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# The identification of susceptibility genes for the onset of Osteoarthritis

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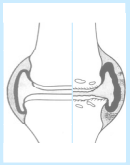
## Osteoarthritis complex disease

- Heterogeneity between radiographic / clinical features
- Age related disease with high prevalence
- Poor treatment: pain relief, joint replacement
- Heritability 40-70% depending on the joint location (hip, knee, hand)

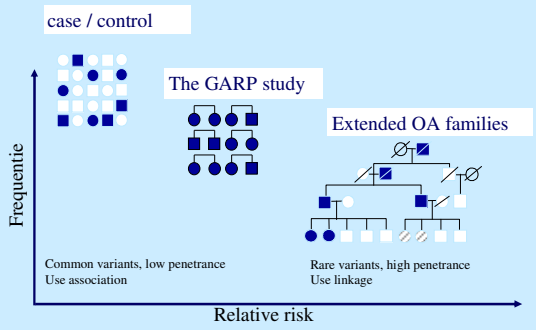


## Genetic determinants of the onset and progression of Osteoarthritis

Molecular Epidemiology, Leiden

- Pathway identification / etiology
- Classification and prognosis patients
- New disease modifying drugable targets

## Genetic research designs




## Genetics osteoARtritis and Progression

The GARP study

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

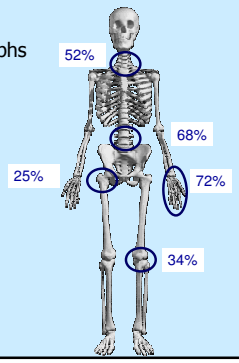
Inclusion:

≥ 2 joints OA

Progression:

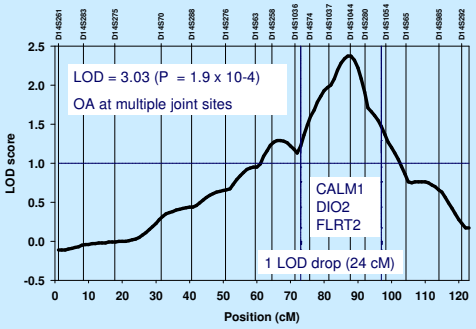
2 jr: 100 pairs

5 jr: 200 pairs




## Linkage on chromosome 14q32

Garp study genome wide scan.



## DIO2, OA susceptibility gene

The Garp study, combined linkage association

| *Gene* | *SNP reference* | *allele* | *alias* | *MAF* | *P-value* |
|---|---|---|---|---|---|
| *DIO2* | **rs12885300** | **C>T** | ORFaGly3Ala | **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** |

## Replication, OA susceptibility gene

DIO2 haplotype rs12885300-rs225014 C-c

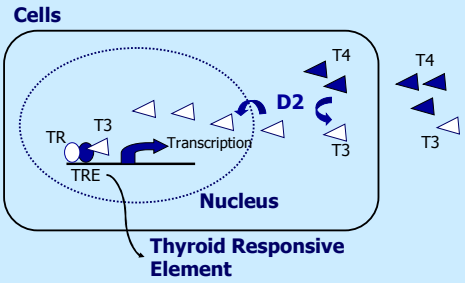
Female cases severe hip OA

| *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.

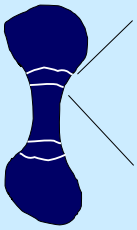
## Function Deiodinase 2 (D2)




Intracellular T3 level triggers gene expression in specific tissues such as brain, muscle and growth plate.

## D2 in growth plate

Endochondral ossification



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

**T4** → **D2** → **T3**

➔ **T3 triggers terminal maturation of growth plate chondrocytes**

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

## D2 in OA cartilage

- **Highly up regulated in end stage OA cartilage**

*Ijiri et al. 2008 Arthritis & Rheum 58:2075-87*

## SNP rs225014, non-synonymous, DIO2 92Ala predisposing

- Located in functional instability loop of enzyme
  *(Dentice et al. 2005 Nat Cell Biol 7:698-705)*
- No direct effect on enzyme activity *in vitro*
  *(Zeold et al. 2006 J Biol Chem 281:31538-43)*
- D2 velocity is decreased in tissues of subjects homozygous 92Ala
  *(Canani et al. 2005 J Clin Endocrinol Metab 90:472-8)*

➔ **Functional effect of SNP unclear**

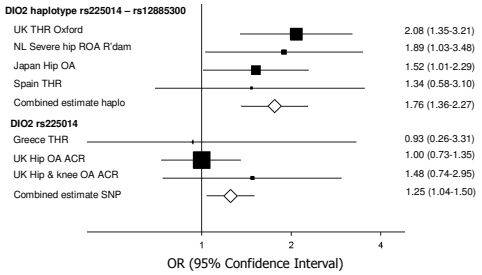
## SNP rs12885300 non-synonymous
DIO2 ORFaGly3 predisposing

- DIO2 contains a long 5'UTR with 3-5 short open reading frames (sORF)
  *(Gereben et al. 2002 Mol Endocrinol 16:1667-79)*
- ORFaAla3 associated with higher DIO2 activity
  *(Coppotelli et al. 2006 Thyroid 16:625-32)*

➔ **OA associated haplotype may result in decrease enzyme activity**

## Genetic follow up DIO2 SNPs
Additional studies

Meta analyses severe hip OA females




## Additional studies DIO2

- SNPs in LD within DIO2
- Genes in the thyroid pathway (DIO1, TSHR, TRA)
- Plasma thyroid levels

## Additional functional studies

Cartilage tissue (Healthy vs OA)
- Immunohistochemistry

Cell lines HEK293
- Explore functional effect of SNP rs225014

DIO2 KO mouse
- Effect of D2 on locomoter apparatus
  (F. Luyten, R. Lories; KU Leuven)

## Recent advances in genetic studies of OA

**Change in our perspective on OA etiology?**

## Recent new OA susceptibility genes

| Location | Phenotype | Gene | Reference |
|---|---|---|---|
| 2q31-32 | THR | FrzB | Loughlin *et al* 04 PNAS 101:9757-62 |
| 9q22.21 | hip & knee OA | ASPN | Kizawa *et al* 05 Nat Genet 37:138-144 |
| 20q11.22 | OA | GDF5 | Miyamoto *et al* 07 Nat Genet 39:529-533 |
| 6p12.1 | THR females | BMP5 | Loughlin *et al* 02 Eur J Hum Genet 10:526-8 |
| 14q32 | GOA, hip OA | DIO2 | Meulenbelt *et al* 08 Hum Mol Genet 17:1867-75 |
| 3p25 | knee OA | DVWA | Miyamoto *et al* 08 Nat Genet 40:944-98 |
| 1q31.1 | knee OA | PTGS2 | Valdes *et al* 08 Am J Hum Genet 82:1231-40 |
| 9q31.3 | knee OA | EDG2 | Mototani *et al* 08 Hum Mol Genet 17:1790–1797 |

## Consistent genetic follow up

| Gene | Additional association | Meta analyses | Ethnic differences |
|---|---|---|---|
| FrzB | +++ / - | | yes |
| ASPN | +++ | ++ | yes |
| GDF5 | ++ | ++ | yes |
| BMP5 | ++ | | |
| DIO2 | +++ | + | no |
| DVWA | ++ / - | + / - | yes |
| COX2/PTGS2 | +++ | | |
| EDG2 | ++ / - | - | yes |

Chapman *et al.* 07 Hum Mol Genet 16:2226-32 (GDF5 meta analyses)
Meulenbelt *et al.* 08 Submitted for publication (DVWA meta analyses)
Gonzalez *et al.* 08 Epub Ann Rheum Dis (EDG2 meta analyses)
Nakamura *et al.* 07 Hum Mol Genet 16:1676-81 (ASPN meta analyses)

## Functional follow up

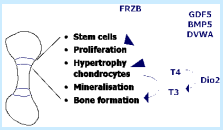
| Gene | Functional studies | |
|---|---|---|
| | *In vitro* | Mouse |
| FrzB | + | + |
| ASPN | ++ | |
| GDF5 | + | + |
| BMP5 | + | |
| DIO2 | | |
| DVWA | + | |
| COX2/PTGS2 | + | |
| EDG2 | + | |

Southam *et al.* 07 Hum Mol Genet 16:2226-32 (GDF5; DAE)
Lories *et al.* 07 A&R 56:4095-4103 (FRZB; KO Mouse)
Masuya *et al.* 07 Hum Mol Genet. 16:2366-75 (GDF5; KO mouse)
Miyamoto *et al.* 08 Nat Genet 40:944-98 (DVWA; *in vitro* cell studies)
Mototani *et al.* 08 Hum Mol Genet 17:1790–1797 (EDG2; *in vitro* cell studies)
Wilkins *et al.* 08 Hum Mol Genet 16:537-46 (BMP5; DAE)
Valdes *et al.* 08 Am J Hum Genet 82:1231-40 (PTGS2; DAE)
Nakajima *et al.* 07 J Biol Chem 44:32185-92 (ASPN; *in vitro* and *in vivo*)

## Common pathway discovered genes?

**FRZB**
**DIO2**
**BMP5**
**GDF5**
**DVWA**

**Skeletal development, endochondral ossification**




**ASPN**
**EDG2**
**PTGS2**
**IL1**
**CRP**

**Inflammation maintenance cartilage**

## Osteoarthritis

Early developmental or age related disease?

## OA as early developmental disease

**Skeletal morphogenesis may predispose to malformation or composition of bones.**

➡ Increased biomechanical stresses, cartilage prone to damage and eventually onset of OA with age

## Healthy cartilage

Chondrocytes

- Maturational arrested
- Highly specific resting cells
- Produce cartilage specific proteins

## Aged and OA cartilage
Chondrocytes

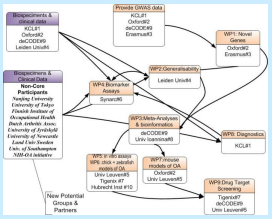
- Loosening of maturational arrest
- Growth plate morphology
- Secrete large amounts of matrix metalloproteinases
- Produce bone specific proteins (COLX, COL1)

➡ Genes involved in early skeletal development may also affect chondrocyte viability and cartilage integrity at adult ages

## TREAT~OA
European collaborative research project (FP7)

**T**ranslational **R**esearch in **E**urope
**A**pplied **T**echnologies for **O**steo**A**rthritis

TreatOA



## TREAT~OA

- Follow up of OA genome wide studies
- Meta analyses across various OA populations
- Functional studies (*In vitro*, Chick, Zebrafish, Mouse)
- Biomarkers
- Diagnostics
- Translation towards (disease modifying) drug target discovery

## Contributors

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