

## **WORKSHOP**

# **Advances in Understanding Early Post-Traumatic Osteoarthritis**

Organizers:

Dominik Haudenschild, PhD

Blaine Christiansen, PhD

Speakers:

Gabriela Loots, PhD

M. Farooq Rai, PhD

Andrew Pitsillides, BSc(Hons) PhD

## **Advances in Identifying Early OA**

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### **Significance and Purpose:**

Joint injuries initiate changes in joint tissue homeostasis that often culminate in osteoarthritis (OA). There is emerging interest in mechanistically investigating events soon after the initial injury that lead to the initiation and progression of OA. The primary purpose of this workshop is to highlight recent advances in the understanding of acute responses to joint injury in different joint tissues. The three presenters have each approached the identification of early OA from unique perspectives in their research. This workshop will bring together these unique perspectives. The goal is to establish a more comprehensive understanding of how the interplay between different tissues during the acute post-injury phase affects the joint.

### **Educational Need:**

Current clinical treatment of joint injuries is focused more on restoring joint stability than it is on preventing degradative osteoarthritic changes in the long term. Often minor joint injuries such as ACL or meniscal tears are not treated clinically for several weeks post-injury. During this time, there are many cellular, molecular, and mechanical changes that can have long-lasting or even permanent damaging effects on the joint. Researchers from diverse fields are helping identify new therapeutic intervention strategies during the acute post-injury phase. There is a concurrent need to educate both patients and clinicians to take preventative measures during these critical early times, to help mitigate the downstream development of OA.

### **Learning Objectives:**

Update attendees on the recent advances in understanding how cellular, molecular, and mechanical changes during the acute post-injury timeframe can affect long-term joint health.

### **Abstract:**

There is no cure for established OA, and joint replacement is often the only solution. Joint injuries, even relatively mild injuries such as ACL or meniscal tear, can increase the lifelong risk for OA by a factor of 10 or more. A strong focus of recent research has been to identify intervention strategies that can decrease the risk of OA after injury. It has become apparent that the acute post-injury phase is important in the downstream development of OA. During this acute phase, events at the cellular and molecular levels can affect the integrity of structures within the joint. While the original damage caused by the injury is unchangeable, the “secondary” cell-mediated injury provides an opportunity for intervention. The goal of this workshop is to convey the recent advances in understanding the early events during the acute post-injury phase. Three speakers have approached this problem from unique and complementary research perspectives. Dr. Farooq Rai will present his findings on molecular signatures, pathways, and phenotypes that appear to be features of pre-OA. Dr. Gaby Loots will present findings from RNAseq analysis of mouse joints shortly after injury, and compare these results across multiple strains of mice with varying susceptibility to OA. Dr. Andrew Pitsillides will present his findings on specific elements of mechanical joint loading at distinct phases of osteoarthritis onset and progression. Discoveries in each of these areas have advanced our understanding of the global changes in the joint that occur during the first hours to days after an injury.

## Genetic Variation Affects Transcriptional Responses to Joint Injury

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Osteoarthritis (OA) represents the most common form of arthritis and is a major cause of physical disability and morbidity in the aging population. The latest census indicates that 50+ million adults in the US bear some form of arthritis and by 2030, these numbers will rise to 67 million. The annual health care burden for OA is \$185 billion based on 2007 data, reflecting its very high prevalence in society and its negative quality of life impact. While the pathways governing early cartilage degeneration in OA remain poorly understood, post-traumatic OA (PTOA) is a form of OA that develops after joint injury in which nature and time of trauma is generally known. Approximately 12% of the overall OA burden can be traced to joint trauma [1]. True prevalence for PTOA may be higher, given the long delay between injury and PTOA (10-15 years). It has been shown that patients undergo total knee replacement at an earlier age if they have a prior history of meniscus or ligament injury [2]. Research suggests that joint injury sets in motion a sequence of events that eventually leads to PTOA. Much interest has recently focused on understanding the pathogenesis of PTOA. A number of genes and pathways have been identified, and many of which are validated as therapeutic targets in preclinical mouse models of PTOA.

Despite this, there is an unmet need to understand the early molecular biology of joint tissues after trauma to trace the onset of PTOA. Our studies in men and mice have led to identification of molecular signatures, pathways and phenotypes that appear to be features of pre-OA (**Fig. 1**).

	Initiation of injury ↓		
Time line (age, years)	Seconds – hours (20-40)	Days – months (40-60)	Months – years (>60)
Disease phenotype	Pre OA (Pre-clinical)	Intermediate OA (Clinical)	End-stage OA (Clinical)
Disease stage	Molecular	Pre-radiographic	Radiographic
Diagnostic tools	Genes, biomarkers	MRI, bone scan, ultrasound	Clinical symptoms, radiographs
Alterations	Homeostatic perturbation	Joint failure (structural changes)	Joint death
Intervention	Arthroscopy	Pain control, joint preservation	Joint replacement

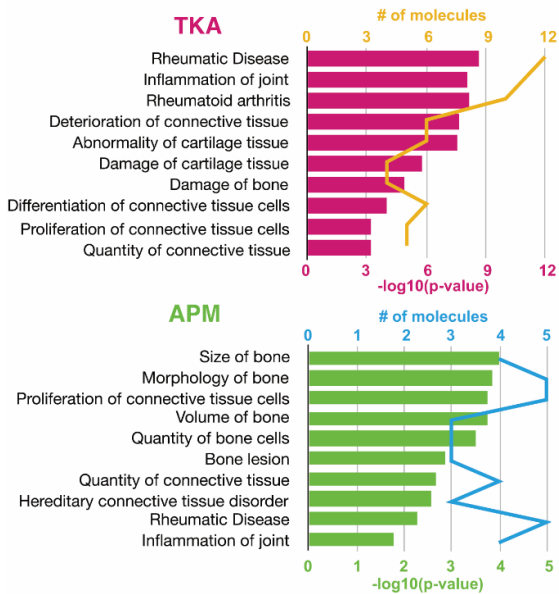
**Fig. 1:** Stages of OA after initial trauma. At molecular level a joint trauma can set off a series of events immediately in the joint beginning with disturbance in joint homeostasis and over time leading to end-stage disease. The focus of research is shifting, albeit with slow pace, from end-stage disease to pre-OA stage. OA = osteoarthritis; MRI = magnetic resonance imaging

### Transcriptional responses to human knee joint injuries

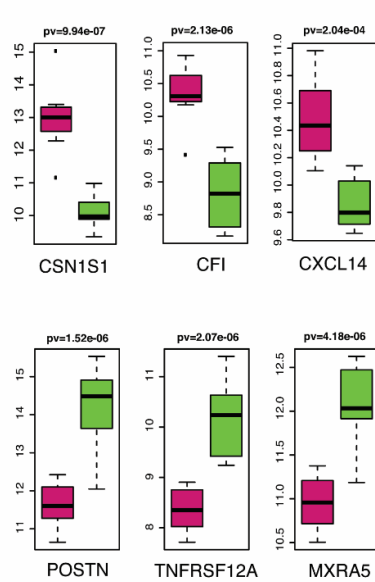
We have chosen to use mRNA (the “transcriptome”) to monitor the metabolic activity of connective tissues such as cartilage, meniscus and ligaments. Using meniscus tear as a model for early arthritic changes in the knee, we performed a microarray comparison between menisci from OA and non-OA joints. We found that a number of transcripts with potential relevance to the pathogenesis of OA were differentially expressed between the two, suggesting that meniscus injury sets the joints in the direction of OA [3] (**Fig. 2**).

In meniscus tear patients, cartilage generally looks normal, however, to determine if we can see molecular signatures that can predict development of OA, our transcriptome analysis of grossly normal cartilage unraveled molecular status of the tissue. When segregated by genetic risk-alleles and OA-associated gene transcripts, we found that cartilage from 30% of patients with meniscus tear express early molecular changes reflective of OA phenotype [4].

A



B



**Fig. 2.** Diseases & development functions of OA-related transcripts highly expressed in TKA samples (yellow line indicates the number of genes identified) and OA-related transcripts highly expressed in APM samples (blue line indicate the number of genes identified). B) A number of selected transcripts that showed differential expression between APM and TKA samples.

**Take home point.** Injury to the meniscus alters the molecular biology of meniscus as well as cartilage and represents early transcriptional changes that overtime result in clinical OA.

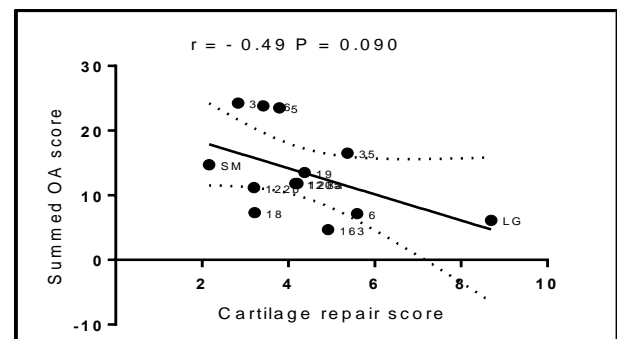
### Genetic variation in susceptibility to PTOA in mice

Our human work indicated that genes involved in extracellular matrix repair were stimulated in some people and under some conditions. This led us to wonder whether the ability to repair cartilage was associated with susceptibility to OA. Because such studies cannot be conducted in humans, we had the opportunity to study pure genetic strains of mice. We used recombinant inbred (RI) strains of mice originally derived from “healer” and “non-healer” mice to generate models of tissue regeneration and degeneration in an effort to discover a gene or a group of genes that contributes to disease pathogenesis. We created three phenotypes in these genetic mouse strains: ear wounds, full-thickness articular injuries of the trochlear groove, and PTOA (both surgical and non-surgical) [5-7]. Genetic heritability and correlations between the healing phenotypes identified super-healer strains [5], with significant protection from PTOA [6].

To gain molecular insight into strain differences, we found a few genes (DNA repair gene e.g. *Xrcc2*, *Pcna*, and Wnt signaling genes e.g. *Axin2*, *Wnt16*) that were significantly correlated with healing phenotypes [8]. Mice undergoing PTOA showed significant heritability for *Xrcc2*, *Il1r12* and *Igf1* (unpublished).

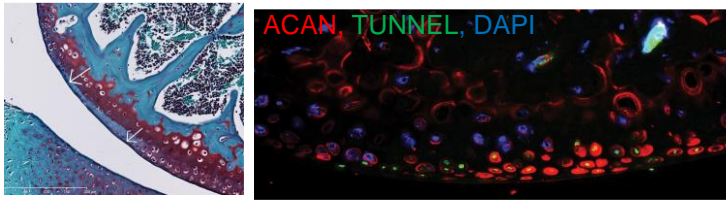
To compare cartilage regeneration and degeneration phenotypes in genetic mouse strains, we found that mice with superior healing potential are protected from PTOA as shown by negative correlation between cartilage regeneration and degeneration scores (**Fig. 3**). We are now interested in studying genes and pathways that are most important to dissect out the mechanism of tissue repair and degeneration (PTOA).

In addition to molecular and genetic studies, we also investigated the early cellular events in the joint following ACL tear in mice undergoing axial tibial compression [7, 9]. Tibial compression disrupted joint stability by rupturing the ACL and instigated a cascade of temporal and topographical features of PTOA. These features included cartilage extracellular matrix loss without proteoglycan replacement, chondrocyte apoptosis



**Fig. 3.** Correlation between cartilage regeneration and degeneration score from RI lines.

at day 5 (**Fig. 4**), synovitis present at day 14, osteophytes, ectopic calcification, and meniscus pathology. These findings provide a plausible model and a whole-joint approach for how joint injury in humans leads to PTOA.

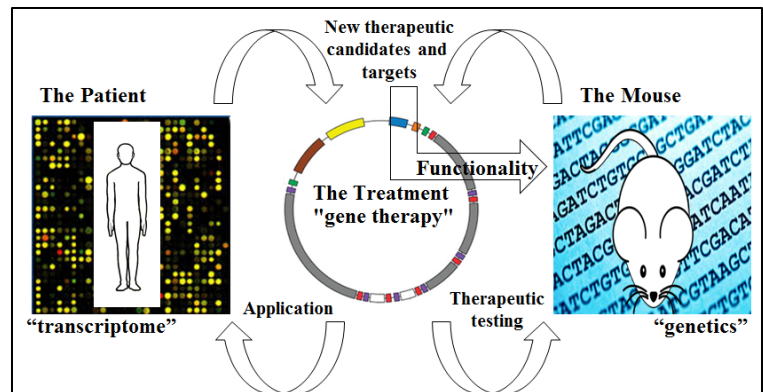


**Fig. 4.** ACL tear results in proteoglycan loss in articular cartilage at the site of impact (left) along with chondrocyte apoptosis and internalization of aggrecan in luciferase of dead cells (right).

**Take home point.** There appears to be common genetic basis between cartilage (tissue) healing and susceptibility to PTOA.

### Translational impact

The information obtained from genetic mouse strains, animal models that reflect common sport injuries in humans and high-throughput technologies for the discovery of new and novel genes in tissues obtained from patients with various injury phenotypes, places us in a unique position to take these findings forward and test various therapeutic candidates in a suitable mouse model (**Fig. 5**).



**Fig. 5:** An overview of human and mouse studies correlate.

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# Injury-induced changes in transcription during the acute phase

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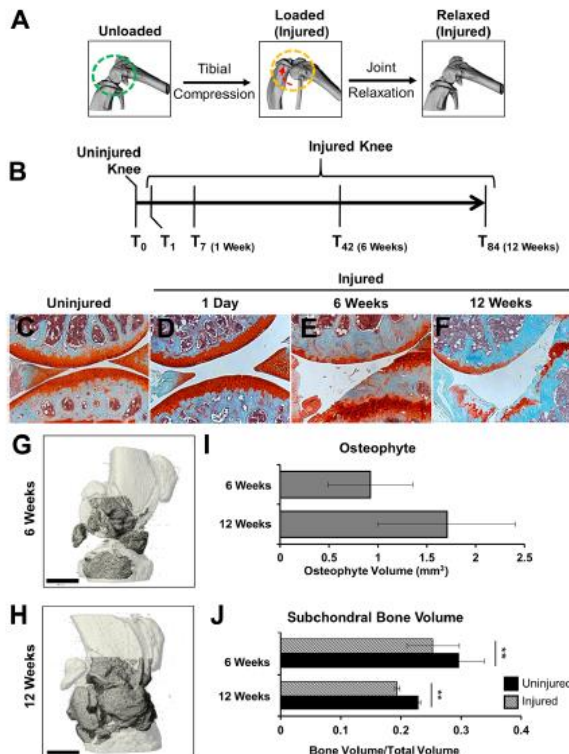
Joint injury causes post-traumatic osteoarthritis (PTOA). About ~50% of patients rupturing their anterior cruciate ligament (ACL) go on to develop PTOA within 1-2 decades after the injury, yet the mechanisms responsible for the development of PTOA after joint injury are not well understood. It is increasingly being recognized that the events triggered by injury and the molecular perturbations shortly after the injury are important in determining whether cartilage degradation will commence and go on to develop into osteoarthritis. Systemic profiling on the joint in health and disease can be used as a discovery tool to identify therapeutic targets as well as to understand the molecular mechanisms contributing to the progression of the disease. Here we will discuss results obtained from RNAseq analysis of injured joints shortly after injury, and compare these results across multiple strains of mice with varying susceptibility to OA.

## Methods

Chang et al. 2017<sup>1</sup> examined whole joint gene expression by RNAseq at 1 day, 1-, 6- and 12-weeks post injury, in a non-invasive tibial compression (TC) overload mouse model of PTOA that mimics ACL rupture in humans (Fig1). C57B/L6 mice underwent injury by applying a TC load (10~12N) to the right knee of 16 weeks old male mice<sup>2</sup>. The data described in this manuscript compare their results to expression data results from DMM injury model of PTOA<sup>3</sup>. RNAseq data from injured and uninjured MRL/MpJ (resistant) and STR/ort (sensitive) strains will also be discussed.

## Results

Change et al. identified 1446 total genes differentially regulated between injured and contralateral joints, where 599, 644, 511 and 201 significant changes were found at 1 day, 1-, 6- and 12- weeks post injury, respectively (Fig 2). Gene ontology analysis of upregulated transcripts at 1-day post injury identified categories corresponding to vasculature development and angiogenesis, cell adhesion, ECM and collagen organization, response to wounding and hypoxia, and a large number of genes were associated with immune responses and inflammation (Table 1).



**Figure 1.** Histological evaluations of tibial compression (TC) OA injury. (A) TC overload leads to joint destabilization through ACL dislocation. The direction of joint displacement is indicated by the red arrow. (B) Time line where mice were injured and joints were collected at 1 day, 1-, 6-, and 12-weeks for either histology or RNA sequencing. Histological assessment of uninjured (C) and injured joints at various time points post injury (D-F) by Safranin-O and Fast Green staining. MicroCT highlight regions (dark gray) of osteophyte formation in 6- (G) and 12- (H) weeks injured joints. (I) Quantification of femoral subchondral trabecular bone formation between injured and uninjured joints. (J) MicroCT quantification of osteophyte formation in injured joints. All histological images were presents were taken at 10X magnification. Scale bar is 1mm; \*\*\*  $p < 0.001$ ; and not significant (ns).

Figure 2. Common differentially up (A) and down (B) regulated genes between every time point post TC injury. The total number of genes per category is in brackets beneath each time point.

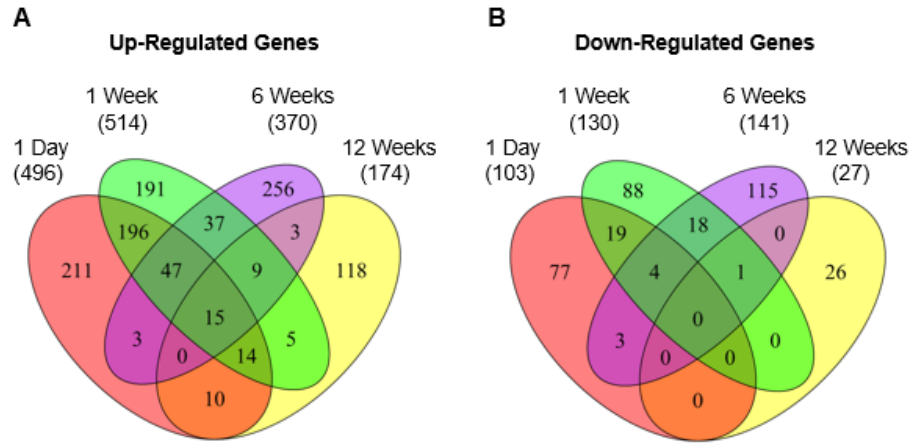
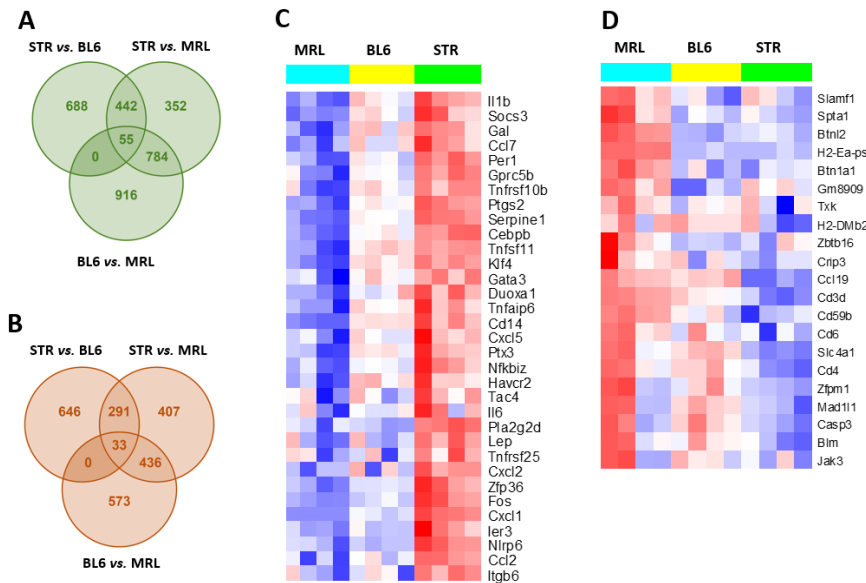


Table 1. Gene Ontology Enrichment Categories at 1 day post injury

GO ID	GO Category	1 Day	
		No. Genes	p-Value
<b>Up-regulated genes</b>			
GO:0001944	Vasculature development	29	5.08E-11
GO:0007155	Cell adhesion	41	4.26E-09
GO:0030198	Extracellular matrix organization	17	5.39E-09
GO:0030199	Collagen fibril organization	7	1.06E-05
GO:0043062	Extracellular structure organization	18	2.64E-07
GO:0042127	Regulation of cell proliferation	25	0.00604
GO:0050654	Chondroitin sulfate proteoglycan metabolic process	4	0.00728
GO:0009611	Response to wounding	27	9.91E-07
GO:0001525	Angiogenesis	14	3.66E-05
GO:0001666	Response to hypoxia	7	0.00581
GO:0006954	Inflammatory response	23	7.64E-08
GO:0006955	Immune response	33	4.93E-07

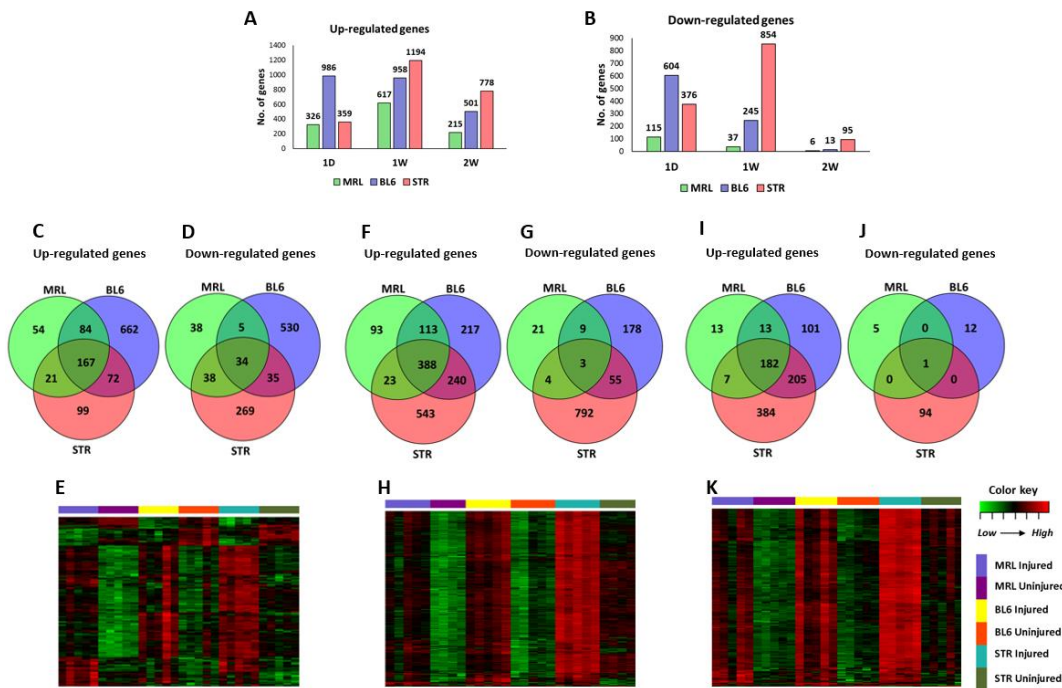
When we compared gene expression among C57B/L6, MRL/MpJ and STR/ort uninjured joints, we observed that the PTOA prone strain exhibited elevated levels of gene expression for transcripts associated with immune/ inflammatory responses (Fig. 3C). In addition, we observed a set of genes associated with T-cell activation to also be highly expressed in the PTOA resistant strain (MRL/MpJ), suggesting T-cell infiltration in the joint, in the absence of injury.



**Figure 3:** Gene expression comparisons among uninjured joints A) Overlap between genes up-regulated in day 0 (uninjured baseline) STR vs. BL6, STR vs. MRL and BL6 vs. MRL. B) Overlap between genes down-regulated in day 0 (uninjured baseline) STR vs. BL6, STR vs. MRL and BL6 vs. MRL. C) Inflammatory response genes highly expressed in STR baseline. D) Genes associated with T cell activation highly expressed in MRLs.

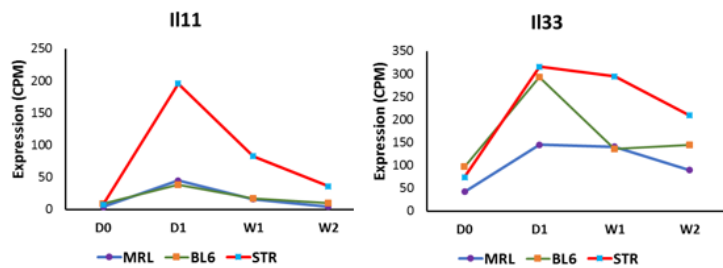
When the analysis was extended to compare gene

expression changes at 1-day, 1-week and 2-weeks post injury, among the 3 strains, an interesting trend developed. The PTOA sensitive strain displayed much more elevated levels of transcripts correlating with immune responses and inflammation, and these levels remained elevated at 1- and 2- weeks post injury, while these genes regressed back to pre-injury levels in MRL/MpJ and C57B/L6 strains (Fig. 4).



**Figure 4.** Overlap between genes up (A) and down-regulated (B) at 1-day post injury. C) Genes up-regulated in all 3 genotypes at 1 day post injury. Most of these genes show highest expression in injured STRs. Overlap between genes up (D) and down-regulated (E) at 1-week post injury. F) Genes up-regulated in all 3 genotypes at 1-week post injury. Most of these genes show highest expression in injured STRs. Overlap

between genes up (G) and down-regulated (H) at 2-weeks post injury. I) Genes up-regulated in all 3 genotypes at 2-weeks post injury. Most of these genes show highest expression in injured STRs.



Two cytokines, Il11 and Il33 are depicted below as an example of persistent elevation in the STR/ort strain which is susceptible to PTOA (Fig.5).

**Figure 5.** Expression profiles in injured joints of Mrl, Bl6 and Str.

Conclusion

RNAseq analysis of injured joints from strains of mice with varying susceptibility to PTOA suggests that early immune responses that persist beyond 1-week post injury may affect the outcomes of PTOA and contribute to the development of OA pathology. Furthermore, subtypes of T-cells may have a beneficial role in PTOA.

References

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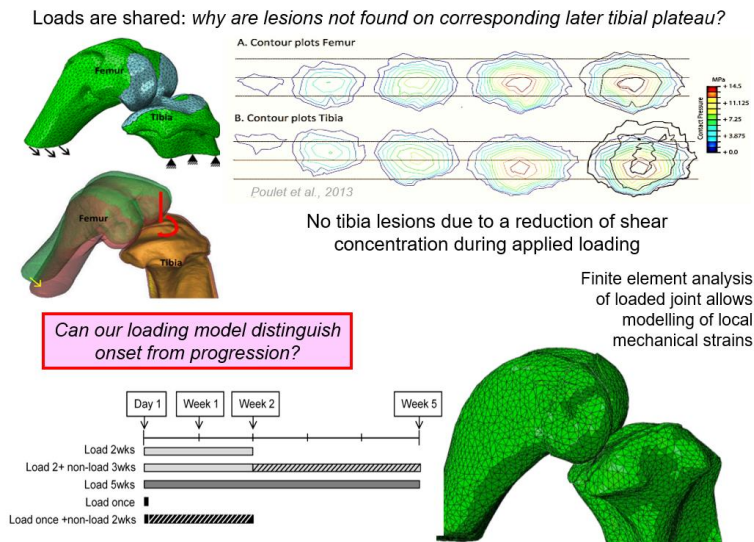
# Differentiating mechanical drivers of trauma susceptibility and OA progression

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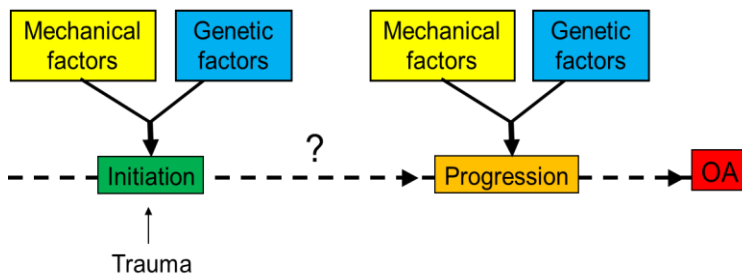
Osteoarthritis is a complex disease with major genetic and mechanical contributions. Attempts to define these contributions have likely been hindered, in part, by incomplete understanding of the roles played by specific elements of the mechanical loading environment, at distinct phases of the disease. For instance, it is often seemingly presumed that predisposition to osteoarthritis - end stage clinically discernible disease - is due to greater articular cartilage susceptibility to mechanical trauma at onset; this is understandable since injurious joint loading can indeed initiate changes in joint tissue homeostasis that often culminate in osteoarthritis. To address whether predisposition to osteoarthritis progression is always linked to greater articular cartilage mechanical trauma susceptibility, it is vital to exploit a model which allows these contributions to be distinguished. In addition, the potential to separate these mechanical drivers of osteoarthritis may help identify new strategies for better defining osteoarthritis heterogeneity and improved clinical disease sub-categorization. It may even move us towards a better appreciation of how genetic and mechanical determinants interact in their contribution to osteoarthritis.

This presentation will introduce, for discussion, three sets of findings from our research using an *in vivo* mouse knee joint loading model. The first findings will discuss data showing that a specific compartment of the mouse joint can be targeted, non-invasively by bespoke loading regimes in order to engender load-induced trauma, and that this does not always lead to inevitable joint deterioration. This will be contextualized within a framework that predicts likely mechanical environment that generates lesions and how other compartments, which share these loads, are likely protected from the onset of articular lesion development *in vivo*.

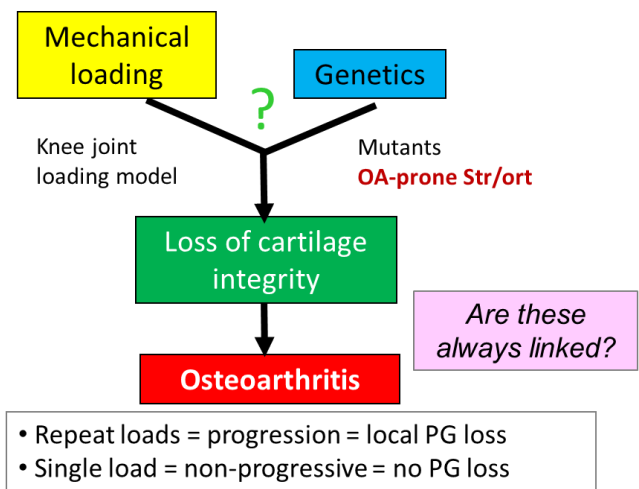


The second will expand upon this by describing the required changes in loading regime that will drive osteoarthritis progression sufficiently, even in the absence of additional applied loads. This aspect will take a whole joint approach and will discriminate between how different tissues of the joint (synovium, subchondral bone, osteophytes and menisci and ligaments), which are characteristic targets in osteoarthritis, respond to such loading regimes. This will introduce the

notion that cross-talk between these tissues is required for osteoarthritis progression but not articular cartilage lesion onset.



Thirdly, the incompletely defined interaction between genetic and mechanical contributions will be addressed by using mice with spontaneous development of osteoarthritis (STR/Ort strain) in our *in vivo* mouse knee joint loading model. These data will address whether there is a distinction between a genetic vulnerability to osteoarthritis development and modified articular cartilage susceptibility to mechanical trauma. They will delve into whether these relationships change before and after osteoarthritis onset and seek to identify whether any specific architectural features of articular cartilage might regulate differential trauma susceptibility. Finally, this set of findings will be extended by new 'real-time' synchrotron scanning data from loaded joints to reveal novel architectural determinants of both the joint's behavior under loading and those that may be predictive of osteoarthritis vulnerability.



Together these discussions should lead to more comprehensive appreciation of the roles played by specific elements of mechanical joint loading, at distinct phases of osteoarthritis onset and progression. They should highlight distinctions between genetic predisposition to osteoarthritis progression and articular cartilage mechanical trauma susceptibility. They will also hopefully identify novel therapeutic targets and, potentially, earlier diagnostic features that reflect the joint's mechanical role.

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