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1

The clinical relevance of genetic susceptibility to osteoarthritis

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Osteoarthritis is a major musculoskeletal cause of disability in the elderly, but current therapeutic approaches are insufficient to prevent initiation and progression of the disease. Genetic studies in humans have identified molecules involved in signalling cascades that are important for the pathology of the joint components. These include the bone morphogenetic protein (BMP) signalling, the wntless-type signalling and the thyroid pathway as well as apoptotic-related molecules. There is emerging evidence indicating that inflammatory molecules related to cytokine production, prostaglandin and arachidonic acid metabolism are also involved in susceptibility to osteoarthritis. All of these pathways are likely targets for pharmacological intervention. Genetic variation also affects pain due to osteoarthritis highlighting molecular mechanisms for pain relief. Moreover, combinations of genetic markers can be used to identify individuals at high risk of osteoarthritis and risk of total joint arthroplasty failure, which should facilitate the application of preventive and disease management strategies.

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Osteoarthritis (OA) is the most common joint disorder and the leading cause of disability in the elderly in the US and Europe [1]. Physician-diagnosed arthritis occurs in more than 50% of adults older than age 65 years and in more than 30% of adults aged 45–64 years [1].

The loss of articular cartilage is the hallmark of OA [2], but all the joint components, including the ligaments, tendons, capsule, synovial lining and periarticular bone, undergo structural and functional

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alterations during the course of OA progression [3]. Although the pathogenesis of osteoarthritis is not fully understood, it is strongly age related, being rare before 40 years, and rising in frequency with age, such that a large proportion of people over the age of 70 have radiographic evidence of osteoarthritis in some joints [4]. OA is a multifactorial disease with genetic and environmental determinants. All cases are probably affected by both genetics and environment, with a continuous distribution between the extremes of predominantly genetic or predominantly environmental causes [4]. For example, the risk of post-traumatic OA after a meniscal injury of the knee is strongly affected by a family history of osteoarthritis, by the presence of nodal osteoarthritis of the hand (the classical marker of generalised osteoarthritis), by obesity and by sex [4].

How do we know that genetics is important in OA?

Evidence for a genetic predisposition to osteoarthritis was reported as early as the 1940s [5]. The clustering of OA in families has been measured by using the risk ratio for a relative of an affected individual compared with the population prevalence [6]. For affected sib pairs, this sib recurrence risk is termed the lambda sib (λ_s). Large values of λ_s indicate that a gene involved in traits should be easier to map than if λ_s is low [6,7]. Thus, it is possible to identify subjects with clinical disease with severe enough symptoms to lead to total joint replacement (TJR) 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 [8] assessed the prevalence of hip OA in siblings of individuals undergoing total hip replacement (THR) to the prevalence of radiographic hip OA in subjects undergoing intravenous urograms for investigation of a renal problem (i.e., controls). A similar study was carried out using total knee replacement (TKR) as the selection criterion [9]. Similar data, but using self-reported total joint arthroplasty in a smaller data set were found by a study in Oxford [10]. The data shown in Table 1 indicate a strong familial aggregation, although lower than autoimmune arthropathies, not much lower than rheumatoid arthritis and much higher than metabolic disorders such as Type 2 diabetes.

An alternative method to assess the actual genetic contribution to a condition is the use of classical twin studies, which enable investigators to quantify the environmental and genetic factors that contribute to a trait or disease. Comparing the resemblance of identical twins for a trait or disease with the resemblance of non-identical twins offers the first estimate of the extent to which genetic variation determines variation of that trait or heritability. 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 body mass index (BMI). 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. Classical twin studies and familial aggregation studies have also investigated the genetic contribution to cartilage volume and progression of disease (see MacGregor *et al.* [16]).

Table 1
Familial aggregation of osteoarthritis and of other disorders.

Type of disorder	Condition	Ascertained via	Sibling recurrence risk λ_s	Data from reference:
Autoimmune arthropathies	Rheumatoid arthritis	Sibs with condition	5.0–8.0	[11,12]
	Juvenile arthritis	“	15	[13]
	Systemic lupus erythematosus	“	30	[12]
Metabolic osteoarthritis	Type 2 diabetes	“	1.2–1.6	[14]
	Knee OA (TF and/or PF)	“	2.08	[9]
	TKR	“	4.81	[10]
	Anteromedial OA	Sibs with UKR	3.21	[15]
	Hip osteophytes grade 3	Sibs with THR	4.27	[8]
	THR	“	1.87–8.53	[8,10]
	Hip KL grade >= 3	“	4.99	[8]
Hip JSW <= 1.5 mm	“	5.07	[8]	

TKR = total knee replacement, THR = total hip replacement, UKR = unicompartmental knee replacement, KL = Kellgren – Lawrence grade, JSW = joint space width, PF = patellofemoral TF = tibiofemoral.

Linkage analyses

Genetic linkage occurs when a locus involved in the trait of interest (in this case OA) and alleles at nearby markers are inherited jointly. At least five genome-wide linkage scans exist in the literature based on small families or twins of affected relatives collected in the U.K., Finland, Iceland and the US (see Lee *et al.* [17] for references). These genome-wide linkage scans were performed on patients ascertained for hip, knee or hand OA and have identified a large number of relatively broad genomic intervals that may harbour OA susceptibility in chromosomes 2, 4, 6, 7, 11, 16, 19 and the X chromosome. Recently, Lee and co-workers [17] conducted a meta-analysis of OA whole-genome scans from 893 families with 3000 affected individuals taking part in three studies (Iceland, U.K. and US). Their analysis provided summarised linkage loci of OA across whole-genome scan studies and, based on their data, they concluded that genetic regions in 7q34–7q36.3, 11p12–11q13.4, 6p21.1–6q15, 2q31.1–2q34 and 15q21.3–15q26.1 were the most likely to harbour OA susceptibility genes. However, to date, no susceptibility genes for large joint OA have been identified directly by linkage alone.

Genetic associations

Genetic association studies provide a means of quantifying the effects of specific gene variants on disease occurrence. If a genetic association is present, a particular allele, genotype at a given polymorphic locus will be seen more often than expected by chance in an individual affected by the disease. With this type of analysis, the frequencies of genotypes or alleles are compared usually in a classical cases-control design, although sometimes association studies are carried also in family settings.

How does genetic variation influence risk of OA?

Genetic variation can influence susceptibility to OA through diverse pathways and at different stages (see Fig. 1), and a select list of genes found to be associated with OA in various studies is presented in Table 2.

A comprehensive list of all the genetic associations with OA reported to date is beyond the scope of this review but in addition to the genes listed in Table 2, several others have been reported, such as extracellular matrix molecules such as COL2A1 [18], MATN3 [19] or COMP [18]. A more extensive list can be found at Valdes and Spector [20]. Here, we review some of those genes that are particularly clinically relevant, along with the molecular mechanisms involved in the pathology of OA.

The articular cartilage forms a biomechanical unit with the subchondral and cortical bone to attenuate forces through joints, particularly following impact loading. Hence, genes underlying the biology of articular cartilage are of the most likely ones to affect genetic risk of joint damage in OA. Besides the known features of the articular cartilage, thickening, sclerosis and osteophyte formation of the subchondral bone are very common in OA. Changes in the articular cartilage cause impairment in the absorption of shocks that it should normally attenuate, impacting the subchondral bone and subsequently destroying it and leading to secondary changes such as sclerosis and osteophyte formation [46]. Therefore, genetic variations at molecules involved in bone remodelling are also involved in susceptibility to OA. Genetic susceptibility of risk of OA is thus due, at least in part, through genetic control of skeletal shape and development. Studies in animal models have shown that both skeletal development and skeletal shape are under tight genetic control and some studies have indicated a role for skeletal shape in the risk of OA. For example, Lane and colleagues [47], examining baseline and 8-year follow-up radiographs, found that an abnormal centre-edge angle and acetabular dysplasia were each associated with increased risk of incident hip osteoarthritis. More recently, Doherty and colleagues [48] reported that both the femoral head shape and the femoral neck shaft angle of affected hips as well as of contralateral hips are very strongly associated with risk of hip OA, indicating that a non-spherical femoral head shape not only occurs as a consequence of OA, but itself may be a morphologic risk factor for development of hip OA.

Several of the genes known to control skeletal development in animal model systems, such as bone morphogenetic proteins (BMPs) and Wnt signalling genes, have indeed been associated with risk of OA in animal models and in humans, although whether this is due to an effect on skeletal shape has not been investigated. Wnt and BMPs are expressed in many overlapping tissues and dual regulation by

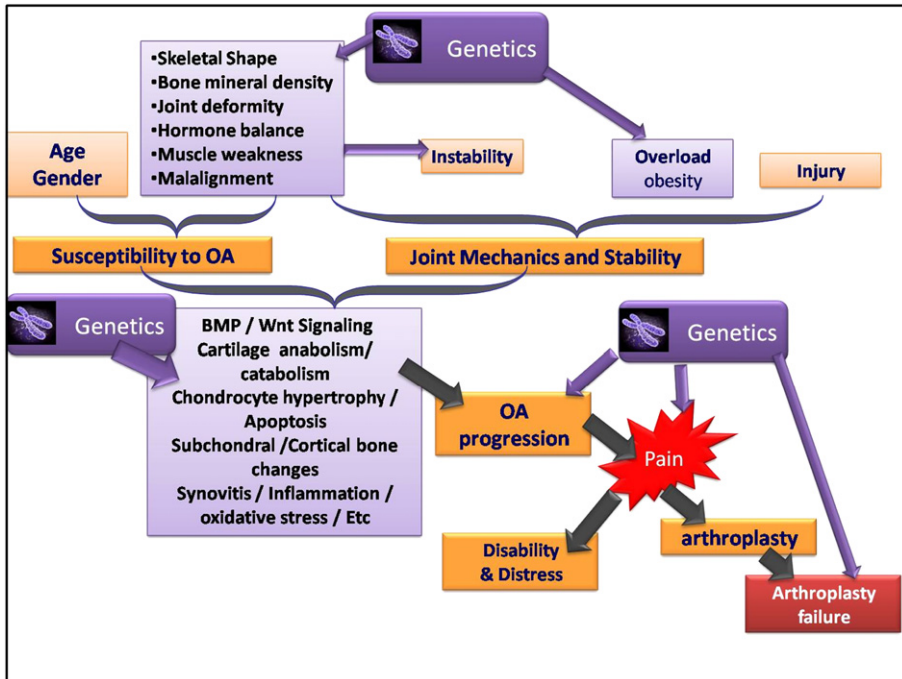


Fig. 1. Role of genetic variation in susceptibility to osteoarthritis.

Wnt and BMPs appear to be frequent in mammalian development [49]. In addition, there is substantial cross-talk between these two pathways. These genes are also involved in bone remodelling and in chondrocyte biology and are therefore primary candidates for influencing risk of OA.

BMP and Wnt signalling

Wnt (wingless-like MMTV integration site family members) proteins belong to a protein family that regulates embryonic development and cell differentiation, proliferation and migration, and disease in many organs including the joints and bone. Signalling is initiated by the binding of the Wnt ligand to receptor molecules of the Frizzled family and to lipoprotein receptor-related proteins (LRP) 5 and 6 [50]. Through several cytoplasmic relay components, the signal is transduced to the cell nucleus to activate transcription of Wnt target genes. Broadly speaking, two types of Wnt protein are recognised: β -catenin-dependent canonical Wnts such as Wnt1 and Wnt3a, and the so-called non-canonical Wnts that are independent of or inhibit β -catenin signalling. Both types of Wnt molecules are involved in cartilage biology in animal models [51]. However, only variation at genes in the canonical pathway has been investigated as possible risk factors for OA.

Several studies have explored the relationship between OA and two polymorphisms in the *FRZB* gene: the Arg200Trp and Arg324Gly variants (SNP IDs rs7775 and rs288326), with some studies finding an association with hip OA (e.g., Lane *et al.* [43].) and knee OA [18] but others failing to find any association despite having sufficient statistical power [52]. A recent large meta-analysis of 10 independent Caucasian cohorts found evidence for an association of rs288326 with risk of hip OA (odds ratio (OR) = 1.12 95% confidence interval (CI): 1.02–1.23, $p < 0.016$) but not with hand or knee OA [27].

The role of genetic polymorphisms at the Wnt ligand receptors *LRP5* and *LRP6* genes has also been explored but no clear association with OA has emerged [52]. More recently, we have described that a molecule shown to regulate Wnt signalling *in vitro* (PHAPI encoded by *ANP32A*) is associated with hip OA [41].

Table 2

Selected published genetic associations with OA and biochemical pathways involved.

Pathway	Symbol	Gene name	References(s)	Trait associated with	Known or putative function with
BMP	<i>ASPN</i>	Asporin	[18,22,23]	Hip OA/Knee OA	Cartilage extracellular protein that regulates the activity of TGF β
BMP	<i>BMP2</i>	Bone morphogenetic protein 2	[24,25]	Knee OA	Growth factor involved in chondrogenesis and osteogenesis
BMP	<i>CILP</i>	Cartilage intermediate layer protein	[18–24]	Knee OA,	Inhibits TGF β 1-mediated induction of cartilage matrix genes
BMP	<i>GDF5</i>	Growth differentiation factor 5	[26–29]	Hip OA	Member of the BMP family, regulator of growth and differentiation
BMP/Wnt Inflammation	<i>OPG</i> <i>PLA2G4A</i>	Osteoprotegerin Cytosolic phospholipase A2	[24,25] [30]	Knee OA Knee OA	Regulation of osteoclastogenesis Catalyzes the rate-limiting step in the production of pro-inflammatory eicosanoids and free radicals
Inflammation	<i>PTGS2</i>	Cyclooxygenase 2	[24,25,30,31]	Knee OA, spine OA	COX-2-produced prostaglandin-E2 modulates cartilage proteoglycan degradation in OA
Inflammation	<i>IL1 gene cluster</i>	Interleukin (IL-)1 alpha, IL- beta and IL-1 receptor antagonist	[32–35]	Hip/knee/ Hand OA	Regulation of metalloproteinase gene expression in synovial cells and chondrocytes
Inflammation	<i>IL6</i>	Interleukin 6	[36–38]	Hip/knee/ Hand OA	Pro-inflammatory cytokine, involved in the cartilage degradation but also induces ILRa
Inflammation	<i>IL10</i>	Interleukin 10	[39,40]	Knee/Hand OA	Anti-inflammatory cytokine inhibits the synthesis of IL-1
Other	<i>VDR1</i>	Vitamin D receptor	[18,24]	Knee OA	Nuclear receptor, mediates effects of vitamin D whose serum levels affect incidence severity and progression of OA
Wnt, other	<i>ANP32A</i>	Acidic leucine-rich nuclear phosphoprotein 32 (pp32 or PHAPI)	[41]	Hip OA	Regulator of apoptosis and of Wnt signaling
Other	<i>ADAM12</i>	A disintegrin and metalloproteinase domain 12	[24,25]	Knee OA	Metalloprotease involved in osteoclast formation and cell-cell fusion
thyroxin	<i>DIO2</i>	Iodothyronine-deiodinase enzyme type 2	[42]	Hip OA, Generalized OA	Thyroxin signalling: Regulates intracellular levels of active thyroid hormones in target tissues
Wnt	<i>FRZB</i>	Secreted frizzled-related protein 3	[18,27,43]	Hip/knee OA, GOA	Wnt antagonist and modulator of chondrocyte maturation
Wnt	<i>LRP5</i>	Low- Density Lipoprotein Receptor-Related Protein 5	[44]	Knee OA	Receptor involved in Wnt signalling via the canonical beta-catenin pathway
Other	<i>COMT</i>	Catechol-O-methyltransferase	[45]	Pain in hip OA	Key regulator of pain perception

Another important signalling cascade involved in joint biology is the BMPs. BMPs are members of the transforming growth factor (TGF- β) superfamily of signal molecules that mediate many diverse biological processes. BMPs trigger cellular responses mainly through the Smad pathway, although the signal molecules can also activate the mitogen-activated protein kinase (MAPK) pathway [53]. In model organisms, a remarkable array of long-distance, modular regulatory elements surrounding the genes, which 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 [54].

One gene related to TGF- β signalling associated with OA is asporin. Kizawa and co-workers [21] reported an association between osteoarthritis and variation at the asporin gene, *ASPN*, in Japanese populations and a more modest association with this gene was subsequently reported in various Caucasian studies [22,23]. *In vitro* studies revealed that the *ASPN* transcript is overexpressed in cartilage from OA cases relative to controls and asporin was shown to bind to TGF- β *in vitro* and to inhibit measures of chondrogenesis in murine chondrocytic cell lines [21].

Another BMP-related gene implicated in risk of knee OA is *BMP2* [24,25]. A crucial role for *BMP2* in chondrogenesis and chondrocyte hypertrophy has been observed in animal models [49]. Polymorphisms in the *BMP2* gene have been found to be associated with knee OA in the U.K. population, but their role in hip OA or in other populations has not been investigated yet.

Regulatory elements from the growth and differentiation factor 5 gene (*GDF5*), belonging also to the BMP family, can be used to inactivate other genes in joints, making it possible to identify genes and signals required for maintenance or repair of articular cartilage [46].

An association with hip and knee OA of a single nucleotide polymorphism (SNP) (rs143383, T/C) located in the 5'-UTR of *GDF5* was reported in Japanese and in Chinese case-control cohorts [26]. The major T allele of the SNP was common in Asian populations, with frequencies >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 U.K. relative to controls with a very modest OR of 1.10. A smaller effect has also been observed in European samples (e.g., Evangelou *et al.* [27], and Valdes *et al.* [28]). The same polymorphism has also been associated with height and fracture risk [29].

A recent large-scale meta-analysis found an OR of 1.15 (95% CI: 1.09–1.22; $p = 9.4 \times 10^{-7}$) for knee OA for the T allele, with no significance between-study heterogeneity across sample sets. Estimates of effect sizes for hip and hand OA were similar to that for knee OA, but a large between-study heterogeneity was observed, and statistical support was only borderline for hip ($p = 0.016$) and absent for hand ($p = 0.17$) OA [27].

Animal models of OA have also shown that both BMP and Wnt signalling pathways are related to different OA phenotypes in mice. For example, Smad3 is one of the signalling mediators of TGF β 1. Mutant mice homozygous for a targeted disruption of Smad3 exon 8 develop a degenerative joint disease resembling human OA, as characterised by progressive loss of articular cartilage, formation of large osteophytes and decreased production of proteoglycans [55]. To date, however, no studies on the role of genetic variation of Smad3, or any of the Smads, on the risk of human OA have been published.

In fact, a systematic study of the role of BMP and Wnt-related genes has not been undertaken (see Research Agenda). The availability of data from genome-wide association scans may help shed light on the extent to which genetic variation in these pathways influences risk of OA.

Indirectly related to BMP signalling is also the thyroid hormone pathway. The deiodinase, iodothyronine, type II gene (*DIO2*) has been recently identified as an OA susceptibility gene. Combined linkage and association analysis of candidate genes in the chromosomal region (14q23) of linkage revealed that this gene explained part of the linkage present at this locus. An association with a specific haplotype was further observed in U.K., Dutch and Japanese OA studies [42]. *DIO2* encodes an intracellular enzyme in the thyroid pathway, responsible for the local bioavailability of the thyroid hormone in specific tissues, including the growth plate. It converts inactive thyroid pro-hormone (T₄) to active T₃ hormone, which, in turn, binds to thyroid receptors in the nucleus and activates thyroid hormone-responsive genes. The active thyroid hormone, triiodothyronine (T₃), plays an essential role in the control of chondrocyte proliferation and differentiation, inhibiting BMP-2-induced growth of mouse rib [56].

Apoptosis and cell death

The important role of apoptosis in OA has been demonstrated in both *in vitro* and *in vivo* models. A significantly higher proportion of apoptotic chondrocytes is found in samples from OA patients than in those from age-matched controls (see Kim and Blanco [57] for review). OA chondrocyte death includes both apoptosis and necrosis [58]. Two main pathways of cell death have now been elucidated: a death-receptor-mediated pathway and a mitochondrial-mediated pathway. The death receptors are a subset of the tumour necrosis factor (TNF) receptor family of cell-surface molecules such as Fas. The mechanisms through which stimulation of Fas by the Fas ligand initiates apoptosis have been extensively investigated, and it has been shown that cell death in primary OA chondrocytes is mediated by the Fas pathway [58]. Mitochondrial DNA (mtDNA) variation has been associated with risk of OA of the knee [59] and of the hip [60]. Given the role of mtDNA damage on mitochondria-driven apoptosis, it is possible to speculate that the involvement of mtDNA haplotypes on OA risk may relate to apoptotic pathways. More recently, we reported that genetic variation at the *ANP32A* gene, which encodes a tumour-suppressor molecule that plays a regulatory role in apoptosis and interferes with canonical Wnt signalling *in vitro* is associated with hip OA in women [41]. Further research into the role of genetic variation in apoptotic signalling molecules in the susceptibility of OA is still needed.

Inflammation, synovitis and pain

From a clinical perspective, a key aspect of genetic involvement in OA is through pain. Cartilage is aneural, so whatever its role in the pathogenesis of joint damage may be, it cannot be the tissue that directly generates pain [4]. By contrast, subchondral bone, periosteum, synovium, ligaments and the joint capsule are all richly innervated and contain nerve endings that could be the source of nociceptive stimuli in OA [61]. Imaging studies at the knee joint have shown a correlation between pain and both synovitis and subchondral bone changes, suggesting that these two tissues could be sources of pain in OA [4,62].

OA is not considered as a classical inflammatory arthropathy due to the absence of neutrophils in the synovial fluid and the lack of systemic manifestations of inflammation [3]. Nevertheless, synovial inflammation is implicated in many of the signs and symptoms of OA, including joint swelling and effusion [63]. The low-grade OA synovitis is cytokine-driven, although the levels of pro-inflammatory cytokines are lower than in rheumatoid arthritis. In particular, tumour necrosis factor- α (TNF- α) and interleukin (IL-1) have been suggested as key players in OA pathogenesis [64] both in synovial inflammation and in activation of chondrocytes. These cytokines can stimulate their own production and they can induce synovial cells and chondrocytes to produce IL-6, IL-8 and leucocyte inhibitory factor. Very importantly, progression of tibiofemoral cartilage damage is more severe in patients with synovial inflammation [65]. Although, unfortunately, no studies on the genetic susceptibility to synovitis have been carried out to date, an individual's inflammatory response is known to be under genetic control [66] and, in fact, a number of variants in genes encoding for cytokines, in particular, IL-1 and IL-6, have been reported to be associated with risk of OA (see Table 2).

In addition to inducing the synthesis of matrix metalloproteinases (MMPs) and other proteinases by chondrocytes, IL-1 and TNF- α increase the synthesis of prostaglandin E₂ (PGE₂) by stimulating the expression or activities of cyclooxygenase (COX)-2, microsomal PGE synthase-1 (mPGES-1), secreted phospholipase A₂ (sPLA₂) and cytosolic phospholipase A₂ (cPLA₂). The only genome-wide scan published to date identified variants in the 5' region of *PTGS2* (the gene encoding the COX-2 enzyme) and *PLA2G4A* (the gene encoding cytosolic PLA₂ α) as significantly associated with the risk of knee OA in women from five different Caucasian cohorts [30]. The data did not allow us to determine whether the association observed was due to the gene encoding COX-2 or cytosolic cPLA₂ α or both. The likely involvement of cPLA₂ in risk of OA is of particular relevance as cPLA₂ α catalyses the rate-limiting step in the production of pro-inflammatory eicosanoids and free radicals and is a likely regulator of cell death and apoptosis [67]. Current thinking is that the role of this enzyme in apoptosis may be restricted to certain instances where particular stimuli and/or cell types are involved. In cells exposed to stimuli that promote non-apoptotic forms of cell death, cPLA₂ α may act to protect the cells from injury [68].

Pain is, in final analysis, the endpoint which leads to distress, disability and total joint replacement. The catechol-O-methyltransferase encoded by the *COMT* gene is a major degrading enzyme in

the metabolic pathways of catecholaminergic neurotransmitters such as dopamine and norepinephrine [69]. This enzyme has been shown to be a key regulator of pain perception, cognitive function and affective mood. The *COMT* Val158Met polymorphism is responsible for differential pain sensitivity in humans, working in part by modulating the endogenous μ -opioid system [70]. Recently, van Meurs and co-workers [45] have shown that in a large OA database (in which there was only a modest overall relationship between radiographic changes and pain levels), the *COMT* Val158Met is associated with hip pain. They found that carriers of the 158Met *COMT* variants had an almost threefold higher risk ($P = 0.02$) of hip pain as compared with carriers of the Val/Val genotype. As noted by the authors, this effect was fully driven by the female carriers. Female carriers of the 158Val allele were 4.9-fold more likely to have pain (95% confidence interval 1.6–14.8, $P = 0.005$), while radiographic damage to the hip was present in carriers and non-carriers of the 158Val allele. The clinical relevance of this finding is worth stressing, because drugs primarily targeting the pathway regulated by the *COMT* enzyme, namely the norepinephrine and/or the dopamine catecholaminergic systems (e.g., duloxetine) have been shown to be safe and effective in treating chronic musculoskeletal pain syndromes such as fibromyalgia [71]. Hence, understanding the molecular pathways involved in OA pain can have direct clinical significance for the choice of pharmacotherapy used to treat OA symptoms.

Total joint arthroplasty failure

Total joint replacement for end-stage OA is an effective surgical intervention. Unfortunately, wear debris, primarily generated from the prosthetic joint articular surface, remains the major factor limiting the survival of joint implants. Aseptic loosening of joint implants can affect patients 10–20 years after joint replacement surgery and is a disabling condition that includes inflammatory and osteolytic processes [72]. To date, at least two studies have investigated the role of genetic variation on osteolysis and aseptic loosening and found polymorphisms in the *MMP1* gene [73] and the *IL1* and *IL6* genes [74] to be associated with the risk of arthroplasty failure.

Genome-wide association studies

Genome-wide association studies (GWAS), if successful, can find variants in specific genes or narrow genomic regions, which are associated with the presence or severity of a specific clinical condition. This approach does not require any knowledge on the part of the scientist regarding the biological mechanism underlying a genetic association, thus enabling the discovery of novel molecular mechanisms involved in disease aetiology.

To date, a pooled large-scale (500 000 markers) GWAS has been published [30] on knee OA. The variants identified by this scan that 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. The importance of such molecules in OA has already been discussed above. Although not yet published, the first non-pooled GWAS for knee, hip and hand OA has already been carried out in the Rotterdam study considering 1,341 OA cases and 3,496 controls and replicated in several independent cohorts from Europe and the US. This study has found that a gene cluster mapping to chromosome 7q is strongly and reproducibly associated with risk of knee OA (van Meurs, personal communication). Although no obvious candidates are located to this genomic region, a transcription factor involved in Wnt signalling regulation maps to it. Several other GWAS are also being carried out at the time of this writing on various OA-related traits, including a U.K. study funded by the arthritis research campaign investigating THR and TKR, and several large cohorts (e.g., Rotterdam, Framingham, TwinsUK and Iceland) with radiographic OA features are also currently being tested in a combined meta-analysis by the European consortium TREAT-OA.

Relevance of genetic studies to the clinician

Severe osteoarthritis is the cause of huge economic and personal burden and is the main cause of the increasing need for joint replacements [75]. Current therapeutic approaches for dealing with OA

are largely insufficient to prevent initiation and progression of disease. The impact and outcome of physiological and pathological processes in the various elements of the joint are determined by molecular signalling pathways. The rapid and accumulating advances in our knowledge of these pathways involved in osteoarthritis susceptibility are therefore likely to have an impact on clinical practice. For instance, the important role of Wnt and BMP signalling identifies these pathways as potential therapeutic targets. The role of thyroid hormone regulation in genetic risk of OA is a particularly attractive therapeutic target, as drugs to intervene in this pathway have been in use for other therapeutic indications for a long time and are well characterised in terms of their safety and pharmacokinetics (e.g., Eisenberg and Distefano [76]).

Unravelling the role that inflammation and apoptosis play in cartilage degeneration and pain is of great value for designing pharmacological interventions. However, the role of these pathways is not limited to cartilage damage; they also appear to influence the risk of total joint replacement failure.

In addition, understanding the molecular causes underlying pain in OA is another fundamental aspect where human genetics can contribute. Although pharmacotherapy aimed at pain relief may not address cartilage damage, improving the quality of life and diminishing the disability derived from OA should be paramount as goals in the study of this disease. The identification of molecular pathways involved in disease is, nevertheless, not the only clinical use of genetic studies.

Prognosis and diagnosis

The rises in the prevalence of OA and the associated expected increased disease burden have intensified the search for disease-modifying treatments. The effective application of such treatments, once they become available, is dependent, in part, on our ability to apply them at the early stages of disease. Yet, the identification of OA before it becomes evident on radiographs remains a challenge. Thus, 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 fairly modest, with most variants carrying ORs between 1.1 and 1.6 (e.g., *GDF5*), which is typical of genetic risks for complex diseases. One obvious question, then, is if an individual carries risk variants at several genes, does his or her risk of OA increase in proportion. We have investigated this possibility by computing a genetic risk variable combining variants from 10 different genes that had been implicated in the risk of 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 [77]. 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 of 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, each making a small contribution.

In summary, osteoarthritis is characterised by focal areas of damage to the articular cartilage, centred on load-bearing areas, associated with osteophytes, changes in the subchondral bone, variable degrees of mild synovitis and thickening of the joint capsule [4]. Severe osteoarthritis is the cause of huge economic and personal burden and is the main cause for joint replacements, [75] but current therapeutic approaches are insufficient to prevent initiation and progression of disease. Genetic studies in humans have identified molecules involved in signalling cascades that are important for the pathology of articular cartilage. These include the BMP and TGF- β pathways, the wntless-type signalling pathway, the thyroid pathway and apoptotic and mtDNA damage-related molecules. A genetic variant in *GDF5* has emerged as the most reproducible genetic association with OA. Synovitis and inflammation are also involved in the aetiology of OA and inflammatory molecules related to cytokine, prostaglandin and arachidonic acid metabolism are associated with susceptibility to OA. All of these pathways are likely intervention targets for pharmacological therapy. In addition, genetic variation also influences the failure of total joint replacement due to aseptic loosening. By selecting sets of genetic variants associated with risk of disease or with progression of OA, it is becoming possible to identify individuals at high risk of disease who should benefit the most from preventive strategies, thus improving monitoring and management of this disease.

Practice points

Importance of genes to osteoarthritis:

- The risk of osteoarthritis is both genetically and environmentally determined
- Genetic studies have identified specific polymorphisms associated with OA and clinical endpoints related to OA

Molecular pathways and therapeutic targets:

- Genes in specific signalling cascades involved in joint biology, such as Wnts and BMPs, thyroid and apoptotic pathways are among those involved in the risk of OA. These pathways are potential therapeutic targets to treat joint damage.
- Genes in inflammatory pathways, in particular, cytokines and eicosanoid-related enzymes, also determine risk of OA
- Variation at an enzyme in a specific pain sensitivity mechanism (catecholaminergic) is associated with pain in OA, suggesting this as an interventional target for symptoms of OA

Joint Replacement Failure

- Polymorphisms in cytokine and metalloproteinase genes have been implicated in the risk of osteolysis and joint arthroplasty failure

Prognosis and Diagnosis

- Combinations of gene markers can be incorporated into mathematical models to identify patients at high future risk of severe OA, suggesting these as diagnostic/prognostic tests for disease management and monitoring

Research agenda

- A thorough analysis should be carried out of the genetic associations of genes in pathways involved in joint damage, inflammation and pain associated with OA including gene–gene and gene–environment interactions
- Focus on the role of genetic variation, not only on risk of radiographic or even clinical OA, but, more importantly, on the risk of disease progression and aseptic loosening will help understand the determinants leading to severe disease and to joint arthroplasty failure
- Future studies should focus on evaluating the diagnostic value (sensitivity and specificity) of genetic marker combinations that can be shown to consistently affect susceptibility to hip and knee OA, and can also be shown to consistently affect progression towards severe OA

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