The Aging Process and Epigenetics: Relationship with OA

Louise N Reynard

louise.reynard@ncl.ac.uk

Newcastle University

NHS National Institute for Health Research

Arthritis Research UK

Providing answers today and tomorrow

D-BOARD

CIMA

Talk outline

- **Background - epigenetic alterations as a hallmark of aging**
- **Epigenetic changes in the aging joint - review of the current literature**
- **Is cartilage aging accelerated in OA cartilage?**
- **Identification of DNA methylation changes that occur in aging cartilage and OA**
- **Conclusions**

The Hallmarks of Aging

Cell

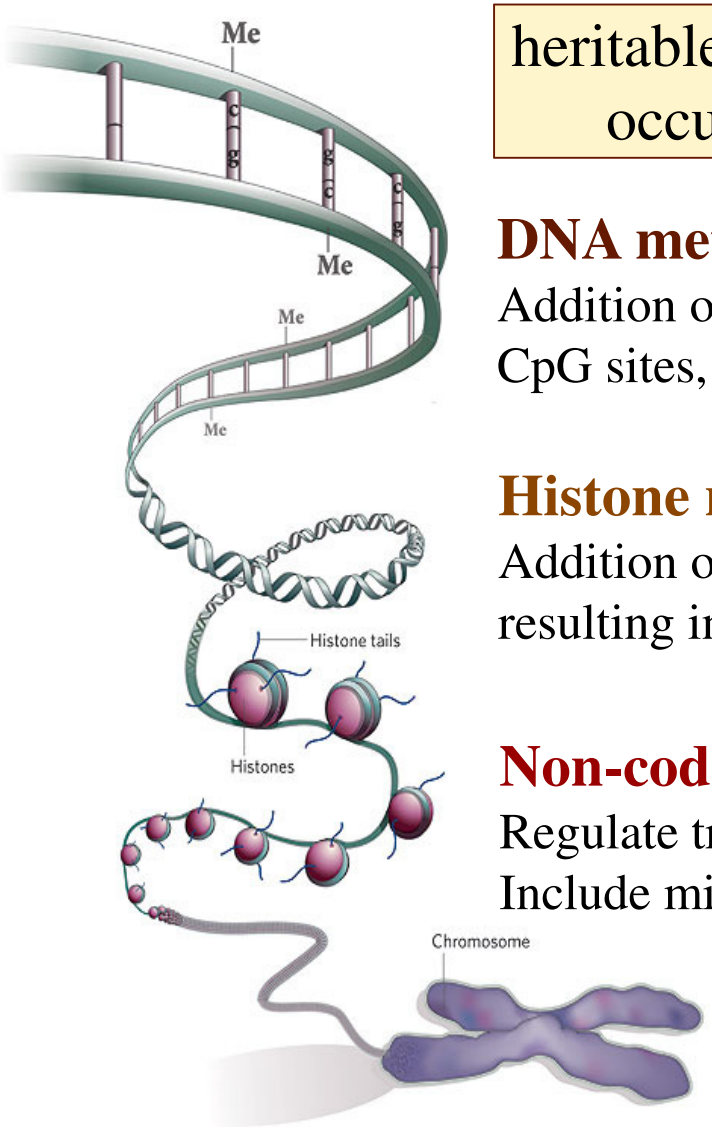
The Hallmarks of Aging

Leading Edge
Review

Carlos López-Otín,[1] Maria A. Blasco,[2] Linda Partridge,[3,4] Manuel Serrano,[5,*] and Guido Kroemer[6,7,8,9,10]

DNA methylation
Chromatin marks
non-coding RNAs

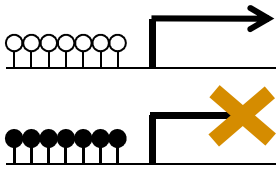
What is epigenetics?



heritable changes in gene expression/phenotype that occur without changes in the DNA sequence

DNA methylation

Addition of methyl groups to CpG sites, altering TF binding



transcription

repression

Histone modifications

Addition of molecules to the 'tails' of histones, resulting in changes to chromatin accessibility

Non-coding RNAs

Regulate transcription, splicing and translation.
Include microRNAs and long non-coding RNAs e.g. *XIST*

What epigenetic changes occur with aging?

DNA methylation

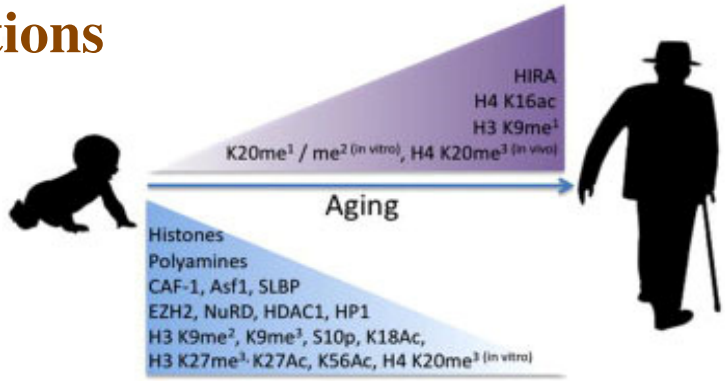
- Genome-wide demethylation (global hypomethylation)
- Promoter-specific increase in methylation (local hypermethylation)

e.g. *OP1/BMP7* in cartilage

Histone modifications

- Changes in histone modifying enzymes
- Global changes in levels of specific histone modifications e.g. H3K9me, H4K16ac
- Changes to specific gene promoters

e.g *NFAT2C* in mouse cartilage



Feser and Tyler, *FEBS Lett. 2011*

Non-coding RNAs

- Altered miR-320c, miR-193b, miR-199a-3p in human cartilage with age
- Increased miR-21 in aged horse cartilage

Hypothesis

Age-related loss of normal epigenetic modifications is a possible mechanism for the late onset of common human diseases *Bjornsson et al., 2004*

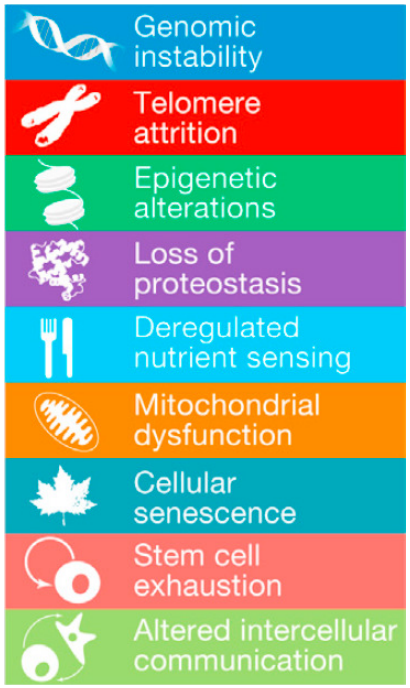
Talk outline

- Background - epigenetic alterations as a hallmark of aging
- **Epigenetic changes in the aging joint - review of the current literature**
- Is cartilage aging accelerated in OA cartilage?
- Identification of DNA methylation changes that occur in aging cartilage and OA
- Conclusions

Do epigenetic alterations occur in the aging joint?

The Hallmarks of Aging

López-Otin., et al 2013



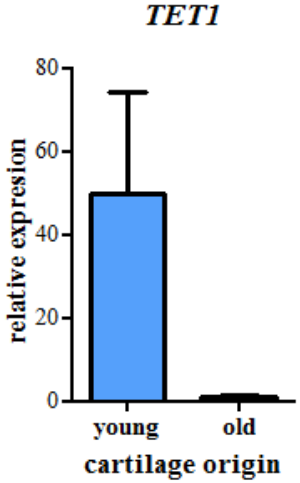
Epigenetic alterations are one of the hallmarks of aging

The other hallmarks of aging occur in the aging joint and in OA

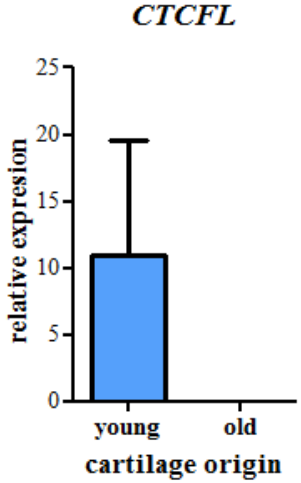
So what about epigenetic alterations?

Expression studies suggest epigenetic changes do occur

RNAseq analysis of cartilage from
- young horses (4yrs)
- old horses (>15yrs)[1]



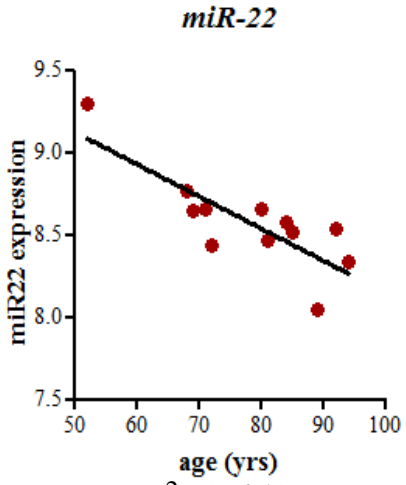
P=0.0008



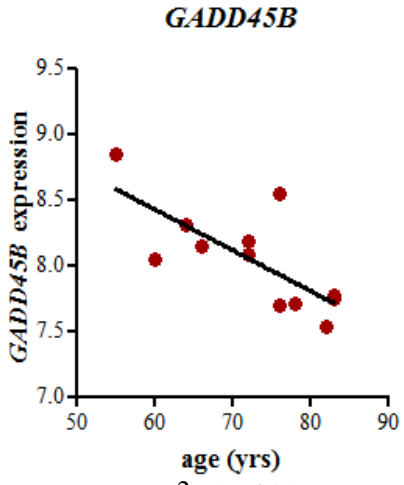
P=0.0017

HORSE[1]	gene	function
	CTCFL	methylation sensitive transcriptional repressor
	HEY2	interacts with histone deacteylase complexes
	HIST4	core nucleosome histone 4
	miR-21	miRNA; targets SMAD7, BMPRII, TGFBII
	POLE2	DNA polymerase subunit
	TET1	catalyses DNA demethylation

Linear regression of microarray data of human control and osteoarthritic hip cartilage[2]



r^2=0.659
P=0.0013



r^2=0.549
P=0.0058

HUMAN[2]	gene	function
	CITED2	transciptional coregulator, interacts with p300
	GADD45B	DNA demethylation
	H2AFJ	core histone H2A
	miR-22HG	miRNA; tagets *HDAC4*, *BMP7*, *PPARA*
	TUG1	long non-coding RNA
	ZBTB16	interacts with histone deacteylase complexes

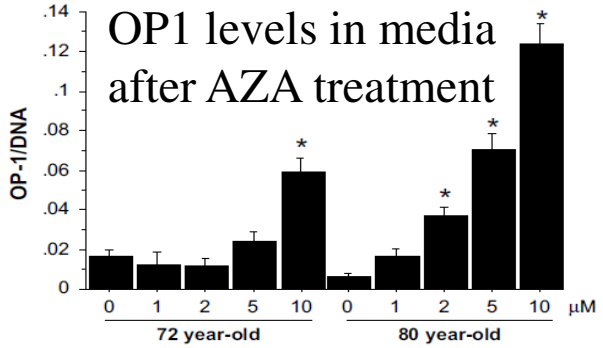
1. Peffers et al 2013; data provided by Mandy Peffers

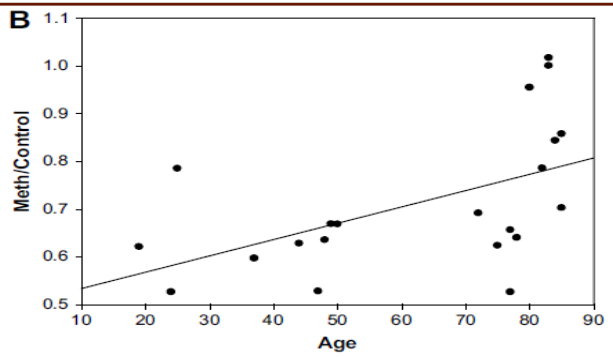
The *BMP7* promoter becomes hypermethylated with age

BMP7/OP-1 transcription is repressed by DNA methylation in chondrocytes

AZA induced demethylation increases OP-1 levels

Loeser et al., 2009





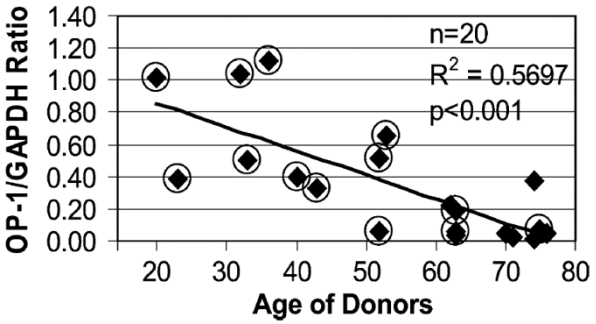
Chondrocyte *BMP7/OP-1* promoter methylation increases with age

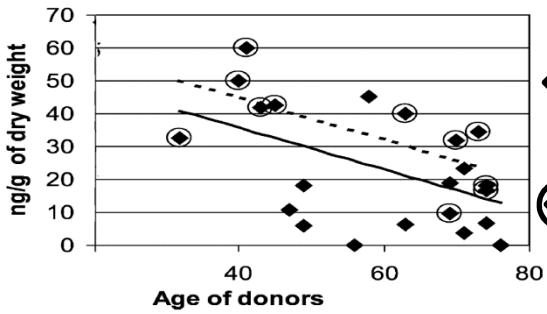
n=22 r^2=0.277 p=0.014

Loeser et al., 2009

BMP7/OP-1 mRNA and protein levels decrease with age

mRNA





protein

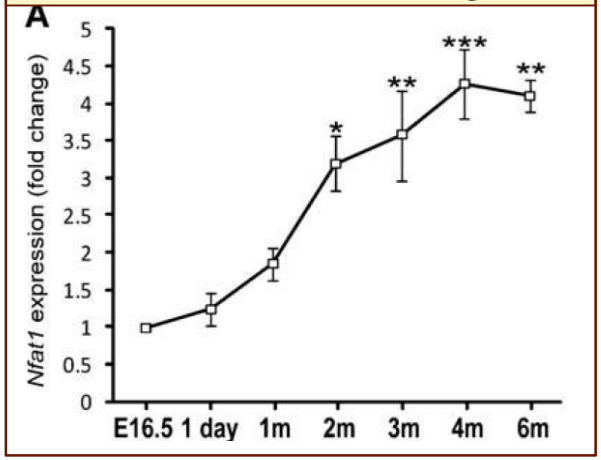
◆ Cartilage
R^2=0.245, p<0.02

◈ Normal cartilage
R^2=0.479, p<0.02

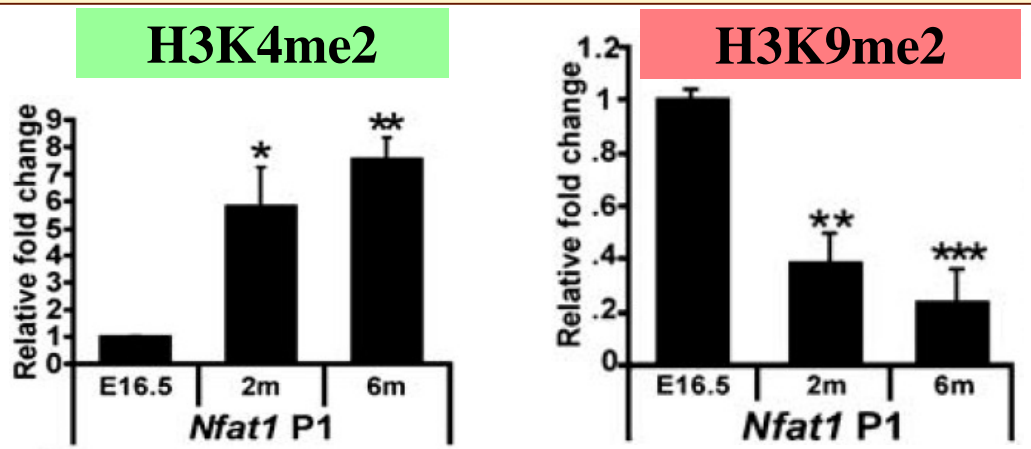
Chubinskaya et al., 2002

Age-dependent expression of *NFAT2C* in mouse cartilage is regulated by dynamic histone methylation

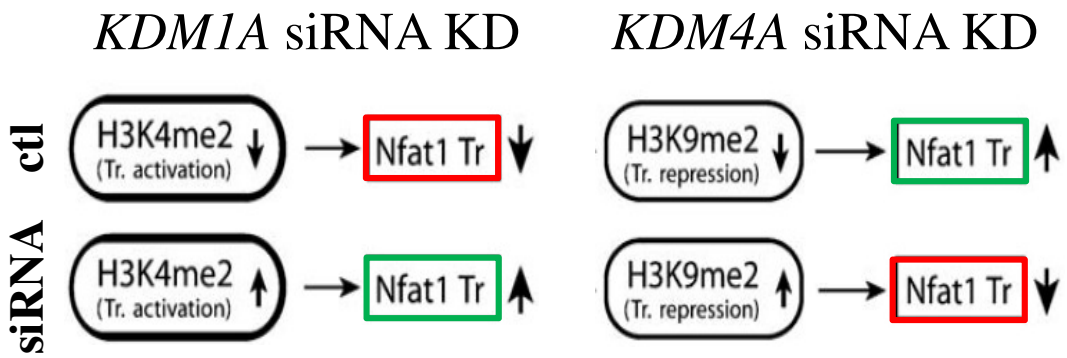
NFAT2C expression increases with age



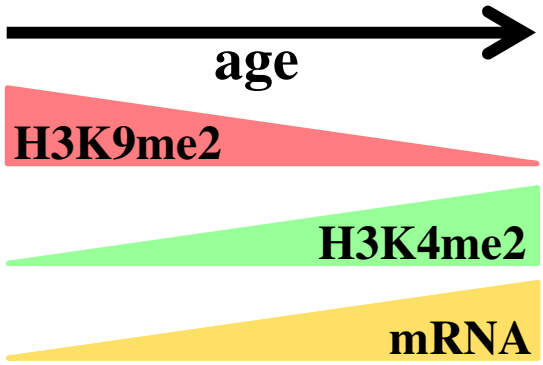
NFAT2C promoter H3K4me2 increases and H3K9me2 decreases with age



H3K4me2 and H3K9me2 regulate *NFAT2C*



NFAT2C promoter



Rodova et al., 2011

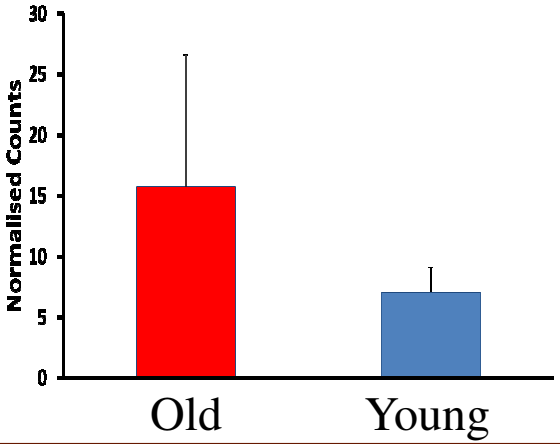
miR-21 is up regulated in cartilage with age and in OA

miR-21 increases with age

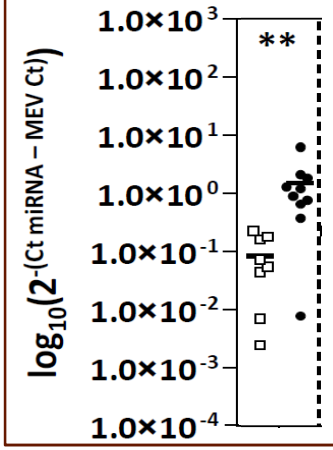
RNA-seq of normal cartilage from

- 4 young horses (4yrs)
- 4 old horses (>15yrs)

Peffers et al., 2013
Transcriptomic signatures in cartilage ageing



miR-21 increases in OA



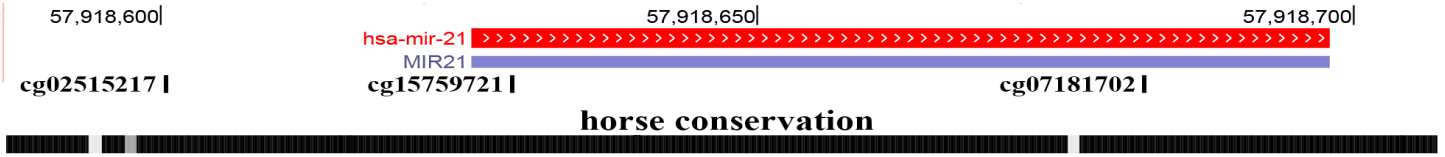
Human hip cartilage

- Control
- OA

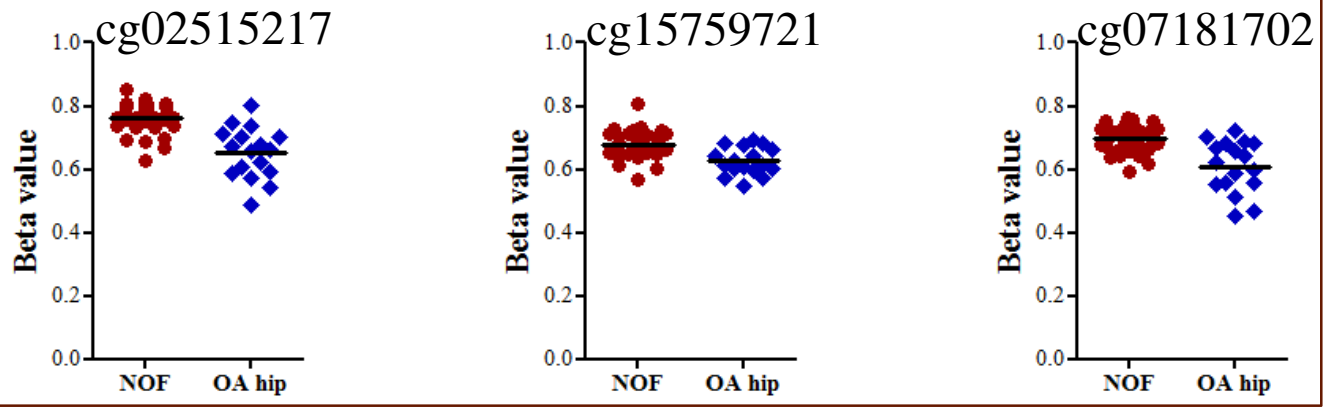
Hannah Elliott
David Young
unpublished

The *miR-21* locus is demethyated in human OA hip cartilage

***miR-21* locus**



- Control hip n=42
- OA hip n=17



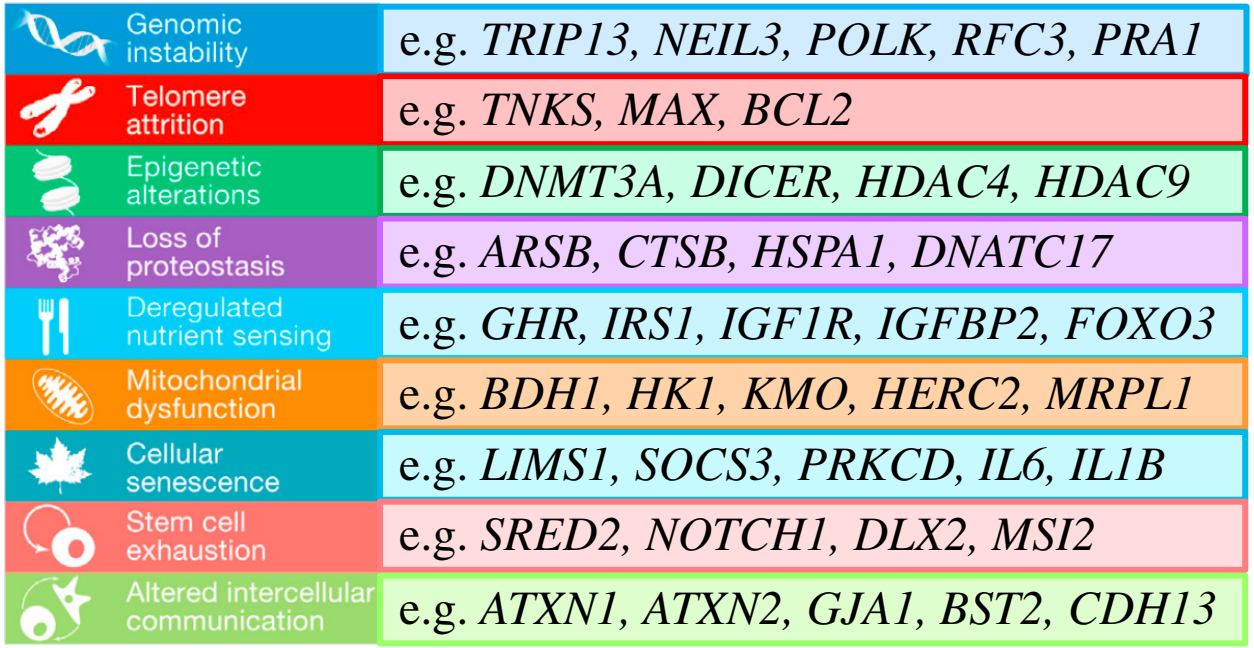
Talk outline

- Background - epigenetic alterations as a hallmark of aging
- Epigenetic changes in the aging joint - review of the current literature
- **Is cartilage aging accelerated in OA cartilage?**
- Identification of DNA methylation changes that occur in aging cartilage and OA
- Conclusions

Some genes involved in aging processes have altered methylation in OA cartilage

Whole genome methylation analysis using the Illumina Infinium Human Methylation450 BeadChip

- measures methylation at ~480,000 CpG sites throughout genome
- Identified differentially methylated CpGs between control hip (n=42) and OA hip cartilage (n=17)#



Suggests the aging process may be altered in OA cartilage

#10% methylation difference, Benjamini-Hochberg adjusted p value <0.01

DNA methylation can predict age

Multi-tissue predictor of age developed using 8000 samples from 82 methylation datasets of 51 healthy tissues and cell types

Methylation level of 353 CpG sites form an 'aging clock' that predict age

Horvath *Genome Biology* 2013, **14**:R115
http://genomebiology.com/2013/14/10/R115

Genome Biology

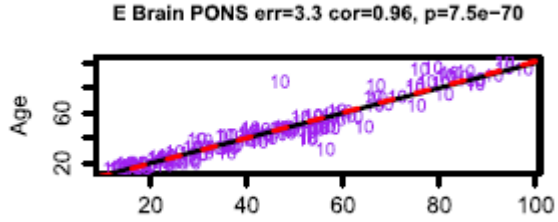
RESEARCH Open Access

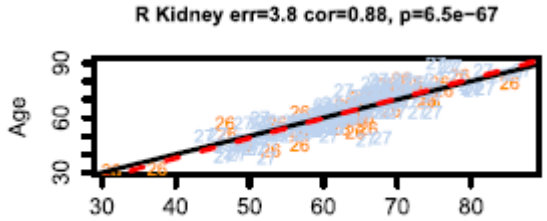
DNA methylation age of human tissues and cell types

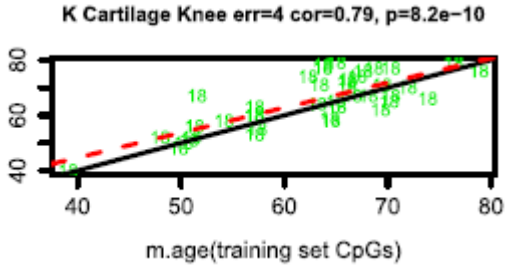
Steve Horvath[1,2,3]

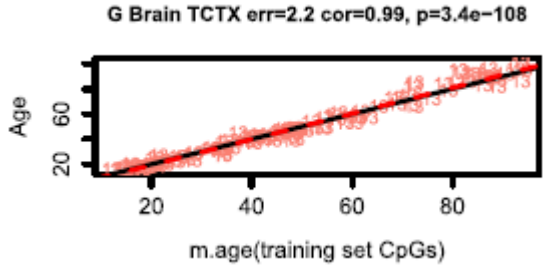
Biological processes clock CpGs are involved in

- Cell Death and Survival
- Cellular Growth and Proliferation
- Lipid Metabolism
- Molecular Transport
- Small Molecule Biochemistry
- Immune Cell Trafficking
- Hematological System Development and Function
- Organismal Development
- Embryonic Development
- Tissue Development









Epigenetic aging is accelerated in several diseases

Obesity accelerates epigenetic aging of human liver

Steve Horvath[a,b,1], Wiebke Erhart[c], Mario Brosch[d], Ole Ammerpohl[e], Witigo von Schönfels[f], Markus Ahrens[f], Nils Heits[f], Jordana T. Bell[g], Pei-Chien Tsai[g], Tim D. Spector[g], Panos Deloukas[h,i,j], Reiner Siebert[e], Bence Sipos[k], Thomas Becker[f], Christoph Röcken[l], Clemens Schafmayer[f,2], and Jochen Hampe[d,2]

Aging Cell (2015) **14**, pp491–495

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]

RESEARCH ARTICLE

Acceleration of Age-Associated Methylation Patterns in HIV-1-Infected Adults

Tammy M. Rickabaugh[1☯], Ruth M. Baxter[2☯], Mary Sehl[1,3], Janet S. Sinsheimer[2,3,4], Patricia M. Hultin[5], Lance E. Hultin[1], Austin Quach[2], Otoniel Martínez-Maza[5,6], Steve Horvath[2], Eric Vilain[2‡], Beth D. Jamieson[1‡*]

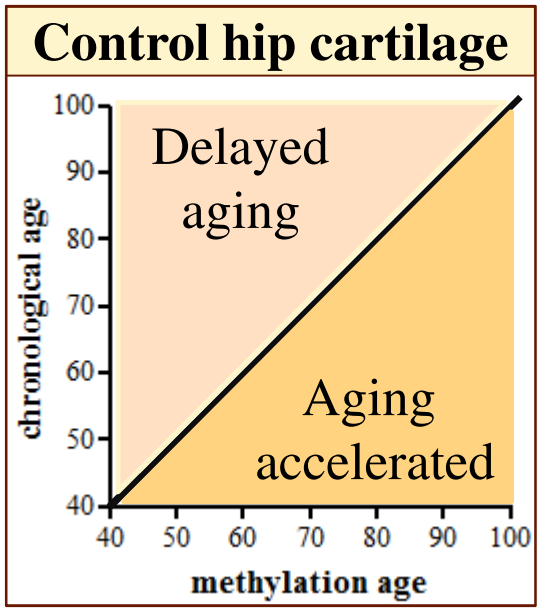
Marioni *et al. Genome Biology* (2015) 16:25
DOI 10.1186/s13059-015-0584-6

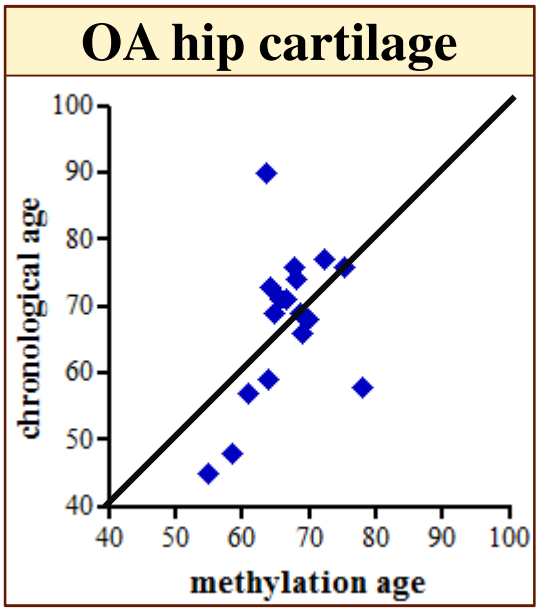
RESEARCH Open Access

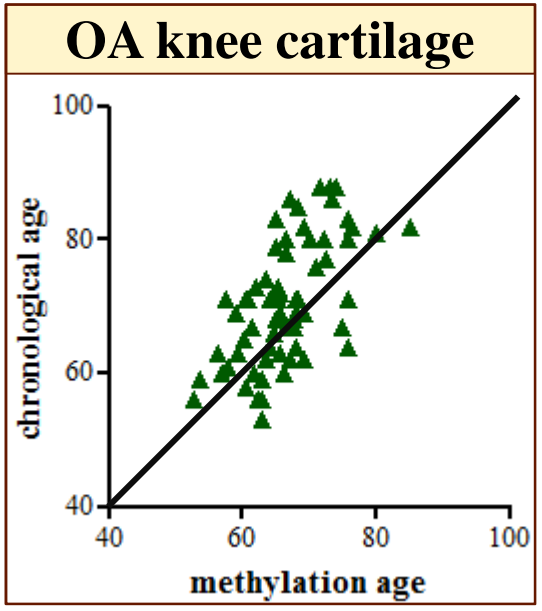
DNA methylation age of blood predicts all-cause mortality in later life

Riccardo E Marioni[1,2,3†], Sonia Shah[3,4†], Allan F McRae[3,4†], Brian H Chen[5,6†], Elena Colicino[7†], Sarah E Harris[1,2],

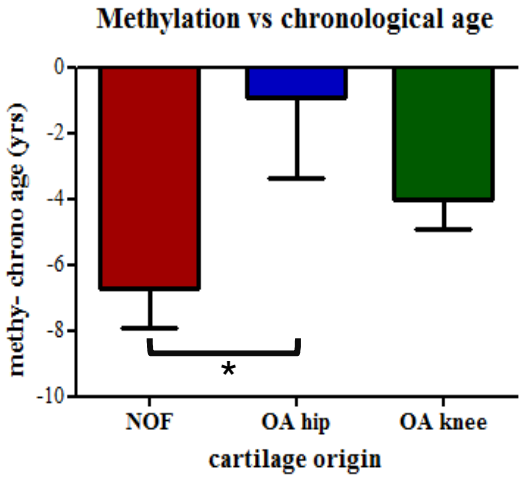
Is aging altered in OA cartilage?

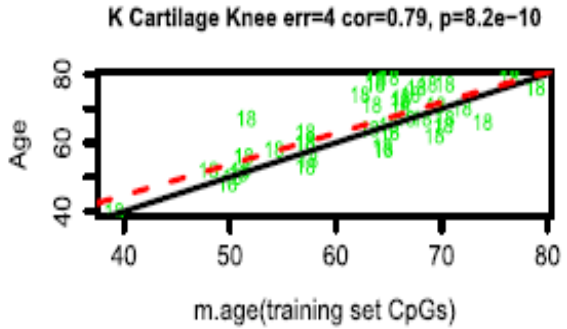




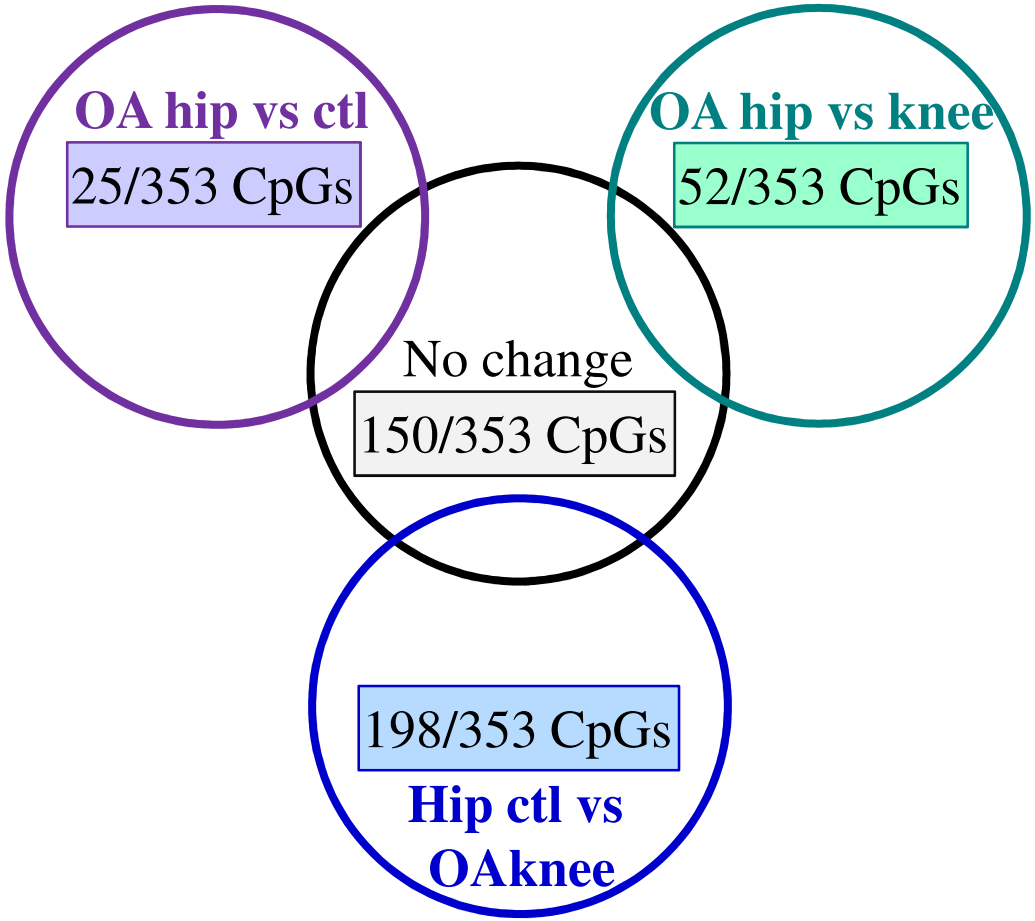


Cartilage type	n number	Actual age	meth age	meth – actual
control hip	42	77.8yrs	71.1yrs	-6.7yrs
OA hip	17	67.5yrs	66.6yrs	-0.9yrs
OA knee	63	70.6yrs	66.5yrs	-4.0yrs





Why does OA hip cartilage have accelerated epigenetic aging relative to normal hip and OA knee cartilage?



There is altered methylation of clock CpG sites in OA hip and knee cartilage (>0.05 beta, p<0.01)

Why does OA hip cartilage have accelerated epigenetic aging relative to normal hip and OA knee cartilage?

OA hip vs hip control

25/353 CpGs
2 hypermethylated in OA
23 hypomethylated in OA

Annotation	P value
cell motility	0.001
cellular physiological process	0.003
axis	0.009
chemotaxis	0.009
regulation of physiological process	0.01
regulation of biological process	0.02
cell differentiation	0.02
regulation of apoptosis	0.03
regulation of programmed cell death	0.03

Hip control vs OA knee

198/353 CpGs
163 hypermethylated in ctl
35 hypomethylated in ctl

Annotation	P value
regulation of physiological process	0.002
regulation of biological process	0.003
positive regulation of physiological process	0.004
apoptosis	0.01
programmed cell death	0.01
cell death	0.02
death	0.02
regulation of nucleic acid metabolism	0.02
regulation of cellular physiological process	0.01
positive regulation of NF-kappaB cascade	0.03
regulation of cellular process	0.03
regulation of metabolism	0.03

OA hip vs OA knee

52/353 CpGs
35 hypermethylated in hip
17 hypomethylated in hip

Annotation	P value
regulation of biological process	0.01
regulation of physiological process	0.01
chromatin modification	0.02
intercellular junction assembly	0.02
regulation of transcription	0.02
regulation of nucleic acid metabolism	0.03
intercellular junction assembly/maintenance	0.03

GATHER gene annotation tool
http://gather.genome.duke.edu/

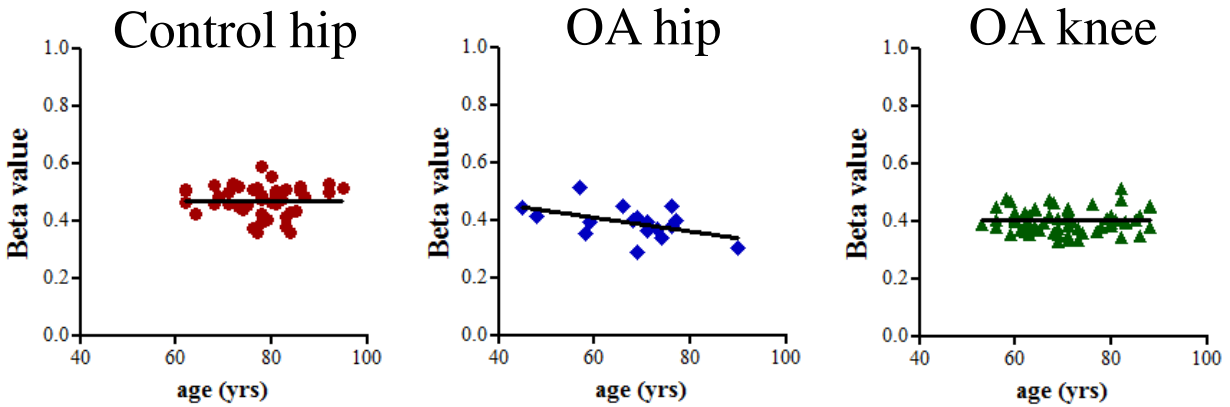
There is altered methylation of clock CpG sites in OA cartilage

Clock CpGs are aberrantly methylated in OA cartilage

cg02388150 in *SFRP1*

Increases with age

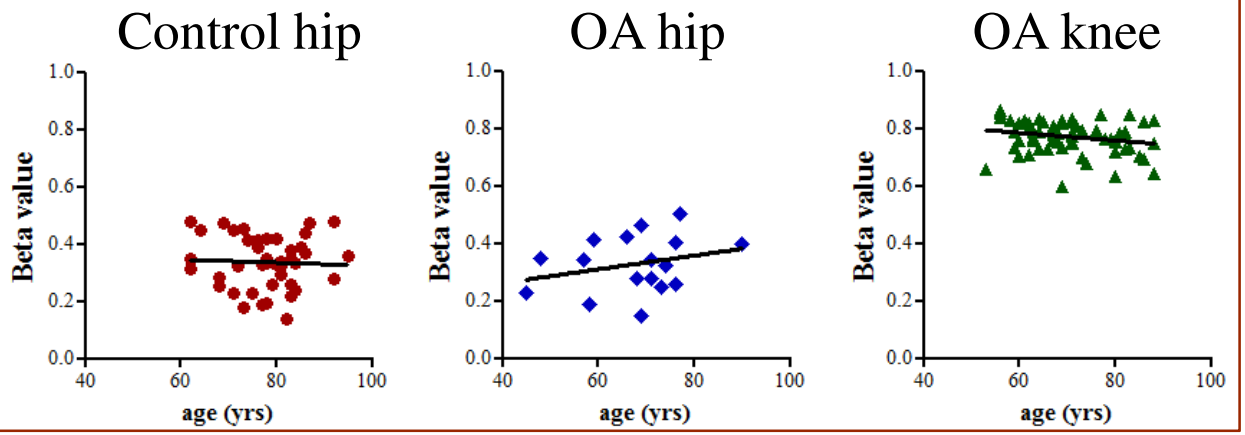
	beta value
Ctl hip mean	0.469
OA hip mean	0.390
OA knee mean	0.402



cg10281002 in *TBX5*

Increases with age

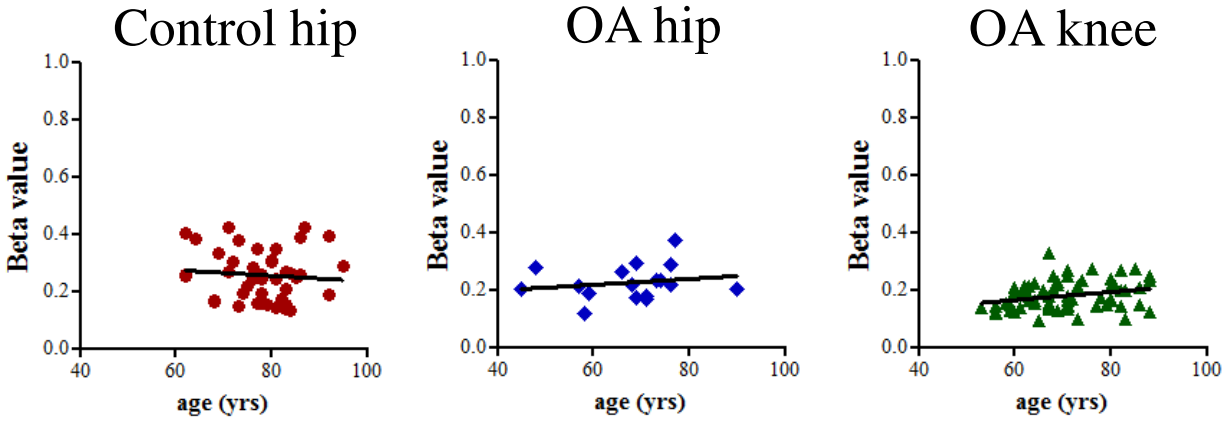
	beta value
Ctl hip mean	0.337
OA hip mean	0.328
OA knee mean	0.772



Age does not affect methylation of several clock CpG sites

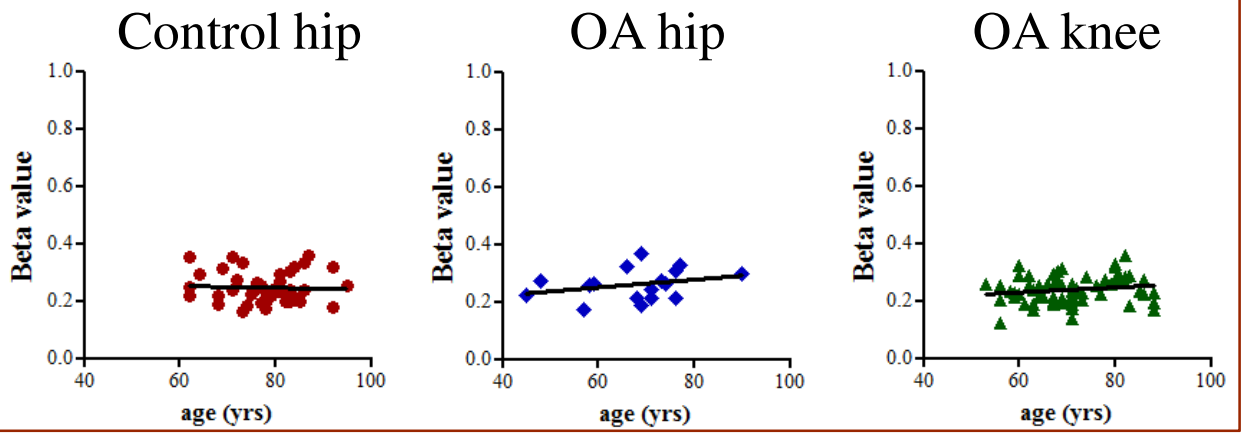
cg2637251 in *TFAP2E*

cg2637251 methylation should **increase** with age



cg01820374 in *LAG3*

cg01820374 methylation should **decrease** with age



Talk outline

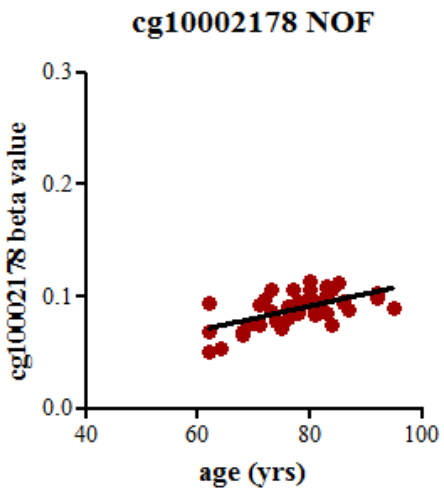
- Background - epigenetic alterations as a hallmark of aging
- Epigenetic changes in the aging joint - review of the current literature
- Is cartilage aging accelerated in OA cartilage?
- **Identification of DNA methylation changes that occur in aging cartilage and OA**
- Conclusions

Does methylation change with age in cartilage?

Identified CpG sites whose methylation changes with age using linear regression of M values for

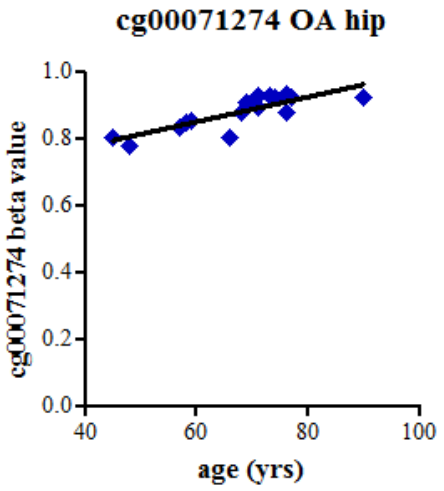
- 42 hip controls samples (aged 62-95yrs)
- 17 OA hip samples (aged 45-90yrs)
- 63 OA knee samples (aged 53-88yrs)

238 control hip CpGs



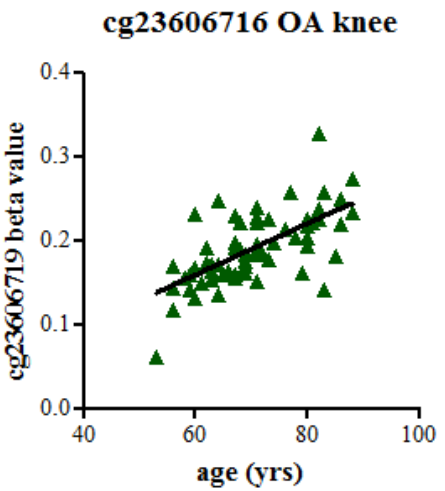
r^2=0.345
P<0.0001

650 OA hip CpGs



r^2=0.692
P<0.0001

556 OA knee CpGs



r^2=0.42
P<0.0001

Majority of CpGs that change with age are cartilage subtype-specific

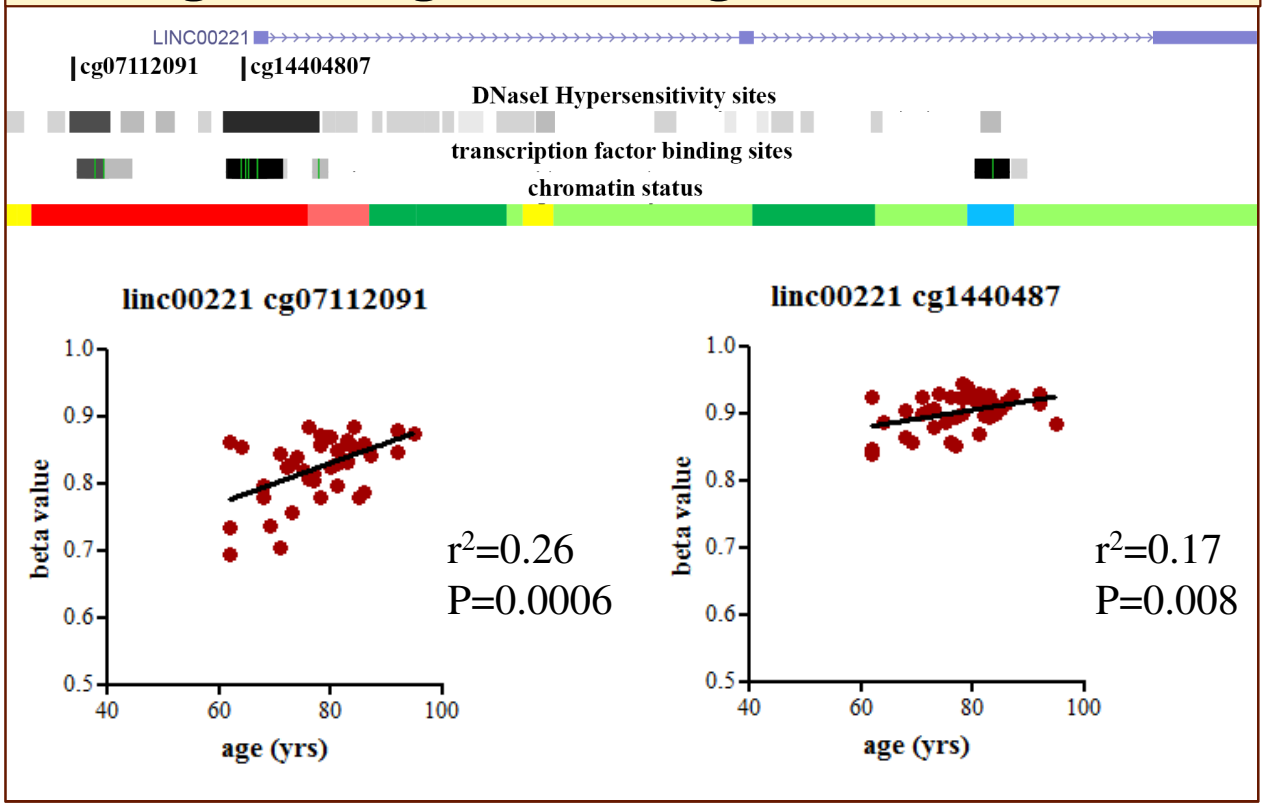
238 CpG sites change with age in normal hip cartilage

CpG sites are enriched in genes involved in organogenesis and ECM-receptor interaction

- 82 CpG sites in 69 genes decrease with age
- 156 CpG sites in 106 genes increase with age

Annotation	p value
Gene ontology	
organogenesis	0.008
organ development	0.008
antigen presentation	0.01
morphogenesis	0.01
germ cell development	0.02
KEGG pathways	
peptidoglycan biosynthesis	0.01
nitrogen metabolism	0.01
Transcription factors	
AP1	0.002
E2F1	0.003
FOXJ2	0.0005
COMP1	0.005
MAZR	0.007
OCT1	0.009
KROX	0.01
NF-kappaB	0.01
CEBPdelta	0.01

e.g. the long non-coding RNA linc00221



GATHER gene annotation tool http://gather.genome.duke.edu/

There are 650 age CpG sites in OA hip cartilage

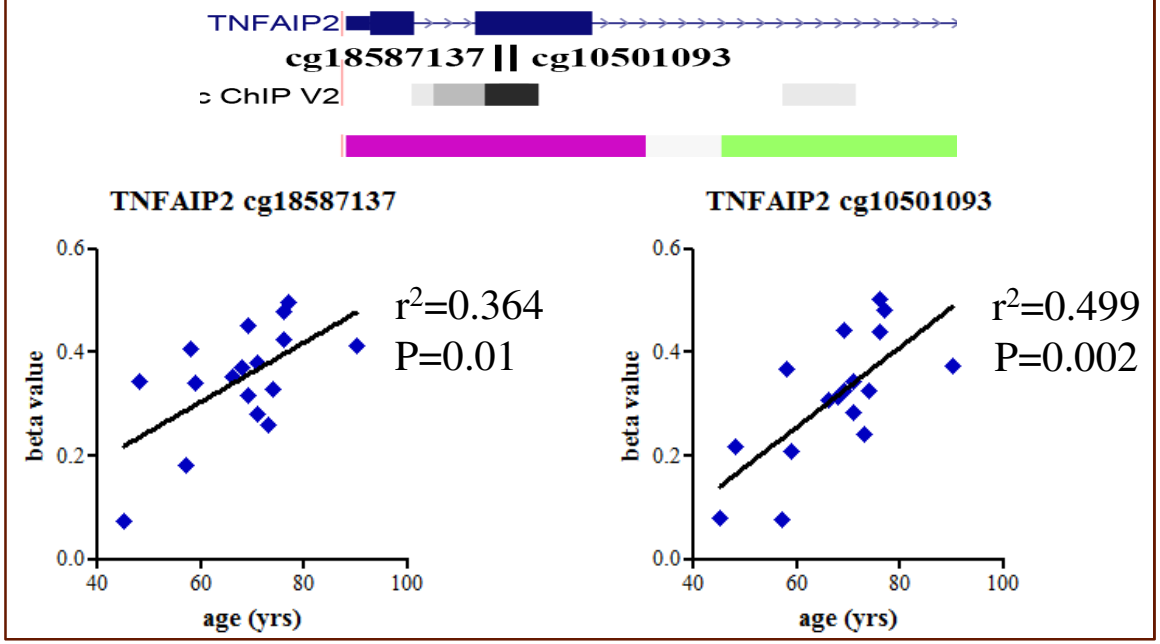
Annotation	p value
Gene ontology	
transcription from PolII promoter	0.0008
negative regulation of transcription	0.003
cytoskeleton organisation	0.006
establishment/maintenance of polarity	0.01
vesicle fusion	0.01
vesicle mediated transport	0.02
protein localisation	0.02
regulation of cellular process	0.03
cofactor catabolism	0.03
Transcription factors	
FOX	<0.0001
PIT1	<0.0001
E2F1	<0.0001
FOXD3	<0.0001
CDC5c-MYC	<0.0001
FOXO4	<0.0001
NFKB	<0.0001
HNF3A	<0.0001
GATA3	0.0001
CEBPdelta	0.0002
MRF2	0.0003
HNF3B	0.0004
SP3	0.0005

GATHER gene annotation tool
http://gather.genome.duke.edu/

- 179 CpG sites in 141genes decrease with age
- 471 CpG sites in 318 genes increase with age

Enriched for genes involved in transcription, cell polarity and cytoskeleton organisation

e.g. TNFα inducible gene *TNFAIP2*

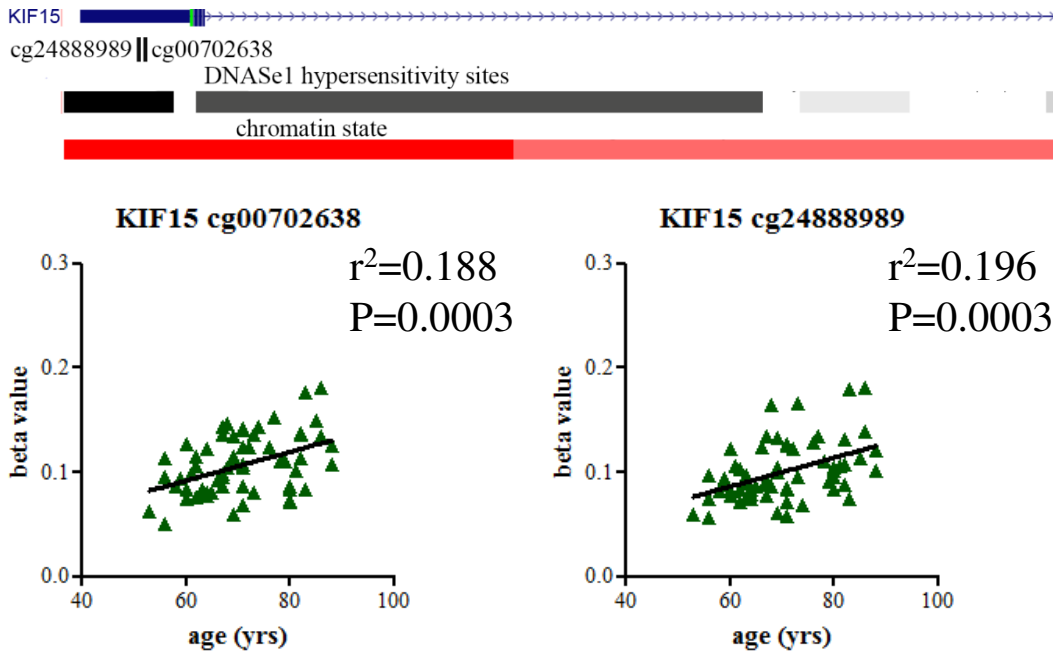


556 CpG sites change with age in OA knee cartilage

- 90 CpG sites in 73 genes decrease
- 466 CpG sites in 325 genes increase

CpG sites are enriched in genes involved in transcription, metabolism and development

e.g. DNA binding protein gene *KIF15*

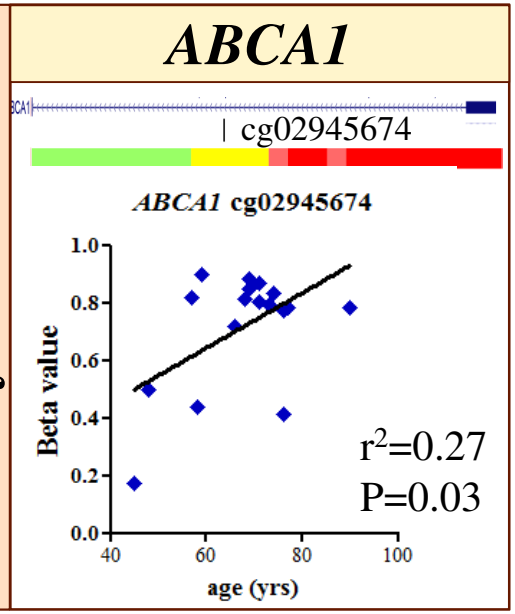


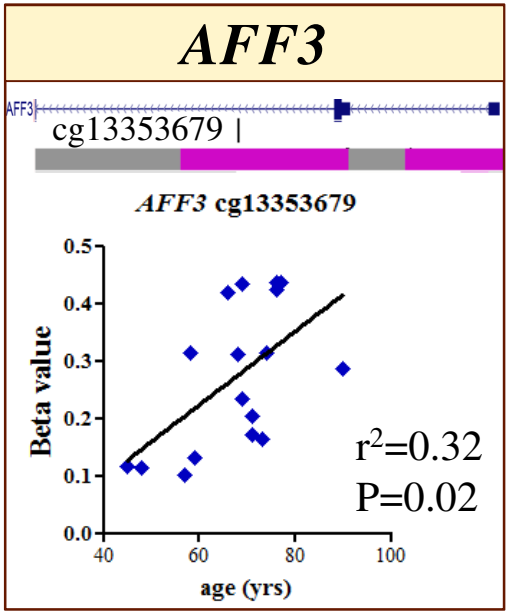
Annotation	p value
Gene ontology	
regulation of transcription	<0.0021
regulation of metabolism	0.0003
development	0.0008
regulation of physiological process	0.0009
morphogenesis	0.003
organogenesis	0.01
unfolded protein response	0.02
Frizzled signalling pathway	0.03
KEGG pathways	
phoshatidylinositol signalling system	0.01
Transcription factors	
KROX	0.0002
E2F1	0.0007
c-Ets-1	0.002
HNF3B	0.004
CDX2	0.004
SP3	0.01
OLF1	0.01
FOXD3	0.01
MAZ	0.02
FOXJ2	0.02
GATA1	0.02
ELK1	0.03
HNF1	0.03

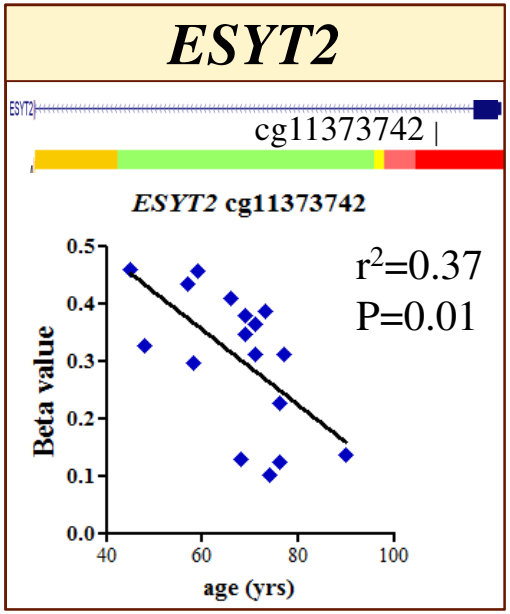
GATHER gene annotation tool
http://gather.genome.duke.edu/

Overlap of genes with age-related methylation and expression changes

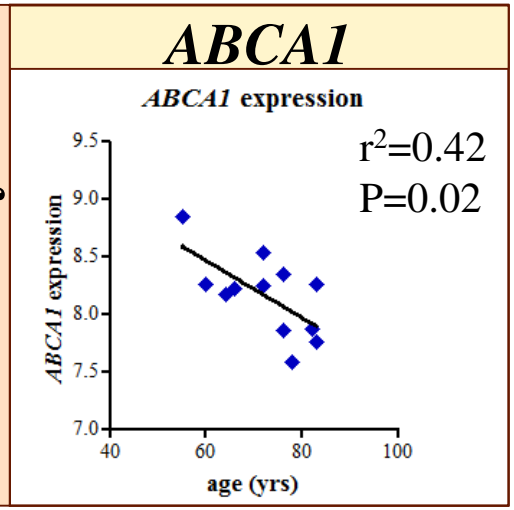
Methylation data

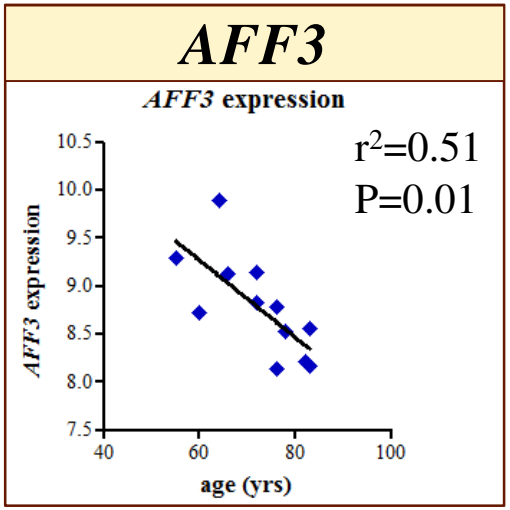


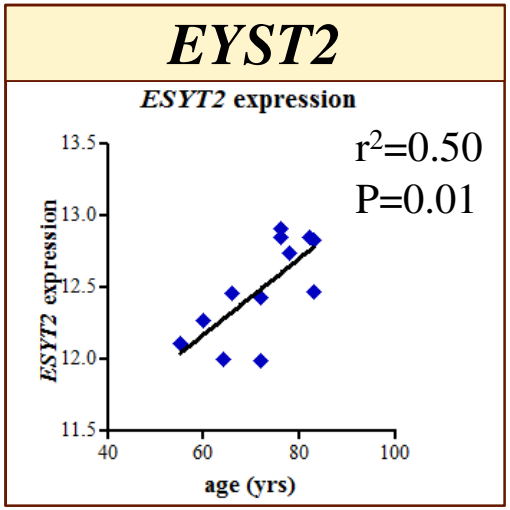




Microarray data







Talk outline

- Background - epigenetic alterations as a hallmark of aging
- Epigenetic changes in the aging joint - review of the current literature
- Is cartilage aging accelerated in OA cartilage?
- Identification of DNA methylation changes that occur in aging cartilage and OA
- **Conclusions**

Conclusions

- **Epigenetic alterations are one of the hallmarks of aging**

- **There are few studies of age-related epigenetic changes in joint tissues**

BUT

- **Gene-specific methylation and chromatin age-related changes have been reported in cartilage, as have changes in expression of specific miRNAs**

- **Normal hip cartilage is epigenetically younger than OA hip cartilage based on the Horvath clock predictor**

- **Methylation at multiple CpG sites change with age in cartilage**

- **CpG sites whose methylation change with age are different between normal and OA cartilage and between hip and knee cartilage.**

Acknowledgments

Andrew J Skelton

David Young

Hannah Elliott

Mandy Peffers, University of Liverpool

Peter Clegg, University of Liverpool

John Loughlin

Bioinformatics Support Unit, Newcastle University

Staff at RVI and Freeman Hospitals, Newcastle