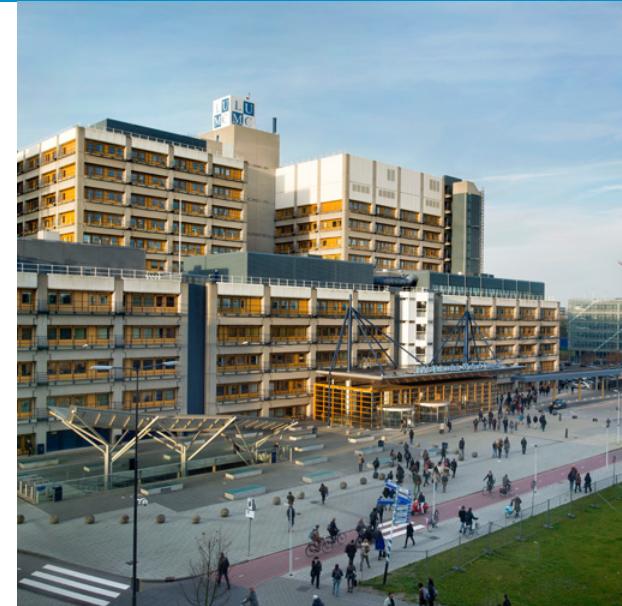


# Osteoarthritis

From *DIO2* risk gene discovery to  
preclinical drug studies

Ingrid Meulenbelt  
Molecular Epidemiology  
LEIDEN, THE NETHERLANDS



# Balance between heritability and environment

**complex genetic**

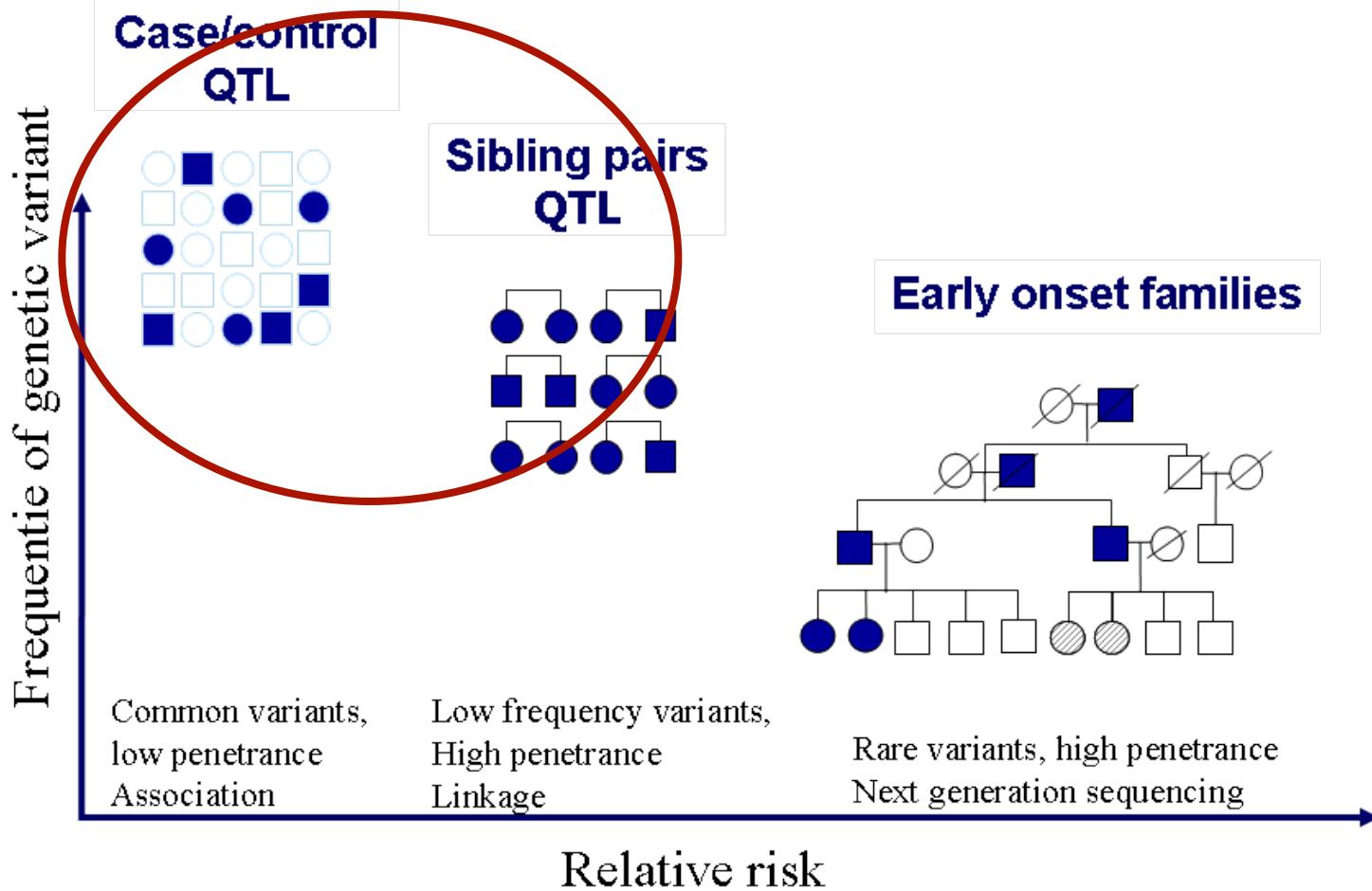


**100% environment**

**100 % genetic**

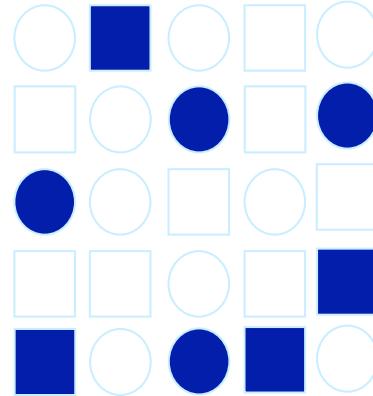
**Determines genetic study design**

# Depending on study design



# Association analyses case/controls

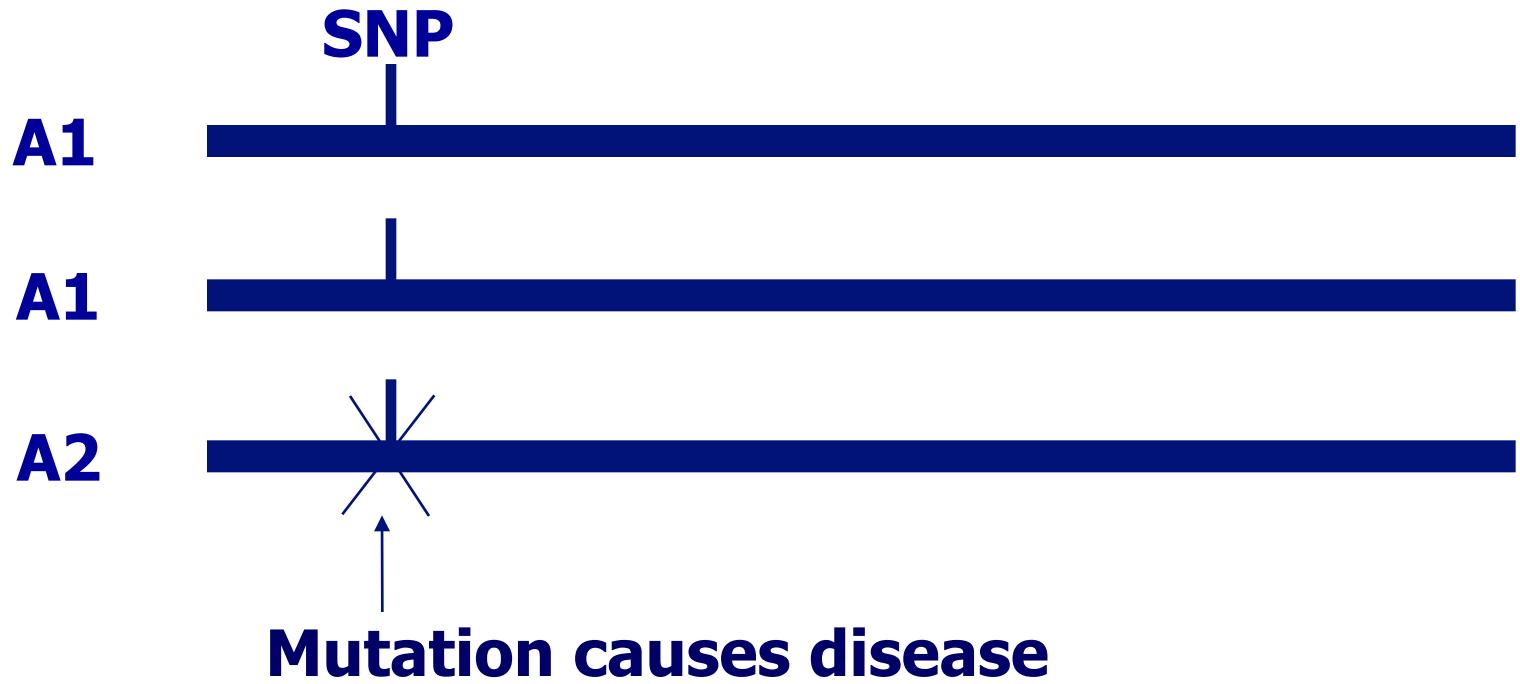
- Cases and controls
- Complex phenotype (common)
- Model free
- Sharing of alleles between cases versus controls
- Use SNPs close together



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

# Direct approach

## Functional mutation



**A2 cases >> A2 controls**



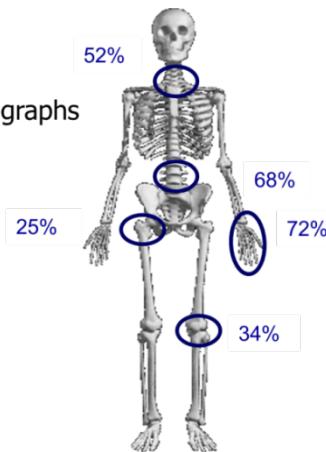
# **Discovery driven analyses, novel underlying disease pathways**

# 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%



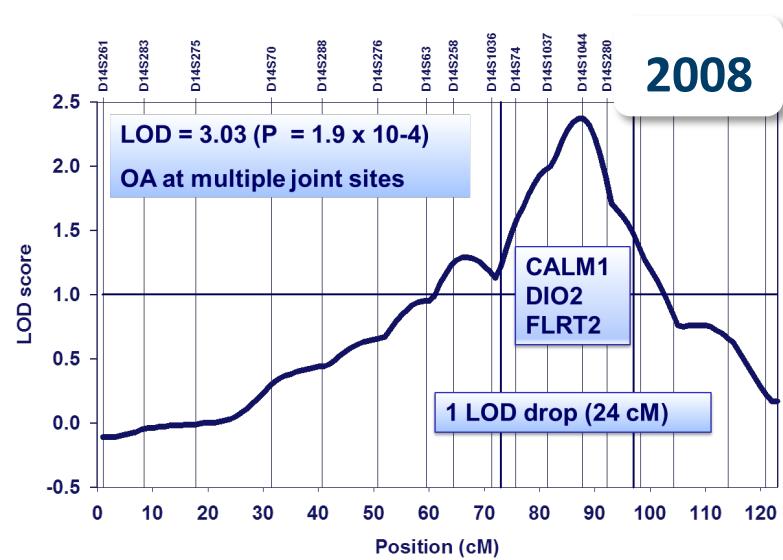
Inclusion:

≥ 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

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

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

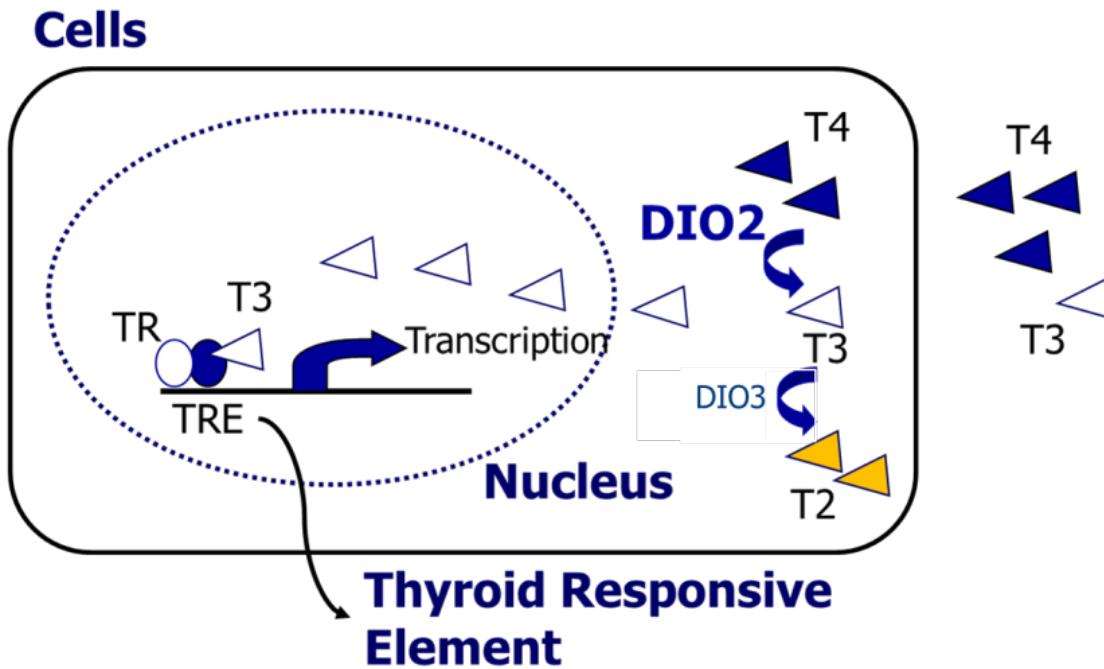
Female cases severe hip OA

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

\*Random effect meta-analyses

# Identification of *DIO2* as OA susceptibility locus

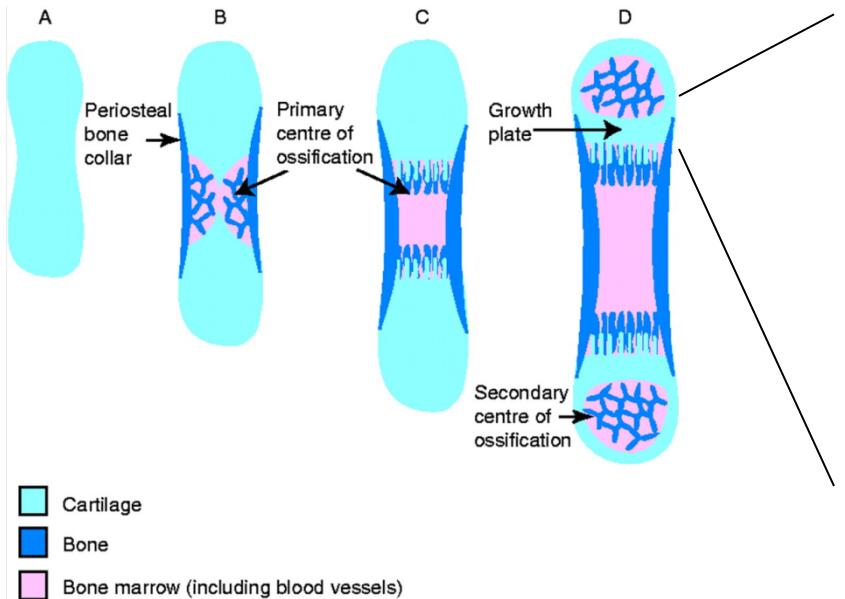
The GARP study



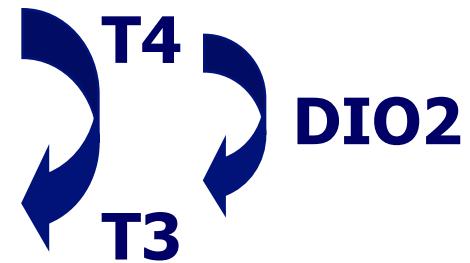
Initial study to indicate thyroid signaling conferring risk to osteoarthritis

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**



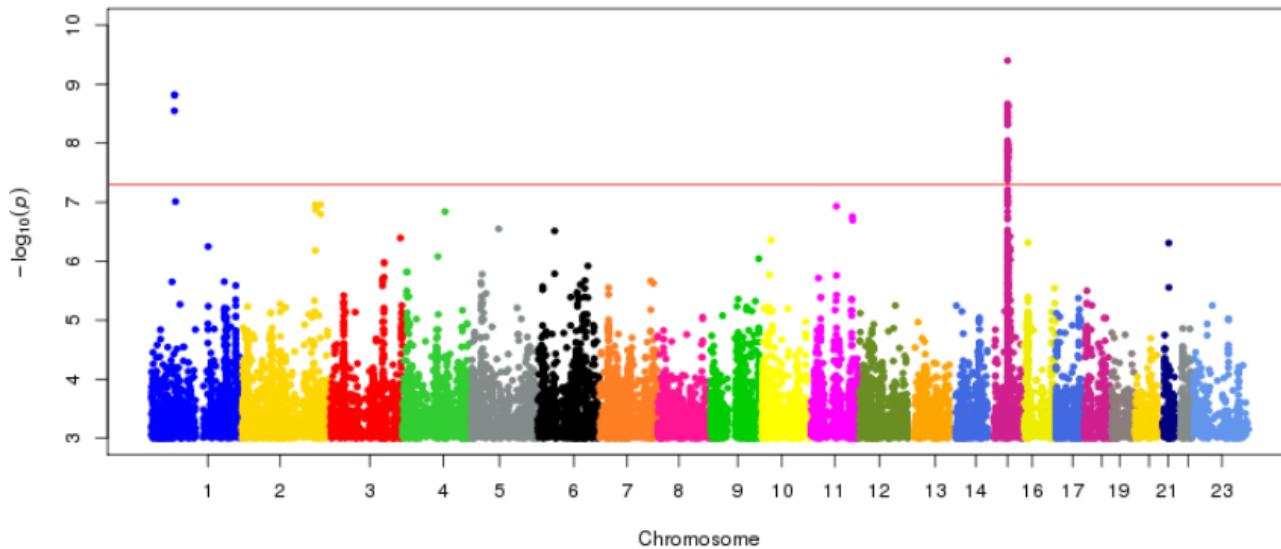
E J Mackie et al. J Endocrinol 2011;211:109-121

**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

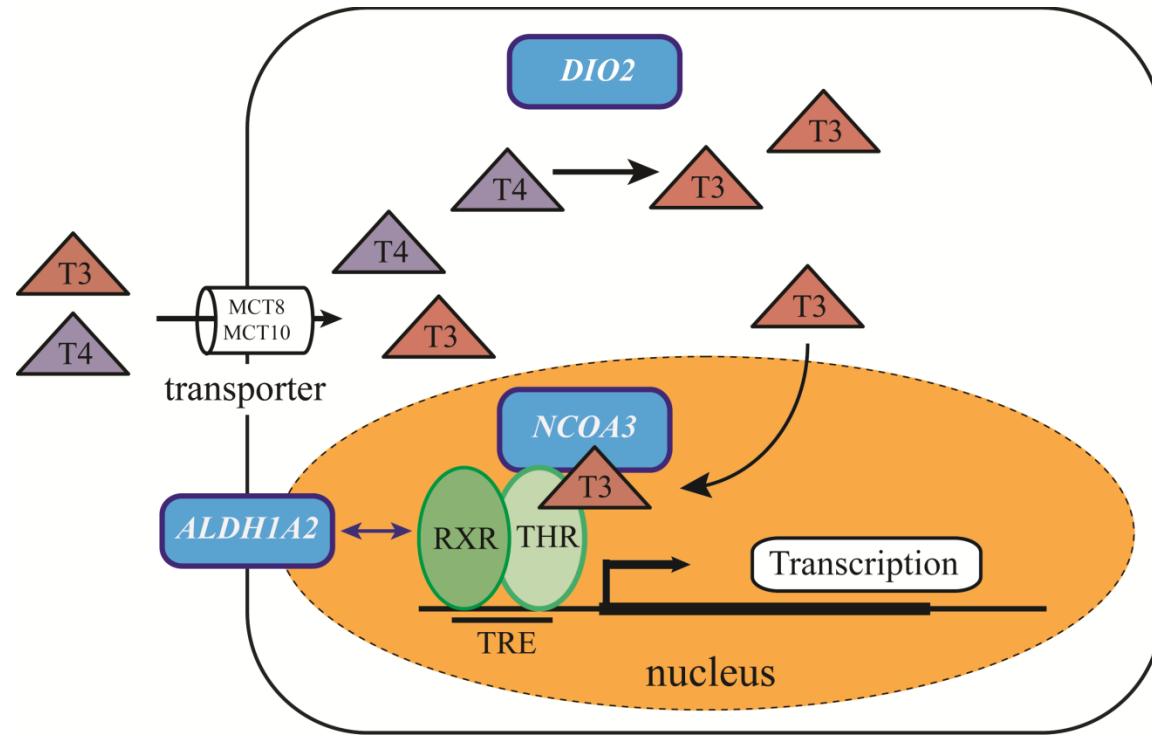
# Additional OA genes in thyroid pathway

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

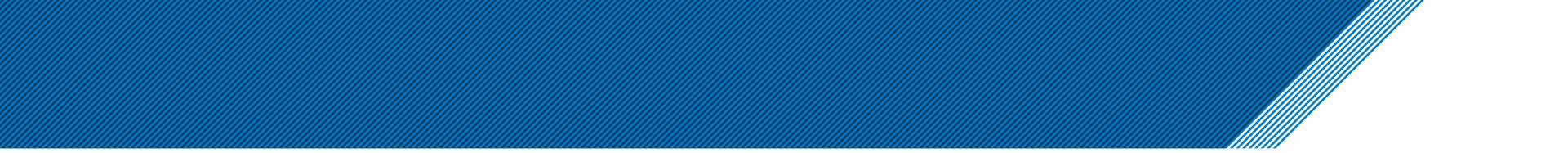


Styrkarsdottir et al. 2014 Nat Genet 46: 498-502  
Evangelou et al. 2014 Ann Rheum Dis 73:2130-6

# Intracellular levels of active thyroid



**Intracellular thyroid signaling may be a common underlying osteoarthritis pathway**



# **How to study effect of DIO2 as risk gene of Osteoarthritis**

# What would be your primary questions?

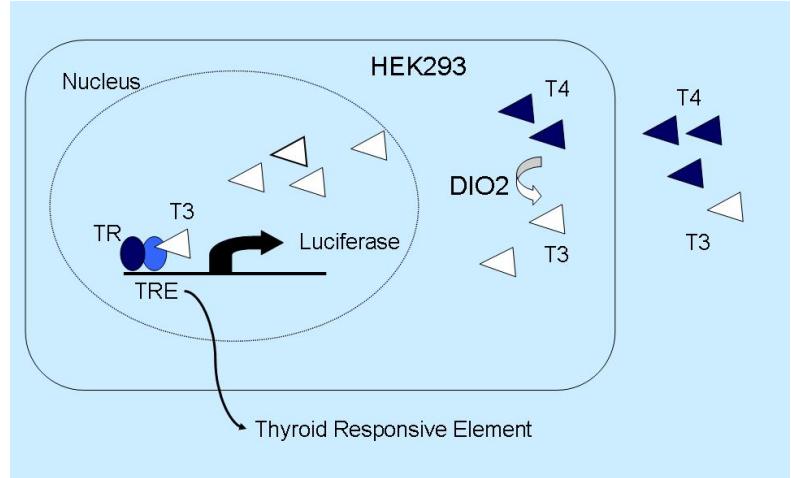
**SNP:**

Does the SNP directly affect protein function?

# Effect of SNP on protein function

Cell lines HEK293

- Effect of SNPs rs225014 (Thr92Ala) on conversion of T4 -> T3



**Although the DIO2 SNP rs225014 is coding, non-synonymously it does not affect enzyme activity**

# What would be your primary questions?

**SNP:**

Does the SNP directly affect protein function?

# What would be your primary questions?

**SNP:**

Does the SNP directly affect protein function?

Does the SNP affect expression of positional gene?

# What would be your primary questions?

## SNP:

Does the SNP directly affect protein function?

Does the SNP affect expression of positional gene?

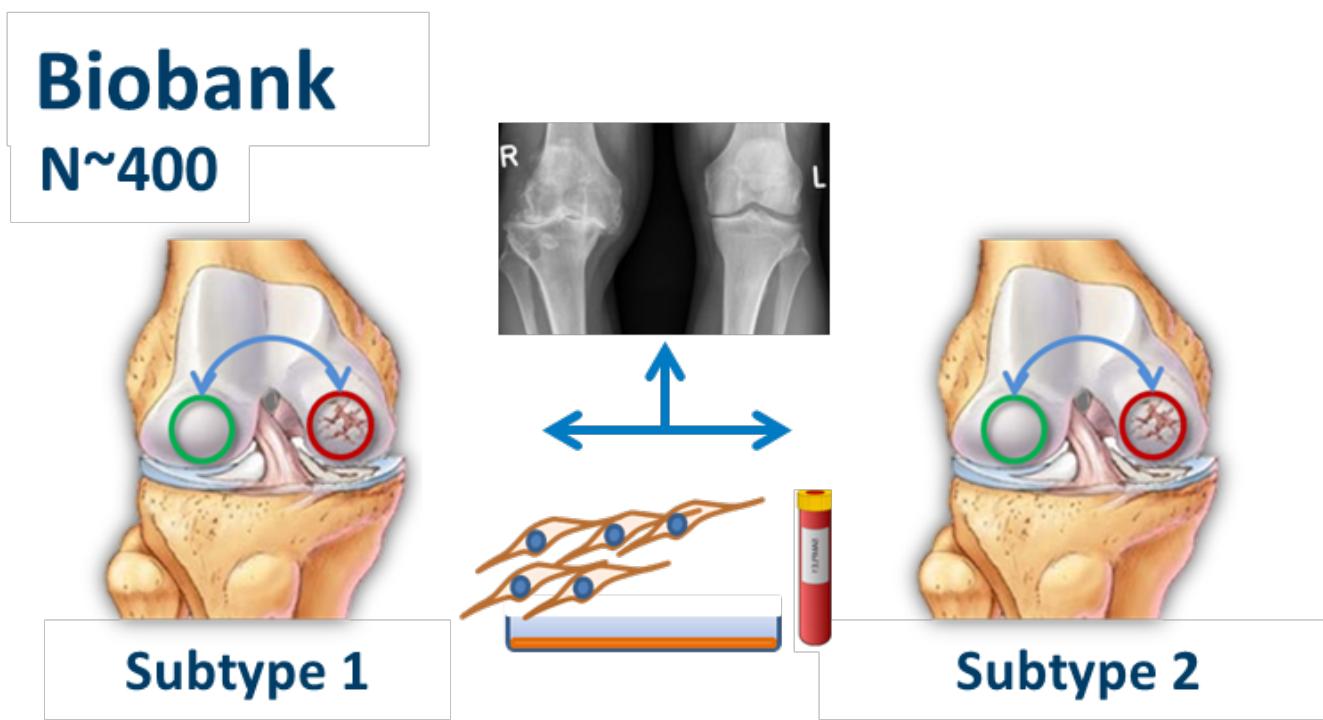
## Gene:

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)



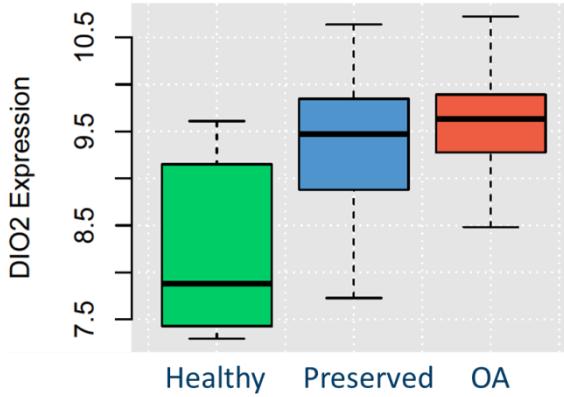
Collection of joint tissues of OA patients: **preserved** and **lesioned** cartilage, DNA, RNA, blood and cells (MSCs and primary chondrocytes).

# 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

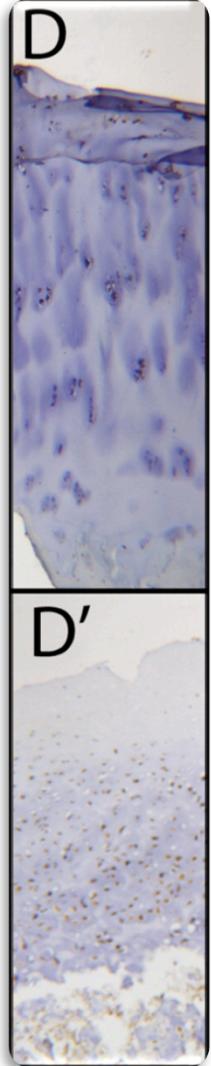
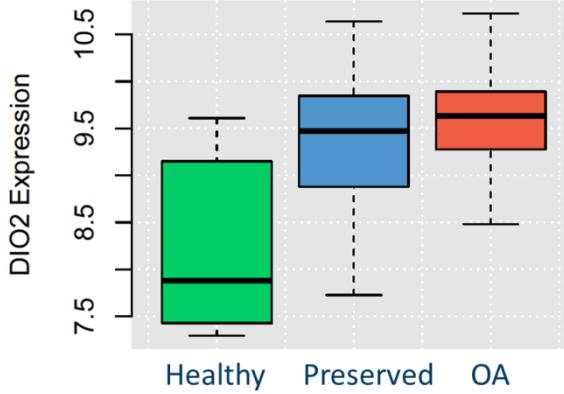
**DIO2 mRNA expression high in OA cartilage compared to healthy**

Ijiri et al. 2010

2012

**DIO2 protein expression up regulated in OA affected cartilage**

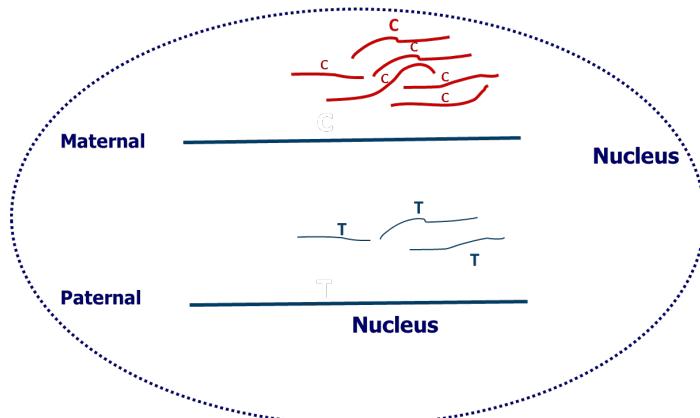
Bos et al. 2012



# Does the SNP affect expression of positional gene?

Test functional relevance of susceptibility SNPs:

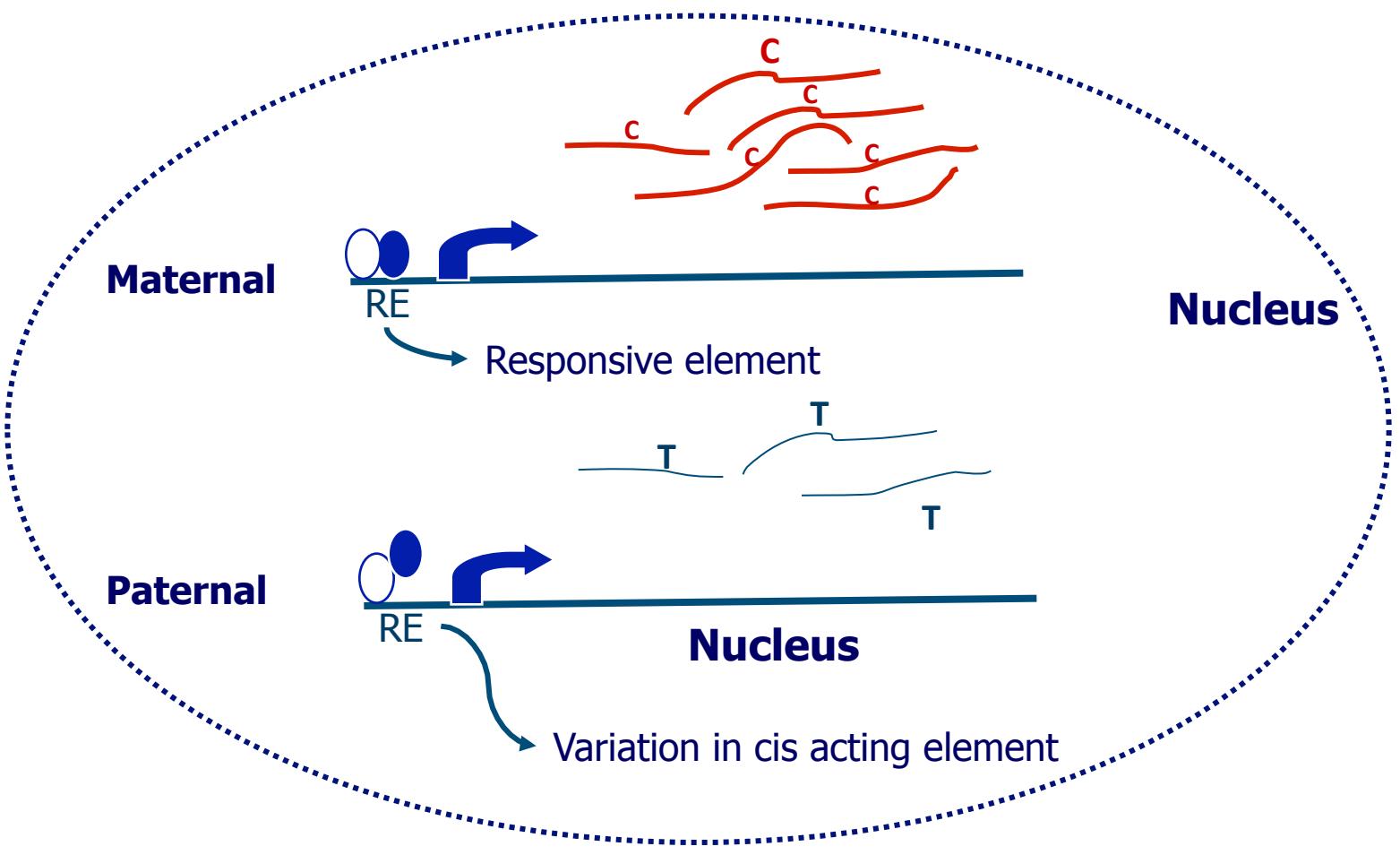
- SNPs in regulatory elements
- Differences in RNA stability
- Epigenetic mechanisms
- Tissues



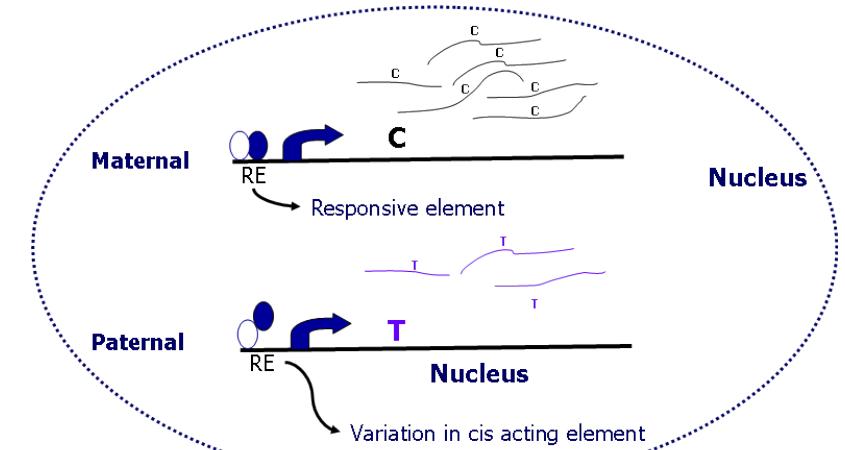
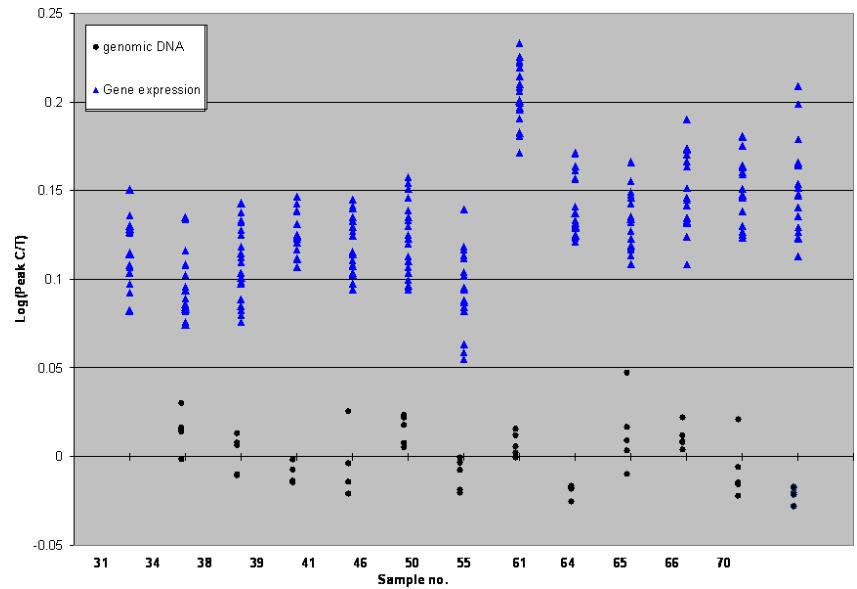
**Subtle changes in expression regularly underlie complex diseases.**

# 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

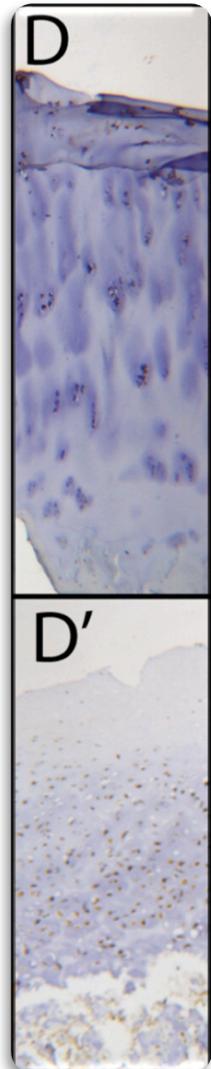
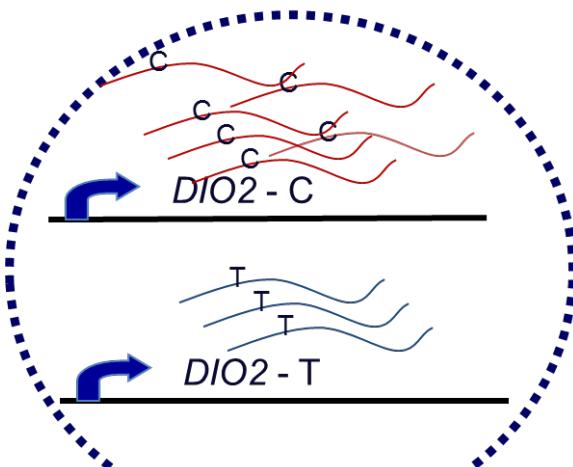
2010

*DIO2* mRNA expression  
absent in healthy and  
high in OA cartilage  
Ijiri et al. 2010

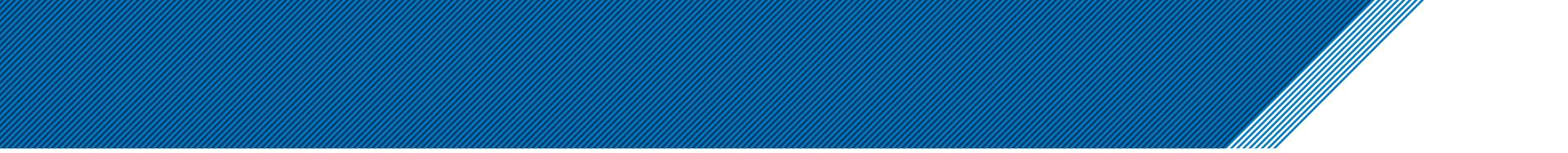
2012

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

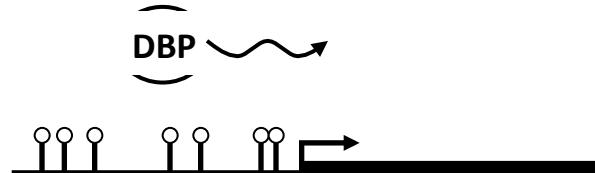
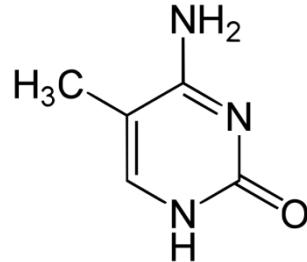
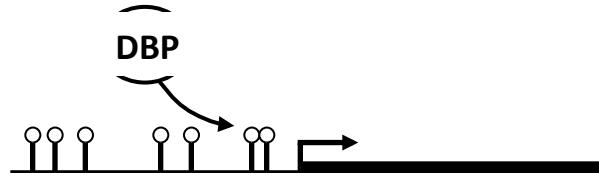
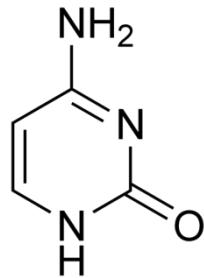


## **How is differential expression with disease state of *DIO2* regulated in cartilage**

# Epigenetics – DNA methylation

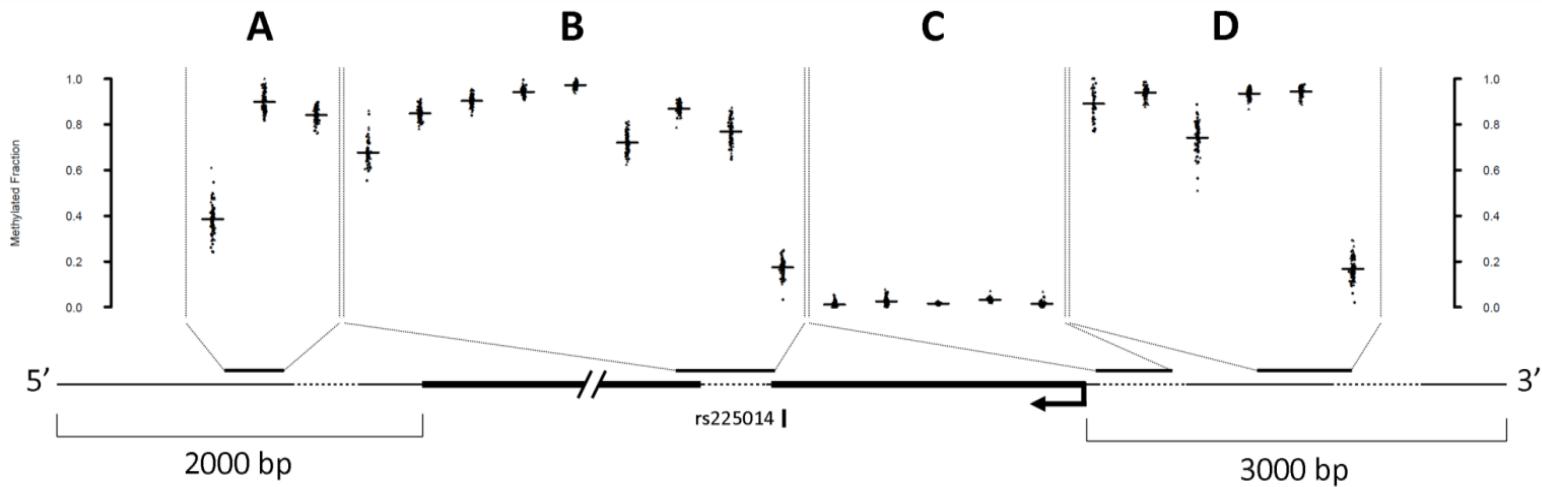
Mechanisms to adapt to environmental changes such as mechanical stress, disease and age.

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



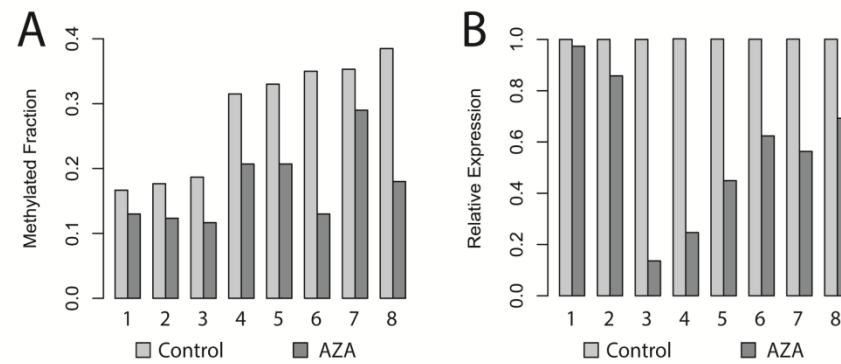
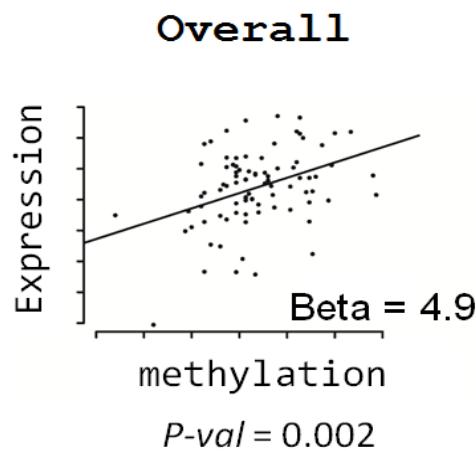
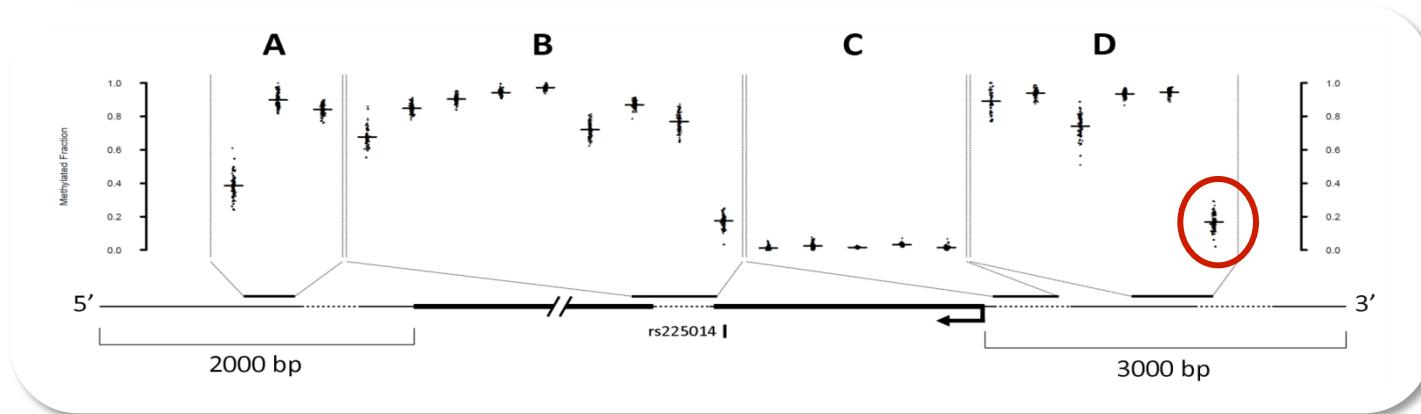
# Regulation of *DIO2* expression

Gene targeted methylation at CpG sites (Epityper, Sequenom)

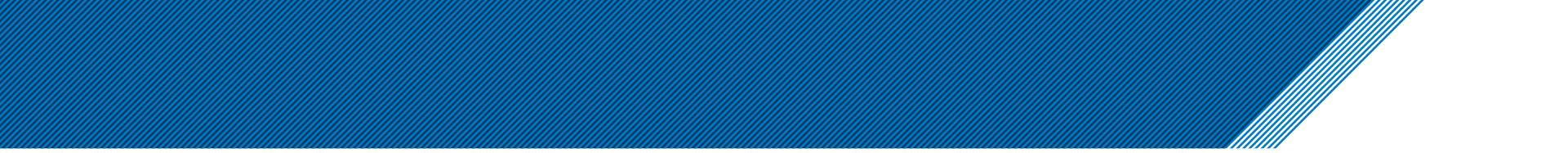


# Regulation of *DIO2* expression

Gene targeted methylation at CpG sites (Epityper, Sequenom)



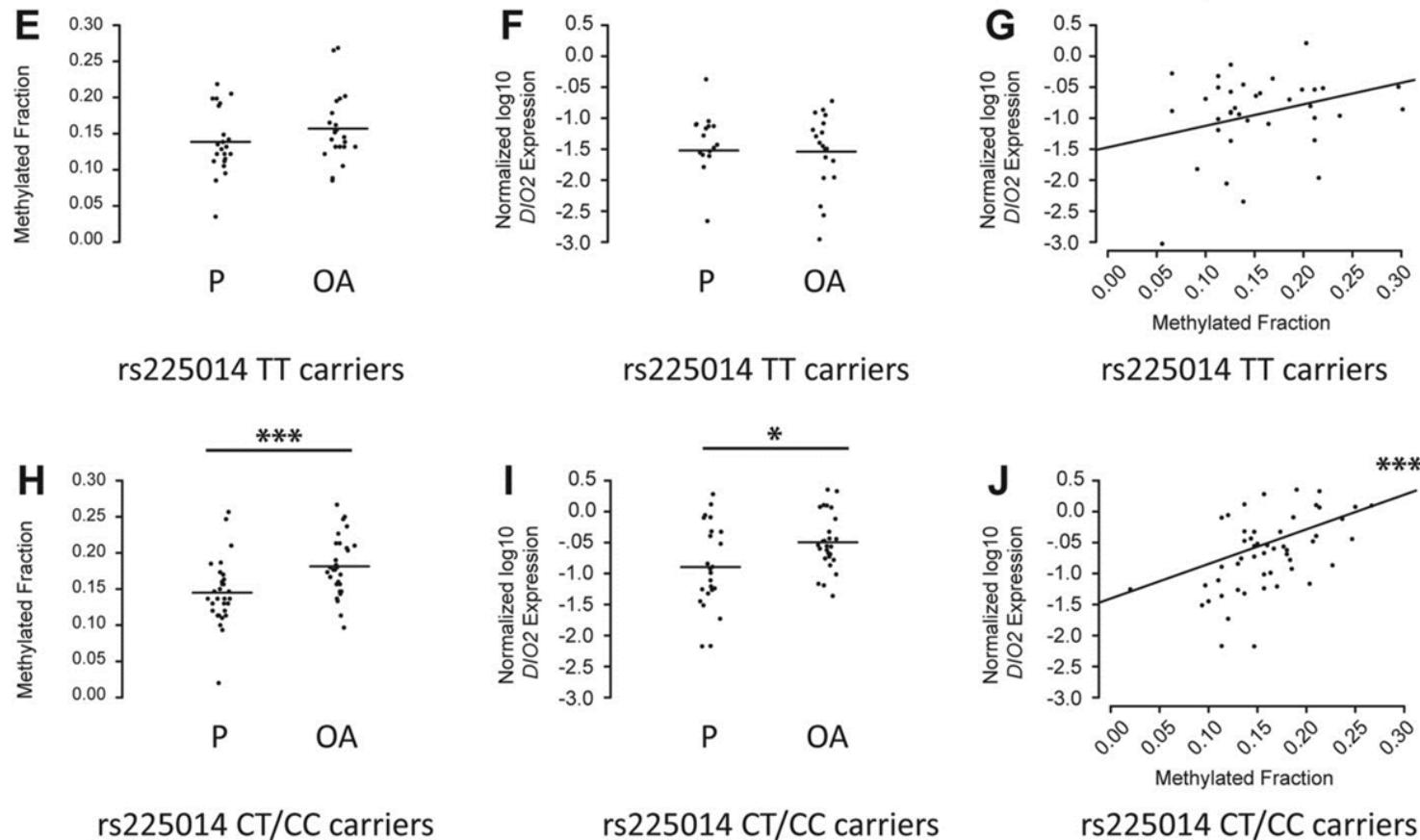
**DIO2 expression in articular cartilage is modulated by methylation at CpG ~-2000 bp**



**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.**

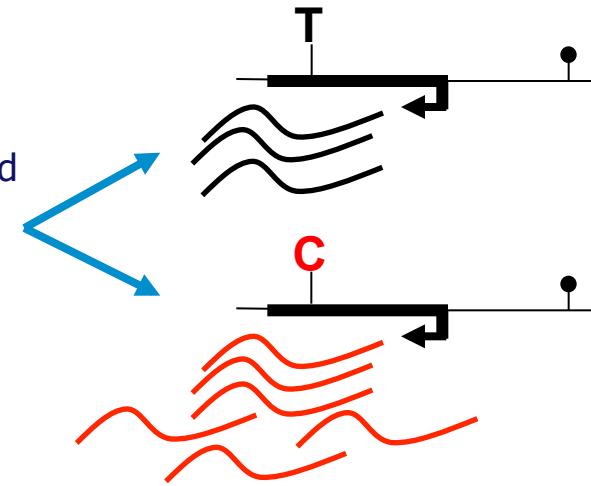
Healthy

DIO2 unmethylated



Osteoarthritic

DIO2 methylated



**Allelic imbalanced expression of RNA; marks effect and direction of effect of the genetic risk allele.**

**Expression of gene in relevant disease tissue; marks a possible function of gene in this tissue.**

**Responsive to disease process; a marks pathophysiological process**

*All based on associations, what about causality*

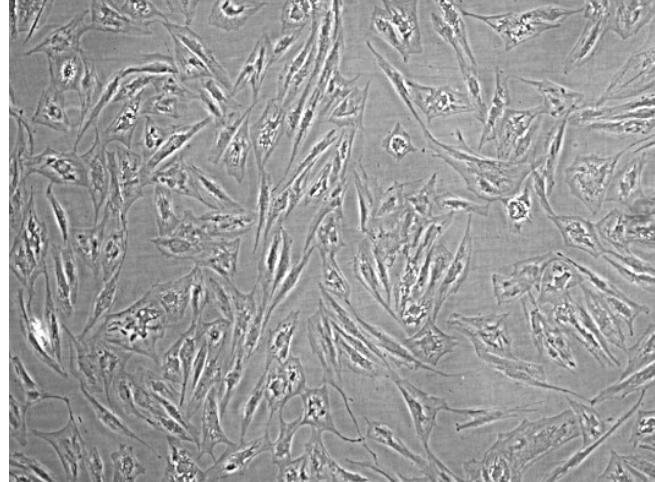


# **What is direct effect of DIO2 upregulation in cartilage?**

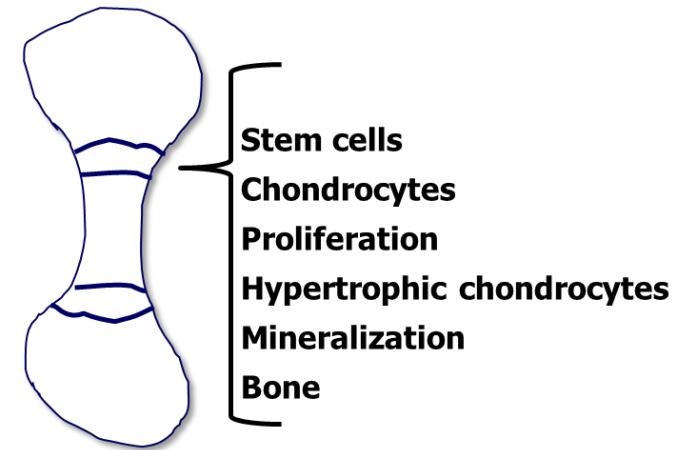
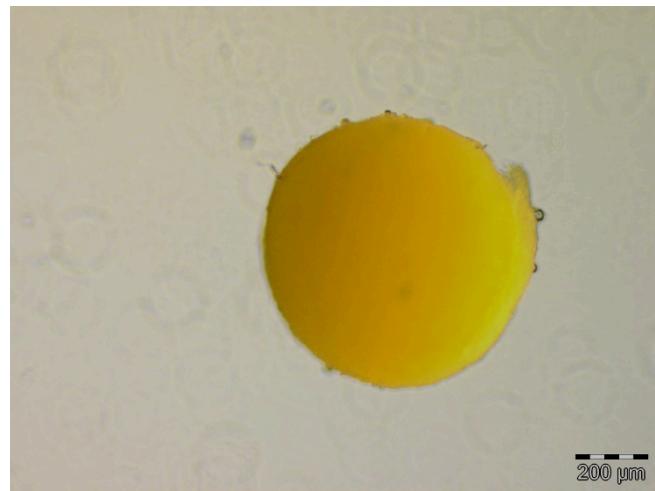
# Human *in vitro* chondrogenesis model

## Stemcells, primary chondrocytes

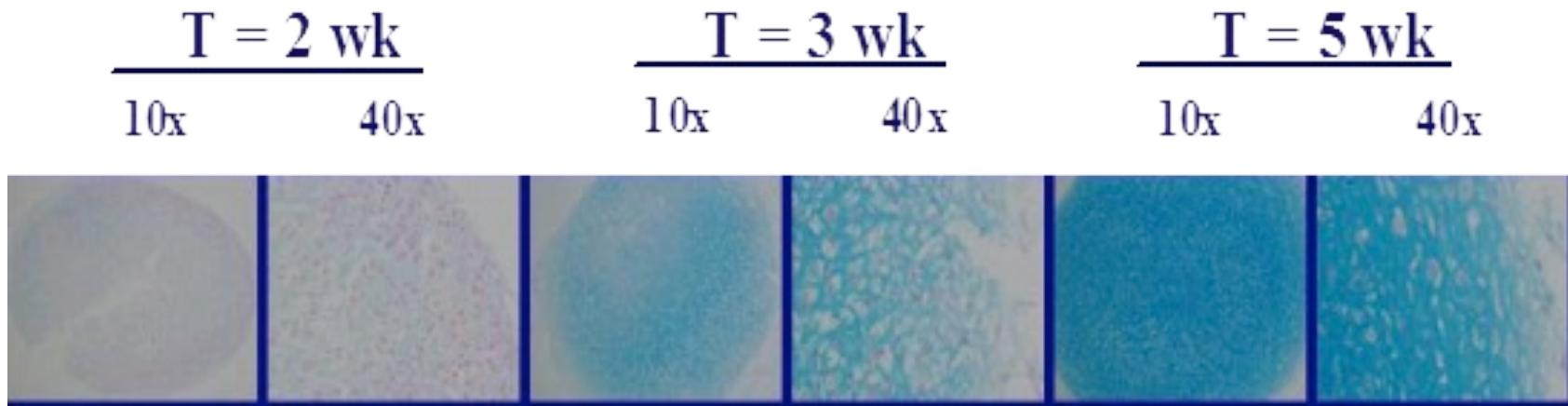
Growing cells  
(monolayer)



Chondrocyte pellet  
cartilage formation



# *In vitro* chondrogenesis model Stemcells, primary chondrocytes

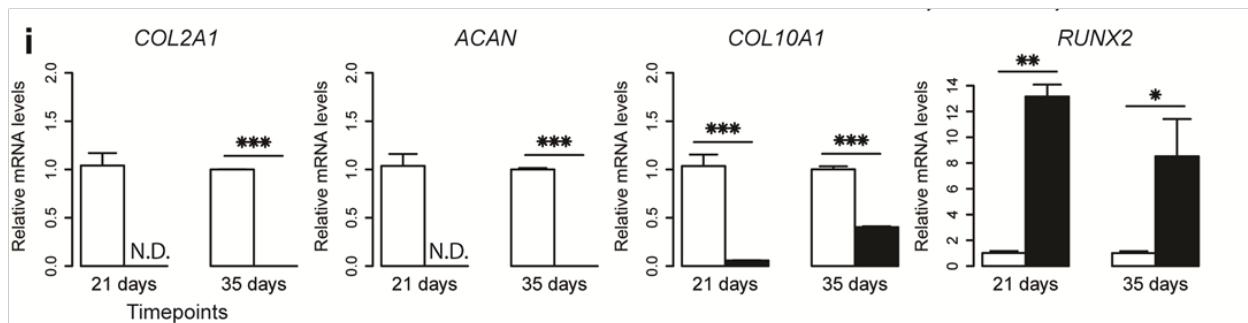
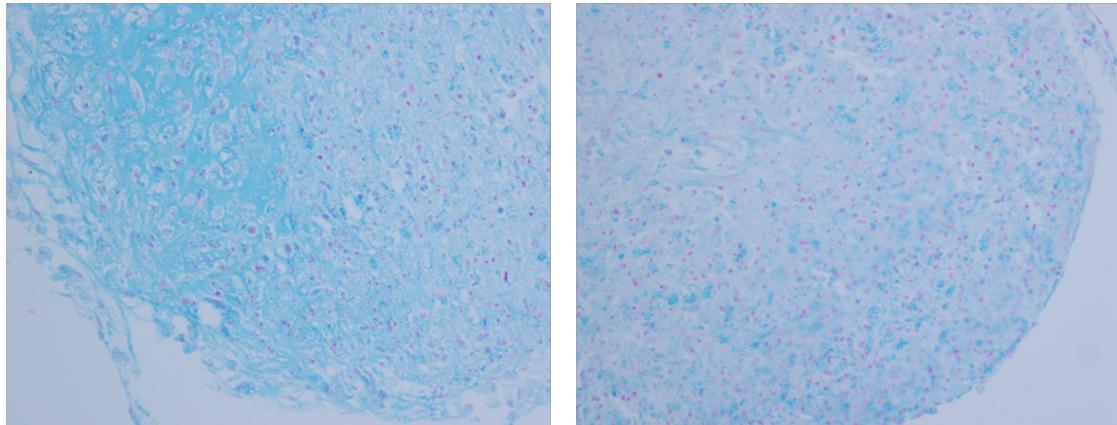


*In vitro* chondrogenesis model using human bone marrow derived mesenchymal stem cells. Alcian blue staining

**What is direct effect of DIO2 upregulation**

# BM-MSC based *in vitro* chondrogenesis model

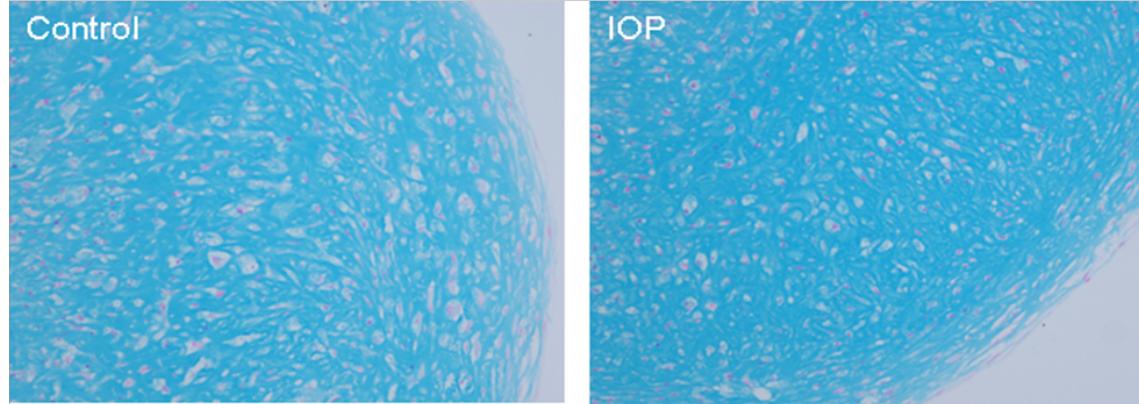
## Overexpression of DIO2



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

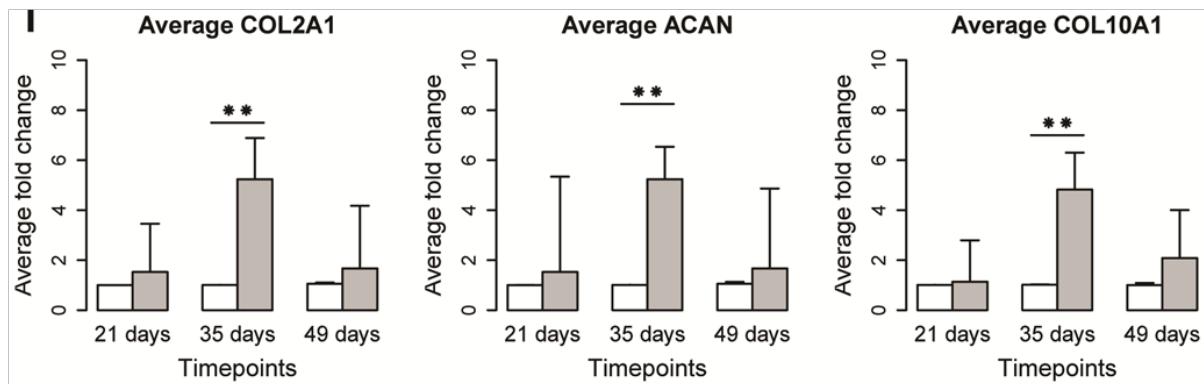
# BM-MSC based *in vitro* chondrogenesis model

## 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 .**

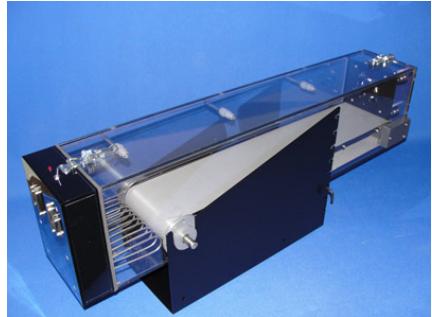
**Beneficial effect of DIO2 on cartilage matrix deposition**

**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)



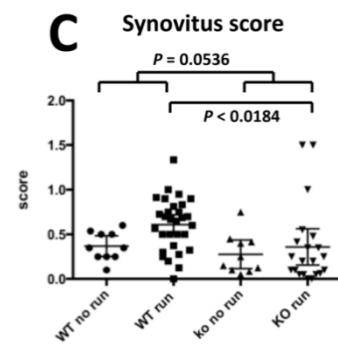
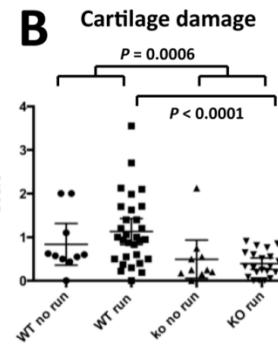
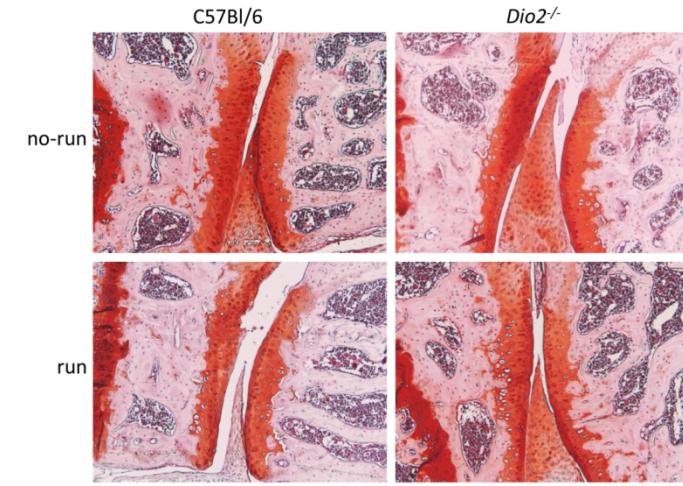
For 3 weeks -> 5 days/week -> 60 min/day  
(60min at 11m/min, 5° incline)



Induce cartilage damage

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

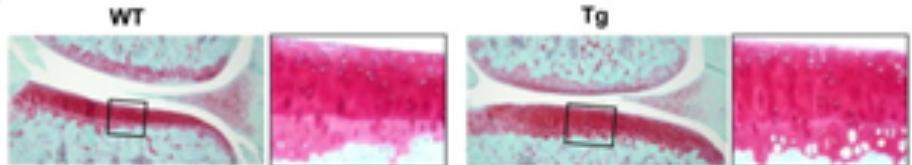
Bomer et al. Ann Rheum Dis. (2015)



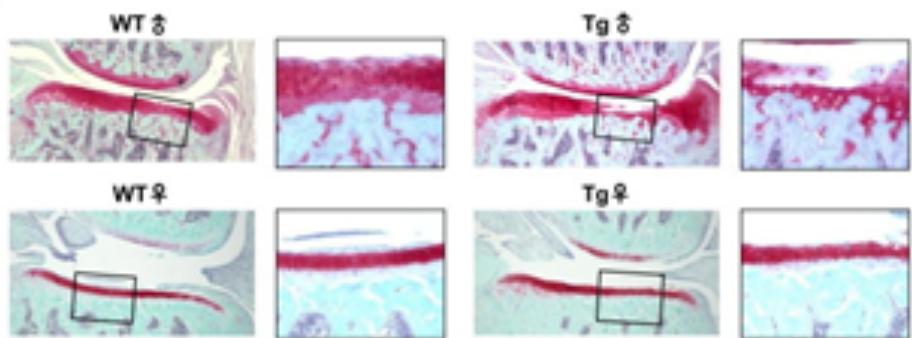
2015

# Tissue specific induction *DIO2* in rats

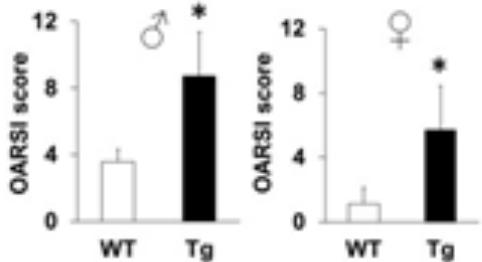
A



B



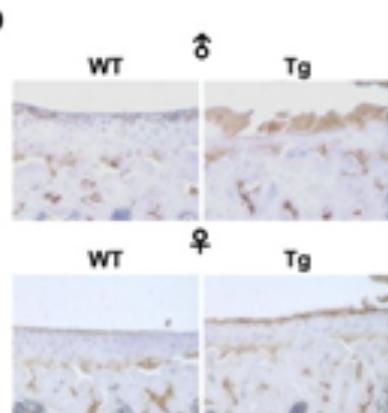
C



**Destruction of cartilage only  
upon applying OA model**

Nagase et al. Ann Rheum Dis 2013

2013



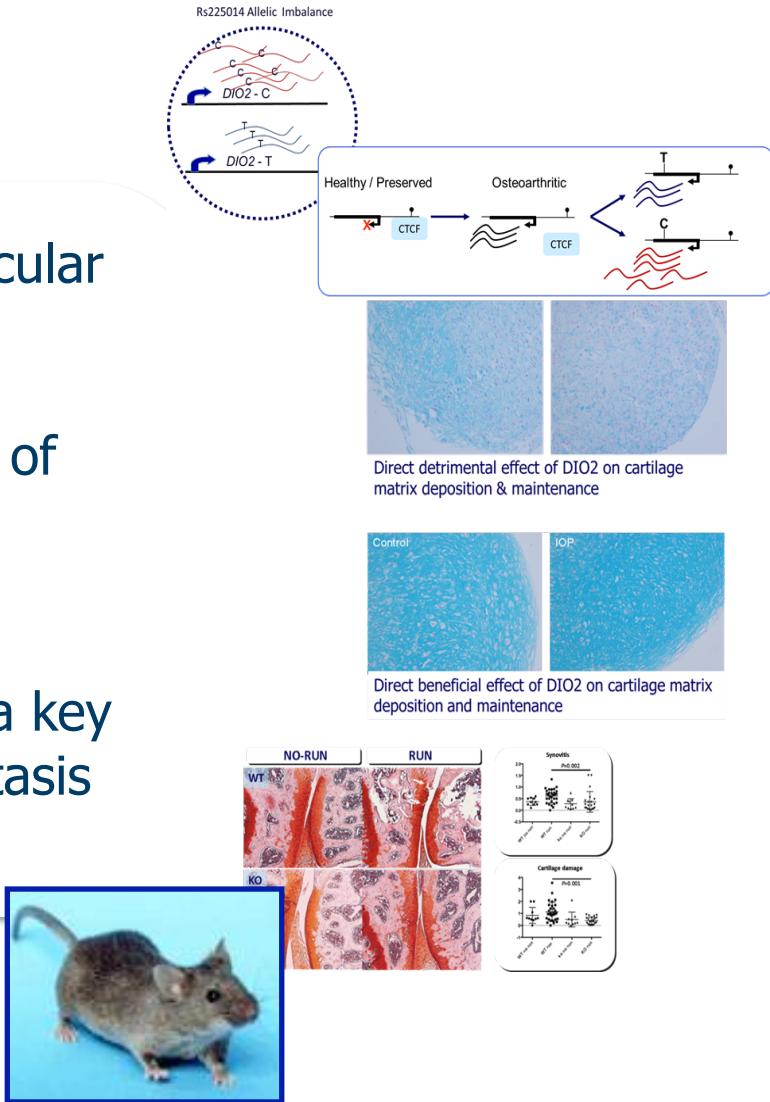
## *Conclusions *in vitro* animal studies DIO2*

**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**

# 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



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



# Acknowledgements

*LUMC Molecular Epidemiology*

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*LUMC Rheumatology*

M. Kloppenburg

*LUMC Pathology*

JVM Bovee



B B M R I • N L



IDEAL FP7/2007-2011 n° 259679