

Brief Review of Genomics in Osteoarthritis

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Received: April 12, 2015 Revised: May 2, 2015

Accepted: May 6, 2015

Published online: August 23, 2015

ABSTRACT

Osteoarthritis (OA), the most common form of arthritis around the world, is a multifactorial degenerative joint disorder characterized by articular cartilage degradation, subchondral bone sclerosis, osteophyte formation, synovial membrane inflammation and affection of extraarticular tissue as tendon, ligaments and articular capsule. Although various factors have been recognized as essential in OA development, there is a strong hereditary component and is considered to be a polygenic disease. The identification of genes associated with osteoarthritis can help reveal biological mechanisms that may lead to development of new therapeutic targets or biomarkers for early detection and risk stratification. The goal of this brief review is to offer an overview of the current status of genetics of osteoarthritis.

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Key words: Osteoarthritis; Genome; Genomic studies

Arellano RD, Arguello JR, Moran-Martinez J, Garcia-Marin AY,

González-Galarza FF, Guzman DD, Sánchez JLC. Brief Review of Genomics in Osteoarthritis. *International Journal of Orthopaedics* 2015; 2(4): 341-346 Available from: URL: <http://www.ghrnet.org/index.php/ijo/article/view/1321>

INTRODUCTION

Over the last years genomics research has played a significant role in the field of personalized medicine and clinical practice. According to recent studies, humans have been shown to have an increase in life expectancy over the last decades; thus, the study of our genome provides a useful tool to assist the medical community in early diagnosis and treatment^[1].

At present, one of the most popular (and costly effective) tools for the study of the human genome has been the use of genomic wide association studies (GWAS). These and other novel techniques have assisted researchers in the discovery of genes associated with different pathologies^[2,3].

Osteoarthritis (OA) is the most common joint disease in humans, with approximately 27 million people diagnosed only in the United States in 2008. However, despite the high prevalence of this disease and its major impact in public health, currently little is known about its etiology, risk of progression and there is no definitive treatment^[4]. It is known that around 35% of people suffering from OA are occupationally active showing the negative impact of the disease and implications in public health^[5,6]. Although OA is considered a disease associated to age, wear and tear of the cartilage of synovial joints have been shown to have a clear genetic component^[7]. Hence, the contribution of genetics to OA has been estimated at 65% for knee, 60% for hip and 39% for the hand^[8].

Despite the fact that OA is considered as a complex disease, genetic factors exert a strong influence on its development and progression. Several familial studies have shown that the heritability of the disease ranges from 39% to 78%^[9-11]. This genetic influence contributes to the complexity of the disease which is characterized by a great variability in clinical characteristics and biological processes involved in the initiation, progression and response to treatment.

Thus, genomics analyses play an important role in the investigation of complex diseases such as OA^[12].

The identification of genes associated with OA may help in the understanding of the underlying biological mechanisms and facilitate the development of new treatment options, as well as early detection and risk stratification. The study of different single nucleotide polymorphisms (SNPs) and its association with OA has provided a greater understanding of the pathophysiology. The large number of investigated genes reflects the complex nature of OA. However, it has been difficult to replicate studies due to the ethnicity, age, sex, and small sample sizes providing limited statistical significance in many cases.

Currently, different techniques such as chain-termination sequencing (Sanger) and the use of polymerase chain reaction (PCR) methods continue to have a role in the understanding of genes involved in OA; however, some mutations may be overlooked when using these methods. Thus, different techniques such as microarrays or next generation sequencing methods may allow researchers to evaluate multiple genetic variations in a short period of time and relatively low cost. Here we review some of the genes and their corresponding polymorphisms that have been associated to OA using these technologies^[13].

BRIEF HISTORY OF GENETIC INFLUENCE IN OSTEOARTHRITIS

Linkage studies

Genetic linkage occurs when a locus involved in a particular trait of interest and nearby alleles are inherited together^[21]. By performing a genome-wide linkage scan in sibling pairs in osteoarthritis from large joints, one of the most significant developments was made by Meulenbelt et al identified DIO2, encoding the D2 enzyme determining the availability of local active thyroid, as a new susceptibility gene for OA^[22].

Candidate gene studies

Candidate gene studies in OA have also been used in screening large populations for genetic mutations potentially involved in the development of OA. The most promising candidates are involved in growth and differentiation pathways necessary for the development and maintenance of synovial joints.

From the candidate gene studies performed in hip and knee, multiple associations have been found with varying degrees of certainty. For example, FRZB gene, encodes secreted frizzled-related protein, which is a soluble antagonist of wingless (wnt) signaling has been associated with hip OA. This involves Wnt signaling through β -catenin, which is important for the development of cartilage and bone. This gene has been further associated with OA in animal models^[23-26].

Besides COL2A1 gene associated with chondrodysplasia, some other structural genes encoding extracellular proteins, such as COL9A2, COL11A1, COL11A221-23 and COMP (cartilage oligomeric matrix protein gene) have been associated with the disease. Moreover, mutations in the COL1A1 gene are associated with a reduced risk of hip osteoarthritis in women^[27-31].

Proteolytic activity on type II collagen and aggrecan is an early event in the articular degenerative process. Therefore, the matrix metalloproteinase (MMP-3) has been considered as the enzyme responsible for aggrecan degradation.

Studies of OA performed in mouse models demonstrated that the

enzymatic activity of ADAMTS-5 induced aggrecan breakdown in articular cartilage after physical activity or injury. However, an association analysis of SNPs in ADAMTS-5 in osteoarthritis of the hip, hand and knee did not found such association. To date, only one cartilage degrading enzyme, disintegrin ADAM 12, has been associated with OA. However, ADAM12 is not apparently involved in the development or progression of OA^[32-34].

The role of apoptosis in OA has been demonstrated in *in vitro* and *in vivo* models. A significantly higher proportion of apoptotic chondrocytes were found in samples from patients with osteoarthritis unlike age-matched controls^[35]. Two major pathways have been proposed in programmed cell death: (1) mediated by the receptor for apoptosis and (2) mitochondrial pathway. In the first case, cell death receptor belongs to tumor necrosis factor family (TNF) such as Fas. The mechanism whereby the stimulation of Fas ligand to Fas initiates apoptosis has been investigated extensively^[36].

The variations in mitochondrial DNA (mtDNA) have been associated with the risk of knee and hip OA^[37,38].

Given the role for mtDNA damage in apoptosis, it is possible to speculate on the role that some mtDNA haplotypes play in cell death. Recently, it was reported that a genetic variation in the ANP32A gene plays a regulatory role in apoptosis and interferes with Wnt signaling *in vitro* and in women with hip OA^[39].

Other genes involved in the OA

Estrogen receptor α (ER α), important mediator in the signal transduction pathway, is expressed in various cells such as human articular chondrocytes^[40,41]. Some alterations have been described in this gene that may favor the development of OA^[42]. Several epidemiological studies in women suggest that estrogen loss may be associated with an increased prevalence of osteoarthritis in the knees and hips, which helps explain the differences in prevalence of the condition according to sex^[43]. Similarly, studies have shown a link between vitamin D levels in serum and the progression of knee osteoarthritis, while others reflect the relationship between certain polymorphisms in the gene coding for the D vitamin and susceptibility to developing osteoarthritis in different joints^[44].

Genome wide association studies (GWAS)

Next generation sequencing (NGS) methods have revolutionized the fields of clinical genomics research, allowing transcriptome, exome and whole genomes sequencing in a fast and 'economical' manner. This will undoubtedly create predictive models of disease for early diagnosis and develop for a more effective individualized treatment plans in clinical practice. By scanning the entire genome in individuals with and without OA, it is possible to find variations associated with the disease. This type of sequencing techniques can be used to test the association of polymorphisms of a single nucleotide (SNP) with OA and other complex diseases.

It has been possible to identify some variants significantly associated with OA of the knee or hip, especially in people of Asian and European countries, see table 1.

Some of these variants are located on chromosome 13q34 near the MCF2L8 (Day) gene, on chromosome 7q22 (Evangelou, Kerkhoff), the rs143383 polymorphism in the gene GDF5 (Miyamoto, Valdes, Sanna), DVWA (HLA) class II/III and BTNL2 (Miyamoto, Nakajima), 9q33 (ASTN2), 6q14 (FILIP1/SEN6), 12p11 (KLHDC5/PTHLH) and 12q23 genes (CHST11) (arcOGEN) that were significantly associated with susceptibility to hip OA and knee. According to HUGE navigator 88 genes have been associated with knee OA^[45-54].

Table 1 Genes associated to OA.

Gene	Snpmutation	Function/Protein	Disease
COL2A1, COL11A1, COL11A2 ^[17-20,27-31]	Single base mutation at position 519 of the $\alpha 1$ (II) chain to a codon for cysteine	Synthesis of type II collagen	Chondrodysplasia, family osteoarthritis.
DIO2 ^[22]	Minor allele of rs225014 and common allele of rs12885300	Regulates thyroid hormone	Large joints OA
FRZB ^[23-26]	rs288326, rs7775	Wnt antagonist	Hip OA
ADAM12 ^[32-34]	rs3740199, rs1278279, rs1044122, rs1871054	Cartilage degrading enzyme	Osteoarthritis
ANP32A ^[37-39]	rs7164503	Apoptosis/Wnt signaling	Osteoarthritis mtDNA damage
VDR1 ^[44]	Allele aa	Vitamin D signaling	Hand OA
COMP gene ^[60]	c.2152C>T mutation in exon 18	Mutation	Family early onset OA
ITPR2 ^[63]	rs10842750	Degeneration of articular cartilage, degradation of the cartilage matrix, necrosis and apoptosis of chondrocytes	Kashin Beck Disease (KBD)
S100A9 (alarmin) ^[65,66]		Stimulate the formation of osteophytes	OA progression

In a study performed in Asian and European populations, Panoutsopoulou and Zeggini have highlighted the importance of defining phenotypes of patients (especially radiographic severity scale of Kellgren and Lawrence OA) which should be considered when interpreting the findings, as subjectivity in interpretation may confuse the findings in GWAS^[55].

The common limitations of GWAS studies have been small sample sizes, the heterogeneity of the populations studied and associated costs by including large populations, so that replication of positive results has been limited.

Meta-analysis in multiple cohorts seeks to better determine the statistical power of studies using GWAS. In a recent meta-analysis of 9 GWAS, researchers found that from the 199 genes suggested as candidates associated with OA, only 2 showed significant association: COL11A1 (encoding an alpha chains of collagen type 11) and VEGF. Levels of vascular endothelial growth factor (VEGF) in the synovial fluid and plasma have been correlated with disease severity in knee osteoarthritis. A single nucleotide polymorphism in the COL11A1 gene has been associated with susceptibility to lumbar disc herniation^[56,57].

In contrast to the study of biomarkers in serum and synovial fluid, the study of genetic variations to predict the risk of development and progression of the disease would be useful in order to adopt preventive measures by clinicians^[58]. Also, whole genome or exome can be sequenced to detect variants associated with complex diseases and family study of OA. In these cases, the most notable successes in sequencing the whole genome or exome have included cases of severe early-onset OA or cases of segregation in families, as in the case of spondyloepimetaphyseal dysplasia with joint laxity, an autosomal dominant skeletal disorder with early OA, which has been identified 5 variants of KIF22 gene^[59].

In a study by Mu *et al.* a linkage signal was found in the sixth generation of a Taiwanese family with early onset of osteoarthritis. They identified a mutation of non-conserved substitution COMP gene. It is noteworthy that 21 out of the 26 mutation-carriers had early onset osteoarthritis and the 5 people remaining were too young to have developed symptoms. This mutation is located in a highly conserved region of the gene and was not detected in 96 samples from reference subjects from Taiwan^[60].

Another example of the potential usefulness of GWAS is in the Kashin-Beck disease (KBD). This disease is an osteochondropathy characterized by degeneration of articular cartilage, degradation of the cartilage matrix, necrosis and apoptosis of chondrocytes. This pathology usually involves children ranging in ages 3-12 years, and patients experience pain, severe joint deformity and OA.

In a study by Lu *et al.* in a large cohort of 10,823 patients from 1,361 Han Chinese families, a family group of patients with KBD with

estimated first-degree relatives of 41.76% heritability was reported. However, even if genetic basis of KBD is not yet understood, some association studies of candidate genes and KBD found association between the disease and HLA-DRB1 gene^[61,62].

Zhang *et al.* have reported a GWAS study in subjects with KBD in which their results strongly suggest that ITPR2 is a new susceptibility gene for KBD, suggesting a mechanism for ITPR2 potential contribution in chondrocyte apoptosis in patients with KBD^[63].

Microarray analysis

Analyses of the expression of different genes by microarrays have been carried out in different studies, increasing our knowledge of their influence on OA. The analysis of gene expression profiles in different tissues of the synovial joint of subjects with OA has revealed various pathways related to the inflammatory process and cartilage metabolism angiogenesis and the Wnt signaling pathway.

Some genes such as TREM1 and S100A9 related to the inflammatory process, genes for MMP-3 and 9, Cathepsin H and S as part of the catabolic pathway of cartilage and Wnt-5A and LRP5 encoding a protein involved in angiogenesis were found with high regulation of inflammation in synovial tissue.

Genes such as COL1A1, COL5A1, PLOD2 and LOX (related to transforming growth factor beta, TGF- β) have been associated with fibrosis of OA from the observation of increased expression evidenced by microarray^[64].

Likewise, studies of gene expression have been made in subchondral bone and osteophytes. First, Chou *et al.* identified 972 different genes overexpressed in chondrocytes of tibial plateau, with and without OA without, showing a network related to expression and bone mineralization such as COL3A1 collagen, BMP1 gene BMP7, POSTN, WISP1, HTRA1, SOST, ITGA11, and angiogenesis-related genes, as ANGPTL1; also some under-expressed genes have been found to be associated with cellular metabolism, proliferation and differentiation such as NMB, LEP, CHRDL2, GRB14, CIDEC, CIDEA, BTG2, PLAC8, and PRDM16. S100A9 gene (alarmin) could be a gene of interest, since it has been shown to stimulate the formation of osteophytes in a model of experimental OA progression and predicts the occurrence of osteophytes in patients with early OA^[65,66].

Finally, the apoptosis pathway in chondrocytes and OA cases have been studied from gene expression profiles in families, and revealed, CASP3 and FAS genes as well as other 694 genes differentially expressed in cases and controls^[67].

EPIGENETICS AND OSTEOARTHRITIS

Some mechanisms of action from which genes influence the development of OA have been postulated, as in the case of the ASPN

gene. However, this is not completely understood^[68]. At present, methylation is one of the most studied epigenetic mechanisms that cells use to control gene expression in OA.

There are many ways by which gene expression is controlled including DNA methylation. It is known that DNA methylation occurs in cytosine bases by the enzyme DNA methyltransferase (DNMT). Methylated cytosine residues are located immediately adjacent to a guanine nucleotide, resulting in two methylated cytosine residues that are located diagonally to each other in the linear sequence of bases along the length of the DNA strand^[69].

Thus, several studies have reported different DNA methylation patterns in OA. For example, reduced methylation at specific CpG sites has been associated with an increased expression of MMP-3, 9 and 13 and ADAMTS-4. In addition, it has been suggested epigenetic regulation of iNOS (inducible nitric oxide synthase enzyme) in OA after observing CpG methylation sites in iNOS enhancer^[70].

A DNA methylation analysis conducted by Fernández Tajés *et al.* in a group of cases and controls with OA showed a difference in the methylation status in both groups; such difference confirmed an increased expression of inflammatory response genes. Consistent differences in methylation profiles have been shown not only in the expression of OA-associated genes, but with disease progression^[71].

ROLE OF MICRORNA'S IN OA

MicroRNAs (miRNAs) are small non-coding sequences, single-stranded, in lengths ranging from 18 to 24 nucleotides, which negatively regulate expression of target genes in post-transcriptional step by binding to specific sequences within target messenger RNA (mRNA)^[72].

A number of studies of large-scale microarray miRNA's have shown differences in over/under expression of different micro RNA's in subjects with and without OA. This is the case study of Park *et al.* In this study it was noted that the micro RNA miR-127-5p was underexpressed in knee OA with increased expression of MMP-13 compared to normal knees. MicroRNA 127-5p, suppressed the expression of MMP-13 induced IL-1 β , whereas the anti-miR-127-5P promoted the increase in production of MMP-13, suggesting that miR-127-5P may be an important regulator of MMP-13 expression in chondrocytes^[73].

In another study, miR-148a was under-expressed in chondrocytes of knee OA compared with normal knees. Overexpression decreased COL10A1, MMP-13 and increased ADAMTS5 and COL2A1 expression. Deposited matrix by chondrocytes contained more proteoglycans and collagen, particularly type II collagen, emphasizing its potential role as a disease modifier^[74].

CONCLUSION

Although only a few loci have been associated with OA, GWAS have proven to be a useful tool for the discovery of new genetic factors, especially when applied to large and well characterized cohorts.

Integration of functional genomics, gene expression studies with microarrays based epigenetic are essential for detection of gene variations of OA; this will allow us a better understanding of OA, identifying signaling pathways regulated during development and progression of the disease and a better understanding not only of its pathophysiology, but the biochemical pathways that may become targets for targeted therapies.

Certainly, knowledge of genes involved in the initiation and progression of OA, as well as those involved in the ability to repair

the joint tissues, will be very important for the development of new treatment options primarily in early stages of the disease or even before the disease starts. Personalized medicine will allow individual sequencing in order to tailor treatment according genetic profile of each person.

CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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