Egyptian Rheumatology and Rehabilitation

RESEARCH Open Access

Genetic polymorphisms of interleukin-16 in Egyptian patients with primary knee osteoarthritis

Eman Abdel Razek Hafez[1*], Reham Magdi Shaat[1], Ola Mohamed Gharbia[1], Shereen Aly Machaly[1], Ola Ali El- Emam[2], Nermin Youssef Abo El –Kheir[2], Narmin Saied[2], Alaa Abo Nour[2], Sherif Elkhanishy[3], Rasha Hassan[4] and Heba El Shehawy[2]

Abstract

Background The pro-inflammatory cytokine, interleukin 16 (IL-16), has been shown to be secreted in low levels in knee osteoarthritis (KOA). The aim of the study was to examine the relationship between IL-16 polymorphisms and the risk of KOA in the Egyptian population, as well as the clinical and radiographic severity of KOA.

Results IL16 rs11556218 thymidine triphosphate (T) T G (guanosine triphosphate), GG, TG + GG genotypes, and G allele (odd ratio (OR) = 0.315; 95% confidence interval (CI) = 0.191–0.518; $P < 0.001$; OR = 0.363; 95% CI = 0.162–0.815, $P = 0.014$; OR = 0.323; 95% CI = 0.202–0.519, $P < 0.001$; OR = 0.480; 95% CI = 0.338–0.683, $P < 0.001$ respectively); rs4778889 cytidine triphosphate (C) T,CC, TC + CC genotypes, and C allele (OR = 0.519, 95% CI = 0.319–0.844, $P = 0.008$; OR = 0.309, 95% CI = 0.105–0.916, $P = 0.034$; OR = 0.485, 95% CI = 0.304–0.775, $P = 0.002$; OR = 0.537, 95% CI = 0.365–0.791, $P = 0.001$ respectively); and rs4072111 CT, TT, CT + TT genotypes, and T allele (OR = 0.537, 95% CI = 0.323–0.893, $P = 0.017$, OR = 0.316, 95% CI = 0.096–0.843, $P = 0.049$, OR = 0.502, 95% CI = 0.309–0.816, $P = 0.005$; OR = 0.534, 95% CI = 0.353–0.809, $P = 0.004$ respectively) were associated with a decreased KOA risk, and they were significantly associated with decreased the Western Ontario and McMaster Universities Arthritis Index (WOMAC) and the Kellgren-Lawrence (K/L) scores.

Neither IL-16 serum levels nor IL-16 polymorphisms were associated with the susceptibility to KOA. Low KOA risk was associated with the haplotypes GTC and TCT.

Conclusion There was no correlation between serum IL-16 levels and KOA susceptibility or IL-16 polymorphisms. GTC and TCT haplotypes were associated with low KOA risk. The variant alleles rs11556218GG, TG + GG; rs4778889 CC, TC + CC; and rs4072111 TT, CT + TT were associated with a reduced risk of KOA.

Keywords Knee osteoarthritis, IL16, Single nucleotide polymorphism

*Correspondence:
Eman Abdel Razek Hafez
emanhafez@mans.edu.eg; emanhafez22@gmail.com
[1] Department of Rheumatology and Rehabilitation, Faculty of Medicine, Mansoura University, Mansoura, Egypt
[2] Department of Clinical Pathology, Faculty of Medicine, Mansoura University, Mansoura, Egypt
[3] Department of Forensic and Toxicology, Faculty of Medicine, Mansoura University, Mansoura, Egypt
[4] Department of Microbiology, Faculty of Medicine, Mansoura University, Mansoura, Egypt

Background

KOA is a complex disease that affects nearly 10% of individuals aged 55 years old or more, with the characteristic degradation of articular cartilage, often ending as joints disability [1]. OA has many associated risk factors, including age, obesity, trauma, diet, smoking habits, and hormone therapy [2–4]. The pathogenicity of OA is still unrecognized and requires more explanation.

Pathogenesis of synovitis and cartilage destruction related to OA can be attributed to the role of inflammatory cytokines [5, 6]. Individual variability of cytokine concentrations can explain the susceptibility variations and disease severity which in turn are basically attributed to single nucleotide polymorphisms (SNPs) in cytokine encoding genes [7].

Pro-inflammatory cytokines, including TNF, IL-1β, IL-6, IL-8, IL-15, IL-17, IL-18, and IL-21, were previously involved in OA pathophysiology [8]. For example, in a study in 2019, IL-15 has been shown to be related to the severity of KOA [9]. In addition, another study investigated the level of IL 33 in RA in comparison with OA but concluded that there was no relation between IL33 level and OA [10]. IL-16 had not been considered to be involved in OA until 2015, when two Chinese papers reported that some IL-16 gene polymorphisms had an impact on KOA susceptibility [11, 12].

IL-16 is a cytokine having a pro-inflammatory features including chemoattraction and modulation of T cell activation [13] and is a crucial mediator in inflammatory and autoimmune diseases in addition to growth and progression of tumor [14, 15]. The IL-16 gene is located on chromosome 15q26.3 [16] and is initially translated into a precursor protein consisting of 631 amino acids, which is cleaved by caspase-3 to make the active C-terminal domain containing 121 amino acids [17, 18].

IL-16 is a CD4-specific ligand. Thus, it selectively activates CD4 + T cells, macrophages, monocytes, and eosinophils by binding with the molecule CD4 [12]. In addition, IL-16 can increase the production of inflammatory cytokines, such as TNF-α, IL-1β, IL-6, and IL-15, leading to inflammatory response [19, 20].

Many IL-16 gene SNPs have been thoroughly studied. A prevalent SNP in IL-16 gene is rs4778889 T/C, situated 295 bp upstream from the start site of transcription and linked with modified levels of gene expression. Two additionally SNPs, rs11556218 T/G and rs4072111 C/T, are situated in an exon region, and their single-nucleotide changes would result in an amino acid replacement; the first leads to an asparagine (Asn) to lysine (Lys) replacement in exon 6 of the IL-16 gene, and the second results in a serine (Ser) to proline (Pro) replacement [21].

IL16 has been identified to be secreted from OA synovial fibroblasts at low concentrations [22]. For that and during the OA inflammatory process, IL-16 might be a prospective mediator. Since the IL-16 production could be genetically controlled, it is reasonable to hypothesize a potential relationship between IL-16 gene polymorphisms and KOA risk.

The objective of this research was to investigate the association of IL-16 polymorphisms with susceptibility to KOA and the impact of SNPs on IL16 serum levels as well as on clinical and radiologic severity in patient with KOA in the Egyptian population.

Methods

Study subjects

This is a case–control study conducted on 150 patients with the diagnosis of primary KOA consecutively selected from the Rheumatology and Rehabilitation outpatient clinic at the Mansoura University hospital, between March 2016 and April 2017, and 150 controls selected from healthy volunteers without evidence of OA, visiting the same hospital for regular check-up. Approval of the study by the institutional research board, Mansoura University, Faculty of Medicine, was received (code R/16.05.39). A written informed consent was obtained from all participants.

sInclusion criteria

The diagnosis of KOA was assessed according to the American College of Rheumatology clinical criteria (clinical and radiographic) [23].

Exclusion criteria

Autoimmune or systemic inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus, spondyloarthropathies, gouty arthritis, and septic arthritis, patients with previous traumatic knee injury, and all secondary KOA were excluded from the study.

Data collection and clinical examination

Baseline clinical data was achieved through interviewing the included participants, demographic characteristics, history of associated medical conditions, review of systems, and history of knee trauma or surgery. Full general and local musculoskeletal examination with stress on local knee examination for diagnosis and assessment of KOA was performed. Weight (kg) measurement was performed on a calibrated scale, and a stadiometer was used to measure standing height. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2).

Evaluation of OA

For the evaluation of pain, stiffness, and function from the Western Ontario and Mc-Master University (WOMAC), OA index scoring was used in patients with OA [24]. Total WOMAC is the sum of the results of the three subscales. WOMAC of high rating indicates more pain and stiffness and serious restriction of function.

Laboratory assessment

DNA extraction and measurement [11]

For analyzing DNA, a peripheral 2 mL of venous blood was obtained from each participant, and an anticoagulant (EDTA-Na2) was added; the sample was stored at – 20 °C. DNA extraction was done by QIAGEN kit for extraction, and the OD 260/280 ratio was measured by spectrophotometer. Good DNA purity could be considered with an OD 260/280 ratio between 116 and 210, with the inclusion of the sample in the study. A temperature of – 70 °C was used for DNA storage.

Primer design and PCR amplification [25]

Primer sequences for each SNP were as follows: rs4778889T/C: 5′-CCATGTCAAAACGGTAGCCTCAAGC-3′ and 5′-CTCCACA CTCAAAGCCTTTTGTTCCTATGA-3′ rs4072111C/T: 5′-TTCAGGTAC-AAACC CAGCCAGC-3′ and 5′-CAC TGTG ATC CCGGTC CAGTC-3′ rs11556218T/G: 5′-TGTGA-CAATCACAG CTTGCCTG-3′ and 5′-GCTCAGGTTC-ACAGAGTGTTT CCATA-3′

A volume of 25 µL was used in PCR amplification reactions. The total volume contains 1.0 µL of template DNA, 12.5 µL hot start master mix, 1.0 µL of forward primers, 1.0 µL of reverse primer, and 11.3 µL ionized water.

The conditions of polymerase chain reaction-restriction fragment length (PCR) reaction at rs11556218T/G were as follows: denaturation of samples was at 95 °C for 5 min, then its procession for 30 denaturation cycles at 95 °C for 45 s, annealing at 60 °C for 45 s and its extension at 72 °C for 1 min, and ending with a final cycle of extension at 72 °C for 5 min. The rs4072111C/T and rs4778-889 T/C annealing temperatures were 67 °C and 63 °C, respectively.

Restriction digestion and gel electrophoresis [26]

Restriction endonucleases *BsmA*I, *Nde*I, and *Ahd*I were used for rs4072111C/T, rs11-556218 T/G, and rs4778889T/C, respectively. 1.2 µL of the restriction endonuclease was required for the digestion of 10 µL of PCR amplification product, and each product was treated in a 37° C water bath for 16 h. The resultant product was subjected to electrophoresis through running on 2% agarose gel and then imaged. For genotype verification, Generay Biotech sequenced the amplified and digested DNA products.

Serum IL-16 levels [27]

Serum samples were collected from both patients and healthy controls. After blood sampling, clotting of the serum was allowed for 30 min at 4 °C before being centrifugated at 3000 rpm for 10 min at 4 °C. Isolation and storage of the total serum was carried out at – 20 °C until its further use. For detection of serum IL-16 concentrations, a sandwich enzyme-linked immunosorbent assay (ELISA) was used. ELISA with the same batch of reagents was used according to the manufacturer's instructions. The minimum concentration of detection for IL-16 was 5 pg/mL with 10% intra-assay coefficients of variation.

Radiological assessment

Plain radiographs of both knees were obtained for every patient in a semiflexed weight bearing anteroposterior and lateral radiographs views. For grading of OA, the Kellgren-Lawrence (KL) score was used [28].

Sample size consideration

For the evaluation of the statistical power of the sample, the CaTS software (Center for Statistical Genetics, University of Michigan [29]) was used. The power of the sample size was calculated to be 80% regarding the next circumstances: 150 patients, 150 healthy controls, the prevalence of disorder is 12%, average allelic frequency of 39.7%, significance level of 0.05.

Statistical analysis

Collected data was reviewed, coded, tabulated, and introduced to a PC using the Statistical Package for the Social Sciences (IBM SPSS Version 20.0.). Data was presented and analyzed according to the nature of data obtained for each parameter. For parametric numerical data, mean and standard deviation ($\pm$SD) were selected, while median and range were used for non-parametric numerical data and frequency and percentage of non-numerical data. Normality of data distribution was tested by using the Kolmogorov–Smirnov test. The Student T test was carried out for assessing the statistical significance of the mean difference between two study groups. The statistical significance of the difference of a non-parametric variable between two study groups was assessed by Mann–Whitney test (U test). For comparison between more than two study group ordinal variables, the Kruskal–Wallis test was used to assess the statistical significance. Chi-square test was carried out for examining the relationship between two qualitative variables. Significance of the results obtained was judged at $P \leq 0.05$. Fisher's exact test was used when the expected count is less than 5 in more than 20% of cells for examining the relationship between two qualitative variables. Deviations from Hardy–Weinberg equilibrium expectations were determined using the chi-square test. Odds ratio and 95% confidence interval were calculated. Linear regression analysis was conducted for prediction of confounders. The HaploView program (version 4.2) was applied for the estimation of the haplotypes

and linkage disequilibrium (LD), through using the expectation maximization (EM) algorithm. The code of colors in the HaploView plot follows the standard color scheme for HaploView: white, $|D'|<1$, LOD < 2; shades of pink/red, $|D'|<1$, LOD ≥ 2; blue, $|D'|=1$, LOD < 2; red, $|D'|=1$, LOD ≥ 2.

NB. (Logarithm of likelihood odds ratio [LOD]; (a measure of confidence in the value of D′).

Results

Demographic data, IL16, WOMAC, and K/L score of KOA patients are shown in Table 1. The mean age of OA cases was 49.5 years; they were 19 males and 131 females. Healthy controls had younger age and matched gender and BMI compared to OA cases. The serum level of IL16 was insignificantly different between KOA cases and healthy controls. The median WOMAC was 60; median K/L score was 2.

The comparison of IL16 SNPs between KOA patients and the control group is presented in Table 2. This sample of participants was randomly selected from a population in Dakahleya Governorate in lower Delta, Egypt. Applying Hardy–Weinberg equation indicated that rs11556218, rs4778889, and rs4072111 genotypes in the control group as well as in the case group were in the Hardy–Weinberg equilibrium. rs11556218 TG, GG, TG + GG genotypes, and G allele; rs4778889 CT, CC, CT + CC genotypes, and C alleles; and rs4072111 CT, TT, CT + TT genotypes, and T allele showed significant lower frequency in KOA when compared to the control groups, with significantly protective effect against OA development among healthy control subjects.

A comparison between rs11556218, rs4778889, and rs4072111 haplotypes in OA cases and controls is shown in Table 3. We performed the current analyses with data derived from the chromosomal region 15q25.1 on chromosome 15. The application of the HaploView program (version 4.2) was performed to estimate the haplotypes and linkage disequilibrium (LD), which uses the expectation maximization (EM) algorithm. TTC haplotype showed highest frequency, while TCC showed lowest frequency in cases and control. Taking TTC haplotype as a reference, GTC and TCT haplotypes showed significantly lower frequency in OA patients when compared to the control group, with significantly protective effect against OA development within healthy control subjects.

The association between rs11556218 genotypes with clinical and radiographic features of KOA patients is presented in Table 4.

rs11556218GG, TG + GG; rs4778889 CC, TC + CC; and rs4072111 TT, CT + TT genotypes were significantly associated with lower WOMAC and Kellgren. Serum IL16 did not differ significantly between different genotypes.

Regression analysis was conducted for prediction of factors affecting IL16 level, using age, gender, and BMI as confounders. None was associated with prediction of IL16 level in Table 5.

Linkage disequilibrium (LD) between rs11556218 and rs4778889 in the control was 40%; in OA, it was 78%; LD between rs11556218 and rs4072111 in the control was 19%; in OA, it was 69%; LD between rs4778889 and rs4072111 in the control was 1%; in OA, it was 67% (Fig. 1).

Discussion

Different inflammatory elements are engaged in OA pathologically. In OA, there is a loss of enhanced chondrocyte anti-inflammatory and synthetic activity due to enhanced degrading activity [30, 31]. The enhanced synthetic activity is restricted to the far deep

Table 1 Demographic data, IL16, and clinical and radiological features in OA cases and control groups

Data		Groups		
		Control ***N*** **= 150**	**Cases** ***N*** **= 150**	***P***
Age	Mean ± SD	39.6 ± 8.7	49.5 ± 9.1	***< 0.001**
Sex				
Males	***N* (%)**	20 (13.3%)	19 (12.7%)	0.864
Females	***N* (%)**	130 (86.7%)	131 (87.3%)	
BMI (kg/m^2)	Mean ± SD	31.2 ± 4.7	31.8 ± 4.8	0.327
IL16 (pg/mL)	Median (range)	213.5 (29.3–455.7)	200.5 (23.7–484.5)	0.147
WOMAC	Median (range)	-	60 (8–96)	-
K/L score	Median (range)	-	2 (1–4)	-

BMI Body mass index, *WOMAC* Western Ontario and McMaster Universities Arthritis Index, *K/L score* Kellgren-Lawrence

* Significance $P \leq 0.05$

Table 2 Comparison of IL16 SNPs between OA patients and controls

Genotype		Groups						
		Control *N* = 150		Cases *N* = 150		*P*	OR	95% CI
		N	%	*N*	%			
rs11556218	***TT***	49	32.7	90	60.0	-	1	**(reference)**
	TG	83	55.3	48	32.0	***< 0.001**	0.315	0.191–0.518
	GG	18	12.0	12	8.0	***0.014**	0.363	0.162–0.815
	TG + GG	101	67.3	60	40.0	***< 0.001**	0.323	0.202–0.519
	T	181	60.3	228	76.0	-	1	**(reference)**
	G	119	39.7	72	24.0	***< 0.001**	0.480	0.338–0.683
	HW p	0.056		0.133				
rs4778889	***TT***	75	50.0	101	67.3	-	1	**(reference)**
	CT	63	42.0	44	29.3	***0.008**	0.519	0.319–0.844
	CC	12	8.0	5	3.3	***0.034**	0.309	0.105–0.916
	TC + CC	75	50.0	49	32.7	***0.002**	0.485	0.304–0.775
	T	213	71.0	246	82.0	-	1	**(reference)**
	C	87	29.0	54	18.0	***0.001**	0.537	0.365–0.791
	HW p	0.807		0.938				
rs4072111	***CC***	87	58.0	110	73.3	-	1	**(reference)**
	CT	53	35.3	36	24.0	***0.017**	0.537	0.323–0.893
	TT	10	6.7	4	2.7	***0.049**	0.316	0.096–0.843
	CT + TT	63	42.0	40	26.7	***0.005**	0.502	0.309–0.816
	C	227	75.7	256	85.3	-	1	**(reference)**
	T	73	24.3	44	14.7	***0.004**	0.534	0.353–0.809
	HW p	0.619		0.614				

HW p Hardy–Weinberg *p* value, *OR* Odds ratio, *CI* Confidence interval

* Significance $P \leq 0.05$

Table 3 Analysis of rs11556218, rs4778889, and rs4072111 haplotypes in OA cases and controls

Haplotype	rs11556218	rs4778889	rs4072111	Control	Cases	*p*	OR	95% CI
TTC	T	**T**	**C**	0.401	0.688	-	1	**(reference)**
GTC	G	**T**	**C**	0.267	0.092	***< 0.001**	0.282	0.177–0.449
TCT	T	**C**	**T**	0.155	0.044	***< 0.001**	0.250	0.132–0.474
GCT	G	**C**	**T**	0.047	0.062	0.398	1.381	0.679–2.809
GCC	G	**C**	**C**	0.046	0.058	0.519	1.227	0.594–2.537
GTT	G	**T**	**T**	0.037	0.028	0.558	0.810	0.331–1.983
TCC	T	**C**	**C**	0.043	0.016	*0.051	0.374	0.132–1.063

OR Odds ratio, *CI* Confidence interval

* Significance $P \leq 0.05$

layers of the cartilage, allowing the degradation imbalance to continue near the synovial border in the deep layer. In the end, malfunction and apoptosis of chondrocyte restrict the capacity for reaction and accelerate OA progression [30, 31].

Therefore, we postulated that the susceptibility to OA may be modulated by IL-16 polymorphisms.

In our study, there were no significant differences between OA cases and healthy controls as regards sex and BMI, but the mean age was significantly older in OA cases ($P < 0.001$). However, none of these factors was associated with prediction of IL16 level as demonstrated by multivariate regression analysis. This makes the relation between OA and IL16 more reliable.

Table 4 Association of IL16 level, WOMAC, and K/L score with IL16 genotypes

Genotypes		Variables					
		IL16		WOMAC		Kellgren	
		Median	Range	Median	Range	Median	Range
rs11556218	**TT (*N* = 90)**	210.8	55.3–484.5	71	53–96	3	2–4
	TG (*N* = 48)	155.9	23.7–418.4	44	30–53	2	1–2
	GG (*N* = 12)	274	55.7–460.4	25	8–30	1	1–1
	TG + GG (*N* = 60)	155.9	23.7–460.4	43	8–53	2	1–2
	***P*[1]**	0.328		***** **< 0.001**		***** **< 0.001**	
	***P*[2]**	0.338		***** **< 0.001**		***** **< 0.001**	
rs4778889	**TT (*N* = 101)**	207	23.7–484.5	69	8–96	2.5	11–4
	TC (*N* = 44)	189.1	29.6–424.4	43	22–56	2	1–2
	CC (*N* = 5)	291.9	96.9–460.4	21	8–53	1	1–2
	TC + CC (*N* = 49)	194	29.6–460.4	43	8–56	2	1–2
	***P*[3]**	0.593		***** **< 0.001**		***** **< 0.001**	
	***P*[4]**	0.890		***** **< 0.001**		***** **< 0.001**	
rs4072111	**CC(*N* = 110)**	203.7	23.7–484.5	67.5	29–96	2.5	1–4
	CT(*N* = 36)	227.1	29.6–424.4	36.5	19–56	2	1–2
	TT(*N* = 4)	123.9	96.9–150.8	30.5	8–53	1.5	1–2
	CT + TT(*N* = 40)	155.9	29.6–424.4	36.5	8–56	2	1–2
	***P*[5]**	0.298		***** **< 0.001**		***** **< 0.001**	
	***P*[6]**	0.971		***** **< 0.001**		***** **< 0.001**	

P[1] comparison between rs11556218 TT, TG, GG, *P*[2] comparison between rs11556218 TG + GG and TT, *P*[3] comparison between rs4778889 TT, TC, CC, *P*[4] comparison between rs4778889 TC + CC and TT, *P*[5] comparison between rs4072111 CC, CT, TT, *P*[6] comparison between CT + TT and CC

* Significance $P \leq 0.05$

Table 5 Regression analysis for prediction of factors affecting IL16 level

Variables	*B*	*P*
Age	1.426	0.141
Sex	3.500	0.521
BMI	1.586	0.289

B regression coefficient

* Significance $P \leq 0.05$

In this study, the prevalent IL-16 SNP namely rs4778889 and two other SNPs (rs4072111 and rs11556218) were chosen for the assessment of their association with KOA patients and healthy controls.

Our study revealed that participants carrying the rs11556218 G allele showed lower risk to KOA development in comparison with those carrying T allele. Also, the participants carrying rs4778889 C allele were less prone to develop OA than those carrying T

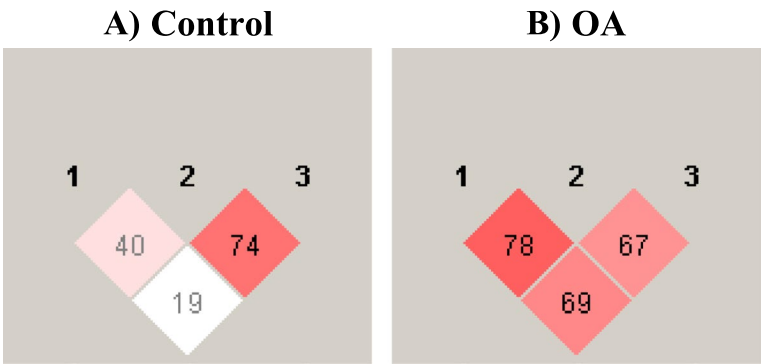

Fig. 1 Linkage disequilibrium between (1) rs11556218, (2) rs4778889, and (3) rs4072111 in **A** control and **B** OA groups

allele. Individuals carrying rs4072111 T allele were less prone to develop KOA than those carrying C allele. These results goes with the study of Luo and his colleagues [12], who studied the same genes in a Chinese population.

In contrast to our study, Liu et al. [11] reported an association between rs4072111 CT and the increased risk of OA, but rs11556218 TC genotype was associated with reduced risk. This discrepancy could be explained by the differences in ethnic origins of the studied populations, the presence of other SNPs within IL-16 gene associated with the susceptibility to OA, or variable exposure to other risk factors, such as tobacco smoking and heavy drinking in the former two studies.

A haplotype is recognized as a collection of SNPs on a single chromatid that is probably considered inherited together more frequently than previously proposed by chance in a block pattern due to existing linkage disorder [32].

This study revealed that there is a significant association between GTC and TCT haplotypes and the reduced risk of KOA.

In contrast to our study, Luo et al. [12] found that GCC and TTT haplotypes were significantly associated with elevated risk of KOA. Also, Liu and his colleagues [11] found that TTT haplotype was associated with increased risk, while the GCC haplotype was associated with decreased risk of KOA.

When we studied linkage disequilibrium, there was LD between rs11556218 and rs4778889 which was higher in KOA patients; LD between rs11556218 and rs4072111 was higher in KOA, but LD between rs4778889 and rs4072111was higher in the control group. Liu et al. [11] found linkage disequilibrium between rs4778889 SNP and rs11556218 which were similar to our results.

When we studied the association between IL16 SNPs and clinical and radiographic data, only rs11556218 GG, TG + GG, rs4072111 TT, and CT + TT were significantly associated with lower WOMAC and K/L scores which denote that individuals carrying these genotypes could be at less susceptibility to develop severe KOA than other genotypes.

In the study of Luo et al. [12] for evaluation of the impact of *IL-16* polymorphisms on KOA risk stratified by sex, they found that there is a clear strong association in female patient subgroups, but they could not find an explanation and revealed that this finding was unexpected, which could be explained by higher number of female patients compared to males, those genes located on chromosome 15 and not linked to sex chromosomes, or might also be the result of estrogen-related effects; estrogen can interact with IL-16 and reduce the possibility of developing KOA.

In our study, the serum levels of IL-16 did not show any significant differences between OA patients with different genotypes and the control group. Similarly, in a study by Gupta et al. [31] on 90 Malaysian patients, no significant difference was found between the serum levels of IL16 in the healthy controls and KOA patients, suggesting the probability of other influences on IL-16 concentrations apart from genetics. This can be explained by the relatively small sample size; more data from additional patients will provide further confirmation which would be provided by additional patient data.

Luo et al. [12] revealed that serum IL16 levels were higher in KOA patients than in controls but not associated with IL16 gene polymorphisms. Those results go with our study except for serum levels of IL16 which were not significantly different from the control group. This discrepancy in the results might be attributed to the lower exposure to risk factors, such as tobacco smoking and heavy alcohol drinking in the former two studies.

The limitation of the study

Three limitations of this study should be addressed. First, the relatively small size of our patients makes the power of study statistics insufficient. Therefore, further studies on larger samples are needed. Second is the study population confined to the Mansoura city population; thus, the findings may not be universal to other populations. More studies on other ethnic groups would be valuable. Third, only three SNPs in the IL-16 gene were investigated. Identifications of more SNPs and studying their association with KOA would be interesting.

Conclusion

There is a relationship between IL16 polymorphisms and the risk of KOA. We found that IL16 rs11556218 TG, GG, TG + GG genotypes, and G allele; rs4778889 CT, CC, CT + CC genotypes, and C alleles; and rs4072111 CT, TT, CT + TT genotypes, and T allele are associated with significant lower risk of KOA. Serum levels of IL-16 is not associated with the susceptibility to KOA. The haplotypes GTC and TCT are associated with low KOA risk. The variant alleles rs11556218GG, TG + GG; rs4778889 CC, TC + CC; and rs4072111 TT, CT + TT are associated with lower WOMAC and K/L score. There is no association between IL-16 polymorphisms and IL-16 serum levels.

Abbreviations

KOA	Knee osteoarthritis
IL-16	Interleukin 16
WOMAC	Western Ontario and McMaster Universities Arthritis Index
K/L scores	Kellgren-Lawrence
PCR	Polymerase chain reaction-restriction fragment length
ELISA	Enzyme-linked immunosorbent assay

A	Adenosine triphosphate
C	Cytidine triphosphate
G	Guanosine triphosphate
T	Thymidine triphosphate
LD	Linkage disequilibrium

Acknowledgements
All authors wish to thank and appreciate all cooperative patients who participated in this study.

Authors' contributions
Dr. E. A. H. performed the design of the work, data collection, and analysis; besides, she has drafted and revised the work. Dr. R. M. S. is responsible for the work design, data collection, and interpretation of data and has drafted and revised the work. Dr. O.M.G. is responsible for the data collection. Dr. S.A.M. is responsible for the design of the work. Dr. O.A.E. performed the design of the work. Dr. N.Y.A. is responsible for the design of the work. Dr. N.S. performed the design of the work and drafted the work. Dr. A. A. N. performed the design of the work and drafted the work. Dr. S. E. performed the design of the work and drafted the work. Dr. R. H. performed the design of the work and drafted the work. Dr. H. E. performed the design of the work and drafted the work. All authors have read and approved the final manuscript.

Funding
This study was totally funded by all authors.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study protocol was reviewed and approved by the local committee for medical research of the Faculty of Medicine in Mansoura University, code R/16.05.39. Informed written consents were provided by all patients sharing in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 19 August 2022 Accepted: 6 February 2023
Published online: 23 February 2023

References

1. Scott D, Kowalczyk A (2008) Osteoarthritis of the knee. Am Fam Physician 77:1149–1150
2. Sowers M (2001) Epidemiology of risk factors for osteoarthritis: systemic factors. Curr Opin Rheumatol 13:447–451
3. Bierma-Zeinstra SM, Koes BW (2007) Risk factors and prognostic factors of hip and knee osteoarthritis. Nat Clin Pract Rheumatol 3:78–85
4. Jiang L, Tian W, Wang Y, Rong J, Bao C, Liu Y et al (2012) Body mass index and susceptibility to knee osteoarthritis: a systematic review and meta-analysis. Joint Bone Spine 79:291–297
5. Goldring SR, Goldring MB (2004) The role of cytokines in cartilage matrix degeneration in osteoarthritis. Clin Orthop Relat Res 427 Suppl:S27–S36. https://doi.org/10.1097/01.blo.0000144854.66565.8f
6. Brooks P (2003) Inflammation as an important feature of osteoarthritis. Bull World Health Organ 81:689–690
7. Honsawek S, Deepaisarnsakul B, Tanavalee A, Yuktanandana P, Bumrungpanichthaworn P, Malila S et al (2011) Association of the IL-6 -174G/C gene polymorphism with knee osteoarthritis in a Thai population. Genet Mol Res 10:1674–1680
8. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H (2011) Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. Nat Rev Rheumatol 7:33–42
9. Ibrahim IK, Saba EKA, Saad NLM et al (2019) Relation of interleukin-15 with the severity of primary knee osteoarthritis. Egyptian Rheumatology and Rehabilitation 46:313–320
10. Farag AMA, El-Gazzar NM, Abo El Hawa MA, Attia MAS (2017) Study of interleukin 33 in rheumatoid arthritis versus osteoarthritis patients. ERAR 44:159–163
11. Liu Z, Ma L, Qiu S, Jia T (2015) Genetic polymorphisms of interleukin-16 are associated with susceptibility to primary knee osteoarthritis. Int J Clin Exp Med 8(1):1401–1405
12. Luo SX, Li S, Zhang XH, Zhang JJ, Long GH, Dong GF et al (2015) Genetic polymorphisms of interleukin-16 and risk of knee osteoarthritis. PLoS ONE. https://doi.org/10.1371/journal.pone.0123442
13. Smith AJP, Humphries SE (2009) Cytokine and cytokine receptor gene polymorphisms and their functionality. Cytokine Growth Factor Rev 20:43–59
14. Moss SF, Blaser MJ (2005) Mechanisms of disease: inflammation and the origins of cancer. Nat Clin Pract Oncol 2(2):90–113. https://doi.org/10.1038/ncponc0081
15. Lu H, Ouyang W, Huang C (2006) Inflammation, a key event in cancer development. MCR 4(4):221–233. https://doi.org/10.1158/1541-7786.MCR-05-0261
16. Kim HS (1999) Assignment of human interleukin 16 (IL16) to chromosome 15q263 by radiation hybrid mapping. Cytogenet Cell Genet 84(1–2):93. https://doi.org/10.1159/000015224
17. Baier M, Bannert N, Werner A, Lang K, Kurth R (1997) Molecular cloning, sequence, expression, and processing of the interleukin 16 precursor. Proc Natl Acad Sci USA 94(10):5273–5277. https://doi.org/10.1073/pnas.94.10.5273
18. Zhang Y, Center DM, Wu DM, Cruikshank WW, Yuan J, Andrews DW, Kornfeld H (1998) Processing and activation of pro-interleukin-16 by caspase-3. J Biol Chem 273(2):1144–1149. https://doi.org/10.1074/jbc.273.2.1144
19. Kai H, Kitadai Y, Kodama M, Cho S, Kuroda T, Ito M, Tanaka S, Ohmoto Y, Chayama K (2005) Involvement of proinflammatory cytokines IL-1beta and IL-6 in progression of human gastric carcinoma. Anticancer Res 25(2A):709–713
20. Shanmugham LN, Petrarca C, Frydas S, Donelan J, Castellani ML, Boucher W, Madhappan B, Tete' S, Falasca K, Conti P, Vecchiet J (2006) IL-15 an immunoregulatory and anti-cancer cytokine. Recent advances. J Exp Clin Cancer Res CR 25(4):529–536
21. Nakayama EE, Wasi C, Ajisawa A, Iwamoto A, Shioda T (2000) A new polymorphism in the promoter region of the human interleukin-16 (IL-16) gene. Genes Immun 1(4):293–294. https://doi.org/10.1038/sj.gene.6363672
22. Weis-Klemm M, Alexander D, Pap T, Schützle H, Reyer D, Franz JK, Aicher WK (2004) Synovial fibroblasts from rheumatoid arthritis patients differ in their regulation of IL-16 gene activity in comparison to osteoarthritis fibroblasts. Cell Physiol Biochem 14(4–6):293–300. https://doi.org/10.1159/000080339
23. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, Christy W, Cooke TD, Greenwald R, Hochberg M (1986) Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum 29(8):1039–1049. https://doi.org/10.1002/art.1780290816
24. Bellamy N, Buchanan WW, Goldsmith CH, Campbell J, Stitt LW (1988) Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. J Rheumatol 15(12):1833–1840
25. Gao LB, Liang WB, Xue H, Rao L, Pan XM, Lv ML, Bai P, Fang WL, Liu J, Liao M, Zhang L (2009) Genetic polymorphism of interleukin-16 and risk of nasopharyngeal carcinoma. Clin Chim Acta 409(1–2):132–135. https://doi.org/10.1016/j.cca.2009.09.017
26. Qin X, Peng Q, Lao X, Chen Z, Lu Y, Lao X, Mo C, Sui J, Wu J, Zhai L, Yang S, Li S, Zhao J (2014) The association of interleukin-16 gene polymorphisms with IL-16 serum levels and risk of nasopharyngeal carcinoma in

a Chinese population. Tumour Biol 35(3):1917–1924. https://doi.org/10.1007/s13277-013-1257-2
27. Luo SX, Li S, Zhang XH, Zhang JJ, Long GH, Dong GF, Su W, Deng Y, Liu Y, Zhao JM, Qin X (2015) Genetic polymorphisms of interleukin-16 and risk of knee osteoarthritis. PloS One 10(5):e0123442. https://doi.org/10.1371/journal.pone.0123442
28. Kellgren JH, Lawrence JS (1957) Radiological assessment of osteo-arthrosis. Ann Rheum Dis 16(4):494–502
29. Skol AD, Scott LJ, Abecasis GR, Boehnke M (2006) Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. Nat Genet 38(2):209–213. https://doi.org/10.1038/ng1706
30. Pelletier JP, Martel-Pelletier J, Abramson SB (2001) Osteoarthritis, an inflammatory disease: potential implication for the selection of new therapeutic targets. Arthritis Rheum 44(6):1237–1247. https://doi.org/10.1002/1529-0131(200106)44:6%3c1237::AID-ART214%3e3.0.CO;2-F
31. Das Gupta E, Ng WR, Wong SF, Bhurhanudeen AK, Yeap SS (2017) Correlation of serum cartilage oligomeric matrix protein (COMP) and interleukin-16 (IL-16) levels with disease severity in primary knee osteoarthritis: a pilot study in a Malaysian population. PloS One 12(9):e0184802. https://doi.org/10.1371/journal.pone.0184802
32 Sandell LJ, Aigner T (2001) Articular cartilage and changes in arthritis. An introduction: cell biology of osteoarthritis. Arthritis Res 3(2):107–113. https://doi.org/10.1186/ar148

Publisher's Note