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Original Article

Association of TNF-α-308 G > A and −238G > A polymorphisms with knee osteoarthritis risk: A case-control study and meta-analysis

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A R T I C L E I N F O

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A B S T R A C T

Objective: A comprehensive search on electronic databases was conducted to identify all eligible studies of TNF-α polymorphisms and knee osteoarthritis (OA).
Methods: Eight studies on TNF-α -308 G > A and three on TNF-α -238G > A polymorphism were identified.
Results: Overall, the pooled ORs indicated that neither TNF-α -238G > A nor -238G > A polymorphism was associated with knee OA risk. Similarly, in the stratified analysis by ethnicity, no significant association was found.
Conclusion: This meta-analysis results inconsistent with the previous meta-analyses showed that the TNF-α -308 G > A and −238G > A polymorphisms may not be associated with the susceptibility to knee OA.

1. Introduction

Osteoarthritis (OA) is a progressive and irreversible degenerative condition, that affecting synovial joints, most frequently the knees, hips, hands, and spine. [1–3] OA, one of the most common causes of disability, affects more than 100 million people worldwide.[4,5] Radiological studies revealed that more than 20 million people in the US suffer from knee OA.[5] A wide array of factors including biomechanical, biochemical, and genetic factors predisposition are known to be involved in the list of causative agents of OA. [1,6,7] Researchers continue to search for immunological and genetic clues OA. Patients with OA have high levels of TNF-α in the synovial fluid and it plays an important role in inflammation and joint destruction that are hallmarks of OA.[8]

TNF-α has an extremely broad spectrum of biological activities.[9] It is a cytokine that plays an important role in acute inflammation and immune responses.[10] It is stimulates cytokine production, which enhances the expression of adhesion molecules and activation of neutrophils.[11] Increasing amount of evidence from clinical studies shows that the blood levels of TNF-α correlate with development and progression of different diseases.[12]

The *TNF-α* gene is located on chromosome 6p21.3, within the class III region of HLA, contains four exons, and spans approximately 3 kbp.[13] Several single nucleotide polymorphisms have been identified in the promoter region of human TNF-α gene[14]. Among these, two common polymorphism in the promoter including −308 G > A and −238G > A has been studied intensively. To date, a few studies based on different ethnicities have reported conflicting evidence regarding the association of −308 G > A and −238G > A polymorphisms in TNF-α gene with risk of knee OA. Thus, to summarize more reliable and large-scale evidence on whether TNF-α-308 G > A and −238G > A polymorphisms are associated with knee OA susceptibility we have performed a meta-analysis.

2. Materials and methods

2.1. Case-control study

2.1.1. Study population

The study protocol was approved by the Ethics Committee and all participants signed written informed consent. From January 2014 to September 2017, a total of 110 patients with radiographically confirmed knee OA and, 120 age and sex control subjects who had no

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Table 1
Comparisons of genotypes and alleles frequencies of TNF-α −308 G > A and -238G > A polymorphisms in melanoma cases and controls.

Polymorphism	Cases (n = 110)	Control (n = 120)	OR (95% CI)	p-value
TNF-α-308 G > A				
Genotypes				
GG	79 (71.8)	85 (70.8)	1.00	
AG	30 (27.2)	33 (27.5)	0.989 (0.553–1.766)	0.969
AA	1 (0.9)	2 (1.6)	0.541 (0.048–6.054)	0.618
Allele				
G	203 (92.3)	194 (80.8)	1.00	
A	17 (7.7)	46 (19.2)	0.353 (0.196–0.637)	0.001
TNF-α -238G > A				
Genotypes				
GG	91 (82.7)	105 (87.5)	1.00	
AG	19 (17.3)	14 (11.7)	1.581 (0.750–3.330)	0.228
AA	0 (0.0)	1 (0.8)	0.360 (0.015–8.942)	0.533
Allele				
G	201 (91.4)	224 (93.3)	1.00	
A	19 (8.6)	16 (6.7)	1.323 (0.663–2.643)	0.427

OR: Odds Ratio; CI: Confidence Interval.

symptoms or signs of OA or related disease were recruited to this case-control study. Most of the patients were residing in the central region of Iran and though some of participants were from north western of Iran. The diagnosis of knee OA was based on the criteria of the American College of Rheumatology, which included primary OA with any symptoms and radiographic signs of OA according to the Kellgren–Lawrence (KL) grading system.[15]

2.1.2. Genotyping

Genomic DNA from each participant was isolated from 4 ml EDTA anti-coagulated whole blood using Genomic DNA Mini Kit (Qiagen Inc., Hilden, Germany) based on the instructions of the protocol. Extracted DNA was labeled and suspended in Tris buffer and stored at −20 °C until used. The TNF-α-308 G > A and −238G > A polymorphisms were determined using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP-PCR) as described by before. Primers forward (5′-AGGCAATAGGTTTTGAGGGCCAT-3′) and reverse (5′-TCCTCCCTGCTCCGATTCCG-3′) were used to amplify the 107-bp DNA fragment of the TNFα−308 A > G polymorphism. In addition, to identify the TNF-α -238G > A polymorphism, the following primers were used: forward (5′-AGAAGACCCCCCTCGGAACC-3′) and reverse (5′-ATCTGGAGGAAGCGGTAGTG-3′). All PCR was carried out in 50 µl containing 0.1 µg of DNA, 5 µl of 10x buffer, 5 µl of 50 mM MgCl2, 1 µl of 10 mM dNTPs, 5 µM of each primers, 2.5U Taq DNA polymerase. PCR conditions were 5 min for initial denaturation at 95 °C; 35 cycles at 95 °C for 1 min for denaturation, 30 s at 65 °C for annealing and 30 s at 72 °C for extension, followed by 5 min at 72 °C for final extension. The PCR products for TNF-α −308 G > A and −238G > A polymorphisms was digested with enzyme NcoI (Frementase, Vilnius, Lithuania) and MspI (Roche diagnostics, Swiss) restriction enzymes for 3 h at 37 °C, respectively. The products were electrophoresed on a 3% agarose gel.

2.1.3. Statistical analysis

The distribution of the genotype frequencies of TNF-α −308 G > A and −238G > A polymorphisms for patients and healthy control were compared using the chi-squared test. Moreover, the Odds ratio (OR) with 95% confidence interval (CI) for knee OA susceptibility was also calculated. The distribution of the genotypes in the control population was tested for Hardy–Weinberg equilibrium (HWE) using a goodness-of-fit Chi-square test. All statistical tests were two-sided and were performed with SPSS software version 29 (Chicago Illinois). All comparisons were considered to be statistically significant at $P < 0.05$.

2.2. Meta-analysis

2.2.1. Search strategy

We performed a systematic literature search in PubMed, Embase, ISI Web of Science, Cochrane Library, and Chinese National Knowledge Infrastructure until April 2018. Searching tasks were independently performed by 2 researchers. The search strategy involved the combination of the following keywords: (osteoarthritis OR knee OR knee OA) AND (tumor necrosis factor-alpha OR TNF-α OR TNFSF2 OR Cachectin) AND (−308 G > A OR rs1800629) AND (−238G > A OR rs361525) AND (genetic OR polymorphism OR variant OR mutation). There was no restriction on time period, sample size, population, language, or type of report. The reference lists of relevant articles were also manually searched to acquire additional eligible original articles and to supplement the yield of initial search in the databases.

Table 2
Characteristics of the individual studies included in the meta-analysis.

First Author	Country (Ethnicity)	SOC	Genotyping Method	Case/Control	Cases					Control					MAFs	HWE
					Genotypes			Alleles		Genotypes			Alleles			
TNF-α −308 G > A					GG	AG	AA	G	A	GG	AG	AA	G	A		
Moos 2000[23]	Germany(Caucasian)	HB	RFLP-PCR	55/240	36	18	1	90	20	166	74	0	406	74	0.154	0.004
Sezgin 2008[24]	Turkey(Caucasian)	HB	NS	151/84	121	26	4	268	34	72	12	0	156	12	0.071	0.480
Han 2012[25]	Korea(Asian)	PB	RFLP-PCR	301/291	79	188	34	346	256	258	33	0	549	33	0.056	0.305
Ji 2013[26]	China(Asian)	PB	TaqMan	200/305	143	50	7	336	64	253	50	2	556	54	0.088	0.781
Munoz-Valle 2014[27]	Mexico(Mixed)	PB	RFLP-PCR	50/100	44	6	0	94	6	93	7	0	193	7	0.035	0.716
Vnukov 2016[28]	Russia(Caucasian)	NS	NS	117/94	84	31	2	199	35	65	26	3	156	32	0.170	0.839
Abdel Galil 2017[29]	Egypt(African)	NS	TaqMan	210/210	180	25	2	386	34	115	82	13	314	106	0.257	0.748
Sobhan 2018	Iran(Asian)	PB	RFLP-PCR	110/120	79	30	1	203	17	85	33	2	194	46	0.191	0.909
TNF-α -238 G > A					**GG**	**GA**	**AA**	**G**	**A**	**GG**	**GA**	**AA**	**G**	**A**		
Ji 2013[26]	China(Asian)	PB	TaqMan	200/305	186	14	0	386	14	282	23	0	587	23	0.037	0.493
Munoz-Valle 2014[27]	Mexico(Mixed)	PB	RFLP-PCR	50/100	47	3	0	97	3	89	11	0	189	11	0.055	0.560
Sobhan 2018	Iran(Asian)	PB	RFLP-PCR	110/120	91	19	0	201	19	105	14	1	224	16	0.066	0.493

SOC: Source of Control; HB: Hospital Based; PB: Population Based; NS: Not Stated; PCR-RFLP: Polymerase Chain Reaction-Restriction Fragment Length Polymorphism; MAF: Minor Allele Frequency; HWE: Hardy–Weinberg Equilibrium.