



Universiteit  
Leiden  
The Netherlands

## On the relation between genetic variation and osteoarthritis

Hollander, W. den

### Citation

Hollander, W. den. (2018, March 29). *On the relation between genetic variation and osteoarthritis*. Retrieved from <https://hdl.handle.net/1887/60908>

Version: Not Applicable (or Unknown)

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/60908>

**Note:** To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The following handle holds various files of this Leiden University dissertation:  
<http://hdl.handle.net/1887/60908>

**Author:** Hollander, W. den

**Title:** On the relation between genetic variation and osteoarthritis

**Issue Date:** 2018-03-29

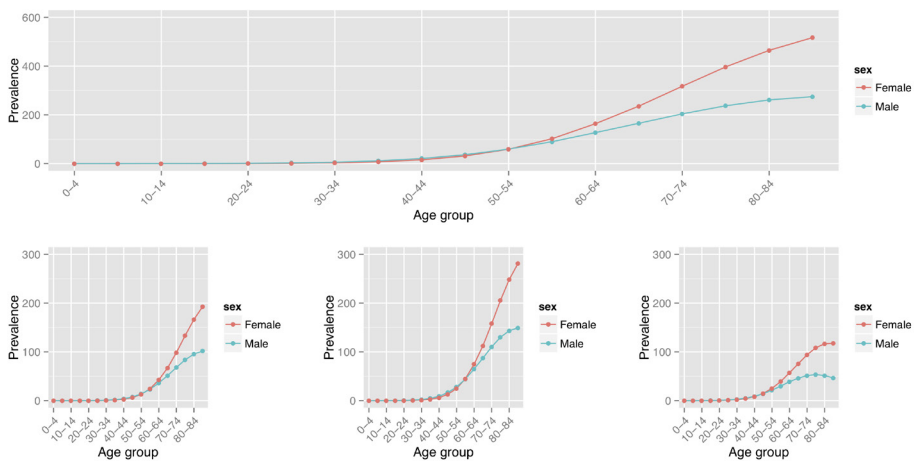
# 1

## GENERAL INTRODUCTION



## OSTEOARTHRITIS

Osteoarthritis (OA) is a prevalent, degenerative musculoskeletal disease that affects all articular joints, although it is most prominent among hip, knee and the phalangeal joints (1). In 2011 it was estimated that over 1,1 million people in the Netherlands (7%) were visiting a general practitioner due to OA related complaints (2). While present across the entire population, OA predominantly affects the elderly, which is reflected by a disease prevalence under 65 years of age of 3.6% and 3.9% for men and women, whereas above 65 years of age these numbers increase to 20.9% and 36.2%, respectively (**Figure 1**) (2). Hence, in light of the increasing proportion of elderly in western civilization, OA's burden on our society is advancing and will likely keep doing so in the near future.



**Figure 1.** Estimated osteoarthritis prevalence per 1000 inhabitants among the Dutch population in 2011, stratified by gender. (A) Overall prevalence of osteoarthritis, measured in all joints. (B) Prevalence of hip osteoarthritis. (C) Prevalence of knee osteoarthritis. (D) Prevalence of hand osteoarthritis. Data from Nationaal Kompas Volksgezondheid 2011 (2).

The disease is principally portrayed by gradual degeneration of cartilage in articular joints, although, in recent years it has become apparent that multiple joint tissues, such as the subchondral bone and synovial membrane, are involved as well (3). Multiple risk factors have been described, and encompass, but are not limited to age, gender (4), body mass index (BMI) (5), joint injuries (6) and genetic predisposition with heritability estimates ranging from 40% to 60% (7). It manifests itself as stiff and painful joints due to joint space narrowing (8), calcified articular cartilage (9), formation of bony outgrowths called osteophytes (10) and remodelling of subchondral bone (11). Presently, no treatment other than pain relief exists and patients are ultimately required to undergo total joint replacement (TJR) surgery to guarantee proper functioning of the joint. While TJR is an effective treatment for end-stage patients, gaining a deeper understanding of the actual biological mechanisms that underlie and/or accompany OA pathophysiology will arguably aid future efforts in developing disease modifying treatments.

Although multiple joint tissues have been implicated in OA pathophysiology (12–14), articular cartilage is allegedly the pivotal tissue affected by the disease. While wear and tear plays a substantial role in cartilage breakdown and thus OA progression, degradation and calcification of the tissue is additionally actively mediated by chondrocytes (15). Chondrocytes, which are thought of to be the single cell type residing in articular cartilage (16), are responsible for maintenance of the extracellular matrix (ECM) and do so by actively breaking down, remodelling and repairing the ECM using a wide variety of both catabolic as well as anabolic proteins and enzymes when they are required to do so (17–21). In order to do so properly throughout life, it is crucial for chondrocytes that after tissue homeostasis is restored upon physical stresses and consequent microtrauma's, they return to their resting state to await reactivation when required. However, chondrocytes residing in OA affected articular cartilage seem to have escaped this perpetual but appropriate cycle of reactivation and resting (15,21,22). Specifically, as OA chondrocytes dedifferentiate, start dividing, form columnar structures and actively start calcifying the ECM. They appear to have lost their articular phenotype and have entered a process that, interestingly, resembles endochondral ossification during fetal development (15,21–26).

### **OSTEOARTHRITIS ASSOCIATED GENETIC VARIATION**

Due to the substantial genetic component of OA, marked by high heritability estimates (7), in recent years, significant efforts have been made to elucidate the complex genetic architecture of the disease (27–46). However, the search for putative genetic variation that might predispose for or protect against the disease has emerged as a considerable challenge. The hereditary nature of OA is therefore still incompletely understood. Association analysis of genetic variation, traditionally performed among candidate genes and more recently by linkage analysis and genome wide association (GWA) studies, has identified a multitude of loci that are involved in OA pathophysiology. Specifically, the presence of common point mutations or single nucleotide polymorphisms (SNPs) in genes such as *GDF5* (31,39,41–43), *FRZB* (39,44,45), *ALDH1A2* (36), *DOT1L* (40,46), *GNL3* (47) and *DIO2* (32) has been associated with in- or decreased prevalence of OA among carriers. As a result, OA is designated a so-called genetically complex disease, i.e. there is no single, common genetic variant that determines whether respective carriers develop generalized OA (48–50). In that regard, uncommon genetic variants, often segregating by Mendelian nature within affected families, have been linked directly with the development of specific OA subtypes (e.g. chondrocalcinosis OA caused by a read-through mutation in the *TNFRSF11B* gene (51)). Whereas these monogenetic forms of OA are generally due to high-impact mutations, the associated effect sizes of SNPs associated with generalized forms of OA are relatively small (50), and as such leading to missed heritability for OA in general.

The nature by which these relatively small genetic effects arise seems to be due modest downstream effects of the associated genes. While these OA associated genetic variations rarely induce structural differences in the resulting protein product, alleles of common OA susceptibility SNPs do frequently mark transcriptional differences of genes in close proximity. Traditionally, SNPs that affect gene expression levels have been addressed by so-called expression quantitative trait locus (eQTL) analysis (52–54), which identified a magnitude of SNPs that affect gene expression of proximal genes marked by allele correlated expression levels. Albeit successful in doing so, eQTL studies require substantial sample sizes to reach statistical significance. In that regard, it has been shown that assessing the extent of imbalance of expressed alleles within heterozygous individuals was shown to be an effective alternative to identify genetic variation that affects gene expression levels using significantly less samples (36,42,55–59). Imbalanced expression of alleles of heterozygous carriers, generally referred to as an allelic imbalance (AI), allele specific expression (ASE) and/or allelic expression (AE), occurs when two alleles of a certain SNP are not expressed to equal extent. Because eQTLs frequently are tissue specific (60–62) and the fact that articular cartilage biobanks are generally of limited size, measuring AI of OA susceptibility SNPs in articular cartilage has gained increasing interest in recent years (36,42,55–59). Multiple common SNP alleles associated with OA have been reported to mark AI in articular cartilage and as such provided mechanistic insight into the putative biological mechanism that underlies the statistical genetic association. For example, it was shown that the risk allele C of rs225014, located in exon 3 of OA susceptibility gene *DIO2*, is expressed to higher extent compared to the wild type allele T in articular cartilage of heterozygous carriers of the SNP (57). Similar results have been reported for other robust OA susceptibility genes such as *GDF5* (42,56) and *ALDH1A2* (36), as well as for genes proximal to SNPs of which it is currently not completely certain which genes are respectively affected (*SPCS1* (55), *GNL3* (55), *COL11A1* (58)).

Nonetheless, as a consequence of these relative small effects governed by generalized OA associated SNPs, attempts to reduce missing heritability for OA is to some extent obstructed in even the largest GWA studies. In parallel, generalized OA arguably comprises a spectrum of subtypes (e.g. affected joint, osteophyte formation and/or presence of joint space narrowing) which might have just partly overlapping genetic determinants. As subtypes are generally combined in GWA studies, true association signals will be diluted into the noisy background. And although testing millions of alleles for association with larger sample sizes and/or deeper phenotypes will presumably yield novel OA associated loci, another worthwhile approach might be reducing the vast number of statistical tests, and thus lowering the multiple testing correction penalty. Selecting for SNPs that are more likely to confer susceptibility, for example by exclusion of SNPs that do not reside in proximity of genes that are involved in articular joint tissue homeostasis, might prove beneficial in this sense.

## DISEASE ASSOCIATED GENE EXPRESSION LEVELS

A well described hallmark of ongoing OA is the substantial amount of differentially expressed genes in OA affected cartilage when compared to control cartilage (18,63–72). And while identification of these has traditionally been performed in a gene targeted fashion, advances in transcriptome wide measurements, such as microarray and sequencing technologies, have markedly extended the number of genes that seem to partake in OA pathophysiology. For example, genes involved in extra cellular matrix (ECM) anabolism (e.g. *COL11*, *AGC*, *CILP*, *PRG4*) (17,18), catabolism (e.g. *MMP3*, *MMP9*, *ADAMTS5*) (73), inflammation (e.g. *IL11*, *IL1*, *TREM1*) (73) and development (e.g. *DKK3*, *DIO2*, *GDF5*, *FRZB*) (73,74) have repeatedly been observed to be expressed at different levels in OA affected cartilage. Dynamic expression and subsequent silencing of these genes is arguably required during normal life to cope with everyday physical stresses, consequent microtraumas and to maintain articular cartilage homeostasis in general. Seen with OA pathophysiology, however, it seems as if gene transcription is no longer balanced and tends to favour cartilage catabolism, consequently leading to gradual degradation and calcification of the tissue. In his regard, chondrocytes residing in OA affected articular cartilage appear to be constitutively active and no longer able to return to their proposed resting state. Worth mentioning in this context, are the apparent differences between gene expression profiles of distinct joints. Nonetheless, irrespective of the joint of interest, from a pathway point of view OA associated expression profiles reflect the aforementioned shift towards catabolism when compared to unaffected cartilage.

Nevertheless, despite the valuable observations that have resulted from (semi) transcriptome wide profiling of OA affected articular cartilage, it is at the current time not possible to designate the apparent transcriptomic disbalance as either pathologically causal and putatively driving the disease, or consequential and merely constituting a biomarker. While it is eminent that changes in gene expression are compulsory to effectuate degradative remodelling of the ECM, it can be expected that the resulting consequences will in turn affect gene transcription rates in the respective chondrocytes. In human OA research this proposed feedback mechanism opposes a challenge when interpreting results generated from transcriptomic experiments, whereas it is not encountered as such in the context of genetic association analyses. In an attempt to bridge this gap (and while doing so refrain from complex clinical translation of animal and *in-vitro* experiments), studying the underlying mechanisms that might regulate gene expression in human articular cartilage *in-vivo* might just help us in understanding the complexity at hand.



## EPIGENETICS IN OSTEOARTHRITIS

Epigenetic mechanisms are traditionally described as heritable modifications of the genome without affecting the actual nucleotide sequence (75,76), although in recent years they have emerged as cellular mechanisms that serve as dynamic regulators of gene expression (77–79). Nonetheless, despite the ambiguous inheritance of epigenetic modifications, its strong correlation with the proximal nucleotide sequence, gene expression levels as well as tissue specificity (80) make it highly applicable for OA research. While a broad spectrum of epigenetic modifiers and levels exist, such as histone modifications (81) and small non-coding RNA expression (82–84), DNA methylation appears studied most intensively (68,85–89). Addition of a methyl group to the 5th carbon atom of cytosines in cytosine-guanine residues (CpGs) within the genomic DNA is known to correlate with gene expression levels, likely due to interference with binding of DNA binding proteins (DBPs) to the genomic DNA (79). Although gene expression is regulated by a vast number of mechanisms, binding of DBPs such as transcription factors is pivotal herein. Additionally, DNA methylation levels at specific positions along the genome are known to be highly tissue specific, partly due to mitotic inheritance of the respective somatic layers during development, but presumably also to maintain cellular differentiation in adult tissues (80). Hence, in light of the strong relation with gene expression as well as constituting distinct tissue profiles, studying DNA methylation seems an appealing endeavour to further dissect the molecular genetic facets of OA (3,90).

Early studies assessing DNA methylation in OA affected articular cartilage have revealed that DNA methylation levels of specific CpGs in or near known OA associated genes often reflect the disease status of the respective tissue. For example, DNA methylation levels of CpGs near multiple matrix metalloproteinases (*MMP3*, *MMP9* and *MMP13*) (91–93), *GDF5* (86,87), *SOX9* (94,95), *IL1* (93,96), *NOS2* (97) and *COL9* (98) were shown to be significantly different between OA affected and unaffected articular cartilage. Notably, these differences in methylation levels coincided with a significant difference in expression levels of the respective genes as well. More recently, multiple studies have reported on (semi-)genome wide DNA methylation profiles of articular cartilage in the context of OA, in part due to the development of affordable genome wide DNA methylation arrays (85,88). By comparing DNA methylation at the methylome level between OA affected and unaffected articular cartilage, numerous differentially methylated CpGs have been reported and confirmed (85,88). However, it is presently still unclear if, and to what extent these CpGs correlate with gene expression levels in articular cartilage. While it was generally accepted that increased methylation or hypermethylation marks decreased transcription of respective genes, an increasing number of CpGs have been reported of which hypermethylation correlates with increased expression. Interestingly, the canonical view of transcriptional downregulation alongside hypermethylation is often observed for CpGs that reside in transcriptional promoters or enhancers, whereas CpGs that do so in opposite

direction are generally located between the transcription start and ending sites of genes (77,79,81). Consequently, given that interpreting cross-sectional DNA methylation differences is challenging on its own, doing so without simultaneous and quantitative knowledge of the transcriptome appears to considerably impede our ability to do so accurately.

### **SUPERIMPOSING CAUSALITY AND/OR DIRECTIONAL EFFECT**

In light of the descriptive nature of the aforementioned transcriptomic and methylomic experiments (i.e. cross-sectional data points), we are inherently refrained from implying causality. This statistical impediment affects *in-vivo* human OA research in multiple ways. Firstly, when we study OA transcriptomic and/or methylomic profiles as described earlier, we cannot differentiate between drivers and markers of the ongoing pathophysiological processes. Secondly, if our interest goes out to understanding the relationship between DNA methylation and gene expression in articular cartilage, we encounter a proposed regulatory feedback loop that prevents us from stating a directional relationship between the two. Within these boundaries, however, identification of CpGs for which their methylation levels quantitatively correlate both with the presence of particular alleles as well as with proximal gene expression, would be indicative of a regulatory relation that propagates from genome to methylome to transcriptome.

### **AIM AND CONTENT OF THIS THESIS**

The here presented thesis aims to address some of the challenges and concerns outlined in the previous sections. As such, the following chapters will elaborate on a number of specific scientific challenges to which the OA research field is currently opposed to.

As candidate gene approaches have resulted in the successful identification of multiple OA predisposing SNPs that exert their disease association through AI in articular cartilage, we have assessed AI on a genome wide scale in articular cartilage. By simultaneously utilizing both transcript sequence as well as abundance information derived in RNA sequencing data generated from human articular cartilage, **chapter 2** describes a transcriptome wide approach to detect novel SNPs that mark AI in articular cartilage. Subsequently in **chapter 3**, we reveal that the generated data and results in chapter 2 can be used to support ongoing OA GWA studies.

Multiple reports on OA susceptibility SNPs that mark imbalanced expression in articular cartilage of their respective genes have been published, as our group has done so for rs225014 (*DIO2*). Given these observations, we were interested in the regulatory mechanisms that might underlie the reported AI and investigated the role of epigenetic regulation of *DIO2* expression by DNA methylation in knee and hip articular cartilage. **Chapter 4** describes the relation between the alleles of rs225014,

proximal DNA methylation and expression of *DIO2* in articular cartilage. Given the insightful results obtained in chapter 4, we next addressed DNA methylation in articular on a semi-methylome wide scale. In **chapter 5** we integrate methylomic and transcriptomic data derived from preserved and paired OA lesioned articular cartilage and discuss the associations in the context of the genetic background. As part of quality control we observed, to some extent by serendipity, distinct clustering of samples upon dimension reduction of the DNA methylation data described in chapter 5, independent of gender and affection status. **Chapter 6** expands hereon and describes apparent joint specific epigenomic profiles. Consequently, motivated by both the increasing body of literature on DNA methylation research in OA, as well as our own efforts, **chapter 7** summarizes the current status and the putative future perspectives of DNA methylation research in OA.

## REFERENCES

1. Ray MB. Osteoarthritis. *PostgradMedJ*. 1937;13 (143):311–20.
2. Nationaal Kompas Volksgezondheid. 2011.
3. Tsezou A. Osteoarthritis year in review 2014: genetics and genomics. *OsteoarthritisCartilage*. 2014 Dec;22(1522–9653):2017–24.
4. Holla JF, Steultjens MP, van der Leeden M, Roorda LD, Bierma-Zeinstra SM, den Broeder AA, et al. Determinants of range of joint motion in patients with early symptomatic osteoarthritis of the hip and/or knee: an exploratory study in the CHECK cohort. *OsteoarthritisCartilage*. 2011 Apr;19(1522–9653):411–9.
5. Elliott KS, Chapman K, Day-Williams A, Panoutsopoulou K, Southam L, Lindgren CM, et al. Evaluation of the genetic overlap between osteoarthritis with body mass index and height using genome-wide association scan data. *AnnRheumDis*. 2013 Jun;72(1468–2060):935–41.
6. Schenker ML, Mauck RL, Ahn J, Mehta S. Pathogenesis and prevention of posttraumatic osteoarthritis after intra-articular fracture. *JAmAcadOrthopSurg*. 2014 Jan;22(1067–151X (Print)):20–8.
7. Valdes AM, Spector TD. The genetic epidemiology of osteoarthritis. *CurrOpinRheumatol*. 2010 Mar;22(1531–6963):139–43.
8. Benichou OD, Hunter DJ, Nelson DR, Guermazi A, Eckstein F, Kwok K, et al. One-year change in radiographic joint space width in patients with unilateral joint space narrowing: data from the Osteoarthritis Initiative. *Arthritis Care Res(Hoboken)*. 2010 Jul;62(2151–4658):924–31.
9. Fuerst M, Bertrand J, Lammers L, Dreier R, Echtermeyer F, Nitschke Y, et al. Calcification of articular cartilage in human osteoarthritis. *Arthritis Rheum*. 2009 Sep;60(0004–3591 (Print)):2694–703.
10. Damen J, Schiphof D, Wolde ST, Cats HA, Bierma-Zeinstra SM, Oei EH. Inter-observer reliability for radiographic assessment of early osteoarthritis features: the CHECK (cohort hip and cohort knee) study. *OsteoarthritisCartilage*. 2014 Jul;22(1522–9653):969–74.
11. Nevitt MC, Zhang Y, Javadi MK, Neogi T, Curtis JR, Niu J, et al. High systemic bone mineral density increases the risk of incident knee OA and joint space narrowing, but not radiographic progression of existing knee OA: the MOST study. *AnnRheumDis*. 2010 Jan;69(1468–2060):163–8.
12. Man GS, Mologhianu G. Osteoarthritis pathogenesis - a complex process that involves the entire joint. *J Med Life* . 2014;7 (1):37–41.
13. Beekhuizen M, Bastiaansen-Jenniskens YM, Koevoet W, Saris DB, Dhert WJ, Creemers LB, et al. Osteoarthritic synovial tissue inhibition of proteoglycan production in human osteoarthritic knee cartilage: establishment and characterization of a long-term cartilage-synovium coculture. *Arthritis Rheum*. 2011 Jul;63(1529–0131):1918–27.
14. Yuan XL, Meng HY, Wang YC, Peng J, Guo QY, Wang AY, et al. Bone-cartilage interface crosstalk in osteoarthritis: potential pathways and future therapeutic strategies. *OsteoarthritisCartilage*. 2014 Aug;22(1522–9653):1077–89.
15. Sun MM, Beier F. Chondrocyte hypertrophy in skeletal development, growth, and disease. *Birth Defects ResCEmbryoToday*. 2014 Mar;102(1542–9768):74–82.
16. Sophia Fox AJ, Bedi A, Rodeo SA. The Basic Science of Articular Cartilage: Structure, Composition, and Function. *Sports Health* . 2009 Nov;1 (6):461–8.
17. Aigner T, Fundel K, Saas J, Gebhard PM, Haag J, Weiss T, et al. Large-scale gene expression profiling reveals major pathogenetic pathways of cartilage degeneration in osteoarthritis. *Arthritis Rheum*. 2006 Nov;54(0004–3591 (Print)):3533–44.
18. Peffers M, Liu X, Clegg P. Transcriptomic signatures in cartilage ageing. *Arthritis ResTher*. 2013;15(1478–6362):R98.
19. Goldring MB, Marcu KB. Cartilage homeostasis in health and rheumatic diseases. *Arthritis ResTher*. 2009;11(1478–6362):224.
20. Ratnayake M, Ploger F, Santibanez-Koref M, Loughlin J. Human chondrocytes respond discordantly to the protein encoded by the osteoarthritis susceptibility gene GDF5. *PLoSOne*. 2014;9(1932–6203):e86590.
21. Thomas CM, Whittles CE, Fuller CJ, Sharif M. Variations in chondrocyte apoptosis may explain the increased prevalence of osteoarthritis in some joints. *RheumatolInt*. 2011 Oct;31(1437–160X):1341–8.
22. Goldring MB. Chondrogenesis, chondrocyte differentiation, and articular cartilage metabolism in health and osteoarthritis. *TherAdvMusculoskeletDis*. 2012 Aug;4(1759–7218):269–85.

23. Saito T, Fukai A, Mabuchi A, Ikeda T, Yano F, Ohba S, et al. Transcriptional regulation of endochondral ossification by HIF-2 $\alpha$  during skeletal growth and osteoarthritis development. *NatMed*. 2010 Jun;16(1546–170X):678–86.
24. Meulenbelt I, Bos SD, Chapman K, van der Breggen R, Houwing-Duistermaat JJ, Kremer D, et al. Meta-analyses of genes modulating intracellular T3 bio-availability reveal a possible role for the DIO3 gene in osteoarthritis susceptibility. *AnnRheumDis*. 2011 Jan;70(1468–2060):164–7.
25. Shao YY, Wang L, Ballock RT. Thyroid hormone and the growth plate. *RevEndocrMetab Disord*. 2006 Dec;7(1389–9155 (Print)):265–71.
26. Mueller MB, Tuan RS. Functional characterization of hypertrophy in chondrogenesis of human mesenchymal stem cells. *Arthritis Rheum*. 2008 May;58(0004–3591 (Print)):1377–88.
27. Xiong DH, Liu XG, Guo YF, Tan LJ, Wang L, Sha BY, et al. Genome-wide association and follow-up replication studies identified ADAMTS18 and TGFBR3 as bone mass candidate genes in different ethnic groups. *AmJHumGenet*. 2009 Mar;84(1537–6605):388–98.
28. Panoutsopoulou K, Southam L, Elliott KS, Wrayner N, Zhai G, Beazley C, et al. Insights into the genetic architecture of osteoarthritis from stage 1 of the arcOGEN study. *AnnRheumDis*. 2011 May;70(1468–2060):864–7.
29. Yerges-Armstrong LM, Yau MS, Liu Y, Krishnan S, Renner JB, Eaton CB, et al. Association analysis of BMD-associated SNPs with knee osteoarthritis. *JBone MinerRes*. 2014 Jun;29(1523–4681):1373–9.
30. Evans DS, Cailotto F, Parimi N, Valdes AM, Castano-Betancourt MC, Liu Y, et al. Genome-wide association and functional studies identify a role for IGFBP3 in hip osteoarthritis. *AnnRheumDis*. 2014 Jun 13;(1468–2060).
31. Valdes AM, Evangelou E, Kerkhof HJ, Tamm A, Doherty SA, Kisand K, et al. The GDF5 rs143383 polymorphism is associated with osteoarthritis of the knee with genome-wide statistical significance. *AnnRheumDis*. 2011 May;70(1468–2060):873–5.
32. Meulenbelt I, Min JL, Bos S, Riyazi N, Houwing-Duistermaat JJ, van der Wijk HJ, et al. Identification of DIO2 as a new susceptibility locus for symptomatic osteoarthritis. *HumMolGenet*. 2008 Jun 15;17(1460–2083):1867–75.
33. Meulenbelt I, Bijkerk C, Breedveld FC, Slagboom PE. Genetic linkage analysis of 14 candidate gene loci in a family with autosomal dominant osteoarthritis without dysplasia. *JMedGenet*. 1997 Dec;34(0022–2593 (Print)):1024–7.
34. Meulenbelt I, Chapman K, Dieguez-Gonzalez R, Shi D, Tsezou A, Dai J, et al. Large replication study and meta-analyses of DVWA as an osteoarthritis susceptibility locus in European and Asian populations. *HumMolGenet*. 2009 Apr 15;18(1460–2083):1518–23.
35. Evangelou E, Kerkhof HJ, Styrkarsdottir U, Ntzani EE, Bos SD, Esko T, et al. A meta-analysis of genome-wide association studies identifies novel variants associated with osteoarthritis of the hip. *AnnRheumDis*. 2013 Sep 4;(1468–2060).
36. Styrkarsdottir U, Thorleifsson G, Helgadóttir HT, Bomer N, Metrustry S, Bierma-Zeinstra S, et al. Severe osteoarthritis of the hand associates with common variants within the ALDH1A2 gene and with rare variants at 1p31. *NatGenet*. 2014 May;46(1546–1718):498–502.
37. Day-Williams AG, Southam L, Panoutsopoulou K, Rayner NW, Esko T, Estrada K, et al. A Variant in MCF2L Is Associated with Osteoarthritis. *AmJHumGenet*. 2011 Sep 9;89(1537–6605):446–50.
38. Kerkhof HJ, Lories RJ, Meulenbelt I, Jonsdóttir I, Valdes AM, Arp P, et al. A genome-wide association study identifies an osteoarthritis susceptibility locus on chromosome 7q22. *Arthritis Rheum*. 2010 Feb;62(0004–3591 (Print)):499–510.
39. Evangelou E, Chapman K, Meulenbelt I, Karassa FB, Loughlin J, Carr A, et al. Large-scale analysis of association between GDF5 and FRZB variants and osteoarthritis of the hip, knee, and hand. *Arthritis Rheum*. 2009 Jun;60(0004–3591 (Print)):1710–21.
40. Evangelou E, Valdes AM, Castano-Betancourt MC, Doherty M, Doherty S, Esko T, et al. The DOT1L rs12982744 polymorphism is associated with osteoarthritis of the hip with genome-wide statistical significance in males. *AnnRheumDis*. 2013 Jul;72(1468–2060):1264–5.
41. Valdes AM, Spector TD, Doherty S, Wheeler M, Hart DJ, Doherty M. Association of the DVWA and GDF5 polymorphisms with osteoarthritis in UK populations. *AnnRheumDis*. 2009 Dec;68(1468–2060):1916–20.
42. Miyamoto Y, Mabuchi A, Shi D, Kubo T, Takatori Y, Saito S, et al. A functional polymorphism in the 5' UTR of GDF5 is associated with susceptibility to osteoarthritis. *NatGenet*. 2007 Apr;39(1061–4036 (Print)):529–33.
43. Chapman K, Takahashi A, Meulenbelt I, Watson C, Rodriguez-Lopez J, Egli R, et al. A meta-analysis of Euro-

- pean and Asian cohorts reveals a global role of a functional SNP in the 5' UTR of GDF5 with osteoarthritis susceptibility. *HumMolGenet.* 2008 May 15;17(1460–2083):1497–504.
44. Valdes AM, Loughlin J, Oene M V, Chapman K, Surdulescu GL, Doherty M, et al. Sex and ethnic differences in the association of ASPN, CALM1, COL2A1, COMP, and FRZB with genetic susceptibility to osteoarthritis of the knee. *Arthritis Rheum.* 2007 Jan;56(0004–3591 (Print)):137–46.
  45. Loughlin J, Dowling B, Chapman K, Marcelline L, Mustafa Z, Southam L, et al. Functional variants within the secreted frizzled-related protein 3 gene are associated with hip osteoarthritis in females. *ProcNatlAcadSciUSA.* 2004 Jun 29;101(0027–8424 (Print)):9757–62.
  46. Castano Betancourt MC, Cailotto F, Kerkhof HJ, Cornelis FM, Doherty SA, Hart DJ, et al. Genome-wide association and functional studies identify the DOT1L gene to be involved in cartilage thickness and hip osteoarthritis. *ProcNatlAcadSciUSA.* 2012 May 22;109(1091–6490):8218–23.
  47. Zeggini E, Panoutsopoulou K, Southam L, Rayner NW, Day-Williams AG, Lopes MC, et al. Identification of new susceptibility loci for osteoarthritis (arcOGEN): a genome-wide association study. *Lancet.* 2012 Sep 1;380(1474–547X):815–23.
  48. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. *Nature.* 2009;461 (7265):747–53.
  49. Jordan JM, Kraus VB, Hochberg MC. Genetics of osteoarthritis. *CurrRheumatolRep.* 2004 Feb;6(1523–3774 (Print)):7–13.
  50. Kloppenburg M, Kwok WY. Hand osteoarthritis—a heterogeneous disorder. *NatRevRheumatol.* 2012 Jan;8(1759–4804):22–31.
  51. Ramos YF, Bos SD, van der Breggen R, Kloppenburg M, Ye K, Lameijer EW, et al. A gain of function mutation in TNFRSF11B encoding osteoprotegerin causes osteoarthritis with chondrocalcinosis. *AnnRheumDis.* 2014 Apr 17;(1468–2060).
  52. Peters JE, Lyons PA, Lee JC, Richard AC, Fortune MD, Newcombe PJ, et al. Insight into Genotype-Phenotype Associations through eQTL Mapping in Multiple Cell Types in Health and Immune-Mediated Disease. *PLoS Genet.* 2016;12 (3):e1005908.
  53. Westra HJ, Franke L. From genome to function by studying eQTLs. *BiochimBiophysActa.* 2014 May 4;(0006–3002 (Print)).
  54. Westra H-J, Peters MJ, Esko T, Yaghootkar H, Schurmann C, Kettunen J, et al. Systematic identification of trans eQTLs as putative drivers of known disease associations. *Nat Genet.* 2013;45 (10):1238–43.
  55. Gee F, Clubbs CF, Raine E V, Reynard LN, Loughlin J. Allelic expression analysis of the osteoarthritis susceptibility locus that maps to chromosome 3p21 reveals cis-acting eQTLs at GNL3 and SPCS1. *BMCMedGenet.* 2014;15(1471–2350):53.
  56. Egli RJ, Southam L, Wilkins JM, Lorenzen I, Pombo-Suarez M, Gonzalez A, et al. Functional analysis of the osteoarthritis susceptibility-associated GDF5 regulatory polymorphism. *Arthritis Rheum.* 2009 Jul;60(0004–3591 (Print)):2055–64.
  57. Bos SD, Bovee J V, Duijnisveld BJ, Raine E V, van Dalen WJ, Ramos YF, et al. Increased type II deiodinase protein in OA-affected cartilage and allelic imbalance of OA risk polymorphism rs225014 at DIO2 in human OA joint tissues. *AnnRheumDis.* 2012 Jul;71(1468–2060):1254–8.
  58. Raine E V, Dodd AW, Reynard LN, Loughlin J. Allelic expression analysis of the osteoarthritis susceptibility gene COL11A1 in human joint tissues. *BMCMusculoskeletDisord.* 2013;14(1471–2474):85.
  59. Bos SD, Suchiman HE, Kloppenburg M, Houwing-Duistermaat JJ, le Graverand MP, Seymour AB, et al. Allelic variation at the C-reactive protein gene associates to both hand osteoarthritis severity and serum high sensitive C-reactive protein levels in the GARP study. *AnnRheumDis.* 2008 Jun;67(1468–2060):877–9.
  60. Price AL, Helgason A, Thorleifsson G, McCarrroll SA, Kong A, Stefansson K. Single-tissue and cross-tissue heritability of gene expression via identity-by-descent in related or unrelated individuals. *PLoS Genet.* 2011;7 (2).
  61. Petretto E, Mangion J, Dickens NJ, Cook SA, Kumaran MK, Lu H, et al. Heritability and tissue specificity of expression quantitative trait loci. *PLoS Genet.* 2006;2 (10):1625–33.
  62. Huang J, Chen J, Esparza J, Ding J, Elder JT, Abecasis GR, et al. eQTL mapping identifies insertion- and deletion-specific eQTLs in multiple tissues. *Nat Commun.* 2015;6(May):6821.
  63. Estrada K, Styrkarsdottir U, Evangelou E, Hsu YH, Duncan EL, Ntzani EE, et al. Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. *NatGenet.* 2012 May;44(1546–1718):491–501.

64. Raine E V, Wreglesworth N, Dodd AW, Reynard LN, Loughlin J. Gene expression analysis reveals HBP1 as a key target for the osteoarthritis susceptibility locus that maps to chromosome 7q22. *AnnRheumDis*. 2012 Dec;71(1468–2060):2020–7.
65. Upton AR, Holding CA, Dharmapatni AA, Haynes DR. The expression of RANKL and OPG in the various grades of osteoarthritic cartilage. *RheumatolInt*. 2012 Feb;32(1437–160X):535–40.
66. Nagase H, Nagasawa Y, Tachida Y, Sakakibara S, Okutsu J, Suematsu N, et al. Deiodinase 2 upregulation demonstrated in osteoarthritis patients cartilage causes cartilage destruction in tissue-specific transgenic rats. *OsteoarthritisCartilage*. 2013 Mar;21(1522–9653):514–23.
67. Kostopoulou F, Gkretsi V, Malizos KN, Iliopoulos D, Oikonomou P, Poultsides L, et al. Central role of SREBP-2 in the pathogenesis of osteoarthritis. *PLoSOne*. 2012;7(1932–6203):e35753.
68. Takahashi A, de Andrés MC, Hashimoto K, Itoi E, Oreffo ROC. Epigenetic regulation of interleukin-8, an inflammatory chemokine, in osteoarthritis. *Osteoarthr Cartil*. 2015;23 (11):1946–54.
69. Ijiri K, Zerbinì LF, Peng H, Otu HH, Tsuchimochi K, Otero M, et al. Differential expression of GADD45beta in normal and osteoarthritic cartilage: potential role in homeostasis of articular chondrocytes. *Arthritis Rheum*. 2008 Jul;58(0004–3591 (Print)):2075–87.
70. Duval E, Bigot N, Hervieu M, Kou I, Leclercq S, Galera P, et al. Asporin expression is highly regulated in human chondrocytes. *MolMed*. 2011;17(1528–3658):816–23.
71. Lisignoli G, Toneguzzi S, Grassi F, Piacentini A, Tschon M, Cristino S, et al. Different chemokines are expressed in human arthritic bone biopsies: IFN-gamma and IL-6 differently modulate IL-8, MCP-1 and rantes production by arthritic osteoblasts. *Cytokine*. 2002 Dec 7;20(1043–4666 (Print)):231–8.
72. Logar DB, Komadina R, Prezelj J, Ostanek B, Trost Z, Marc J. Expression of bone resorption genes in osteoarthritis and in osteoporosis. *JBone MinerMetab*. 2007;25(0914–8779 (Print)):219–25.
73. Lambert C, Dubuc JE, Montell E, Verges J, Munaut C, Noel A, et al. Gene expression pattern of cells from inflamed and normal areas of osteoarthritis synovial membrane. *Arthritis Rheumatol*. 2014 Apr;66(2326–5205):960–8.
74. Leijten JC, Emons J, Sticht C, van GS, Decker E, Uitterlinden A, et al. Gremlin 1, frizzled-related protein, and Dkk-1 are key regulators of human articular cartilage homeostasis. *Arthritis Rheum*. 2012 Oct;64(1529–0131):3302–12.
75. Eccleston A, DeWitt N, Gunter C, Marte B, Nath D. Epigenetics. *Nature* . 2007;447 (7143):395–395.
76. Bird A. Perceptions of epigenetics. *Nature* . 2007;447 (7143):396–8.
77. Bell JT, Pai AA, Pickrell JK, Gaffney DJ, Pique-Regi R, Degner JF, et al. DNA methylation patterns associate with genetic and gene expression variation in HapMap cell lines. *Genome Biol* . 2011;12 (1):R10.
78. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat Rev Genet* . 2012;13 (7):484–92.
79. Muers M. Gene expression: Disentangling DNA methylation. *Nat Rev Genet* . 2013;14 (8):519.
80. Sliker RC, Bos SD, Goeman JJ, Bovee J V, Talens RP, van der Breggen R, et al. Identification and systematic annotation of tissue-specific differentially methylated regions using the Illumina 450k array. *EpigeneticsChromatin*. 2013;6(1756–8935):26.
81. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *NatGenet*. 2003 Mar;33 Suppl(1061–4036 (Print)):245–54.
82. Mendell JT, Olson EN. MicroRNAs in stress signaling and human disease. *Cell*. 2012 Mar 16;148(1097–4172):1172–87.
83. Zhang L, Yang M, Marks P, White LM, Hurtig M, Mi QS, et al. Serum non-coding RNAs as biomarkers for osteoarthritis progression after ACL injury. *OsteoarthritisCartilage*. 2012 Dec;20(1522–9653):1631–7.
84. Barter MJ, Young DA. Epigenetic mechanisms and non-coding RNAs in osteoarthritis. *CurrRheumatolRep*. 2013 Sep;15(1534–6307):353.
85. Fernandez-Tajes J, Soto-Hermida A, Vazquez-Mosquera ME, Cortes-Pereira E, Mosquera A, Fernandez-Moreno M, et al. Genome-wide DNA methylation analysis of articular chondrocytes reveals a cluster of osteoarthritic patients. *AnnRheumDis*. 2014 Apr;73(1468–2060):668–77.
86. Reynard LN, Bui C, Syddall CM, Loughlin J. CpG methylation regulates allelic expression of GDF5 by modulating binding of SP1 and SP3 repressor proteins to the osteoarthritis susceptibility SNP rs143383. *HumGenet*. 2014 May 27;(1432–1203).
87. Reynard LN, Bui C, Canty-Laird EG, Young DA, Loughlin J. Expression of the osteoarthritis-associated gene GDF5 is modulated epigenetically by DNA methylation. *HumMolGenet*. 2011 Sep 1;20(1460–2083):3450–

- 60.
88. Rushton MD, Reynard LN, Barter MJ, Refaie R, Rankin KS, Young DA, et al. Characterization of the cartilage DNA methylome in knee and hip osteoarthritis. *Arthritis Rheumatol*. 2014 May 16;(2326–5205).
  89. Delgado-Calle J, Fernandez AF, Sainz J, Zarrabeitia MT, Sanudo C, Garcia-Renedo R, et al. Genome-wide profiling of bone reveals differentially methylated regions in osteoporosis and osteoarthritis. *Arthritis Rheum*. 2013 Jan;65(1529–0131):197–205.
  90. Loughlin J, Reynard LN. Osteoarthritis: Epigenetics of articular cartilage in knee and hip OA. *Nat Rev Rheumatol*. 2015;11 (1):6–7.
  91. Roach HI, Yamada N, Cheung KSC, Tilley S, Clarke NMP, Oreffo ROC, et al. Association between the abnormal expression of matrix-degrading enzymes by human osteoarthritic chondrocytes and demethylation of specific CpG sites in the promoter regions. *Arthritis Rheum*. 2005;52 (10):3110–24.
  92. Bui C, Barter MJ, Scott JL, Xu Y, Galler M, Reynard LN, et al. cAMP response element-binding (CREB) recruitment following a specific CpG demethylation leads to the elevated expression of the matrix metalloproteinase 13 in human articular chondrocytes and osteoarthritis. *FASEB J*. 2012;26 (7):3000–11.
  93. Hashimoto K, Otero M, Imagawa K, De Andrés MC, Coico JM, Roach HI, et al. Regulated transcription of human matrix metalloproteinase 13 (MMP13) and interleukin-1 $\beta$  (IL1B) genes in chondrocytes depends on methylation of specific proximal promoter CpG sites. *J Biol Chem*. 2013;288 (14):10061–72.
  94. Im G-I, Kim K-I, Park S-Y. Increased methylation status of SOX9 gene promoter in human osteoarthritic cartilage. *Ann Rheum Dis*. 2013;71.
  95. Kim K II, Park YS, Im G II. Changes in the epigenetic status of the SOX-9 promoter in human osteoarthritic cartilage. *J Bone Miner Res*. 2013;28 (5):1050–60.
  96. Hashimoto K, Oreffo ROC, Gibson MB, Goldring MB, Roach HI. DNA demethylation at specific CpG sites in the IL1B promoter in response to inflammatory cytokines in human articular chondrocytes. *Arthritis Rheum*. 2009;60 (11):3303–13.
  97. Andrés MC, Imagawa K, Hashimoto K, Gonzalez A, Roach HI, Goldring MB and Oreffo ROC, et al. Loss of methylation in CpG sites in the proximal coding region and NF-B enhancer elements of iNOS are responsible for gene induction in human articular chondrocytes. *Bone*. 2011;48:S155.
  98. Imagawa K, De Andrés MC, Hashimoto K, Itoi E, Otero M, Roach HI, et al. Association of reduced type IX collagen gene expression in human osteoarthritic chondrocytes with epigenetic silencing by DNA hypermethylation. *Arthritis Rheumatol*. 2014;66 (11):3040–51.





