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# Genetic markers of osteoarthritis: early diagnosis in susceptible Pakistani population

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## Abstract

**Background and aim:** Osteoarthritis (OA) is a multiple factorial disease with unidentified specific markers. The alternate method such as biochemical and genetic markers for the diagnosis of osteoarthritis is an undeniable need of the current era. In the present study, we aimed to investigate the association of interleukin-6 (IL-6)(IL-6-174G/C), transforming growth factor- $\beta$ 1 (TGF-beta1-29C/T), and calmodulin 1 gene-16C/T (CALM1-16C/T) polymorphism in clinically definite Pakistani OA patients and matching controls.

**Methods:** The study design was based on biochemical analysis of OA via serum hyaluronic acid (HA) enzyme-linked immunosorbent assay (ELISA) test and genetic analysis based on amplification refractory mutation system (ARMS) PCR. Statistical evaluations of allele probabilities were carried through chi-squared test. This study includes 295 subjects including 100 OA patients, 105 OA susceptible, and 90 controls.

**Results:** HA levels obtained were distinct for all the populations: patients with a mean value of  $\pm$  5.15, susceptible with mean value of  $\pm$  2.27, and control with mean value of  $\pm$  0.50. The prevalent genotypes in OA were GG genotype for IL-6-174G/C, CT genotypes for TGF  $\beta$ 1-29C/T, and TT genotype for CALM1-16C/T polymorphism. A significant *P* value of 0.0152 is obtained as a result of the comparison among the patients and controls on the number of individuals possessing the disease-associated genotypes.

**Conclusions:** The positive association of GG genotype for IL-6-174G/C, TT genotype for CALM1-16C/T polymorphism in OA while high prevalence of CT TGF  $\beta$ 1-29 C/T genotypes in susceptible population in our study group implies these polymorphisms can serve as susceptible marker to OA and genetic factors for screening OA patients in Pakistan. There might be other factors that may influence disease susceptibility. However, further investigations on larger population are required to determine the consequences of genetic variations for prediagnosis of OA.

**Keywords:** Hyaluronic acid, Genotype, Polymorphism, ARMS PCR

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## Background

OA is one of the most common forms of arthritis, characterized by destruction of cartilage and subsequently the subchondral bone. This degenerative joint disease results in bones rubbing against each other as well as formation of osteophytes, leading to symptoms such as pain, swelling, and restrictive movement of joints. Sclerosis, cysts, and synovial inflammation manifest as a result of the condition. The disease is more prevalent in women than in men, with the estimates of 18.0% women and 9.6% men, above the age of 60 worldwide, experiencing its symptoms [1]. In Pakistan, 3.6% in rural and 3.1–4.6% in urban parts of Northern Pakistan were found diagnosed by knee OA [2]. For the rest of the areas of Pakistan, the data available on epidemiology is scarce.

The etiology is also poorly understood. Since it is a multifactorial disease, studies have discovered a number of causative factors including obesity, genetic predisposition, bone density, trauma, and occupational injuries [3].

Diagnosis is carried out on the basis of symptoms, arthroscopy, X-rays, and MRI imaging [4, 5]. While X-ray is the most common mechanism used, there are some drawbacks. Early stages of OA are often unnoticeable. There is a frequent non-correlation among the degree of symptom of pain and dysfunction experienced by the patient and the stage of OA depicted through the image. Moreover it has less sensitivity and is less precise [4]. The other methods also lack sensitivity and specificity [5]. Thus, there is a need of availability of better diagnostic techniques which are not only able to detect OA in its initial stages but also determine susceptibility in population [6].

This role can be played by biomarkers specific to OA [6, 7]. For this purpose, a number of studies have been carried out on different animal models, and potential biomarkers have been identified [7]. A significant discovery has been of HA [8–10]. HA is a major constituent of cartilage matrix and synovial fluid [11]. Its level is increased during the proliferative synovial inflammation and hence by determining its quantity in blood serum or urine, important details can be revealed including the diagnosis of OA [9, 10]. These include duration since the onset and the severities that have developed with it [12].

In addition to biochemical markers, genetic markers have also been studied, and susceptibility genes have been identified, CALM1-16C/T is one of them. In chondrocytes and articular cartilage cultured from OA patients, calmodulin expression was found to be high [13]. This results in increased expression of cartilage matrix genes COL2A1 and AGC1, which mediate chondrocyte differentiation [14, 15]. CALM1-16C/T (rs12885713) is a single nucleotide polymorphism found in the functional core promoter of the calmodulin gene. TT genotype, a recessive model, has been reported to decrease the rate

of transcription of calmodulin [14]. This results in decreased expression of matrix genes, and hence, chondrocytes are unable to respond to mechanical stress. This genotype has been found associated with hip OA (HOA) in the Japanese population and associated to OA in a meta-analysis done using six-control case studies [14, 16]. However, a similar study done on British population and five case-control studies meta-analysis revealed no association with it [17, 18].

Another potential genetic marker reported is IL-6. IL-6 is a pro-inflammatory cytokine whose expression is known to be upregulated during inflammation. Increased IL-6 promotes IL-1 $\beta$ -induced degradation of proteoglycans in the joints, preventing chondrocyte proliferation [19]. It is plausible that this high expression of IL-6 during chondrocyte degradation causes OA. A study showed increased amount of IL-6 in cartilage, serum, and synovial fluid of OA patients [20]. In case of genetic variation in the gene, different types of OA originate, affecting cartilage degradation [21–23]. An example is of IL-6-174G/C in which the G allele at the promoter region gives rise to severe forms of OA [21].

Polymorphisms of TGF  $\beta$ 1 gene have been reportedly associated with increased likelihood of OA [24]. TGF  $\beta$ 1 is a multifunctional growth factor with a significant role in growth and differentiation of cartilage and its matrix metabolism [25, 26]. It is abundantly expressed in articular cartilage and chondrocytes, and under OA conditions its expression increases [27, 28]. Elevated levels of TGF  $\beta$ 1 have been found in the spinal fluid of OA patients [29]. TGF  $\beta$ 1-29C/T (rs1800470) is a polymorphism that has been correlated to OA among Japanese women, to hip OA among adults, to hand OA among Finnish women, and to arthritis in twenty-two case studies meta-analysis [26, 30–32]. While its functional role in pathogenesis remains unclear, there has been some evidence of it affecting TGF  $\beta$ 1 secretion and function in hepatocytes [26, 33]. TT genotype and T allele are the variants that have been reported to increase susceptibility to OA [24].

In this study we investigate the levels of HA and the genotypes of the rest among susceptible population in Pakistan. A combined analysis of these biomarkers will allow us detect susceptibility of OA among population and pave the way for prediagnosis and early treatment; both of which are not possible with the current diagnostic tests.

## Methods

### Pilot study

#### Patients

A retrospective case-control study was conducted with sample size ( $N$ ) 295, of which 95 were regarded as control cases, whereas 100 OA patients and 105 OA susceptible

**Table 1** Sequence of primers used in ARMS

SNPs	Primer	Sequence
TGF β1-29C/T (rs1800470)	T allele	5'AGGCGTCAGCACCAGTAG3'
	C allele	5'ACCACACCAGCCCTGTTC3'
	Reverse (common primer)	5'TAGCAGCAGCAGCAGCA3'
	Internal control (reverse)	5'GCATCTTGCTCTGTGCAGAT3'
	Internal control (forward)	5'TGCCAAGTGGAGCACCCAA3'
IL-6-174G/C (rs1800796)	T Allele	5'GGATTATGAAGAAGGTAATACTA3'
	C Allele	5'CACGAAATTTGAGGATGG3'
	Reverse (common primer)	5'ACAACAGCCCCTCACAGG3'
	Internal control (reverse)	5'CAACTTCATCCACGTTACCC3'
	Internal control (forward)	5'ACACAACCTGTGTTCACTAGC3'
CALM1-16C/T (rs12885713)	T allele	5'GCACCATATATATATCGCGAGGT3'
	C allele	5'GCACCATATATATATCGCGAGC3'
	Reverse (common primer)	5'ACTCCCGACCTACCATGGT3'
	Internal control (reverse)	5'GCATCTTGCTCTGTGCAGAT3'
	Internal control (forward)	5'TGCCAAGTGGAGCACCCAA3'

cases according to exclusion/inclusion criteria. Sample size was validated by using G\*power Software version 3.1.9.2 for Windows. The study included unrelated consecutive adult (≥ 18 years of age) who gave a written informed consent. Cases were defined as patients with radiological verification, susceptible population were those without radiological verification yet showing symptoms of OA and having supportive family history, and the controls were healthy people. Samples were collected from Allied Hospital Faisalabad and PIMS Islamabad. The study was approved by the Institutional Review Board committee, National University of Sciences and Technology, Islamabad, Pakistan (Date/ IRB No: 15-10-2019-/05).

**Biochemical testing**

Sandwich-based ELISA Kit (TECO® HAPLUS) was used for biochemical analysis.

**DNA extraction**

Genomic DNA was extracted by phenol chloroform method at Atta-ur-Rahman School of Applied Biosciences

(ASAB), National University of Sciences and Technology (NUST), Islamabad, Pakistan, and stored at 4 °C. Gel electrophoresis was carried out on 1% agarose gel to confirm the presence of extracted DNA.

**Design of primers**

Primers for IL-6-174G/C were designed by using the Primer3 Software, while the sequences for CALM1-16C/T and TGF β1-29C/T were obtained from literature [15, 34]. These sequences are mentioned in Table 1.

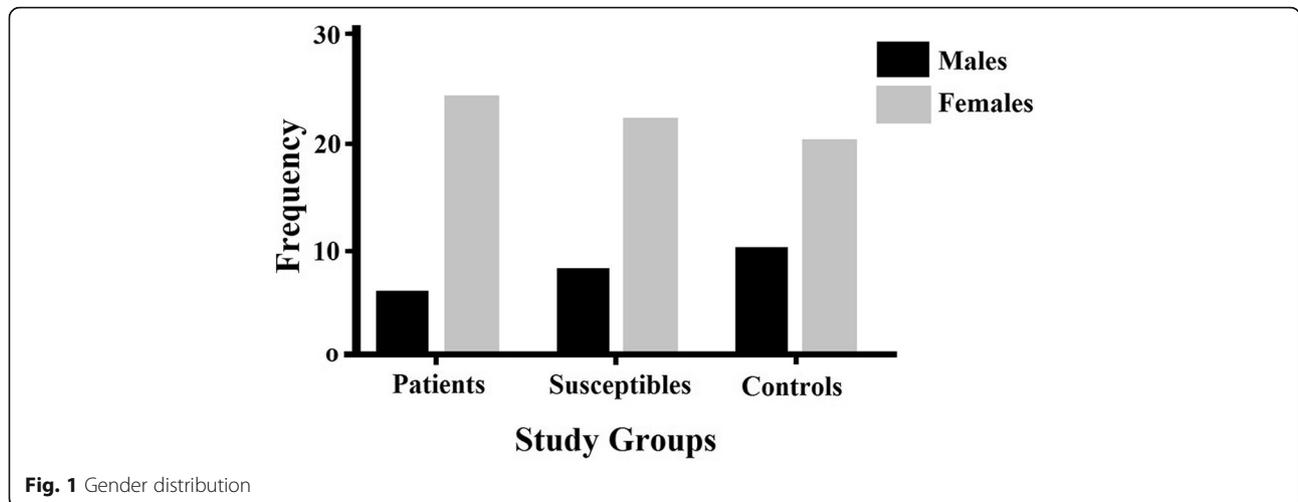
**Genetic analysis**

ARMS PCR was used. The conditions used in PCR are shown in Table 2. Denaturation, annealing, and extension were repeated 30, 32, and 35 times for IL-6-174G/C, CALM1-16C/T, and TGF β1-29C/T respectively.

Two percent agarose gel was used to analyze the PCR product of ARMS. A 100 bp ladder was also loaded alongside the PCR products for comparison of size. The results were analyzed by Wealtec dolphin-doc gel analysis systems.

**Table 2** PCR conditions for IL-6-174G/C, CALM1-16C/T, and TGFB1-29C/T

	IL-6-174G/C		CALM1-16C/T		TGFB1-29C/T	
	Temperature/°C	Time	Temperature/°C	Time	Temperature/°C	Time
Denaturation	94	03:00	95	15:00	95	10:00
Denaturation	94	00:30	95	00:30	95	00:15
Annealing	57	00:50	60	01:30	57	00:50
Extension	72	00:40	72	01:30	72	00:40
Final Extension	72	03:00	72	10:00	72	05:00



**Statistical analysis**

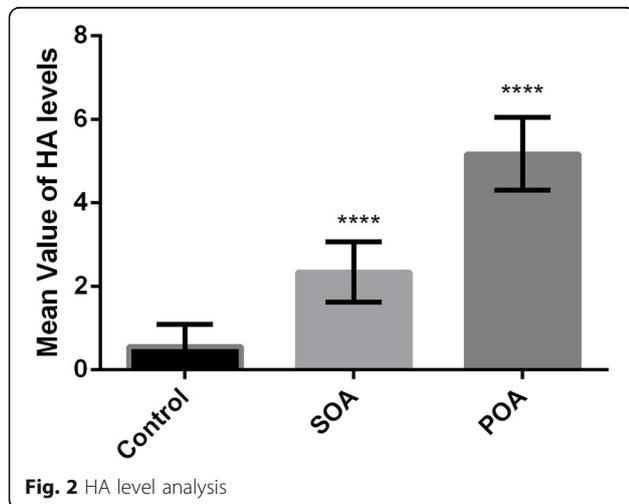
Statistical analysis was done using the Graph Pad Prism 7 software. The probabilities of alleles were calculated using the Chi-square (Fisher’s exact test). A probability of less than 0.05 was taken as significant.

**Results**

From a total of 295 individuals, about 73% were females while 27% were males. Within three groups of patients, susceptible, and control, the females dominated by approximately 80%, 73%, and 67% respectively as shown in Fig. 1.

Their HA serum levels were tested, and the mean values calculated were distinct for each group: patients with a mean value of ± 5.15, susceptible with mean value of ± 2.27, and control with mean value of ± 0.50 (Fig. 2).

Distributions of different genotypes for all polymorphisms are shown in Tables 3, 4, and 5.



A comparison was also carried out between genotypes of all the polymorphisms. A significant *P* value is <0.05 is obtained as a result of the comparison among the patients and controls. TT and GG genotypes of CALM1-16C/T and IL-6-174G/C respectively depicted a strong association with OA, while low prevalence of TT genotype of TGF β1-29C/T is associated with OA as compared to control group (Fig. 3).

**Discussion**

OA is currently classified as a heritable as well as non-heritable disabling condition. The disease is marked by multiple factors responsible for the onset and gradual loss of the articular cartilage. The etiology of OA is poorly understood, and pre-diagnosis is difficult because specific markers are not identified [35]. The aim of this study was to find out genetic biomarker for a combined profile analysis based on sub-tests to diagnose or pre-diagnose OA and find an alternate diagnostic method instead of X-ray analysis. For this purpose, ARMS PCR of IL-6-174G/C, TGF β1-29C/T, and CALM1-16C/T was carried out.

The prevalence of OA is relatively more in the female population as compared to the male population. The trend of the patients more likely being females resulted in the possible susceptibility in the same gender; thus, the samples for the susceptible population were collected mainly comprising of the middle-aged females. The main objective of the study was to find a diagnostic and prognostic test for OA in the susceptible population.

Expression of HA was found to be high in serum or urine of OA patients in comparison to the control subjects. Hence, HA holds the potential of being a predictive biochemical marker [9, 10]. Results obtained of the HA test show the significant levels in patients with a mean value of ± 5.15, in susceptible mean value of ±

**Table 3** Distribution of different genotypes of TGF  $\beta$ 1-29/CT

Genotypes	%		
	Patients	Susceptible	Control
CC	27.59	31.03	13.33
TT	24.14	41.38	56.67
CT	48.28	27.59	30.00

2.27, and in controls mean value of  $\pm 0.50$ . These results thus help determine the current state of OA or its progression in susceptible population. The greater the value of HA, the more is the likelihood of OA. The levels in the susceptible population are likely to rise with the progression of OA; monitoring them over the years can help analyze the disease probability.

The results of CALM1-16C/T genotype frequency distribution are such that the TT genotype was prevalent in the patient and susceptible population. Possessing two copies (i.e., a recessive model) of the T-allele of SNP rs12885713 was a particular risk factor in Japanese, with a *P* value of 0.00036, and in five case-control study with *P* value of 0.12 [15, 18]. Thus, it could be proposed that the TT genotype is responsible for disease presence and prediction in the susceptible population. The results show the similar trends in the diseased and susceptible population for TT genotype; however, the CC genotype was more abundant in the population under study. The likely inference is that CC genotype is associated with disease in Pakistani population or that the population size for the study is relatively small to study the prevalence efficiently.

TGF- $\beta$ 1-29C/T is involved in OA pathogenesis and the development of the musculoskeletal system as well.

In some populations, genotypes TC or CC of TGF- $\beta$ 1-29C/T are more prevalent [36–38]. The CC genotype has positive association to OA in some of the populations according to the previous studies [37]. According to the present study, CC genotype is prevalent in OA patients, CT genotype is prevalent in susceptible population, and TT genotype is most prevalent in control population and least in the patient population.

In IL-6-174G/C allele frequency distribution analysis, the GG genotype is more prevalent in OA. IL-6-174G/C recessive model was also found to be associated to risk of KOA in Chinese Han population with *P* < 0.001 and to OA in an Indian population with *P* < 0.001 [39, 40]. The genetic variability of IL-6-174G/C contributes distal inter phalangeal OA with G allele at the IL-6-174G/C promoter region being responsible for its severe form [21]. This study represents highest frequency of GG genotype in case of OA patients followed by the susceptible population.

Association of CALM1-16(TT), IL-6-174(GG), and TGF  $\beta$ 1-29(TT) polymorphic genotypes was determined in OA. Their increased prevalence of CALM1-16(TT) and IL-6-174(GG) while low prevalence of TGF  $\beta$ 1-29(TT) might be responsible for the OA. The study thus holds immense potential for the formulation of an effective diagnostic and prognostic technique.

**Table 4** Distribution of different genotypes of IL-6-174 G/C

Genotypes	%		
	Patients	Susceptible	Control
CC	36.67	33.33	42.86
GG	53.33	51.85	25.00
CG	10.00	14.81	32.14

**Table 5** Distribution of different genotypes of CALM1-16C/T

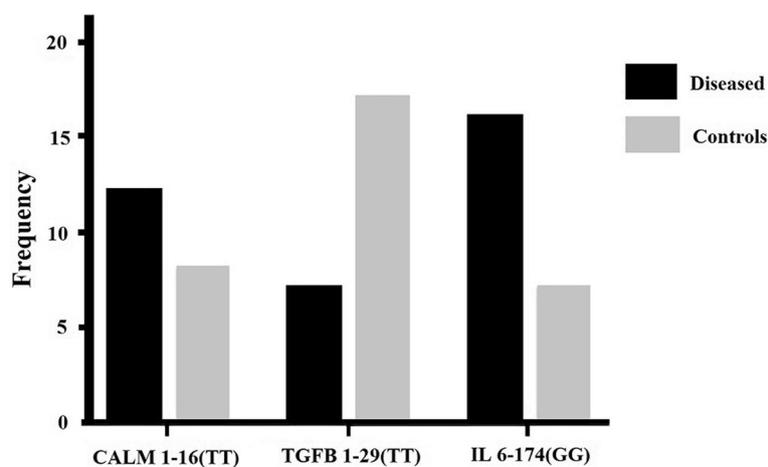
Genotypes	%		
	Patients	Susceptible	Control
CC	48.15	33.33	40.74
TT	44.44	44.44	29.63
CT	7.40	22.22	29.63

**Conclusions**

Radiography along with assessment of pain and restlessness is known as the hallmark for the initiation of OA. Though a great deal has been done to identify some reliable biomarkers, only few of these biomarkers have been used in clinical settings.

Our research shows the positive association of GG genotype for IL-6-174G/C, TT genotype for CALM1-16C/T polymorphism in OA while high prevalence of CT genotype for TGF β1-29 C/T in susceptible population. The research signifies the role of the proposed genetic markers in detection of OA. It means the combined analysis would be helpful in the diagnosis and prediction in susceptible population. The HA levels were also very distinctive in the different populations: patients with a mean value of ± 5.15, susceptible with mean value of ± 2.27, and controls with mean value of ± 0.50; thus, susceptibility can be identified before the disease occurs analyzing this data. The high number of the study participants could get

more generalized data as part of the future prospects. The trends followed by OA in Pakistani population are relatable to other world populations in terms of IL-6-174G/C and TGF β1-29C/T. The purpose of the study was to devise a prediagnostic test for OA detection. Patients will benefit from the early identification of the OA which will also help in selecting duration of treatment. This can become an effective screening method for the OA in Pakistani population because genetic changes are more robust and are present since birth, so they can be identified at an early age among Pakistani populations. Moreover, as an endpoint representative of the degradative process during OA, biomarkers must be assessed as potential therapeutic candidates for a new drug development regime for OA. Early diagnosis of OA using biomarkers will help physicians to not only develop a strategy for treating OA at early stages but will even prove beneficial in reducing the cost of treatment for patients.



**Fig. 3** Comparison among genotypes of CALM1-16C/T, TGF β1-29C/T, and IL-6-174G/C associated with OA disease

### Abbreviations

ARMS: Amplification refractory mutation system; DNA: Deoxyribonucleic acid; IL-6: Interleukin-6; PCR: Polymerase chain reaction; SNP: Single nucleotide polymorphism; CALM1: Calmodulin 1 gene; TGF  $\beta$ 1: Transforming growth factor- $\beta$ 1

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### Authors' contributions

Conceptualization: Yasmin Badshah and Hunza Hayat. Methodology: Hunza Hayat and Maria Shabbir. Formal analysis and investigation: Yasmin Badshah and Hunza Hayat. Writing—original draft preparation: Zoha fatma and Shafiq ur Rehman. Writing—review and editing: Yasmin Badshah and Shafiq ur Rehman. Funding acquisition: Maria Shabbir, Yasmin Badshah. Resources: Asad Burki and Sidra Khan. Supervision: Yasmin Badshah. Validation: Maria Shabbir and Shafiq ur Rehman. Visualization: Yasmin Badshah and Sidra Khan. All co-authors take full responsibility for the integrity of the study and all parts of the manuscripts. The authors read and approved the final manuscript.

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### Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

### Ethics approval and consent to participate

The study was carried out after approval from the Institutional Review Board of Atta-ur-Rahman School of Applied Biosciences (IRB-ASAB). All the procedures were performed by the guidelines provided by the ethical review board. Written informed consent was obtained from the patient before the specimen was taken.

### Consent for publication

Written informed consent was obtained from the patient for the publication of this study.

### Competing interests

The authors declare no competing interest.

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### References

- WHO World Health Organization: The WHO register. <https://www.who.int/chp/topics/rheumatic/en/> (2020). Accessed 20 Feb 2019.
- Das SK, Farooqi A. Osteoarthritis. *Best Pract Res Clin Rheumatol*. 2008;22:657–75.
- Haq I, Murphy E, and D. J. Osteoarthritis *Postgrad Med J*. 2003;79:377–83.
- Rousseau J-C, Delmas PD. Biological markers in osteoarthritis. *Nat Rev Rheumatol*. 2007;3:6.
- Hunter DJ, Felson DT. Osteoarthritis. *BMJ*. 2006;332:7542.
- Chyu MC, von Bergen V, Brismée JM, Zhang Y, Yeh JK, Shen CL. Complementary and alternative exercises for management of osteoarthritis. *Arthritis*. 2011;2011:1–10.
- Baker K, Grainger A, Niu J, Clancy M, Guerzani A, Crema M, Hughes L, Buckwalter J, Wooley A, Nevitt M, Felson DT. Relation of synovitis to knee pain using contrast-enhanced MRIs. *Ann Rheum Dis*. 2010;69(10):1779–83.
- Van Spil WE, DeGroot J, Lems WF, Oostveen JC, Lafeber FP. Serum and urinary biochemical markers for knee and hip-osteoarthritis: a systematic review applying the consensus BIPED criteria. *Osteoarthr Cartil*. 2010;18(5):605–12.
- Laurent TC, Laurent UB, Fraser JR. Serum hyaluronan as a disease marker. *Ann Med*. 1996;28(3):241–53.
- Turan Y, Bal S, Gurgan A, Topac H, Koseoglu M. Serum hyaluronan levels in patients with knee osteoarthritis. *Clin Rheumatol*. 2007;26(8):1293–8.
- <https://www.quidel.com/research/elisa-kits/tecomedical-human-hyaluronic-acid-elisa>. Accessed 13 Feb 2019.
- Inoue R, Ishibashi Y, Tsuda E, Yamamoto Y, Matsuzaka M, Takahashi I, Danjo K, Umeda T, Nakaji S, Toh S. Knee osteoarthritis, knee joint pain and aging in relation to increasing serum hyaluronan level in the Japanese population. *Osteoarthr Cartil*. 2011;19(1):51–7.
- Valdes AM, Loughlin J, Oene MV, Chapman K, Surdulescu GL, Doherty M, Spector TD. Sex and ethnic differences in the association of ASPN, CALM1, COL2A1, COMP, and FRZB with genetic susceptibility to osteoarthritis of the knee. *Arthritis Rheum*. 2007;56(1):137–46.
- Mototani H, Mabuchi A, Saito S, Fujioka M, Iida A, Takatori Y, Kotani A, Kubo T, Nakamura K, Sekine A, Murakami Y. A functional single nucleotide polymorphism in the core promoter region of CALM1 is associated with hip osteoarthritis in Japanese. *Hum Mol Genet*. 2005;14(8):1009–17.
- Poulou M, Kaliakatsos M, Tsezou A, Kanavakis E, Malizos KN, Tzetzis M. Association of the CALM1 core promoter polymorphism with knee osteoarthritis in patients of Greek origin. *Genet Test*. 2008;12(2):263–5.
- Yang H, Hu Z, Zhuang C, Liu R, Zhang Y. Association between the polymorphisms of CALM1 gene and osteoarthritis risk: a meta-analysis based on observational studies. *Bioscience Reports*. 2018;38(5):11–28.
- Loughlin J, Sinsheimer JS, Carr A, Chapman K. The CALM1 core promoter polymorphism is not associated with hip osteoarthritis in a United Kingdom Caucasian population. *Osteoarthr Cartil*. 2006;14(3):295–8.
- Shi J, Gao ST, Lv ZT, Sheng WB, Kang H. The association between rs12885713 polymorphism in CALM1 and risk of osteoarthritis: A meta-analysis of case-control studies. *Medicine*. 2018;97:36(e12235).
- Kaneko S, Satoh T, Chiba J, Ju C, Inoue K, Kagawa J. Interleukin-6 and interleukin-8 levels in serum and synovial fluid of patients with osteoarthritis. *Cytokines, cellular & molecular therapy*. 2000;6(2):71–9.
- Jikko A, Wakisaka T, Iwamoto M, Hiranuma H, Kato Y, Maeda T, Fujishita M, Fuchihata H. Effects of interleukin-6 on proliferation and proteoglycan metabolism in articular chondrocyte cultures. *Cell Biol Int*. 1998;22(9-10):615–21.
- Kämäräinen OP, Solovieva S, Vehmas T, Luoma K, Riihimäki H, Ala-Kokko L, Mäntikkö M, Leino-Arjas P. Common interleukin-6 promoter variants associate with the more severe forms of distal interphalangeal osteoarthritis. *Arthritis research & therapy*. 2008;10(1):R21.
- Nojonen-Hietala N, Virtanen I, Karttunen R, Schwenke S, Jakkula E, Li H, Merikivi R, Barral S, Ott J, Karppinen J, Ala-Kokko L. Genetic variations in IL6 associate with intervertebral disc disease characterized by sciatica. *Pain*. 2005;114(1-2):186–94.
- Pola E, Papaleo P, Pola R, Gaetani E, Tamburelli FC, Aulisa L, Logroscino CA. Interleukin-6 gene polymorphism and risk of osteoarthritis of the hip: a case-control study. *Osteoarthr Cartil*. 2005;13(11):1025–8.
- Liu C, Sun J, Zhang H, Li L. TGF  $\beta$ 1 gene polymorphisms correlate with the susceptibility of osteoarthritis. *Int J Clin Exp Pathol*. 2017;10(8):8780.
- Dickinson ME, Kobrin MS, Silan CM, Kingsley DM, Justice MJ, Miller DA, Ceci JD, Lock LF, Lee A, Buchberg AM, Siracusa LD. Chromosomal localization of seven members of the murine TGF- $\beta$  superfamily suggests close linkage to several morphogenetic mutant loci. *Genomics*. 1990;6(3):505–20.
- Hämäläinen S, Solovieva S, Vehmas T, Luoma K, Leino-Arjas P, Hirvonen A. Genetic influences on hand osteoarthritis in Finnish women—a replication study of candidate genes. *PLoS One*. 2014;9(5):e97417.
- Shur I, Lokiec F, Bleiberg I, Benayahu D. Differential gene expression of cultured human osteoblasts. *J Cell Biochem*. 2001;83(4):547–53.
- Nakajima M, Kizawa H, Saitoh M, Kou I, Miyazono K, Ikegawa S. Mechanisms for asporin function and regulation in articular cartilage. *J Biol Chem*. 2007;282(44):32185–92.
- Schlaak JF, Pfers I, Meyer ZB, Märker-Hermann E. Different cytokine profiles in the synovial fluid of patients with osteoarthritis, rheumatoid arthritis and seronegative spondylarthropathies. *Clin Exp Rheumatol*. 1996;14(2):155.
- Yamada Y. Association of a Leu10  $\rightarrow$  Pro polymorphism of the transforming growth factor- $\beta$ 1 with genetic susceptibility to osteoporosis and spinal osteoarthritis. *Mech Ageing Dev*. 2000;116(2-3):113–23.

31. Kolundžić R, Trkulja V, Mikolaučić M, Kolundžić MJ, Pavelić SK, Pavelić K. Association of interleukin-6 and transforming growth factor- $\beta$ 1 gene polymorphisms with developmental hip dysplasia and severe adult hip osteoarthritis: a preliminary study. *Cytokine*. 2011;54(2):125–8.
32. Liu J, Chen Q, Alkam E, Zheng X, Li Y, Wang L, Fang J. Association between gene polymorphisms of TGF- $\beta$  and Smad3 and susceptibility to arthritis: a meta-analysis. *Expert Rev Clin Immunol*. 2020;16(9):943–54.
33. Ma W, Zha Z, Chen K, Chen H, Wu Y, Ma J, Zeng S, Zhi L, Yao S. Genetic association study of common variants in TGF $\beta$ 1 and IL-6 with developmental dysplasia of the hip in Han Chinese population. *Sci Rep*. 2017;7(1):1–7.
34. Hanafy SM, Abdo A. Impact of single nucleotide polymorphism of TGF- $\beta$ 1 gene (SNP-Codon10) on hepatocellular carcinoma risk in Egyptian patients following HCV infection. *Aust J Basic Appl Sci*. 2011;5(9):1814–21.
35. Singh JA, Saag KG, Bridges SL Jr, Akl EA, Bannuru RR, Sullivan MC, Vaysbrot E, McNaughton C, Osani M, Shmerling RH, Curtis JR. 2015 American College of Rheumatology guideline for the treatment of rheumatoid arthritis. *Arthritis Rheum*. 2016;68(1):1–26.
36. Kreła-Kaźmierczak I, Michalak M, Wawrzyniak A, Szymczak A, Eder P, Łykowska-Szuber L, Kaczmarek-Ryś M, Drwęska-Matelska N, Skrzypczak-Zielińska M, Linke K, Słomski R. The c. 29T > C polymorphism of the transforming growth factor beta-1 (TGF $\beta$ 1) gene, bone mineral density and the occurrence of low-energy fractures in patients with inflammatory bowel disease. *Mol Biol Rep*. 2017;44(6):455–61.
37. Malemud CJ. Anticytokine therapy for osteoarthritis. *Drugs Aging*. 2010;27:2.
38. Tzakas P, Wong BY, Logan AG, Rubin LA, Cole DE. Transforming growth factor beta-1 (TGF $\beta$ 1) and peak bone mass: association between intragenic polymorphisms and quantitative ultrasound of the heel. *BMC Musculoskelet Disord*. 2005;6(1):29.
39. Tzakas P, Wong BY, Logan AG, Rubin LA, Cole DE. Transforming growth factor beta-1 (TGF $\beta$ 1) and peak bone mass: association between intragenic polymorphisms and quantitative ultrasound of the heel. *BMC musculoskeletal disorders*. 2005;6(1):1471–4.
40. Singh M, Mastana S, Singh S, Juneja PK, Kaur T, Singh P. Promoter polymorphisms in IL-6 gene influence pro-inflammatory cytokines for the risk of osteoarthritis. *Cytokine*. 2020;127:154985.

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