Assessing Mitochondrial Morphology of Heart Tissue in the Barth Syndrome Mouse Model

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Abstract

Mutations in the gene tafazzin (*TAZ*) cause mitochondrial deformity. This causation links to Barth syndrome (BTHS), a rare X-linked disorder that is associated with the symptoms of cardiomyopathy, neutropenia, growth retardation, and exercise intolerance in patients. The present study examined the effects of an AAV-mediated delivery of a healthy TAZ transgene on the mitochondrial morphology in the well-characterized doxycycline-induced shRNA-*TAZ* knockdown mouse model of BTHS. In adult and neonatal treated mouse heart tissue, BTHS untreated mouse heart tissue, and wild type mouse heart tissue, area, contact area, and the number of inter-junctions of mitochondria were measured by ImageJ. There was not a significant difference between the averages of the number of inter-junctions, number of inter-junctions to contact area, and number of inter-junctions to contact area normalized by the total area. The data suggested that the number of inter-junctions, the number of inter-junctions to contact area, and the number of inter-junctions to contact area normalized to total area of the treated heart tissue did improve as compared to BTHS untreated heart tissue. Although, the number of inter-junctions to contact area normalized to total area of neonatal treated heart tissue did not improve in comparison to BTHS untreated heart tissue. These findings suggest that the mitochondrial morphology did improve in treated mouse heart tissue, but the small sample data is not conclusive. Additionally, the data suggested the mitochondrial morphology of BTHS untreated heart tissue is characterized by decreased amounts of inter-junctions and increased mitochondrial fragmentation, or smaller total areas of mitochondria.

Keywords: Barth syndrome, cardiomyopathy, inner mitochondrial membrane

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Introduction

Background

Barth syndrome (BTHS) is a rare and commonly fatal X-linked recessive genetic disorder. Dr. Peter G. Barth, a pediatric neurologist, fully described BTHS first in 1983 (Barth et al., 1983). BTHS has an estimated prevalence rate of 1/300,000-400,000 live births (Clarke et al., 2013). The syndrome occurs in individuals around the world and has not appeared to be more common in any one ethnic group than another. BTHS occurs almost exclusively in males and typically affects them from birth or early into childhood. Males have been historically documented to die from the BTHS symptoms, such as heart failure and infection, by three years of age (www.barthsyndrome.org). The survival rate is increasing with improved access to information from an increased amount of research on the topic about diagnoses, medical treatments, and monitoring BTHS.

Causes

BTHS is caused by functionless mutations in the *TAZ* gene (G4.5). The *TAZ* gene encodes for the protein tafazzin. Tafazzin, a nuclear-encoded transference, is expressed in high levels of cardiac and skeletal muscle. The transference is responsible for remodeling monolysocardiolipin, or an immature and nonfunctional molecular configuration of cardiolipin, to mature and functional cardiolipin, an important component of the inner mitochondrial membrane that maintains mitochondrial membrane fluidity, structure, and osmotic stability in muscle. The uniquely localized phospholipid also maintains the mitochondria's ability to induce apoptosis and metabolize energy through the electron transport chain. The electron transport chain is not

able to convert ATP from ADP without the *TAZ* gene resulting in inefficient energy production (Reynolds, 2015).

Principle Diagnostic Characteristic Features

The principal diagnostic characteristic features of BTHS include combinations and varying degrees of dilated cardiomyopathy, underdeveloped skeletal musculature, muscle weakness, fatigue, exercise intolerance, and neutropenia. Weak heart muscle that can cause dilated cardiomyopathy is a result of weak mitochondrial membrane structure and the cardiac cells' lack of ability to produce sufficient energy for the muscles. The muscle commonly exhibits other functional impairments of the heart related to enlargement and noncompaction, including variable myocardial hypertrophy and occasionally left ventricular noncompaction and/or endocardial fibroelastosis. Chronic, cyclic, and intermittent neutropenia is also commonly seen in individuals with BTHS due to a compromised immune system, increasing the risk of mouth ulcers, fevers, and bacterial infections and becoming seriously ill. Sepsis resulting from neutropenia and heart failure are the two leading causes of death in infants with BTHS. Heart failure is the most common principal clinical diagnostic criteria identified at birth (Reynolds, 2015).

Treatment

There is currently no cure for BTHS (Reynolds, 2015). Current standard treatments that temporarily manage high blood pressure and heart failure include diuretics and angiotensin converting enzyme (ACE) inhibitors. These treatments are not optimal for patients and 15% of individuals with heart failure ultimately require heart transplants. Consequently, development of effective therapeutic treatment options for BTHS is of high importance. The Pacak Laboratory is one of many laboratories that currently have ongoing research funded by

grants from the Barth Syndrome Foundation that is attempting to develop a therapeutic treatment. Although BTHS is rare, development of treatments can treat other conditions that are caused by mutations in the *TAZ* gene. Such conditions include familial dilated cardiomyopathy, dilated cardiomyopathy, and isolated noncompaction of left ventricular myocardium without the other principal characteristics of BTHS (ghr.nlm.nih.gov).

Study

Gene therapy is currently being investigated as a therapeutic option for BTHS. BTHS is an ideal candidate for this option since *TAZ* mutations arise from the single gene (www.barthsyndrome.org). Adeno-associated virus (AAV) vectors are a potential treatment that has the ability to transduce the tissues affected by BTHS with a long-term expression of healthy *TAZ* genes. AAV vectors are preferred over other viral vectors because they do not integrate into the genome of the host, which can affect the expression of the *TAZ* gene. The Pacak Laboratory's initial comparison study in mice has yielded results that demonstrate strong *TAZ* expression levels and correction of muscle weakness. The aim of this study is to build upon this pre-clinical data that suggests the strong potential of an AAV-mediated approach to treat BTHS.

Methods

Images

Images of the mitochondria of mouse heart tissue from wild type mice and the well-characterized doxycycline-induced shRNA-*TAZ* knockdown mouse model of BTHS that were treated and not treated with an AAV-mediated delivery of a healthy *TAZ* transgene were captured for this study. The ages of the mice used for the images included neonatal, or up two days of life after birth, and adult, or up to three months of life after birth. There were 3 male and 3 female mice in each group. The neonatal mice were injected in the temporal vein and the adult mice in the jugular vein. The images were captured by electron microscopy at Emory University's Electron Microscopy Core (Gupte, 2015).

Imaging Platform

We evaluated the mitochondrial morphology of these images using ImageJ, an open platform for scientific image analysis. We measured various features of the mitochondria that had been previously observed to be different in BTHS mitochondria and healthy mitochondria to determine if the AAV-mediated delivery of a healthy *TAZ* transgene improved the mitochondrial morphology of the BTHS treated mitochondria (Gupte, 2015).

Procedures Carried Out

Measurements

The total area of mitochondria that are in contact with one another was measured by using the freehanded rounded area tool in ImageJ. The measurement of the first mitochondria was identified as "Area 1" and for the second mitochondria "Area 2". The pixel area of the contact length between each mitochondrion was measured using the freehanded line area tool. The measurement was identified as "Contact Area". The number of cristae junctions between

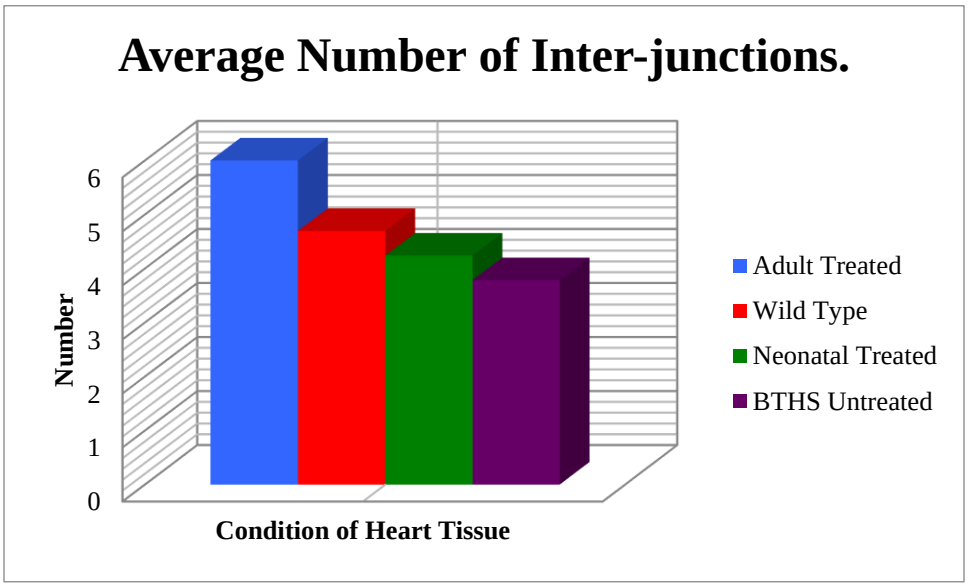
each mitochondrion was counted. The measurement was identified as “Number of Interjunctions”. The scale bar of each image was two μm. The scale bar was measured during each measurement to check that each measurement was being recorded on the same scale and could be accurately compared after the data was collected. Each image was measured at 33.3% magnification to also ensure that the data collected would be able to be accurately compared (Gupte, 2015).

Data Collection and Analysis

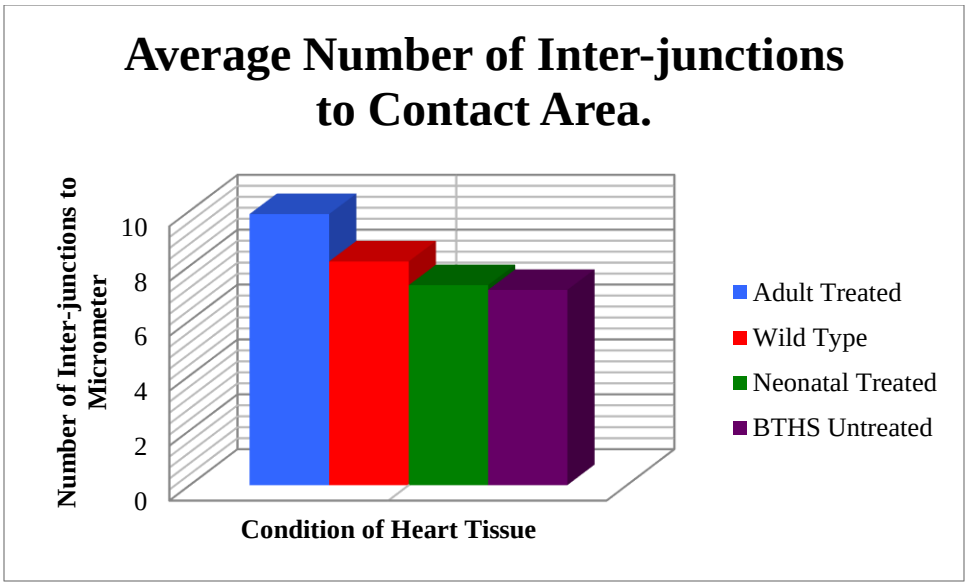
Following the analysis where the condition of the heart tissue was blinded from the researcher, the categorization of the images was revealed. An Excel spreadsheet was created to include each of the measurements collected during the evaluation. The measurements were collected in pixels and converted to micrometers, a more useful measurement for data analysis. The data collected was used to solve the formula, number of junctions/contact area, which was normalized by the total area of the mitochondria to determine if our hypothesis was correct. Additionally, a statistical t-test comparing the untreated tissue and the healthy control was run through Sigma Plot to assess significant differences. (Gupte, 2015)

Results

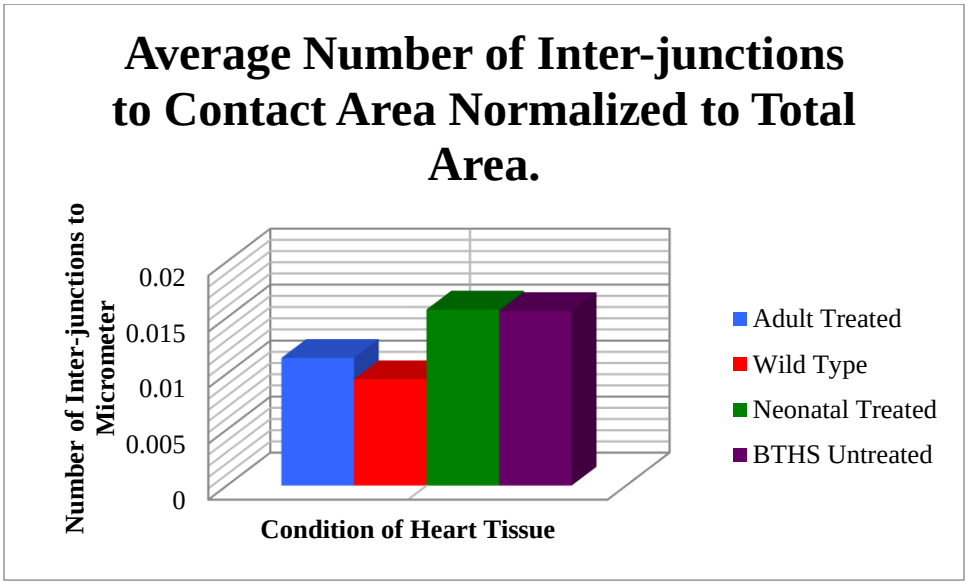
Measurements



Graph 1: The figure illustrates the average number of mitochondrial inter-junctions counted in the groups of adult treated, wild type, neonatal treated, and BTHS untreated heart tissue.



Graph 2: The figure illustrates the average number of mitochondrial inter-junctions counted to the contact area measured in the groups of adult treated, wild type, neonatal treated, and BTHS untreated heart tissue.



Graph 3: The figure illustrates the average number of mitochondrial inter-junctions counted to the contact area measured normalized to the total area measured in the groups of adult treated, wild type, neonatal treated, and BTHS untreated heart tissue.

There was an improvement of the number of inter-junctions, contact length area, and total area of the adult heart tissue that was treated and an improvement on number the inter-junctions and contact area of the neonatal heart tissue. There is a decreasing trend of the average number of inter-junctions from adult treated heart tissue to BTHS untreated heart tissue (Graph 1). There is a decreasing trend of the average number of inter-junctions to contact area from adult treated heart tissue to BTHS untreated heart tissue (Graph 2). There is an increasing and variable trend of the average number of inter-junctions to contact area normalized to the total area from adult treated heart tissue to BTHS untreated heart tissue (Graph 3).

There was no statistical significance difference between the average number of inter-junctions of wild type and BTHS untreated heart tissue in the paired t-test run ($p = 0.085$). There was no statistical significance between the average number of inter-junctions to contact area of wild type and BTHS untreated heart tissue ($p = 0.0641$). There was no statistical significance between the average number of inter-junctions to contact area normalized to total contact area of wild type and BTHS untreated heart tissue ($p = 0.120$).

Discussion

The data collected did not conclusively support or not support the hypothesis of this study, most of the data collected does implicate the hypothesis was supported. Additionally, the data collected, like earlier findings, did suggest that decreased amounts of inter-junctions between mitochondria and increased mitochondrial fragmentation, or smaller total areas of mitochondria, does characterize the mitochondrial morphology of BTHS heart tissue in comparison to wild type heart tissue (www.barthsyndrome.org).

The decreasing trend of the average number of inter-junctions from adult treated heart tissue to BTHS untreated heart tissue suggested that the mitochondrial morphology of adult and neonatal treated heart tissue was improved in comparison to BTHS untreated heart tissue, although neonatal treated heart tissue did not improve as much as the adult heart tissue (Graph 1). Additionally, the trend suggested that wild type heart tissue had an increased amount of mitochondrial inter-junctions in comparison BTHS untreated heart tissue. This suggests that the treatment was successful and the hypothesis was supported.

The decreasing trend of the average number of inter-junctions to contact area from adult treated heart tissue to BTHS untreated heart tissue continued to suggest that the mitochondrial morphology of adult and neonatal treated heart tissue was improved and wild type heart tissue has an increased amount of mitochondrial inter-junctions in a contact area (Graph 2). This continues to suggest that the treatment was successful and the hypothesis was supported.

The increasing and variable trend of the average number of inter-junctions to contact area that was normalized to the total contact area from adult treated heart tissue to BTHS untreated heart tissue suggested that BTHS untreated heart tissue has increased fragmentation in comparison to wild type heart tissue (Graph 3). Additionally, the trend suggested that the

mitochondrial morphology of adult treated heart tissue improved in comparison to BTHS untreated heart tissues. The trend suggests the morphology of the neonatal treated tissue did not improve, which was a deviation from what was expected. The trend might have been different than expected due to measurement error or the mitochondrial morphology of neonatal heart tissue may not be as efficiently improved by the treatment in comparison to adult tissue. This hypothesis could be tested in the future.

The absence of statistically significant differences between the averages suggests that additional data needs to be collected and analyzed before it can be determined if the hypothesis was supported.

This study will be used to guide future research and work on BTHS. Although the data was not conclusive, it can be theoretically applied to the development of future research studies of the treatment.

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