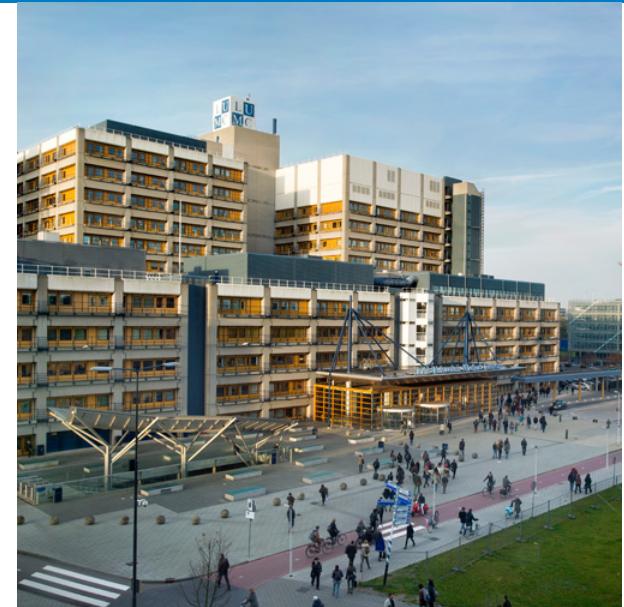
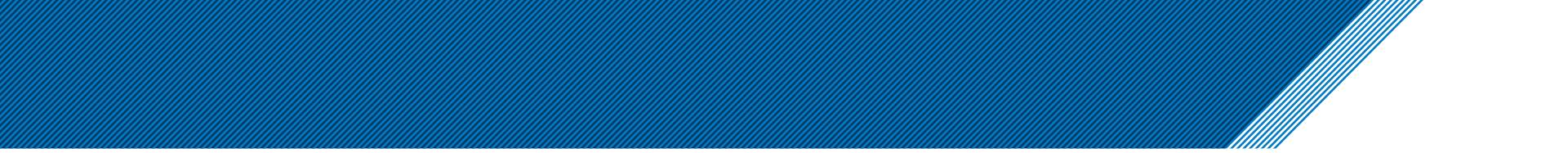


Osteoarthritis

**Functional characterization of the *DIO2*
risk locus towards preclinical drug studies**

Ingrid Meulenbelt
Molecular Epidemiology
LEIDEN, THE NETHERLANDS





Osteoarthritis complex the trait



Osteoarthritis (OA)

- **Degenerative disease of joint tissues**
- **Prevalent**
- **No effective treatment**



Major cause of disability among elderly affecting mobility and hampering daily life activities

Osteoarthritis - risk factors

- **Age**
- **Gender**
- **Bone mineral density**
- **Body mass index**
- **Genetic factors**

Osteoarthritis - radiographs



Figure 1



Figure 2

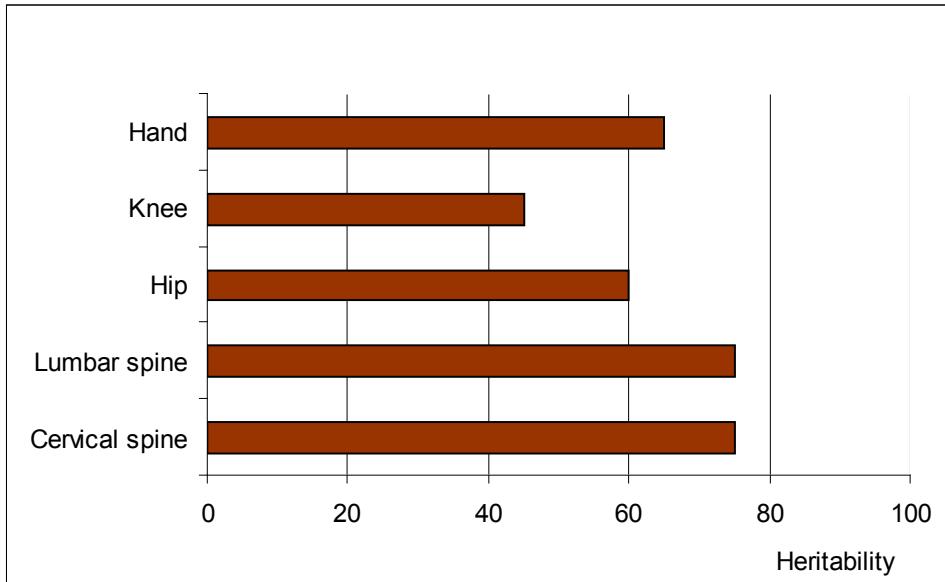
Joint damage: visible on X-ray

Osteoarthritis - Symptoms

- **Stiffness**
- **Pain**
- **Crepitus**
- **Limitation of movement**
- **Invalidity**

→ **Low correlation between symptoms and radiology**

Osteoarthritis - Heritability



Balance between heritability and environment

complex genetic



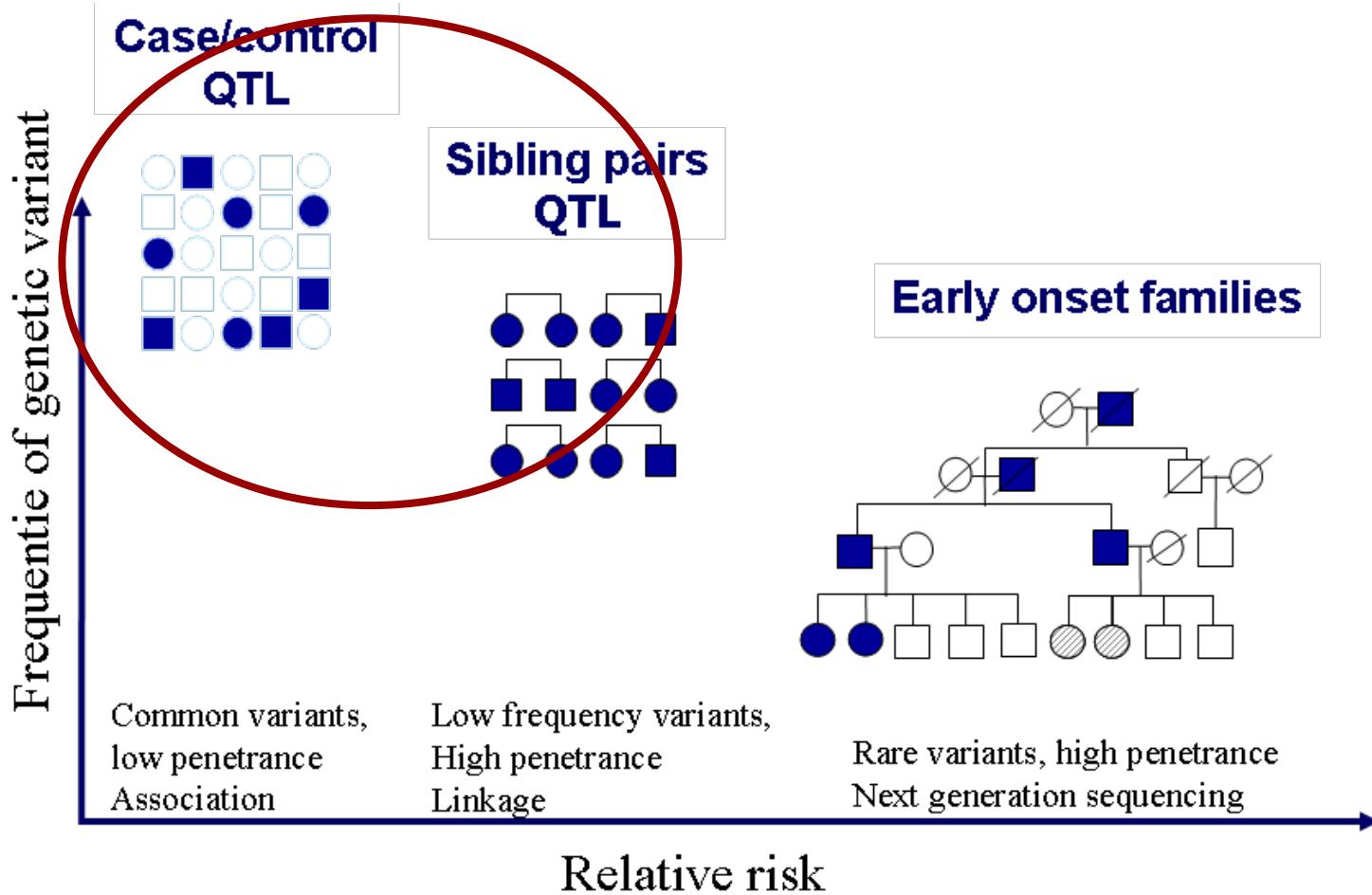
100% environment

100 % genetic

Determines genetic study design

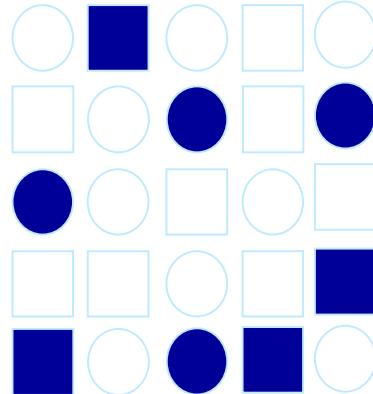
The genetic study design

Depending on study design



Association analyses case/controls

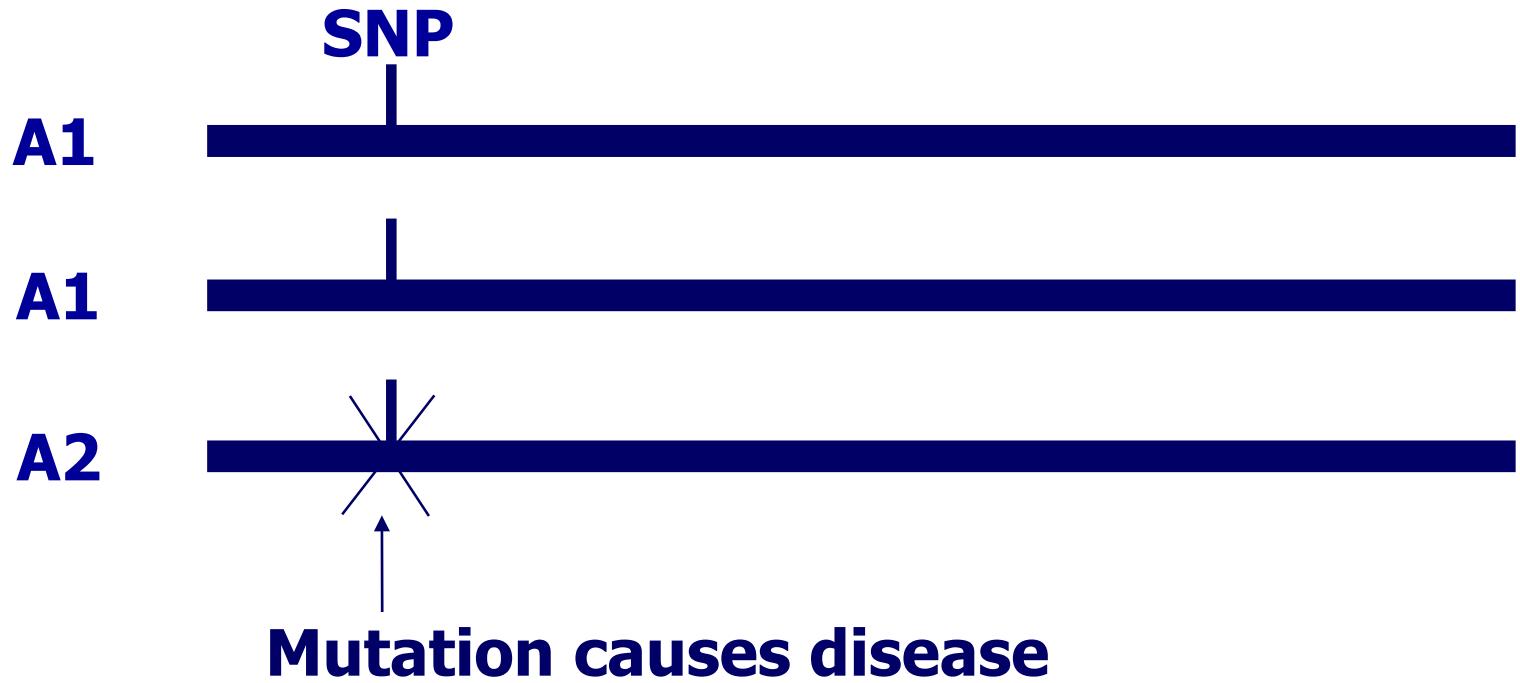
- Cases and controls
- Complex phenotype (common)
- Model free
- Sharing of alleles between cases versus controls
- Use SNPs close together

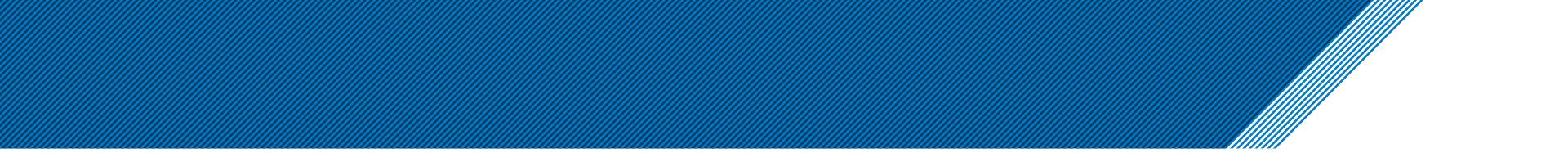


Finding association about changing your risk to develop disease not necessarily about finding genes causing disease.

Direct approach

Functional mutation





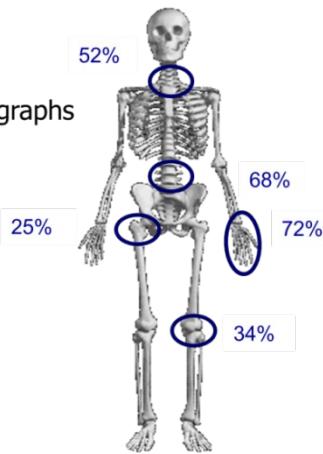
Discovery driven analyses, novel underlying disease pathways

Identification of *DIO2* as OA susceptibility locus

The GARP study

GARP study

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



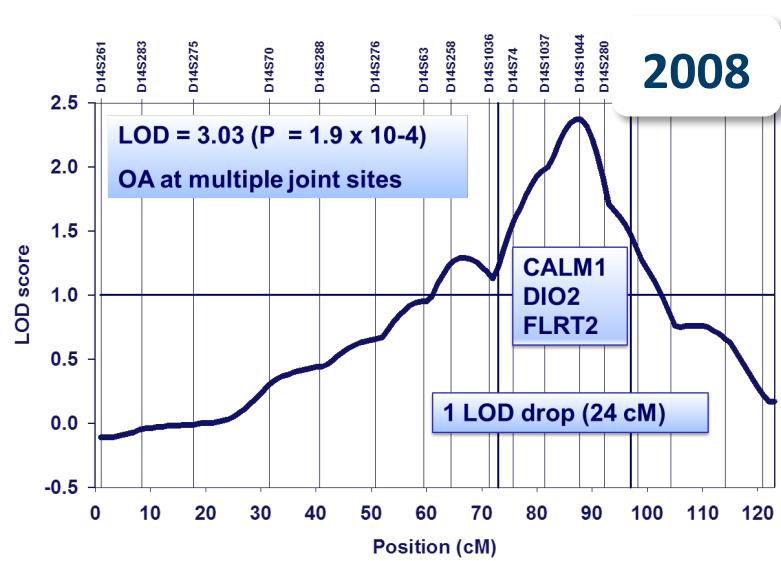
Inclusion:

≥ 2 joints OA

Progression:

2 jr: 100 pairs

5 jr: 200 pairs



Initial study to indicate thyroid signaling conferring risk to osteoarthritis

Meulenbelt et al. 2008 *Hum Mol Genet* 17: 1867-75

DIO2, OA susceptibility gene

The Garp study, combined linkage association

<i>Gene</i>	<i>SNP reference</i>	<i>allele</i>	<i>alias</i>	<i>MAF</i>	<i>P-value</i>
<i>DIO2</i>	rs12885300	C>T	ORFaGly3Asp	0.36	0.04
	rs2267872	G>A		0.09	0.30
	rs225011	T>C		0.43	0.14
	rs225014	T>C	Thr92Ala	0.36	0.006
	rs10136454	C>T		0.02	0.60

Which step in the Freedman et al strategy?

1. Using linkage disequilibrium (LD structure) to find candidate gene

Initial replication, OA susceptibility gene DIO2 haplotype rs12885300-rs225014 C-c

Female cases severe hip OA

<i>Gene</i>	<i>OR Recessive model</i>	<i>P of OR</i>	<i>P value heterogeneity test</i>
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	

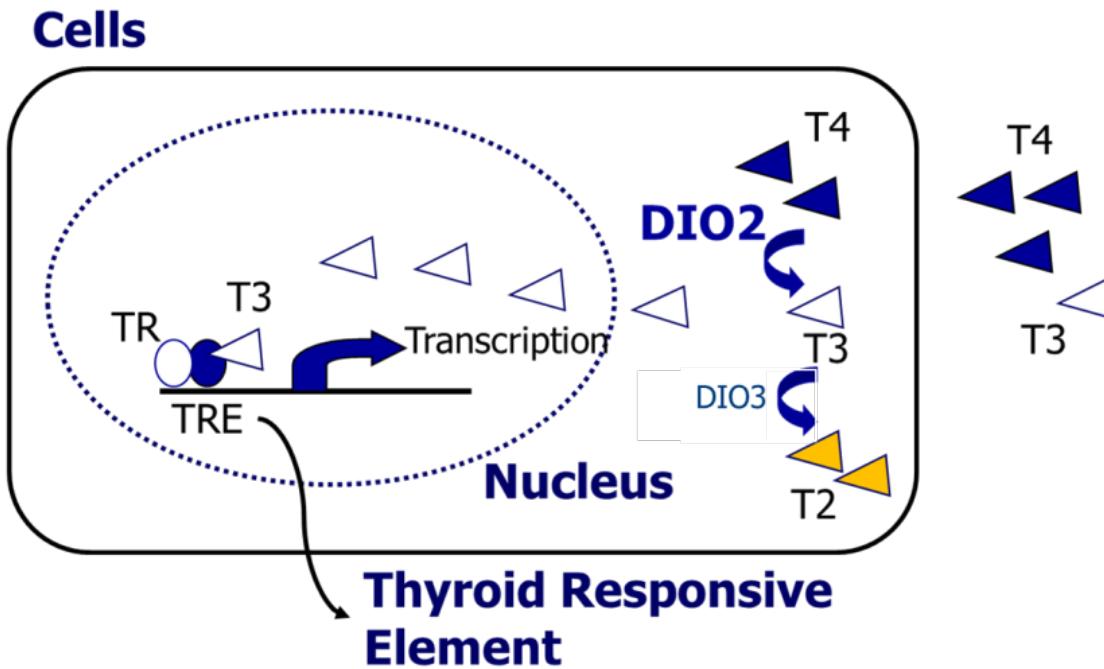
*Random effect meta-analyses

Which step in the Freedman et al strategy?

2. **Used other populations to refine LD regions / association to pinpoint a strong OA risk candidate gene**

Identification of *DIO2* as OA susceptibility locus

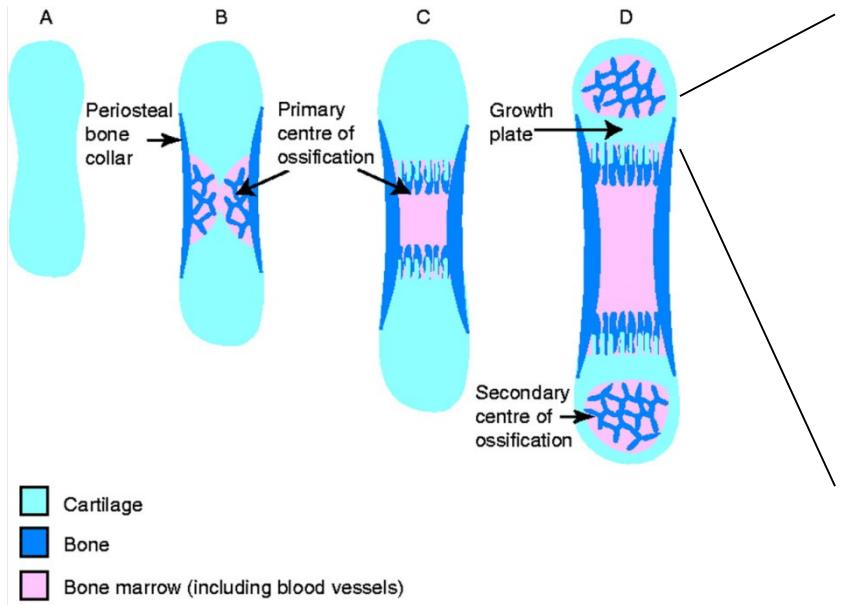
The GARP study



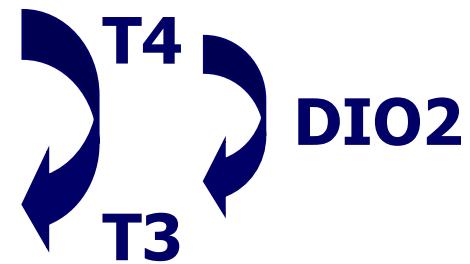
Initial study to indicate thyroid signaling conferring risk to osteoarthritis

Meulenbelt et al. 2008 *Hum Mol Genet* 17: 1867-75

Growth plate; elongation of long bones via endochondral ossification



Stem cells
Growth plate chondrocytes
Proliferation
Hypertrophic
Mineralization
Bone



E J Mackie et al. J Endocrinol 2011;211:109-121

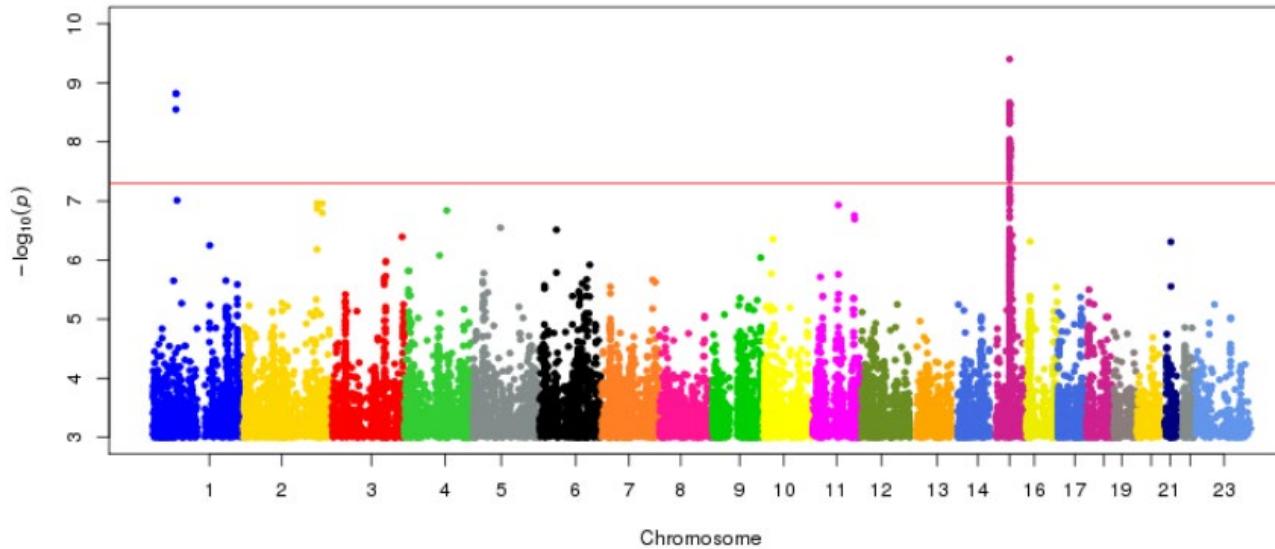
Active intracellular thyroid (T₃) triggers terminal maturation of growth plate chondrocytes to allow transition to bone

Wang et al. 2007 J bone and Min Res. 22; 1988-95



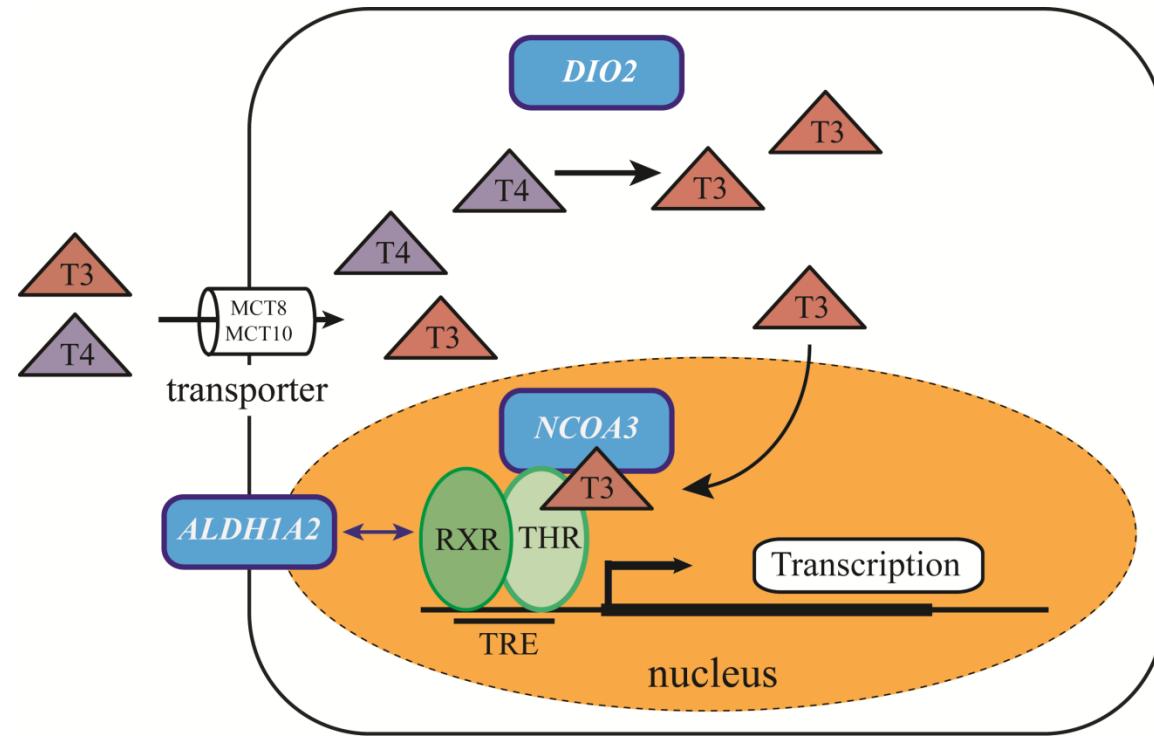
Additional OA genes in thyroid pathway

- Large scale genome wide meta analyses of osteoarthritis;
ALDH1A2, NCOA3



Styrkarsdottir et al. 2014 *Nat Genet* 46: 498-502
Evangelou et al. 2014 *Ann Rheum Dis* 73:2130-6

Intracellular levels of active thyroid



Intracellular thyroid signaling may be a common underlying osteoarthritis pathway

Which step in the Freedman et al strategy?

2. **Used other populations to refine LD regions / association to pinpoint a strong OA risk candidate gene**

The hypothesis

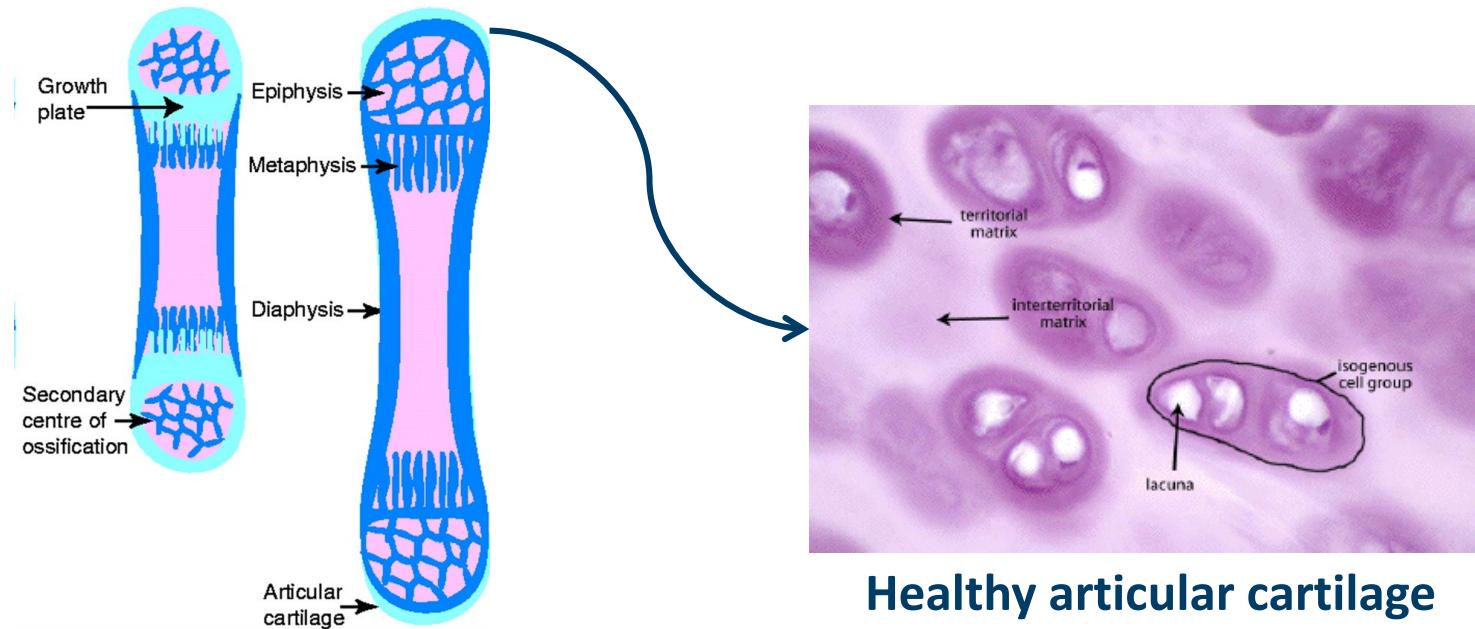
Developmental process of endochondral ossification

Late cause of a late life disease?



Articular chondrocytes

- Highly specialized, post-mitotic cell
- Maturational arrested
- Require phenotypic plasticity and shift between active metabolic and maturational arrested states to respond to environmental challenges.



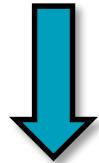


Endochondral ossification and Osteoarthritis

Late life effect of *DIO2*

Late life

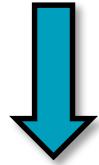
disruption of epigenetic silencing of thyroid
signaling in articular cartilage



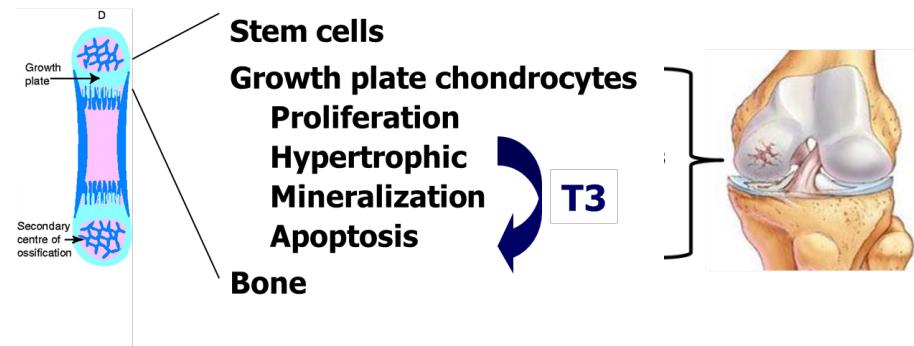
Loosening of maturationally arrested state



Debilitating cartilage signaling

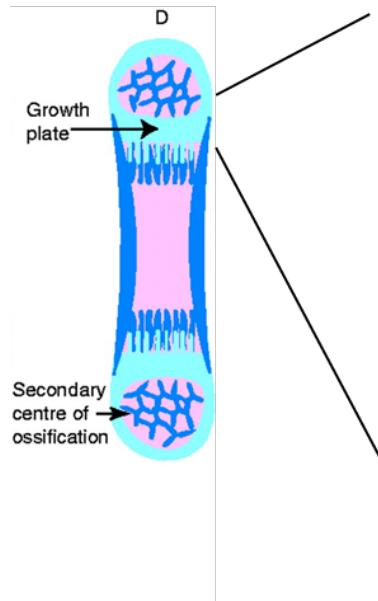


Osteoarthritis
susceptibility

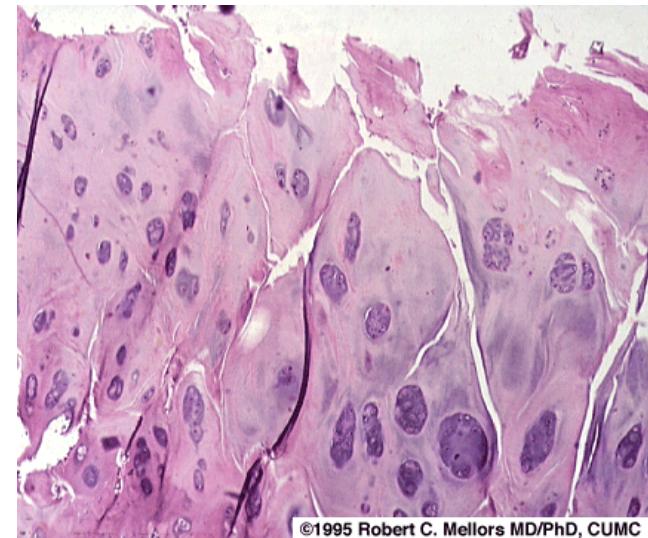


Chondrocytes in OA pathophysiology

- Loss of maturational arrested state and recuperation of growth plate morphology
- Proliferation, while degrading and calcifying the articular cartilage matrix.



Stem cells
Growth plate chondrocytes
Proliferation
Hypertrophic
Mineralization
Bone

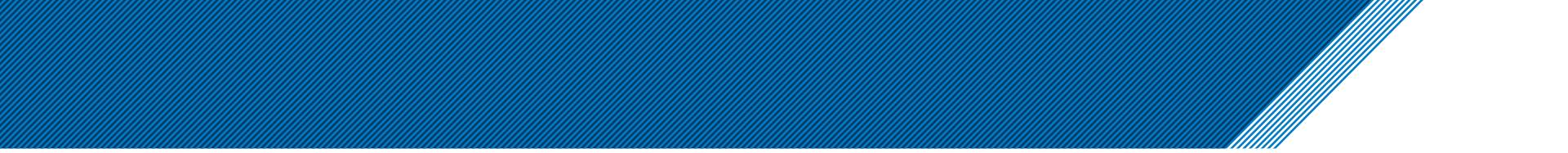


OA affected articular cartilage

OA phenotype in articular cartilage; developmental process of endochondral ossification

The recuperation of thyroid signaling in osteoarthritic cartilage?

But to do functional studies disease tissue and in vitro cell models are necessary



Functional characterization of the DIO2 risk locus

What would be your primary questions?

SNP:

Does the SNP directly affect protein function?

Does the SNP affect expression of positional gene?

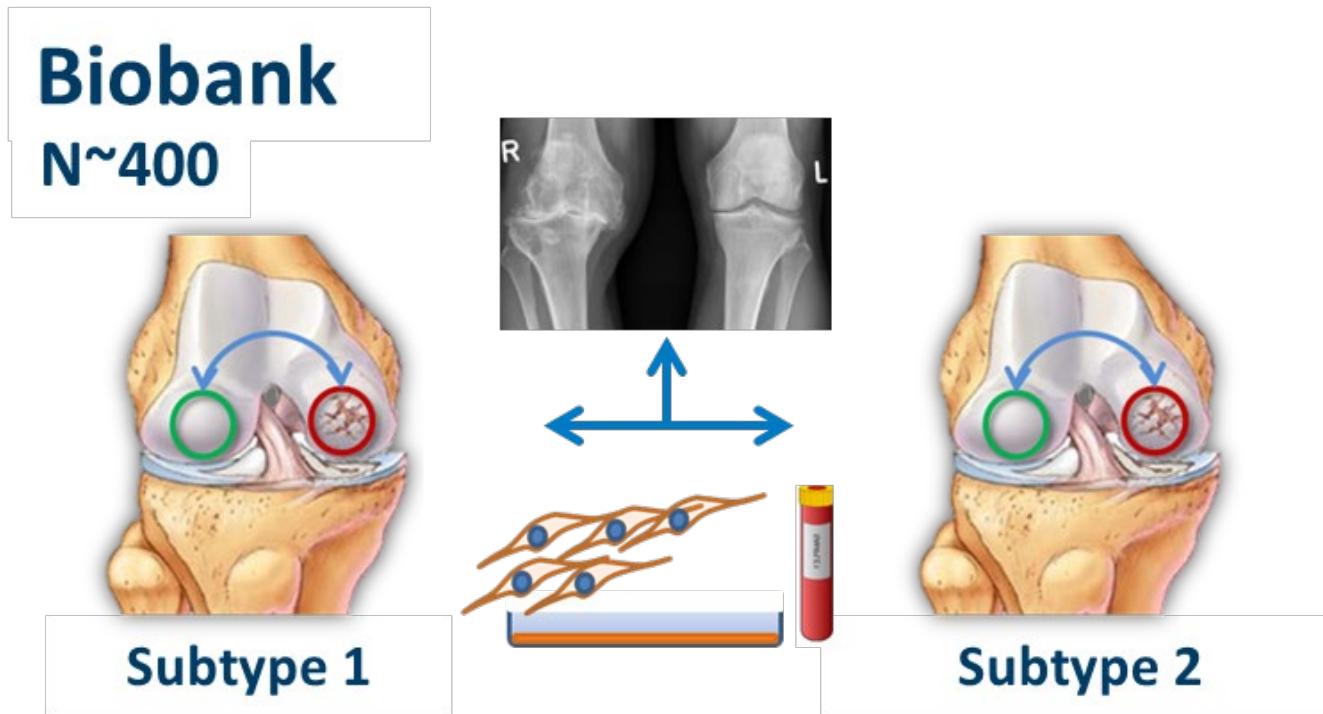
Gene:

Expression gene/protein is disease relevant tissue

Is the gene responsive to disease process

Research Articular osteoArthritis Cartilage

RAAK study (collaboration with Orthopaedics, RGHH Nelissen)



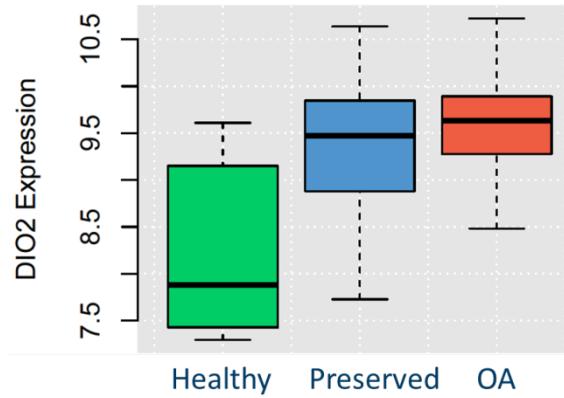
Collection of joint tissues of OA patients: **preserved** and **lesioned** cartilage, DNA, RNA, blood and cells (MSCs and primary chondrocytes).



DIO2 expression in articular cartilage

2010

**DIO2 mRNA expression high in OA
cartilage compared to healthy**
Ijiri et al. 2010





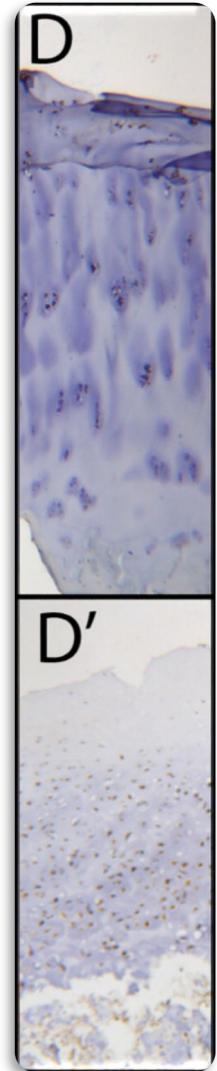
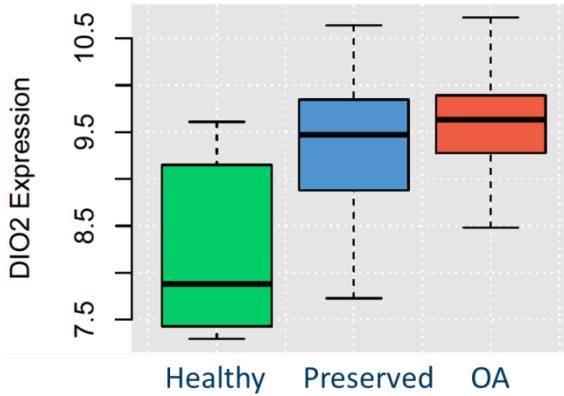
DIO2 protein expression in articular cartilage

2010

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

2012

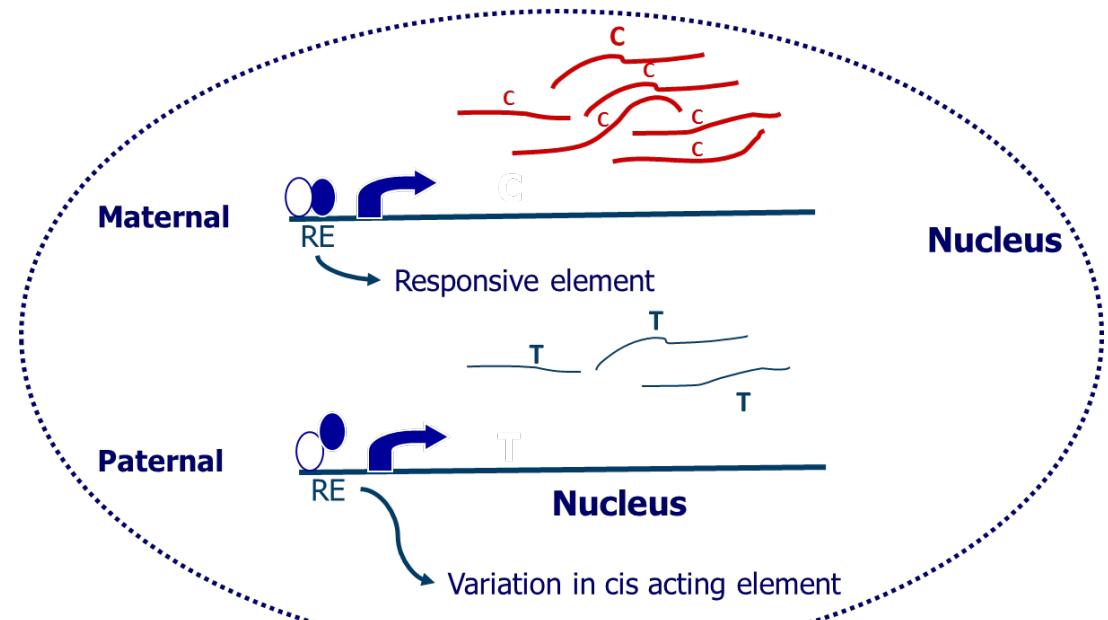
DIO2 protein expression up regulated in OA affected cartilage
Bos et al. 2012



Does the SNP affect expression of positional gene?

Test functional relevance of susceptibility SNPs:

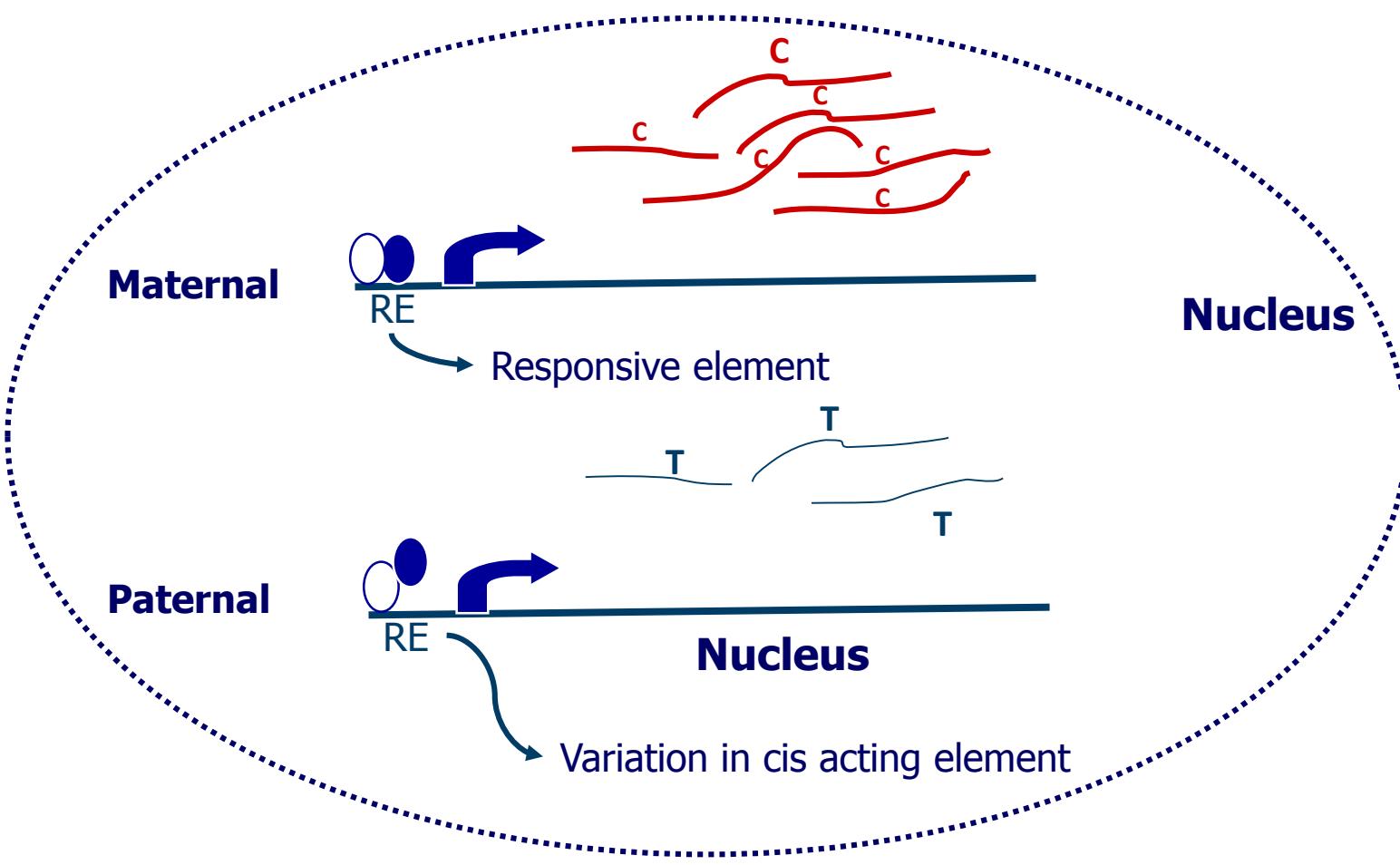
- SNPs in regulatory elements
- Differences in RNA stability
- Epigenetic mechanisms
- Tissues



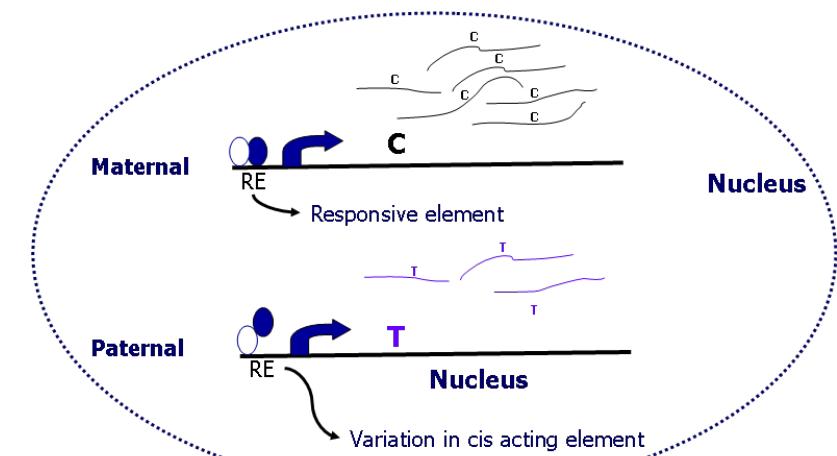
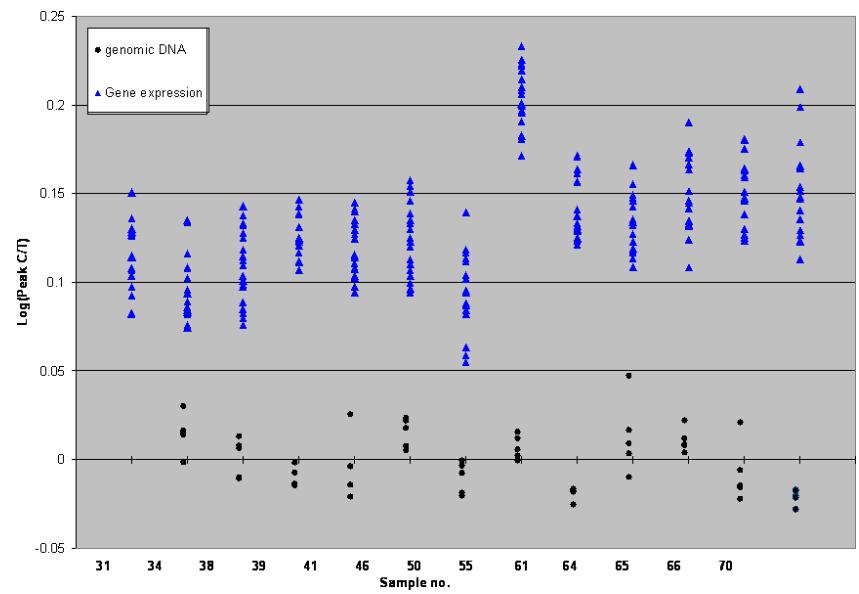
Subtle changes in expression regularly underlie complex diseases.

Differential allelic expression

Genetic variation at *cis*-acting regulator elements



Differential allelic expression DIO2



Significant increased expression of the DIO2 susceptibility allele in OA cartilage

DIO2 in articular cartilage

2010

DIO2 mRNA expression
absent in healthy and
high in OA cartilage

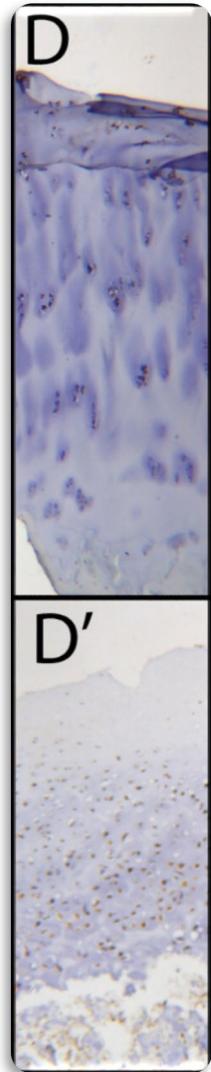
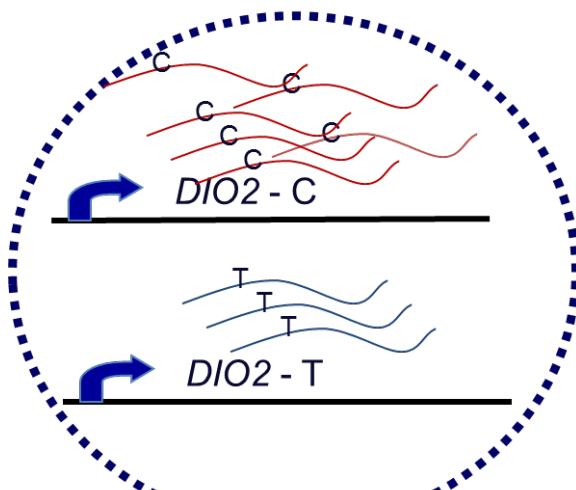
Ijiri et al. 2010

2012

Allelic imbalance &
protein up regulation

Bos et al. 2012

Rs225014 Allelic Imbalance

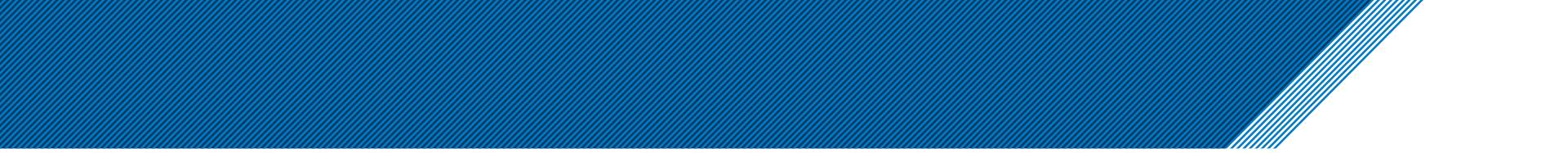


- Potential relevance *DIO2* in OA pathology
- Cis-eQTL function & direction of effect of risk allele

Which step in the Freedman et al strategy?

3. Determined expression levels of nearby genes as a function of genotype at each locus (eQTL).

..in disease relevant tissue!

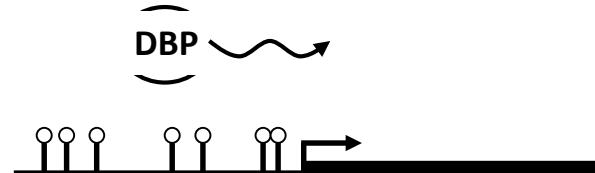
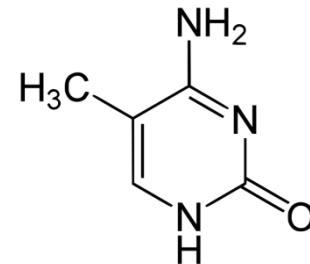
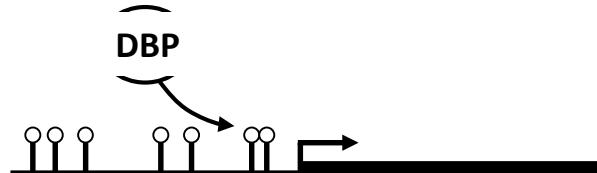
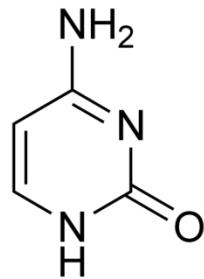


How is differential expression with disease state of *DIO2* regulated in cartilage

Epigenetics – DNA methylation

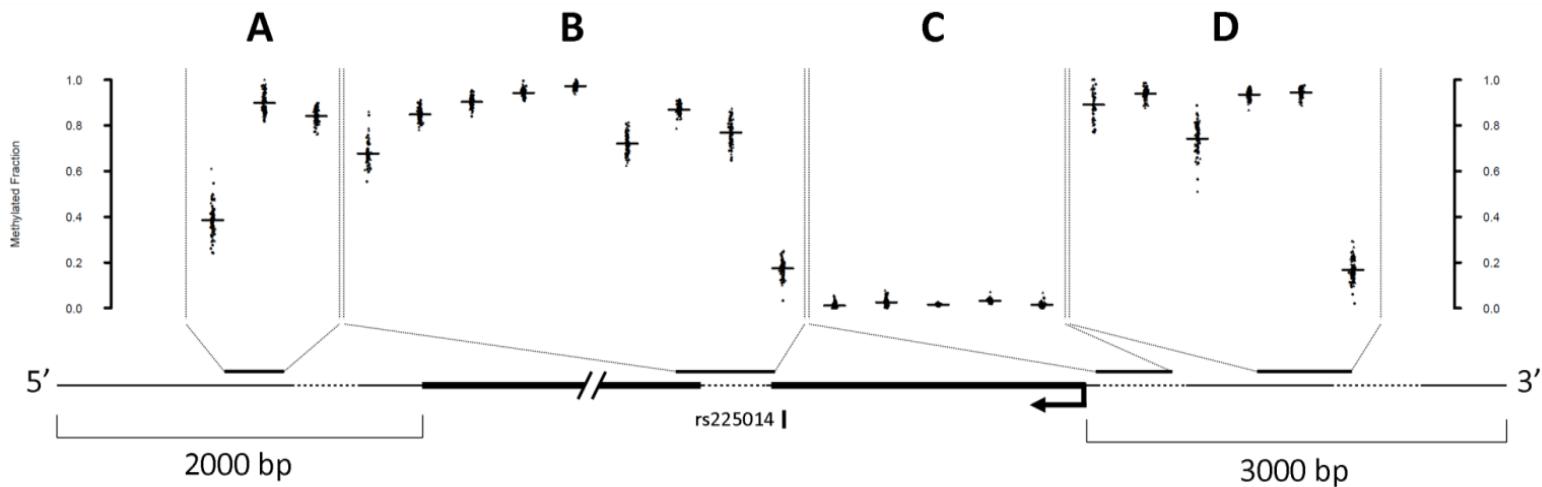
Mechanisms to adapt to environmental changes such as mechanical stress, disease and age.

- miRNAs
- Histon modifications
- DNA methylation
- ...



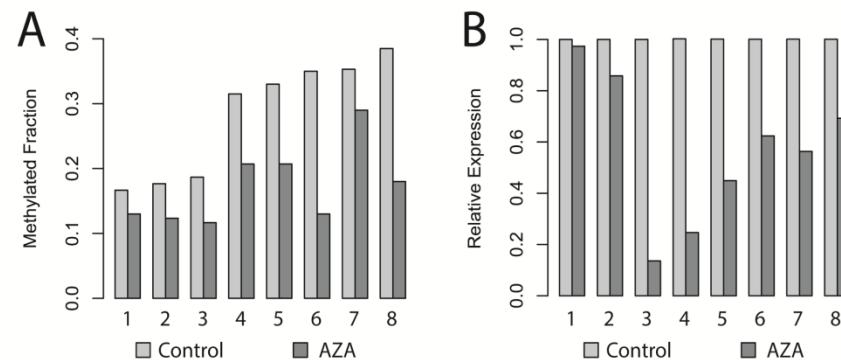
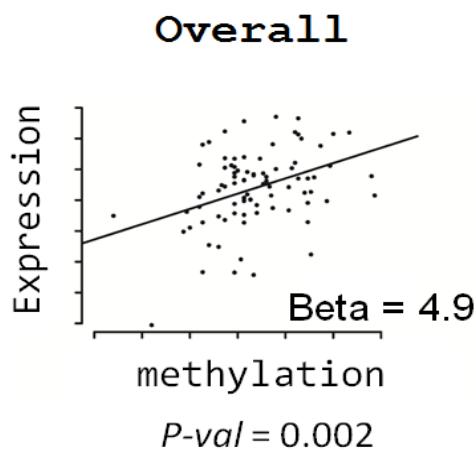
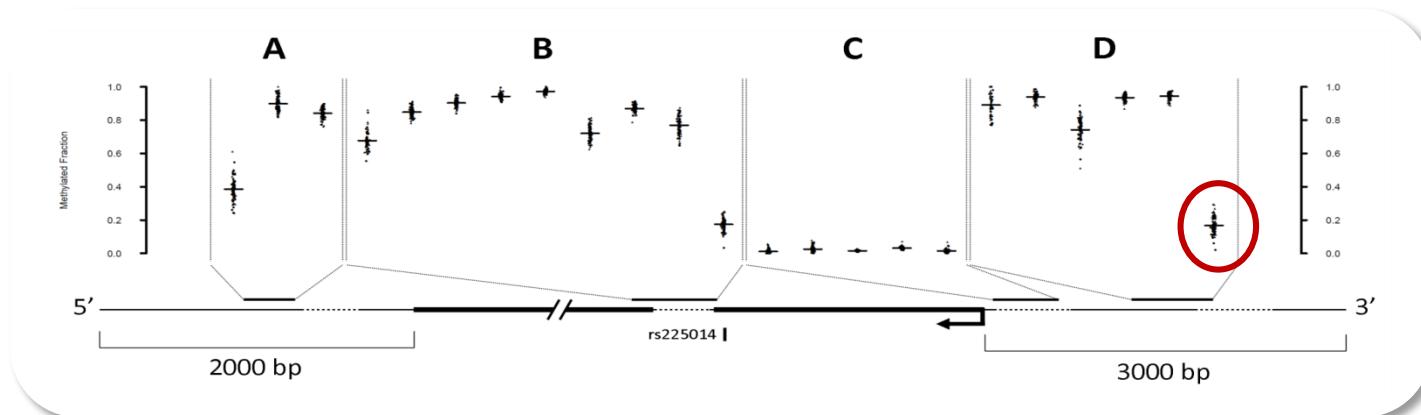
Regulation of *DIO2* expression

Gene targeted methylation at CpG sites (Epityper, Sequenom)

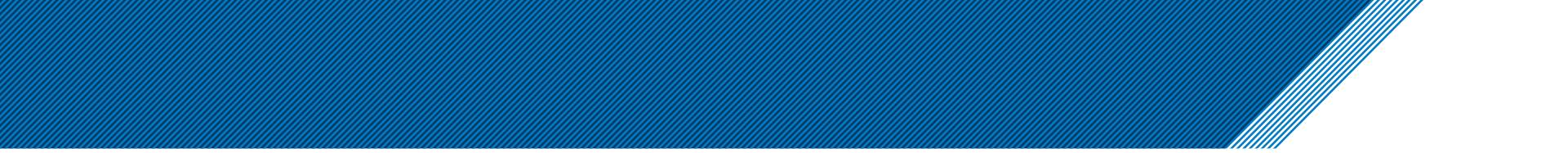


Regulation of *DIO2* expression

Gene targeted methylation at CpG sites (Epityper, Sequenom)



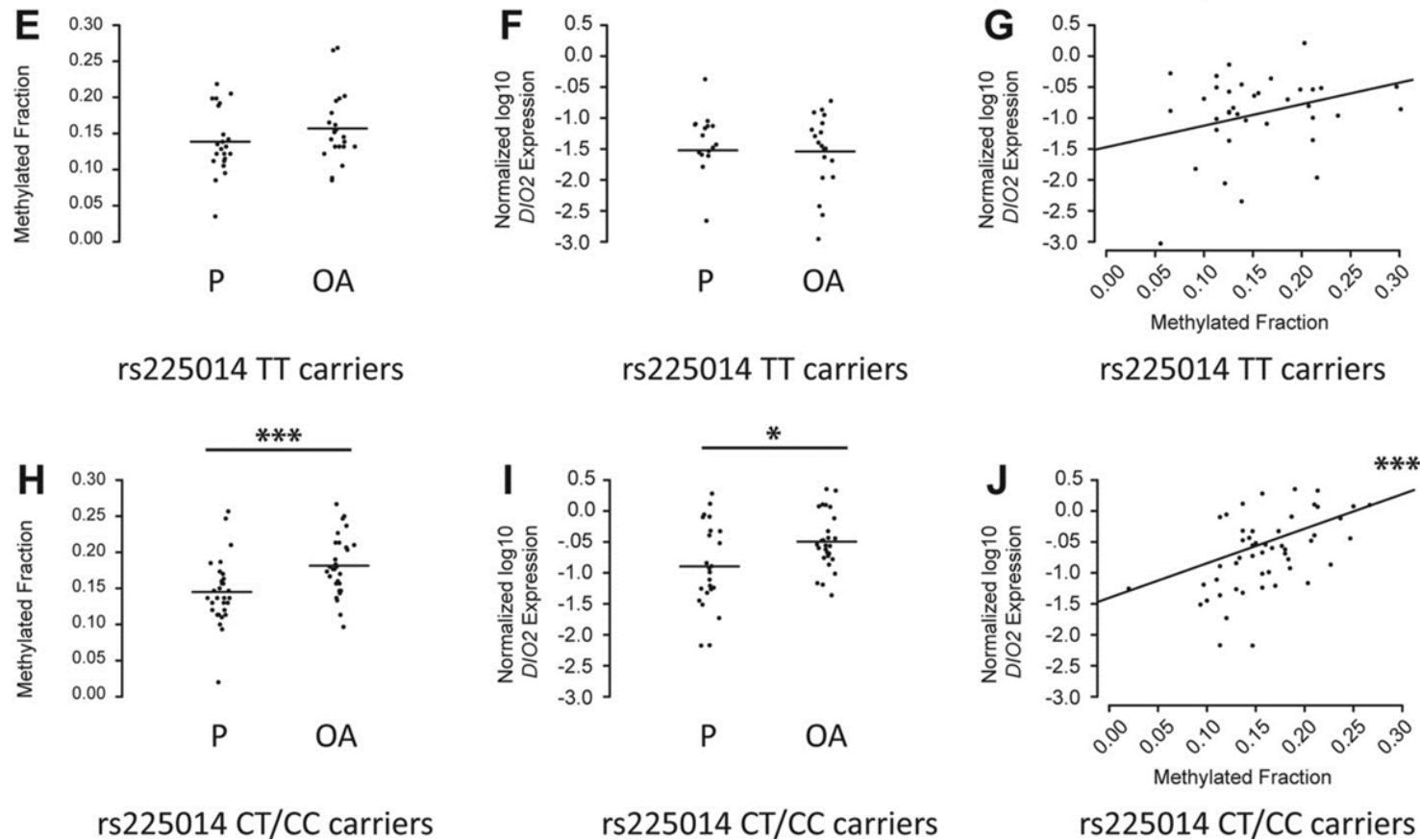
DIO2 expression in articular cartilage is modulated by methylation at CpG ~-2000 bp



Does the *DIO2* rs255014 modulates the effect?

Regulation of *DIO2* expression

Gene targeted methylation at CpG sites (Epityper, Sequenom)



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

Healthy

DIO2 unmethylated



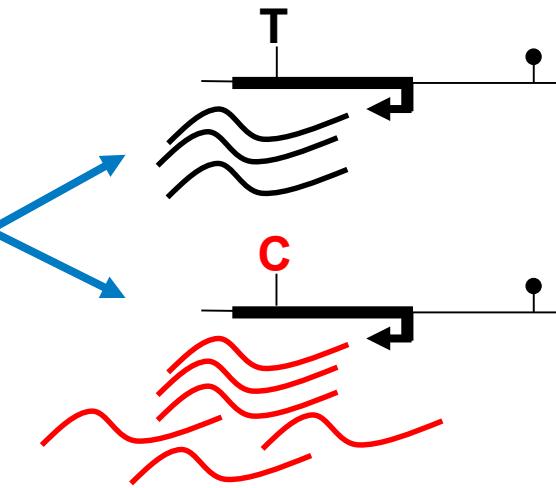
CTCF

Osteoarthritic

DIO2 methylated



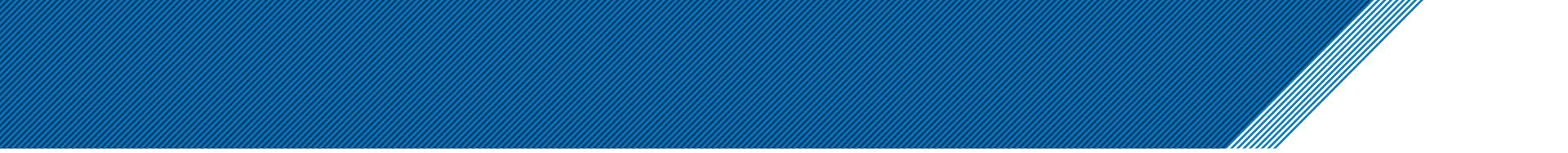
CTCF



Which step in the Freedman et al strategy?

- 4. Characterized gene regulatory regions by multiple techniques**
- 7. Explore epigenetic mechanisms in the context of genome wide genetic polymorphisms**

..in disease relevant tissue!

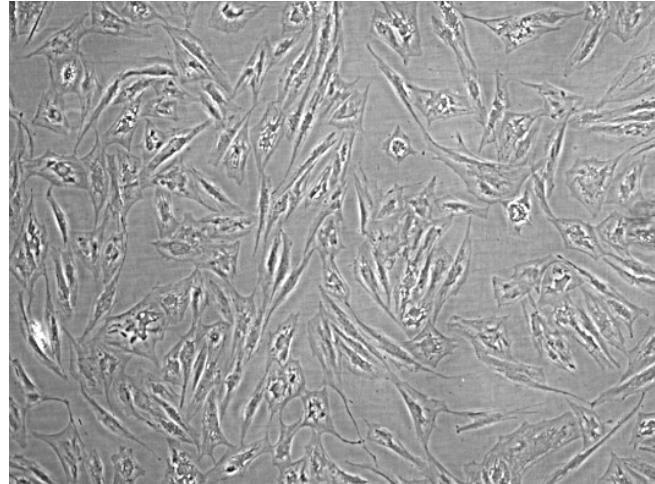


What is direct effect of DIO2 upregulation in cartilage?

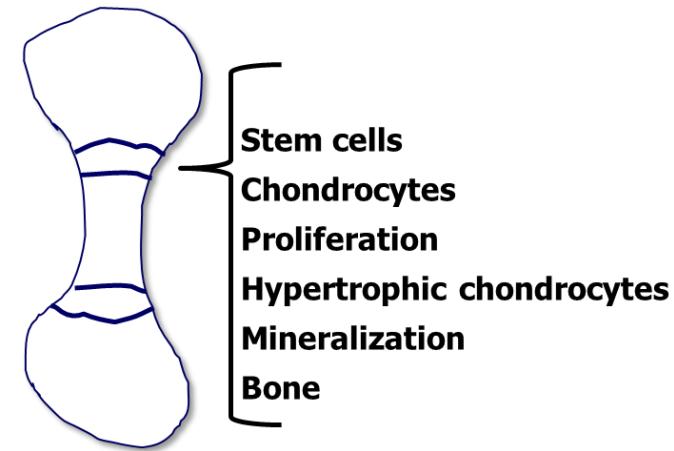
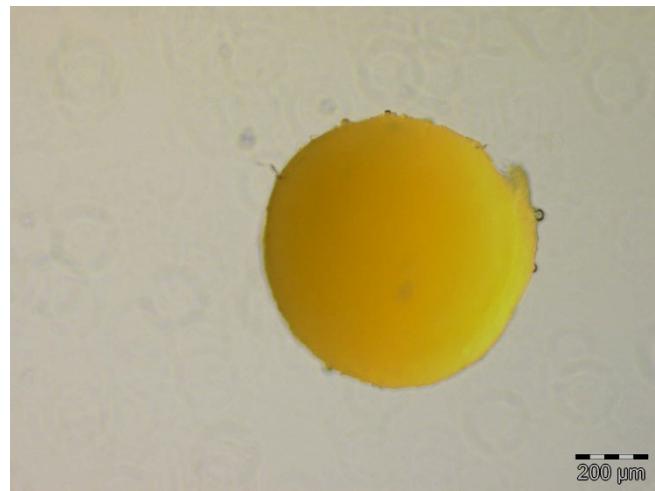
Human *in vitro* chondrogenesis model

Stemcells, primary chondrocytes

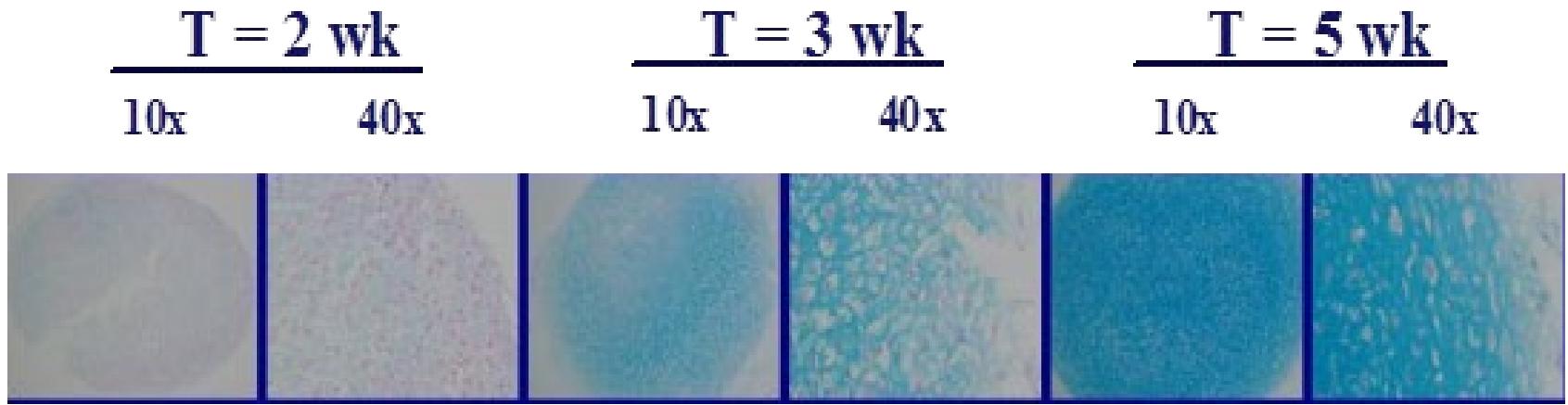
Growing cells
(monolayer)



Chondrocyte pellet
cartilage formation



In vitro chondrogenesis model Stemcells, primary chondrocytes

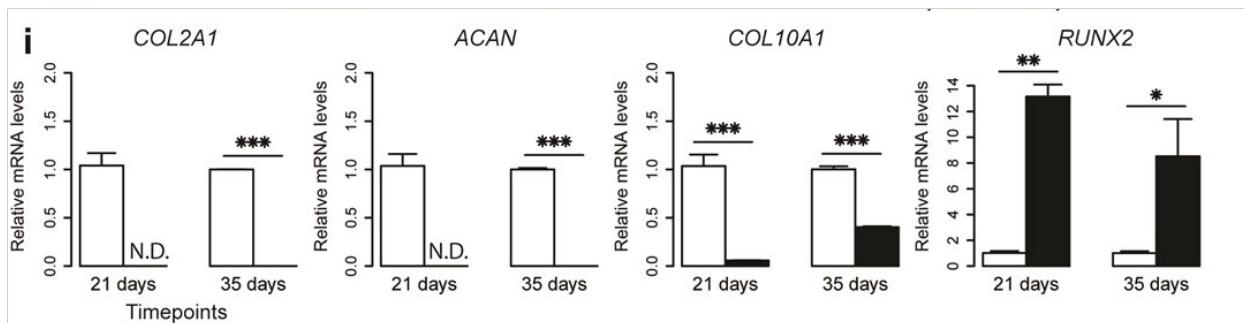
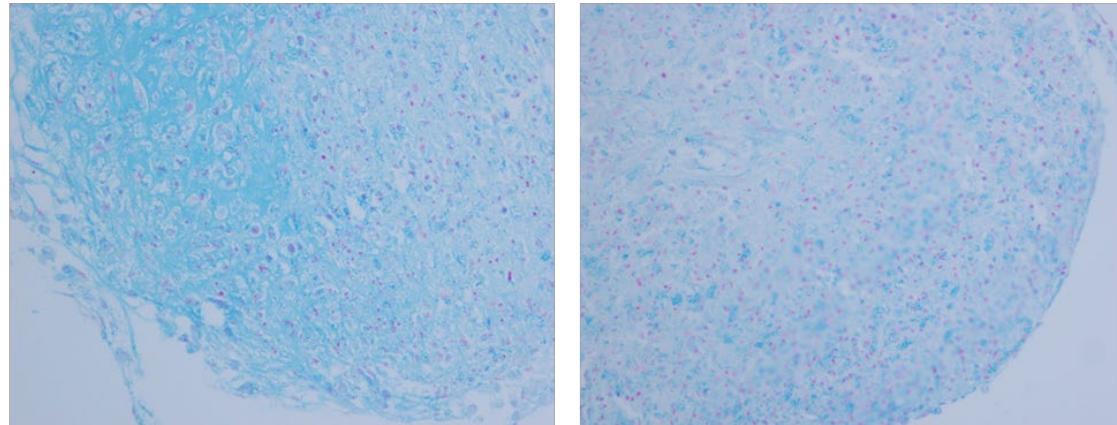


In vitro chondrogenesis model using human bone marrow derived mesenchymal stem cells. Alcian blue staining

What is direct effect of DIO2 upregulation

BM-MSC based *in vitro* chondrogenesis model

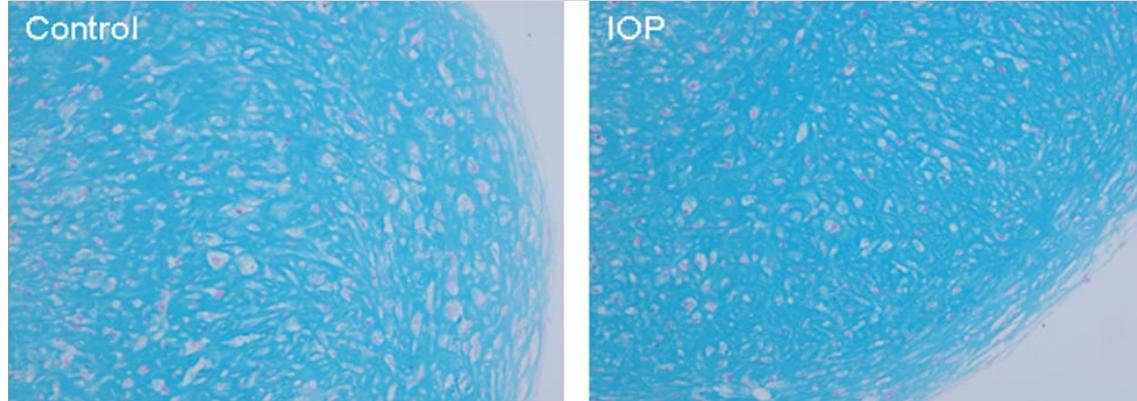
Overexpression of DIO2



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

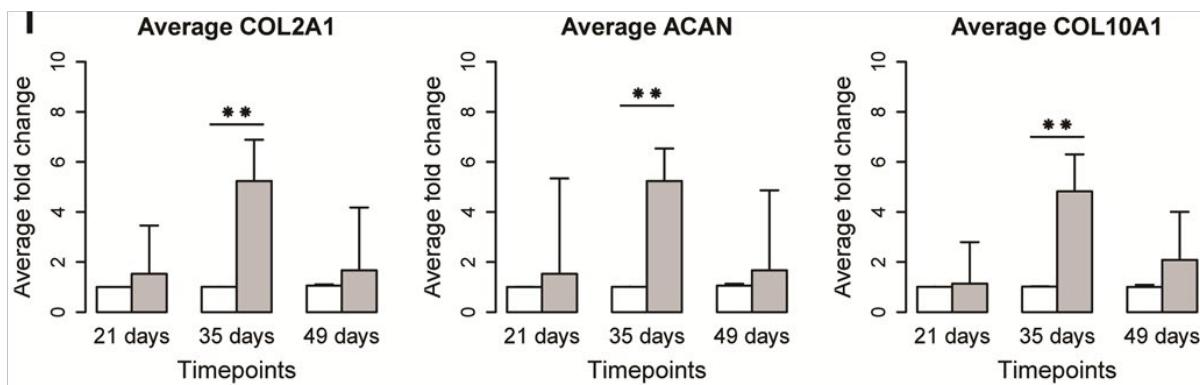
BM-MSC based *in vitro* chondrogenesis model

Inhibition of DIO2 function



IOP = Iopanoic acid, an oral cholecystographic agent, an effective inhibitor of iodothyronine deiodinase and medication for thyrotoxicosis.

Tyer et al. Endocr Pract. 2014 20 (10):1084-1092



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

Conclusions human *in vitro* studies DIO2

Direct detrimental effect of DIO2 on cartilage matrix deposition .

Beneficial effect of DIO2 on cartilage matrix deposition

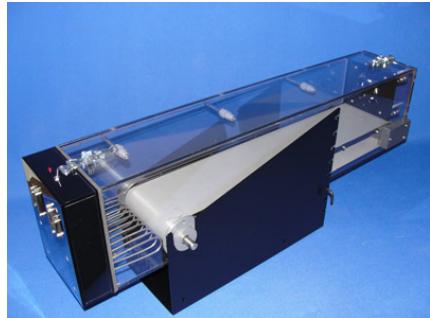
What about *in vivo* studies?



DIO2 Knock-Out model

Collaboration KU Leuven, Leuven, Belgium

Design: Running induced mechanical stress



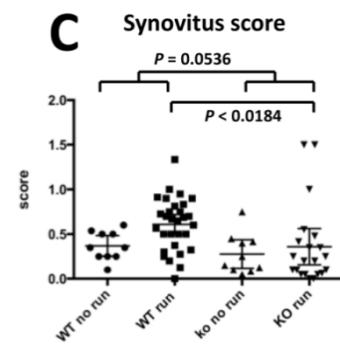
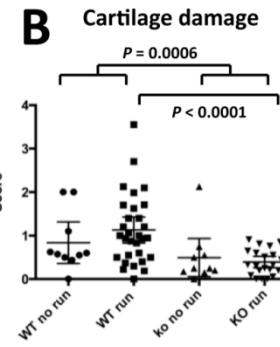
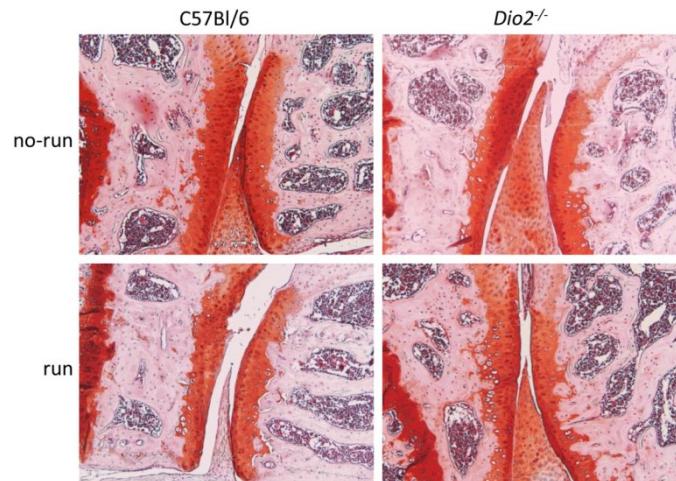
DIO2-KO & Control
(C57BL/6 males)



For 3 weeks -> 5 days/week -> 60 min/day
(60min at 11m/min, 5° incline)



Induce cartilage damage



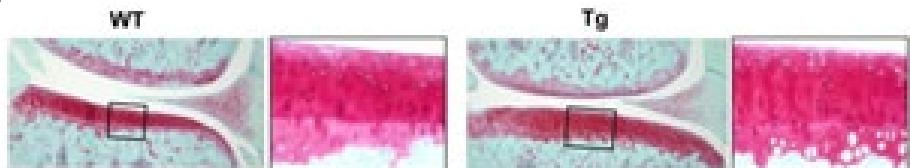
Dio2-/- mice protected against cartilage damage
only upon exercise-induced OA

Bomer et al. Ann Rheum Dis. (2015)

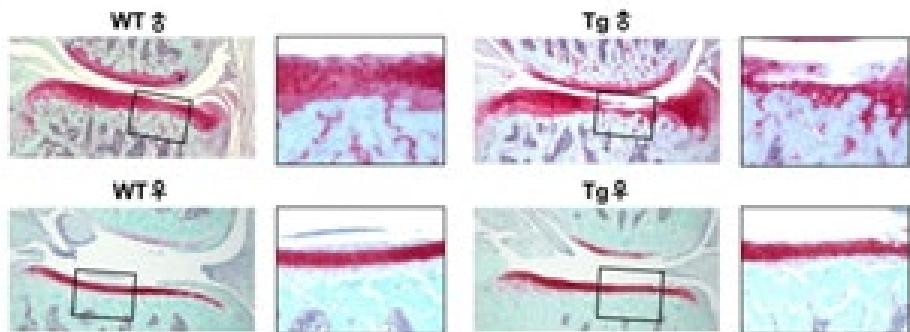
2015

Tissue specific induction *DIO2* in rats

A



B

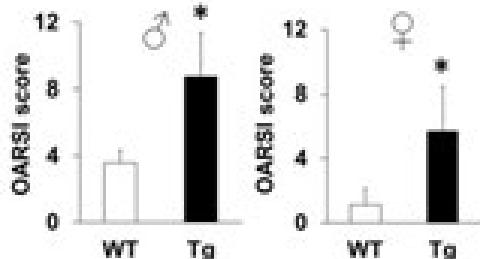


Destruction of cartilage only
upon applying OA model

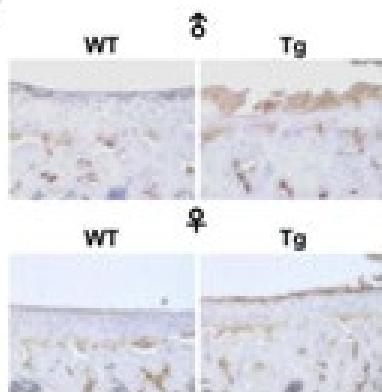
Nagase et al. Ann Rheum Dis 2013

2013

C



D



6. **Multiple experimental manipulations in model systems are needed to progressively implicate transcription units (genes) in mechanisms relevant to the associated loci**

Pharmacological attenuation of thyroid hormone signaling; An evidence based treatment option for Osteoarthritis





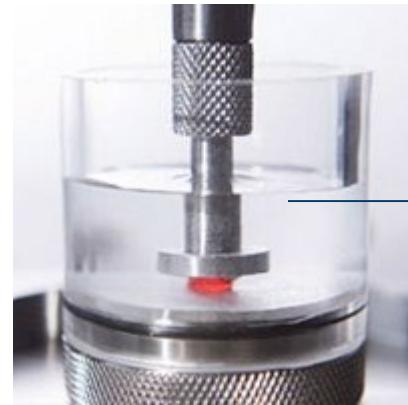
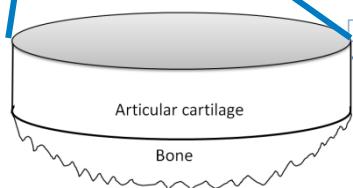
*Conclusions *in vitro* animal studies DIO2*

**Tissue specific upregulation of *DIO2* in rat cartilage
Prone to OA after applying OA model**

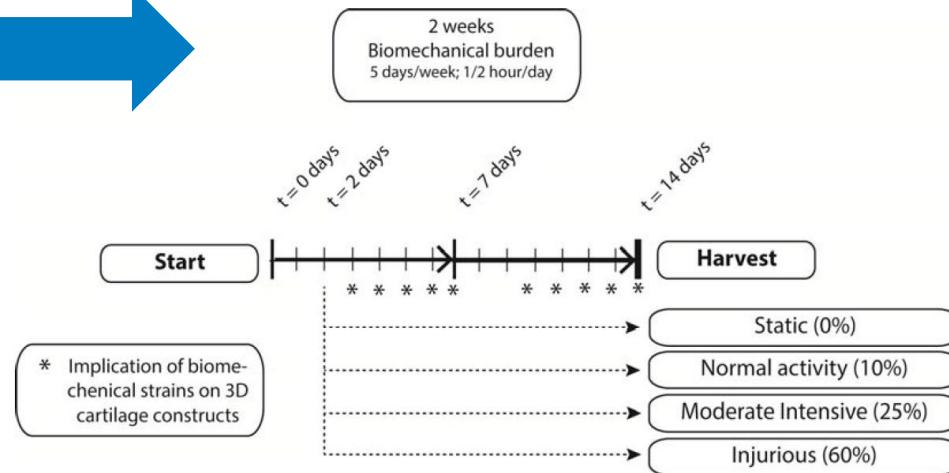
**DIO2 knock out mice protected against cartilage damage
only upon exercise-induced OA**

Objective 1

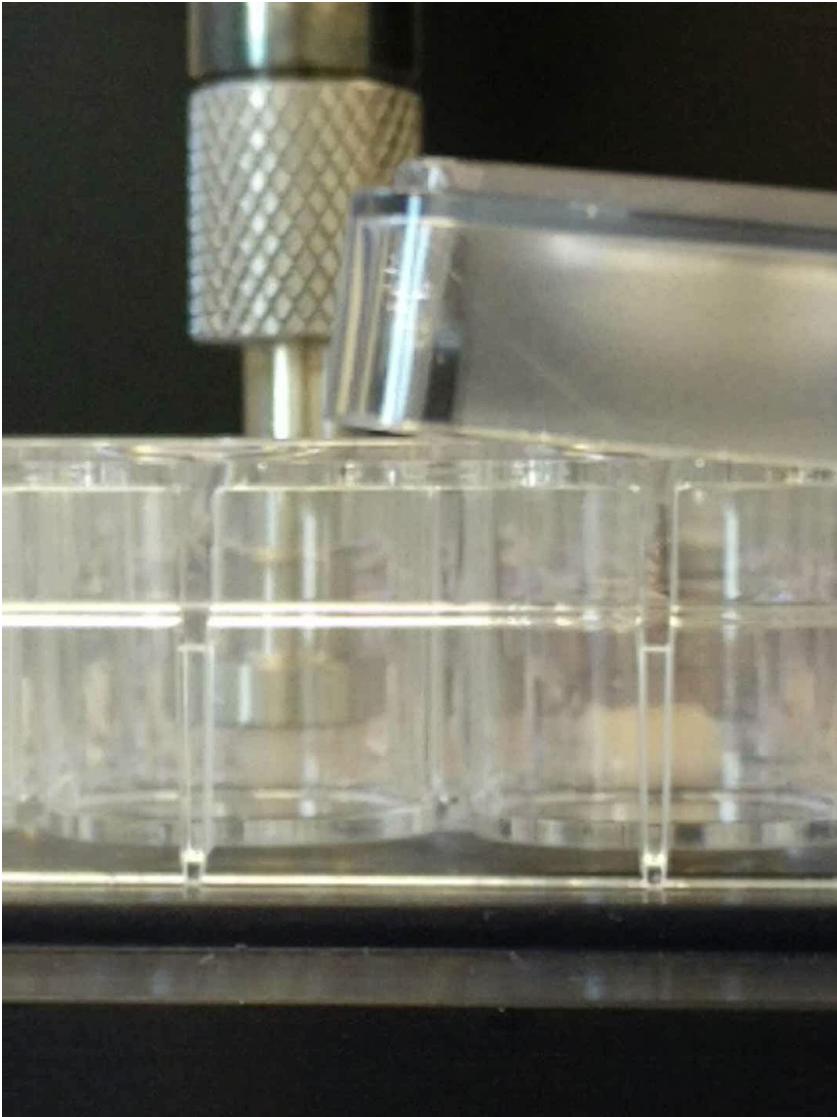
- To accomplish a human *ex vivo* cartilage explant OA model and demonstrate the beneficial effect of IOP herein
 - Cartilage explants collected from unaffected areas
 - Simulate average human loading: 5 days per week



PBS

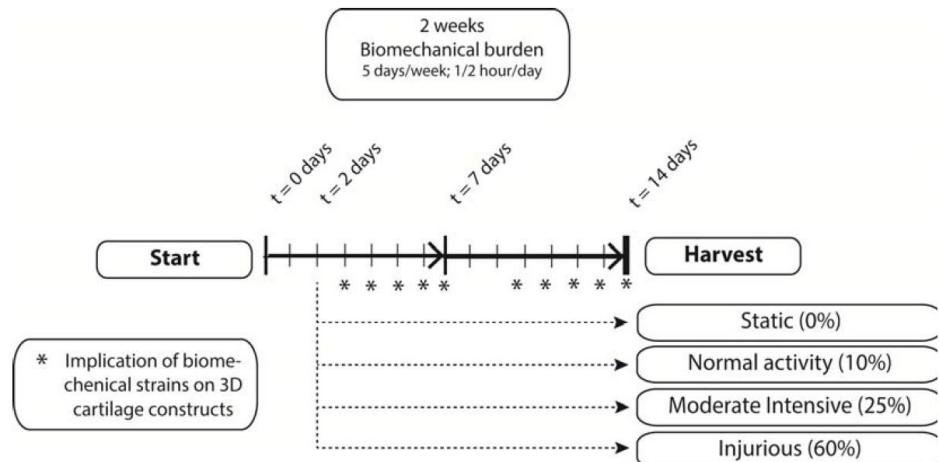
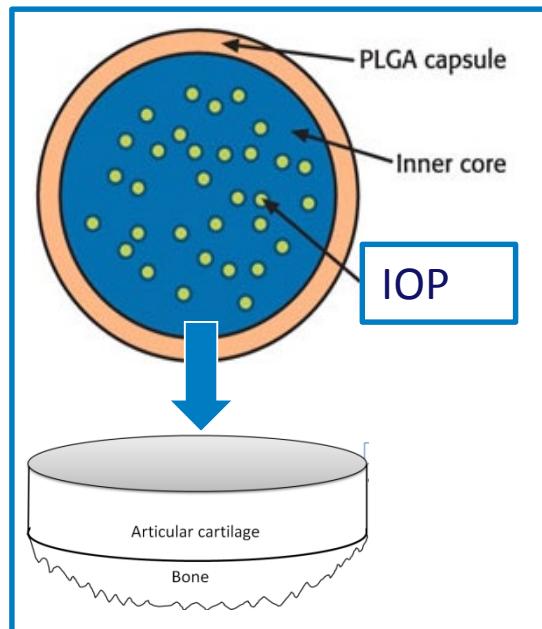


Trigger OA pathophysiology by mechanical loading



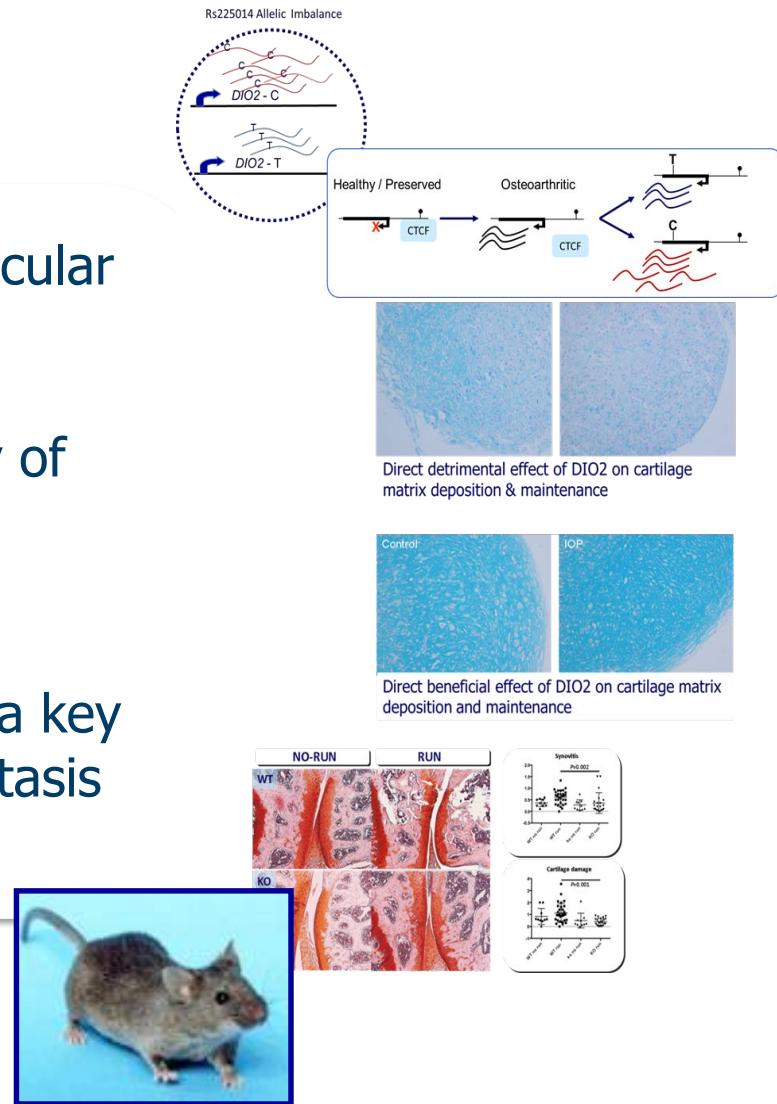
Objective:

- To demonstrate the beneficial effect of IOP carrying PGLA nano-particles in the human *ex vivo* cartilage explant model
 - start IOP treatment at different time points to investigate whether IOP can:
Prevent, Stop or Reverse OA damage



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



Which step in the Freedman et al strategy?

8. Employ (*in vitro*) cell models and tissue reconstructions to evaluate mechanisms using gene perturbations and polymorphic variants.

..in human tissue!