Osteoarthritis (OA) is a complex, multifactorial disease with a significant genetic component that has been established over the last 60 years through epidemiological studies, including sibling-risk, familial aggregation and twin studies as well as population-based cohort studies [1–12]. Taken together, these studies suggest that the genetic contribution to OA accounts for at least half of the variation in susceptibility to disease and that, like other complex diseases, genetic susceptibility to OA is most likely mediated by a number of genes with each contributing a small individual effect and relative risk [13]. Although the precise mechanisms underlying disease initiation and progression have not been fully elucidated, there have been some successes in finding susceptibility genes for OA through a number of avenues such as candidate-gene studies, genome-wide linkage scans, and gene-based association studies [14,15].

A major problem encountered in the genetic investigation of any complex disease, however, is the dissection of true associations from false-positives, as spurious associations can occur even when a study is properly designed [16]. The gold standard in confirming the robustness of a genotype-phenotype association is therefore considered to be replication of that association in multiple independent datasets [17,18]. There have been a number of recent publications of polymorphisms associated with OA [19–24], and in this review, we will focus on three of the most compelling finds to date: FRZB [19], ASPN [20], and GDF5 [22]. Associated variants in these genes not only have a demonstrated functional significance but have also been replicated in multiple independent populations, confirming the credibility of these associations. We will close the review by discussing how these genetic findings may be taken forward into the realm of therapeutic interventions as well as the future for OA genetics as we move into the genomic era.

**FRZB**

One approach to identify genes associated with a complex, multigenic disease such as OA is to carry out a series of genetic studies, with each study further narrowing down the number of candidate genes with a possible role in the disease of interest. This strategy was used to identify polymorphism in the gene FRZB as a risk factor for OA [19]. After an initial genome-wide linkage scan of UK Caucasians identified a broad region on chromosome 2q likely to harbor OA susceptibility, finer mapping using affected sibling-pair families concordant for hip OA narrowed the linkage interval to 8.6 cM. Within this region, eight candidate genes were selected on the basis of expression evidence in tissues of mesenchymal origin during development or adulthood. One of those candidate genes was FRZB, which codes for secreted frizzled-related protein 3 (sFRP3), an extracellular antagonist of Wnt signaling. Wnt family members are involved in a number of developmental and maintenance processes, including bone remodeling [25] and chondrogenesis [26], with sFRP3 shown specifically to have in vivo chondrogenic/osteogenic activity [27].
Following a microsatellite- and a SNP-based association study, FRZB (chromosome 2q32.1) consistently demonstrated a significant association with hip OA in females (p < 0.04) [19]. FRZB contains two nonsynonymous SNPs that code for the substitution of conserved arginine residues: Arg200Trp (C > T) in exon 4 and Arg324Gly (C > G) in exon 6. In a combined cohort of the female probands from the families used in the linkage scan and 558 unrelated female hip OA cases ascertained by joint replacement, the G-allele of the exon 6 SNP showed the strongest association to female hip OA (p = 0.02, odds ratio [OR]: 1.5; 95% confidence interval [CI]: 1.1–2.1) (Table 1). Haplotype analysis revealed that individuals possessing substitutions of the conserved arginine residues in both exon 4 (T-allele) and exon 6 (G-allele) of the same sFRP3 molecule were at an increased risk of developing hip OA (OR: 4.1; 95% CI: 1.6–10.7).

Functionally, possession of both arginine substitutions was shown to decrease the ability of sFRP3 to antagonize Wnt signaling (Figure 1). A study of differential allelic expression (DAE) of FRZB in end-stage OA cartilage samples, however, revealed that only a small percentage (24%) of individuals showed significant differences in allelic output and that genotype at the Arg200Trp, Arg324Gly or three promoter SNPs (rs12469777, rs9288087 and rs4293535) was not correlated with DAE status [28]. Furthermore, a study by Lane et al. found that individuals with hip OA showed increased levels of frizzled-related protein in their serum relative to non-OA controls, but frizzled-related protein levels were not associated with genotype at the Arg200Trp SNP [29]. Overall, these studies indicate that cis-regulatory effects acting on FRZB impacts the OA phenotype is through the arginine substitutions decreasing the ability of sFRP3 to antagonize Wnt signaling.

Subsequent to the initial UK report, a number of follow-up studies have been published. In 2005, Min et al. conducted an association study on both FRZB SNPs (Arg200Trp and Arg324Gly) in a Netherlands population-based cohort scored for radiographic OA status at multiple sites (Rotterdam Study) and in a population of Caucasian probands and their siblings with symptomatic OA at multiple joint sites (GARP Study) [30]. Although neither SNP demonstrated association with female hip OA, the G-allele, which codes for the glycine of the Arg324Gly SNP did show an association with generalized OA at multiple sites (p < 0.05). In another replication study carried out in Caucasian USA females assessed radiographically for hip OA, no association was found between the two FRZB SNPs (Arg200Trp and Arg324Gly) alone and

### Table 1. Summary of the association of the FRZB Arg324Gly (C > G) substitution with OA.

<table>
<thead>
<tr>
<th>Individual study</th>
<th>Allele count (frequency)</th>
<th>G-allele vs C-allele</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G-allele</td>
<td>C-allele</td>
<td>p-value*</td>
</tr>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td>Cases</td>
</tr>
<tr>
<td><strong>Hip OA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UK (females only)</td>
<td>115 (10.4)</td>
<td>56 (7.1)</td>
<td>989 (89.6)</td>
</tr>
<tr>
<td>USA (females only)</td>
<td>100 (8.8)</td>
<td>684 (8.3)</td>
<td>1040 (91.2)</td>
</tr>
<tr>
<td>Dutch (Rotterdam Study)</td>
<td>22 (7.5)</td>
<td>144 (7.6)</td>
<td>272 (92.5)</td>
</tr>
<tr>
<td>Spanish (females only)</td>
<td>52 (14.1)</td>
<td>26 (11.6)</td>
<td>316 (85.9)</td>
</tr>
<tr>
<td>Belgian (females only)</td>
<td>15 (9.9)</td>
<td>46 (9.7)</td>
<td>137 (90.1)</td>
</tr>
<tr>
<td><strong>Knee OA</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Spanish</td>
<td>62 (11.3)</td>
<td>64 (11.1)</td>
<td>486 (88.7)</td>
</tr>
<tr>
<td><strong>Hand OA</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Spanish</td>
<td>56 (11.8)</td>
<td>64 (11.1)</td>
<td>418 (88.2)</td>
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<td><strong>Polyarticular OA</strong></td>
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<td>58 (10.3)</td>
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<td>504 (89.7)</td>
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<tr>
<td>Dutch (GARP Study)</td>
<td>55 (11.2)</td>
<td>144 (7.6)</td>
<td>435 (88.8)</td>
</tr>
</tbody>
</table>

*NS: Not significant (p > 0.05).
†Significant associations (p < 0.05).
CI: Confidence interval; OA: Osteoarthritis; OR: Odds ratio.
OA [29]. The frequencies of the minor alleles of each SNP, however, were elevated in cases compared to controls, which is the same trend as that seen in the studies showing a significant association between these SNPs and OA. In line with the primary UK study and the Netherlands study, possession of the haplotype containing both arginine substitutions was associated with hip OA in the USA population (p < 0.05; OR: 1.5; 95% CI: 1.01–2.22).

A Spanish study in which cases were ascertained by hand OA (n = 242) or by joint replacement due to primary OA of the hip (n = 310) or knee (n = 277) demonstrated no association between the Arg324Gly or the Arg200Trp SNP and OA [31]. When patients were stratified by sex and the occurrence of OA at one or more additional sites to that of the primary complaint, however, a significant trend was observed for Arg324Gly in females whose primary affected joint was the hip (p = 0.008). In contrast to The Netherlands, USA and UK study, there was no significant association between the haplotype consisting of both arginine substitutions and OA, although this could be a result of the low frequency of individuals with such a haplotype and a consequent lack of power to detect such an association.

Recently, differential association of the Arg200Trp SNP has been reported between hip OA cases and hip osteoporosis (OP) cases from a cohort of Belgian female Caucasians [32]. In this study, the frequency of carriers of the T-allele, which codes for the tryptophan of Arg200Trp SNP, showed a significant difference between the two disease groups (p = 0.016). This difference was also observed when the FRZB haplotype frequencies were compared. Intriguingly, these differences were more extreme than those observed between OA cases and controls, implying that the polymorphisms may also modulate the OP phenotype. FRZB has also been implicated in susceptibility to colorectal cancer with individuals homozygous for glycine at the Arg324Gly SNP shown to have a significantly increased risk of developing the disease (p < 0.001; OR: 5.1; 95% CI: 1.74–14.71) [33]. The Belgian report of OA versus OP and the colorectal cancer report show that the coding polymorphisms of FRZB are important in susceptibility to not only OA, but

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**Figure 1. The effect of the FRZB polymorphisms on Wnt signaling.**

(A) Wild-type sFRP3 contains arginines at positions 200 and 324 and is able to bind strongly to Wnt ligands in the extracellular matrix. This leads to a low level of Wnt signaling and a low level of β-catenin in the cytoplasm. (B) When the arginines at positions 200 and 324 have been substituted, with tryptophan and glycine, respectively, sFRP3 has a reduced ability to bind soluble Wnt ligands. This results in an increase in β-catenin in the cytoplasm.
also to other diseases, which is demonstrative of the potential for a polymorphism to impact on more than one phenotype owing to its occurrence in a protein that functions within a pathway which is active in a broad spectrum of cellular and tissue systems.

The collecting evidence does support the role of polymorphisms within FRZB in OA susceptibility, but the causal polymorphism is currently unclear as both the Arg200Trp and Arg324Gly SNPs have shown discrepant associations with OA in different populations. Additionally, both the double mutant protein and the protein containing only the Arg324Gly substitution have been shown to decrease the ability of sFRP3 to antagonize Wnt signaling [19]. The exact stratum of OA in which these polymorphisms assign susceptibility will also need clarification, although the evidence most strongly suggests a predisposition for females with OA at the hip plus other sites. The role of FRZB in OA will be dependent not only on the FRZB polymorphism present, but also on interactions with other genetic susceptibilities and environmental conditions. FRZB is therefore an interesting discovery in OA genetics, and the lack of consensus evidence for the associated polymorphism and exact phenotype produced clearly demonstrates the complexity of unraveling the genetics of complex diseases such as OA.

ASPN

Asporin was first identified in 2001 as a member of the small leucine-rich proteoglycan (SLRP) family, which is a subfamily of the leucine-rich repeat (LRR) superfamily of proteins [34,35]. SLRPs are extracellular matrix (ECM) proteins that function both as structural organizers through interactions with ECM components such as collagens and as modulators of growth factor activities through direct binding of factors such as TGF-β in the ECM [36,37]. Asporin, coded for by the gene ASPN on chromosome 9q22.21, is very similar to biglycan and decorin based on amino acid sequence, except for a unique stretch of repeating aspartic acid residues of variable length at the N-terminus known as the D-repeat [35]. The original ASPN association with OA [20] has been reviewed previously [14,38], and for this review, we will briefly describe the findings from the original report while focusing more on the follow-up studies.

Kizawa et al. identified a number of polymorphisms in the ASPN gene for use in an association study including a repeat polymorphism in exon 2 that codes for the D-repeat [20]. These polymorphisms were initially genotyped in a population-based cohort of Japanese individuals, and the D-repeat was found to show positive association with knee OA with the D14 allele (14 aspartic acid repeats) showing an increased frequency in cases (susceptibility allele) and the D13 allele (13 aspartic acid repeats) showing an increased frequency in controls (protective allele) (Table 2). The significant association of the D14 allele was subsequently confirmed in independent Japanese case-control populations of knee OA cases and hip OA cases. Functionally, asporin was found to inhibit the expression of

<table>
<thead>
<tr>
<th>Individual study</th>
<th>Allele count (frequency)</th>
<th>D14 allele vs others</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D14</td>
<td>Others</td>
<td>p-value</td>
</tr>
<tr>
<td>Japanese (JP)*</td>
<td>91  (8.6)</td>
<td>58 (4.8)</td>
<td>969 (91.4)</td>
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<tr>
<td>UK (UK)</td>
<td>76  (13.7)</td>
<td>190 (12.7)</td>
<td>480 (86.3)</td>
</tr>
<tr>
<td>Greek (GK)</td>
<td>47  (15.2)</td>
<td>53 (13.9)</td>
<td>263 (84.8)</td>
</tr>
<tr>
<td>Spanish (SP)</td>
<td>56  (14.9)</td>
<td>74 (12.6)</td>
<td>320 (85.1)</td>
</tr>
<tr>
<td>Han Chinese (HC)*</td>
<td>41  (9.4)</td>
<td>44 (4.8)</td>
<td>395 (90.6)</td>
</tr>
</tbody>
</table>

**Meta-analysis**

| All studies combined (JP, UK, GK, SP, HC)* | 311 (11.4) | 419 (9.1) | 2427 (88.6) | 4169 (90.9) | 0.0030 | 1.5 | 1.1–1.9 | [45] |
| Asian studies combined (JP, HC)*         | 132 (8.8) | 102 (4.8) | 1364 (91.2) | 2022 (95.2) | 0.0000013 | 2.0 | 1.5–2.6 | [45] |
| European studies combined (UK, GK, SP)    | 179 (14.4) | 317 (12.9) | 1063 (85.6) | 2147 (87.1) | 0.20 | 1.1 | 0.9–1.4 | [45] |

*Significant associations (p < 0.05).
CI: Confidence interval; OR: Odds ratio.
markers of chondrogenesis such as the type II collagen and the aggrecan genes and to bind TGF-β in vitro (Figure 2). Additionally, asporin inhibited the prochondrogenic effects of TGF-β, with the D14 allele showing a significantly enhanced inhibition relative to the D13 allele.

To assess the global significance of the association of the D-repeat polymorphism of ASPN, a number of follow-up studies have been performed. In a UK Caucasian population comprised of OA cases ascertained by joint replacement of a knee, a hip, or both a hip and a knee and non-OA controls, there was no significant susceptibility effect of the D14 allele or protective effect of the D13 allele in the combined cohort [39]. After stratification by sex and affected site, however, marginal association of the D14 allele was detected for males with hip OA (17.4% in cases and 13.1% in controls, p = 0.025, OR: 1.41, 95% CI: 1.05–1.88). In a smaller study conducted on Europeans of Greek origin with knee OA ascertained by total joint replacement, no significant effect of the D14 allele was reported, but the D13 allele showed a significantly enhanced frequency in controls relative to cases (p = 0.002, OR: 0.62; 95% CI: 0.46–0.84), thus confirming the protective role of the D13 allele in this population [40]. In a Spanish Caucasian case-control cohort comprised of patients with knee OA, hip OA or hand OA, the D14 allele and the D13 allele showed no significant association to OA in the combined cohort or when the data were stratified by affected joint [41]. Most recently, the effect of the D-repeat on OA was evaluated in a case-control cohort of Han Chinese with the OA cases determined by symptomatic knee OA confirmed by radiographic evidence [42]. In this population, the D14 allele had a significantly elevated frequency in cases relative to controls (p = 0.0013, OR: 2.04; 95% CI: 1.32–3.15), but there was no significant protective effect of the D13 allele.

These follow-up studies have provided a conflicting picture of the genetic effect of the D-repeat on OA susceptibility, and they may have failed to consistently confirm the effects of the D13 and D14 alleles reported in the original Japanese study for a number of reasons. For example, it is possible that the estimated genetic effect of the D-repeat on OA susceptibility was inflated in the original study, which was reported for initial genetic findings for other complex

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**Figure 2. The effect of the ASPN polymorphisms on TGF-β signaling.**

(A) Asporin containing the D14 repeat can bind strongly to TGF-β in the extracellular matrix, causing a reduction in TGF-β-mediated signaling and a consequent decrease in COL2A1 and AGC1 expression. (B) Asporin containing the D13 repeat can bind less strongly to TGF-β, leading to more TGF-β mediated signaling and increased expression of COL2A1 and AGC1.
diseases [43]. Alternatively, differences in inclusion criteria for OA cases may be responsible for the discrepancy as the Asian cases were recruited by symptomatic OA with radiographic confirmation whereas the European cases were ascertained by need for a total joint replacement [44,45]. Additionally, ethnic differences may play a part as there are significant differences in the frequencies of the D13 allele and the D14 allele not only within the European populations but also between the Asian and European populations [44,45]. Of note, however, is that the majority of the follow-up studies showed a similar trend to that of the original study with an increased D14 frequency and a reduced D13 frequency in cases relative to controls.

Nakamura et al. have recently conducted a meta-analysis on the five published reports (Japan, UK, Greece, Spain and China) on the genetic effect of the ASPN D-repeat in susceptibility to knee OA and hip OA [45]. Meta-analysis has proven a successful and efficient means to quantitatively combine the data from different published studies on one topic to provide an estimate of discrepancy between the studies and to exclude the effects of confounding factors [43,45,46]. When the knee OA data were combined from all studies, the D14 allele was significantly over-represented (p = 0.003) and the D13 allele was significantly under-represented (p = 0.026) in the cases. Significant heterogeneity, however, was detected between these studies (p = 0.047), so the authors stratified the studies into two groups by ethnicity: Asians (Japanese and Chinese) and Europeans (UK, Greek and Spanish). In these stratified populations, the D14 allele showed a significant effect in the Asians (p = 0.0000013) with nonsignificant heterogeneity (p = 0.54), but there was no significant association of the D14 allele in European knee OA (p = 0.20), with nonsignificant heterogeneity between the different European populations (p = 0.90). The D13 allele showed no association in either Asians or Europeans when the data were stratified. When the hip OA data were combined, significant heterogeneity between the populations was detected, and there was no significant positive global association between the D14 allele (p = 0.55) or the D13 allele (p = 0.12) with hip OA.

These results demonstrate that there are significant ethnic differences in the effect of the D-repeat on OA susceptibility, but there does appear to be a genuine effect of the D14 allele on susceptibility to knee OA. The effect is much stronger in Asians than in Europeans and this may be due to gene–gene or gene–environment interactions that are specific to one ethnic group. The meta-analysis revealed no significant association of the D-repeat with hip OA susceptibility, however, and this may be because the initial report was spurious or that the effect of the D-repeat is relatively minor in the pathogenesis of hip OA, so a larger sample size will be needed to reveal the true effect. Alternatively, differences in hip morphometry between Asians and Caucasians may explain why the positive association of the D14 allele to hip OA in the Japanese population was not replicated in European populations. Asians have been reported to demonstrate significantly more shallow acetabular dimensions than Caucasians and this may result in a fundamental difference in the etiology of hip OA between Asian and Caucasian populations [47–49]. Overall, these data attest to the relevance of the D14 allele to knee OA susceptibility, although the genetic effect is significant only for Asian populations. There is a trend towards significant association for the D14 allele with knee OA in European populations, however, which suggests a much weaker genetic effect that may become significant in a larger sample size of Europeans.

**GDF5**

Growth/differentiation factor GDF5 is a member of the bone morphogenetic protein (BMP) family of signaling molecules, which is a subfamily of the TGF-β superfamily of secreted proteins [50]. BMPs are known historically for their ability to induce ectopic bone formation but are now known to function in a variety of physiological roles such as cell proliferation and differentiation, apoptosis and skeletogenesis [51]. GDF5 (also known as cartilage derived morphogenetic protein 1; CDMP1) is active throughout the tissues of the synovial joint both during development and adulthood and has been shown to stimulate chondrogenesis and chondrocyte metabolism [52–54], to induce the formation of tendon and ligament tissue [55], and to facilitate bone repair [56,57]. Mutations in the gene encoding GDF5 (GDF5, chromosome 20q11.22) cause a number of human diseases including brachydactyly type C (MIM 113100) [58], Hunter-Thompson type dysplasia (MIM 201250) [59], chondrodysplasia Grebe-type (MIM 200700) [60], and angello-shaped phalango-epiphysyal dysplasia (MIM 105835) [61], which leads to osteoarthritic changes in the hip [62]. Based on the biological role of GDF5 and its implication in a number of
human chondrodysplasias, GDF5 was investigated as a candidate gene for hip OA susceptibility [22]. Miyamoto et al. initially identified three common GDF5 SNPs by sequencing the exons and their flanking regions and tested these SNPs for association with hip OA in a cohort of 239 cases ascertained by symptomatic hip OA confirmed by radiography and 256 controls [22]. These three SNPs were significantly associated for both genotypes and alleles, and the associations were confirmed in a second independent Japanese case-control cohort of 761 hip OA cases and 728 controls. When the data from the first and second cohorts were combined, the most significant association (p = 1.8 × 10^{-13} for allelic frequency) was for a SNP in the 5’ untranslated region (UTR; rs143383, +104T/C) with the susceptibility allele (+104T) showing an increased frequency in the cases (83.6%) relative to the controls (74.0%) (Table 3). The OR for the susceptibility allele was 1.79 (95% CI: 1.53–2.09). The association of the T-allele of rs143383 with OA was confirmed in an independent Japanese cohort of 718 knee OA cases and 861 controls (p = 0.0021; OR: 1.10; 95% CI: 1.01–1.20) and a Han Chinese cohort of 313 knee OA cases and 485 controls (p = 0.00028; OR: 1.54; 95% CI: 1.22–195). Because rs143383 resides in the 5’ UTR, it was hypothesized that the polymorphism would affect the transcriptional activity of the promoter, so luciferase reporter constructs carrying either allele of rs143383 were generated and assayed for transcriptional activity. The T-allele (OA susceptibility allele) showed significantly lower activity than the C-allele in both chondrogenic and nonchondrogenic cell lines, thus confirming the functionality of this polymorphism and providing a mechanism for OA susceptibility through reduced GDF5 expression (Figure 3).

Based on the evidence for association of rs143383 with OA in two Asian populations, Southam et al. investigated whether this SNP was associated with OA in a broad European cohort composed of UK and Spanish cases (n = 2487), ascertained by need for joint replacement of a hip or a knee or by fulfilling the ACR criteria for hand OA, and age-matched, non-OA controls (n = 2047) [63]. In the combined analysis, the T-allele of rs143383 was significantly associated with OA (p = 0.03; OR: 1.10; 95% CI: 1.01–1.20). As in the Asian study, the T-allele was at a significantly elevated frequency in cases (63.2%) relative to controls (61.0%). Stratification by sex and affected joint (hip, knee or hand OA) did not show an increased significance, implying that no one sex or joint site was responsible for the association. Individuals carrying the T-allele (TT and TC genotypes) showed the strongest significance in the combined European cohort, with carriage rates of 87.2% in cases versus 84.1% in controls (p = 0.004;

<table>
<thead>
<tr>
<th>Table 3. Summary of the association of the GDF5 rs143383 T-allele with OA.</th>
<th>Allele count (frequency)</th>
<th>T-allele vs C-allele</th>
<th>Ref.</th>
</tr>
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<tbody>
<tr>
<td><strong>Individual study</strong></td>
<td><strong>T-allele</strong></td>
<td><strong>C-allele</strong></td>
<td><strong>p-value</strong></td>
</tr>
<tr>
<td><strong>Cases</strong></td>
<td><strong>Controls</strong></td>
<td><strong>Cases</strong></td>
<td><strong>Controls</strong></td>
</tr>
<tr>
<td><strong>Hip OA</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Japanese*</td>
<td>1668 (83.6)</td>
<td>1455 (74.0)</td>
<td>328 (16.4)</td>
</tr>
<tr>
<td>UK</td>
<td>1565 (64.1)</td>
<td>1020 (62.0)</td>
<td>877 (35.9)</td>
</tr>
<tr>
<td>Spanish</td>
<td>361 (59.4)</td>
<td>1441 (60.2)</td>
<td>247 (40.6)</td>
</tr>
<tr>
<td>UK and Spanish combined</td>
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<td><strong>Knee OA</strong></td>
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<tr>
<td>UK and Spanish combined*</td>
<td>3142 (63.2)</td>
<td>2461 (61.0)</td>
<td>1832 (36.8)</td>
</tr>
</tbody>
</table>

*Significant associations (p < 0.05).
CI: Confidence interval; OA: Osteoarthritis; OR: Odds ratio.
OR: 1.28; 95% CI: 1.08–1.51), which suggests that the T-allele exerts a dominant effect on OA susceptibility in Europeans. When the UK data and the Spanish data were analyzed separately, however, no compelling association was detected for the T-allele in either ethnic group, although the T-allele was at an increased frequency in the cases relative to the controls for both groups.

Whereas the Asian study demonstrated that the T-allele mediated a significant reduction in GDF5 promoter activity in vitro, the European study of Southam et al. assessed the effect of this allele on GDF5 expression in vivo using RNA extracted from the cartilage of patients who had undergone joint-replacement surgery for either hip or knee OA. In all of the cartilage samples examined (n = 9), the T-allele showed reduced expression relative to the C-allele, and in the most extreme case, the T-allele showed a 27% reduction in expression compared with the C-allele (p = 0.00007). When the data for all nine patients were combined, the T-allele demonstrated a 12% average reduction in GDF5 expression (p = 0.006). Overall, the T-allele of rs143383 has been shown to be associated with hip and knee OA in two distinct populations (Asians and Europeans) and to mediate reduced GDF5 expression in vivo and in vitro, but like the D14 allele of ASPN, possession of the T-allele appears to be a more significant risk factor for OA susceptibility in Asian populations.

Conclusion: toward an OA-causing pathway
In order to effectively prevent and treat OA, it is paramount to design therapies that target the susceptibilities possessed by an individual. Current treatments tend to focus on symptoms of the end-stage disease rather than on repair or prevention, with joint replacement and analgesics the major avenues for treatment currently utilized. The development of new therapeutics may enable modulation of the properties and activities of cells of mesenchymal origin involved in articular joint biology. The simplest situation for drug prescription would require identification of a target pathway or molecule that is common to a number of susceptibility loci. A small
number of therapies would then be able to treat a maximal number of people, as demonstrated by anti-TNF-α therapy for rheumatoid arthritis. OA shows a range of phenotypes, however, with different progressions, severity and ages of onset complicating the ability to exploit a global treatment regimen. Even if commonalities exist between susceptibility genes, the complex nature of the cross-talk between pathways within the cell, the pleiotropy and redundancy inherent in the function of many proteins, and the existence of tissue-specific activities are all likely to further complicate the identification of broad therapeutics. The three genes discussed in this review provide a demonstration of the difficulties arising in finding a common OA pathway for drug intervention.

Asporin, sFRP3 and GDF5 can all function extracellularly in cell signaling. Both asporin and sFRP3 are antagonists capable of binding to protein activators of signaling, whereas GDF5 is an activator of signaling, capable of binding to its receptors and initiating a multitude of downstream events. Asporin and GDF5 can function directly in TGF-β-related signaling through asporin’s ability to directly bind TGF-β1 and BMP-2 and through GDF5’s ability to activate signaling through BMP receptors [20,64–66]. Whether asporin can bind to and inhibit GDF5 activity is currently unknown. sFRP3 has been reported to bind Wnt5a and to antagonize Wnt5a and Wnt9a [67–69], but there is known cross-talk between the Wnt and TGF-β pathways, as it has been demonstrated that TGF-β1 can initiate nuclear accumulation of β-catenin [70], that BMP-2 can induce expression of soluble Wnts, thus activating Wnt signaling [71], and that GDF5 expression is modulated by Wnt signaling [72]. This cross-talk demonstrates the potential for a few therapeutics to provide global correction for a range of OA susceptibilities. For such an intervention to work, however, all potential interactions would need to be known to allow effective treatment without inadvertently targeting pathways or processes that could have a detrimental effect on OA pathology. Therapies would also need to guarantee a means for the correct intrinsic biological regulation after drug intervention so that people with different genotypes at causal polymorphisms and with different environmental influences can be effectively treated.

The influence of asporin, sFRP3 and GDF5 on gene expression and cell phenotype also illustrates the challenges in teasing out a downstream effector that is common to multiple susceptibility loci. The sFRP3 ligands Wnt5a and Wnt9a are able to enhance chondrogenesis whereas Wnt3a has been shown to inhibit chondrogenesis [73–75]. GDF5 and the asporin ligands BMP-2 and TGF-β1 have all been shown to enhance chondrogenesis and influence repair or development of other tissues of mesenchymal origin although their specific roles and modes of action can vary [76–91]. In monolayer cultures of the prechondrogenic ATDC5 cell line treated with BMP2, TGF-β1 or GDF5, GDF5 induced nodule formation and cellular condensation by day 14 of treatment, while BMP2 and TGF-β1 did not [88]. Moreover, GDF5 caused an increase in COL2A1 expression that continued to rise throughout the time-course of the experiment, whereas BMP2 and TGF-β1 caused an initial enhancement of COL2A1 expression with expression levels decreasing at later time points. In cultures of bovine synovial explants, however, incubation with BMP2, but not TGF-β, induced a chondrocytic phenotype and expression of chondrogenic markers, including COL2A1. The redundancies and the variations in protein function are essential for proper development and homeostasis of the joint, but they add complexity to finding a common target for OA treatments.

In light of the vast interactions between susceptibility genes and the other factors involved in articular joint homeostasis, the most effective path taken by future OA therapies would be individual-specific medication that targets the direct effect of the variants associated with disease. Treatment could potentially involve increasing the amount of functional sFRP3 or GDF5 or decreasing the amount of asporin such that the concentration of the protein is correct for the tissue targeted. The optimal time point at which to treat patients will need to be derived. If treatment should begin once OA symptoms have appeared, it is quite likely that therapies will not only have to target the OA-predisposing locus but also be able to correct the joint changes already mediated through the polymorphism’s artillery of effects. Treating prior to symptom appearance would require surmounting a number of financial and ethical hurdles, especially as polymorphisms increasing susceptibility to OA are not 100% penetrant and are moderated by environmental factors. This is not to cast a negative view on the ability to treat and prevent OA. As our understanding of joint biology increases and further OA-susceptibility loci are identified, the pathways to be followed for drug
therapy should become clearer and we may be able to develop an enlightened view of the elusive OA disease pathway.

Future perspective
Although progress has been made in understanding the genetics of OA, there remains a significant amount of genetic susceptibility to find and to characterize. Further progress has essentially been hindered by the limited power of avenues such as candidate gene-studies and genome-wide linkage scans to identify susceptibility genes for complex traits [16,21]. The completion of the human genome sequencing project (HGSP) [92], however, has precipitated a number of advances that have increased both the power and the resources available to unravel the genetic architecture of complex diseases [18]. For example, projects such as the SNP Consortium have interrogated the consensus sequence published by the HGSP to discover and catalog common variation in the human genome, such as SNPs, which may have direct medical relevance [18,93,94]. More recently, the International HapMap Project was set up to examine these SNPs for common patterns and correlations (linkage disequilibrium) [95].

Throughout the human genome, it is currently estimated that there are 10 million common SNPs (both alleles demonstrating a frequency ≥1%) [95]. SNPs that are physically close to each other are generally strongly correlated with one another (high linkage disequilibrium), and, because of this correlation, the genotyping of a few, carefully chosen SNPs (tag SNPs) from within a particular region will essentially predict the genotypes of the majority of the other common SNPs in that region [95]. Although it is technically unrealistic to genotype every SNP within an individual in the search for disease-causing variants, it is now possible to genotype a select number of SNPs (100,000–500,000) to indirectly cover the genome through linkage disequilibrium between the genotyped SNPs and the ungenotyped SNPs [96,97]. In combination with cost-effective and high-throughput genotyping technology, genomic resources such as the HapMap have opened the door for genome-wide association (GWA) studies, which have proven successful in identifying novel genetic susceptibility loci for complex diseases like OA, as evidenced by the recent work of the Wellcome Trust Case Control Consortium (WTCCC) [17,96,98].

GWA studies like that of the WTCCC succeed on a number of levels. First, because of the large sample sizes (the WTCCC examined ~2000 cases per disease and a common cohort of ~3000 controls), the studies are reasonably powered to detect susceptibility loci with moderate effects (ORs between 1.3 and 1.5). Second, because GWA studies approach the genome ‘agnostically’ with no a priori ideas about the type of the mutation or about a gene’s candidacy for disease involvement, novel disease pathways can be identified. Third, the coverage of the GWA study has the potential to extend into noncoding regions such as promoters, introns and intergenic regions, which have generally been ignored in candidate-gene studies and gene-based association scans that tend to focus on exons and untranslated regions. These noncoding regions, however, have been shown to not only harbor a number of diverse functional elements [99], but also to play important roles in susceptibility to common, complex disease like OA [100].

Many of the major OA research groups have now started to combine their population cohorts to conduct GWA studies. New projects are either underway or have recently received funding. These have adopted a number of acronyms, such as arcOGEN and TREAT-OA that will soon be familiar to those in the field. The cohort sizes are impressive, with several approaching 10,000 cases. There will therefore soon be an abundance of new, and hopefully replicated, loci that can be taken forward for functional analyses which will open new avenues for therapeutic intervention.

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FRZB

- A UK group reported an association of a nonsynonymous SNP coding for the substitution of a conserved arginine in exon 6 of the FRZB gene with female hip osteoarthritis (OA). Possession of the arginine substitution in the encoded protein resulted in a decreased ability of the protein to antagonize Wnt signaling.
- This SNP was also associated with generalized OA at multiple sites in two independent Dutch populations. No association of this SNP was found, however, in USA, Spanish and Belgian female hip OA cohorts, a Spanish hand OA cohort, and a Spanish knee OA cohort.

ASPN

- A Japanese group reported an association of the D14 allele of an aspartic acid repeat polymorphism in the asporin gene (ASPN) with knee OA. The D14 allele was shown to bind more strongly to TGF-β than other asporin alleles, resulting in a significantly enhanced inhibition of the prochondrogenic effects of TGF-β.
- A number of follow-up studies have been carried out in Asian and European populations. Meta-analysis has shown that possession of the D14 allele is a significant risk factor for knee OA in Asian populations, but the genetic effect of the D14 allele is much weaker in European populations.

GDF5

- A Japanese group reported an association of a SNP (+104T/C) in the 5´ untranslated region of the GDF5 gene with hip OA. The T-allele (OA susceptibility allele) showed significantly reduced expression relative to the C-allele in vitro and in vivo.
- This association was replicated in independent Japanese and Chinese knee OA cohorts as well as in a broad European cohort comprised of UK and Spanish hip, knee and hand OA cases and controls.

Conclusion: toward an OA-causing pathway

- These genetic finds have shed some light on the pathophysiology of OA, but because of cross-talk between signaling pathways and the pleiotropy and redundancy of signaling molecules, an OA-causing pathway still remains elusive.

Future perspective

- Genome wide association studies will provide a powerful and comprehensive tool to further interrogate the underlying genetic architecture of OA to confirm previously reported associations and to identify novel OA susceptibility genes.

Bibliography

Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers.


**Original FRZB study.**


**Original ASPN study.**


**FRZB replication study in Caucasian females with hip OA in the USA.**


**FRZB replication study in Dutch individuals with generalized OA at multiple sites.**


**APSN replication study in a cohort of Greek knee OA cases.**


**APSN replication study in cohorts of Spanish knee, hip or hand OA.**


**APSN replication study in a cohort of Han Chinese with knee OA.**


**Meta-analysis of Asian and European ASPN association studies that provides an estimate of the global relevance of ASPN to OA susceptibility.**


**APSN replication study in a cohort of UK Caucasians with knee OA or hip OA.**
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