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Modelling Variability in Gene Expression to Understand Determinants of Human Aging

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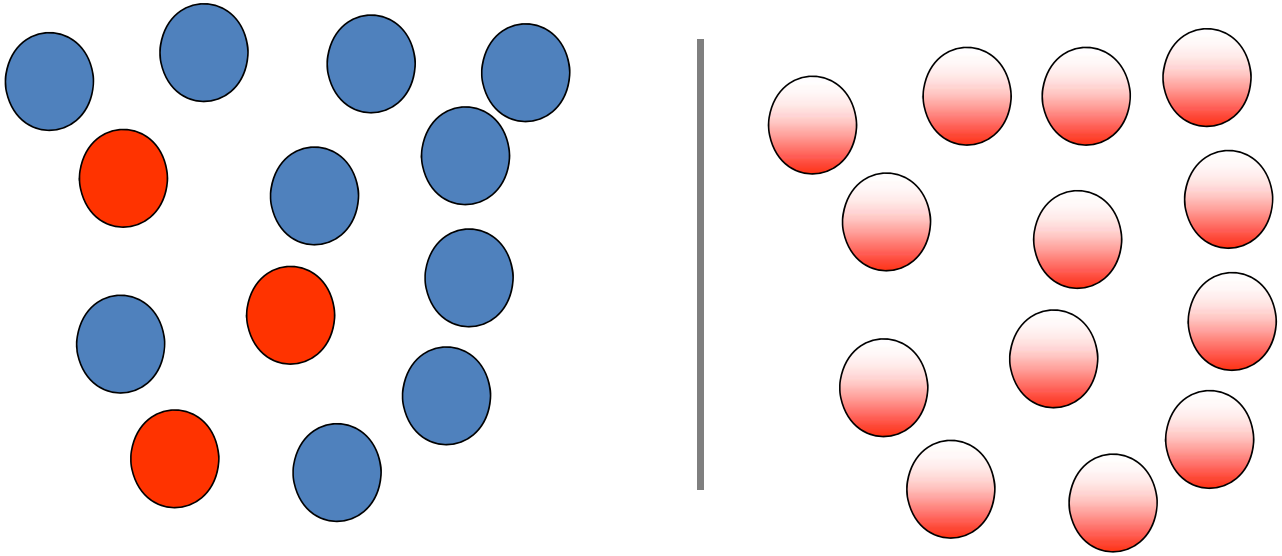
Cell populations are inherently heterogeneous

Ensemble methods like qPCR, microarrays and RNA-sequencing measure the "average" transcriptome which masks single cell behavior.



Single cell sequencing has changed the way we think about transcriptional regulation and revolutionized how we understand biological processes, including aging!

Levsky & Singer. (2003). *Gene expression and the myth of the average cell.* Trends in Cell Biology.

Modeling heterogeneity involves statistics

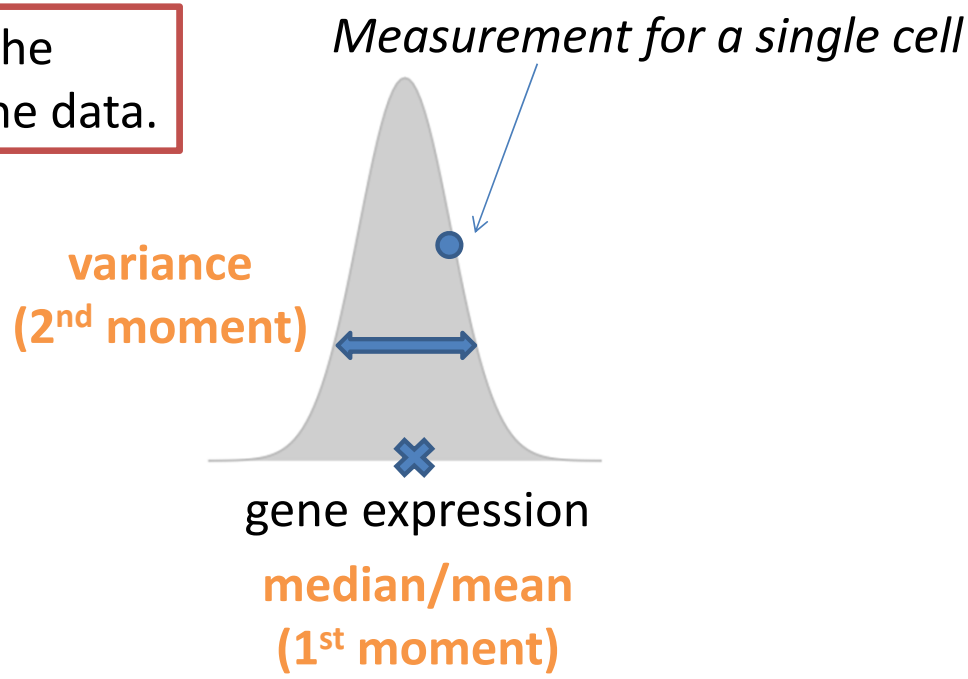
Consider the density of a gene's expression profile in a population of cells:

Statistical moments report on the underlying population structure of the data.

Other **higher moments**:
Skewness (3rd moment)
Kurtosis
Hyperskewness
Hyperflatness

Other features:
bimodality, multi-modes.

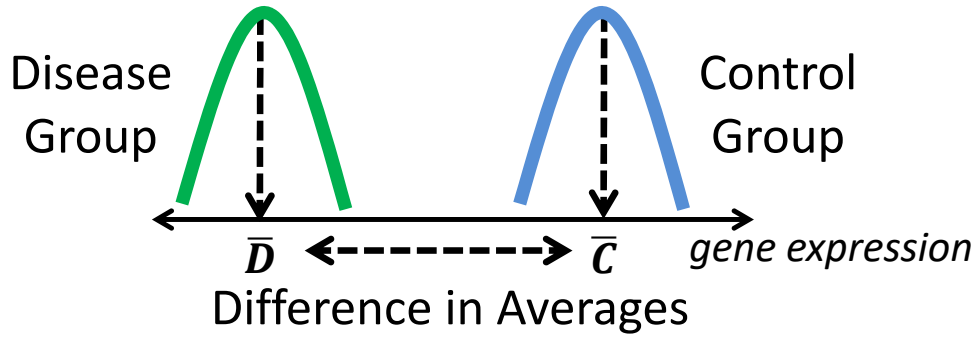
Higher moments are good for identifying extreme values, outliers, and sub-populations.



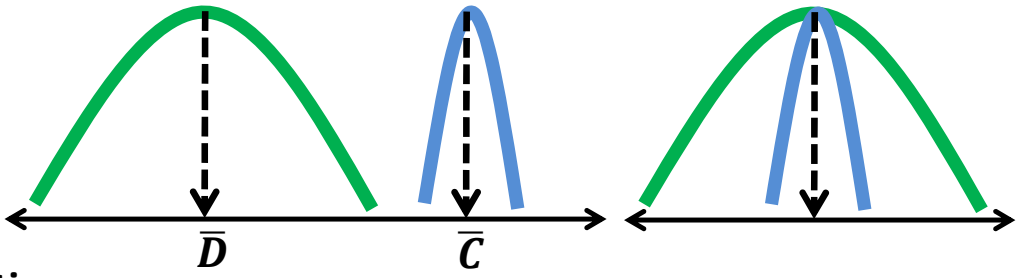
Bioinformatics methods typically focus only on the 1st moment.

Gene expression variance as a population-specific regulatory parameter – what can we learn?

$$T_{(gene)} = \frac{\bar{D} - \bar{C}}{f(Var(D, C))}$$



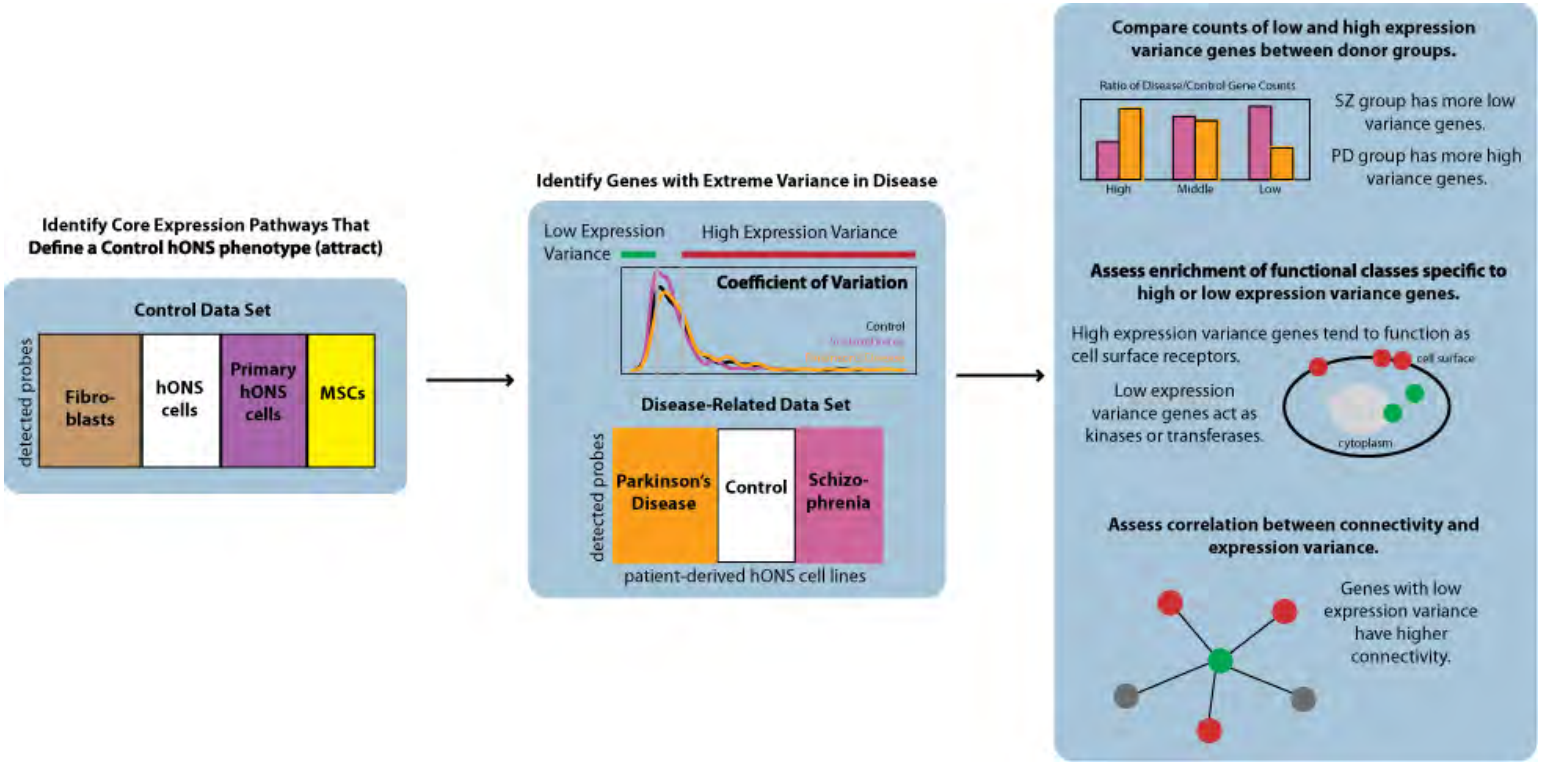
Proof of Principle Exercise



Mar *et al.* (2011) **PLoS Genetics.**

Gene expression variability in human stem cell models of neurological disease

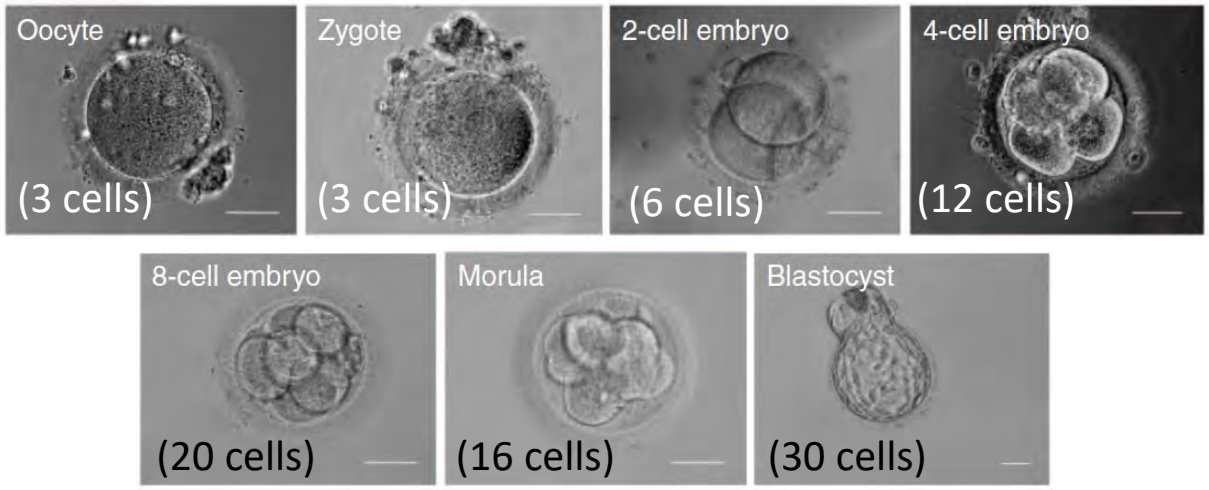
Nasal biopsies from human donors in a larger study on *Parkinson's disease, Schizophrenia* and related controls (Collaboration with **Profs Alan Mackay-Sim & Christine Wells**).



Mar *et al.* (2011) **PLoS Genetics.**

Transcriptional programs control human embryonic development

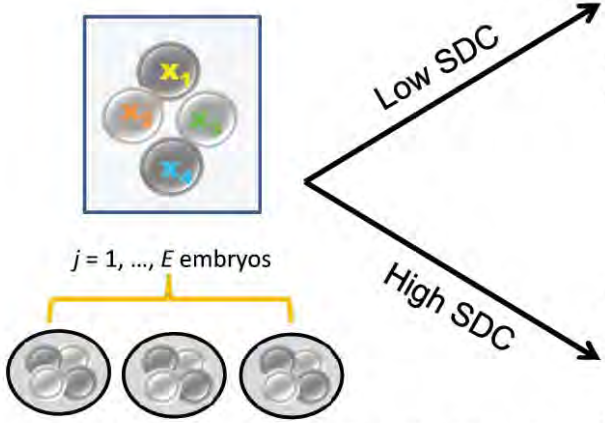
Studying how individual cells in a human embryo alter their transcriptomes during development can help shed light on how embryos grow!



- Single cell RNA-sequencing; 20-60 million 100-bp reads/cell
- 3 embryos for each developmental stage (except morulae with 2 embryos)

Yan et al (2013) **Nature Structural & Molecular Biology.** *Single-cell RNA-Seq profiling of human preimplantation embryos and embryonic stem cells.*

What can variability tell us about transcriptional control in a embryonic cell population?



Low SDC

Stable, Invariant Expression

- Gene is expressed homogeneously.
- Highly predictable
- expression for any cell sampled.

High SDC

Widely distributed, Variable Expression

- Gene is expressed heterogeneously.
- A diversity of expression states that may lead to different cell fates.

SDC$_j$ = inter-cellular variability in the j-th embryo
= Standard Deviation $\{x_1, x_2, x_3, x_4\}$

$$= \frac{1}{N}\sum_{i=1}^{N}(x_i - \bar{x})^2 \quad \text{for } N \text{ cells.}$$

Inter-cellular Variability

$$\mathbf{SDC} = \frac{1}{E}\sum_{j=1}^{E}\frac{1}{N_j}\sum_{i=1}^{N_j}\left(x_{ij} - \bar{x}_j\right)^2 = \frac{1}{E}\sum_{j=1}^{E} SDC_j$$

Inter-embryo Variability

$$\mathbf{SDE} = \frac{1}{E}\sum_{j=1}^{E}\left(SDC_j - \overline{SDC}\right)^2$$

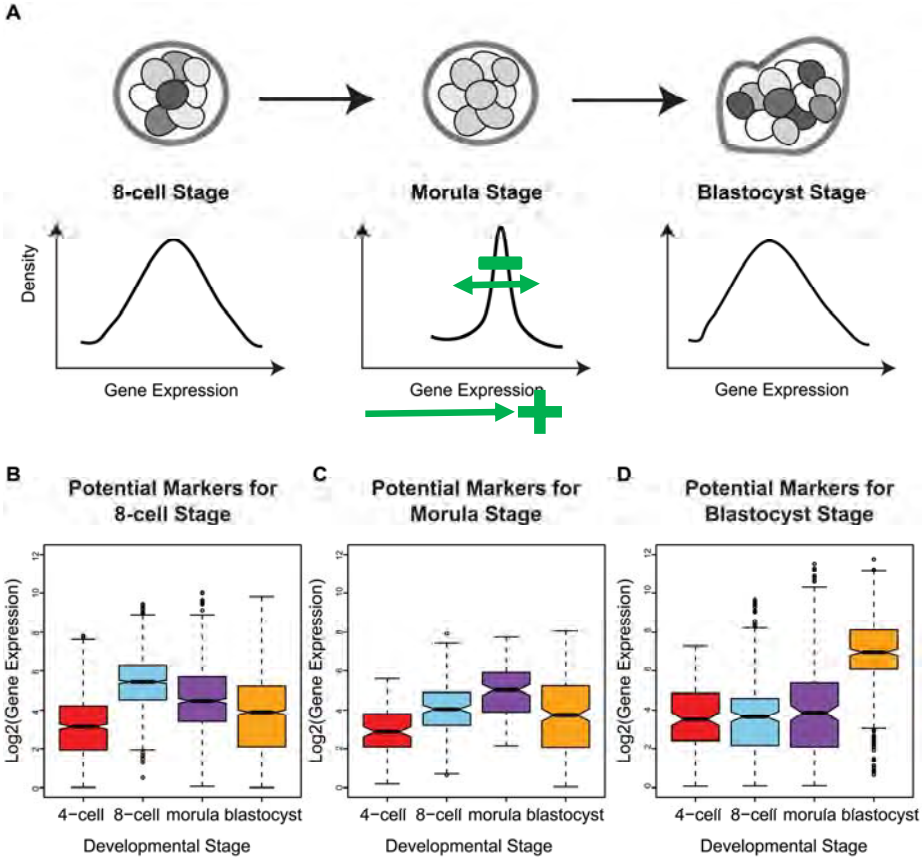
Yu Hasegawa

Hasegawa et al. (2015). ***PLoS Genetics.***

Identifying new stage-specific markers based on variability + average expression

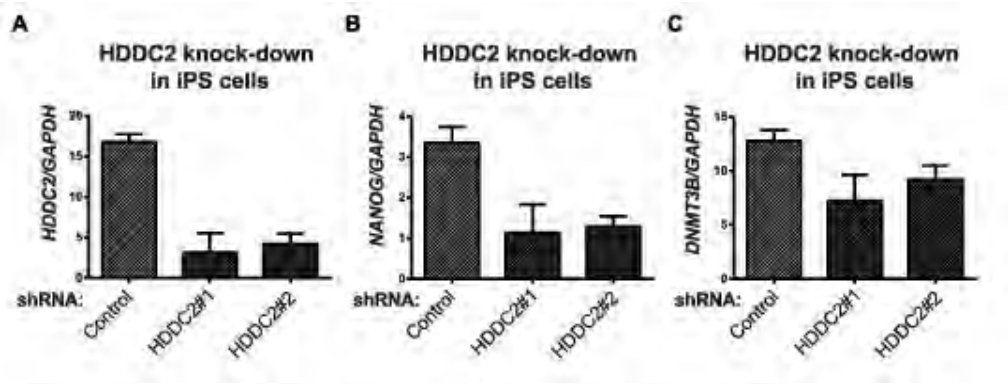
Standard methods for identifying markers are based on average changes in expression.

For single cells, important shifts in expression may be more complex.

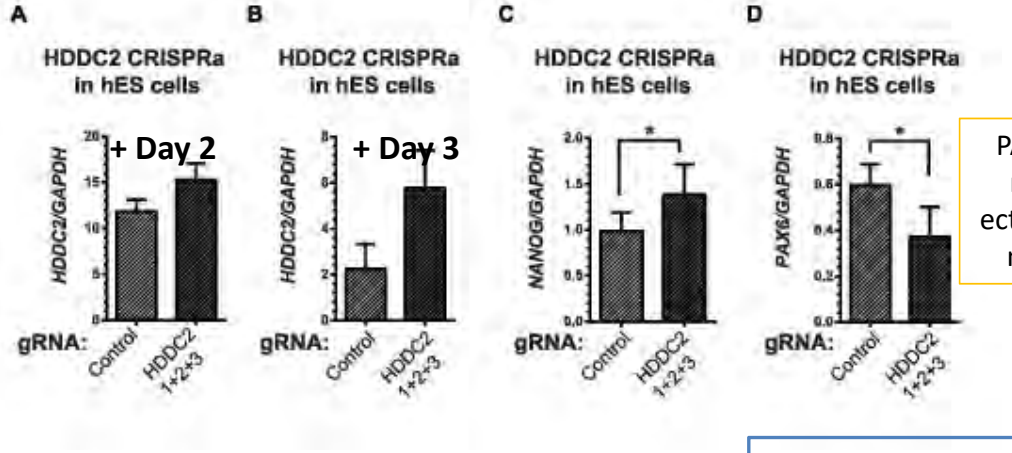


Experimental validation of *HDDC2* in human iPSCs and ESCs indicates a role in pluripotency

shRNA-mediated knockdowns in induced pluripotent stem cells



Early stage neuro-differentiation of ES cells using *HDDC2* over-expression.



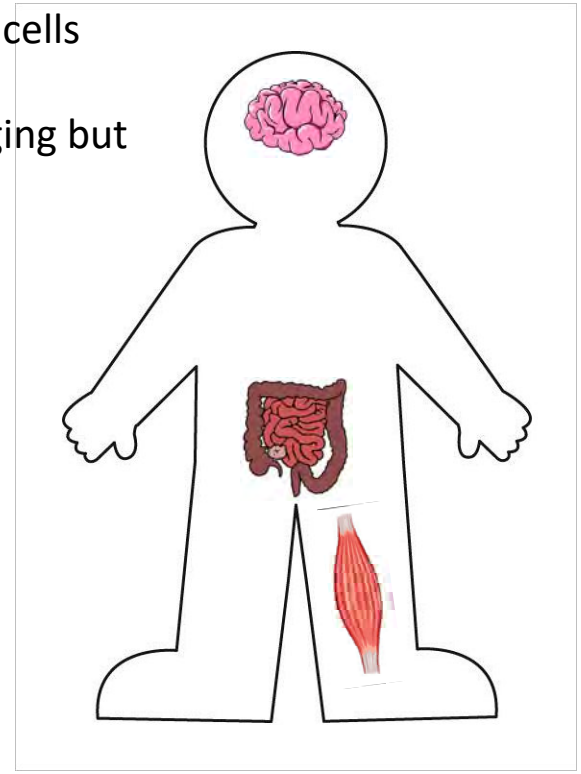
PAX6 is a neuro-ectodermal marker

Hasegawa et al. (2015). ***PLoS Genetics.***

Wolvetang Lab (AIBN/UQ)

Stem cells – reportedly, the “fountain of youth”?

- In the human body, over 200 specialized cell types exist.
- Throughout a lifespan, adult stem cells have two major jobs (1) to replace cells that have died, and (2) to regenerate damaged tissue.
- A general decline in quantity and quality of adult stem cells occurs with aging but this depends on the tissue.



Blood: hematopoietic stem cells have lower regenerative capacity that contributes to lineage-skewing.

Intestines: Intestinal stem cells are associated with increased activation of environmental stress responses.

Muscle: satellite cell frequency decreases with age so that regeneration of skeletal muscle fibres in response to injury is less successful.

Brain: neural stem cells also decline in number with age and may contribute to learning and memory deficits.

Skin: the stability of hair follicle stem cells decline with age, resulting in hair loss; melanocyte stem cells decline in number causing a loss in hairy colour.

Schultz & Sinclair. (2016). *When stem cells grow old: phenotypes and mechanisms of stem cell aging.*

Could heterogeneity be the key to understanding how stem cells age?

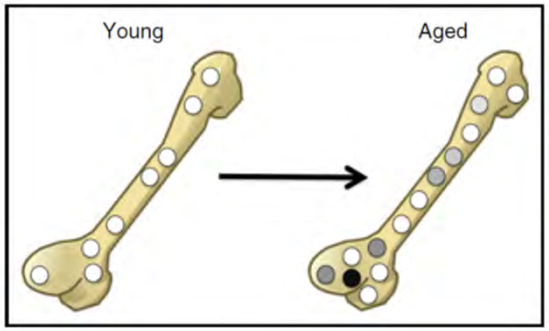
Heterogeneity is being recognized as an important component to aging stem cells.

Martinez-Jimenez et al. (2017). Science. ***Aging increases cell-to-cell transcriptional variability upon immune stimulation.***

Aging increases expression variability in CD4+ T-cells in older versus younger mice.

Bahar et al. (2006). Nature. ***Increased cell-to-cell variation in expression in ageing mouse heart.***

Increased expression variability and genomic damage in the cardiomyocytes in older versus younger mice.



De Haan & Lazare. (2018). Blood. ***Aging of hematopoietic stem cells.***

Variability in the range of regenerative capacity increases in aged HSCs.

Talk Summary

- Heterogeneity in stem cell populations is informative for understanding transcriptional regulation.
- Stem cells undergo (heterogeneous) decline during aging.
- Modelling variability in expression directly may help identify determinants of aging

  e.g. Metformin + single cell transcriptomics.
- Does modelling heterogeneity provide insight for other cell populations relevant to aging?

  For collaborations, please get in touch!

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