Association of cytokine gene polymorphisms with osteoarthritis susceptibility

OTILIA CONSTANTINA ROGOVEANU[1*], DANIELA CALINA[2*], MIHAI GABRIEL CUCU[3], FLORIN BURADA[3], ANCA OANA DOCEA[4], SIMONA SOSOI[3], EMILIAN STEFAN[5], MIHAI IOANA[3] and EMILIA BURADA[3,6]

[1]Department of Physical Medicine and Rehabilitation; [2]Department of Clinical Pharmacy; [3]Human Genomics Laboratory; [4]Department of Toxicology, University of Medicine and Pharmacy of Craiova, 200349 Craiova; [5]Clinic of Orthopaedics and Traumatology, Clinical Hospital CF2, 011464 Bucharest; [6]Department of Physiology, University of Medicine and Pharmacy of Craiova, 200349 Craiova, Romania

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Abstract. Osteoarthritis (OA) is a multifactorial disease characterized by low-grade inflammatory processes that are mediated initially by the cells and factors of the innate immune system. In addition to their key role in inflammation, cytokines contribute to the pathogenesis of OA through angiogenesis and chemotaxis. The purpose of the present case-control study was to investigate a possible association of four cytokine single nucleotide polymorphisms (SNPs), IL-4R -3223C>T (rs2057768), IL-8 -251T>A (rs4073), IL-10 -1082A>G (rs1800896) and TNF -A-308G>A (rs1800629) with OA susceptibility. Genomic DNA was isolated from blood samples collected from 305 Romanian subjects (90 patients with OA and 215 controls) and the genotyping of the SNPs was performed by TaqMan allelic discrimination polymerase chain reaction using predesigned assays. Our data indicated a significant association for IL-4R rs2057768 C>T SNP. The subjects that carried the CT genotype were at a higher risk for OA (OR 2.03, 95% CI: 1.21-3.42, P=0.007) compared with those that had the CC genotype. Furthermore, the carriers of the T allele were at a 1.9 fold higher risk for OA (OR 1.92; 95% CI, 1.17-3.17; P=0.009) in a dominant model. The association remained significant only for knee OA in the subgroups analysis. No correlations were observed between the other remaining SNPs and OA. The results suggested that the IL-4R rs2057768 SNP could contribute to OA susceptibility in the Romanian population, providing novel evidence for the involvement of IL-4/IL-4R pair in the pathogenesis of OA.

Correspondence to: Dr Florin Burada, Human Genomics Laboratory, University of Medicine and Pharmacy of Craiova, 2 Petru Rares, 200349 Craiova, Romania
E-mail: buradaflorin@gmail.com

Dr Anca Oana Docea, Department of Toxicology, University of Medicine and Pharmacy of Craiova, 2 Petru Rares, 200349 Craiova, Romania
E-mail: ancadocea@gmail.com

*Contributed equally

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Introduction

Osteoarthritis (OA) is a chronic disease characterized by progressive degradation and loss of articular cartilage. Although the disease present in various joints, the weight-bearing joints such as knees and hips are commonly affected. Furthermore, alterations in other joint tissues such as ligaments, synovium and subchondral bone are usually noted in OA (1,2). Generally, the symptoms develop gradually over time and the loss of cartilage can lead to decreased joint space, stiffness, pain with increasing loss of function and disability, and finally to the need for surgical joint replacement (3). Nowadays, OA is not only viewed as a degenerative disease of cartilage and different risk factors were associated with OA, including genetic predisposition, sex, obesity, age, diet, occupation, metabolic syndromes, injury and mechanical stress (1,4,5). Accumulating evidence indicates that inflammation has a critical role in OA pathogenesis (6), and recent findings show that the development of OA is in notably driven by low-grade inflammatory processes and mediated mainly by the innate immune system (7). The inflammatory response was identified as the key component, which promoted synovitis as well as progression of cartilage and bone destruction in OA via the secretion of chemokines, cytokines and other molecules, which can be detected in the synovial fluids (8-10). In addition to their evident role in inflammation, cytokines can contribute to the pathogenesis of OA through angiogenesis and chemotaxis (11,12). OA can take years and even decades to develop and radiography is routinely used to help in the diagnosis of OA. However, symptoms often occur before the onset of any radiographic abnormality and further work should be focused on the identification of new biological markers as early indicators of OA risk.

Based on this rational, we have genotyped four single nucleotide polymorphisms (SNPs) located in the promoter region of the following cytokine genes: IL-4R -3223C>T

(rs2057768), IL-8 -251T>A (rs4073), IL-10 -1082A>G (rs1800896) and TNF-A -308G>A (rs1800629). This study was performed in a Romanian population (Eastern European population) in order to establish whether these cytokine gene SNPs are associated with OA susceptibility in this region.

Materials and methods

Subjects. In the current hospital-based case-control study we included 305 Romanian subjects (90 patients diagnosed with OA and 215 controls). All OA cases were diagnosed based on clinical and radiographic signs, arthroscopic or MRI findings with Kellgren-Lawrence grade 2 or more. The patients with history of trauma or skeletal defects were excluded. Matched controls of the same ethnic and geographical origins were recruited among unrelated volunteers. The controls were admitted in the Clinical Hospital CF2 from Bucharest and in the Emergency Clinical County Hospital of Craiova, Romania. The subjects with a positive history of tumor, autoimmune, other inflammatory or chronic infectious symptoms were excluded. Written informed consent from all the participants was obtained and the study was approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova (Craiova, Romania).

SNPs genotyping. Total genomic DNA was isolated from the peripheral blood leukocytes using the Wizard Genomic DNA Purification kit (Promega Corporation, Madison, WI, USA) following the manufacturer's protocol. DNA concentration and purity were determined with UV spectrophotometry. The identification of SNPs was performed by TaqMan allelic discrimination real-time PCR. Validated TaqMan SNP genotyping assays were obtained from Applied Biosystems (Foster City, CA, USA): IL-4R -3223C>T (rs2057768, assay C_2769607_10); IL-8 -251T>A (rs4073, assay C_11748116_10); IL-10 -1082A>G (rs1800896, assay C_1747360_10) and TNF-A -308G>A (rs1800629, assay C_7514879_10). The procedures for the PCR reactions and quality control samples have been described previously (13).

Statistical analysis. The deviation from Hardy-Weinberg equilibrium was tested among controls by χ^2 test. The demographic data between groups were compared using the χ^2 test for sex and the Student's t-test for age. Using unconditional logistic regression analysis under codominant and dominant models, we have calculated the differences in genotype distributions and minor allele frequencies among OA patients and controls based on Odds ratios (OR) and 95% confidence intervals (CIs). The reference group included the subjects that were homozygous for the most common allele. A stratified analysis by joint location was further performed aside from the overall association analysis. SPSS software package (v.17.0; SPSS Inc., Chicago, IL, USA) was used for data analysis and a P-value of less than 0.05 indicated a statistically significant difference.

Results

Subjects characteristics. A total of 90 OA patients (63 women and 27 men, mean age 64.12 years, with a body mass index ≤27) and 215 healthy controls (150 women and 65 men, mean age 62.69 years) were included in the present study. In 54 cases the location was within the knee and in 36 cases within the hip joint. The age and sex distributions of the groups were comparable (Table I).

Table I. Baseline characteristics of the patients and controls.

Characteristic	Osteoarthritis cases (n=90)	Control (n=215)	P-value
Male/female	63/27	150/65	0.97
Age (years)	64.12	62.69	0.26
Location			
Knee	54	-	-
Hip	36	-	-

Cytokine SNPs and risk of overall OA. For each polymorphism, the genotype distributions were consistent with those predicted by the Hardy-Weinberg equilibrium. A significant association was observed for IL-4R -3223C>T (rs2057768) SNP. The subjects carrying CT genotype were at a higher risk for OA (OR 2.03; 95% CI: 1.21-3.42; P=0.007) compared with the more frequently encountered CC genotype. Furthermore, the carriers of T allele were at a 1.9 fold elevated risk for OA in a dominant model (OR 1.92; 95% CI, 1.17-3.17; P=0.009). The T allele was significantly more frequent in patients with OA when the allele frequencies were assessed (OR 1.51; 95% CI, 1.03-2.21; P=0.035).

No correlation was noted between OA cases and controls with regard to IL-8 -251AA, IL-10 -1082GG and TNF-A -308AG genotypes and overall OA risk (Table II). In addition, the carriers of IL-10 -1082 G, IL-8 -251A and TNF-A -308A allele were not associated with an increased risk of OA in a dominant model.

Risk of knee and hip OA by genotype. In a stratified analysis, the only association between OA and cytokine polymorphisms was found for IL-4R -3223C>T polymorphism and was restricted to the knee OA cases. The T carriers exhibited an increased risk (OR 2.31; 95% CI, 1.26-4.26) (Table III; Fig. 1). No significant differences were observed between knee or hip OA and controls in the subgroups analysis for the remaining SNPs (Table III; Fig. 2).

Discussion

In the present observational study, we assessed whether the four promoter SNPs located in the cytokine genes influence the risk of OA in the Romanian population. The polymorphisms were selected based on multiple previous reports that have demonstrated quantitative differences in the transcription and/or expression, and their involvement in other multifactorial diseases, where the inflammatory process plays an important role in pathogenesis.

From the tested SNPs, the only association was detected for IL-4R rs2057768 C>T SNP. This gene encodes the alpha chain of the interleukin-4 receptor that can bind the anti-inflammatory cytokine IL-4. Previous research investigated the association

Table II. Genotype frequencies for cytokine polymorphisms in cases and controls and their association with risk of osteoarthritis.

Polymorphism	Osteoarthritis (n=90) (%)	Control (n=215) (%)	OR (95% CI)	P-value
IL-4R -3223C>T				
CC	39 (43.33)	128 (59.54)	Reference	-
CT	44 (48.89)	71 (33.02)	2.03 (1.21-3.42)	0.007
TT	7 (7.78)	16 (7.44)	1.44 (0.55-3.74)	0.457
T carriers	51 (56.67)	87 (40.46)	1.92 (1.17-3.17)	0.009
IL-8 -251T>A				
TT	22 (24.44)	74 (34.42)	Reference	-
TA	51 (56.67)	103 (47.91)	1.67 (0.93-2.98)	0.084
AA	17 (18.89)	38 (17.67)	1.51 (0.72-3.17)	0.280
A carriers	68 (75.56)	141 (65.58)	1.62 (0.93-2.83)	0.097
IL-10 -1082 A>G				
AA	33 (36.67)	81 (37.67)	Reference	-
AG	43 (47.78)	103 (47.91)	1.02 (0.60-1.76)	0.929
GG	14 (15.56)	31 (14.42)	1.11 (0.52-2.35)	0.787
G carriers	57 (63.33)	134 (62.33)	1.04 (0.63-1.74)	0.868
TNF-A -308 G>A				
GG	73 (81.11)	173 (80.47)	Reference	-
GA	17 (18.89)	40 (18.60)	1.07 (0.54-1.89)	0.982
AA	0 (0.00)	2 (0.93)	-	-
A carriers	17 (20.14)	42 (19.53)	0.96 (0.51-1.79)	0.896

OR, odds ratio; CI, confidence interval; TNF, tumor necrosis factor; IL, interleukin.

Table III. Comparative analysis between genotype frequencies and the risk of osteoarthritis in the joint location subgroups.

Polymorphism	Knee, n=54 (%)	OR (95% CI)	P-value	Hip, n=36 (%)	OR (95% CI)	P-value
IL-4R -3223C>T						
CC	21 (38.89)	Reference		18 (50.00)	Reference	
CT	31 (57.41)	2.66 (1.42-4.97)	0.002	13 (36.11)	1.30 (0.60-2.81)	0.501
TT	2 (3.70)	0.76 (0.16-3.56)	0.728	5 (13.89)	2.22 (0.73-6.80)	0.153
T carriers	33 (61.11)	2.31 (1.26-4.26)	0.006	18 (50.00)	1.47 (0.73-2.99)	0.283
IL-8 -251T>A						
TT	14 (25.93)	Reference		8 (22.22)	Reference	
TA	29 (53.70)	1.49 (0.74-3.01)	0.267	22 (61.11)	1.98 (0.83-4.68)	0.117
AA	11 (20.37)	1.53 (0.63-3.69)	0.342	6 (16.67)	1.46 (0.47-4.51)	0.509
A carriers	40 (74.07)	1.50 (0.77-2.93)	0.234	28 (77.78)	1.84 (0.80-4.23)	0.148
IL-10 -1082A>G						
AA	18 (33.33)	Reference		15 (41.67)	Reference	
AG	27 (50.00)	1.18 (0.61-2.29)	0.625	16 (44.44)	0.84 (0.39-1.80)	0.651
GG	9 (16.67)	1.31 (0.53-3.22)	0.560	5 (13.89)	0.87 (0.29-2.60)	0.804
G carriers	36 (66.67)	1.20 (0.64-2.27)	0.554	21 (58.33)	0.85 (0.41-1.73)	0.648
TNF A -308 G>A						
GG	44 (81.48)	Reference		29 (80.56)	Reference	
GA	10 (18.52)	0.98 (0.46-2.12)	0.965	7 (19.44)	1.04 (0.43-2.55)	0.924
AA	0 (0.00)	-		0 (0.00)	-	
A carriers	10 (18.52)	0.94 (0.44-2.01)	0.865	7 (19.44)	0.99 (0.41-2.42)	0.989

OR, odds ratio; CI, confidence interval; TNF, tumor necrosis factor; IL, interleukin.

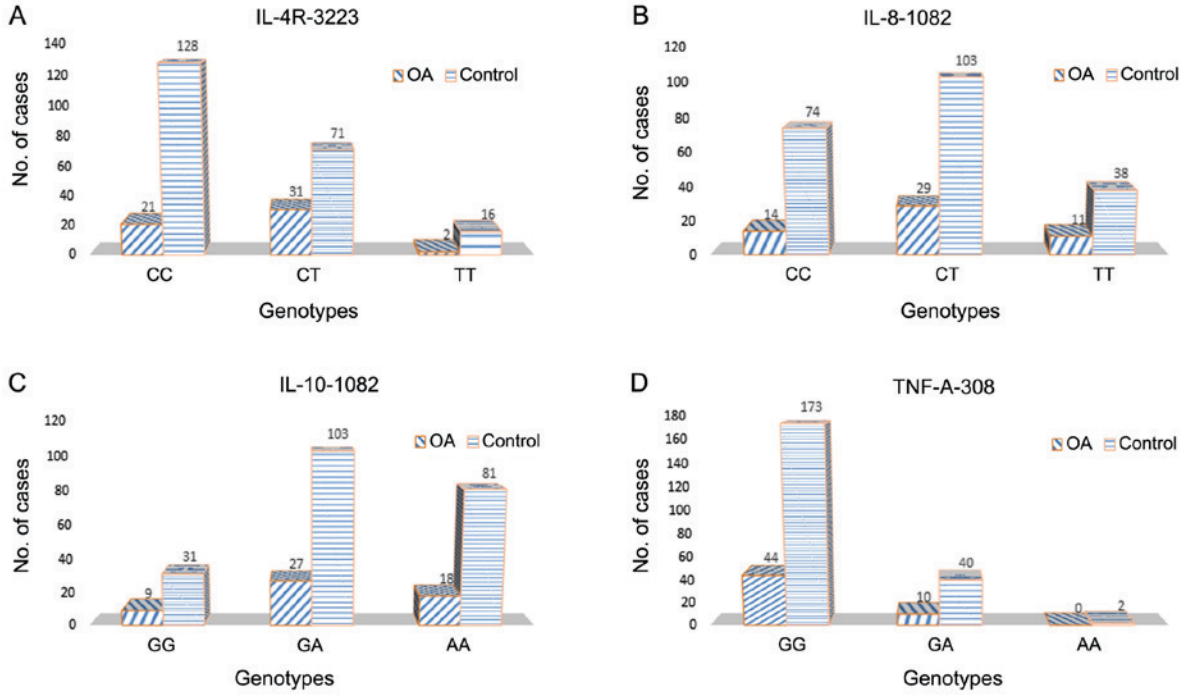

Figure 1. Genotype frequencies of IL-4R -3223C>T (A), IL-8 -251T>A (B), IL-10-1082A>G (C) and TNF-A -308G>A (D) SNPs in controls and cases with knee OA. TNF, tumor necrosis factor; IL, interleukin; OA, osteoarthritis.

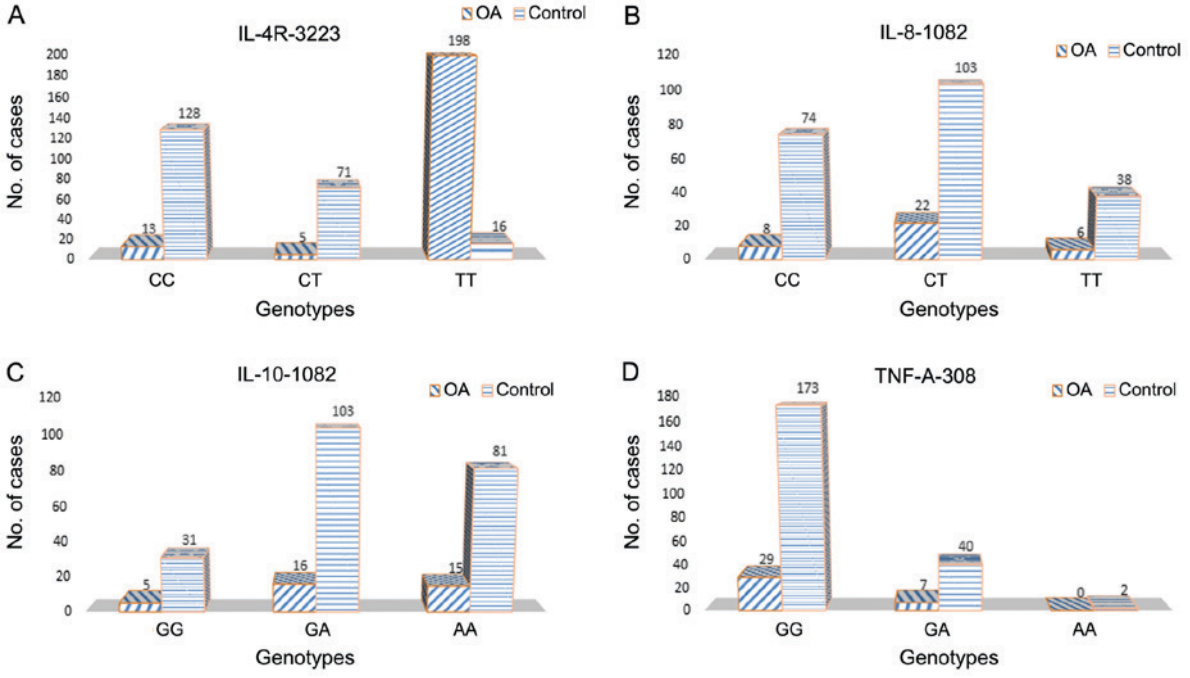

Figure 2. Genotype frequencies of IL-4R -3223C>T (A), IL-8 -251T>A (B), IL-10-1082A>G (C) and TNF-A -308G>A (D) SNPs in controls and cases with hip OA. TNF, tumor necrosis factor; IL, interleukin; OA, osteoarthritis; SNPs, single nucleotide polymorphisms.

between IL-4R SNPs and OA susceptibility in different populations, with inconsistent results. A significant association was found for both rs1805013 and rs1805016 IL-4R SNPs and hip OA in a case-control study including UK Caucasians subjects (14). In contrast to Forster *et al* (14), another UK study was unable to replicate the associations of these two SNPs with hip OA and/or knee OA susceptibility (15). Furthermore, Vargiolu *et al* (16) suggested a positive association in an Italian population between both rs1805013 and rs1805015 located in the IL-4R gene and overall risk for hand OA, while in the subgroups analysis only the association between rs1805013 and non-erosive hand OA was significant. In addition, the possible involvement of the IL-4/IL-4R axis in the pathogenesis of OA was suggested by an association of the IL-4 intron 3 VNTR polymorphism and the incidence of knee OA in a Turkish population (17). No statistically significant associations were

noted between IL-4R SNPs (rs1805015 and rs1805016) and hand OA in a Finnish study (18).

In the present study no correlation was noted between the IL-8 -251T>A (rs4073), IL-10 -1082A>G (rs1800896) and TNF-A -308G>A (rs1800629) SNPs and OA susceptibility. A statistically significant higher frequency of IL-8 -251TT genotype and IL-8 -251T allele was noted in patients diagnosed with OA compared with controls in a Han Chinese population, suggesting that the TT genotype and the T allele of the IL-8 gene at position-251 confer a high risk in OA. In the same study, the IL-8 SNP located at position +781C>T influenced the risk of OA (19). Furthermore, IL-10 -1082A>G, -819T>C and -592A>C gene promoter SNPs were tested in a Chinese Han population and no significant difference was observed in the allele and/or haplotype frequencies between end-stage knee OA and the controls (20). Similar results were obtained in a Dutch population, where no significant association was detected between distal interphalangeal OA and IL-10 SNPs, including IL-10 -1082A>G (21). Moreover, no significant difference in the genotype distribution between OA individuals and controls was observed for the IL-10 -1082A>G gene and the hand OA among Finnish women (18). A positive correlation between IL-10 G microsatellite SNP and idiopathic knee OA was noted in a Greek case-control study, and the carriers of the LL genotype were at 4 times higher risk than the SS genotype (22).

The association between TNF-A gene and OA susceptibility has been widely studied, and the published results remain inconclusive. The TNF-α gene encodes the important pro-inflammatory cytokine TNF-α and different genetic variants located in the promoter region have been associated with altered gene expression, notably at the -308 position (23,24). No association was observed in the present study between the TNF-α -308G>A SNP and the risk of OA, which is consistent with the results from the Turkish Caucasian and Mexican Mestizo population. Both studies included only knee OA (25,26). On the contrary, in the Han Chinese population, the TNF-α -308A allele was found to increase the risk, whereas the TNF-α -238 SNP did not play a role in OA patients (27). Similar findings were reported in the other Asian population, although the risk of OA was significantly higher for carriers of the TNF-α -308A allele in a Korean Population (28). A recent meta-analysis, which included 983 OA cases and 1355 control subjects indicated a significant association between TNF-α -308A allele and the risk of OA. An increased OA risk was found in the recessive genetic model and higher frequency of both AA genotype and GA genotype was observed in OA patients compared with the control population. Interestingly, the association only existed among the Asian population but not among the Caucasian population (29).

The current study has some limitations. Firstly, our findings rely on a small sample and the selection bias cannot be ruled out in this hospital case-control study. OA susceptibility is influenced by multiple interactions between different other genes, which were not evaluated and additional data regarding the biological effects of investigated SNPs are required. Moreover, a subgroup analysis with regard to additional clinicopathological parameters could not be performed for this population. In conclusion, the IL-4R -3223C>T (rs2057768) polymorphism was correlated with OA susceptibility, mainly for the knee OA, in the Romanian population. The present results provide new evidence for the involvement of the IL-4/IL-4R axis in the OA pathogenesis and additional well-designed large studies are required before final conclusions can be drawn.

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Availability data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

OCR, DC, MI and EB contributed to the conception of the study. OCR, AOD and ES collected the clinical data and samples. MGC, FB, SS and MI performed the experiments. MGC, FB, SS and MI performed the data analyses. OCR, DC, AOD and EB contributed significantly to manuscript preparation. OCR, DC, AOD, FB and EB wrote the manuscript. MI, FB and EB helped perform the analysis with constructive discussions. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of University of Medicine and Pharmacy of Craiova approved this study and a written informed consent was provided by the included participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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