Telomere Biology, Vascular Aging and Coronary Artery Disease

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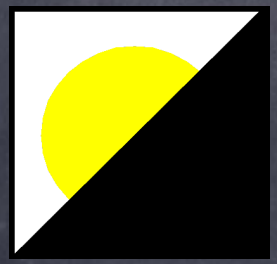
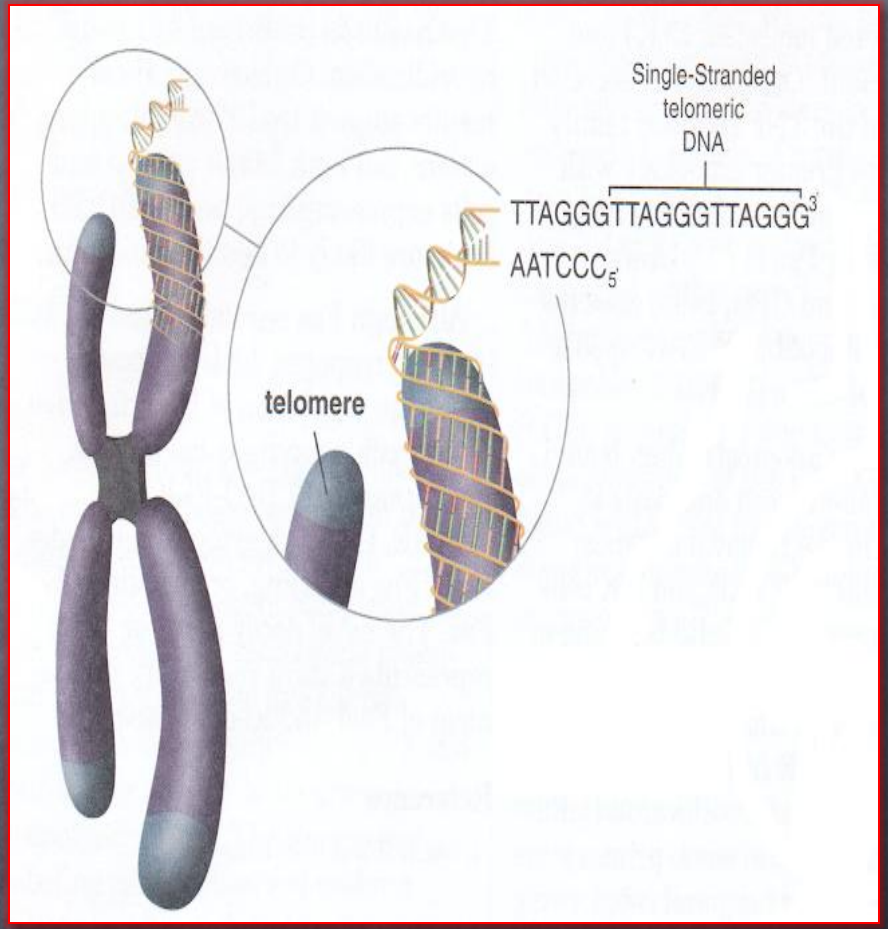


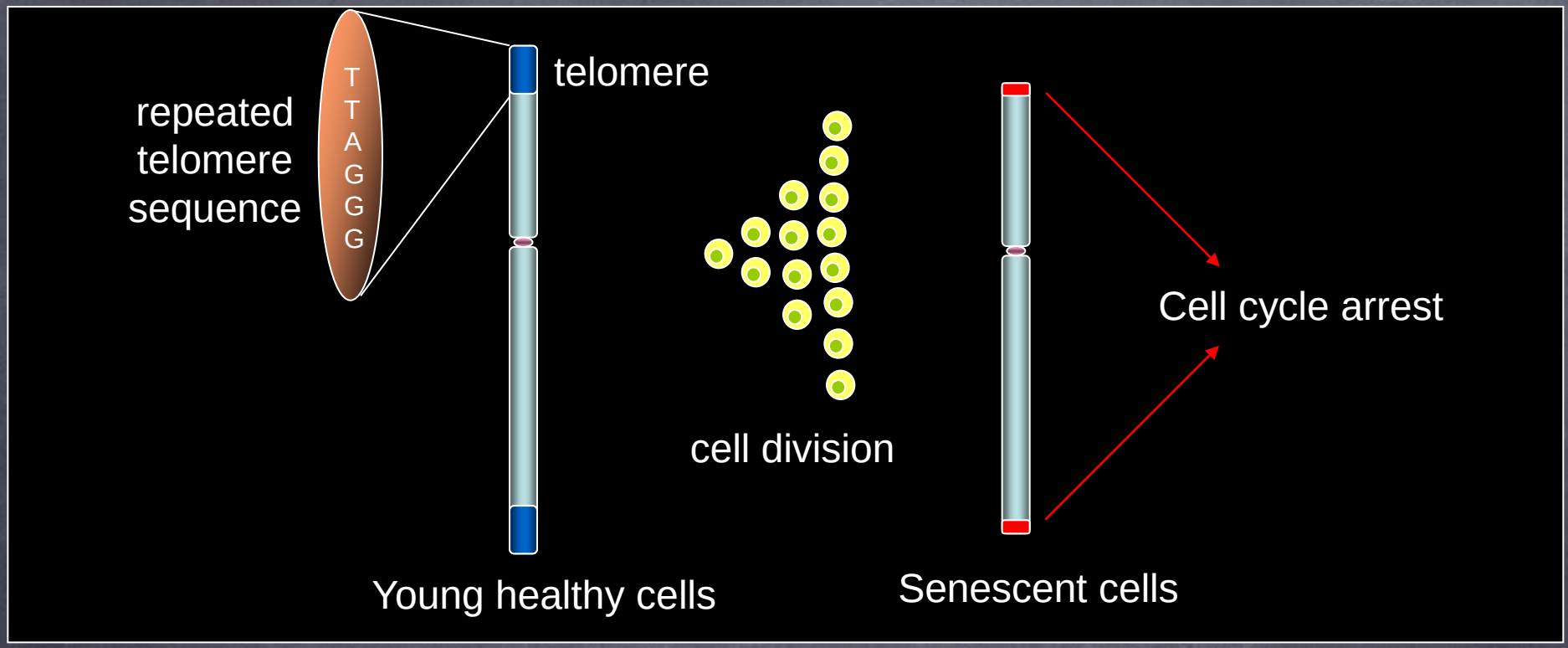
Sir Jules Thorn Trust

Telomere structure: an overview

- Telomeres are the ends of chromosomes
- Human telomeres consist of TTAGGG repeat
- Function to protect the ends of chromosomes

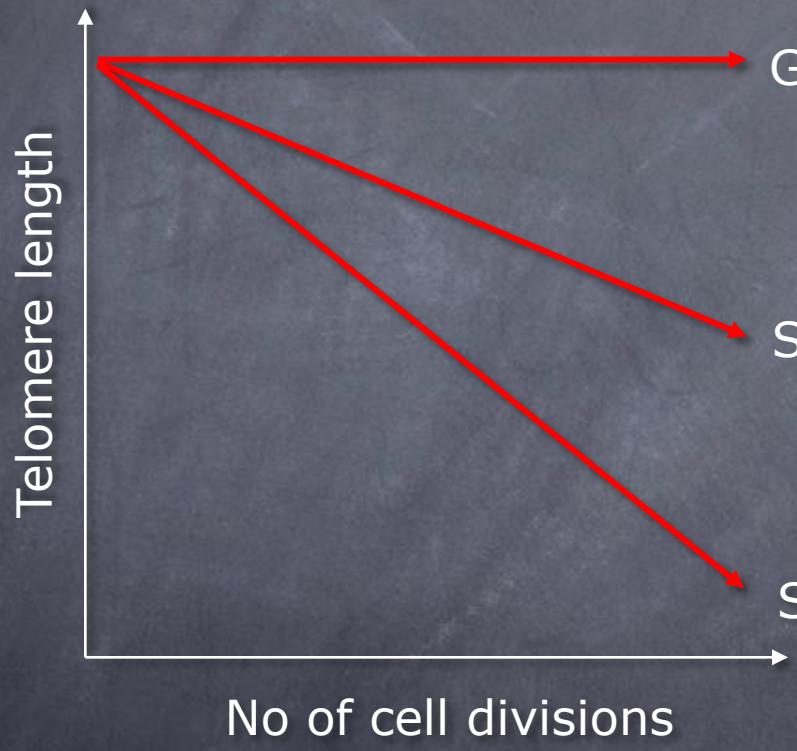


Telomere attrition leads to senescence



Mean telomere length provides a measure of *biological* age

The Telomere End Replication Problem



	Telomerase activity
Germ cell	High
Stem cell	Intermittent
Somatic cell	Low to none

Physiological Ageing

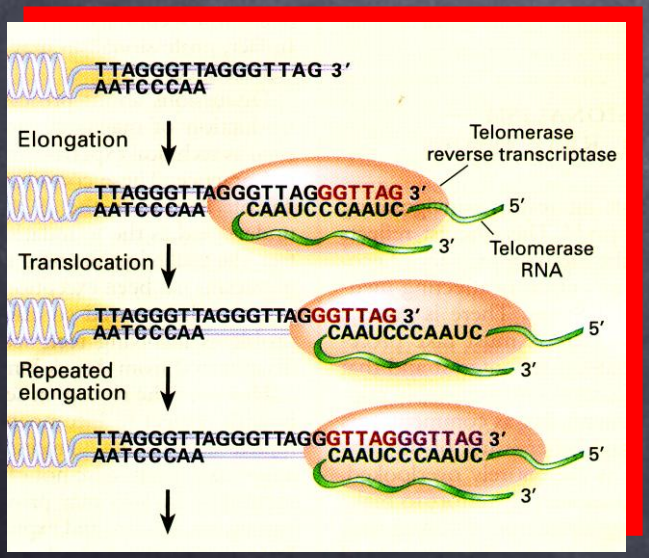
Ageing

Pathological Ageing

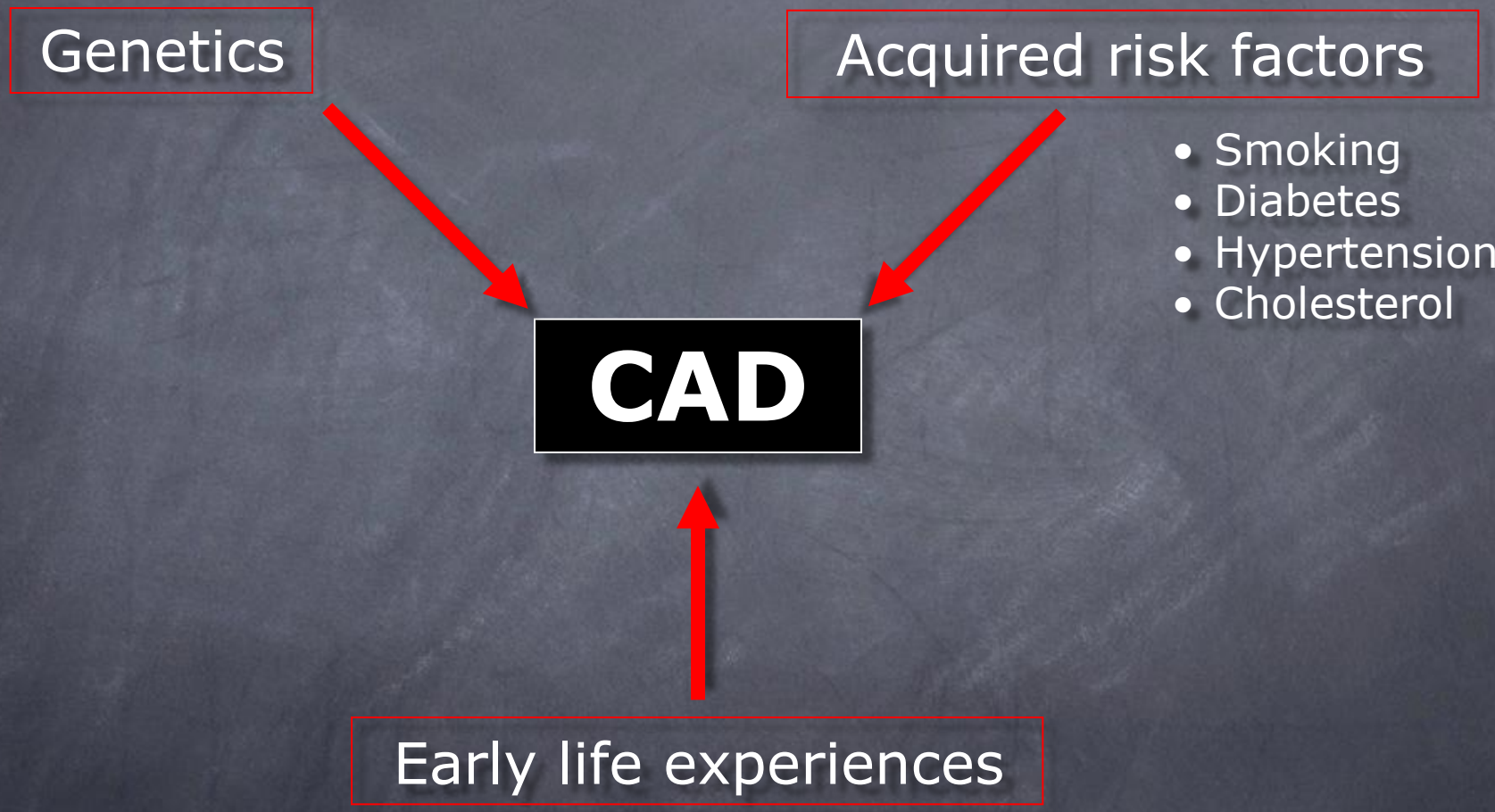
Atherosclerosis

Alzheimer's disease

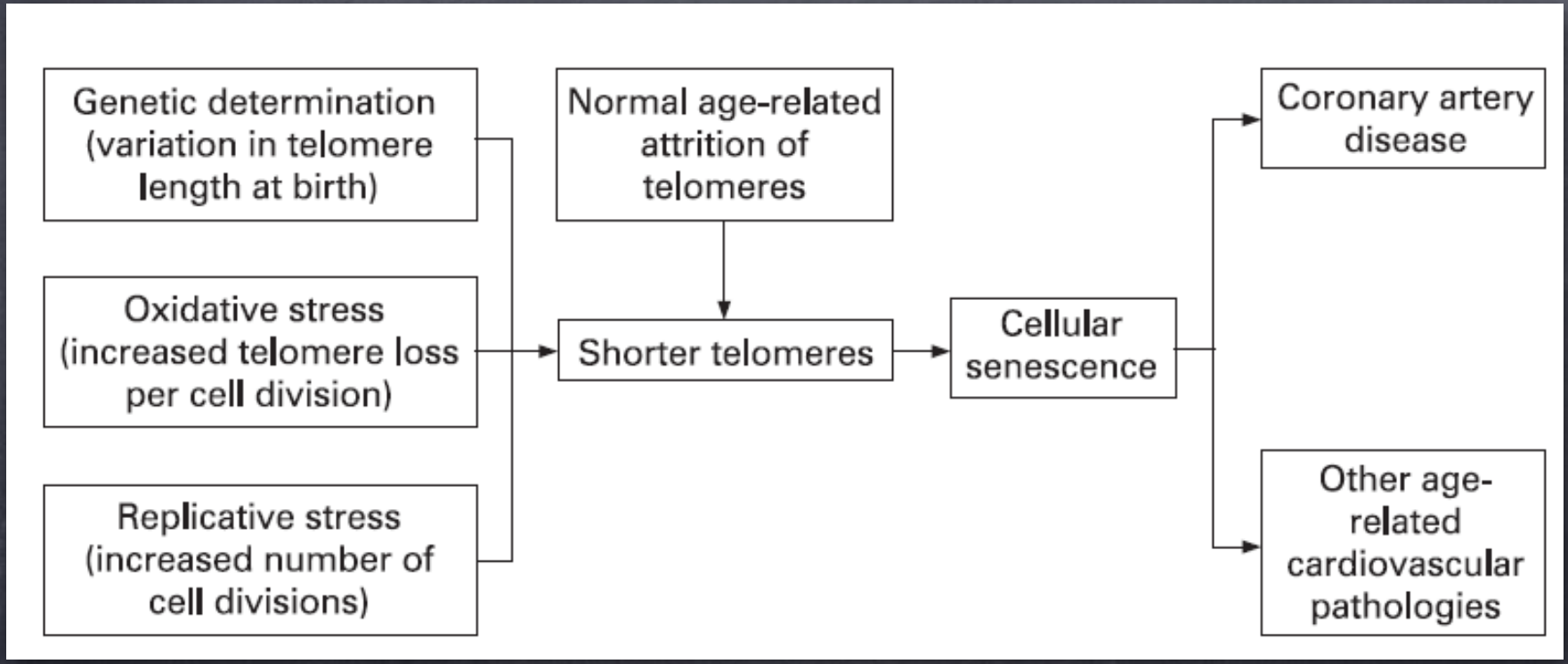
Cellular Senescence



Aetiology of CAD

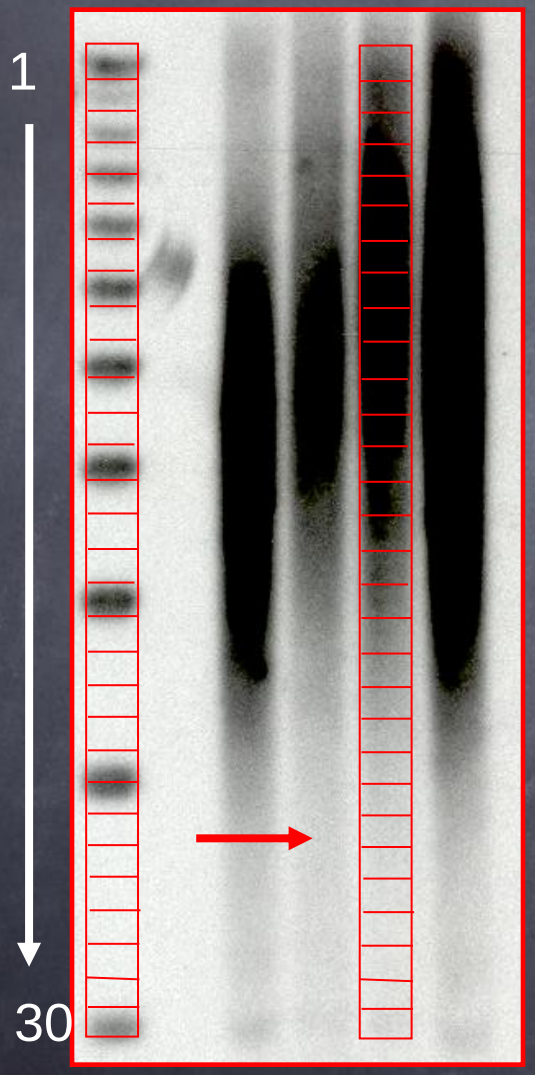


Biological ageing and cardiovascular disease

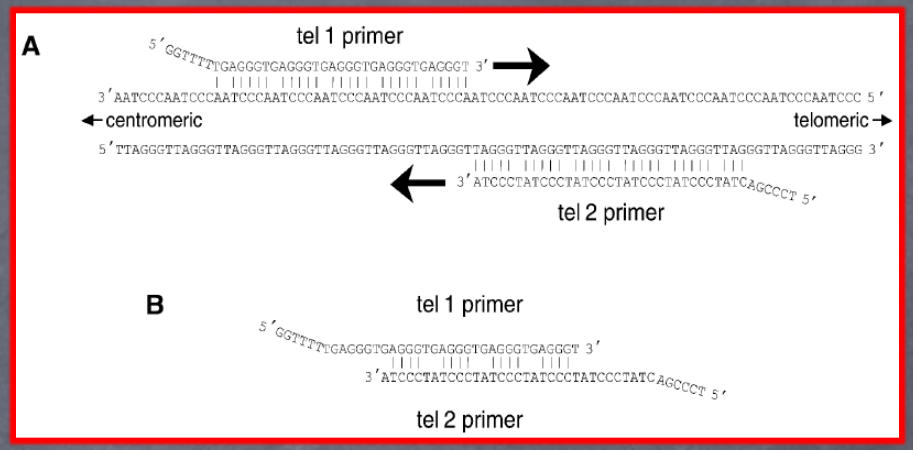


Samani NJ & van der Harst P Heart 2008

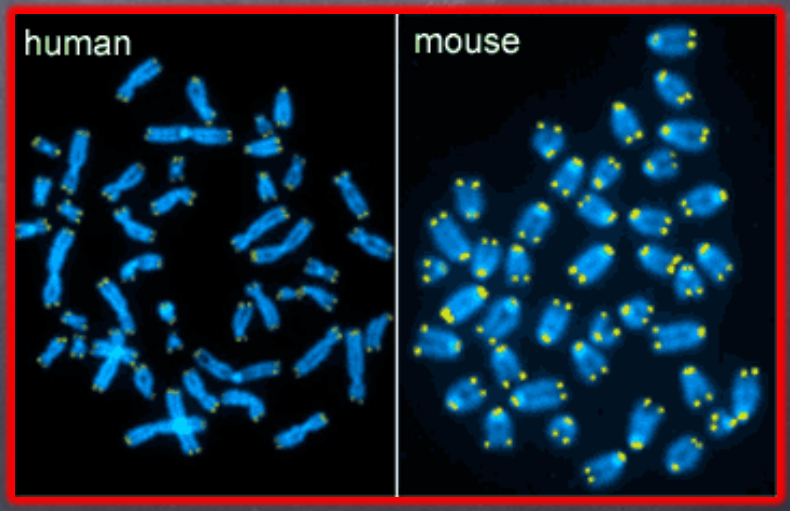
Measurement of telomere length



Southern blot



Real-time PCR



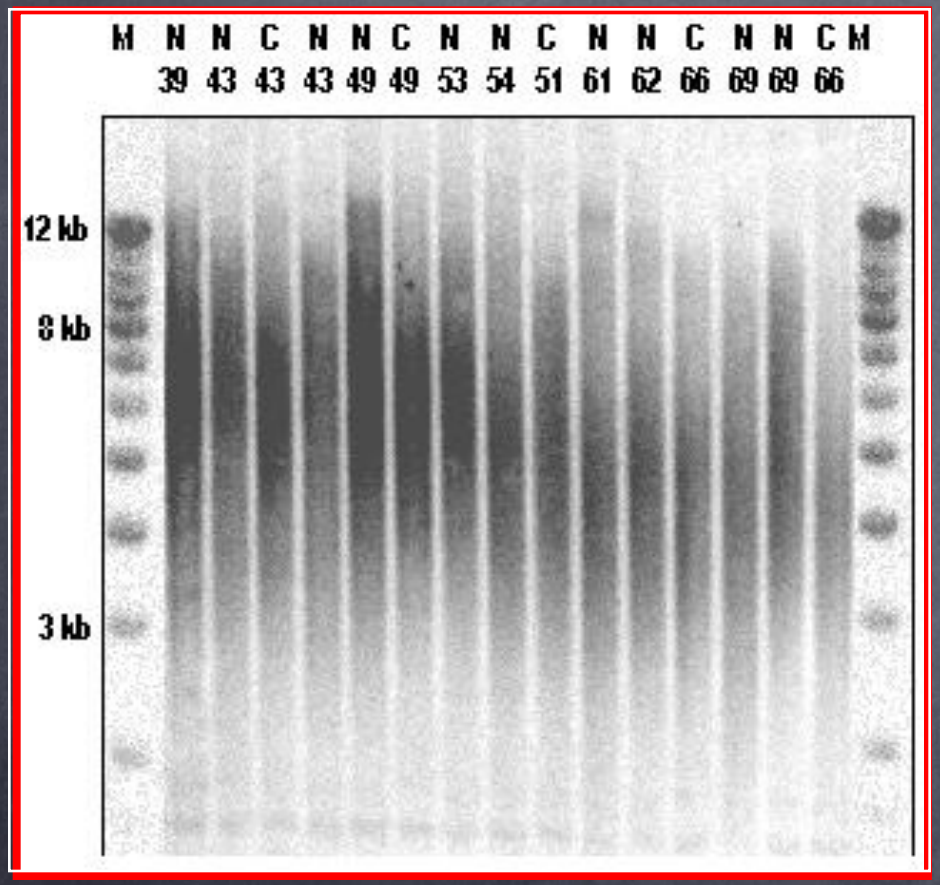
Fluorescence in-situ hybridization

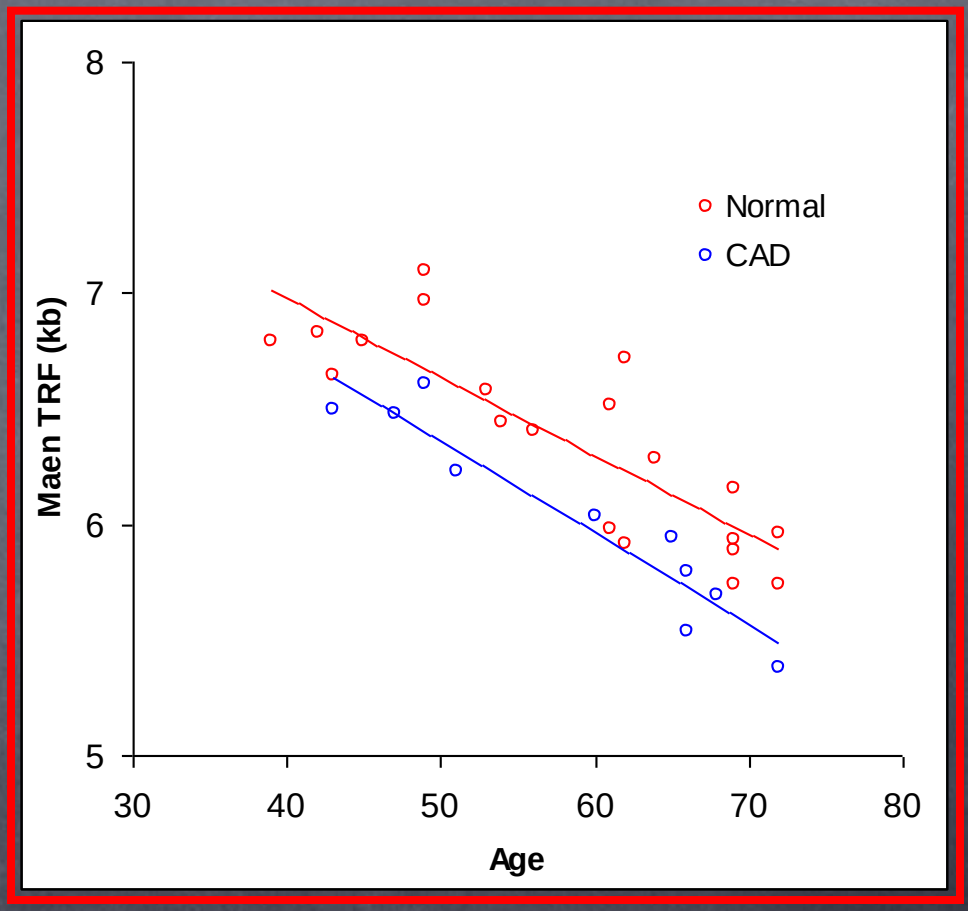
Hypothesis

Coronary artery disease is a disease of premature *biological* ageing

Olovnikov AM "Biological clock" 1973

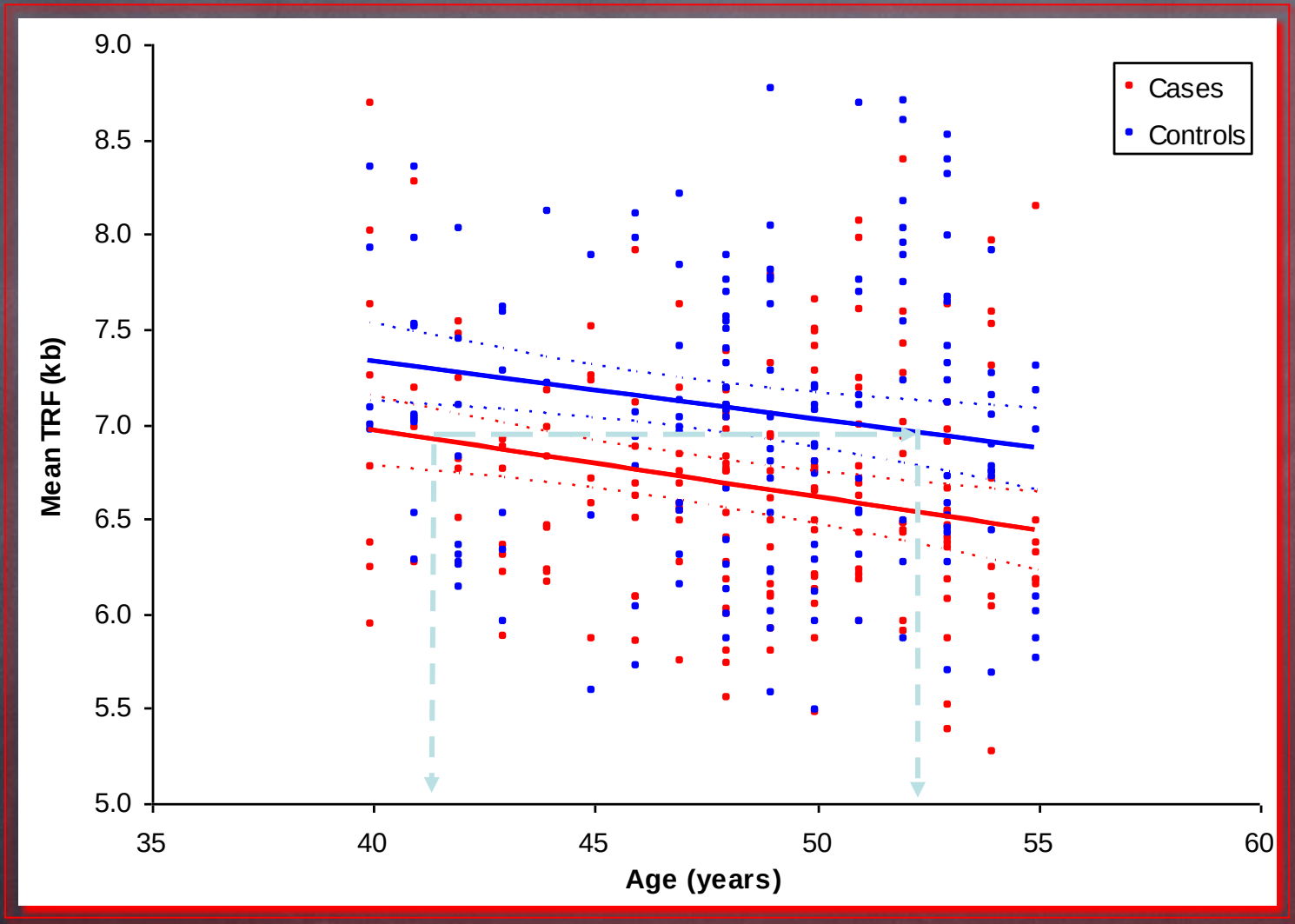
Telomere shortening in atherosclerosis





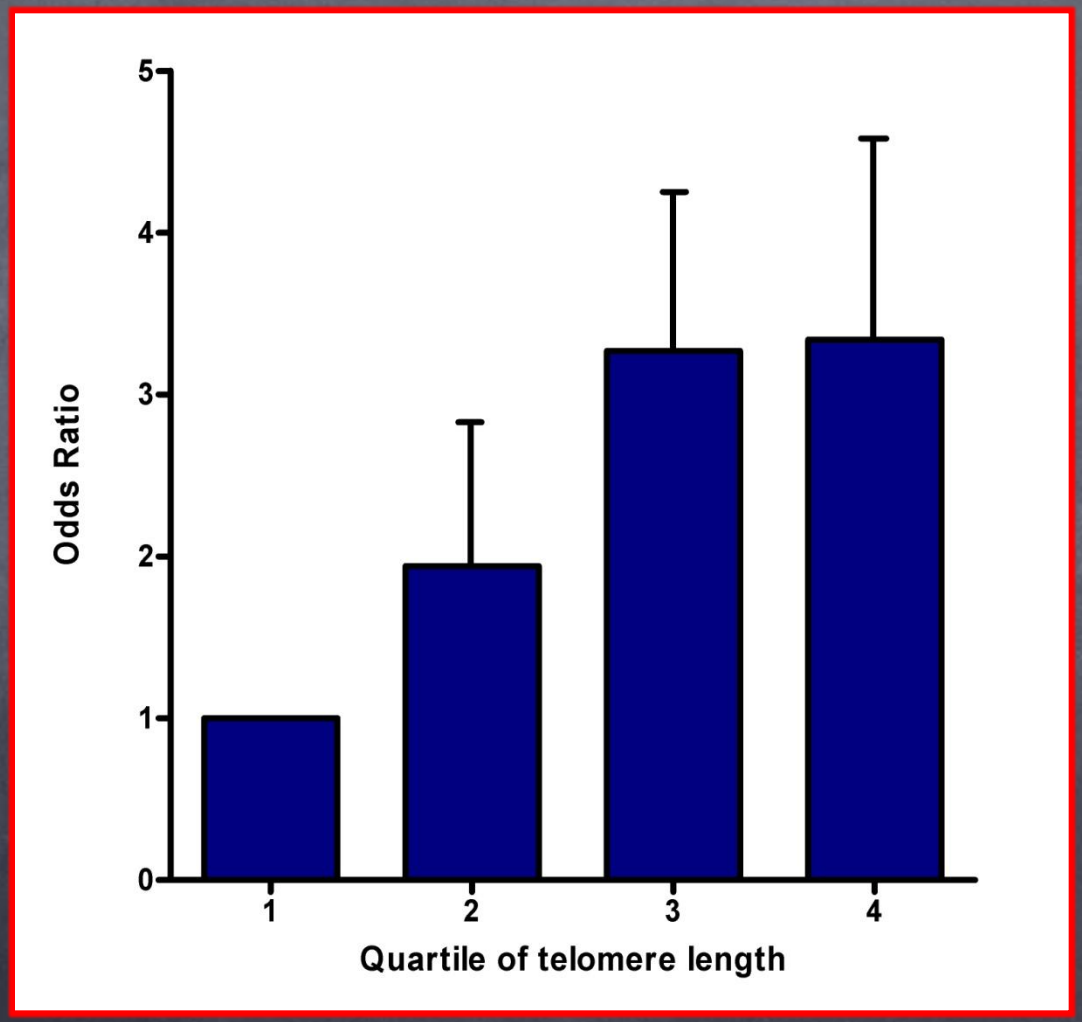
Samani et al. Lancet 2001

Telomere shortening in premature MI



Brouilette et al. ATVB 2003

Risk of MI increases with progressive shortening



Brouilette et al., ATVB, 2002 & 2003.

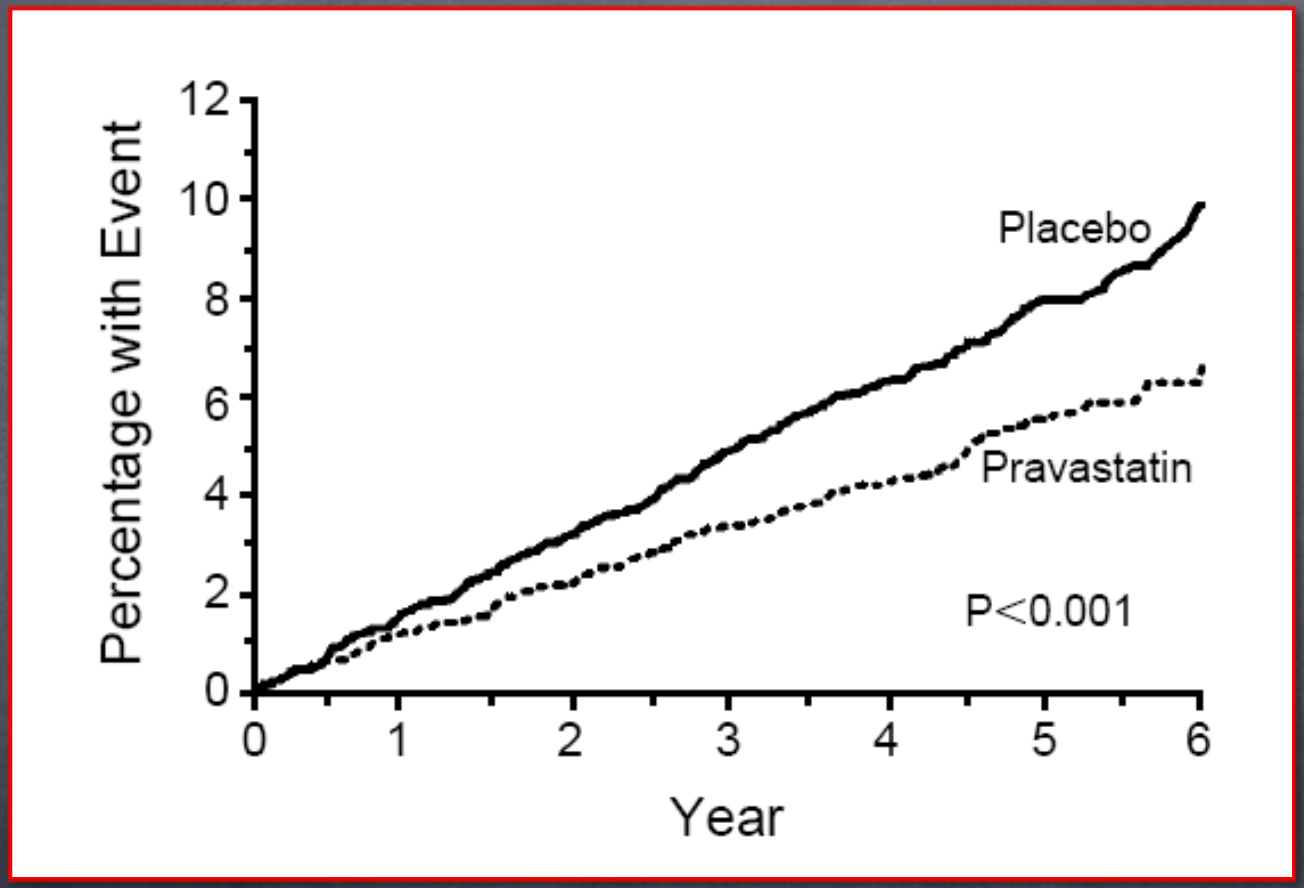
Prospective association of telomere length with CHD

West **o**f **Sco**tland **P**revention **S**tudy (**WOSCOPS**)

6595 males, age 45-65 years, with a mean baseline total cholesterol of 7.0 mmol/l and no history of MI were randomised to pravastatin (20 mg) or placebo

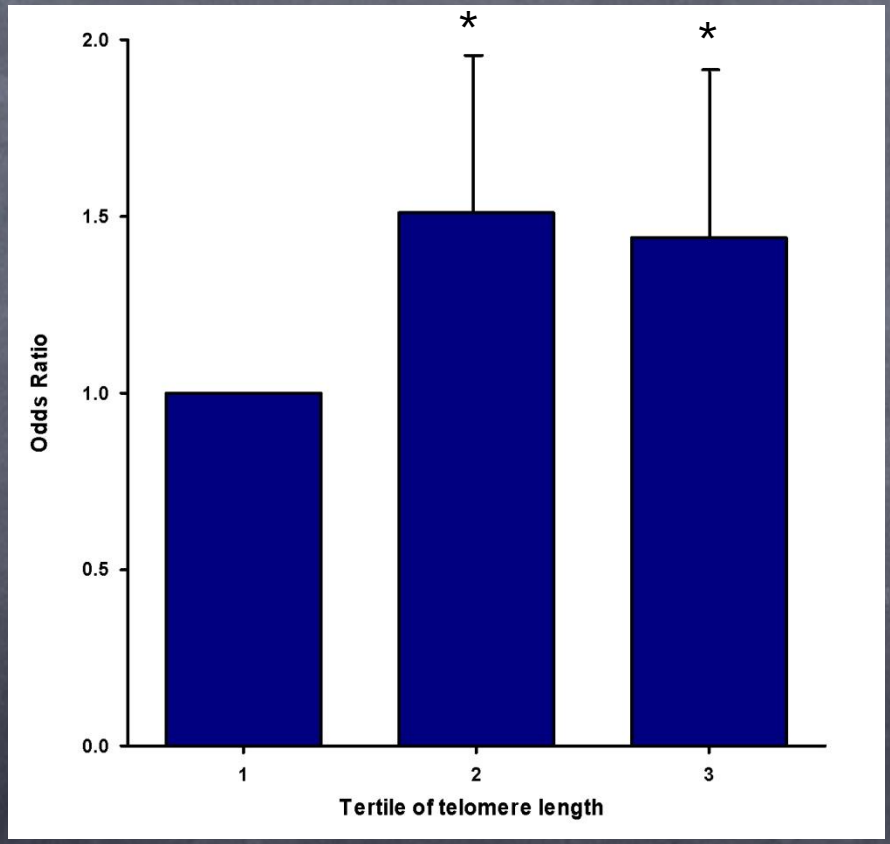
Over a mean follow-up of 4.2 years, 580 subjects developed CHD end-points

Benefit of statin treatment in WOSCOPS



Shepherd J et al. NEJM 1995

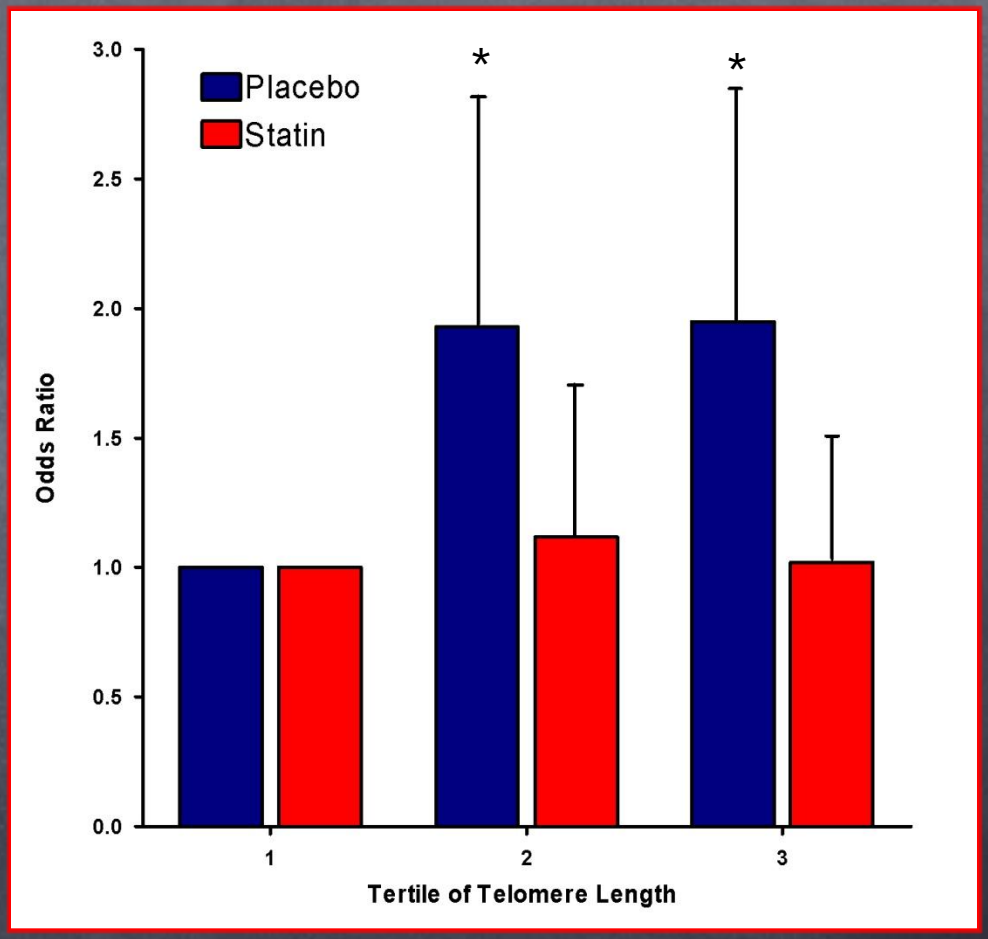
Baseline mean telomere length and risk of CAD in WOSCOPS



Brouilette et al. Lancet, 2007

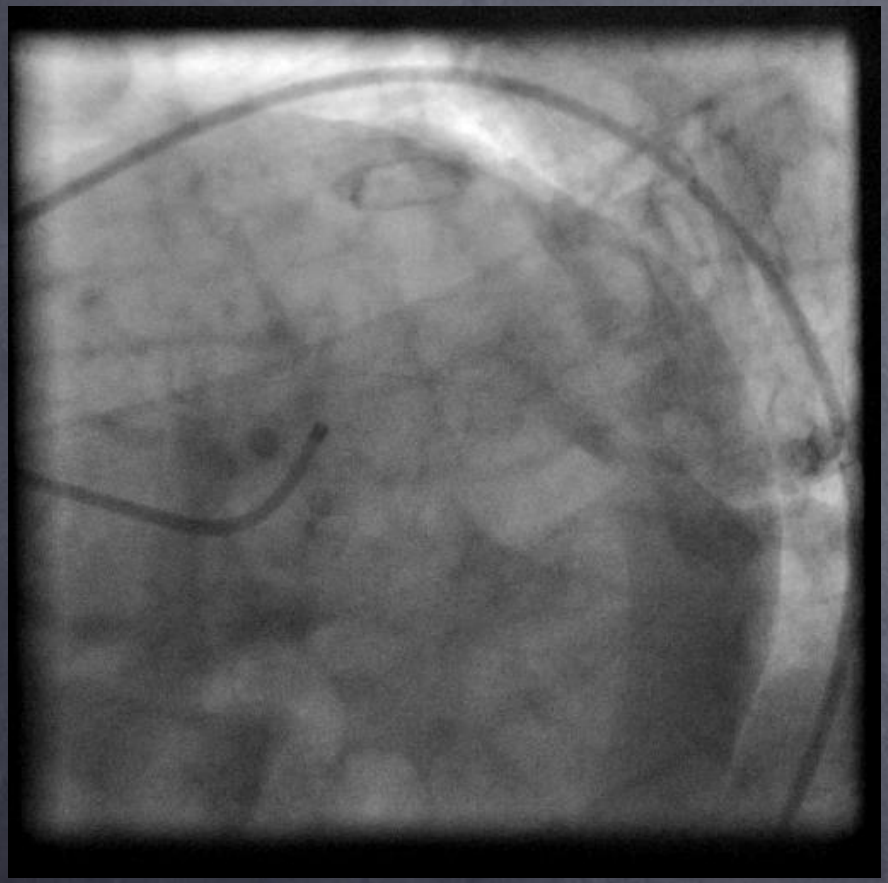
Telomere length, CHD events and benefits from statin treatment

Tertile	*Placebo*	*Statin*
1	62	61
2	118	68
3	109	68

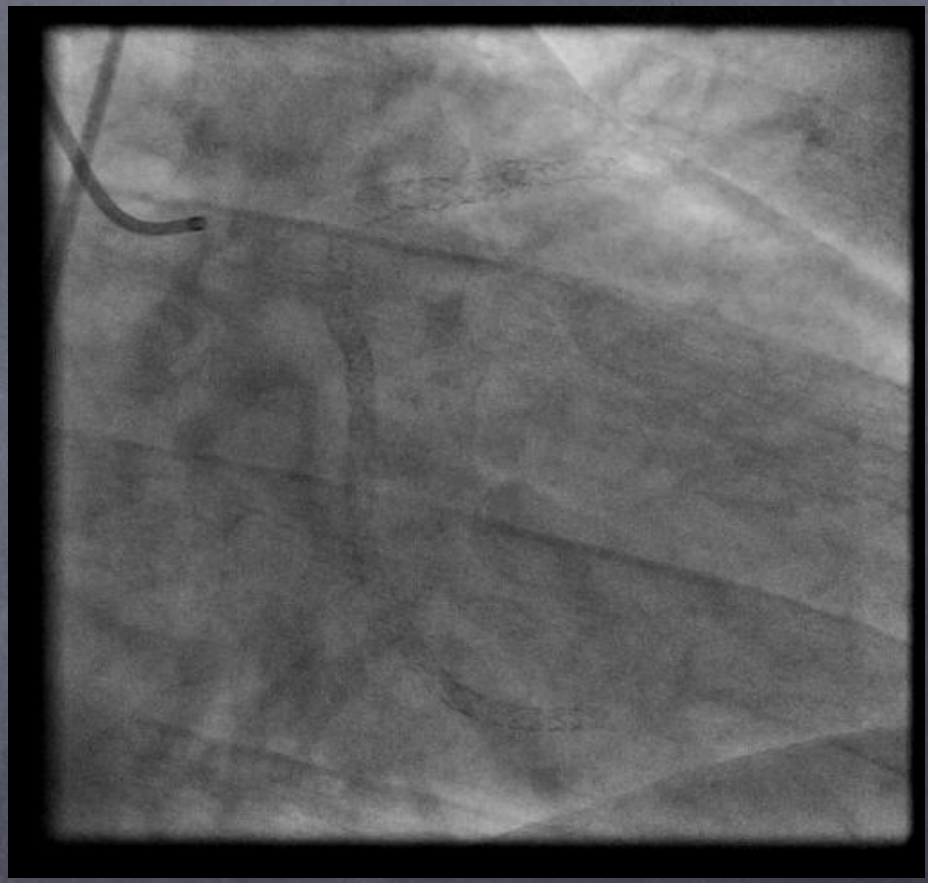


Brouilette et al. Lancet 2007

The coronary heart disease paradox

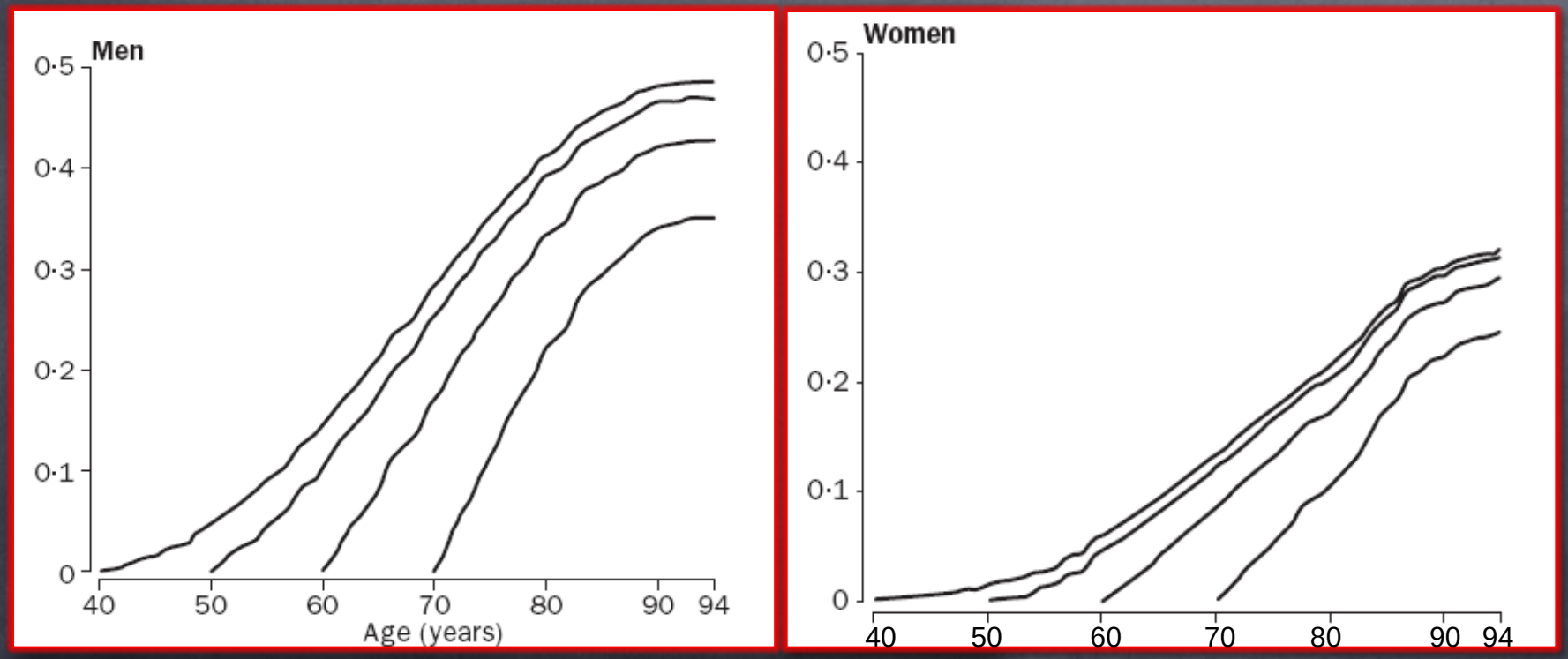


78 year old male



40 year old male

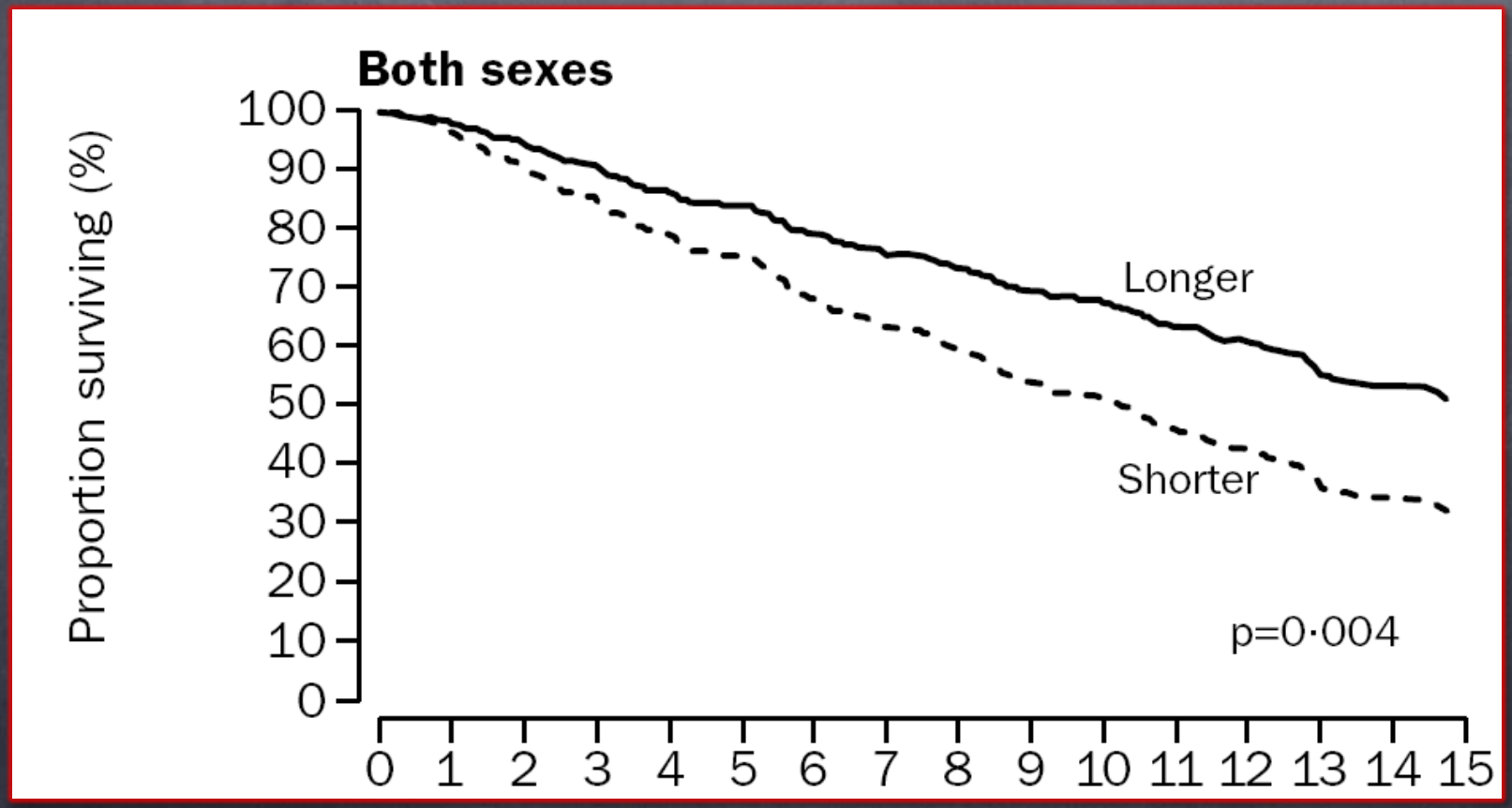
CAD is an age-associated disease but not an inevitable consequence



Lloyd-Jones et al. Lancet 1999

Telomere length and mortality

Association between telomere length in blood and mortality in people aged 60 years or older



Cawthon et al. Lancet 2003

Shorter telomeres and cause-specific mortality in normal subjects 60 years or older

	Number of individuals/ number of deceased	Mortality rate ratio* (95% CI)	p
All-cause mortality†			
Both sexes	143/101	1·86 (1·22–2·83)	0·004
Women	71/46	2·16 (1·07–4·39)	0·033
Men	72/55	1·94 (1·01–3·74)	0·047
Age at blood draw			
Younger than 75 years	93/53	1·96 (1·11–3·48)	0·021
Age 75 years or older	50/48	1·73 (0·93–3·24)	0·086
Cause-specific mortality‡			
Heart†	124/30	3·18 (1·36–7·45)	0·008
Cerebrovascular§	124/15	1·35 (0·36–5·13)	0·660
Cancer§	124/12	1·43 (0·34–6·03)	0·625
Infectious§	124/8	8·54 (1·52–47·9)	0·015
Other known§	124/16	2·15 (0·71–6·50)	0·174
All known causes apart from heart and infectious§	124/43	1·70 (0·82–3·53)	0·156

Cawthon et al. Lancet 2003

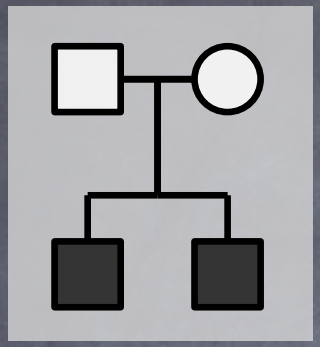
Regulation of telomere length

Genetics – what you start with

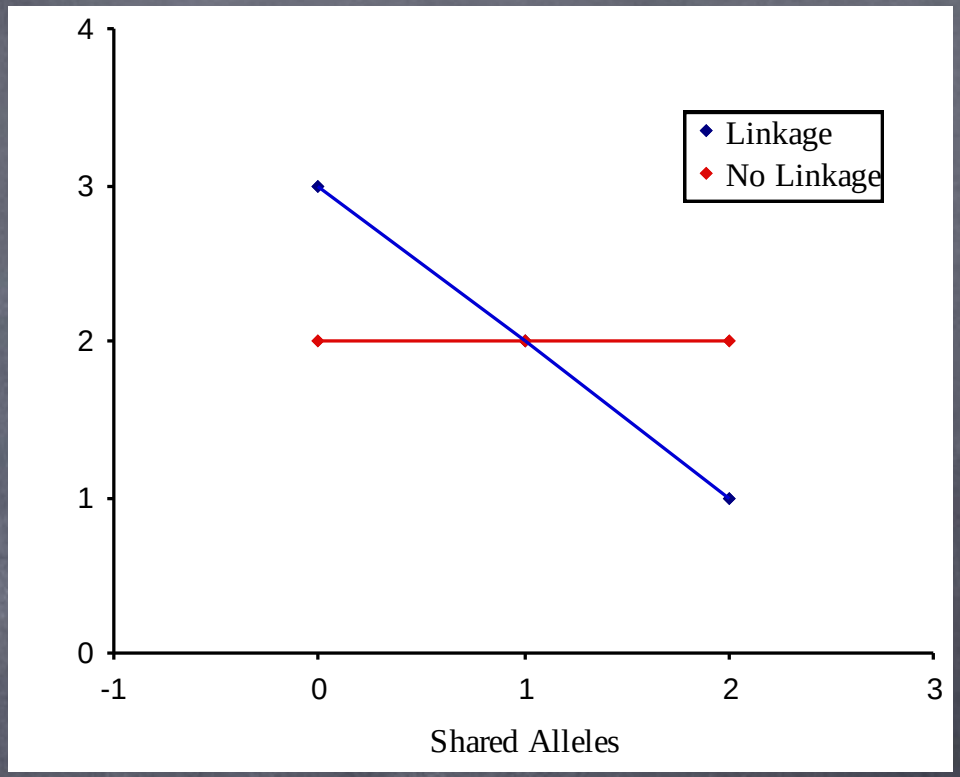
Replicative stress – more cell divisions

Oxidative stress – more loss per division

Telomere length is heritable

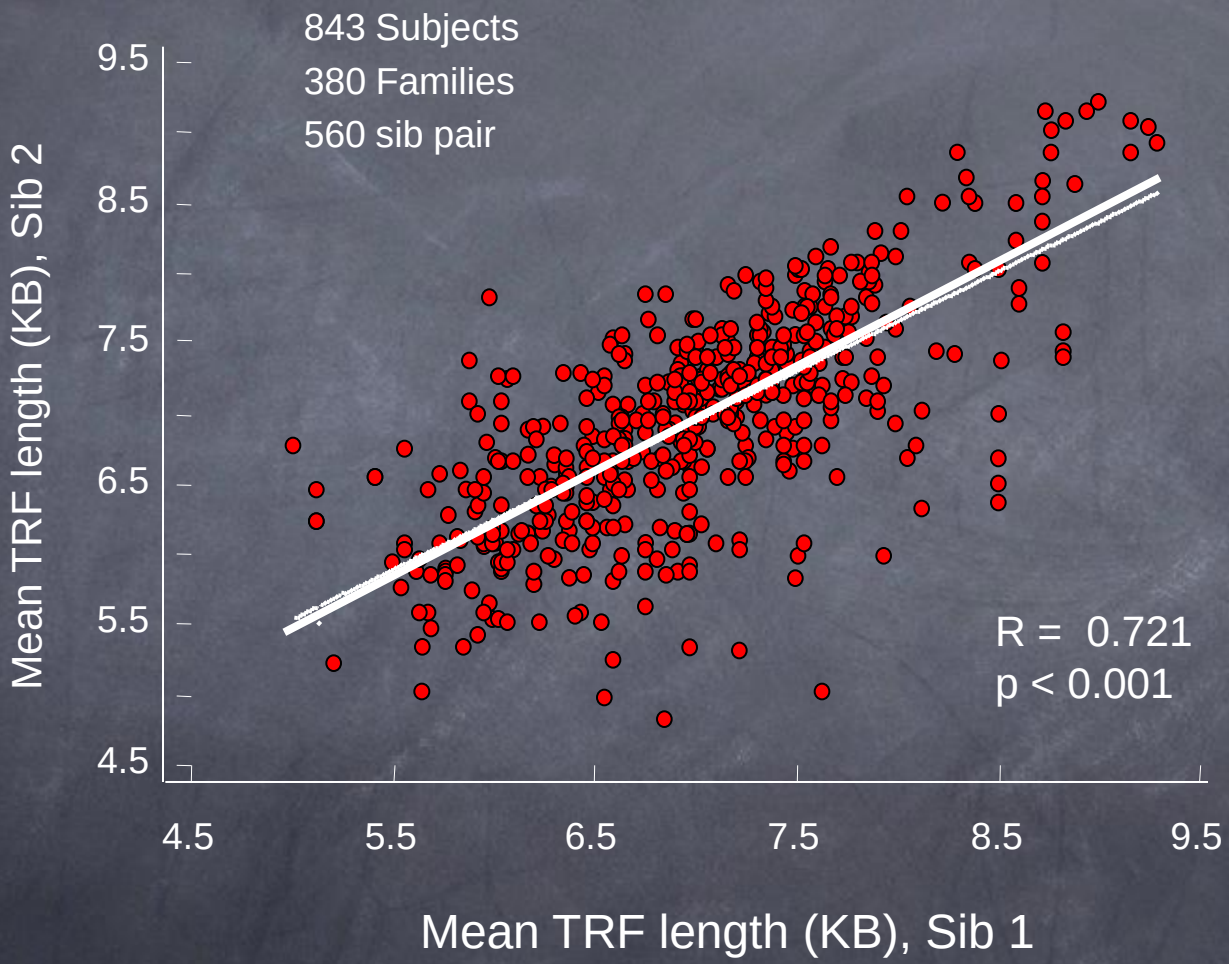


Shared Alleles	*Frequency*
0 alleles	0.25
1 allele	0.5
2 alleles	0.25

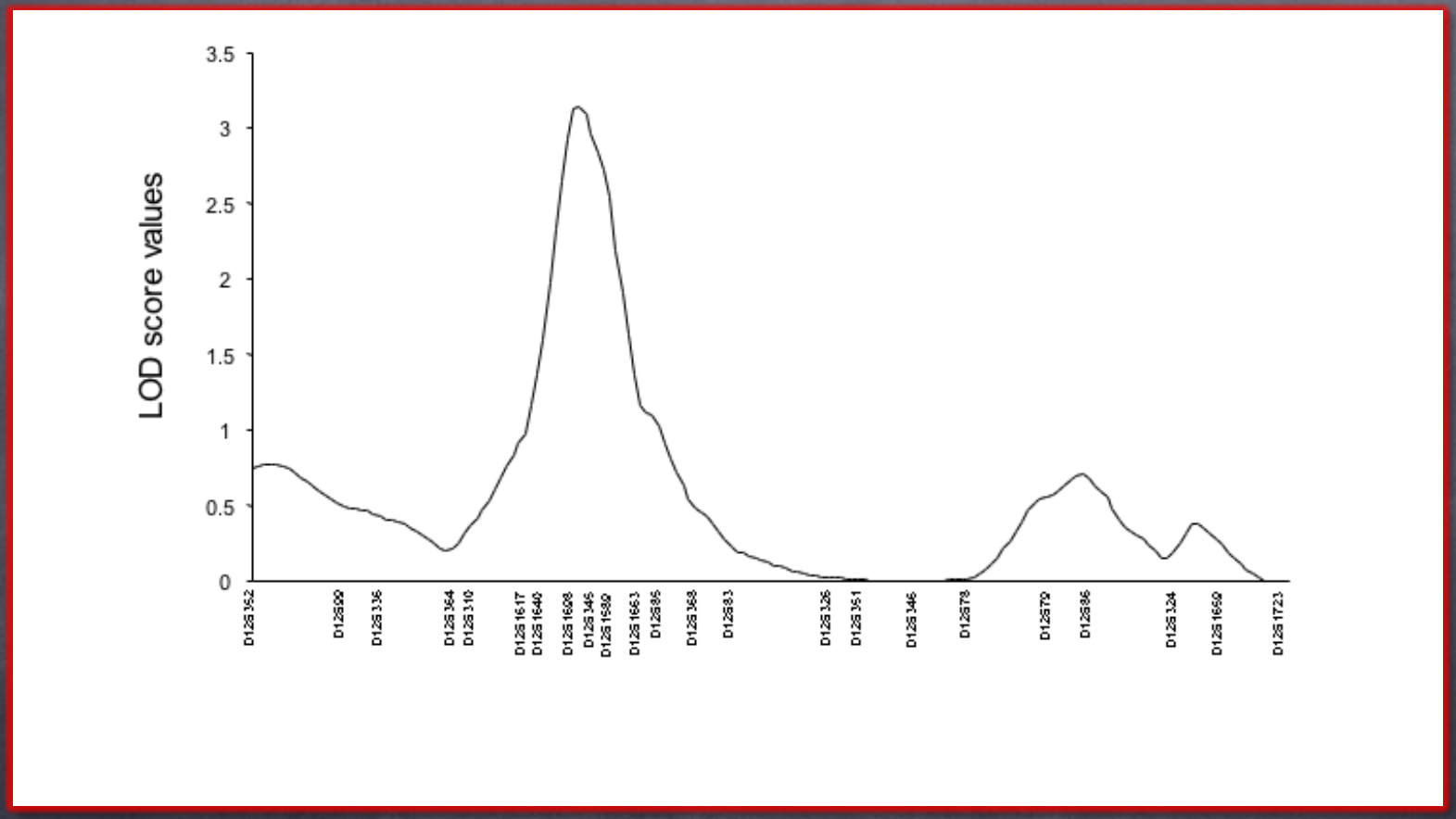


- If marker & QTL (telomere length) are *not* linked, siblings will not differ significantly in length, regardless of the number of shared alleles (red line).
- However, if the marker *is* in linkage, the difference in telomere length between siblings will decrease as they share more alleles (blue line).

Telomere length is heritable



A locus on chromosome 12 determines mean telomere length in humans



The American Journal of Human Genetics

Vasa-Nicotera et al. Am J Hum Genet 2005.

BRIEF COMMUNICATIONS

nature genetics

Common variants near *TERC* are associated with mean telomere length

Veryan Codd[1,11], Massimo Mangino[2,11], Pim van der Harst[1,3,11], Peter S Braund[1], Michael Kaiser[1], Alan J Beveridge[1], Suzanne Rafelt[1], Jasbir Moore[1], Chris Nelson[1], Nicole Soranzo[2,4], Guangju Zhai[2], Ana M Valdes[2], Hannah Blackburn[4], Irene Mateo Leach[3], Rudolf A de Boer[3], Masayuki Kimura[5], Abraham Aviv[5], Wellcome Trust Case Control Consortium[10], Alison H Goodall[1], Willem Ouwehand[6], Dirk J van Veldhuisen[3], Wiek H van Gilst[3], Gerjan Navis[7], Paul R Burton[8], Martin D Tobin[8], Alistair S Hall[9], John R Thompson[8], Tim Spector[2,11] & Nilesh J Samani[1,11]

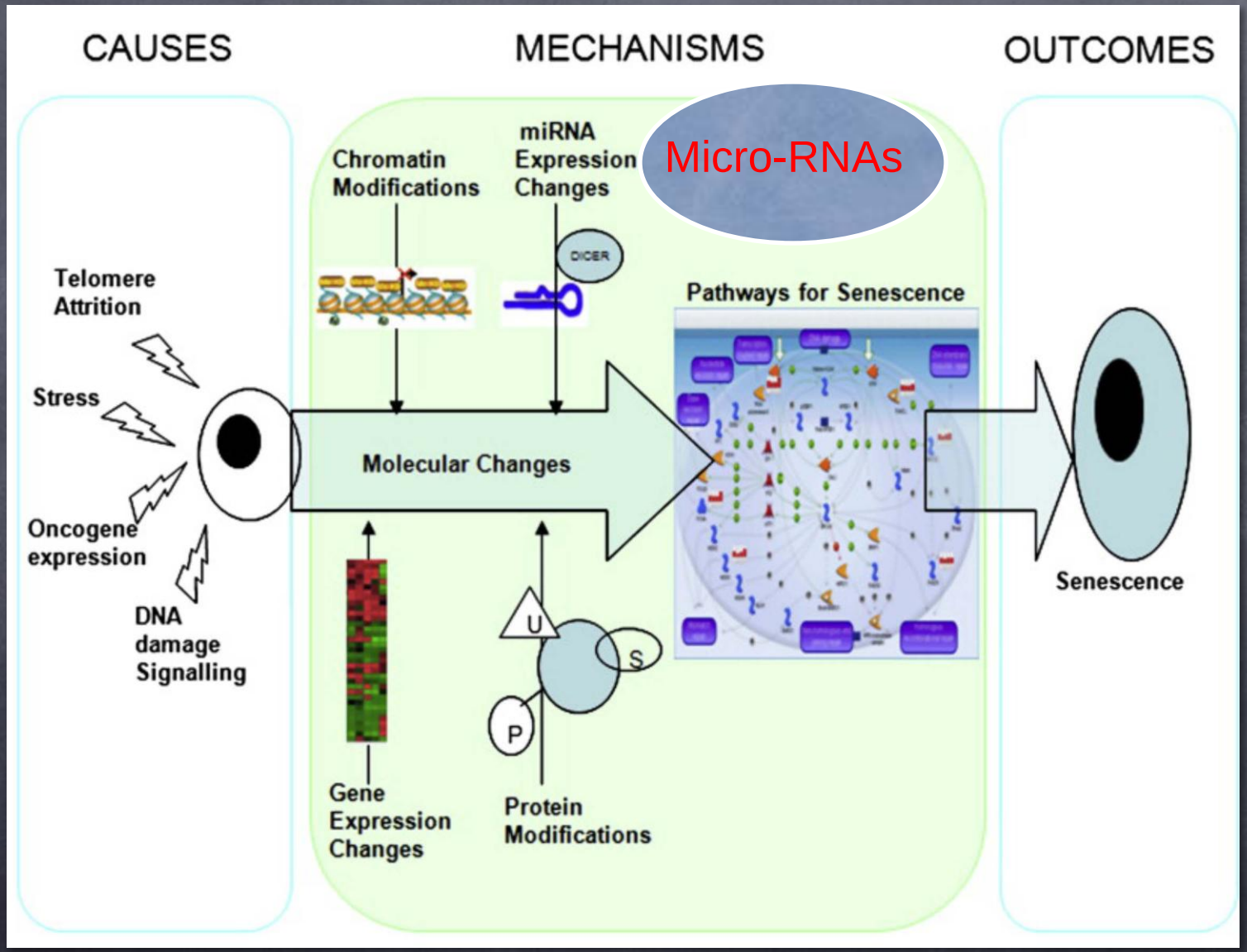
to human chromosomes 3p26.1, 10q26.13, 12q12.22 and 14q23.2 (refs. 3–5). A recent genome-wide association study (GWAS) identified associations of two SNPs on chromosome 18q12.2 with telomere length, although the associations were not at a genome-wide significant level[6]. To identify additional variants that affect telomere length, we undertook genome-wide association analyses in two large European cohorts followed by replication of promising signals in three further European cohorts.

The discovery cohorts comprised 1,487 individuals with coronary artery disease from the British Heart Foundation Family Heart Study (BHF-FHS)[7] and 1,430 United Kingdom Blood Service donors (UKBS)[8] for whom there was genome-wide SNP genotype data available that was generated using the Affymetrix 500K array as part of the Wellcome Trust Case-Control Consortium (WTCCC) study[8]. Further details of

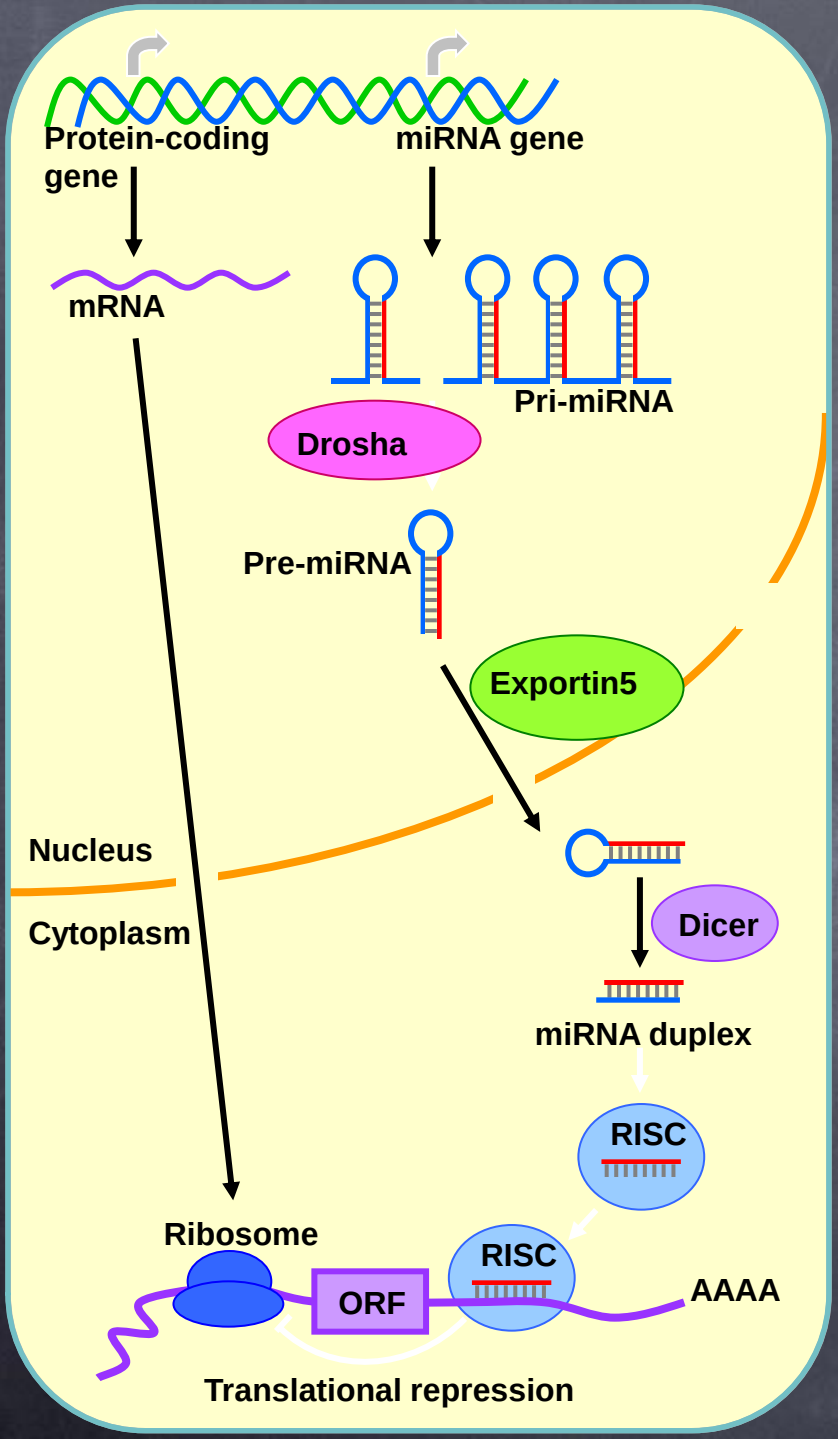
Potential implications of the telomere hypothesis

- Individuals with shorter telomeres have an increased risk of CHD
- CHD risk associated with shorter telomeres is AT LEAST AS GREAT as that with conventional risk factors
- Global structural property of DNA that is inherited i.e. telomere length, may at least partly explain the familial basis
- Variation in telomere length, may explain, in part, the variable age of onset of CHD

Novel mechanisms in modulation of cellular senescence

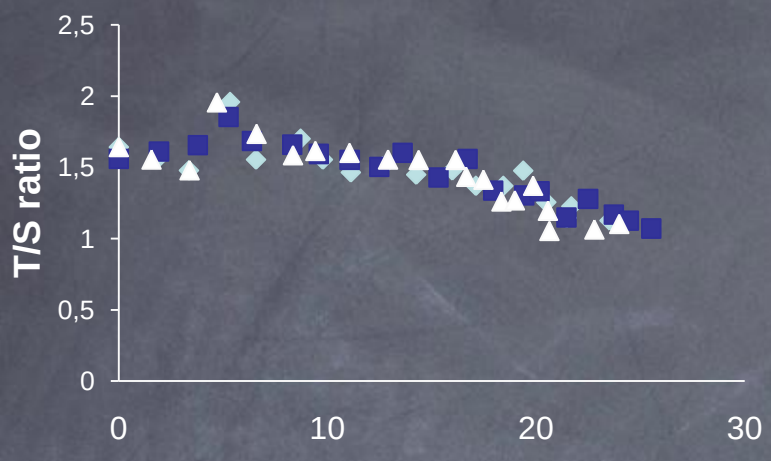


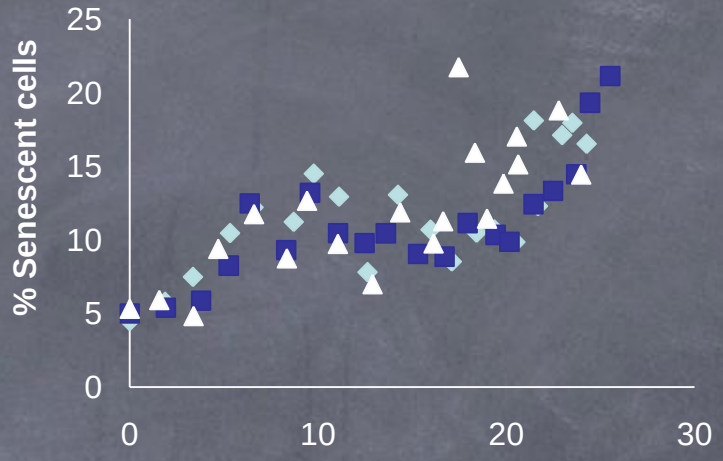
Micro-RNAs

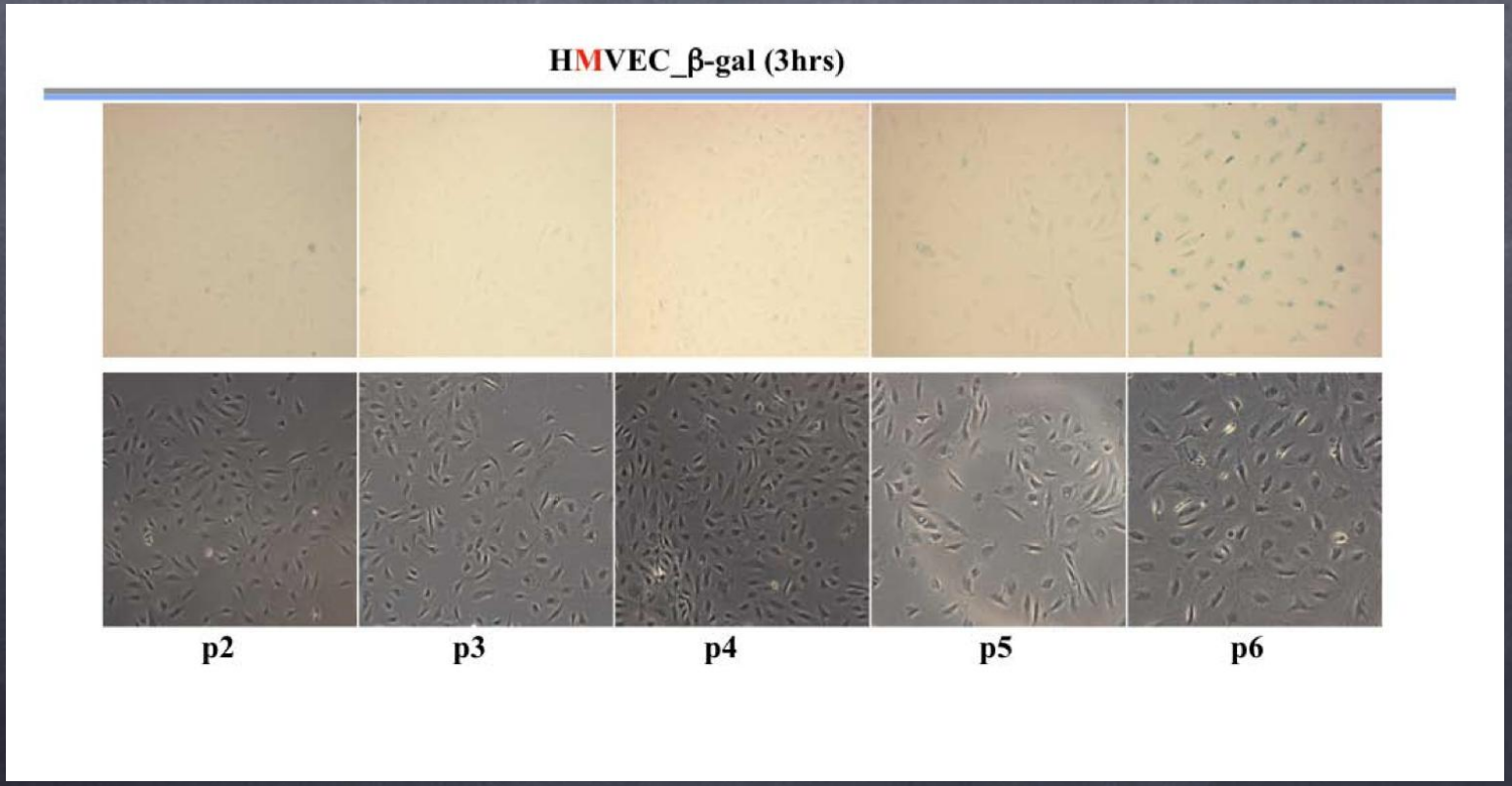


- Short (19-22nt) endogenous non-coding RNA molecules.
- Transcribed from RNA polymerase II promoters.
- Regulate protein expression through interaction with the 3'UTR of target mRNA
- Repression of protein expression or promotion of target mRNA degradation

Model of *in vitro* EC senescence

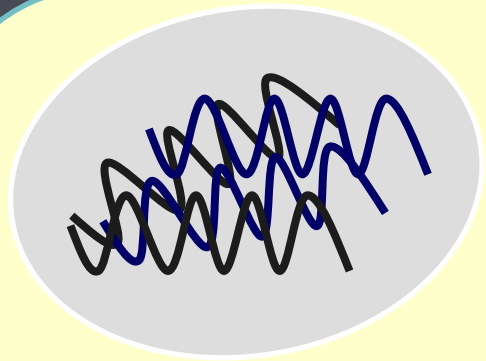




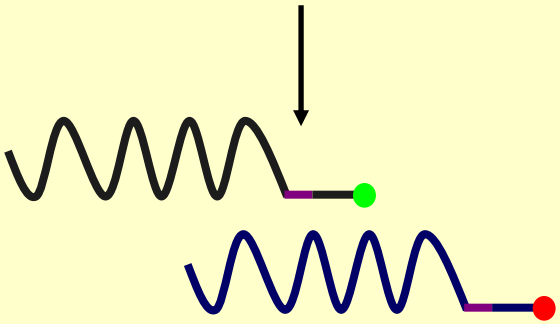


miRNA arrays

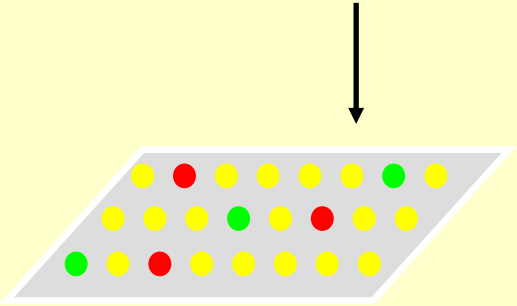
Method



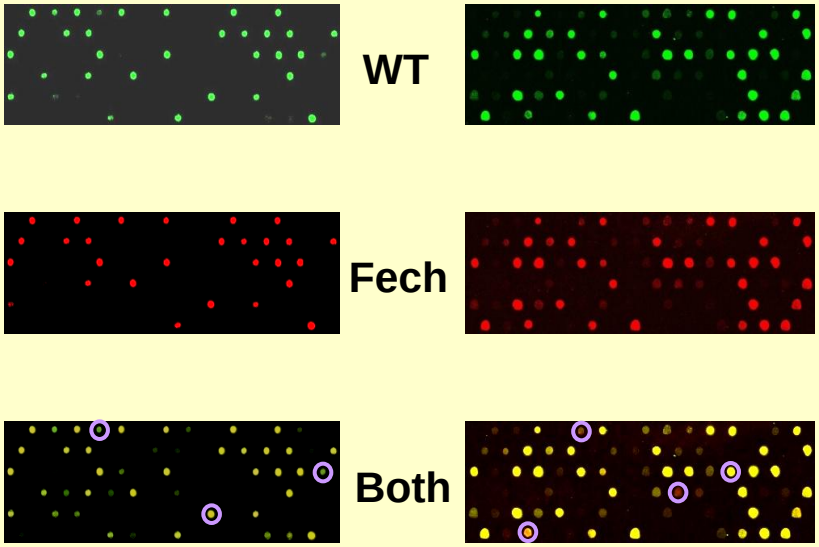
Extract RNA (Tri Reagent, Sigma)



Label RNA (*flash*Tag™ kits, Genisphere)



Hybridise on arrays printed in-house (miRCURY™ LNA probes, Exiqon)



Increased expression

Decreased expression

No change

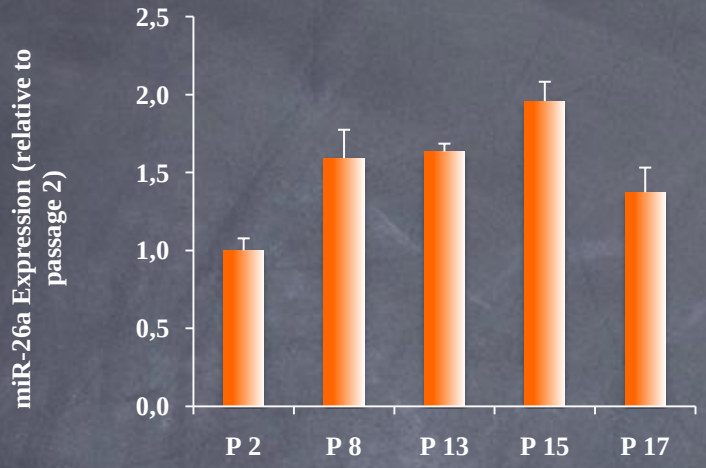
Example of a miRNA array

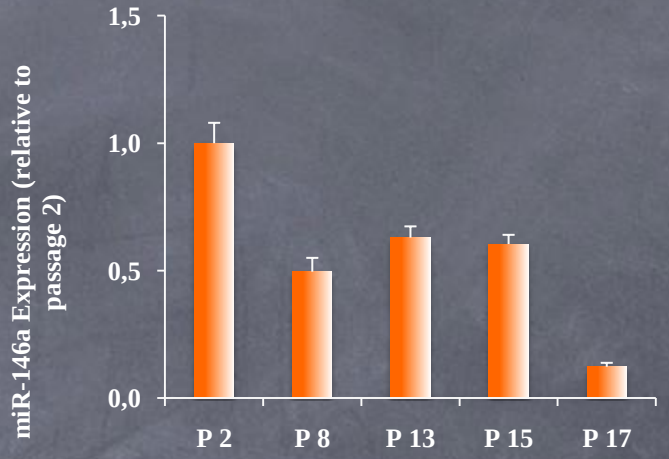
Potential miRNA targets

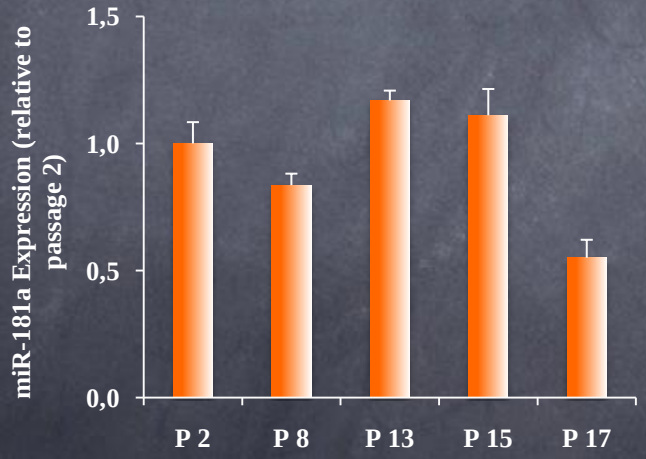
Name	fold change	p value
hsa-miR-26a	1.16136	0.00169
hsa-miR-181a	1.31859	0.00267
hsa-miR-769-3p	1.45341	0.00105
hsa-miR-221	1.52227	0.00484
has-miR-744	1.76016	0.00062
hsa-miR-146a	0.86406	0.00261
hsa-miR-92b	0.72013	0.04975
hsa-miR-200a	0.77659	0.04672
hsa-miR-200b	0.80369	0.02005
hsa-miR-217	0.79781	0.01839

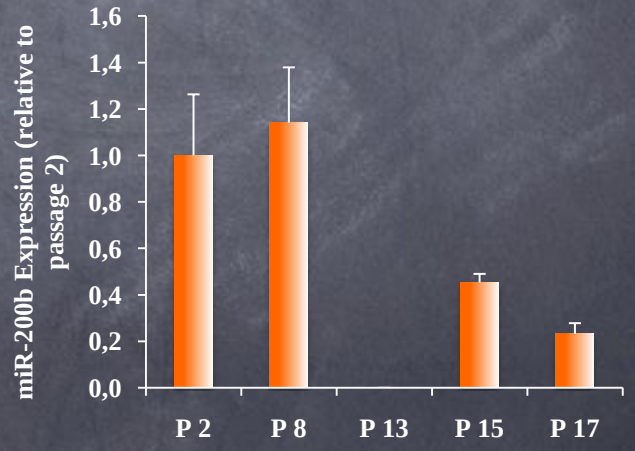
Unpublished data

Validation of selected miRNA









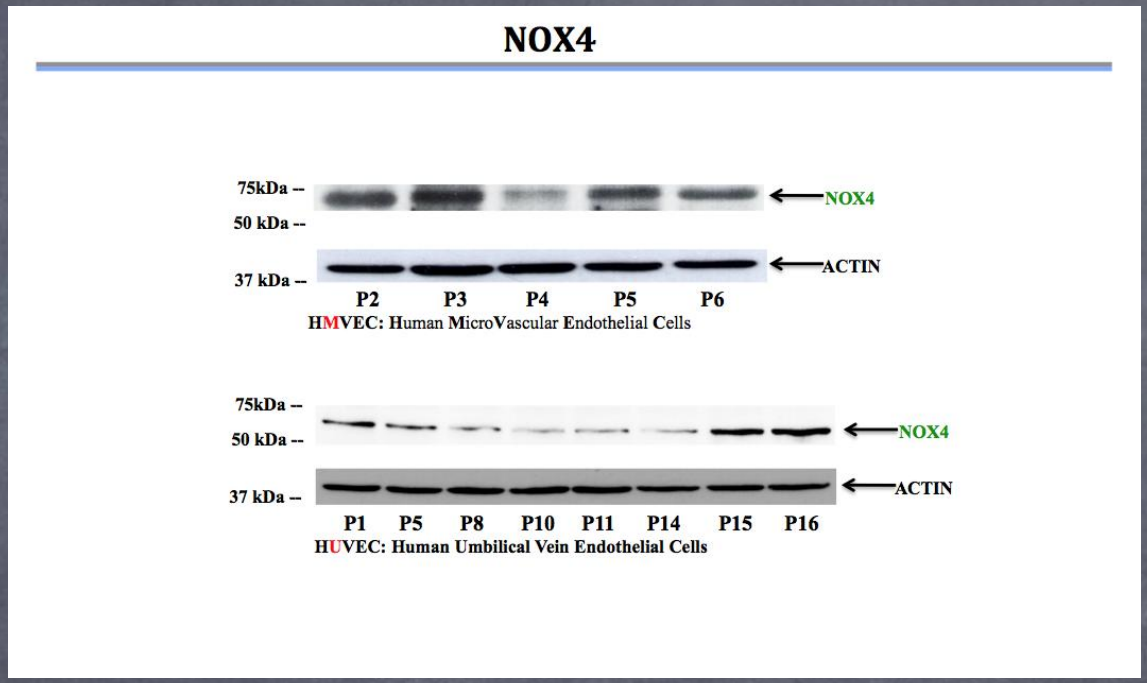
HUVEC

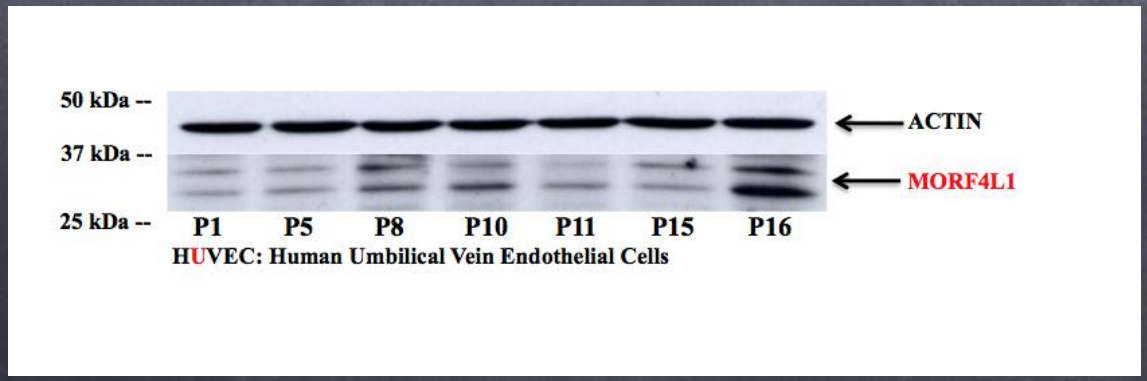
Human **U**mbilical **V**ein **E**ndothelial **C**ells

Potential Target Proteins

miR	Potential Targets	Express in	Function	Select
has-miR-146a	NOX4	HUVEC	Cell senescence	Yes
has-miR-181a	AKT3	HUVEC(Abcam); 3T3- cells, MEFs	Development & Cells Aging	Yes
has-miR-181a	MAPK1	HUVEC	Cell proliferation & apoptosis	Yes
has-miR-221	MORF4L1	Hela cells …….	Cell senescence	Yes. Check the expess in HUVEC
has-miR-26a	CDK6	HEK293 HDFs	Cell Senescence	Yes. Check the expess in HUVEC

Initial expression profile of target proteins





“Every man desires to live long, but no man wishes to be old.”

Jonathan Swift, author & satirist, 1667-1745.

ACKNOWLEDGMENTS

Nilesh J Samani

Scott Brouilette
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