R & the Study of Rare Diseases: Using Government Databases & Molecular Datasets to Set Research Priorities

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Rare diseases

- A rare disease is often defined as a disorder that affects fewer than 200,000 individuals in the United States.
- Over 70% of rare diseases are genetic.
  - Of those, 70% start in childhood and often lead to a substantially reduced life expectancy.
  - Eg: cystic fibrosis, sickle cell anemia, muscular dystrophies, Huntington's disease, some inherited cancer syndromes
- Together, rare diseases affect 25-30 million individuals in the United States.
- What are some good data resources and research approaches for combating rare diseases?

Zebra ribbon: https://commons.wikimedia.org/wiki/File:Zebra_ribbon.svg

(David Richfield)

“When you hear hoofbeats behind you, don't expect to see a zebra”

Theodore Woodward (1914-2005)
Researcher at University of Maryland

Challenges with clinical trials in rare diseases

- It can be very challenging to perform clinical trials in this space:
  - Small number of individuals affected by any one disease
  - Often progressive nature: How do you choose which outcomes to consider?
  - Ethical and implementational challenges with performing research on children.
- The FDA allows for specific flexibilities when evaluating new drugs for the treatment of rare diseases:
  - Eg by allowing the use of biomarkers as surrogate endpoints in some instances.

Zebra ribbon: https://commons.wikimedia.org/wiki/File:Zebra_ribbon.svg

(David Richfield)

Duchenne Muscular Dystrophy (DMD)

- Devastating X-linked single-gene disorder.
  - Mutations are in the *DMD* gene.
  - Different mutations may lead to different phenotypes.
  - Certain therapies target only individuals with specific changes.
- Affects about 1 in 5,000 newborn males.
- Leads to muscle loss and eventual loss of ambulation and death in late teens to early 20s or 30s, generally due to cardiac or respiratory problems.
- Steroid treatment can prolong ambulation but chronic use can cause many problems.

Understanding the clinical trial landscape for DMD

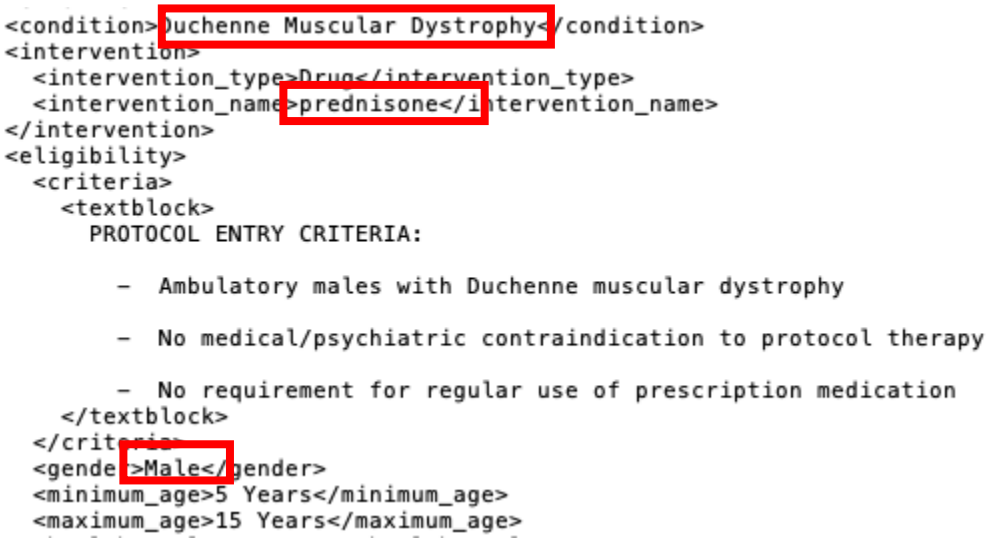
- A few therapies are approved or conditionally approved in US for a subset of individuals, with others in clinical trials.
- Many different trials for DMD patients:
  - Multiple trials are competing over a small number of patients.
  - Other patients may not be served at all (older patients, rarer mutations)

-> Need to assess what trials are performed in DMD space.

- Eventual goals:
  - Develop a product to allow researchers, clinicians, and patients to stay up-to-date with ongoing drug development in the DMD.
  - Prioritize research focusing on individuals who do not currently have many available clinical trial options.

Understanding the clinical trial landscape for DMD

- Summer student Bin Hai (2019) downloaded xml files for DMD-related clinical trials from https://clinicaltrials.gov/, the government database of registered clinical trials.
- Used XML package to extract information from xml files:

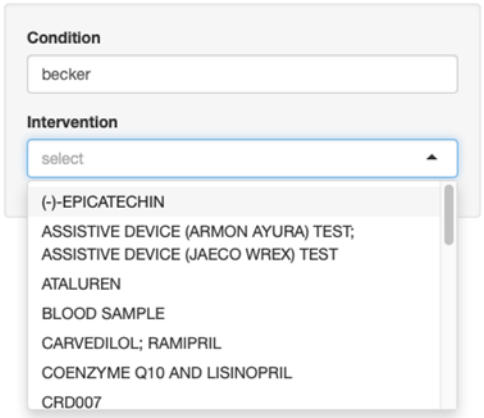


```
<condition>Duchenne Muscular Dystrophy</condition>
<intervention>
  <intervention_type>Drug</intervention_type>
  <intervention_name>prednisone</intervention_name>
</intervention>
<eligibility>
  <criteria>
    <textblock>
      PROTOCOL ENTRY CRITERIA:

        -  Ambulatory males with Duchenne muscular dystrophy

        -  No medical/psychiatric contraindication to protocol therapy

        -  No requirement for regular use of prescription medication
    </textblock>
  </criteria>
  <gender>Male</gender>
  <minimum_age>5 Years</minimum_age>
  <maximum_age>15 Years</maximum_age>
```

- Performed data cleaning, including for redundant drug names

Understanding the clinical trial landscape for DMD

- Used R/shiny to design and created a web interface to explore DMD-related clinical trials https://bh658.shinyapps.io/curation_dmd_clinicaltrials/



Total trials: 36

Gender	count
All	18
Female	1
Male	17

Allocation	count
Non-Randomized	4
Randomized	10
NA	22

Study Rank	NCT ID	Title	Allocation	Condition	Intervention Type	Intervention
213	NCT00005574	Gentamicin Treatment of Muscular Dystrophy	NA	BECKER MUSCULAR DYSTROPHY; DUCHENNE MUSCULAR DYSTROPHY	Drug	GENTAMICIN
254	NCT00104078	Study Evaluating MYO-029 in Adult Muscular Dystrophy	Randomized	BECKER MUSCULAR DYSTROPHY; FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY; LIMB-GIRDLE MUSCULAR DYSTROPHY	Drug	STAMULUMAB
231	NCT00390104	Molecular Analysis of Patients With Neuromuscular Disease	NA	BECKER MUSCULAR DYSTROPHY; DUCHENNE MUSCULAR DYSTROPHY; LIMB-GIRDLE MUSCULAR DYSTROPHY; NEUROMUSCULAR; DISORDER, HEREDITARY	NA	NA
134	NCT00592553	Phase 2b Study of PTC124 in	Randomized	BECKER MUSCULAR DYSTROPHY; DUCHENNE	Drug	ATALUREN

Searching for biomarkers of disease progression and drug response

- Given the difficulty with choosing appropriate outcomes, there has been a focus on finding potential biomarkers of disease progression and drug response.
- We considered metabolomics – the study of metabolites (small biomarkers):
  - Can be detected in various biofluids and tissues.
  - Have various biological functions.
  - Can be influenced by genetics and environmental factors.

Boca SM et al. "Discovery of metabolic biomarkers for Duchenne Muscular Dystrophy within a natural history study."
PLOS ONE, 2016. https://doi.org/10.1371/journal.pone.0153461

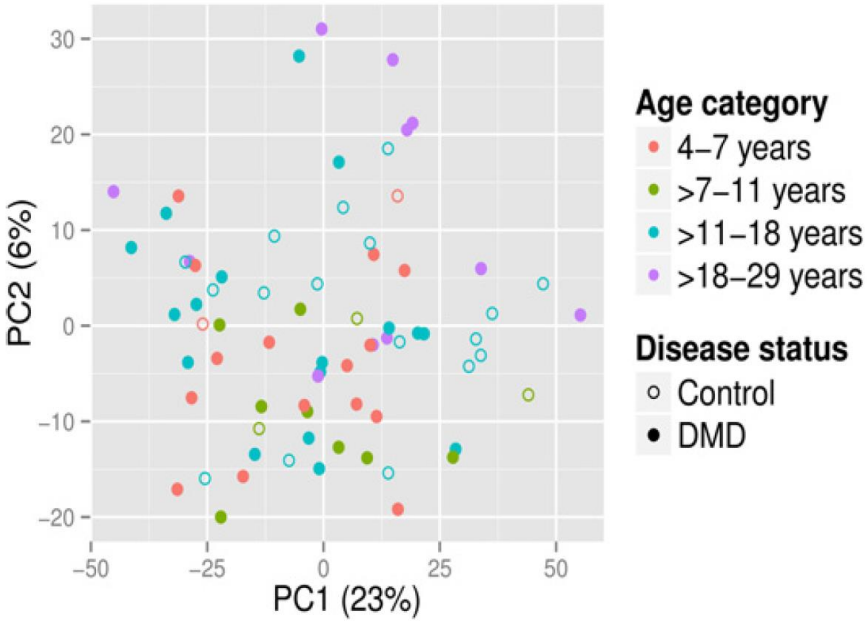
Thangarajh M et al. "Discovery of potential urine-accessible metabolite biomarkers associated with muscle disease and corticosteroid response in the mdx mouse model for Duchenne." *PLOS ONE*, 2019. https://doi.org/10.1371/journal.pone.0219507

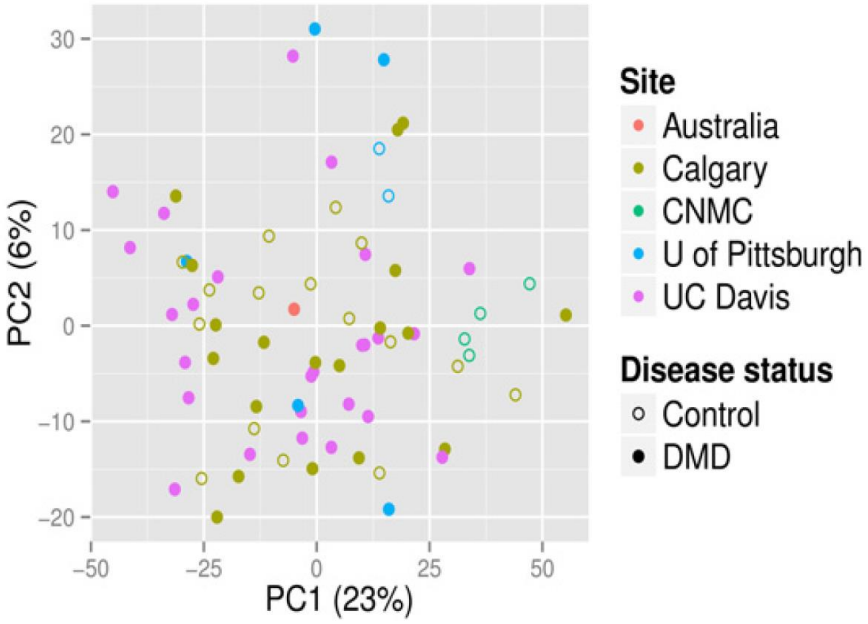
Overview of study

- Use untargeted metabolomics from serum samples of 51 DMD patients and 22 healthy controls.
- A total of 2,203 metabolites detected.

Study site	DMD patients	Healthy controls
Alberta Children's Hospital (Calgary)	19	16
University of California, Davis (UC Davis)	26	0
University of Pittsburgh / Children's Hospital of Pittsburgh of UPMC (U of Pittsburgh)	5	2
Children's National Medical Center (CNMC)	0	4
Children's Hospital at Westmead (Australia)	1	0
Median age in years (minimum, maximum)	11.4 (4, 28.7)	13.7 (6, 17.8)
Total by age category		
4–7 years	15	2
> 7–11 years	8	3
> 11–18 years	17	17
> 18–29 years	11	0
Total	51	22

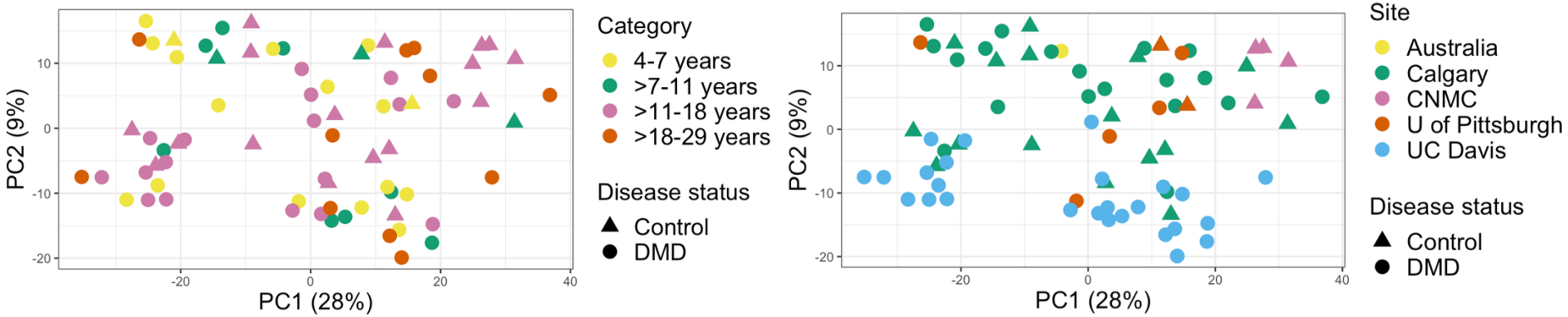
Principal components analysis





Principal components analysis: What I've learned since!

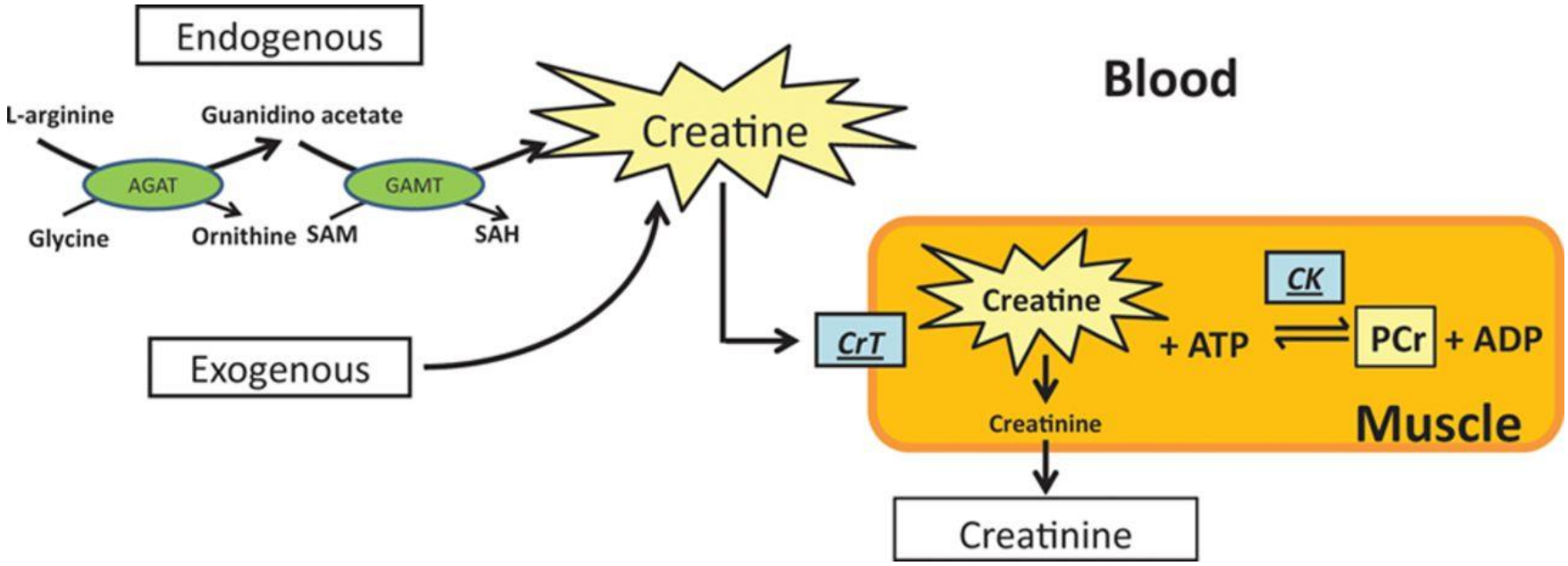
- Use colorblind-friendly palette!
- Don't scale and use same distances on both axes!
  - For example, see Nguyen and Holmes. "Ten quick tips for effective dimensionality reduction." *PLOS Computational Biology*, 2019. https://doi.org/10.1371/journal.pcbi.1006907



Metabolic biomarkers in DMD

- Fourteen metabolites were found significantly altered (FDR of 1%) between DMD patients and healthy controls.
  - Models adjusted for age and site, considered age x disease interaction
- Metabolites validated using MS/MS:
  - Higher in cases:
    - Creatine, L-arginine (likely validated)
  - Higher in controls:
    - Creatinine, 5a-DHT, testosterone sulfate

Creatine pathway and DMD

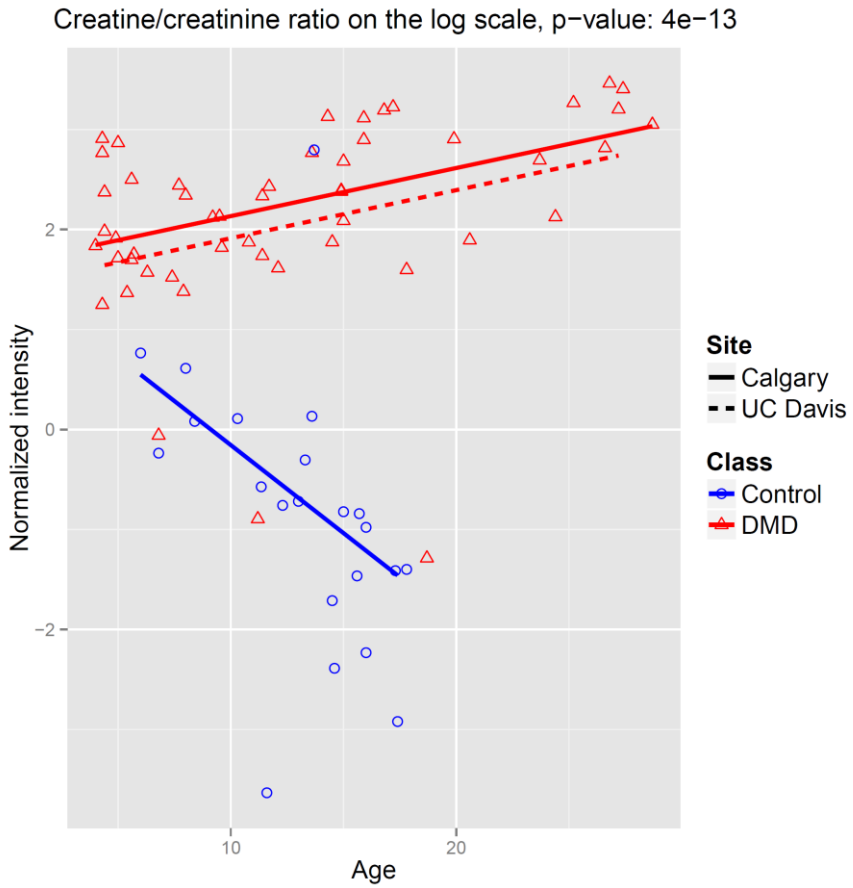


CK = creatine kinase

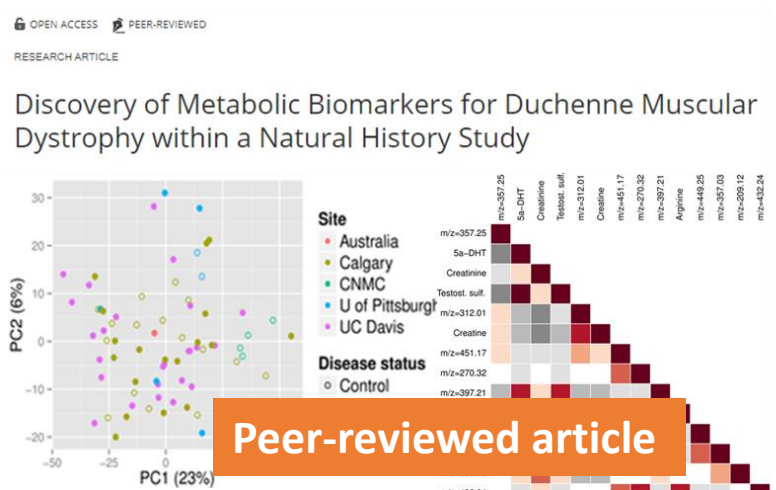
From: https://www.ahajournals.org/doi/full/10.1161/CIRCRESAHA.113.300974

Metabolic biomarkers in DMD

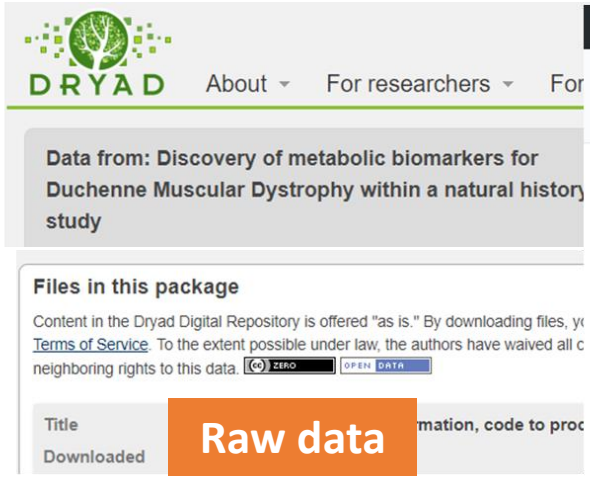
- Also considered the ratio between creatine and creatinine as a possible biomarker:



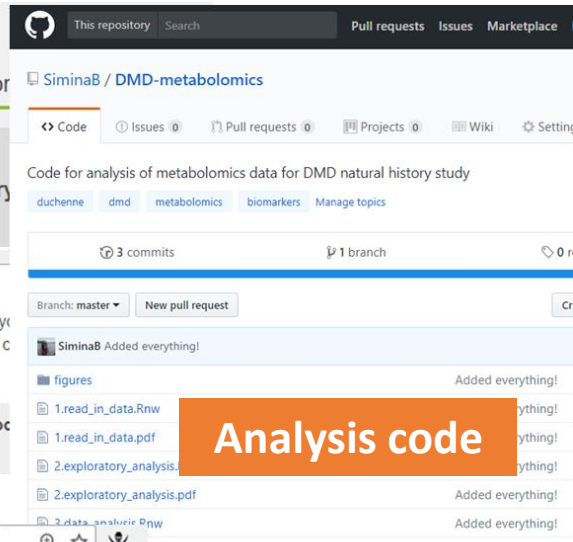
Study Ecosystem: Beyond the paper



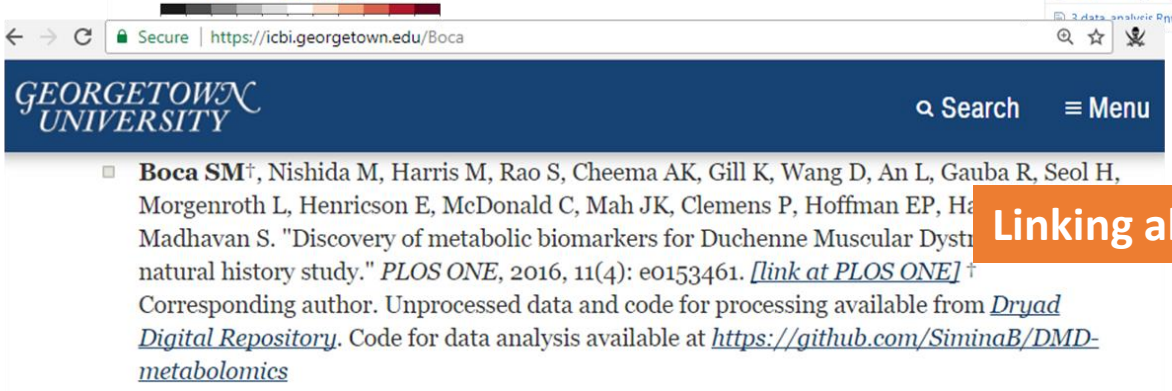
Peer-reviewed article



Raw data



Analysis code



Linking all components

Reproducibility: Powered by

Conclusions

- The 2016 study was the first comprehensive metabolomic study for DMD.
  - Represents one of the first steps towards finding metabolic surrogate biomarkers of disease progression.
  - Adds to the list of possible non-invasive blood circulating biomarkers.
- The 2020 study provided additional biomarkers, which may be related to either the mechanisms of muscle injury in DMD or prednisolone treatment.
- Future goals include:
  - Embedding these types of studies into clinical trials!
  - Connecting this type of study with genetic data and focusing it on the areas of greatest need.

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