

## The Contribution of Genes to Osteoarthritis

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Osteoarthritis (OA) is the most prevalent form of arthritis in the elderly. Primary OA is an idiopathic phenomenon, occurring in previously intact joints, with no apparent initiating factor such as joint injury or developmental abnormalities. The disease is characterized by softening, splitting, and fragmentation (fibrillation) of articular cartilage. This process is usually accompanied by subchondral bone sclerosis, bone cysts, and bony out-growths at the joint margins (osteophytes) [1]. In the United States alone, the prevalence of clinical OA has grown to nearly 27 million, up from an estimate of 21 million for 1995 [2], and is the third most prevalent condition causing work disability [3]. OA may be local (ie, confined to one joint) or generalized [4]. Generalized OA (GOA) refers to the involvement in the disease of at least three joints or a group of joints (eg, the interphalangeal joints). Two types of generalized disease have been described: nodal and nonnodal. The nodal type features Heberden's nodes of the distal interphalangeal joints and predominates in women. The hereditary nature of Heberden's nodes was noted as early as the nineteenth century [5] and by the 1940s it was concluded that the phenotype was inherited as a dominant trait [6]. Further studies established that nodal OA often occurred in the context of OA at multiple sites and even suggested polygenic inheritance of the disease [7].

Understanding the genetic contribution to OA has two important clinical implications. First, by finding genes involved in disease risk or involved in progression, we will better understand the molecular pathogenesis of OA, which may open areas for therapeutic intervention. Second, by identifying sets of genetic variants associated with risk for disease or with progression of OA, it will be possible to detect individuals at high risk and to monitor

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disease progression better. The authors review the current knowledge about the genetic contribution to OA, focusing mostly on the hip and knee and on the specific genetic regions and genes involved.

Several strategies can be used to investigate the role of genetics in OA, including familial aggregation studies, twin studies, linkage analysis, and candidate gene-association studies (Fig. 1) [5,8,9]. Some of these methods have also been applied to risk factors or components of disease, such as cartilage volume, and to some longitudinal traits relating to OA incidence and progression. Nevertheless, the strategies geared toward the identification of the genes and variants actually involved in the disease have, to date, been applied only to radiographic or clinical OA status.

### Familial aggregation

The risk ratio for a relative of an affected individual, compared with the population prevalence, is a measure for familial aggregation of complex diseases [10]. It has been applied extensively in the field of genetic epidemiology to derive the statistical power available for a given condition to detect genetic linkage to a complex genetic disorder [11]. For affected sib-pairs, this sib recurrence risk is termed the lambda sib ( $\lambda_s$ ). Table 1 shows estimates of the sibling recurrence risk for various conditions.

It is possible to identify subjects who have clinically severe disease (eg, severe enough symptoms to lead to total joint arthroplasty [TJA]), and to compare the prevalence of OA in their siblings (who have a genetic exposure) with that in controls who are matched as closely as possible to the siblings. A study in Nottingham [12] compared the prevalence of hip OA in siblings of individuals undergoing total hip replacement (THR) with the

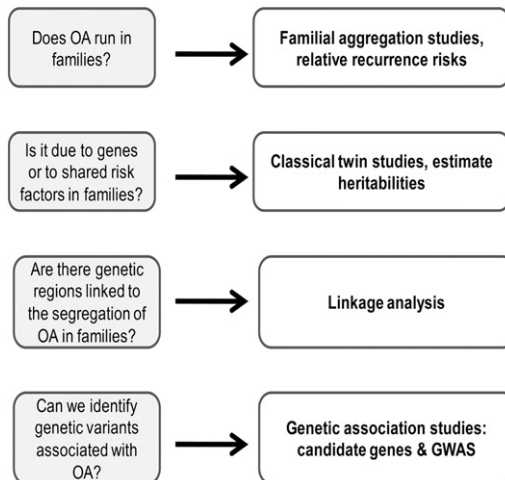


Fig. 1. Types of genetic studies. GWAS, genome-wide association studies.

Table 1  
 Familial aggregation of osteoarthritis and of other disorders

Type of disorder	Condition	How ascertained	Sibling recurrence risk ( $\lambda_S$ )	Reference
Autoimmune	Rheumatoid arthritis	Sibs with condition	5.00	[81]
	Juvenile rheumatoid arthritis	Sibs with condition	15.00	[82]
	Celiac disease	Sibs with condition	7.50–30.00	[83]
Metabolic	Obesity	Sibs with condition	1.60–1.91	[84]
	Hyperglycemia	Sibs with condition	1.39–1.81	[84]
	Type 2 diabetes	Sibs with condition	1.20–1.60	[85]
Cardiovascular	Hypertension	Sibs with condition	1.22–1.34	[84]
Osteoarthritis	Tibiofemoral OA	Sibs with TKR	2.13	[13]
	Patellofemoral OA	Sibs with TKR	1.66	[13]
	Knee OA (TF or PF)	Sibs with TKR	2.08	[13]
	TKR	Sibs with TKR	4.81	[14]
	Anteromedial OA	Sibs with UKR	3.21	[15]
	Hip osteophytes grade 3	Sibs with THR	4.27	[12]
	THR	Sibs with THR	1.87–8.53	[12,14]
	Hip KL grade $\geq 3$	Sibs with THR	4.99	[12]
Hip JSW $\leq 1.5$ mm	Sibs with THR	5.07	[12]	

*Abbreviations:* JSW, joint space width; KL, Kellgren-Lawrence grade; PF, patellofemoral; TF, tibiofemoral; THR, total hip replacement; TKR, total knee replacement; UKR, unicompartmental knee replacement.

prevalence of radiographic hip OA in controls. A similar study was performed using total knee replacement (TKR) as the selection criterion [13]. Similar data using self-reported TJA in a smaller data set were found in a study in Oxford [14]. The data presented in Table 1 indicate a strong familial aggregation, even in comparison with some autoimmune conditions known to have an important genetic component. In addition, familial aggregation of specific knee OA phenotypes, such as anteromedial OA, which correspond to lesions in the tibial plateau and preservation of cartilage of other compartments of the knee, have been reported in populations in the United Kingdom [15]. Unlike other patterns of OA, cartilage degeneration in anteromedial OA is consistent with increased loading, and thus could be assumed to be due mostly to mechanical causes, rather than genetic factors. However, the high familial aggregation reported suggests a genetic contribution, even for this particular type of OA.

#### *Familial aggregation of generalized osteoarthritis and progression of osteoarthritis*

Early studies by Kellgren and coworkers [16] in the 1950s in families of probands who had GOA involving six or more joint groups found a twofold

excess of OA among first-degree relatives compared with population controls; the recurrence risk was highest among the relatives of female probands. These recurrence risks are comparable to those for knee or hip OA shown in Table 1.

Among participants of the Genetics, Arthrosis, and Progression study (GARP) in the Netherlands, where probands were diagnosed with primary OA at multiple sites, familial aggregation has been investigated by assessing concordance rates with their siblings. The odds ratio (OR), adjusted for age, sex, and body mass index (BMI), for siblings to be affected in the same joint sites as the proband were increased in OA of the hand (OR 4.4, 95% confidence interval [CI] 2.0–9.5), hip (OR 3.9, 95% CI 1.8–8.4), spine (OR 2.2, 95% CI 1.0–5.1), hip–spine (OR 4.7, 95% CI 2.1–10.4), and hand–hip (OR 3.4, 95% CI 1.1–10.4). Siblings of probands who had OA in the knee (affected also at other joints) did not have an increased likelihood of knee OA [17]. Familial aggregation of OA progression has also been investigated in the GARP study [18] by evaluating concordance in the change in joint space narrowing (JSN) and osteophyte grade at various anatomic sites. The ORs (95% CI), adjusted for age, sex, and BMI, of a sibling having radiographic progression if the proband had progression were 3.0 (1.2–7.8) for JSN progression and 1.5 (0.6–3.6) for osteophyte progression. A dose–response relationship was found between the amount of increase in JSN total scores among probands and the progression of JSN in siblings.

The reference control population drawn on to estimate the ORs in the GARP study were other probands and their siblings who had OA (although not OA at that specific site, or without progression at that site). Thus, such measures of familial aggregation are not recurrence risks and are not directly comparable with those shown in Table 1. However, the data from the GARP study indicate that, in middle-aged patients who have familial OA at multiple sites, familial aggregation of OA is most striking for hand and hip, and that changes in JSN are significantly correlated between siblings.

Familial aggregation does not result exclusively from genetic factors and may reflect environmental exposures that are shared by family members. If only weak familial aggregation is observed, it is not in itself convincing evidence of the contribution of genetic, as opposed to environmental, factors [19]. An alternative method for assessing the actual genetic contribution to a condition, in this case OA, is the use of classic twin studies, which enable investigators to quantify the environmental and genetic factors that contribute to a trait or disease.

### Classic twin studies

The classic twin study compares resemblances between identical or monozygotic (MZ) and nonidentical or dizygotic (DZ) twins. MZ twins derive from a single fertilized egg and therefore inherit identical genetic material, unlike DZ twins, who, on average, share only 50% of their genetic

material. Comparing the resemblance of MZ twins for a trait or disease with the resemblance of DZ twins offers the first estimate of the extent to which genetic variation determines variation of that trait. If MZ twins resemble each other more than DZ twins do, then the “heritability” of the trait can be estimated from twice the difference between MZ and DZ correlations [20]. In this context, heritability refers specifically to how much of the variance in the distribution of the trait under study might be attributable to genetic, rather than constitutional or environmental, factors that might be shared by individuals from the same family.

The heritability of OA has been calculated in twin sets after adjustment of the data for other known risk factors such as age, sex, and BMI. The correlations of radiographic osteophytes and JSN at most sites and the presence of Heberden’s nodes and knee pain have been found to be higher in the MZ pairs than in the DZ pairs [21]. Such findings show that the influence of genetic factors in radiographic OA of the hand, hip, and knee in women is between 39% and 65%, independent of known environmental or demographic confounding factors. Classic twin studies and familial aggregation studies have also investigated the genetic contribution to cartilage volume and progression of disease.

#### *Genetic contribution to disease progression*

Cartilage loss is the hallmark of established OA [22]. Recent twin and sibling studies have indicated that the heritability of cartilage volume is high (Table 2) [23]. However, comparing the offspring of people who have severe OA to controls, Jones and colleagues [24] were able to identify no difference in cartilage volume, suggesting that it is cartilage loss later in life that influences OA pathogenesis, but not a lower cartilage volume in itself.

Using longitudinal radiograph data, heritability estimates of 62% for progression of osteophytes and 72% for progression of JSN of the knee, independent of age and BMI, have been reported [25]. Genetic influence on radiographic disease progression over 2 years in a separate study was also assessed in a sib-pair design with generalized symptomatic OA (the GARP study) [18]. Moreover, a longitudinal sib-pair study using MRI also demonstrated that longitudinal changes in knee structures of relevance to later OA, such as medial tibial cartilage volume, lateral tibial bone size, and progression of chondral defects, have a high heritability [26]. The MRI sib-pair study and the twin radiograph study found that the heritability of change in medial compartments had a much stronger genetic component than that in lateral compartments. These results highlighted a strong genetic influence on progression of OA and provide a logical basis for the next step in identifying specific genetic factors responsible for incidence and progression of OA.

Unfortunately, to date, few studies have attempted to test candidate genes involved in longitudinal changes [27] (see later discussion). However, the past few years have seen the advent of prospective studies designed

Table 2  
Heritability of various osteoarthritis-associated traits

Trait	Heritability ( $h^2$ )	Data from reference
Radiographic knee OA	39%	[86]
Radiographic hip OA	60%	[21]
Radiographic hand OA	59%	[87]
Femoral cartilage volume	61%	[23]
Tibial cartilage volume	76%	[23]
Patellar cartilage volume	66%	[23]
Change in medial cartilage volume <sup>a</sup>	73%	[26]
Change in lateral cartilage volume <sup>a</sup>	40%	[26]
Change in medial knee osteophyte grade	69%	[25]
Change in lateral knee osteophyte grade	33%	[25]
Change in knee JSN grade	74%	[25]

*Abbreviation:* JSN, joint space narrowing.

<sup>a</sup> From a sib-pair, not a twin study.

specifically to investigate factors affecting the incidence and progression of knee OA. The two most notable examples are the Multicenter Osteoarthritis Study (MOST) [28] and the Osteoarthritis Initiative (OAI) ([www.oai.ucsf.edu](http://www.oai.ucsf.edu)), both of which are funded by the US National Institutes of Health.

These cohorts have recruited individuals who have clinically significant knee OA or are at high risk for developing new clinical knee OA, and are obtaining appropriate images and biospecimens at various time points on these subjects. Although no genetic studies have been performed on these collections yet, these cohorts present an ideal opportunity to investigate the role of genetic variation on the incidence and progression of disease.

Genes may affect the incidence, progression, or severity of OA through several pathways (Fig. 2). It is important to investigate which of the pathways known to affect progression are also influenced by genes. Based on data from multiple high-quality studies, Belo and coworkers [29] concluded that sex, knee injury, quadriceps strength, and regular sport activities are not associated with radiographic progression of knee OA, and knee pain at baseline and radiographic severity of OA at baseline appear to be, at best, only weakly associated with the progression. Evidence from multiple high-quality studies [29] shows that the level of hyaluronic acid in serum and the presence of GOA are associated with radiologic progression of knee OA. Evidence is strong for a genetic contribution to GOA. To date, however, no studies exploring the genetic contribution to serum levels of hyaluronic acid are available. Other factors that have been implicated in knee OA progression include synovial fluid volume, medial bone marrow edema lesions, adduction moment, alignment of the joint (varus/valgus), bone density, low serum levels, and dietary intake of vitamin D, among others. Some of these, such as bone density, are known to be strongly influenced by genes.

Bone is not structurally normal in OA. Periarticular bone in OA has increased turnover, decreased bone mineral content and stiffness, and

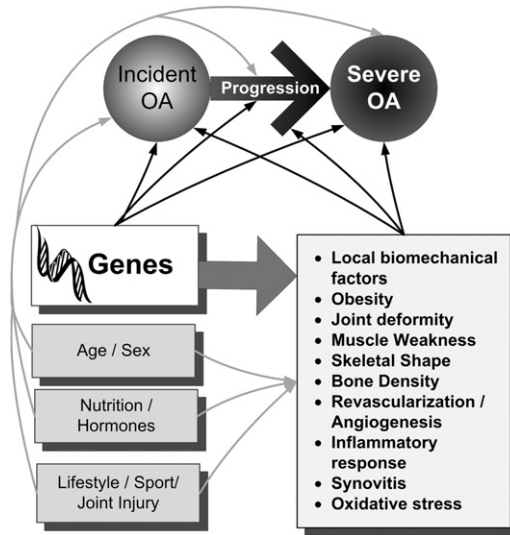


Fig. 2. Mechanisms by which genes can contribute to OA.

decreased trabecular numbers. Individuals who have OA exhibit striking increases in bone mass in affected sites, such as the knee and hip, and in non-synovial sites, such as the lumbar spine [30]. This increase in bone mass is due to an abnormal metabolism of osteoblasts, particularly in the subchondral bone tissue, which appears to be a response to altered local signals [31]. It has been hypothesized [32] that enhanced bone remodeling is the initiating event triggering the cartilage damage. Attempts to repair the cartilage then lead to several biochemical adaptations in bone and cartilage, which may overwhelm these attempts and lead to further sclerosis and damage.

Bone mineral density (BMD) and bone remodeling are under strong genetic control [33], and genetic variation at genes strongly and consistently involved in determining BMD and fractures has also been involved in risk for OA. Most notably, *LRP5* [34] and *OPG* [35] have repeatedly been associated with BMD, as have *VDR* and *ESRI* (Table 3).

Certain alterations in the mechanical environment of the joint adversely affect load distribution. Knee alignment is knee position in reference to the hip and ankle. Alignment at the knee (the hip–knee–ankle angle as measured by full-limb radiography) can either be varus (bowleg), valgus (knock-knee), or neutral [4]. Varus–valgus alignment has been shown to influence the risk for patellofemoral [36] and tibiofemoral OA progression [37].

Malalignment predicts worse surgical outcomes, but its role in the natural history of OA has been minimally considered. The magnitude of the torque that adducts the knee during the stance phase of gait correlates with disease severity in knee OA [4] and may predict the natural rate of disease progression. Further, in a longitudinal MRI-based study, Cicuttini and coworkers

Table 3  
Selected published genetic associations with osteoarthritis

Symbol	Gene name	Reported significant associations	Negative reports	Trait associated with	In linkage region?	Known or putative function
AACT	Alpha 1 antitrypsin	[27,59]	—	Knee OA	No	Natural inhibitor of serine proteinase involved in the degradation of cartilage proteoglycan
ADAM12	A disintegrin and metalloproteinase domain 12	[57,59]	—	Knee OA	No	Metalloprotease involved in osteoclast formation and cell–cell fusion
ASPN	Asporin	[64,88–91]	—	Hip OA, knee OA	No	Cartilage extracellular protein that regulates the activity of TGF- $\beta$
BMP2	Bone morphogenetic protein 2	[27,59]	—	Knee OA	No	Growth factor involved in chondrogenesis and osteogenesis
BMP5	Bone morphogenetic protein 5	[92]	—	Hip OA	Yes	Regulator of articular chondrocyte development
CALM1	Calmodulin 1	[75]	[64,76]	Hip OA	No	Intracellular protein, interacts with proteins involved in signal transduction
CILP	Cartilage intermediate layer protein	[27,64,93]	—	Knee OA, LDD	No	Inhibits TGF- $\beta$ 1–mediated induction of cartilage matrix genes
COL2A1	Type II collagen	[64,94]	—	Knee OA	No	Major cartilage collagen, structural cartilage component
COMP	Cartilage oligomeric matrix protein	[64]	—	Knee OA	No	Cartilage matrix macromolecule
COX2 (PTGS2)	Prostaglandin	[27,59,79,95]	—	Knee OA, spine OA	No	COX-2–produced PGE(2) modulates cartilage proteoglycan degradation in OA
DIO2	Iodothyronine deiodinase enzyme type 2	[96]	—	Hip OA, GOA	No	Regulates intracellular levels of active thyroid hormones in target tissues
ESR1	Estrogen receptor alpha	[27,54,97,98]	—	Knee OA, GOA	No	In chondrocytes, modulator of proteoglycan degradation and matrix metalloprotease mRNA expression

FRZB	Secreted frizzled-related protein 3	[63–67]	[68]	Hip/knee OA, GOA	Yes	Wnt antagonist and modulator of chondrocyte maturation
GDF5	Growth differentiation factor 5	[71,72]	—	Hip OA	No	Member of the bone morphogenetic family, regulator of growth and differentiation
HLA	Human leukocyte antigen system	[99–102]	—	Hand/hip /knee OA, GOA	Yes	Antigen presentation and binding of HLA/antigen complex to the T-cell receptor determining specificity of immune response
IL-1 gene cluster	Interleukin-1 alpha beta and interleukin-1 receptor antagonist	[103–105]	[105] (Knee OA)	Hip/knee OA	Yes	Regulation of metalloprotease gene expression in synovium and chondrocytes
IL-4R	Interleukin-4 receptor	[106]	—	Hip OA	Yes	Putative role in cartilage chondrocyte response to mechanical signals
IL-6	Interleukin-6	[107,108]	—	Hip/knee OA	No	Proinflammatory cytokine, involved in the cartilage degradation but also induces ILRa
IL-10	Interleukin-10	[109,110]	—	Knee/hand OA	No	Anti-inflammatory cytokine inhibits the synthesis of IL-1
LRCH1	Leucine-rich repeats and calponin homology (CH) domain containing 1	[77]	[78]	Hip/knee OA	No	Unknown
LRP5	Low-density lipoprotein receptor-related protein 5	[111]	[68]	Knee OA	Yes	Receptor involved in Wnt signaling by way of the canonical beta-catenin pathway
MATN3	Matrilin 3	[112,113]	—	Hand OA, spine OA	Yes	Extracellular matrix macromolecule
OPG	Osteoprotegerin	[27,59]	—	Knee OA	No	Regulation of osteoclastogenesis
RHOB	Ras homolog gene family, member B	[114]	[115]	Hip OA, knee OA	No	GTPase with tumor suppressor activity (antagonist of the PI3K/Akt pathway)

*(continued on next page)*

Table 3  
(continued)

Symbol	Gene name	Reported significant associations	Negative reports	Trait associated with	In linkage region?	Known or putative function
TXNDC3	Thioredoxin domain containing 3	[114]	[115]	Knee OA	No	Protein disulfide reductase participating in several cellular processes by way of redox-mediated reactions
TNA	Tetranectin	[27,59]	—	Knee OA	No	Plasminogen-binding protein, mediates degradation of extracellular matrix
VDR1	Vitamin D receptor	[27,64,115]	—	Knee OA	No	Nuclear receptor, mediates effects of vitamin D whose serum levels affect incidence severity and progression of OA

*Abbreviations:* ILRa, interleukin 1 receptor antagonist; LDD, lumbar disc degenerative disease; PGE(2), prostaglandin 2; TGF- $\beta$ , transforming growth factor beta.

[38] found that baseline knee angle is associated with the rate of cartilage loss in the knee. Two recent studies, from the United States and Australia, have shown, however, that knee malalignment is not associated with disease incidence and that it is more likely to be a marker of disease progression or severity [39]. On the other hand, in the Rotterdam Study, an increasing degree of varus alignment was associated not only with progression of radiographic knee OA, but also with development of knee OA [37]. However, this association seemed particularly applicable to overweight and obese persons.

Other mechanical factors that may affect risk for incidence or progression of OA include knee laxity [40] and proprioception [4]. Studies assessing the role that genetics play in these factors have not been published to date.

Another possible route of genetic control of risk for OA could be through skeletal shape. Studies in animal models have shown how skeletal development and skeletal shape are under tight genetic control [41], and some studies have indicated a role for skeletal shape in the risk for OA. For example, Lane and colleagues [42], examining baseline and 8-year follow-up radiographs, found that an abnormal center-edge angle and acetabular dysplasia were each associated with an increased risk for incident hip OA, adjusting for age, current weight, BMI, affected side, and investigational site (adjusted OR 3.3, 95% CI 1.1–10.1 for center-edge angle and 2.8, 95% CI 1.0–7.9 for acetabular dysplasia). In a cross-sectional study, Shepstone and coworkers [43] found a statistically significant difference in the shape of the intercondylar notch between the OA and non-OA groups. The observed difference in shape might have been congenital and one that increases the risk for anterior cruciate ligament damage or perhaps alters knee mechanics through some other route and, therefore, is a genuine risk factor for knee OA. However, given the cross-sectional nature of the study, it could also be a result of OA.

In a recent study of hip OA progression in the Rotterdam cohort, significant changes in the shape of the proximal femur occurred within the OA group from baseline to follow-up [44], apparently as a result of OA.

Several of the genes known to control skeletal development in animal model systems, such as bone morphogenetic proteins (BMPs) and Wnt signaling genes (see later discussion), have indeed been associated with risk for OA, but whether this association is due to an effect on skeletal shape has not been investigated.

Another aspect shown to contribute to disease risk and progression is inflammation, in particular, synovitis. A body of evidence is growing that synovial inflammation is implicated in many of the signs and symptoms of OA, including joint swelling and effusion [45]. Histologically, the OA synovium shows hyperplasia with an increased number of lining cells and a mixed inflammatory infiltrate consisting mainly of macrophages [46].

The low-grade OA synovitis is cytokine driven, although the levels of proinflammatory cytokines are lower than in rheumatoid arthritis. In particular, tumor necrosis factor- $\alpha$  and interleukin (IL)-1 have been suggested as key players in OA pathogenesis [47] in synovial inflammation and in

activation of chondrocytes. These cytokines can stimulate their own production and can induce synovial cells and chondrocytes to produce IL-6, IL-8, and leukocyte inhibitory factor. They can also stimulate protease and prostaglandin production. Progression of tibiofemoral cartilage damage is more severe in patients who have synovial inflammation. Ayral and coworkers [48] assessed changes in tibiofemoral cartilage damage over a 1-year period in 422 knee OA patients. Their results indicated that medial chondropathy after 1 year was statistically more severe in the group of patients who had an inflammatory perimeniscal synovial membrane at baseline than in patients who had a normal synovium. An individual's inflammatory response is known to be under genetic control [49] and several variants in genes encoding for cytokines or proteins involved in inflammation have been reported to be associated with OA (see later discussion).

### **Linkage analyses**

In genetics, a locus refers to a particular location on a chromosome or the DNA at that position. It can be present in the population in one or more forms, called alleles. If more than one allele exists for a locus, it is termed polymorphic. When the specific alleles at two or more loci in the same chromosome are being studied, the particular combination of alleles is called a haplotype. A polymorphic locus genotyped solely because its inheritance can be monitored, and not because it may be involved in a clinical or phenotypic trait, is called a genetic marker. A single nucleotide polymorphism (SNP) is a polymorphic locus consisting of a change at a single nucleotide base. When comparing alleles at a locus between two individuals, the alleles may be identical by descent if the allele came from the same parent (or ancestor), or only by state (eg, if one patient obtained the allele from the mother and his/her sibling obtained an identical allele from the father).

Genetic linkage occurs when a locus involved in the trait of interest (in this case OA) and alleles at nearby markers are inherited jointly, which means that the genetic markers and the disease locus map close to each other in the same chromosome. Therefore, genetic markers can be used as tools to track the inheritance pattern of a gene involved in a specific trait or disease. To identify chromosomal regions harboring OA genes, researchers have used pairs of siblings who are both affected with OA. When the proportion of alleles identical by descent that siblings concordant for OA share at a given marker is higher than expected, it is concluded that that marker is close in chromosomal location to an OA-related gene, or that significant linkage with OA has been found.

At least five genome-wide linkage scans have been published to date based on small families or twins related to affected individuals collected in the United Kingdom [50–52], Finland [53], Iceland [54,55], and the United States [56,57]. These genome-wide linkage scans have been performed on patients ascertained for hip, knee, or hand OA and have identified many broad

genomic intervals that may harbor OA susceptibility in chromosomes 2, 4, 6, 7, 11, 16, 19, and the X. Recently, Lee and coworkers [58] conducted a meta-analysis of OA whole-genome scans from 893 families, with 3000 affected individuals taking part in three studies (Iceland, United Kingdom, and United States). Their analysis provided summarized linkage loci of OA across whole-genome scan studies and, based on their data, they concluded that genetic regions in areas such as 7q34–7q36.3, 11p12–11q13.4, 6p21.1–6q15, 2q31.1–2q34, and 15q21.3–15q26.1 were the most likely to harbor OA susceptibility genes.

A summary of the chromosomal regions identified by linkage analyses is presented in Table 4. Some of the intervals identified from genome-wide linkage scans have subsequently been subjected to association analyses, principally candidate-gene-based studies, specifically the IL-1 (*IL1*) gene cluster, chr 2q11–q13; matrilin 3 (*MATN3*), chromosome 2p24.1; IL-4 receptor (*IL4R*), chromosome 16p12.1; secreted frizzled-related protein 3 (*FRZB*), chromosome 2q32.1; and bone morphogenetic protein 5 (*BMP5*), chromosome 6p12.1. These genes have been reviewed in detail by Loughlin [8]. The authors discuss the genetic associations found at these and other genes in the next section (see Table 3).

Table 4

Chromosomal region identified from genome-wide linkage scans that have been performed on families containing osteoarthritis-affected relatives

Chromosome	Cytogenetic location	Genes in region associated with OA	Linkage study	Linkage with trait	Reference or references
1	1p32–p22	—	USA	Hand OA	[56,57]
2	2q12–2q21	IL1	Finland	Hand/knee/hip OA	[53]
	2q31.1–2q34	FRZB	UK	Hip OA	[50,51]
	2p23.2–2p16.2	MATN3	Iceland, USA	Hand OA	[54–57]
3	3p22.2–3p14.1	—	Iceland	Hand OA	[54,55]
4	4q26–4q32.1	—	Finland, Iceland	Hand OA	[53,54]
6	6p21.1–6q15	BMP5, HLA	UK	Hip OA	[50,51]
7	7q34–7q36.3	—	USA	Hand OA	[56,57]
	7p15–7p21	—	Finland	Hand OA	[53]
11	11p12–11q13.4	LRP5	UK	Hip OA	[50,51]
13	13q33.1–13q34	—	USA	Hand OA	[56,57]
15	15q21.3–15q26.1	—	USA	Hand OA	[56,57]
16	16p13.1–16q12.1	IL4R	UK, Iceland	Hip OA	[50,51,54,55]
	16q22.1–q23.1	—	UK	Knee OA/hip OA	[50,51]
19	19q13	—	USA, UK	Hand OA	[50,51,56,57]
X	X cen	—	Finland	Hand OA	[53]

Also listed are those instances in which a gene within a linked interval has subsequently been shown to be associated with osteoarthritis.

## Genetic associations

Genetic association studies provide a means of quantifying the effects of specific gene variants on disease occurrence. It is important to distinguish between a genetic association and the role of a gene or its encoded product in disease. For example, matrix metalloproteases (MMPs) are of key importance in OA, yet genetic variants in these genes have not been reported to be associated with susceptibility to disease, which can be explained if over- or underexpression of the gene encoding MMPs during disease is due to other factors (inflammation, aging, injury) but not to an individual carrying a particular genetic variant. On the other hand, if a variant at a gene is associated with disease risk, the probability is high that the gene is involved in disease pathogenesis.

Early candidate gene studies concentrated on cartilage components such as *COL2A1*, which encodes for the alpha 1 polypeptide chain of type II collagen, the principal collagenous component of articular cartilage. Other extracellular matrix-related genes that were considered included type IX and type XI collagen genes and the aggrecan gene. These studies, however, did not yield convincing evidence to support a role for common, nonsynonymous mutations in cartilage extra cellular matrix (ECM) structural protein genes as risk factors for primary OA [8]. Other candidates were some of the genes associated with osteoporosis and bone density, such as the genes encoding estrogen receptor alpha (*ESR1*) and the vitamin D receptor (*VDR*). Various variants at these two genes have been reported to be associated with OA (see Table 3) [59].

A different approach was taken by the authors' own group. They compared human cDNA libraries from OA-affected and normal cartilage and synovium and selected 22 genes that showed significantly different expression. One or two SNPs per gene were then tested for association with cross-sectional and longitudinal radiographic features of knee OA. SNPs at 9 of the 22 genes were found to be associated with OA in a population-based cohort. Variants at 7 of those 9 were also associated with clinical knee OA in either men or women, or both, in an independent population [59].

Genes falling under linkage peaks have also been tested, with varying results. Replication studies for genes derived from linkage studies have yielded mixed results, with *FRZB* being the gene most replicated in association studies. The genes in Table 3 represent, broadly speaking, at least five different molecular pathways or classes of molecules: inflammation (*IL1*, *IL4R*, *COX2*, *IL6*, *IL10*, *HLA*), ECM molecules (*ASPN*, *MATN3*, *COL2A1*, *COMP*, *CILP*), Wnt signaling (*FRZB*, *LRP5*), BMPs (*BMP2*, *BMP5*, *GDF5*), and proteases or their inhibitors (*ADAM12*, *TNA*, *AACT*), in addition to genes related to modulation of osteocyte or chondrocyte differentiation or proteolytic activity, as would be *ESR1*, *VDR*, and *OPG*. The genes that have been replicated in the largest number of independent populations to date are *GDF5* and *FRZB*; therefore, it is worth discussing in more detail the BMPs and Wnt signaling pathways and their genetic association with OA.

### *Wnt signaling, FRZB variants and gender-specific associations*

Wnt proteins form a family of highly conserved secreted signaling molecules. As currently understood, Wnt proteins bind to receptors of the frizzled and low density lipoprotein related protein families on the cell surface. Through several cytoplasmic relay components, the signal is transduced to the nucleus to activate transcription of Wnt target genes, some of which include TIMP1 (stromelysin, inhibitor of MMPs), RANK ligand, COX-2, osteocalcin, BMP4, NOS2, and FGF [60]. Evidence in the literature shows that the Wnt signaling pathway is involved in cartilage degeneration and OA [61].

The *FRZB* gene is one of the frizzled transmembrane receptors. The loss of tetranectin in *Frzb*(-/-) knockout mice has recently been shown to contribute to cartilage damage by increasing the expression and activity of MMPs; *FRZB* deficiency in mice also resulted in thicker cortical bone, with increased stiffness and higher cortical appositional bone formation after loading, which may contribute to the development of OA by producing increased strain on the articular cartilage during normal locomotion [62].

Several studies have explored the relationship between OA and two polymorphisms in the *FRZB* gene: the Arg200Trp and Arg324Gly variants. In three papers, the relationship between the rare *FRZB* Trp<sup>200</sup>-Gly<sup>324</sup> haplotype (frequency of 0.6%–5.0%) and OA has been examined [63–66]. It was found that female carriers of this haplotype have an increased risk for THR [63,66], severe JSN of the hip [65], and clinical knee OA [64], further supporting a role for *FRZB* variants in OA. In a Dutch study, the association of the Arg324Gly was also seen with GOA, but not with radiographic hip OA [67]. Although strongly associated in women, these variants did not appear to be associated with hip or knee OA in men [63,64], suggesting a gender-specific effect. A meta-analysis for hip or knee OA in women yielded an OR of the effect of less than 1.4 [64].

In contrast, a recent study, sufficiently powered to find evidence of an association between *FRZB* variants and radiographic OA in two large independent cohorts, failed to do so. Kerkhof and coworkers [68] did not study individuals who had symptoms of OA or severe OA requiring TJA, as was done in previous studies, but focused on markers of cartilage degradation and on radiographic features of knee and hip OA. Their data, along with previous results [67], suggest that *FRZB* genetic variants may play a role in OA limited to severe (joint replacement phenotype) or symptomatic OA, but not in radiographic OA. This said, the clinical phenotype is not restricted to persons who have radiographic OA, so its clinical relevance is still inherent.

### *Bone morphogenetic proteins*

BMPs are members of the transforming growth factor (TGF)- $\beta$  superfamily of signal molecules that mediate many diverse biologic processes. BMPs trigger cellular responses mainly through the Smad pathway, although the signal molecules can also activate the mitogen-activated protein kinase

pathway [69]. In model organisms, a remarkable array of long-distance, modular, regulatory elements surrounding the genes that encode BMPs has been identified. These sequences correspond to individual “anatomy” elements that help control the size, shape, and number of individual bones and joints. For example, regulatory elements from the *GDF5* gene can be used to inactivate other genes, specifically in joints, making it possible to identify genes and signals required for maintenance or repair of articular cartilage [70].

Among the BMP genes reported to be associated with OA are *BMP5* (not replicated to date), *BMP2*, which has been associated in two United Kingdom populations, and *GDF5*. An association with hip and knee OA of a single SNP (rs143383, T/C) located in the 5'-UTR of the growth and differentiation factor 5 gene, *GDF5*, was reported in Japanese and Chinese case-control cohorts [71]. The major allele of the SNP, the T allele, was common in the Asian populations, with frequencies of more than 70% in controls, and was at an elevated frequency in OA cases, with ORs ranging from 1.30 to 1.79 for knee and hip cases. In vitro cell transfection studies revealed that the T allele mediated a moderate, but significant, reduction in the activity of the *GDF5* promoter. The same T allele was found to be increased in hip and knee OA cases from Spain and the United Kingdom relative to controls, with a modest OR of 1.10. Although the effect size in the European samples was modest in RNA extracted from the cartilage of OA patients who had undergone joint-replacement surgery, the T allele showed up to a 27% reduction in expression relative to the C allele ( $P < 7 \times 10^{-5}$ ) [72], suggesting that a small, but persistent, imbalance of *GDF5* expression throughout life might render an individual more susceptible to OA.

From the discussion regarding the *FRZB* and *GDF5* genes, it is clear that even genetic variants that are replicated in many populations have fairly modest effects and the associations may be limited to certain phenotypes.

### **What can we expect in the years to come in genetic research in osteoarthritis?**

Genome-wide association studies (GWAS) are a result of the human genome and HapMap projects (<http://www.hapmap.org>) and, if successful, can find variants in specific genes, or narrow genomic regions, that are associated with the presence or severity of a specific clinical condition. The information conveyed by these studies is unlikely to influence clinical practice in the immediate future, yet it represents an important advance in medicine [73]. The GWAS approach enables a genome-wide comparison of gene-variant prevalence between cases and controls, avoiding the need to guess which genes are likely to harbor variants affecting risk. Although the same was true of genome-wide linkage analyses, the association approach uses three orders of magnitude more genetic markers and a more thorough coverage of the genome than the linkage scans did. If

a marker is found to be associated, it will be much closer to, or even in, the gene actually involved in disease.

These studies have the potential to convey novel, unbiased information about the heritable basis of OA at a level of detail that has not been previously possible. The results of such GWAS will tell us that a particular genetic variant is located at a gene or, more likely, is in linkage disequilibrium with a gene, which is important in the pathogenesis of OA. However, this kind of analysis cannot tell us the mechanisms responsible. Some of the other major weaknesses and pitfalls of this approach have been highlighted already [74]. For example, even the newest assays do not cover all genetic variation in the genome; thus, false-positives and false-negatives can be expected. More importantly, to correct for  $5 \times 10^5$  comparisons, large sample sizes are needed. Thus, investigators can find themselves with low power to detect modest effects. Further, an independent replication of findings becomes absolutely necessary. In fact, the only assurance that scientists can have that a genetic association found in this manner is real is to find the same marker to be associated consistently in independent studies.

To date, two large case-control association scans have been reported. Mototani and coworkers [75] tested 72,000 markers for association with hip OA, and identified a variant in the *CALM1* gene to be strongly associated in the Japanese population. However, studies in United Kingdom samples failed to show an association of this variant with hip [76] or knee OA [64]. Spector and coworkers [77] examined 25,000 genic SNPs for association with radiographic knee OA in Caucasians, and identified an SNP in a gene of unknown function. However, association with this gene, *LRCHI*, failed to be replicated in two sufficiently powered large studies from East Asia of knee and hip OA samples from China and Japan [78].

A pooled, large-scale (500,000 markers) GWAS on knee OA has been published recently [79]. The variants identified by this scan, which were subsequently replicated in independent cohorts, fell in the 5' region of the gene encoding the COX-2 and the cytosolic phospholipase enzymes (both involved in prostaglandin synthesis), in the 2q33 linkage region, and near a gene involved in transcriptional repression of thyroid hormone receptors. In addition, a large consortium in the United Kingdom funded by the Arthritis Research Campaign is underway to determine variants associated with TKR and THR in a total of 8000 cases. Other large cohorts (eg, Rotterdam, Framingham, TwinsUK, and Iceland) with radiographic OA features are also currently being tested. Once we identify the individual genes involved, we will probably have a long list of genes relating to various processes that may relate to the health of joint tissues. Because GWAS is a position-based approach, many genes will hold no prior association with OA. These genes might be developmental and not disease specific [9].

A greater understanding of the pathogenesis of OA is not the only valuable contribution of GWAS results. To date, no single large genetic effect has been found. Rather, the increased risks for carrying a predisposing

genetic variant appear to be modest, with most of them having ORs between 1.1 and 1.6 (eg, *GDF5* and *FRZB*). One obvious question is this: If an individual carries risk variants at several genes, does his/her risk for OA increase in proportion? The authors' group has investigated this question by computing a genetic risk variable combining variants from 10 different genes that had been implicated in risk for knee or hip OA in other populations. When the top and bottom quartiles of this variable were used, the ORs became 8.68 (95% CI 5.20–14.49,  $P < 2 \times 10^{-16}$ ) for women and 5.06 (95% CI 3.10–8.27,  $P < 1 \times 10^{-10}$ ) for men [80]. The ORs obtained using the genetic risk variable were comparable to those reported for obesity or knee injury by some studies. Such data indicate that it is possible to identify individuals at high risk for knee OA by combining genotype data from several loci, and that the genetic risk for knee OA is likely to be due to the sum of many loci making a small contribution each. The same may hold true for hip or GOA. The next step, once GWAS results appear, will be gene–gene and gene–environment interaction studies that will be needed to enlarge our understanding of the manner in which the individual genes implicated in OA exert their effect. Fully understanding the genetic basis for OA will require the availability of large cohorts of well-characterized individuals who have hip OA, knee OA, or multiple joint OA [5], such as the OAI and MOST.

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