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INTERLEUKIN-16 RS4778889 POLYMORPHISM AND ITS INTERACTION WITH INTERLEUKIN-10 RS1800896 POLYMORPHISM INCREASE THE RISK FOR KNEE OSTEOARTHRITIS IN THE LEBANESE POPULATION

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Abstract

To investigate the effect Interleukin-16 (IL-16) and Interleukin-10 (IL-10) polymorphisms, and their interaction, on knee osteoarthritis (KOA) risk in the Lebanese population. Kompetitive Allele Specific PCR (KASP) genotyping assay was performed to determine IL-16 rs4778889, rs11556218, and rs4072111 and IL-10 rs1800896 polymorphisms in 118 patients diagnosed with KOA (≥ 2 points on Kellgren-Lawrence (K&L) radiological classification scale) and 70 controls matched for age and gender (K&L score ≤ 1). After adjusting for age, gender, presence of metabolic disorders, smoking and drinking status, our findings suggest that rs4778889 TT genotype increases the risk for KOA compared to the combined CC and TC genotypes (OR=2.131, 95% CI 1.037 – 4.379, p = 0.04) and that the T allele increases KOA risk compared to the C allele (OR=1.8, 95% CI 1.008 – 3.212, p = 0.047). No significant associations with the disease risk were found for the other studied polymorphisms (p > 0.05). Our data suggest that there is an interaction between IL-16 rs4778889 and IL-10 rs1800896 (p = 0.010). IL-16 rs4778889 TT genotype increases the risk for KOA only among individuals carrying IL-10 rs1800896 GG or GA genotypes (OR=4.821, 95% CI 1.847 – 12.583). None of the IL-16 haplotypes was associated with KOA risk in our study population (p > 0.05). Our findings suggest that IL-16 rs4778889 T allele is associated with KOA and that there is an interaction between this polymorphism and IL-10 rs1800896 with regard to KOA.

Keywords

Polymorphism, Gene-gene interaction, Interleukin-16, Interleukin-10, Knee Osteoarthritis

1. INTRODUCTION

Osteoarthritis (OA) is a widespread rheumatic disease affecting globally around 303 million individuals in 2017 (GBD 2017 Disease and Injury Incidence and Prevalence Collaborators, 2019). Additionally, knee osteoarthritis (KOA), which is one of the most common sites of the disease, is a major contributor to disabilities worldwide (Cross et al., 2014). Understanding the pathology of the disease and identifying the potential risk factors is of high importance in order to develop preventive strategies against this debilitating disease. Recent data suggest the involvement of low-grade inflammation, with related disturbances in cytokine levels, in the development of OA (Robinson et al., 2016). Among the recently studied cytokines, in relation to KOA, are interleukin-16 (IL-16) and interleukin-10 (IL-10) (Luo et al., 2015; Das Gupta et al., 2017; El-Ali at el., 2021; Imamura et al., 2015; Barker et al., 2021). IL-16 is a pleiotropic cytokine implicated in chemoattraction and modulation of T cell activation (Smith et al., 2009). Previous studies revealed its role in various inflammatory conditions (Lichtenauer et al., 2015; Schernthaner et al., 2017). On the other hand, interleukin-10 (IL-10) inhibits the production of pro-inflammatory mediators (Smith et al., 2009). Many studies have associated elevated serum levels of IL-10 with inflammatory diseases, where it is hypothesized to reduce disease induced inflammation (Peng et al., 2021; Braga et al., 2021). The role of IL-10 and IL-16 in the development of KOA is still unfolding with several studies yielding conflicting results (Luo et al., 2015; Das Gupta et al., 2017; El-Ali at el., 2021; Imamura et al., 2015; Barker et al., 2021). In addition to exploring the independent function of each cytokine in OA development, recent studies are exploring the effect of the balance between various pro-inflammatory and anti-inflammatory cytokines on the risk of developing the disease, and some suggest that the ratio between selected pro-inflammatory and anti-inflammatory cytokines might better predict the risk of OA than the level of each interleukin separately (Barker et al., 2021; Suyasa et al., 2017).

On the other hand, the production of interleukins is affected by various polymorphisms in their corresponding genes. Hence, several authors have investigated the association between polymorphisms in interleukin genes and the risk of developing OA (Shao et al., 2020; Badshah et al., 2021). However, because the balance between pro inflammatory and anti-inflammatory cytokines might better predict the risk of the disease, one can hypothesize that a polymorphism in a single gene coding for a single interleukin cannot fully explain the differences in disease susceptibility between individuals. Accordingly, there is a need to elucidate all the genetic variations involved in the development of KOA as well as the effect of the interaction between these variations on the risk of developing the disease.

Three common single nucleotide polymorphisms (SNPs) were identified in the gene coding for IL-16: rs4778889, rs11556218, and rs4072111. The first one is a located in the promoter region and was previously suggested to affect expression levels, whereas the latter two SNPs are located in the exon region and would result in amino acid substitution (Burkat et al., 2006; Luo et al., 2015;). Additionally, a common SNP in the promoter region of IL-10 coding gene (rs1800896) was associated with altered expression levels (Smith et al., 2009). Several studies have associated these IL-16 and IL-10 SNPs with the risk of various inflammatory diseases including rheumatic diseases (de Souza et al., 2020; Souza et al., 2020; Liu et al., 2018; Braga et al., 2021). However there are only few studies examining their effect on the risk of KOA none of which was conducted in the Lebanese population. Additionally, to the best of our knowledge, the interaction between IL-16 and IL-10 polymorphisms was not examined in relation to KOA previously.

Our study examined the association between each of the IL-10 rs1800896 and IL-16, rs11556218T, rs4778889 and rs4072111 polymorphisms and the risk of KOA in Lebanese population. Furthermore the study assessed the effect of the interaction between these polymorphisms on the risk of KOA.

2. METHODS

2.1 Study Design

Institutional review board at Beirut Arab University (2017H-0032-S-P-0216) has approved this case-control study design. A total of 118 patients diagnosed by radiological evaluation with primary KOA, using Kellgren-Lawrence (K&L) radiological classification scale, were recruited from five health care centers and hospitals in North Lebanon and

classified in the cases group (Kellgren et al., 1957). Only patients who score ≥ 2 points on K&L radiological classification scale were included in this group. Additionally, among the subjects visiting the health centers for regular check-ups during the same period of time, we recruited 70 healthy subjects with no radiological signs of KOA or with doubtful radiographic KOA (K&L score 1) and classified them as controls. Cases and controls were matched for age and gender. The exclusion criteria were the presence of any other types of arthritis, any systemic inflammatory or autoimmune disorder, or previous traumatic knee injury. All participants signed an informed consent before being enrolled in the study. Additionally, all study participants filled a questionnaire including data on demographic characteristics, medical history and lifestyle behaviors.

2.2 Blood Samples, DNA Extraction and Genotyping

Genomic DNA was extracted from the whole blood samples using FlexiGene DNA kit (Cat. No./ID:51206) (Qiagen, Hilden, Germany) according to manufacturer's instructions. Genotyping was performed using KASP (Kompetitive Allele Specific PCR) genotyping assays at LGC group (LGC Biosearch Technologies, Hoddesdon, UK). KASP is a reliable fluorescence-based assay that allows accurate biallelic scoring of SNPs.

2.3 Statistical Analysis

The sample size was calculated based on a previously published study (Bujang et al., 2018). Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA). Goodness-of-fit chi-square test was used to assess Hardy-Weinberg equilibrium. Chi-square test and *t*-test were used to compare the characteristics of knee osteoarthritis patients and controls. Odds ratios (OR) with 95% confidence intervals (CI) were estimated using binary logistic regression to test the association between the genotypes and alleles of interest and the risk of KOA as well as the gene-gene interaction in KOA. Odds ratios were adjusted for age, gender, smoking, alcohol consumption and presence of metabolic diseases (diabetes, hypertension, dyslipidemia, hyperthyroidism, hypothyroidism, osteoporosis, uric acid and kidney disease). Haplotype analysis and the calculation of degree of pairwise linkage disequilibrium were performed using the Haploview software package (version 4.2). Chi-square test was used to compare the distribution of different haplotypes between cases and controls and OR with 95% confidence intervals (CI) were calculated to test the association between different haplotypes and risk of KOA. A two-tailed p-value ≤ 0.05 was deemed statistically significant in all analyses.

3. RESULTS

Genotype frequencies were in Hardy Weinberg equilibrium for all studied polymorphisms.

The characteristics of study participants are summarized in Table 1. Metabolic disorders were more frequent among cases than controls ($p < 0.001$). No significant differences for gender, mean age, smoking and drinking status between cases and controls were detected ($p > 0.05$).

Table 1: Characteristics of study participants

Variables	Cases	Controls	P-value
Age (mean±SD §)	60.06±10.55	57.71±7.03	0.1
Gender (N=188)			
Male N(%)	32 (27.1%)	14 (20%)	0.297
Female N(%)	86 (72.9%)	56 (80%)	
Smoking (N=187)			
Yes	52 (44.4%)	38 (54.3%)	0.227
No	65 (55.6%)	32 (45.7%)	
Alcohol consumption (N=187)			
Yes	2 (1.7%)	6 (8.6%)	0.054
No	115 (98.3%)	64 (91.4%)	
Presence of metabolic diseases (N=188)			
Yes	77 (65.3%)	27 (38.6%)	<0.001*
No	41 (34.7%)	43 61.4%)	

§ SD: Standard deviation * p-value ≤ 0.05

For rs4778889, among controls, the frequency of TT genotype was 54.1% versus 45.9% for the combined CC and TC genotypes under the dominant model. On the other hand, the frequency of TT genotype was 67.9% and that of the combined CC and TC genotypes was 32.1% among cases. After adjusting for age, gender, presence of metabolic disorders, smoking and alcohol consumption, the TT genotype showed an increased risk for developing KOA compared to the combined CC and TC genotypes (OR, 2.131; 95% CI, 1.037 – 4.379, p = 0.04). Additionally, in controls, the frequency of the T allele was 26.2% versus 73.8% for the C allele and in cases, the frequency of the T allele was 18.3% versus 81.7% for the C allele. The T allele increased the risk of KOA compared to the C allele (OR, 1.8; 95% CI, 1.008 – 3.212, p = 0.047) after adjusting for age, gender, presence of metabolic disorders, smoking and alcohol consumption. No significant difference in the risk of developing KOA was observed between the various genotypes and alleles of IL 10 rs1800896, IL-16 rs11556218 and IL-16 rs4072111 (p > 0.05) (As indicated in Table 2).

Table 2: Association between selected IL-10 and IL-16 polymorphisms and the risk of knee osteoarthritis

	Controls n (%)	Cases n(%)	OR § (95% CI) §§	p-value
IL-10 rs1800896 (n=171)				
GG	11(19%)	21(18.6%)	0.987 (0.38 – 2.561)	0.978
AA	21 (36.2%)	38 (33.6%)	0.792 (0.364 – 1.724)	0.556
GA	26 (44.8%)	54 (47.8%)	1	
Dominant model				
AA	21 (36.2%)	38 (33.6%)	1	
GG+GA	37 (63.8%)	75 (66.4%)	1.258 (0.609 – 2.598)	0.535
Recessive model				
GG	11 (19%)	21 (18.6%)	1	
AA+GA	47(81%)	92 (81.4%)	0.957 (0.613 – 1.492)	0.845
Alleles				
G	48(41.4%)	96 (42.5%)	1	
A	68 (58.6%)	130 (57.5%)	0.875 (0.533 – 1.435)	0.597
IL-16 rs11556218 (n=173)				
TT	54 (90%)	100 (88.5%)	1	
TG	6 (10%)	13 (11.5%)	1.224 (0.7 – 2.138)	0.478
Alleles				
T	114 (95%)	213 (94.2%)	1	
G	6 (5%)	13 (5.8%)	1.437 (0.499 – 4.140)	0.502
IL-16 rs4778889 (n=173)				
CC	4 (6.6%)	5 (4.5%)	1	
TT	33 (54.1%)	76 (67.9%)	2.352 (0.526 -10.515)	0.263
CT	24 (39.3%)	31 (27.7%)	1.122 (0.243 – 5.175)	0.883
Dominant Model				
TT	33(54.1%)	76 (67.9%)	2.131 (1.037 – 4.379)	0.040*
CC+TC	28 (45.9%)	36 (32.1%)	1	
Recessive model				
CC	4 (6.6%)	4 (3.6%)	1	
TT + TC	57 (93.4%)	108 (96.4%)	1.888 (0.421 – 8.459)	0.406
Alleles				
C	32 (26.2%)	41 (18.3%)	1	
T	90 (73.8%)	183 (81.7%)	1.8 (1.008 – 3.212)	0.047*
IL-16 rs4072111 (n=169)				
CC	53 (88.3%)	97 (89%)	1	
CT	7 (11.7%)	12 (11%)	0.846 (0.495 – 1.445)	0.541
Alleles				
C	113 (94.2%)	206 (94.5%)	1	
T	7 (5.8%)	12 (5.5%)	0.734 (0.262 – 2.052)	0.555

§ OR odds ratio; §§ CI confidence interval; OR are adjusted for age, gender, presence of metabolic disorders, smoking and alcohol consumption; * p-value ≤ 0.05

On the other hand, an interaction between IL-10 rs1800896 and IL-16 rs4778889 genotypes affects the risk of developing KOA (p = 0.010). Individuals with IL-16 rs4778889 TT genotype showed 4.8 times higher risk of developing KOA only among carriers of IL-10 rs1800896 GG or GA genotypes (OR, 4.821; 95% CI, 1.847 – 12.583) (As indicated in Table 3).

Table 3: Interaction between IL-10 rs1800896 and IL-16 rs4778889 in relation to knee osteoarthritis

		OR § (95% CI) §§	P-value
IL10 rs1800896	IL-16 rs4778889		0.010*
AA	TT	0.701 (0.188 – 2.605)	
	CC + TC	1	
GG + GA	TT	4.821 (1.847 – 12.583)	
	CC + TC	1	

§ OR odds ratio; §§ CI confidence interval; OR are adjusted for age, gender, presence of metabolic disorders, smoking and alcohol consumption; * p-value ≤ 0.05

There is a strong evidence of linkage disequilibrium between rs11556218 and rs4778889 (D'=1, r^2=0.208). The SNPs of interest in IL16 gene composed five haplotypes, none of which was associated with an increased risk of KOA (p > 0.05) (As indicated in Table 4).

Table 4: Association of IL-16 haplotypes with knee osteoarthritis

Haplotype	rs4072111	rs4778889	rs11556218	Total frequency	OR § (95% CI) §§	p-value
CTT	C	T	T	0.751	1	-
CCT	C	C	T	0.154	0.5838 (0.3207 – 1.0627)	0.0782
CCG	C	C	G	0.039	1.2079 (0.3683 – 3.9615)	0.7553
TTT	T	T	T	0.036	1.0871 (0.3256 – 3.6295)	0.8920
TCG	T	C	G	0.015	0.9663 (0.1736 – 5.3791)	0.9688

*§ OR odds ratio; §§ CI confidence interval; * p-value ≤ 0.05*

4. DISCUSSION

Our study aimed at investigating the relationship between each of three IL-16 and one IL-10 polymorphisms and the risk of KOA in the Lebanese population. We found an association between the T allele in IL-16 rs4778889 and the increased risk of developing KOA. Moreover, we found that the interaction between IL-10 rs1800896 and IL-16 rs4778889 can influence the risk of developing KOA although IL-10 rs1800896 did not show an association with KOA in our study population. IL-16 can stimulate the secretion of various inflammatory cytokines including TNF-α and IL-6 that have been implicated in the development of KOA (Mathy et al., 2000; Mabey et al., 2016; Min et al., 2017). Data concerning the role of IL-16 in KOA is still controversial. Luo et al., found higher circulating levels of IL-16 in KOA patients compared to controls (Luo et al., 2015). However, these results were not confirmed by subsequent studies (das Gupta et al., 2017; El-Ali et al., 2021). These conflicting results might be due to the different genetic backgrounds of the studied populations which might influence the secretion levels of IL-16 and subsequently its role in KOA. Therefore, one can hypothesize that IL-16 polymorphisms can potentially be associated with the risk of developing KOA. Previous studies have associated these polymorphisms with various inflammatory diseases including cancer, cardiovascular diseases and periodontitis (de Souza et al., 2020; Souza et al., 2020). Our results on IL-16 rs4778889 polymorphism suggest that individuals carrying the TT genotype have an increased risk of KOA compared to the combined

CC and TC genotypes and that the T allele increases the risk for KOA compared to the C allele. These results conform to the results of a previously published study by Luo et al. on the Chinese population. Yet, Liu et al. could not find an association between this polymorphism and KOA, despite the fact that both studies were done on the same population (Luo et al., 2015; Liu et al., 2015).

On the other hand, we were not able to find an association between IL-16 rs11556218 and rs4072111 polymorphisms and KOA as in the previous studies. Liu et al., 2015 showed that rs11556218 TG genotype decreases the risk for KOA compared to the TT genotype and rs4072111 CT genotype increases the risk for KOA compared to the CC genotype (Liu et al., 2015). On the other hand, Luo et al., 2015 revealed a decreased risk in the carriers of the rs11556218 G allele compared to the carriers of the T allele and a decreased risk in the carriers of the rs4072111 T allele compared to the carriers of the C allele (Luo et al., 2015). In our total sample, the frequency of the rs11556218 G allele was very low compared to the T allele (G allele: 5.5% and T allele: 94.5%) and the frequency of the rs4072111 T allele was also very low compared to the C allele (T allele: 5.6% and C allele: 94.4%). Hence, these markers were uninformative in the Lebanese population, which might explain the inconclusive results of their association with KOA. This also might justify the absence of association between the various IL-16 haplotypes and KOA in our study compared to previous studies (Luo et al., 2015; Liu et al., 2015). Therefore, further studies with larger sample size from Lebanon are needed to study this association.

IL-10 s1800896 was previously associated with various inflammatory diseases including ankylosing spondylitis and rheumatoid arthritis, however, our results and the existing body of literature show no association between this SNP and the risk of OA (Braga et al., 2021, Liu et al., 2018; Hämäläinen et al., 2014). IL-10 is known inhibit the production of proinflammatory mediators (Smith et al., 2009). Two previous studies revealed higher circulating levels of IL-10 in KOA patients whereas a recent study found that IL-10 levels were lower among patient with severe KOA compared to patients with moderate KOA (Imamura et al., 2015; El-Ali at el., 2021; Barker et al., 2021). The effect of the balance between various pro-inflammatory and anti-inflammatory cytokines on the risk of the disease might be a possible explanation for these conflicting results. Indeed, two studies that have examined IL-10/TNF-α and IL-6/IL-10 ratios in relation to OA yielded interesting results (Barker et al., 2021; Suyasa et al., 2017). Subsequently, one can speculate that interaction between various polymorphisms in genes coding for pro-inflammatory and anti-inflammatory cytokines might better predict the risk of OA than each polymorphism separately. In this regard, an earlier study revealed that IL-10 rs1800896 is not associated with the risk of hand OA, but the interaction between this SNP and TNFα rs1799964 or TNFα rs1800630 can affect the risk of the disease (Hämäläinen et al., 2014). Similarly, our results show no association between IL-10 rs1800896 and KOA, however there is a significant effect of the interaction between this SNP and the IL-16 rs4778889 on the risk of developing the disease. It is possible that these polymorphisms are affecting the balance between IL-10 and IL-16 cytokine levels, which might be influencing the risk of KOA knowing that these SNPs were previously associated with alteration in IL-16 and IL-10 expression levels (Burkat et al., 2006; Smith et al., 2009). However, it is worth noting that although Luo et al. associated the studied IL-16 polymorphisms with KOA and found higher circulating levels of IL-16 in KOA patients compared to controls, they couldn't find a relationship between these polymorphisms and circulating levels of IL-16 among cases and controls (Luo et al., 2015). Therefore, further studies would certify how exactly these SNPs are affecting the transcription levels of the corresponding proteins in KOA patients and subsequently how they are affecting IL-10/IL-16 ratios, elucidating the effect of the balance between these interleukins and the risk of KOA.

5. CONCLUSION

In conclusion, our study showed an association between IL-16 rs4778889 and KOA, as the T allele increases the risk to develop the disease compared to the C allele. Moreover, our results suggest an interaction between IL-16 rs4778889 and IL-10 rs1800896 genotypes in relation with the disease. Further studies, with larger sample size collected from various regions in Lebanon are needed in order to confirm our results. Our findings, can be used in the development of preventive strategies as they can be used to identify individuals at risk of KOA.

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