Gene profiling in connective tissues

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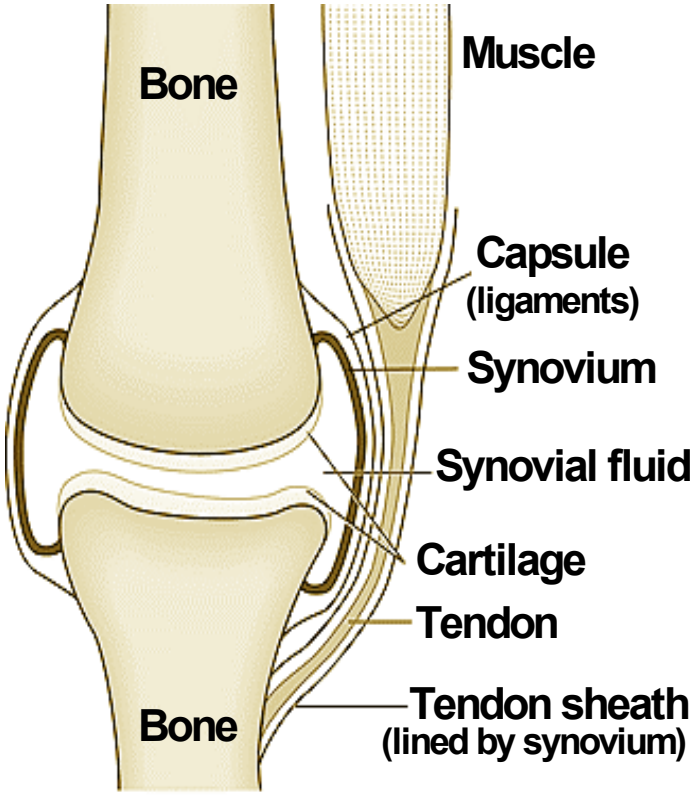
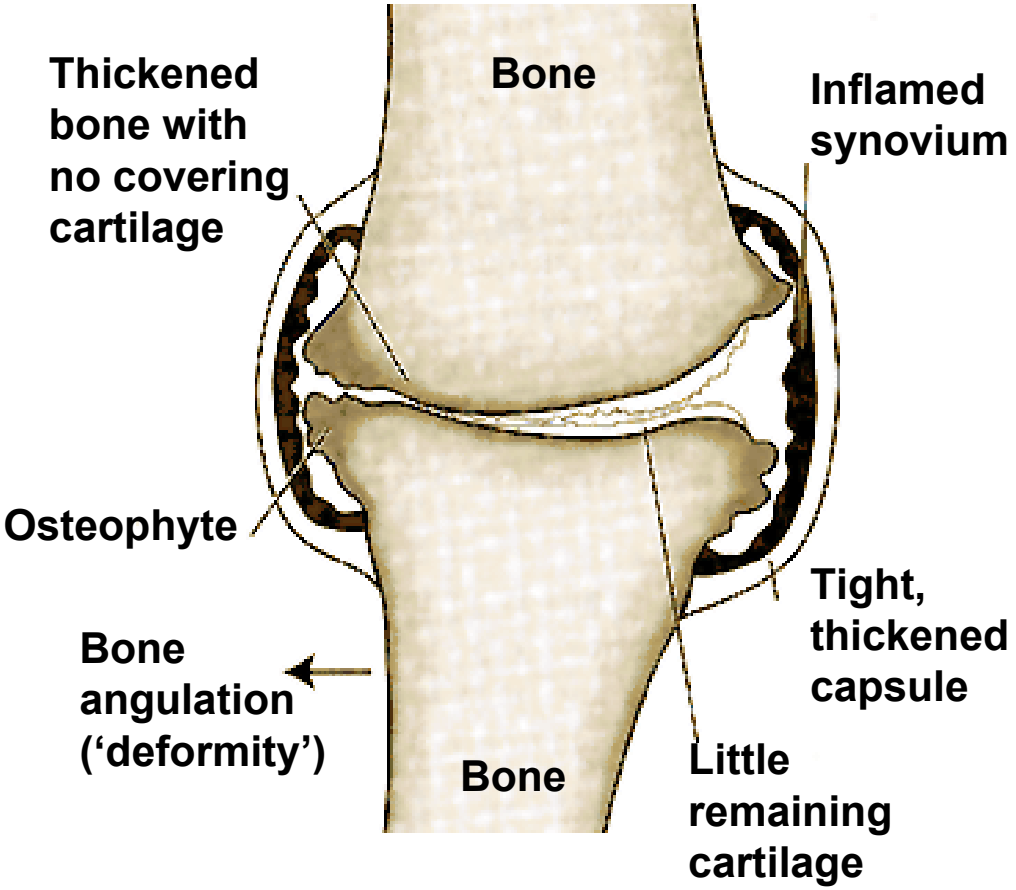
- introduction / osteoarthritis
- Dupuytren's disease

Joint pathology - osteoarthritis



Normal joint



Osteoarthritic joint

Metalloproteinases and their inhibitors

Matrix metalloproteinases

23 human enzymes

(Collagenases MMP-1, -8, -13, -2, -14?)

ADAMTSs

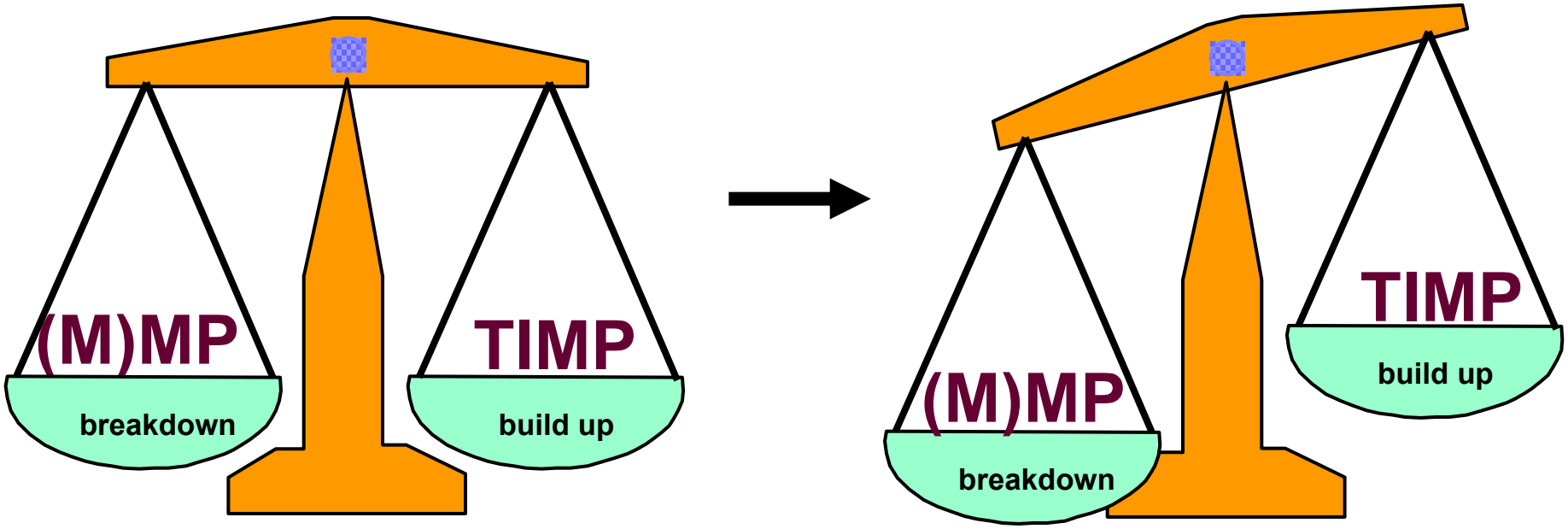
19 human enzymes

(Aggrecanases ADAMTS-1, -4, -5, -8, -9, -15?)

TIMPs

4 human inhibitors

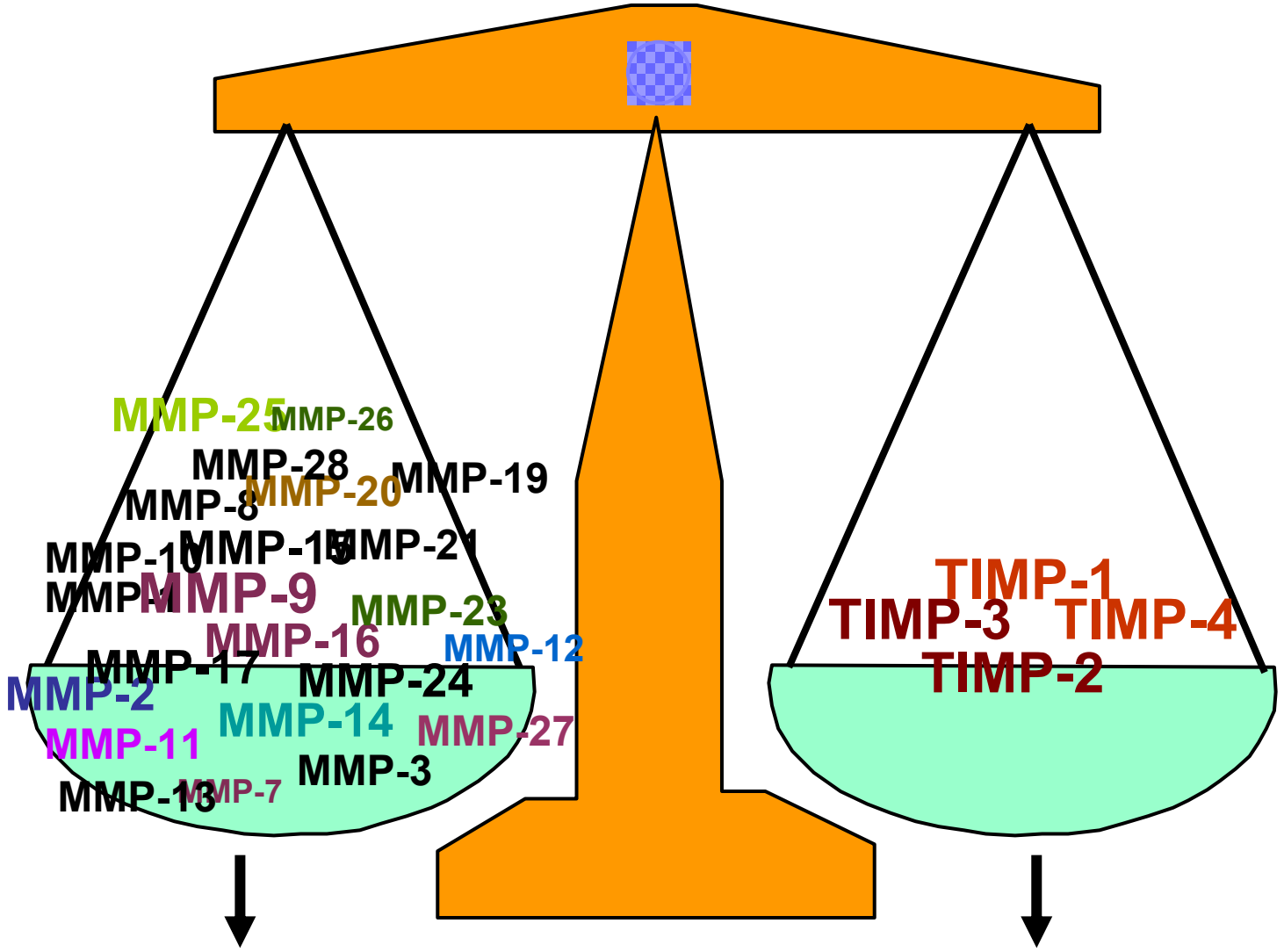
The balancing act of cartilage turnover…



Cartilage turnover

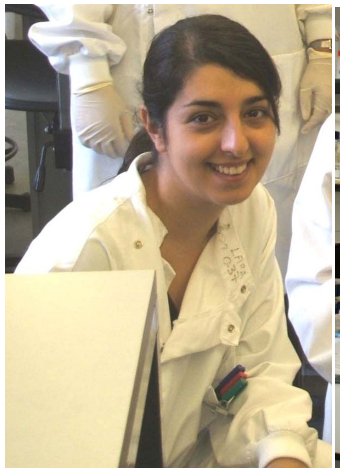
Cartilage degradation

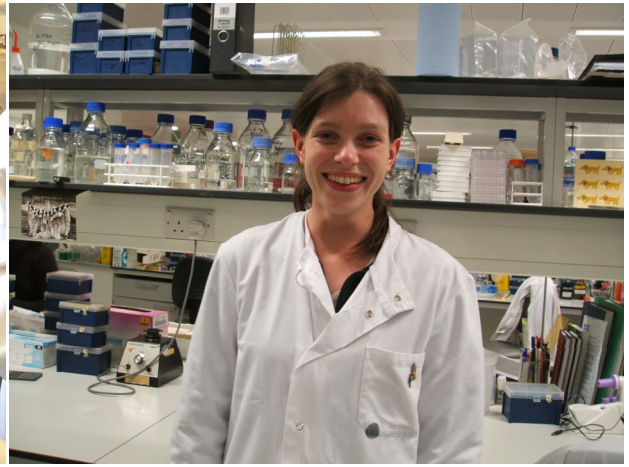
The balancing act of cartilage turnover…



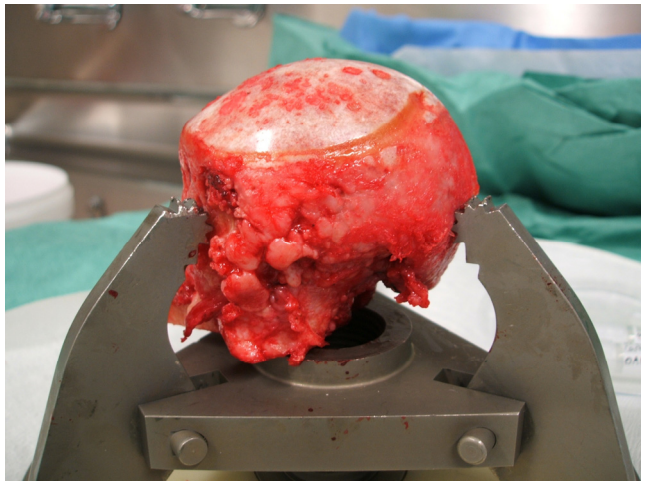
What are the key metalloproteinases regulated in osteoarthritic joint tissues?

Lara Kevorkian, Rose Davidson



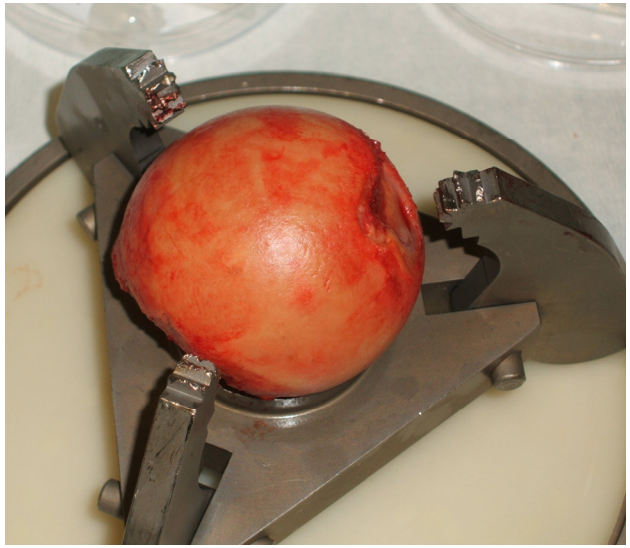


Cartilage samples



Osteoarthritis (OA)

femoral head from THR for osteoarthritis (n=18, age 38-81)

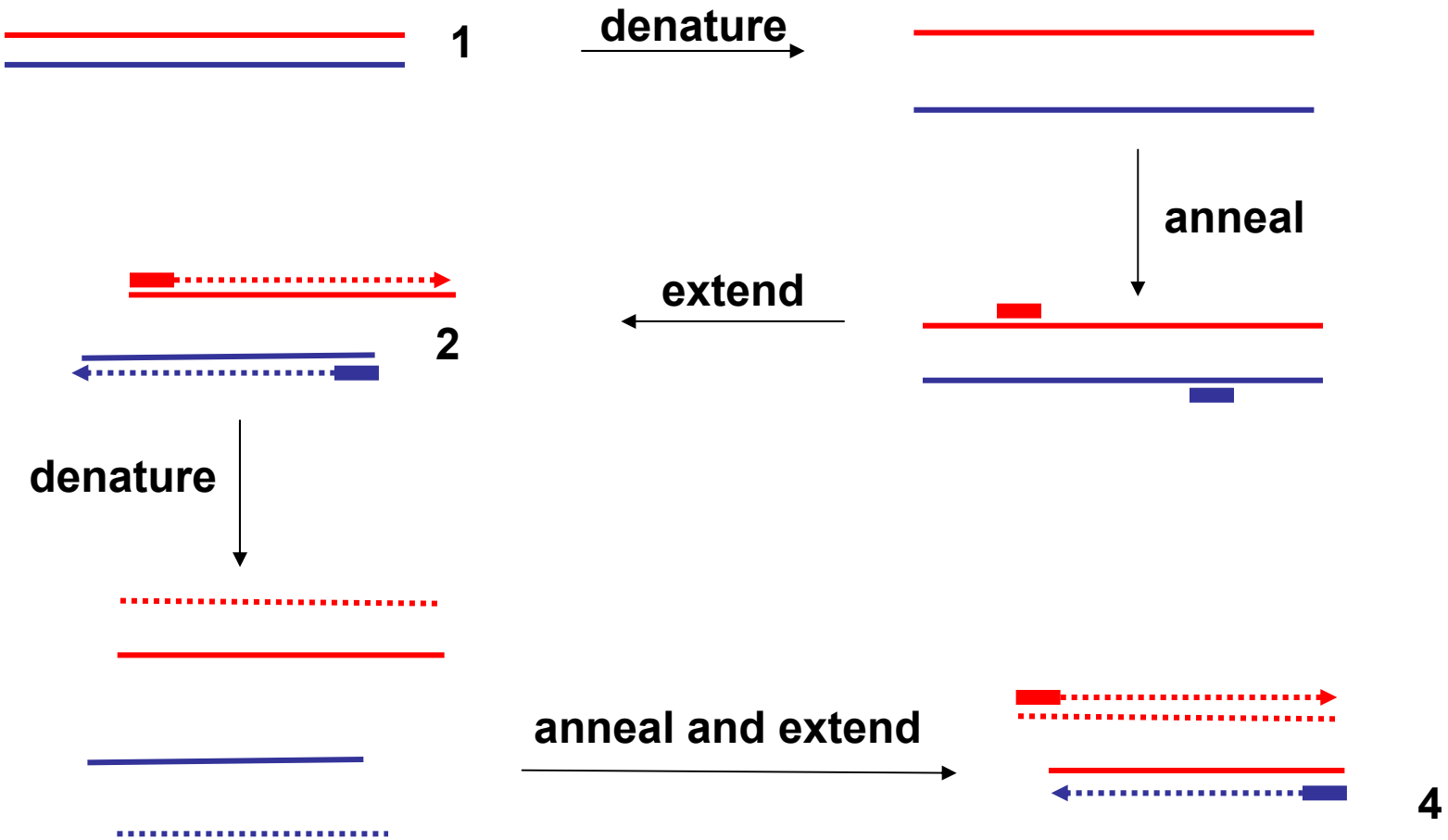


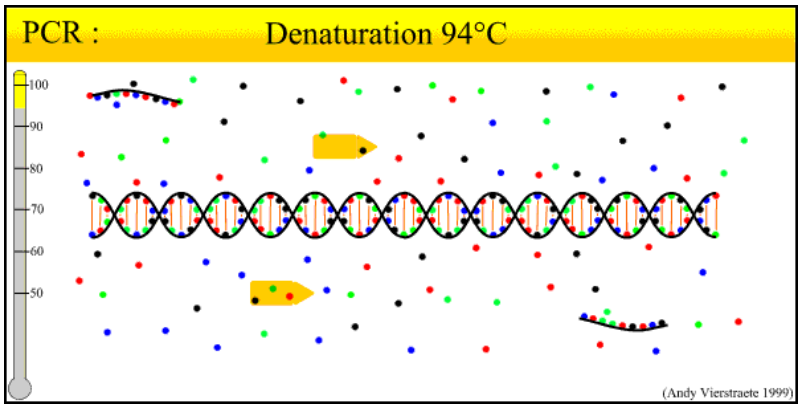
‘Normal’

femoral head from THR following fracture to the neck of femur ‘NOF’ (n=15, age 52-93)

(Simon Donell, Clare Darrah, Adele Cooper - NNUH)

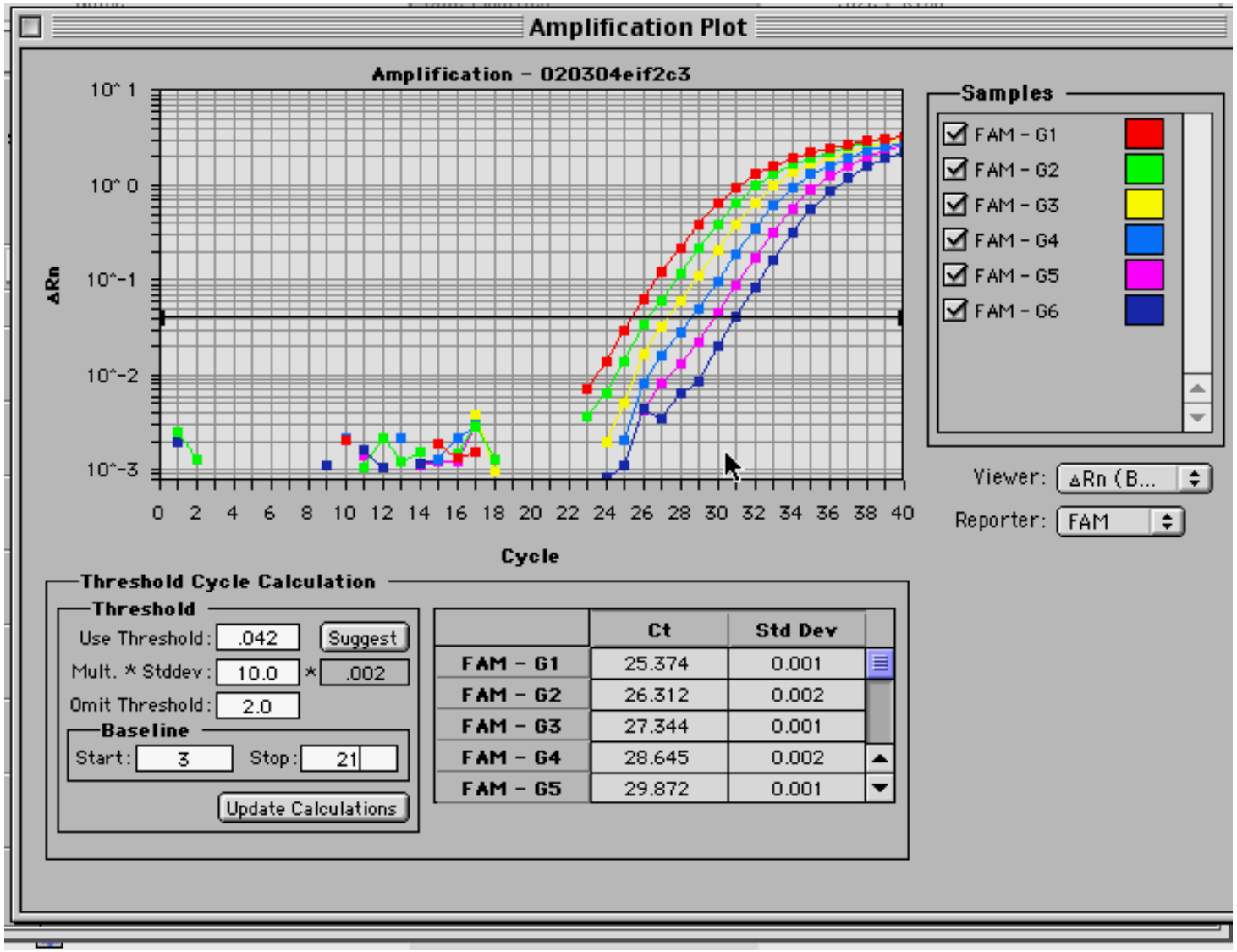
Polymerase chain reaction (PCR)



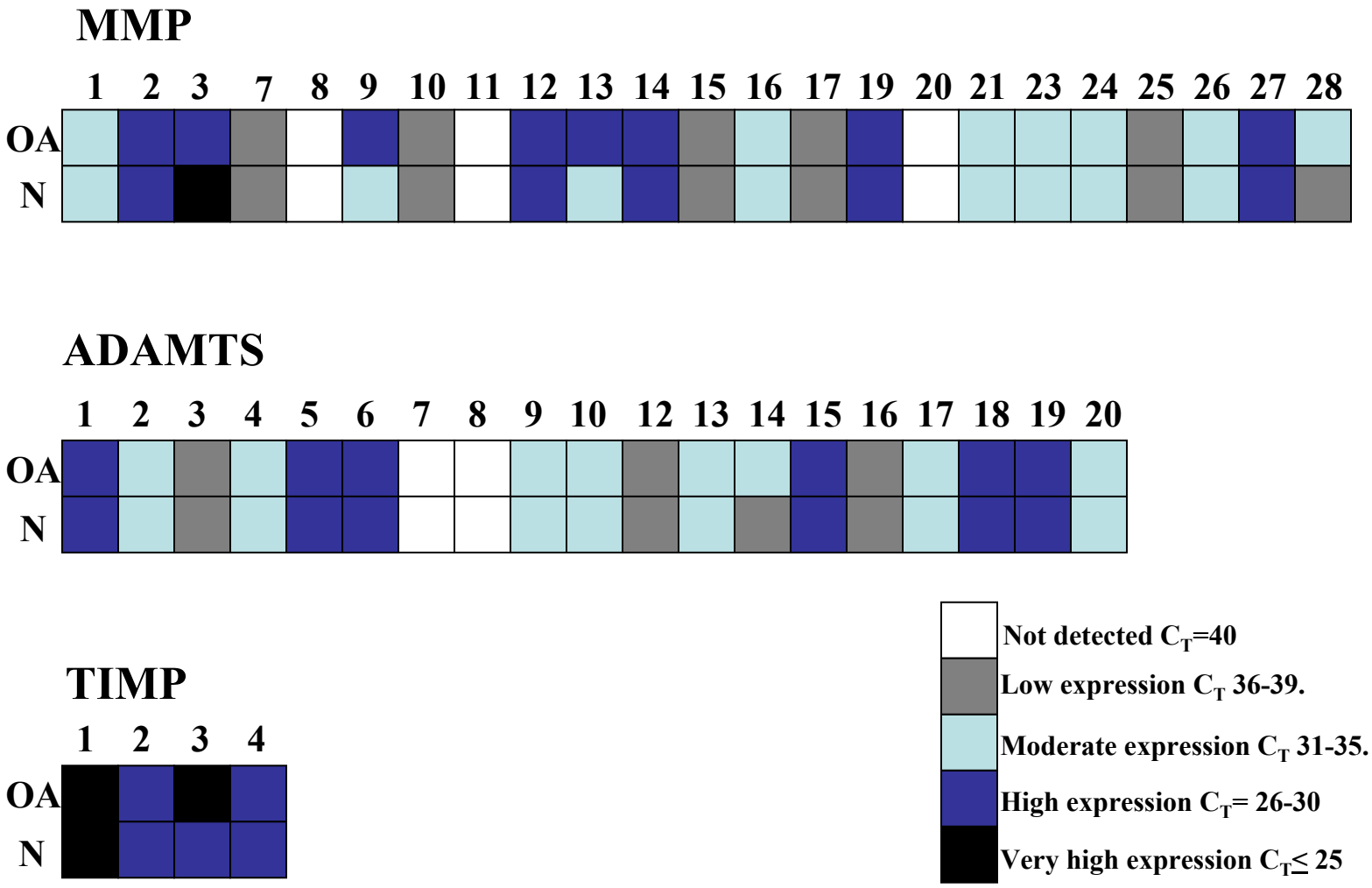


http://users.ugent.be/~avierstr/principles/pcrani.html

‘Taqman’ quantitative real-time PCR

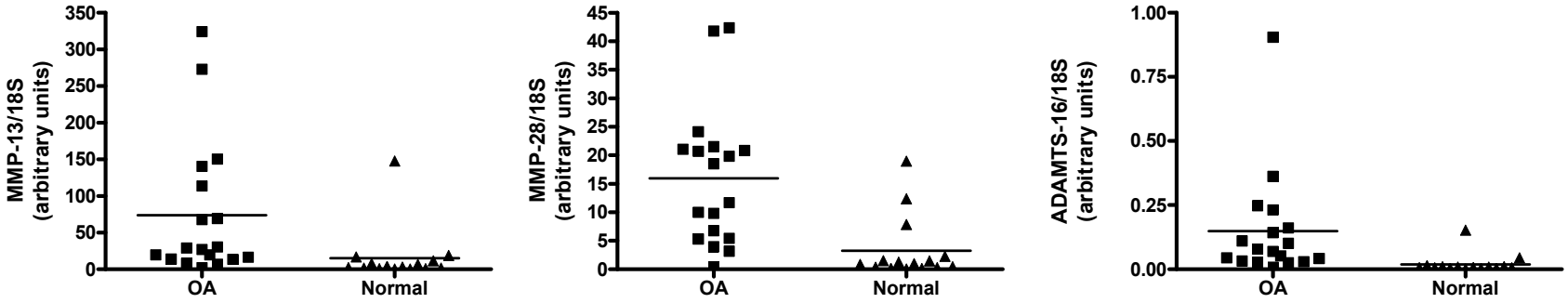


Expression profile of MMP, ADAMTS and TIMP family in normal vs. OA cartilage (mean Ct values)



Genes that are up-regulated in OA

P< 0.001



MMP13 ***MMP28*** ***ADAMTS16***

Collagenase: cleaves cartilage collagen

Unknown function

Kevorkian et al (2004) A&R 50:131

Metalloproteinase expression profiling in synovium

P value	Synovium	
	↑ in OA	↓ in OA
<0.05	***MMP9*** ***MMP16*** ***ADAMTS10***	***ADAMTS1*** ***ADAMTS5***
<0.01	***MMP11*** ***MMP13*** ***ADAMTS2*** ***ADAMTS8*** ***ADAMTS13***	
<0.0001	***MMP28*** ***ADAMTS16*** ***ADAMTS17*** ***TIMP2***	***MMP10*** ***ADAMTS4*** ***ADAMTS9***

Davidson et al (2006) ART 8:R124

Conclusions

- **First expression profiles to assay all *MMP*s, *ADAMTS*s and *TIMP*s in cartilage and synovium**

- ***MMP28* and *ADAMTS16* expression is significantly increased in end-stage OA**

Hypothesis

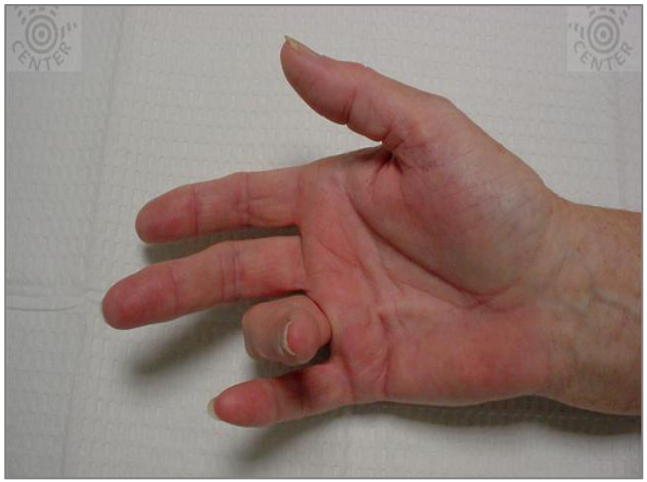
MMP-28 and ADAMTS-16 play key roles in the metabolism of joint tissues and the destruction of cartilage in osteoarthritis

Dupuytren's disease

Phillip Johnston

Background

- **Dupuytren's Disease**
  - fibroproliferative disorder of palmar fascia
  - altered extracellular matrix turnover

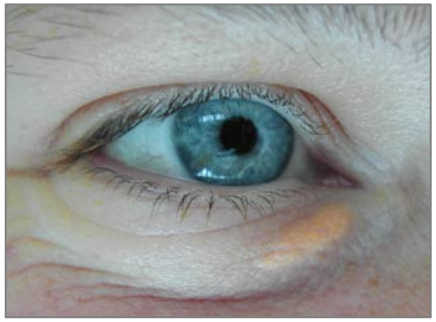


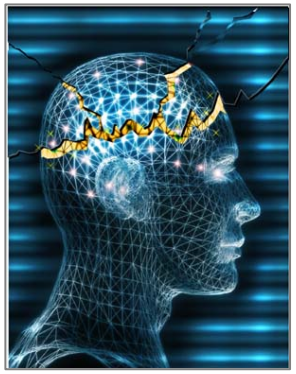
Aetiology

- Alcohol
- Smoking
- Manual work
  - Vibration tools
- Injury

Aetiology

- Diabetes
- Epilepsy
- Hyperlipidaemia
- Rheumatoid disease
- Lederhosen's





Prevalence

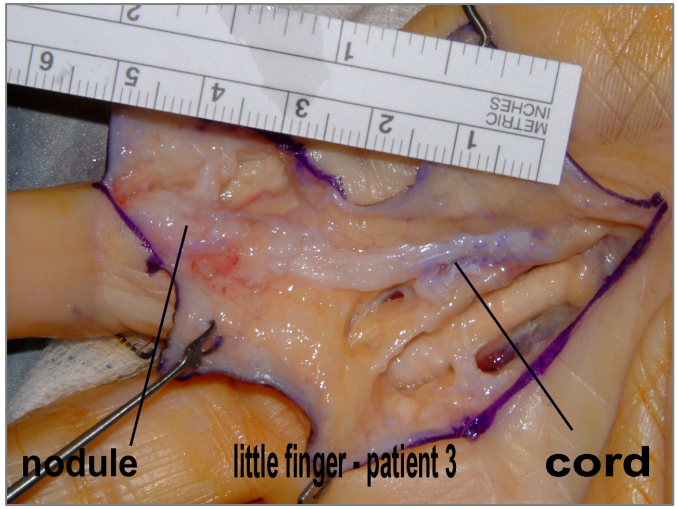
- Mainly men of north-European descent
  - 2 - 42% depending on study population
- Approx. 2 million people in the UK are thought to have Dupuytren's disease

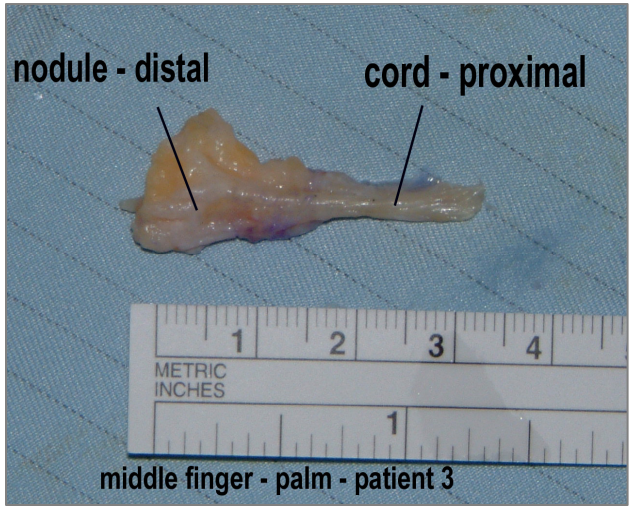
Metalloproteinases and DD

- Extracellular matrix deposition and remodelling
- Broad spectrum MMP inhibitors in cancer clinical trials showed toxicity described as ‘musculoskeletal syndrome’
  - Dupuytren's disease and frozen shoulder

Samples

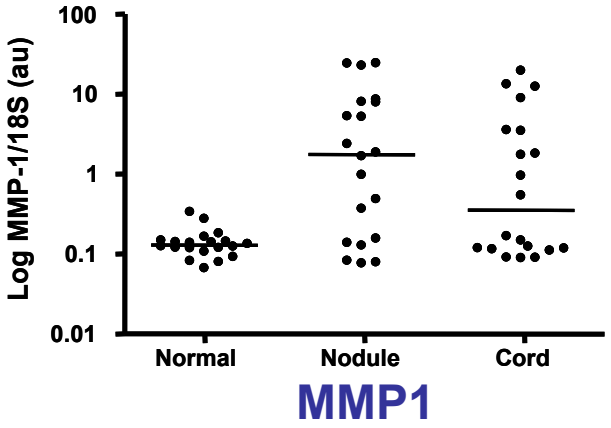
- 20 patients with Dupuytren's disease
  - Samples divided cord / nodule
- 20 controls (palmar fascia from carpal tunnel decompression)

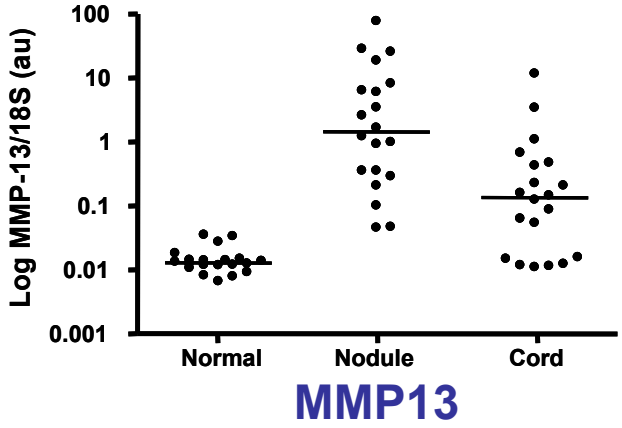


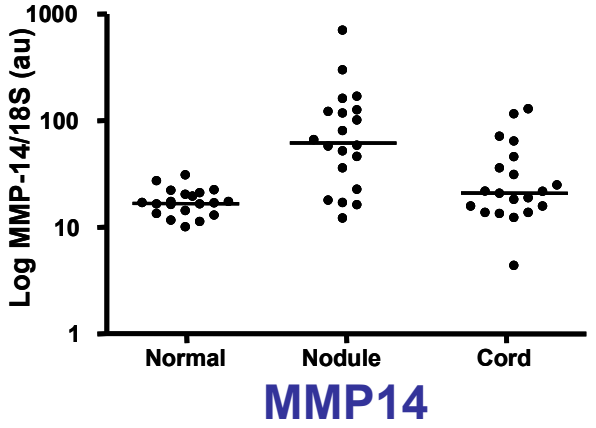


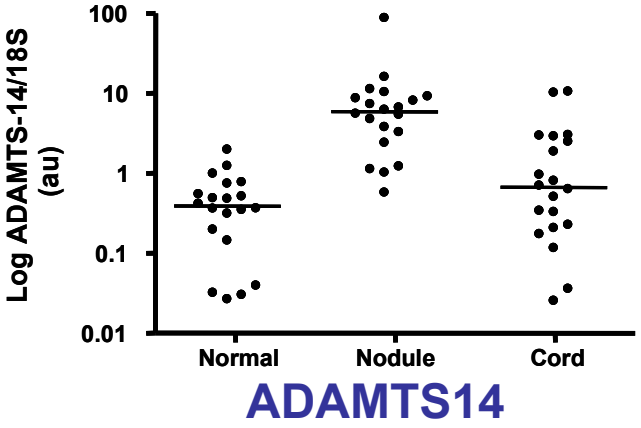
(Adrian Chojnowski - NNUH)

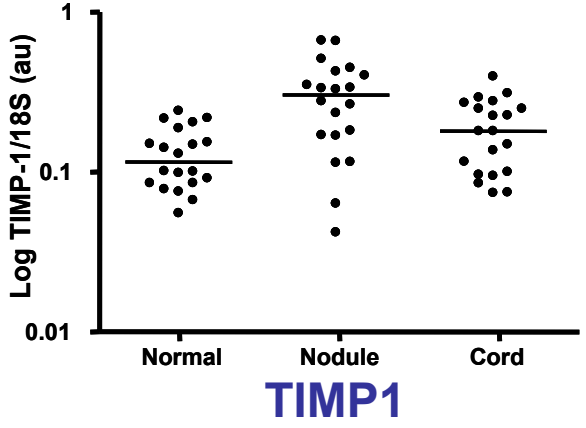
Results











Johnston et al (2007) J Hand Surg [Am] 32:343-351.

Conclusions

- first expression profiles to assay all *MMP*s, *ADAMTS*s and *TIMP*s in DD
- a number of key genes are regulated

Hypothesis

- increased collagen biosynthesis mediated by increased ADAMTS-14
- increased TIMP-1 blocking MMP-1 and MMP-13-mediated collagenolysis
- contraction enabled by MMP-14 mediated pericellular collagenolysis which may escape inhibition by TIMP-1

Correlation with clinical outcomes

- 22 patients; primary fasciectomy for DD
- Clinical scores
  - Pre op
  - 3 months post op (interim)
  - 14 months (11 – 16) post op (final)
- Range of movement
- Grip strength
- DASH (disabilities of the arm, shoulder and hand)
- MHQ (Michigan Hand Outcome Questionnaire)
- Vancouver Scar Scale

Correlations

- **Reduction in fixed flexion deformity**
  - **(overall)**
    - **MMP-13 (p = 0.062, R = 0.463)**
    - **ADAMTS-5 (p = 0.058, R = 0.467)**
    - **ADAMTS-14 (p = 0.034, R = 0.517)**
    - **ADAMTS-16 (p = 0.032, R = 0.521)**

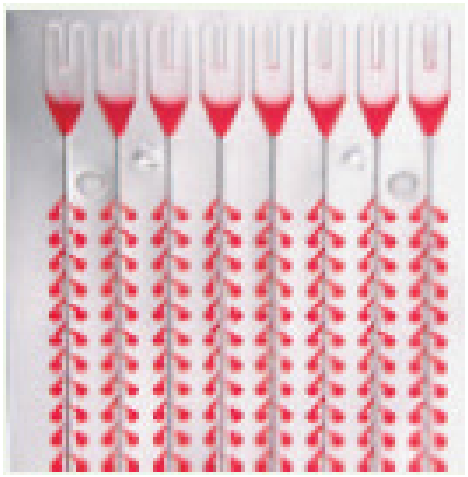
- **Reduction in fixed flexion deformity**
  - **(interim to final follow up)**
    - **MMP-13 (p = 0.005, R = 0.727)**
    - **MMP-14 (p = 0.006, R = 0.714)**
    - **ADAMTS-14 (p = 0.003, R = 0.752)**

Conclusions

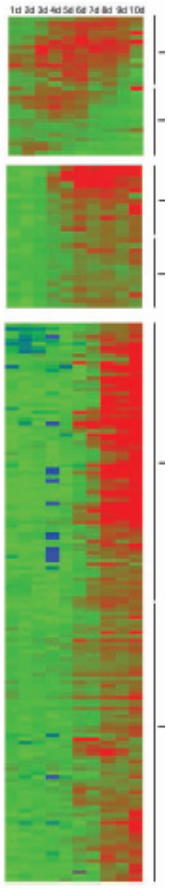
- potential to predict disease progression

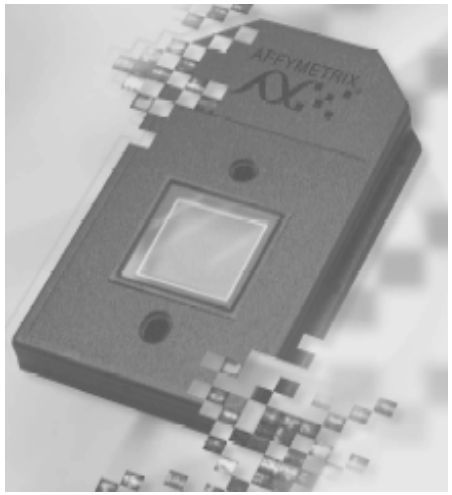
Summary

- **Focused expression profiling in human connective tissues can help build hypotheses on disease mechanisms**



ABI microfluidic card





Affymetrix GeneChip

Acknowledgements

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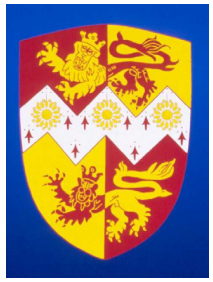

Institute of Orthopaedics

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