In Vivo Disease Modeling

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Outline

- What is *in vivo* disease modeling?
- Utility of *in vivo* disease models
- What makes a good *in vivo* disease model?
- Examples of disease models (🐭, 🐶, 🐟, 🐵…)
  - What made it a good model;
  - What methods were used;
  - Implication of the results.
- Ethics
- References

What is *in vivo* disease modeling?

Use live animals to study disease

- *in vivo*
  - (Latin for "within the living") ⟶ complex biological system
- Modeling
  - devise a simplified description (of a system or process) to assist calculations and predictions.

    Definitions from Oxford Languages

in vitro (Latin for "within the glass") In vitro models are defined as synthetic experimental systems that contain living human cells, mimic tissue, and organ-level physiology in vitro by taking advantage of recent advances in tissue engineering and microfabrication.

Utility of *in vivo* disease models

- Understand disease transmission (infectious disease);
- Understand disease progression;
- Understand disease mechanism;
  - Identify disease causing genes/mutations;
  - Study the signaling pathway or gene regulatory networks that underlie the disease;
  - Identify genetic modifiers of disease phenotype and potential treatment
- Drug and vaccine development;

What makes a good *in vivo* disease model?

- Genetic diversity with well curated resources;
- Produce large amount of offsprings;
- Short life span;
- Tractable experimental systems;
- Cheap and easy to maintain;
- Resemble human physiology or disease symptoms

What makes 🐭 a good *in vivo* disease model?

- Genetic diversity with well curated resources;
- Produce large amount of offsprings; (3-14 per litter. 5-10 litters per year)
- Short life span; (26-30 months)
- Tractable experimental systems;
- Cheap and easy to maintain;
- Resemble human physiology or disease symptoms (in some cases…)

Hutchinson-Gilford Progeria Syndrome/accelerated aging

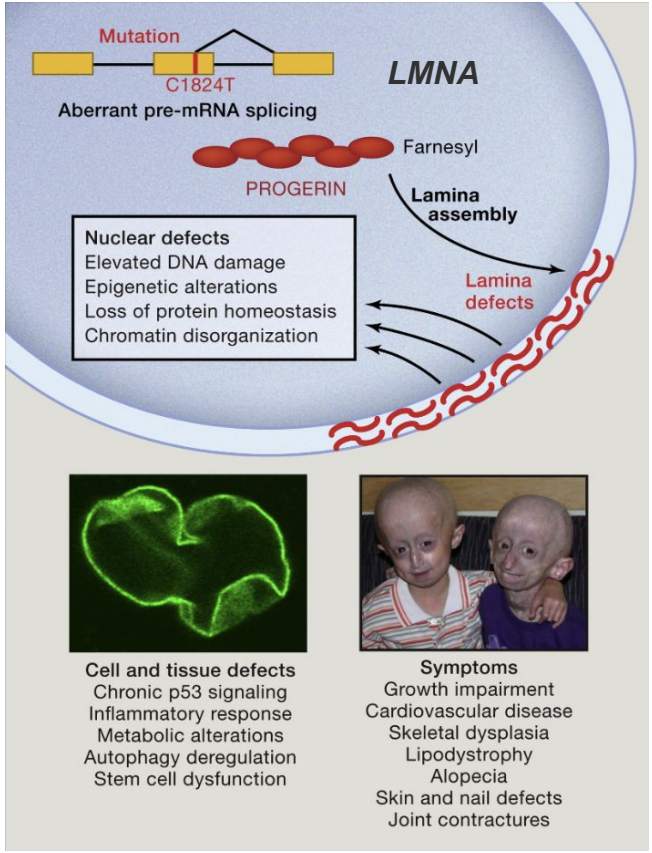


PHOTO BY LEAH FASTEN

TIMELINE HIGHLIGHTS 1999-2021: OUR HISTORY, OUR FUTURE...

1999

March: The Progeria Research Foundation (PRF) is incorporated as a Massachusetts non-profit organization. On June 9, 1999, the Board of Directors holds its first meeting.

August: PRF awards its 1st research grant. To date, PRF has awarded 77 grants totaling over $8.4 million. The projects have allowed innovative research in Progeria to thrive.

November: PRF launches its website, a comprehensive resource of information on Progeria for researchers, families of children with Progeria and their physicians, and the general public. Together with social media sites (nearly 1 million Facebook followers!), millions around the world follow PRF's progress regularly.

2000

January: PRF launches its International Patient Registry, maintaining centralized information on children and families living with Progeria. This program assures rapid distribution of new information that may benefit the children.

October 17, 2000: PRF is instrumental in securing Progeria-specific guidelines for the National Institutes of Health (NIH) in the 2000 Children's Health Act. As a result, NIH has regularly supported Progeria research efforts, including all PRF scientific workshops, a first-ever natural history study of children with Progeria, the Progeria Triple Drug Trial, and preclinical research.

2001

November: PRF holds its premier workshop in Bethesda, Maryland. PRF has organized 13 successful scientific conferences that have brought together scientists and clinicians from all over the world to share their expertise and cutting-edge scientific data, and foster collaboration in the fight against Progeria.

2002

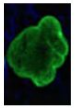
January: The PRF Genetics Consortium is formed with the goal of finding the gene for Progeria, which was accomplished ten months later!

March: PRF launches the Cell & Tissue Bank at Rhode Island Hospital, providing researchers the biological tools needed to advance Progeria research. The Bank contains thousands of samples and cell lines, distributed to hundreds of laboratories in over two dozen countries.

The PRF Medical & Research Database is created, to help learn more about the disease and provide treatment recommendations.

2003

May: **PROGERIA GENE DISCOVERED!** Using cells from the PRF Cell & Tissue Bank, PRF Genetics Consortium members Drs. Maria Eriksson and Francis Collins lead the Progeria gene discovery, published in the journal Nature.



June: In the wake of the gene discovery, PRF launches its Progeria Diagnostics Program, enabling earlier diagnosis, fewer misdiagnoses and early medical intervention.

2004

August: PRF awards its 10th research grant, funding the creation of a Progeria mouse. Animal models provide essential preclinical testing for new drugs.

2005

November: PRF's 1st chapter opens in California. Chapters, as well as thousands of other volunteers worldwide, help raise awareness and conduct local fundraising events in support of PRF's mission.

2006

June: PRF launches a public service announcement (PSA) campaign featuring voices of long-time PRF supporters Ted Danson and Mary Steenburgen. PRF has since created many PSAs with other celebrities, including Boston Bruins players and Dave Matthews.

February/July: PRF-funded studies find that farnesyltransferase inhibitors (FTIs) are a potential treatment for Progeria, as they prevent some signs of disease in Progeria mice. These and other studies, along with data analyzed from PRF's Medical & Research Database, pave the way for a clinical drug trial using the FTI lonafarnib.

2007

April: Boston Children's Hospital (BCH) launches the first-ever Progeria Clinical Drug Trial, funded and co-coordinated by PRF, bringing hope of a possible treatment for children with Progeria.

2008

October: In a stunning display of progress with the FTI drug, and providing further confirmation of how Progeria research may help millions with heart disease, an NIH study finds that FTI's prevent the most devastating effect of Progeria in mice: cardiovascular disease.

2009

onepossible. The Progeria Research Foundation

April: PRF's first annual 'ONEpossible' campaign successfully reaches our $100,000 goal. To date, ONEpossible supporters have raised $2 million towards making a cure POSSIBLE!

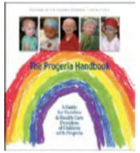
October: PRF partners with Spectrum and GLOBALHealthPR to launch "Find the Other 150", a global campaign to find all children with Progeria so they can get the support they need.

FIND THE OTHER 150

To date, along with other public awareness efforts, PRF has found hundreds of children with Progeria from more than 65 countries.

2010

April: PRF publishes the first Progeria Handbook: A Guide for Families & Health Care Providers of Children with Progeria.



September: A landmark study led by PRF's medical director, in partnership with NIH, shows that the Progeria-causing protein progerin exists and increases in everyone as we age. The study confirms that by examining one of the rarest diseases, we gain crucial insight into the heart disease that affects millions, and the aging affecting us all.

2012

September: **FIRST-EVER TREATMENT FOR PROGERIA DISCOVERED!** PRF makes history, publishing trial results showing that every child experienced improvement in one or more areas, including the vital cardiovascular system. With a definitive finding that a drug (lonafarnib) altered the Progeria disease process, the quest for a cure is stronger than ever.

2013

January: HBO Film's *Life According to Sam (LATS)* debuts at the Sundance Film Festival. Featuring Sam Berns and his parents (PRF co-founders) Drs. Leslie Gordon and Scott Berns, this riveting documentary about love, life and hope for children with Progeria received an Emmy, Peabody and Christopher Awards, and ten festival awards.

December: Sam presented his wildly popular TEDx talk, "My Philosophy for a Happy Life." Both this and LATS have vastly raised awareness of Progeria and the importance of PRF's mission, resulting in an unprecedented surge in supporters, social media presence and general interest in these remarkable children. Today, Sam's talk is the second most viewed TEDx talk, with over 85 million views!

2014

July: While PRF continues to search for more effective drug candidates, the PRF-funded extension and expansion of the clinical trial gives every child with Progeria access to lonafarnib, the drug that could give them longer and healthier lives.

The drug trial expansion allowed Prachi, age 4, from India to receive lonafarnib.

2015

PRF continues its remarkable pace of progress, working on a multitude of research-related projects and achieving a record-high $1 million investment in research grants this year.

July: PRF and Merck make lonafarnib available to the research community through the PRF Cell & Tissue Bank, fostering more basic science studies to further investigate the effects of lonafarnib on Progeria.

2016

April: Children begin to enroll in a new, 2-drug trial with lonafarnib plus everolimus, with the hope that the two drugs together will be even more effective than lonafarnib alone.

NEW DRUG, AND NEW HOPE FOR CHILDREN WITH PROGERIA.

Zoey (left) and Carley enroll in the PRF-funded 2-drug trial at Boston Children's Hospital.

2017

Phase 1 of the 2-drug trial is completed, and Phase 2 begins. Phase I determined the best dose of everolimus to give the children. Phase 2 determines whether the combination therapy is effective. Children from 27 countries, speaking 20 languages, come to Boston to participate.

Mio, 13 years old traveled from Japan to Boston to participate in the 2-drug trial.

2018

April: In a spectacular development, a new study published in The Journal of the American Medical Association (JAMA) shows that lonafarnib extends lifespan in children with Progeria. This is the first evidence that lonafarnib improves the children's survival – a remarkable feat that demonstrates our work is resulting in longer, stronger lives for them.

May: On the heels of the JAMA study, PRF partners with Eiger BioPharmaceuticals to pursue U.S. Food and Drug Administration (FDA) approval of lonafarnib, marking the first Progeria therapy to be submitted to the FDA.

JAMA The Journal of the American Medical Association

2019

December: PRF's biopharmaceutical partner, Eiger, submits a New Drug Application (NDA) to the FDA for lonafarnib, a major step toward approval as the first-ever treatment for Progeria.

2020

November: PRF holds its 10th international scientific workshop, with over 370 registrants from 30 countries coming together on Zoom to share the latest findings in Progeria research and hear from some of the families being helped by their work.

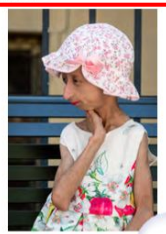
FDA APPROVAL FOR LONAFARNIB (now branded as 'Zokinvy') is granted! Progeria now joins fewer than 5% of rare diseases with an FDA-approved treatment.

FDA

2021

January and March: Breakthrough studies on Progeria mouse models, co-funded by PRF and co-authored by PRF's Medical Director Dr. Leslie Gordon, are published in the journal *Nature and Nature Medicine*. One study shows that genetic editing in mice can correct the mutation that causes Progeria, improves several key disease symptoms, and dramatically increases lifespan in the mice. The other studies used RNA therapies to reduce the toxic protein, progerin, and improved survival in the mice. Additional preclinical studies are needed to investigate these results, which we hope will one day lead to a clinical trial.

nature



We've come so far
In such a short time.
With your continued support,
Together, we *WILL* cure Progeria!

Mission

To discover treatments and the cure for Progeria and its aging-related disorders, including heart disease.

Progeria Research Foundation
FOR THE CHILDREN ♥ FOR THE CURE

Mouse model for Hutchinson-Gilford Progeria Syndrome

In vivo base editing rescues Hutchinson–Gilford progeria syndrome in mice

https://doi.org/10.1038/s41586-020-03086-7

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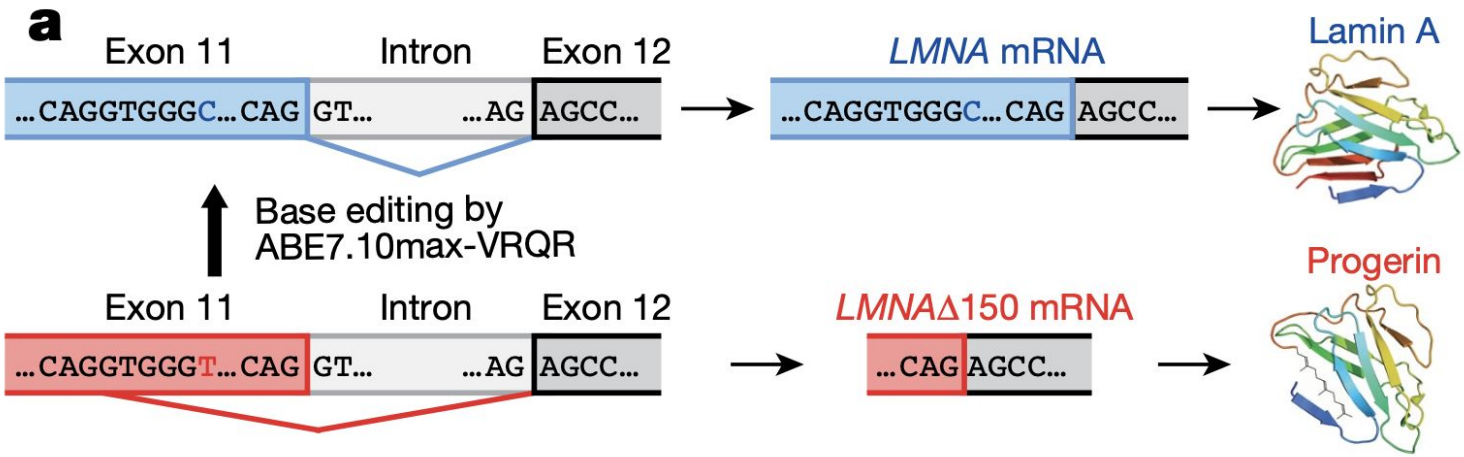
Luke W. Koblan[1,2,3,13], Michael R. Erdos[4,13], Christopher Wilson[1,2,3], Wayne A. Cabral[4], Jonathan M. Levy[1,2,3], Zheng-Mei Xiong[4], Urraca L. Tavarez[4], Lindsay M. Davison[5], Yantenew G. Gete[6], Xiaojing Mao[6], Gregory A. Newby[1,2,3], Sean P. Doherty[5], Narisu Narisu[4], Quanhu Sheng[7], Chad Krilow[4], Charles Y. Lin[8,9,12], Leslie B. Gordon[10,11], Kan Cao[6], Francis S. Collins[4]✉, Jonathan D. Brown[5]✉ & David R. Liu[1,2,3]✉



In vivo base editing rescues Hutchinson–Gilford progeria syndrome in mice

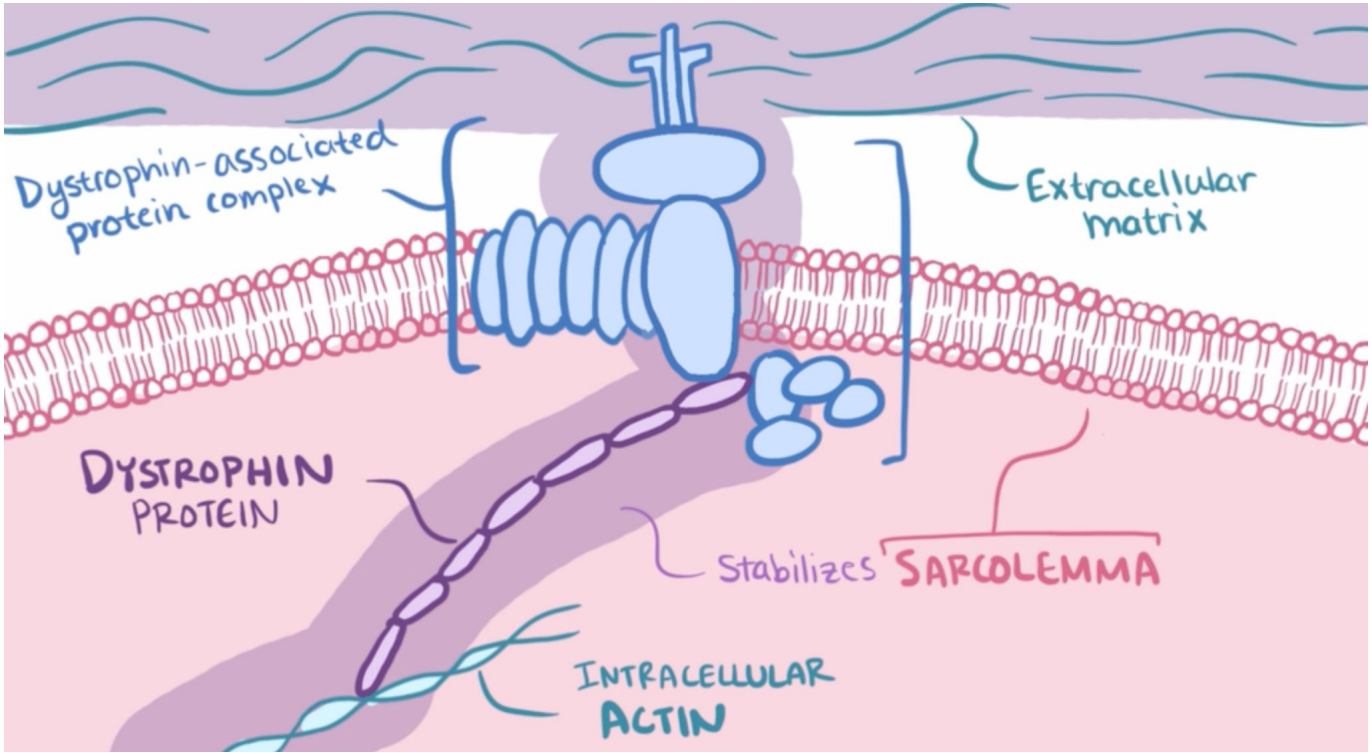
Going forward

- Best result to date in the mouse model;
- a number of the longest-living treated mice developed liver tumors — a known long-term complication when using adeno-associated viruses (AAV) to deliver genes into mice;
- Base editors may be less effective in primates (61% *in vivo* gene editing efficacy in the liver of mice compared to 26% in primates).

🐭 offers the most comprehensive set of tools for preclinical research (if the disease pathology mimics human disease well).

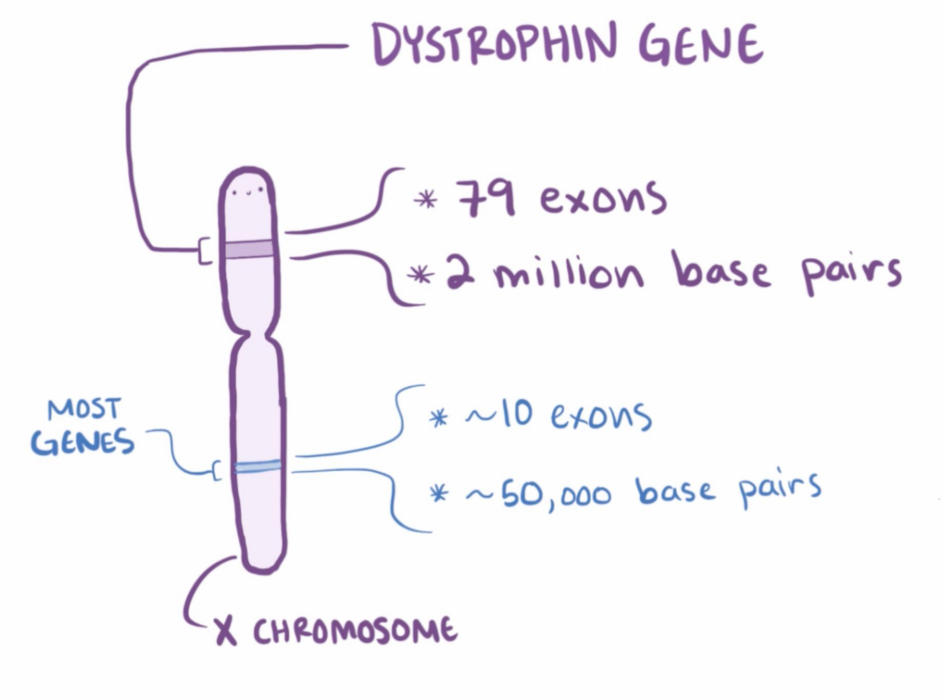
Duchenne Muscular Dystrophy (DMD)

DMD is caused by loss-of-function mutations in the Dystrophin gene



Merck Manual for the Professional/Duchenne and Becker Muscular Dystrophy

DMD is a x-linked recessive disease



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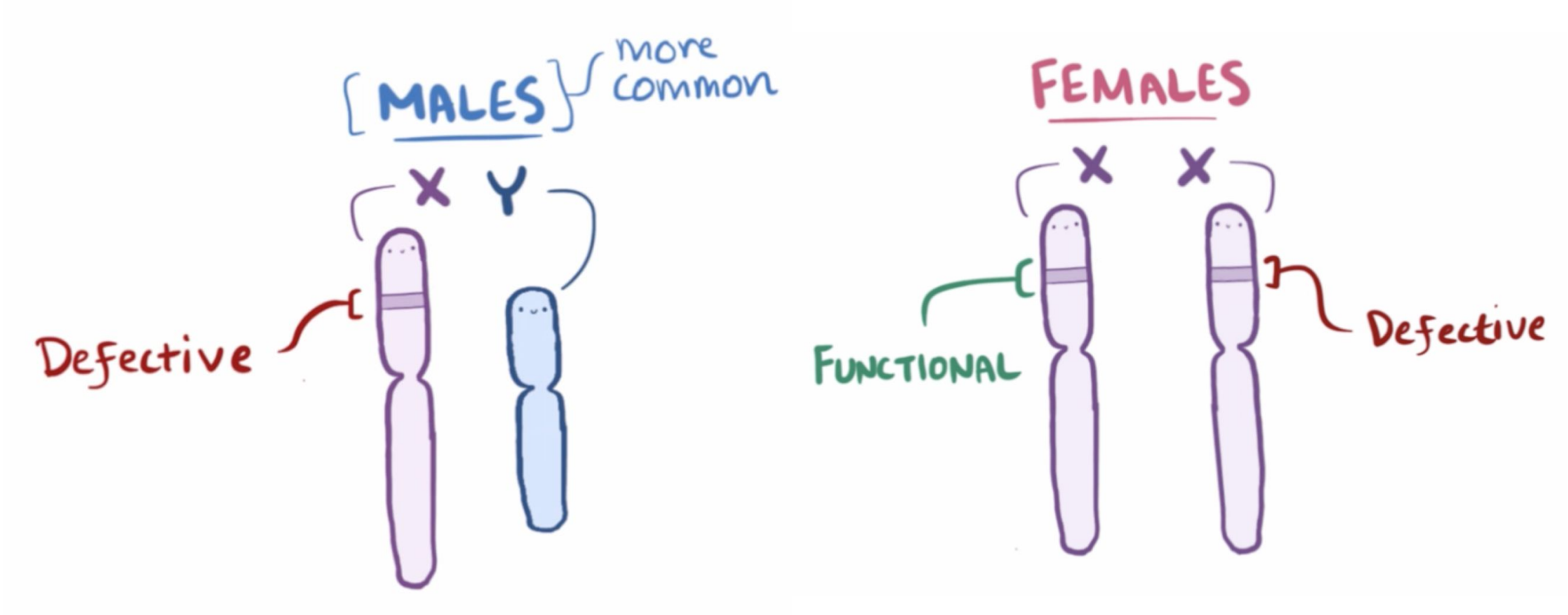
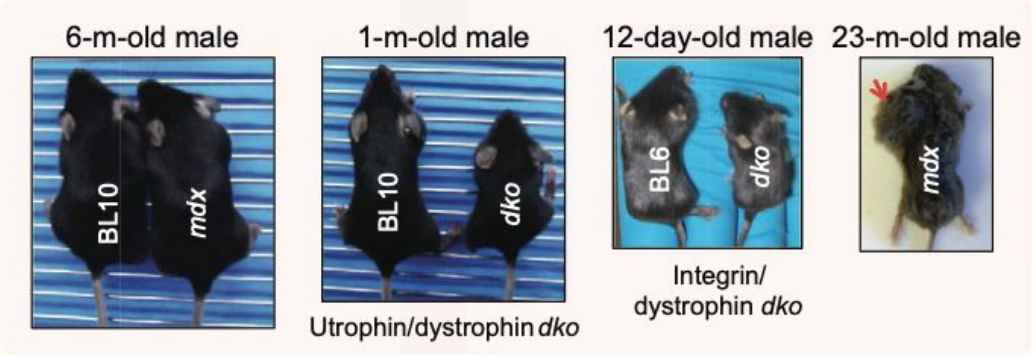
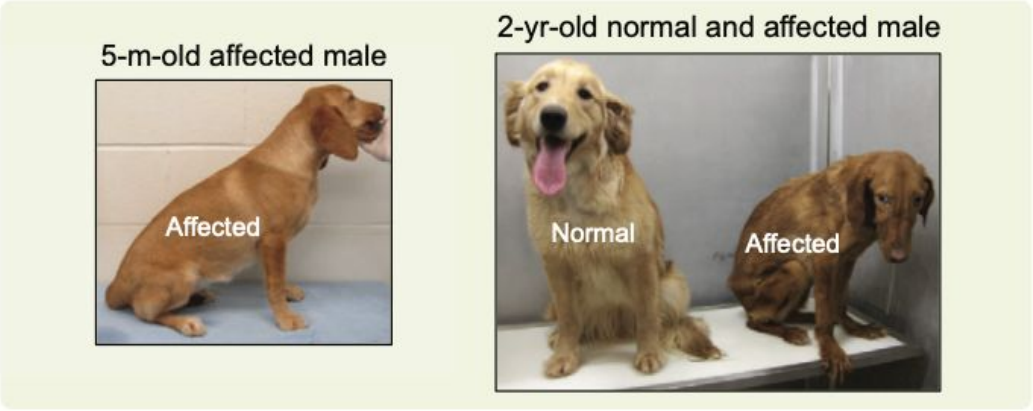
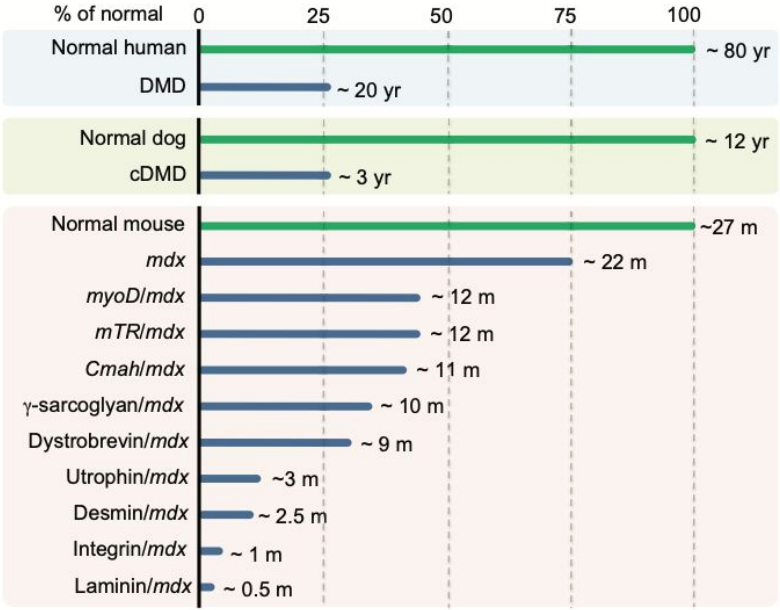


Merck Manual for the Professional/Duchenne and Becker Muscular Dystrophy

Animal models for DMD

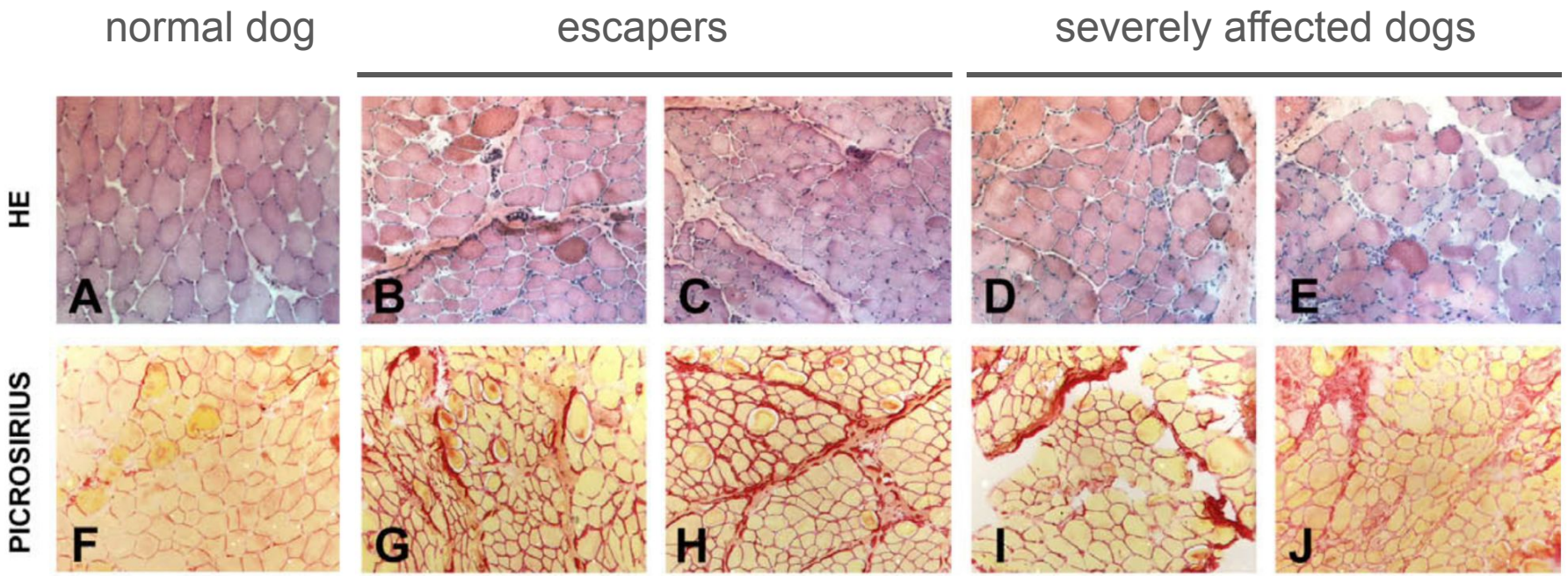




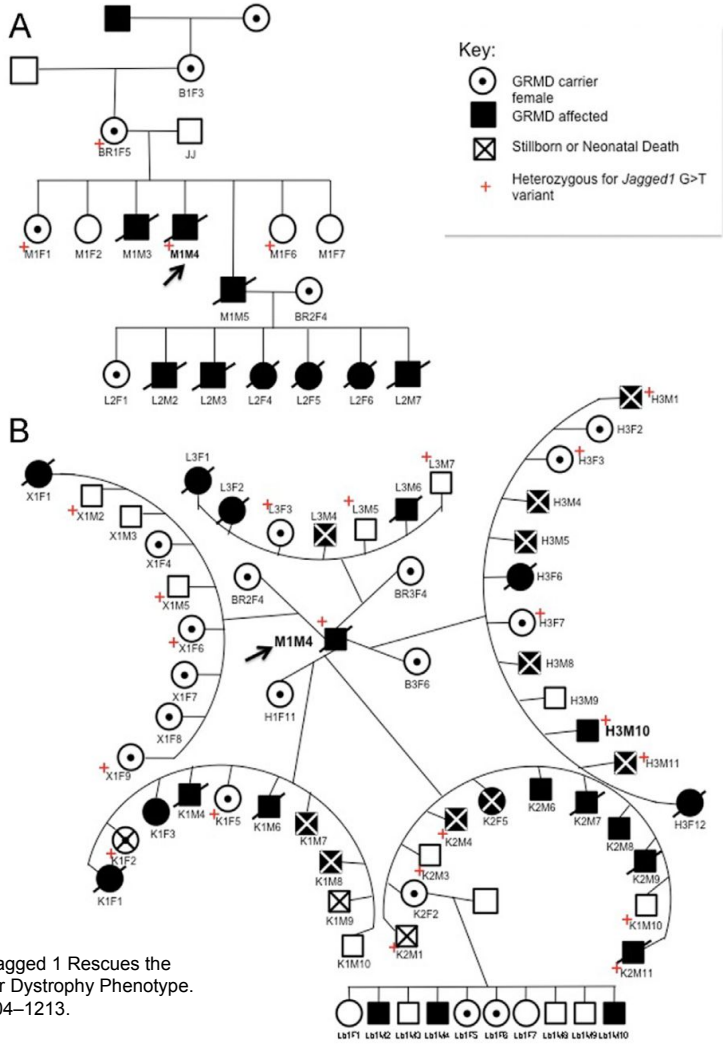


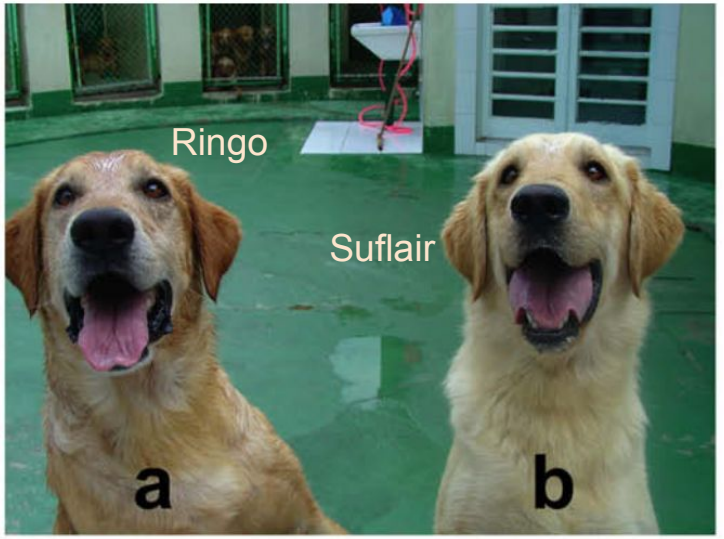
McGreevy, J.W. et al., 2015. Animal models of Duchenne muscular dystrophy: from basic mechanisms to gene therapy. *Disease models & mechanisms*, 8(3), pp.195–213.

Escapers showed same muscular dystrophic process as severely affected dogs



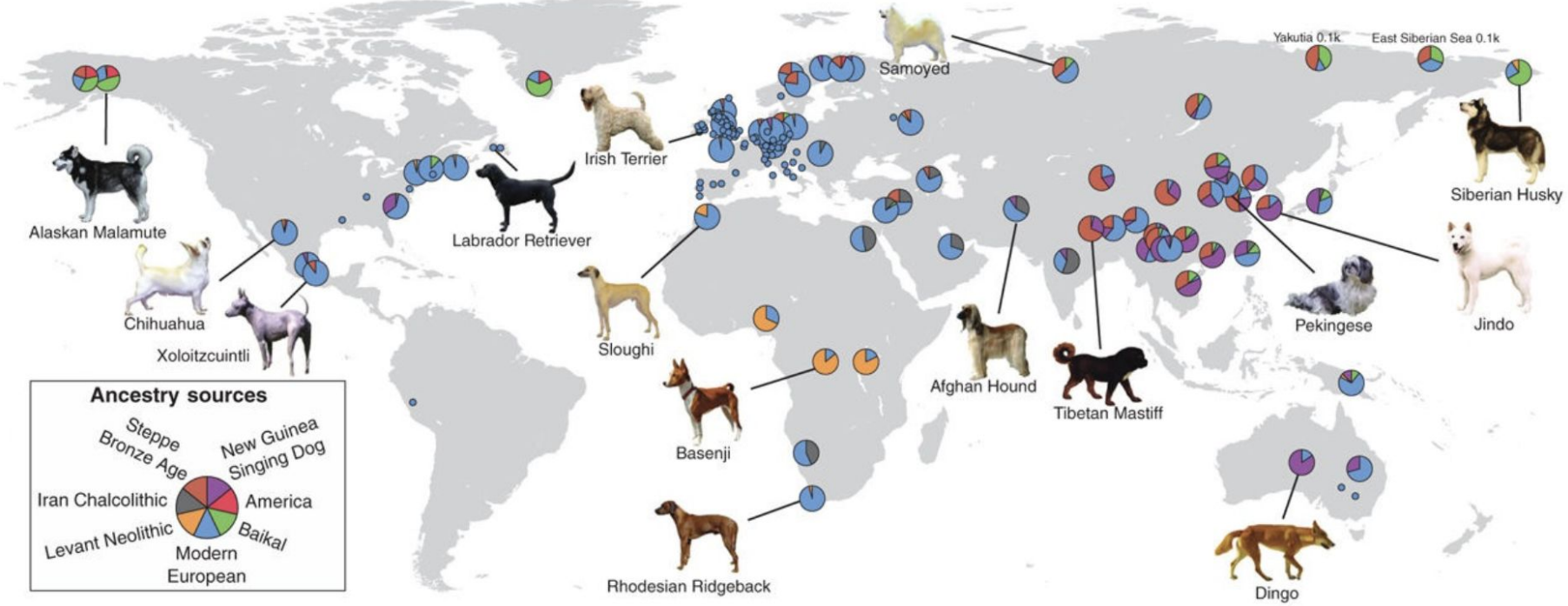
E. Zucconi et al./Neuromuscular Disorders 20 (2010) 64–70





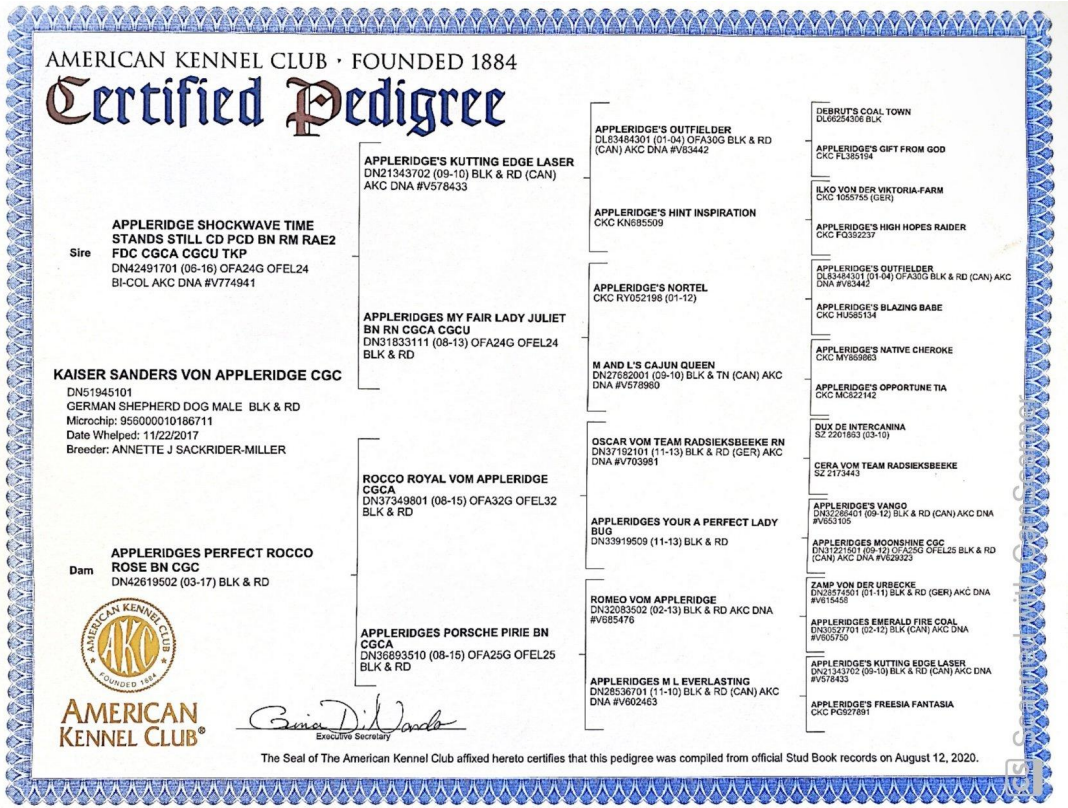
Vieira, N.M. et al., 2015. Jagged 1 Rescues the Duchenne Muscular Dystrophy Phenotype. *Cell*, 163(5), pp.1204–1213.

Dog breeds around the world provide genetic diversity



Today's dogs can trace their ancestry to canines that lived up to 11,000 years ago. (Bergstrom et al. / Science)

Dog breeds are genetically isolated populations within the same species



AMERICAN KENNEL CLUB · FOUNDED 1884

Certified Pedigree

KAISER SANDERS VON APPLERIDGE CGC
DN51945101
GERMAN SHEPHERD DOG MALE BLK & RD
Microchip: 956000010186711
Date Whelped: 11/22/2017
Breeder: ANNETTE J SACKRIDER-MILLER

Sire APPLERIDGE SHOCKWAVE TIME STANDS STILL CD PCD BN RM RAE2 FDC CGCA CGCU TKP
DN42491701 (06-16) OFA24G OFEL24
BI-COL AKC DNA #V774941

- APPLERIDGE'S KUTTING EDGE LASER
  DN21343702 (09-10) BLK & RD (CAN) AKC DNA #V578433
  - APPLERIDGE'S OUTFIELDER
    DL83484301 (01-04) OFA30G BLK & RD (CAN) AKC DNA #V83442
    - DEBRUT'S COAL TOWN
      DL66254306 BLK
    - APPLERIDGE'S GIFT FROM GOD
      CKC FL385194
  - APPLERIDGE'S HINT INSPIRATION
    CKC KN685509
    - ILKO VON DER VIKTORIA-FARM
      CKC 1055755 (GER)
    - APPLERIDGE'S HIGH HOPES RAIDER
      CKC FQ392237
- APPLERIDGES MY FAIR LADY JULIET BN RN CGCA CGCU
  DN31833111 (08-13) OFA24G OFEL24 BLK & RD
  - APPLERIDGE'S NORTEL
    CKC RY052196 (01-12)
    - APPLERIDGE'S OUTFIELDER
      DL83484301 (01-04) OFA30G BLK & RD (CAN) AKC DNA #V83442
    - APPLERIDGE'S BLAZING BABE
      CKC HU585134
  - M AND L'S CAJUN QUEEN
    DN27682001 (09-10) BLK & TN (CAN) AKC DNA #V578980
    - APPLERIDGE'S NATIVE CHEROKEE
      CKC MY859883
    - APPLERIDGE'S OPPORTUNE TIA
      CKC MC822142

Dam APPLERIDGES PERFECT ROCCO ROSE BN CGC
DN42619502 (03-17) BLK & RD

- ROCCO ROYAL VOM APPLERIDGE CGCA
  DN37349801 (08-15) OFA32G OFEL32 BLK & RD
  - OSCAR VOM TEAM RADSIEKSBEEKE RN
    DN37192101 (11-13) BLK & RD (GER) AKC DNA #V703981
    - DUX DE INTERCANINA
      SZ 2201893 (03-10)
    - CERA VOM TEAM RADSIEKSBEEKE
      SZ 2173443
  - APPLERIDGES YOUR A PERFECT LADY BUG
    DN33919509 (11-13) BLK & RD
    - APPLERIDGE'S VANGO
      DN32286401 (09-12) BLK & RD (CAN) AKC DNA #V653105
    - APPLERIDGES MOONSHINE CGC
      DN31221501 (09-12) OFA25G OFEL25 BLK & RD (CAN) AKC DNA #V628323
- APPLERIDGES PORSCHE PIRIE BN CGCA
  DN36893510 (08-15) OFA25G OFEL25 BLK & RD
  - ROMEO VOM APPLERIDGE
    DN32083502 (02-13) BLK & RD AKC DNA #V685476
    - ZAMP VON DER URBECKE
      DN29574501 (01-11) BLK & RD (GER) AKC DNA #V615458
    - APPLERIDGES EMERALD FIRE COAL
      DN30527701 (02-12) BLK (CAN) AKC DNA #V605750
  - APPLERIDGES M L EVERLASTING
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    - APPLERIDGE'S KUTTING EDGE LASER
      DN21343702 (09-10) BLK & RD (CAN) AKC DNA #V578433
    - APPLERIDGE'S FREESIA FANTASIA
      CKC PC927891

AMERICAN KENNEL CLUB®

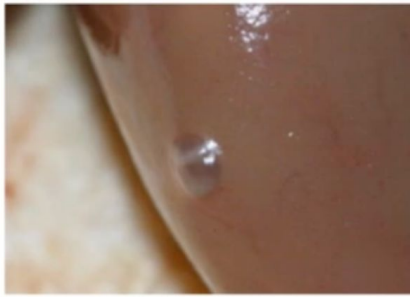
Executive Secretary

The Seal of The American Kennel Club affixed hereto certifies that this pedigree was compiled from official Stud Book records on August 12, 2020.

Canine Hereditary Multifocal Renal Cystadenocarcinoma and Nodular Dermatofibrosis (RCND)

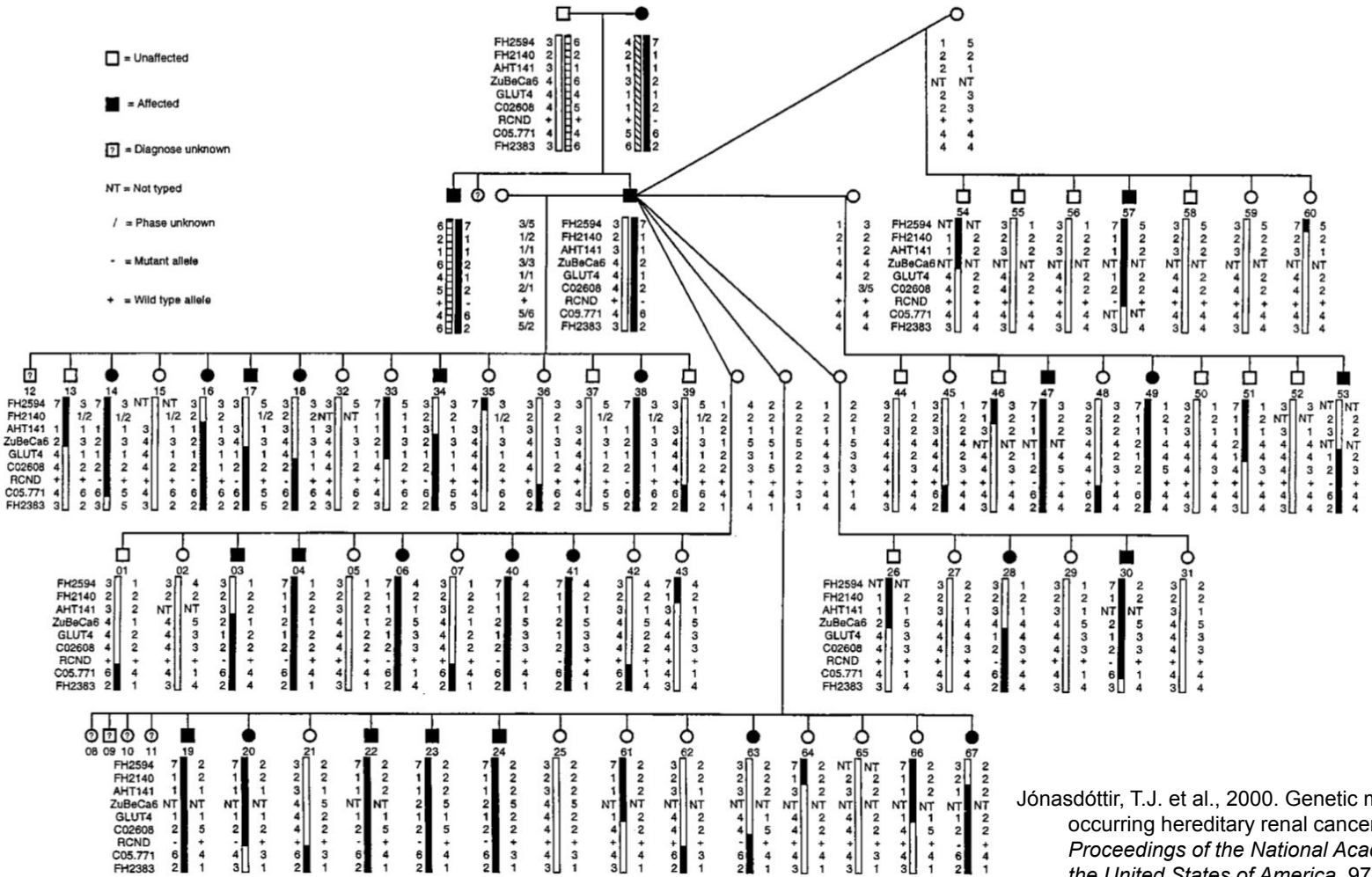
German Shepherds

- Autosomal dominant form of kidney cancer
- Early diagnosis by observation of microscopic renal cysts
- Skin-fibrofolliculomas or trichodiscomas
- Lung-cysts and pneumothorax
- Kidney -7 fold increase in risk for renal cell carcinoma tumors



Renal cortical cyst in 8 week puppy

Jonasdottir et al., 2000 PNAS; Lingaas et al., 2003 Hum Mol Genet

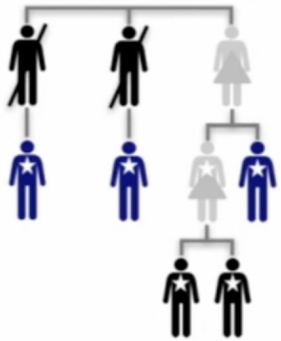


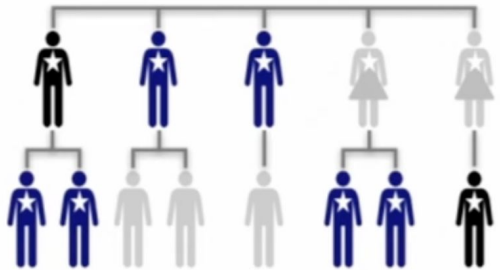
Jónasdóttir, T.J. et al., 2000. Genetic mapping of a naturally occurring hereditary renal cancer syndrome in dogs. *Proceedings of the National Academy of Sciences of the United States of America*, 97(8), pp.4132–4137.

Birt-Hogg Dube Syndrome

Conclusions

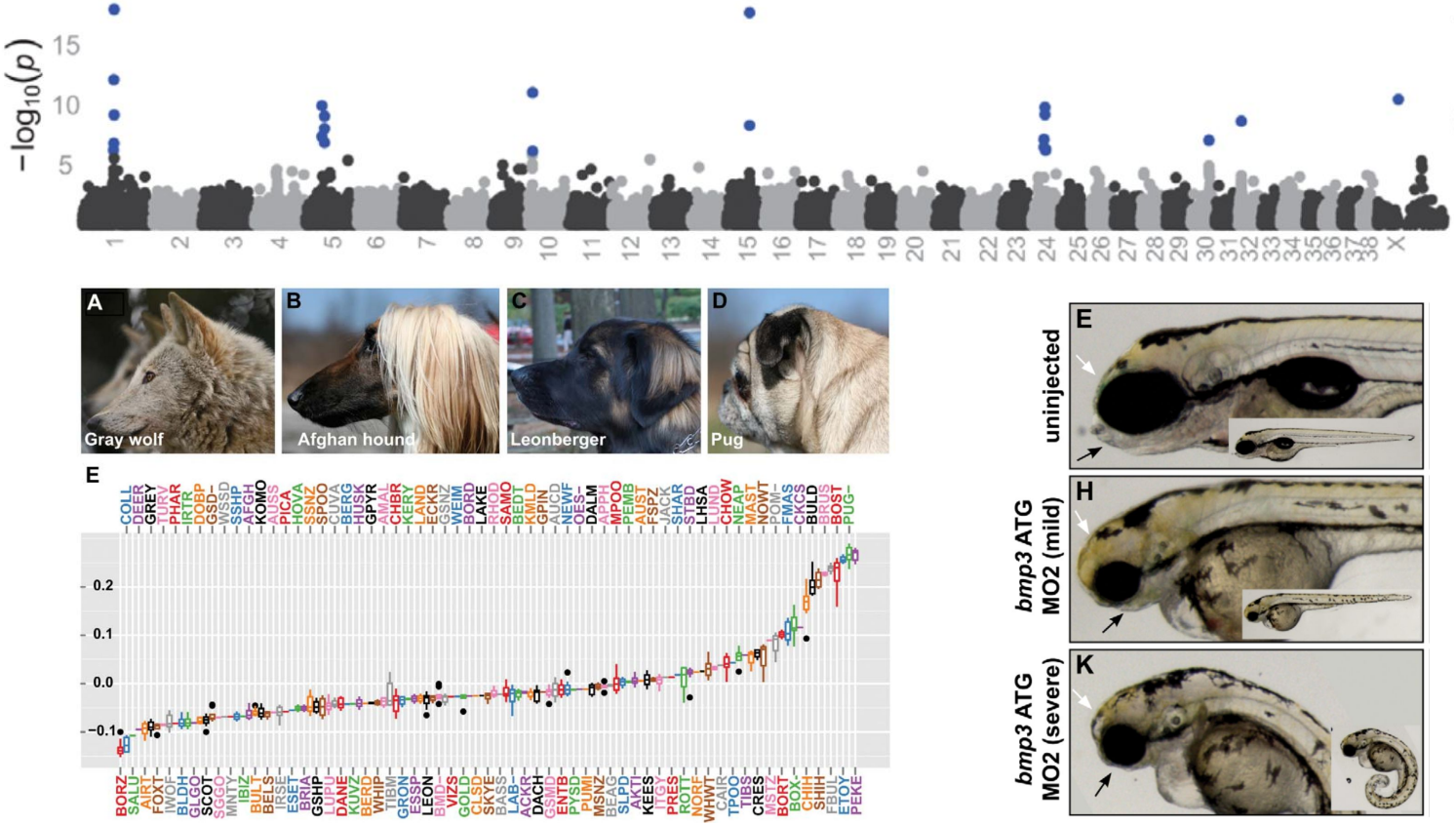
- Disease caused by germline mutations in the *folliculin* gene, which encodes a tumor suppressor.
- Signaling link between folliculin, mTOR pathway and cancer susceptibility.
- The locus found first in canine genetic study (not human), but the gene causes the human disorder Birt-Hogg Dube Syndrome
- Such pedigrees are unusual in canine studies, impossible to find in human genetic studies, especially in cancer.





Jonasdottir et al., 2000 PNAS; Lingaas et al., 2003 Hum Mol Genet

offers powerful disease pedigree and population genetic resources



Schoenebeck, J.J. et al., 2012. Variation of BMP3 contributes to dog breed skull diversity. *PLoS genetics*, 8(8), p.e1002849.

Further readings related to the journal club paper

Duchenne Muscular Dystrophy: Advancements Research in the Pipeline

FDA Approves First Gene Therapy for Treatment of Certain Patients with Duchenne Muscular Dystrophy

Sarepta Therapeutics/Duchenne: A Rare Genetic Neuromuscular Disease

Uffelmann, E., Huang, Q.Q., Munung, N.S. et al. Genome-wide association studies. Nat Rev Methods Primers 1, 59 (2021). https://doi.org/10.1038/s43586-021-00056-9

Understanding QQ Plots

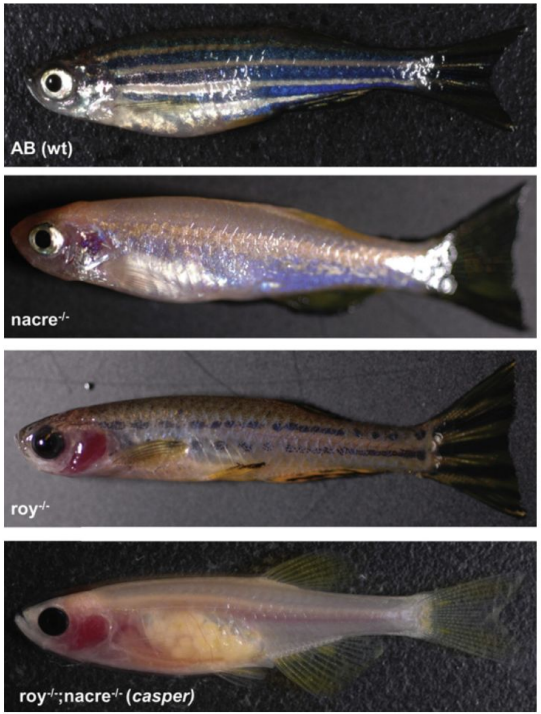
LOD SCORE

Ostrander, E.A., 2012. Both Ends of the Leash — The Human Links to Good Dogs with Bad Genes. The New England journal of medicine, 367(7), pp.636–646.

Dog Genes Tell Surprising Tales - Dr. Elaine Ostrander

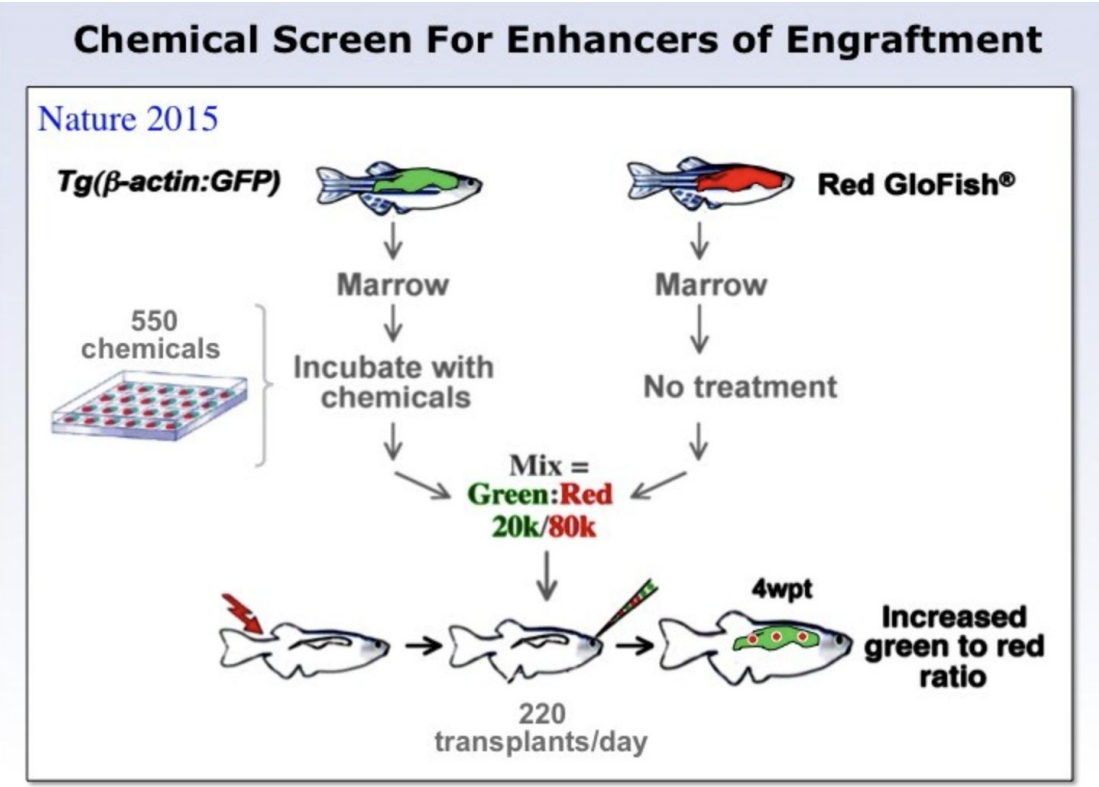
Zebrafish 🐟, a transparent model

- The zebrafish is a member of the minnow family of fish.
- The zebrafish embryo is transparent, it develops outside of its mother, and its development from eggs to larvae happens in just three days.
- Easy to maintain. Not very susceptible to disease;
- 20 - 200 offspring in a single breeding;
- Good model for chemical genetics approaches for drug screen.

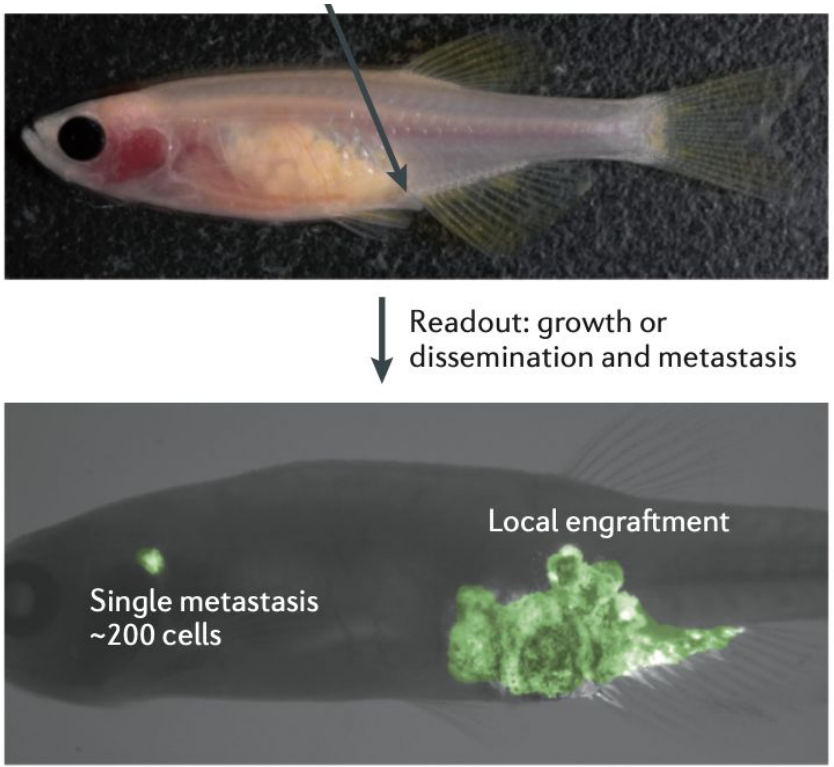


White, R.M. et al., 2008. Transparent adult zebrafish as a tool for in vivo transplantation analysis. *Cell stem cell*, 2(2), pp.183–189.

Zebrafish model for bone marrow transplantation



Zebrafish model for cancer metastasis



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Mouse lemur 🐵

- Mouse lemurs are the smallest primates that are about twice the size of a mouse and live exclusively on Madagascar;
- Genetic diversity in wild populations;
- Closely resemble human physiology.

Ho, C.L.A., Fichtel, C. & Huber, D., 2021. The gray mouse lemur (Microcebus murinus) as a model for early primate brain evolution. *Current opinion in neurobiology*, 71, pp.92–99.

The Mouse Lemur, a Genetic Model Organism for Primate Biology, Behavior, and Health

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The Mouse Lemur, a Genetic Model Organism for Primate Biology, Behavior, and Health

Ethics

- Diseased animals breed for research purposes;
- Protocols and guidelines to manage pain and suffering of animals;
- Improve veterinary medicine;
- The most used animal models are what we feel comfortable killing in mass.

Summary

- Different animal models offer different things;
- Understand the model systems;
- Clarify your question and pick a model that would help you address the clinical need;
- Plan your experiments well.

UCSF BROAD STEM CELL CENTER

UCSF Department of Surgery

Getting to Patients

with Alexander Fay, MD, PhD

Parnassus Campus, Health Sciences West
Presentation: **HSW-301 (4 – 5:30 PM)**
Reception: **HSW Lobby (5:30 – 6 PM)**

Tuesday, Sept 3
4 – 6 PM

Gene Therapy for Duchenne Muscular Dystrophy

with a Patient Perspective

Dr. Alexander Fay, pediatric neurologist with special training in neuromuscular diseases, and his patient will discuss a Duchenne muscular dystrophy therapy, followed by Q&A session moderated by Dr. Julia Brown, Assistant Professor, UCSF Bioethics Program. Reception to follow.

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