Senior Presentation 2020

Karen Jackson

The Effect of Rapamycin Dosing on A Murine Model of Congenital Muscular Dystrophy Over a Trial of Six Months

Introduction

Muscular Dystrophy

- **Muscular dystrophy**: collective name for diseases causing progressive muscle weakness and atrophy (1)
- **Genetic** disorder (2)
  - Sex-linked
  - Predominantly affects males
  - Spontaneous mutation

Introduction

Symptoms & Pathology

- Generally affects young boys
- Symptoms range from moderate to severe
  - Severe symptoms (4):
    - Scoliosis
    - Breathing issues
    - Cardiovascular diseases

https://www.ccc1011.com/scoliosis

Introduction

Affected Genes

Fukuyama Congenital Muscular Dystrophy

- FKTN

Congenital Muscular Dystrophy with or without Cognitive Impairment (5, 7)

- DOLK
- DPM1
- DPM2
- DPM3
- FKRP
- FKTN,
- POMT1
- POMT2
- POMGnT1

Limb Girdle Muscular Dystrophy with or without Cognitive Impairment

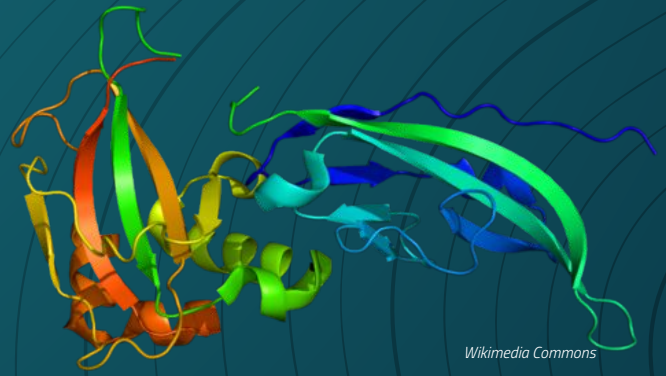
- FKRP
- FKTN
- POMT1
- POMT2
- POMGNT1
- ISPD

Introduction

Dystroglycanopathies

- **Dystroglycanopathies** are a subset of muscular dystrophy characterized by interruptions in the processing of **α-dystroglycan** (6)

Links extracellular matrix and cytoskeleton



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Introduction

Literature Review

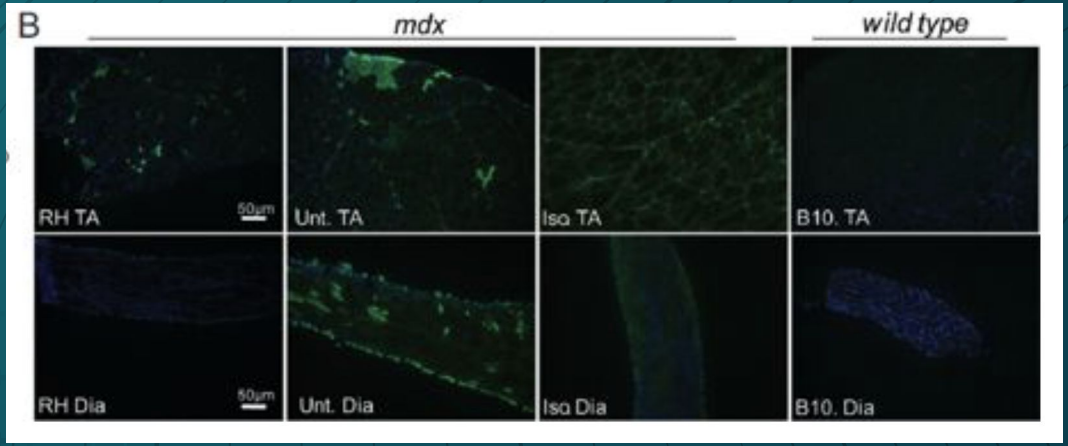
- **Successful Myoblast Allotransplantation In Mdx Mice Using Rapamycin**
  - Vilquin, J.-T., Asselin, I., Guérette, B et al. (1995)

- **Rapamycin nanoparticles target defective autophagy in muscular dystrophy to enhance both strength and cardiac function**
  - Kristin P. Bibee, Ya-Jian Cheng, James K. Ching et al. (2014)

Introduction

Literature Review

- **Rapamycin Ameliorates Dystrophic Phenotype in mdx Mouse Skeletal Muscle**
  - Saman Eghtesad, Siddharth Jhunjhunwala, et al (2011)

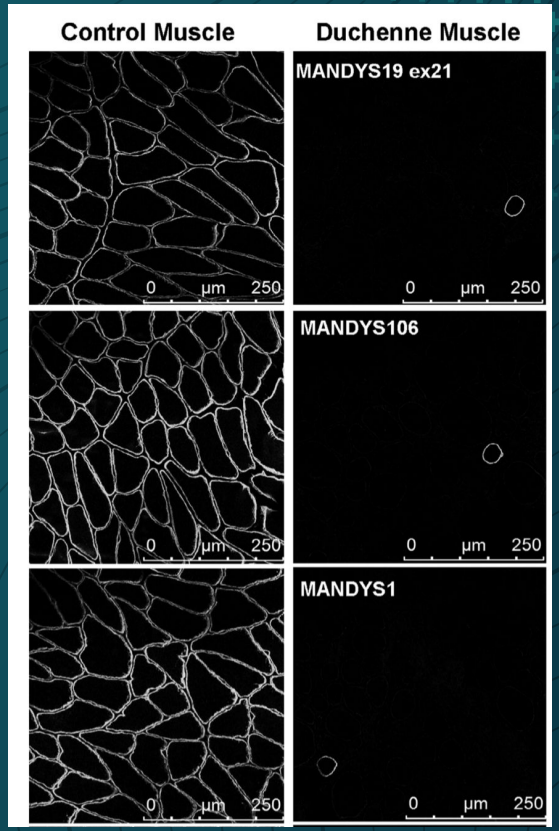


Saman Eghtesad, Siddharth Jhunjhunwala, et all (2011)

Literature Review

- **Monoclonal antibodies for clinical trials of Duchenne muscular dystrophy therapy.**
  - Lam, L., Man, N., & Morris, G. (2014)

- **Measuring clinical effectiveness of medicinal products for the treatment of Duchenne muscular dystrophy**
  - Lynn, S., Aartsma-Rus et al (2015)



Lam, L., Man, N., & Morris, G. (2014)

Gap in the Research

How can the progression of muscular dystrophy, especially dystroglycanopathy, be slowed or stopped?

https://meduniverse.com/knowledge-gap-2/

Hypothesis

Rapamycin dosage will decrease muscular dystrophy progression in fukutin-deficient mice

- Rapamycin inhibits mTOR, a cell-cycle regulating protein
- Fukutin-deficient mice are prone to dystroglycanopathy

https://meduniverse.com/knowledge-gap-2/

Methodology: Mice

- Myd mice obtained from Jackson Laboratories
  - Maintained at SUNY Binghamton
- Ear clips taken at birth
- Body weights collected at five weeks

https://www.jax.org/strain/009088

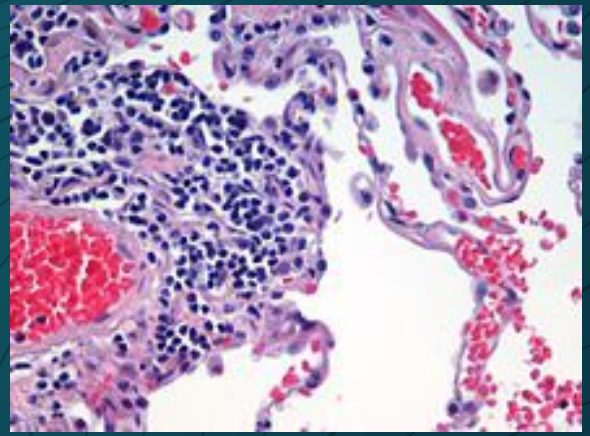
Methodology: Measurements

- Body mass
- Muscle torque
- Blood tests
  - ALT and BUN levels
- Qualitative observations

https://www.shutterstock.com/search/lab%2Bmouse?page=5&section=1&sort=newest&image_type=vector&search_source=base_related_searches

Methodology: Tissue Harvest

- Mice euthanized at 8 weeks and iliopsoas (*ilio)* muscle harvested
- Sectioned with microtome-cryostat
- Stained with H&E and fluorophores
  - Alexa Fluor far-red, blue, and green



https://ko.wikipedia.org/wiki/H%26E_%EC%97%BC%EC%83%89

Methodology: Microscopy

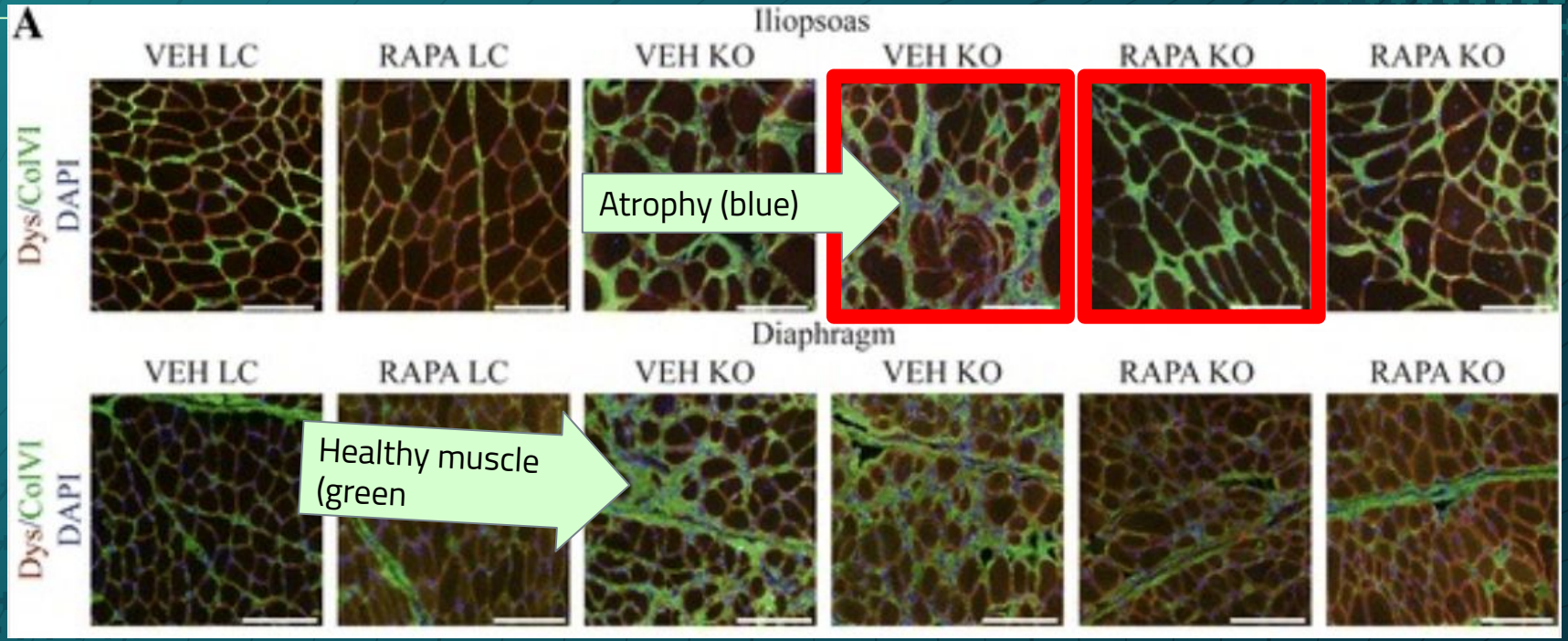
- X71 inverted epifluorescent microscope with camera
- Sections compiled manually *and* with image manip software

https://www.tendersontime.com/blogdetails/supply-epifluorescence-microscope-25238/

Methodology: Data Analysis

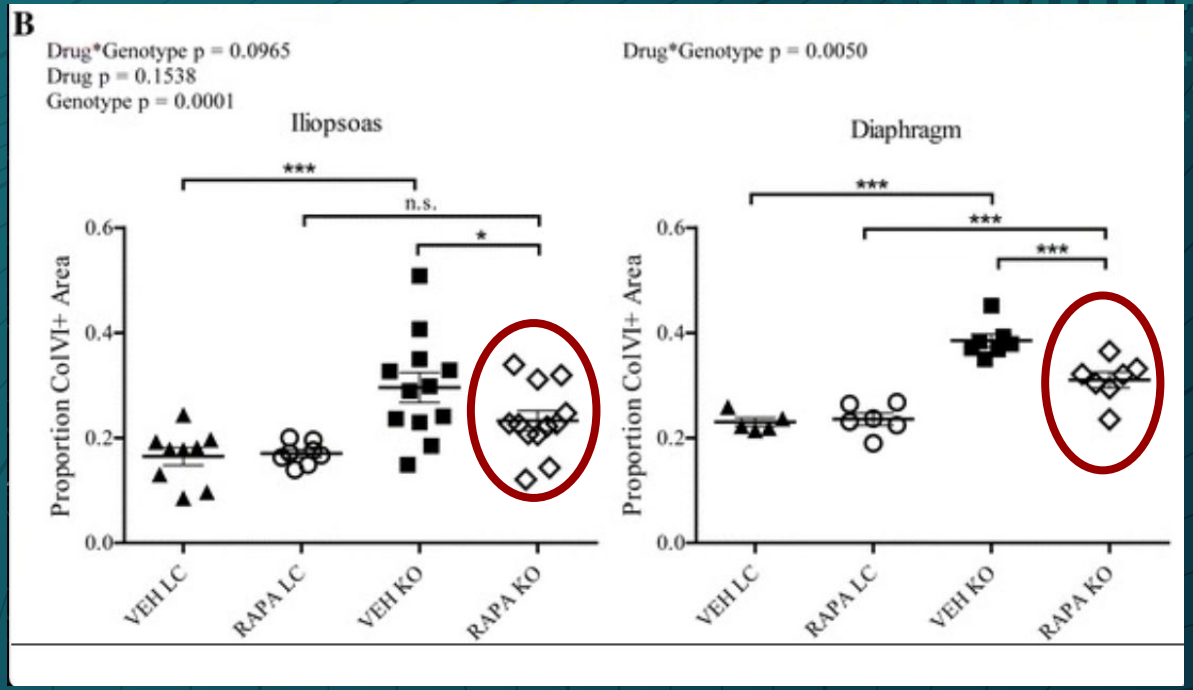
- Fiber size distributions compared by two-way ANOVA
- Differences between study groups determined by two-tailed Student's t test

Results


A
Iliopsoas
VEH LC
RAPA LC
VEH KO
VEH KO
RAPA KO
RAPA KO
Dys/ColVI
DAPI
Atrophy (blue)
Diaphragm
VEH LC
RAPA LC
VEH KO
VEH KO
RAPA KO
RAPA KO
Dys/ColVI
DAPI
Healthy muscle
(green

Results

Initial four weeks showed decreased fibrosis in rapamycin treated mice



Results

- Qualitative behavioral observations show improvement in cohort of mice
  - Study ongoing
- Four week two-way ANOVA showed ***$p < 0.05$**

https://www.dkfindout.com/us/animals-and-nature/rodents/mice/

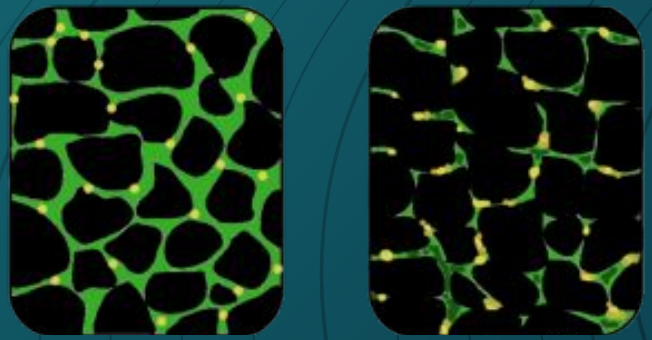
Discussion

- Initial four-week study showed marked improvement in dosed mice
  - Increased muscle torque
  - Decreased myopathy
- Preliminary results are similar

https://www.kentscientific.com/

Limitations

- Incomplete dystrophin knockouts
  - Embryonic lethal mutation requires use of a conditional knockout
- Variances between individual mice



https://providencecanhelp.com/medical-conditions/muscular-dystrophy/

Applications

- Quality of life improvements
- Reduction of wheelchair usage
- Further therapeutic development

https://kdsmartwheelchair.com/product/standard-kd-smart-wheelchair/

Conclusions

Significance

- Potential human use in the future
  - Must be studied more before clinical trials
- Interactions with other organ systems must be closely monitored

Future Research

- Impacts of rapamycin on kidney and liver health
- Dosing schedule improvements

https://research.umn.edu/inquiry/post/%E2%80%98dirty%E2%80%99-mice-may-be-better-models-human-biology

Acknowledgements

- My mentor Dr. Aaron Beedle and the staff at SUNY Binghamton
- Ms. Gillian Rinaldo and my science research peers
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The Effects of Autophagy Induction on the Replication Rate of ZIKV

Introduction

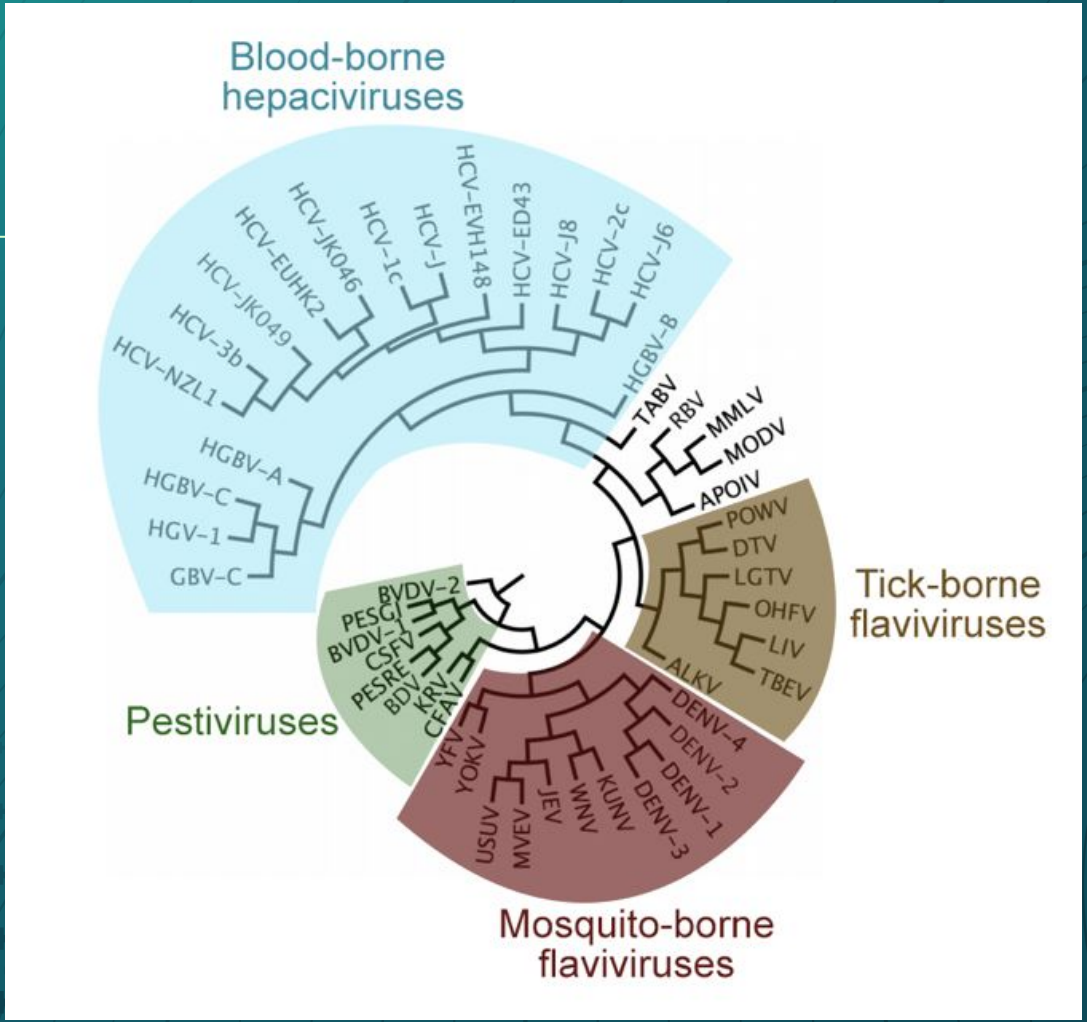
Zika Fever

- Zika virus (ZIKV) causes Zika fever when contracted
  - Had an unprecedented number of cases in the past few years
- South America and Africa most severely affected
  - Epidemic began to move into northern countries
- Spreads via the A. aegypti mosquito
  - can also transmit via sexual intercourse and exchange of fluids

Introduction

Symptoms & Pathology

- Causes relatively inconsequential symptoms for the majority of people infected
  - Flu-like symptoms and occasional hematospermia
  - Distinctive macropapular rash
- Linked to the development of microcephaly, which contributes to:
  - Epilepsy and seizures
  - Intellectual disabilities
  - Poor motor control



Zika genealogy

Introduction

Virology

- ZIKV is a single-stranded RNA virus with a normal cytoplasmic replication cycle
- Replicates in the cytoplasm, but has been found in the nuclei
- Approximately six hours post-infection, the vacuoles and mitochondria of a host cell swell until cell death is induced by paraptosis
- New viral particles then exit the dead cell

Introduction

Autophagy

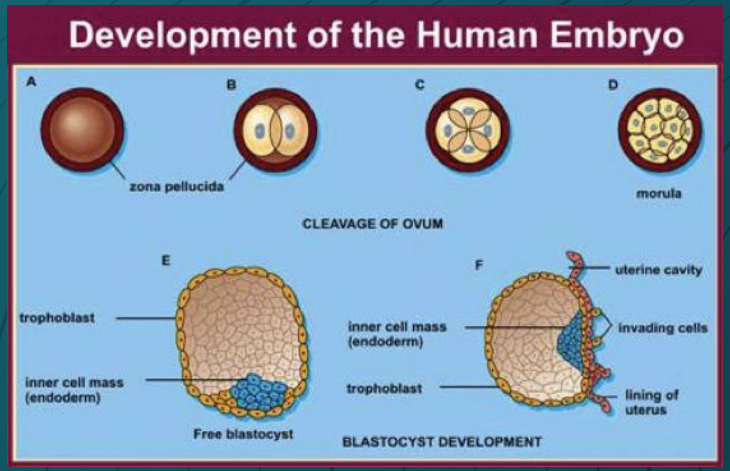
- "Cellular suicide"
  - Key in regulating homeostasis
- Assists in the bactericidal process, but may also suppress the innate immune response and contribute to RNA virus replication
- Regulated by the ATG12-ATG5 complex

Gap in the Research

How does the presence of the Atg5-Atg12 protein complex affect ZIKV replication?

Hypothesis

Increased levels of Atg5-Atg12 will lead to increased viral replication in trophoblasts.



Literature Review

- A new virus had been isolated from rhesus monkey serum in the Zika forest, and it bore little resemblance to previously known viruses and therefore was new.
  - *Dick, G., et al. (1953)*
- There are two strains of Zika hailing from Africa and America, sharing up to 90% of their genomes.
  - *Baronti, et al. (2014)*
- Zika could potentially transmit through blood transfusion.
  - *Musso, D., et al. (2014)*
- Zika's primary transmission method was via mosquitoes.
  - *Bogoch, I. et al. (2016)*

Literature Review

- Zika had the potential for sexual transmission.
  - *Foy, B. D. et al. (2016)*
- Zika was a possible implicating factor in the development of microcephaly.
  - *Talan, J. et al (2016)*
- Zika was declared a public health emergency by the Centers for Disease Control and Prevention.
  - *Gulland, A. (2016)*
- Both Zika and Dengue virus benefit from the induction of autophagy.
  - *J. U. Jung et al (2017)*

Methodology

Trophoblast Culture

- Trophoblasts were the main cell line
  - Among the first embryonic cells to develop
- Incubated at 37c in a CO2 incubator
  - Roughly body temperature
- Grown in RPMI medium treated with 10% of FBS and pen-strep antibiotic
- ATG12 knockout generated and incubated in similar conditions

Methodology

Vero Cell Culture

- Vero cells cultured in DMEM medium treated with 10% FBS and pen-strep antibiotic
- Incubated at 37c in the CO2 incubator.
- ZIKV propagated in Vero cells for 72 hrs
- Viral particles isolated by extracting the Vero cells from the medium

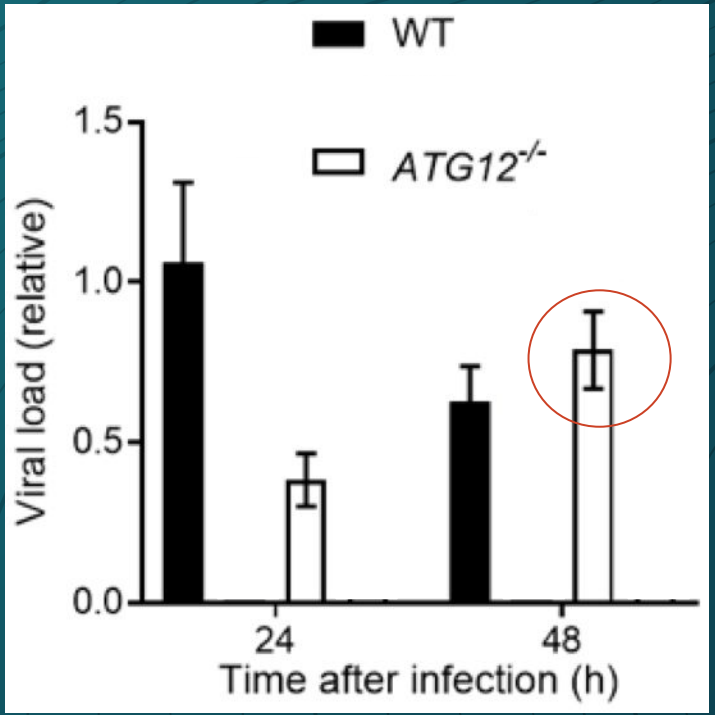
Methodology

Viral Infection

- Cells cultured to 90% confluence
- Medium of the trophoblast removed
- Cells treated with cold PBS for one minute
- Both wild type and the ATG12 knockout cells seeded in a 24-well plate and incubated until reaching confluence
- Medium removed and cells treated with Zika virus in all wells for 3 hours

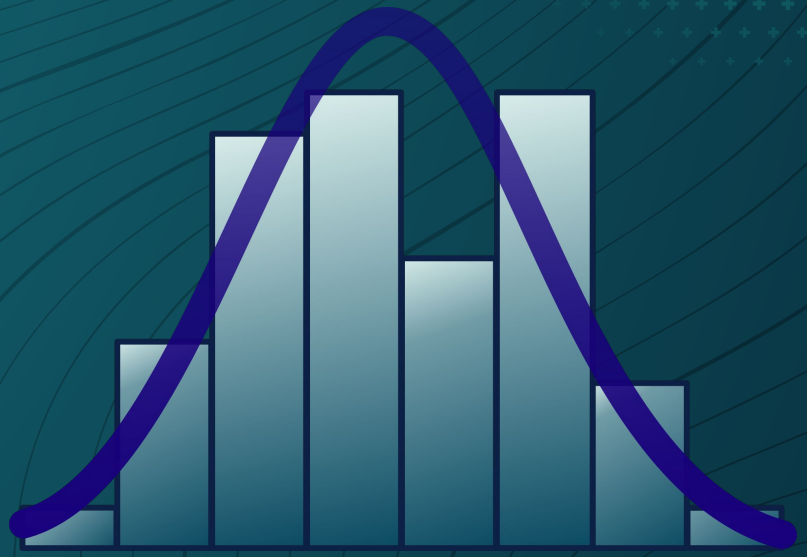
Results

Viral Infection



Discussion

- Relative viral load was higher in the wild type than the knockout
  - No significant difference between the types at the 48 hour mark
- Initial viral load decreased over time
  - Lower at the 48-hour mark



https://en.wikipedia.org/wiki/Analysis_of_variance

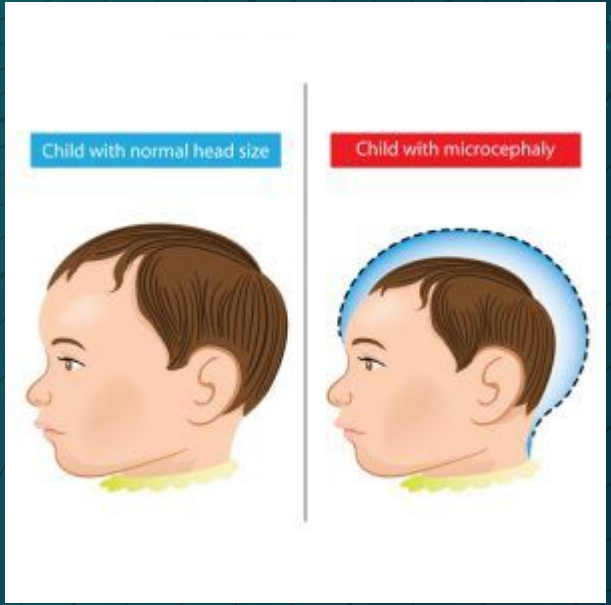
Conclusions

- **Cells that did not undergo autophagy due to the removal of the ATG5-ATG12 knockout gene had a decreased viral load**
  - This supports the hypothesis

http://globalhealthcare.net/en/

Applications

- **Theraputic targets**
  - Pharmaceuticals that could potentially decrease rate of microcephaly development during gestation
  - Pharmaceuticals that decrease a patient's chance of becoming infected to begin with
  - Overall prevention of microcephaly and other severe symptoms



https://www.thurswell.com/michigan-birth-injury-lawyer/microcephaly/

Future Research

- Impact of halting viral replication on microcephaly development
  - Both animal and human models
- Therapeutic development using methods that halt viral replication
- Effect on pen-strep antibiotic on results

https://www.thurswell.com/michigan-birth-injury-lawyer/microcephaly/

Acknowledgements

- Dr. Penghua Wang and Mohammed Marghini
- New York Medical College
- Ms. Gillian Rinaldo
- My Science Research peers
- My parents, my sister, and the rest of my family

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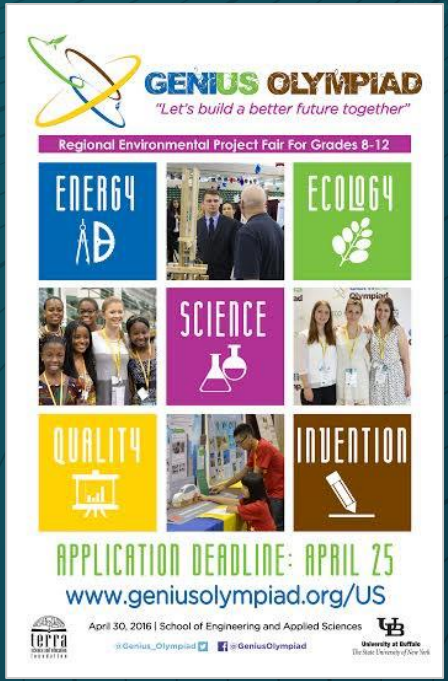
Other Projects and Events

GENIUS Olympiad

- Arts and sciences competition focused on ecology and futurism
- A platform for international discussion about current environmental problems

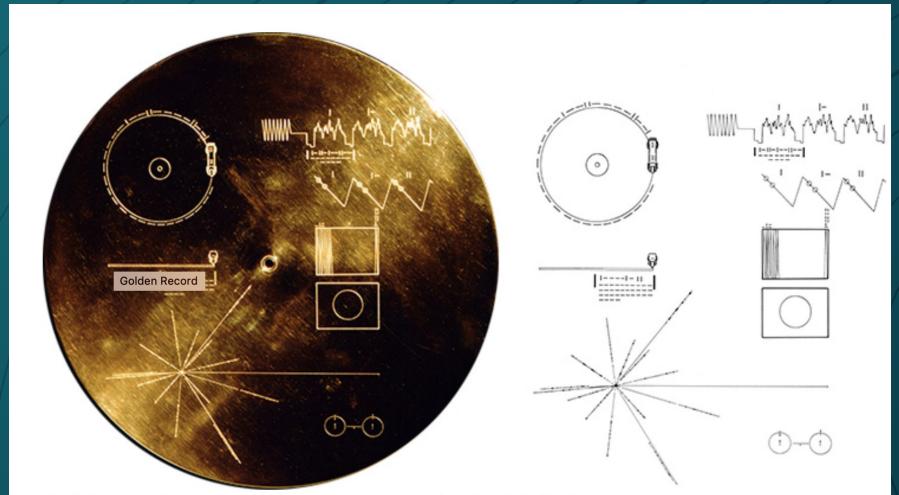


Zoonotic jumps

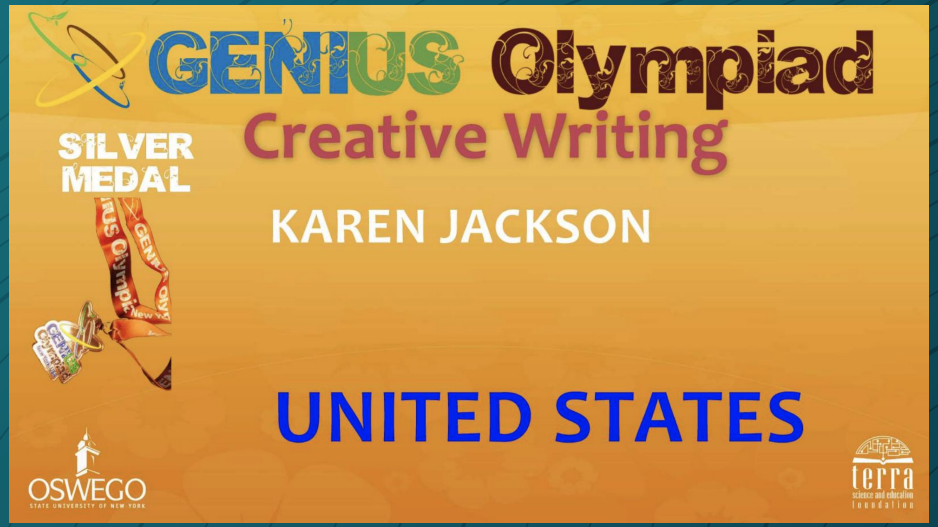
- When a disease “jumps” from an animal to a human, adapting to infect and kill people instead
- Can happen as a result of environmental damage
  - Habitat loss results in more human/animal interaction, resulting in a higher chance of diseases “jumping over”
  - Happened with AIDS, avian flu, bubonic plague, and other diseases

Golden Record

- A phonograph record launched on the Voyager containing information about the human species
- Intended for spacefaring civilizations to find in the distant future



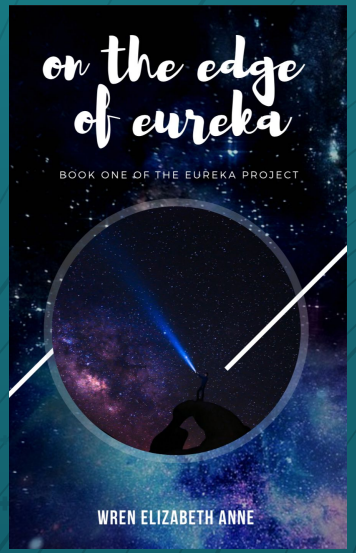
The Golden Record cover shown with its extraterrestrial instructions. Credit: NASA/JPL

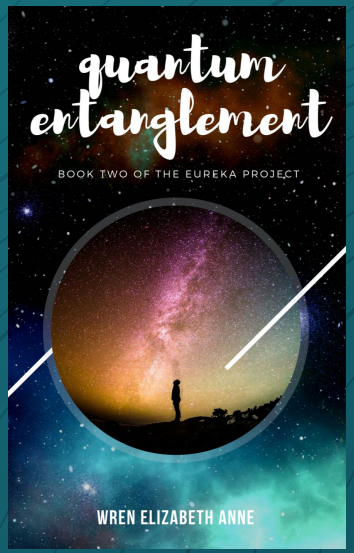

GENIUS Olympiad
Creative Writing
SILVER MEDAL
KAREN JACKSON
UNITED STATES
OSWEGO
STATE UNIVERSITY OF NEW YORK
terra
science and education
foundation

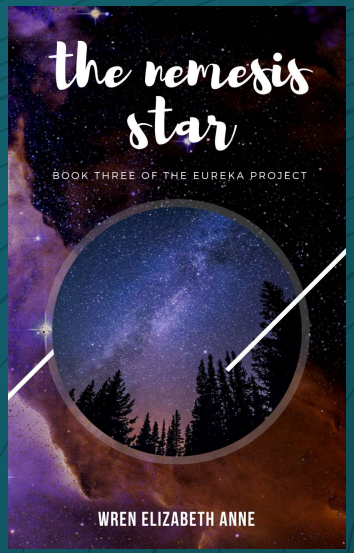

CONGRATULATIONS GRADUATES
OLYMPIAD

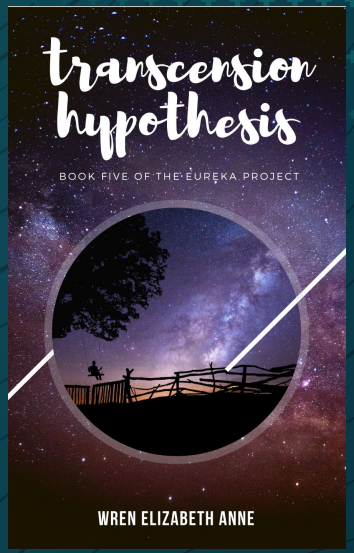

GENIUS
Olympiad

Writing Projects









Writing Projects

- Optogenetics—using light to control neurons
- Biocybernetics—biology as understood in comparison to technology
- Artificial blood
  - PFC, antibiotic, and salt suspended in water as an emulsion
  - Ex vivo erythrocyte production: using stem cells to grow new blood

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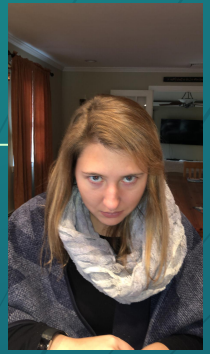


FIRE STORM


Happy Birthday
HODGE INDUSTRIES
Brian


Science National Honor Society

Future Plans