
Copyright:
© 2015 The Authors. Published by Elsevier Ltd and Osteoarthritis Research Society International. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

DOI link to article:
http://dx.doi.org/10.1016/j.joca.2015.01.003

Date deposited:
03/11/2015
The role of inflammation-related genes in osteoarthritis

E.L. Rogers, L.N. Reynard, J. Loughlin*

Newcastle University, Institute of Cellular Medicine, Newcastle upon Tyne, UK

Introduction

Over the last decade the volume of data derived from the genomic analysis of osteoarthritis (OA) has escalated almost exponentially, with the application of genome-wide association scans (GWASs), comprehensive epigenetic analyses and powerful gene expression studies. For the majority of these investigations, the OA phenotype used has been severe disease in the absence of a secondary cause, such as joint trauma, and in which the disease is so painful and debilitating that joint replacement has been undertaken. Alternative phenotypes have included injury models, in which the disease is a secondary cause, such as joint trauma, and in which the disease is so painful and debilitating that joint replacement has been undertaken. Alternative phenotypes have included injury models, in particular meniscal tears that can increase the risk of OA development. This latter model has been particularly investigated in gene expression studies. For the majority of these investigations, the OA phenotype used has been severe disease in the absence of a secondary cause, such as joint trauma, and in which the disease is so painful and debilitating that joint replacement has been undertaken. Alternative phenotypes have included injury models, in particular meniscal tears that can increase the risk of OA development. This latter model has been particularly investigated in gene expression analyses.

For a genetic association study in which trans-generational heritable DNA changes are being measured as opposed to somatic mutations, the cell source of the genotyped DNA is irrelevant. Blood is the tissue often used in these studies due to its relative ease of accessibility, although saliva is now a common alternative. For epigenetic and gene expression studies the source of the nucleic acid (DNA and RNA, respectively) can, however, be critical, since alterations in epigenetic status or gene expression levels can be cell or tissue-type specific with the added variability of possible temporal effects. In this regard OA has an advantage over many other common diseases in that joint replacement surgery provides researches with ready access to relevant tissues, albeit at an end stage of the disease process. Due to the central nature of cartilage in the OA disease process, the cartilage chondrocyte has been the preferred cell for many of the epigenetic and gene expression studies so far performed. The increasing realization, however, that OA is a disease of the whole joint has encouraged the analysis of a wider range of joint tissues and cell types. This has the advantage that a more comprehensive approach is being undertaken in the molecular analysis of the disease but the complication that, unlike cartilage, joint tissues such as synovium, fat pad and subchondral bone contain multiple cell types, adding heterogeneity and therefore a further level of complexity to the interpretation of data derived from these tissues.

* Address correspondence and reprint requests to: J. Loughlin, Newcastle University, Institute of Cellular Medicine, Musculoskeletal Research Group, 4th Floor Catherine Cookson Building, The Medical School, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK. Tel: 44-191-208-7178; Fax: 44-191-208-5455. E-mail address: john.loughlin@ncl.ac.uk (J. Loughlin).

© 2015 The Authors. Published by Elsevier Ltd and Osteoarthritis Research Society International. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Here we shall review results from the latest OA genetic, epigenetic and gene expression studies and, where relevant, we shall also refer to earlier studies where they help to make a point.

OA genetics and inflammation-related genes

The use in the not too distant past of underpowered case—control cohorts in OA genetic studies threw up a large number of genetic associations. When these studies involved the targeting of candidate genes, as they invariably did, several pathways emerged that looked very promising. Amongst these were a number of genetic associations. When these studies involved the targeting of gene expression to the HLA class II/III region was identified in a meta-analysis of Japanese and Europeans. The European contribution to genetic studies in this area involved a GWAS of knee OA in Japanese patients that reported an association to ALDH1A2 (P-value for the association of 3.99 \( \times 10^{-10} \)), which codes for an enzyme involved in retinoic acid synthesis, and hip and/or knee OA GWAS in European cohorts that reported associations to a range of genes including CHST11 (P = 1.64 \( \times 10^{-8} \)), which codes for an enzyme that sulfates proteoglycan. From amongst the OA association signals reported from GWAS none encompass a gene coding for a protein involved in inflammation.

A recent meta-analysis of OA European candidate gene studies confirmed that the selection of candidates in OA genetic studies is in fact an extremely fallible exercise, with only the type XI collagen gene COL11A1 and the vascular endothelial growth factor gene VEGF demonstrating any semblance of association to the disease from among the 199 candidates examined.39 However, even their association P-values were much less compelling than the signals that have emerged so far from the GWAS studies, with values >1 \( \times 10^{-6} \). The inflammation-related genes amongst the 199 candidates studied included those coding for tumor necrosis factor and the NF-κB protein complex, as well as several coding for interleukins and their receptors and antagonists. None of these merited particular comment in that report.

This overwhelming lack of support for inflammation as a repository of OA genetic risk is tempered by one study, reported in 2010 and involving a GWAS of knee OA in Japanese patients.41 Here an association to the HLA class II/III region was identified, with a P-value of 6.73 \( \times 10^{-8} \) in the Japanese and a P-value of 5.10 \( \times 10^{-9} \) in a meta-analysis of Japanese and Europeans. The European contribution to this P-value was, however, quite modest, whilst subsequent attempts to replicate the signal in both Asians and Europeans has proven fruitless.42,43 It appears possible therefore that this association is restricted to the Japanese cohort that was the source of the initial discovery.

So what to conclude? Apart from this Japanese study, there is at present no compelling evidence that DNA polymorphism within or near to genes that code for proteins involved in inflammation is a risk factor for OA. That, however, is not the end of the story. The GWAS and meta-analyzed candidate studies performed to date have focused on common DNA polymorphisms, with minor allele frequencies >10%. It is therefore possible that future analyses of less common polymorphisms will uncover associations to inflammatory genes. It is also the case that the case—control sample sizes studied to date are too small to detect particularly small-effect risk alleles (odds ratios < 1.1). Since OA is known to be highly polygenic and lacks large effect loci it will be the case that as more powerful OA GWAS studies are undertaken new risk alleles will be identified. It may be that inflammatory genes will be amongst these new discoveries. Finally, the analysis of alternative OA phenotypes (for example, different compartments of the knee rather than studying knee OA as a single entity) may refine the association studies and uncover association signals in inflammatory genes relevant to particular OA subsets.

At present, however, the conclusion to draw is that there is no link between OA genetic risk alleles and inflammation-related genes.

OA epigenetics and inflammation-related genes

Epigenetic modifications are a mitotically heritable means by which the cell can regulate gene expression in the absence of an alteration in the sequence of the DNA. There are three known epigenetic mechanisms: DNA CpG methylation, histone modifications and microRNAs (miRNAs). The first two alter the accessibility of the DNA within the chromatin, influencing the binding of trans-acting factors and thereby modulating the initiation and level of gene transcription. miRNAs regulate the stability of mRNA transcripts and therefore regulate translation rather than transcription.

Several informative review and commentary articles have been published recently on the role of epigenetics in OA.14–18 Most OA epigenetic studies have focused on candidate loci, often those coding for catabolic regulators of cartilage homeostasis. As more high-throughput technologies have been developed, particularly the use of arrays for CpG methylation analysis, a more genome-wide approach has been adopted, akin to changes observed in genomics with the shift from candidates to GWAS. Epigenetic effects on inflammatory genes in OA have been convincingly demonstrated using both the candidate gene and genome-wide approaches, and below we shall provide examples for each of these.

Candidate studies

Altered CpG methylation of the promoter of the pro-inflammatory interleukin-1β gene IL1B, with concurrent changes in its expression, has been consistently demonstrated.19,20 These experiments involved the direct study of chondrocytes from OA patients and in vitro analyses including the forced de-methylation of the chondrocyte DNA with the agent 5-azacytidine. The role of histone modifications in OA has been investigated by examining the effect of histone deacetylase inhibitors (HDACi) on cartilage explants and chondrocytes from OA patients. These studies have revealed that histone acetylation/deacetylation modulates cartilage catabolism genes, in particular the matrix metalloproteinases (MMPs), in response to IL1.21 The potential utility of this discovery has been demonstrated in the destabilization of the medial meniscus (DMM) mouse OA model, with HDAC inhibition attenuating the disease process.22

Genome-wide approaches

Most miRNA studies performed in OA have started with a microarray-based approach to aagnostically assess which miRNAs are up or down regulated in the disease, followed by a more targeted analysis. A classic example of this is the discovery that miR140 expression is reduced in OA cartilage and is also down-regulated following IL-1β stimulation of chondrocytes.14,15 Subsequent studies have demonstrated that other miRNAs induce or are induced by inflammatory proteins, including miRNAs 145, 127-5p and 146a-5p.26–28

Genome-wide CpG methylation analyses in OA have been performed using arrays that enable the investigation of up to several hundred thousand CpGs. There has so far been six such methylation analyses reported, five investigating DNA extracted from hip or knee cartilage27–31 and one investigating DNA extracted from trabecular bone.32 Of the five cartilage studies numerous comparisons were made, including hip vs knee, OA vs non-OA, and cartilage adjacent to the lesion vs intact cartilage distal to the lesion.
Hypo and hypermethylated CpGs were identified and gene ontology analysis performed to identify gene families and pathways demonstrating significant clustering. Four of the five studies reported inflammation-related genes as among the most significant pathways identified. Intriguingly, two of these four studies reported that knee OA patients and hip OA patients each segregate into clusters based on the differential methylation of inflammation-related genes. These results suggest that OA patients can be stratified on the basis of the methylation status of these genes in their cartilage, implying that inflammation is relevant to the disease in some but not necessarily all OA patients. The trabecular bone study compared OA samples with those from osteoporotic patients. Inflammation was not one of the pathways identified as demonstrating differential CpG methylation between the two groups.

### OA gene expression and inflammation-related genes

Through the application of gene-expression microarrays, transcriptomic analyses are identifying unique expression patterns of inflammation-related genes in OA, not only in comparison with non-OA controls, but also according to the stage of disease progression, and in the study of healthy and diseased regions of the same tissue. Below we review the gene expression data with respect to these three categories.

### OA-specific differences in inflammation-related gene expression

Clear differences in inflammatory gene expression have been identified in the OA joint compared to non-OA controls, with one of the most overtly inflamed tissues being the synovium. Inflammation is less pronounced in the OA synovium in comparison to rheumatoid arthritis (RA) but correlates with both pain and structural decline. While the precise cause of synovium inflammation (synovitis) remains to be defined, gene-expression microarray analysis shows increased expression of complement effector genes and a decrease of complement inhibitors in the OA synovium compared to controls.

Further to this, IL-1β has been associated with many of the pathological features of OA. Although IL-1R, encoding the IL-1 receptor, is not differentially expressed in normal compared to OA synovial fibroblasts, IL-1R is upregulated at the protein level in OA. IL-1β signaling has been shown to modulate expression of 909 out of 3459 genes in primary human articular chondrocytes, including significant induction of numerous chemokines and inflammatory mediators, such as the genes IL11 and CCL5. This post-transcriptional upregulation of IL-1R in OA is therefore likely to cause a significant alteration in inflammatory gene expression. In line with this, comparison of OA cartilage to microscopically intact cartilage from individuals without OA identified a distinct upregulation of chemokine and cytokine genes in OA cartilage, including IL8 and LIF.

Expression changes in inflammatory-associated genes in cartilage have been shown to display commonality between knee and hip OA when compared to non-diseased controls. For example, differential expression of genes in the TREM1 signaling pathway, involved in amplification of the inflammatory response, occurs in both knee and hip OA articular cartilage when compared with controls. In addition to localized tissue-specific differences in gene expression, systemic elevation of inflammatory gene expression is also present in OA. Microarray analysis of peripheral blood leukocytes (PBLs) from OA patients and non-OA controls identified differential expression of 173 genes, including many inflammatory genes. Furthermore, a subgroup of OA patients displayed increased expression of IL1B of >2 fold when compared with controls. Not only does this provide support for systemic inflammation in OA, but it also supports the notion that a subgroup of OA patients may exist, for whom inflammation is a key disease driver.

**Early changes in inflammatory gene expression**

There is an expanding body of research reporting differential inflammatory gene expression in relation to disease progression. The unique inflammatory transcriptome in early OA is of particular interest as this precedes structural joint damage and as such, may potentially provide an option for effective therapeutic intervention. The study of early OA is troublesome, given that clinical symptoms generally do not occur until cartilage destruction has already begun. Consequently, studies often focus on groups with highly increased disease risk. The Multicentre Osteoarthritis Study (MOST) longitudinal case-control study reported that meniscal tears are a key risk factor for developing radiographic OA. This suggests that joint trauma can catalyze the molecular changes underpinning later OA development. Microarray analysis of patients undergoing arthroscopic meniscectomy following a meniscal tear identified 266 genes to be differentially expressed in inflamed synovium compared with synovium without inflammation. Chemokines and chemokine receptors, required in innate immune activation, demonstrated the highest upregulation in inflamed synovium. In particular, expression of CCL5, a C–C chemokine able to recruit innate immune cells such as lymphocytes, was found to associate with severity of inflammation, as validated by real-time PCR. CCL5 induces IL-6 expression in a concentration-dependent manner in synovial fibroblasts via interaction with the CCR5 receptor, which displayed elevated mRNA expression in OA compared to normal synovial fibroblasts. As such, it is suggested that this early upregulation of inflammatory chemokine expression is pivotal in inducing cytokine expression, which may be required to instigate the processes leading to downstream OA-pathology.

The unique early chemokine signature in the damaged joint is highly dynamic. A pilot analysis of gene expression in synovial fluid from individuals who had suffered meniscal injury identified significant changes in gene expression dependent on duration of injury. Molecular characterization of synovial cell pellets and cell-free supernatant, by microarray and RNA-sequencing, respectively, revealed a distinct inflammatory expression pattern according to injury duration. In cell pellets, there was increased expression of inflammatory genes in the longer injury duration group (>2 months since injurious event), with the top 5% of genes with the greatest average expression belonging to inflammatory pathways. The cell supernatant analysis identified 764 differentially expressed genes, with SLCA2A9 displaying the greatest differential expression. While not directly involved in inflammatory pathways, this regulator of glucose homeostasis is induced by OA-associated inflammatory cytokines, such as IL-1β, and may have a role in chondrocyte survival. This therefore indicates that even at the very earliest stage of injury, altered inflammatory gene expression may have downstream functional implications.

Analysis of synovium from patients with confirmed early-stage OA, evidenced by knee pain and cartilage abnormality with a Kellgren–Lawrence (K–L) score of <2, supports these molecular findings. In synovial membrane biopsies obtained during arthroscopic meniscectomy, TNF, IL1B and IL6 gene expression levels in both synovial membrane and synovial fluid were at comparable levels in early OA and end-stage disease synovium. This again demonstrates that changes in the inflammatory gene expression profile are an early event in OA pathogenesis.

**Focal differences in inflammatory gene expression: damaged vs undamaged tissue**

As discussed, gene expression data has provided evidence for tissue-wide inflammatory gene dysregulation in OA. Further to this, a distinct inflammatory gene expression pattern is also apparent at
a focal level in diseased tissues. This may hold the key to establishing why specific regions of cartilage are affected in OA.

A microarray comparison of lesioned and unlesioned cartilage in individuals with knee OA found 754 genes to be differentially expressed, with an enrichment of inflammatory genes\(^5\). Interestingly, genes involved in the complement system, such as C2, were found to be upregulated in unlesioned cartilage, possibly reflecting altered responsiveness of the lesioned regions. A more powerful microarray study identified differential expression of 1717 genes in 33 lesioned and unlesioned knee-OA cartilage pairs, including many inflammatory genes\(^6\). CD55, coding for a regulator of the complement response, was one of the most differentially expressed genes. Increased gene expression was reflected by elevated protein levels, suggestive of downstream functional consequences of abnormal focal inflammatory gene regulation. This confirmed a previous in-joint microarray analysis of five patients undergoing knee arthroplasty\(^7\). A total of 411 differentially expressed genes were identified, yet CD55 was one of only six genes consistently upregulated in the lesioned group, as validated by quantitative real-time PCR in five additional specimen pairs. Increased CD55 expression in damaged tissue may initially seem as somewhat of a paradox, as it is a negative regulator of the complement system. However, loss of CD55 in mice ameliorates symptoms in a collagen-induced model of RA, with significant restriction in inflammatory symptoms such as paw swelling\(^8\). CD55 is known to function as a ligand for CD97, present on leukocytes, although the inflammatory implications of the CD55–CD97 interaction remain to be established. Given that OA presents from a genetically diverse disease population, this reproducible evidence implies core inflammatory genes to be integral to disease pathology.

In light of the fact that all tissues in the joint display pathologic changes in OA, it is to be expected that focal inflammatory gene dysregulation is not limited to cartilage. Microarray analysis of inflamed vs macroscopically normal synovium from 12 patients with knee OA identified 886 genes to be differentially expressed, with an enrichment of inflammatory genes. TREM1 demonstrated particularly increased expression in inflamed synovium, validated by western blot analysis\(^9\). Earlier research comparing lesioned and unlesioned cartilage from knee OA also identified TREM1 to be significantly upregulated in damaged cartilage\(^10\). More commonly associated with RA, TREM1 expression is induced by activation of toll like receptor 4 (TLR4) in response to acute inflammation, and results in expression of pro-inflammatory cytokines including TNF-α and IL-1β\(^11\). Together, these data provide evidence of focal dysregulation of inflammatory genes in damaged OA tissues, with a level of commonality between different tissue types. This may indicate that early universal dysregulation of a small group of inflammatory genes in damaged tissue is essential to the global pathologic events in the progression of OA.

**Genes and OA treatments**

Current guidelines recommend topical non-steroidal anti-inflammatory drugs (NSAIDs) and paracetamol as a first-line treatment for OA ahead of oral NSAIDs, with the Osteoarthritis Research Society International (OARS) recommending topical NSAID use in those who do not respond to paracetamol\(^12\). This reflects the risk of cardiovascular and gastrointestinal adverse events posed by NSAIDs, which is significantly greater when taken orally\(^13,14\). Conflicting data has been published when comparing the effectiveness of NSAIDs and paracetamol for patients with severe pain, which could be argued to be suggestive of a variable inflammatory contribution in different OA groups\(^15,16\). As such, further research characterizing the role of inflammation in OA may have important bearing on future treatment stratification.

The ability therefore to identify at a genetic level OA patients for whom inflammation is a core disease modulator may prove fundamental in advancing pharmacologic intervention and facilitating the formation of differential treatment pathways. This would protect patients less likely to respond well to oral anti-inflammatory treatment from the risk of significant adverse effects, while improving the risk-benefit ratio in the inflammatory subgroup, in favor of increased benefits. The risks of adverse effects from NSAIDs are also increased by extended periods of use\(^17,18\). Therefore, the future potential to intervene during early stages of the disease when aberrant inflammation is initiated may create the opportunity for short-term intervention, halting disease progression while minimizing adverse effects.

**Conclusions**

From an epigenetic and gene expression perspective, inflammation-related genes have a clear role in the OA disease process. Most epigenetic and gene expression studies performed to date have focused on cartilage or synovial tissue. Future analyses should expand the repertoire to other tissues of the synovial joint and should also increase sample sizes from the tens of patients studied so far to the hundreds. This will provide the comprehensive coverage and power needed to establish the clearest picture possible of exactly how inflammation-related genes contribute to OA initiation and development. The recent discovery of a circulating miRNA correlating with OA progression\(^19\) and of the differential expression between OA and healthy age-matched controls of peripheral blood genes\(^20\) highlights that a joint-centric approach to OA may also have to be rethought.

One of the most exciting finds from the epigenetic analyses is that OA patients can be stratified into those in whom inflammation-related genes are actively involved in OA and those in whom they appear not to be. This has clear relevance to the design of clinical trials for testing disease-modifying treatments and to the potential re-purposing of anti-inflammatory drugs: the right treatment for the right patient.

From a genetic perspective inflammation-related genes are not relevant to the disease, with no compelling evidence that DNA polymorphism at these genes contributes to OA susceptibility. As noted earlier, this picture may change as ever more powerful genetic association studies are performed. The fact that genes have already received quite considerable attention it is hard to imagine that they will emerge as major repositories of OA genetic risk.

**Author contributions**

All authors were involved in drafting the article and revising it critically for important intellectual content, and all authors approved the final version to be published.

**Role of the funding source**

The funding bodies had no role in the design of the study, data collection, analysis and interpretation of the data, the writing of the manuscript or in the decision to submit the manuscript.

**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**Acknowledgements**

Our research is funded by Arthritis Research UK (19824), by the NIHR Newcastle Biomedical Research Centre (20028), by the European Union Seventh Framework Program (FP7/2007–2013).
under grant agreement number 305815 (D-BOARD), by the MRC–Arthritis Research UK Centre for Integrated research into Musculoskeletal Ageing (CIMA) (2023), and by the Dr William Harker Foundation (19953).

References


36. Sadouk MB, Pelletier JP, Tardif G, Kiansa K, Cloutier JM, Martel-Pelletier J. Human synovial fibroblasts coexpress IL-1 receptor type I and type II mRNA. The increased level of the IL-1 receptor in osteoarthritic cells is related to an increased level of the type I receptor. Lab Invest 1995;73:347–55.


