Identification of novel genetic variations affecting osteoarthritis patients

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Abstract

Background: Osteoarthritis (OA) is a progressive joint disease characterized by gradual degradation of extracellular matrix (ECM) components in the cartilage and bone. The ECM of cartilage is a highly specified structure that is mainly composed of type II collagen and provides tensile strength to the tissue via aggrecan and proteoglycans. However, changes in the ECM composition and structure can lead to loss of collagen type II and network integrity. Several risk factors have been correlated with OA including age, genetic predisposition, hereditary factors, obesity, mechanical injuries, and joint trauma. Certain genetic association studies have identified several genes associated with OA using genome-wide association studies (GWASs).

Results: We identified several novel genetic variants affecting genes that function in several candidate causative pathways including immune responses, inflammatory and cartilage degradation such as SELP, SPN, and COL6A6.

Conclusions: The approach of whole-exome sequencing can be a promising method to identify genetic mutations that can influence the OA disease.

Keywords: Osteoarthritis, Whole-exome sequencing, Single nucleotide variants

Background

Osteoarthritis (OA) of the knee, which is characterized by gradual degradation of articular cartilage and subchondral bone, is the most common joint disease occurring worldwide [1, 2]. Approximately 27 million individuals in the USA alone are estimated to be diagnosed with OA which affects about 10% of adults over age 55 years old [2, 3]. Various risk factors have been associated with OA including genetic predisposition, obesity, gender, mechanical injuries, physical workload, and joint trauma. However, age is the most common factor and affects especially the weight bearing joints, which is often under mechanical stress [4]. The progression of OA is a consequence of mechanical, biological, biochemical, and molecular factor interactions. What correlation? This correlation can disturb the normal series of synthesis and degradation of articular cartilage chondrocytes and extracellular matrix (ECM) components, and subchondral bone [5, 6]. In normal cartilage ECM there is a balance between synthesis and degradation that sustain dynamic equilibrium which can be disrupted if microenvironment changes in the ECM composition and structure result in type II collagen loss [7].

Inflammatory stimuli are associated with cartilage ECM changes in OA and play an essential role in the pathogenesis of synovium inflammation and cartilage degeneration through inducing the catabolic activities of chondrocytes [8, 9]. These Inflammatory factors and cytokines include interleukins family (IL) (IL-1, IL-4, IL-6, IL-17, IL-18), and tumor necrosis factor-α (TNF-α) [10–14]. For instance, the inflammatory cytokine IL-1β...
is highly expressed in chondrocytes and inhibiting ECM synthesis by decreasing the expression SOX9 [15, 16]. This response ultimately decreases the expression of collagen type II and aggrecan in articular cartilage that lead to reducing the production of chondrogenic ECM [7].

The genetic predisposition to OA is not fully understood. A number of family studies have investigated the genetic nature of OA. Familial mutations were identified in the type II collagen gene (COL2A1) [17]. Mutations affecting collagen IX gene, and COL9A1 have been linked to OA of the hip [18]. Recently, various genetic association studies have identified several genes associated with OA using genome-wide association studies (GWASs). These genes include GDF5, DUS4L, Ch7q22, and COL9A1 [19–22]. However, the genetic association between single nucleotide polymorphisms and OA disease remain controversial.

In this study, a pilot whole-exome sequencing analysis was performed on osteoarthritis cases in order to identify candidate gene mutations associated with OA in Saudi Arabia.

**Methods**

**Patients**

Peripheral blood was obtained from five end-stage osteoarthritis patients with age range of 46–70 years old. The collection of these samples were approved by the King Abdulaziz University Hospital’s ethical committee (No.11–557). Informed consent was collected from all donors prior to participation in this study.

**Whole-exome sequencing**

Genomic DNA was extracted from peripheral blood samples of five OA cases using QIAamp DNA blood mini kit according to the manufacture procedure (Qiagen Inc, USA). Briefly, three micrograms of genomic DNA were fragmented using the Covaris S2 system. Exome capture was performed using the Agilent’s SureSelect Whole-Exome Enrichment kit (v4). Fragment libraries were prepared from the captured exomes for sequencing on the SOLiD 5500 platform (Applied Biosystems). Fragments were sequenced in single reads of 50 bp. Reads were aligned to the UCSC hg19 reference sequence of the human genome using the LifeScope analysis pipeline which was also utilized for variants identification and annotation.

**PCR amplification and sanger sequencing**

DNA was amplified using custom oligonucleotides primers listed in Table 1. Polymerase chain reaction reactions (PCR) was performed to amplify the selected primers. The PCR reactions were conducted using Go Taq Green Master Mix (Promega, USA). The reactions conditions were performed at one denaturation cycle at 95°C for 15 min followed by 35 cycles of denaturation at 95°C for 30 s, and annealing at 52°C for 30 s and extension at 72°C for 30 s. The final step was done at 72°C for 10 min. To perform Sanger sequencing, purification of PCR products using ethanol precipitation were required prior to the cycle sequencing reaction. Purified PCR products were labeled with BigDye Terminator kit v3.1 (ABI Biosystem, USA) for cycle sequencing according to the manufacture procedure. After the second purification for the PCR products, the sequencing analysis was performed on the automated sequencer from Applied Biosystems 3500 DNA Analyzer.

**Results**

Whole-exome sequencing of 5 OA patients revealed several novel mutations that are predicted to be damaging to protein function (SIFT). Identified variants were prioritized according to their ontology grouping in ECM and/or inflammation (Table 2). A novel c.381G > T, p.Asn127Lys mutation in the collagen type VI alpha 6 (COL6A6) gene in another OA patient. This heterozygous c.2263T > C, p.Ile1137Thr mutation was also validated by Sanger sequencing. Other single nucleotide variants were identified that had no minor allele frequency reported by dbSNP. However, they were also found by the ExAC project albeit at a very low frequency (Fig. 1). These genes were identified that impact cartilages extracellular matrix (ECM) organization and cartilage development.

**Table 1 Primers used for validation of the single nucleotide variations identified by whole-exome sequencing**

| Gene   | Forward primer (5’→3’) | Reverse primer (5’→3’) |
|--------|------------------------|------------------------|
| SELP   | CTCGTTAAACCAATGCGG     | TACACCTCTTACTACTGT     |
| FIGNL1 | ATGGTAGCAATGCGACAGCG   | GAGATGCTATTCCTAGTGG    |
| SPN    | TCTGTTGCTGAGGCGGAGG    | CATCCTAAGCACTGCCAGAC   |
| USP36  | CTCGTGCTAGGAGGAGGAGG   | CTCAGTGGCCACAGATGGG    |
| TNRC6B | TCTGGCAGAGAAGAGGGCT    | ACCCGTTTAGTGGTGGTCC    |
| HSPG2  | GACTCTGCTGATGCGATGCT   | ACACAGGTTGCTGACAGAC    |
| SUSDS  | AGTTGACTGCTGCTGAGTGG   | CTAGTTACTCCTGACAGCC    |
| ITGA8  | GGAAGGCTATATCGATCGAG   | GGTTAGAAAGCTGTGGTCTC   |
| PXN    | CATCAAAGAGACCTCCAC     | CCACTGTCGAGACTTCTAG    |
| NLRP6  | AGCACTGAGGCTGTCGTG     | GTCGGCGCTGATGTCGGCG    |
| COL2A1 | CTTAGTCCAGAGCAGCTGG    | GGCTAGCACAGGCTAGCTG    |
| COL6A6 | CATCGGTGCTGCTCACTCAG   | CTGTTGCTACAGGCTAGTGG   |
| GAS6   | GGGACGCGAGCAGATGCGA    | CCCAGGCTGAGTACGGAG     |
including heparan sulfate proteoglycan2 (HSPG2), sushi domain containing 5 (SUSD5), integrin subunit alpha 8 (ITGA8), paxillin (PXN), collagen type II alpha 1 (COL2A1), and fidgetin-like 1 (FIGNL1). Moreover, our results revealed some mutations associated in the inflammatory response and immune response such as pyrin domain containing 6 (NLRP6) and growth arrest specific 6 (GAS6); ubiquitin specific peptidase 36 (UPS36), sialophorin (SPN), and trinucleotide repeat containing 6B (TNRC6B) respectively (Table 2).

Discussion
To identify the predisposition genetic factors and the association of these candidate genes with the disease, we conducted a pilot whole-exome sequencing on 5 OA patients from Saudi Arabia. The result of the exome sequence analysis identified several nucleotide variants affecting candidate genes that could likely contribute in OA susceptibility and involved in crucial pathways including cartilage degradation and development, inflammatory, and immune response [5].

The finding of mutations in these genes functionally interrelated to cartilage ECM organization and cartilage signaling pathway may have functional consequences. One of the identified mutations in this study is the p.Arg4174Cys mutation found in the HSPG2 gene. This gene plays multiple roles in the extracellular matrix organization involved in cartilage development, enchondral bone morphogenesis, and chondroitin sulfate metabolic process. Additionally, it is responsible for glycosaminoglycan (GAG) biosynthetic and catabolic process that maintains the expression and aggregation of mature chondrogenic markers [23]. SUSD5 harbors the p.Thr466Met mutation in one OA patient from our cohort. This gene influences the

| Gene   | Chromosome position | AA change    | Nucleotide change | Protein ID   | dbSNP         | ExAC frequency |
|--------|---------------------|--------------|-------------------|--------------|---------------|----------------|
| HSPG2  | 1,22154535,G,A      | p.Arg4174Cys | c.4391C>T         | ENSP00000363827 | rs199899258   | 0.00005066     |
| SUSD5  | 3,33194727,G,A      | p.Thr466Met  | c.629C>T          | ENSP00000308727 | rs377664152   | 0.00009122     |
| ITGA8  | 10,15628601,G,A     | p.Ala785Val  | c.1063C>T         | ENSP00000367316 | rs371802080   | 0.0000248      |
| PXN    | 12,12066050,T,C     | p.Tyr181Cys  | c.605A>G          | ENSP00000267257 | rs371118243   | 0.0001199      |
| COL2A1 | 12,48371204,G,A     | p.Arg1058Cys | c.1487C>T         | ENSP00000369889 | rs148350640   | 0.00002493     |
| COL6A6 | 3,130293232,T,C     | p.Ile1137Thr | c.2263T>C         | ENSP00000351310 | rs200274210   | 0.006855       |
| FIGNL1 | 7,50513244,A,G      | p.Met581Thr  | c.674T>C          | ENSP00000349356 | rs200453649   | 0.000173       |
| USP36  | 17,76803235,T,C     | p.Arg631Gly  | c.1123A>G         | ENSP0000041214 | rs112843316   | 0.0000082      |
| SPN    | 16,29676061,C,T     | p.Arg338Cys  | c.400C>T          | ENSP00000353238 | rs200681097   | 0.000485       |
| TNRC6B | 22,40661502,C,T     | p.Glu423Val  | c.1833G>T         | ENSP00000401946 | rs201057205   | 0.0003241      |
| SELP   | 1,169586366,G,T     | p.Asn127Lys  | c.381G>T          | ENSP00000263686 | NA            | NA             |
| NLRP6  | 11,281256,G,T       | p.Ala508Ser  | c.892G>T          | ENSP00000309767 | rs373174851   | 0.000101       |
| GAS6   | 13,114535300,G,A    | p.Pro415Leu  | c.721C>T          | ENSP00000349062 | NA            | NA             |

![Fig. 1 Selected chromatograms showing the Sanger confirmation of identified variants in our samples](image-url)
hyaluronic acid binding, a core component in articular cartilage [24]. Another mutation was identified is the p.Ala785Val mutation in ITGA8, and the p.Tyr181Cys mutation in PXN gene. Both ITGA8 and PXN genes are important in cellular matrix adhesion, ECM organization, and transforming growth factor beta receptor regulation [25, 26]. Most importantly, SNPs variants in cartilage related genes that impacts cartilage development, cartilage condensation, ECM and collagen fibril organization, and collagen catabolic process has been associated in our OA patients. That includes the p.Arg1058Cys mutation in COL2A1, and the p.Ile1137Thr mutation in COL6A6. In addition, the results of this study showed the p.Met581Thr mutation in FIGNL1 gene; a gene that influences the regulatory role in osteoblast proliferation and differentiation [27].

The result of this exome analysis revealed other mutations related to the inflammatory response such as NLRP6 and GAS6; and immune response such as USP36, SPN, and TNRC6B. The identified mutation in UPS36 gene is p.Arg631Gly. This gene is a known member of the ubiquitin specific protease family that regulates the immune signaling pathway [28]. The p.Arg338Cys mutation in SPN was also identified in our patients, and the influence of this gene can affect cellular defense responses and the regulation of the tumor necrosis factor (TNF) pathway [29]. The mutation found in TNRC6B gene is p.Gly423Val; this gene not only act in the innate immune response, but also regulates fibroblast growth factor receptor signaling pathway [30]. Furthermore, the results showed some other mutations involved in the inflammatory response. Interestingly, we identified a novel SNV variation in the SELP, p.Asn127Lys, which has not been reported previously. The function of SELP is to stimulate leukocytes adhesion in the site of injury, which could explain the presence of inflammatory markers IL family, and TNF in OA patients [31]. The p.Ala508Ser mutation in NLRP6 gene, and the p.Pro415Leu mutation in the GAS6 gene were identified in our screen; both genes regulates the inflammatory response [32, 33]. However, GAS6 specifically regulates tumor necrosis factor-mediated signaling pathway and macrophage cytokine production [33].

Conclusions

OA is a progressive joint disease characterized by gradual degradation of (ECM) components in the cartilage and bone. Several risk factors have been associated with OA such as genetic predisposition and molecular factors. In this study, a pilot whole-exome sequencing was performed and we identified several genes candidate genes associated with OA in Saudi Arabia that function in various causative pathways including immune response, inflammatory and cartilage degradation. Despite the small sample size of this group, a hypothesis could be formulated that states the importance of personalized genetic screening of OA patients in order to understand the individual's genetic makeup potentially responsible for susceptibility towards OA.

Abbreviations

OA: Osteoarthritis; ECM: Extracellular matrix; SNPs: Single nucleotide polymorphisms

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Declaration

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Availability of data and materials

Not applicable.

Authors’ contributions

MAG, AMA, MAE, AD and MMA participated in the study design. MA, MAG, AG, MK and HSA performed data collection, DNA extraction and sequencing studies. KG, HA, RK, MAE and AD analyzed data, interpreted the results and drafted the manuscript. MAG, AMA, MMA, and MFA participated in critical review, editing and finalization of manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

This study was approved by the Research Committee of the Biomedical Ethics Unit, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia. Participants in the study provided their consent prior to sample collection.

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