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Genetics in Osteoarthritis Knee

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Abstract

Osteoarthritis (OA) is a debilitating joint disorder with a complex pathogeny wherein diverse factors interact, causing a process of deterioration of the articular cartilage and the subchondral bone. It can be primary or secondary but has common clinical, radiological, and pathological manifestations. Unfortunately, there are no curative or preventive options available for this disease. The knee is the most common site to develop OA among all synovial joints. Both environmental and genetic factors play an essential role in the initiation of the disease. Identifying the genes underlying the genetic background could give new insights into the pathophysiology of knee osteoarthritis (KOA) and could potentially lead to new drug targets. Several genes involving developmental processes or maintenance of cartilage and bone are found to be associated with KOA susceptibility and progression. Understanding the gene functions has improved the knowledge towards the disease pathogenesis. So, it will be of interest to investigate the role of gene-gene interaction in the disease.

Keywords: KL grade, knee osteoarthritis, single nucleotide polymorphism, VAS, WOMAC

1. Introduction

Osteoarthritis (OA) is the most common degenerative arthritis caused by the breakdown of articular cartilage [1]. The prevalence of OA is high and expected to increase in the coming years [2]. Results of some epidemiologic studies indicate that the incidence of symptomatic OA is about 8–9% in China [3]. OA is a multifactorial joint disorder in which growing age, genetic factors; hormonal as well as mechanical factors are significant contributors to its onset and progression. The molecular mechanism underlying the cartilage degeneration is poorly understood [4]. American College of Rheumatology defines Osteoarthritis as a heterogenous group of condition that leads to joint symptoms and signs which are associated with defective integrity of articular cartilage, in addition to related changes in the underlying bone at joint margin [5]. OA is primarily a non-inflammatory disorder of movable joint characterized by an imbalance between the synthesis and degradation of articular cartilage, leading to the classic pathological change of wearing away and destruction of cartilage [6]. OA affects nearly 21 million people in the United States, accounting for 25% of the visits to primary care physicians and half of all having NSAID (Non- Steroid Anti Inflammatory drugs) prescriptions. It is estimated that about 80% of the population was having radiographic evidence of Osteoarthritis by the age of 65 years, although only about 60% of these were

symptomatic [7]. Epidemiological profile of Osteoarthritis in India is not clear, but it is estimated that more than 30-40% of the Indian population suffers from Osteoarthritis over the age of 50 years (www.wrongdiagnosis.com).

Osteoarthritis is considered to be of two types:

1. Primary
2. Secondary

2. Primary osteoarthritis

Primary OA is a chronic degenerative condition of mobile joints due to an unknown cause. This may result due to aging because few people do not show any clinical or functional signs of the diseases in the late 90s. The proteoglycan and water content of the cartilage reduce with the advancement of age, hence the toughness of cartilage and increasing the susceptibility of collagen fibers to degenerate [8]. Mild inflammation around the joint capsule may occur in OA as compared to rheumatoid arthritis. This inflammation is in response to the small particles of the debris produced by this cartilage breakage and then attempted clearance by the scavengers cells located in joint lining [8]. New bone outgrowths called 'spurs' or osteophytes may form on the margins of the joint, possibly in an attempt to improve the congruence of the articular cartilage surfaces. Some of these bone changes, along with low-grade inflammation, may cause pain and mobility.

3. Secondary osteoarthritis

This type of OA is caused by other factors or disease, but the resulting pathological changes are the same as for primary OA.

Leading causes of secondary Osteoarthritis:

- Accidental injury to joints [9].
- Inflammatory diseases [7] (such as Perthes disease, Lyme disease) and all chronic form of arthritis (e.g. gout, pseudogout and rheumatoid arthritis). In gout, uric acid crystals cause cartilage degradation at a faster pace [10].
- Healed infection of the joints.
- Sports injuries [11]

According to Creamer *et al.* [12], Knee Osteoarthritis (OA) is a significant cause of disability, particularly in older people. The factors determining disability remain unclear. A study was conducted to assess the impact of clinical and psychosocial variables on function in knee Osteoarthritis and to develop models to account for observed variance in self-reported disability. It was conducted that function in symptomatic knee Osteoarthritis is determined more by pain and obesity than by structural changes as seen on plain X-ray. Hunter and Felson [13] said that Osteoarthritis had been known believed to be a disease of articular cartilage since ages, but the current concept is entirely different. Hunter and Felson explained that OA is a structural and functional impairment of synovial joints resulting in a range

of disease. OA entails the whole joint including the subchondral bone, menisci, ligaments, periarticular muscle, capsule and synovium.

Osteoarthritis (OA) is a chronic degenerative disorder of multifactorial etiology characterized by loss of articular cartilage, hypertrophy of bone at the margins, subchondral sclerosis and range of biochemical and morphological alterations of the synovial membrane and joint capsule. It may be either Primary Osteoarthritis or Secondary Osteoarthritis. Primary OA is mostly related to aging. It can present as localized, generalized or as erosive Osteoarthritis. Another disease or condition causes secondary OA [14].

Osteoarthritis (OA) is a disease of the musculoskeletal system that primarily involves the joints of the knee, hip, spine, hand and foot. OA is estimated to affect 40% of people >70 years of age [15], making it more prevalent than any other form of arthritis [16]. The Framingham Knee Osteoarthritis study suggests that knee osteoarthritis increases in prevalence throughout the elderly, more so in women than in men [17]. Females are found to have more severe OA, more number of joints are involved, and have more symptoms and increased hand and knee OA [18]. Osteoarthritis (OA) is the most common cause of musculoskeletal disability related to aging and is characterized by late-onset degeneration of articular cartilage [19]. OA is one of the leading causes of disability and dysfunction in the elderly population [20]; it has been estimated that the total cost for arthritis, including OA, is over 2% of the United States gross domestic product [21].

Prevalence of OA: Osteoarthritis (OA) is the second most common rheumatological problem and is the most frequent joint disease with a prevalence of 80% in the population having radiographic evidence. About 60% of radiographically evidenced subjects are symptomatic [22]. In India, more than 41.1% of the population suffers from Osteoarthritis beyond the age of 50 years [23]. This is the most common cause of locomotor disability in the elderly [24]. More than 20 million people are affected with OA in the United States, including 10% of adults of age 50 years. It has been estimated that 2% of women and 1.4% of men develop radiographic OA per year, but approx half of these individual show symptoms [19].

Etiology of OA: OA is a multifactorial disease with both genetic and environmental determinants, and all cases are probably affected by both, with a continuous distribution between the extremes of genetic or predominantly environmental causes [15]. The pathophysiology of OA is complex and do not comprehend with its clinical feature. The disease is rare before the age of 40 years but frequency increases with age as large no. of individual with ≥ 70 years demonstrate radiographic evidence of OA in some joints. All cases are probably affected by both genetics and environment, with a continuous distribution between the extremes of predominantly genetic or predominantly environmental causes [15].

Role of genes in OA: Role of genetics is emerging as an important etiological factor in recent times. More than 65 genes are associated with knee osteoarthritis (KOA) in different populations, and Indian population candidate genes like CALM-1 [25], VDR gene polymorphism [26], GDF-5 [22], SMAD-3, BMP-5, CCL2, COL2A1 [27] and COL2A1, CRT11 [28] are associated with KOA. In recent years many studies have been conducted on KOA to investigate its association with SNPs. Genome-Wide Association Study is performed on a large scale to identify the role of different loci in the development of the disease depending on the number of variants used. To date, more than ten loci (LSP1P3, GDF5, CHST11, FTO, GNL3, ASTN2, SENP6, PTHLH, TP63, CDC5L and CHST11) have been found associated with KOA through GWAS in European, Asian and Caucasians populations [29, 30]. Candidate gene studies have been responsible for identifying several susceptible loci for OA. GDF5, ASPN, FRZB and PTGS2 are few other genes which have been identified this way. These genes

continue to be the subject of functional studies and further genetic replication in independent populations [31–36].

Valdev et al. [37] reported an association between an amino acid variant in the TRPV1 gene and risk of symptomatic KOA for the first time. This amino acid has been implicated in pain sensitivity previously. The observation that the genotype implicated in lower pain sensitivity is significantly associated with a lower risk of painful OA. After adjustment for confounding variables (age, sex, BMI and radiographic severity) the difference between symptomatic and asymptomatic OA also achieves statistical significance.

Knee OA subjects showed individual characteristics in their expression of PBMC gene. A set of 173 genes was identified to diagnose Knee OA cases. The sensitivity and specificity were 89% and 76% respectively [38]. Besides, they observed that patients with symptomatic KOA could be categorized into two distinct groups based on the level of inflammatory gene expression (e.g., IL-1 β , IL-8, COX-2). The differential overexpression of these inflammatory genes in KOA subclasses was validated using qPCR ($P < 0.0001$) in 2 cohorts from NYUHJD and one cohort from Duke University.

Yerges Armstrong et al. [39] identified four SNPs significantly associated with radiographic KOA. The strongest signal ($p = 0.0009$) maps to 12q3, which contains a gene coding for SP7. Additional loci map to 7p14.1 (TXNDC3), 11q13.2 (LRP5) and 11p14.1 (LIN7C). The allele associated with higher BMD was also associated with higher odds of KOA in all four loci. This meta-analysis demonstrated that several GWAS identified BMD SNPs are nominally associated with prevalent radiographic KOA and further supports the hypothesis that BMD or its determinants may be a risk factor contributing to OA development.

A study by Shi-Xing Luo et al. [40] on IL-16 showed a significant association of SNP rs4778889 with altered gene expression levels as well as two other SNPs (rs11556218 and rs4072111). The latter two SNPs are located in an exon region, and their single nucleotide change results in an amino acid substitution. This was the first study to investigate the association of IL16 polymorphism with KOA risk, and a significant effect was observed IL16 rs1155218. Polymorphism represented an Asn to Lys substitution in exon 6 of the gene. It was mentioned that individual with rs11549465 C allele was at lower risk to develop the disease than those with T allele. Javier Fernández-Torres et al. [41] found that the SNP rs11549465 located in the exon 12 within the HIF1A gene was associated with KOA in Mexican patients. Their results showed that the presence of the CC homozygous variant or C allele represents potential risk factors for development of KOA. On the contrary to this, they detected that the heterozygous variant of CT or T allele of the rs11549465 polymorphism of the HIF1A gene (in comparison with the homozygous carriers) play a protective role against the disease.

Rui Zhang et al. [32] demonstrated that SNP rs143383 of GDF5 is a compelling risk factor for both knees and hand OA and provide further support for GDF5 in etiology of OA. A recent study by Kwo Wei Ho et al. [42] suggested that the COL11A2, a collagen-encoding gene, may play a role in pain sensitization after the development of OA. In a case-control study, Haohuan Li et al. [43] investigated the association between EN1 rs4144782 and susceptibility to KOA in a Chinese population. A significant association of SNP was observed with increased risk of KOA. The results reconfirmed the close connection between BMD and OA.

Indian scenario: Besides our studies, not much work has been done on the association of SNPs in the Indian population. Studies published from our laboratory on genetic polymorphism in KOA demonstrated an association of BMP5, CCL2, COL2A1, IL1B, SMAD3, GDF5, ESR- α , CALM1 and COMP genes with the development of KOA. Background of these genes is given below.

4. Bone morphogenetic protein (BMP5) gene

BMP5 is a member of the TGF- β superfamily of secreted proteins whose family members are involved in synovial joint development and joint tissue homeostasis [44]. Hahn *et al.* [45] reported that BMP5 was found on human chromosome 6p12.1.

BMPs were originated as protagonists of bone formation and growth. They have a major role in morphogenesis of a variety of vertebrate tissues and organs. They stimulate all proliferation and matrix synthesis for differentiation of chondrocytes. BMP5, in particulate, are known to regulate ovarian development [8, 46], cardiac development [47], and limb bud development [48] with a well-defined role in the differentiation of chondrocytes through the promotion of cell proliferation and matrix synthesis [49, 50]. Southam *et al.* [51] found that Polymorphisms located within the transcribed region of *BMP5* and its proximal promoter had previously been excluded for association with OA. Nevertheless, there is increasing evidence; however, that polymorphisms in regulatory elements involved in gene transcription play an important role in conferring susceptibility to complex disease traits [52].

The studies carried out in multiple organisms demonstrated its role in the regulation of various episode of embryonic development which includes dorsal-ventral and left-right axis formation, mesenchymal-epithelial interactions, and differentiation of many specific tissues including lung, gut, kidney, hair, teeth, cartilage, and bone [7]. BMPs can trigger the entire process of cartilage and bone formation when implanted at ectopic sites in adult animals [53] and are usually expressed in and around early cartilage and bone precursors during embryonic development [54–57]. Moreover, mutations in different BMP genes block the formation of particular skeletal features, showing that BMPs are also required for the normal formation of skeletal tissue [58].

BMP1 is a protease which takes part in the maturation of fibrillar collagens whereas the other BMPs are secreted molecules of TGF-beta family [59, 60].

BMPs influence the normal development and repair of the synovial joint; therefore alterations in the activity of these molecules could affect the arthritic phenotype [61, 62]. Using genetic association analysis, we have tested BMP5 as the chromosome 6 OA susceptibility gene. Zuzarte-Luis *et al.* [63] provided evidence for a role of this BMP member in the development of limb autopodium through the activation of Smad proteins and MAPK p38. Previous studies demonstrate that secreted signaling molecules in the bone morphogenetic protein (BMP) family play a vital role in both formation and repair of skeletal structures. These molecules are expressed both in early skeletal precursors and in the surface perichondrium and periosteum layers that surround growing cartilage and bone [64].

Wilkins *et al.* [65] identified an SNP and a functional microsatellite associated with OA. It has been exhibited that various alleles of microsatellite are behind the modified transcriptional activity of BMP5 promoter suggestive of *cis*-regulation of *BMP5*, is involved in OA susceptibility.

5. Chemokine (C-C motif) ligand 2 (CCL2) gene

Chemokines are small, secreted proteins that stimulate the directional migration of leukocytes and mediate inflammation (Baggiolini *et al.*, 1997). These are a family of heparin-binding cytokines known for this chemotactic activity. Four subfamilies of chemokines have been identified based on the juxtaposition of cysteine residues in the protein's N-terminus. These families have been named C, C-C, C-X-C, and C-X3-C [66].

Sozzani *et al.* [67] said that the C-C and C-X-C chemokines represent two significant subgroups. The C-X-C chemokines include IL-8 and growth-related oncogene α . The C-C chemokine family comprises families of monocyte chemoattractant proteins (e.g. MCP-1, MCP-2, MCP-3), macrophage inflammatory proteins (e.g., MIP-1a and MIP-1b), and a chemokine designated RANTES (regulated upon activation, normal T cell expressed and secreted). As their names suggest, these chemokines act predominantly on mononuclear cells (e.g., T cells, monocytes, and macrophages).

CCL2; previously known as monocyte chemoattractant protein-1, MCP-1 is a chemoattractant that belongs to the CC chemokine subfamily. Mehrabian *et al.* [68] localized the gene for monocyte chemotactic protein-1 (CCL2) to chromosome 17. Corrigan *et al.* 2001 proposed that CCL2 in the synovial membrane serves to recruit macrophages and perpetuate inflammation in the joints of patients with rheumatoid arthritis. CCL2 plays a crucial role in host defense by recruiting monocytes and macrophages at the site of inflammation [69].

CCL2 production is inducible in various types of cells, including synoviocytes [70]. In several studies, increased expression of CCL2 was observed in patients with inflammatory diseases, including neuropsychiatric syndromes of systemic lupus erythematosus, rheumatoid arthritis, OA and degenerative and inflammatory arthropathies, including gout [71–73].

Reports have demonstrated the involvement of chemokines in cartilage abnormalities in OA [74–76]. Reported studies suggests that CCL2 is involved in inflammatory diseases such as RA and OA, However, in previous studies, the CCL2 gene polymorphism (22510A/G) did not show any association with Spanish and Korean RA [77, 78]. However, Park *et al.* [79] investigate that significant association between polymorphisms of the CCL2 gene and primary knee OA patients in a Korean population.

6. Collagen, type II, alpha 1 (COL2A1) gene

Solomon *et al.* [80] suggest that the human type II collagen gene, COL2A1, has been assigned to chromosome 12.

Law *et al.* [81, 82] said a cosmid clone containing the entire human type II alpha one collagen gene (COL2A1) was used as a probe in the Southern analysis of DNA from a panel of human/hamster somatic cell hybrids containing different portions of human chromosome 12. Two of the hybrids exhibited a similar terminal deletion q14.3----qt, but one was positive for the gene while the other was negative. Therefore, the gene must reside in the region q14.3.

Holderbaum *et al.* [83, 84] referred a single base change resulting in the substitution of Cys for Arg at position 519 of the type II collagen triple helix is a predisposing factor in the pathogenesis of a precocious-onset form of familial Osteoarthritis associated with a mild chondrodysplasia. Cartilage obtained at the time of total knee replacement in a patient with the Arg-Cys519 mutation was used to investigate the expression of Col2A1 alleles. Using PCR assisted amplification of mRNA with specific amplification of a region of Col2A1 message encompassing exons 31-34, followed by single-strand conformation polymorphism and sequence analyses, we have found transcription products of both mutant and normal type II collagen alleles. Further analysis of the sequence of these exons provides evidence that the Arg-Cys519 mutation arose independently in at least two of the three known affected families. The presence of both mutant and normal alleles of Col2A1 in cDNA derived from cartilage obtained from this patient suggests that Cys519-containing type II collagen may continue to be produced even in advanced stages of Osteoarthritis.

On the other hand, mutations in Type II procollagen (COL2A1) can cause a hereditary form of the joint disorder with a broad spectrum of phenotypes ranging from primary OA with mild chondrodysplasia, mild spondyloepiphyseal dysplasia and osteonecrosis to severe generalized OA, including achondrogenesis and hypochondrogenesis [85].

The COL2A1 gene appears to play a crucial role in OA pathogenesis because the protein encoded is the most abundant in articular cartilage. However, results of the studies searching for a relationship between COL2A1 and OA are controversial, due to that an association with specific genotypes has been reported in some studies, whereas others have denied this. We found no association between OA of the knee and COL2A1 gene polymorphism in the overall sample. Nevertheless, when the association was analyzed according to radiologic grade, a significant relationship was denoted in OA grade 4 with allele p (Pp/pp) [OR (95% CI) 4.1 (1.2;14.6)] independently of gender, age, and BMI; this indicates that in Mexican Mestizo population, a COL2A1 gene polymorphism is associated with advanced stages of OA of the knee [86–89].

The study of Mu *et al.* [90] does not fully support that COL2A1 could be implicated in primary OA of other non-Asian ethnic groups since ethnic variability in gene susceptibility is very well documented. The clinical features of early-onset OA, mild clinical phenotypes and one patient with osteonecrosis of the femoral head strongly suggested that COL2A1 may also be the underlying cause of OA in our family. Linkage analysis and direct sequencing of COL2A1, however, clearly rule out this possibility. Kannu *et al.* [91] have been described COL2A1 mutations in association with bilateral hip disease and Osteoarthritis in the second decade of life but without ocular abnormalities or short stature. Xu *et al.* [92] investigate the relationships between two COL2A1 single nucleotide polymorphisms (SNPs; T2088C and G4006A) and Osteoarthritis (OA) in Han Chinese women.

7. Interleukin 1 beta (IL1B) gene

Loughlin *et al.* [93] said that IL-1 is the primary catabolic cytokine of the OA joint and can stimulate the synthesis of several proteinases, which can result in the breakdown of cartilage extracellular matrix proteins. The 2 IL-1 genes (*IL1A* and *IL1B*) and the gene encoding IL-1Ra (*IL1RN*) are located on chromosome 2q13 within a 430-kb genomic fragment [94]. Loughlin *et al.* 2002 reported that IL-1R antagonist (IL-1Ra) competes with IL-1 for binding to the IL-1 receptors and can act as an inhibitor of cartilage loss. When the catabolic and anabolic activities of the cytokines are balanced, cartilage integrity is maintained. If there is an imbalance favoring catabolism, however, cartilage destruction can proceed, resulting in OA. It is, therefore, reasonable to propose that a proportion of the genetic susceptibility to OA may be encoded for by variation in the activity of interleukins and that for chromosome 2q this susceptibility could reside within the IL-1 gene clusters. Stern *et al.* [95] reported that IL1B 5810 G > A SNP genotypes marker were not in Hardy-Weinberg equilibrium ($p < 0.05$ in both non-erosive and erosive hand OA subgroups). Statistically significant association with the IL1B 5810 AA genotype was found in the erosive hand OA subgroup (relative risk 3.8, $p = 0.007$). This IL1B 5810 AA genotype association was also significant between erosive and non-erosive hand OA subjects (relative risk 4.01, $p = 0.008$). As expected, significant linkage disequilibrium was present between IL1B 5810 SNP and IL1A (–)889 SNP, other IL1B SNPs, and the nearest IL1RN SNP examined. The IL1B 5810A allele occurs most frequently on haplotypes with the SNP alleles IL1B 1423C, IL1B 1903 T, IL1B 5887C, and IL1A (–)889C. Genotypes at null loci failed to show evidence suggesting

population stratification that might account for the spurious association. Sezgin *et al.* [95] said that some researchers had suggested an association between the IL-1 gene cluster and the occurrence of OA.

Previously, Moos *et al.* [96] investigated the distribution of polymorphic alleles of four different genes encoding TNF-alpha, IL1RN, IL1B and IL-6 in the knee or hip OA patients with controls. The analysis of genotype frequencies for the IL1B gene, more OA patients than controls was homozygous for allele 2, although any significant differences for the TNF-alpha, IL1RN and IL-6 polymorphisms were found.

8. SMAD3 (SMAD family member 3) gene

Smad3 was found on human chromosome 15q22.33. The classic TGF- β mediated signaling pathway involves Smad activation. Smads are a family of intracellular proteins that comprise three classes of signaling molecules: - receptor-associated Smads (2 and 3 for TGF- β , 1, 5, and 8 for BMP signaling), the co-factor Smad4, and the inhibitory Smads (6 and 7) [97]. The receptor-activated SMADs include SMADs 1, 2, 3, 5, and 8. SMADs 2 and 3 respond to TGF- β and activins [97, 98], whereas SMAD1, 5, and 8 function in BMP signaling pathways [99–101]. The receptor-associated Smads bind to the type I receptor, and on ligand binding and activation, are phosphorylated and released into the cytoplasm. The activated receptor-associated Smads form a trimeric heterodimer with the co-factor Smad4, translocate to the nucleus, and influence gene transcription [102].

Yao *et al.* [103] reported that Smad3 gene mutation is a possible predisposing factor for human OA and found gene mutation in OA, providing insight into the function of SMAD3 mediated TGF- β signals in the development of OA and also suggested that Smad3 gene mutation may be a risk factor for genetic susceptibilities to OA.

Ferguson *et al.* [104] has established that the TGF- β Smads inhibit chondrocyte maturation, whereas the BMP-related Smads accelerate maturation. Many studies have shown that transforming growth factor- β (TGF- β) signals function as crucial regulators in bone formation, remodeling and maintenance. [105]. Micheal *et al.* [106] have shown that loss of Smad3 results in impaired immune responses, accelerated wound healing decreased bone density, OA and access to colon cancer.

Yang *et al.* [107] showed that Smad3-mediated TGF- β signals are essential for maintaining articular cartilage in the quiescent state by repressing chondrocyte differentiation and controlling matrix molecule synthesis. Consequently, impairment of TGF- β signals due to Smad3 disruption results in phenotypes resembling human Osteoarthritis.

Wu *et al.* [108] suggested that Loss of Smad3 appears to enhance bone morphogenetic protein signaling in the articular chondrocytes, leading to hypertrophy and OA-like changes. The observation further supports the crucial role of Smad3 that Smurf2 overexpression leads to dephosphorylation of Smad3 and is associated with a spontaneous OA phenotype in transgenic mice.

Also, Cherlet *et al.* [109] reported that Smad3 levels are lower in women than in men, which is consistent with other data showing that estrogens inhibit SMAD3 transcriptional activity. Nevertheless, Valdes *et al.* found that the genetic association of the SMAD3 intronic SNP with OA was significant in both men and women and that effect sizes were remarkably similar between sexes, confirming the robustness of the result. Valdes *et al.* [109] reported that four SNPs (rs266335, rs12901499, rs6494629, and rs2289263) were found to be nominally significantly associated with

knee OA ($P < 0.05$), but only 1 of them, rs12901499, was nominally associated also with hip OA ($P < 0.021$) and also observed that the major allele G was found at a higher frequency among OA patients than among controls [109–112].

9. Growth differentiation factor-5 (*GDF-5*)

Hotten *et al.* [9] determined that the *GDF5* gene contains two exons. Miyamoto *et al.* [113] reported that the gene encoding growth differentiation factor-5 (*GDF-5*) is associated with Osteoarthritis in Asian populations. An SNP in the 5' untranslated region (UTR) of *GDF-5* showed significant association with hip osteoarthritis in 2 independent Japanese populations. This association was replicated for knee osteoarthritis in Japanese and Han Chinese populations. This SNP, located in the *GDF-5* core promoter, exert allelic differences on transcriptional activity in chondrogenic cells, with the susceptibility allele showing reduced activity. The findings implicated *GDF-5* as a susceptibility gene for Osteoarthritis and suggested that decreased *GDF-5* expression is involved in the pathogenesis of Osteoarthritis. [2] also isolated and characterized human *GDF5*, which they designated *CDMP1*, as well as human *GDF6* (*CDMP2*). *GDF6* is predominantly expressed at sites of skeletal morphogenesis. Al-Yahyaee *et al.* [114–117] identified two mutations in the *GDF5* gene: a silent 1137A-G transition encoding lysine and a 1-bp deletion, 1144delG, predicting a frame shift resulting in loss of the biologically active C terminus of the protein. Thomas *et al.* [118] found that heterozygotes for the C400Y mutation had phenotypes resembling brachydactyly types A1, A4, or C.

10. Estrogen receptor alpha (*ESR- α*) gene

The estrogen receptor (*ESR- α*) is a ligand-activated transcription factor composed of several domains essential for hormone binding, DNA binding, and activation of transcription. Alternative splicing results in several *ESR- α* mRNA transcripts, which differ primarily in their 5' untranslated regions. The translated receptors show less variability [4, 119]. Ponglikitmongkol *et al.* [120, 121] showed that the human *ESR- α* gene is more than 140 kb long. It contains eight exons, and the position of its introns has been highly conserved, being, for example, remarkably similar to those of one of the chicken thyroid hormone receptor genes. Sputnik *et al.* [122] reported that *ESR α* isoform is a ligand-activated transcription factor composed of several essential domains for hormone binding and activation of transcription. *ESR α* is an essential mediator in the signal transduction pathway. Jin *et al.* [123] reported that Estrogen receptors (*ESR- α*) are known to play an essential role in the pathophysiology of Osteoarthritis. To investigate *ESR α* gene polymorphisms for its association with primary knee osteoarthritis, they conducted a case-control association study in patients with primary knee osteoarthritis and healthy individual in the Korean population. Jin *et al.* (2004) investigated the association between haplotypes of three polymorphism in *PVU II* in intron 1 (IVS1-397 T/C), Xba I in intron 1 (IVS1-351A/G) and Big I in exon 8 (exon8 229G/A) of *ESR- α* gene and primary knee OA in the Korean population, first two SNP in intron one also investigated by Bergink *et al.* [124] in Rotterdam population. Ushiyama *et al.* [125] found on *ESR- α* gene association between a genotype of *PVU II* and Xba I polymorphisms in Intron 1 and generalized Osteoarthritis with a severe radiographic change in the Japanese population.

11. Calmodulin 1 (*CALM1*) gene

Rhyner et al. [126] found that the *CALM1* gene contains six exons spread over about 10 kb of genomic DNA. The exon-intron structure was identical to that of *CALM3*. A cluster of transcription-start sites was identified 200 bp upstream of the ATG translation-start codon, and several putative regulatory elements were found in the 5' flanking region, as well as in intron 1. A short CAG trinucleotide repeat region was identified in the 5-prime untranslated region of the gene. Motoani et al. [127] identified susceptibility genes for Osteoarthritis in a large-scale case-control association study using gene-based single-nucleotide polymorphism (SNPs) in a Japanese population. In two independent case-control populations, they found a significant association ($p = 9.8 \times 10^{-7}$) between hip osteoarthritis and an SNP (IVS 3–293 C>T) located in intron 3 of the calmodulin (Cam) 1 gene (*CALM-1*). *CALM 1* was expressed in cultured chondrocytes, and articular cartilage and its expression were increased in Osteoarthritis. Subsequent linkage – disequilibrium mapping identified five SNPs showing significant equivalent to IVS 3–293 C>T. One of these (–16 C>T) is located in the core promoter region of *CALM 1*. Functional analysis indicated that the susceptibility – 16 T allele decreases *CALM 1* transcription in-vitro and in-vivo. Inhibition of CAM in chondrogenic cells reduced the expression of the major cartilage matrix genes *col 2a1* and *Agc 1*. These results suggested that the transcriptional level of *CALM-1* was associated with susceptibility for hip osteoarthritis through modulation of chondrogenic activity. Their findings revealed that the *CALM-1* mediated signaling pathway is chondrocytes as a novel potential target for the treatment of Osteoarthritis. Loughlin et al. [128] studied in a Caucasian population using a cohort of 1672 individuals and concluded that *CALM-1* core promoter polymorphism is not a risk factor for Osteoarthritis.

12. Cartilage oligomeric matrix protein (*COMP*) gene

Briggs et al. [129] demonstrated that the *COMP* gene contains 19 exons. Exons 4–19, which encode the EGF-like (type II) repeats, calmodulin-like (type III) repeats (CLRs), and the C-terminal domain, correspond in sequence and intron location to the thrombospondin genes, whereas exons 1–3 are unique to *COMP*. Mabuchi et al. [130] reported that hereditary osteochondral dysplasia produces severe early-onset OA Among them are Pseudoachondroplasia (PSACH) & multiple epiphyseal dysplasias (MED) both of which are caused by a mutation in the *COMP* gene. Therefore *COMP* may be a susceptibility gene for OA. For these reasons, Mabuchi et al. [131] hypothesized that Osteoarthritis is a common disorder may be at the mild end of the phenotypic gradation produced by *COMP* mutations. They ascertained the sequences of the exons and exon-intron boundaries and identified 16 polymorphisms in the *COMP* gene. Using five polymorphisms spanning the entire *COMP* gene (–1417 C/G in promoter region with $P = 0.29$, c.279C/A in exon 4 with $P = 0.19$, IVS5 + 76 T/C with $P = 0.74$, IVS16–45C/T with $P = 0.19$ and IVS18–40 T/C with $P = 0.93$), Mabuchi et al. [132], examined the association of this gene in Japanese patients with Osteoarthritis of the knee and hip joints. Genotype and allele frequencies of the polymorphisms were significantly different between osteoarthritis and control groups, and with the help of this study, they hypothesize that comp gene is a candidature gene for OA Song et al. [133] identified mutations in the *COMP* gene in 9 of 9 Korean patients with PSACH and 3 of 5 Korean patients with MED. Three of the eight mutations identified were novel. Deere et al. identified 12 mutations in the *COMP* gene, including ten novel mutations in 12 patients with PSACH. The site of the mutations emphasized the importance of the calcium-binding domains and

the globular domain to the function of COMP. Kennedy *et al.* reported three SNPs in COMP, first in exon-11, nucleotide change A>G at c1156 with allelic frequency 0.03, second in exon -16, nucleotide change G>A at c1755 with allelic frequency 0.05, and last third one is in 3'UTR, Nucleotide change A>G at c2289 with allelic frequency 0.005 which are associated with OA [133].

CALM1 gene showed a significant association of SNP with the disease. CALM1 gene intronic SNP (rs3213718) was present in our population, and its occurrence was significantly affecting the disease.

A case-control study of 600 subjects showed a significant association of the +104 T/C GDF5 polymorphism with KOA and with individual clinical symptoms of the disease. A study done by **Srivastava et al.** reported that genetic polymorphisms affecting KOA vary between genders and indicate a role for BMP5, COL2A1, CCL2, and IL1B in North Indian Population. Another case-control study of 499 KOA cases and 458 controls exhibited an association between rs1470527, rs9382564 polymorphisms of BMP5 gene with KOA. This association was validated by haplotype analysis. Further, the association between KOA and rs1470527 polymorphism was more robust in both the genders and age groups. However, association with rs9382564 was stronger only in female patients and either gender aged >55 years. Moreover, our data showed a significant association of both SNPs with VAS and WOMAC clinical scores.

Furthermore, a genetic study conducted in our laboratory on SNPs rs921126 (BMP5) and rs12901499 (SMAD3) showed a significant association between these SNPs and risk of KOA. It was found that the risk increased with age (>55) in both the genders. We also conducted a pilot study on VDR gene polymorphism and its association with KOA. It was found that Taq1 polymorphism influenced the clinico-radiological response to vitamin D supplementation in KOA subjects with insufficient 25(OH) vitamin D levels. Validation of the results is in process on large population with insufficient 25(OH) vitamin D levels.

An extensive literature search, we could find only one more study conducted in Indian population other than ours, by Subramanyam et al. They studied the association of rs73297147 and rs73771337 polymorphisms in COL2A1 and CRT1L1 genes with primary KOA in South Indian population, and a significant association was observed.

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