Osteoarthritis a complex disease with high social economic burden

Osteoarthritis (OA)

- Degenerative joint disease
- Prevalent
- No effective treatment

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

Impediments OA disease management

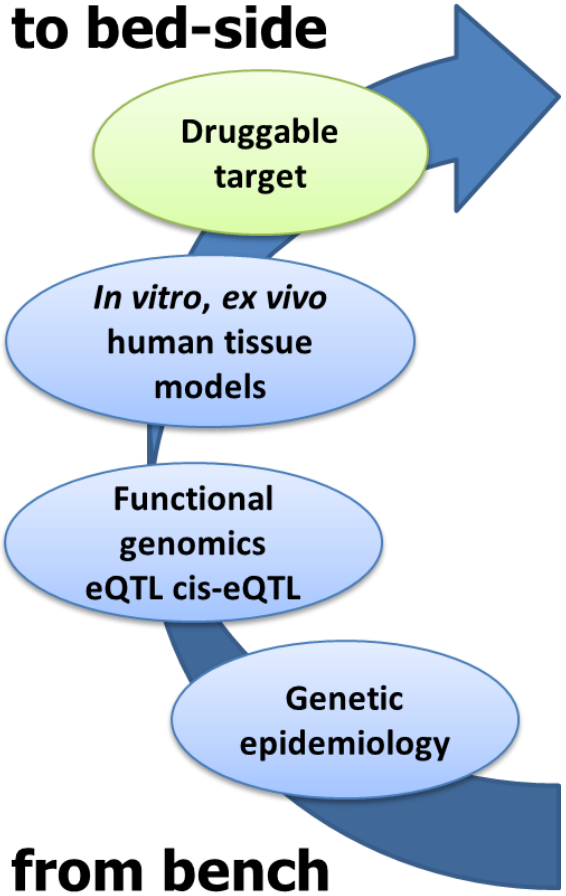
- **Complex heterogeneous disease**
- **Little insight in disease etiology**
- **Difficult diagnosis**

Severe backlog in development of effective disease modifying OA therapies

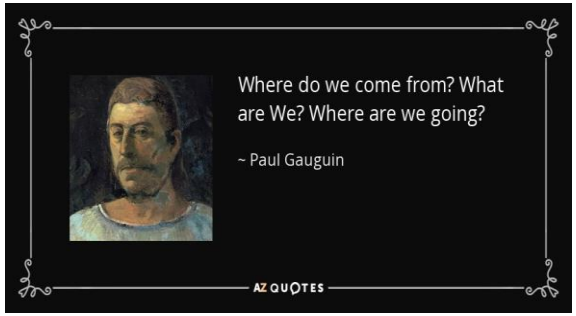
Which step in the Freedman et al strategy?

1. Which type of study is powerful in identifying underlying disease mechanisms?

Overview



- **Novel underlying disease pathways**
- **Novel druggable targets**



Heritability and the genetic study design

Balance between heritability and environment

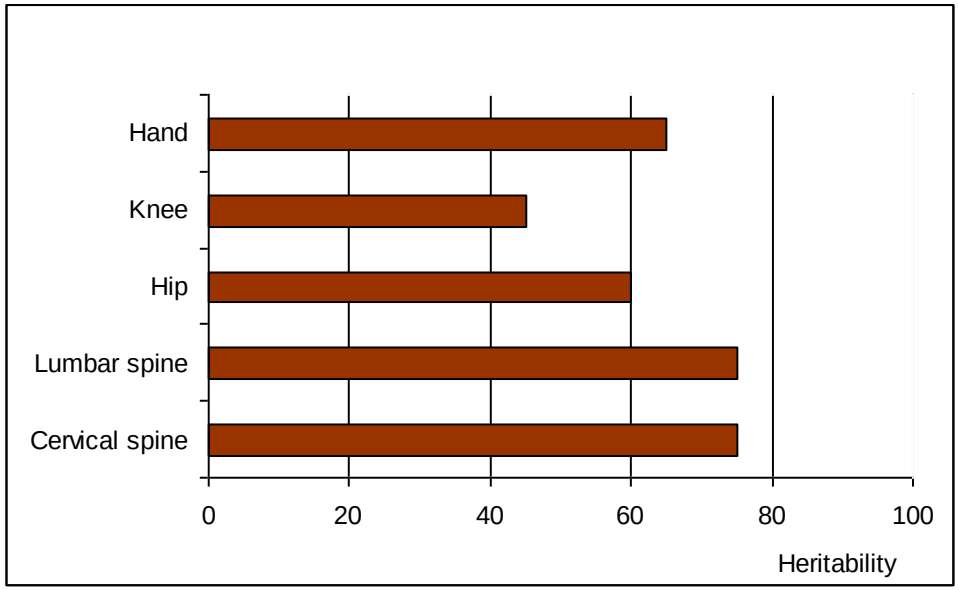
complex genetic

100% environment

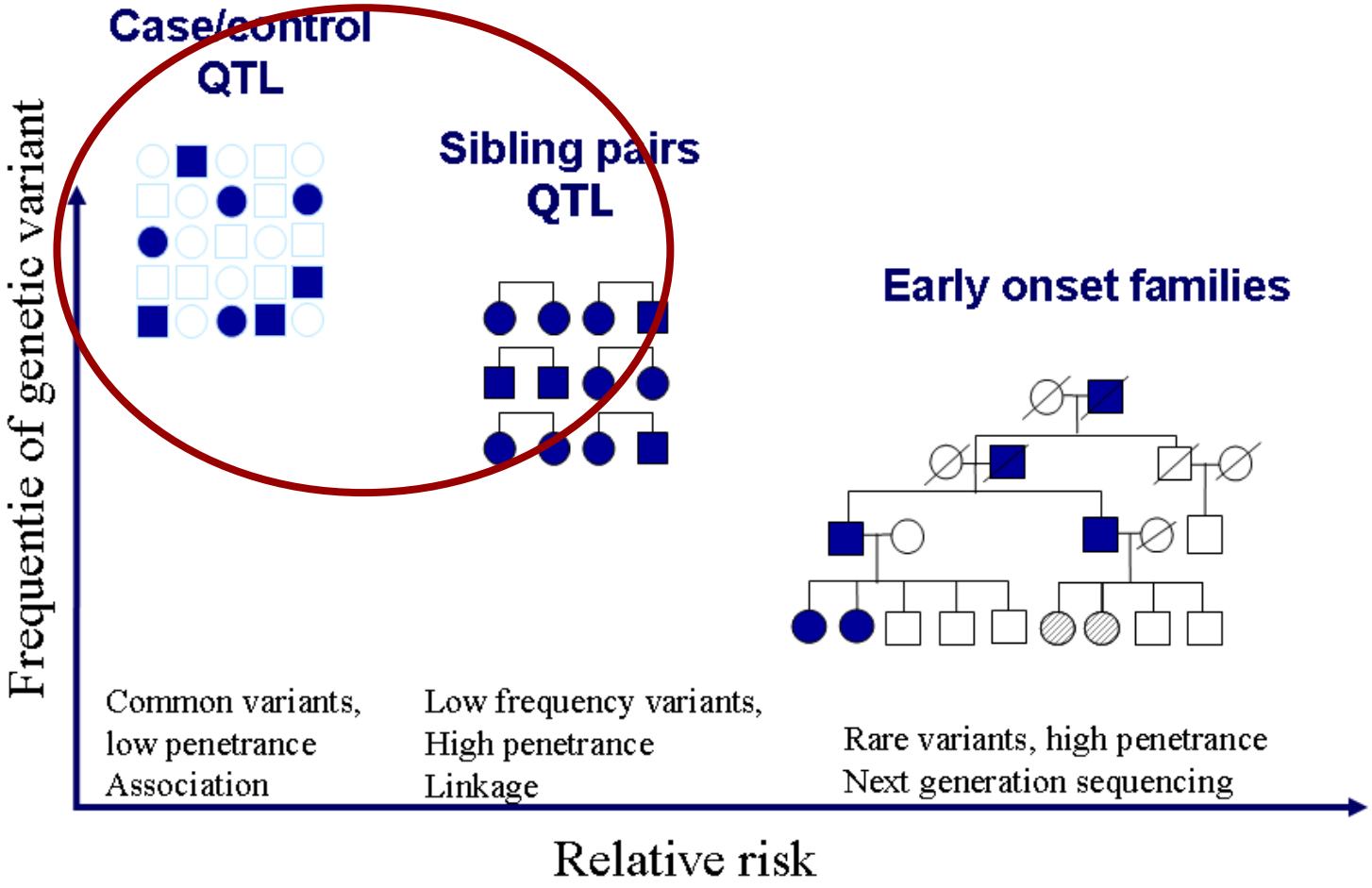
100 % genetic

Determines genetic study design

Osteoarthritis - Heritability

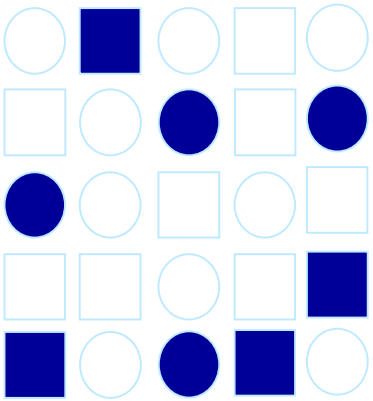


Study design depends on inheritance pattern



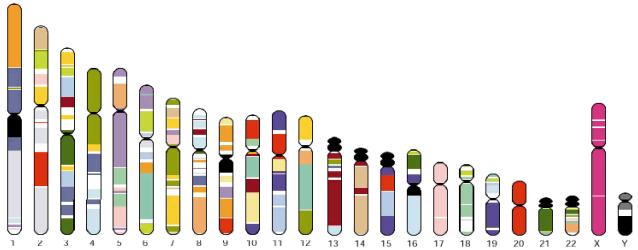
Association analyses case/controls

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



Finding association is about finding genes / pathways changing your risk to develop disease

Possible genetic approaches



Candidate genes

Hypothesis driven - Educated guess

Genome wide scan

Discovery driven - Acknowledged lack of knowledge

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%

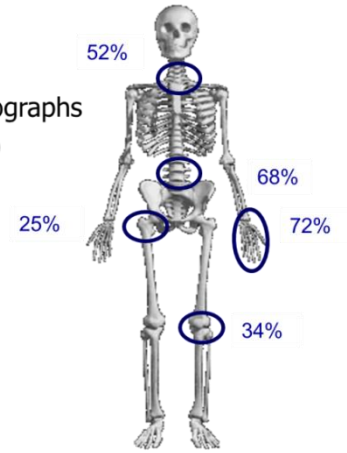
Inclusion:

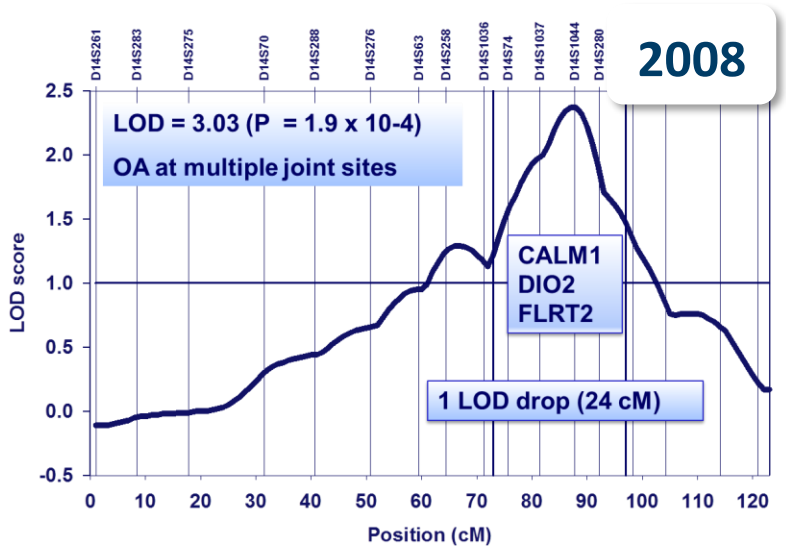
≥ 2 joints OA

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

How to delineate the risk locus?

Which step in the Freedman et al strategy?

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

BOX 1 STRATEGIES TO PROGRESS FROM TAG SNP TO MECHANISM

1) Target resequencing efforts using linkage disequilibrium (LD) structure.

may be limited by functio
methods in rat are promis

DIO2, OA susceptibility gene
The Garp study, combined linkage association

Gene	*SNP reference*	*allele*	*alias*	*MAF*	*P-value*
DIO2	**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**

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

Are we convinced of this locus being a strong risk gene?

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**

BOX 1 STRATEGIES TO PROGRESS FROM TAG SNP TO MECHANISM

1) Target resequencing efforts using linkage disequilibrium (LD) structure.

2) Use other populations to refine LD regions (for example African ancestry with shorter LD and more heterogeneity).

may be limited by functio
methods in rat are promis
expression analysis.
ii) Use chromatin associat
regulatory regions to deter

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

Female cases severe hip OA

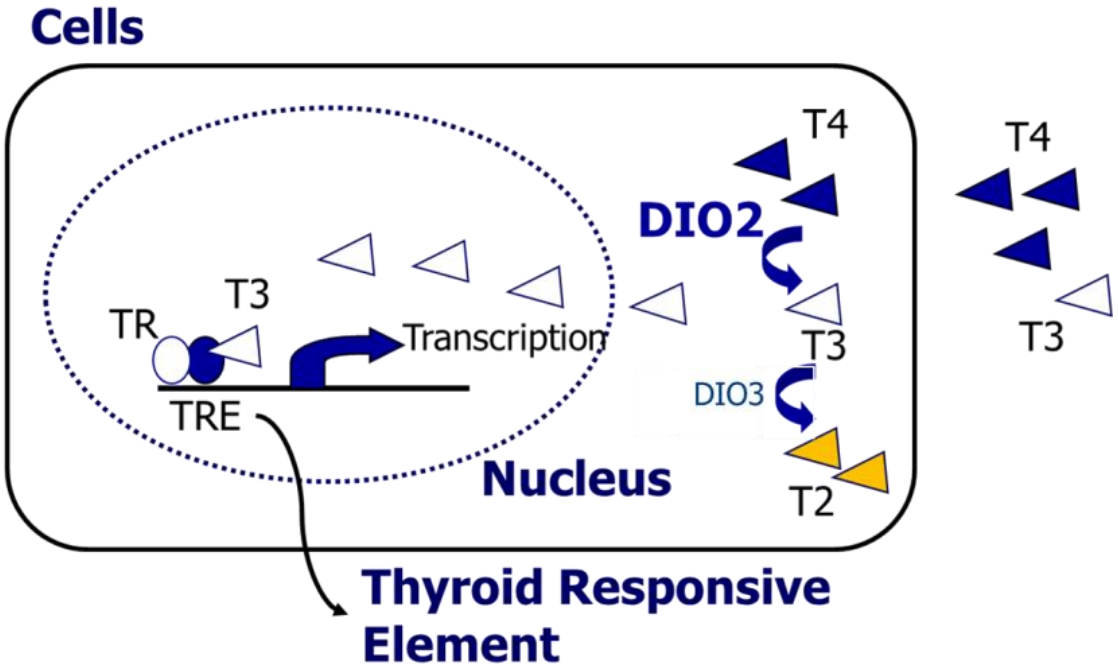
Gene	*OR Recessive model*	*P of OR*	*P value heterogeneity test*
All*	**1.8 (1.4-2.3)**	**$2x10^{-5}$**	**0.6**
UK (Oxford)	**2.1 (1.4-3.2)**	**0.001**	
NL (R'dam)	**1.9 (1.0-3.5)**	**0.040**	
Japan (Riken)	**1.5 (1.0-2.3)**	**0.047**	

*Random effect meta-analyses

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

Identification of *DIO2* as OA susceptibility locus

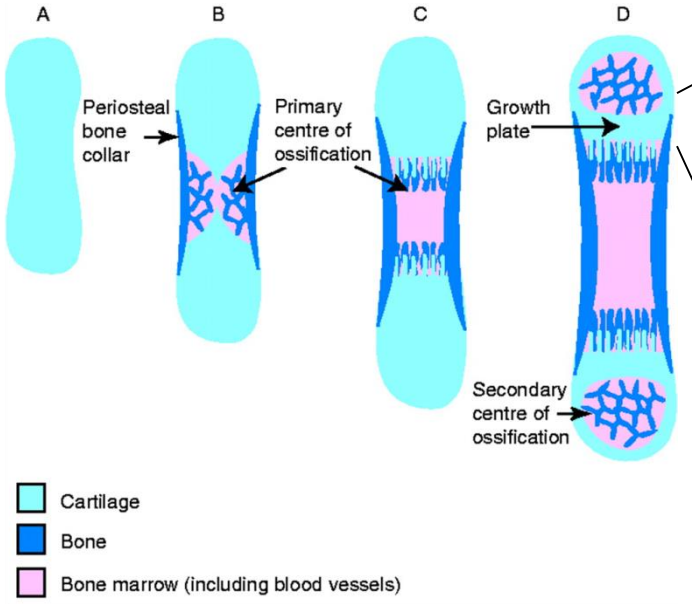
The GARP study



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



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

Stem cells

Growth plate chondrocytes

- **Proliferation**
- **Hypertrophic**
- **Mineralization**

Bone

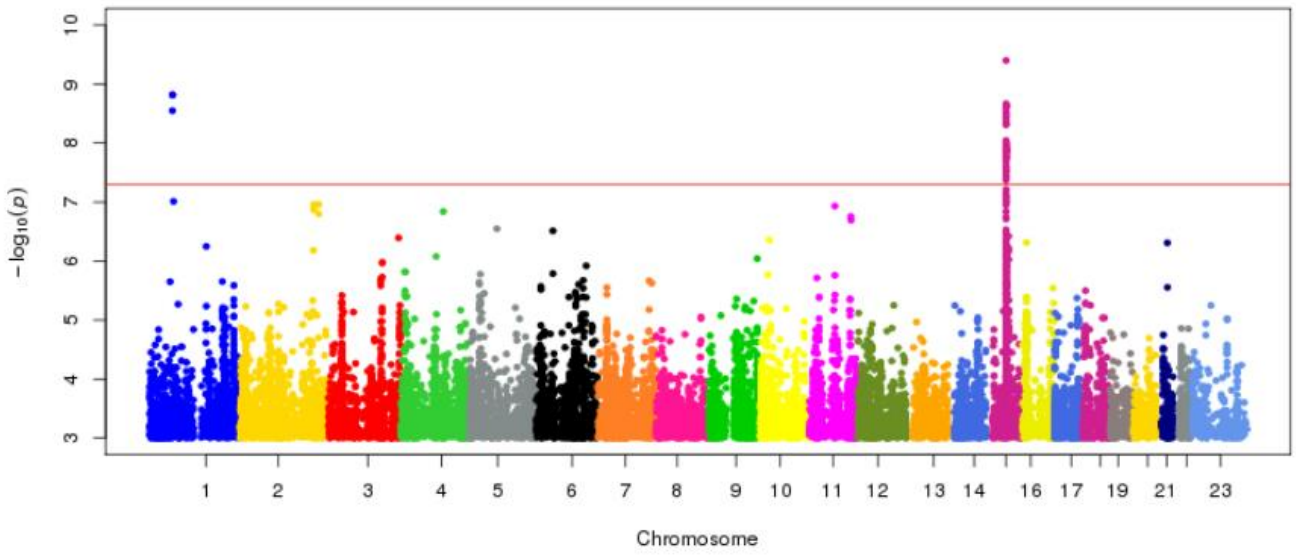
T4 → **DIO2** → **T3**

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

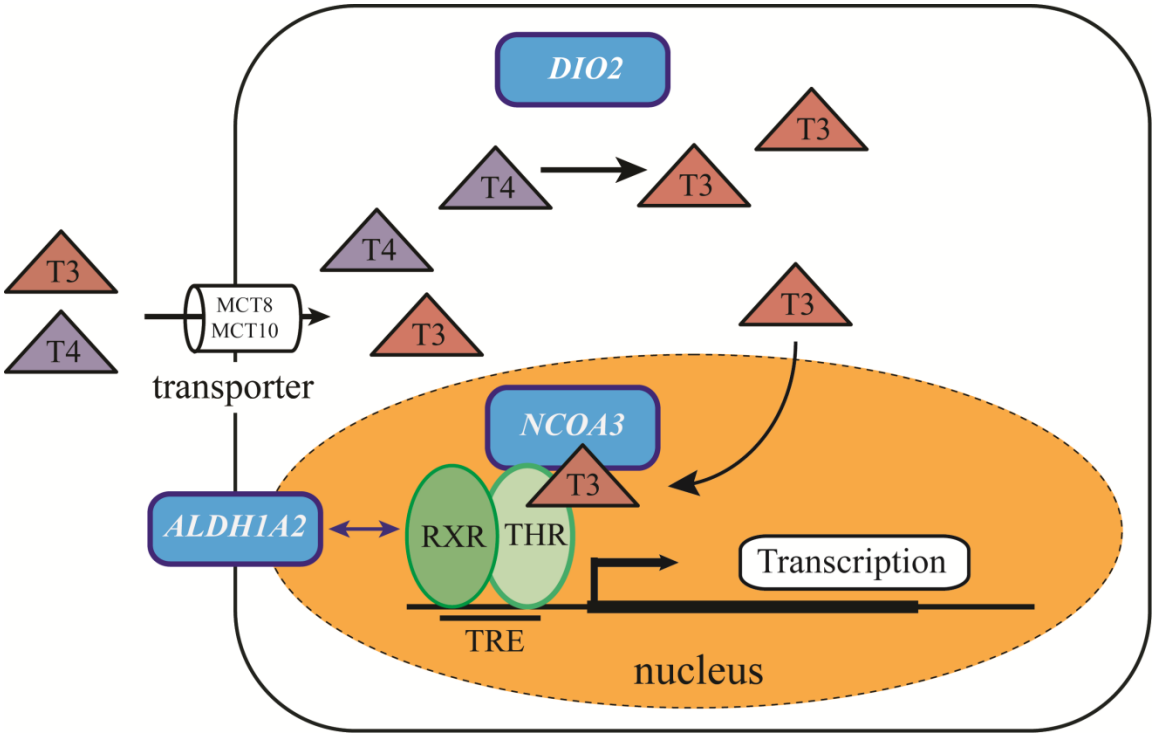
Additional OA genes in thyroid pathway

- 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

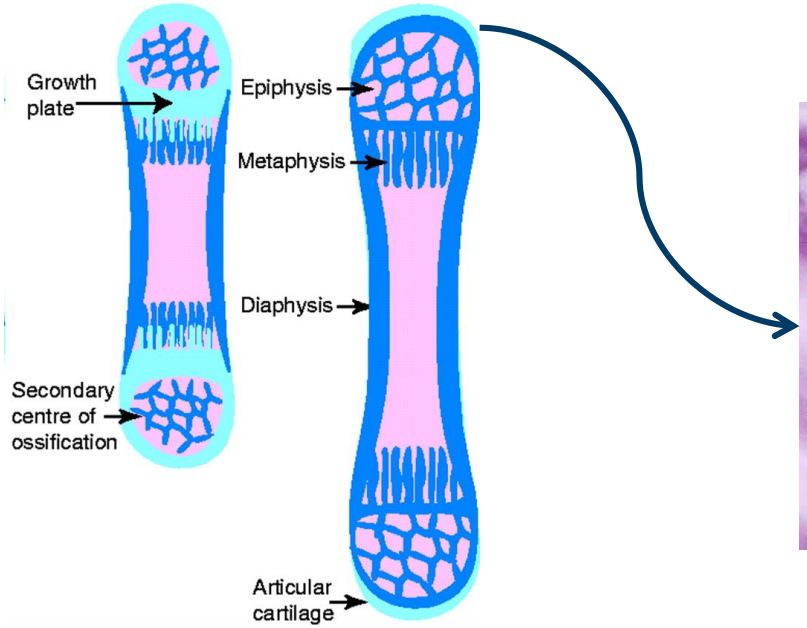
Formulate hypothesis

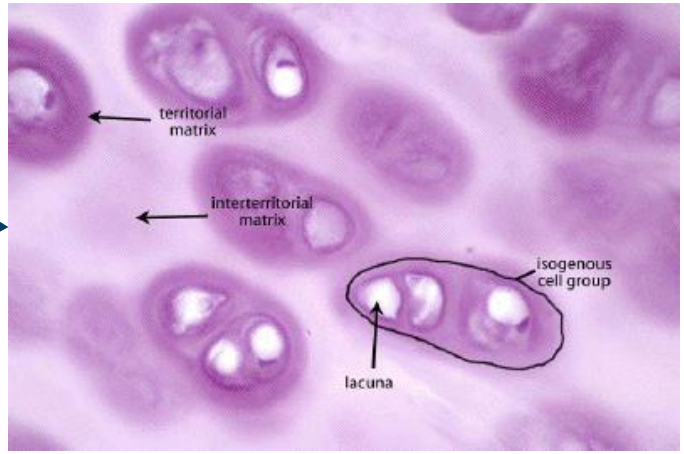
Developmental process of endochondral ossification

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.





Healthy articular cartilage

Endochondral ossification and Osteoarthritis
Late life effect of *DIO2*

Late life
disruption of epigenetic silencing of thyroid signaling in articular cartilage

↓

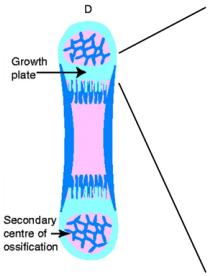
Loosening of maturational arrested state

↓

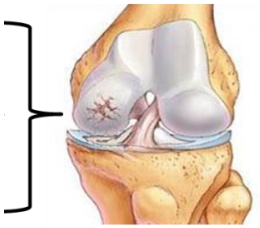
Debilitating cartilage signaling

↓

Osteoarthritis susceptibility

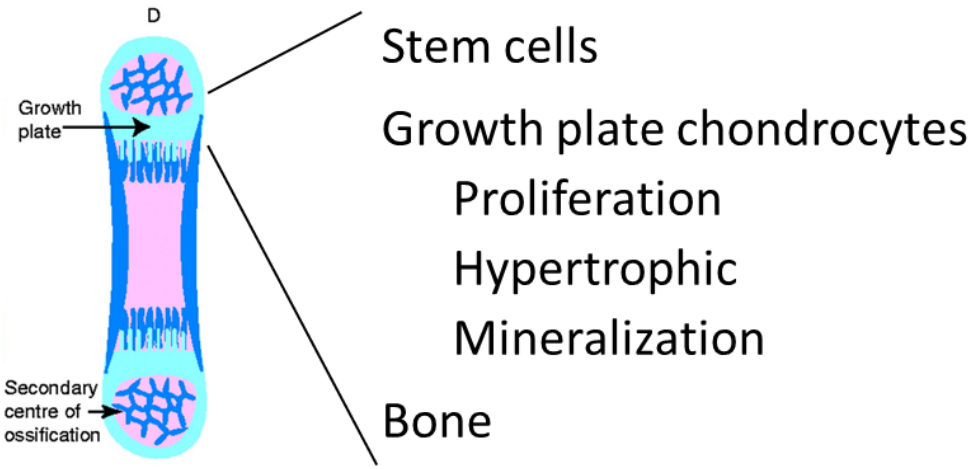


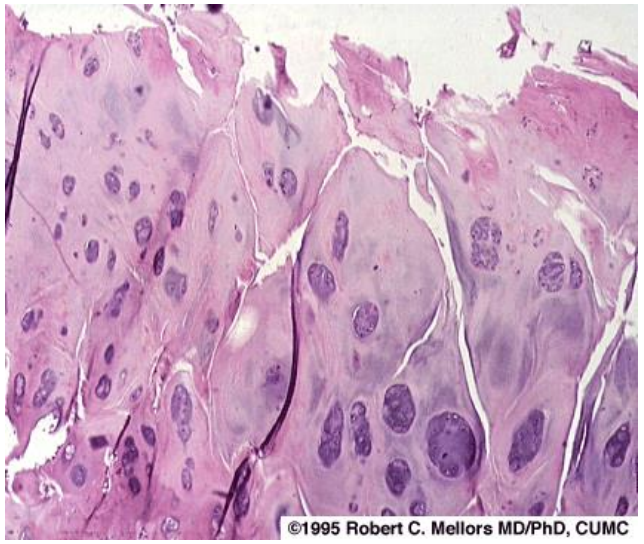
Stem cells
Growth plate chondrocytes
Proliferation
Hypertrophic
Mineralization
Apoptosis
T3
Bone



Chondrocytes in OA pathophysiology

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





OA affected articular cartilage

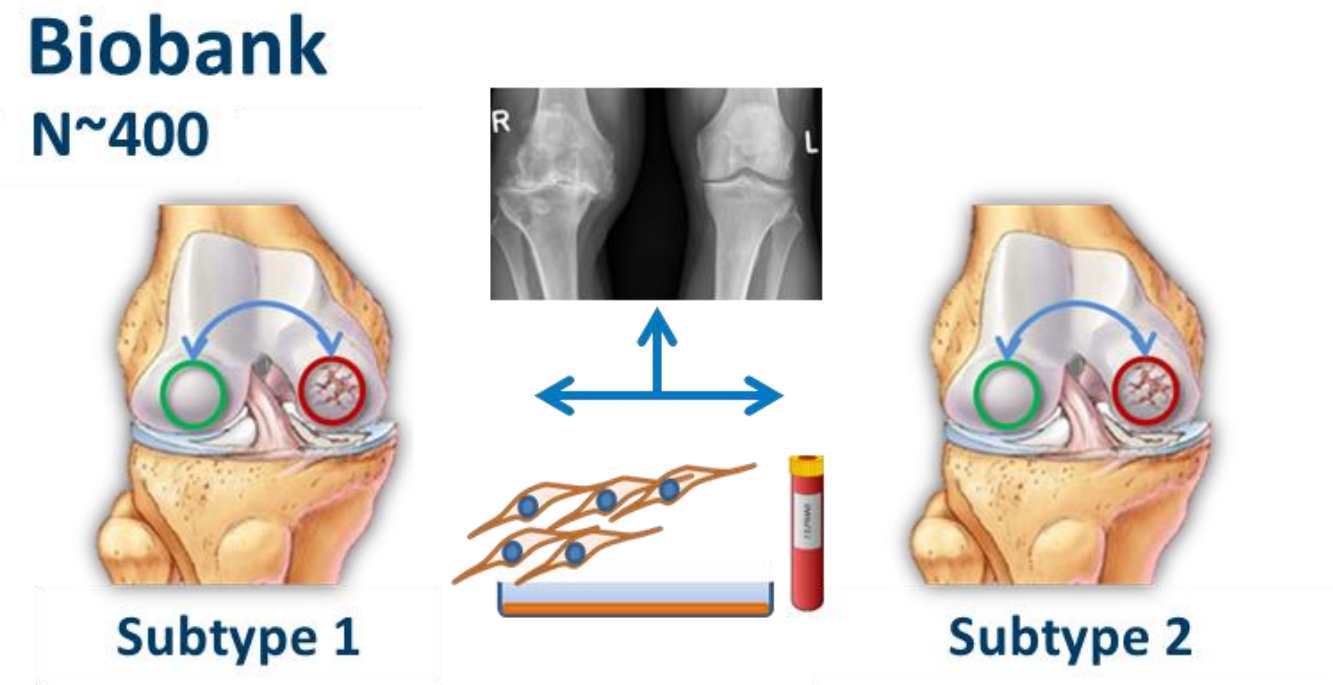
OA phenotype in articular cartilage; developmental process of endochondral ossification

The recuperation of thyroid signaling in osteoarthritic cartilage?

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

Research Articular osteoArthrits 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).

Functional characterization of the DIO2 risk locus

What would be your primary questions?

Gene:

Expression gene/protein is disease relevant tissue

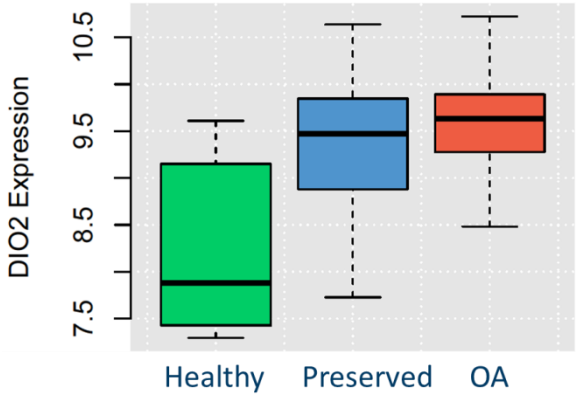
Is the gene responsive to disease process

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

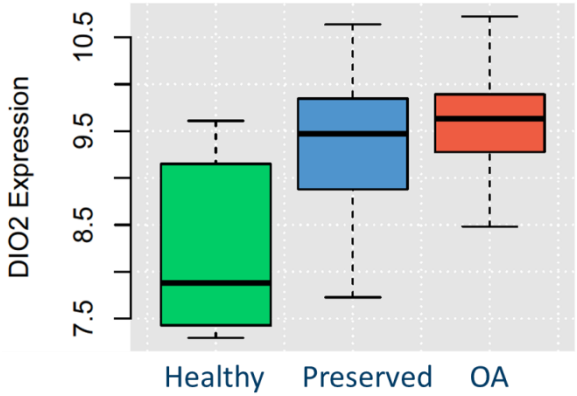
DIO2 mRNA **expression high in OA cartilage compared to healthy**

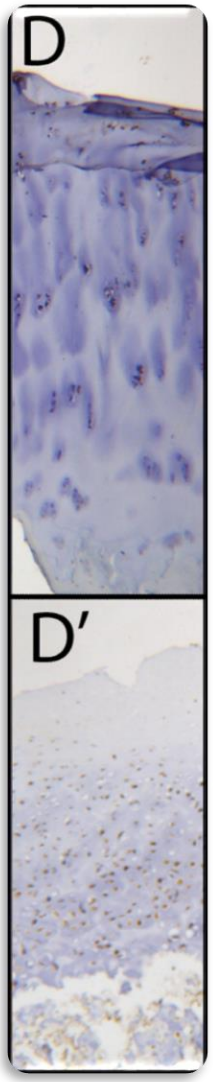
Ijiri et al. 2010

2012

DIO2 protein expression up regulated in OA affected cartilage

Bos et al. 2012

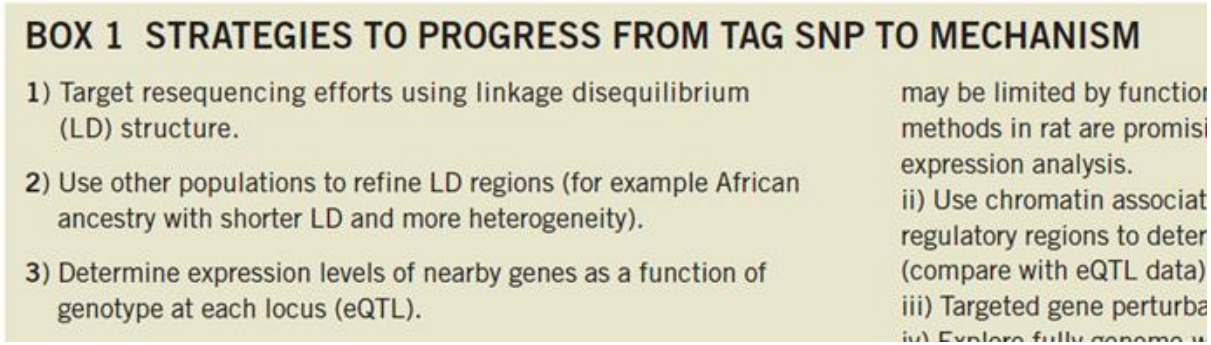




What would be your next question?

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

..in disease relevant tissue!



BOX 1 STRATEGIES TO PROGRESS FROM TAG SNP TO MECHANISM

1) Target resequencing efforts using linkage disequilibrium (LD) structure.

2) Use other populations to refine LD regions (for example African ancestry with shorter LD and more heterogeneity).

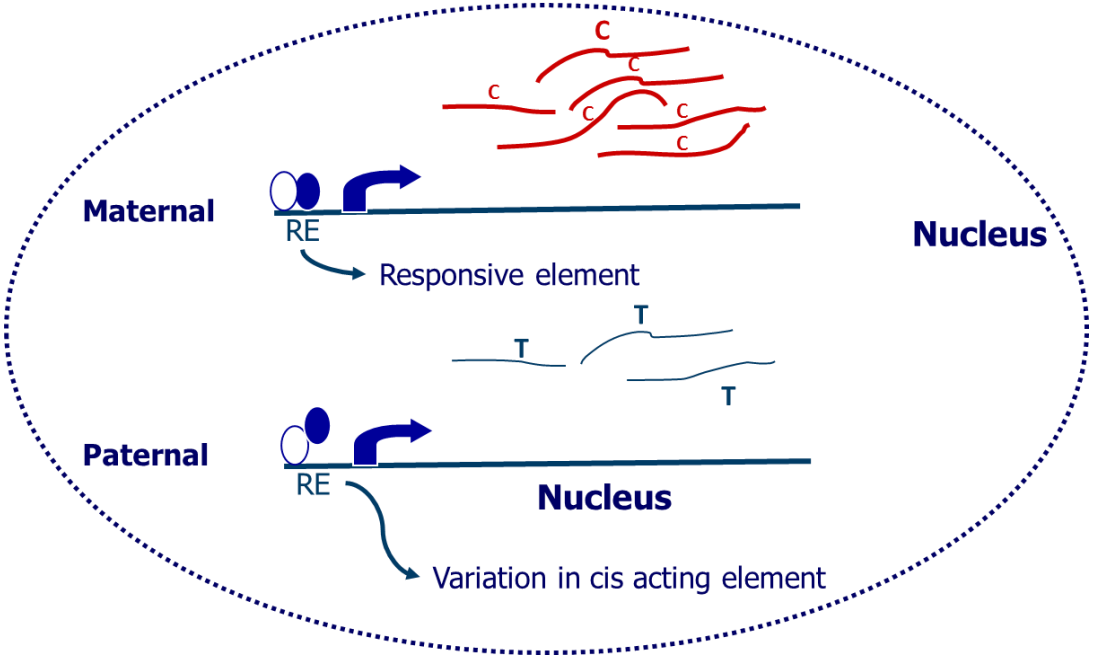
3) Determine expression levels of nearby genes as a function of genotype at each locus (eQTL).

may be limited by functio
methods in rat are promis
expression analysis.
ii) Use chromatin associat
regulatory regions to deter
(compare with eQTL data)
iii) Targeted gene perturba

Does the SNP affect expression of positional gene?

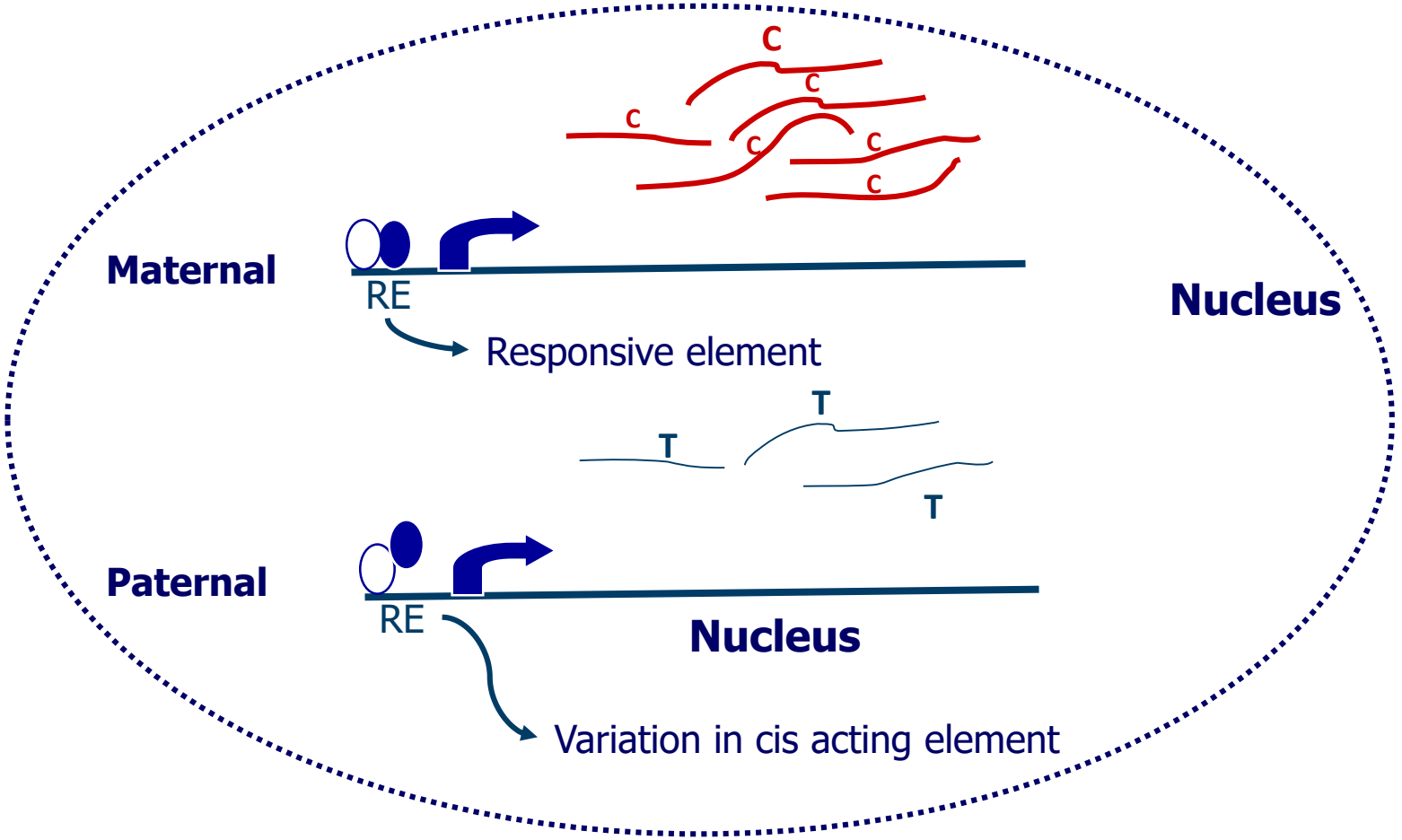
Test functional relevance of susceptibility SNPs:

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



Subtle changes in expression regularly underlie complex diseases.

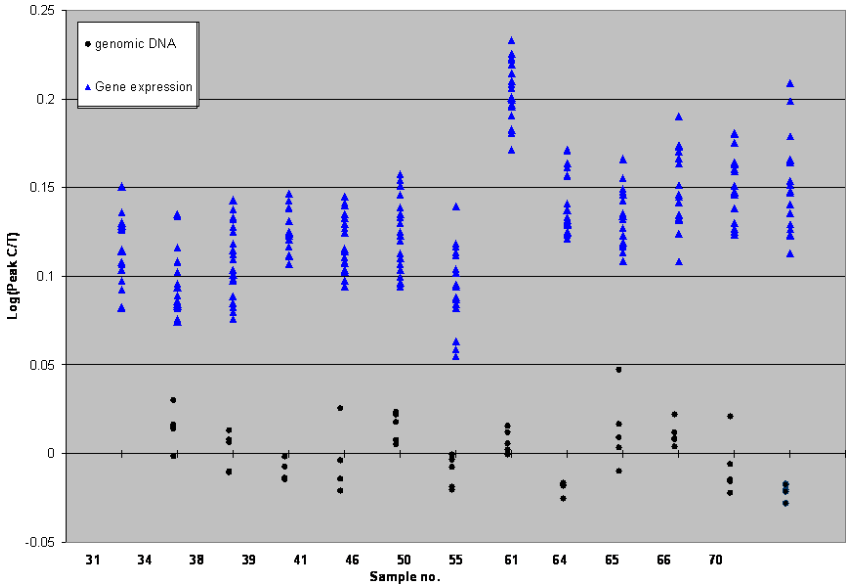
Differential allelic expression
Genetic variation at *cis*-acting regulator elements

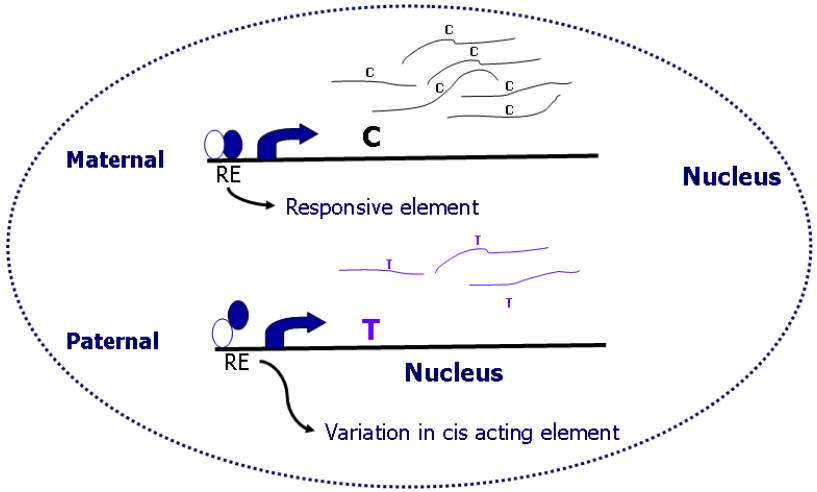


Bos et al. Ann Rheum Dis. 2012

Measurable with coding SNPs

Differential allelic expression DIO2





Significant increased expression of the DIO2 susceptibility allele in OA cartilage

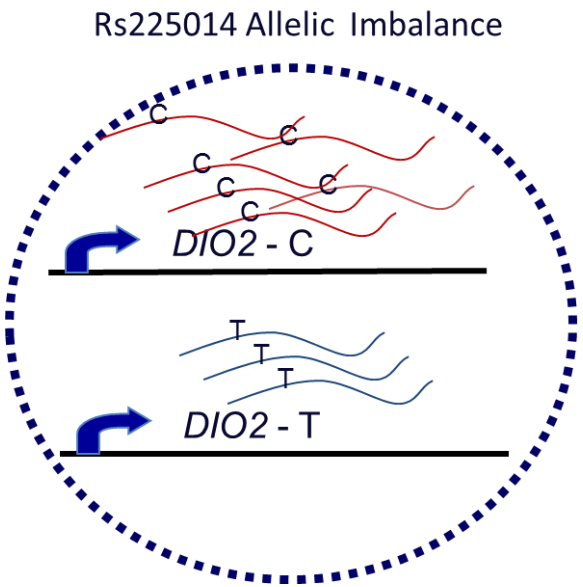
DIO2 in articular cartilage

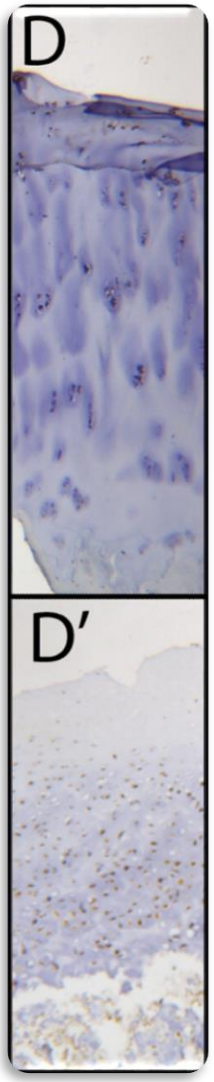
2010

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





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

Which next step in the Freedman et al strategy?

4. Characterized gene regulatory regions by multiple techniques

..in disease relevant tissue!

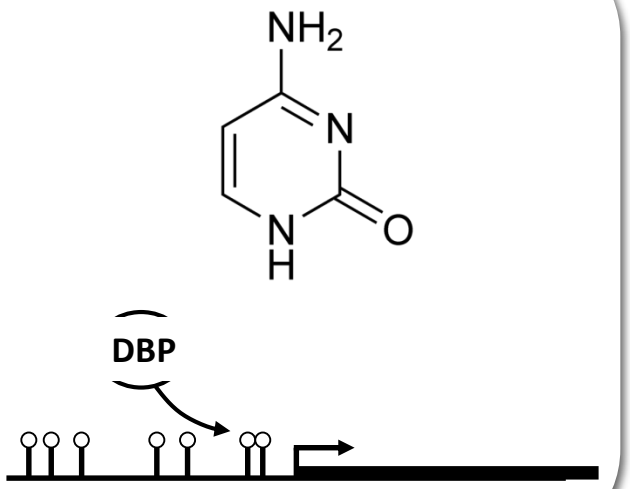
BOX 1 STRATEGIES TO PROGRESS FROM TAG SNP

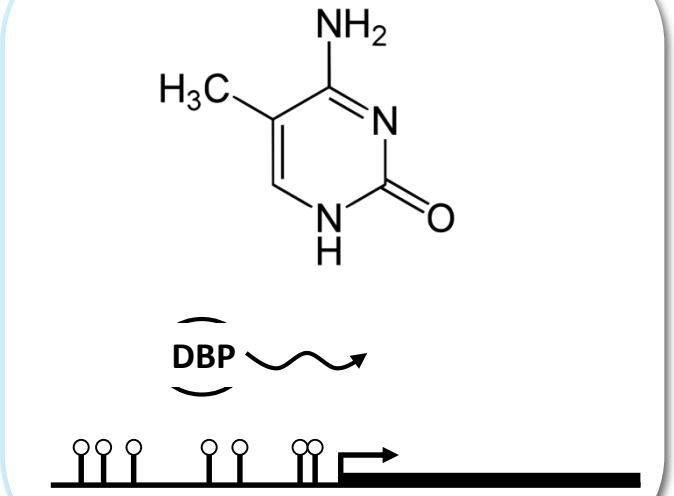
1) Target resequencing efforts using linkage disequilibrium (LD) structure.
2) Use other populations to refine LD regions (for example African ancestry with shorter LD and more heterogeneity).
3) Determine expression levels of nearby genes as a function of genotype at each locus (eQTL).
4) Characterize gene regulatory regions by multiple empirical techniques bearing in mind that these are tissue and context specific.

Epigenetics – DNA methylation

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

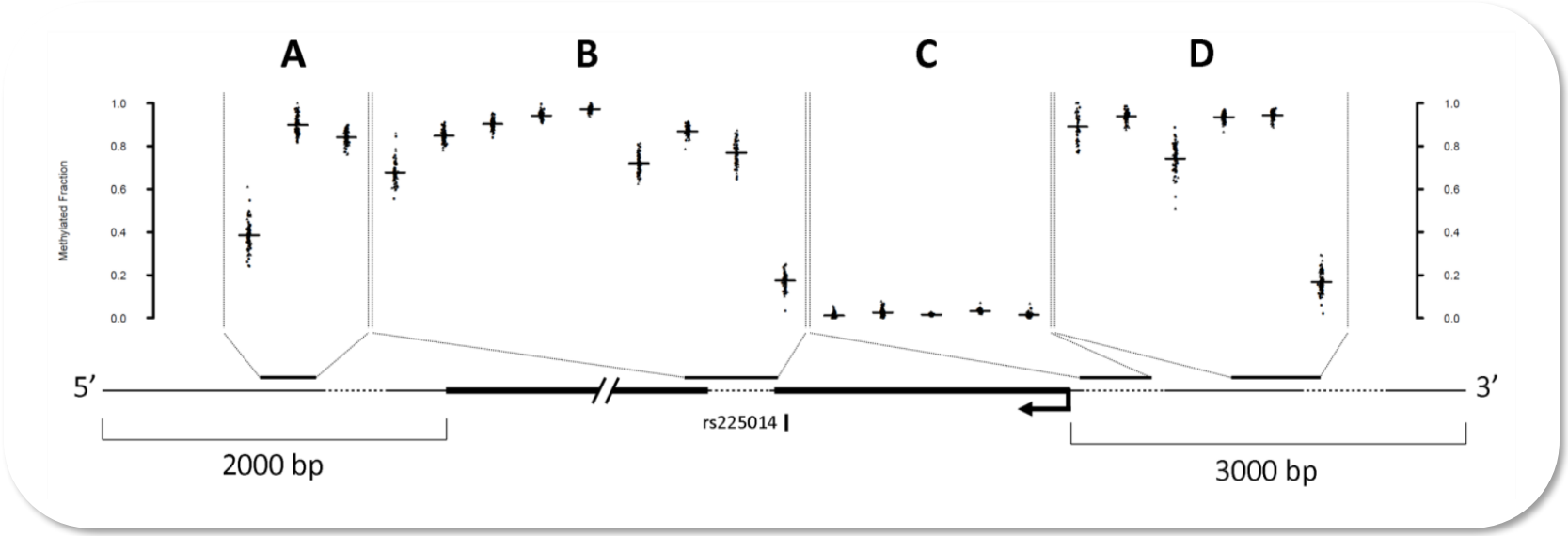
- **miRNAs**
- **Histon modifications**
- **DNA methylation**
- **…**





Regulation of *DIO2* expression

Gene targeted methylation at CpG sites (Epityper, Sequenom)



N. Bomer, W den Hollander and YFM Ramos et al. ARD 2015

Regulation of *DIO2* expression

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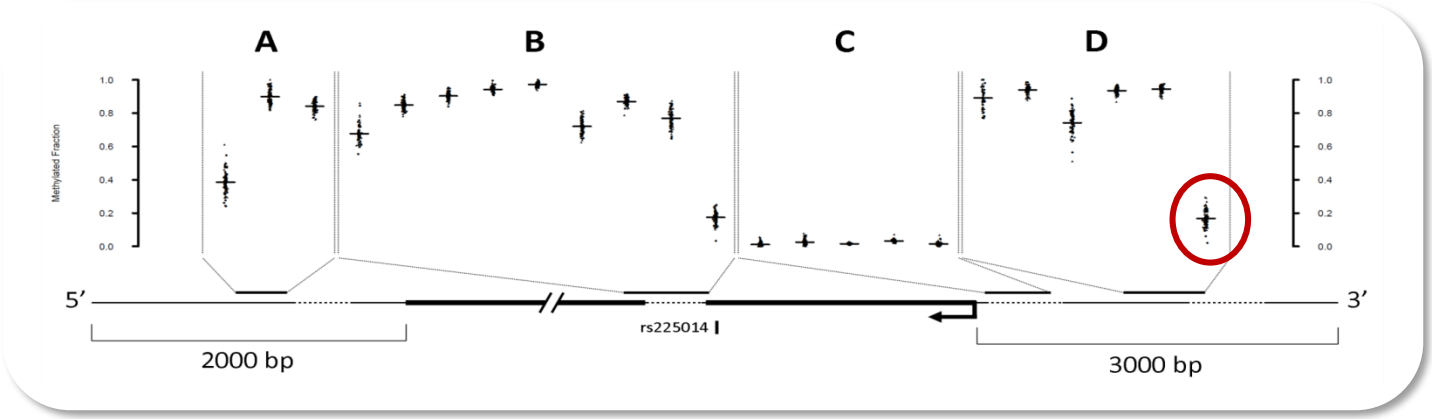

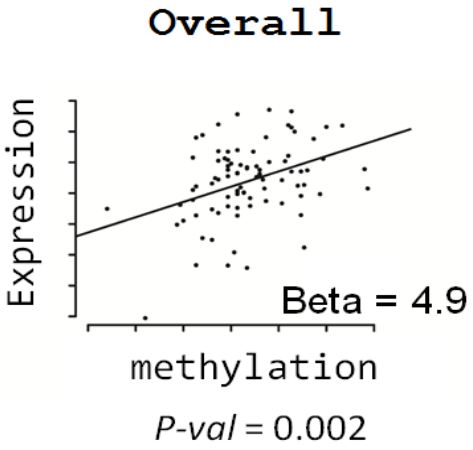

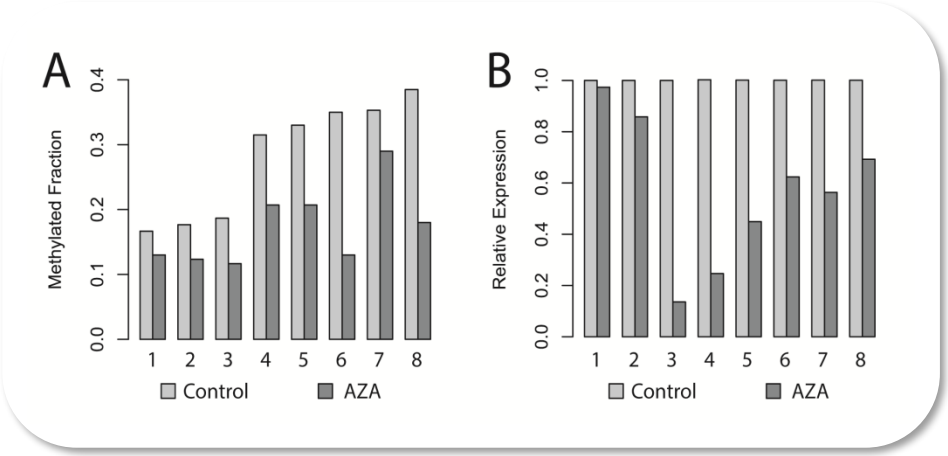

DIO2 expression in articular cartilage is modulated by methylation at CpG ~-2000 bp

N. Bomer, W den Hollander and YFM Ramos et al. ARD 2015

Which next 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!

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1) Target resequencing efforts using linkage disequilibrium (LD) structure.
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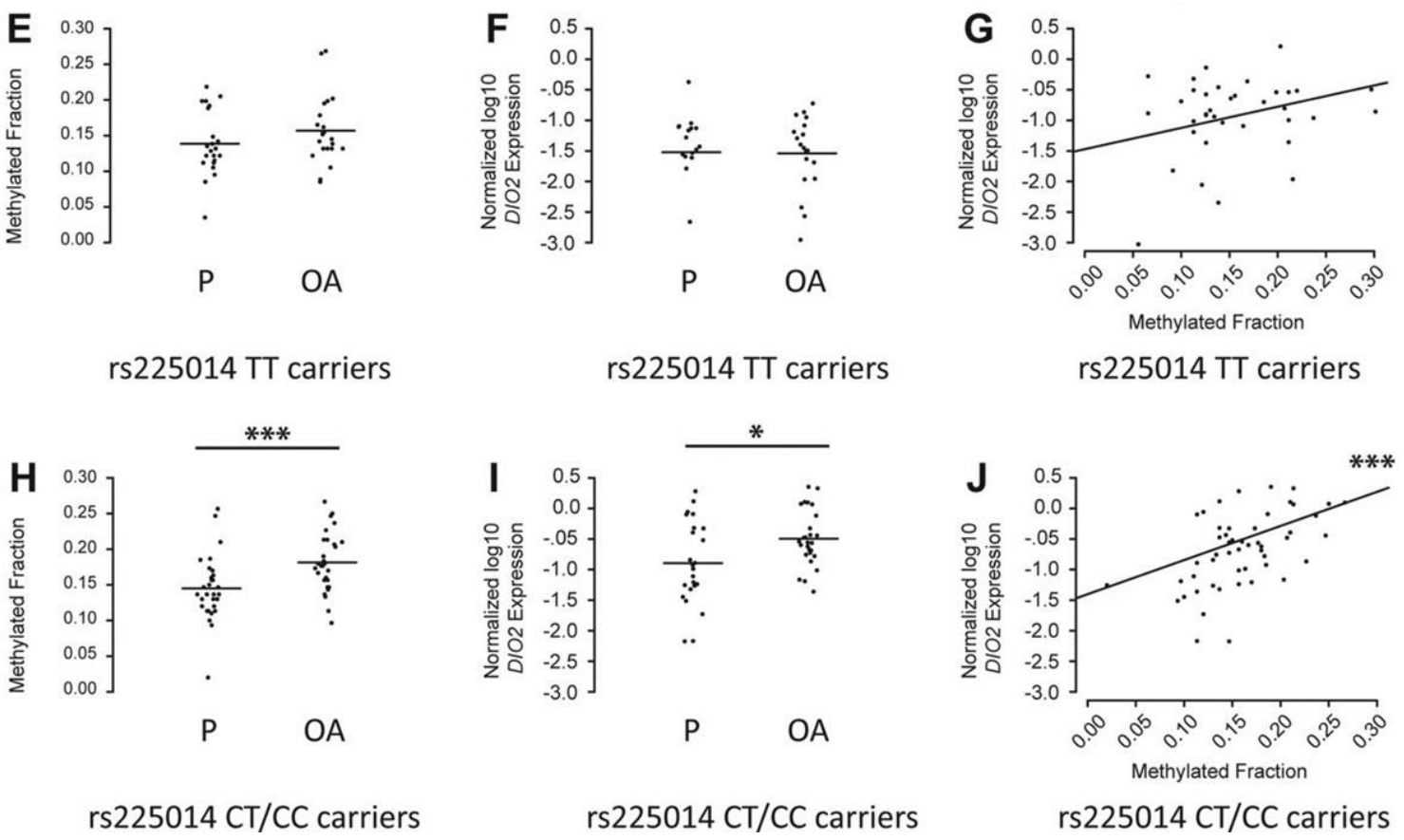
4) Characterize gene regulatory regions by multiple empirical techniques bearing in mind that these are tissue and context specific.

Does the *DIO2* rs255014 modulates the effect?

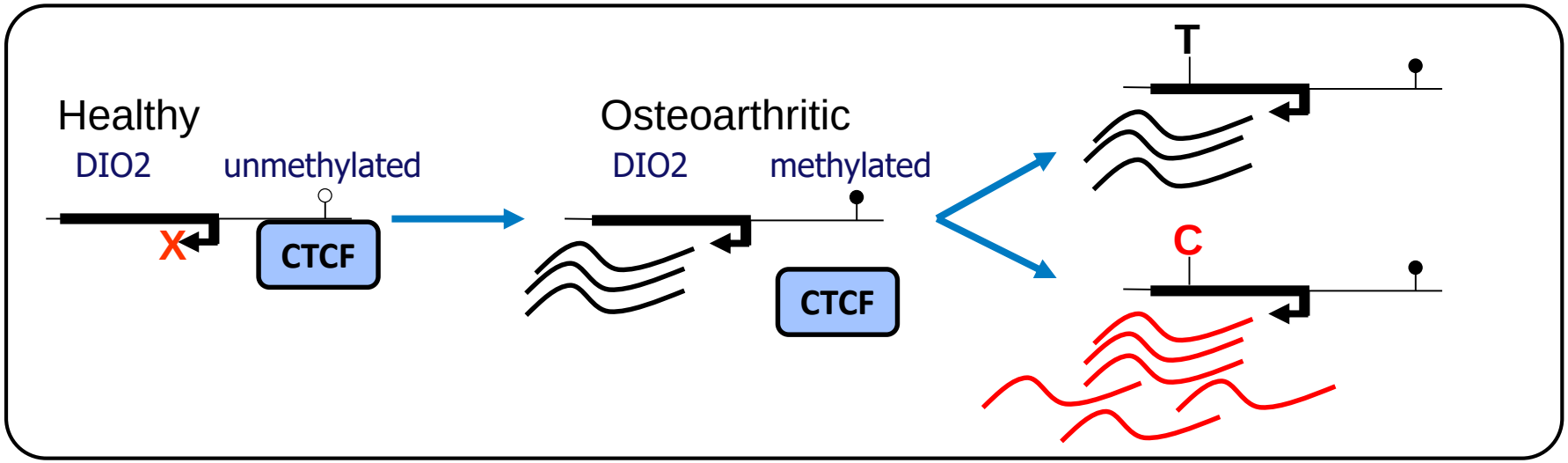
Regulation of *DIO2* expression

Gene targeted methylation at CpG sites (Epityper, Sequenom)



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

*N. Bomer, **W den Hollander** and YFM Ramos et al. ARD 2015*


Healthy
DIO2
unmethylated
X
CTCF
Osteoarthritic
DIO2
methylated
CTCF
T
C

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!

BOX 1 STRATEGIES TO PROGRESS FROM TAG SNP TO MECHANISM

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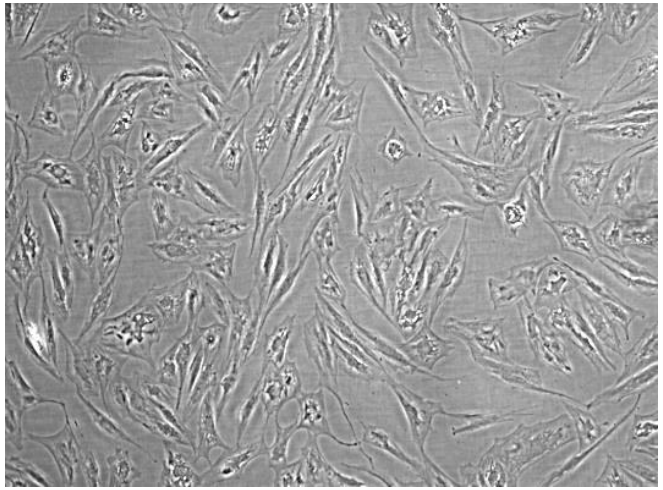
Human 3D *in vitro* model of cartilage

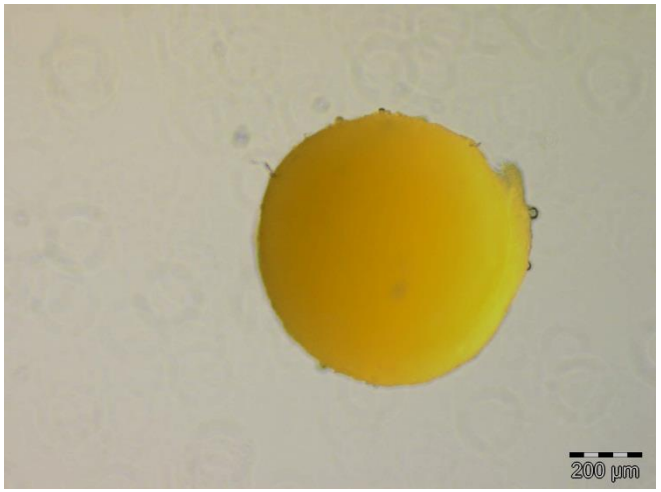
What is direct effect of DIO2 upregulation?

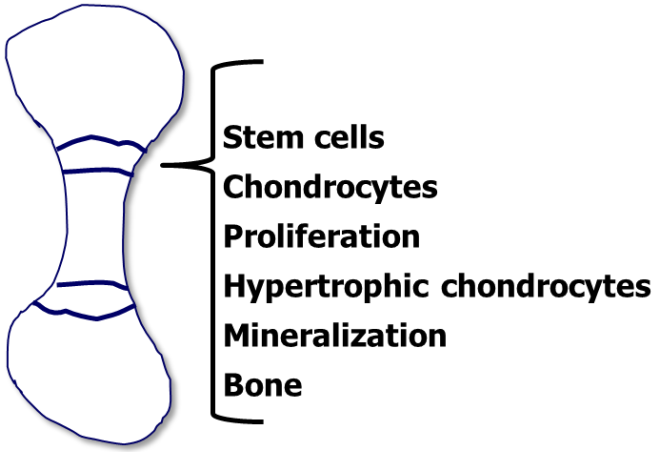
Human *in vitro* chondrogenesis model
Stemcells, primary chondrocytes

Growing cells (monolayer)

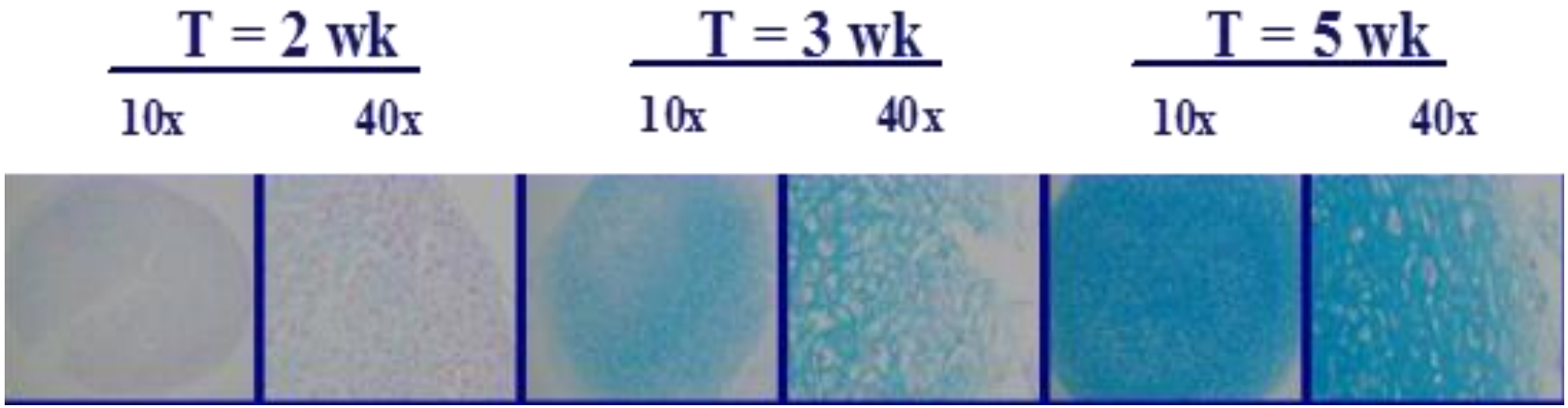


Chondrocyte pellet cartilage formation





In vitro chondrogenesis model Stemcells, primary chondrocytes



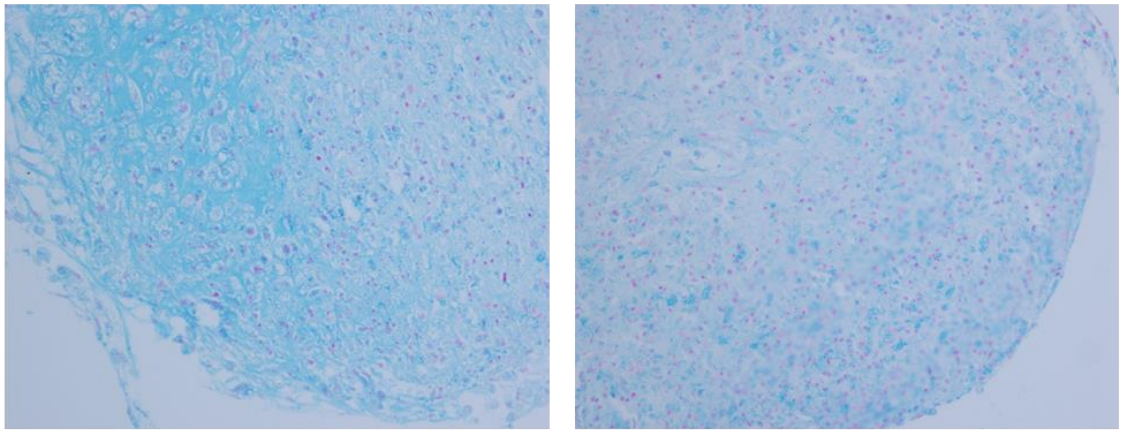
In vitro chondrogenesis model using human bone marrow derived mesenchymal stem cells. Alcian blue staining

What is direct effect of DIO2 upregulation on cartilage integrity?

***N. Bomer**, W den Hollander and **YFM Ramos** et al. ARD 2015*

BM-MSC based *in vitro* chondrogenesis model

Overexpression of DIO2

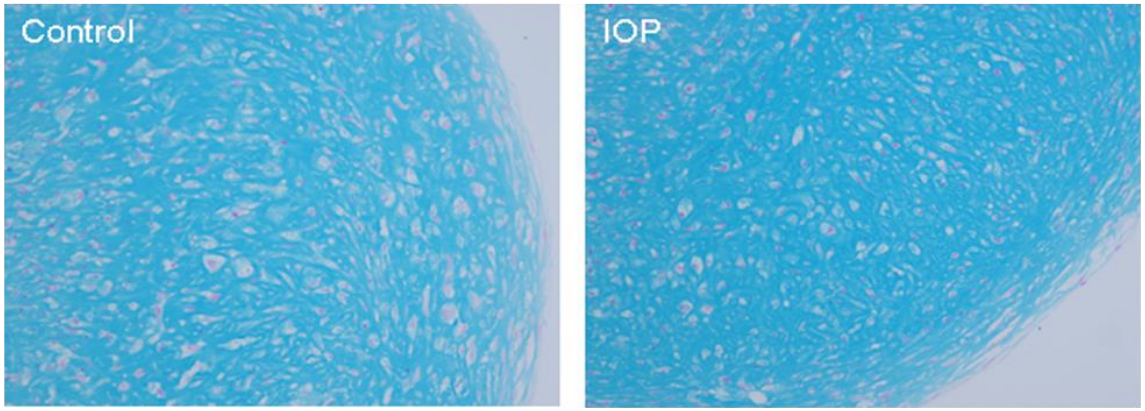


Direct detrimental effect of DIO2 on cartilage matrix deposition
Destruction without early hypertrophy (COLX)

N. Bomer, *W den Hollander and* ***YFM Ramos*** *et al. ARD 2015*

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

N. Bomer, *W den Hollander and* ***YFM Ramos*** *et al. ARD 2015*

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?

Which step in the Freedman et al strategy?

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

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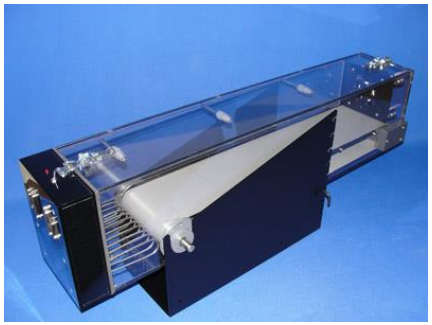
DIO2 Knock-Out model

Collaboration KU Leuven, Leuven, Belgium

Design: Running induced mechanical stress



DIO2-KO & Control
(C57BL/6 males)

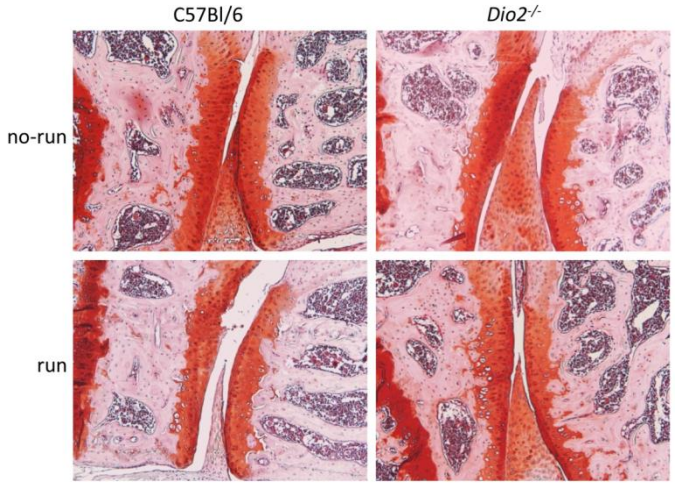
+

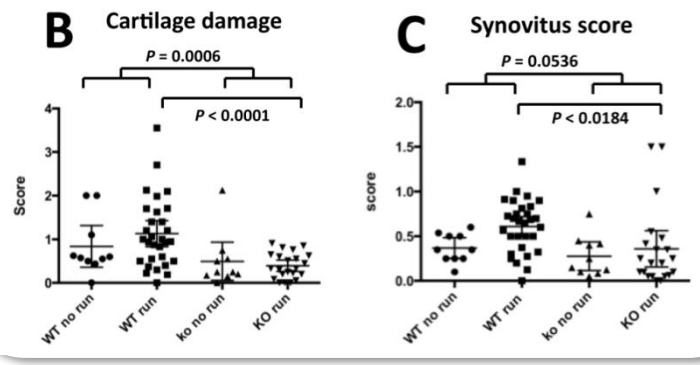
For 3 weeks -> 5 days/week -> 60 min/day

(60min at 11m/min, 5 incline)

↓

Induce cartilage damage



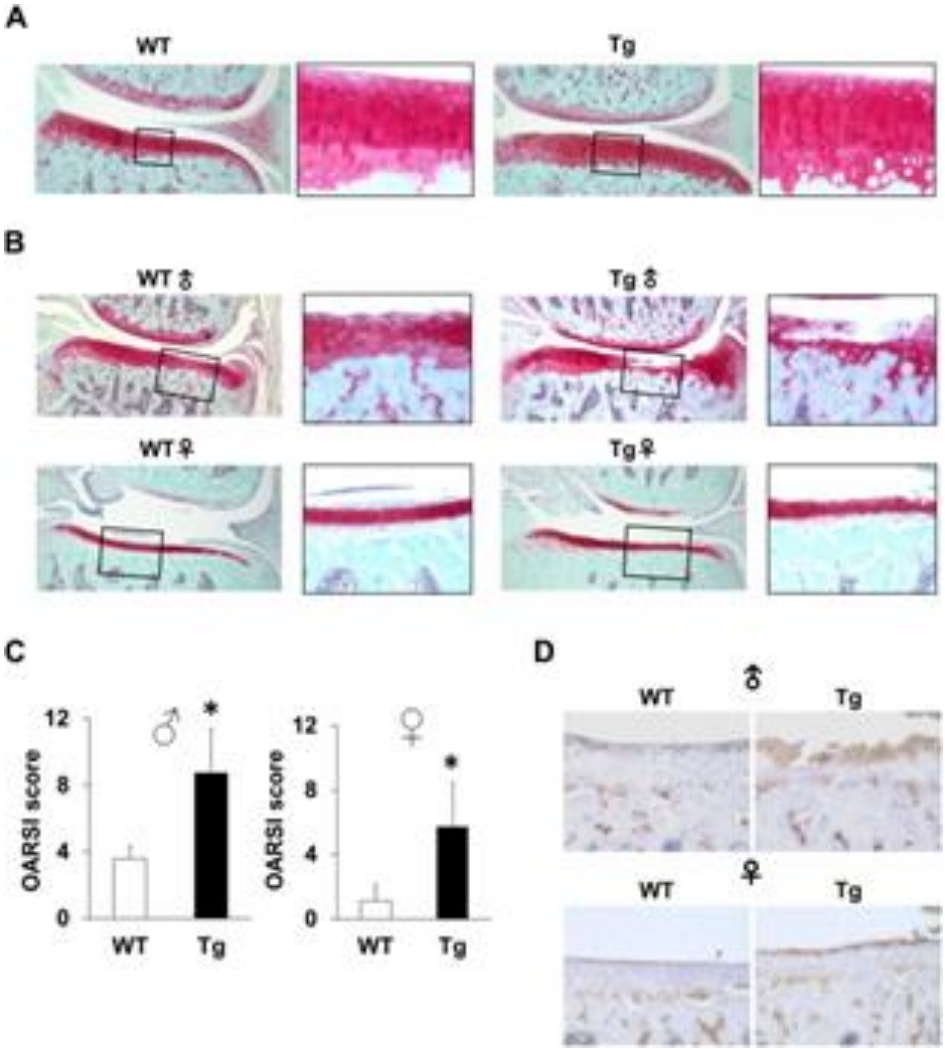


Dio2-/- mice protected against cartilage damage only upon exercise-induced OA

Bomer et al. Ann Rheum Dis. (2015)

2015

Tissue specific induction *DIO2* in rats



Destruction of cartilage only upon applying OA model

Nagase et al. Ann Rheum Dis 2013

2013

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

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Dutch Arthritis Association Grant

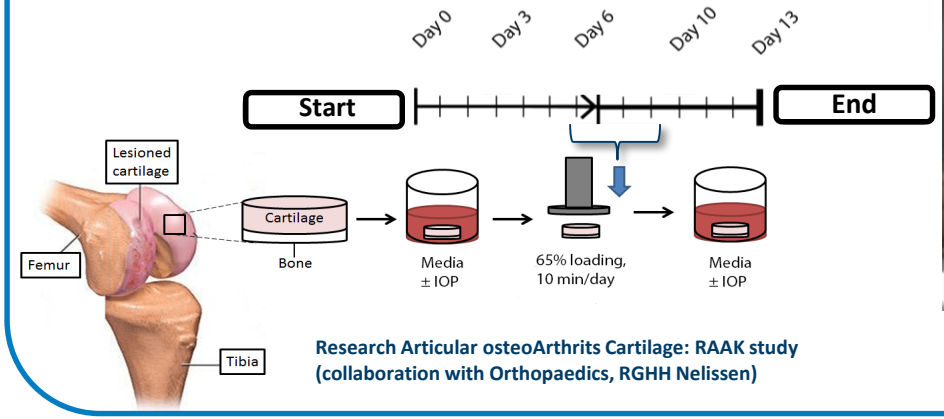
Pharmacological attenuation of thyroid hormone signaling; An evidence-based treatment option for Osteoarthritis

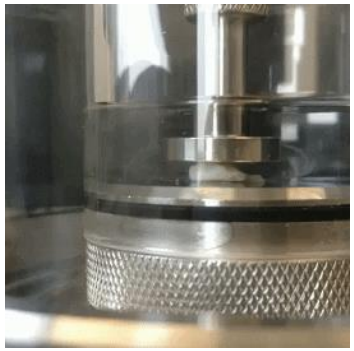
Reumafonds
Sterk in beweging

The human explant model

DutchArthritisSociety
ReumaNederland

Pre-clinical human *ex vivo* osteochondral explant model





Research Articular osteoArthrits Cartilage: RAAK study
(collaboration with Orthopaedics, RGHH Nelissen)

Readout of model:

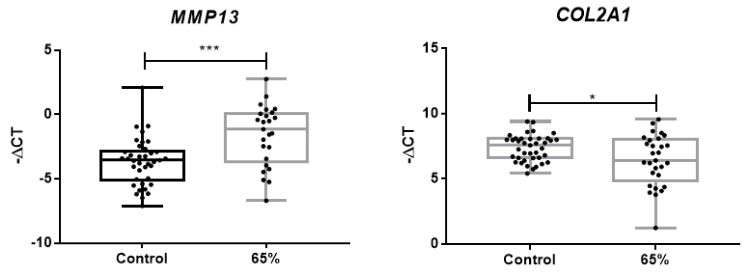
- Gene expression
- Sulphated GAG loss
- Cartilage structure
- Mechanical properties

Model optimization – mechanical loading

Mechanical stress induces detrimental changes to the cartilage:

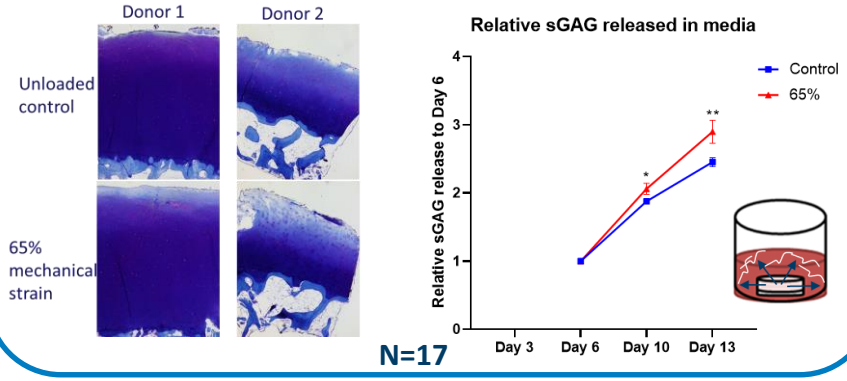
- Increased detrimental gene expression (*MMP13*)
- Reduced matrix producing gene expression (*COL2A1*)
- Increased loss of matrix (sGAG) from cartilage

Gene expression



N=17

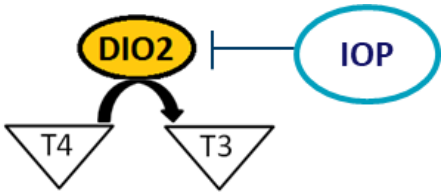
Matrix loss



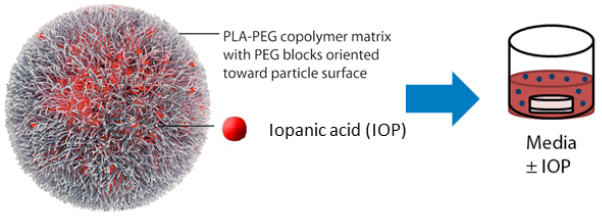
N=17

Model optimization – drug delivery

Inhibition of DIO2



Nanoparticles for delivery of IOP



(In collaboration with Radiology (TNI), dr. L. Cruz)

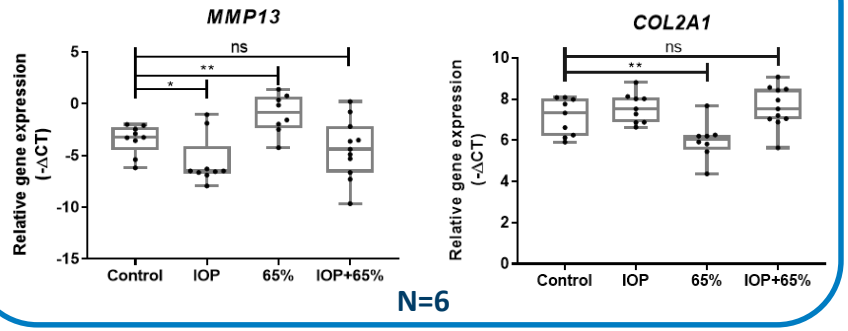
Does inhibition of thyroid signaling prevent mechanical induced detrimental changes?

Can IOP inhibit mechanical induced damage?

Inhibition of thyroid signaling (IOP) alleviated these detrimental changes to the cartilage

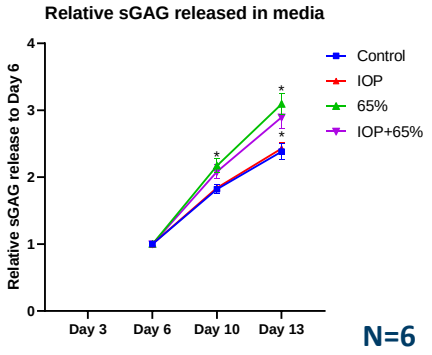
Nanoparticles containing IOP show similar results on gene expression

Gene expression



N=6

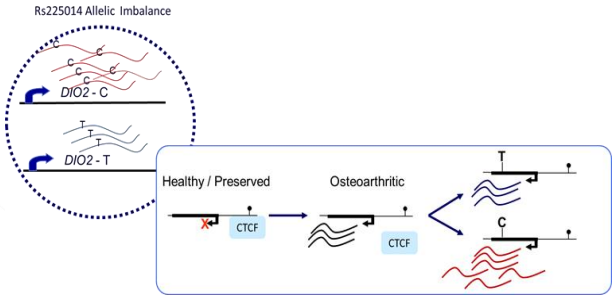
Matrix loss

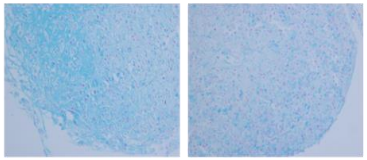


N=6

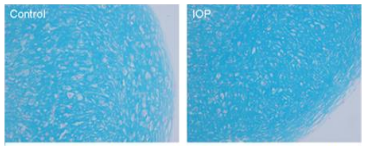
In summary

- 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

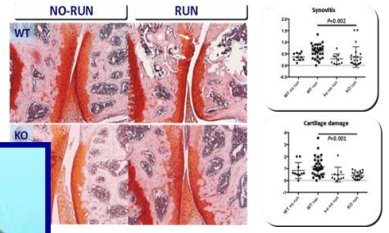




Direct detrimental effect of DIO2 on cartilage matrix deposition & maintenance



Direct beneficial effect of DIO2 on cartilage matrix deposition and maintenance



Osteoarthritis research group