# Modeling the genetic and mechanical interplay in osteoarthritis: from in vitro systems to mechanistic insights

Bloks, N.G.C.

## Citation

Bloks, N. G. C. (2025, October 22). *Modeling the genetic and mechanical interplay in osteoarthritis: from in vitro systems to mechanistic insights*. Retrieved from https://hdl.handle.net/1887/4279635

PETER M.G COX

*1980*

# CHAPTER 1

# Introduction

# Introduction

## Osteoarthritis

Osteoarthritis (OA) is a complex, degenerative disease of the articular joints in which the etio-pathophysiology revolves around environmental factors such as injurious mechanical strains, obesity, sex, and genetic factors. With a global prevalence of 7.6% in 2020 and an increase of 134% of years lived with disability from 1990 to 2020 (*1*), OA is now ranked as the 14th disease in terms of years lived with disability. The projected increase in the prevalence of knee and hip OA in 2050 is 74.9% and 78.6% respectively. The pathological progression of OA involves a gradual deterioration of articular cartilage, synovial thickening, osteophyte formation, and alterations in subchondral bone structure. Clinically, this manifests as chronic pain, joint stiffness, instability, swelling, and narrowing of joint spaces. Together with the societal burden of OA increasing, there is a pressing need to understand the underlying mechanisms that lead to the onset and progression of OA. Eventually this knowledge will facilitate disease-modifying treatment development.

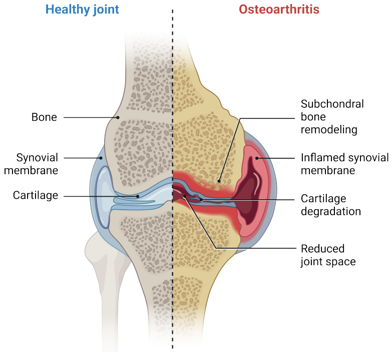

**Figure 1 | Overview of pathophysiology of Osteoarthritis.** Schematic overview of OA pathophysiology such as bone remodeling, inflammation, and cartilage degeneration.

## Articular Cartilage in health and disease

### Articular cartilage

Articular cartilage covers the ends of long bones which functions, under normal physiologic circumstances, as a nearly frictionless load-bearing surface. (*2, 3*) The main constituents of cartilage are water (>70%), an extracellular matrix (ECM), and a single cell type the chondrocyte. The ECM consists of a dense type II collagen network with proteoglycans, but also several other collagens like collagen type VI and other non-collagenous proteins. (*4*) Generally, collagens contain a triple helical domain of

Gly-X-Y repeats, embedded between other domains such as the Von Willebrand Factor, providing binding sites for interaction with other ECM proteins, such as proteoglycans. (*5, 6*) Proteoglycans in cartilage generally consist of a core protein, such as aggrecan, to which multiple negatively charged glycosaminoglycans are linked that facilitate trapping of water. The chondrocytes are post-mitotic cells that reside in articular cartilage in a maturational arrested state, and that are directly surrounded by a pericellular matrix. In a healthy steady state, chondrocytes respond to external stimuli, such as mechanical loading, by providing minimal turnover of collagens (*7*) and replacing sGAGs. (*8*) Nonetheless, due to the postmitotic, metabolic resting state, chondrocytes are generally ill-qualified to perform repair activities upon injurious environmental events. (*4-6*) The organizational structure of the articular cartilage ECM differs between the layers of the articular cartilage and reflects the various adaptations to mechanical forces (**Figure 2A**) In the superficial layer, the collagen fibers are orientated transversely to resist the shear forces during articulation. (*9, 10*) The intermediate layer is, to a lesser extent, exposed to shear forces, and as such the fibers are oriented more randomly to facilitate resistance to compressive forces. The deep zone is predominantly exposed to compressive forces and as such the collagen fibers are predominantly perpendicularly oriented. (*9*) The negatively charged proteoglycans attract water, thereby creating a structure with unique compressive and lubricative properties. Upon compression of the articular cartilage, water molecules associated with the negatively charged sGAGs are extruded from the ECM, thereby increasing the negative charge density and thus osmotic pressure. When the mechanical compression is released, the ECM again attracts water and other soluble molecules through the negatively charged sGAGs via osmosis, thereby returning to the equilibrium state. As such, under physiological joint loading conditions, chondrocytes are exposed to a variety of biomechanical cues such as compression, stretch, shear, and pressure, as well as ionic effects. (*7, 11*)

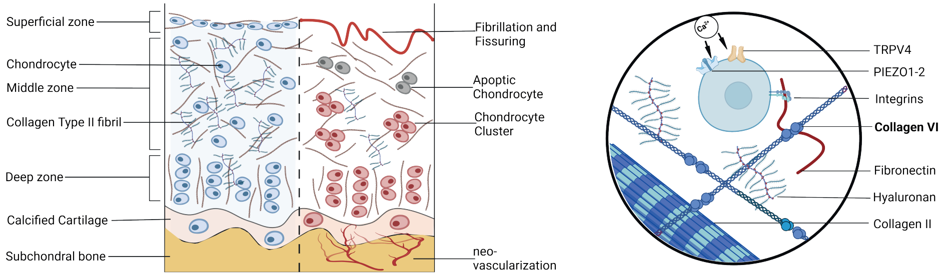

**Figure 2 | Overview of structure and composition of cartilage and the chondron (A)** Articular cartilage extracellular matrix components and structure. left healthy, right OA affected **(B)** Schematic representation of the chondrocyte and the pericellular matrix (chondron), containing proteoglycans, collagen type VI, Fibronectin interacting with mechanosensors such as integrins, and regulating ion-channels such as TRPV4

## Pericellular Matrix

The pericellular matrix (PCM) encompasses a region, approximately 2-4 μm thick, that directly surrounds chondrocytes, and that is biochemically and biomechanically distinct from the ECM (**Figure 2B**). It is rich in proteoglycans and collagen types II, VI, and IX, but defined particularly by the presence of Fibronectin and collagen VI. (*12-14*) The PCM with the chondrocyte forms a separate functional unit also referred to as the chondron. Together, the chondron controls the transduction of mechanical and biochemical cues from the ECM towards the chondrocyte and vice versa. (*13-15*) It has been shown that the transduction of these signals between the chondrocyte and the ECM is of critical importance in regulating the development and maintenance of cartilage. This is because changes in signal transduction between ECM via PCM to chondrocytes are known to have significant effects on proteoglycan metabolism, gene expression, and response to growth factors. (*16-21*) In addition to the biological function of the PCM, it also plays an important biomechanical role in articular cartilage. It has been shown to function as a mechano-transducer (*22, 23*), while also modulating the strains imposed on chondrocytes upon mechanical loading. (*13, 24-26*)

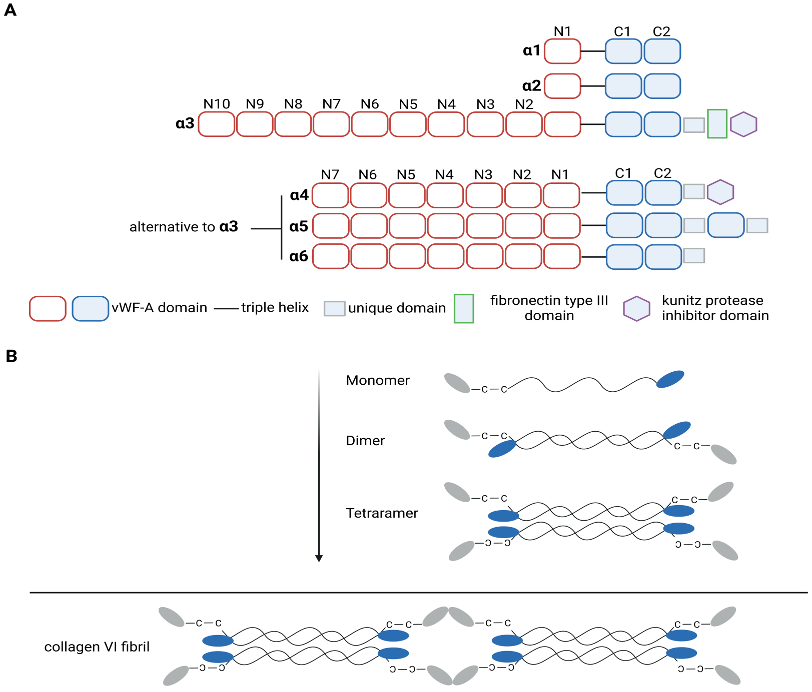

**Figure 3 | Schematic representation of collagen VI. (A)** the six different collagen VI monomers with their respective domains. **(B)** Assembly of collagen monomers that together form a collagen VI fibril.

### Collagen VI

In healthy articular cartilage, the primary distinguishing characteristic of the PCM is the presence of collagen type VI (*12, 27*) It has been hypothesized that type VI collagen forms a flexible network that anchors the chondrocyte to the PCM (*15, 23, 28-33*) Collagen type VI is encoded by six different genes (*COL6A1-A6*). (*34*) The domain structures of these six collagen VI chains are represented in **Figure** 3 Generally, the collagen type VI subunits contain large globular N-termini comprising von Willebrand Factor A domains. Each chain has a 336 amino acid GLy-X-Y repeat triple helix and a globular C-terminus composed of several different types of protein modules. Studies on the COLVI alpha 3 unit show that portions of these N-terminus project away from the triple helical structure and are well positioned to interact with other ECM components. (*35*) Indeed, it has been found that collagen VI interacts with other PCM and ECM components such as fibronectin and hyaluronan biglycan, decorin, fibromodulin, hyaluronan, fibronectin, perlecan, and heparin but has a lower affinity with collagens I and II. (*5, 6, 34, 36, 37*) Importantly, collagen VI also interacts with the integrin receptor which is a well-known mechano-transducer, and as such is likely directly involved in this mechano-transduction pathway relevant for chondrocyte health. (*38-40*) In several diseases, modification of collagen VI is associated with alterations in tissue biomechanics, (*41, 42*) where upregulation of collagen VI is associated with increased tissue stiffness. (*42-44*) Moreover, deletion of collagen VI subunit α1 in mice resulted in a loss of PCM properties, altered mechanotransduction, and early-onset hip OA. (*15, 23*) These findings suggest that altered concentrations and arrangement of collagen VI is likely to affect the mechanical properties and physicochemical conversion of mechanical strains in the PCM. (*15*)

### Mechanical loading

Mechanical loading plays a critical role in regulating the chondrocytes' cellular differentiation, growth, and balance between anabolic and catabolic signaling. (*45, 46*) The response of chondrocytes depends on a multitude of factors, such as frequency, strain, strain rate as well as loading history. Physiological mechanical cues, typically within the range of 10-20% compression, are known to contribute to an anabolic healthy chondrocyte state (*11*) enhancing the synthesis of ECM molecules, such as proteoglycans and collagens. In contrast, supra- or hyper-physiologic mechanical loading fails to enhance matrix production and increases a catabolic response. This catabolic response can lead to (micro) damage of articular cartilage (*47*) thereby disrupting the delicate post-mitotic silent phenotype of chondrocytes toward a detrimental catabolically active chondrocyte with hypertrophic phenotype. Indeed, chondrocytes that have been exposed to an injurious mechanical load, fail to recuperate their anabolic response to physiologic mechanical loading. (*48*) These persistently altered responses to mechanical environmental cues can potentially be explained by an altered epigenetic state of chondrocytes in interaction with a loss of mechanical properties of the cartilage. Since the chondrocyte has an inherent low repair capability, the mechanical properties and structure of cartilage are affected at each damaging event thereby reducing the amount of mechanical loading that the cartilage can sustain. (*49*) While repair processes in response to injury are (in part) driven by the release of growth factors that are sequestered to the PCM and released upon mechanical loading. (*50*), hyper-physiologic mechanical cues can induce the onset of inflammatory signaling in chondrocytes, such as the prostaglandin pathway. (*51-54*) As such, mechanical forces are a critical environmental factor for maintaining joint homeostasis by regulating chondrocyte phenotypes and their anabolic, catabolic, and inflammatory responses.

Mechano-transduction in chondrocytes is in large part mediated by mechanosensitive ion channels. Two main ion channels in chondrocytes are the transient receptor potential cation channel subfamily V member 4 (TRPV4) and the PIEZO channels, piezo-type mechanosensitive ion channel components 1 and 2 ( PIEZO1 and PIEZO2) (*8, 23, 55-61*) that are activated by both mechanical stretching of the cell membrane and osmotic pressure. *TRPV4* is characterized as a pro-anabolic channel, with a relatively low activation threshold whereas PIEZO channels are typically characterized as pro-catabolic channels that are activated with higher activation thresholds. (*62*) The mechanical properties of the pericellular matrix (PCM) play a critical role in this mechanosensing system by modulating the local strain fields surrounding the chondrocyte and, consequently, the activation of these mechanosensitive channels. (*13*)

### OA Pathophysiology in cartilage

The onset of OA pathophysiology in cartilage is characterized by an increase in water content and an irreversible loss of stiffness of the PCM, preceding the loss of stiffness of the ECM. (*63, 64*) Concomitantly, chondrocytes lose their maturational arrested state, become activated, and increase synthetic activity and production of proteoglycans. (*63-65*) Once the disease progresses chondrocytes lose their maturational arrested state and start to proliferate. (*66-68*) Thereby, they start to lose their balance between anabolic and catabolic signaling, by increasing the expression of catabolic enzymes such as matrix metalloproteinases (MMPs) such as MMP13, as well as disintegrin and metalloproteinase with thrombospondin motifs such as ADAMTS5, while reducing the expression of anabolic proteins such as aggrecan (*ACAN*) and collagen II (*COL2A1*). (*69*) The resulting degradation of proteoglycans and collagens progressively compromises both ECM and PCM integrity, leading to deteriorating biomechanical properties. This creates a detrimental feedback loop where impaired mechanical properties expose chondrocytes to excessive strains during normal loading, triggering further catabolic responses. Additionally, the normal PCM-restricted distribution of type VI collagen becomes disrupted, with expression found throughout the ECM in osteoarthritic cartilage. (*30-33*)

### Molecular pathophysiology of OA

Owing to the vast number of joint replacement surgeries, OA-relevant joint tissues are readily available. Moreover, with advancing next-generation -omic technology the OA pathophysiological process has been characterized at various levels of molecular information. A notable study that facilitated such studies is the research in articular osteoArthritis research (RAAK) study, in which transcriptome and methylome wide landscapes of paired preserved and lesioned OA cartilage were determined. These studies enabled the detection of relevant OA disease pathways (*69, 70*) patient stratification (*71*) as well as target identification for further modeling toward potential druggable target identification. (*70, 72*)

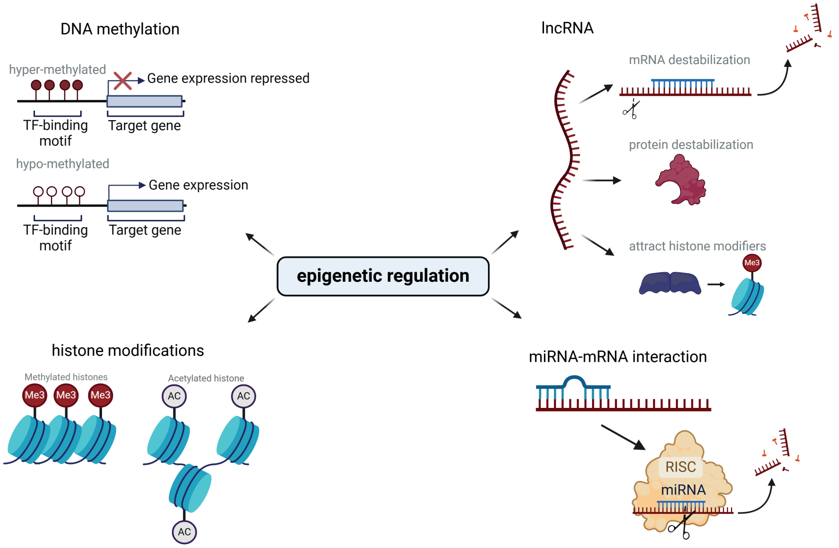

**Figure 4 | Epigenetic regulation of gene expression.** Non-exhaustive overview of mechanisms by which gene expression is regulated by DNA methylation, Histone modifications, and lncRNA as well as miRNAs.

### Epigenetics in OA

The postmitotic and metabolically inactive chondrocyte relies heavily on epigenetic mechanisms to regulate temporal changes in gene expression in response to environmental challenges such as injurious mechanical stress. (*73*) Epigenetics is defined as any acquired change in gene expression that is not accompanied by changes in DNA sequence. (*74, 75*) Important levels of epigenetic regulation include DNA methylation, non-coding RNAs, such as micro-RNAs (miRNAs) and long noncoding RNAs (lncRNAs) (82–84)suggesting an especially significant role for these small RNAs in pathologic settings. Here, we review emerging principles of miRNA regulation of stress signaling pathways and apply these concepts to our understanding of the roles of miRNAs in disease. These discussions further highlight the unique challenges and opportunities associated with the mechanistic dissection of miRNA functions and the development of miRNA-based therapeutics **(Figure 4)** (*76*)

DNA Methylation occurs at cytosine within cytosine-guanine (CpG) dinucleotides and is involved in processes that require long-term stable set points of gene expression by controlling transcription binding site accessibility.(*76*) Environmental challenges such as disease or injurious mechanical stress have been thought to bring about long-lasting alterations in DNA methylation that eventually could impair cellular performance. (*77, 78*) While DNA methylation studies in OA have thus far focused on patient stratification (*79*) and differential methylation patterns (*80, 81*), the RAAK study integrated transcriptome- and methylome-wide data from the same OA cartilage samples. (*67*) This allowed to uncovering of transcriptionally active CpG sites that mark the OA pathophysiological process in cartilage and revealed that differential methylation between preserved and lesioned OA cartilage affects genes involved in the development and ECM maintenance, potentially reactivating endochondral ossification. Notable examples include CD44, which regulates matrix assembly and mechano-transduction, and ITGA5, which mediates cellular responses to mechanical stress. (*82-87*)

Non-coding RNAs comprise two main classes: microRNAs (miRNAs, <22 nucleotides) and long non-coding RNAs (lncRNAs, >200 nucleotides). MiRNAs play a role in post-transcriptional regulation of gene expression by binding to complementary sequences in the 3'UTR of target mRNAs, thereby inhibiting translation and reducing mRNA stability. (*88*) In the RAAK study we uncovered the miRNA-mRNA interactome of the OA pathophysiological process in cartilage by combining transcriptome-wide mRNA and miRNA data from the same OA cartilage samples. Subsequent pathway enrichment analysis revealed that genes involved in nervous system development are likely mediated by miRNA regulatory mechanisms (*70*) lncRNAs, on the other hand, are highly tissue-specific yet have a much broader set of functions. They are involved in gene expression regulation by affecting the stability of mRNAs, but they can also act upon the transcriptional machinery by interfering with chromatin modification. (*89*)

Moreover, they can affect protein stability, and some lncRNAs also act as molecular decoys or sponges for miRNAs. (*90*) Despite the significant advantage of RNA sequencing technologies, annotating lncRNAs has been a challenge since their sequence-function relationship is poorly understood. (*91*) By integrating two machine learning methods, the coding potential assessment tool (CPAT) and the LncFinder R package, a new RNA sequencing pipeline was established that allowed robust detection and exploration of lncRNAs in RnA in OA cartilage samples from the RAAK study. (*92*) This demonstrated that cis-acting lncRNAs represent a substantial portion of known lncRNAs with varying positional relationships to their target genes. Moreover, it was shown that *in vitro* functional validation of this *cis*-regulation of such target genes is effectively studied by lnaGAPmers. (*92*)

**Genetic studies of OA**

OA has a polygenic genetic component, with many genes contributing to OA disease risk. (*93*) Identification of these OA risk genes may provide valuable insight into the underlying disease pathways. (*94*) To identify which genes determine OA etiology, genetic studies have been performed that focus primarily on two types of genetic study designs.

First, comprehensive genome-wide association studies (GWAS) are suited to identify common genetic OA risk variants, with relatively low penetrance and effect sizes. These SNPs generally cause risk to OA by inducing slight changes in levels of expression of positional genes *in cis*, so-called expression quantitative trait loci (eQTLs). (*70, 95*) Moreover, the majority of functional risk SNPs are located in regulatory regions of gene expression and are often modified by methylation at CpG sites. (*96, 97*) As a result, it is generally accepted that genetic risk variants act via subtle changes in gene expression. While short-term exposure to these small changes in gene expression may be benign, life-long exposure to subtle gene expression changes will increase the risk of developing OA. (*98-100*) The largest GWAS meta-analysis currently performed identified a total of 100 independent SNPs associated with general OA and different subtypes such as hip OA, knee OA, and hand OA. These SNPs were mapped to cartilage-relevant genes such as *IL11, COL27A1,* and *TNC.* (*101*)

Second, genetic linkage studies often combined with exome sequencing are being performed in both sibling pair studies (*102*) and early-onset families. (*18, 103, 104*) In these familial OA cases, OA generally occurs earlier in life and affects multiple joint sites simultaneously. (*18, 103, 105*) Generally, the genetic variants identified are rare with high penetrance and co-segregate to the OA phenotype in families via Mendelian inheritance patterns. The functional variants in these studies are readthrough, missense, or non-sense and directly affect protein function. A notable example is a genetic variant in *FN1*, *TNFRSF11B* causing early onset in extended early-onset families. (*18, 103*) Affected sibling-pair linkage studies, are particularly suited to identify low-frequency variants, with high penetrance. (*106*) such as the GARP study consisting of affected sibling pairs with symptomatic OA at multiple joint sites. (*105*) A notable example herein is the discovery of a genetic variant in *DIO2* (*102*) where functional follow-up studies demonstrated that this gene confers risk to OA via affecting matrix deposition and cartilage calcification. (*107*)

### Functional studies: from genetic susceptibility toward understanding OA pathophysiology

While multiple compelling OA susceptibility loci have been identified, only a limited amount of these loci have been studied for their biological relevance and functional effects, and none have incorporated the interaction of genetic variance with environmental risk factors. (*108*) Identifying and understanding the causal pathways by which OA susceptibility loci confer risk to the onset and development of OA requires functional follow-up of these genetic studies, preferably in human biomimetic tissue models where OA is induced by physiological relevant perturbations.

### *In vitro* Models

Multiple *in vitro* model systems are available for studying OA, such as two-dimensional cell cultures, three-dimensional pellet cultures, and three-dimensional multi-tissue co-cultures. Two-dimensional *in vitro* models allow for an easy culture of OA-relevant cells such as chondrocytes, osteocytes, and bone-marrow-derived mesenchymal stromal cells, that can be easily exposed to perturbations such as cytokines, or lentiviral induction or inhibition of genes of interest. (*108*) While regulatory pathways can be determined using this approach, the *in vivo* relevance remains limited as the regulation of chondrocytes heavily relies on cellular interaction with cellular niches such as the PCM. Additionally, primary cell sources such as human primary articular chondrocytes have a tendency to dedifferentiate and lose their tissue-specific cellular phenotype. To better maintain chondrocyte phenotype, one can transfer primary chondrocytes to a 3D environment either by offering a hydrogel scaffold (*8, 11, 62*) or allowing cells to deposit matrix. (*108, 109*) In this respect it was shown that primary chondrocytes upon centrifugation are prone to *in vitro* chondrogenesis and readily grow their neo-cartilage tissue constructs. (*109-111*) While the precise orientation and structure of the ECM and PCM in these models are different from those *in vivo*, they enable the study of chondrocyte-matrix interactions. However, their limited expansion capability restricts their use in studying specific genetic variations. To circumvent this issue, one can use human induced pluripotent stem cell (hiPSC) derived disease models. These hiPSCs are generated by reprogramming adult somatic cells into a pluripotent state capable of differentiating into any cell type. (*112*) For OA disease modeling, hiPSCs provide distinct advantages through their unlimited self-renewal capacity, and suitability for genetic engineering via CRISPR-Cas9. They also enable the generation of patient-specific cell lines and can reliably differentiate into functional chondrocytes. When differentiated using established protocols, hiPSCs can generate neo-cartilage that closely resembles tissue produced by primary chondrocytes. (*113*) Such a hiPSC-derived cartilage model has already been used for functional follow-up of a genetic OA susceptibility gene FN1 that was identified in an early onset family. This study revealed the FN1 variant's effects on both chondrogenic potential and PCM protein interaction.

### *Ex vivo* Models

Human aged *ex vivo* osteochondral explants using tissues from OA patients undergoing joint replacement surgeries are considered a reliable biomimetic preclinical OA model at the tissue level. Major strengths of this model are that it constitutes full-thickness aged, preserved, human osteochondral explants that retain the interaction between bone and cartilage of heterogeneous disease states of OA patients while complying with the societal concept of refinement, reduction, and replacement of animal studies. Moreover, OA-related damage can be triggered by hypertrophy (T3), inflammation (IL1ß), or mechanical injurious stress to explore personalized treatment modalities. (*54, 114-116*) While these are clear advantages of this model, genome engineering to e.g. mimic changes in gene expression is not possible, thereby limiting the use of this model for functional follow-up studies of genetic risk loci.

### *In vivo* Models

*In vivo* models are still being used in OA research as they harbor the complexity of the articular tissue and tissue-cross talk at the organism level. A knockout mouse model of *COL6A1* showed accelerated development of OA joint degeneration, as well as other musculoskeletal abnormalities such as delayed secondary ossification and reduced bone mineral density (BMD). (*15*) Additionally, these mice also showed compromised PCM mechanical properties, which highlights the need for further follow-up on the mechano-transduction pathways related to this PCM protein. Several established *in vivo* animal models examine altered joint loading patterns; anterior cruciate ligament (ACL) transection, which increases joint laxity, meniscectomy which alters joint loading distribution, and running regimes which cause mechanical overload. Using these models upregulation of matrix-degrading enzymes such as *ADAMTS5* (*117*) and *MMP13* have been identified. (*118*) Additionally, using a knockout mouse model of DIO2, it has been shown that a lack of DIO2 protects against cartilage damage in running-induced OA. (*119*) The disadvantage of these *in vivo* models is that they lack control over mechanical parameters such as strain magnitude, rate, and loading frequency. Additionally, there is a societal pressure to reduce the use of animal studies in science. Hence alternative models need to be developed that incorporate the complexity of the tissue with methods for introducing environmental risk factors.

## Outline of thesis

Multiple genetic studies have found high-impact causal genes and putative OA-associated genetic loci that have highlighted important etiological OA pathways. This thesis aims to bridge the gap between the identification of OA-associated genes and an in-depth understanding of disease mechanisms through tailored functional follow-up studies. We employ next-generation disease modelling techniques and use multi-modal genome-wide -omics data as readout. Moreover, to study gene- and environmental risk factor interaction, we incorporated hyper-physiological mechanical loading in our models.

Hereto, in **chapter 2,** an *in vitro* model was optimized enabling the implementation of mechanical loading in functional follow-up of genetic studies. In **chapter 3**, we identified the optimal cell source for the generation of sustainable hiPSC-derived neo-cartilage constructs by comparing epigenetically regulated transcriptome between hiPSCs derived from different cell types and human primary articular chondrocytes. This comparison allowed us to evaluate whether the epigenetic memory of articular chondrocytes could result in superior hiPSC-derived *in vitro* chondrogenesis. In **chapter 4**, we identified the effect of mechanical loading on DNA methylation-driven epigenetic regulation as a potential long-lasting regulator of transcriptome-wide response to environmental stimuli. In **chapter 5**, we detected and characterized a high-impact genetic variant in *COL6A3.* Using CRISPR-edited hiPSCs we studied both the general role of collagen VI in articular cartilage in osteoarthritis and the specific effects of this variant. By implementing the model developed in chapter 2, we determined how this genetic variant interacts with mechanical loading as an environmental risk factor.

## References

1. J. D. Steinmetz, G. T. Culbreth, L. M. Haile, Q. Rafferty, J. Lo, K. G. Fukutaki, J. A. Cruz, A. E. Smith, S. E. Vollset, P. M. Brooks, Global, regional, and national burden of osteoarthritis, 1990–2020 and projections to 2050: a systematic analysis for the Global Burden of Disease Study 2021. *The Lancet Rheumatology* **5**, e508-e522 (2023).
2. K. S. Halonen, C. M. Dzialo, M. Mannisi, M. S. Venalainen, M. de Zee, M. S. Andersen, Workflow assessing the effect of gait alterations on stresses in the medial tibial cartilage - combined musculoskeletal modelling and finite element analysis. *Sci Rep* **7**, 17396 (2017).
3. G. W. Greene, X. Banquy, D. W. Lee, D. D. Lowrey, J. Yu, J. N. Israelachvili, Adaptive mechanically controlled lubrication mechanism found in articular joints. *Proc Natl Acad Sci U S A* **108**, 5255-5259 (2011).
4. Y. Luo, D. Sinkeviciute, Y. He, M. Karsdal, Y. Henrotin, A. Mobasheri, P. Onnerfjord, A. Bay-Jensen, The minor collagens in articular cartilage. *Protein Cell* **8**, 560-572 (2017).
5. D. A. Keesler, T. L. Slobodianuk, C. E. Kochelek, C. W. Skaer, S. L. Haberichter, V. H. Flood, Fibronectin binding to von Willebrand factor occurs via the A1 domain. *Res Pract Thromb Haemost* **5**, e12534 (2021).
6. U. Specks, U. Mayer, R. Nischt, T. Spissinger, K. Mann, R. Timpl, J. Engel, M. L. Chu, Structure of recombinant N-terminal globule of type VI collagen alpha 3 chain and its binding to heparin and hyaluronan. *EMBO J* **11**, 4281-4290 (1992).
7. K. M. Heinemeier, P. Schjerling, J. Heinemeier, M. B. Moller, M. R. Krogsgaard, T. Grum-Schwensen, M. M. Petersen, M. Kjaer, Radiocarbon dating reveals minimal collagen turnover in both healthy and osteoarthritic human cartilage. *Sci Transl Med* **8**, 346ra390 (2016).
8. W. Lee, R. J. Nims, A. Savadipour, Q. Zhang, H. A. Leddy, F. Liu, A. L. McNulty, Y. Chen, F. Guilak, W. B. Liedtke, Inflammatory signaling sensitizes Piezo1 mechanotransduction in articular chondrocytes as a pathogenic feed-forward mechanism in osteoarthritis. *Proc Natl Acad Sci U S A* **118**, (2021).
9. A. R. Poole, T. Kojima, T. Yasuda, F. Mwale, M. Kobayashi, S. Laverty, Composition and structure of articular cartilage: a template for tissue repair. *Clin Orthop Relat Res* **391**, S26-33 (2001).
10. J. Becerra, J. A. Andrades, E. Guerado, P. Zamora-Navas, J. M. López-Puertas, A. H. Reddi, Articular Cartilage: Structure and Regeneration. *Tissue Engineering Part B: Reviews* **16**, 617-627 (2010).
11. R. J. Nims, L. Pferdehirt, N. B. Ho, A. Savadipour, J. Lorentz, S. Sohi, J. Kassab, A. K. Ross, C. J. O'Conor, W. B. Liedtke, B. Zhang, A. L. McNulty, F. Guilak, A synthetic mechanogenetic gene circuit for autonomous drug delivery in engineered tissues. *Sci Adv* **7**, eabd9858 (2021).
12. C. A. Poole, S. Ayad, J. R. Schofield, Chondrons from articular cartilage: I. Immunolocalization of type VI collagen in the pericellular capsule of isolated canine tibial chondrons. *J. Cell Sci.* **90**, 635-643 (1988).
13. J. B. Choi, I. Youn, L. Cao, H. A. Leddy, C. L. Gilchrist, L. A. Setton, F. Guilak, Zonal changes in the three-dimensional morphology of the chondron under compression: the relationship among cellular, pericellular, and extracellular deformation in articular cartilage. *J Biomech* **40**, 2596-2603 (2007).
14. R. M. Schinagl, D. Gurskis, A. C. Chen, R. L. Sah, Depth-dependent confined compression modulus of full-thickness bovine articular cartilage. *J. Orthop. Res.* **15**, 499-506 (1997).
15. L. G. Alexopoulos, I. Youn, P. Bonaldo, F. Guilak, Developmental and osteoarthritic changes in Col6a1-knockout mice: biomechanics of type VI collagen in the cartilage pericellular matrix. *Arthritis Rheum* **60**, 771-779 (2009).
16. J. C. Adams, F. W. Watt, Regulation of development and differentiation by the extracellular matrix. *Development* **117**, 1183-1198 (1993).
17. N. Boudreau, C. Myers, M. J. Bissel, From laminin to lamin: regulation of tissue-specific gene expression by the ECM. *Trends Cell Biol.* **5**, 1-4 (1995).
18. M. van Hoolwerff, A. Rodriguez Ruiz, M. Bouma, H. E. D. Suchiman, R. I. Koning, C. R. Jost, A. A. Mulder, C. Freund, F. Guilak, Y. F. M. Ramos, I. Meulenbelt, High-impact FN1 mutation decreases chondrogenic potential and affects cartilage deposition via decreased binding to collagen type II. *Sci Adv* **7**, eabg8583 (2021).
19. E. Ruoslahti, Y. Yamaguchi, Proteoglycans as modulators of growth factor activities. *Cell* **64**, 867-869 (1991).
20. L. Macri, D. Silverstein, R. A. Clark, Growth factor binding to the pericellular matrix and its importance in tissue engineering. *Adv Drug Deliv Rev* **59**, 1366-1381 (2007).
21. J. D. Sandy, J. R. O'Neill, L. C. Ratzlaff, Acquisition of hyaluronate-binding affinity in vivo by newly synthsized cartilage proteoglycans. *Biochem. J.* **258**, 875-880 (1989).
22. R. E. Wilusz, F. Guilak, High resistance of the mechanical properties of the chondrocyte pericellular matrix to proteoglycan digestion by chondroitinase, aggrecanase, or hyaluronidase. *J. Mech. Behav. Biomed. Mater* **38**, 183-197 (2014).
23. N. A. Zelenski, H. A. Leddy, J. Sanchez-Adams, J. Zhang, P. Bonaldo, W. Liedtke, F. Guilak, Type VI Collagen Regulates Pericellular Matrix Properties, Chondrocyte Swelling, and Mechanotransduction in Mouse Articular Cartilage. *Arthritis Rheumatol* **67**, 1286-1294 (2015).
24. L. G. Alexopoulos, L. A. Setton, F. Guilak, The biomechanical role of the chondrocyte pericellular matrix in articular cartilage. *Acta Biomater* **1**, 317-325 (2005).
25. E. Kim, F. Guilak, M. A. Haider, The dynamic mechanical environment of the chondrocyte: a biphasic finite element model of cell-matrix interactions under cyclic compressive loading. *J. Biomech. Eng.* **130**, 061009 (2008).
26. E. Kim, F. Guilak, M. A. Haider, An axisymmetric boundary element model for determination of articular cartilage pericellular matrix properties in situ via inverse analysis of chondron deformation. *J. Biomech. Eng.* **132**, 031011 (2010).
27. G. M. Lee, C. A. Poole, S. S. Kelley, J. Chang, B. Caterson, Isolated chondrons: a viable alternative for studies of chondrocyte metabolism in vitro. *Osteoarthritis Cartilage* **5**, 261-274 (1997).
28. J. Marcelino, C. A. McDevitt, Attachment of articular cartilage chondrocytes to the tissue form of type VI collagen. *Biochim Biophys Acta* **1249**, 180-188. (1995).
29. A. F. Sherwin, D. H. Carter, C. A. Poole, J. A. Hoyland, S. Ayad, The distribution of type VI collagen in the developing tissues of the bovine femoral head. *Histochem. J.* **31**, 623-632. (1999).
30. L. Hambach, D. Neureiter, G. Zeiler, T. Kirchner, T. Aigner, Severe disturbance of the distribution and expression of type VI collagen chains in osteoarthritic articular cartilage. *Arthritis Rheum* **41**, 986-996. (1998).
31. C. A. McDevitt, J. A. Pahl, S. Ayad, R. R. Miller, M. Uratsuji, J. T. Andrish, Experimental osteoarthritic articular cartilage is enriched in guanidine soluble type VI collagen. *Biochem Biophys Res Commun* **157**, 250-255. (1988).
32. O. Pullig, G. Weseloh, B. Swoboda, Expression of type VI collagen in normal and osteoarthritic human cartilage. *Osteoarthritis Cartilage* **7**, 191-202. (1999).
33. B. Swoboda, O. Pullig, T. Kirsch, B. Kladny, B. Steinhauser, G. Weseloh, Increased content of type-VI collagen epitopes in human osteoarthritic cartilage: quantitation by inhibition ELISA. *J Orthop Res* **16**, 96-99. (1998).
34. M. Cescon, F. Gattazzo, P. Chen, P. Bonaldo, Collagen VI at a glance. *J. Cell Sci.* **128**, 3525-3531 (2015).
35. N. Beecher, A. M. Roseman, T. A. Jowitt, R. Berry, H. Troilo, R. A. Kammerer, C. A. Shuttleworth, C. M. Kielty, C. Baldock, Collagen VI, conformation of A-domain arrays and microfibril architecture. *J Biol Chem* **286**, 40266-40275 (2011).

36. C. M. Kielty, S. P. Whittaker, M. E. Grant, C. A. Shuttleworth, Type VI collagen microfibrils: evidence for a structural association with hyaluronan. *J. Cell Biol.* **118**, 979-990. (1992).
37. W. B. Stallcup, K. Dahlin, P. Healy, Interaction of the NG2 chondroitin sulfate proteoglycan with type VI collagen. *J. Cell Biol.* **111**, 3177-3188. (1990).
38. R. F. Loeser, Chondrocyte integrin expression and function. *Biorheology* **37**, 109-116 (2000).
39. R. F. Loeser, Integrin-mediated attachment of articular chondrocytes to extracellular matrix proteins. *Arthritis Rheum* **36**, 1103-1110 (1993).
40. C. A. McDevitt, J. Marcelino, L. Tucker, Interaction of intact type VI collagen with hyaluronan. *FEBS Lett.* **294**, 167-170 (1991).
41. P. Bonaldo, P. Braghetta, M. Zanetti, S. Piccolo, D. Volpin, G. M. Bressan, Collagen VI deficiency induces early onset myopathy in the mouse: an animal model for Bethlem myopathy. *Hum Mol Genet* **7**, 2135-2140. (1998).
42. A. Rauch, R. A. Pfeiffer, U. Trautmann, Deletion or triplication of the alpha 3 (VI) collagen gene in three patients with 2q37 chromosome aberrations and symptoms of collagen-related disorders. *Clin. Genet.* **49**, 279-285. (1996).
43. M. R. Griffiths, M. Shepherd, R. Ferrier, D. Schuppan, O. F. James, A. D. Burt, Light microscopic and ultrastructural distribution of type VI collagen in human liver: alterations in chronic biliary disease. *Histopathology* **21**, 335-344. (1992).
44. U. Specks, A. Nerlich, T. V. Colby, I. Wiest, R. Timpl, Increased expression of type VI collagen in lung fibrosis. *Am. J. Respir. Crit. Care Med.* **151**, 1956-1964. (1995).
45. F. Guilak, Biomechanical factors in osteoarthritis. *Best Pract Res Clin Rheumatol* **25**, 815-823 (2011).
46. T. Hodgkinson, D. C. Kelly, C. M. Curtin, F. J. O'Brien, Mechanosignalling in cartilage: an emerging target for the treatment of osteoarthritis. *Nat Rev Rheumatol* **18**, 67-84 (2022).
47. E. Houtman, M. Tuerlings, J. Riechelman, E. Suchiman, R. J. P. van der Wal, R. Nelissen, H. Mei, Y. F. M. Ramos, R. Coutinho de Almeida, I. Meulenbelt, Elucidating mechano-pathology of osteoarthritis: transcriptome-wide differences in mechanically stressed aged human cartilage explants. *Arthritis Res Ther* **23**, 215 (2021).
48. M. S. Ashwell, M. G. Gonda, K. Gray, C. Maltecca, A. T. O'Nan, J. P. Cassady, P. L. Mente, Changes in chondrocyte gene expression following in vitro impaction of porcine articular cartilage in an impact injury model. *J Orthop Res* **31**, 385-391 (2013).
49. D. A. Doré, T. M. Winzenberg, C. Ding, P. Otahal, J.-P. Pelletier, J. Martel-Pelletier, F. M. Cicuttini, G. Jones, The association between objectively measured physical activity and knee structural change using MRI. *Annals of the rheumatic diseases* **72**, 1170-1175 (2013).
50. T. L. Vincent, Targeting mechanotransduction pathways in osteoarthritis: a focus on the pericellular matrix. *Curr Opin Pharmacol* **13**, 449-454 (2013).
51. T. L. Vincent, Mechanoflammation in osteoarthritis pathogenesis. *Semin Arthritis Rheum* **49**, S36-S38 (2019).
52. T. T. Chowdhury, O. O. Akanji, D. M. Salter, D. L. Bader, D. A. Lee, Dynamic compression influences interleukin-1β-induced nitric oxide and prostaglandin E2 release by articular chondrocytes via alterations in iNOS and COX-2 expression. *Biorheology* **45**, 257-274 (2008).
53. B. Fermor, J. B. Weinberg, D. S. Pisetsky, M. A. Misukonis, C. Fink, F. Guilak, Induction of cyclooxygenase-2 by mechanical stress through a nitric oxide-regulated pathway. *Osteoarthritis Cartilage* **10**, 792-798 (2002).
54. M. Gosset, F. Berenbaum, A. Levy, A. Pigenet, S. Thirion, J. L. Saffar, C. Jacques, Prostaglandin E2 synthesis in cartilage explants under compression: mPGES-1 is a mechanosensitive gene. *Arthritis Res Ther* **8**, R135 (2006).
55. W. Lee, F. Guilak, W. Liedtke, Role of Piezo Channels in Joint Health and Injury. *Curr Top Membr* **79**, 263-273 (2017).
56. W. Lee, H. A. Leddy, Y. Chen, S. H. Lee, N. A. Zelenski, A. L. McNulty, J. Wu, K. N. Beicker, J. Coles, S. Zauscher, J. Grandl, F. Sachs, F. Guilak, W. B. Liedtke, Synergy between Piezo1 and Piezo2 channels confers high-strain mechanosensitivity to articular cartilage. *Proc Natl Acad Sci U S A* **111**, E5114-5122 (2014).
57. L. O. Romero, A. E. Massey, A. D. Mata-Daboin, F. J. Sierra-Valdez, S. C. Chauhan, J. F. Cordero-Morales, V. Vasquez, Dietary fatty acids fine-tune Piezo1 mechanical response. *Nat Commun* **10**, 1200 (2019).
58. M. R. Servin-Vences, M. Moroni, G. R. Lewin, K. Poole, Direct measurement of TRPV4 and PIEZO1 activity reveals multiple mechanotransduction pathways in chondrocytes. *Elife* **6**, (2017).
59. S. Fu, H. Meng, S. Inamdar, B. Das, H. Gupta, W. Wang, C. L. Thompson, M. M. Knight, Activation of TRPV4 by mechanical, osmotic or pharmaceutical stimulation is anti-inflammatory blocking IL-1beta mediated articular cartilage matrix destruction. *Osteoarthritis Cartilage* **29**, 89-99 (2021).
60. A. L. McNulty, H. A. Leddy, W. Liedtke, F. Guilak, TRPV4 as a therapeutic target for joint diseases. *Naunyn Schmiedebergs Arch. Pharmacol.* **388**, 437-450 (2015).
61. C. J. O'Conor, H. A. Leddy, H. C. Benefield, W. B. Liedtke, F. Guilak, TRPV4-mediated mechanotransduction regulates the metabolic response of chondrocytes to dynamic loading. *Proc Natl Acad Sci U S A* **111**, 1316-1321 (2014).
62. A. Savadipour, R. J. Nims, N. Rashidi, J. M. G. Castorena, R. H. Tang, G. K. Marushack, S. J. Oswald, W. B. Liedtke, F. Guilak, Membrane stretch as the mechanism of activation of PIEZO1 ion channels in chondrocytes. *Proceedings of the National Academy of Sciences of the United States of America* **120**, e2221958120 (2023).
63. R. E. Wilusz, S. Zauscher, F. Guilak, Micromechanical mapping of early osteoarthritic changes in the pericellular matrix of human articular cartilage. *Osteoarthritis Cartilage* **21**, 1895-1903 (2013).
64. D. R. Chery, B. Han, Q. Li, Y. Zhou, S. J. Heo, B. Kwok, P. Chandrasekaran, C. Wang, L. Qin, X. L. Lu, D. Kong, M. Enomoto-Iwamoto, R. L. Mauck, L. Han, Early changes in cartilage pericellular matrix micromechanobiology portend the onset of post-traumatic osteoarthritis. *Acta Biomater* **111**, 267-278 (2020).
65. P. Lorenzo, M. T. Bayliss, D. Heinegard, Altered patterns and synthesis of extracellular matrix macromolecules in early osteoarthritis. *Matrix Biol* **23**, 381-391 (2004).
66. P. M. van der Kraan, W. B. van den Berg, Chondrocyte hypertrophy and osteoarthritis: role in initiation and progression of cartilage degeneration? *Osteoarthritis Cartilage* **20**, 223-232 (2012).
67. W. den Hollander, Y. F. Ramos, N. Bomer, S. Elzinga, R. van der Breggen, N. Lakenberg, W. J. de Dijcker, H. E. Suchiman, B. J. Duijnisveld, J. J. Houwing-Duistermaat, P. E. Slagboom, S. D. Bos, R. G. Nelissen, I. Meulenbelt, Transcriptional associations of osteoarthritis-mediated loss of epigenetic control in articular cartilage. *Arthritis Rheumatol* **67**, 2108-2116 (2015).
68. B. Rolauffs, J. M. Williams, M. Aurich, A. J. Grodzinsky, K. E. Kuettner, A. A. Cole, Proliferative remodeling of the spatial organization of human superficial chondrocytes distant from focal early osteoarthritis. *Arthritis Rheum* **62**, 489-498 (2010).
69. Y. F. Ramos, S. D. Bos, N. Lakenberg, S. Bohringer, W. J. den Hollander, M. Kloppenburg, P. E. Slagboom, I. Meulenbelt, Genes expressed in blood link osteoarthritis with apoptotic pathways. *Ann Rheum Dis* **73**, 1844-1853 (2014).
70. R. Coutinho de Almeida, Y. F. M. Ramos, A. Mahfouz, W. den Hollander, N. Lakenberg, E. Houtman, M. van Hoolwerff, H. E. D. Suchiman, A. Rodriguez Ruiz, P. E. Slagboom, H. Mei, S. M. Kielbasa, R. Nelissen, M. Reinders, I. Meulenbelt, RNA sequencing data integration reveals an miRNA interactome of osteoarthritis cartilage. *Ann Rheum Dis* **78**, 270-277 (2019).

71. R. Coutinho de Almeida, A. Mahfouz, H. Mei, E. Houtman, W. den Hollander, J. Soul, E. Suchiman, N. Lakenberg, J. Meessen, K. Huetink, R. G. H. H. Nelissen, Y. F. M. Ramos, M. Reinders, I. Meulenbelt, Identification and characterization of two consistent osteoarthritis subtypes by transcriptome and clinical data integration. *Rheumatology* **60**, 1166-1175 (2021).
72. J. Steinberg, R. A. Brooks, L. Southam, S. Bhatnagar, T. I. Roumeliotis, K. Hatzikotoulas, E. Zengini, J. M. Wilkinson, J. S. Choudhary, A. W. McCaskie, E. Zeggini, Widespread epigenomic, transcriptomic and proteomic differences between hip osteophytic and articular chondrocytes in osteoarthritis. *Rheumatology (Oxford)* **57**, 1481-1489 (2018).
73. M. B. Goldring, K. B. Marcu, Epigenomic and microRNA-mediated regulation in cartilage development, homeostasis, and osteoarthritis. *Trends Mol. Med* **18**, 109-118 (2012).
74. A. Eccleston, F. Cesari, M. Skipper, Transcription and epigenetics. *Nature* **502**, 461-461 (2013).
75. P. A. Jones, Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nature Reviews Genetics* **13**, 484-492 (2012).
76. R. Jaenisch, A. Bird, Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat. Genet* **33 Suppl**, 245-254 (2003).
77. E. W. Tobi, J. J. Goeman, R. Monajemi, H. Gu, H. Putter, Y. Zhang, R. C. Slieker, A. P. Stok, P. E. Thijssen, F. Muller, E. W. van Zwet, C. Bock, A. Meissner, L. H. Lumey, P. Eline Slagboom, B. T. Heijmans, DNA methylation signatures link prenatal famine exposure to growth and metabolism. *Nat Commun* **5**, 5592 (2014).
78. B. T. Heijmans, E. W. Tobi, A. D. Stein, H. Putter, G. J. Blauw, E. S. Susser, P. E. Slagboom, L. H. Lumey, Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc Natl Acad Sci U S A* **105**, 17046-17049 (2008).
79. W. den Hollander, Y. F. Ramos, S. D. Bos, N. Bomer, R. van der Breggen, N. Lakenberg, W. J. de Dijcker, B. J. Duijnisveld, P. E. Slagboom, R. G. Nelissen, I. Meulenbelt, Knee and hip articular cartilage have distinct epigenomic landscapes: implications for future cartilage regeneration approaches. *Ann Rheum Dis* **73**, 2208-2212 (2014).
80. M. D. Rushton, D. A. Young, J. Loughlin, L. N. Reynard, Differential DNA methylation and expression of inflammatory and zinc transporter genes defines subgroups of osteoarthritic hip patients. *Ann. Rheum. Dis* **74**, 1778-1782 (2015).
81. M. A. Jeffries, M. Donica, L. W. Baker, M. E. Stevenson, A. C. Annan, M. B. Humphrey, J. A. James, A. H. Sawalha, Genome-wide DNA methylation study identifies significant epigenomic changes in osteoarthritic cartilage. *Arthritis Rheumatol* **66**, 2804-2815 (2014).
82. W. Knudson, G. Chow, C. B. Knudson, CD44-mediated uptake and degradation of hyaluronan. *Matrix Biol* **21**, 15-23 (2002).
83. L. Mellor, C. B. Knudson, D. Hida, E. B. Askew, W. Knudson, Intracellular domain fragment of CD44 alters CD44 function in chondrocytes. *J Biol Chem* **288**, 25838-25850 (2013).
84. O. Nagano, D. Murakami, D. Hartmann, B. De Strooper, P. Saftig, T. Iwatsubo, M. Nakajima, M. Shinohara, H. Saya, Cell-matrix interaction via CD44 is independently regulated by different metalloproteinases activated in response to extracellular Ca(2+) influx and PKC activation. *J Cell Biol* **165**, 893-902 (2004).
85. M. Almonte-Becerril, L. I. Gimeno, O. Villarroya, M. Benito-Jardon, J. B. Kouri, M. Costell, Genetic abrogation of the fibronectin-alpha5beta1 integrin interaction in articular cartilage aggravates osteoarthritis in mice. *PLoS One* **13**, e0198559 (2018).
86. M. E. Candela, C. Wang, A. T. Gunawardena, K. Zhang, L. Cantley, R. Yasuhara, Y. Usami, N. Francois, M. Iwamoto, A. van der Flier, Y. Zhang, L. Qin, L. Han, M. Enomoto-Iwamoto, Alpha 5 Integrin Mediates Osteoarthritic Changes in Mouse Knee Joints. *PLoS One* **11**, e0156783 (2016).
87. T. T. Chowdhury, R. N. Appleby, D. M. Salter, D. A. Bader, D. A. Lee, Integrin-mediated mechanotransduction in IL-1 beta stimulated chondrocytes. *Biomech Model Mechanobiol* **5**, 192-201 (2006).
88. R. Coutinho de Almeida, Y. F. M. Ramos, I. Meulenbelt, Involvement of epigenetics in osteoarthritis. *Best Pract Res Clin Rheumatol* **31**, 634-648 (2017).
89. L. Statello, C.-J. Guo, L.-L. Chen, M. Huarte, Gene regulation by long non-coding RNAs and its biological functions. *Nat Rev Mol Cell Bio* **22**, 96-118 (2021).
90. D. Holoch, D. Moazed, RNA-mediated epigenetic regulation of gene expression. *Nature Reviews Genetics* **16**, 71-84 (2015).
91. B. Uszczynska-Ratajczak, J. Lagarde, A. Frankish, R. Guigó, R. Johnson, Towards a complete map of the human long non-coding RNA transcriptome. *Nat Rev Genet* **19**, 535-548 (2018).
92. M. van Hoolwerff, P. I. Metselaar, M. Tuerlings, H. E. D. Suchiman, N. Lakenberg, Y. F. M. Ramos, D. Cats, R. Nelissen, D. Broekhuis, H. Mei, R. C. de Almeida, I. Meulenbelt, Elucidating Epigenetic Regulation by Identifying Functional cis-Acting Long Noncoding RNAs and Their Targets in Osteoarthritic Articular Cartilage. *Arthritis Rheumatol* **72**, 1845-1854 (2020).
93. K. Panoutsopoulou, L. Southam, K. S. Elliott, N. Wrayner, G. Zhai, C. Beazley, G. Thorleifsson, N. K. Arden, A. Carr, K. Chapman, P. Deloukas, M. Doherty, A. McCaskie, W. E. Ollier, S. H. Ralston, T. D. Spector, A. M. Valdes, G. A. Wallis, J. M. Wilkinson, E. Arden, K. Battley, H. Blackburn, F. J. Blanco, S. Bumpstead, L. A. Cupples, A. G. Day-Williams, K. Dixon, S. A. Doherty, T. Esko, E. Evangelou, D. Felson, J. J. Gomez-Reino, A. Gonzalez, A. Gordon, R. Gwilliam, B. V. Halldorsson, V. B. Hauksson, A. Hofman, S. E. Hunt, J. P. Ioannidis, T. Ingvarsson, I. Jonsdottir, H. Jonsson, R. Keen, H. J. Kerkhof, M. G. Kloppenburg, N. Koller, N. Lakenberg, N. E. Lane, A. T. Lee, A. Metspalu, I. Meulenbelt, M. C. Nevitt, F. O'Neill, N. Parimi, S. C. Potter, I. Rego-Perez, J. A. Riancho, K. Sherburn, P. E. Slagboom, K. Stefansson, U. Styrkarsdottir, M. Sumillera, D. Swift, U. Thorsteinsdottir, A. Tsezou, A. G. Uitterlinden, J. B. van Meurs, B. Watkins, M. Wheeler, S. Mitchell, Y. Zhu, J. M. Zmuda, E. Zeggini, J. Loughlin, Insights into the genetic architecture of osteoarthritis from stage 1 of the arcOGEN study. *Ann. Rheum. Dis* **70(5)**, 864-867 (2011).
94. G. Aubourg, S. J. Rice, P. Bruce-Wootton, J. Loughlin, Genetics of osteoarthritis. *Osteoarthritis Cartilage* **30**, 636-649 (2022).
95. R. Coutinho de Almeida, M. Tuerlings, Y. Ramos, W. Den Hollander, E. Suchiman, N. Lakenberg, R. Nelissen, H. Mei, I. Meulenbelt, Allelic expression imbalance in articular cartilage and subchondral bone refined genome-wide association signals in osteoarthritis. *Rheumatology (Oxford)* **62**, 1669-1676 (2023).
96. S. J. Rice, K. Cheung, L. N. Reynard, J. Loughlin, Discovery and analysis of methylation quantitative trait loci (mQTLs) mapping to novel osteoarthritis genetic risk signals. *Osteoarthritis Cartilage* **27**, 1545-1556 (2019).
97. S. J. Rice, F. Beier, D. A. Young, J. Loughlin, Interplay between genetics and epigenetics in osteoarthritis. *Nat Rev Rheumatol* **16**, 268-281 (2020).
98. D. Serre, S. Gurd, B. Ge, R. Sladek, D. Sinnett, E. Harmsen, M. Bibikova, E. Chudin, D. L. Barker, T. Dickinson, J. B. Fan, T. J. Hudson, Differential allelic expression in the human genome: a robust approach to identify genetic and epigenetic cis-acting mechanisms regulating gene expression. *PLoS Genet* **4**, e1000006 (2008).
99. S. D. Bos, J. V. Bovee, B. J. Duijnisveld, E. V. Raine, W. J. van Dalen, Y. F. Ramos, R. van der Breggen, R. G. Nelissen, P. E. Slagboom, J. Loughlin, I. Meulenbelt, Increased type II deiodinase protein in OA-affected cartilage and allelic imbalance

of OA risk polymorphism rs225014 at DIO2 in human OA joint tissues. *Ann Rheum Dis* **71**, 1254-1258 (2012).

100. W. den Hollander, I. Pulyakhina, C. Boer, N. Bomer, R. van der Breggen, W. Arindrarto, R. Couthino de Almeida, N. Lakenberg, T. Sentner, J. F. J. Laros, P. A. C. t Hoen, E. P. E. Slagboom, R. Nelissen, J. van Meurs, Y. F. M. Ramos, I. Meulenbelt, Annotating Transcriptional Effects of Genetic Variants in Disease-Relevant Tissue: Transcriptome-Wide Allelic Imbalance in Osteoarthritic Cartilage. *Arthritis Rheumatol* **71**, 561-570 (2019).
101. C. G. Boer, K. Hatzikotoulas, L. Southam, L. Stefansdottir, Y. Zhang, R. Coutinho de Almeida, T. T. Wu, J. Zheng, A. Hartley, M. Teder-Laving, A. H. Skogholt, C. Terao, E. Zengini, G. Alexiadis, A. Barysenka, G. Bjornsdottir, M. E. Gabrielsen, A. Gilly, T. Ingvarsson, M. B. Johnsen, H. Jonsson, M. Kloppenburg, A. Luetge, S. H. Lund, R. Magi, M. Mangino, R. Nelissen, M. Shivakumar, J. Steinberg, H. Takuwa, L. F. Thomas, M. Tuerlings, O. C. arc, H. A.-I. Pain, A. Consortium, C. Regeneron Genetics, G. C. Babis, J. P. Y. Cheung, J. H. Kang, P. Kraft, S. A. Lietman, D. Samartzis, P. E. Slagboom, K. Stefansson, U. Thorsteinsdottir, J. H. Tobias, A. G. Uitterlinden, B. Winsvold, J. A. Zwart, G. Davey Smith, P. C. Sham, G. Thorleifsson, T. R. Gaunt, A. P. Morris, A. M. Valdes, A. Tsezou, K. S. E. Cheah, S. Ikegawa, K. Hveem, T. Esko, J. M. Wilkinson, I. Meulenbelt, M. T. M. Lee, J. B. J. van Meurs, U. Styrkarsdottir, E. Zeggini, Deciphering osteoarthritis genetics across 826,690 individuals from 9 populations. *Cell* **184**, 4784-4818 e4717 (2021).
102. I. Meulenbelt, J. L. Min, S. Bos, N. Riyazi, J. J. Houwing-Duistermaat, H. J. van der Wijk, H. M. Kroon, M. Nakajima, S. Ikegawa, A. G. Uitterlinden, J. B. van Meurs, W. M. van der Deure, T. J. Visser, A. B. Seymour, N. Lakenberg, R. van der Breggen, D. Kremer, C. M. van Duijn, M. Kloppenburg, J. Loughlin, P. E. Slagboom, Identification of DIO2 as a new susceptibility locus for symptomatic osteoarthritis. *Hum Mol Genet* **17**, 1867-1875 (2008).
103. Y. F. Ramos, S. D. Bos, R. van der Breggen, M. Kloppenburg, K. Ye, E. W. Lameijer, R. G. Nelissen, P. E. Slagboom, I. Meulenbelt, A gain of function mutation in TNFRSF11B encoding osteoprotegerin causes osteoarthritis with chondrocalcinosis. *Ann Rheum Dis* **74**, 1756-1762 (2015).
104. A. Rodríguez Ruiz, M. van Hoolwerff, S. Sprangers, E. Suchiman, T. Schoenmaker, P. Dibbets-Schneider, J. L. Bloem, R. Nelissen, C. Freund, C. Mummery, V. Everts, T. J. de Vries, Y. F. M. Ramos, I. Meulenbelt, Mutation in the CCAL1 locus accounts for bidirectional process of human subchondral bone turnover and cartilage mineralization. *Rheumatology (Oxford)*, (2022).
105. N. Riyazi, I. Meulenbelt, H. M. Kroon, K. H. Ronday, M. P. Hellio Le Graverand, F. R. Rosendaal, F. C. Breedveld, P. E. Slagboom, M. Kloppenburg, Evidence for familial aggregation of hand, hip, and spine but not knee osteoarthritis in siblings with multiple joint involvement: the GARP study. *Ann. Rheum. Dis* **64(3)**, 438-443 (2005).
106. G. R. Abecasis, W. O. Cookson, L. R. Cardon, The power to detect linkage disequilibrium with quantitative traits in selected samples. *Am. J. Hum. Genet* **68**, 1463-1474 (2001).
107. N. Bomer, W. den Hollander, Y. F. Ramos, S. D. Bos, R. van der Breggen, N. Lakenberg, B. A. Pepers, A. E. van Eeden, A. Darvishan, E. W. Tobi, B. J. Duijnisveld, E. B. van den Akker, B. T. Heijmans, W. M. van Roon-Mom, F. J. Verbeek, G. J. van Osch, R. G. Nelissen, P. E. Slagboom, I. Meulenbelt, Underlying molecular mechanisms of DIO2 susceptibility in symptomatic osteoarthritis. *Ann Rheum Dis* **74**, 1571-1579 (2015).
108. Y. F. Ramos, I. Meulenbelt, Implementation of Functional Genomics for Bench-to-Bedside Transition in Osteoarthritis. *Curr Rheumatol Rep* **17**, 53 (2015).
109. M. Tuerlings, G. M. C. Janssen, I. Boone, M. van Hoolwerff, A. Rodriguez Ruiz, E. Houtman, H. E. D. Suchiman, R. J. P. van der Wal, R. Nelissen, R. Coutinho de Almeida, P. A. van Veelen, Y. F. M. Ramos, I. Meulenbelt, WWP2 confers risk to osteoarthritis by affecting cartilage matrix deposition via hypoxia associated genes. *Osteoarthritis Cartilage* **31**, 39-48 (2023).
110. N. Bomer, H. W. den, H. Suchiman, E. Houtman, R. C. Slieker, B. T. Heijmans, P. E. Slagboom, R. G. Nelissen, Y. F. Ramos, I. Meulenbelt, Neo-cartilage engineered from primary chondrocytes is epigenetically similar to autologous cartilage, in contrast to using mesenchymal stem cells. *Osteoarthritis. Cartilage* **24**, 1423-1430 (2016).
111. A. Rodríguez Ruiz, M. Tuerlings, A. Das, R. Coutinho de Almeida, H. E. D. Suchiman, R. G. H. H. Nelissen, Y. F. M. Ramos, I. Meulenbelt, The role of TNFRSF11B in development of osteoarthritic cartilage. *Rheumatology* **61**, 856-864 (2021).
112. K. Takahashi, S. Yamanaka, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663-676 (2006).
113. S. S. Adkar, C. L. Wu, V. P. Willard, A. Dicks, A. Ettyreddy, N. Steward, N. Bhutani, C. A. Gersbach, F. Guilak, Step-Wise Chondrogenesis of Human Induced Pluripotent Stem Cells and Purification Via a Reporter Allele Generated by CRISPR-Cas9 Genome Editing. *Stem Cells* **37**, 65-76 (2019).
114. E. Houtman, M. van Hoolwerff, N. Lakenberg, E. H. D. Suchiman, E. van der Linden-van der Zwaag, R. Nelissen, Y. F. M. Ramos, I. Meulenbelt, Human Osteochondral Explants: Reliable Biomimetic Models to Investigate Disease Mechanisms and Develop Personalized Treatments for Osteoarthritis. *Rheumatol Ther* **8**, 499-515 (2021).
115. M. Ebrahimi, S. Ojanen, A. Mohammadi, M. A. Finnila, A. Joukainen, H. Kroger, S. Saarakkala, R. K. Korhonen, P. Tanska, Elastic, Viscoelastic and Fibril-Reinforced Poroelastic Material Properties of Healthy and Osteoarthritic Human Tibial Cartilage. *Ann Biomed Eng* **47**, 953-966 (2019).
116. J. B. Fitzgerald, M. Jin, D. Dean, D. J. Wood, M. H. Zheng, A. J. Grodzinsky, Mechanical compression of cartilage explants induces multiple time-dependent gene expression patterns and involves intracellular calcium and cyclic AMP. *J Biol Chem* **279**, 19502-19511 (2004).
117. S. S. Glasson, R. Askew, B. Sheppard, B. Carito, T. Blanchet, H. L. Ma, C. R. Flannery, D. Peluso, K. Kanki, Z. Yang, M. K. Majumdar, E. A. Morris, Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. *Nature* **434**, 644-648 (2005).
118. C. B. Little, A. Barai, D. Burkhardt, S. Smith, A. Fosang, Z. Werb, M. Shah, E. Thompson, Matrix metalloproteinase 13–deficient mice are resistant to osteoarthritic cartilage erosion but not chondrocyte hypertrophy or osteophyte development. *Arthritis and Rheumatism: Official Journal of the American College of Rheumatology* **60**, 3723-3733 (2009).
119. N. Bomer, F. M. Cornelis, Y. F. Ramos, W. den Hollander, L. Storms, R. van der Breggen, N. Lakenberg, P. E. Slagboom, I. Meulenbelt, R. J. Lories, The effect of forced exercise on knee joints in Dio2−/− mice: type II iodothyronine deiodinase-deficient mice are less prone to develop OA-like cartilage damage upon excessive mechanical stress. *Annals of the rheumatic diseases*, annrheumdis-2014-206608 (2014).