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POSTER ABSTRACTS

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Cognitive debriefing of the Barth Syndrome-Symptom Assessment (BTHS-SA)

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OBJECTIVES: Barth Syndrome (BTHS) is a rare, X-linked genetic condition experienced primarily by males, resulting from mutations in the TAZ gene and is characterized by a variety of symptoms. The BarTH Syndrome-Symptom Assessment (BTHS-SA), a patient-reported outcome measure, captures patient perspectives on the severity of common BTHS symptoms. Adult (≥ 16 years) and adolescent (12-15 years) versions of the BTHS-SA were developed from interviews with individuals with BTHS and/or their caregivers. Cognitive debriefing interviews (CDIs) were conducted to evaluate the extent to which the BTHS-SA instructions, items, response options and relevance were understood by individuals with BTHS.

METHODS: The BTHS-SA daily questionnaire was designed to evaluate disease-defining symptoms of BTHS in adolescents (9 items) and adults (8 items), respectively. Each age-based version of the questionnaire uses a 24-hour recall period and a five-point verbal response scale. CDIs were conducted (adolescents and adults with BTHS; target N=12 each age group). CDIs are semi-structured, one-on-one interviews with trained researchers who prompt participants to complete and provide feedback on the interpretability and relevance for either version of the BTHS-SA. Transcribed interviews are serially coded and analyzed. Findings are summarized to determine potential revisions.

RESULTS: CDIs were conducted with twelve adults and ten adolescents with BTHS (N=22). Most participants were white males (n=20 [90.9%]; n=2 [9.1%] were Black or African American) from 12.5-34.9 years of age (mean 18.8 years, standard deviation [SD] = ± 6.4). For the majority of participants (n=20, 90.9%), the self-reported onset of BTHS symptoms occurred within the first five years of life. Interview data indicate that adult and adolescent participants can interpret the instructions, recall period, item concepts, and response options for the BTHS-SA as intended and that the item concepts are relevant to their experience. The addition of new concepts/item revisions was not supported. Final results (incorporating 2 more adolescent participant CDIs) will be presented in a manuscript.

CONCLUSIONS: Accurate measurement of BTHS symptoms is important in understanding the burden of illness and new-treatment effects. The CDI results show that the BTHS-SA is understandable and relevant to the experiences of individuals with BTHS. The results, with data collected from concept elicitation interviews, will be used to support the BTH-SA as a content valid questionnaire to assess treatment benefit in clinical trials for adolescents and adults with BTHS.

Understanding downstream cellular effects of TAZ deficiency in a novel CRISPR edited cellular model

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Barth syndrome (BTHS) is a genetic disorder characterized by features such as cardiomyopathy, skeletal myopathy, and intermittent neutropenia. BTHS is caused by defects in tafazzin (TAZ), an acyltransferase involved in the final remodeling step of cardiolipin (CL), which results in increased monolysocardiolipin (MLCL) and decreased mature CL on the inner mitochondrial membrane.

In order to study the downstream cellular effects of TAZ deficiency and to identify novel pathways of cellular pathogenesis, we developed and characterized a novel cellular model in HEK293 cells. CRISPR/Cas9-gRNA was used to edit the HEK293 cells within exon 2 of TAZ. A resultant 45bp deletion was identified in 3 individually isolated clones, which were then combined at a 1:1:1 ratio to create our TAZ deficient model, TAZ Δ 45. Characterization of TAZ Δ 45, showed a decrease in mature CL, a shift towards unsaturated CL, and an increase in MLCL.

For initial discovery, we performed untargeted proteomics on TAZ Δ 45 and TAZ WT cells. Cellular proteins were analyzed on an Orbitrap Fusion mass spectrometer and acquired data was searched against the SwissProt Human database by Mascot. We identified 8087 unique protein signatures, of which 241 proteins were significantly upregulated in TAZ Δ 45 compared to WT and 83 proteins were significantly downregulated in TAZ Δ 45 compared to WT.

We analyzed proteins that were 1.25-fold higher or 0.8 fold lower than WT expression using the bioinformatics resource DAVID (Database for Annotation, Visualization and Integrated Discovery) and identified 38 enriched KEGG pathways, 27 of which were significantly enhanced for our proteins of interest ($p < 0.05$). Collectively these pathways represent both mitochondrial and extramitochondrial bioenergetic adaptations, which functionally overlap with more common conditions including Non-alcoholic fatty liver disease (NAFLD), Alzheimer's Disease, and Parkinson's Disease. We then analyzed the genes encoding the differentially expressed proteins for enriched transcription factor sites using the bioinformatics tool PASTAA (Predicting Associated Transcription Factors From Annotated Affinities). This analysis identified a significant increase in genes regulated by transcription factors involved in similar cellular bioenergetic adaptations. This includes n-Myc, which has some regulatory control over the tricarboxylic acid (TCA) cycle, and transcription factors associated with NAFLD.

We also performed semi-targeted, quantitative LC-MS analysis of 24 bioenergetic metabolites, which provides functional support of our proteomics analysis, including metabolites associated with glycolysis, oxidative phosphorylation and the TCA cycle. A greater understanding of the downstream cellular effects will potentially uncover metabolic predictors of clinical status as well as identify targets for disease-specific therapies.

***Drosophila tafazzin* mutants have impaired exercise capacity**

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Cardiolipin (CL) is a mitochondrial phospholipid that helps maintain the normal structure of the inner mitochondrial membrane and stabilize the protein complexes of the electron transport chain to promote efficient ATP synthesis. Tafazzin, an acyl-transferase, is required for synthesis of the mature form of CL. Mutations in the tafazzin (TAZ) gene are associated with a human disorder known as Barth syndrome (BTHS). Symptoms of BTHS often include muscle weakness and exercise intolerance. Previous work demonstrates *Drosophila* TAZ mutants exhibit motor weakness resulting in reduced flying and climbing abilities. However, *Drosophila* TAZ mutants' response to exercise has not been examined. In this study, we examined the baseline exercise capacity of TAZ mutant flies, and their ability to adapt to exercise training. Prior to training, TAZ mutants demonstrated reduced endurance, flight, and climbing capabilities relative to control flies. After training, exercised TAZ mutants' endurance and flight ability did not improve. Although cardiac phenotypes are observed in human patients, no obvious cardiac phenotype was observed in *Drosophila* TAZ mutants. In the future, endurance may be a useful screening tool to identify genetic modifiers of tafazzin.

Exploring the sign and symptom experience of Barth syndrome in adult and adolescent populations

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OBJECTIVES: Barth Syndrome (BTHS) is a rare genetic condition with a variable expression of symptoms. This research aimed to better understand BTHS signs/symptoms in adolescent (15 years of age and younger) and adult populations (16 years of age and older).

METHODS: Concept elicitation interviews (CEIs) were conducted with adolescent (n=18) and adult (n=15) patients and caregivers (N=33) to identify and describe the signs/symptoms that characterize the BTHS experience. In open-ended, face-to-face interviews with trained researchers, subjects discussed their condition-related experiences. Transcribed interviews were coded and analyzed, and results were summarized in two BTHS conceptual models for each the adolescent and adult populations.

RESULTS: A total of 57 BTHS sign/symptom concepts were reported across samples (N=33) with saturation analyses confirming adequacy of sample size for each. Adolescents most frequently reported signs/symptoms of fatigue/tiredness (n=17, 94.4%), cardiomyopathy (n=14, 77.8%), muscle weakness (n=14, 77.8%), eating small quantities (n=11, 61.1%), and physical developmental delay (n=10, 55.6%). Fatigue/tiredness (n=9, 50.0%), headache (n=4, 22.2%), eating difficulty (n=3, 16.7%), and muscle weakness (n=3, 16.7%) were rated as the most bothersome symptoms. Adolescents most frequently reported muscle weakness (n=10, 88.9%) and fatigue/tiredness (n=8, 44.4%) as important symptoms to improve with treatment. Adults (n=15) most frequently reported signs/symptoms of fatigue/tiredness (n=15, 100.0%), cardiomyopathy (n=13, 86.7%), muscle weakness (n=12, 80.0%), neutropenia (n=12, 80.0%), and infection (n=9, 60.0%). Fatigue/tiredness (n=7, 46.7%), muscle weakness (n=6, 40.0%), and neutropenia (n=3, 20.0%) were rated as the most bothersome symptoms. Adults most frequently reported fatigue/tiredness (n=13, 86.7%) and muscle weakness (n=9, 60.0%) as important symptom treatment targets.

CONCLUSIONS: Though there were some differences, results suggest symptomatic similarities in the experience of BTHS as an adolescent and as an adult and, moreover, that both patient populations would find treatment meaningful if it targeted and reduced fatigue/tiredness and muscle weakness.

An unexpected lack of regulation of superoxide/H₂O₂ production rates in isolated heart and skeletal muscle mitochondria from a mouse model of Barth syndrome

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Barth Syndrome (BTHS) is an X-linked disorder caused by mutations in tafazzin gene and is characterized by loss of cardiolipin and severe cardiomyopathy. Recent advances in BTHS research suggest that mitochondrial superoxide/H₂O₂ production contributes to the cardiomyopathy observed in different BTHS models. Mitochondrial superoxide/H₂O₂ production is not a single process; there are at least eleven sites in mitochondria that can produce superoxide/H₂O₂ at significant rates. These sites are associated with the mitochondrial electron transport chain and matrix substrate oxidation. Which of these sites misbehave in BTHS and start to produce aberrant levels of oxidants is unknown. Here, we measured the maximum capacity of superoxide/H₂O₂ production from each site in mitochondria isolated from heart and skeletal muscle of tafazzin-deficient mice (tazkd) at 3, 7 and 12 months of age. Strikingly, the rates of superoxide/H₂O₂ production were overall undistinguishable between tazkd mice and their wild-type littermates in both heart and skeletal muscle across the time points analyzed. In a complementary approach, mitochondrial superoxide/H₂O₂ production was measured ex vivo under uninhibited conditions. Ex vivo rates of superoxide/H₂O₂ production were determined during the oxidation of a complex mixture of substrates mimicking either heart or skeletal muscle cytosol as appropriate and were found to be indistinguishable between wild-type and tazkd mice. Although superoxide/H₂O₂ production was not altered, we consistently measured decreased FAD-linked respiration in mitochondria isolated from tazkd mice along with a mild decrease in abundance of mitochondrial oxidative phosphorylation (oxphos) proteins. We conclude that the maximum capacity and ex vivo rates of superoxide/H₂O₂ production are not increased in mitochondria isolated from heart and skeletal muscle from tazkd mice, despite changes in oxphos activity and protein composition. Therefore, in this mouse model it seems unlikely that mitochondrial oxidants contribute to the development of cardiomyopathy. It may be prudent to contemplate alternative mechanisms that may link loss of tafazzin to cardiomyopathies and recognize potential shortcomings of this particular mouse model to resemble BTHS, especially considering the differences in the onset of disease.

AAV-mediated TAZ gene replacement restores mitochondrial and cardioskeletal function in Barth syndrome

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Barth syndrome (BTHS) is a rare mitochondrial disease that affects heart and skeletal muscle and has no curative treatment. It is caused by recessive mutations in the X-linked gene TAZ, which encodes tafazzin. To develop a clinically relevant gene therapy to restore tafazzin function and treat BTHS, three different adeno-associated virus serotype 9 (AAV2/9) vectors were tested to identify the optimal promoter (cytomegalovirus, desmin, or a native tafazzin promoter) in a double stranded AAV vector for TAZ expression following intravenous administration of 1×10^{13} vector genomes/kilogram to a mouse model of BTHS as either neonates or adults. Biodistribution of vector genomes, TAZ transcript and protein expression, mouse activity assessments, fatigue in response to exercise, muscle strength, cardiac function, mitochondrial structure, and electron transport chain complex activity assays were evaluated to measure the extent of functional improvement in treated mice. The desmin promoter (Des) provided strong TAZ expression levels to cardiac and skeletal muscle, improved mitochondrial structure, and resulted in significant functional improvement in every assessment. This study provides substantial support for translation of an AAV9-mediated TAZ gene replacement strategy to the clinic using a Des promoter for BTHS.

Aberrant cardiolipin metabolism is associated with cognitive deficiency and hippocampal alteration in tafazzin knockdown mice

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Cardiolipin (CL) is a key mitochondrial phospholipid essential for mitochondrial energy production. CL is remodeled from monolysocardiolipin (MLCL) by the enzyme tafazzin (TAZ). Loss-of-function mutations in the gene which encodes TAZ results in a rare X-linked disorder called Barth syndrome (BTHS). The mutated TAZ is unable to maintain the physiological CL:MLCL ratio, thus reducing CL levels and affecting mitochondrial function. BTHS is best known as a cardiac disease, but has been acknowledged as a multi-syndrome disorder, including cognitive deficits. Since reduced CL levels has also been reported in numerous neurodegenerative disorders, we examined how TAZ deficiency impacts cognitive abilities, and the function of hippocampal neurons and glia in TAZ knockdown (TAZ kd) mice. We have identified for the first time the profile of changes that occur in brain phospholipid content and composition of TAZ kd mice. The brain of TAZ kd mice exhibited reduced TAZ protein expression, reduced total CL levels and a 19-fold accumulation of MLCL compared to wild-type littermate controls. TAZ kd brain exhibited a markedly distinct profile of CL and MLCL molecular species. Interestingly, despite normal motor function, TAZ kd mice showed significant memory deficiency based on novel object recognition tests. This result correlated with reduced synaptophysin expression and derangement of the neuronal CA1 layer in hippocampus. Finally, brain microglial cells showed increased activation in TAZ kd mice compared to littermate controls. Collectively, our findings demonstrate that TAZ-mediated remodeling of CL contributes significantly to the expansive distribution of CL molecular species in the brain, plays a key role in maintaining normal cognitive function, and identifies the hippocampus as a potential therapeutic target for BTHS.

How do women adapt to being a Barth syndrome carrier? Results of a mixed methodological study of psychological adjustment and reproductive options

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Background: Barth syndrome (BS) is an X-linked cardiomyopathy characterized by pediatric onset, neutropenia, and skeletal myopathy caused by mutations in tafazzin (TAZ). Carriers are unaffected but navigate psychological and increasingly complex reproductive challenges.

Methods: To identify and describe common psychological and reproductive challenges and explore the role of social and familial support, we conducted semi-structured telephone interviews of 28 adult carriers recruited through the Barth Syndrome Foundation. Interviews were recorded, transcribed, double-coded, and analyzed for common themes. In phase 2 reproductive choices made were quantified and psychosocial adjustment measured via questionnaires.

Results: While guilt was experienced by most, but not all, mothers and grandmothers, relationships among carriers ameliorated distress by normalizing guilt. In contrast, participants held starkly different views of advanced reproductive technologies. For a few, any prenatal testing was unacceptable, but for many considering reproductive options was both morally and practically/financially challenging. Most mothers who considered pre-implantation or pre-natal diagnosis after having an affected child described a difficult decision-making process, likening affected embryos/fetuses to their sons. A few reached decisions that conflicted with long-held beliefs. In contrast to the strong mutual support carriers reported regarding the medical and practical aspects of BS, nearly all were wary of discussing reproductive planning. While nearly all stressed that their choices should not be normative for or influence others, most feared damaging relationships by broaching these personal and potentially political topics.

Conclusions: Enhanced support for reproductive decision-making may best come from sources outside traditional genetic support groups. With the increasingly prominent role of patient organizations in not only aiding families but also directing the research agenda, our data highlight potential fault lines leaders may encounter as members confront the potentially polarizing issues surrounding reproductive technology and reproductive choices.

Cardiolipin deficient cells require NAD generated from the fermentation pathway

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Background: Cardiolipin (CL) is a mitochondrial membrane phospholipid. Perturbation of the CL remodeling gene tafazzin leads to the X-linked disorder Barth syndrome (BTHS). In the yeast *Saccharomyces cerevisiae*, disruption of the CL synthesis gene *CRD1* or the tafazzin gene *TAZ1* leads to mitochondrial dysfunction, including defects in the electron transport chain (ETC) complexes.

The ETC carries out oxidative phosphorylation to generate ATP, accompanied by generating NAD from NADH. NAD is an important coenzyme that is synthesized from tryptophan and aspartic acid (*de novo*) or from precursors such as niacin. The recycling of NAD from NADH is carried out mainly by the ETC and subordinately by the yeast ethanol fermentation pathway or the mammalian lactate fermentation pathway. We **hypothesized** that the ETC defects of CL mutants lead to decreased NAD levels. To replenish the NAD required by glycolysis, CL mutants upregulate the ethanol fermentation pathway.

Methods: **1) SGA.** A *MAT α can1 Δ crd1 Δ* mutant was crossed to the array of deletion mutants (*MAT a*) that are linked to *KanMX4*. Double mutants were selected after sporulation. Growth (fitness) of the mutants was used to determine genetic interactions. **2). Cell growth.** Cultures at the stationary phase were diluted to A₅₅₀=0.5. After 1:10 serial dilution, 5 μ l of each dilution were spotted on synthetic complete plates and incubated at 30°C or 38°C. **3). NAD/NADH determination.** 200 μ l fresh cultures at the mid-log phase were treated in boiling ethanol (2.1ml ethanol buffered with 0.2ml 1M HEPES-NaOH, pH7.5) for three min. Samples were chilled on ice, followed by vacuum drying. Dried samples were suspended in 400 μ l H₂O before centrifugation at 5,000 x g for 10 min at 4°C. The supernatants were used for the NAD/NADH-Glo™ Assay (Promega). **4). RT-PCR.** Cultures in the mid-log phase were harvested. RNA was isolated and purified using the RNeasy Plus mini kit (QIAGEN). cDNA was synthesized using a cDNA synthesis kit (Roche). RT-PCR was performed using Brilliant III Ultra-Fast SYBER Green qPCR Master Mix (Agilent Technologies).

Results: **1). Lack of NAD is deleterious to *crd1 Δ* .** Deletion of NAD synthesis pathway genes *BNA7* or *NMA2* in *crd1 Δ* leads to decreased growth. *crd1 Δ* showed decreased growth on niacin⁻ medium at 38°C, which was rescued by supplementation with nicotinamide riboside (NR). **2). *crd1 Δ* has decreased NAD/NADH ratio at elevated temperature.** After transfer to 38°C for two hr, the NAD/NADH ratio of *crd1 Δ* decreased by 21.22% (p=0.017), while WT did not exhibit significant decrease. **3). Alcohol dehydrogenase (Adh) function is important for CL mutants.** Deletion of *ADH2* or *ADH5* decreases the growth of *crd1 Δ* . *crd1 Δ* and *taz1 Δ* exhibit increased transcription of *ADH1*, *ADH2*, and *ADH3* at 38°C.

Conclusions: The negative synthetic interactions between *CRD1* and NAD synthesis genes indicate the importance of NAD to *crd1 Δ* . This is supported by the growth defect of *crd1 Δ* on niacin⁻ medium, which is rescued by supplementation with a NAD precursor, NR. Moreover, *crd1 Δ* exhibits decreased NAD/NADH ratio compared to WT at elevated temperature. The demand of *crd1 Δ* for NAD precursors suggests a defect in NAD synthesis and/or recycling. To address the need for NAD, *crd1 Δ* and *taz1 Δ* increase transcription of alcohol dehydrogenase genes. We predict that this is accompanied by increased ethanol levels that can be lowered by supplementation with NR. Understanding the regulation of NAD and the benefits of NR in CL mutants may shed light on the pathogenesis and treatment of BTHS.

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Cardiolipin is required for optimal activation of pyruvate dehydrogenase, synthesis of acetyl-CoA, and TCA cycle function

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Background: Cardiolipin (CL) is a unique phospholipid that is localized primarily in the membranes of mitochondria and plays diverse roles in cellular functions. Mutations in the gene *tafazzin* encoding the transacylase for CL remodeling lead to the life-threatening disorder, Barth syndrome (BTHS). However, the mechanisms linking CL deficiency to the pathology observed in BTHS is unknown. Our previous studies indicate that CL deficiency in the yeast CL synthase mutant (*crd1Δ*) leads to decreased activities of TCA cycle enzymes aconitase and succinate dehydrogenase (SDH). In addition, yeast *crd1Δ* cells exhibit decreased acetyl-CoA levels. We proposed that the TCA cycle is also defective in the tafazzin knock-out (TAZ-KO) C2C12 mouse cell line. To test this, we analyzed metabolic flux of [U-¹³C] glucose in TAZ-KO cells.

Methods:

1. **[U-¹³C] glucose flux analysis in TAZ-KO cells.** Cells were grown at 37°C in a humidified incubator with 5% CO₂. For flux analysis, cells were incubated in serum-free medium containing 1 g/L [U-¹³C] glucose for 1 h. Cells were collected and MS analysis was performed.
2. **Pyruvate dehydrogenase (PDH) and SDH activity assay.** Mitochondrial PDH activity was measured spectrophotometrically by determining the reduction of NAD⁺ to NADH, coupled to the reduction of a reporter dye to yield a colored reaction product with an increase in absorbance at 450 nm at 37°C (Biovision). SDH activity was measured as the velocity of 2,6-dichlorophenolindophenol (DCPIP) reduction, corresponding to a decrease in absorbance at 600 nm.
3. **Pyruvate assay.** Intracellular pyruvate levels were quantified using a pyruvate assay kit (Cayman Chemical). In brief, pyruvate from cell extracts was converted to acetyl phosphate, hydrogen peroxide (H₂O₂), and carbon dioxide. H₂O₂ reacts stoichiometrically with 10-acetyl-3,7-dihydroxyphenoxazine (ADHP) to produce the fluorescent compound resorufin, which has an excitation wavelength between 530-540 nm and an emission wavelength between 585-595 nm.

Results:

1. **Decreased flux of [U-¹³C] glucose to acetyl-CoA in TAZ-KO cells.** The percentages of ¹³C-labeled acetyl-CoA, citrate/isocitrate, malate, succinate, fumarate, glutamate, and aspartate were decreased in TAZ-KO cells relative to wild type cells.
2. **CL activates PDH.** PDH activity was decreased by about 50% in TAZ-KO mitochondria, and CL restored activity of PDH in mutant mitochondria to wild type levels. Incubation of CL with purified PDH complex resulted in increased enzymatic activity.
3. **Increased pyruvate in TAZ-KO cells.** TAZ-KO cell extracts exhibited increased levels of pyruvate.
4. **Decreased SDH activity in TAZ-KO cells.** Mitochondria isolated from TAZ-KO cells exhibited a 40% decrease in SDH activity compared to the control. By comparison, activity of the TCA cycle enzyme malate dehydrogenase was not affected in mutant mitochondria.

Conclusions: These findings indicate that CL plays an important role in the regulation of TCA enzyme activities and cellular metabolism. CL is required for optimal activation of PDH, mediated by pyruvate dehydrogenase phosphatase, suggesting potential targets for treating BTHS.

Raising children with Barth syndrome: Impact on the family

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Background: Barth syndrome (BTHS) is a rare disorder with an onset in infancy. Most previous studies have investigated the natural history and clinical features of BTHS, while the impact of a child's health conditions on their parents' daily lives and parenting occupations has received less research. To increase understanding of the impact of raising a child with BTHS, this study compared parental quality of life (QoL), family functioning, and family cohesion between families with and without children with BTHS.

Method: A cross-sectional design was used for this study. Parents of children between the ages of 4 and 19 with BTHS ($n = 35$), and parents of age-matched unaffected children ($n = 35$) participated in this study. Both groups of parents completed the questionnaires measuring parental QoL, family functioning, and family cohesion. Parental QoL and family functioning were examined using the PedsQLTM Family Impact Module, and family cohesion was assessed using the Family Adaptability and Cohesion Evaluation Scale-IV; higher scores on each instrument indicate better functioning. Independent-samples t test was conducted to compare parental QoL, family functioning, and family cohesion between the parents of children with BTHS and parents of unaffected children.

Results: The results of the independent-samples t test showed that the parental QoL ($t(68) = 2.821, p < .05$) and family functioning ($t(68) = 2.410, p < .05$) scores of the unaffected group were significantly higher than the BTHS group. However, in family cohesion, the score of the affected group was not significantly different from the BTHS group ($t(68) = 1.546, p = .127$). In addition, there was a tendency that the mean of the family cohesion in the BTHS group ($m = 3.02, sd = 1.01$) was higher than that of the unaffected group ($m = 2.67, sd = 0.87$).

Conclusions: The findings of this study show that parental QoL and family functioning of families of children with BTHS were negatively affected by their child's health. However, the mean score of the cohesion in the families of children with BTHS was higher than that of the families of unaffected children, possibly indicating that families of children with BTHS are successfully working together to meet the challenges they face. The findings suggest the need for development of effective strategies to support parents' and families' occupations and positive family functioning. This study provides empirical evidence important for strengthening evidence-based approaches supporting parents and families.

Assessing olfactory functions in patients with Barth syndrome

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Background: Barth syndrome is a rare X-linked disease affecting less than 200 individuals worldwide. Several comorbidities have been associated with the pathology and, among those, cardiac myopathy and neutropenia are the most life threatening. The appropriate nutritive support is important to sustain the everyday life of Barth syndrome patients given the chronic fatigue they experience. Since they often prefer salty and fried food, and avoid vegetables and fruits, their eating habit and food preferences do not always provide the proper amounts of vitamins and amino acids. It has been indeed reported that Barth syndrome patients have altered taste sensitivity. As olfaction also contributes to food consumption and flavor perception, we decided to investigate their olfactory abilities.

Methods: We used the “Sniffin’ sticks’ extended test” (odor threshold, odor discrimination and odor identification) to explore the Barth syndrome patient olfactory abilities.

Results: We found no significant difference in any of the tested olfactory abilities between the group of Barth syndrome patients and the healthy controls. Nevertheless, we observed the tendency to improve the scores in neutropenic patients or under medication with G-CSF (33% of Barth subjects) that is worth to discuss.

Conclusions: Altered food preference of Barth boys could not be easily explained with an altered olfactory perception.

A novel role for cardiolipin remodeling in mitigating the effects of cardiolipin peroxidation

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Cardiolipin (CL) is a unique phospholipid that is localized almost exclusively within the mitochondrial membranes where it is synthesized. CL interacts with many mitochondrial proteins and plays important roles in mitochondrial function. Newly synthesized CL undergoes acyl remodeling to produce CL species enriched with unsaturated acyl groups. Loss of the CL remodeling enzyme tafazzin leads to Barth syndrome. Cld1 is the only identified CL-specific phospholipase in yeast and is required to initiate the CL remodeling pathway. In addition to its role in CL remodeling, deacylation of CL results in the generation of mitochondria-derived free fatty acids, which may modulate various cellular functions. In higher eukaryotes, peroxidation of CL, yielding CLO_x, has been implicated in the cellular signaling events that initiate apoptosis. CLO_x can undergo enzymatic hydrolysis, resulting in the release of various lipid mediators with diverse signaling properties. Our previous findings suggested the possibility that *CLD1* expression is upregulated in response to oxidative stress, and that one of the physiological roles of CL remodeling is to remove peroxidized CL. We have further determined previously that yeast cells can incorporate oxidizable polyunsaturated fatty acids (PUFAs) into CL. To exploit the powerful yeast model to study functions of *CLD1* in CL peroxidation, we expressed the *H. brasiliensis* Δ^{12} -desaturase gene in yeast, which then synthesized PUFAs that are incorporated into CL species. Using LC-MS based redox phospholipidomics, we identified and quantified the molecular species of CL and other phospholipids in *cld1* Δ vs WT cells. Multiple species of mono-hydroperoxy-CL and di-hydroperoxy-CL were detected in the Δ^{12} -desaturase-expressing cells. Loss of *CLD1* in these cells led to a dramatic decrease in chronological life span and increased levels of mono-hydroperoxy-CLs, particularly among the highly unsaturated CL species, including tetralinoleoyl-CL. In addition, purified Cld1 exhibited a higher affinity for CLO_x, and treatment of cells with H₂O₂ increased *CLD1* expression in the logarithmic growth phase. Taken together, these data suggest that *CLD1* expression is required to mitigate oxidative stress. The findings from this study contribute to our overall understanding of CL remodeling and its role in mitigating oxidative stress. This study also indicates that oxidation of mitochondrial CL may have arisen early in evolution and provides a framework for future studies to define the mechanisms underlying CL peroxidation and its cellular significance.

Management of disease impacts and goal setting in rare, severe, pediatric health conditions

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Socioemotional selectivity theory describes how individuals with limited life expectancies and poor functional health (e.g., older adults) often prioritize short-term, emotion-based goals above the longer-term goals that younger, healthy individuals may target, such as knowledge acquisition. Children born with severe, degenerative, incurable chronic illnesses experience a discrepancy between the “on-time” expectation that adolescents and young adults prioritize future-oriented goals and the “off-time” emotion-based goal-setting that is most relevant in the context of their physical limitations and shortened life expectancies. This research evaluates goal-setting in Barth Syndrome (BTHS), a rare genetic condition among males characterized by cardiac complications, fatigue, weakness, infection, and growth delay experienced from birth. Few men with BTHS survive to midlife. Hourlong face-to-face interviews were conducted with 33 individuals with BTHS (ages 2 to 34) and/or their caregivers. Participants reported prioritizing short-term, emotion-based goals focused on symptom control and personal relationships, as opposed to longer-term goals such as pursuit of higher education or professional careers. Individuals with BTHS selected limited goals (e.g., modified participation in activities) and compensated for their lack of abilities (e.g., by using assistive devices). Participants primarily reported using secondary coping mechanisms (emotion management and downward comparisons) to manage the impacts to physical functioning and risk of early mortality. Boys learned how to practice goal selection from their caregivers who helped to manage their participation in activities. Individuals with BTHS reckon with the limited feasibility and value associated with investing in longer-term activities focused on knowledge acquisition, more closely resembling older adults than their age-peers.

Legitimization of chronic illness at the intersection of severity, visibility, and control over symptoms: A case study of Barth syndrome

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Individuals with unapparent, severe symptoms that cannot be treated or controlled may struggle to gain legitimacy for their health condition. These individuals must make their health status known to others to mitigate others' doubts and negative perceptions (e.g., that one is lazy or demotivated) and to obtain the necessary institutional support to manage the impacts of their condition. Research thus far has primarily focused on commonly experienced, less severe pediatric or adult-onset illnesses, as opposed to life-limiting and life-threatening early-onset, rare health conditions. Studies report that males with chronic illnesses tend to disassociate from their condition, aiming to appear healthy, rather than seeking to openly manage their illness. This research evaluated how individuals with Barth Syndrome (BTHS) experience and aim to legitimize their condition. BTHS is a rare and severe condition in males characterized by largely unapparent symptoms including cardiomyopathy, fatigue, muscle weakness, neutropenia, and growth delay. The risk of mortality in BTHS remains high, however, overall survival with the condition has improved due to advancements in its diagnosis and management. Therefore, a growing number of individuals with BTHS will require institutional support as their health outcomes improve. Thirty-three open-ended interviews were conducted face-to-face with an international sample of individuals with BTHS and/or their caregivers. Each 60-minute interview was audio-recorded, transcribed, and anonymized. Transcripts were analyzed using a qualitative data analysis program. The analytic approach was deductive (i.e., informed by prior theory regarding the role of symptom severity, visibility, and control in the social and institutional legitimization of illness) as well as inductive (i.e., informed by the responses elicited from participants in this sample). It was hypothesized that individuals with BTHS would publicly manage and make their condition known to others to mitigate negative perceptions and promote the legitimization of BTHS. Interviews were conducted with participants ≤ 15 years of age ($n=18$, mean age=8.6 years, $SD=\pm 3.9$, range 2.5-15.0) and/or their caregivers, and with individuals ≥ 16 years of age ($n=15$, mean age=22.9 years, $SD=\pm 5.8$, range 16.0-34.0). Almost all participants reported being Caucasian. Social legitimization of BTHS was informed by the severity, visibility, and uncontrollability of the condition. The symptoms of BTHS are severely limiting on a daily basis and life-threatening, requiring preventive management (e.g., guarding against infection and heart complications). Symptom severity made it unrealistic and dangerous for individuals to pass as healthy (e.g., participation in physical activities such as contact sports was not possible or too risky). The public management of BTHS-related physical impacts (e.g., the use of assistive devices to facilitate walking) made the otherwise unapparent symptoms of weakness and fatigue visible to others. This confirmed one's status as being sick rather than lazy or disinterested. Individuals with BTHS and their caregivers attempted to make others aware of the uncontrollable, severe nature of the condition to promote understanding of and support for BTHS. The process by which individuals aim to legitimize unapparent chronic health conditions is informed by the intersecting experience and levels of disease severity, visibility, and control as is evidenced by the case of BTHS.

Studying Barth syndrome's pathomechanism using high throughput screens in yeast

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Tafazzin is a mitochondrial protein that is involved in the metabolism of cardiolipin (CL) by functioning as a phospholipid-lysophospholipid transacylase. Mutations in the tafazzin gene are associated with a number of clinical disorders including Barth syndrome (BTHS). The tafazzin gene is highly conserved from yeast to human. Deletion of the yeast orthologue TAZ1 results in a decrease in the total cellular amounts of CL, increased levels of mono lyso CL (MLCL), and compromised functions of mitochondria. Similar alterations were also observed in fibroblasts and platelets from patients with BTHS or from animal models. Currently, the biochemical mechanisms underlying the mitochondrial dysfunction in BTHS remain uncharacterized. To understand better the cellular effects of losing Taz1 activity, we screened in yeast cells for multi-copy suppressors of the *taz1Δ* growth defect on ethanol-containing medium. Importantly, we identified the branched-chain amino acid transaminases (BCATs), Bat1 and Bat2 as multicopy suppressors of the *taz1Δ* growth defect. Along this line, supplying *taz1Δ* cells with the branched-chain amino acid valine or leucine improved their growth on ethanol. Surprisingly, overexpression of Bat1 or Bat2 does not restore the reduced membrane potential, the altered stability of respiratory chain complexes, and the defective accumulation of MLCL species in cells devoid of Taz1. Hence, our findings suggest that metabolism of amino acids has an important role in cells lacking Taz1 function and thus shed new light on the pathomechanism of Barth syndrome. These results also hint to a potential usage of branched-chain amino acids as a therapy avenue to treat patients with Barth syndrome.

An appraisal of the tafazzin Knockdown Mouse Model of Barth syndrome: What have we learned?

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Disclosure: This work was supported by the Barth Syndrome Foundation.

Background & Objective. Barth syndrome (MIM 302060) is a mitochondrial disorder in which patients are afflicted by cardiomyopathy, skeletal myopathy, neutropenia, and growth delay. Mutations in a mitochondrial phospholipid transacylase (tafazzin) lead to cardiolipin deficiency and mitochondrial dysfunction. A mouse model of Barth syndrome was developed almost 10 years ago: This model relies on a doxycycline-inducible shRNA to knock down expression of tafazzin mRNA (“TAZKD”). The goals of any model include a deeper understanding of disease pathogenesis, and translational insights into potential therapies. Our objective was to review published data from the TAZKD mouse to determine its contributions to our pathogenetic understanding of and potential treatment strategies for, Barth syndrome.

Methods. We focused on the following questions: 1) Does the mouse mimic the clinical syndrome? 2) Has this model led to a deeper understanding of disease pathogenesis? 3) Have potential therapies been suggested by this model?

Results.

1) Clinical syndrome: Two distinct cardiac phenotypes have been reported, depending on the doxycycline induction protocol used to knock down TAZ mRNA: 1) perinatal lethality due to severe diastolic dysfunction associated with ventricular noncompaction, and 2) a mild, adult-onset dilated cardiomyopathy. Cardiolipin profiles showed a deficiency in the normal tetralinoleoyl cardiolipin and a marked increase in monolysocardiolipin. Skeletal muscle strength is reduced in this mouse model. Although basal resting metabolic rates are unaffected, TAZKD mice exhibited markedly impaired oxygen consumption during exercise. Mitochondrial and muscle fiber ultrastructural abnormalities are present. These physiological, biochemical, and ultrastructural abnormalities mirror those in Barth patients. However, TAZKD mice have not demonstrated any significant ventricular arrhythmias, nor has this model exhibited growth delay or a known neutropenia.

2) Disease pathogenesis: Tafazzin deficiency destabilizes respiratory chain complexes by disrupting supercomplex assembly. Proteomic analysis of TAZKD mouse hearts demonstrated reduced components of the mitochondrial respiratory chain; marked metabolic remodeling, including disruptions in the interactions between the electron transport chain and fatty acid oxidation enzymes; and reduced mitochondria-bound myoglobin, potentially disrupting intracellular oxygen delivery to the OXPHOS system. Clinical metabolomics data from Barth patients showed similar metabolic aberrations. Disruption of supercomplex assembly with cardiac-specific loss of succinate dehydrogenase in the TAZKD mouse model was also confirmed in Barth patient cardiomyocytes.

3) Potential therapies: The PPAR pan-agonist bezafibrate ameliorated the cardiomyopathy in TAZKD mice, while increasing mitochondrial biogenesis. A clinical trial is now underway in the UK to test bezafibrate in Barth syndrome patients.

Conclusion. The TAZKD mouse model of Barth syndrome has led to important insights into disease pathogenesis and potential therapeutic targets.

A multi-faceted approach to enhance pill-swallowing ability in children and adults with Barth syndrome

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Objective: Individuals with Barth syndrome (BTSH) present with sensory and motor deficits that influence their ability to swallow medications in solid form. The purpose of this study was to evaluate the effectiveness of a brief, multi-faceted pill swallowing program that used evidence-based training methods.

Methods: Sixteen individuals with BTSH, ages 6-34, participated in the training. Possible intervention strategies included a behavioral approaches, adaptive strategies, or positioning strategies. Pill swallowing milestones were ranked on a scale from 0-12; participants were scored pre-training, immediately post-training, and at 6-months post-training.

Results: Fourteen out of the 16 participants demonstrated improvement in their pill swallowing ability. Overall, there was a statistically significant change in pill swallowing ability from pre-training to post-training, and these changes were maintained after six months.

Conclusions: This study suggests that a brief multi-faceted training approach, led by trained professionals, may be effective for helping individuals with sensory and motor deficits learn to swallow pills independently.

Dietary intake and hedonic preferences for sodium in Barth syndrome

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Background: Individuals with Barth syndrome (BTHS) present with decreased activity tolerance and increased fatigability. Recent evidence suggests that differences in metabolic function in BTHS negatively impact the production of aerobic energy for activity and maintenance of muscle mass. Recommendations on a metabolically beneficial diet are complicated by selective eating behaviors often seen in individuals with BTHS. While known to be selective eaters with a preference for salty foods, there is limited evidence on specific food preferences and dietary intake for individuals with BTHS. The purpose of this study was to explore “liking” and consumption frequencies among food groups and to determine hedonic “liking” and perception of sodium intensity.

Methods: A non-experimental, cross-sectional design was employed which compared the BTHS group to age-matched controls. A food inventory was adapted to compare food consumption frequencies and liking across seven categories. A taste test was conducted to explore liking and perception of different salt concentrations.

Results: Overall individuals with BTHS did not consume salty foods more frequently than their peers, though sodium rich foods appear to make up a larger portion of the daily diets compared to other foods like vegetables, fruits, and grains. Findings from our structure taste test indicated that the BTHS population had a slightly higher hedonic preference for sodium infused foods (broth) than their age-matched peers but had similar sodium discrimination abilities.

Conclusion: This study was the first to systematically test ubiquitous reports of a high sodium preference in the BTHS population. Results confirmed prior clinical and family reports of a restricted dietary intake in individuals with BTHS, and a higher than typical preference for foods with high salt content. More research is needed to determine the health implications of a sodium rich diet for individuals with BTHS.

AAV9-TAZ gene replacement ameliorates cardiac TMT proteomic profiles in a mouse model of Barth syndrome

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Barth syndrome (BTHS) is a rare mitochondrial disease that causes severe cardiomyopathy and has no disease modifying therapy. It is caused by recessive mutations in the gene TAZ, which encodes tafazzin. Tafazzin is an acyltransferase that remodels cardiolipin (CL) – an inner mitochondrial membrane lipid. To identify novel mechanistic pathways involved in BTHS and evaluate the effects of gene therapy on proteomic profiles, we performed a multiplex tandem mass tagging (TMT) quantitative proteomics analysis to compare protein expression profiles from heart lysates isolated from BTHS, healthy wild-type (WT), and BTHS treated with adeno-associated virus TAZ gene replacement (AAV9-TAZ) as neonatal or adult mice. A total of 197 proteins with ≥ 2 unique peptides were identified. Of these, 91 proteins were significantly differentially expressed in BTHS compared to WT controls. Cause-effect relationships between tafazzin deficiency and altered protein profiles were confirmed through demonstrated significant improvements in expression levels following administration of AAV9-TAZ. This study provides support for translation of AAV9-TAZ gene therapy to the clinic and identifies novel mechanistic pathways involved in the pathophysiology of BTHS.

Unraveling new potential therapeutic targets in iPS-CM model of Barth syndrome

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There is a lack of effective specific therapies for cardiological complications in Barth syndrome due to the limited understanding of cellular pathophysiology and myocardial metabolism in human. Here, we established isogenic control and TAZ (frameshift c.517delG) induced pluripotent stem cell-derived cardiomyocytes (iPS-CM) as a cellular model of Barth Syndrome and applied targeted gene expression, metabolic profiling and fates of ¹³C labeled substrates to explore TAZ induced functional abnormalities. Regularly beating control and TAZ-iPS-CMs were obtained by differentiation from corresponding iPS by Wnt/ β catenin pathway modulation under fully defined conditions. All cells displayed sarcomeric organization as indicated by α -actinin and troponin I (TNNI3) co-staining and appearance of z lines. Both control and TAZ-iPSCM survive in glucose-depleted conditions and used lactate to maintain active metabolism for several days.

Our molecular findings suggest impairment in Ca²⁺ handling as was determined by decreased sarco/endoplasmic reticulum Ca²⁺ATPase (SERCA2a) gene expression. TAZ-iPSCMs also exhibit high fatty acid protein (FABP4) expression levels and elevated free fatty acid that may cause a detrimental effect on cardiomyocyte's contraction.

To determine the effect of TAZ mutation on cellular metabolism, we cultured iPSCM with ¹³C labeled substrates and traced ¹³C incorporation to the relevant metabolites. Both control and TAZ-iPS-CM exhibit detectable 3-methylglutaconic acid (3MGC) levels and same fractional synthesis rate. ¹³C incorporation pattern suggest a possible metabolic origin of 3-MGC. Under low glucose conditions, TAZiPS-CMs demonstrate alteration in citric acid cycle intermediates levels and mild remodeling in fatty acid utilization as a carbon source for citrate synthesis. These results can be attributed to TAZ-induced mitochondrial dysfunction, whereas TAZ-iPS-CMs are seeking to utilize oxygen in a more efficient way.

Our findings also indicate flux of glucose through hexosamine pathway (HBP). TAZ-iPSCM demonstrated decreased fractional synthesis and a low total pool of uridine diphosphate-N-acetylGlucose amine (UDP-GlnNAc) a final product of HBP and a substrate for glucosamine (O-GlcNAc), a posttranslational modifier of cytosolic and nuclear proteins. In conclusion, our study suggests SERCA and FABP4 as new potential therapeutic targets for the cardiac manifestation of Barth syndrome and reveal some metabolic remodeling implicated to TAZ (frameshift c.517delG) mutation.

Increased anaerobic metabolism during exercise in Barth syndrome may result from augmented liver glycogenolysis

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Individuals with Barth syndrome (BTHS) have altered metabolic functioning arising from a mutation in the tafazzin gene leading to an impairment of the acyltransferase activity in making tetralinoleic cardiolipin, the most abundant phospholipid in inner-mitochondrial membrane. As an effect, BTHS patients have exercise intolerance and show an excessive reliance on anaerobic metabolism resulting in high blood lactate concentrations at low exercise intensities. Increased anaerobic metabolism during exercise are supportive of enhanced gluconeogenesis to meet energy demand, however this has not yet been evaluated in BTHS. We, therefore, hypothesized that the increased anaerobic metabolism observed in BTHS patients during exercise is a result of enhanced gluconeogenesis. Using the BTHS mouse model, liver $2\text{H}/^{13}\text{C}$ metabolic flux analyses were performed on male tafazzin knockdown mice (TazKD, $n=9$) and wildtype (WT, $n=7$) littermates during an acute exercise bout. Mice were catheterized to perform infusions and blood draws, infused with stable isotopes of $[6,6-2\text{H}_2]$ glucose and $[^{13}\text{C}_3]$ propionate, and underwent an acute exercise bout. TazKD mice exhibited exercise intolerance with not all mice able to perform the 30 min exercise bout. Blood samples from mice at 30 min of exercise or exhaustion were analyzed via gas chromatography-mass spectrometry to determine metabolic differences. Blood glucose was significantly higher in Taz KD after acute exercise. There was a trend for increased endogenous glucose production in TazKD ($p=0.064$) compared to WT when assessing flux from glucose-6-phosphate to glucose after exercise. Further, there was a significant increase in the flux of glycogen to glucose-6-phosphate in the TazKD ($p<0.05$) after exercise. These data suggest that tafazzin knockdown results in increased liver glycogenolysis after acute exercise, and that this metabolic substrate may be preferentially used to support increased anaerobic metabolism during exercise, which is observed in humans with BTHS. Future analyses will assess mechanisms of exercise-induced glycogen depletion in liver.

Cardiolipin controls oxidative phosphorylation through lactate signaling

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Mammalian tissues that are highly oxidative such as heart and skeletal muscle contain cardiolipin (CL) molecules with linoleate (LA)-rich side chains, whereas highly glycolytic tissues such as brain and lens tend to have alternate side chains such as oleate. In the cardiac disease states of heart failure and Barth syndrome, CL is also LA depleted suggesting an increase in the dependence on glycolysis. Previous studies have shown that the presence of LA side chains on CL allows the side chains to tightly pack such that electrons can efficiently flow through the CL during electron transport. Alternate side chains may lead to the escape of electrons causing increased reactive oxygen species (ROS) and slowing oxidative phosphorylation (OxPhos). However, the factor that controls CL remodeling in tissues, disease states, or development has not been determined. We hypothesize that lactate, which is generated during glycolysis, may be a signaling molecule that can slow the degree of LA remodeling in CL and therefore slow the rate of OxPhos.

Neonatal rat ventricular myocytes (NRVMs) were treated with 10 or 20 mM lactate for 72 to 96 hours and CL molecular species were determined via electrospray ionization mass spectrometry. Mitochondrial respiratory control ratio and ROS production were measured using Oroboros O2K high resolution respirometry, OxPhos rates were determined using a Seahorse apparatus, and mRNA levels of CL synthase (CLS), tafazzin (TAZ), and monolysocardiolipin acyltransferase (MLCL AT) were determined using qRT PCR. CL molecular species and mitochondrial OxPhos were also examined from heart and liver from 2 day old, 2 week old, and adult rats.

The ratio of tetralineoyl CL (L4CL) to tetraoleoyl CL (O4CL) was significantly decreased with lactate treatment in NRVMs. Lactate also caused a significant decrease in the mitochondrial respiratory control ratio and increase in ROS as well as a decrease in OxPhos. mRNA for CLS was increased and for the remodeling enzymes MLCL AT and TAZ was decreased. L4CL/O4CL in 2 day and 2 week rat hearts was significantly decreased compared to adult heart and the mitochondrial OxPhos was lower in the young hearts compared to adult rat heart. In liver, L4CL/O4CL was unchanged during development.

In conclusion, lactate causes upregulation of gene expression of CLS and downregulation of remodeling enzymes resulting in less LA side chains on CL and leading to lower rates of OxPhos and higher ROS production. During early development of the rat heart, but not liver, CL has less LA and lower mitochondrial OxPhos rates. Future experiments have been designed to test lactate levels during development.

Understanding the life experience of Barth syndrome from the perspective of older individuals

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BACKGROUND: Individuals with Barth Syndrome (BTHS) who survive to adulthood have historically experienced shortened life expectancy. Given this, little is known regarding the life experiences of older individuals with BTHS. Specifically, there is a paucity of information regarding the symptom experience and progression of the condition over time, as well as the long-term impact of BTHS on individuals' daily physical functioning and quality of life. As greater numbers of individuals with BTHS survive past infancy due to advancements in diagnosis and disease management, understanding the progression of BTHS over time is becoming increasingly important.

OBJECTIVES: The purpose of this research is to explore the lived experience of BTHS over time from the perspective of older men with the condition, and specifically, to gather information on the potential degenerative and disabling nature of BTHS in adulthood. To achieve this goal, in-depth interviews with older men with BTHS are being conducted and qualitatively analyzed. Interviews are designed to elicit information regarding symptom onset and progression, diagnosis and management of BTHS, and impact to daily life and overall quality of life (e.g., ability to work, life independence, coping strategies for symptom experience).

METHODS: Independent review board approval for the study was obtained in April 2018, and interview conduct with individuals living in Europe and the United States commenced in May 2018. Individuals selected for interview participation were identified in collaboration with the Barth Syndrome Foundation. Trained researchers from Adelphi Values are currently conducting semi-structured interviews, either via telephone or in-person, with approximately six individuals over 35 years of age with BTHS. Each interview will last approximately 60 minutes in duration and will be audio-recorded, transcribed verbatim and anonymized, and qualitatively coded and analyzed.

RESULTS: Qualitative data from the interviews will be analyzed to inform the important and relevant themes reported by individuals regarding life experience of BTHS in adulthood.

CONCLUSIONS: Conclusions drawn from analyses of these interview data will serve to inform current understanding of the disease experience of BTHS over time and help to characterize the relevant health outcomes and needs of older individuals with BTHS.

Neutropenia clue in bone marrow of TAZ deficient mouse model

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Barth syndrome is an X-linked inherited disorder characterized with cardiomyopathy, skeletal myopathy, and neutropenia. In1997, Yakisan et al^[1] described a high incidence of bone mineral loss in a series of patients with severe congenital neutropenia. Until 2003, different degrees of osteoporosis/ osteopenia have been reported in 47.9% of 236 children and adult patients with severe neutropenia (ANC: absolute neutrophil count < 500 mm³) through bone density measurements^[2]. We utilize 1-year-old male TAZ KD mouse model and focus on bone marrow hematopoietic progenitor differentiation relevant to bone density, to investigate their interconnection and pathological mechanism for Barth neutropenia.

First of all, in micro CT scan, less trabecular bone volume (Tb BV/TV, %) in TAZ KD femurs has been found as a functionally important deficiency compared to age-matched WT femurs (2.7379±0.9540 vs. WT 4.5251±1.5658, n=8-12, *t*<0.01). Nevertheless, trabecular bone mineral density (BMD) demonstrated not only bone volume but also bone density decreased in the TAZ femurs (BMD: 0.0523±0.0016 vs. WT 0.0556±0.0040, n=8-12, *t*<0.05).

Secondly, we found neutropenia indeed occurred in bone marrow of TAZ KD male mice based on hematopoietic cell counting on per smear head area of bone marrow smear. For diagnosis, bone marrow can be described quantitatively^[3] with erythroid to neutro-myeloid cell ratio (TAZ KD: 4.4566±1.1489, WT: 1.8708±0.7795, n=2-3, *t*<0.05) and immature to mature neutrophil cell ratio (TAZ KD: 0.5499±0.0149, WT: 0.9733±0.2929, n=2-3), detail results seen in Table below.

Bone marrow hematopoietic cells (BM smear counting cell number)	1yo TAZ KD male mice		1yo WT male mice		<i>t test</i> (WT vs TAZKD)
	Ave±SD (n=3)	(%)	Ave±SD (n=2)	(%)	
Neutrophil myeloblast	85.3±76.1	0.97 ↓	516.5±316.1	5.88	0.147656
Neutrophil metamyeloblast	73.7±64.5	0.84 ↓	323.5±4.95	3.69	<u>0.010352</u>
Banded neutrophil	362.3±134.6	4.13 ↓	619.0±107.5	7.05	<u>0.054362</u>
Segmented neutrophil	948.3±148.0	10.8 ↓	1603.0±691.6	18.26	0.201733
Pro-monocyte & monocyte	719.0±293.6	8.19 ↑	261.0±135.8	2.97	<u>0.051703</u>
Pro-eusinophil & eusinophil	110.3±56.1	1.26 ↑	23.5±20.5	0.27	<u>0.051248</u>
Pro-, poly-, ortho-erythroblasts	6375.3±594.9	72.62	5379.5±712.1	61.28	0.123922

Similarly and more severely, 4-month old TAZ KO male mice presented neutropenia in 4 femur/tibia bones-derived bone marrow hematopoietic cell counting on per entire bone marrow smears (total cell counting per 60,000-100,000). Erythroid to neutro-myeloid cell ratio (TAZ KO-1: 13.7336, WT: 7.1462) showed almost a half of neutron-myeloid cells to WT's, whereas growth-retarded TAZ KO-2 mouse was even worse with only a half of total bone marrow cells compared to WT and TAZ KO-1 (3.825x10⁷ vs. 7.38x10⁷ and 5.625x10⁷), thereby both erythroid and neutro-myeloid deficiencies.

Thirdly, we cultured 4-month-old mouse bone marrow cells at 3x10⁶/well of 24-well plate supplied with 10% FBS-DMEM-GlutaMax-penicillin-streptomycin medium and removed non-anchored suspended hematopoietic cells next day. Surprisingly, on day 19, we found that at least 8-22 nuclei giant osteoclast-like cells were existed in TAZ KO wells much more than at least 8 nuclei giant cells in WT wells. On day 22, more and more 8-22 nuclei giant osteoclast-like cells appeared in TAZ KO wells but rarely or none in WT wells. The concentrated culture of TAZ KO bone marrow cells survived those osteoclast-like cells but not in WT groups, due to TAZ deficiency. It might be a reason why TAZ bone mineral density went down, in addition to Barth neutropenia.

In short, all of preliminary evidence above lead to an interconnection between bone density-bone marrow and innate immune system for Barth neutropenia. On the other hand, why almost normal-level of mature neutrophils with extreme low-level of immature neutrophils existed in TAZ KO bone marrow, would be critical for molecular mechanism of Barth neutropenia.

References

[1] Yakisan E et al. High incidence of significant bone loss in patients with severe congenital neutropenia (Kostmann's syndrome). *J. Pediatr.* 1997; 131:592-7.
[2] Dale, DC et al. Severe chronic neutropenia: treatment and follow-up of patients in the Severe Chronic Neutropenia International Registry. *Am. J. Hematol.* 2003; 72:82-93.
[3] Skokowa, J, Dale, DC, and Welte K et al. Severe congenital neutropenia. *Nat. Rev. Dis. Primers.* 2017; 3:17032.