

UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA

DOCTORAL SCHOOL

PhD THESIS

Abstract

**IMPLICATION OF SOME CYTOKINES
POLYMORPHISM IN OSTEOARTHRITIS**

PhD Thesis Advisor

MIHAI CRUCE PhD, Professor

PHD STUDENT

ȘTEFAN EMILIAN

Craiova

2014

Introduction

Osteoarthritis (OA) is the most frequent musculoskeletal disease, which is defined as a progressive and invalidating affection. It is a chronic long term disease, which produces joint degradation, patients complaining of joint stiffness, pain and causes mobility deficiency. (1)

There are two types of osteoarthritis: primary and secondary.

Primary osteoarthritis is a chronic degenerative disease which is influenced by aging, yet not caused by it. There are solid facts which point out that the genetic factor is involved in approximately 60% of all cases of OA. (2, 3)

Secondary osteoarthritis tends to appear early in life, based on a specific cause, usually an injuries, diabetes or obesity. Even though it has a different etiology from the primary form, both symptoms and pathology are identical. (2, 3)

Osteoarthritis as well as musculoskeletal disease are frequently met in general population.(4)

10-15% of the total population over 60 years has a different degree of osteoarthritis. (5) Between the member states of the European Union, the prevalence of osteoarthritis varies between 2.8% in Romania and 18.3% in Hungary. (6)

In the actual context our study wants to assess the association between the frequency with which the polymorphisms of the genes encoding some cytokines appear and susceptibility to Osteoarthritis.

Key words: Osteoarthritis, cytokines, SNP, genotype, susceptibility.

I. State of knowledge

Chapter 1. EPIDEMIOLOGY AND RISK FACTORS.

In this chapter are presented recent data related to incidence and prevalence of Osteoarthritis to both National and European level. Moreover, there will be a estimation of the future evolution of this affection. With the increase of elderly persons in the general population, so will increase the prevalence of chronic diseases and from musculoskeletal group we refer to osteoarthritis. (7)

According to some recent studies, the number of persons over 60 will be tripled in the next 40 years, which will mean that by 2050 will pass 20 percent of the general population meaning approximately 130 million. It is expected that 15 percent of that (meaning 40 million persons) will be affected by osteoarthritis. (5)

In this chapter there are also presented the risk factors associated with this affection: age, obesity, joint injuries, sex.

Chapter 2. HOMEOSTASIS OF JOINT CARTILAGE IN HEALTY SUBJECTS AND PATIENTES WITH OSTEOARTHRITIS.

Adult joint cartilage it is a tissue which lacks blood vessels composed mostly of a special matrix formed out of collagen, proteoglycans and non-collagen proteins. In this matrix the chondrocytes represent the sole cellular component.

Countless in vitro or in vivo studies during the last two decades have confirmed the fact that these joint chondrocytes are able to respond to mechanic injuries, joint instability due to genetic factors and biological stimuli like cytokines and growth and differential factors, which in turn contribute to structural modification at cartilage matrix level.(8)

Stress induced intracellular signaling, catabolic cytokines and necrosis factor alpha (TNF- α) appearance, and other inflammatory mediators produced by synovial cells and chondrocytes, induces enzyme degradation, matrix metalloproteinase (MMPs) and ADAMTS family activation. Matrix degradation products can regulate these cellular events through feedback. (9)

Our study is based on one of the risk factors for osteoarthritis, the genetic factor. As so we have assessed the association level between some cytokines, like IL-1B, IL-1RN, IL-4R, IL-8, IL-10, polymorphisms and that of the necrosis factor alpha (TNF- α), and susceptibility, or on the contrary, resistance to osteoarthritis.

I. ORIGINAL CONTRIBUTION

Chapter 3. Material and Methods

Including subjects in study

We have taken 2 study groups from CF2 Hospital in Bucharest. One of the groups was diagnosed with osteoarthritis and the other is the healthy control groups. All subjects have been informed about the study and have signed consent.

Sample collection

Blood samples were collected on EDTA from all subjects in a total volume of 3ml.

DNA Isolation

For isolating DNA from whole blood we have used the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI).

This method follows three simple steps. The protocol begins with cell lysis solution and nucleic lysis solution. Next is an optional step which requires RNA digestion using RN-ease. It is followed by removing proteins from the solutions by

precipitating in a saline environment. DNA which remains in the solution is transferred to a new tube and precipitated in isopropanol. The next phase is a wash in ethanol followed by air dry and rehydration of the DNA.

Genotyping using Real-Time PCR

SNPs genotyping was performed in Laboratorul de Genetică Moleculară al UMF Craiova, which has the necessary technology for this process.

TaqMan®SNP Genotyping Assays allow for genotyping of nucleotide polymorphisms using the 5' nuclease assay for amplifying and detecting specific alleles in purified genomic DNA samples. Each assay genotypes individuals for a specific polymorphism. Each TaqMan®Genotyping Assay contains two primers for amplifying the sequence of interest and two TaqMan®MGB probes for allele detection. The presence of two probes in each reaction allows for genotyping two possible variant alleles at the polymorphic site in a DNA target sequence. The genotyping assay determines the presence or absence of a polymorphism based on the change in fluorescence of the dyes associated with the probes.

Statistical analysis

We used Microsoft®Office Excel® 2007 and Genex Pro 4.4.2.308© to correlate results from Real-Time PCR and the parameters of the study.

For descriptive analysis of the results we have used Microsoft Access 2007. With this program we have designed the data base for the subjects, and this data based was afterwards used to extract the parameters. We have used statistical indicators such as: standard deviation, average.

Chapter 4. Results and Discussions

We have 302 subjects included in this study out of which 90 were diagnosed with osteoarthritis and 302 healthy controls.

IL-1B -31T>C Polymorfism

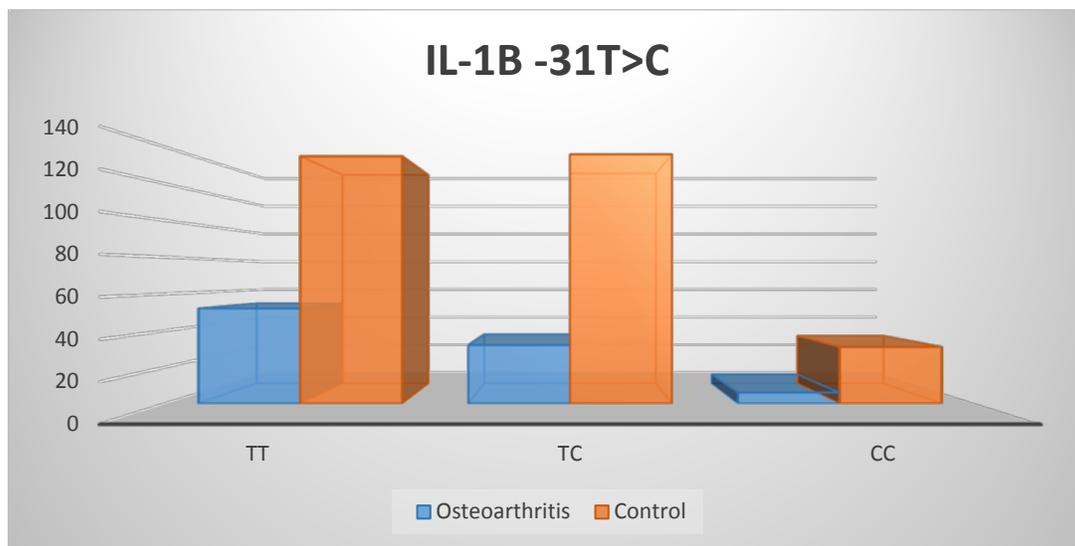


Figure. 1: Genotype frequency for IL-1B -31T>C polymorfism

In this study the results have shown that IL-1B -31T>C polymorphism, shown in figure 1, it is not associated with an increase risk to develop osteoarthritis. We continued the with a stratified study based on the joint localization of osteoarthritis in which we obtained again no association between this polymorphism and the disease.

IL1-RN +2018T>C polymorfism

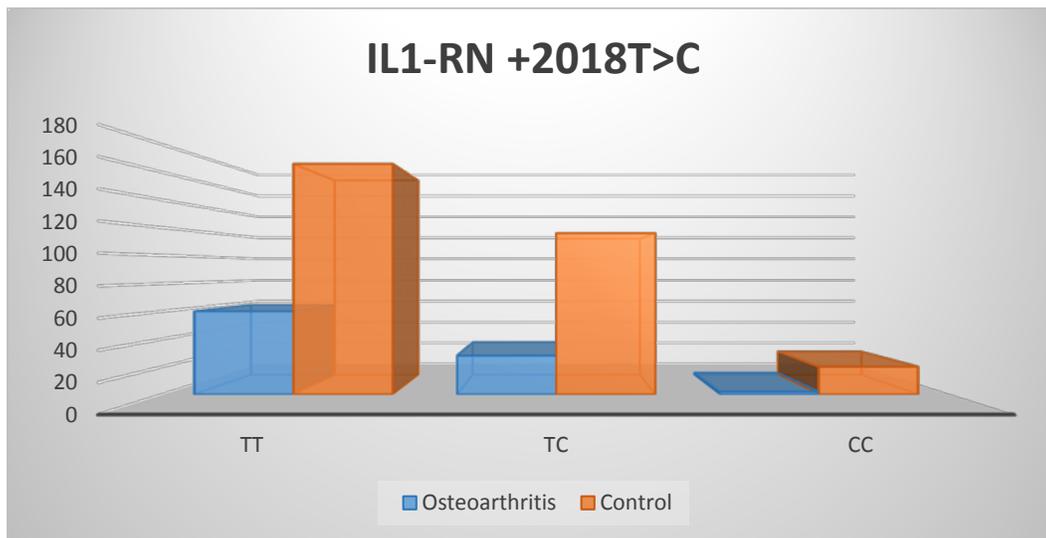


Figure. 2: Genotype frequency for IL1-RN +2018T>C polymorfism

Through comparative analysis of the genotypes, with genotype TT as the reference, we have obtained that IL1-RN +2018T>C polymorphism has no significant statistical association with osteoarthritis.

IL-1B -511C>T polymorfism

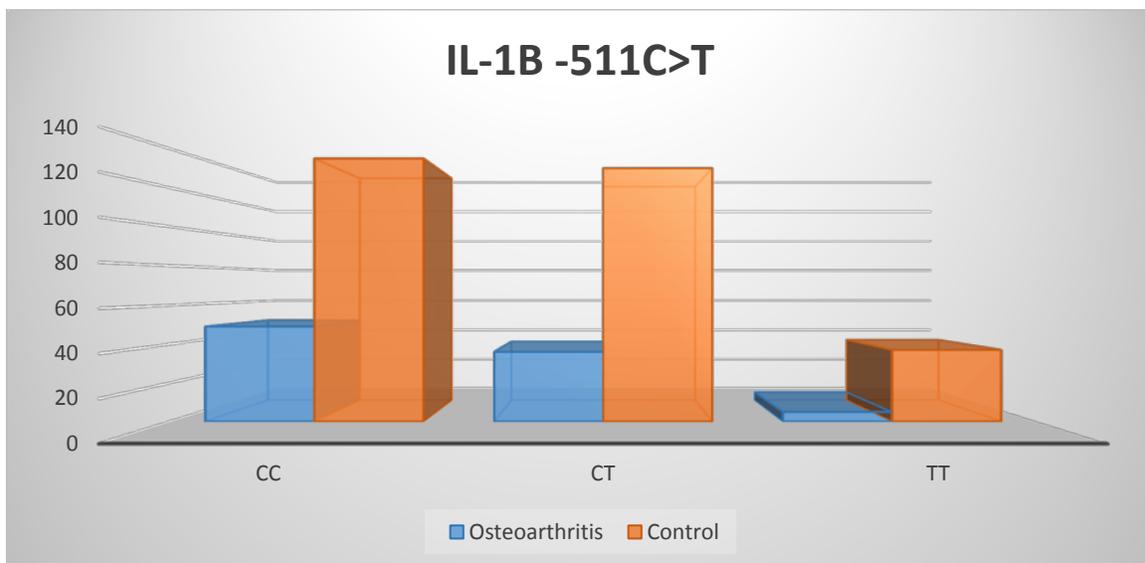


Figure. 3: Genotype frequency for IL-1B -511C>T polymorfism

Comparing the results obtained for the genotypes with the reference genotype CC, we have obtained that IL-1B -511C>T polymorphism has no significant involvement in conferring susceptibility to osteoarthritis. When we have stratified our analysis based on the localization of the affection, even though we have obtained no association, our results showed that carriers of T allele (genotypes CT and TT) appear to be more protected to developing osteoarthritis than those with the CC genotype.

IL-4R -3223C>T polymorphism

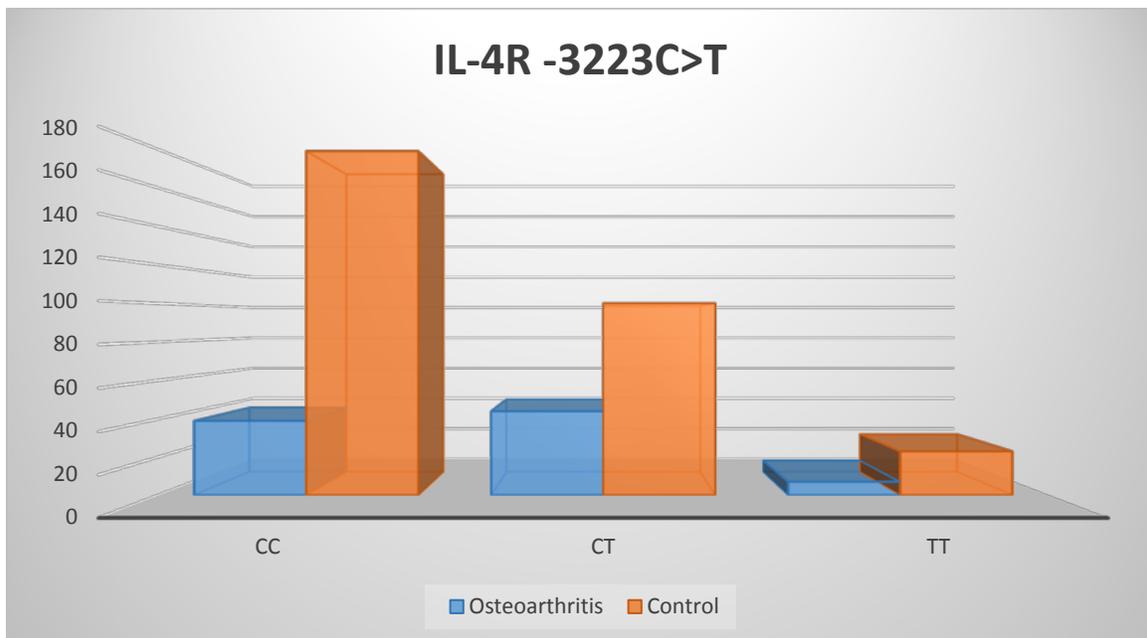


Figure. 4: Genotype frequency for IL-4R -3223C>T polymorphism

Based on a comparative analyze of the genotypes, with genotype CC as the reference, we have obtained that **IL-4R -3223C>T polymorphism has a high association with susceptibility to osteoarthritis, for CT genotype (heterozygous)**. Continuing with the stratified analysis we have obtained that for the knee joint we have a very high association between this polymorphism and susceptibility to osteoarthritis, while for the hip joint we have no association whatsoever.

IL-8 -251T>A polymorfism

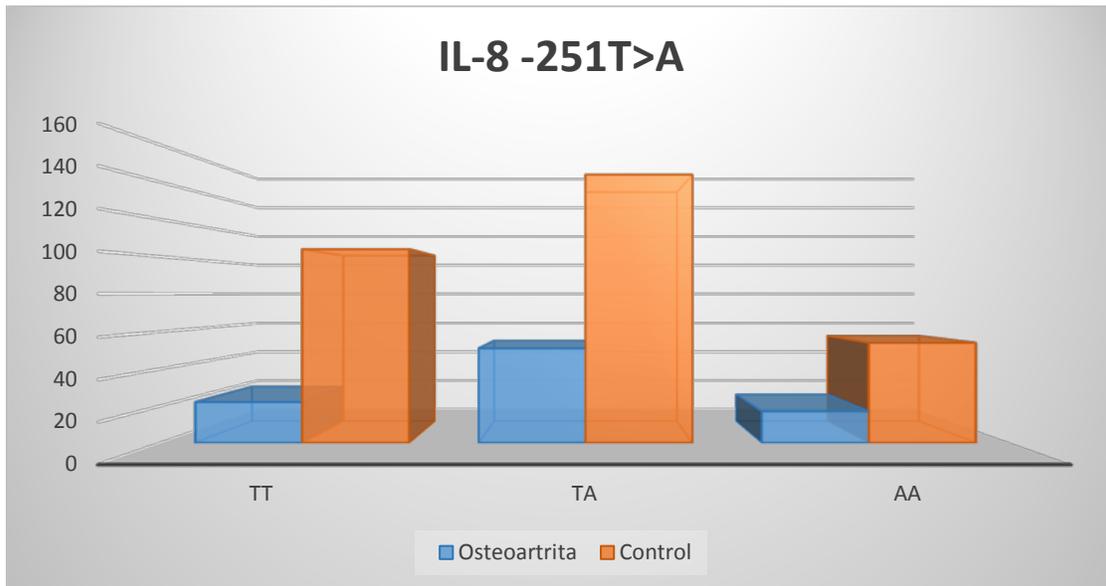


Figure. 5: Genotype frequency for IL-8 -251T>A polymorfism

As it can be seen in the figure above, we have found no significant association between IL-8 -251T>A polymorphism and susceptibility to osteoarthritis.

IL-10 -1082A>G polymorfism

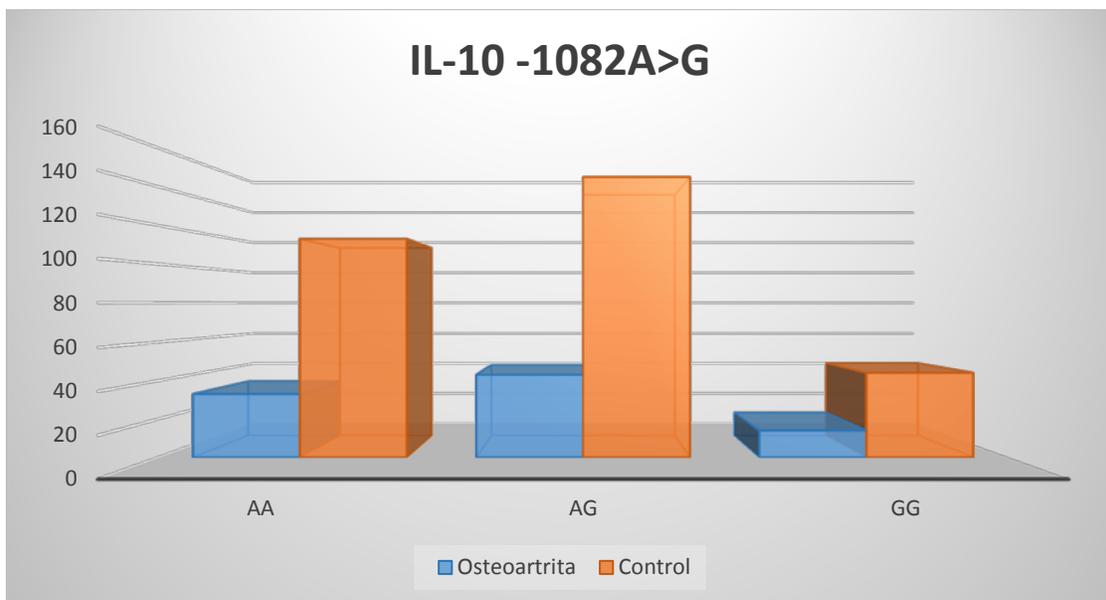


Figure. 6: Genotype frequency for IL-10 -1082A>G polymorfism

Our results, obtained through comparative analysis of the genotypes, showed that for IL-10 -1082A>G polymorphism we have no association with the risk to develop osteoarthritis.

TNF-A -308G>A polymorfism

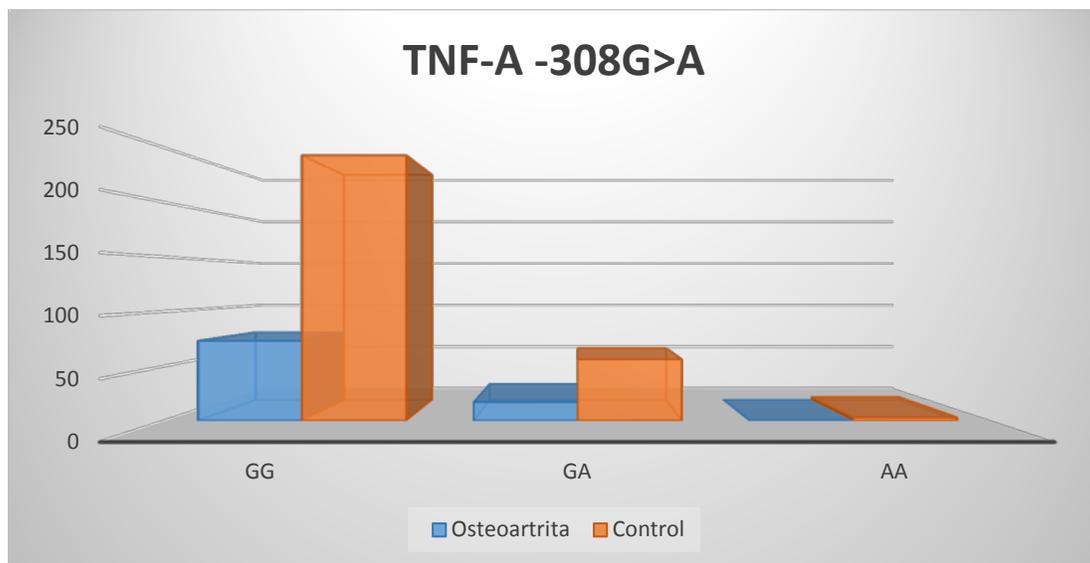


Figure. 7: Genotype frequency for TNF-A -308G>A polymorfism

For the last part of our study we have found no association for TNF-A -308G>A polymorphism and susceptibility to osteoarthritis neither on the general group or the stratified analysis based on location of the affection.

Our results which conclude the implication of the analyzed polymorphisms and their implication in rendering susceptibility to osteoarthritis are confirming other results in the literature. (10-16)(10-16)

Chapter 6. CONCLUSIONS

1. We have concluded that for IL-4R -3223C>T polymorphism the presence of CT genotype is associated with twice the risk of developing osteoarthritis. In dominant model, carriers of T allele T (CT and TT genotypes), have a risk of 1.5 higher of developing the disease.

2. Based on the stratified analysis on localization of osteoarthritis, we have concluded that IL-4R -3223C>T polymorphism is highly associated with a two and a half higher risk of developing knee osteoarthritis.
3. For the first time in literature we have concluded that carriers of allele T for IL-1B -511C>T polymorphism have a lower risk to develop osteoarthritis. We have, of course, to confirm these findings by continuing the study mainly by enlarging the groups.
4. We the exceptions mentioned above, all other polymorphism in our study have shown no association with increased risk of developing osteoarthritis.
5. A final conclusion would that even though our groups are not that numerous they respect Hardy-Weinberg equilibrium. Moreover most our results are confirmed by literature.

References

1. Haq I, Murphy E, Dacre J. Osteoarthritis. Postgraduate Medical Journal. 2003;79(933):377-83.
2. Bijlsma JW, Berenbaum F, Lafeber FP. Osteoarthritis: an update with relevance for clinical practice. Lancet. 2011;377(9783):2115-26.
3. Hochberg MC, Altman RD, April KT, Benkhalti M, Guyatt G, McGowan J, et al. American College of Rheumatology 2012 recommendations for the use of nonpharmacologic and pharmacologic therapies in osteoarthritis of the hand, hip, and knee. Arthritis care & research. 2012;64(4):465-74.
4. Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions,

1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *The Lancet*. 2013;380(9859):2197-223.

5. Lim K, Lau CS. Perception is everything: OA is exciting. *International journal of rheumatic diseases*. 2011;14(2):111-2.

6. Organization WH. *Chronic Diseases and Health Promotion: Chronic Rheumatic Conditions*.(2011). 2012.

7. Fejer R, Ruhe A. What is the prevalence of musculoskeletal problems in the elderly population in developed countries? A systematic critical literature review. *Chiropr Man Therap*. 2012;20(1):31-.

8. Goldring MB, Goldring SR. Osteoarthritis. *Journal of cellular physiology*. 2007;213(3):626-34.

9. Goldring MB, Marcu KB. Cartilage homeostasis in health and rheumatic diseases. *Arthritis Res Ther*. 2009;11(3):224.

10. Jotanovic Z, Etokebe GE, Mihelic R, Kaarvatn MH, Mulac-Jericevic B, Tijanic T, et al. IL1B -511(G>A) and IL1RN (VNTR) allelic polymorphisms and susceptibility to knee osteoarthritis in Croatian population. *Rheumatology international*. 2012;32(7):2135-41.

11. Ni H, Shi D, Dai J, Qin J, Xu Y, Zhu L, et al. Genetic polymorphisms of interleukin-1beta (-511C/T) and interleukin-1 receptor antagonist (86-bpVNTR) in susceptibility to knee osteoarthritis in a Chinese Han population. *Rheumatology international*. 2009;29(11):1301-5.

12. Moxley G, Meulenbelt I, Chapman K, van Duijn CM, Slagboom PE, Neale MC, et al. Interleukin-1 region meta-analysis with osteoarthritis phenotypes. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2010;18(2):200-7.

13. Kerkhof HJ, Doherty M, Arden NK, Abramson SB, Attur M, Bos SD, et al. Large-scale meta-analysis of interleukin-1 beta and interleukin-1 receptor

antagonist polymorphisms on risk of radiographic hip and knee osteoarthritis and severity of knee osteoarthritis. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2011;19(3):265-71.

14. Attur M, Wang HY, Kraus VB, Bukowski JF, Aziz N, Krasnokutsky S, et al. Radiographic severity of knee osteoarthritis is conditional on interleukin 1 receptor antagonist gene variations. *Annals of the rheumatic diseases*. 2010;69(5):856-61.

15. Lv C, Xu X, Wang J, Zhang Z, Zhang D, Guo C, et al. Combined effect of cytokine gene polymorphisms on end-stage knee osteoarthritis from Chinese Han population. *Rheumatology international*. 2012;32(11):3625-9.

16. Sezgin M, Barlas IO, Ankarali HC, Altintas ZM, Turkmen E, Gokdogan T, et al. Tumour necrosis factor alpha -308G/A gene polymorphism: lack of association with knee osteoarthritis in a Turkish population. *Clinical and experimental rheumatology*. 2008;26(5):763-8.