Basel Life Sciences Week: Aging & Drug Discovery
Basel, September 24, 2015

Aging Research: 1985-2015

George M. Martin, MD
Professor of Pathology Emeritus
University of Washington

Outline

- **Replicative senescence**: from Alexis Carrel to Leonard Hayflick to Liz Blackburn and the First Nobel Prize for basic research on the biology of aging
- What we have learned about the role of **nuclear genomic instability** since 1985
- What we have learned about the role of **mitochondria** in biological aging since 1985
- Learning how to “wake up” **stem cells**
- Genomes, Epigenomes, Transcriptomes, Metabolomes and Expososomes may lead to a new era of Precision Geroscience, but wait – we will still have to deal with **stochastic events** in aging!

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The Nobel Prize in Physiology or Medicine 2009

Elizabeth Blackburn

Carol Greider

Jack Szosak

"for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase"

Early telomerase inactivation accelerates aging independently of telomere length

Z Xie et al., Cell. 2015
Liz Blackburn Lab

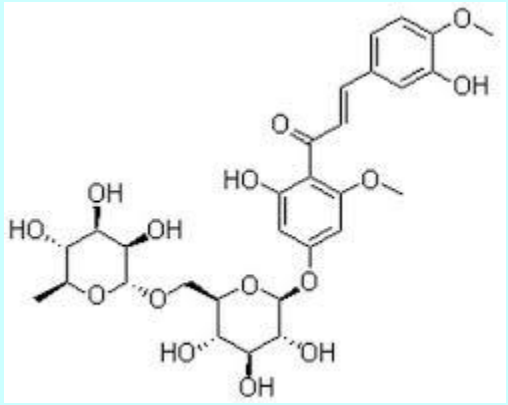
Early after telomerase inactivation (ETI) mother cell aging is accelerated in yeast

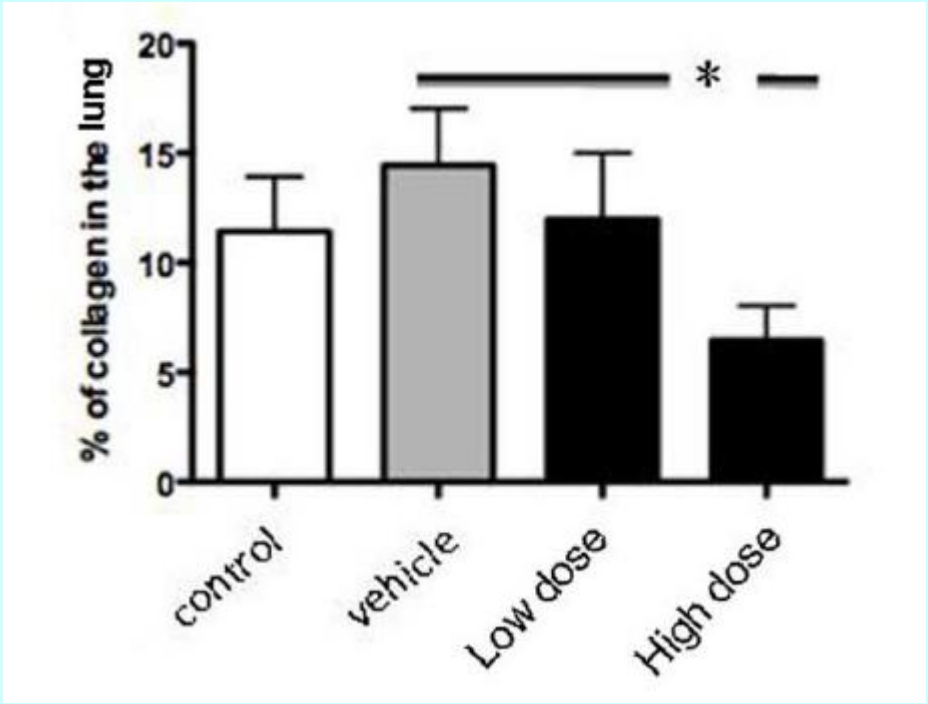
Accelerated aging occurs before critical telomere shortness induced senescence

ETI mother cells show heterogeneous cell cycles that track with lifespan reduction

ETI acceleration of mother cell aging results from transient DNA damage response

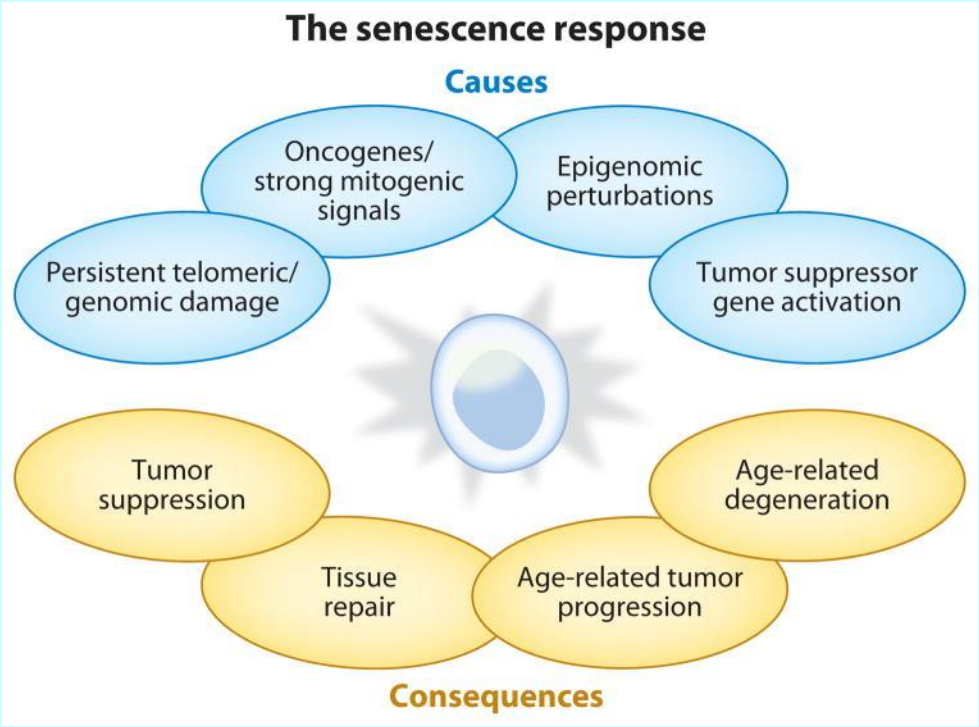
Cycloastragenol (TA65;GRN665;TAT2), an Activator of Telomerase, Suppresses Lung Damage in a Murine Model of Idiopathic Pulmonary Fibrosis





PLoS One. 2013;8(3):e58423. doi: 10.1371

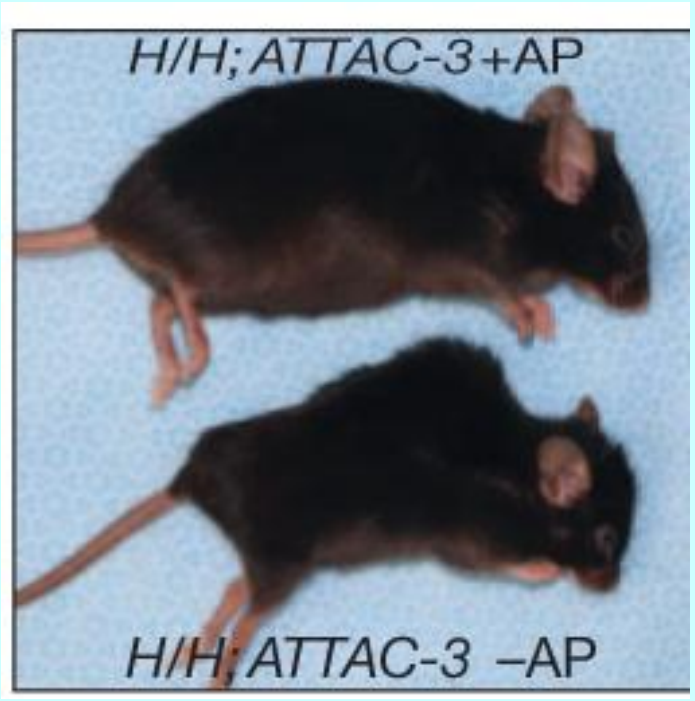
SASP
Senescence-Associated Secretory Phenotype



J Campisi Annu Rev Physiol. 2013; 75: 685–705

Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders

Jan van Duersen



DJ Baker et al., Nature. 2011 Nov 2; 479(7372): 232–236.

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ESTIMATED ENDOGENOUS DNA DAMAGE IN MAMMALIAN CELLS

Damage	*Events per cell/day*	*Reference*
Single-strand break	55,200	*Tice and Setlow, 1985*
Depurinations	12,000	*Lindahl, 1977*
	13,920	*Tice and Setlow, 1985*
Depyrimidinations	600	*Lindahl, 1977*
	696	*Tice and Setlow, 1985*
06-methylguanine	3,120	*Tice and Setlow, 1985*
Cytosine deamination	192	*Tice and Setlow, 1985*
Glucose-6-phosphate adduct	2.7	*Bucala, et at, 1985*
Thymine glycol	270	*Saul et at, 1987*
Thymidine glycol	70	*Saul et at., 1987*
Hydroxymethyluracil	620	*Saul et at., 1987*
8-oxo-G	178	*Richter et al., 1988*
		Shigenaga et at, 1989
Unidentified methyl adduct	unknown	*Park and Ames, 1988a, b*
Interstrand cross-link	8,0	*Bernstein and Bernstein, 1991*
Double-strand break	8,8	*Bernstein and Bernstein, 1991*
DNA-protein cross-link	unknown	*Bernstein and Bernstein, 1991*

Thanks to Wil Bohr for this slide

Segmental Progeroid Syndromes Document Genomic Instability as a Mechanism of Aging

Werner Syndrome, Age 39
Control: GM Martin, Age 64

Chief Complaint: Ankle ulcers
Recent myocardial infarct
Thyroid nodule
Osteoporosis
Premature graying of hair
Ocular cataracts
Tightness of skin
Loss of peripheral sub-cut. tissue
Weak, high-pitched voice
Short stature

Compound heterozygote for *WRN* helicase null mutations

Evidence of Genomic Instability in Werner Syndrome

- Variegated translocation mosaicism
- Elevation mutation rates at *HPRT* in skin fibroblasts and lymphocytes
- Mutator phenotype in host cell ligation assay
- Mutator phenotype at the *GYPA* locus in RBC
- Accelerated loss of telomeric DNA
- Enhanced oncogenesis in vivo
- Instability of heterochromatin

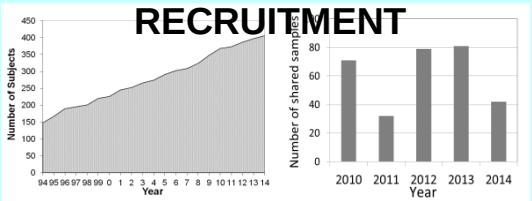
International Registry of Werner Syndrome

Shon Soosman[1], Fuki M. Hisama[2], Lin Lee[1], George M. Martin[1], Junko Oshima[1]
Departments of [1]Pathology and [2]Medicine, University of Washington, Seattle, WA

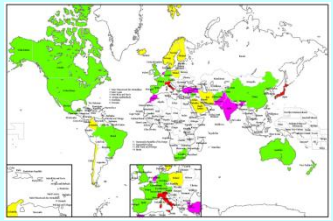
ABSTRACT

The International Registry of Werner Syndrome was (www.wernersyndrome.org) was established in 1988 with the original purpose of collecting Werner syndrome (WS) cases samples for positional cloning of *WRN* gene. The Registry also serves as a valuable resource for biological materials derived from patients or developed within our Registry. Approximately 20% of clinically diagnosed WS cases do not carry *WRN* mutations; these are operationally categorized as “Atypical Werner Syndrome (AWS)”. Our Registry has expanded its scope from WS to the search for causative mutations and mechanisms responsible for the broader range of progeroid syndromes from all over the world. Newly found loci highlight major roles in DNA repair and replication. Those findings continue to support the concept of genomic instability as a major mechanism of biological aging.

PATIENT RECRUITMENT

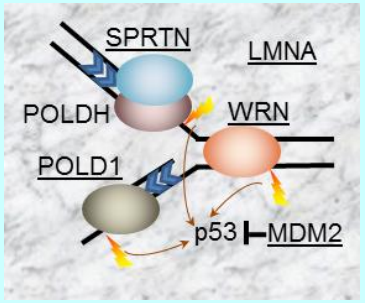


Total number of subjects in the Registry including family members (left) and numbers of biological materials (right).

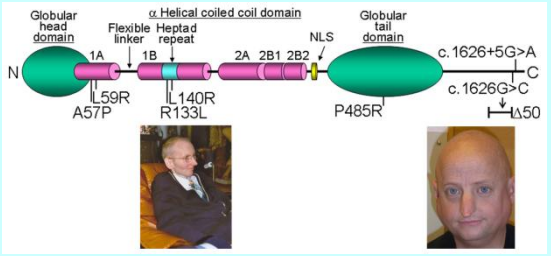


Countries with WRN mutants, founder mutations, ethnic-specific mutations, and AWS

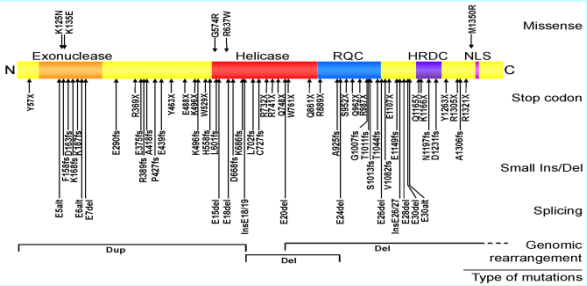
NOVEL AWS MUTATIONS



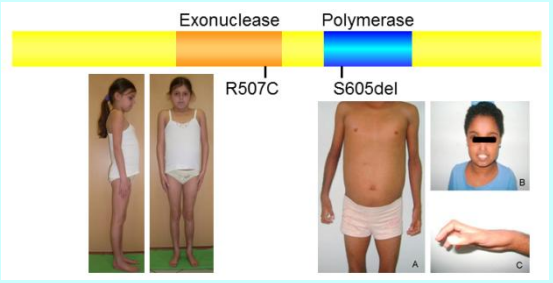
LMNA MUTATIONS



WRN



POLD1 MUTATIONS



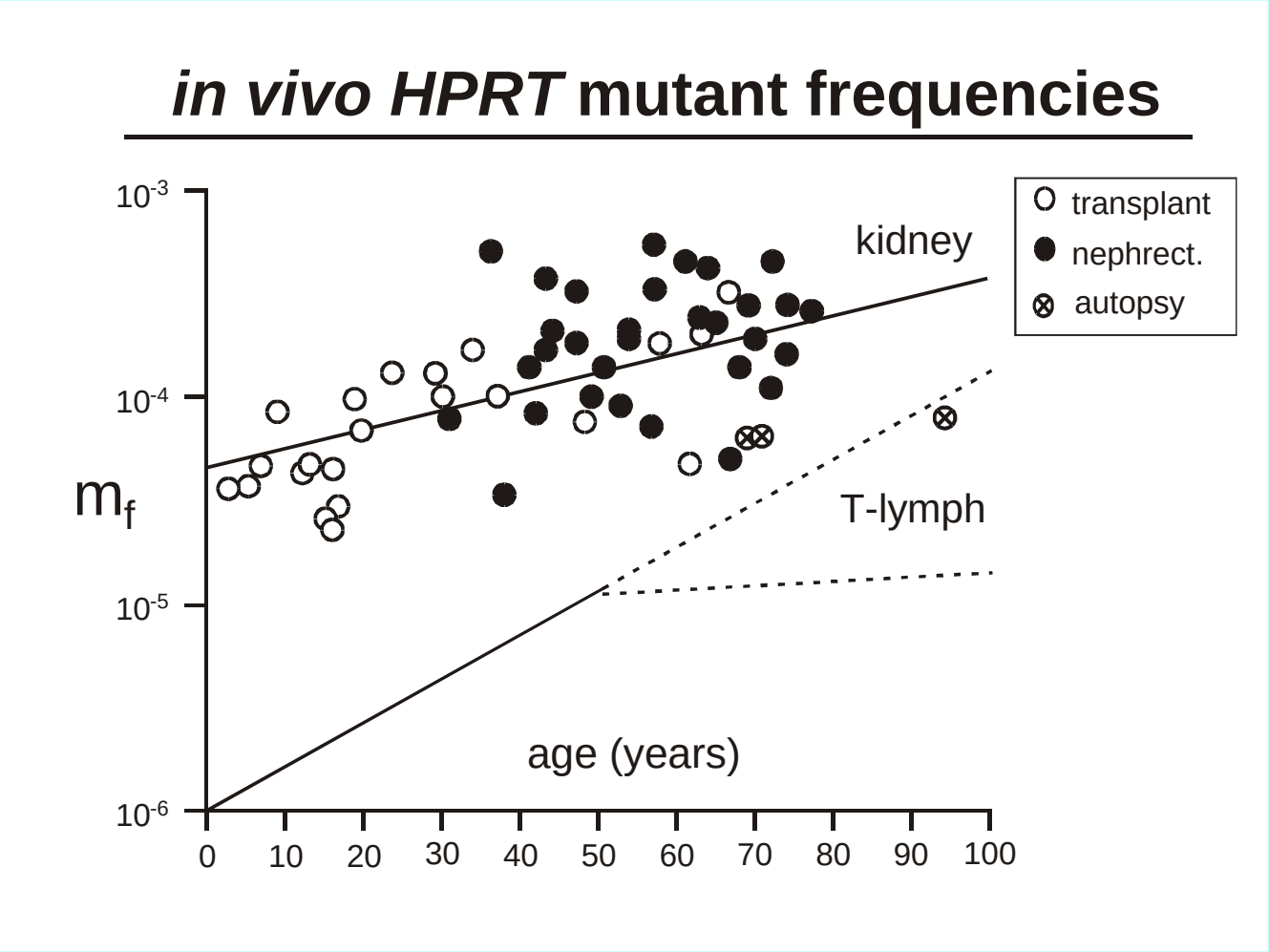
REFERENCE

Oshima J, Hisama FM. Search and Insights into Novel Genetic Alterations Leading to Classical and Atypical Werner Syndrome Gerontology. 2014;60(3):239-46. PMID: 24401204

Lessel D et al. Atypical Aicardi-Goutieres syndrome: Is the WRN locus a modifier? Am J Med Genet A. 2014 Oct;164A(10):2510-3. PMID: 24989684

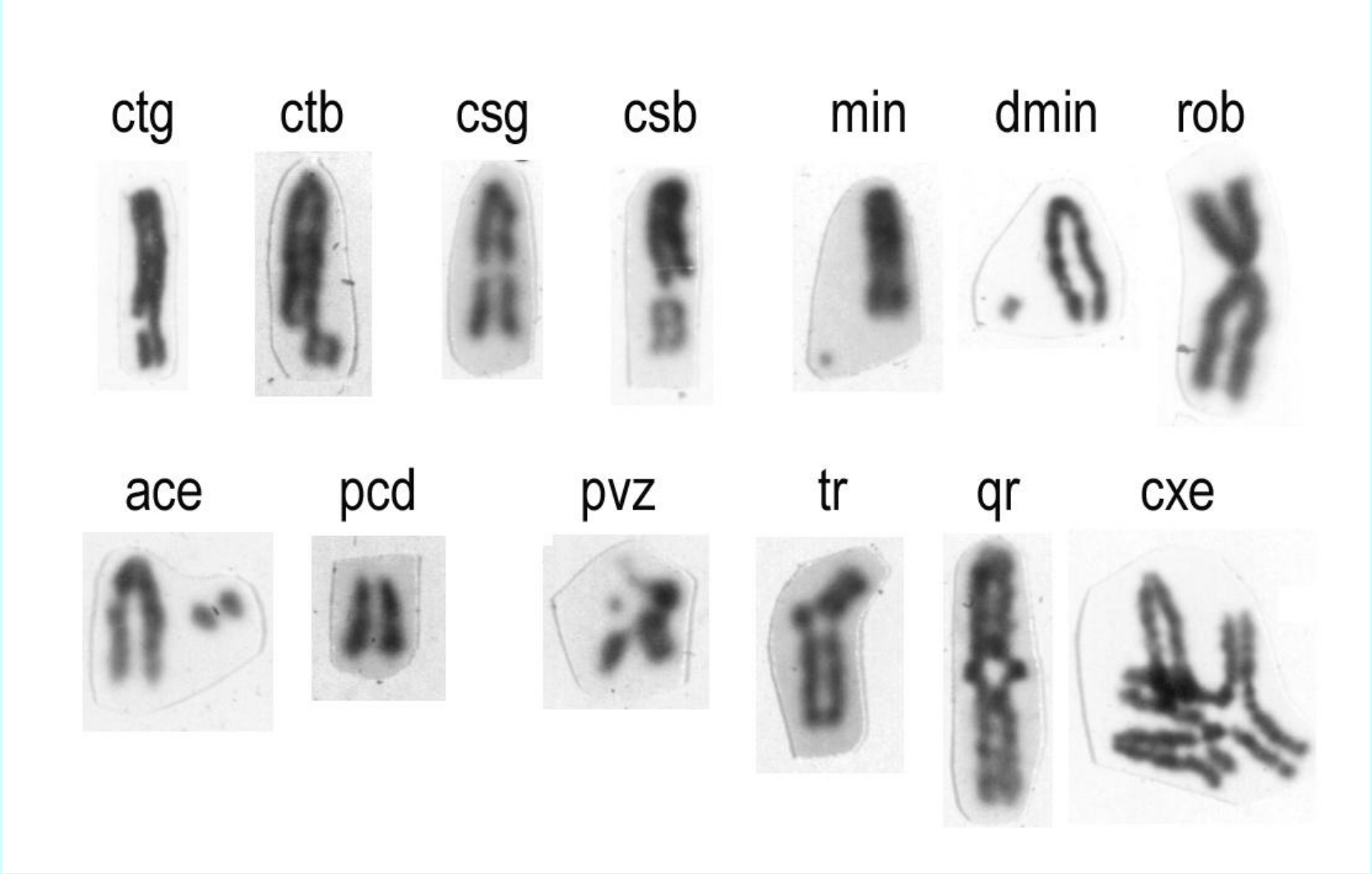
Lessel D et al. Mutations in SPRTN cause early onset hepatocellular carcinoma, genomic instability and progeroid features. Nature Genetics. 2014b;46(11):1239-44. PMID: 25261934.

The Frequencies of Non-Leaky *HPRT* Mutations in Renal Epithelial Cells Increase Exponentially With Age and Are Higher Than Reported for T cells

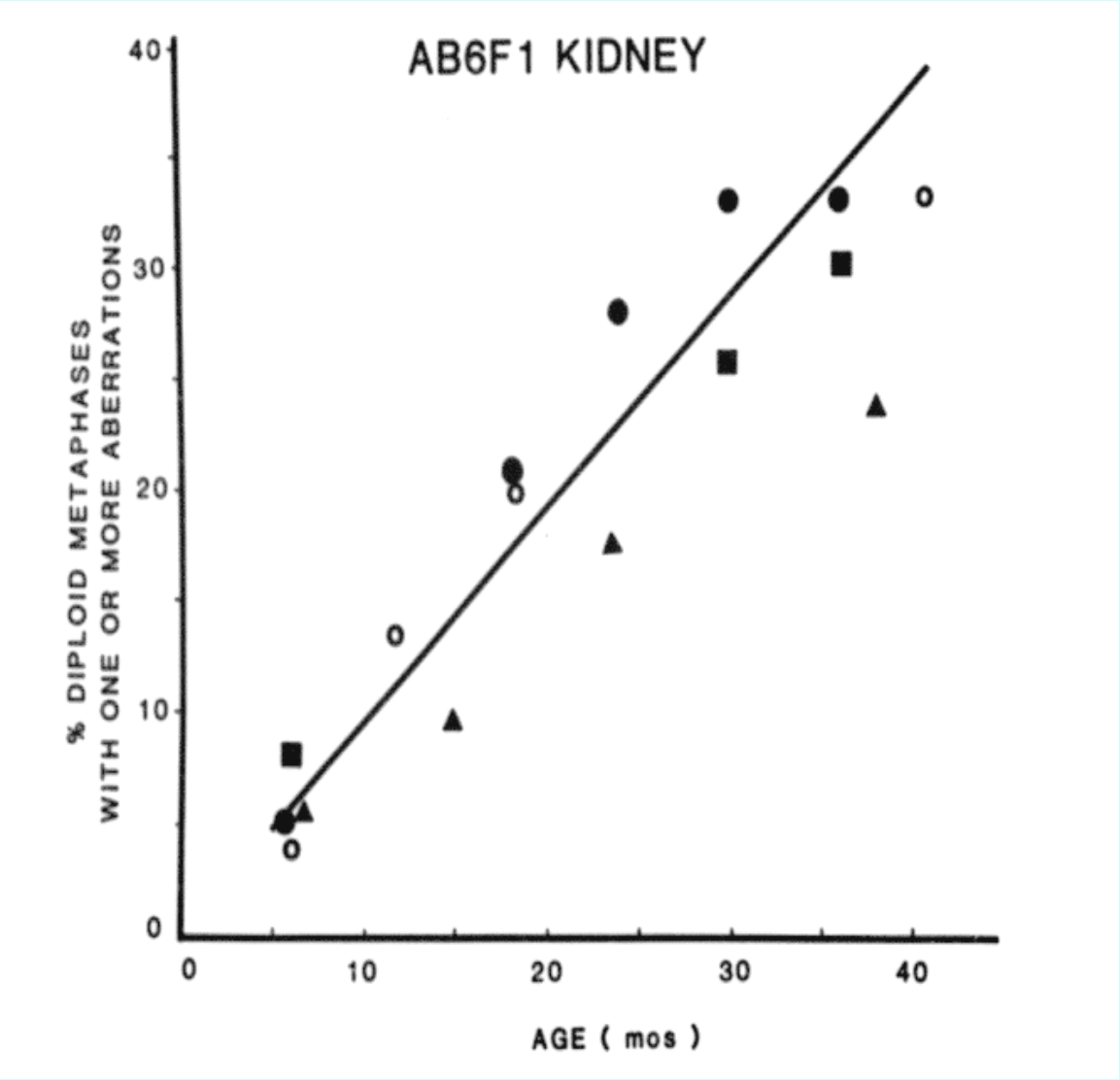


RJ Monnat, after GM Martin et al., Human Mol Genet 5:215, 1996

Chromosomal Aberrations in First Metaphases of Renal Epithelial Cells Cultured from Ageing Mice



GM Martin et al., 1985



GM Martin et al., Israeli J Med Sci 21:296, 1985 & unpublished

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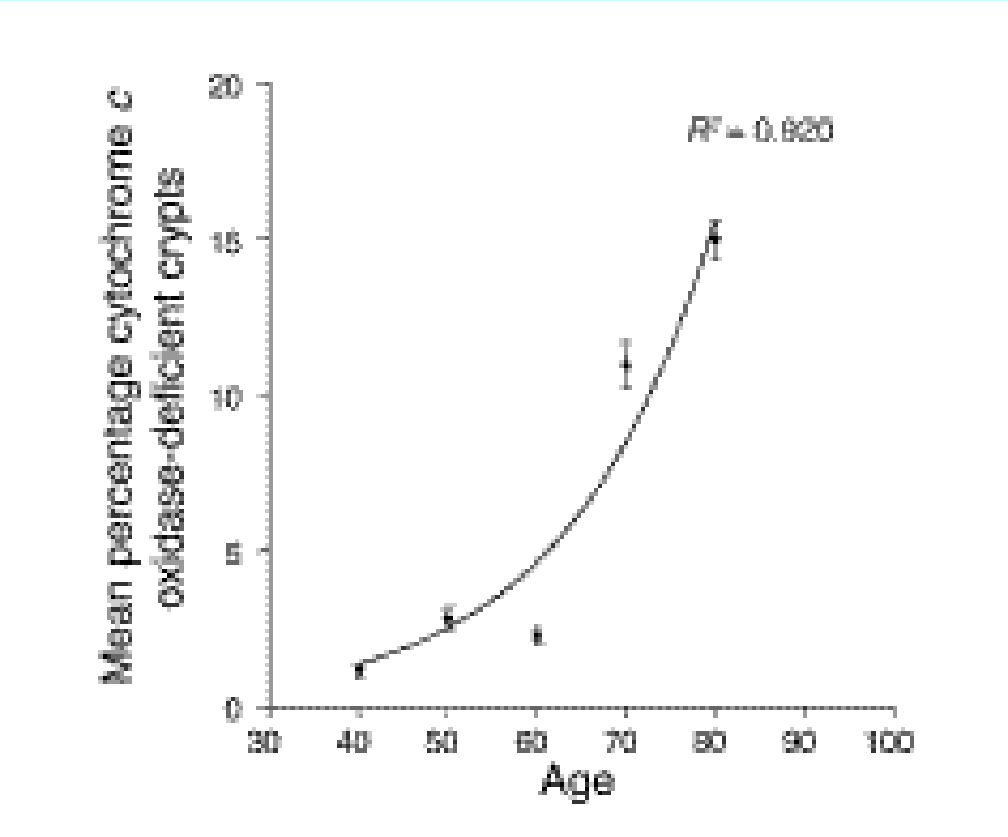
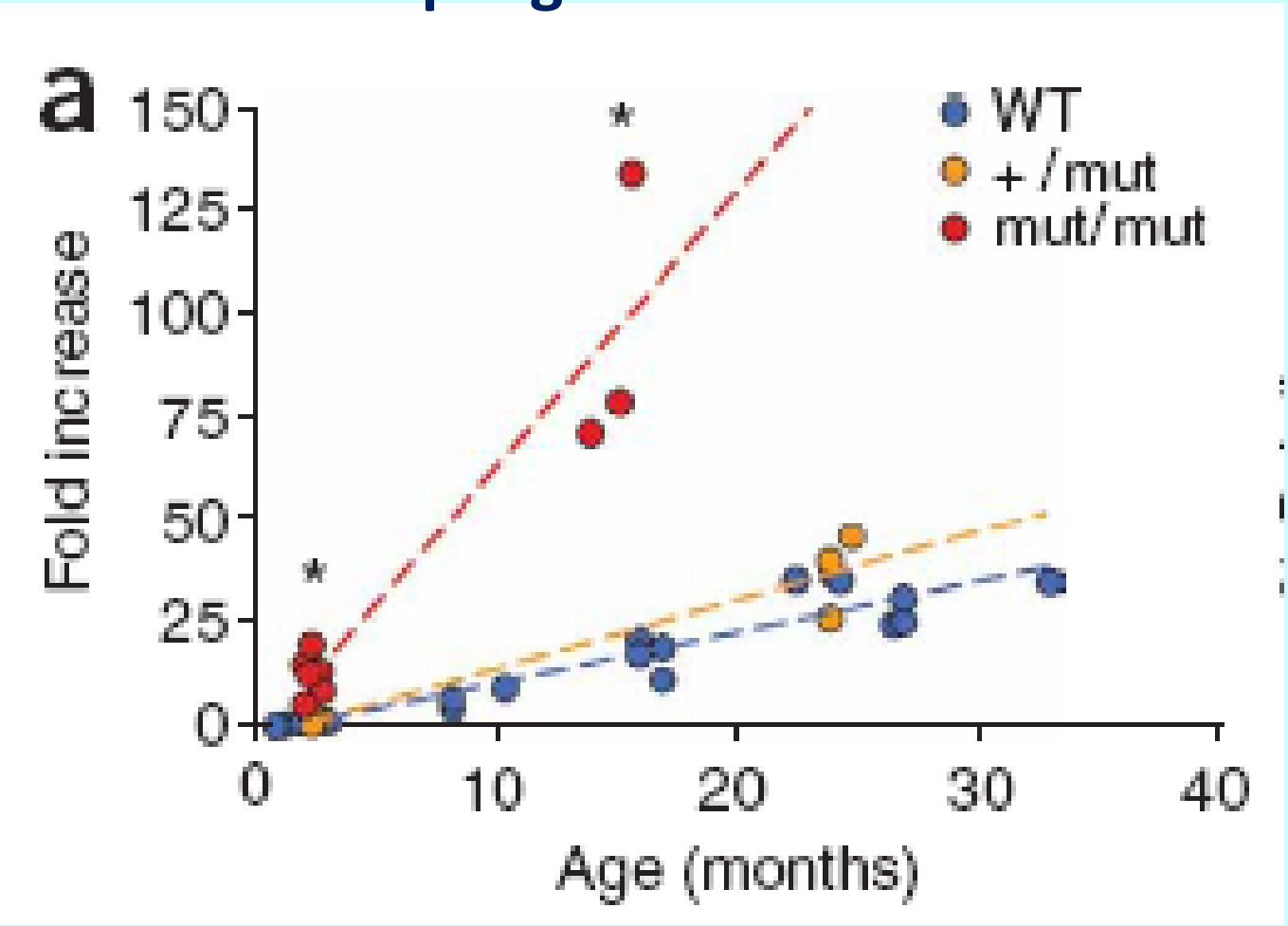
Exponential Increases in Cytochrome C Oxidase Deficient Colonic Crypts



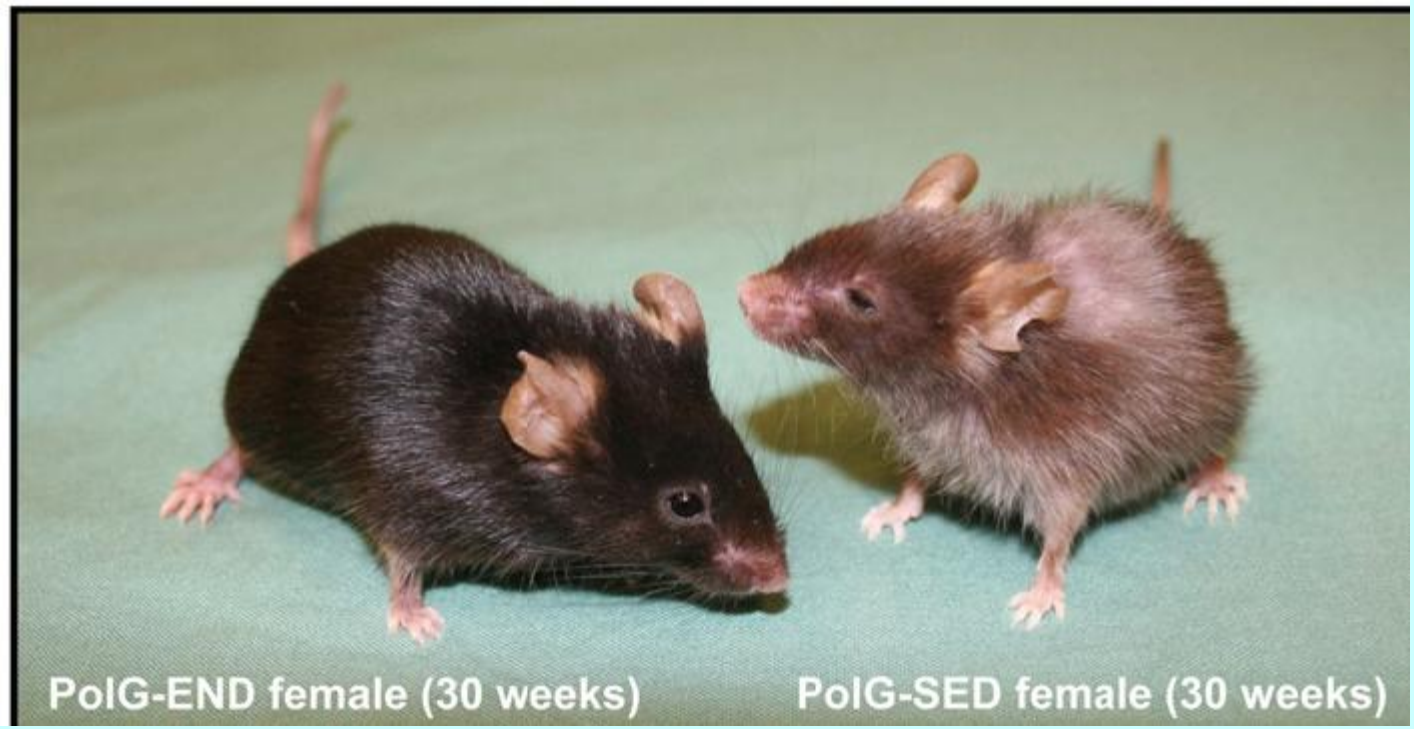
RW Taylor et al., J Clin Invest 2003

Mitochondrial Deletions May Play Key Roles In the Genesis of Senescent Phenotypes (brains of DNA pol gamma mutant vs. wt mice)



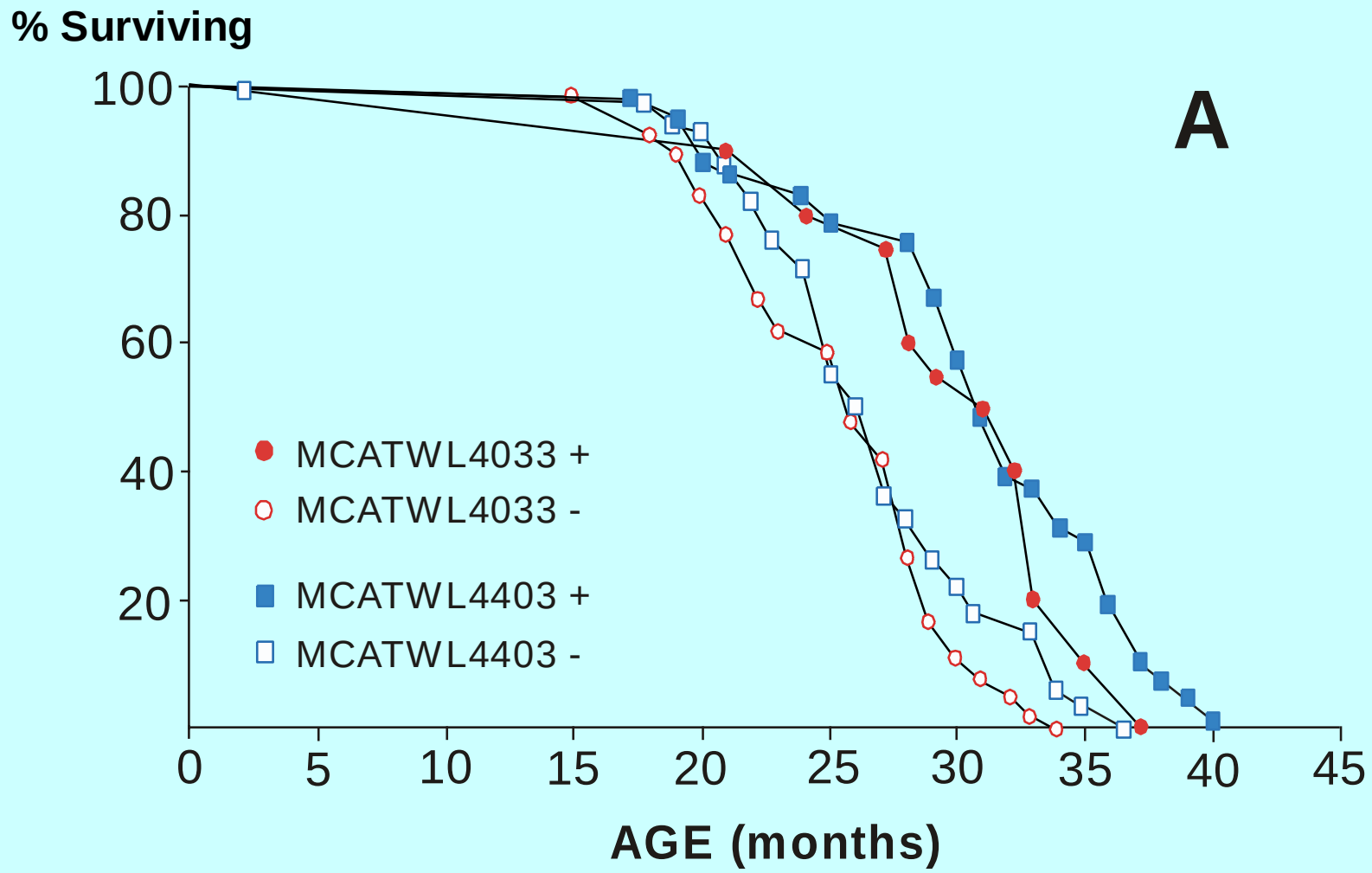
M Vermulst et al., Nature Genet 40:392, 2008

The Miracle of Exercise!
Five Months of Endurance Exercise 3X per Week
In Mitochondrial Mutant Mice



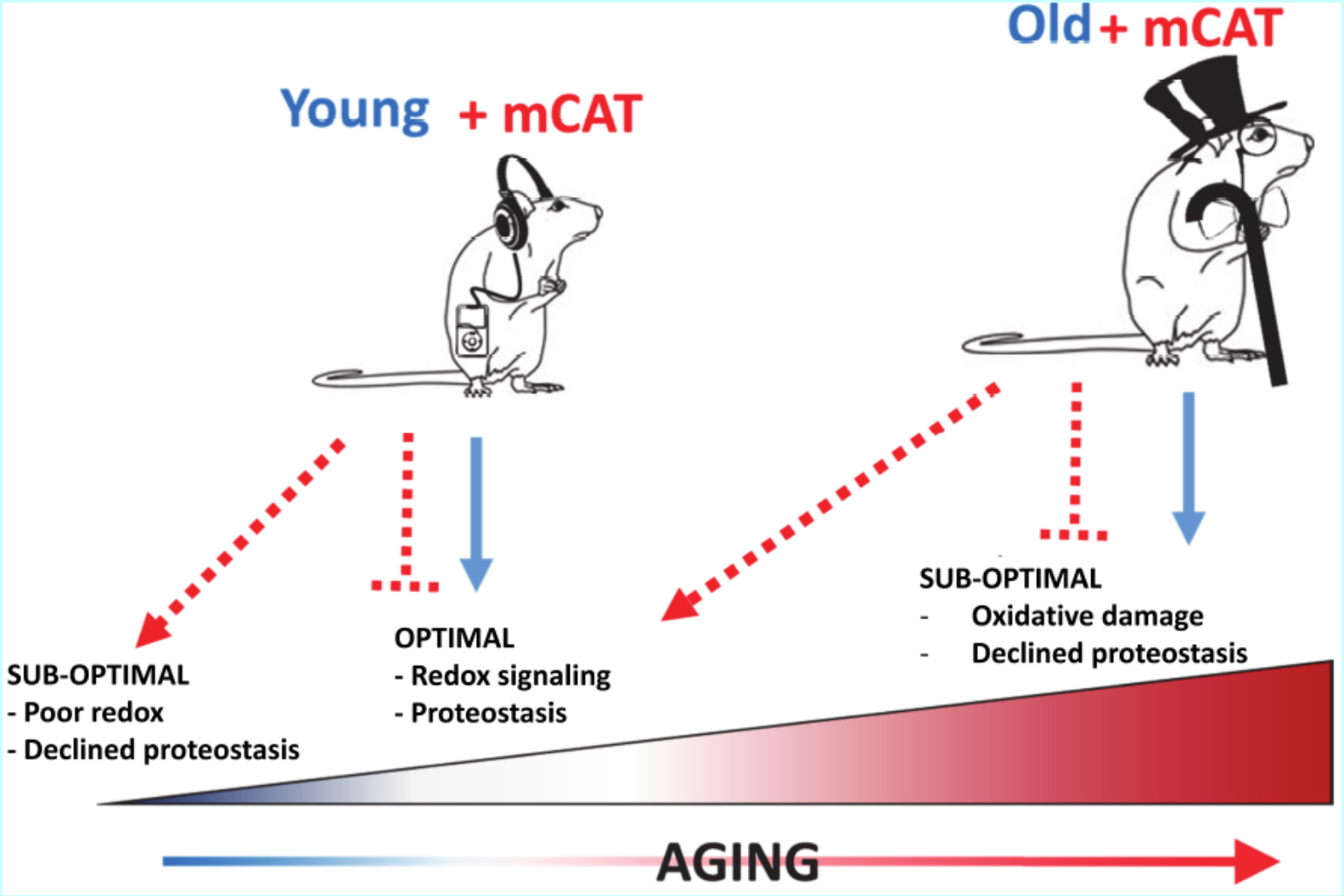
Survival Curves of Two Independent Lines of Transgenic Mice Expressing Human Catalase in Mitochondria

(Even good alleles can escape the force of natural selection)



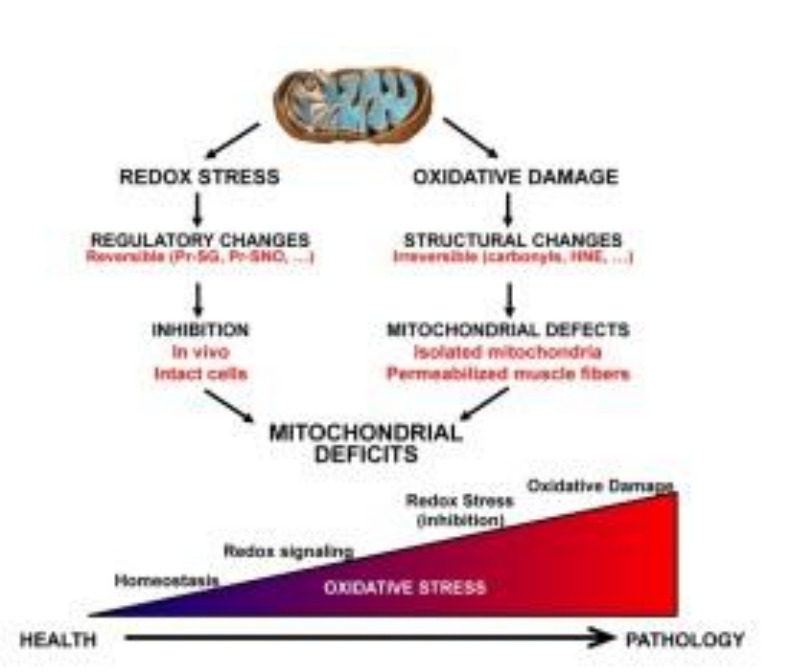
S Schriner et al. Science 2005

Expression of Human Catalase in Mitochondria of Old Mice Enhances Lifespan & Healthspan, but the Opposite Result is Seen in Young Mice: "Reverse Antagonistic Pleiotropy"

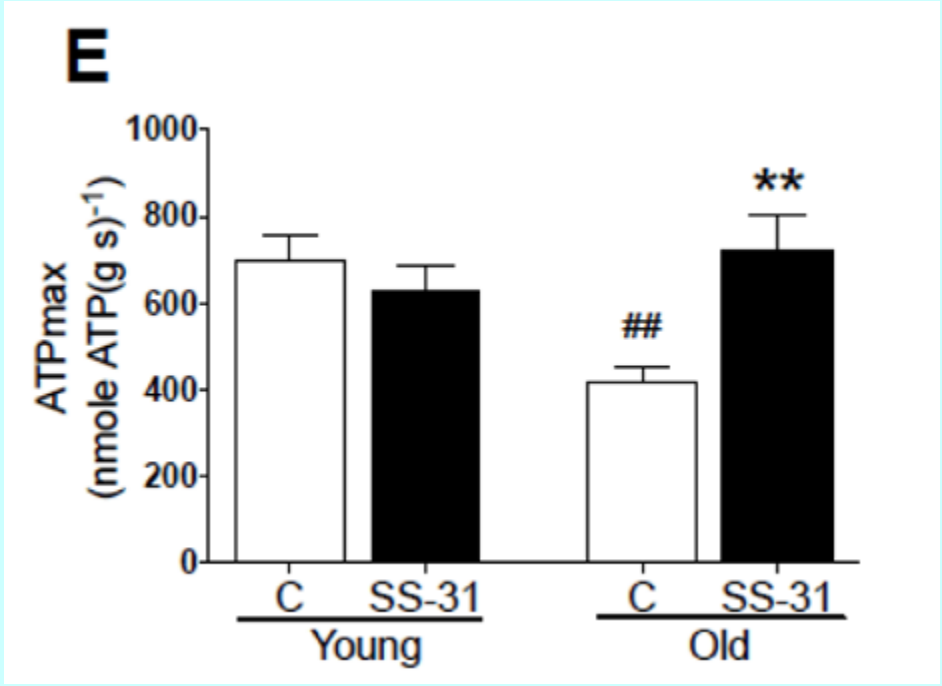


N Basisty et al., submitted, 2015 (Rabinovitch lab)

SS-31, a Tetrapeptide that Binds to the Cardiolipins of the Mitochondrial Inner Cell Membrane, Improves Mitochondrial Functions After 1 Hour



DJ Marcinek & MP Siegel Aging, 2014



MP Siegel et al., Aging Cell, 2013

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Rejuvenation of aged progenitor cells by exposure to a young systemic environment

Tom Rando

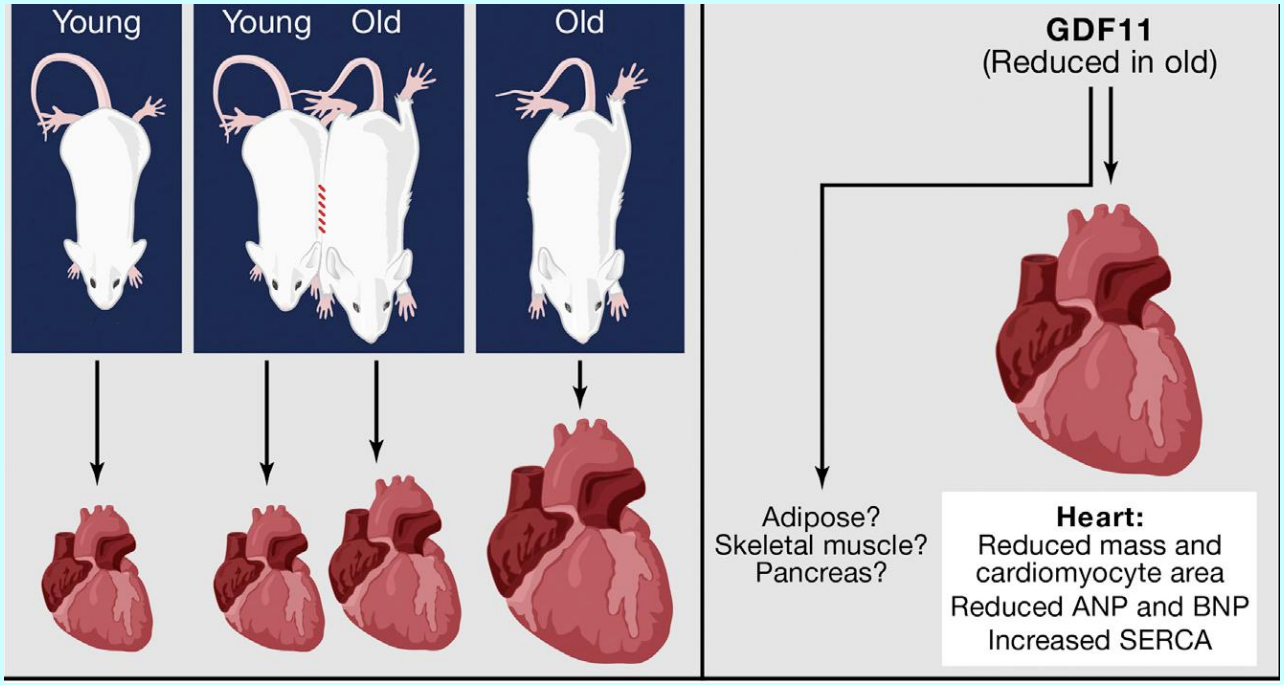
Irena Conboy

Heterochronic parabiosis
The young mouse should look green (GFP)

IM Conboy et al, Nature 433, 760-764 (17 February 2005)

Growth Differentiation Factor 11 is a Circulating Factor that Reverses Age-Related Cardiac Hypertrophy

Amy Wagers

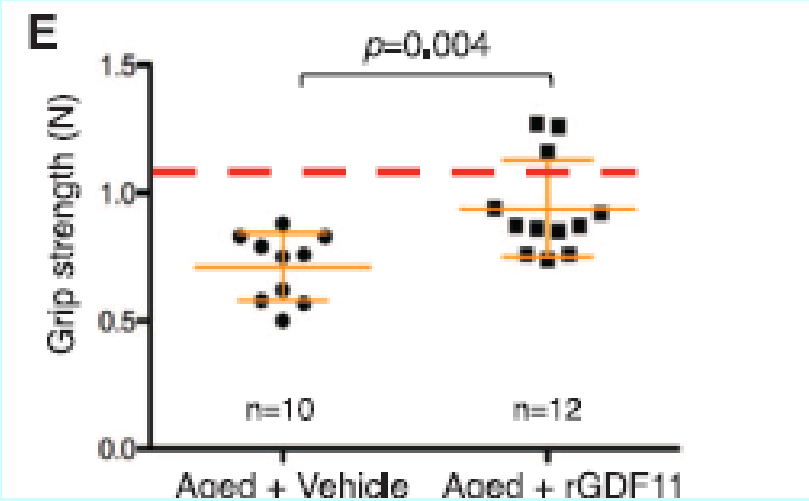


LA. Leinwand1, BC. Harrison, Cell 2013, Commentary

FS Loffredo et al., Cell, 2013 May 9;153(4):828-39

Restoring Systemic GDF11 Levels Reverses Age-Related Dysfunction in Mouse Skeletal Muscle

"…restoration of aged satellite cell function by this factor is coincident with reversal of accumulated DNA damage"



M Sinha et al., Science. 2014 May 9;344(6184):649-52

Adult hippocampal neural stem and progenitor cells regulate the neurogenic niche by secreting VEGF

Tony Wyss-Coray

"We show here a previously unidentified functional role of undifferentiated neural stem and progenitor cells in the adult hippocampus as secretory cells that help maintain their own neurogenic niche by secreting large, biologically relevant quantities of the essential growth factor, VEGF. These findings suggest that the function of adult neurogenesis may include the secretome of undifferentiated stem and progenitor cells."

ED Kirby et al., Proc Natl Acad Sci U S A. 2015 Mar 31;112(13):4128-33.

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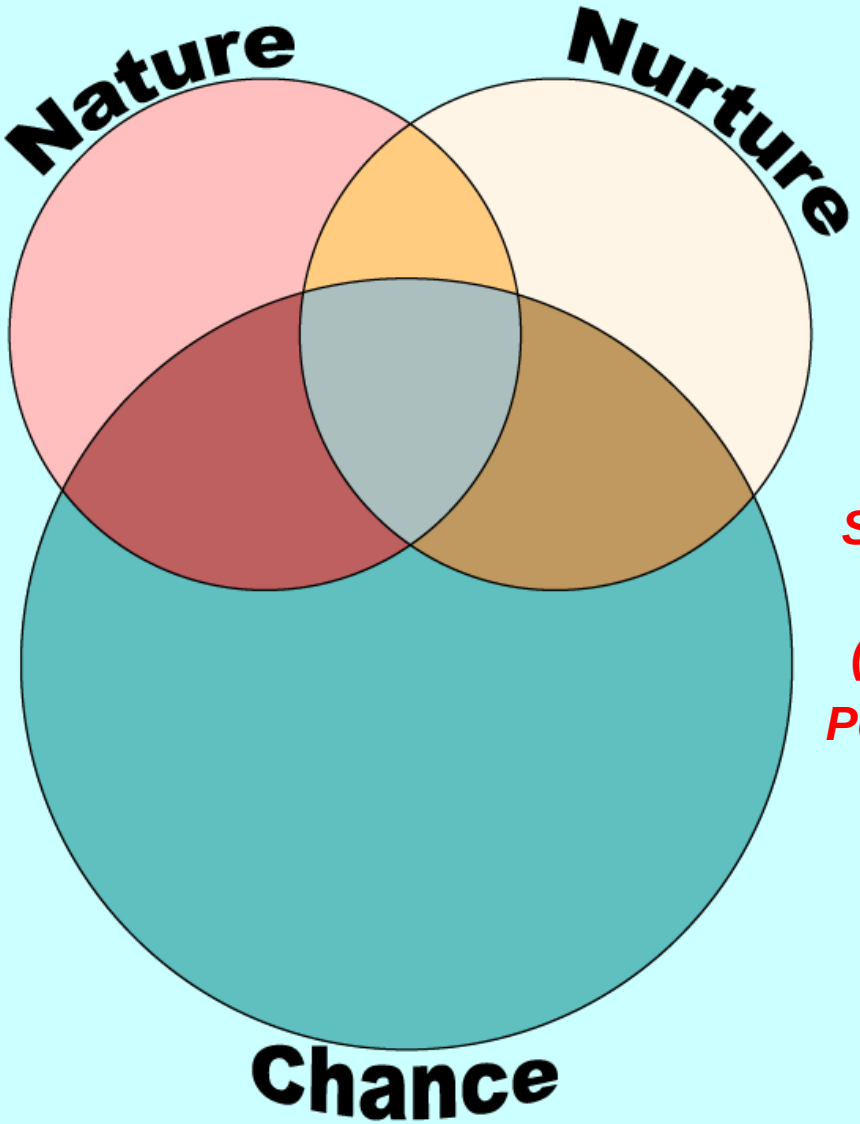
INTRA-Specific Variations in Healthspan & Lifespan: *Chance*



Does CHANCE Dominate?

Mechanisms:

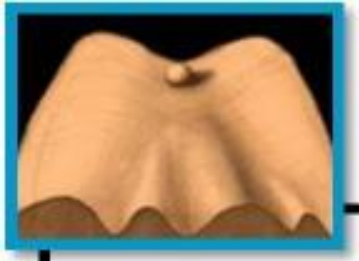
Stochastic Drifts in Gene Expression:

(Epigenetic and/or Post-transcriptional)

Splicing Variants

Somatic Mutation

Random Clonal Monoallelic Expression

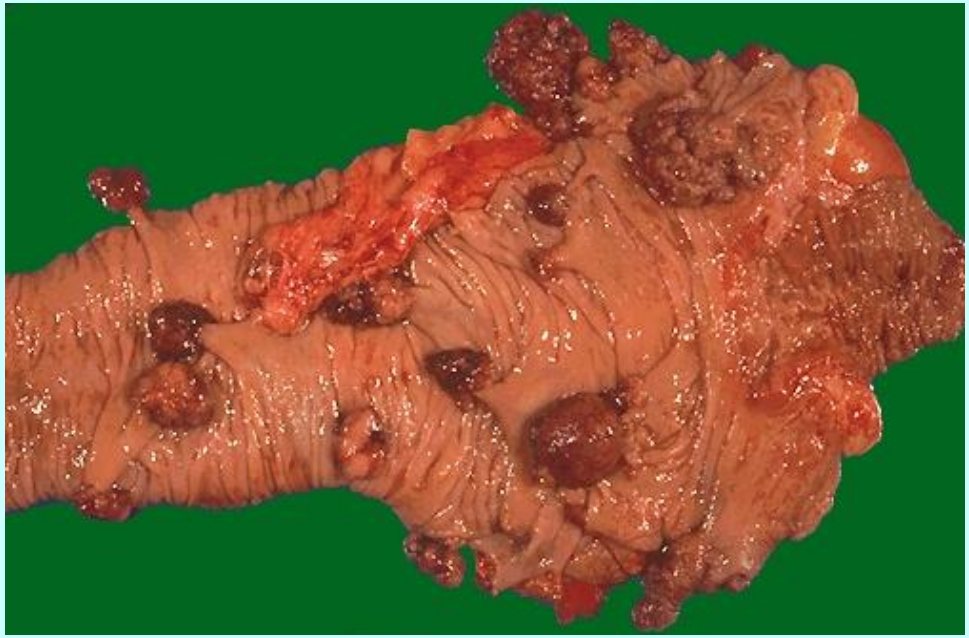


Post Developmental BUMPS in the Waddington Landscape

Alzheimer Brain

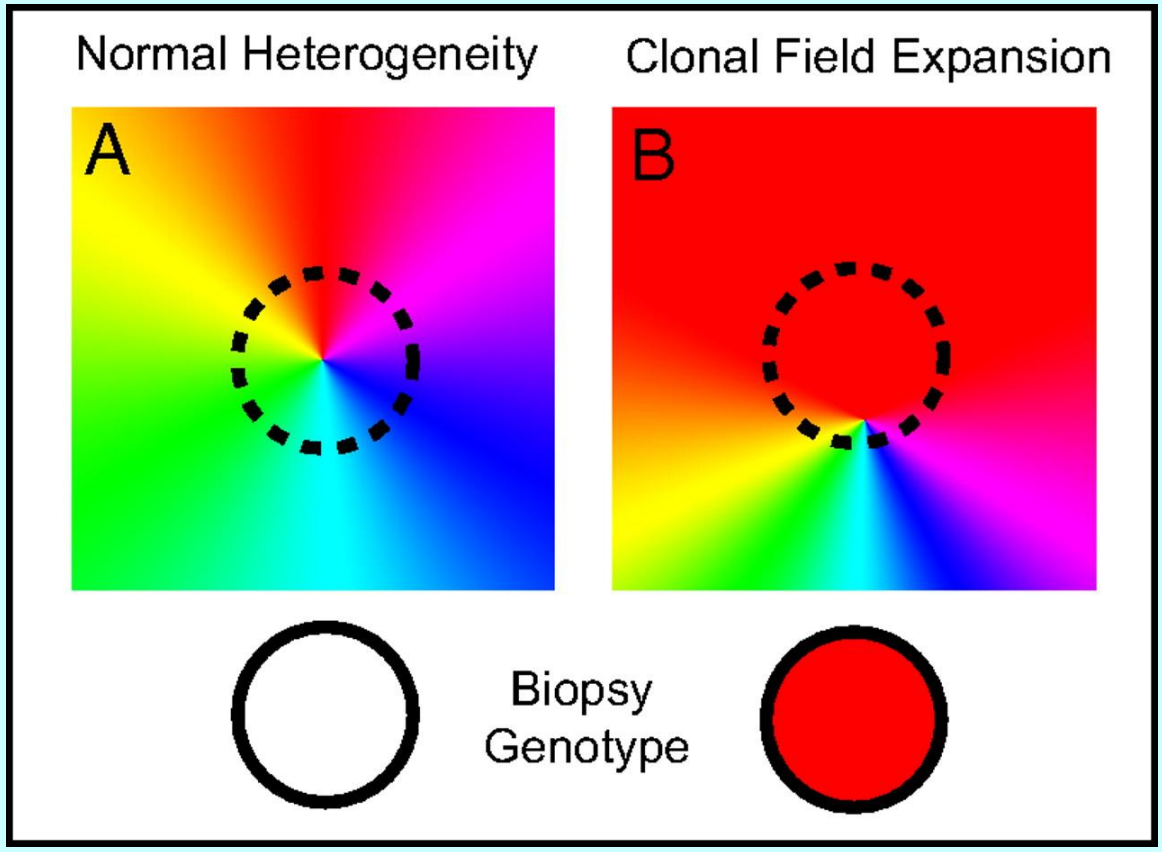
		Synthesis of Amyloid Beta				Degradation of Amyloid Beta		
		α	β	Y	GSAP	IDE	Neprilsin	ACAT1
Young	Microregion 1	↑	↓	↓	↓	↓	↑	↓
	Microregion 2	↑	↓	↓	↓	↑	↑	↑
	Microregion 3	↑	↓	↑	↑	↑	↑	↓
Middle Aged	Microregion 1	↑	↓	↓	↓	↓	↑	↓
	Microregion 2	↑	↑	↓	↓	↑	↓	↑
	Microregion 3	↓	↓	↑	↑	↑	↓	↓
Old	Microregion 1	↑	↓	↑	↑	↓	↑	↑
	Microregion 2	↑	↑	↓	↓	↑	↓	↓
	Microregion 3	↓	↑	↑	↑	↓	↓	↑

Quasi-Stochastic Distributions of Adenomatous Polyps of Colon



Multiple adenomatous polyps of cecum from patient with Familial Polyposis. At least one lesion is likely to have evolved into an adenocarcinoma

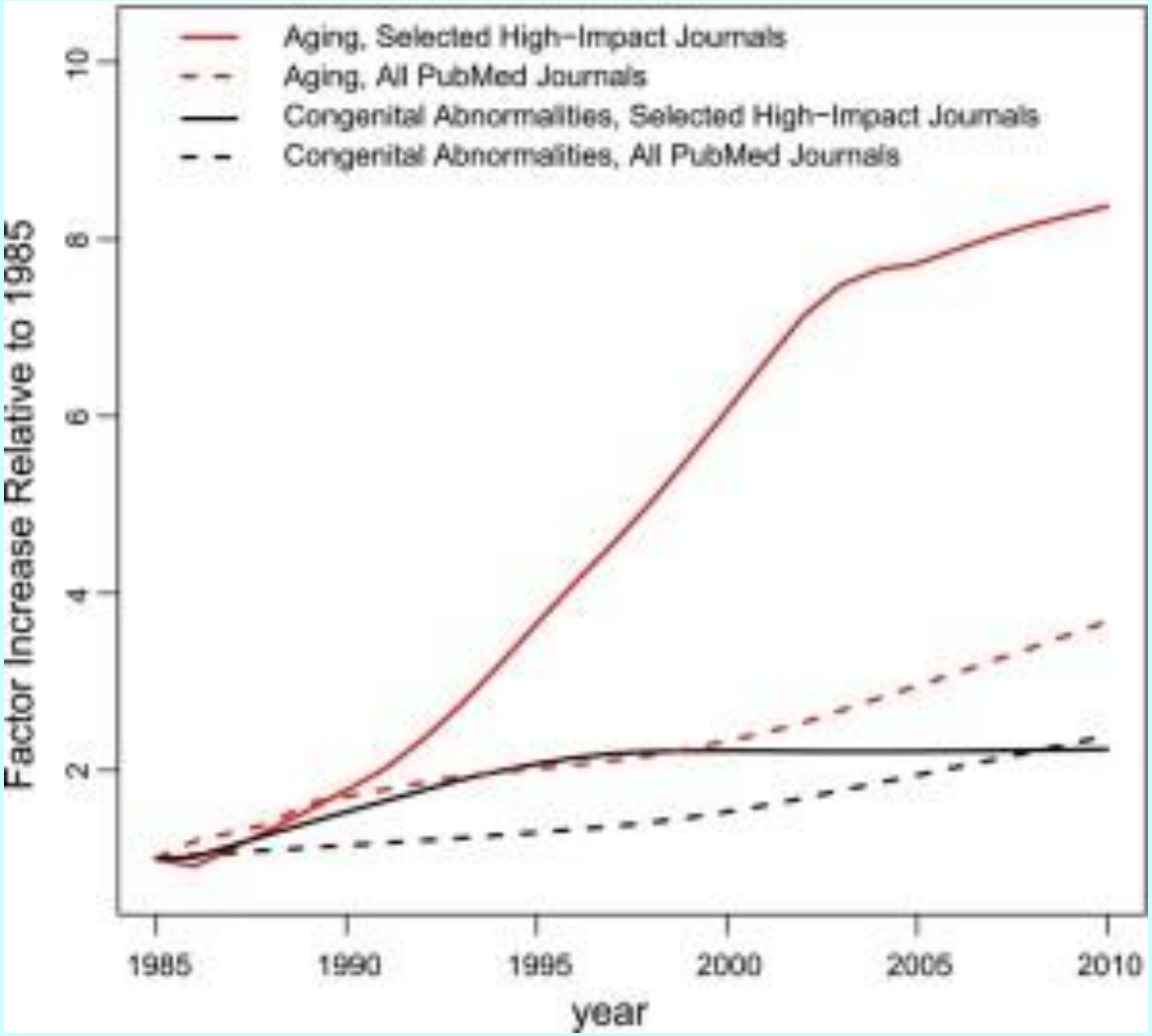
Normal Mucosa Surrounding Colonic Adenocarcinomas in Ulcerative Colitis Patients Display Clonal Expansions of Neutral Mutations



Salk J J et al. PNAS 2009;106:20871-20876

The Remarkable Rate of Increase in Publications of Papers on the Biology of Aging in Cell, Nature, Science & PNAS

(but it may have begun to taper off)



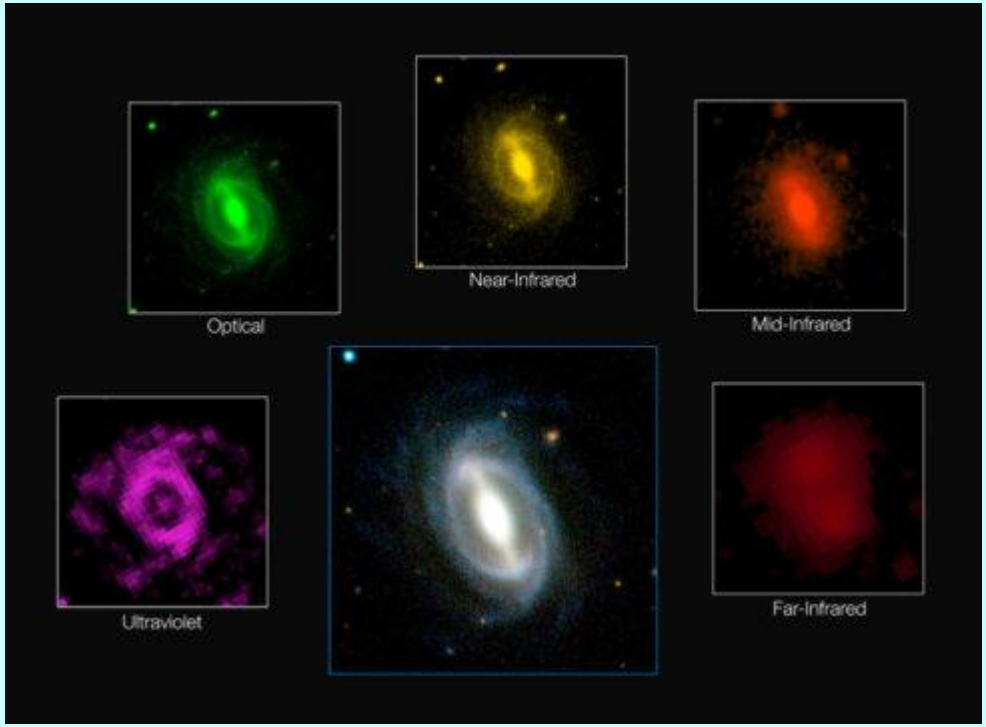
GM Martin FASEB J, 2011

Thanks for your attention!

University of Washington

The energy produced in a section of the Universe today is only about half what it was two billion years ago. This fading is occurring across all wavelengths, from the ultraviolet to the far infrared.

The Universe is slowly dying



http://www.sciencedaily.com/releases/2015/08/150810162511.htm

1963

Return to Research on the Werner Syndrome at the University of Washington

Arno Motulsky

Charles Epstein
At Unibomber Hearing

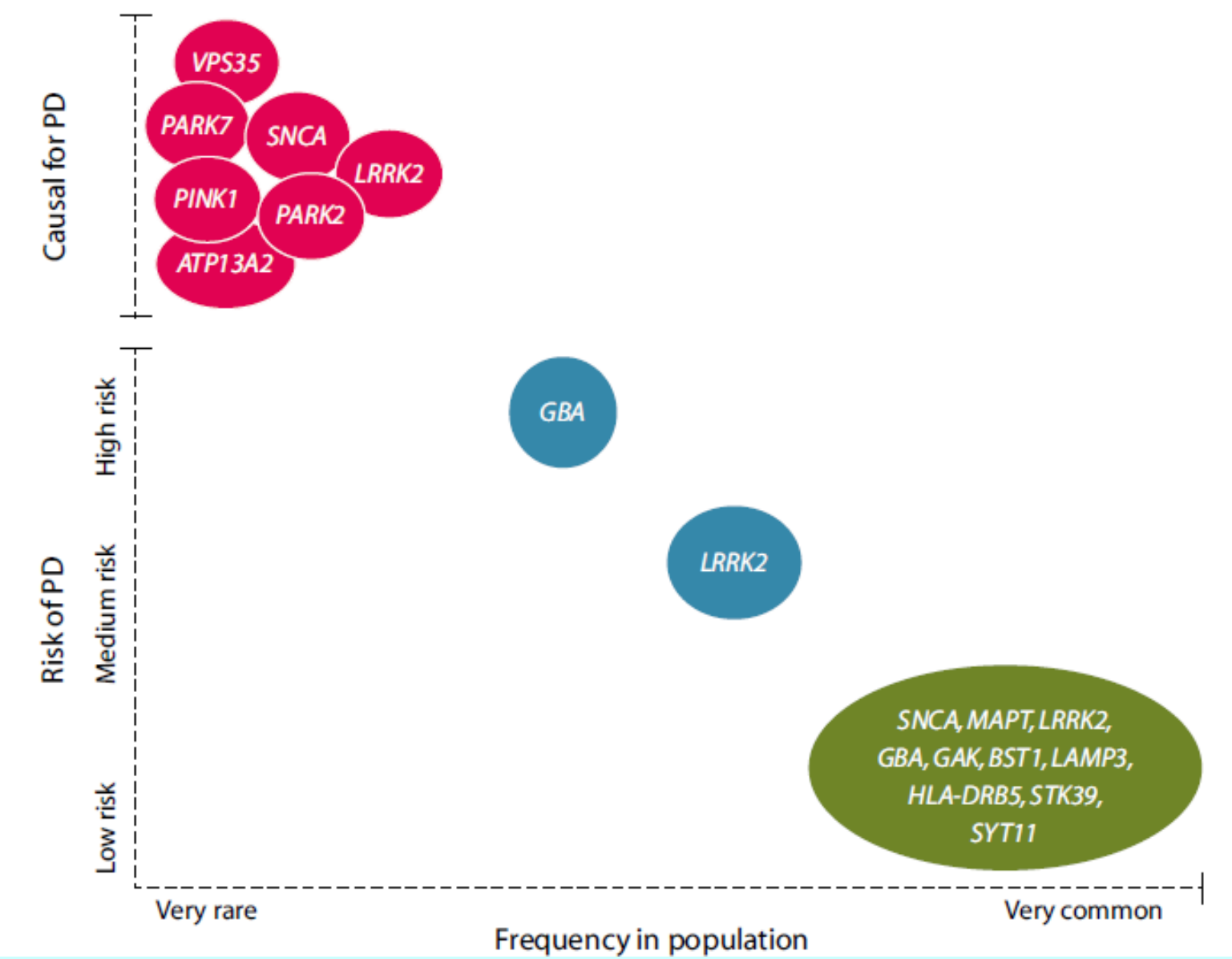
Werner Syndrome,
Ages 15 and 48

Leonard Hayflick

- Replicative senescence of normal diploid human cells

Hayflick & Moorhead, 1961

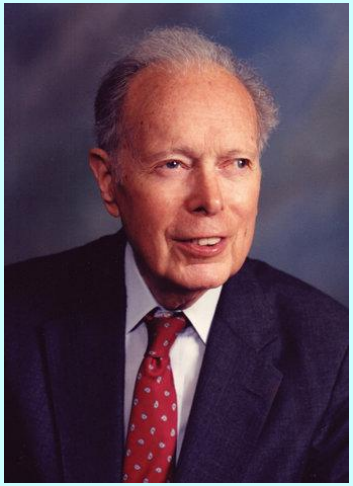
Very Rare Mutations Are More Likely to be Causal in PD



MP van der Brug et al., Science Translational Medicine, 2015

NY Times Obituary, Nov. 28, 2014
Denham Harman, 98

Correction: December 5, 2014



An obituary on Saturday about the research biochemist Denham Harman, author of the influential paper “Free Radical Theory of Aging,” referred incorrectly to the form in which glucose becomes a free radical in the breathing process. It is a molecule, not a cell.