Comparison of matrix deposition and biomechanical responses in tissue engineered cartilage model of musculoskeletal ageing

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Introduction

Pseudoachondroplasia (PSACH) is an autosomal dominant skeletal dysplasia characterised by short stature and delayed ossification (Fig 1A)[(1)]. PSACH patients present with several musculoskeletal complications including a mild myopathy, joint dislocations and early onset osteoarthritis, making it a good model to study the **effect of extracellular matrix (ECM) changes on premature musculoskeletal ageing** (Fig 1B,C)[(1,2)]. It results from mutations in a gene encoding Cartilage Oligomeric Matrix Protein (COMP). Wild type COMP is a bridging molecule within cartilage ECM, and interacts with structural molecules such as type II collagen and aggrecan[(1)]. T585M is a mutation in the C-terminal globular domain, resulting in the failure to export COMP from the cell[(1,3)]. Chondrocyte mechanosensing via integrins and primary cilia of dynamic compression leads to an upregulation of structural ECM molecule synthesis[(4)]. It has been hypothesised that COMP mutations affect the ECM ultrastructure and biomechanical properties of cartilage which impact on chondrocyte mechanosensing and signalling (Fig 1D).

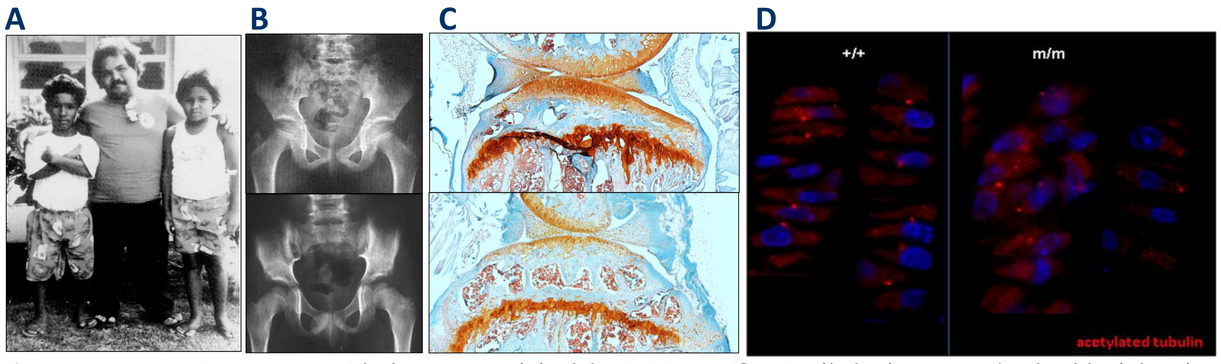

Figure 1. PSACH patient present with short stature (A); delay in joint ossification (*below*) compared to healthy (*above*) (X-rays at 7 years, (B)); early onset osteoarthritis (*below*) compared to wild type (*above*) (Safranin O staining of mouse cartilage at 7 months (=human 30 y, (C)), due to abnormal matrix deposition and primary cilia misalignment (D). *(source: K Piróg; M Briggs)*

Methods

- $10x10^6$/ml chondrogenic ATDC5 cells (un-transfected, WT-COMP and T585M-COMP) were cultured in 2% agar constructs in DMEM/F12 supplemented with 5% FBS, 1% insulin/transferrin selenium, 1% non-essential amino acids, 1% Penicillin/Streptomycin, ascorbic acid, BMP7, TGFβ3 and nystatin
- the tissue engineered constructs were grown for 6 days then compressed at 10kPa for 30min every day for 14 days using the Flexcell FX500 compression system (DunnLabortechnik Ltd)
- samples were harvested at days 1, 7, 14 and 21 of the experiment and fixed in 4% paraformaldehyde (for histology) or dissolved in QG+RTL+β-mercaptoethanol (Qiangen; RNA)
- type I and II collagen primary antibodies (1:200 in 1% BSA/PBS; Abcam), and Biotinylated Goat Anti-Polyvalent secondary antibodies (Abcam) were used for immunohistochemical analysis of the constructs
- qPCR was performed using SYBR Green (ABI) on cDNA generated from 50ng of extracted RNA, using the MasterMix kit, to assess gene expression levels of: **stem cell (Sox9), chondrocyte (*Col2a1* and *Acan*), dedifferentiation (*Col1a1*), hypertrophy (*Col10a1*) and mechanoresponsive cartilage (*Ihh*) markers.** Expression of each marker was normalised to ***18S*** levels
- Student t-test was used for statistical analysis of data at $P<0.05$ significance

Results

Gene expression and protein levels in 2% agar culture of ATDC5 cells indicate chondrogenic differentiation and mechanoresponsiveness

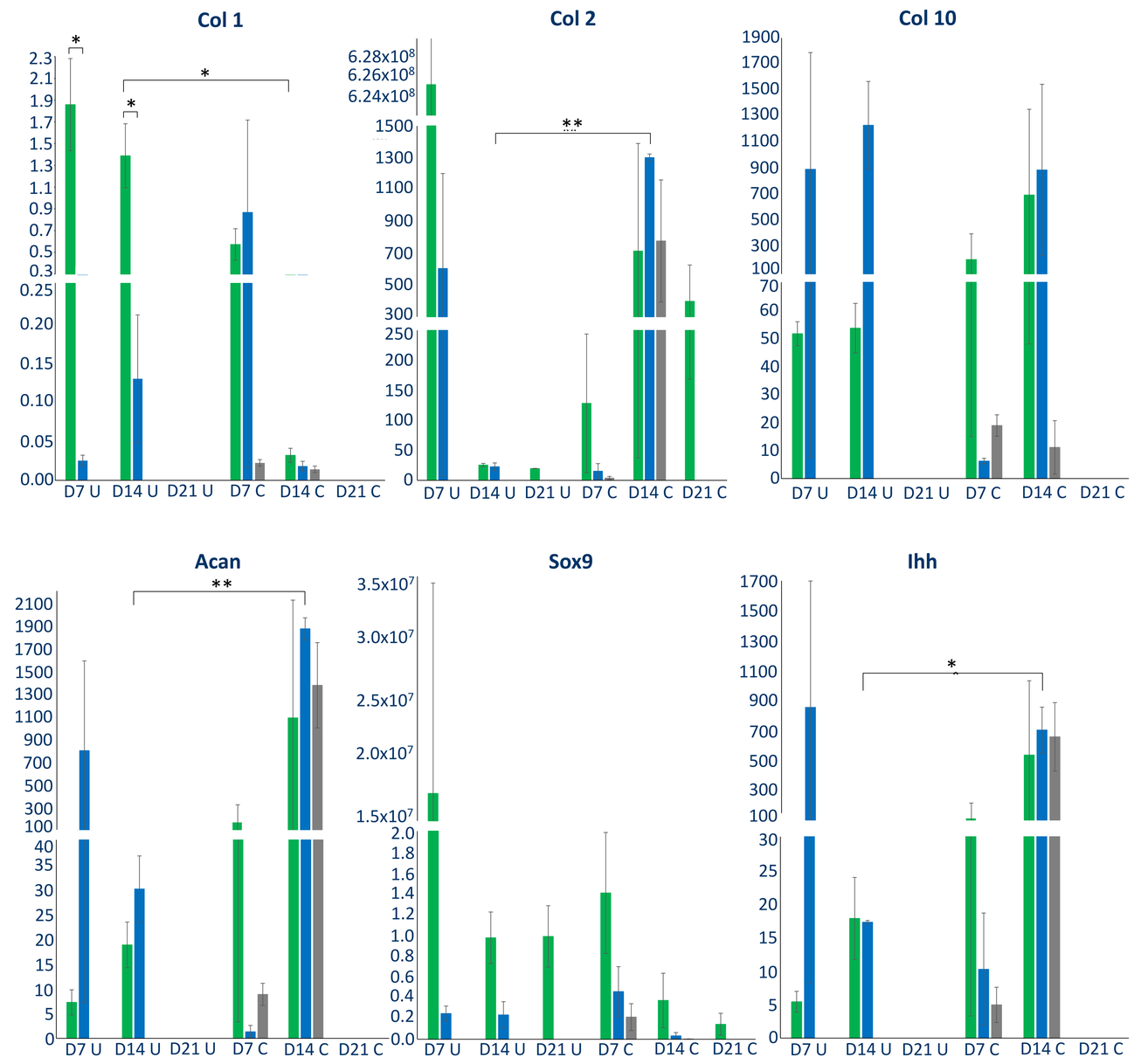

Figure 2. Gene expression of each gene marker, from qPCR analysis of cDNA derived from extracted RNA. U represents uncompressed and C represents compressed. Significance: * $p<0.05$; ** $p<0.01$
Green bars = Un-transfected; *Blue bars* = Wild type COMP; *Grey bars* = T585M Mutant COMP

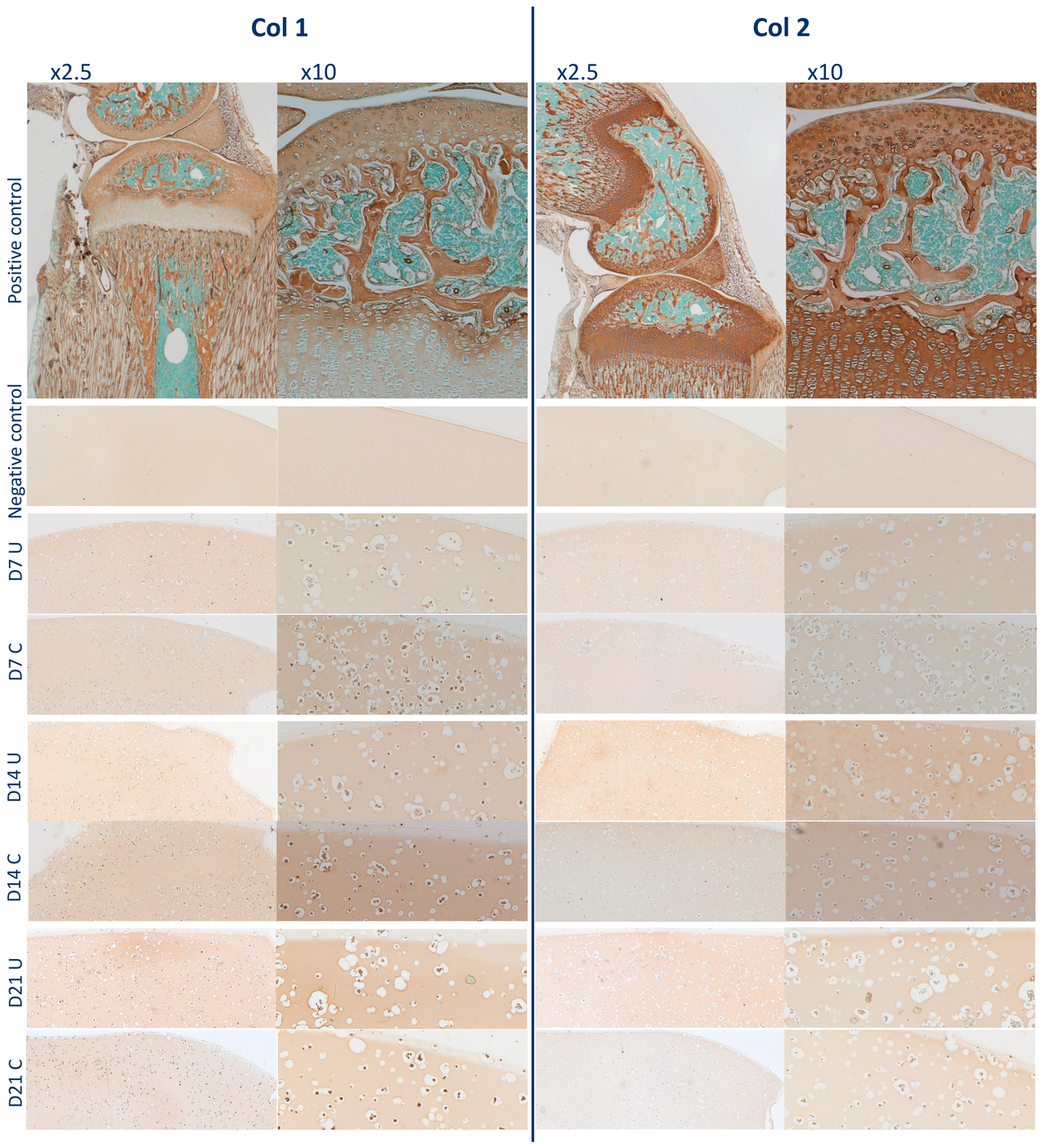

Figure 3. Immunohistochemistry staining; positive control (mouse tibia at 3 weeks) and negative control (agar with no primary antibody). U represents uncompressed and C represents compressed.

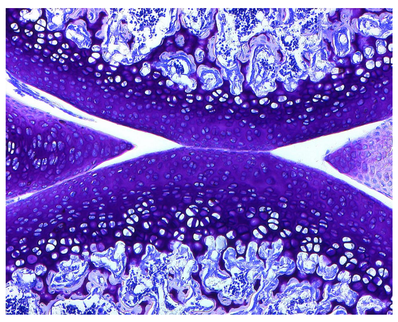

Figure 4. Toluidine Blue staining; positive control (mouse tibia at 3 weeks) x10 magnification

Discussion

Gene expression of chondrogenic markers increased in ATDC5 cells upon compression, whilst *Col1* (dedifferentiation) and *Sox9* (stem cell marker) expression decreased. Interestingly, levels of *Col1*, *Col2*, *Acan* and *Ihh* expression increased upon compression of wild type COMP ATDC5 whilst *Col10* and *Sox9* expression decreased. Compared with wild type, expression of all markers was decreased in T585M COMP cells upon compression, indicating cell stress. Due to the large standard errors in some of the qPCR data, further replicates of each condition would be needed to reduce the standard errors.

References

1. Piróg KA, Irman A, Young S, et al. PLoS One. 2014;9(2):e85145
2. Posey KL, Alcorn JL, Hecht JT. Matrix Biology. 2014;37:167-173
3. Chen TL, Posey KL, Hecht JT and Vertel BM. Journal of Cellular Biochemistry. 2008;103: 778-787
4. Sanchez-Adams J, Leddy HA, McNulty AL, O'Conor CJ, Guilak F. Curr Rheumatol Rep. 2014;16(10):451