

The Genetic Epidemiological Landscape of Hip and Knee Osteoarthritis: Where Are We Now and Where Are We Going?

Eleni Zengini, Chris Finan, and J. Mark Wilkinson

ABSTRACT. Osteoarthritis (OA) is a complex disease that affects the whole joint, with multiple biological and environmental factors contributing to its development. The heritable component for primary OA accounts for ~50% of susceptibility. So far, candidate gene studies and genome-wide association scans have established 18 OA-associated loci. These findings account for 11% of the heritability, explaining a rather small fraction of the genetic component. To further unravel the genetic architecture of OA, the field needs to facilitate more precise phenotypic definitions, high genome coverage, and large sample metaanalyses, expecting the identification of rare and low frequency variants with potentially higher penetrance, and more accurate methods for calculating phenotype-genotype correlation. Expression analysis, epigenetics, and investigation of interactions can also help clarify the implicated transcriptional regulatory pathways and provide insights into further novel pathogenic OA mechanisms leading to diagnostic biomarker identification and new, more focused therapeutic disease approaches. (J Rheumatol First Release December 1 2015; doi:10.3899/jrheum.150710)

Key Indexing Terms:

OSTEOARTHRITIS GENETIC STUDIES SINGLE-NUCLEOTIDE POLYMORPHISM

Osteoarthritis (OA) is a musculoskeletal disorder that causes degradation of synovial joints. It is the most common joint disease, and most frequently affects the hip, knee, spine, and the small joints of the hand and the foot. OA causes loss of articular cartilage, formation of osteophytes, changes in subchondral bone, and synovitis¹. It is a complex disease that affects the whole joint, with multiple biochemical, genetic, biological, morphological, biomechanical, and environmental factors contributing to disease occurrence^{1,2}. The heritable contribution to primary OA susceptibility has been established by twin and sibling studies to be about 50% (range 40–65%), and is transmitted in a non-Mendelian manner³. OA typically has its onset in mid-to-later life, indicating that age is a susceptibility risk factor. OA is more common in women, particularly following menopause. Sex differences may be partly explained by the suggested involvement of

estrogen in cartilage metabolism⁴. OA is also thought to be associated with body mass index, joint injury, and lifestyle¹. OA has a large effect on society. Psychological and physical disability lead to a substantial economic burden that is expected to grow with the increasing prevalence of OA⁵.

The prevalence of hip and knee OA varies by ethnicity. Asian populations show a higher prevalence for knee OA and a lower prevalence for hip OA in the Chinese and Japanese compared with populations of European descent⁶. These differences in OA prevalence may also indicate genetic heterogeneity. The interplay between genetic and environmental factors in OA is not fully understood and only a small fraction of the genetic component has been explained. The purpose of our perspective article is to review the current genetic epidemiological landscape of OA and to explore future opportunities to further unravel its genetic architecture.

From the Dromokaiteio Psychiatric Hospital of Athens, Athens, Greece; Department of Human Metabolism, University of Sheffield, Sheffield; Wellcome Trust Sanger Institute, Hinxton, UK.

E. Zengini, BSc, PhD Student, Dromokaiteio Psychiatric Hospital of Athens, and Department of Human Metabolism, University of Sheffield; C. Finan, PhD, Research Excellence Fellow, PhD, Wellcome Trust Sanger Institute; J.M. Wilkinson, FRCS (Tr&Orth), PhD, Professor of Orthopaedics, Department of Human Metabolism, University of Sheffield, Honorary Consultant Orthopaedic Surgeon, Sheffield Teaching Hospitals National Health Service Foundation Trust.

Address correspondence to Dr. J.M. Wilkinson, Department of Human Metabolism, The Medical School, Beech Hill Road, Sheffield, S10 2RX, UK. E-mail: j.m.wilkinson@sheffield.ac.uk

Accepted for publication September 23, 2015.

CURRENT FINDINGS IN OA GENETICS

Genetic architecture of OA. As with other common complex disorders, the genetic architecture of OA is underpinned by variants covering a wide range of frequencies and effect sizes⁷. Most established OA-associated variants are represented by common single-nucleotide polymorphism (SNP) with minor allele frequency (MAF) > 5% that have moderate to small effect sizes (OR ~1.1–1.3). The small number of OA loci established to date account for only a small fraction of disease heritability (10.7%; Figure 1)³. It is also possible to estimate the proportion of the genetic component explained

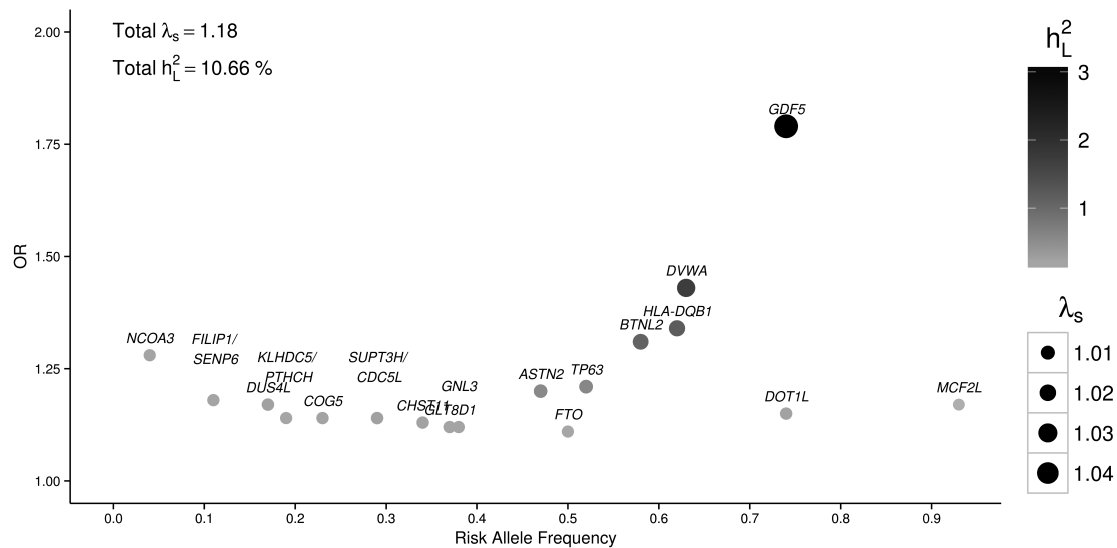


Figure 1. Effect size and RAF of established hip and knee OA-associated variants. Most SNP are common (RAF 0.04 to 0.93) with moderate to small effect sizes (OR 1.11 to 1.79) as reported in the respective studies. The sibling relative risk ratio (λ_s) for each SNP is shown by the different size of each circle. The darker color of the circles shows the percentage of variance explained on the liability scale (h^2_L ; calculated assuming 10% prevalence of OA). Totals of λ_s and h^2_L are shown on the left top of the plot. RAF: risk allele frequency; OA: osteoarthritis; SNP: single-nucleotide polymorphism.

by all variants using genome-wide association study summary statistics, with approaches such as Restricted Maximum Likelihood and Phenotype correlation genotype correlation regression^{8,9}. An application in case-control studies suggests that common variants could explain at least half the heritability for common diseases. Low frequency and rare variants (MAF 1–5% and MAF < 1%, respectively), epigenetic changes, structural variants, and gene-environment interactions may also contribute to the missing heritability of OA¹⁰.

Established OA loci. Several linkage scans have been conducted for OA, but most have failed to establish replicating allelic loci. Numerous candidate gene studies based on the (to date) limited known biological etiology of OA have been conducted and have also been irreproducible³. Multiple factors, including lack of study power, account for this lack of replication. Exceptionally, *GDF5* that has been associated repeatedly with OA was originally discovered in gene-centric studies. *GDF5* affects chondrogenesis and joint element formation during skeletal development, which are consistent with the protein expression in the cartilage and joint interzone¹¹. A candidate gene study first reported that rs143383 (in the 5' untranslated region) was associated with hip OA in 2 independent Japanese populations (OR 1.79, $p = 1.8 \times 10^{-13}$)¹². The SNP is associated with reduction in *GDF5* transcription in chondrogenic cells, which results in reduced levels of protein expression. This locus was also later found to be associated with OA in European populations¹³. Genetic variation in *GDF5* was also found to be associated with height¹⁴. It is also suggested that lower levels of *GDF5* in mice include mechanisms of altered loading and changes in

subchondral bone, exhibiting developmental failure of the condyles and the articular ligaments¹⁵ (Table 1).

Genome-wide association studies (GWAS) examine hundreds of thousands of SNP across the genome and seek to establish associations with the risk of complex diseases and traits. The insights afforded by these studies can help improve our understanding of the key pathways involved in disease pathogenesis, and may lead to novel approaches to disease diagnosis, treatment, prevention, prognostic markers, and personalized (precision) therapy. In the field of complex disease, thousands of SNP have been robustly associated with disease risk at genome-wide statistical significance ($p \leq 5 \times 10^{-8}$)¹⁶. Several small- and medium-scaled GWAS have been carried out for OA.

A GWAS identified a missense variant, rs7639618, in *DVWA* that is associated with knee OA, and replicated at a genome-wide significant level ($p = 7.3 \times 10^{-11}$) in a Japanese and combined Japanese and Han Chinese cohorts in 2008¹⁷. *DVWA* is a 276 amino acid protein that binds to β -tubulin, modulating its chondrogenic function¹⁸. The gene was also found to be more highly expressed in cartilage than in other human tissues, suggesting an involvement of *DVWA* in the metabolism of cartilage in humans (Table 1).

A further GWAS for knee OA in Japanese participants identified association with further SNP rs7775228 (OR 1.34, 95% CI 1.21–1.49, $p = 2.43 \times 10^{-8}$) and rs10947262 (OR 1.32, 95% CI 1.19–1.46; $p = 6.73 \times 10^{-8}$) in a region containing HLA class II/III genes (*HLA-DQB1* and *BTNL2*), and was replicated in Europeans¹⁹, indicating that immunologic mechanisms may also contribute to OA pathogenesis.

The Treat-OA consortium GWAS reported and replicated

Table 1. Polymorphisms associated with hip and knee OA.

SNP	Risk Allele	Nearest Gene(s)	OR	p	Risk AF	Joint	Sex Specificity	Ethnic Group	Suggested Functional Relevance to OA
rs143383-T		<i>GDF5</i>	1.79	1.8×10^{-13}	0.74	Hip	Both	Asian	Affects chondrogenesis and joint element formation
rs143383-T		<i>GDF5</i>	1.16	8.3×10^{-9}	N/A	Knee	Both	European	
rs7639618-G		<i>DVWA</i>	1.43	7.3×10^{-11}	0.63	Knee	Both	Asian	Involvement in metabolism of cartilage Immunologic mechanisms involvement
rs7775228-T		<i>HLA-DQB1</i>	1.34	2.4×10^{-8}	0.62	Knee	Both	Asian	
rs10947262-C		<i>BTNL2</i>	1.31**	$5.1 \times 10^{-9**}$	0.58**	Knee	Both	Asian, European	Expression in OA joint environment
rs3815148-C*		<i>COG5</i>	1.14	8×10^{-8}	0.23	Knee	Both	European	
rs4730250-G		<i>DUS4L</i>	1.17	9.2×10^{-9}	0.17	Knee	Both	European	
rs11842874-A		<i>MCF2L</i>	1.17	2.1×10^{-8}	0.93	Knee and hip	Both	European	
rs12982744-C		<i>DOT1L</i>	1.15 [†]	$2.2 \times 10^{-8†}$	0.74 [‡]	Hip	Males	European	
rs6094710-A		<i>NCOA3</i>	1.28	7.9×10^{-9}	0.04	Hip	Both	European	Expression in OA cartilage, involvement in regulation of hormones associated with bone metabolism, role in chondrocyte mechanotransduction
rs6976-T		<i>GLT8D1</i>	1.12	7.2×10^{-11}	0.37	Knee and hip	Both	European	Unknown
rs11177-A		<i>GNL3</i>	1.12	1.3×10^{-10}	0.38	Knee and hip	Both	European	Unknown
rs4836732-C		<i>ASTN2</i>	1.2	6.1×10^{-10}	0.47	Hip	Females	European	Unknown; regulates neuronal protein
rs9350591-T		<i>FILIP1, SENP6</i>	1.18	2.4×10^{-9}	0.11	Hip	Both	European	Unknown; <i>COL12A1</i> (close located) gene affects bone formation
rs10492367-T		<i>KLHDC5, PTHLH</i>	1.14	1.5×10^{-8}	0.19	Hip	Both	European	Affects skeletal development
rs835487-G		<i>CHST11</i>	1.13	1.6×10^{-8}	0.34	Hip	Both	European	Implication in cartilage development
rs12107036-G*		<i>TP63</i>	1.21	6.7×10^{-8}	0.52	Knee	Females	European	Unknown; involvement in facial shape development
rs8044769-C*		<i>FTO</i>	1.11	6.9×10^{-8}	0.5	Knee and hip	Females	European	Affects OA through obesity
rs10948172-G*		<i>SUPT3H, CDC5L</i>	1.14	7.9×10^{-8}	0.29	Knee and hip	Males	European	Unknown; <i>RUNX2</i> (in LD and close located) gene has a regulatory role in bone development

* SNP just below genome-wide significance. ** Values of combined replication. [†] Values of combined datasets. [‡] Risk allele frequency in Europeans from the 1000 genomes database (www.1000genomes.org). OA: osteoarthritis; SNP: single-nucleotide polymorphism; AF: allele frequency; LD: linkage disequilibrium; N/A: not available.

an association of rs3815148 in *COG5* on chromosome 7q22 with knee and hand OA in Europeans ($p = 8 \times 10^{-8}$)²⁰. SNP in *COG5* were in strong linkage disequilibrium (LD) with variants in 5 further neighboring genes (*PRKAR2B*, *HPB1*, *GPR22*, *DUS4L*, and *BCAP29*), complicating the identification of the functionally relevant genes. *Gpr22* expression was absent in mice without OA, but present in mice with OA²⁰. In a large metaanalysis and replication study, an SNP (rs4730250) in *DUS4L* was associated with knee OA at a genome-wide significant level ($p = 9.2 \times 10^{-9}$), indicating that any of these highly linked genes may contribute to the risk of developing knee OA²¹. Validated expression of these genes in the joint environment was also confirmed by functional analysis and gene expression studies using cartilage tissues from OA cases and controls, suggesting that *HBP1* could be prioritized as a potential biomarker²² (Table 1).

The *DOT1L* gene was initially associated with the hip OA endophenotype joint space width ($p = 1.1 \times 10^{-11}$)²³. Functional analysis in mice identified a role for *Dot1L* in chondrogenesis²³. The same locus was previously associated with height and skeletal development²². A GWAS using individuals of European origin identified an association of

rs12982744 with hip OA in men ($p = 7.8 \times 10^{-9}$, OR 1.17, 95% CI 1.11–1.23)²⁴.

An additional SNP (rs6094710) situated near *NCOA3* was established by a large metaanalysis of hip OA GWAS (OR 1.28, 95% CI 1.18–1.39, $p = 7.9 \times 10^{-9}$)²⁵. *NCOA3* demonstrates reduced expression in OA-affected articular cartilage when compared with macroscopically unaffected cartilage of the same joint. The involvement of *NCOA3* expression in the setting of OA remains unclear and needs to be investigated, although involvement in hormonal regulation of bone turnover, such as thyroid hormones and steroids, or the suggested involvement of the gene in chondrocyte mechanotransduction are possibilities²² (Table 1).

The arthritis research council Osteoarthritis GENetics (arcOGEN) consortium carried out a 2-stage GWAS, culminating in the largest GWAS in OA to date. The initial hip and knee OA GWAS (sample size: 3177 cases and 4894 controls) and replication performed by the arcOGEN study in the United Kingdom did not identify any loci at genome-wide significance level, underlining the need for larger sample sizes and stricter phenotype definitions of OA given the complexity and heterogeneity of the disease⁷.

Following imputation of the initial GWAS and further metaanalysis, an SNP (rs11842874) in *MCF2L* was associated with knee OA at genome-wide significance (OR 1.17, 95% CI 1.11–1.23, risk allele frequency 0.93, $p = 2.1 \times 10^{-8}$)²⁶. *MCF2L* has been reported to be involved in cell motility of the nervous system, indicating that the gene affects nociception²². Functional studies in zebrafish concluded that expression of *MCF2L* is involved in skeletal system development²⁷ (Table 1).

The final larger-scaled arcOGEN GWAS identified the association of 5 novel loci with OA at genome-wide significance level and 3 additional novel loci at borderline significance. Almost 80% of the OA cases studied had undergone hip and/or knee arthroplasty, contributing to a severe OA phenotype definition²⁸.

The strongest 2 signals established by this study were rs6976 (*GLT8D1*) and rs11177 (located in *GNL3*), both on chromosome 3p21.1 and in almost perfect LD with each other (OR for both SNP was 1.12, 95% CI 1.08–1.16, rs6976 $p = 7.2 \times 10^{-11}$, and rs11177 $p = 1.3 \times 10^{-10}$). These 2 loci are associated with both hip and knee OA in both men and women. The association was stronger in subjects with total joint replacement (TJR), illustrating the need to use stricter phenotype OA definitions. Further functional analysis is needed to unravel the underlying mechanisms²⁸ (Table 1).

An intronic SNP (rs4836732) situated in *ASTN2* was associated with hip OA in women who had undergone arthroplasty (OR 1.20, 95% CI 1.13–1.27, $p = 6.11 \times 10^{-10}$), also pointing out that stricter phenotype definition can empower analysis²⁸. *ASTN2* has been suggested to be involved in the regulation of the neuronal protein *ASTN1*, but the contributory involvement to OA mechanisms needs to be investigated (Table 1).

A further intronic SNP, rs835487 located in the *CHST11* gene, was found to be associated with hip OA in TJR subjects with an OR of 1.13 (95% CI 1.09–1.18, $p = 1.64 \times 10^{-8}$) for both sexes combined²⁸. *CHST11* is differentially expressed in OA-affected and unaffected cartilage, and the gene is implicated in cartilage development. A further signal at rs9350591 was located between *FILIP1* and *SENP6*. The signal reached genome-wide significance for hip OA (OR 1.18, 95% CI 1.12–1.25, $p = 2.42 \times 10^{-9}$)²⁸. Although there is no suggested related function of *FILIP1* or *SENP6* in OA etiology yet, *COL12A1* resides nearby and is known to be involved in bone formation²⁹. An additional signal rs10492367, which lies between *KLHDC5* and *PTHLH*, was also established as a risk locus for hip OA (OR 1.14, 95% CI 1.09–1.20, $p = 1.48 \times 10^{-8}$)²⁸. *PTHRP* has been reported to be involved in mouse skeletal development³⁰ (Table 1).

Three further variants were identified as associated with OA, but narrowly missed genome-wide significance in the arcOGEN study: the intronic variant rs8044769, located in the fat mass- and obesity-associated *FTO* gene, more highly associated in women; the intronic variant rs12107036 in

TP63, more highly associated in women who had undergone TJR; and rs10948172, which lies between *SUPT3H* and *CDC5L*, more highly associated in men²⁸. *FTO* is an established obesity risk locus³¹. The phenotypic overlap between OA and obesity has been genetically substantiated³², and the involvement of *FTO* in OA has been shown to be mediated through obesity³³. The functional involvement of *TP63* in the pathogenesis of OA is unclear, although it is reported to be involved in facial shape development³⁴. The functional involvement of *SUPT3H* and *CDC5L* remain undefined in OA, although *RUNX2*, a closely located gene in extended LD, plays a regulatory role in bone development³⁵ (Table 1). *Success of genetic locus detection in OA versus the inflammatory arthritides*. In contrast to OA, the primary signal in the autoimmune arthritides, such as rheumatoid arthritis (RA) and juvenile idiopathic arthritis, resides in the MHC at the HLA loci. Numerous further loci genome-wide have been identified, many of which overlap across rheumatic diseases but not with them³⁶. The main reasons behind these differences are thought to be the different etiopathology of OA, increased heterogeneity of the condition, and the relatively smaller GWAS conducted to date. For example, the largest metaanalysis of RA GWAS to date reached 29.9K individuals with disease compared with 7.5K patients with OA in the largest OA GWAS to date³⁷.

Epigenetics of OA. There is existing evidence that implicates the involvement of epigenetic gene expression regulatory mechanisms in the pathophysiology of OA. Several genome-wide methylation studies for hip and knee OA have been conducted to date and most focus on the cartilage^{38,39,40,41,42}. These studies demonstrate differentially methylated regions that include genes that have been associated with OA and distinct clustering of hip and knee samples. The concordance of these studies' results is limited. Further epigenetic research could enable understanding of cartilage engineering mechanisms and lead to novel epigenetic-based therapeutic strategies for OA treatment.

FUTURE PROSPECTS

OA phenotypic definition and endophenotypes. The site- and sex-specific heterogeneity of OA is reflected in the associated genetic heterogeneity. Of the 18 established OA susceptibility loci, only 1 has been associated with both knee and hand OA, 5 with both knee and hip OA, 7 with hip OA only, and 6 with knee OA only (Table 1). Similarly, 13 loci have been associated with OA in both male and female subjects, 3 in women only, and 2 in men only. The examination of narrower, more homogeneous phenotypic definitions of OA such as disease severity (e.g., arthroplasty), combined with radiographic severity grading and disability measurements, specific morphological variations, anatomic differences of patterns of the affected joints, and stratification by sex are expected to further empower the identification of the genetic

determinants of OA. Bone geometry is also thought to be genetically determined and may be a contributory factor^{43,44}. Pistol grip deformity (femoral head shape) and neck shaft angle have been associated with the hip OA phenotype⁴⁵. There is also an association between joint deformities quantified by measuring morphologic indices including alpha angle, triangular index height, lateral center edge angle, and extrusion index on pelvic radiographs with the risk of hip arthroplasty and an endstage OA phenotype⁴⁶. These findings underline the importance of further exploration of the genetic basis of well-defined OA-associated endophenotypes. For example, the OA-associated locus in the *DOTL1* gene was first discovered through its association with cartilage thickness (measured by joint space width on radiographs) in a relatively small population²³. The outcomes from the arcOGEN study also suggest that use of precise endophenotypes has the potential to enhance the detection of relevant associations²⁸.

Technological and analytical advances. In keeping with other common and complex diseases, the total of 18 OA-associated variants in Asian and European populations are represented by common SNP with moderate to small effect sizes. Some of the established signals do not replicate in all ethnic groups tested so far, even when the power of the replication cohorts is sufficiently high. These findings explain only a modest fraction of the heritable component of OA (Figure 1), although the application of genome-wide methods to assess the proportion of genetic variance explained by SNP is expected to increase this estimate⁸.

The use of high-throughput genotyping and sequencing approaches along with more advanced imputation (for example, using the Haplotype Reference Consortium data: www.haplotype-reference-consortium.org) will enable the interrogation and identification of variants from the full allelic frequency spectrum. Low frequency (MAF 1–5%) and rare variants (MAF < 1%) may have larger effect sizes and point to more functionally relevant regions and causal genes. The bigger the biological effect a gene has, the more likely it is to provide important clues about the underlying disease process. There are several examples of rare variants contributing to complex disease⁴⁷ and it is likely that OA susceptibility will also be underpinned by variants across the MAF spectrum. In addition, the focus of OA genetics studies to date has been on biallelic sequence changes. It is likely that copy number variation may also be involved. The decreasing cost of high-depth whole genome sequencing is poised to make high-throughput, large-scale genetic studies possible in the near future.

Integrative analysis. Systematic functional genomics approaches in relevant tissues will help characterize the molecular landscape of OA alongside genetics approaches. To understand and treat this multifunctional and complex chronic disease, analytical approaches need to model disease biology from a holistic perspective⁴⁸. Integrative data

analysis including targeted approaches such as transcriptomics, proteomics, metabolomics, and methylomics can help in this regard. These large heterogeneous datasets require integration and support by advanced software methodology and novel analysis tools⁴⁹. This approach necessitates multidisciplinary research⁵⁰, although studies using integrated molecular genome-wide profiling of relevant tissue have thus far been limited. Integrating the range of different approaches in OA research is the next step to unravel the underlying mechanisms of the whole joint failure.

Over the last few years there has been a profound transformation in the ability to identify genes that contribute to musculoskeletal disorders. The OA susceptibility loci established to date point to several biological pathways, but further functional analysis is needed to identify the causal genes and determine their involvement. Identification of the missing heritability could be enhanced through the use of advanced genotyping technologies, imputation, stricter OA phenotypic definitions, and the establishment of larger and better-powered studies and metacollaborations. Systematic integrative analysis is also expected to lead to a profound transformation in the ability to holistically understand this complex disease. The future opportunities of OA genetic research are brightened by all these novel approaches.

ACKNOWLEDGMENT

We are grateful to Eleftheria Zeggini for commenting on the manuscript.

REFERENCES

1. Felson DT, Lawrence RC, Dieppe PA, Hirsch R, Helmick CG, Jordan JM, et al. Osteoarthritis: new insights. Part 1: the disease and its risk factors. *Ann Intern Med* 2000;133:635-46.
2. Felson DT, Lawrence RC, Hochberg MC, McAlindon T, Dieppe PA, Minor MA, et al. Osteoarthritis: new insights. Part 2: treatment approaches. *Ann Intern Med* 2000;133:726-37.
3. Panoutsopoulou K, Zeggini E. Advances in osteoarthritis genetics. *J Med Genet* 2013;50:715-24.
4. Schicht M, Ernst J, Nielitz A, Fester L, Tsokos M, Guddat SS, et al. Articular cartilage chondrocytes express aromatase and use enzymes involved in estrogen metabolism. *Arthritis Res Ther* 2014;16:R93.
5. Cross M, Smith E, Hoy D, Nolte S, Ackerman I, Fransen M, et al. The global burden of hip and knee osteoarthritis: estimates from the Global Burden of Disease 2010 study. *Ann Rheum Dis* 2014;73:1323-30.
6. Xu L, Nevitt MC, Zhang Y, Yu W, Alibadi P, Felson DT. [High prevalence of knee, but not hip or hand osteoarthritis in Beijing elders: comparison with data of Caucasian in United States]. [Article in Chinese] *Zhonghua Yi Xue Za Zhi* 2003;83:1206-9.
7. Panoutsopoulou K, Southam L, Elliott KS, Wrayner N, Zhai G, Beazley C, et al; arcOGEN Consortium. Insights into the genetic architecture of osteoarthritis from stage 1 of the arcOGEN study. *Ann Rheum Dis* 2011;70:864-7.
8. Golan D, Lander ES, Rosset S. Measuring missing heritability: inferring the contribution of common variants. *Proc Natl Acad Sci U S A* 2014;111:E5272-81.
9. Lee SH, Wray NR, Goddard ME, Visscher PM. Estimating missing heritability for disease from genome-wide association studies. *Am J Hum Genet* 2011;88:294-305.

10. Panoutsopoulou K, Tachmazidou I, Zeggini E. In search of low-frequency and rare variants affecting complex traits. *Hum Mol Genet* 2013;22:R16-21.
11. Buxton P, Edwards C, Archer CW, Francis-West P. Growth/differentiation factor-5 (GDF-5) and skeletal development. *J Bone Joint Surg Am* 2001;83-A Suppl 1:S23-30.
12. 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. *Nat Genet* 2007;39:529-33.
13. 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. *Ann Rheum Dis* 2011;70:873-5.
14. Sanna S, Jackson AU, Nagaraja R, Willer CJ, Chen WM, Bonnycastle LL, et al. Common variants in the GDF5-UQCC region are associated with variation in human height. *Nat Genet* 2008;40:198-203.
15. Masuya H, Nishida K, Furuichi T, Toki H, Nishimura G, Kawabata H, et al. A novel dominant-negative mutation in Gdf5 generated by ENU mutagenesis impairs joint formation and causes osteoarthritis in mice. *Hum Mol Genet* 2007;16:2366-75.
16. Welter D, MacArthur J, Morales J, Burdett T, Hall P, Junkins H, et al. The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. *Nucleic Acids Res* 2014;42:D1001-6.
17. Miyamoto Y, Shi D, Nakajima M, Ozaki K, Sudo A, Kotani A, et al. Common variants in DVWA on chromosome 3p24.3 are associated with susceptibility to knee osteoarthritis. *Nat Genet* 2008;40:994-8.
18. Nakajima M, Miyamoto Y, Ikegawa S. Cloning and characterization of the osteoarthritis-associated gene DVWA. *J Bone Miner Metab* 2011;29:300-8.
19. Nakajima M, Takahashi A, Kou I, Rodriguez-Fontenla C, Gomez-Reino JJ, Furuichi T, et al. New sequence variants in HLA class II/III region associated with susceptibility to knee osteoarthritis identified by genome-wide association study. *PLoS One* 2010;5:e9723.
20. Kerkhof HJ, Lories RJ, Meulenbelt I, Jonsdottir I, Valdes AM, Arp P, et al. A genome-wide association study identifies an osteoarthritis susceptibility locus on chromosome 7q22. *Arthritis Rheum* 2010;62:499-510.
21. Evangelou E, Valdes AM, Kerkhof HJ, Styrkarsdottir U, Zhu Y, Meulenbelt I, et al; Translation Research in Europe Applied Technologies for Osteoarthritis (TreatOA). Meta-analysis of genome-wide association studies confirms a susceptibility locus for knee osteoarthritis on chromosome 7q22. *Ann Rheum Dis* 2011;70:349-55.
22. Rodriguez-Fontenla C, Gonzalez A. Genetics of osteoarthritis. *Rheumatol Clin* 2015;11:33-40.
23. Castaño Betancourt MC, Cailotto F, Kerkhof HJ, Cornelis FM, Doherty SA, Hart DJ, et al. Genome-wide association and functional studies identify the DOTIL gene to be involved in cartilage thickness and hip osteoarthritis. *Proc Natl Acad Sci U S A* 2012;109:8218-23.
24. Evangelou E, Valdes AM, Castano-Betancourt MC, Doherty M, Doherty S, Esko T, et al. The DOTIL rs12982744 polymorphism is associated with osteoarthritis of the hip with genome-wide statistical significance in males. *Ann Rheum Dis* 2013;72:1264-5.
25. 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. *Ann Rheum Dis* 2014;73:2130-6.
26. Day-Williams AG, Southam L, Panoutsopoulou K, Rayner NW, Esko T, Estrada K, et al. A variant in MCF2L is associated with osteoarthritis. *Am J Hum Genet* 2011;89:446-50.
27. Yoshizawa M, Sone M, Matsuo N, Nagase T, Ohara O, Nabeshima Y, et al. Dynamic and coordinated expression profile of dbl-family guanine nucleotide exchange factors in the developing mouse brain. *Gene Expr Patterns* 2003;3:375-81.
28. arcOGEN Consortium; arcOGEN Collaborators, Zeggini E, Panoutsopoulou K, Southam L, Rayner NW, et al. Identification of new susceptibility loci for osteoarthritis (arcOGEN): a genome-wide association study. *Lancet* 2012;380:815-23.
29. Izu Y, Sun M, Zwolanek D, Veit G, Williams V, Cha B, et al. Type XII collagen regulates osteoblast polarity and communication during bone formation. *J Cell Biol* 2011;193:1115-30.
30. Yuan Q, Sato T, Densmore M, Saito H, Schüller C, Erben RG, et al. Deletion of PTH rescues skeletal abnormalities and high osteopontin levels in Klotho^{-/-} mice. *PLoS Genet* 2012;8:e1002726.
31. Thorleifsson G, Walters GB, Gudbjartsson DF, Steinthorsdottir V, Sulem P, Helgadóttir A, et al. Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. *Nat Genet* 2009;41:18-24.
32. 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. *Ann Rheum Dis* 2013;72:935-41.
33. Panoutsopoulou K, Mestrury S, Doherty SA, Laslett LL, Maciewicz RA, Hart DJ, et al. The effect of FTO variation on increased osteoarthritis risk is mediated through body mass index: a mendelian randomisation study. *Ann Rheum Dis* 2014;73:2082-6.
34. Liu F, van der Lijn F, Schurmann C, Zhu G, Chakravarty MM, Hysi PG, et al. A genome-wide association study identifies five loci influencing facial morphology in Europeans. *PLoS Genet* 2012;8:e1002932.
35. Komori T. Regulation of bone development and extracellular matrix protein genes by RUNX2. *Cell Tissue Res* 2010;339:189-95.
36. Kurkó J, Besenyei T, Laki J, Glant TT, Míkecz K, Szekanecz Z. Genetics of rheumatoid arthritis - a comprehensive review. *Clin Rev Allergy Immunol* 2013;45:170-9.
37. Okada Y, Wu D, Trynka G, Raj T, Terao C, Ikari K, et al. Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature* 2014;506:376-81.
38. den Hollander W, Ramos YF, Bos SD, Bomer N, van der Breggen R, Lakenberg N, et al. Knee and hip articular cartilage have distinct epigenomic landscapes: implications for future cartilage regeneration approaches. *Ann Rheum Dis* 2014;73:2208-12.
39. 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;66:3040-51.
40. Fernández-Tajes J, Soto-Hermida A, Vázquez-Mosquera ME, Cortés-Pereira E, Mosquera A, Fernández-Moreno M, et al. Genome-wide DNA methylation analysis of articular chondrocytes reveals a cluster of osteoarthritic patients. *Ann Rheum Dis* 2014;73:668-77.
41. Jeffries MA, Donica M, Baker LW, Stevenson ME, Annan AC, Humphrey MB, et al. Genome-wide DNA methylation study identifies significant epigenomic changes in osteoarthritic cartilage. *Arthritis Rheumatol* 2014;66:2804-15.
42. 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;66:2450-60.
43. Karasik D, Zhou Y, Cupples LA, Hannan MT, Kiel DP, Demissie S. Bivariate genome-wide linkage analysis of femoral bone traits and leg lean mass: Framingham study. *J Bone Miner Res* 2009;24:710-8.
44. Lanyon P, Muir K, Doherty S, Doherty M. Influence of radiographic phenotype on risk of hip osteoarthritis within families. *Ann Rheum Dis* 2004;63:259-63.
45. Doherty M, Courtney P, Doherty S, Jenkins W, Maciewicz RA, Muir K, et al. Nonspherical femoral head shape (pistol grip

- deformity), neck shaft angle, and risk of hip osteoarthritis: a case-control study. *Arthritis Rheum* 2008;58:3172-82.
46. Nicholls AS, Kiran A, Pollard TC, Hart DJ, Arden CP, Spector T, et al. The association between hip morphology parameters and nineteen-year risk of end-stage osteoarthritis of the hip: a nested case-control study. *Arthritis Rheum* 2011;63:3392-400.
47. Saint Pierre A, Génin E. How important are rare variants in common disease? *Brief Funct Genomics* 2014;13:353-61.
48. Joyce AR, Palsen BØ. The model organism as a system: integrating 'omics' data sets. *Nat Rev Mol Cell Biol* 2006;7:198-210.
49. Li-Pook-Than J, Snyder M. iPOP goes the world: integrated personalized Omics profiling and the road toward improved health care. *Chem Biol* 2013;20:660-6.
50. Tieri P, de la Fuente A, Termanini A, Franceschi C. Integrating Omics data for signaling pathways, interactome reconstruction, and functional analysis. *Methods Mol Biol* 2011;719:415-33.