



LEIDEN UNIVERSITY MEDICAL CENTER

Genetic Links Between Development and Osteoarthritis: *DIO2* risk gene

Dept. Medical Statistics and Bioinformatics

Section Molecular Epidemiology

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Genome wide linkage scan

GARP study

GARP study

- 188 sibling pairs + 4 trios
- OA; ACR criteria and radiographs
- Age: 60 yrs (range 43-79)
- Female: 82%

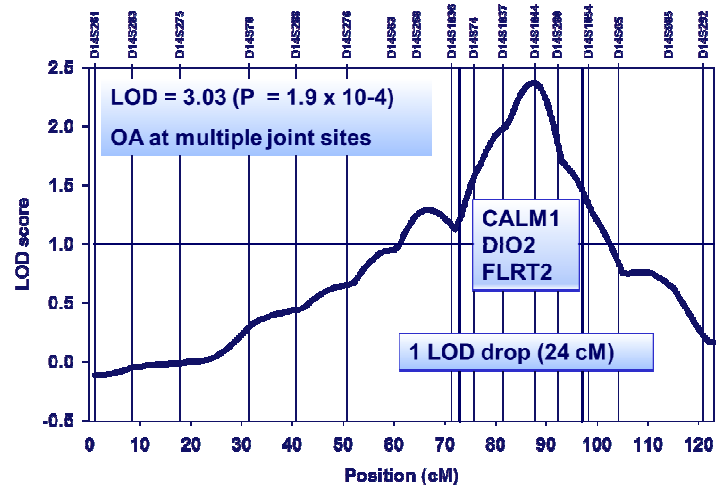
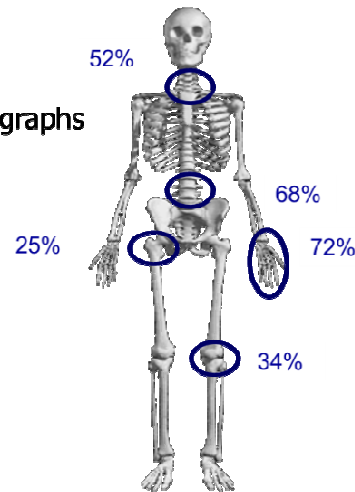
Inclusion:

≥ 2 joints OA

Progression:

2 jr: 100 pairs

5 jr: 200 pairs



Replication female cases severe hip OA

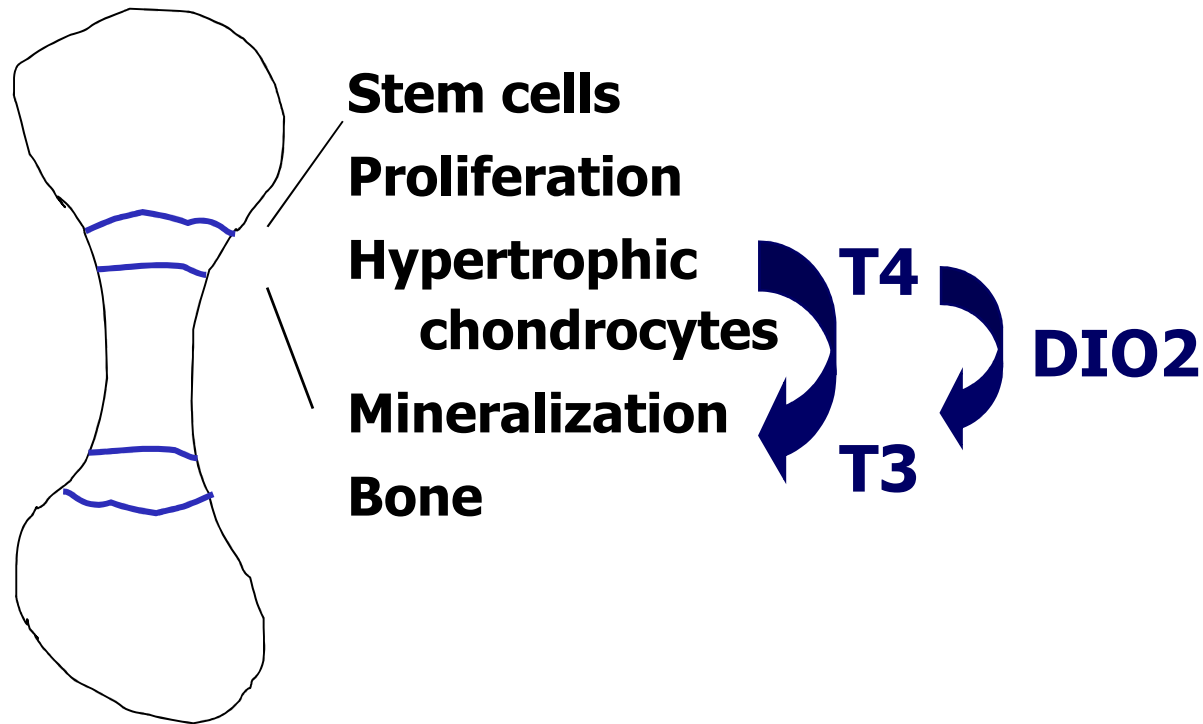
DIO2 haplotype rs12885300-rs225014 C-c

| Gene | OR Recessive model | P of OR | P value heterogeneity test |
|---------------|--------------------|--------------------|----------------------------|
| All* | 1.8 (1.4-2.3) | 2x10 ⁻⁵ | 0.6 |
| UK (Oxford) | 2.1 (1.4-3.2) | 0.001 | |
| NL (R'dam) | 1.9 (1.0-3.5) | 0.040 | |
| Japan (Riken) | 1.5 (1.0-2.3) | 0.047 | |

*Random effect meta-analyses

***DIO2* in growth plate**

Endochondral ossification



T3 triggers terminal maturation of growth plate chondrocytes

Osteoarthritis

Early developmental or age related disease?

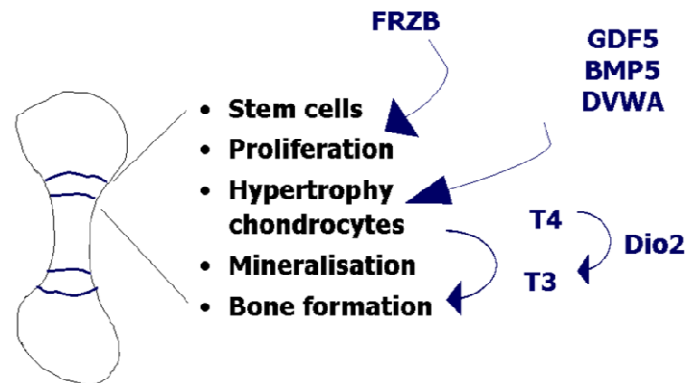


Susceptibility to more common OA

Pool of compelling OA genes

- Early genetic studies (e.g. GDF5, DIO2, SMAD3)
- Large scale genome wide meta analyses (e.g. CHST111, DOT1L, NCOA3)

Skeletal development, endochondral ossification



Endochondral ossification; common pathway underlying OA etiology

Endochondral Ossification and Osteoarthritis

Early life effect of *DIO2*

Early life

Disruption of endochondral
ossification genes



Altered skeletal morphogenesis
Suboptimal joint shape

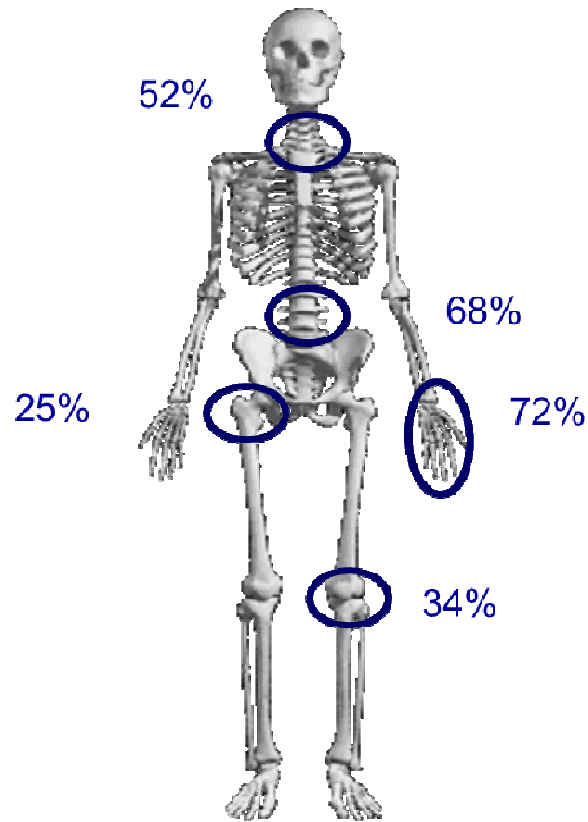


**Osteoarthritis
susceptibility**



Shape modeling within GARP subjects

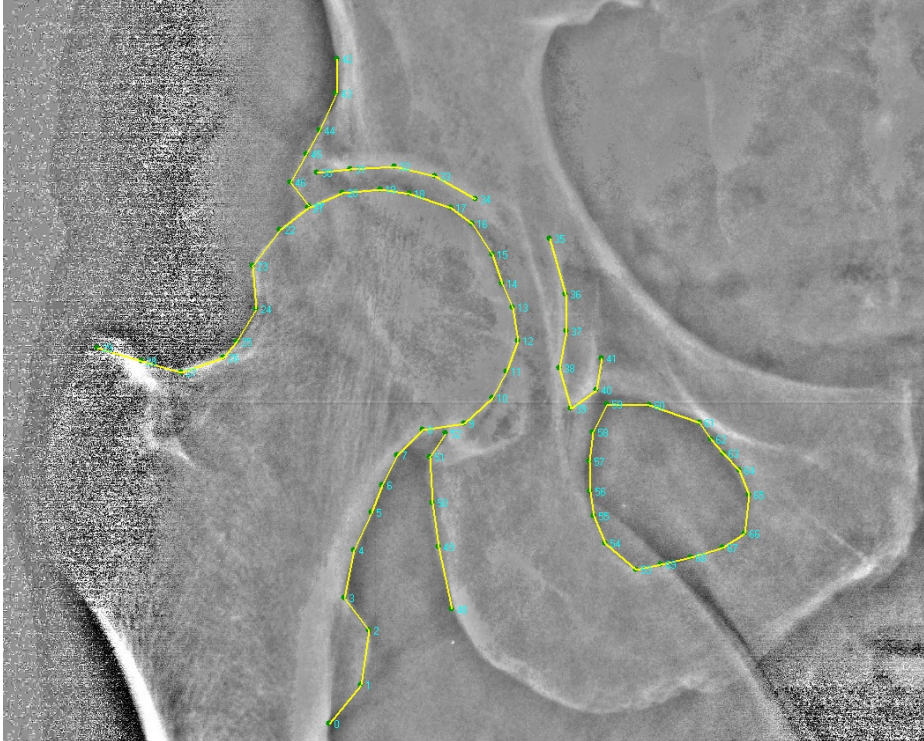
Collaboration E. Waarsing, H.H. Weinans (Rotterdam)



Study design allows investigation of hip joint shape with and without OA

Shape modeling within GARP subjects

Collaboration E. Waarsing, H.H. Weinans (Rotterdam)

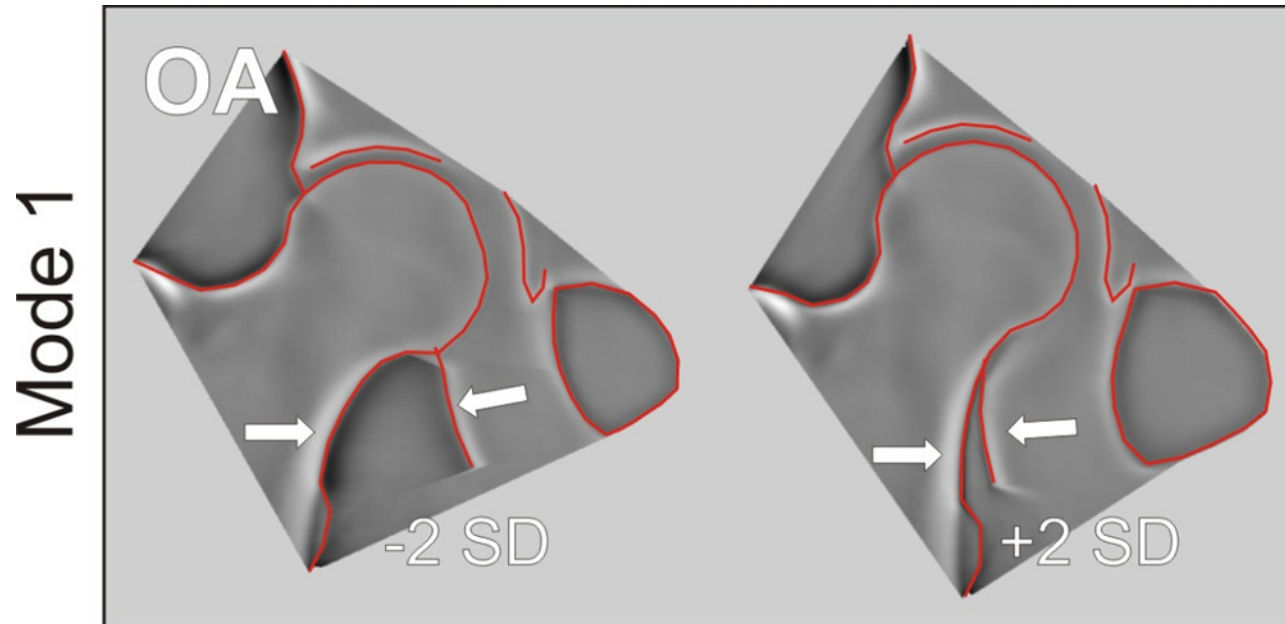


Define shape with 70 points around the hip joint.

Association with Osteoarthritis?

Mode 1 within GARP subjects

Collaboration E. Waarsing, H.H. Weinans (Rotterdam)



Mode 1 was characterized by a high within person correlation
 Association with OA for wide standing position or small pelvis, $P = 2 \times 10^{-4}$

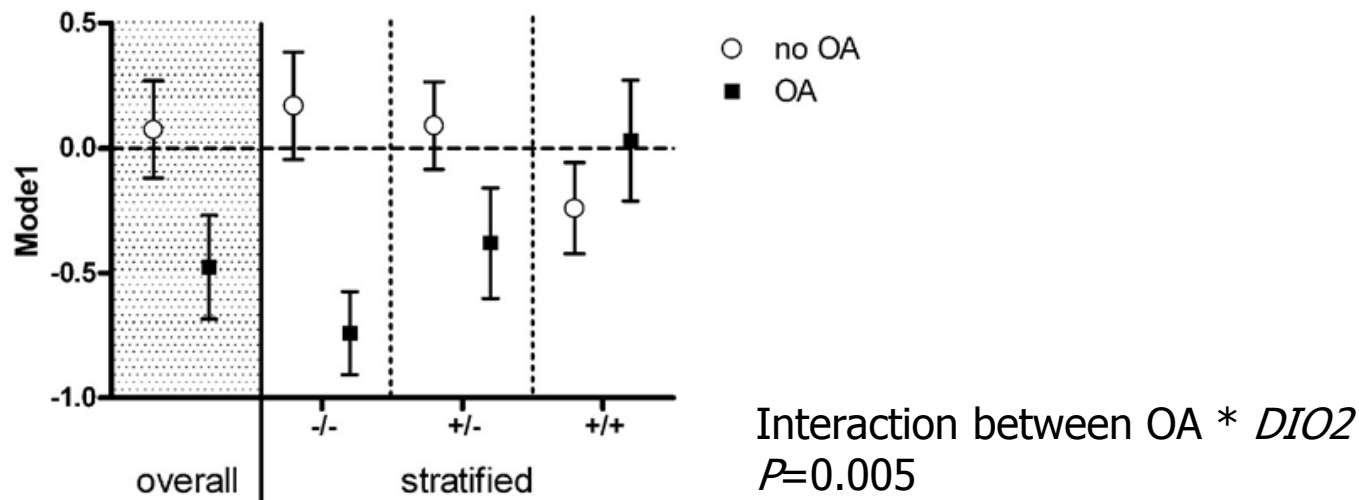
Interaction with *DIO2* genotypes?

Mode 1 within GARP subjects

Collaboration E. Waarsing, H.H. Weinans (Rotterdam)

Mode1

DIO2 rs12885300



DIO2 risk allele carriers are more vulnerable to biomechanical stress caused by suboptimal hip shape

Endochondral Ossification and Osteoarthritis

Late life effect of *DIO2*

Early life disruption of
EO genes



Altered skeletal morphogenesis
Suboptimal joint shape

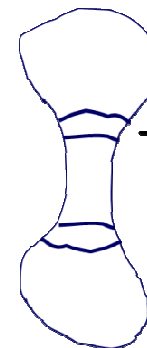


Osteoarthritis
susceptibility

Late life activation of
EO genes



Cartilage
degradation/mineralization



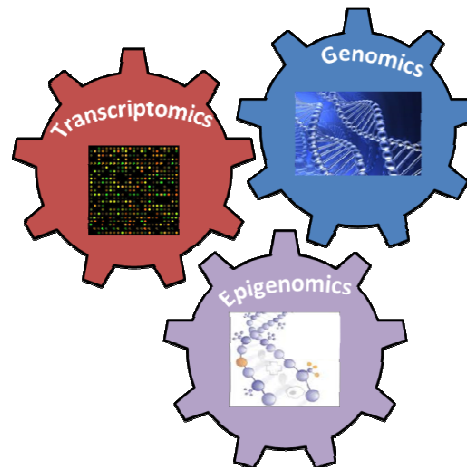
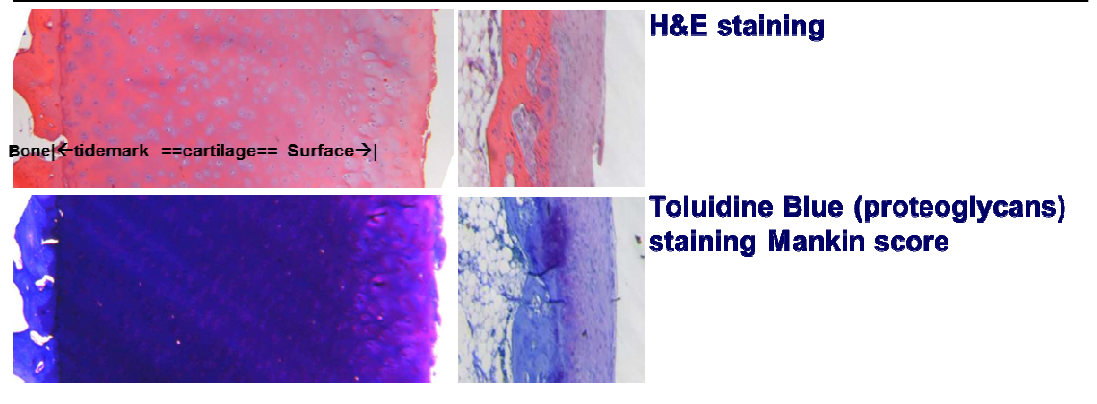
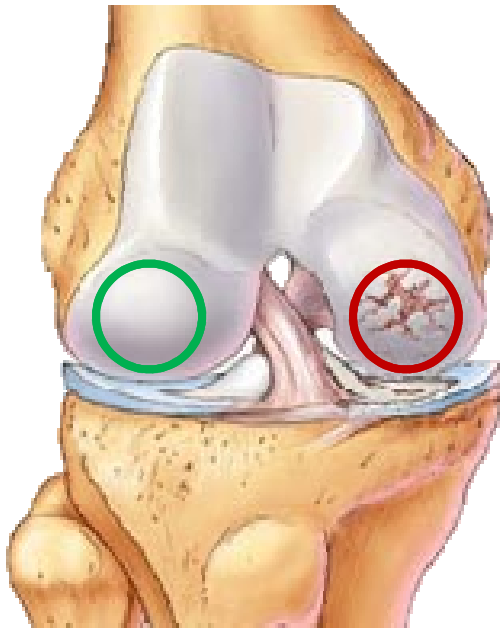
Stem cells
Chondrocytes
Proliferation
Hypertrophic chondrocytes
Mineralization
Bone



Experimental set up; the RAAK Study

Dept. Orthopedics (RGHH Nelissen)

Collection of joint tissues of OA patients: **preserved** and **lesioned** cartilage, DNA, RNA, blood and cells (MSCs and primary chondrocytes).



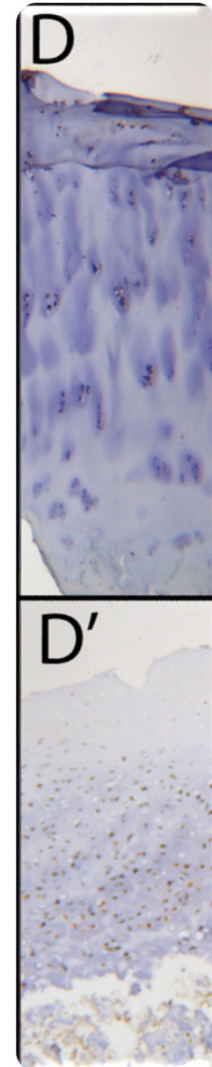
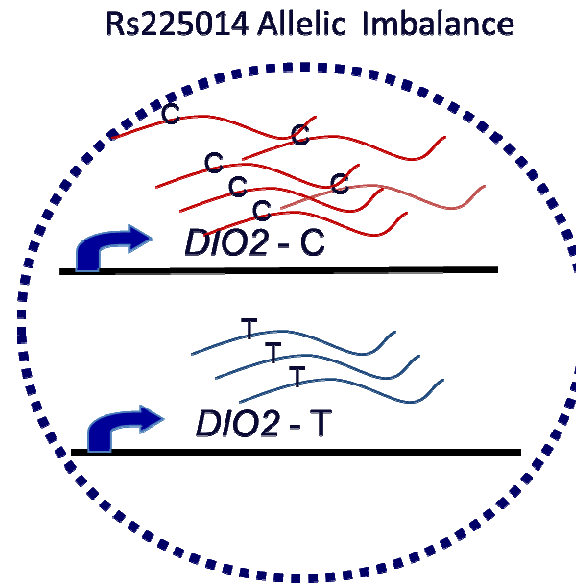
DIO2 in articular cartilage

2010

DIO2 mRNA expression
high in OA cartilage
Ijiri et al. 2010

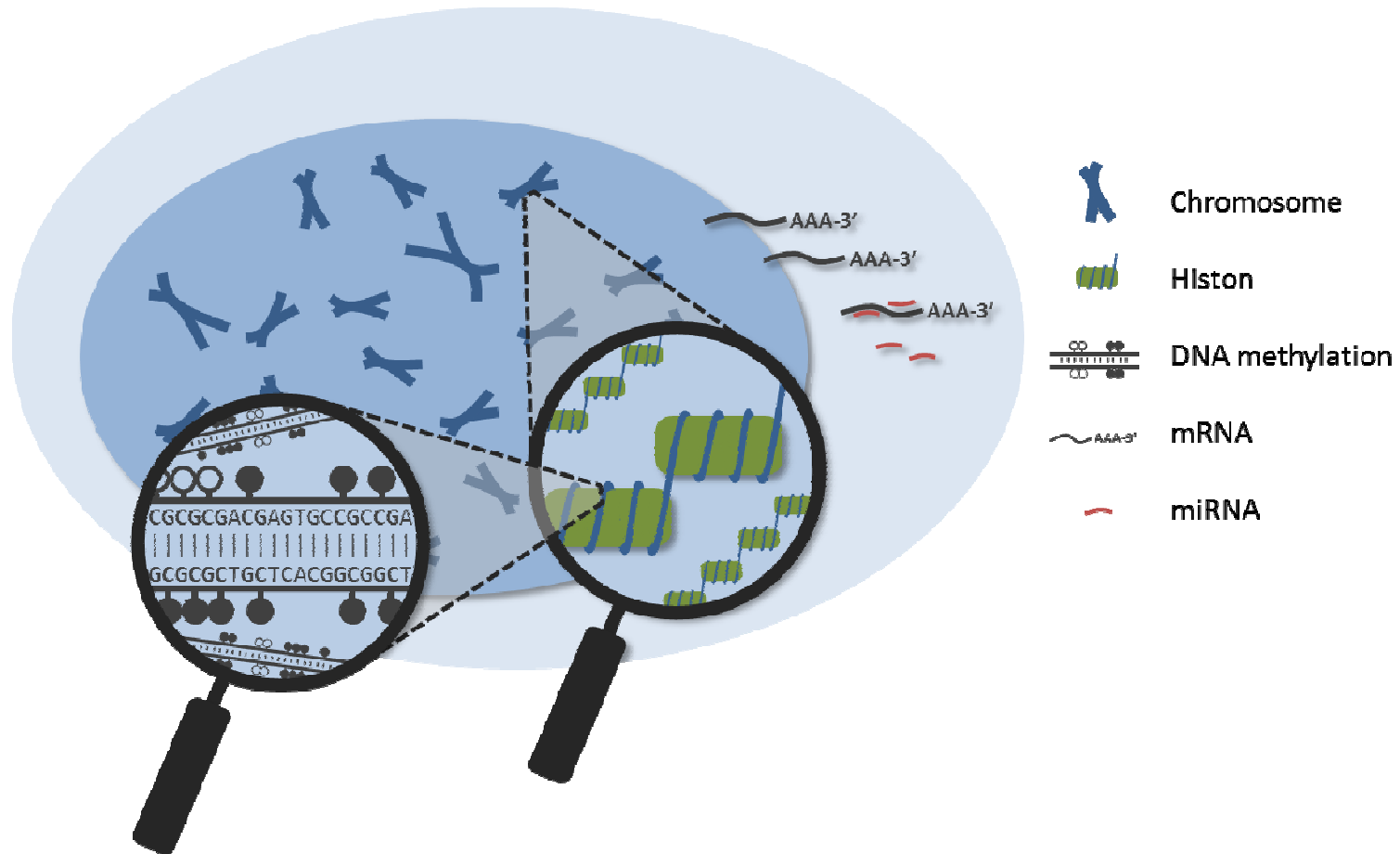
2012

Allelic imbalance &
protein up regulation
Bos et al. 2012



- Potential relevance *DIO2* in OA pathology
- Cis-eQTL function & direction of effect of risk allele

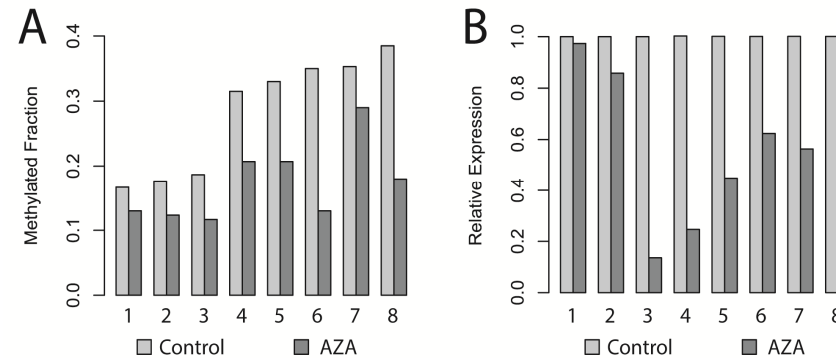
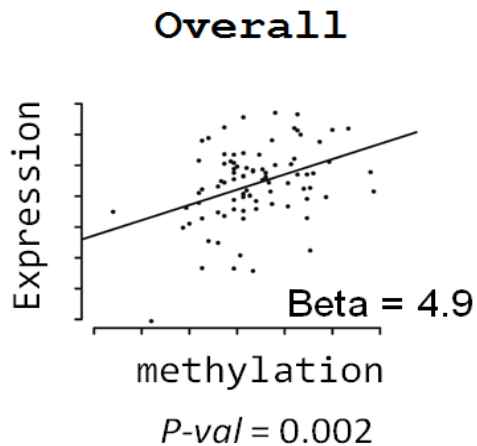
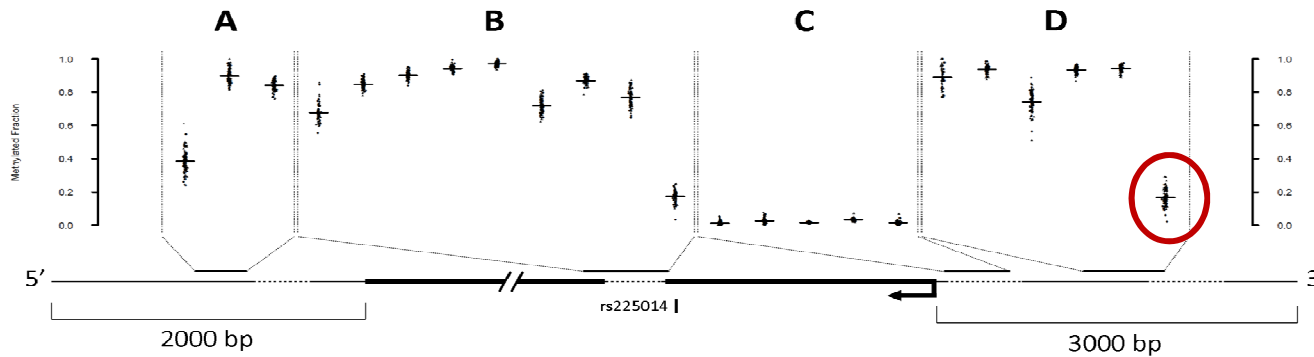
Regulation *DIO2* expression



Methylation at CpG sites allows cells to dynamically adjust expression of genes in adaptation to changing environment

Regulation of *DIO2* expression

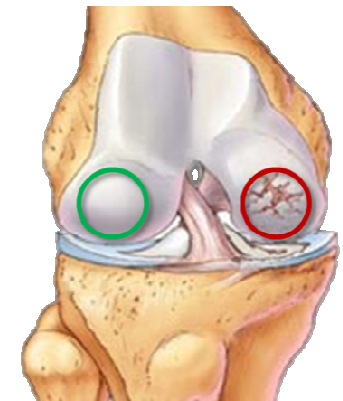
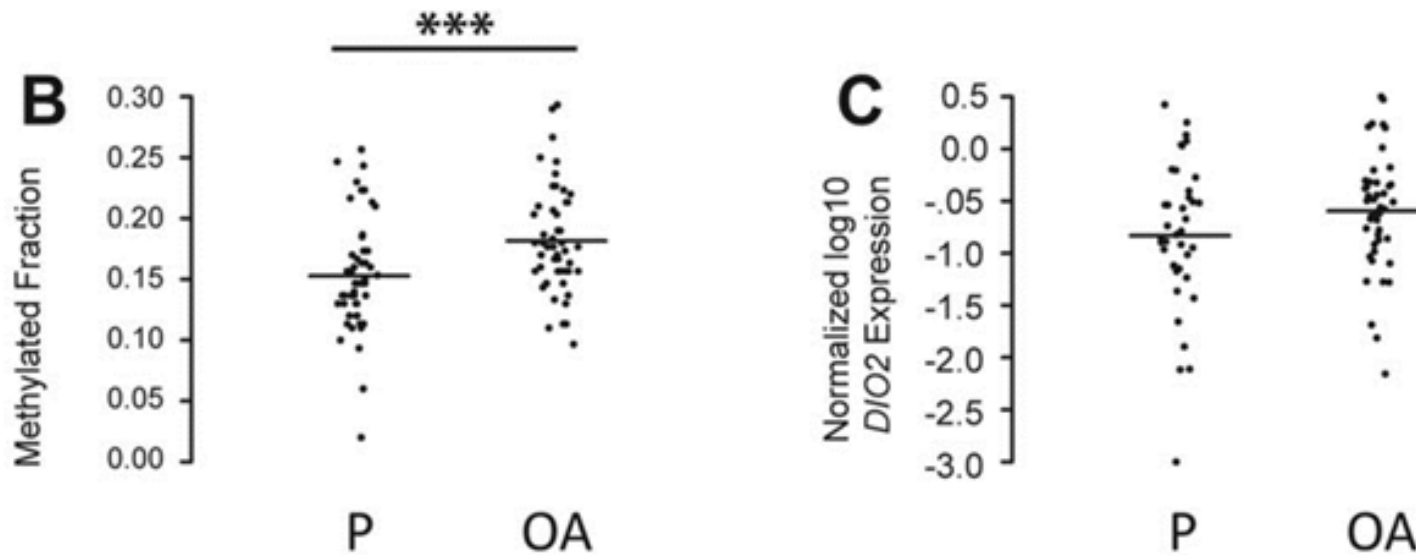
Gene targeted methylation at CpG sites (Epityper, Sequenom)



- DIO2* expression in articular cartilage is **modulated by methylation** at CpG ~-2000 bp

Regulation of *DIO2* expression

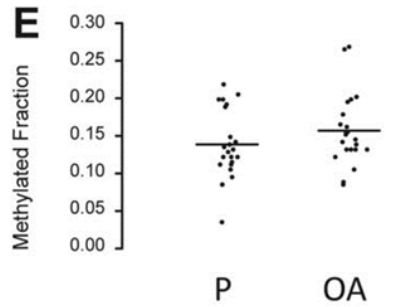
Gene targeted methylation at CpG sites (Epityper, Sequenom)



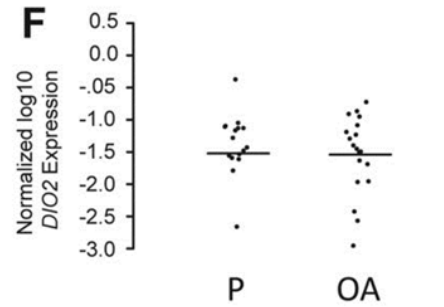
- DIO2* expression in articular cartilage is epigenetically regulated by methylation at an **OA sensitive** CpG site

Regulation of *DIO2* expression

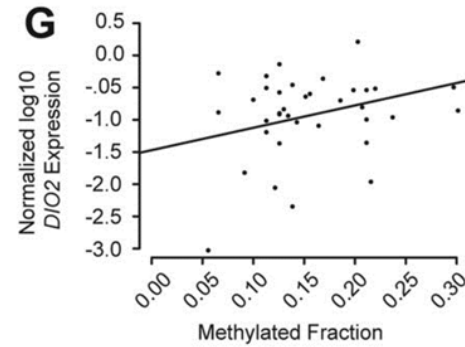
Gene targeted methylation at CpG sites (Epityper, Sequenom)



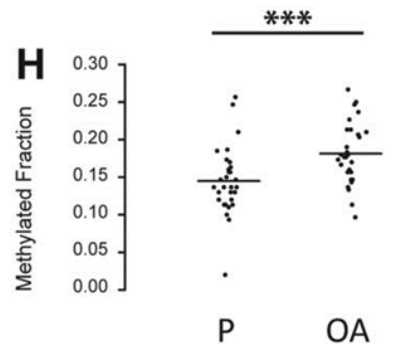
rs225014 TT carriers



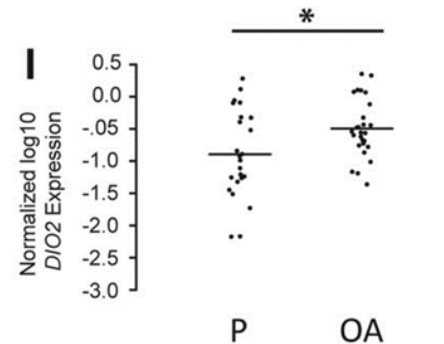
rs225014 TT carriers



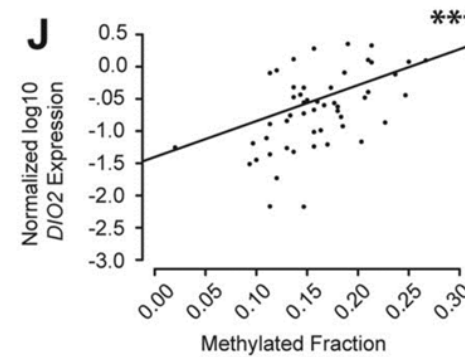
rs225014 TT carriers



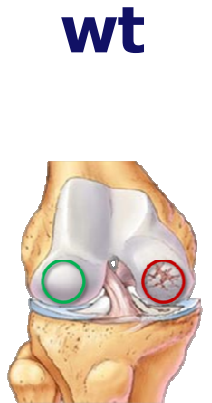
rs225014 CT/CC carriers



rs225014 CT/CC carriers



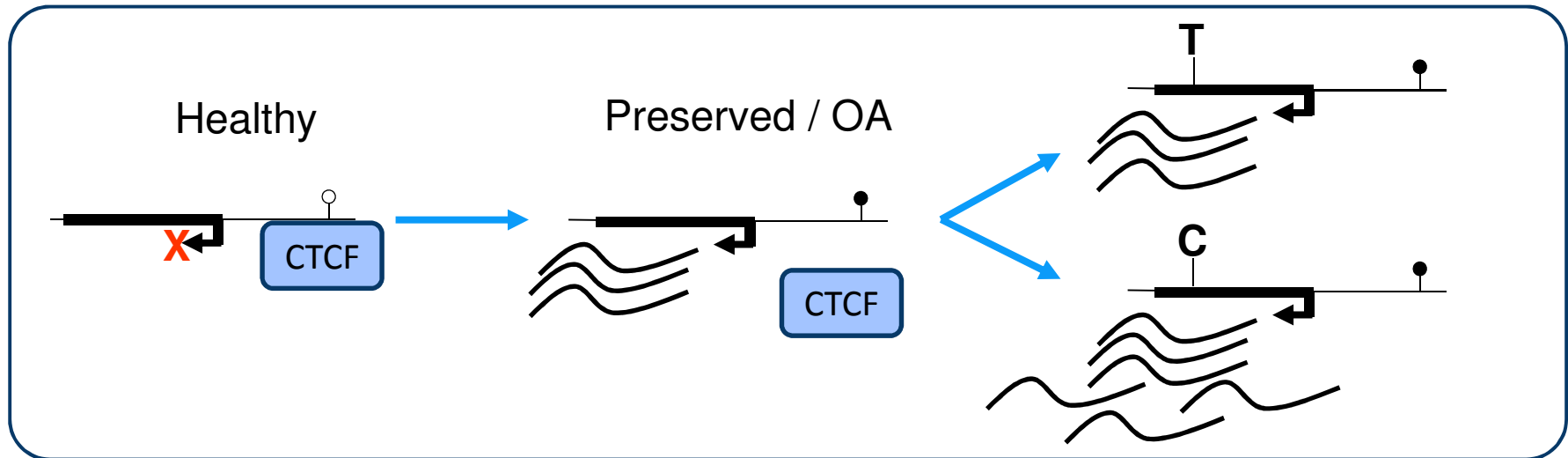
rs225014 CT/CC carriers



risk

- DIO2* expression is more sensitive to methylation changes in rs225014 risk allele carriers.

In summary



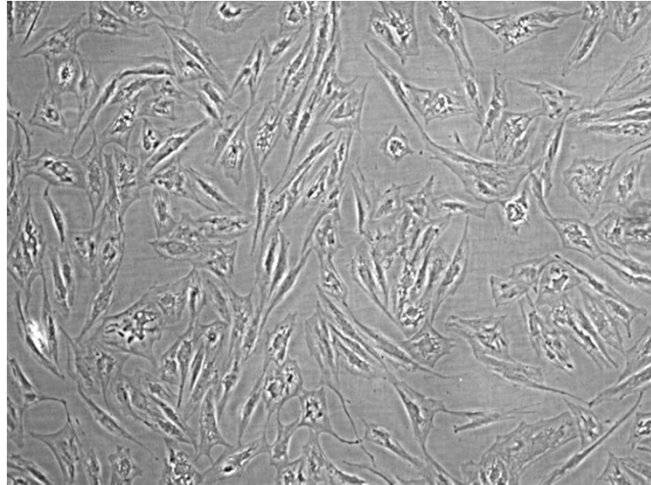
- *DIO2* is epigenetically regulated by CpG methylation in articular cartilage, likely mediated via CTCF.
- *DIO2* expression is more sensitive to methylation changes in rs225014 risk allele carriers.

What is direct effect of *DIO2* upregulation in cartilage?

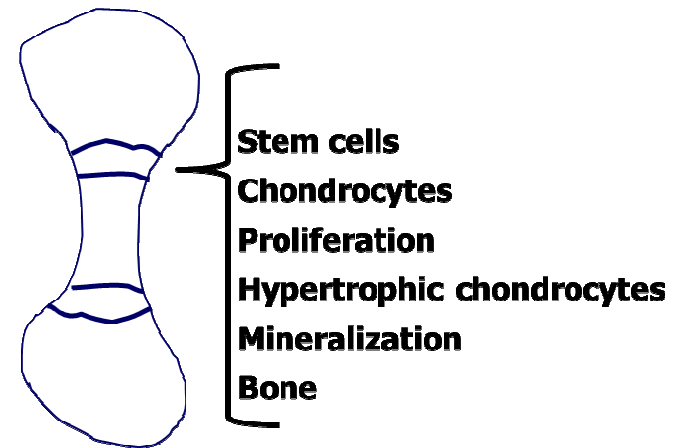
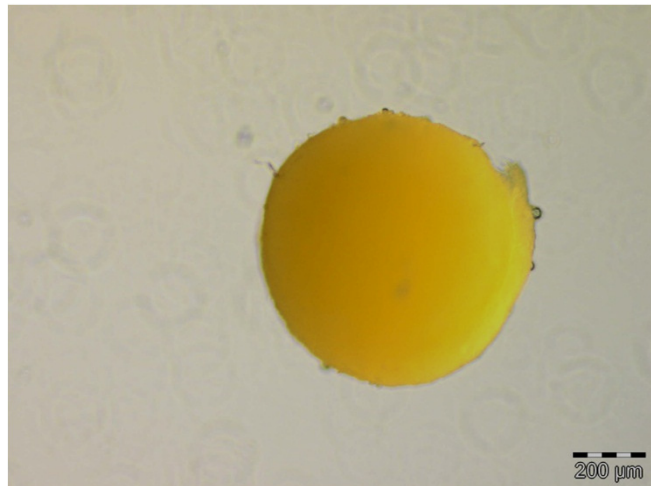
In vitro chondrogenesis model

Stemcells, primary chondrocytes

Growing cells
(monolayer)

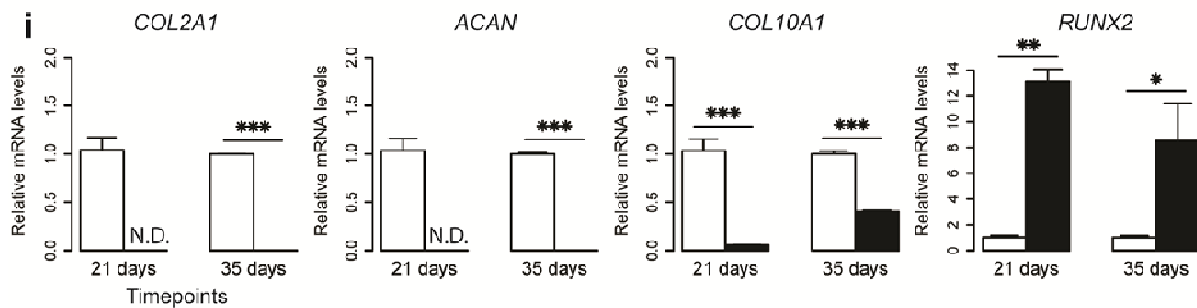
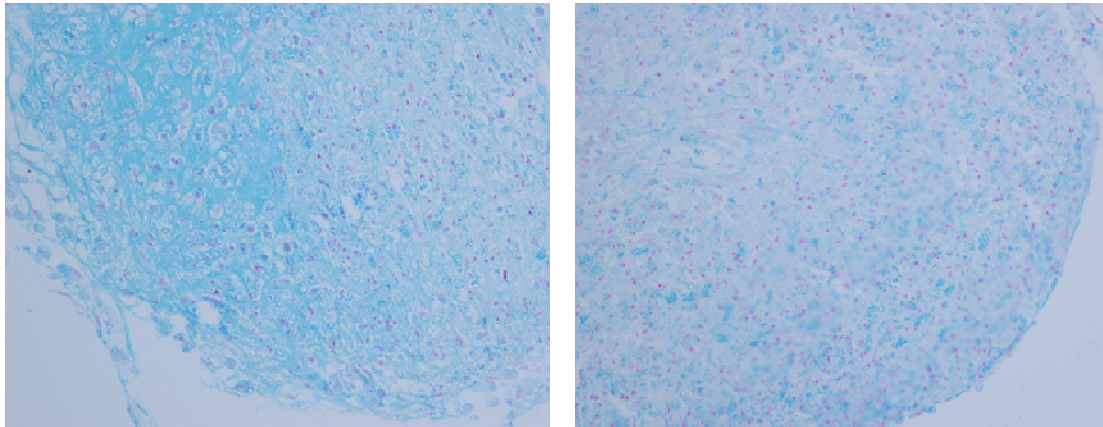


Chondrocyte pellet
cartilage formation



BM-MSC based *in vitro* chondrogenesis model

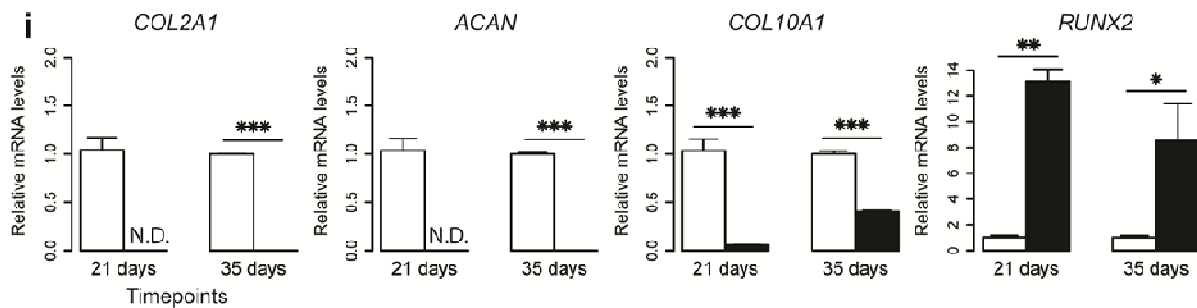
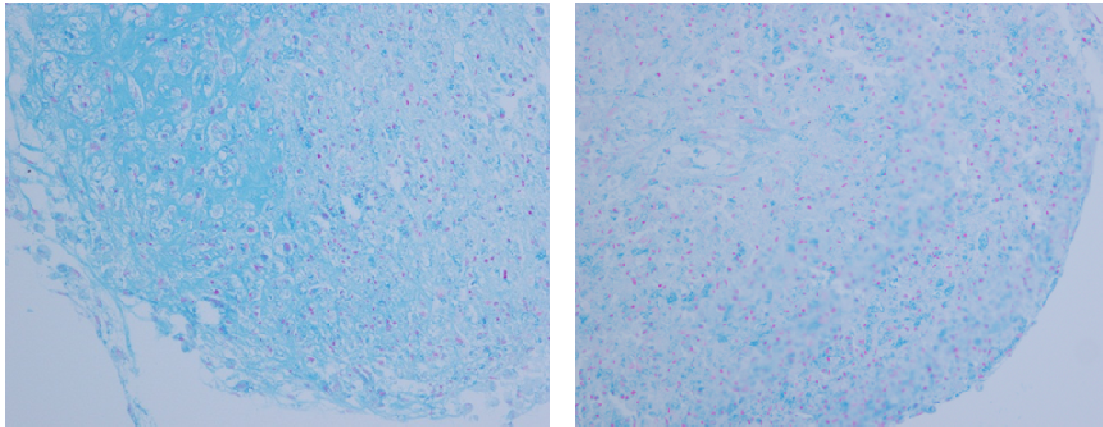
Overexpression of DIO2



- Direct detrimental effect of DIO2 on cartilage matrix deposition
- Destruction without early hypertrophy (COLX)

BM-MSC based *in vitro* chondrogenesis model

Overexpression of DIO2

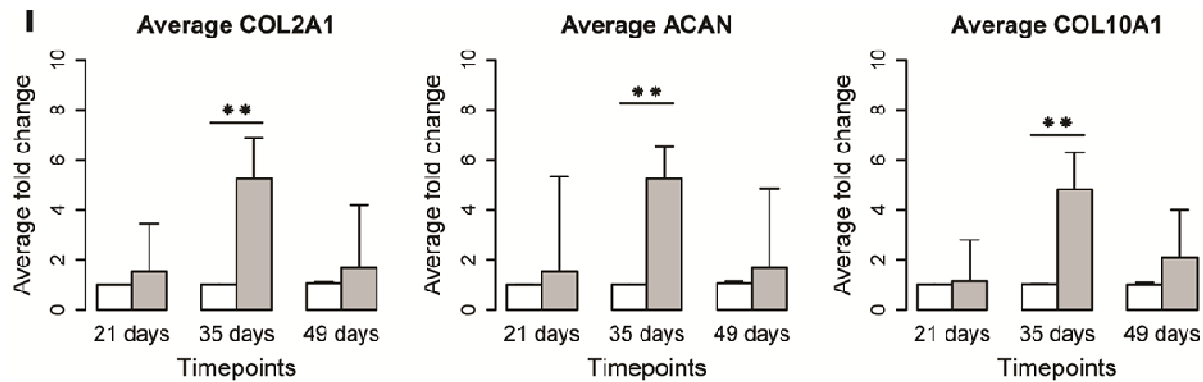
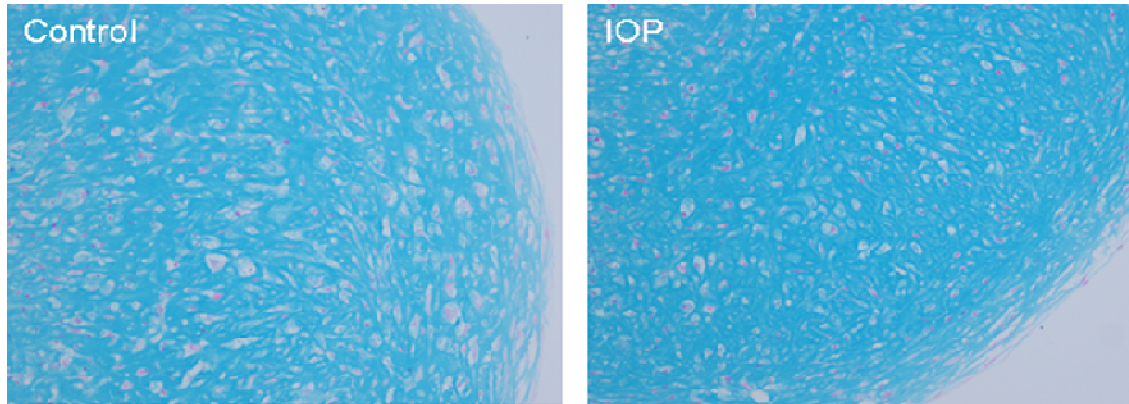


DIO2 up-regulation
rats cartilage
destruction
Nagase et al. 2013

- Direct detrimental effect of DIO2 on cartilage matrix deposition
- Destruction without early hypertrophy (COLX)

BM-MSC based *in vitro* chondrogenesis model

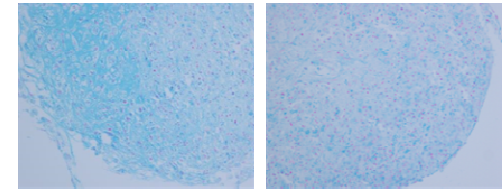
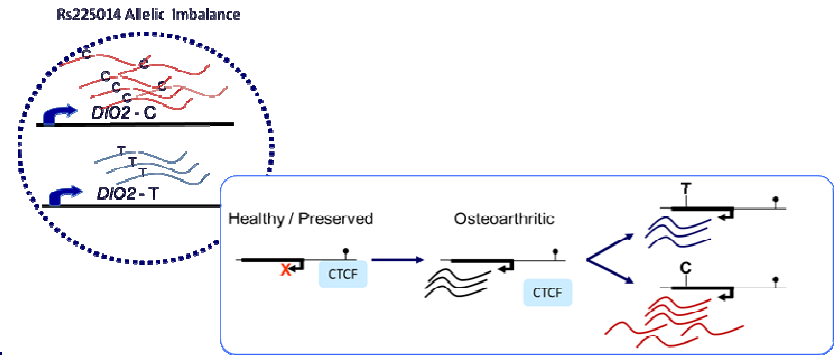
Inhibition of DIO2 function



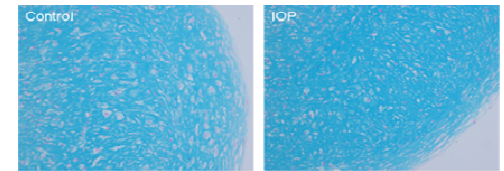
- Beneficial effect of DIO2 on cartilage matrix deposition
- Early hypertrophy (COLX), no destruction

In summary

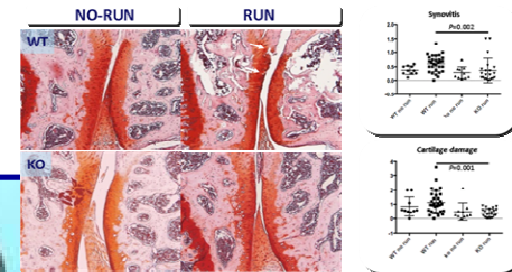
- Risk allele modulates epigenetically regulated transcription of *DIO2* in articular cartilage
- *DIO2* up-regulation affects propensity of chondrocytes to undergo terminal maturation.
- Attenuating thyroid signaling may be a key factor in securing joint tissue homeostasis and a likely druggable target



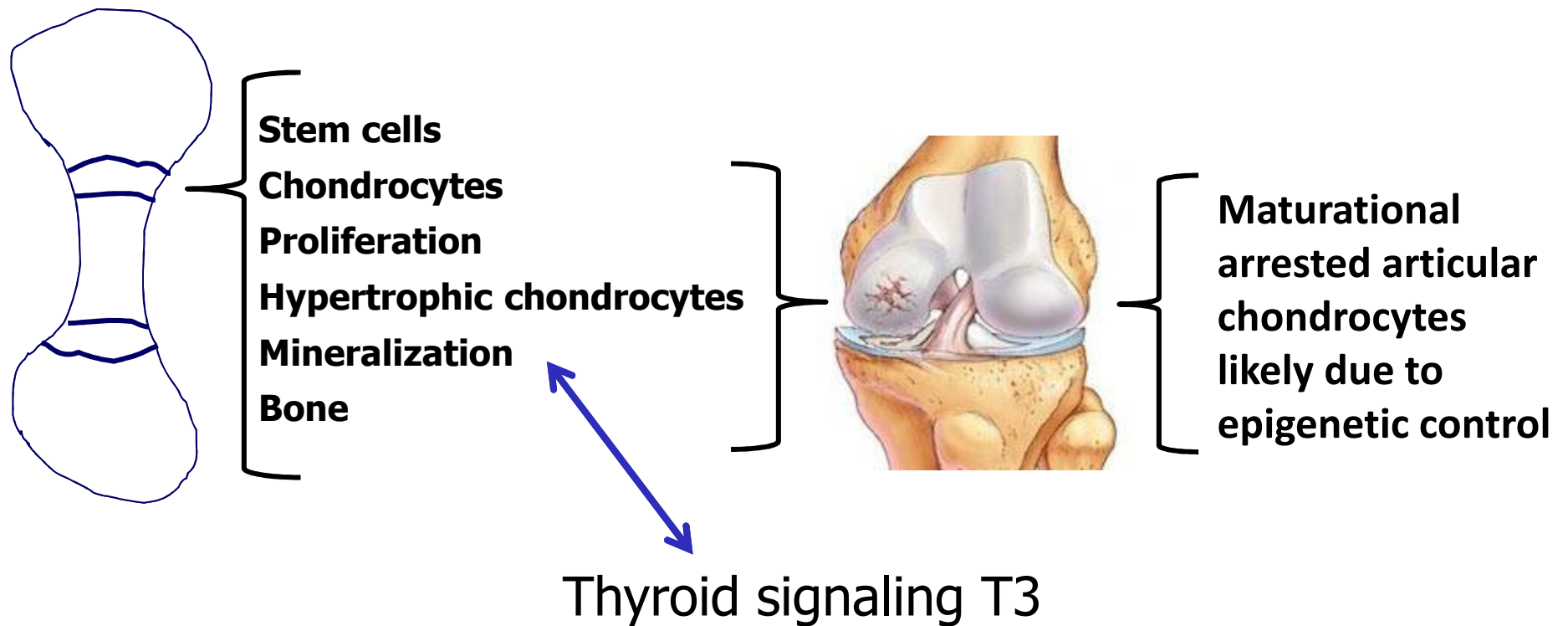
Direct detrimental effect of *DIO2* on cartilage matrix deposition & maintenance



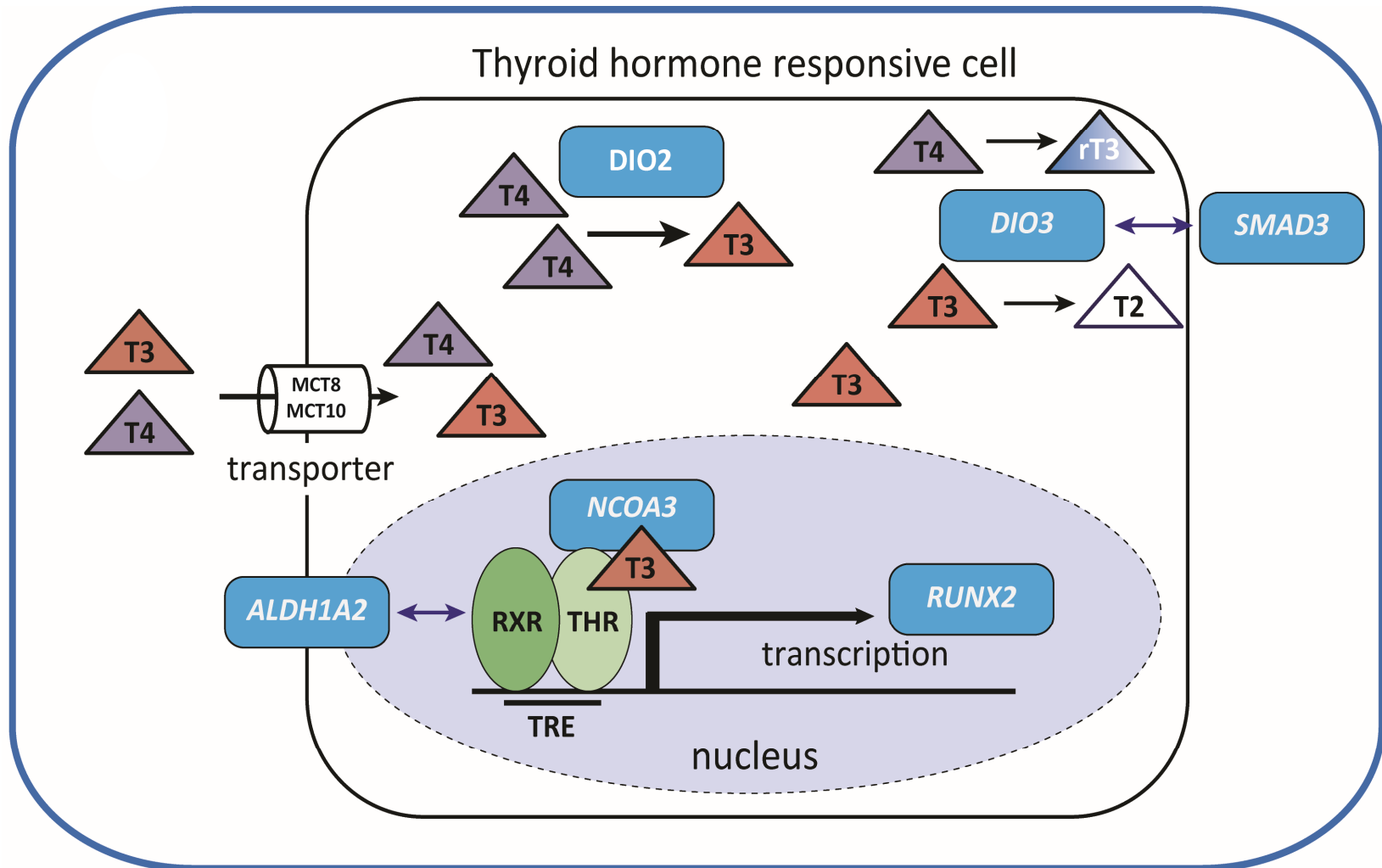
Direct beneficial effect of *DIO2* on cartilage matrix deposition and maintenance



Genetic Link Between Development and Osteoarthritis



Genetic Link Between Development and Osteoarthritis; thyroid signalling





Acknowledgements

LUMC Molecular Epidemiology

Osteoarthritis group

I. Meulenbelt

W. Den Hollander

N. Bomer

Y. Ramos

LUMC Orthopedy

RGHH Nelissen

LUMC Rheumatology

M. Kloppenburg

LUMC Pathology

JVM Bovee



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Netherlands Consortium for
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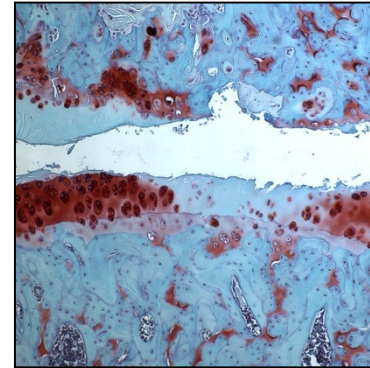
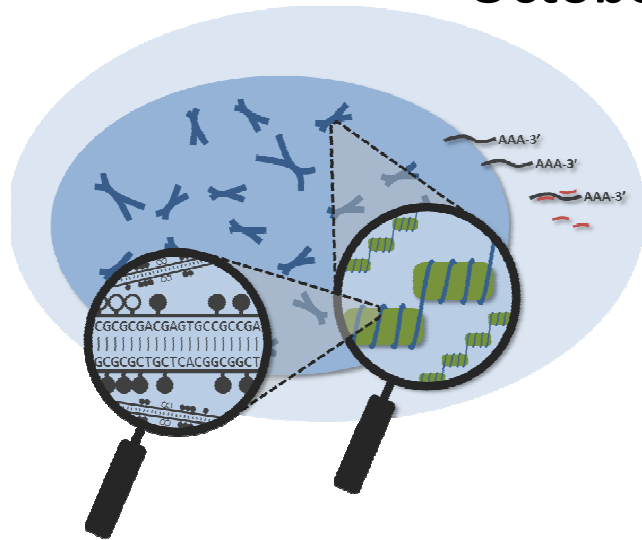
IDEAL FP7/2007-2011n° 259679

1st Osteoarthritis Epigenetics Workshop

Royal Netherlands Academy of Arts and Sciences

Amsterdam, The Netherlands

October 20th-21st 2015



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<http://www.molepi.nl/research/osteoarthritis/workshop>

Supported by The Dutch Arthritis Foundation and OARSI