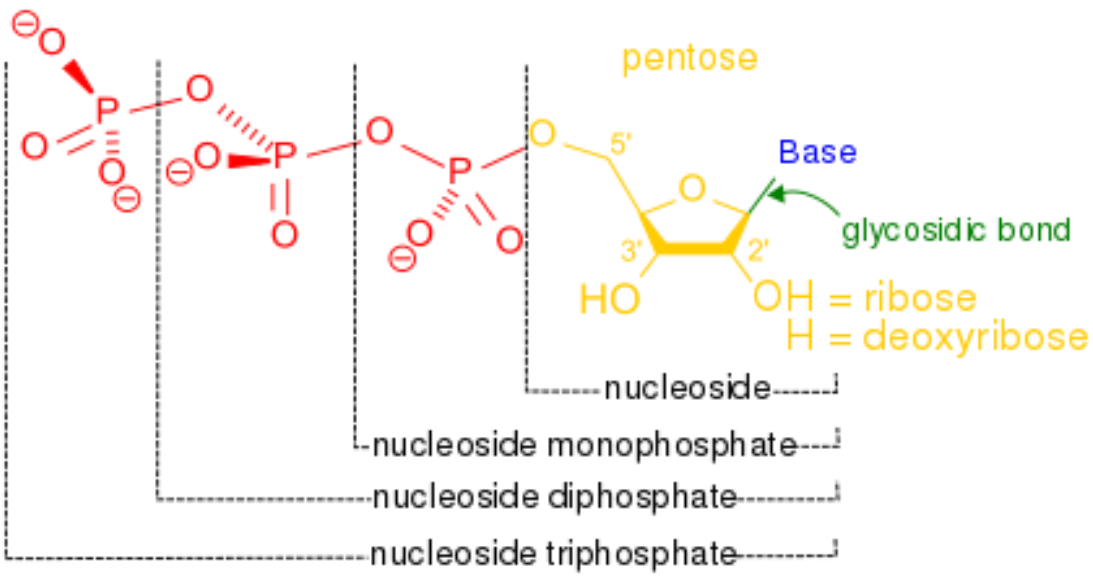
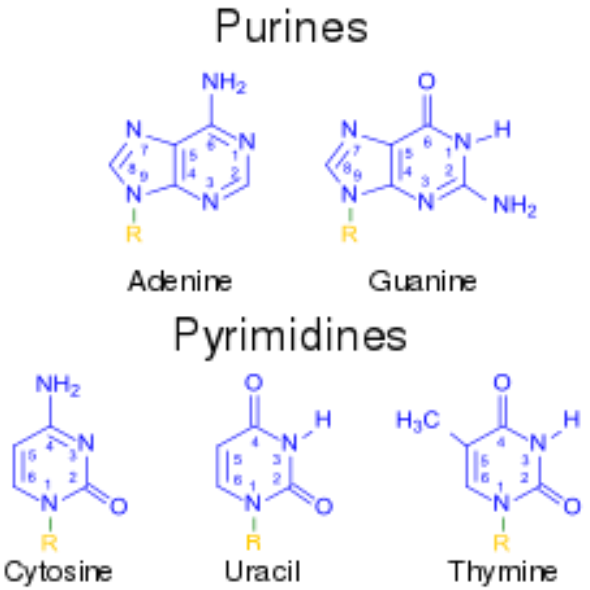
Genetics of complex traits: genetics of human longevity as a case

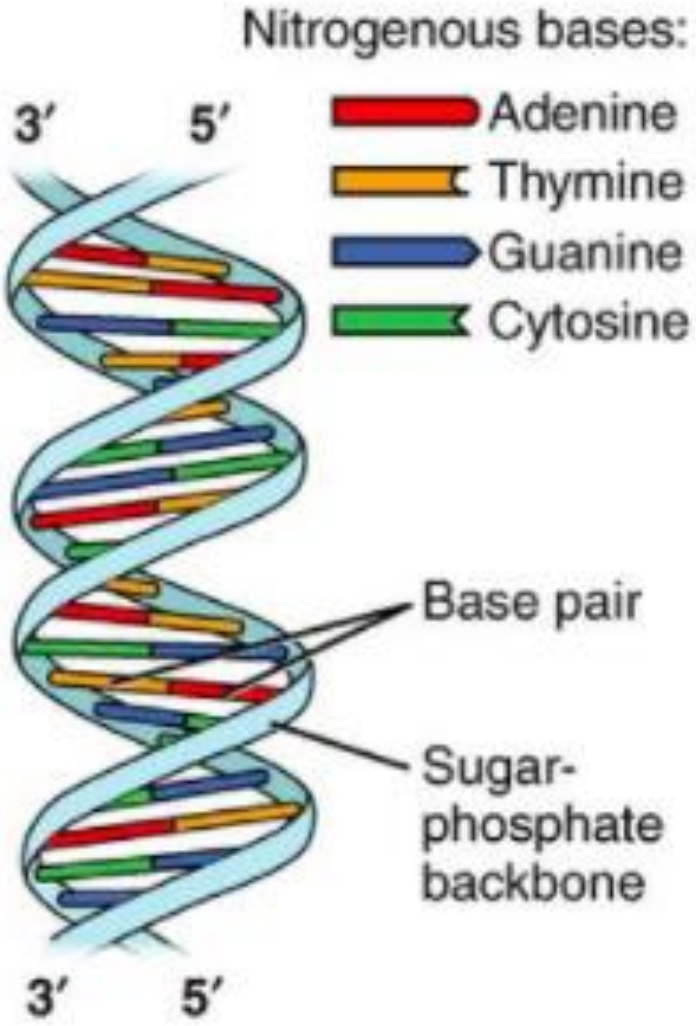
$$P = G \times E$$

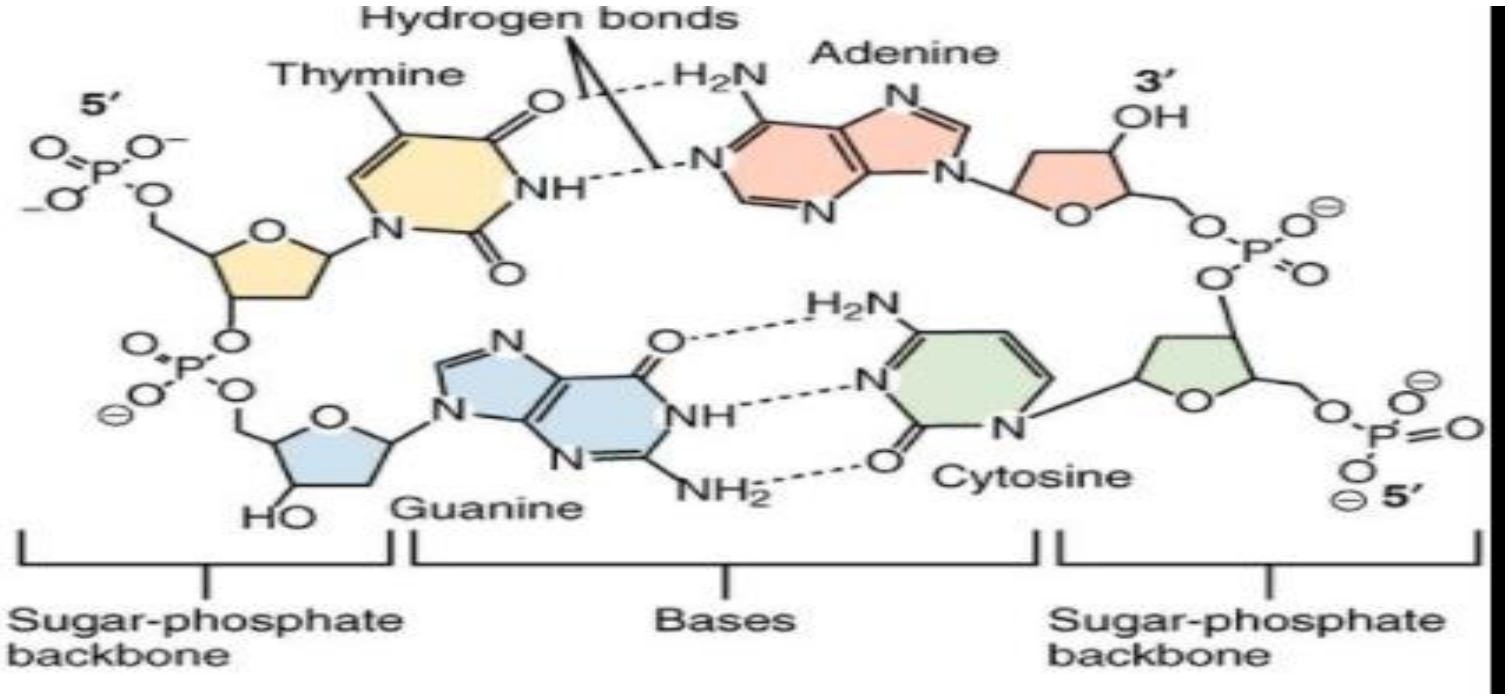
$$P = G \times E$$

NUCLEOTIDES





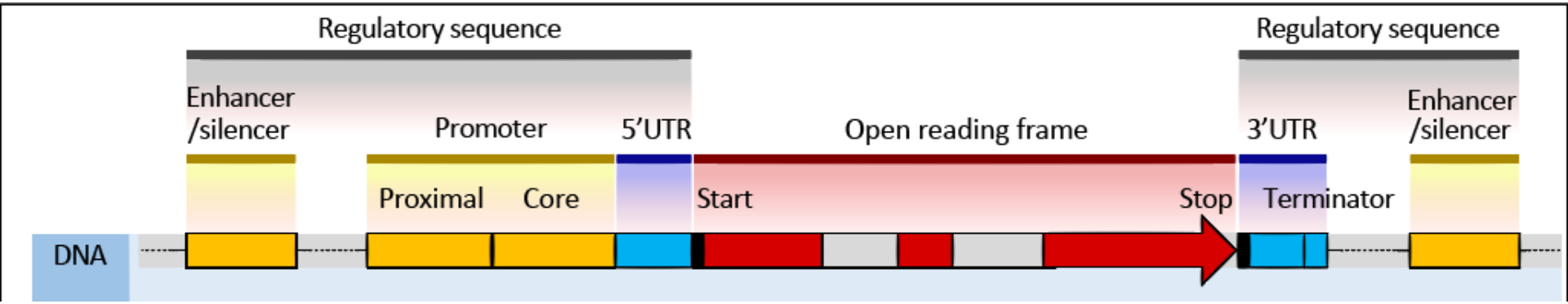

Nitrogenous bases:
Adenine
Thymine
Guanine
Cytosine
3′
5′
Base pair
Sugar-
phosphate
backbone
3′
5′


Hydrogen bonds
Thymine
Adenine
5′
3′
OH
Cytosine
Guanine
HO
5′
Sugar-phosphate backbone
Bases
Sugar-phosphate backbone

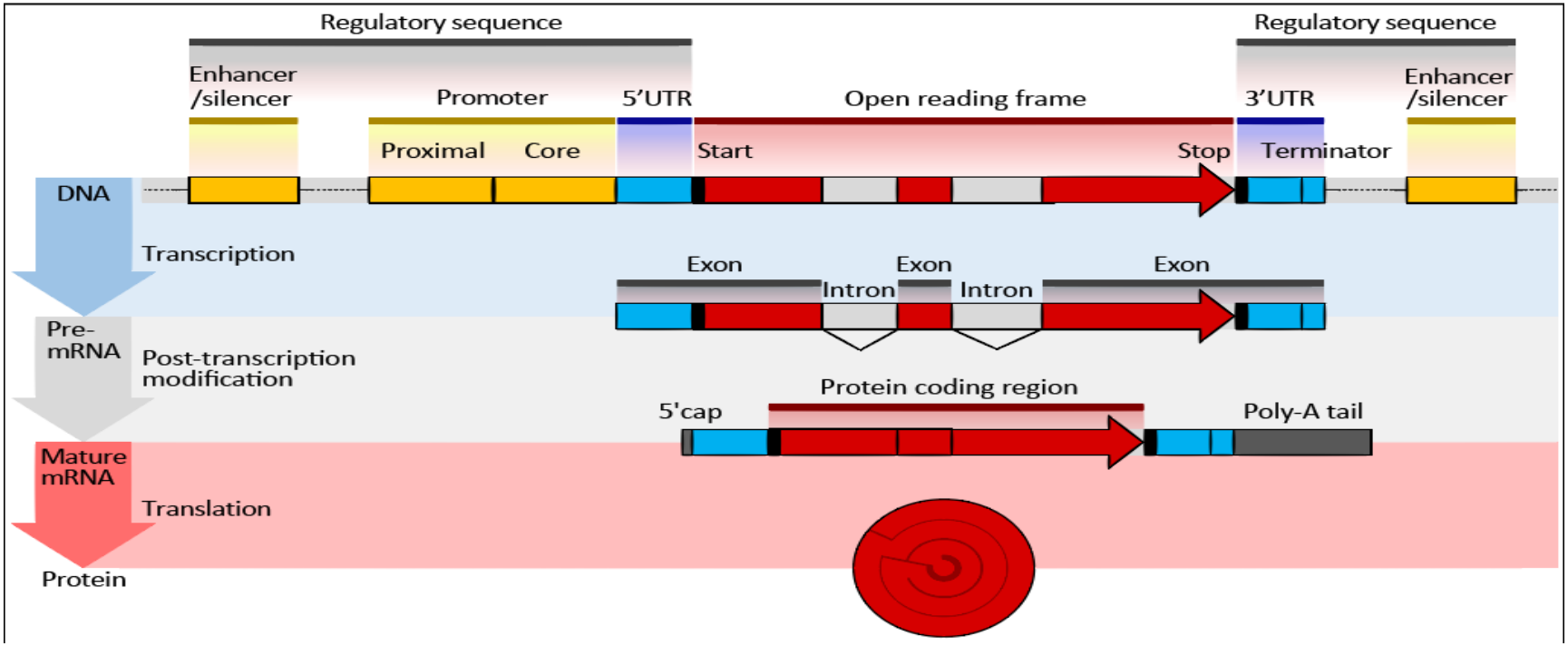
GENOME ORGANIZATION

- DNA is organised in long linear molecules
- The human genome is composed by more than 3 billions of nucleotides (6 billions if we consider the diploid state)
- In each cells of an individual we found 2 copy of 23 DNA molecules, named chromosomes
  (44 autosomic chromosomes + 2 sex chromosomes)
- 1,5% of the genome encodes for genes (about 30K genes)
- 98.5% of the genome is constituted by not coding regions, characterised by low complexity sequence

GENE


Regulatory sequence
Regulatory sequence
Enhancer /silencer
Promoter
5'UTR
Open reading frame
3'UTR
Enhancer /silencer
Proximal
Core
Start
Stop
Terminator
DNA

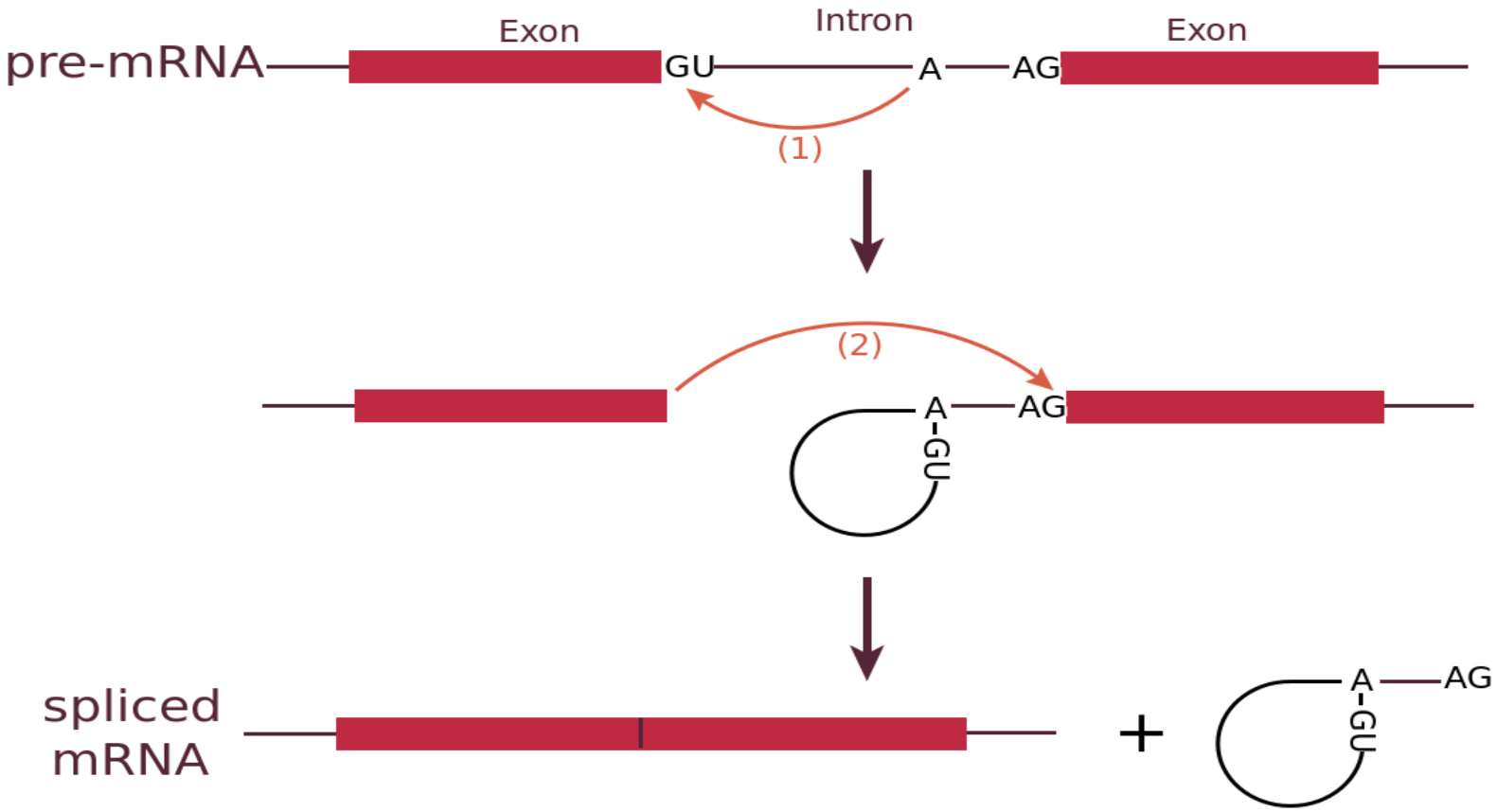
GENE EXPRESSION



Modificazioni Post-Trascizionali

Splicing

Per splicing si intende il meccanismo di rimozione degli introni dal filamento di RNA immaturo e l'associazione degli esoni così da costituire la sequenza codificante(CDS).



Modificazioni Post-Trascizionali

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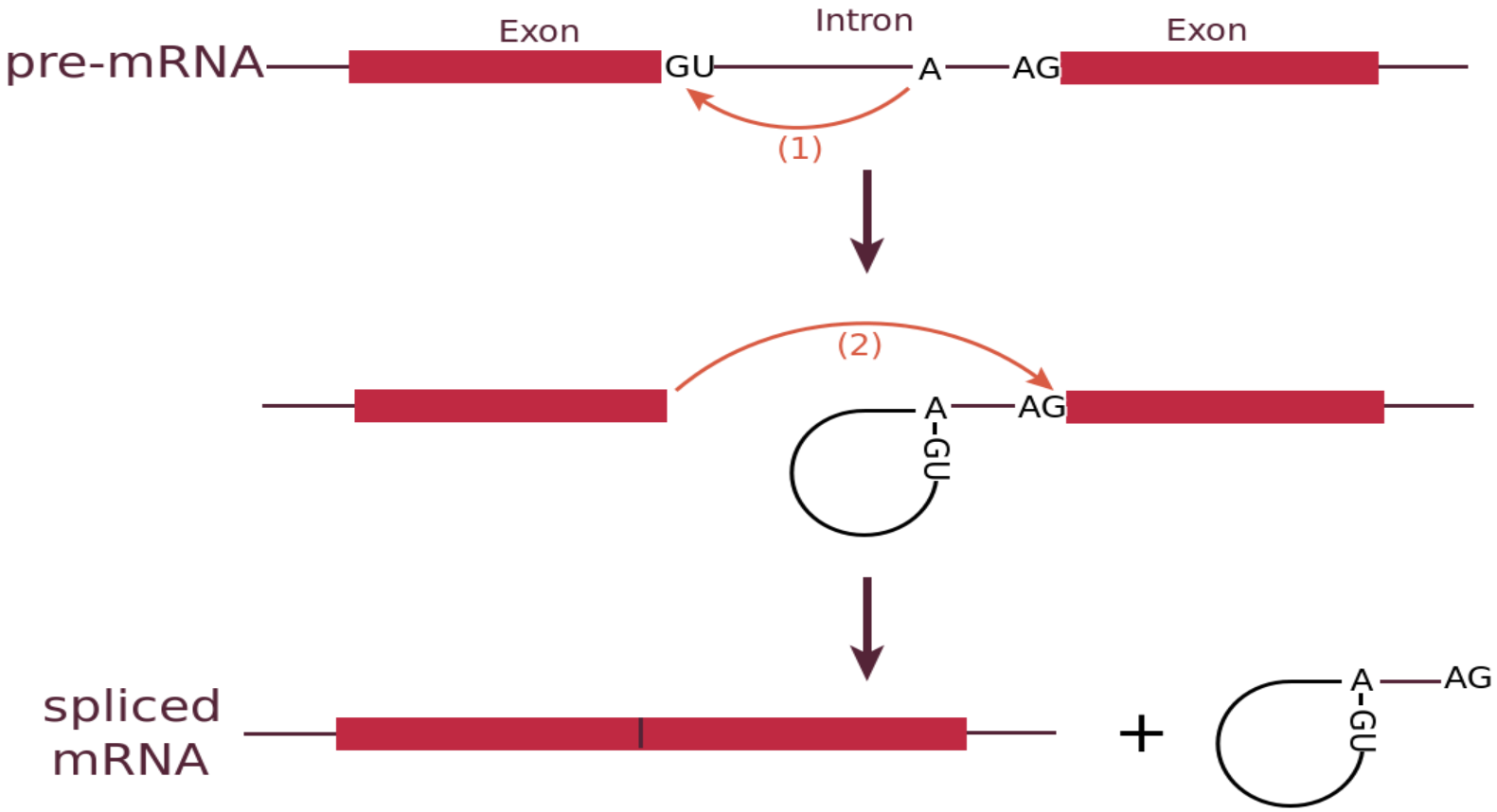
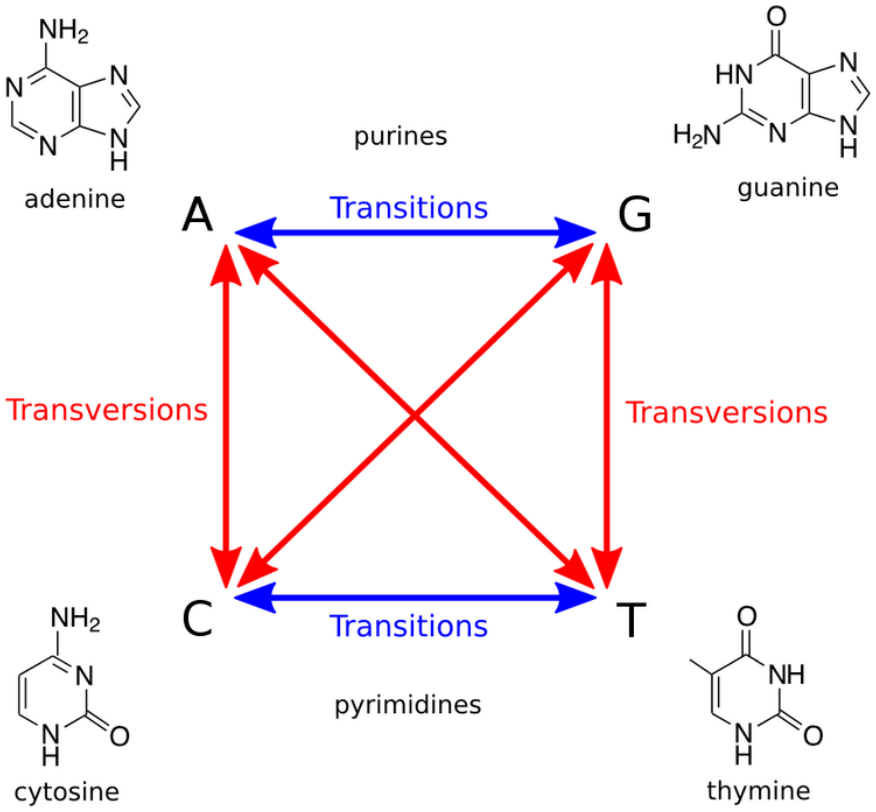
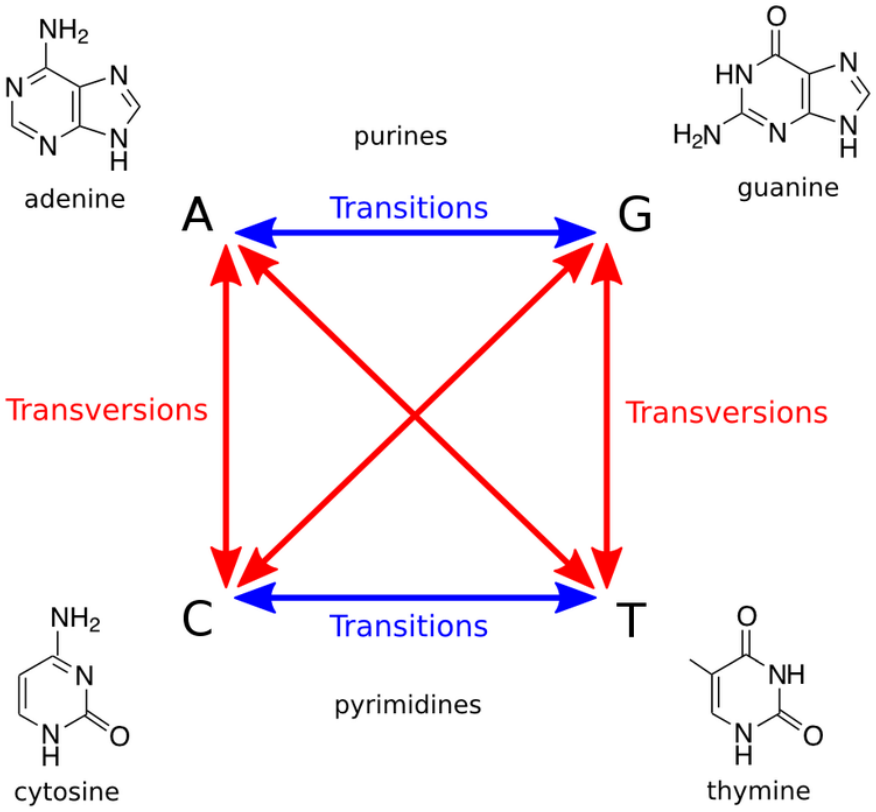


POINT MUTATIONS



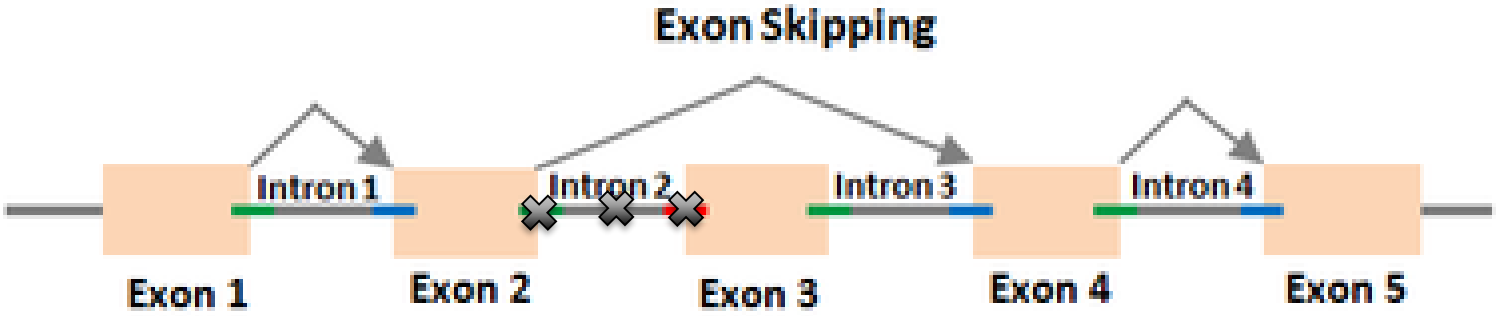
POINT MUTATIONS



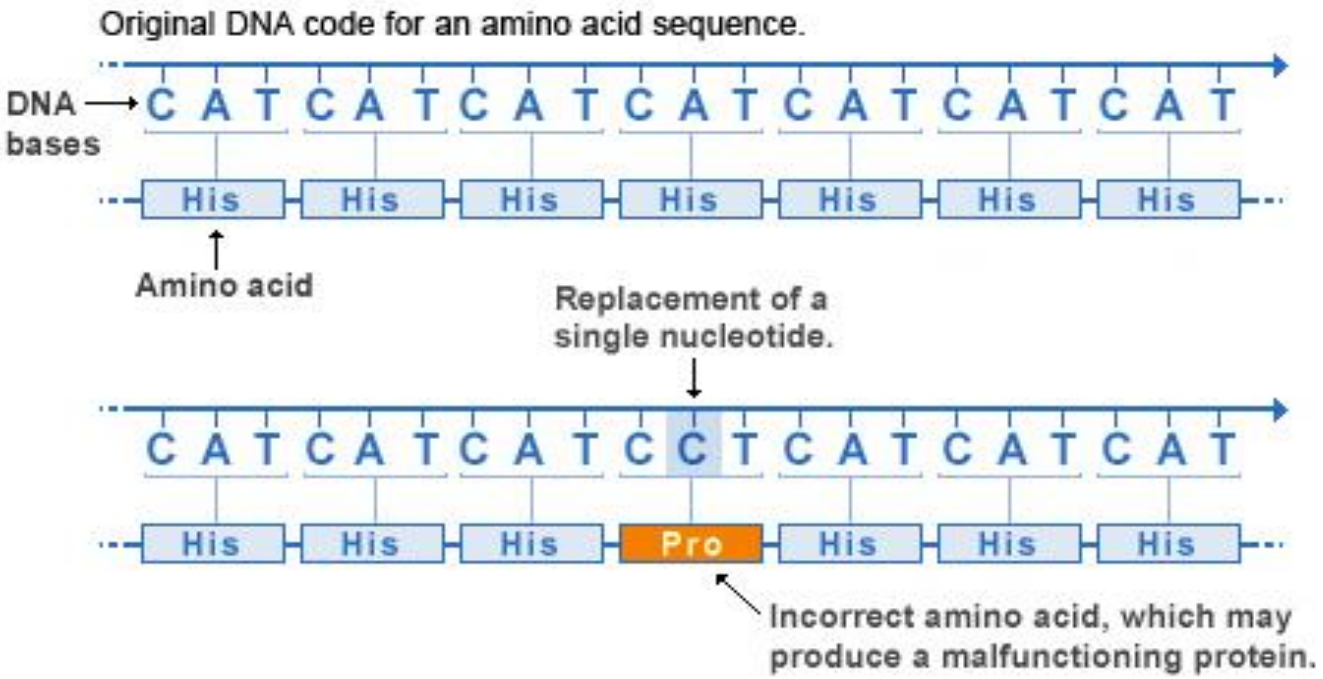
SILENT MUTATIONS

1st base	2nd base								3rd base
	T		C		A		G		
T	TTT	(Phe/F) Phenylalanine	TCT	(Ser/S) Serine	TAT	(Tyr/Y) Tyrosine	TGT	(Cys/C) Cysteine	T
	TTC		TCC		TAC		TGC		C
	TTA	(Leu/L) Leucine	TCA		TAA[B]	Stop (*Ochre*)	TGA[B]	Stop (*Opal*)	A
	TTG		TCG		TAG[B]	Stop (*Amber*)	TGG	(Trp/W) Tryptophan	G
C	CTT		CCT	(Pro/P) Proline	CAT	(His/H) Histidine	CGT	(Arg/R) Arginine	T
	CTC		CCC		CAC		CGC		C
	CTA		CCA		CAA	(Gln/Q) Glutamine	CGA		A
	CTG		CCG		CAG		CGG		G
A	ATT	(Ile/I) Isoleucine	ACT	(Thr/T) Threonine	AAT	(Asn/N) Asparagine	AGT	(Ser/S) Serine	T
	ATC		ACC		AAC		AGC		C
	ATA		ACA		AAA	(Lys/K) Lysine	AGA	(Arg/R) Arginine	A
	ATG[A]	(Met/M) Methionine	ACG		AAG		AGG		G
G	GTT	(Val/V) Valine	GCT	(Ala/A) Alanine	GAT	(Asp/D) Aspartic acid	GGT	(Gly/G) Glycine	T
	GTC		GCC		GAC		GGC		C
	GTA		GCA		GAA	(Glu/E) Glutamic acid	GGA		A
	GTG		GCG		GAG		GGG		G

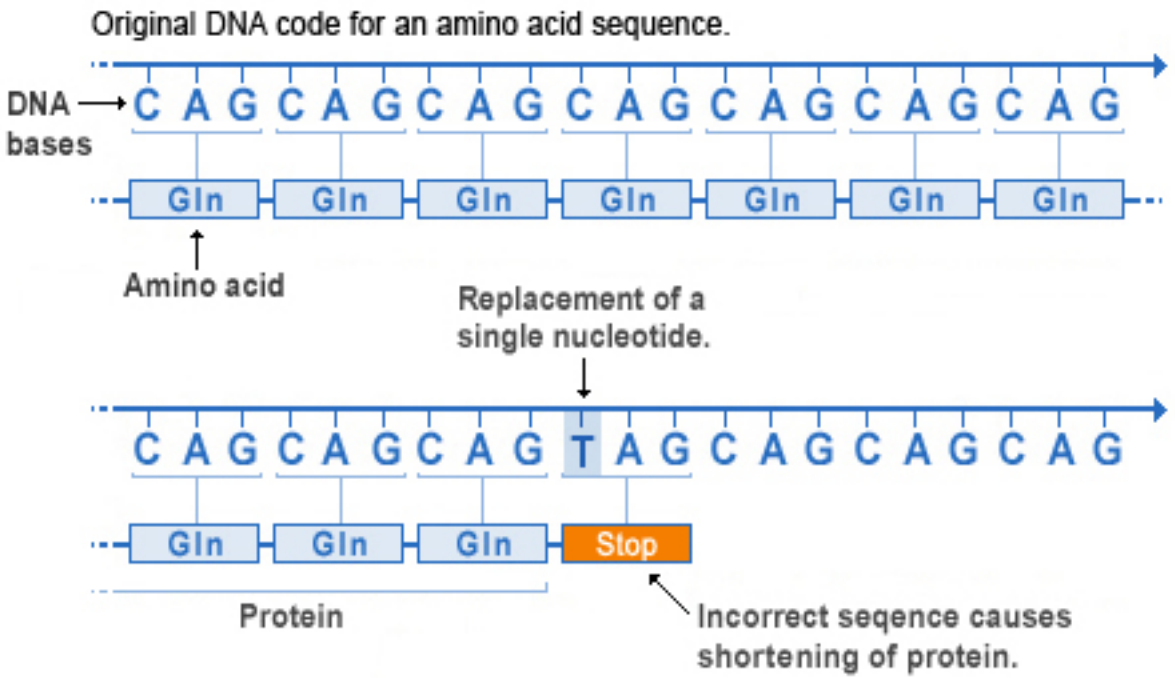
Exon skipping



Missense mutation



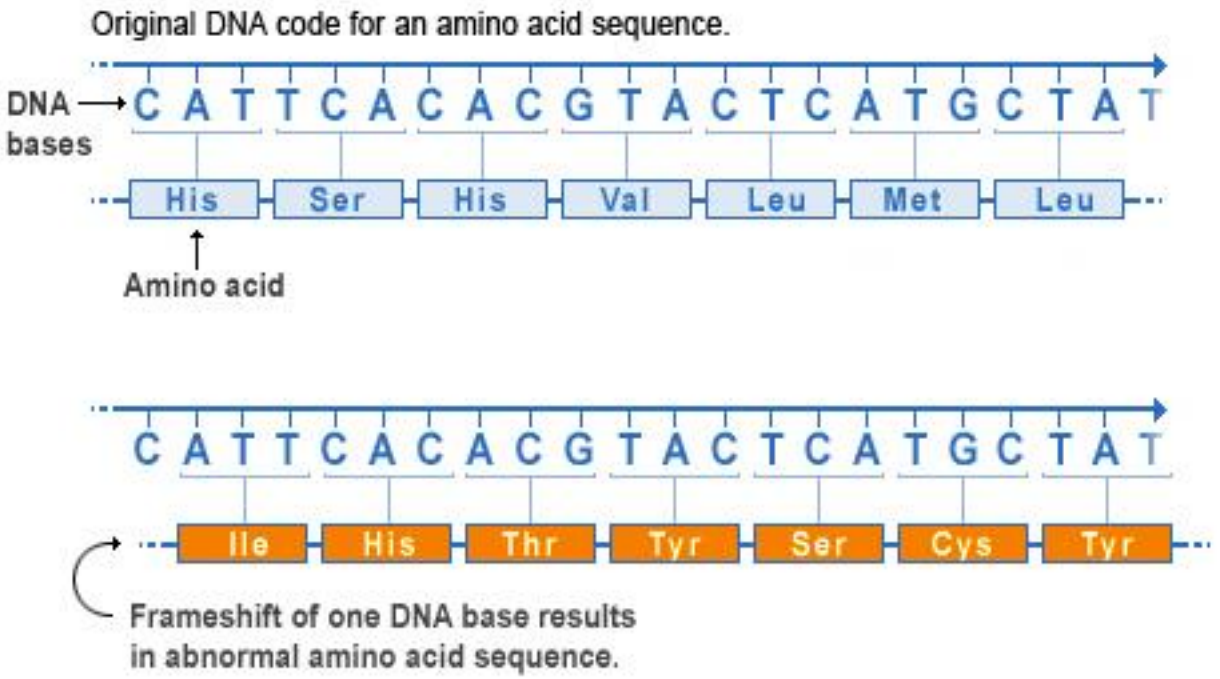
Nonsense mutation



U.S. National Library of Medicine

https://ghr.nlm.nih.gov/primer/mutationsanddisorders/possiblemutations

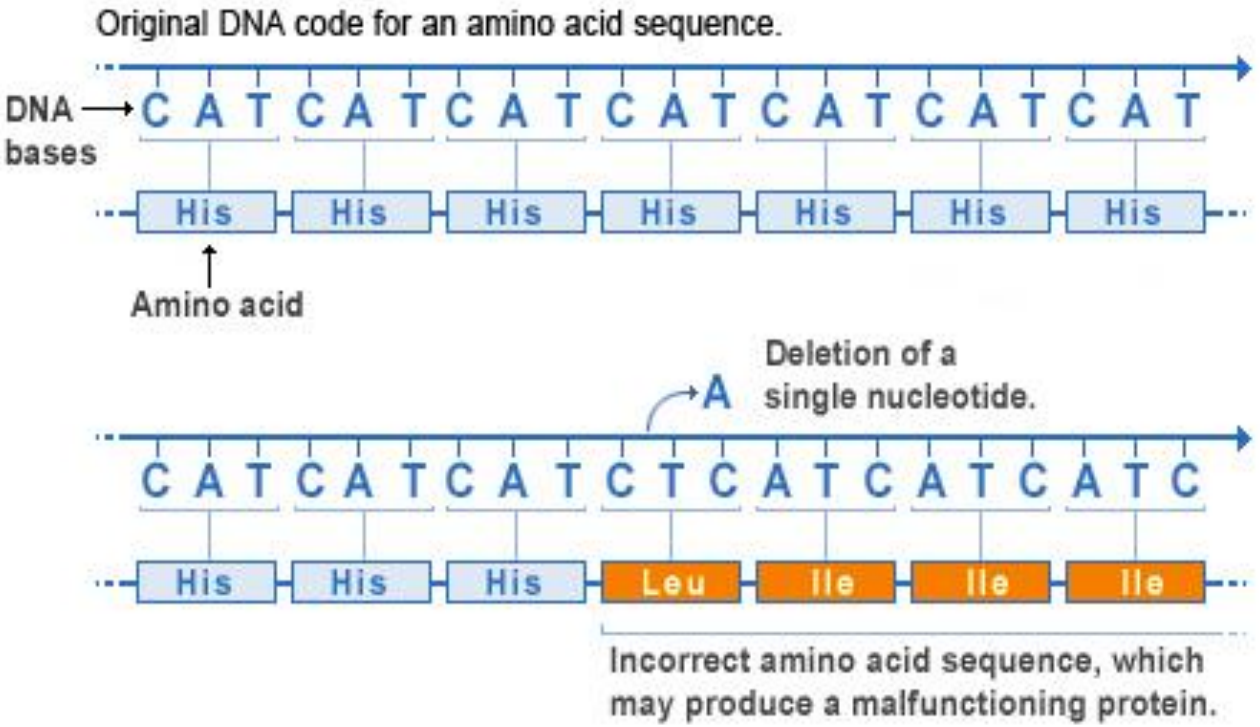
Frameshift mutation



U.S. National Library of Medicine

https://ghr.nlm.nih.gov/primer/mutationsanddisorders/possiblemutations

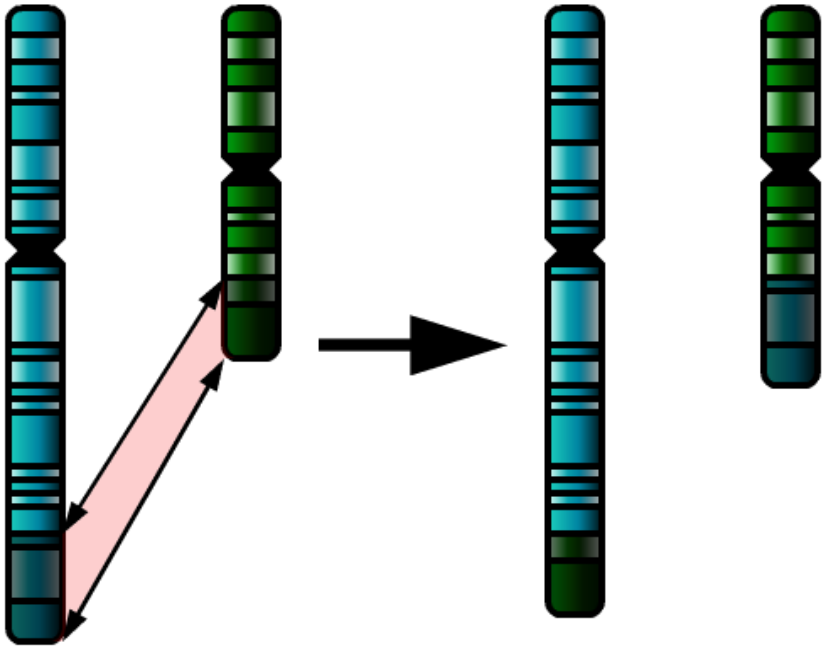
Deletion mutation



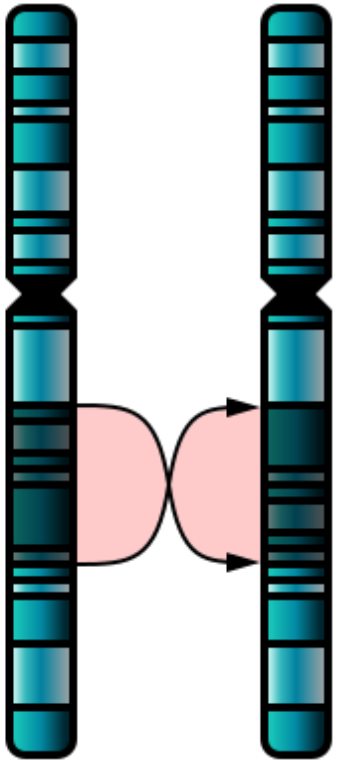
U.S. National Library of Medicine

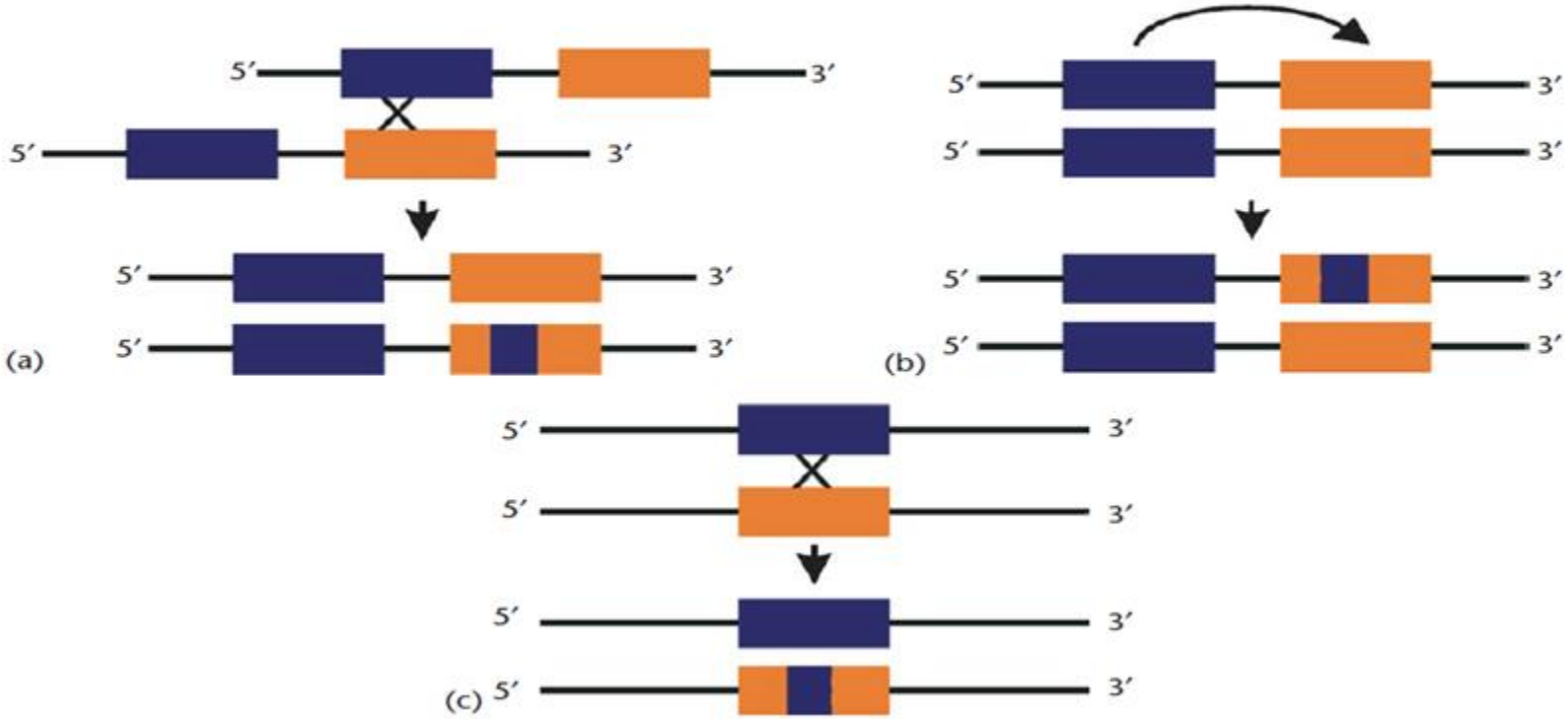
https://ghr.nlm.nih.gov/primer/mutatio
sanddisorders/possiblemutations

2. **Traslocazione**
   Trasferimento di una regione di un cromosoma ad un altro

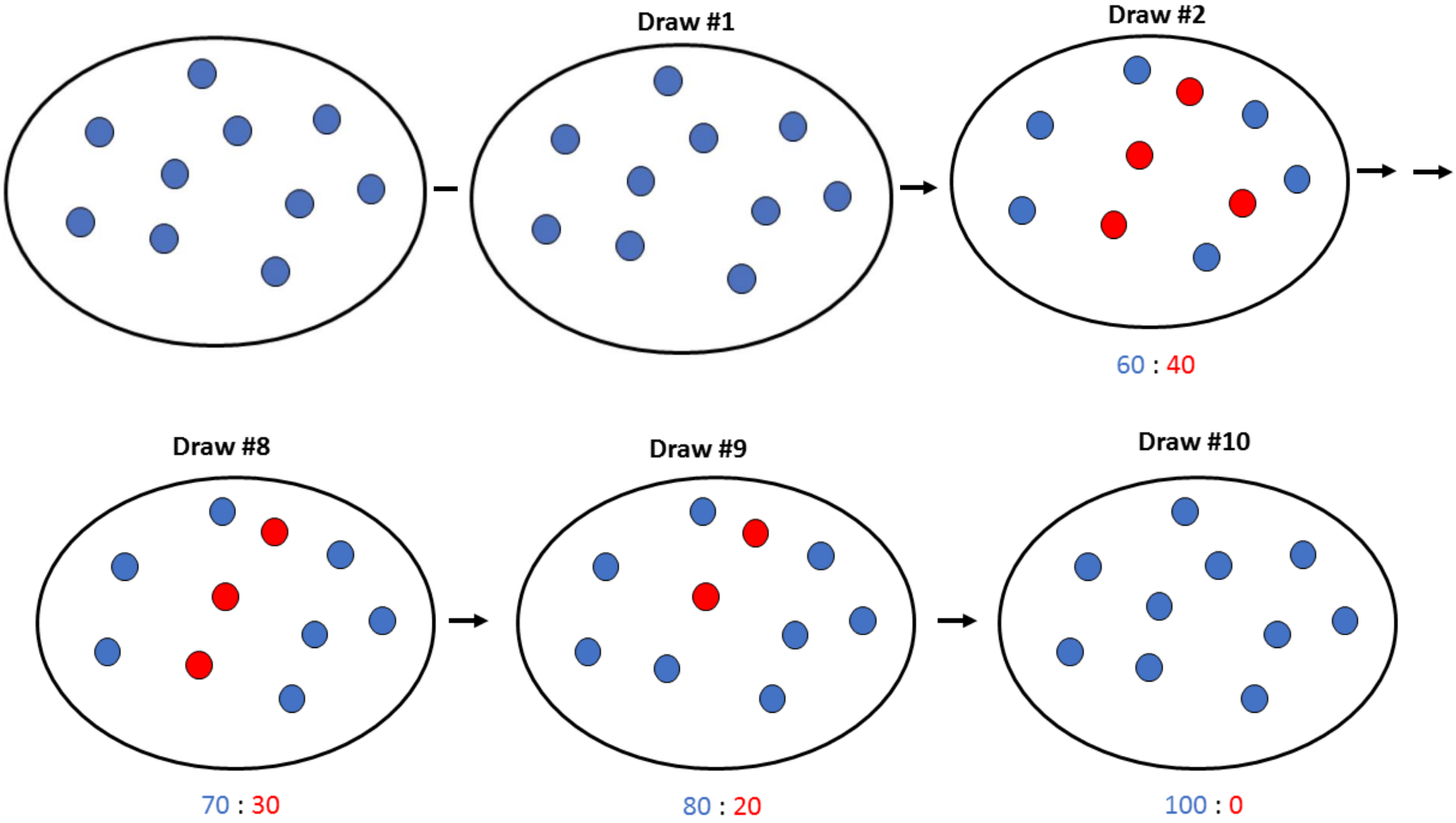


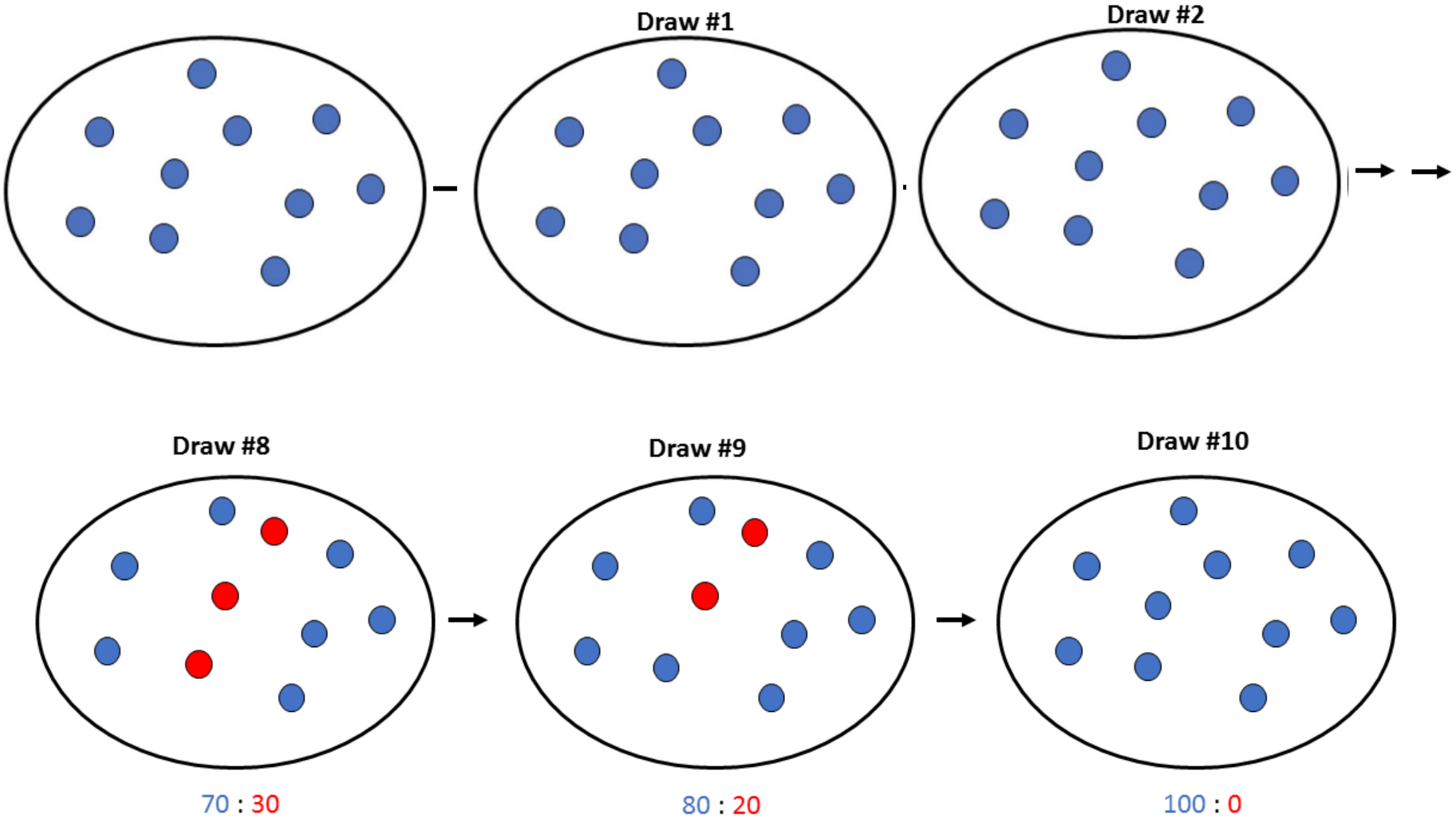
3. Inversions

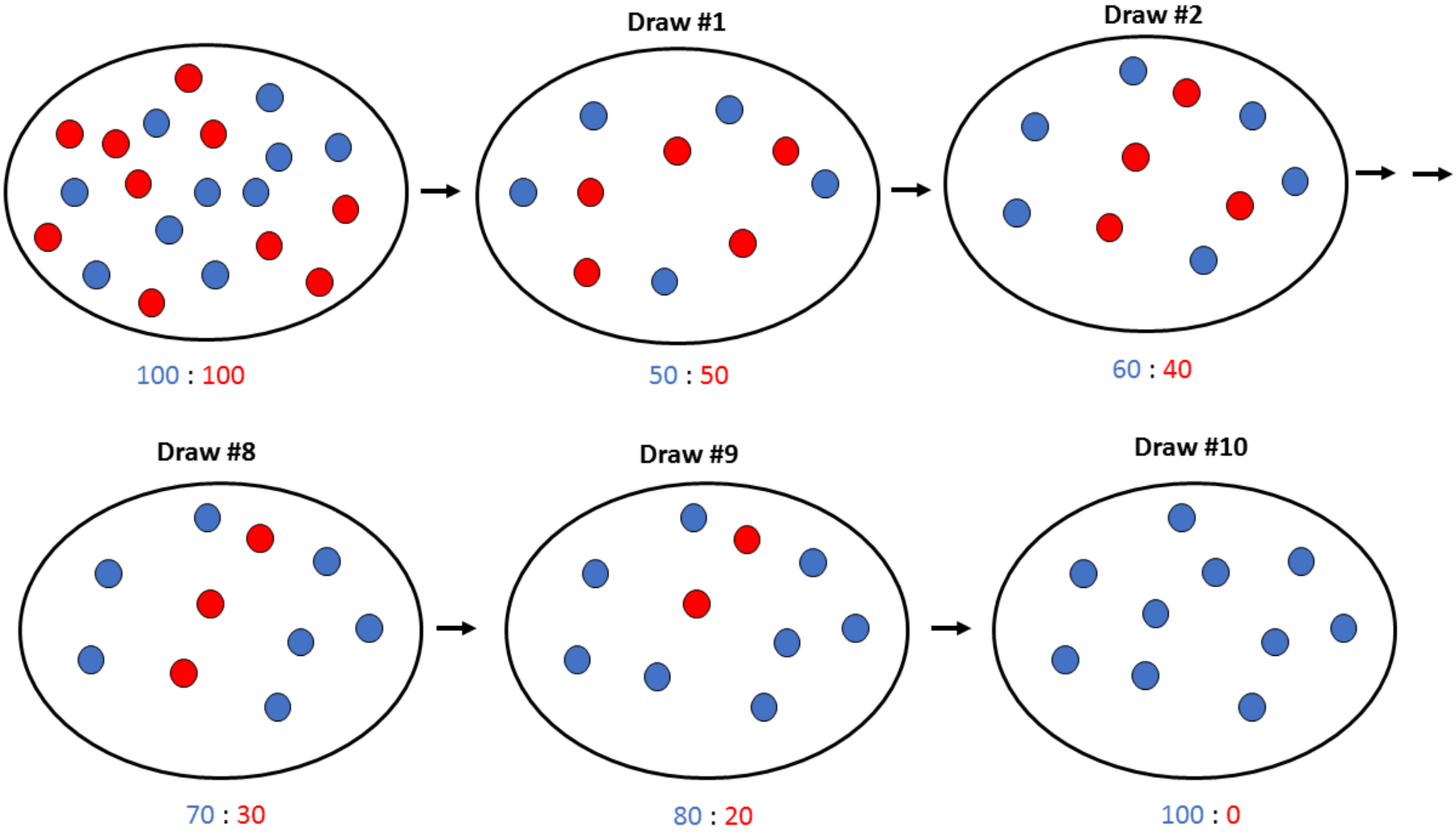


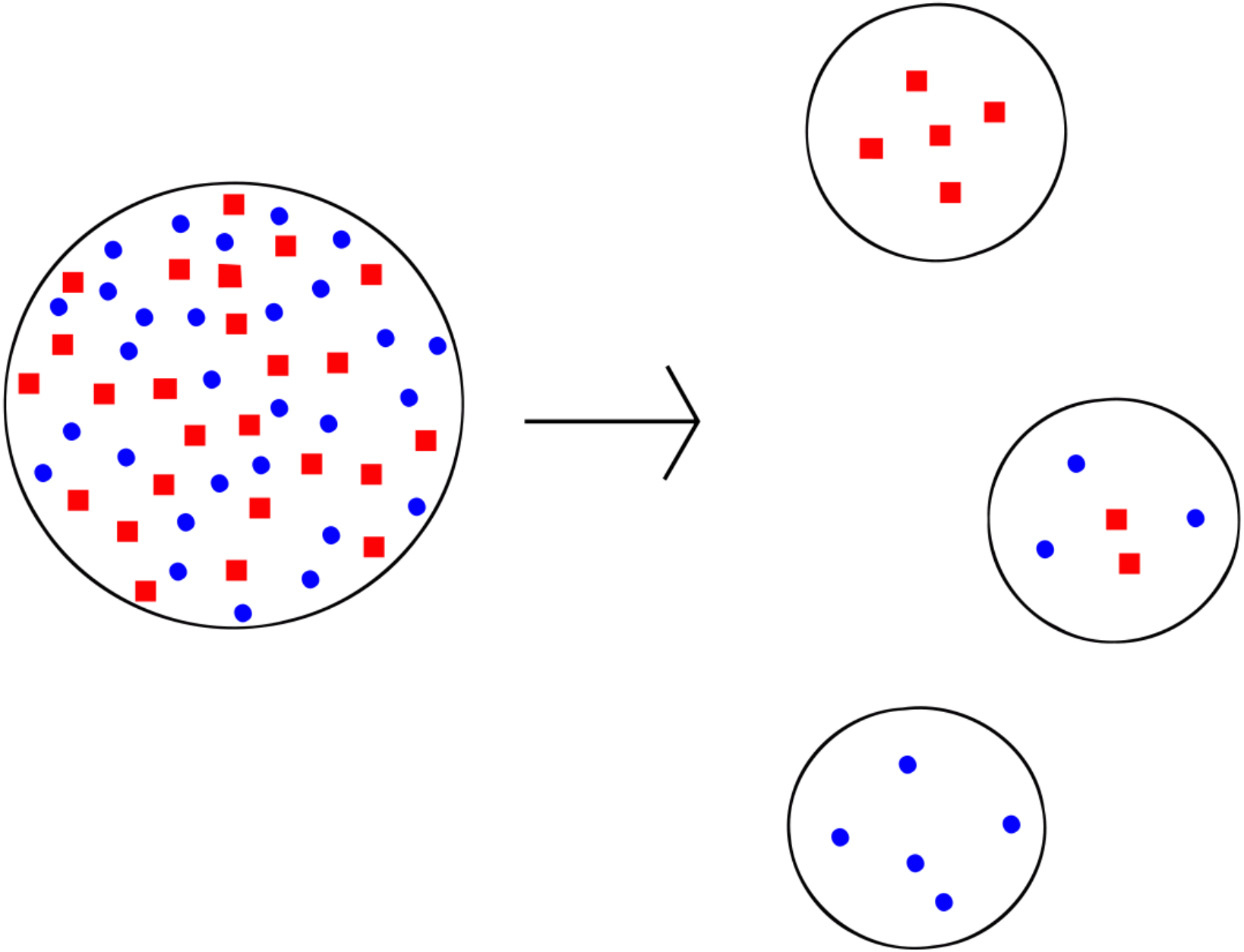


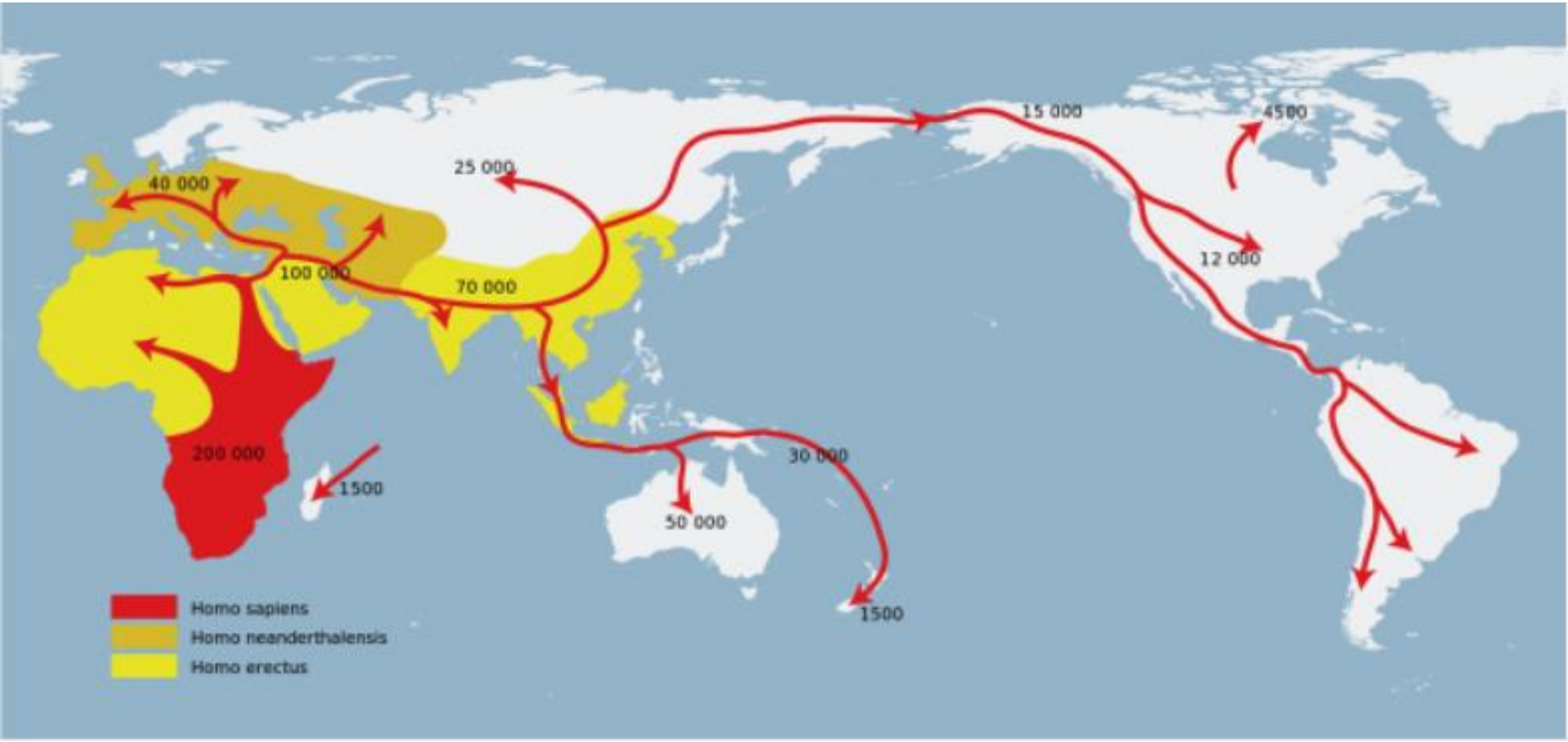
MUTATION FATE

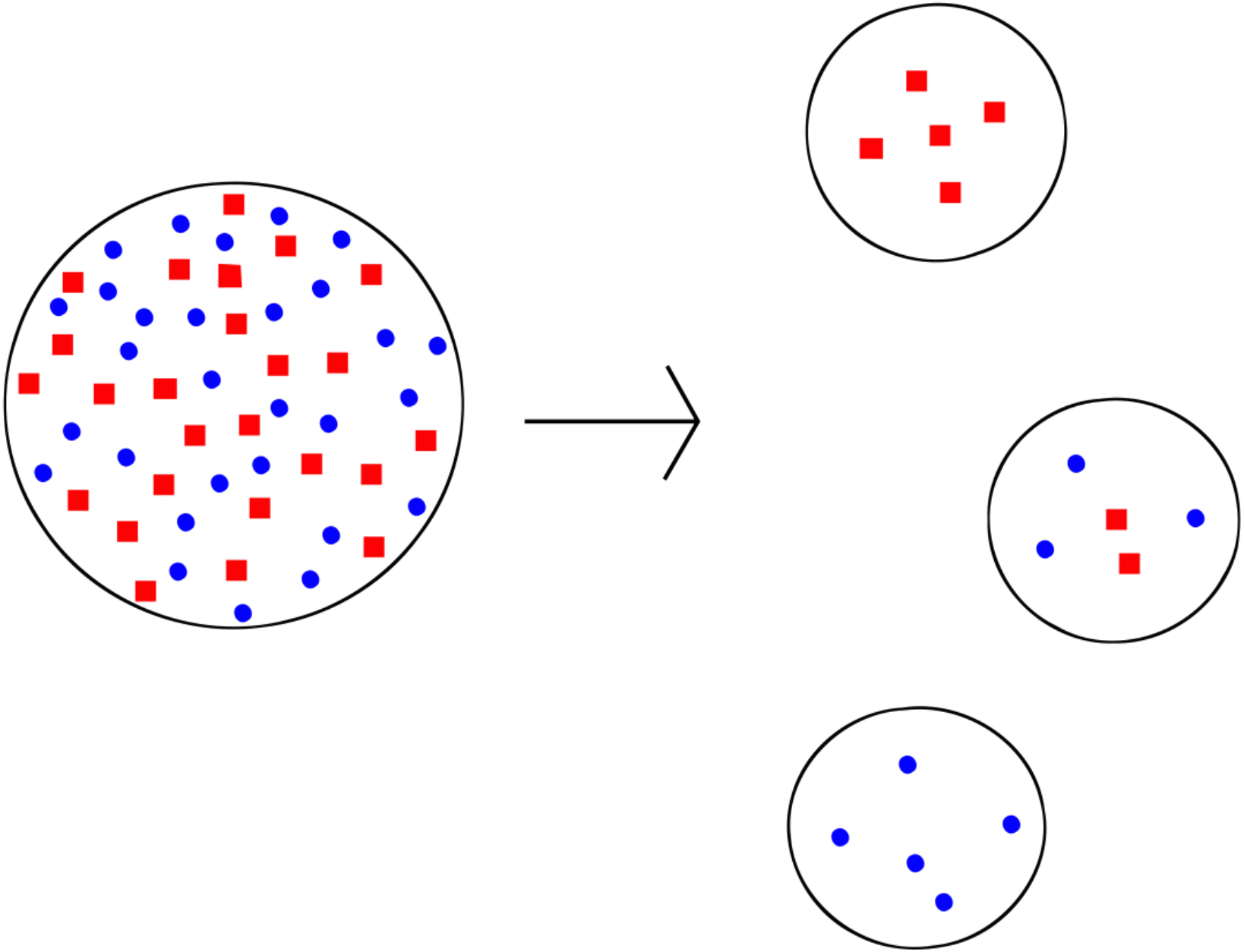

Draw #1
Draw #2
60 : 40
Draw #8
Draw #9
Draw #10
70 : 30
80 : 20
100 : 0


Draw #1
Draw #2
Draw #8
Draw #9
Draw #10
70 : 30
80 : 20
100 : 0


Draw #1
Draw #2
100 : 100
50 : 50
60 : 40
Draw #8
Draw #9
Draw #10
70 : 30
80 : 20
100 : 0




40 000
100 000
200 000
1500
25 000
70 000
50 000
30 000
1500
15 000
12 000
4500
Homo sapiens
Homo neanderthalensis
Homo erectus



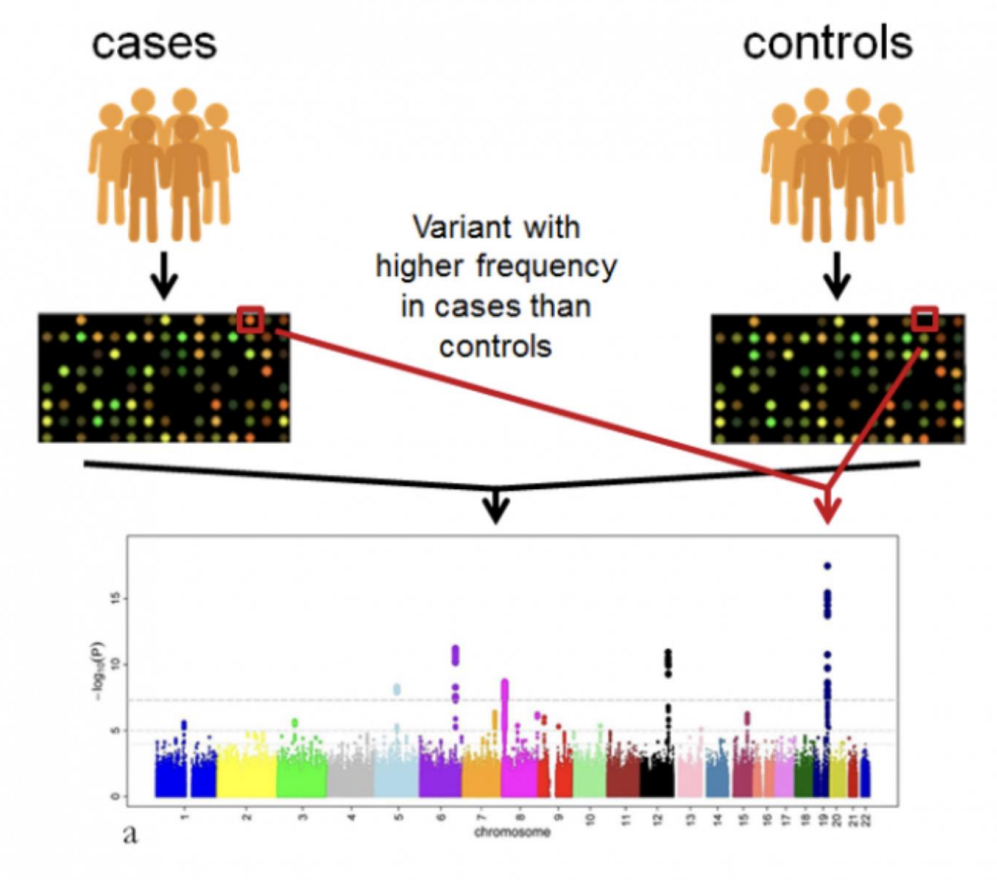
TECHNOLOGIES TO ASSESS DNA VARIABILITY

GWAS

SNPs MICROARRAY

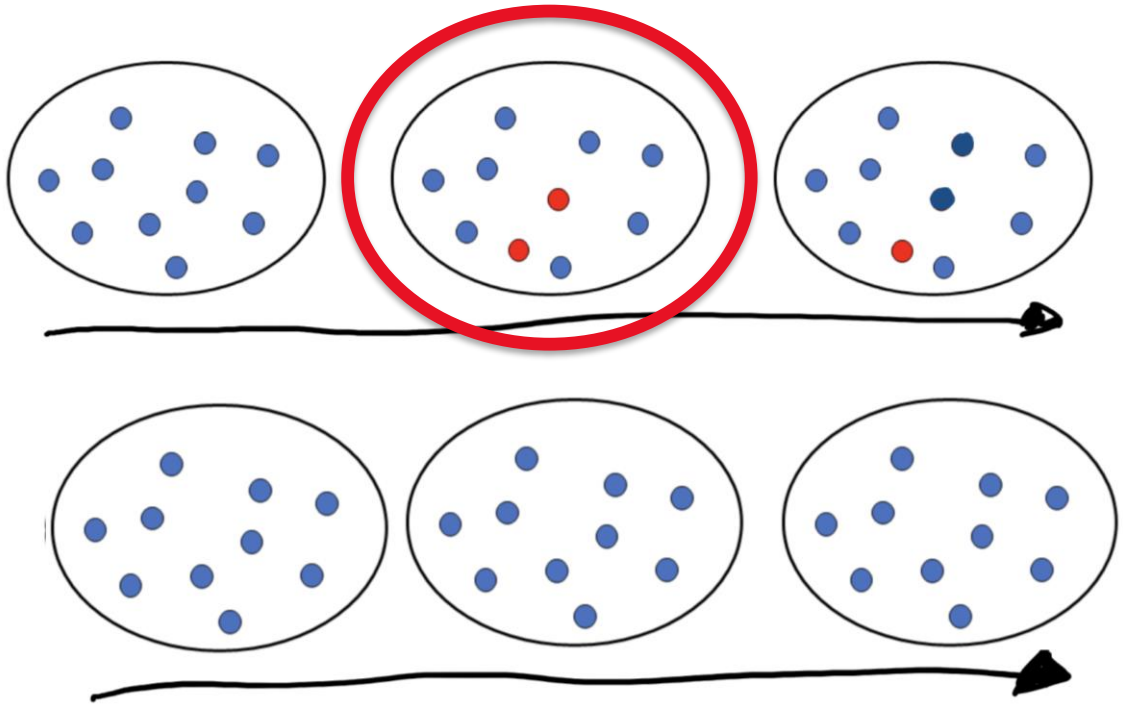
COMMON VARIANTS COMMON DISEASES

COMMON VARIANTS COMMON DISEASES



NEXT GENERATION SEQUENCING

- WHOLE GENOME RE-SEQUENCING
- DATA ON RARE AND COMMON VARIANTS

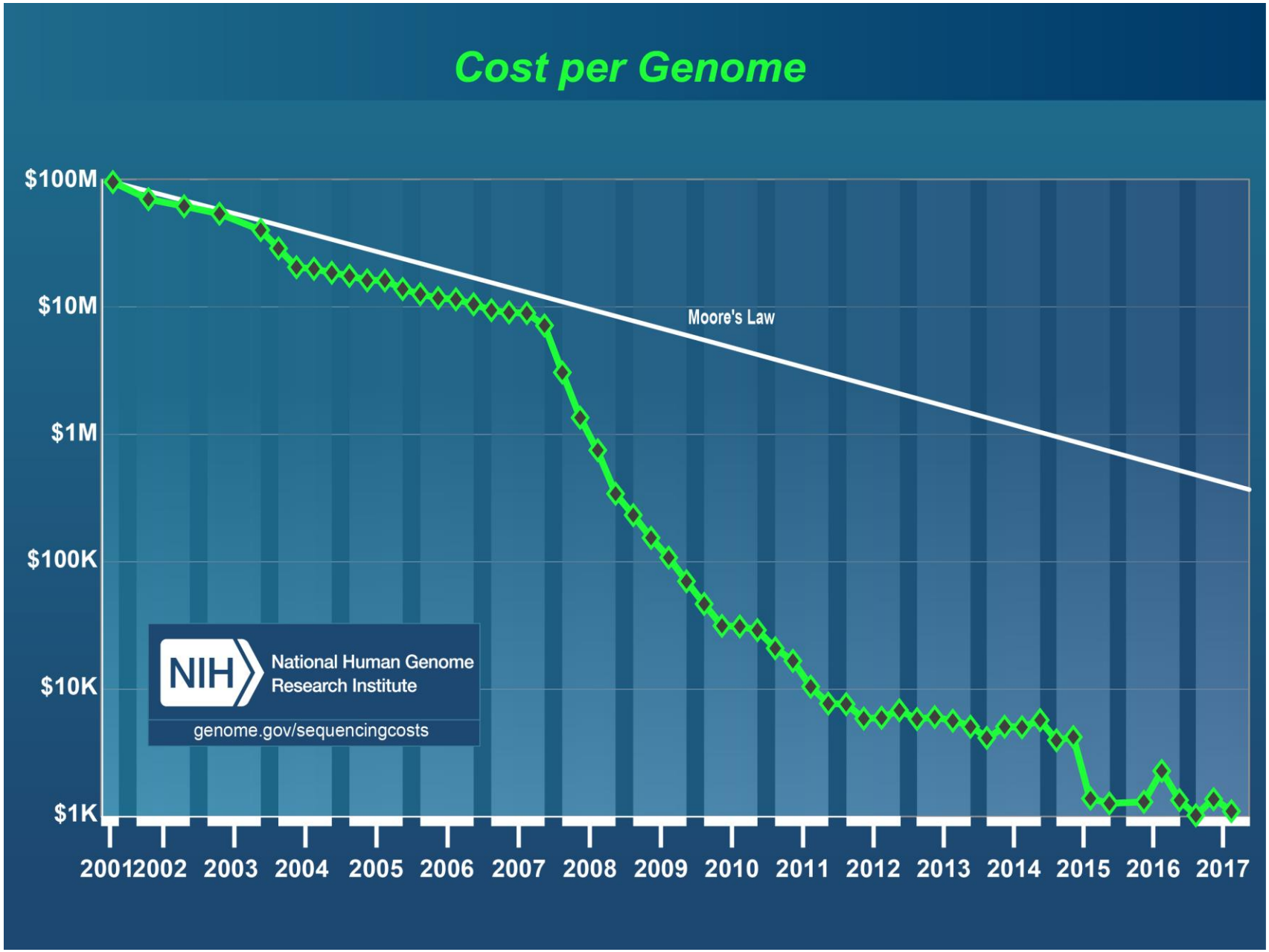


Changes in sequencing cost

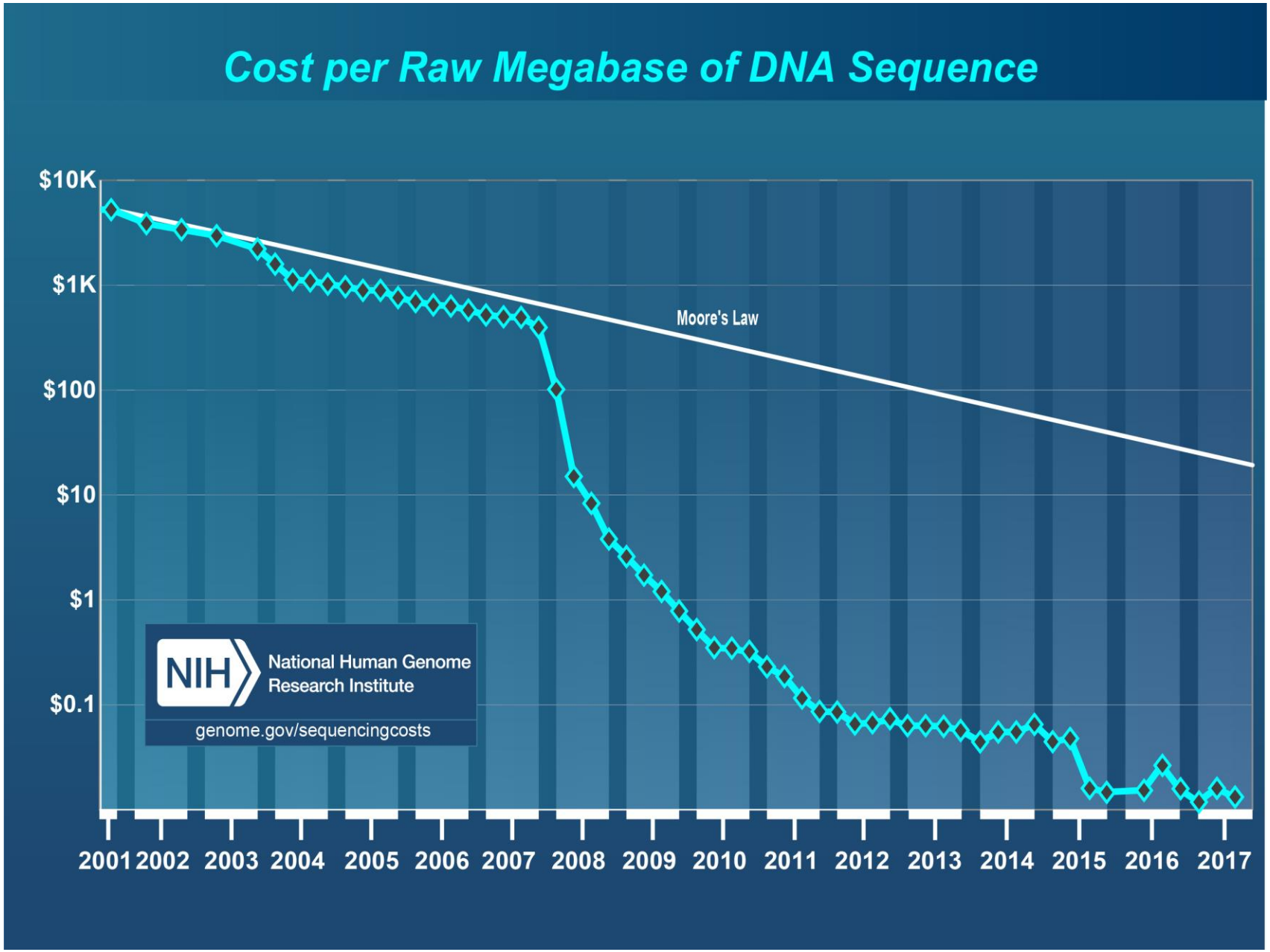
"A draft' human genome sequence was produced over a 15-month period (from April 1999 to June 2000).
The estimated cost for generating that initial 'draft' human genome sequence is ~$**300 million** worldwide."

"The estimated cost for advancing the 'draft' human genome sequence to the 'finished' sequence was ~$**150 million** worldwide."

Wetterstrand KA. DNA Sequencing Costs: Data from the NHGRI Genome Sequencing Program (GSP) Available at: www.genome.gov/sequencingcostsdata. Accessed [date of access].



Wetterstrand KA. DNA Sequencing Costs: Data from the NHGRI Genome Sequencing Program (GSP) Available at: www.genome.gov/sequencingcostsdata. Accessed [date of access].



Wetterstrand KA. DNA Sequencing Costs: Data from the NHGRI Genome Sequencing Program (GSP) Available at: www.genome.gov/sequencingcostsdata. Accessed [date of access].

"In mid-2015 the cost to generate a high-quality 'draft' whole human genome sequence was just above $**4,000**."

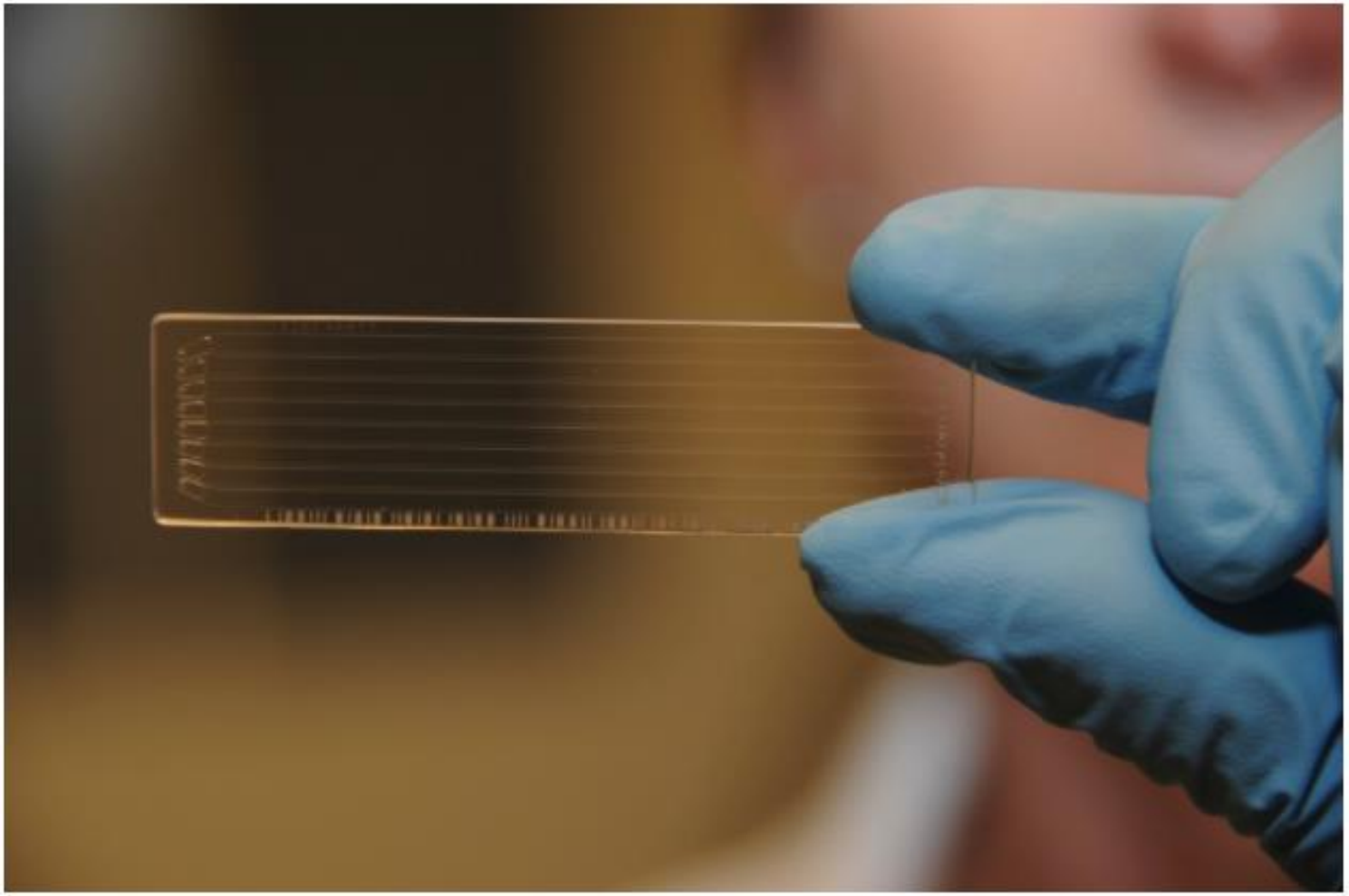
"By late in 2015, that figure had fallen below $**1,500**. "

Wetterstrand KA. DNA Sequencing Costs: Data from the NHGRI Genome Sequencing Program (GSP) Available at: www.genome.gov/sequencingcostsdata. Accessed [date of access].

Illumina wants to sequence your whole genome for $100

Sarah Buhr @sarahbuhr / 2 years ago

Comment



THE GENETICS OF HUMAN AGING/LONGEVITY

Why is it so difficult ?

THE GENETICS OF HUMAN LONGEVITY

In no species other than humans do **cultural, social, and biological factors interact with each other** in modulating complex phenotypes such as aging, longevity and age-related diseases

THE GENETICS OF HUMAN LONGEVITY

several non mutually exclusive possibilities:

- 90+/100+ do not have "disease-risk" gene variants
- 90+/100+ do have disease-risk gene variants but their lifestyle /environment was not permissive
- 90+/100+ have "protective" gene variants for age-related diseases

THE GENETICS OF HUMAN LONGEVITY

several non mutually exclusive possibilities:

- 90+/100+ do not have "disease-risk" gene variants (WRONG)
- 90+/100+ do have disease-risk gene variants but their lifestyle /environment was not permissive (YES, some evidence)
- 90+/100+ have "protective" gene variants for age-related diseases (YES few evidence)

THE GENETICS OF HUMAN LONGEVITY

burning questions:

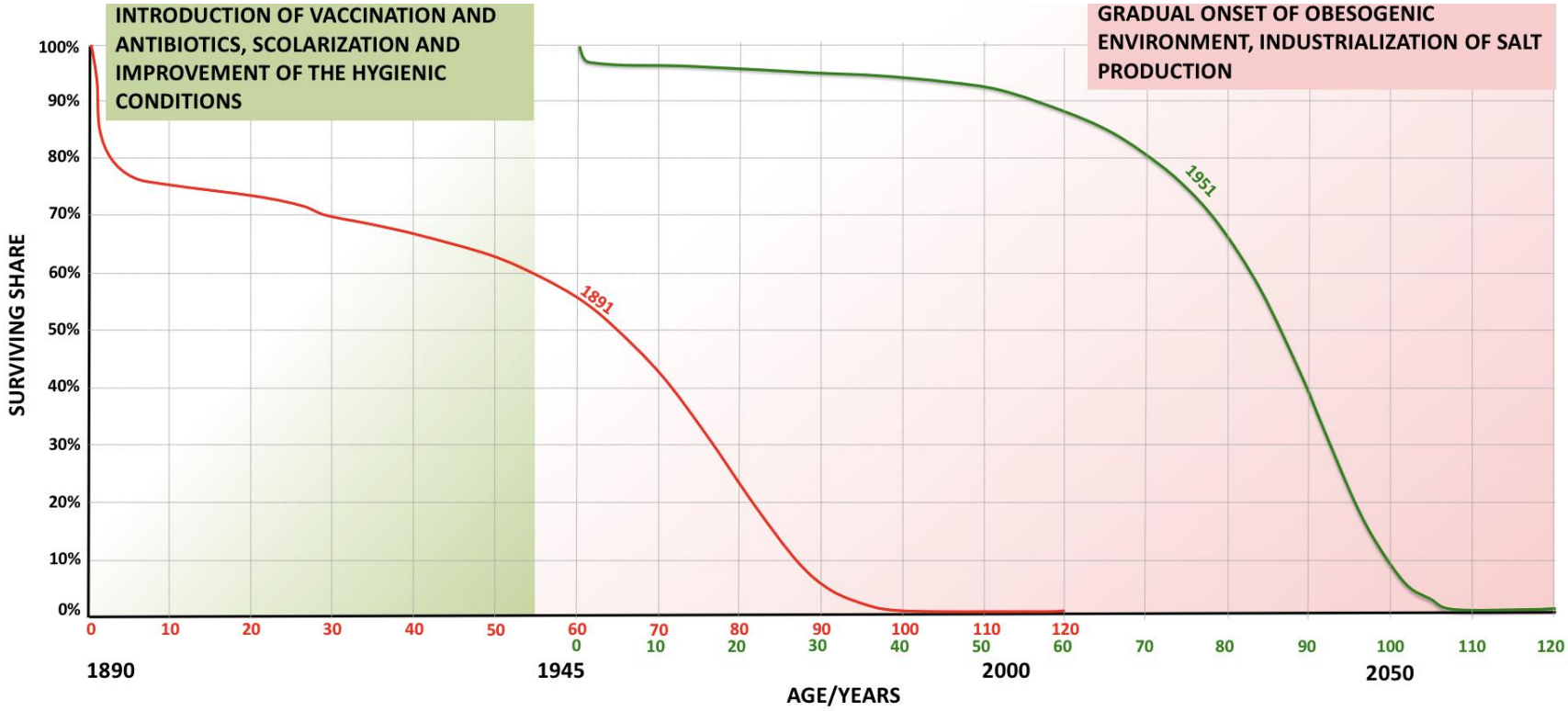
if and how much is

- population-specific ?
- gender-specific ?
- "public" or "private" ?

THE GENETICS OF HUMAN LONGEVITY

MODELS:

- CENTENARIANS
- FAMILIAL LONGEVITY
- PARENTAL LONGEVITY
- AGE RELATED DISEASES


INTRODUCTION OF VACCINATION AND ANTIBIOTICS, SCOLARIZATION AND IMPROVEMENT OF THE HYGIENIC CONDITIONS
GRADUAL ONSET OF OBESOGENIC ENVIRONMENT, INDUSTRIALIZATION OF SALT PRODUCTION
SURVIVING SHARE
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1891
1951
0 10 20 30 40 50 60 70 80 90 100 110 120
0 10 20 30 40 50 60 70 80 90 100 110 120
1890
1945
2000
2050
AGE/YEARS

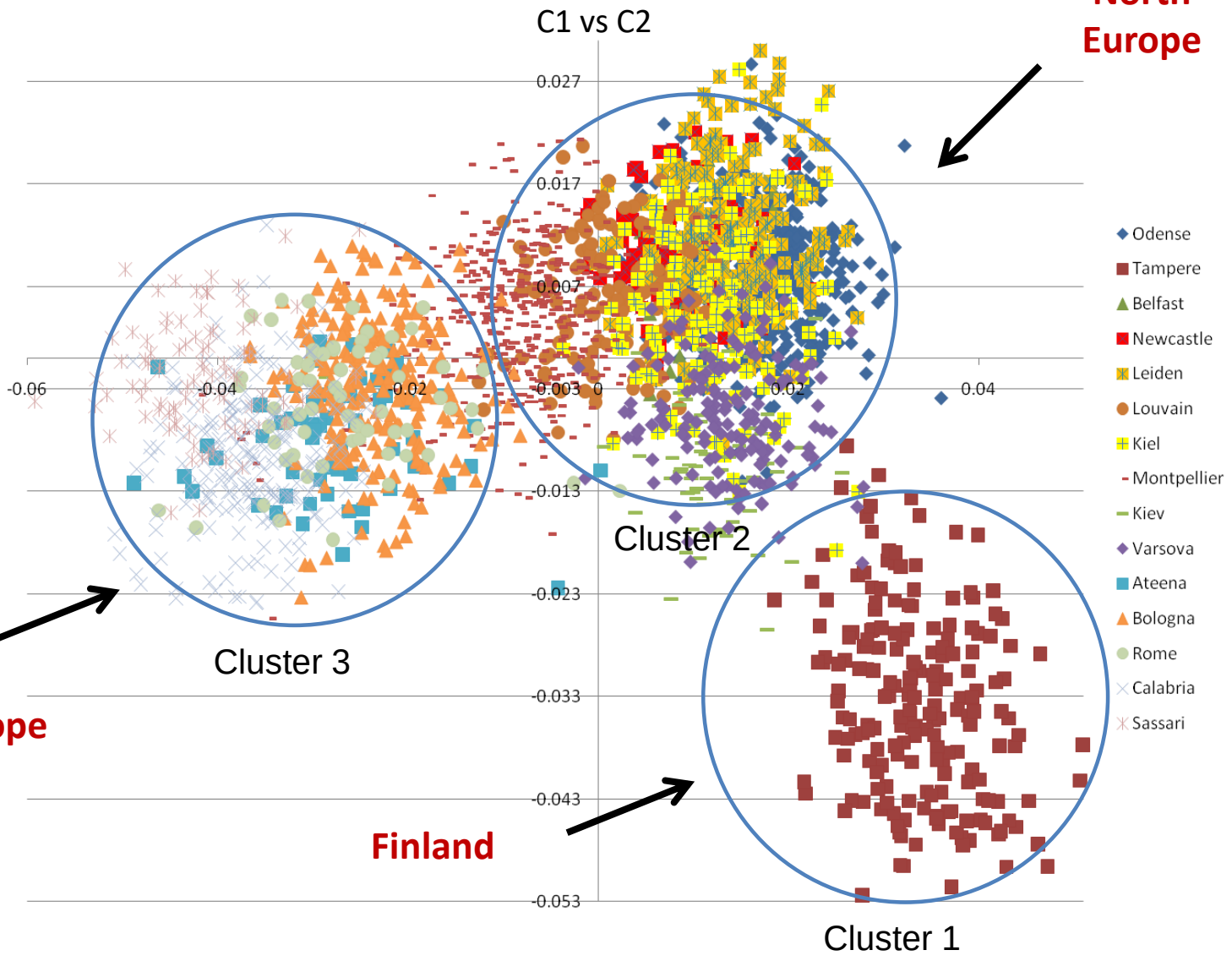
The genetics of human aging and longevity

- Human populations are characterized by **specific gene pools** that arise from the particular group's history in terms of chance (genetic drift) and environment (natural selection); furthermore, humans are unique in having linked **cultural and biological inheritance**.

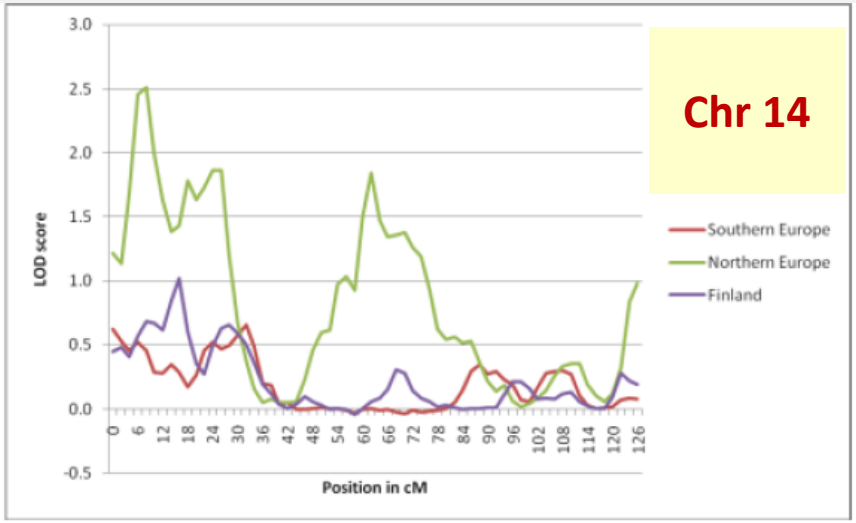
AGING CELL, Jan 2013

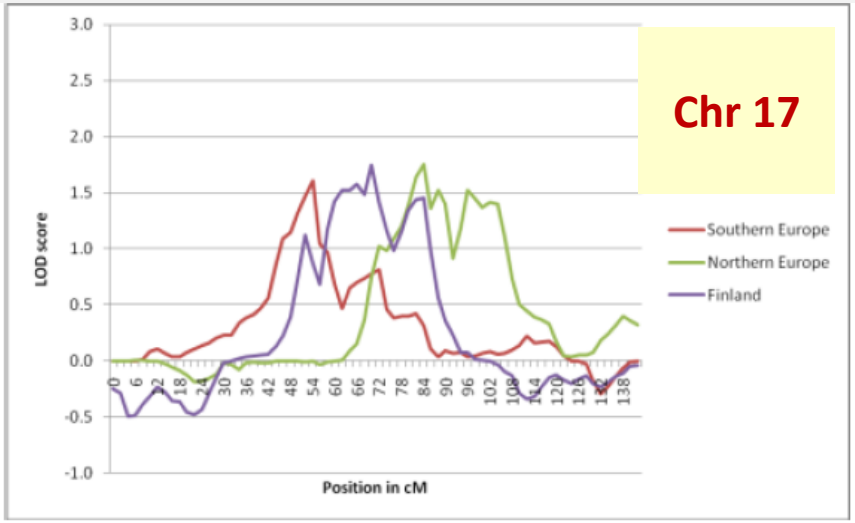
- Genome-wide linkage analysis for human longevity: Genetics of Healthy Ageing Study Marian Beekman, Hélène Blanché, Markus Perola, Anti Hervonen, Vladyslav Bezrukov, Ewa Sikora, Frederieke Flachsbart, Lene Christiansen, Anton J.M. De Craen, Thomas B.L. Kirkwood, Irene Maeve Rea, Michel Poulain, Jean-Marie Robine, Silvana Valensin, Maria Antonietta Stazi, Giuseppe Passarino, Luca Deiana, Efstathios S. Gonos, Lavinia Paternoster, Thorkild I.A. Sørensen, Qihua Tan, Quinta Helmer, Erik B. Van den Akker, Joris Deelen, Francesca Martella, Heather J. Cordell, Kristin L. Ayers, James W. Vaupel, Outi Törnwall, Thomas E. Johnson, Stefan Schreiber, Mark Lathrop, Axel Skytthe, Rudi G.J. Westendorp, Kaare Christensen, Jutta Gampe, Almut Nebel, Jeanine J. Houwing-Duistermaat, P. Eline Slagboom and Claudio Franceschi, The GEHA Consortium

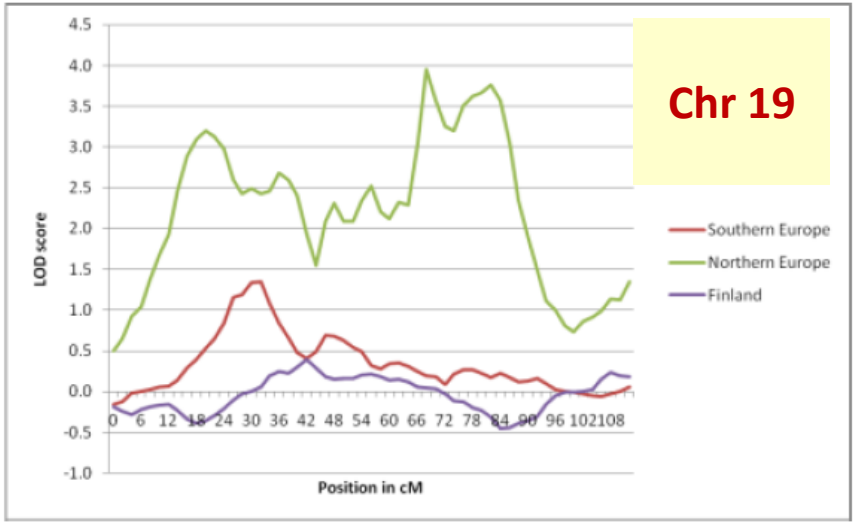
GEHA linkage analysis
identify 3 geographical clusters



4 Linkage regions in the 3 GEHA geographical clusters







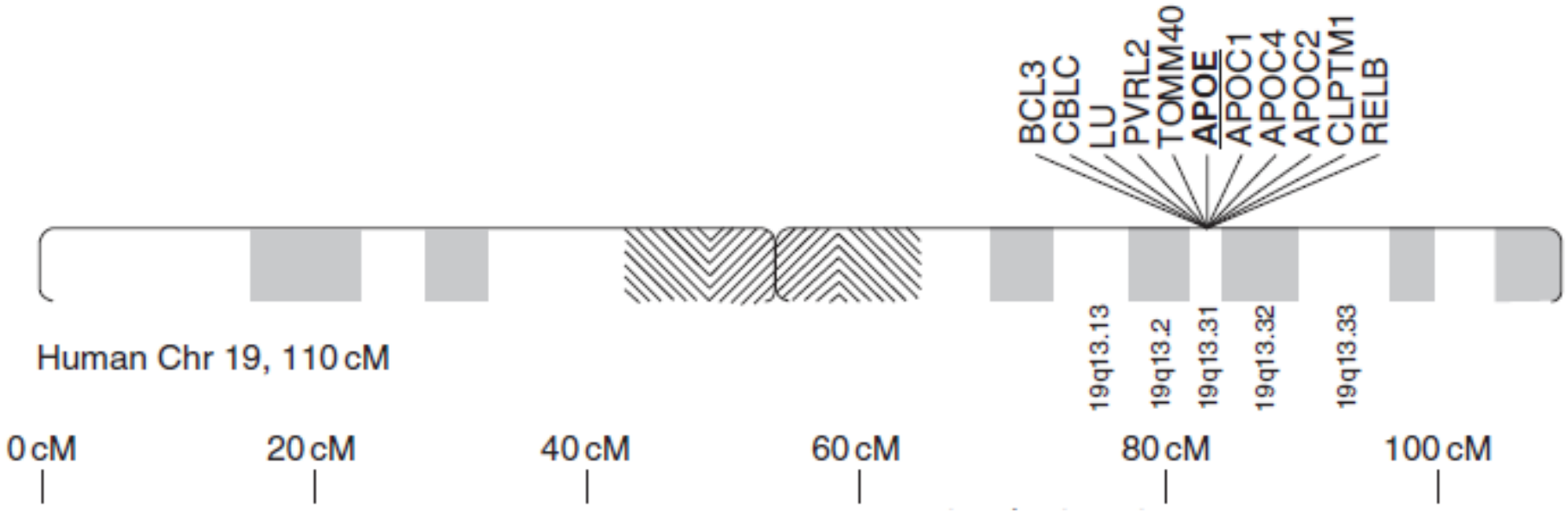
The 3 European regions (populations) contributed differently to the 3 peaks emerged in the linkage study (LOD score >3)

The “bad” SNP(s)

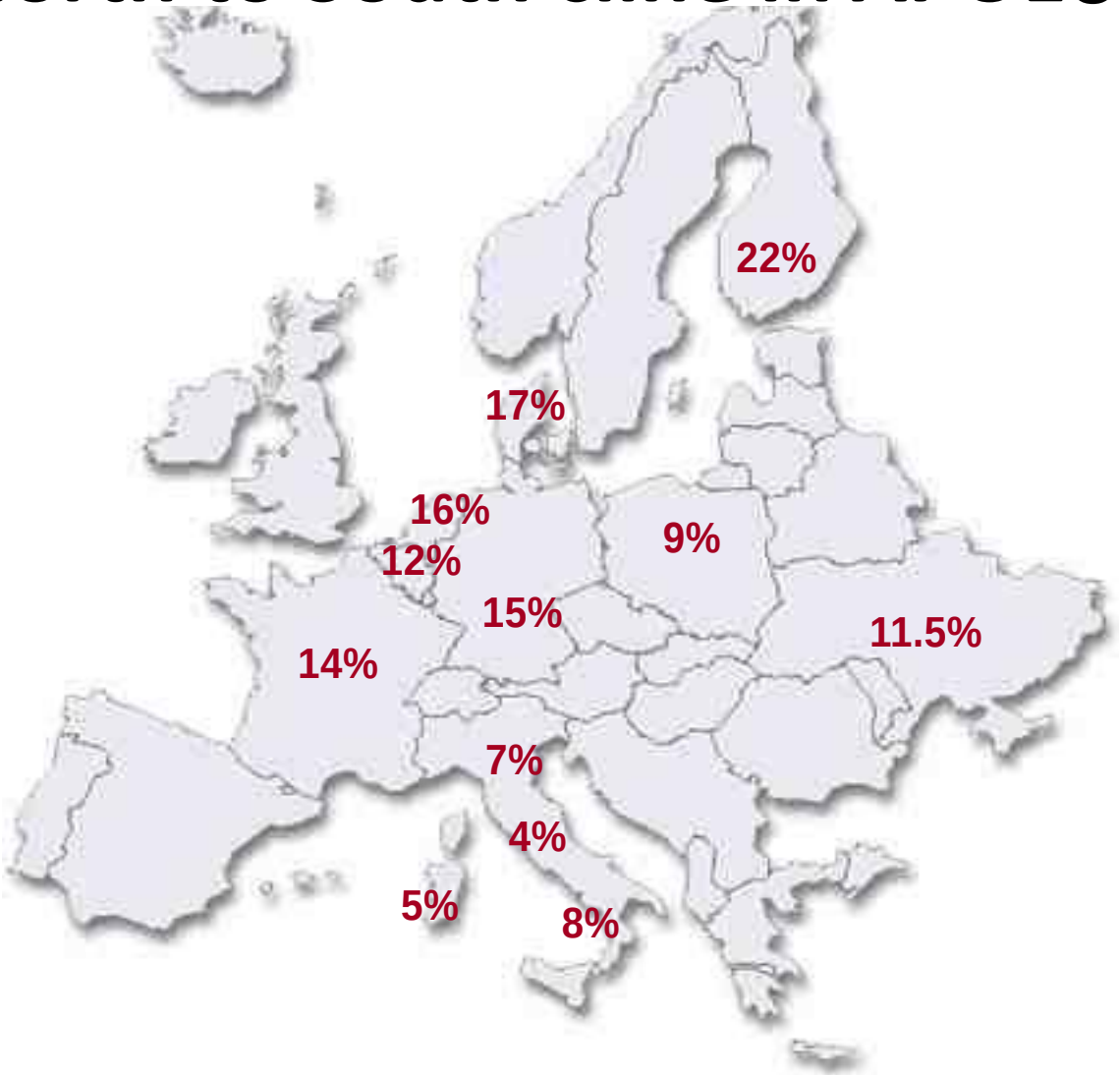
The previous **GEHA linkage study** on 2250 90+ sibships identifyed **genetic linkage with familial longevity at the *APOE* gene locus**.

Moreover, **the GEHA GWAS** showed that **rs4420638** (19q13.11-q13.32) at ***TOMM40/APOE/APOC1* gene locus** had a significant **negative** association with familial longevity (p value=9.6×10^{-8})

Human chromosome 19 *APOE* locus



In European populations there is a North to South cline in *APOE*ε4 allele



All 8,067 subjects recruited within the GEHA Project have been genotyped for APOE variants

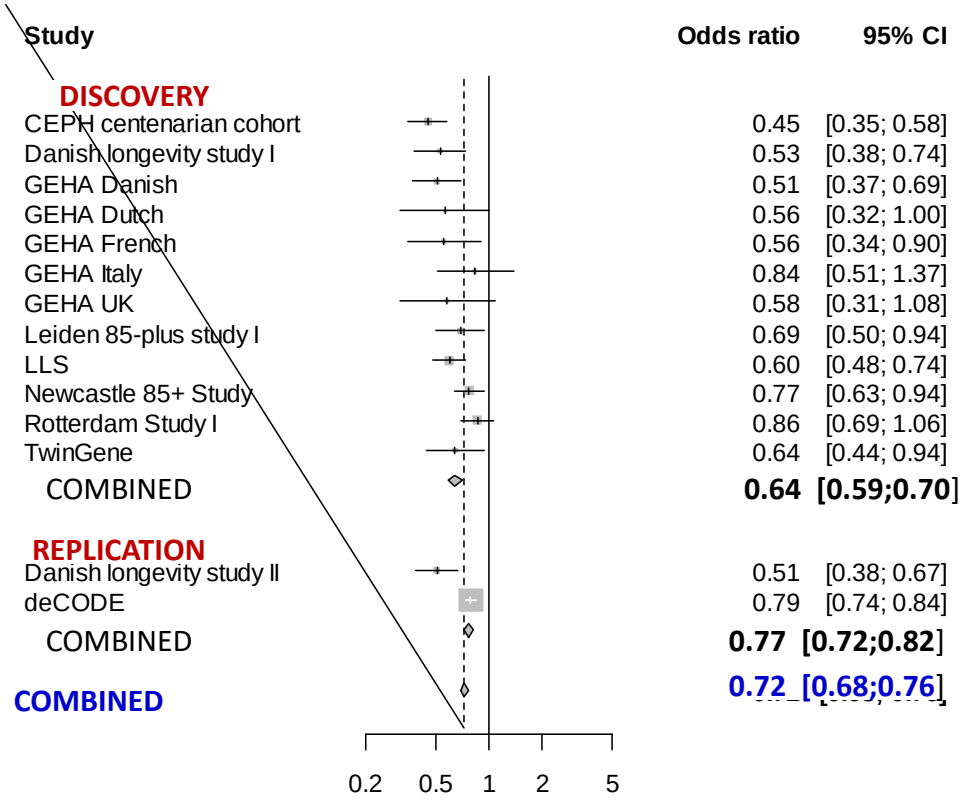
- **Genome-wide association meta-analysis of human longevity identifies a novel locus conferring survival beyond 90 years of age**.
- **Deelen J**, Beekman M, Uh HW, Broer L, Ayers KL, Tan Q, Kamatani Y, Bennet AM, Tamm R, Trompet S, Guðbjartsson DF, Flachsbart F, Rose G, Viktorin A, Fischer K, Nygaard M, Cordell HJ, Crocco P, van den Akker EB, Böhringer S, Helmer Q, Nelson CP, Saunders GI, Alver M, Andersen-Ranberg K, Breen ME, van der Breggen R, Caliebe A, **Capri M, Cevenini E**, Collerton JC, Dato S, Davies K, Ford I, Gampe J, **Garagnani P**, de Geus EJ, Harrow J, van Heemst D, Heijmans BT, Heinsen FA, Hottenga JJ, Hofman A, Jeune B, Jonsson PV, Lathrop M, Lechner D, Martin-Ruiz C, McNerlan SE, Mihailov E, Montesanto A, Mooijaart SP, Murphy A, Nohr EA, Paternoster L, Postmus I, Rivadeneira F, Ross OA, **Salvioli S,** Sattar N, Schreiber S, Stefánsson H, Stott DJ, Tiemeier H, Uitterlinden AG, Westendorp RG, Willemsen G, Samani NJ, Galan P, Sørensen TI, Boomsma DI, Jukema JW, Rea IM, Passarino G, de Craen AJ, Christensen K, Nebel A, Stefánsson K, Metspalu A, Magnusson P, Blanché H, Christiansen L, Kirkwood TB, van Duijn CM, **Franceschi C**, Houwing-Duistermaat JJ, **Slagboom PE**.
- Hum Mol Genet. 2014 Mar 31. [Epub ahead of print]

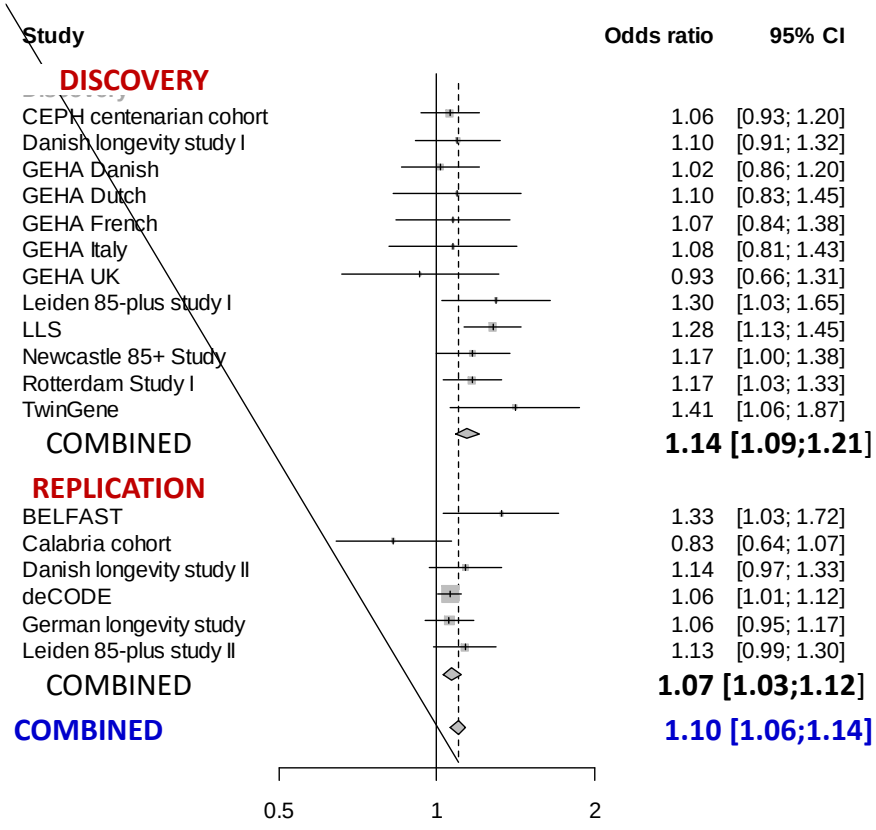
The discovery and replication studies involved a total of 187170 subjects (85+, 90+ and controls)

The prospective study for mortality involved 34103 subjets (30-105 yrs)

Forest plots for rs4420638 and rs2149954

Forest plots representing the odds ratios with 95% CI of rs4420638 and rs2149954 for the cohorts analyzed in the discovery and replication phase (≥ 90 years). The size of the boxes represents the sample size of the cohort.





rs4420638

chr 19q13.32

rs2149954

chr 5q33.3

The new "good" SNP

rs2149954 (T) is associated with:

1. **lower all-cause mortality**
2. **lower mortality risk for cardiovascular disease**
3. **a decreased risk for coronary artery disease**
4. **lower diastolic and systolic blood pressure**

Sci Rep. 2016; 6: 21243.
Published online 2016 Feb 25

www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

Novel loci and pathways significantly associated with longevity

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Published: 25 February 2016

Yi Zeng[1,2,†], Chao Nie[3,*], Junxia Min[4,*], Xiaomin Liu[3,*], Mengmeng Li[5], Huashuai Chen[1,6], Hanshi Xu[3], Mingbang Wang[3], Ting Ni[7], Yang Li[8], Han Yan[8], Jin-Pei Zhang[8], Chun Song[8], Li-Qing Chi[8], Han-Ming Wang[8], Jie Dong[8], Gu-Yan Zheng[8], Li Lin[5], Feng Qian[5], Yanwei Qi[3,9], Xiao Liu[3], Hongzhi Cao[3], Yinghao Wang[3], Lijuan Zhang[3], Zhaochun Li[3], Yufeng Zhou[3], Yan Wang[3], Jiehua Lu[10], Jianxin Li[10], Ming Qi[4], Lars Bolund[3,11], Anatoliy Yashin[12], Kenneth C. Land[12], Simon Gregory[13], Ze Yang[14], William Gottschalk[15], Wei Tao[16], Jian Wang[3,17], Jun Wang[3,18], Xun Xu[3], Harold Bae[19], Marianne Nygaard[20], Lene Christiansen[20], Kaare Christensen[20], Claudio Franceschi[21], Michael W. Lutz[15], Jun Gu[16], Qihua Tan[20], Thomas Perls[22], Paola Sebastiani[23], Joris Deelen[24], Eline Slagboom[24], Elizabeth Hauser[13], Huji Xu[5], Xiao-Li Tian[8,†], Huanming Yang[3,17,†] & James W. Vaupel[25]

GENETICS
of Chinese, European and USA 100+

- **2178 Han Chinese centenarians** (mean age 102.7 years) and **2299 mid-age controls** (mean age 48.4 years)
- **Compared with European 90+ sibs** of EC-funded GEHA project (Genetics of Healthy Ageing 2005-2010; Coordinator: C. Franceschi) and **100+ of New England Centenarian (NEC) study**

Two top loci emerged as genome-wide significant

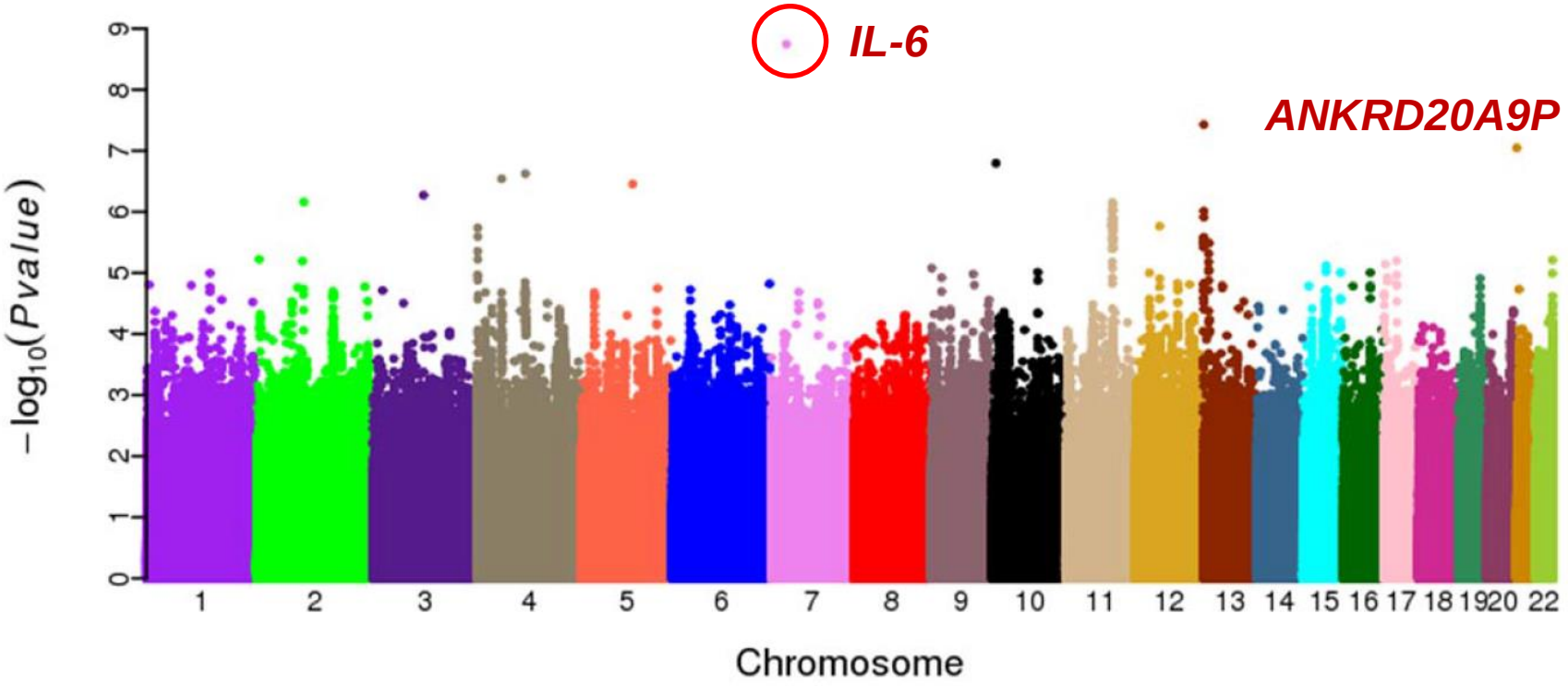
rs2069837, chr 7p15.3, *IL-6*, *P* =1.80x10^{-9}

rs2069837- *IL-6* alone explained 1.0% of the variance

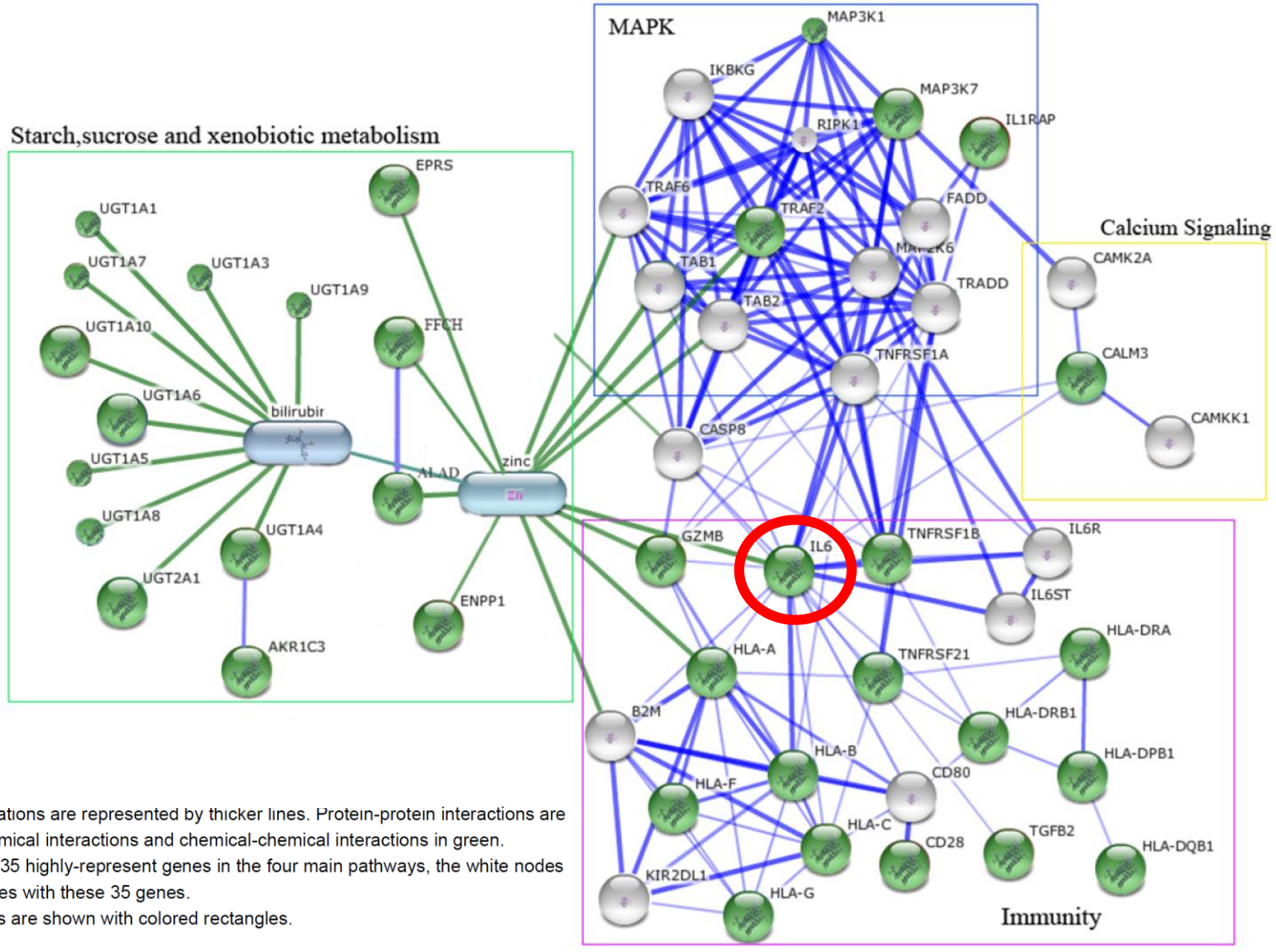
rs2440012, chr 13q12.12, *ANKRD20A9P*, *P* =3.73x10^{-8}

rs2149954 (T) in chr 5q33.3 was confirmed

ANKRD20A9P is a pseudogene affiliated with the long non-coding RNAs (lncRNA) class.



Gene and Pathway Networks in Human Longevity



Notes: (1) Stronger associations are represented by thicker lines. Protein-protein interactions are shown in blue, protein-chemical interactions and chemical-chemical interactions in green.
(2) The green nodes mark 35 highly-represent genes in the four main pathways, the white nodes mark highly interacted genes with these 35 genes.
(3) The four main pathways are shown with colored rectangles.

Zeng et al., 2016

The genetics of human aging and longevity

- *Sci. Aging Knowl. Environ.*, 28 June 2006
  Vol. **2006**, Issue 10, p. pe20
  PERSPECTIVES

  The Unusual Genetics of Human Longevity

  Giovanna De Benedictis, and Claudio Franceschi
- **Abstract**

  … we discuss some recent findings (**the impact of geography and demography,…homozygosity,** the role of **the nuclear-mitochondrial genome cross-talk**) and **new ideas, such as the concept of A COMPLEX ALLELE TIMING as a pivotal process in modulating the probability of achieving longevity**

THE GENETICS OF HUMAN LONGEVITY

- **"Complex allele timing" the same allele can have different effect at different ages.**

Moreover,

- **the same gene can have pleiotropic effects and participate in different pathways.**
- **Epistasis, i.e. gene-gene interactions**

“age remodelling”

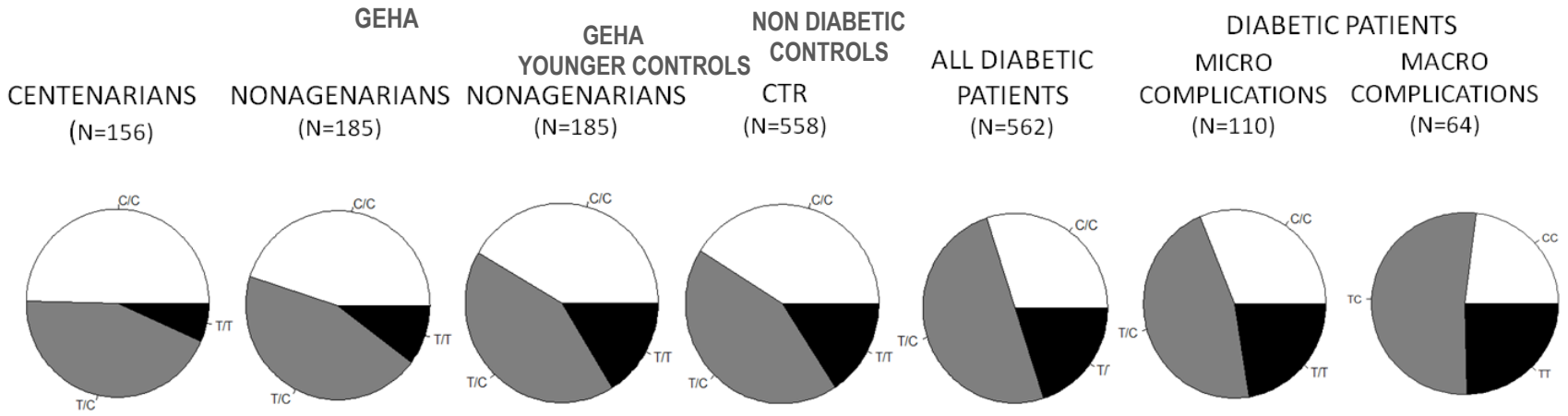
- The physiological changes that characterize the bodies of old people result from **adaptive strategies** (“remodelling”) at the molecular and cellular levels aimed at compensating for damage that accrued over time.
  - In such an adaptive process, **the cell microenvironment** plays a pivotal role (see heterochronic parabiosis).

The Extreme Phenotypes Approach: 100+ *vs* T2D

candidate gene variants associated with T2D and its complications in a total of 1,646 subjects (including n. 156 100+ and n.185 90+)

TCF7L2, DDAH1, IRS1, TERC, IGF2BP, APM1, hTERT, EPO, CAT, KCNJ11, KCNQ1, HIF-1α, FTO

RESULTS of the rs7903146 in the TCF7L2 gene



rs 7903146 – TCF7L2							
	Genotypes frequencies (%)						
CC	49.7	45.1	41.6	41.1	30.0	29	23.4
TC	43.6	44.6	42.2	43.0	50.0	50	53.1
TT	6.7	10.3	16.2	15.8	20.0	21	23.4
	Allele frequencies (%)						
C	71.5	67.4	62.7	62.6	55	54.3	51.8
T	28.5	32.6	37.3	37.4	45	45.7	48.2

Garagnani et al., 2013

Type 2 Diabetes *TCF7L2* Risk Genotypes Alter Birth Weight: A Study of 24,053 Individuals

Rachel M. Freathy, Michael N. Weedon, Amanda Bennett, Elina Hyppönen, Caroline L. Relton, Beatrice Knight, Beverley Shields, Kirstie S. Parnell, Christopher J. Groves, Susan M. Ring, Marcus E. Pembrey, Yoav Ben-Shlomo, David P. Strachan, Chris Power, Marjo-Riitta Jarvelin, Mark I. McCarthy, George Davey Smith, Andrew T. Hattersley, and Timothy M. Frayling

To conclude, we have shown that maternal *TCF7L2* type 2 diabetes–risk genotypes at *rs7903146* are associated with increased offspring birth weight, probably through impaired maternal insulin secretion.

Am. J. Hum. Genet. 2007;80:1150–1161.

Ageing Research Reviews 18 (2014) 53–73

Contents lists available at ScienceDirect

Ageing Research Reviews

journal homepage: www.elsevier.com/locate/arr

Review

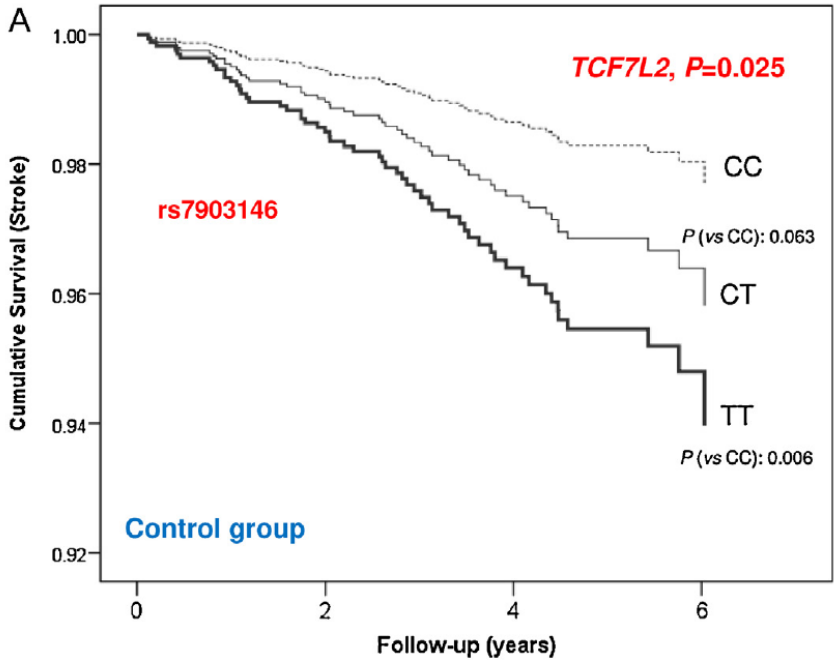
Aging and cardiovascular diseases: The role of gene–diet interactions

Dolores Corella[a,b], José M. Ordovás[c,d,e,*]

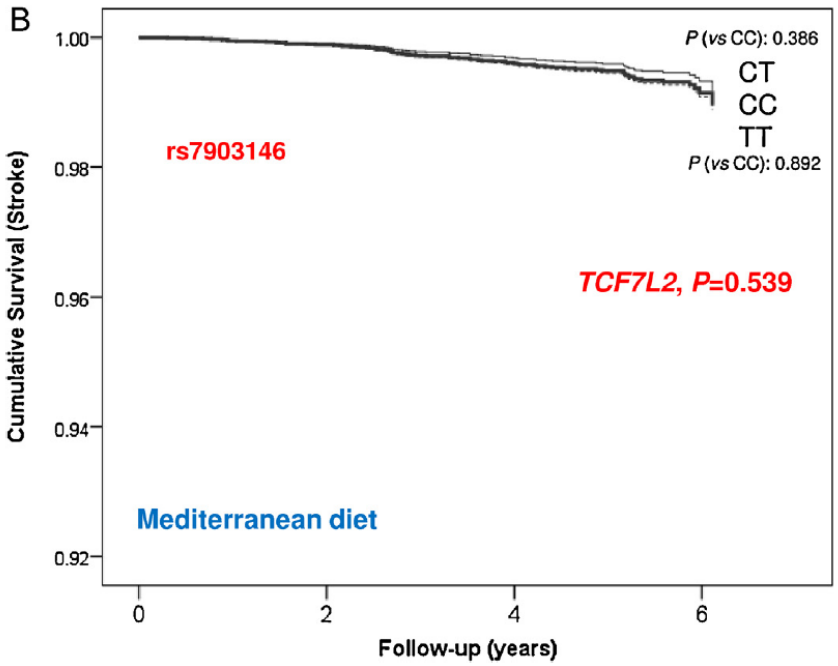
- **TCF7L2-rs7903146 T-allele carriers have a higher risk of cardiovascular diseases.**

- **Strict adherence to Mediterranean diet is capable of counteracting the genetic risk of stroke.**

- Our study on Italian centenarians (Garagnani et al., 2013) shows that **rs7903146 behaves as an important genetic marker related to longevity**



Panel A shows cumulative stroke free-survival by TCF7L2-rs7903146 genotypes (CC, CT and TT) in subjects in the control group (n = 2291)



Panel B shows cumulative stroke free-survival in subjects allocated in the Mediterranean diet Intervention groups (n = 4827) and who had a strict adherence to the Mediterranean diet

THE GENETICS OF LONGEVITY
IS THE RESULT OF THE INTERACTION
AMONG

MANY GENOMES

" the *H. sapiens* metagenome"

the *H. sapiens* metagenome

nuclear DNA x mtDNA

x microbiomes

(malleable and modifiable)

x environment/lifestyle

mtDNA variants in aging and longevity

Aging Cell 2013 Open Access

mtDNA = 16.569 bp

N Raule, F Sevini, S Li, A Barbieri, F Tallaro, L Lomartire, D Vianello, J Moilanen, THE GEHA Group, K Majamaa, M Perola, TE Johnson, L Bolund, H Yang, G Passarino, C Franceschi

The co-occurrence of mtDNA mutations on different oxidative phosphorylation subunits, not detected by haplogroup analysis, affects human longevity and is population specific.

Owing the unprecedented number of subjects analysed (>2200 90+ and the same number of younger controls) and the high number of complete mtDNA sequences (1300) it was possible to show:

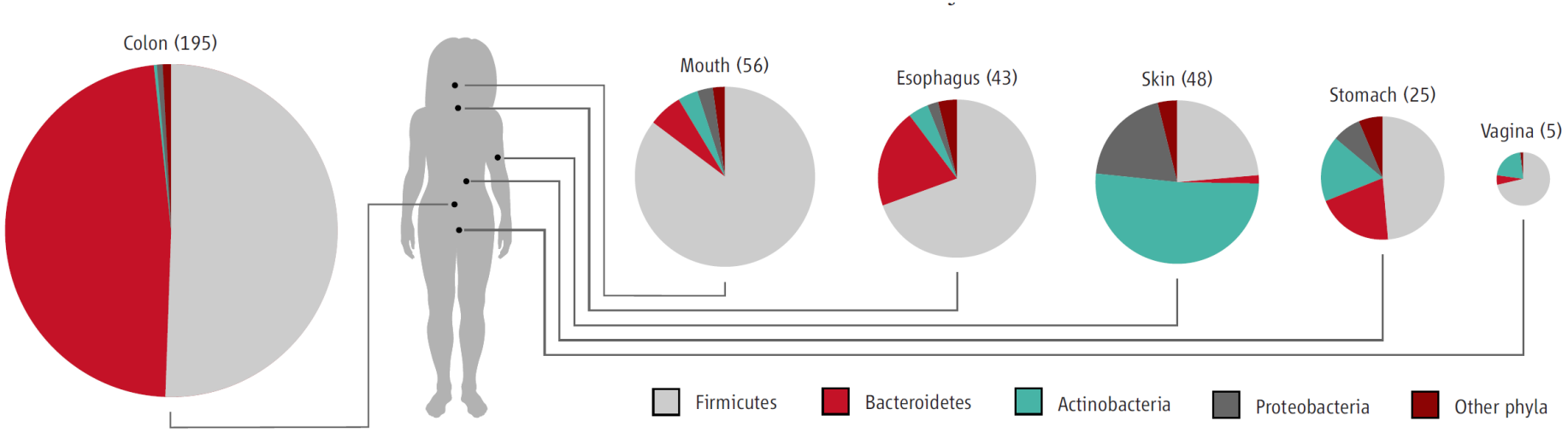
1. a beneficial effect of mutations in the subunits of OXPHOS complex I

2. a detrimental effect of mutations when they are present concomitantly in subunits of complex I and III and complex I and V

3. positive effects of mutations in tRNA genes, which had previously been found to be associated to a number of disorders

Invisible Partners

bacteria in the different body sites



SCIENCE VOL 330 17 DECEMBER 2010

GUT MICROBIOTA a very complex bacterial ecosystem

- **10 to 100 trillion of bacteria**
- **their collective genome (microbiome) contains >3 million non-redundant microbial genes, >100 times the genes of the human genome**

Each human has a **"metagenome"** (human genome + microbiome) and has to be considered a **"metaorganism**

- ***Firmicutes*** **65%**
- ***Bacteroidetes*** **25%**
- ***Proteobacteria*** **8%**
- ***Actinobacteria*** **5%**
- ***Fusobacteria*** **1%**

Gut Microbiome Metagenomics (faeces) & Metabolomics (serum & urine) in 100+ and their offspring

In collaboration with

Patrizia Brigidi
University of Bologna
Serge Rezzi & Sebastiano Collino
NIHS, Nestlè Institute of Health Science, Lausanne

OPEN ACCESS Freely available online

PLoS one

Through Ageing, and Beyond: Gut Microbiota and Inflammatory Status in Seniors and Centenarians

Elena Biagi[1]*, Lotta Nylund[2,3], Marco Candela[1], Rita Ostan[4], Laura Bucci[4], Elisa Pini[4], Janne Nikkïla[3], Daniela Monti[5], Reetta Satokari[2], Claudio Franceschi[4], Patrizia Brigidi[1], Willem De Vos[3,6]

1 Department of Pharmaceutical Sciences, University of Bologna, Bologna, Italy, **2** Functional Foods Forum, University of Turku, Turku, Finland, **3** Division of Microbiology and Epidemiology, Department of Basic Veterinary Medicine, University of Helsinki, Helsinki, Finland, **4** Department of Experimental Pathology and CIG-Interdepartmental Center L. Galvani, University of Bologna, Bologna, Italy, **5** Department of Experimental Pathology and Oncology, University of Florence, Florence, Italy, **6** Laboratory of Microbiology, Wageningen University, Wageningen, The Netherlands

PLoS One 2010

CONCLUSIONS

The fecal microbiota of Centenarians

↓ **BIODIVERSITY**

↓ *Clostridium* cluster XIVa*

↓ Bifidobacteria

Rearrangement of *Clostridium* cluster IV*

↑ Facultative anaerobes, including Bacilli and Proteobacteria (“pathobionts”)

* **butyrate producer**

www.impactaging.com AGING, December 2013, Vol 5 N 12

Research Paper

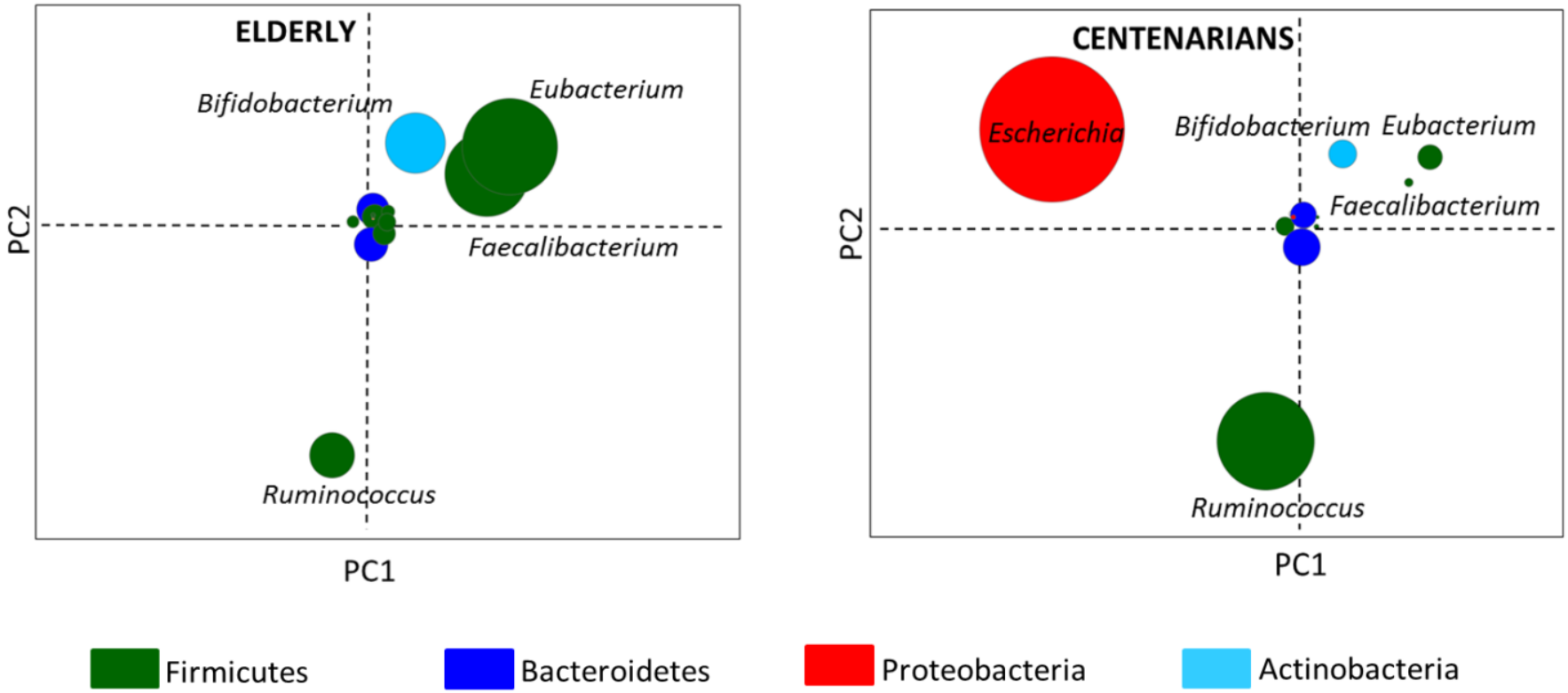
Functional metagenomic profiling of intestinal microbiome in extreme ageing

Simone Rampelli [1], Marco Candela [1], Silvia Turroni [1], Elena Biagi [1],Sebastiano Collino[2], Claudio Franceschi [3], Paul W O'Toole [4], and Patrizia Brigidi [1]

By **Illumina shotgun sequencing** of the fecal microbial DNA from the centenarians, elderly and young people, we generated **a total of 214.6 million paired-end reads**, with an average of **23.841 million (± 0.067 SD) reads per subject**.

Taxonomic fingerprint of aging

The genera *Escherichia* and *Ruminococcus* were over-represented in centenarians, whereas *Faecalibacterium*, *Eubacterium* and *Bifidobacterium* were more abundant in elderly.

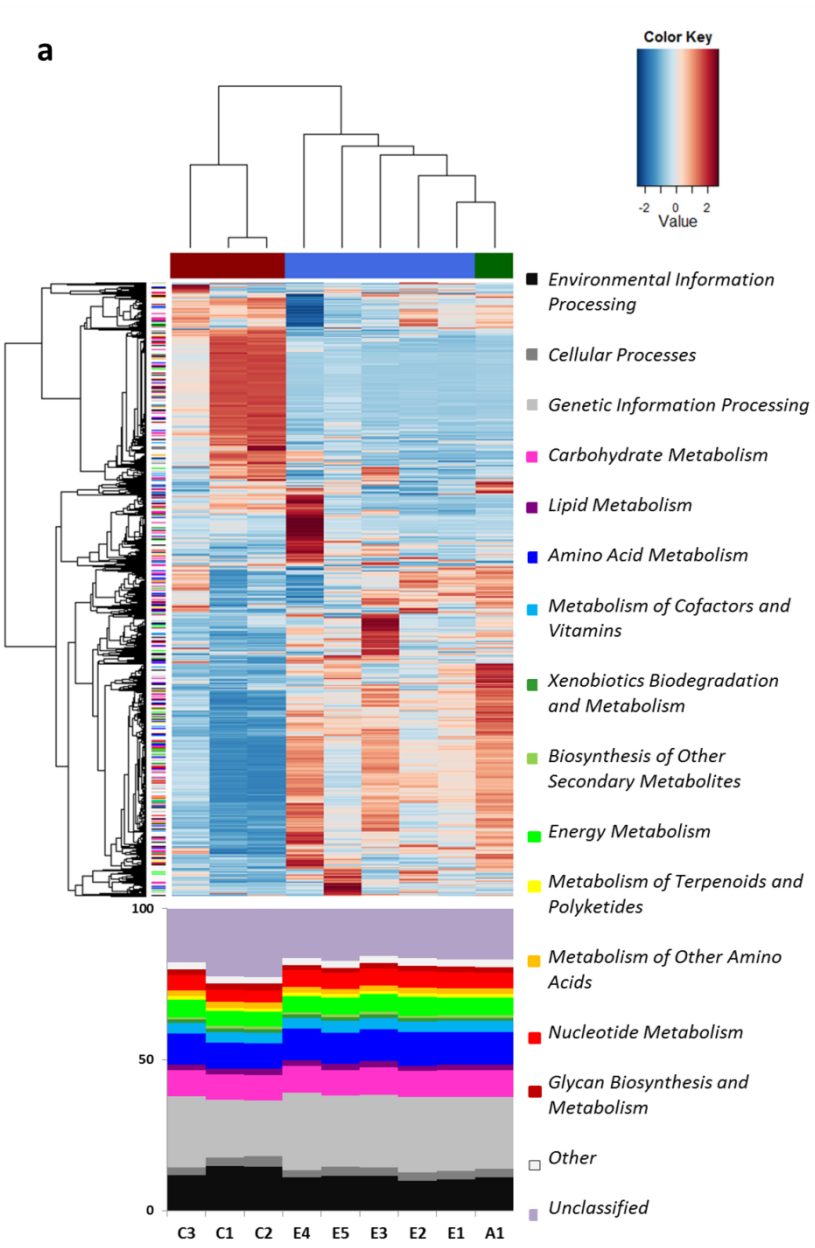


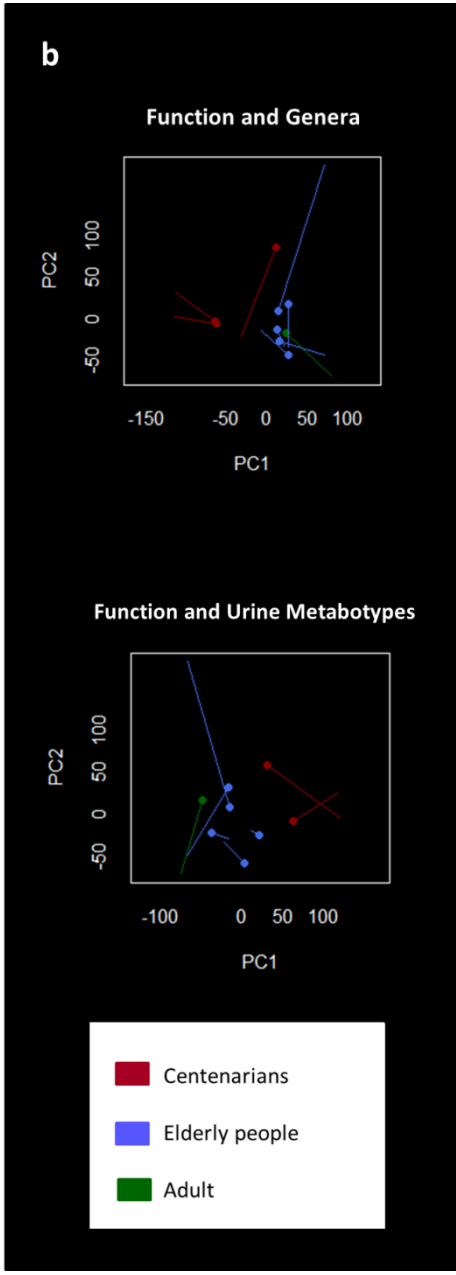
Rampelli *et al.*, AGING 2013

Metagenome function analysis separates centenarians from the other subjects in agreement with genus and urine metabolite clustering.

a, Hierarchical Ward-linkage clustering based on the Pearson correlation coefficients of the abundance of KO genes, filtered for KO gene subject presence ≥ 1 in at least 8/9 subjects. KO genes are clustered in the vertical tree and color-coded according to the first level of KEGG classification or the second level for functions concerning metabolism. 2719 KO genes confidently classified in the KEGG database are visualized.
The bottom panel shows the relative abundance of the KEGG categories.

b, Procrustes analysis combining Euclidean PCoA of functional microbiota (non-circle end of lines) with either Euclidean PCoA based on the genus dataset (circle-end of lines; upper graph) [8], or Euclidean PCoA based on the spectra of urine metabolites (circle-end of lines; lower graph).





OPEN ACCESS Freely available online

PLOS ONE

Metabolic Signatures of Extreme Longevity in Northern Italian Centenarians Reveal a Complex Remodeling of Lipids, Amino Acids, and Gut Microbiota Metabolism

Sebastiano Collino[1]*, Ivan Montoliu[2], François-Pierre J. Martin[1], Max Scherer[1], Daniela Mari[3,4], Stefano Salvioli[5,6], Laura Bucci[5,6], Rita Ostan[5,6], Daniela Monti[7], Elena Biagi[8], Patrizia Brigidi[8], Claudio Franceschi[5,6], Serge Rezzi[1]

1 Proteomics and Metabonomics, Nestlé Institute of Health Sciences SA, Campus EPFL, Quartier de l'innovation, Lausanne, Switzerland, **2** Applied Mathematics, NESTEC SA, Nestlé Research Center, Lausanne, Switzerland, **3** Department of Medical Sciences, University of Milan, Milan, Italy, **4** Geriatric Unit Ca' Grande Foundation Maggiore Policlinico Hospital, Milan, Italy, **5** Department of Experimental Pathology, University of Bologna, Bologna, Italy, **6** Interdepartmental Centre L. Galvani, University of Bologna, Bologna, Italy, **7** Department of Experimental Pathology and Oncology, University of Florence, Florence, Italy, **8** Department of Pharmaceutical Sciences, University of Bologna, Bologna, Italy

PLOS ONE March 2013 | Volume 8 | Issue 3 | e56564

A total of 457 individuals:

N= 143 centenarians

N= 220 offspring of centenarians

N= 73 offspring of non long-lived parents

N= 21 young subjects

www.impactaging.com AGING, January 2014, Vol. 6, No 1

Research Paper

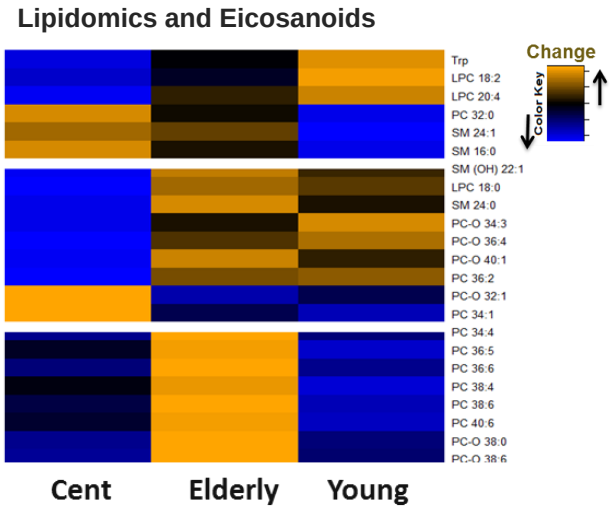
Serum profiling of healthy aging identifies phospho- and sphingolipid species as markers of human longevity

Ivan Montoliu[1], Max Scherer[2], Fiona Beguelin[2], Laeticia DaSilva[2], Daniela Mari[3,4], Stefano Salvioli[5,6] ,François-Pierre J. Martin[2], Miriam Capri[5,6], Laura Bucci[5,6],Rita Ostan[5,6],Paolo Garagnani[5,6], Daniela Monti[7], Elena Biagi[8], Patrizia Brigidi[8], Martin Kussmann[2,9,10], Serge Rezzi[2], Claudio Franceschi[5,6], and Sebastiano Collino[2]

[1]NESTEC SA, Nestlé Research Center, Vers-chez-les-Blanc, CH-1000 Lausanne 26, Switzerland;
[2]Nestlé Institute of Health Sciences SA, Campus EPFL, Molecular Biomarkers Core, Quartier de l'innovation, CH-1015 Lausanne, Switzerland;
[3]Department of Medical Sciences, University of Milan, Milan, Italy;
[4]Geriatric Unit IRCCS Ca' Grande Foundation Maggiore Policlinico Hospital, Milan, Italy;
[5]Department of Experimental, Diagnostic and Specialty Medicine Experimental Pathology, University of Bologna, Via S. Giacomo 12, 40126 Bologna, Italy;
[6]Interdepartmental Centre "L. Galvani" CIG, University of Bologna, Piazza di Porta S. Donato 1, 40126 Bologna, Italy;
[7]Department of Clinical, Experimental and Biomedical Sciences, University of Florence, Viale Morgagni 50, 50134 Florence, Italy;
[8]Department of Pharmaceutical Sciences, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy;
[9]Faculty of Life Sciences, Ecole Polytechnique Fédérale Lausanne (EPFL), Lausanne, Switzerland;
[10]Faculty of Science, Interdisciplinary NanoScience Center (iNANO), Aarhus University, Aarhus, Denmark;

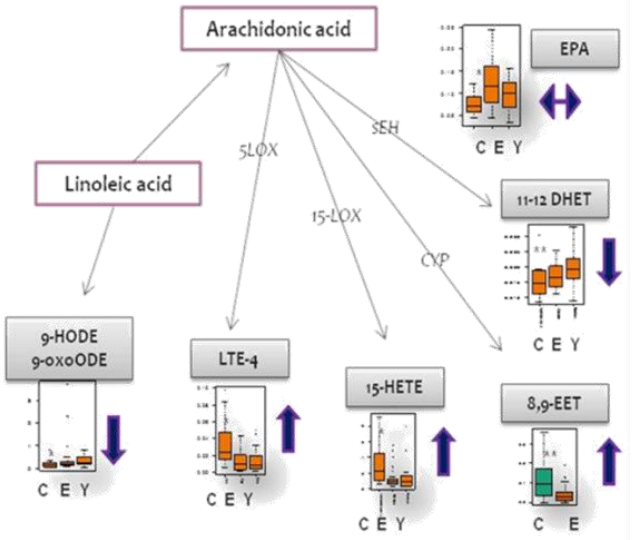
Metabonomic and lipidomic biomarkers of human aging and longevity

Healthy Ageing is characterized by an unique capacity to adapt/respond to aging-induced accumulation of oxidative and chronic inflammatory conditions



- **Decrease concentration of AA (Trp)**
- Decrease concentration GPL (LPCs)
- Increase concentration of sphingolipids (SM)
- Different membrane FA composition/integrity (PC/PE ratio) with lower MUFA/PUFA ratio

- **Increase proinflammatory synthesis (Leukotrines)**
- **Activation anti-inflammatory mechanism (HETE, EET)**
- **Reduction of oxidative stress (9-HODE,9-oxoODE)**



For the first time, a metabolic phenotype of centenarians is reported: a good reference model for human longevity and healthy ageing

Collino et al. PLOS ONE 2013

Age-Trajectories of Metabolomic Biomarkers (information molecules !)

A G E

Metabolomic Biomarkers of Aging and Longevity

- **monotonically increased or decreased with age :**

- **decreased concentrations of Tryptophan (Trp) & lysophospatidylcholines (LPC 18:2, LPC 20:4);**
- **increased levels of PC 32:0 and sphingomyelins (SM 24:1, SM 16:0);**

- **remaining largely until age 70 and undergoing significant changes in centenarians**

- **decreased concentration in sphingomyelins and specific glycerophospholipids (SM-*OH* 22:1, LPC 18:0, SM 24:0, PC-*O* 34:3, PC-*O* 36:4, PC-*O* 40:1, PC 36:2);**
- **increased concentration in specific glycerophospholipids (PC-*O* 32:1, PC-*O* 34:1)**

- **remarkably similar in young subjects and centenarians**

- **specific glycerophospholipids (PC34:4, PC36:6, PC 36:5, PC 38:4, PC 38:6, PC 40:6, PC-*O* 38:0, PC-*O* 38:6).**

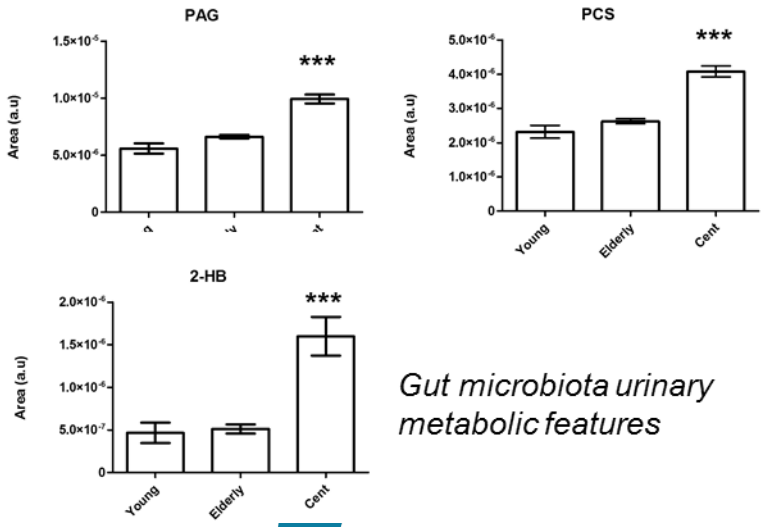
Collino et al., PLoS ONE 2013

Specific metabolites of gut microbiota in the urine

Centenarian metabolic signature

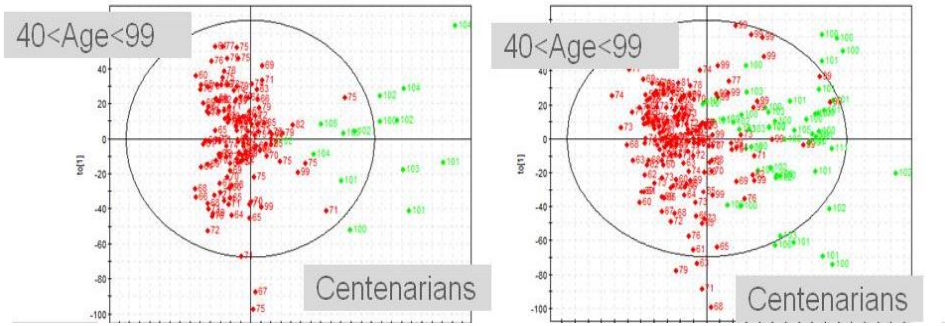
➢ Phenylacetylglutamine (PAG)

p-cresol sulfate (PCS), (2HB)2-hydroxybenzoate



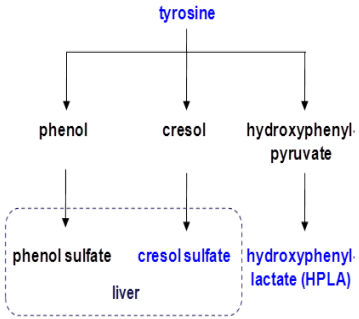
Gut microbiota urinary metabolic features

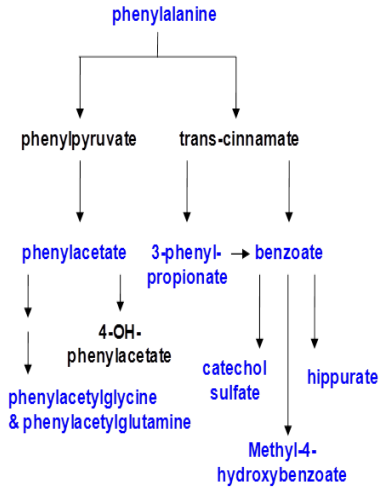
Urine metabolic profile



Males

Females





Collino, et al.PLOS ONE 2013

in centenarians we found relatively

- lower amount of glycerophosphocholine (GPC)
- higher amounts of N-acetylglycoproteins (NAC), glutamine, citrate, creatinine, and phenylalanine

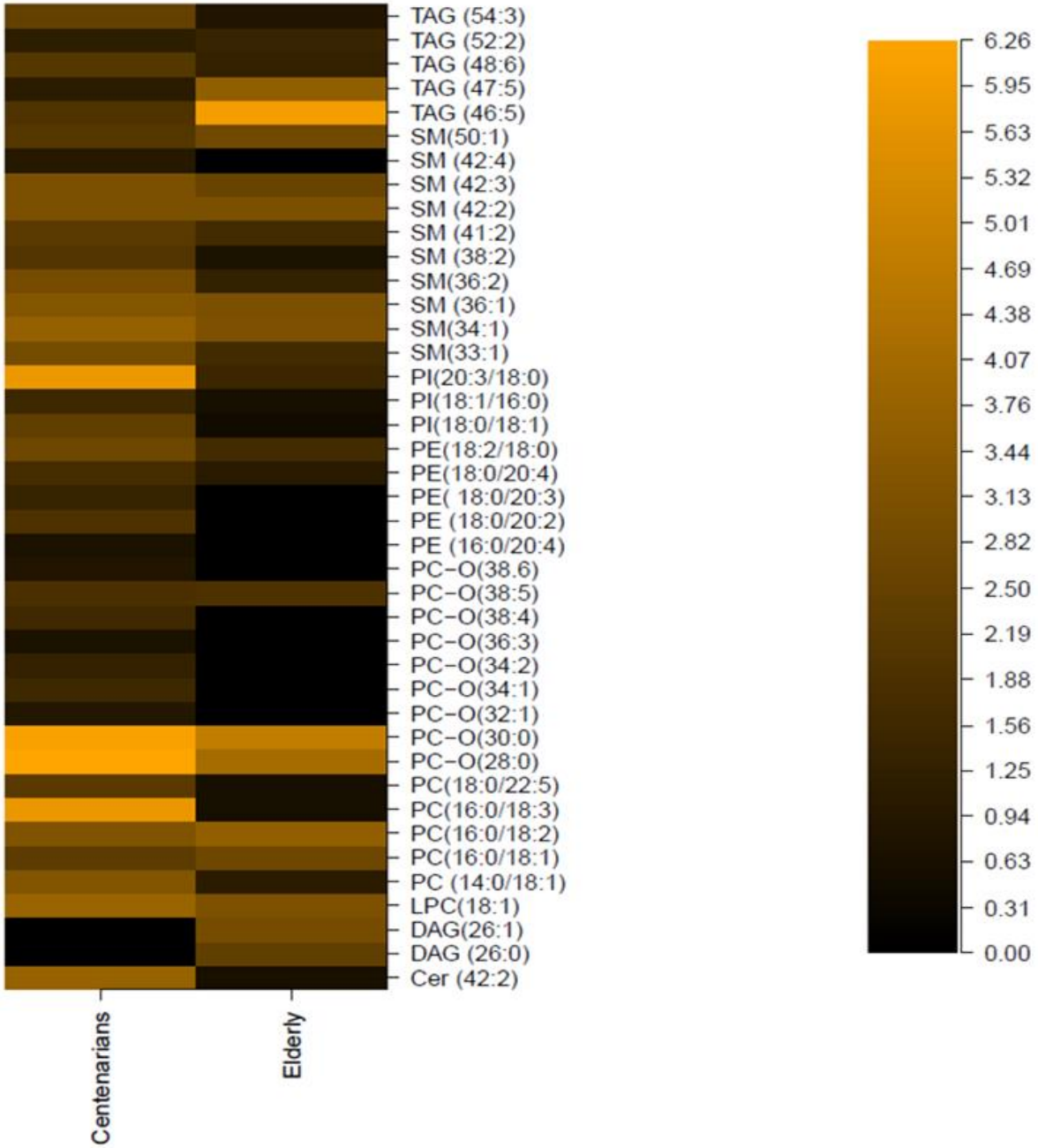
metabolomics profiling revealed that longevity can be viewed as a distinct metabolomic phenotype marked by changes in amino acids and lipid metabolism.

LIPIDOMICS: 12 different lipid classes (174 species) were analysed and quantified:

- triacylglycerol TAG (n=30),
- sphingomyelin SM (n=25),
- lysophosphatidylcholine LPC (n=7),
- phosphatidylcholine PC (n=34),
- ether phosphatidylcholine PC-O (n=19),
- ceramides Cer (n=6),
- phosphatidylethanolamine PE (n=14),
- phosphatidylethanolamine based ether PE-O (n=9),
- lysophosphatidylethanolamine LPE (n=3),
- phosphatidylinositol PI (n=7),
- phosphatidic acid PA (n=1),
- diacylglycerol DAG (n=19).

Differences in mean (μM) standardized lipids value between elderly and centenarians.

Shot gun lipidomics approach shows unique changes in lipids biosynthesis in centenarians, with 41 lipid species associated to longevity.



Current Biology
Report

The continuous remodeling with age of the gut microbiota (GM)

Gut Microbiota and Extreme Longevity

Elena Biagi,[1,*] Claudio Franceschi,[2,3,4] Simone Rampelli,[1] Marco Severgnini,[5] Rita Ostan,[2,3] Silvia Turroni,[1] Clarissa Consolandi,[5] Sara Quercia,[1] Maria Scurti,[2,3] Daniela Monti,[6] Miriam Capri,[2,3] Patrizia Brigidi,[1] and Marco Candela[1,*]

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[2]DIMES-Department of Experimental, Diagnostic and Specialty Medicine, Alma Mater Studiorum, University of Bologna, Bologna 40126, Italy
[3]CIG-Interdepartmental Centre "L. Galvani," Alma Mater Studiorum, University of Bologna, Bologna 40126, Italy
[4]IRCCS, Institute of Neurological Sciences of Bologna, Bologna 40139, Italy
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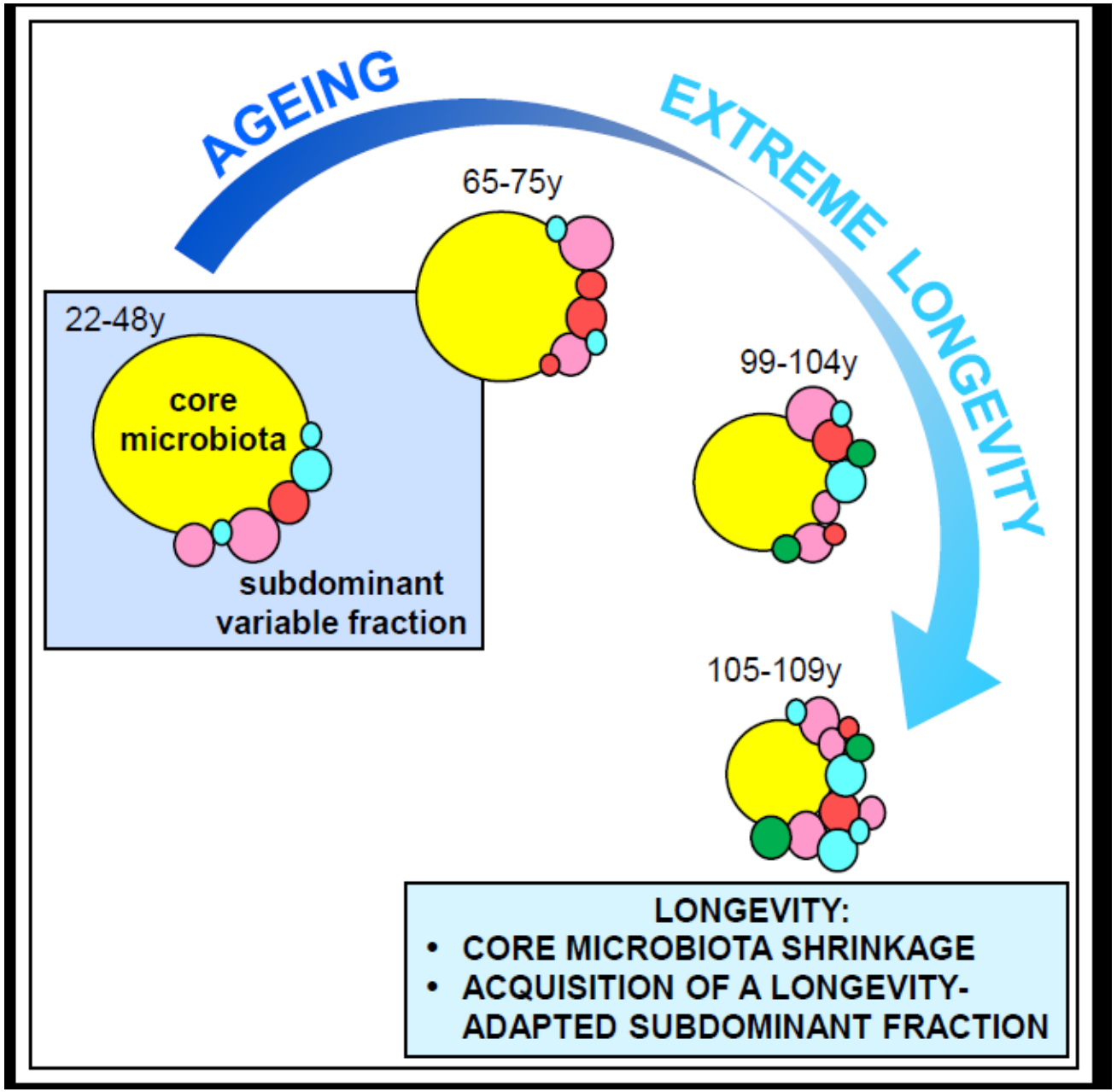
[6]Department of Clinical, Experimental and Biomedical Sciences, University of Florence, Florence 50134, Italy

Current Biology 26, 1–6 June, 2016

There is a GM signature of aging and longevity

The adaptive remodeling of gut microbiota with age from 22 to 109 years



Biagi et al., *Curr Biol* 2016

Unexpected INCREASE of GM DIVERSITY in Italian, Chinese and Japanese centenarians

Diversity	GM diversity in Centenarians according to:			
	Biagi et al., 2016	Wang et al., 2015	Kong et al., 2016	Odamaki et al., 2016
Simpson reciprocal index of diversity	↑			
Alpha diversity (Chao index)	↑	↑	↑	↑
Shannon index	↑	=	↑	↑

In chronic age-associated diseases the GM diversity decreases

Santoro *et al.*, Cell Mol Life Sci 2017

www.impactaging.com AGING, December 2013, Vol 5 N 12

Research Paper

Functional metagenomic profiling of intestinal microbiome in extreme ageing

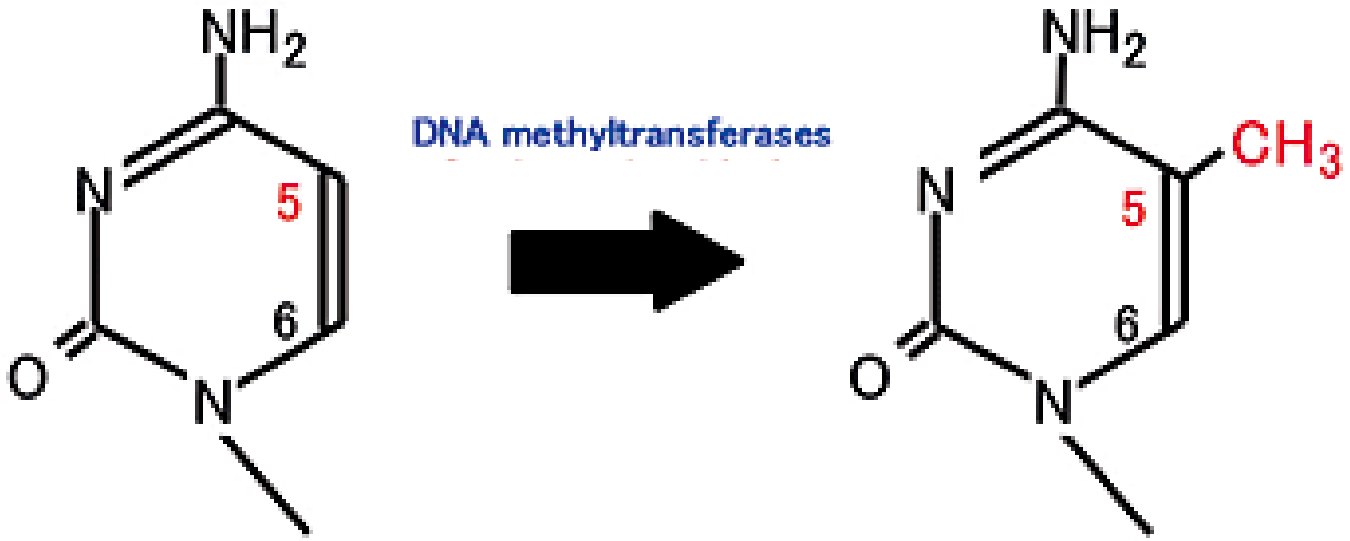
Simone Rampelli [1], Marco Candela [1], Silvia Turroni [1], Elena Biagi [1],Sebastiano Collino[2], Claudio Franceschi [3], Paul W O'Toole [4], and Patrizia Brigidi [1]

By **Illumina shotgun sequencing** of the fecal microbial DNA from the centenarians, elderly and young people, **a total of 214.6 million paired-end reads**, with an average of **23.841 million (± 0.067 SD) reads per subject**, were generated.

We observed an age-related increased abundance of genes involved in the tryptophan metabolism pathway, in agreement with the reduction of tryptophan found in serum of centenarians [*Collino et al, 2013*].

EPIGENETICS:
DNA METHYLATION

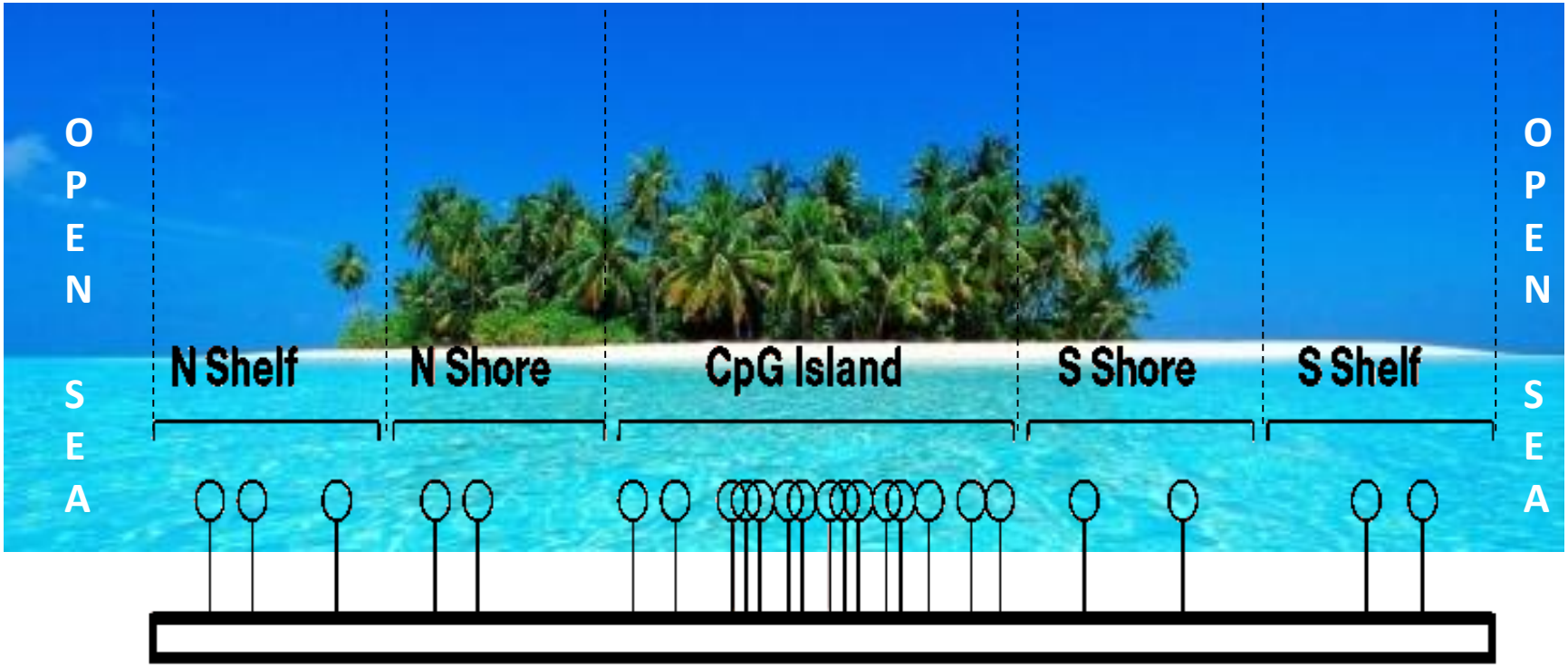
Methylation of Cytosine



5'–CpG–3'

3'–GpC–5'

Creates new phenotypes (sensitive to lifestyle, diet, stressors…) without changing the DNA sequence



Illumina *Infinium* HumanMethylation450 BeadChip (480,000 CpG/genoma)

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DNA methylation profile

of 105+, Offspring and Controls

Illumina *Infinium* HumanMethylation450 BeadChip (480,000 CpG/genoma)

	Milano **	Bologna *	Calabria ***	TOTAL	Mean Age (± std)	Male (N)	Female (N)
105+	29	33	20	**82**	105.5 ± 1.7	18	64
Offspring	28	22	13	**63**	69.8 ± 7.2	22	25
Controls	17	16	14	**47**	71.6 ± 8.0	26	37
TOTAL	74	71	47	**192**			

* PI: Prof. Claudio Franceschi, DIMES, UNIBO

** PI: Prof. Daniela Mari, DIP. DI SCIENZE CLINICHE E DI COMUNITA', UNIVERSITÀ DI MILANO

***PI: Prof. Giuseppe Passarino, DIP. DI BIOLOGIA, ECOLOGIA E SCIENZE DELLA TERRA, UNIVERSITÀ DELLA CALABRIA

DATA ANALYSIS:

Paolo Garagnani, Chiara Pirazzini, Steve Horvath

THE EPIGENETIC CLOCK

Using data of more than 8000 samples present in 82 databases on DNA methylation data obtained by Illumina platforms (Infinium 450K and 27K) Steve Horvath identified in the whole genome **353 CpGs** whose methylation level is a

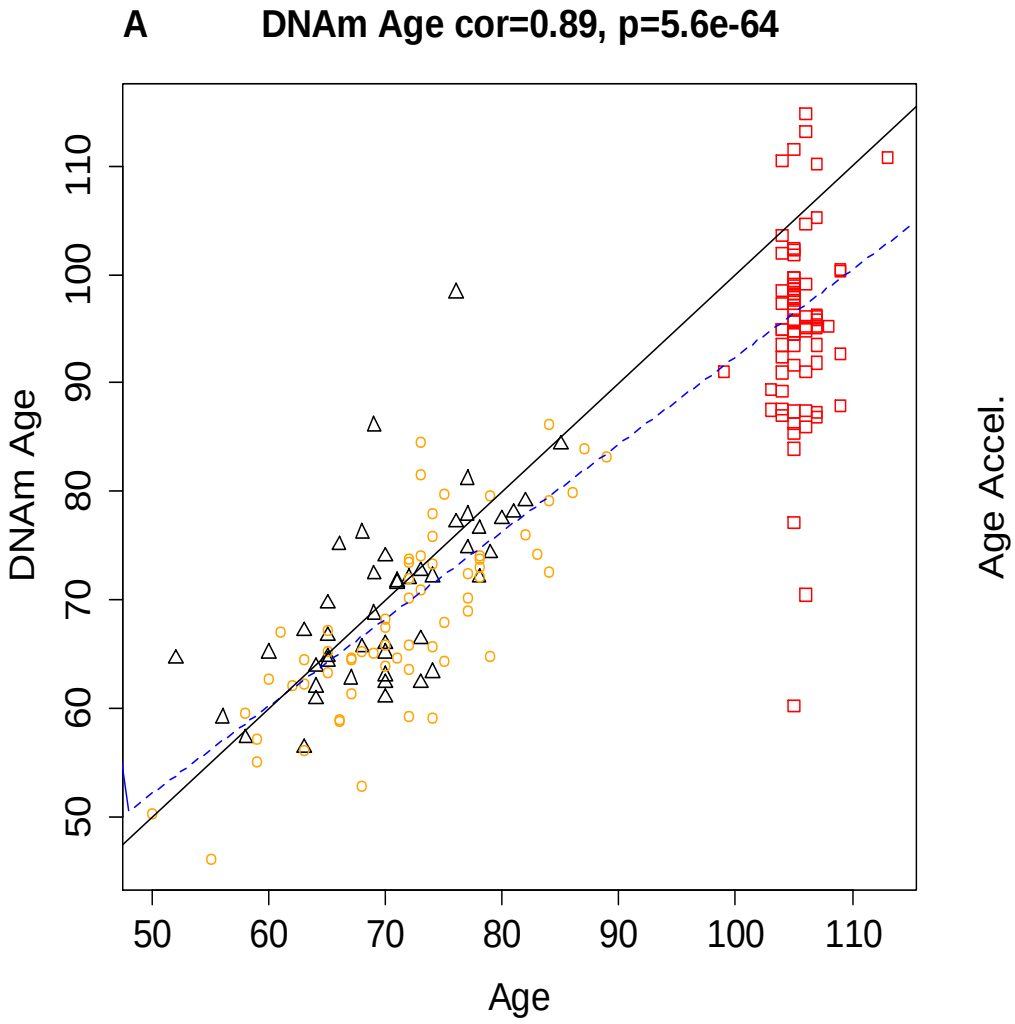
MULTI-TISSUES PREDICTOR OF AGE

which allows to estimate epigenetic age *versus* chronological age, *i.e*. the DNA METHYLATION AGE

(DNAm Age)

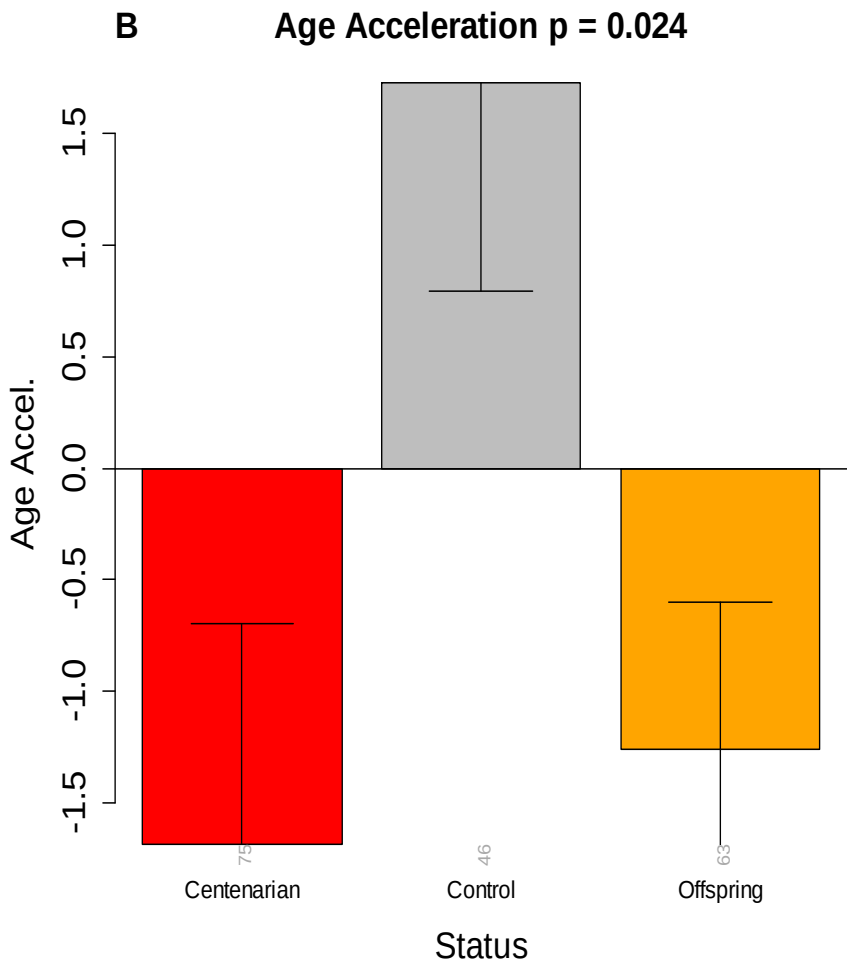
DNA methylation age of human tissues and cell types
Steve Horvath
Genome Biology 2013, 14:R115

The black line corresponds to y=x.



The dashed blue line corresponds to a regression line through the samples who are younger than 90 years old.

DNAm age *versus* chronological age. AGE ACCELERATION is defined as the (vertical) difference of each sample to the blue regression line.



- **105+ are on average 10.8 years younger than expected**
- **controls are only -0.09 years younger than expected (not significantly different from zero).**
- **offspring are 5.2 years younger than controls.**

Aging Cell (2015) pp1–5

Accelerated epigenetic aging in Down syndrome

Steve Horvath,[1,2,*] Paolo Garagnani,[3,4,5,*] Maria Giulia Bacalini,[3,4,6] Chiara Pirazzini,[3,4] Stefano Salvioli,[3,4] Davide Gentilini,[7] Anna Maria Di Blasio,[7] Cristina Giuliani,[8] Spencer Tung,[9] Harry V. Vinters[9] and Claudio Franceschi[4,10]

Here, we utilize a quantitative molecular marker of aging (known as the epigenetic clock) to demonstrate that trisomy 21 significantly increases the age of blood and brain tissue (on average by 6.6 years, $P = 7.0 \times 10^{-14}$).

Age acceleration for DS:

Leukocytes: 3.9 years

Brain: 11.5 years

Whole blood: 4.6 years

Buccal epithel: 2.8 years

Different methylated regions (DMR) between 32 mothers and their offspring using the Illumina *Infinium* HumanMethylation450 BeadChip (480,000 CpG/genome)

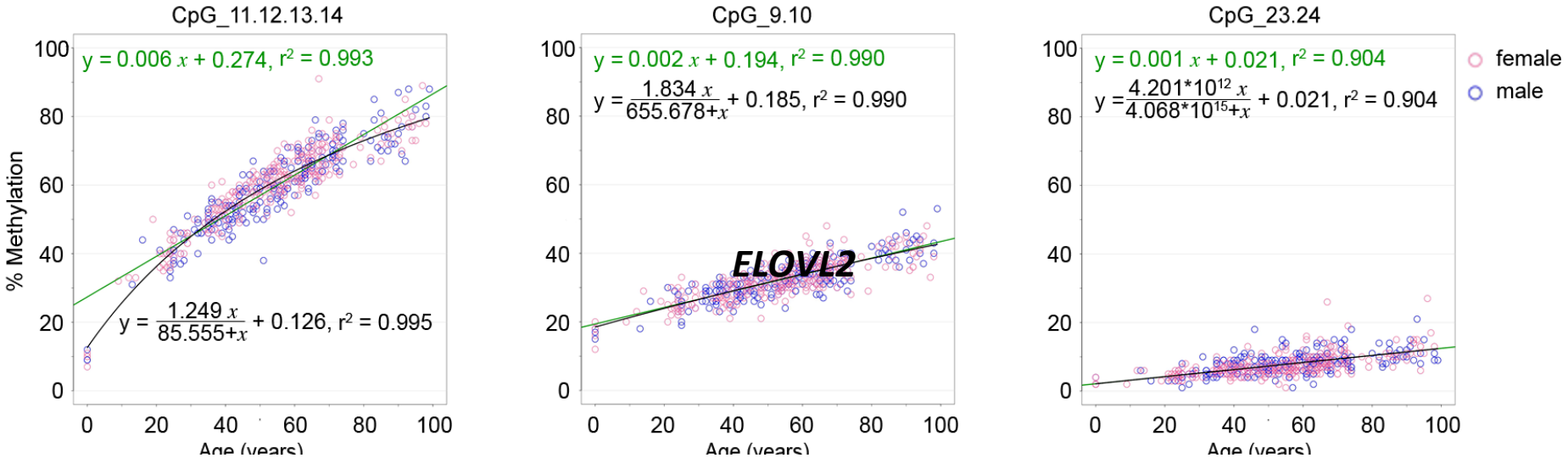
The top 5 loci mapped within the CpG islands of 3 genes:

ELOVL2 (cg16867657), ***FHL2*** (cg06639320, cg22454769, cg24079702) and ***PENK*** (cg16419235).

The CpG islands of *ELOVL2*, *FHL2* and *PENK* resulted **hypermethylated in mothers respect to the offspring cohort** with no sex- or family-associated differences in methylation levels.

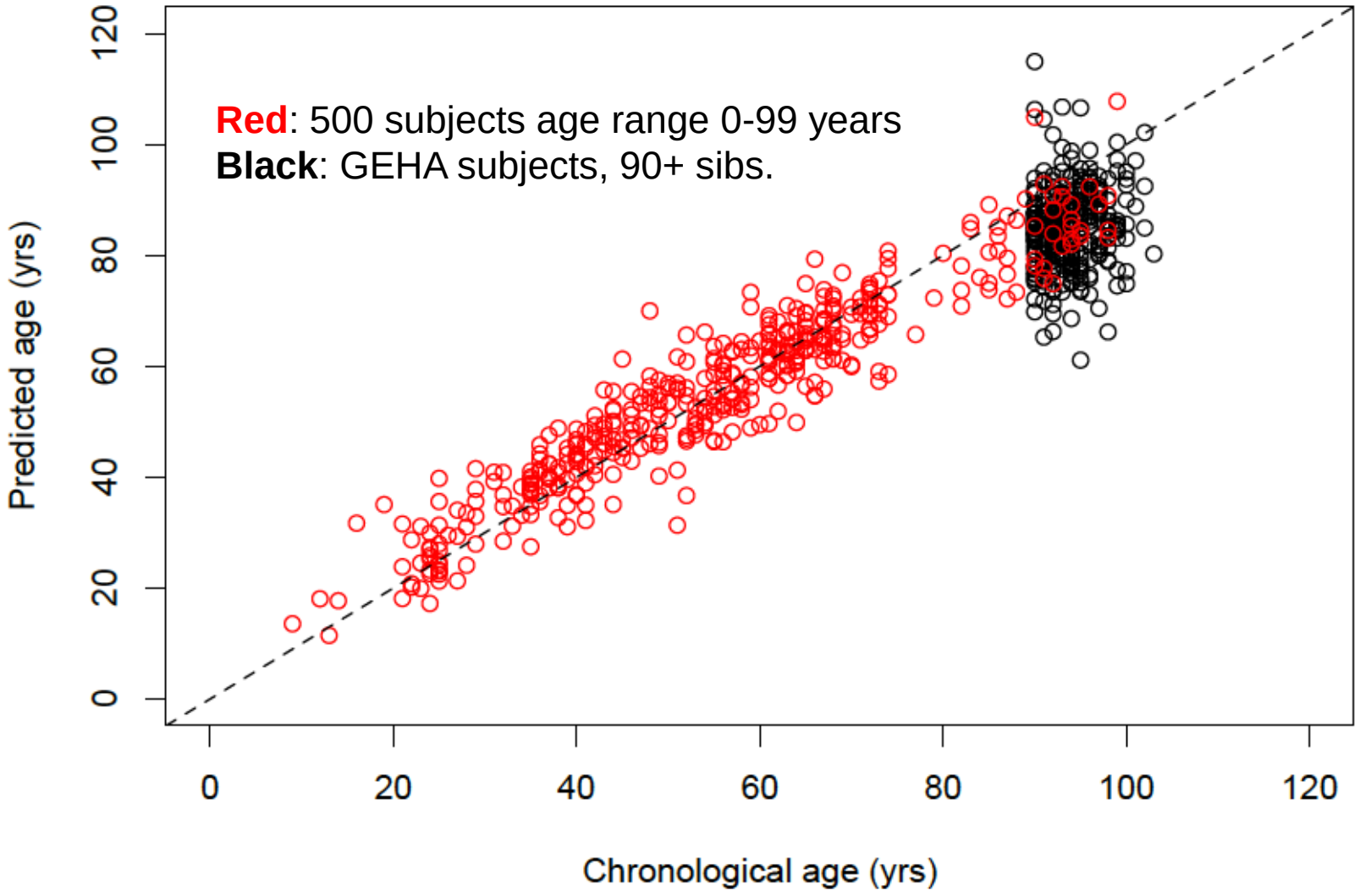
***Garagnani et al.*, AGING CELL 2012**

Methylation of *ELOVL2*, *FHL2* & *PENK* CpG islands in 501 healthy subjects from newborns to 100+ (Sequenom EpiTYPER)



ELOVL2 the most powerful single biomarker of aging

Garagnani *et al.*, AGING CELL 2012



DNAm age of n. 330 GEHA 90+ sibs
(methylation of ELOVL2 and FHL2 genes) is on average
8.8 years younger
than their chronological age (average 93.76 years)

OPEN ACCESS Freely available online

PLOS ONE

Metabolic Signatures of Extreme Longevity in Northern Italian Centenarians Reveal a Complex Remodeling of Lipids, Amino Acids, and Gut Microbiota Metabolism

Sebastiano Collino[1]*, Ivan Montoliu[2], François-Pierre J. Martin[1], Max Scherer[1], Daniela Mari[3,4], Stefano Salvioli[5,6], Laura Bucci[5,6], Rita Ostan[5,6], Daniela Monti[7], Elena Biagi[8], Patrizia Brigidi[8], Claudio Franceschi[5,6], Serge Rezzi[1]

1 Proteomics and Metabonomics, Nestlé Institute of Health Sciences SA, Campus EPFL, Quartier de l'innovation, Lausanne, Switzerland, **2** Applied Mathematics, NESTEC SA, Nestlé Research Center, Lausanne, Switzerland, **3** Department of Medical Sciences, University of Milan, Milan, Italy, **4** Geriatric Unit Ca' Grande Foundation Maggiore Policlinico Hospital, Milan, Italy, **5** Department of Experimental Pathology, University of Bologna, Bologna, Italy, **6** Interdepartmental Centre L. Galvani, University of Bologna, Bologna, Italy, **7** Department of Experimental Pathology and Oncology, University of Florence, Florence, Italy, **8** Department of Pharmaceutical Sciences, University of Bologna, Bologna, Italy

PLOS ONE March 2013 | Volume 8 | Issue 3 | e56564

A total of 457 individuals:

N= 143 centenarians

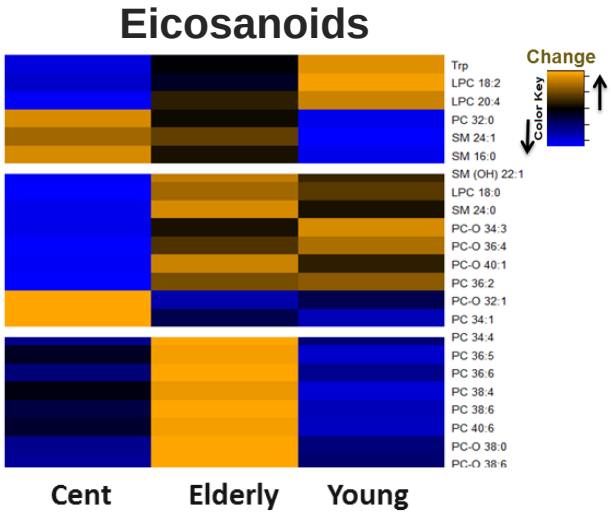
N= 220 offspring of centenarians

N= 73 offspring of non long-lived parents

N= 21 young subjects

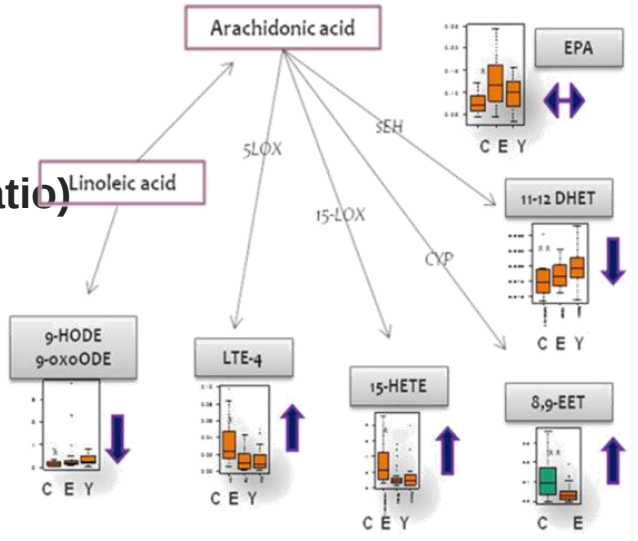
Metabolomic biomarkers of human aging and longevity

A peculiar metabolic phenotype of centenarians is reported for the first time and assumed as a GOLD STANDARD for human healthy ageing and longevity



- **Decreased concentration of AA (Trp)**
- Decreased concentration GPL (LPCs)
- Increased concentration of sphingolipids (SM)
- Different membrane FA composition/integrity (PC/PE ratio) with lower MUFA/PUFA ratio

- Increased proinflammatory synthesis (Leukotrines)
- Activation anti-inflammatory mechanism (HETE, EET)
- Reduction of oxidative stress (9-HODE,9-oxoODE)



Collino et al. PLOS ONE 2013