Genetic Links Between Development and Osteoarthritis: \textit{DIO2} risk gene

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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

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

*Random effect meta-analyses

Identification of DIO2 as a new susceptibility locus for symptomatic osteoarthritis
**DIO2** in growth plate

Endochondral ossification

- Stem cells
- Proliferation
- Hypertrophic chondrocytes
- Mineralization
- Bone

T3 triggers terminal maturation of growth plate chondrocytes

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

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
Define shape with 70 points around the hip joint.
Mode 1 was characterized by a high within person correlation with OA for wide standing position or small pelvis, \( P = 2 \times 10^{-4} \)

Interaction with \textit{DIO2} genotypes?

Mode 1 within GARP subjects
Collaboration E. Waarsing, H.H. Weinans (Rotterdam)

**Mode 1**

Interaction between OA * \( DIO2 \) 
\( P=0.005 \)

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

Endochondral Ossification and Osteoarthritis

Late life effect of \textit{DIO2}

Early life disruption of EO genes

- Altered skeletal morphogenesis
- Suboptimal joint shape

Late life activation of EO genes

- Cartilage degradation/mineralization

Osteoarthritis susceptibility
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 \textit{DIO2} expression

Gene targeted methylation at CpG sites (Epityper, Sequenom)

- \textit{DIO2} expression in articular cartilage is \textbf{modulated by methylation} at CpG \textasciitilde-2000 bp

N. Bomer, \textbf{W den Hollander} and YFM Ramos et al. ARD 2014
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

N. Bomer, **W den Hollander** and YFM Ramos et al. ARD 2014
Regulation of \textit{DIO2} expression

Gene targeted methylation at CpG sites (Epityper, Sequenom)

- \textit{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
Stem cells, primary chondrocytes

Growing cells (monolayer)

Chondrocyte pellet cartilage formation

Stem cells
Chondrocytes
Proliferation
Hypertrophic chondrocytes
Mineralization
Bone
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 2014
BM-MSC based *in vitro* chondrogenesis model

**Overexpression of DIO2**

![Images of histological sections and graphs showing gene expression over time](image)

- 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 2014

**DIO2 up-regulation**

- Rats cartilage destruction
- Nagase et al. 2013
BM-MSC based *in vitro* chondrogenesis model

**Inhibition of DIO2 function**

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

*N. Bomer, W den Hollander and YFM Ramos et al. ARD 2014*
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
Genetic Link Between Development and Osteoarthritis

Stem cells
Chondrocytes
Proliferation
Hypertrophic chondrocytes
Mineralization
Bone

Maturational arrested articular chondrocytes likely due to epigenetic control

Thyroid signaling T3
Genetic Link Between Development and Osteoarthritis; thyroid signalling
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http://www.molepi.nl/research/osteoarthritis/workshop

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