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Original Research

Predictive role of cytokines IL-10, IL-12 and TNF-α gene polymorphisms for the development of osteonecrosis of the femoral head in the Chinese Han population

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Abstract: The progressive neurodegenerative process in osteonecrosis of the femoral head (ONFH) is accompanied by chronic inflammation, including activation of microglia and astrocytes that express pro-inflammatory cytokines. Up to now, numerous studies of genetic epidemiology have assessed the association of anti- and pro-inflammation cytokines gene polymorphisms and risk of ONFH in different populations, but contradictory results were obtained due to the genetic heterogeneity of in different populations. In the study, 250 ONFH patients and 228 matched healthy controls from Shandong Province were recruited to evaluate the influence of interleukin-10 (IL-10) rs1800872, IL-12 rs3212227 and tumor necrosis factor-(TNF-α) rs1800629 polymorphism in ONFH. Single nucleotide polymorphism (SNP) locus was genotyped using the PCR-RFLP method. The genotypic and allele frequencies of TNF-α and IL-10 did not show significant difference between ONFH patients and normal controls. However, the frequencies of wild (AA) and homozygous mutant (CC) genotype of IL-12 rs3212227 in controls were more than that in ONFH patients, but that of the heterozygous genotype was more in patients with ONFH. Thus, our findings indicate that IL-12 rs3212227 AC genotype confer genetically susceptibility to ONFH in the Chinese Han population.

Key words: Osteonecrosis of the femoral head; IL-10; IL-12; TNF-α; Polymorphism.

Introduction

Osteonecrosis of the femoral head (ONFH) is a kind of bone disorder that usually affects middle-aged men between 30-50 years of age. Various factors have been implicated in the development of ONFH (1, 2). However, the exact pathogenesis of non-traumatic ONFH is largely unknown, although it has been associated with corticosteroid usage, alcoholism, infections, marrow infiltrating diseases, and coagulation defects (3). Therefore, it is believed that ONFH is a multifactorial disease in some cases that is associated with a genetic predilection as well as exposure to certain risk factors. The mentioned risk factors include corticosteroid use, alcohol intake, smoking and various chronic diseases, such as renal disease, hematological disease, inflammatory bowel disease, post-organ transplantation and hypertension(4).

Cytokines are redundant secreted proteins with growth, differentiation and activation functions that regulate immune cell function as well as trafficking. The cytokine produced in response to an immune insulting determines whether an immune response can be produced, and whether this response can be humoral, cytotoxic, cell-mediated or allergic. A cascade of responses to synergizing cytokines can be seen in an immune response for an optimal immune function. Cytokines could drive a completely different function depending on the cellular source, target as well as the state of specific immune response.

Up to now, numerous findings have provided evidence that the inflammatory process is an important pathological factor associated with ONFH (5-7). In recent years, the effects of the genetic polymorphisms which widely exist in human tissues with the diseases have increasingly drew the attention of the scientists and there have been many researches involving the association between single nucleotide polymorphism (SNP) and ONFH. Nucleotide variations in genes encoding anti- and pro-inflammatory cytokines, such as interleukin-10 (IL-10), interleukin-12 (IL-12) and tumor necrosis factor-α (TNF-α) (7) may affect their biological activities on the occurrence of ONFH. The balance between osteoclasts and osteoblasts determine the rate of bone remodeling, which was regulated by several molecular pathways. Certain cytokines such as IL-12, TNF-α and other pro-inflammatory cytokines stimulate osteoclasts differentiation and activation. Also it is well known that TNF-α acts on osteoblasts or bone marrow cells to synthesize and release cytokines, directly associating with osteoclasts proliferation and maturation (8, 9).

It was demonstrated that SNPs within the IL-10 promoter region including -1082C/A (rs1800896), -819 C/T (rs1800871) and -592C/A (rs1800872) (10, 11). Biologically active IL-12 consists of two functional subunits (p35 and p40), which are encoded by the IL-12A and IL-12B genes, respectively. The most studied SNPs within the IL-12 promoter region including -1188A/C (rs3212227) in the IL-12B gene and -277G/A (rs568408) and -564T/G (rs2243115) in the IL-12A

(12). The most studied SNPs within the TNF-α promoter region including TNF-α −238G/A (rs361525), −308G/A (rs1800629), −857C/T (rs1799724), −863C/A (rs1800630), and −1031T/C (rs1799964) (13). In order to evaluate the association between inflammation cytokines and ONFH, we analysis the influence of gene polymorphisms in IL-10 rs1800872, IL-12 rs3212227 and TNF-α rs1800629 on the susceptibility to ONFH patients in Chinese Han population.

Materials and Methods

Ethics Statement

The Medical Ethics Committee of Shandong Provincial Hospital affiliated to Shandong University approved this study. Written informed consents conforming to the tenets of the Declaration of Helsinki were obtained from each participant prior to the study.

Participants

A total of 250 unrelated patients with ONFH and 228 unrelated control subjects were consecutively enrolled in the department of joint surgery, Shandong Provincial Hospital affiliated to Shandong University from 2012 to 2016. ONFH diagnoses were established by the evidence of osteonecrosis through magnetic resonance imaging (MRI) in Stage 1 of the Association Research Circulation Osseous (ARCO) classification system and plain radiographs in Stages 2, 3, and 4. Control subjects were defined in the following way: they had no hip pain, and anteroposterior and frog leg lateral pelvic radiographs did not show any lesions with a sclerotic margin or subchondral collapse consistent with ONFH. Cases with a demonstrable history of direct trauma or the possibility of a combination of many causes were excluded. Steroid-induced osteonecrosis was defined by a history of taking prednisolone (1800 mg) or an equivalent over 4 weeks with nephritic syndrome, systemic lupus erythematosus, rheumatoid arthritis, allergic asthma, or organ transplantation. Alcohol-induced osteonecrosis was diagnosed by the consumption of more than 400 ml of pure ethanol per week, or alcohol induced fatty liver and liver cirrhosis.

Genotyping

Genome DNA from whole blood cells of each sample was extracted using Blood Genomic DNA Miniprep Kit (Axygen, USA) according to the manufacturer's instructions. DNA samples were stored at -20 °C until analysis. Genotyping for the IL-10 -592C/A (rs1800872), IL-12 -1188 A/C (rs3212227) and TNF-α -308G/A (rs1800629) polymorphisms in genomic DNA were performed using the polymerase chain reaction (PCR) and restriction fragment length polymorphism (PCR-RFLP). The genomic regions encompassing polymorphism were amplified using the following primers: IL-10 F: 5'-ACT TTTCCCCCTAGTTGTGTCTTTC-3', R: 5'-AGAATGAGCCTCAGACATCTCCAGT-3', IL-12 F: 5'-GGC ATTCTCTTCCAGGTTCTG-3', R: 5'-CCATGGCAACTTGAGAGCTG-3' and TNF-α F: 5'-AGGCAATAGGTTTTGAGGGCCAT-3', R: 5'-TCCCTGCTCCGATTCCG-3'. PCR products were generated in a 10 μL reaction volume vessel containing 50 ng genomic DNA, 1×PCR buffer, 2 mmol/L $MgCl_2$, 0.2 mmol/L of each dNTP, 1 μmol/L of each primer, and 0.25 U Taq DNA polymerase (Invitrogen Corporation, Carlsbad, CA). Cycling conditions consisted of an initial denaturation step at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds and a final elongation step at 72°C for 1 minute. Then, PCR products were digested with 2 U *NcoI* restriction enzyme at 37°C, in line with the manufacturer's instructions (New England BioLabs, Ipswich, MA). IL-10, the amplified fragment of 204 bp was cleaved into two fragments of 24 and 180 bp. The uncut product of 204 bp was identified as CC genotype; and the CA genotype was revealed by two fragments of 24 and 180 bp, and the AA genotype was revealed by a fragment of 180 bp. The IL-12 PCR products resulted in either two fragments of 173 and 70 bp (allele C) or a single fragment of 243 bp (allele A). Finally, the -308G allele contains a *NcoI* restriction site not presented in the -308A allele. Thus, in the presence of -308G allele, the PCR product (107 bp) is cut into 2 fragments of 80 and 27 bp in length.

Assay of serum IL-10, IL-12 and TNF-α levels

The serum level of IL-12, IL-10 and TNF-α were determined by ELISA Quantikine Human immunoassay kits (Biosource, USA). The lower limit of detection ranged from 4 to 6 pg/mL. Assay was carried out according to the manufacturer' s instructions.

Statistical analysis

Data were statistically described in terms of mean ± standard deviation (SD), or frequencies (number of cases) and percentages as required depending on their distribution. The Hardy–Weinberg equilibrium (HWE) was assessed for each variant to identify the deviation. The differences of the genotypes and alleles of detected genes between patients and normal controls were evaluated via Pearson Chi-square test. Exact test was used instead when the expected frequency is less than 5. The odds ratio (OR) and 95% confidence intervals (95% CI) were calculated. Unpaired Student's *t* test or Mann-Whitney tests were used for comparisons between two groups. Statistical analysis of data was performed using the SPSS software package 18.0 (SPSS Inc. USA). *P*-value less than 0.05 was considered to be statistically significant.

Results

In the study, 250 ONFH patients (110 males and 140 females) and 228 healthy controls (112 males and 116 females) were screened for IL-10 rs1800872, IL-12 rs3212227 and TNF-α rs1800629 polymorphisms using the PCR-RFLP method. No statistically significant differences were observed in the distributions of age (age of ONFH patients compared with that of control subjects at examination) and gender between ONFH patients and control subjects (Table 1).

Firstly, the frequencies of genotypes and alleles of IL-10 rs1800872, IL-12 rs3212227 and TNF-α rs1800629 were detected in both ONFH patients and controls. Hardy-Weinberg Expectation (HWE) of rs1800872, rs3212227 and rs1800629 in patients and controls were

listed in Table 2, and the results showed that allele distributions of detected SNPs were not deviated from HWE in both ONFH patients and controls. IL-10 rs1800872 in the study population were as follows:7.5% CC, 33.6% CA and 59.2% AA for the ONFH patients and 4.9 %CC, 30.3 %CA, and 64.6% AA for the controls, indicating that the genotypes distributions were similar between the ONFH patients and controls. Also, genomic analysis did not reveal a difference between ONFH patients and healthy controls in allele frequency at the -592 positions for the IL-10 gene promoter (rs1800872). Similarly, the genotypic and allele frequencies of TNF-α rs1800629 did not show significant difference between ONFH patients and normal controls. Then, genotype and allele frequencies of rs1800629 were detected in ONFH patients and normal controls (Table 2). The genotypic and allele frequency of rs1800629 between ONFH patients and controls did not show significant difference. However, the Power is less than 0.6, indicating a larger sample size is needed.

However, the frequencies of wild (AA) and homozygous mutant (CC) genotype IL-12 rs3212227 genotypes in ONFH patients and controls were found more than those in controls, but the frequency of the heterozygous genotype was higher (60.15%) in patients with ONFH. A significant risk of ONFH was observed for AC genotype of IL-12. The genomic analysis did not reveal differences in allele frequency of the IL-12 (A/C) gene between ONFH patients and healthy controls. Furthermore, results revealed that in ONFH patients (Figure 1), the rs3212227 AC genotypes exhibited a significantly higher IL-12 serum levels than the AA and CC genotypes (54.21±1.63 vs. 48.43±1.52, P<0.001).

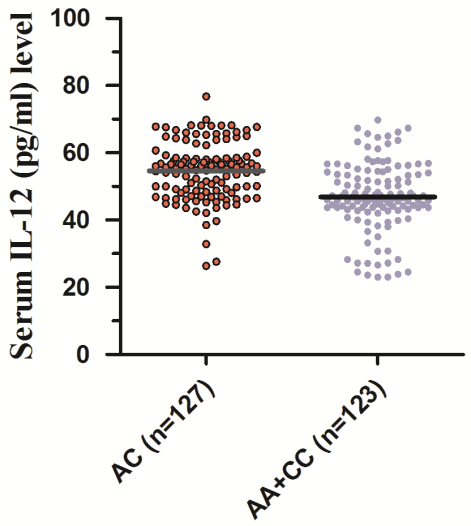

Figure 1. Comparison of serum levels of IL-12 in ONFH patients group with the genotypes of rs3212227 polymorphisms. AC genotype of rs3212227 in ONFH patients (n=127), and AA and CC genotypes of rs3212227 in patients with ONFH (n=123). Student t test was performed. IL-12 serum levels in the rs3212227 AC genotypes were significantly higher than the AA and CC genotypes (54.21±1.63 vs. 48.43±1.52, P<0.001).

Table 1. Characteristics of healthy controls and patients with osteonecrosis of the femoral head (ONFH).

Characteristics	Case	Control	*P*-value
Male [n (%)]	62 (23.71)	46 (18.83)	0.542
Age (years)	39.94 ± 8.120	38.46 ± 6.257	0.715
BMI (kg/m^2)	25.70 ± 4.69	25.55 ± 4.69	0.073
Cholesterol (mg/dL)	211.65 ± 41.80	162.87 ± 37.50	<0.001
Triglyceride (mg/dL)	133.23 ± 70.79	111.73 ± 53.20	<0.001
HDL-C (mg/dL)	54.14 ± 12.29	62.25 ± 13.16	<0.001
LDL-C (mg/dL)	128.35 ± 34.68	103.83 ± 35.41	<0.001

Table 2. Genotype and allele frequencies of IL-10 rs1800872, IL-12 rs3212227 and TNF-α rs1800629, and Pearson's chi-square test in ONFH patients and normal controls.

Genotype/Allele	Patients (n=250)	Controls (n=228)	*P*-value	OR (95% CI)
IL-10 rs1800872	HWE*P= 0.39	HWE P= 0.21		
GG	149	148	0.621	0.905 (0.526-1.320)
GC	83	69	0.057	0.822 (0.781-1.484)
CC	18	11	0.132	1.560 (0.556-2.412)
G	381	365	0.334	0.841 (0.498-1.192)
C	119	91		
IL-12 rs3212227	HWE*P= 0.22	HWE P= 0.71		
AA	108	83	0.0531	0.805 (0.526-1.220)
AC	127	140	**0.021**	3.749 (1.104-5.417)
CC	15	5	0.0539	0.972 (0.653-1.521)
A	343	306	0.235	0.471 (0.328-0.981)
C	157	150		
TNF-α rs1800629	HWE*P= 0.32	HWE P= 0.51		
GG	174	171	0.539	0.769 (0.542-1.392)
GA	68	51	0.931	1.558 (0.787-2.010)
AA	8	6	0.330	3.458 (0.347-32.543)
G	416	393	0.336	0.713 (0.524-1.267)
A	84	63		

* Chi-square test for deviation from the Hardy–Weinberg equilibrium (a value of P< 0.001 was regarded as a deviation from the HWE).

Discussion

For the etiology of ONFH, many scientists suggested that osteocyte apoptosis was the main cause. Apoptosis, also known as programmed cell death, was well-organized cell death and controlled by genes (14, 15). It serves as a crucial factor in the bone formation during bone growth and bone remodeling. The signaling pathways related to apoptosis includes cytoplasmic, mitochondrial and endoplasmic reticulum pathways. Multiple factors are involved in the regulation of apoptosis (16). Considerable effort has been invested in understanding the effect of cytokines on bone formation of osteoblasts and the bone resorption of osteoclasts (17, 18). It is now apparent that the activities of osteoblast and osteoclasts are interrelated. Activity and bone resorption of osteoclasts, appear to be modulated by cytokines released by osteoblasts. Polymorphisms in cytokine genes may result in inter-individual variation in transcriptional regulation, and thus in differential cytokine production. It has been widely hypothesized that genetic variants of cytokines could have phenotypic relevance and the potential influence of an individual's internal micro-environment (19, 20).

The gene SNPs have been thought to alter expressions of certain genes. Thus, SNPs could be associated with an altered risk of multiple diseases (21-23). Up to now, the important role of inflammatory cytokines during ONFH development and prognosis is increasingly attracting interest around the world. Several lines of evidence point to the involvement of IL-10 in pathogenesis of ONFH (24). In the current study, evidence of an association between the IL-10 rs1800872 polymorphism and ONFH were not found in the China Han Population. This specific genotype is associated with elevated serum levels of IL-10, and having in mind the inhibition role of IL-10 on other cytokines. Thus, it could be hypothesized that especially the control group could benefit from this IL-10 excess, since this molecule has a restrictive role on other cytokines and their roles (25).

IL-12 is an important pro-inflammatory cytokine that plays a key role in the development and progress of diseases (26). Variation in the DNA sequence lead to altered IL-12 production, which can alter individual's susceptibility to cancer. The IL-12 3'UTR A>C polymorphism is a functionally important SNP that alters IL-12 production and it has been a reported potential biomarker for risks of numerous diseases including hepatitis, psoriasis, Barrett's esophagus, asthma and ONFH (27, 28). More importantly, genetic variation in IL-12 was revealed to affect susceptibility to multiple sclerosis, another neurodegenerative disease with evident inflammatory responses. Considering the potential role of IL-12 in ONFH pathogenesis as well as the involvement of IL-12 polymorphisms in the predisposition to many inflammatory diseases, increased frequency of IL-12 rs3212227 AA and CC homozygous genotypes among controls was observed, but that of the heterozygous AC genotype was higher in patients with ONFH. Thus, a significant risk of ONFH was observed for AC genotype of IL-12 rs3212227. A cascade of responses to cytokines has been shown in an immune response in ONFH (9). We found ONFH is associated with IL-2 SNP, but the role of IL-12 in ONFH remains not clear.

TNF-α gene is located in the class III region of the human major histocompatibility complex (MHC) on chromosome 6p21 (29). Among the SNPs identified in TNF-α, TNF-α rs1800629 is most extensively studied. The A allele of this polymorphism can lead to high binding affinity of nuclear factors to the TNF promoter, resulting in a high level of transcription activity and secretion levels of TNF-α. Therefore, it was advised to have a significant functional effect (30). In the present study, the results indicated that the genotypes distributions of TNF-α rs1800629 in ONFH patients were almost similar to that in the controls. In previous studies, the rs1800629 was mainly investigated in the Caucasian population and African American patients with a variation of A allele frequency from 13% to 29% with an OR=1.05 from all Caucasian studies (31, 32). Recently, Wang and colleagues reported that the single analysis of this SNP in Asian population with frequency of A allele (11%) is slightly minor in respect to Caucasian sample, but with a positive result (OR=1.67) probably due to the very rare A allele frequency in the controls (7%) (33). TNF-α 308 promoter polymorphism is a biallelic G to A polymorphism, and the TNF-α A allele is associated with increased levels of TNF-α in plasma. Although we have not tested whether TNF-α rs1800629 was associated with level of TNF-α in plasma in Chinese Han population in the present study, our results indicate a lack of association between TNF-α rs1800629 and the risk in ONFH in Chinese Han population. The Power is less than 0.6, indicating a larger sample size is needed. We will study whether the detected SNP responsible for TNF-α level in the future." This is added in the results and discussion section now. In addition, the contradictory results of TNF-α SNP in our study with the literatures probably due to the genetic, clinical and population heterogeneity. TNF-α and epidermal growth factor (EGF) are well-known stimuli of cyclooxygenase (COX)-2 expression, and TNF-α stimulates trans-activation of EGF receptor (EGFR) signaling to promote the survival in colon epithelial cells.

Moreover, our study was a hospital-based case-control study, selection bias could not be fully excluded. With the limitations of sample size and SNP variety, it is difficult to completely illuminate the interaction of genes and environment factors. At present, there are few studies in this field. More systematic studies with multiple environment factors needs to be conducted, which will contribute to uncover the effects of gene polymorphisms and environmental factors on ONFH in Chinese Han population. In summary, our findings indicated that IL-12 rs3212227 AC genotype confers genetically susceptibility to ONFH in Chinese Han population.

References

1. Glueck CJ, Freiberg RA, Oghene J, Fontaine RN, Wang P. Association between the T-786C eNOS polymorphism and idiopathic osteonecrosis of the head of the femur. J Bone Joint Surg Am 2007; 89:2460-8.

2. Song Y, Du Z, Yang Q, Ren M, Sui Y, Wang Q, Wang A, Zhao H, Wang J, Zhang G. Associations of IGFBP3 Gene Polymorphism and Gene Expression with the Risk of Osteonecrosis of the Femoral Head in a Han Population in Northern China. DNA Cell Biol 2016; 35:836-44.

3. Zhong DN, Wu JZ, Li GJ. Association between CYP2C8 (rs1934951) polymorphism and bisphosphonate-related osteonecrosis of the jaws in patients on bisphosphonate therapy: a meta-analysis. Acta Haematol 2013; 129:90-5.
4. Sarasquete ME, Garcia-Sanz R, Marin L, Alcoceba M, Chillon MC, Balanzategui A, Santamaria C, Rosinol L, de la Rubia J, Hernandez MT, et al. Bisphosphonate-related osteonecrosis of the jaw is associated with polymorphisms of the cytochrome P450 CYP2C8 in multiple myeloma: a genome-wide single nucleotide polymorphism analysis. Blood 2008; 112:2709-12.
5. Beksac K, Sonmez C, Cetin B, Kismali G, Sel T, Tuncer Y, Kosova F. Evaluation of proinflammatory cytokine and neopterin levels in women with papillary thyroid carcinoma. The International journal of biological markers 2016; 31:e446-e50.
6. Lumachi F, Basso SM, Orlando R. Cytokines, thyroid diseases and thyroid cancer. Cytokine 2010; 50:229-33.
7. Kubaszek A, Pihlajamaki J, Komarovski V, Lindi V, Lindstrom J, Eriksson J, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, et al. Promoter polymorphisms of the TNF-alpha (G-308A) and IL-6 (C-174G) genes predict the conversion from impaired glucose tolerance to type 2 diabetes: the Finnish Diabetes Prevention Study. Diabetes 2003; 52:1872-6.
8. Mozzati M, Martinasso G, Maggiora M, Scoletta M, Zambelli M, Carossa S, Oraldi M, Muzio G, Canuto RA. Oral mucosa produces cytokines and factors influencing osteoclast activity and endothelial cell proliferation, in patients with osteonecrosis of jaw after treatment with zoledronic acid. Clin Oral Investig 2013; 17:1259-66.
9. Hungerford MW, Mont MA. [Potential uses of cytokines and growth factors in treatment of osteonecrosis]. Orthopade 2000; 29:442-8.
10. Oleksyk TK, Shrestha S, Truelove AL, Goedert JJ, Donfield SM, Phair J, Mehta S, O'Brien SJ, Smith MW. Extended IL10 haplotypes and their association with HIV progression to AIDS. Genes and Immunity 2009; 10:309-22.
11. Dmello DM, Ariyanto I, Estiasari R, Halstrom S, Gaff J, Lee S, Price P. Polymorphisms in IL10 may alter CD4 T-cell counts in Indonesian HIV patients beginning antiretroviral therapy. Hum Immunol 2017; 78:387-90.
12. Zheng Y, Wang M, Tian T, Liu K, Liu X, Zhai Y, Lin S, Yang P, Li S, Dai Z, et al. Role of interleukin-12 gene polymorphisms in the onset risk of cancer: a meta-analysis. Oncotarget 2017; 8:29795-807.
13. Zhang P, Wu X, Li G, He Q, Dai H, Ai C, Shi J. Tumor necrosis factor-alpha gene polymorphisms and susceptibility to ischemic heart disease: A systematic review and meta-analysis. Medicine (Baltimore) 2017; 96:e6569.
14. Di Martino MT, Arbitrio M, Guzzi PH, Leone E, Baudi F, Piro E, Prantera T, Cucinotto I, Calimeri T, Rossi M, et al. A peroxisome proliferator-activated receptor gamma (PPARG) polymorphism is associated with zoledronic acid-related osteonecrosis of the jaw in multiple myeloma patients: analysis by DMET microarray profiling. Br J Haematol 2011; 154:529-33.
15. Zeng Z, Wang B, Pan H. Relation between osteonecrosis of the femoral head and PAI-1 4G/5G gene polymorphism: a meta-analysis. Int J Clin Exp Med 2015; 8:20337-42.
16. Miller KD, Masur H, Jones EC, Joe GO, Rick ME, Kelly GG, Mican JM, Liu S, Gerber LH, Blackwelder WC, et al. High prevalence of osteonecrosis of the femoral head in HIV-infected adults. Ann Intern Med 2002; 137:17-25.
17. Bond J, Adams S, Richards S, Vora A, Mitchell C, Goulden N. Polymorphism in the PAI-1 (SERPINE1) gene and the risk of osteonecrosis in children with acute lymphoblastic leukemia. Blood 2011; 118:2632-3.
18. Yin JM, Liu Z, Zhao SC, Guo YJ, Liu ZT. Relationship between the Apolipoprotein AI, B gene polymorphism and the risk of non-traumatic osteonecrosis. Lipids Health Dis 2014; 13:149.
19. Gross CE, Sershon RA, Frank JM, Easley ME, Holmes GB, Jr. Treatment of Osteonecrosis of the Talus. JBJS Rev 2016; 4.
20. Liu B, Cao Y, Wang D, Yao G, Bi Z. Vascular endothelial growth factor -634G/C polymorphism associated with osteonecrosis of the femoral head in a Chinese population. Genet Test Mol Biomarkers 2012; 16:739-43.
21. Kakkoura MG, Demetriou CA, Loizidou MA, Loucaides G, Neophytou I, Marcou Y, Hadjisavvas A, Kyriacou K. Single-nucleotide polymorphisms in one-carbon metabolism genes, Mediterranean diet and breast cancer risk: a case-control study in the Greek-Cypriot female population. Genes & nutrition 2015; 10:453.
22. Papewalis C, Jacobs B, Wuttke M, Ullrich E, Baehring T, Fenk R, Willenberg HS, Schinner S, Cohnen M, Seissler J, et al. IFN-alpha skews monocytes into CD56+-expressing dendritic cells with potent functional activities in vitro and in vivo. Journal of immunology 2008; 180:1462-70.
23. Solini A, Cuccato S, Ferrari D, Santini E, Gulinelli S, Callegari MG, Dardano A, Faviana P, Madec S, Di Virgilio F, et al. Increased P2X7 receptor expression and function in thyroid papillary cancer: a new potential marker of the disease? Endocrinology 2008; 149:389-96.
24. Stanciu AE, Serdarevic N, Hurduc AE, Stanciu MM. IL-4, IL-10 and high sensitivity-CRP as potential serum biomarkers of persistent/recurrent disease in papillary thyroid carcinoma with/without Hashimoto's thyroiditis. Scandinavian journal of clinical and laboratory investigation 2015; 75:539-48.
25. Farahat O, Salah M, Mokhtar A, Abouelfetoh F, Labib D, Baz H. The association of promoter gene polymorphisms of the tumor necrosis factor-alpha and interleukin-10 with severity of lactic acidosis during liver transplantation surgery. Transplant Proc 2012; 44:1307-13.
26. Firouze Moqadam I, Najafi S, Mohammadzadeh M, Zare Bidoki A, Yousefi H, Farhadi E, Tonekaboni A, Meighani G, Mohammadzadeh M, Amirzargar AA, et al. Lack of Association between Interleukin-12 Gene Polymorphisms and Recurrent Aphthous Stomatitis. Avicenna J Med Biotechnol 2016; 8:200-1.
27. Grzegorzewska AE, Wobszal PM, Mostowska A, Jagodzinski PP. Antibodies to hepatitis B virus surface antigen and interleukin 12 and interleukin 18 gene polymorphisms in hemodialysis patients. BMC Nephrol 2012; 13:75.
28. Navaglia F, Basso D, Zambon CF, Ponzano E, Caenazzo L, Gallo N, Falda A, Belluco C, Fogar P, Greco E, et al. Interleukin 12 gene polymorphisms enhance gastric cancer risk in H pylori infected individuals. J Med Genet 2005; 42:503-10.
29. Daniels SE, Bhattacharrya S, James A, Leaves NI, Young A, Hill MR, Faux JA, Ryan GF, le Souef PN, Lathrop GM, et al. A genome-wide search for quantitative trait loci underlying asthma. Nature 1996; 383:247-50.
30. Schluter B, Erren M, Schotte H, Junker R, Rust S, Assmann G. The mutagenically separated polymerase chain reaction is a rapid and reliable method for genotyping of the tumour necrosis factor-alpha promoter polymorphism (-308 G/A). Clinica chimica acta; international journal of clinical chemistry 2002; 320:135-8.
31. Horneff G, Klein A, Oommen PT, Hospach A, Foeldvari I, Feddersen I, Minden K. OP0219 update on malignancies in children with juvenile idiopathic arthritis in the German BIKER Registry. Clinical and experimental rheumatology 2016; 34:1113-20.
32. Jedlickova H, Durcanska V, Vasku V. Paraneoplastic Scleroderma: Are There Any Clues? Acta dermatovenerologica Croatica : ADC 2016; 24:78-80.
33. Lv N, Shan Z, Gao Y, Guan H, Fan C, Wang H, Teng W. Twist1

regulates the epithelial-mesenchymal transition via the NF-kappaB pathway in papillary thyroid carcinoma. Endocrine 2016; 51:469-77.