and mitochondrial physiology at the levels of mitochondrial dysfunction and altered morphology, warranting rigorous clinical trial evaluation to objectively determine whether DCA will improve the survival, function, and quality of life of human subjects with FBXL4 disease.

Abstract #: 2022PA-0000000061

Presenter: Prabhjot Kaur
Title: Modeling Human Mitochondrial Dihydrolipoamide Dehydrogenase Disease (DLD) in C. elegans and Evaluating Therapeutic Reagents

Authors: Prabhjot Kaur[1], Chynna N Broxton[1], Eiko Nakamaru-Ogiso[1], Vernon Anderson[1], Marni J Falk[1,2]
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Background: Dihydrolipoamide dehydrogenase (DLD) deficiency is a rare autosomal recessive genetic disorder having the highest carrier rate found amongst the Ashkenazi Jews, with a disease frequency of 1:35,000–1:48,000. DLD is the E3 subunit of three mitochondrial enzyme complexes; pyruvate dehydrogenase (PDH), alpha-ketoglutarate dehydrogenase (α-KGDH) and branched chain 2- oxoacid dehydrogenase (BCKDH). DLD dysfunction in any of these processes can cause multisystemic symptoms to occur in patients, including, Leigh syndrome, lactic acidemia, liver dysfunction, various neurologic deficits, metabolic decompensation with stressors, and failure to thrive. DLD disease symptoms are primarily attributed to dysfunction of the PDH complex, however the full biochemical functional impact remains unknown. Due to this, current therapies are non-specific and generally ineffective. Here, feeding RNA interference (RNAi) knockdown was used to deplete DLD protein expression in C. elegans, characterize disease phenotypes, and test candidate therapeutics.
Methods: Mutations that delete dld-1 expression are embryonic lethal in C. elegans. Therefore, feeding RNAi was used to variably deplete DLD-1 protein in N2 Bristol wild-type (WT) worms (N2). Depletion of DLD-1 was achieved by feeding worms with bacteria expressing the RNAi clone LLC1.3, which encodes a double-stranded RNA (dsRNA) against dld-1 when induced by isopropyl-β-D-thiogalactoside (IPTG) and dramatically reduces DLD-1 protein levels in C. elegans. DLD-1 deficient C. elegans were characterized by DLD-1 protein expression, brood size, animal length, neuromuscular function, mitochondrial physiology, mitochondrial unfolded protein response (UPRmt), and lifespan. Therapeutic effects of empiric treatments commonly used in human DLD disease were tested on mitochondrial stress response.
Results: RNAi from early development induced a 90% decrease in DLD-1 protein expression in a synchronous young adult worm population. DLD-1 deficient worms displayed a 90% reduction in brood size and a 23% reduction in animal length observable at adult day 3. DLD-1 deficient animals displayed a 50% reduction in neuromuscular function assessed by chemotaxis assay. UPRmt was substantially induced, with a 30-fold increased induction of a HSP6:GFP reporter. Total cellular ATP levels were decreased by 55% in the DLD-1 deficient worms. RC complexes I, II, and IV enzyme activities were not significantly different in the dld-1(RNAi) worms relative to WT control worms. A 2.8-fold increase of pyruvate and 1.9-fold decrease in lactate tissue levels were found in dld-1(RNAi) worms relative to WT. UPRmt induction was significantly reduced in DLD-1 deficient C. elegans by 30% with dichloroacetate (DCA) and by 25% with thiamine (vitamin B1). Combinatorial DCA + thiamine therapies showed synergistic effect, with 50% reduction in UPRmt induction.
Conclusion: Overall, we demonstrate that RNAi inhibition of DLD-1 expression and activity in C. elegans provides a robust invertebrate animal model of DLD disease. DLD-1

deficient animals had many gross phenotypes, including decreased brood size, shortened adult length, and impaired neuromuscular function. They also displayed complex mitochondrial pathophysiology, with dramatically increased UPRmt induction, increased tissue pyruvate and decreased tissue lactate, and substantial ATP deficiency despite normal RC enzyme activities. Importantly, DCA and thiamine therapies significantly reduced their UPRmt, with synergistic effects when combined together. These findings suggest that evaluation of DCA and thiamine is warranted in objective clinical treatment trials of human patients with DLD deficiency.

Abstract #: 2022PA-0000000063

Presenter: Manuela Lavorato, PhD, Research Assistant Professor
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Title: Neuromuscular activity and mitochondrial ultrastructure investigation of a novel zebrafish model of Dihydrolipoamide (DLD) Deficiency
Introduction: Dihydrolipoamide dehydrogenase (DLD) deficiency is an autosomal recessive mitochondrial disorder with multi-system dysfunction caused by impaired activity of the E3 (DLD) subunit of three mitochondrial matrix enzyme complexes: pyruvate dehydrogenase (PDHc), αketoglutarate dehydrogenase and branched chain α-ketoacid dehydrogenase. To date, most DLD disease symptoms have been attributed to dysfunction of PDHc. However, full understanding of the relative mechanistic contributions is still being elucidated and, current therapies for DLD deficiency are non-specific and generally ineffective. We have previously created a genetic zebrafish (D. rerio, vertebrate) model of DLD deficiency which showed developmental delay at larval stage, reduced protein expression, enlarged liver and low survival rate. In this study we evaluated the neuromuscular activity in homozygous zebrafish larvae, conducted in-depth analysis of mitochondrial ultrastructure in high-energy demand tissues and we tested the efficacy of therapeutic agents on the zebrafish liver disease phenotype.
Methods: Swimming activity was assessed in 6 days post-fertilization (dpf) dldh -/- and dldh +/+ (WT) zebrafish larvae using the Zebrabox tracking system and Zebralab software (ViewPoint Life Sciences). At 7 dpf, dldh-/- zebrafish larvae were fixed in 6% glutaraldehyde in 0.1 M cacodilate and processed for ultrastructural studies. Ultrathin sections were obtained to analyze mitochondrial ultrastructure of the liver, intestine and skeletal tail muscle. In-depth statistical analysis was performed to analyze mitochondrial ultrastructure. Therapeutic agents were administered to dldh - /- and dldh +/+ (WT) zebrafish larvae beginning at 2dpf and liver size was assessed at 8dpf using ImageJ quantification. RESULTS: dldh-/- zebrafish larvae showed impaired neuromuscular activity with 50 % reduced swim activity ($p< 0.0001$). A significant positive association was seen between the reframing subscale on the F-COPES with the CD-RISC-25 Scale (Spearman's Correlation Coefficient of 0.58, $P < 0.01$). This important

finding suggests that cognitive reframing may be possible to improve with targeted interventions, and thus improve resilience.
Conclusions: Overall, coping in adults with PMD is poor, with significant life stressors reported by affected individuals. Further, coping and resilience skills in PMD subjects are significantly reduced, as quantified on the validated measures F-COPES and CD-RISC-25. In an effort to determine whether PMD patients' resilience and coping can be improved, an NAMDC-funded pilot award (NIH, PI E.M. McCormick) was granted to study a targeted psychosocial intervention that is now under active planning.

Abstract # 2022PA-0000000070

Presenter: Amel Karaa
Title: Genetic subgroup learnings from the MMPOWER-3 trial: Elamipretide improved six-minute walk test in individuals with mtDNA replisome disorders
Authors: Amel Karaa[1], Michelangelo Mancuso[2], Bruce Cohen[3], Marni J. Falk[4], Amy Goldstein[4], Mary Kay Koenig[5], Michio Hirano[6], John Vissing7
Institutions: [1]Genetics Unit, Massachusetts General Hospital, Boston, MA 02114; [2]University of Pisa, Pisa, Italy; [3]Akron Children's Hospital, Akron, OH; [4]Mitochondrial Medicine Frontier Program, Division of Human Genetics, Department of Pediatrics, Children's Hospital of Philadelphia and University of Pennsylvania Perelman School of Medicine, Philadelphia, PA; [5]University of Texas McGovern Medical School, Department of Pediatrics, Center for the Treatment of Pediatric Neurodegenerative Disease, Houston, Texas; [6]Chief of the Neuromuscular Division, Columbia University Medical Center Neurology, New York, NY; [7]Copenhagen Neuromuscular Center, Rigshospitalet University of Copenhagen, Copenhagen, Denmark
Abstract Introduction: Primary mitochondrial myopathy (PMM) is a genetic disorder of the mitochondria, adversely affecting predominantly, but not exclusively, skeletal muscle, leading to decline in quality of life. Several clinical trials designed to identify novel treatments for primary mitochondrial myopathy are either complete or in progress. The mitochondria-targeting peptide elamipretide was recently assessed in a Phase 3, randomized, double-blind, placebo-controlled clinical trial for the treatment of subjects (N=218) with primary mitochondrial myopathy (MMPOWER-3). Enrolled subjects had a variety of pathogenic variants in either nuclear (nDNA) or mitochondrial DNA (mtDNA) genes that caused their myopathy. Overall, the trial did not meet the primary endpoints in the highly heterogeneous study cohort. Here, we report results of genetic subgroup, post-hoc analyses subsequently performed on the per protocol population.
Methods: Using the MMPOWER-3 per protocol population in subjects who successfully completed the full trial duration, elamipretide effects on change from baseline in the six-minute walk test (6MWT) were examined as a function of gene variants.
Results: Individuals with primary mtDNA pathogenic variants or single deletions represented 74% of the entire trial population. Those with mtDNA variants receiving elamipretide showed no significant effects on 6MWT when compared to placebo. Participants with MT-TL1 pathogenic variant (approximately 33% of the mtDNA cohort, all with the m.3243A>G most common mutation) who were randomized to the placebo

arm increased 42.4±13.4 meters in 6MWT at week 24. This group also had lower mtDNA mutation heteroplasmy in blood (34%). After 24- weeks of elamipretide, subjects with nDNA gene mutations walked significantly farther than placebo counterparts (25.5 ± 8.0 versus 0.3 ± 7.7 meters, respectively; $p<0.05$). Further analysis revealed that the majority of the nDNA cohort was comprised of subjects with pathogenic variants in genes required for mtDNA genome maintenance (the mtDNA replisome, n=51 subjects). 6MWT at week 24 in subjects with replisome mutations changed 25.2 ± 8.7 with elamipretide versus 2.0 ± 8.6 meters in placebo ($p<0.05$). This effect was even more prominent in individuals with replisome mutations and a comorbidity of progressive external ophthalmoplegia (PEO; n=32), with elamipretide-treated subjects walking 37.3 ± 10.7 meters versus -8.0 ± 9.5 meters in placebo at 24 weeks ($p<0.05$). Pharmacokinetic analyses in the nDNA cohort showed a positive correlation between plasma elamipretide concentration and 6MWT improvement.
Conclusions: Overall, these findings highlight the challenge of developing therapies for the broadly heterogeneous class of mitochondrial disease. The subgroup analysis demonstrated that 6MWT in the mtDNA cohort was confounded by a 'placebo effect', disproportionally driven by subjects with MT-TL1 pathogenic variants. In contrast, no placebo effect was observed in the pathogenic nDNA gene cohort. The MMPOWER-3 basket trial design clearly introduced significant heterogeneity and directly highlights the importance of considering genetic subgroups in developing future trials and treatments for individuals with PMM. While the observation of improvement in 6MWT in the nDNA cohort with replisome disorders is encouraging, efforts are currently underway to confirm this positive benefit and findings. Furthermore, future studies are planned to explore the efficacy of elamipretide in patients with nDNA-encoded mtDNA maintenance-related replisome disorders.

Abstract # 2022PA-0000000079

Presenter: Sonal Sharma, MD
Title: Novel development of magnetic resonance imaging (MRI) to study structural anatomic differences in diverse organs of primary mitochondrial disease adult zebrafish animal models
Authors: Sonal Sharma[1], Sergey Magnitsky[2], Emily Reesey[1], Mitchell Schwartz[1], Suraya Haroon[1,3], Manuela Lavorato[1,3], Sherine Chan[4], Christoph Seiler[5], Benjamin Wilkins[6], Daniel Martinez[6], Marni J. Falk[1,3]
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Abstract Introduction: Zebrafish (Danio rerio) is a valuable preclinical animal model for primary mitochondrial disease (PMD), beneficial for studying global phenotypic changes

in stable transgenic mutants, developing new biomarkers, and optimizing new therapies. Here, we report novel magnetic resonance imaging (MRI) methods we developed to create the first high resolution anatomical atlas of wild-type (WT) adult zebrafish as well as age-matched transgenic zebrafish mutants (surf1-/- , opa1+/- , and fbxl4-/-), analyze organ volumes, and compare their growth in different age groups.
Methods: Fifteen WT adult zebrafish, nine surf1-/- mutants, three opa1+/- mutants, and three fbxl4-/- mutants were studied between ages 5 and 31 months. MRI acquisition was obtained with a 9.4 T vertical bore magnet in the CHOP small animal imaging core facility. A 9-month-old WT zebrafish animal was submitted for H&E staining in the CHOP histology core facility. MR and histological images were directly compared to enable reliable organ identification. A 3D mask was created of each animals' brain, spinal cord, liver, kidneys, heart, ears, eyes, optic nerve and swim bladder, enabling calculation of all organ volumes. P values were determined for normalized organ sizes to animal size of each mutant strain using a paired students t-test with a 0.05 significance level. Results: A linear increase in organ volume was noted in WT fish that followed aging progression across five age groups (5, 8-9, 12-14,16-18, 31 months). Several organ-specific differences in the mitochondrial disease old surf1-/- mutants were seen, including larger ear volume at 12-14 months (P=0.005), larger brain volume at 19 months (P=0.006), and maller spinal cord volume of 19 months (P=0.09). In addition, brain volume of 16–18-month-old opa1+/- mutants was larger (P=0.022) compared to age matched WT fish. No significant differences in organ volumes were seen in other organs of the opa1+/- , surf1-/- or fbxl4-/- mutants relative to WT controls.
Conclusion: We have developed a novel, high-resolution MRI imaging methodology to quantify adult zebrafish organ volumes and anatomy in fixed animals. This approach can now be readily used as a first-line three-dimensional test to screen for global organ involvement in novel transgenic zebrafish disease lines, which represents a major advance over traditional histological staining methods by maintaining organ structure and 3D topography. Based on detailed volumetric analyses performed in 3 primary mitochondrial disease mutant lines showing specific organ differences, further functional assessment is planned to evaluate auditory function in surf1-/- mutants as well as brain and spinal cord tissue pathology in surf1-/- and opa1+/- mutants. Future research will focus on defining MRI signaling patterns of dysfunction in diverse organs, as well as developing live animal NMR imaging methods to permit metabolite imaging in primary mitochondrial disease zebrafish animal models.

Abstract#: 2022PA-0000000082

Presenter: Ryan Mendel and Suraiya Haroon
Title: Single large-scale mitochondrial deletion (SLSMD) disease model in C. elegans demonstrates increased UPRmt response phenotype despite low heteroplasmy levels
Authors: Ryan Mendel[1], Yi Cheng[1], Neal Mathew[1], Eiko Ogiso[1], Marni J. Falk[1,2], and Suraiya Haroon[1,2]
Institutions: [1]Mitochondrial Medicine Frontier Program, Division of Human Genetics, Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA.

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Abstract background: Kearns-Sayre syndrome (KSS) and Pearson Syndrome (PS) are mitochondrial diseases caused by single large-scale mitochondrial DNA (mtDNA) deletions (SLSMD). SLSMD diseases, 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 mtDNA deletions that are stable and transgenerational. As such, naturally occurring models help advance understanding of disease progression and enable therapeutic development. Specifically, the uaDf5 C. elegans model harbors a 3.1 kilobase mtDNA deletion of 11 genes that encode 7 tRNAs and 4 proteins. Here, we characterized the uaDf5 model in the context of SLSMD disease and developed an assay to perform high-throughput drug screen to identify new therapeutic targets.
Methods: We assessed the heteroplasmy level, or frequency of mutant mtDNA genomes that harbor the SLSMD, in the uaDf5 model using SYBR Green and Taqman quantitative-PCR (qPCR) methods. Mitochondrial, fecundity and neuromuscular fitness of these mutants were respectively quantified using a genetic fluorescent mitochondrial stress reporter, brood size count, and body movement in liquid (thrashing assay). The fluorescent reporter-based assay of mitochondrial stress induction was used to screen potential therapies. To validate therapeutic leads, six different SLSMD disease human patient cell line models are being characterized under metabolic stress conditions to assess their survival (ATP levels), heteroplasmy levels (qPCR), mitochondrial mass (MitoTracker Green fluorescence), and mitochondrial membrane potential (Tetramethylrhodamine, methyl ester, TMRM fluorescence).
Results: Although prior publications reported an average of 60% SLSMD heteroplasmy in uaDf5 animals, after rigorous multigenerational assessment, the highest heteroplasmic strain maintained on average 20% heteroplasmy. This strain did not have a discernible neuromuscular defect assessed by thrashing, and only very minor defect in fecundity assessed by progeny count. However, uaDf5 heteroplasmic worms, had significantly increased mitochondrial stress by both large particle flow cytometry (COPAS vision bio sorter) and a high content imaging platform (CX5, Thermo) imaging. We observed that the induced level of mitochondrial stress correlated with heteroplasmy level, suggesting that SLSMD heteroplasmy causes mitochondrial stress. We have used this phenotype to develop a high-throughput assay to screen an FDA-approved drug library of ~2500 compounds. SLSMD patient and control human fibroblast cell line studies are ongoing.
Conclusions: uaDf5 mutant worms are an informative animal model in which to study SLSMD heteroplasmy mechanistic effects and preclinical therapeutic modeling. However, the SLSMD heteroplasmy level in these animals is lower than previously published. By developing a uaDf5 SLSMD strain that expresses a mitochondrial stress fluorescent marker, we were able to establish a high throughput method to screen drug libraries to identify therapeutic leads for SLSMD disease. Candidate therapeutic leads will be studied for their effects to reduce SLSMD heteroplasmy levels in the uaDf5 worm model, and further validated in human SLSMD fibroblasts from KSS and PS patients.

Abstract #: 2022PA-0000000086

Presenter: Suraiya Haroon

Title: N-Acetylcysteine and cysteamine bitartrate prevent azide-induced neuromuscular decompensation by restoring glutathione balance in two novel surf1-/- zebrafish deletion models of Leigh's Syndrome

Authors: Suraiya Haroon[1,2#], Heeyong Yoon[1#], Christoph Seiler[3], Bruce Osei-Frimpong1, Jie He[4], Rohini M. Nair[4], Neal Mathew[1], Leonard Burg[1], Chavali Venkata[2], Vernon E. Anderson[1], Eiko Nakamaru-Ogiso[1,2], Marni J. Falk[1,2*]. (# equal contribution)

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Abstract background: SURF1 deficiency causes Leigh syndrome (LS), a mitochondrial disorder typified by stress induced metabolic strokes, neurodevelopmental regression, and progressive multisystem dysfunction. These severe symptoms arise in childhood and the prognosis is very poor. Unfortunately for patients with SURF1 diseases, as with most mitochondrial diseases, there are no effective treatments or FDA approved therapies. One major roadblock to therapeutic development is the lack of effective animal models to screen novel therapies. Here, we describe two novel surf1-/- zebrafish knockout models generated by CRISPR/Cas9 technology and two prophylactic therapies that can prevent severe disease symptoms in these zebrafish models of SURF1 disease.

Methods: Complex IV levels and activity were assessed in the larvae of both surf1-/- zebrafish models using various methods since reduced complex IV activity is a major hallmark of LS. The fitness of the larvae was assessed by several neuromuscular behavioral response assays, including swim activity. Stress tolerance was assessed in both surf1-/- models by exposing the larvae to low levels of a complex IV inhibitor, sodium azide. Lastly, we screened nine compounds that affect a range of biological pathways to identify possible in their ability to ameliorate the phenotypes induced by sodium azide. In the adults, we performed histological analyses to characterize the severe morphological defect in the adult eyes.

Results: The surf1-/- zebrafish models contain a deletion in the surf1 gene that result in unstable truncated proteins which effectively result in surf1 protein knockout. The gross larval morphology, fertility, and survival into adulthood appear unaffected in both surf1-/- mutants, however, there are gross adult-onset ocular anomalies and classical biochemical hallmarks of human SURF1 disease in the larvae, including reduced complex IV expression and enzymatic activity, and increased tissue lactate. The surf1-/- larvae also demonstrate stressor hypersensitivity to the complex IV inhibitor, azide, which exacerbate their complex IV deficiency and further reduce super complex formation. This stressor also induces acute neurodegeneration typical of LS including brain death, impaired neuromuscular responses, reduced swimming activity, and absent heartrate. Remarkably, prophylactic treatment of surf1-/- larvae with either cysteamine bitartrate or Acetylcysteine, but not other antioxidants, significantly improve animal resiliency to

stressor-induced brain death, swimming and neuromuscular dysfunction, and loss of heartbeat. Mechanistic analyses demonstrate that cysteamine bitartrate pretreatment does not improve complex IV deficiency, ATP deficiency, or increased tissue lactate but does restore glutathione balance in surf1-/- animals.
Conclusions: Overall, two novel surf1-/- zebrafish models recapitulate the gross biochemical hallmarks of LS, including reduced complex IV activity and azide stressor hypersensitivity that was associated with glutathione deficiency. The severe phenotypes induced by the stressors are preventable by cysteamine bitartrate or N-acetylcysteine therapy.

Abstract #: 2022PA-0000000092

Presenter: Amy Goldstein MD
Title: Improving Acute Care for Mitochondrial Disease: Development of an Inpatient Clinical Care Pathway in the EPIC Electronic Medical Record Platform.
Authors: Amy Goldstein[1,2], Rebecca Ganetzky[1,2], Matt Demczko[1], Cassandra Pantano[1], [1,]Zarazuela Zolkipli-Cunningham[1,2], Sonal Sharma[1,2], Marni J. Falk[1,2]
Institution: [1]Mitochondrial Medicine Frontier Program, Division of Human Genetics, Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA, [2]University of Pennsylvania Perelman School of Medicine, Philadelphia, PA.
Abstract introduction: Children with mitochondrial disease often have severe, multi-systemic symptoms that prompt emergent evaluation at acute care medical facilities. Given the rare nature of these inherited metabolic disorders, many emergency room physicians and hospitalists are not familiar with mitochondrial disease, the many possible complications that may arise, and expert consensus defined standard of care clinical interventions. Our aim was to develop specific evidence-based guidelines that help educate front-line clinicians at the time of patient encounters to aid in the rapid identification and optimal management of acute symptomatology seen in primary mitochondrial disease. The long-term goal of these electronic medical record support tools is to improve patient outcomes and reduce physician anxiety in caring for these children.
Methods: Published and unpublished acute care guidelines from several mitochondrial centers and organizations, including Newcastle upon Tyne Hospital, Bambino Gesu Hospital (Italy), and the Mitochondrial Medicine Society (US) were reviewed to identify a streamlined set of expert consensus guidelines. Key subspecialists within our hospital were consulted to review suggested guidelines from pediatric emergency medicine acute care intensivists, and hospitalist primary care physicians. Subsequently, our health systems' informatics team partnered to build an electronic medical record Care Pathway in EPIC, including recommended order sets for common labs to obtain at the time of acute medical care in the emergency department and/or inpatient hospital setting.
Results: The Mitochondrial Care Pathway was launched in Spring 2022 on the CHOP EPIC web system. Within our health system, we will monitor its use metrics, including number of Web page visits and long-term ability to improve standardization across primary mitochondrial disease patient clinical evaluation for acute medical concerns, diagnosis, treatment, admission, and disposition. These recommended acute care

guidelines have also been made publicly accessible for reference by other clinicians outside of our health system when caring for a patient with mitochondrial disease at the following Web link: Emergency Department and Inpatient Mitochondrial Clinical Pathway | Children's Hospital of Philadelphia (chop.edu)
Conclusion: The CHOP Mitochondrial Medicine Clinical Care Pathway is available through the EPIC electronic medical record system and through public links for clinicians and mitochondrila disease families to access at the time of acute medical care evaluation by emergency department and inpatient hospital setting. The long-term goal of this Care Pathway is to provide centralized treatment plans that are easily accessible in real-time, in an effort to reduce morbidity, mortality and acute care costs in the complex primary mitochondrial disease patient cohort.

Abstract#: 2022PA-0000000108

Presenter: Zahra Tara and Suraiya Haroon
Title: Exploiting mitophagy to develop therapies for OPA1 disease in Worm with validation in zebrafish and human fibroblast cell line translational models.
Authors: Zahra Tara[1], Erin Haus[1], Christie Campbell[1], Seinn Wei[1], Ryan Mendel[1], Neal Mathew[1], Christoph Seiler[2], Sherine Chen[3], Eiko Ogiso[1], Marni J Falk[1,4], and Suraiya Haroon[1,4]
Institutions: [1]Mitochondrial Medicine Frontier Program, Division of Human Genetics, Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA. [2]Aquatics Core Facility, The Children's Hospital of Philadelphia, Philadelphia, PA. [3]Medical University of South Carolina, Charleston, South Carolina. [4]Department of Pediatrics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA.
Abstract background: Pathogenic OPA1 variants lead to progressive vision loss, with 20% of OPA1 patients developing additional symptoms such as motor/sensory neuropathy, ataxia, myopathy, and sensorineural hearing loss. OPA1 is a mitochondrial GTPase that facilitates mitochondrial fusion, respiratory chain super complex stabilization, and mitochondrial DNA (mtDNA) stability. OPA1 dysfunction induces fragmented mitochondria, which are susceptible to bioenergetic dysfunction and subsequent degradation via mitophagy. One approach to prevent the continual cycling of mitochondrial degradation and biogenesis is to modulate mitophagy, which could reduce ATP use, preserve mitochondrial mass, and stabilize mtDNA content. Here, we report the development of a humanized C. elegans (worm) model for OPA1 disease to screen candidate therapies. Therapeutic leads will be validated using multiple outcomes in three evolutionarily distinct species, which includes two worms, a zebrafish, and two patient cell models.
Methods: The two mutant worm strains in this study carries missense mutations in the GTPase domain. The strain developed by CRISPR/Cas9 technology carries a R289Q mutation, homologous to the disease-causing allele R290Q in humans. The strain obtained from a public repository carries a V328I mutation. The mutants were evaluated in diverse aspects of mitochondrial physiology, including mtDNA content (qPCR),

mitochondrial function (Seahorse respiration), and unfolded mitochondrial stress response (UPRmt) induction (genetic fluorescent reporter). Organismal fitness was also assessed by fecundity, development, and neuromuscular function (thrashing). UPRmt induction is being used to screen potential therapies currently in empiric use for different mitochondrial diseases, known mitophagy modulating drugs, and a commercial drug library of 2,500 FDA-approved and natural product compounds. Therapeutic leads will be validated in OPA1-/- zebrafish vision defects and in two patient cell lines (R290Q+/- and I403T+/-) at the level of cell survival (CellGlo) and mitochondrial physiology, which includes mtDNA content, mitochondrial content (MitoTracker Green), and mitochondrial membrane potential (TMRM).

Results: Both OPA1 mutant worm strains showed significant defects in mitochondrial physiology at the level of mtDNA content and respiration, as well as on organismal fitness at the levels of fecundity, development, and thrashing activity. The UPRmt was also significantly increased in both worm strains. Control drugs that partially rescue animal thrashing activity and mitochondrial stress were identified, with screening of 60 mitophagy modulating compounds and an FDA-approved and natural product compound library now in progress. A quantitative visual defect in the OPA1-/- zebrafish larvae has been identified by optokinetic response assay, which will be used to screen candidate therapies identified in the worm OPA1 mutant models. The human cell lines demonstrated defects in mtDNA content, mitochondrial respiration, and mitochondrial content.

Conclusions: Overall, we describe a novel worm (R289Q), a known worm (V328I) and two human patient cell (R289+/- and I403T+/-) models of OPA1 disease that display deficient mtDNA content and mitochondrial respiration dysfunction. Both worm mutant strains show impaired fecundity, development, and neuromuscular function. The zebrafish OPA1-/- model exhibits impaired vision, a hallmark of OPA1 disease. Compound screens are underway in the worm models, where therapeutic leads will be validated in the zebrafish and human fibroblast cell lines to help develop novel therapies for OPA1 disease.