

NLRP3 gene polymorphisms and expression in rheumatoid arthritis

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Received June 20, 2020; Accepted December 3, 2020

DOI: 10.3892/etm.2021.10544

Abstract. The present study aimed to investigate the association between the single-nucleotide polymorphisms (SNPs) rs4612666 and rs10754558 in the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) gene and the susceptibility to rheumatoid arthritis (RA) in a Han Chinese population. mRNA expression of NLRP3, apoptosis-associated speck-like protein (ASC), and caspase-1 were determined in peripheral blood mononuclear cells (PBMCs) and neutrophils using reverse-transcription quantitative PCR. The results demonstrated that the C allele at rs4612666 locus and the G allele at rs10754558 locus were associated with significantly increased risk of RA. A statistical significance was also revealed in the dominant model (CC+CT vs. TT: OR=1.549; 95% CI=1.120-2.144; and GG + GC vs. CC: OR=2.000; 95% CI=1.529-2.616; P<0.05). Additionally, the mRNA expression of NLRP3, ASC and caspase-1 in PBMCs and neutrophils derived from patients with RA were significantly upregulated compared with the controls. Furthermore, the mRNA levels of NLRP3, ASC and caspase-1 in PBMCs and neutrophils from patients with active RA were notably increased compared with patients in remission. NLRP3 expression was positively correlated with the levels of

C-reaction protein, erythrocyte sedimentation rate and disease activity score of 28 joint counts. Overall, the current study indicated that the NLRP3 rs4612666 and rs10754558 loci were associated with susceptibility to RA. In addition, the results of the present study demonstrated that the high expression of NLRP3 could serve a critical role in the pathogenesis of RA.

Introduction

Rheumatoid arthritis (RA) is one of the most common inflammatory autoimmune diseases in the world, and is mainly characterized by synovitis and joint destruction, which ultimately leads to disability. Approximately 1% of the worldwide population suffers from RA (1). The exact mechanism underlying RA pathogenesis remains unclear; however, accumulating evidence has suggested that genetic and environmental factors may be associated with the development of RA.

The pro-inflammatory cytokines IL-1 β and IL-18 serve vital roles in bone resorption and cartilage destruction in RA (2). The NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3), which is a member of the nod like receptor (NLR) family, contains a nucleotide-binding domain (NOD), and is predominantly expressed in peripheral blood leukocytes (3,4). In response to a variety of signals, including pathogens and danger signals, NLRP3 rapidly forms NLRP3 inflammasome combined with the apoptosis-associated speck-like protein (ASC) and the caspase-1 protease (5). As a result, the NLRP3 inflammasome promotes ASC polymerization and activation of caspase-1, which in turn induces the secretion of pro-inflammatory cytokines such as IL-1 β and IL-18, eventually leading to pyroptosis (6,7). Previous evidence has also suggested that mutations in the NLRP3 gene may be associated with the excessive release of IL-1 β (8). Polymorphisms in the NLRP3 gene have been associated with some common diseases such as type-1 diabetes, inflammatory bowel disease and gout (9-11). Furthermore, studies have demonstrated that mutations in NLRP3 may be involved in the development of RA in different populations (12-15). In addition, Choulaki *et al* (16) revealed that NLRP3 was upregulated in peripheral blood cells derived from patients with RA, thus suggesting that NLRP3 could serve a crucial role in RA. However, to the best of our knowledge, the association of NLRP3 polymorphisms with susceptibility to RA in Chinese Han patients, and the expression levels of NLRP3, ASC and

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Abbreviations: NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; RA, rheumatoid arthritis; PBMCs, peripheral blood mononuclear cells; ASC, apoptosis-associated speck-like protein; NLR, nod like receptor; SNPs, single-nucleotide polymorphisms; RF, rheumatoid factor; A-CCP, anti-cyclic citrullinated peptide antibodies; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; DAS28, disease activity score of 28 joint counts; HWE, Hardy-Weinberg equilibrium

Key words: NOD-, LRR- and pyrin domain-containing protein 3, polymorphism, expression, rheumatoid arthritis

caspase-1 in peripheral blood mononuclear cells (PBMCs) and neutrophils have not yet been investigated. Therefore, the current study aimed to investigate the association between two common NLRP3 single-nucleotide polymorphisms (SNPs) and RA in a case-control study. In addition, the effect of these SNPs on NLRP3 expression was also evaluated. Therefore, the NLRP3 mRNA expression levels were determined in PBMCs and neutrophils from RA and healthy individuals.

Materials and methods

Patients. A total of 934 individuals were recruited from the Second Hospital of Anhui Medical University, Anhui Province, China, between August 2016 and March 2018. The main characteristics of the patients included were shown in Table I. Among them, 402 were diagnosed with RA and classified according to the American Rheumatism Association 1987 revised criteria (17). The inclusion criteria for patients with RA were as follows: i) Definite diagnosis of RA; ii) treated only with conventional disease-modifying antirheumatic drugs such as methotrexate or/and leflunomide. The patients suffering from other inflammatory/autoimmune diseases or cancer were excluded from the present study. A total of 532 healthy individuals were included. Exclusion criteria included if patients exhibited a different inflammatory/autoimmune disease or cancer or had a family history of RA. Blood samples from 532 age-, sex-, and residential area-matched healthy subjects, who underwent a health check-up, were collected. During this period, the expression of NLRP3, ASC and caspase-1 were determined in 49 randomly selected RA cases, including 32 active and 17 inactive patients and in 30 healthy control individuals.

All clinical characteristics, including rheumatoid factor (RF), anti-cyclic citrullinated peptide antibodies (A-CCP), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), disease activity score of 28 joint counts (DAS28) (18). Sex, age and disease duration were recorded in detail. In patients with RA, DAS28 values of ≤ 2.6 and > 2.6 were considered to indicate inactive and active RA disease (19). The current study protocol was approved by the Ethics Committee of the Second Affiliated Hospital of Anhui Medical University, and all experiments were carried out in accordance with the declaration of Helsinki. Finally, informed consent was obtained from all study participants or their families if the patients were considered incapable of providing consent.

SNP selection and genotyping. The SNPs rs4612666 and rs10754558 were selected due to their involvement in other diseases, including gastric cancer (20), type 1 diabetes (9), inflammatory bowel disease (10) and primary gouty arthritis (11). The genomic DNA was isolated from 1 ml of peripheral blood using the AxyPrep DNA Purification kit (Axygen; Corning, Inc). The primers designed were as follows: rs4612666 forward-5'-TGGAGTTTCAGTTCTGCAAG-3' and reverse 5'-ATGGACAAGGAAGCACCCG-3'; rs10754558 forward 5'-TTGAGCCTTCTTGGTAGGAGTGG-3' and reverse 5'-GAATAGAAAGATAGCGGGAATG-3'. The PCR amplification in Sanger sequencing was performed in a 30 μ l volume solution which contained 15 μ l 2x Green Master Mix, 0.5 μ l genomic DNA, 0.75 μ l of each primer and 13 μ l

Nuclease-Free Water. The conditions of PCR were as follows: Initial denaturation at 95°C for 5 min, followed by 35 cycles at 95°C for 15 sec, with annealing at 50°C for 30 sec and extension at 72°C for 30 sec, followed by a final extension at 72°C for 10 min with 1 cycle. The genotyping of NLRP3 rs4612666 and rs10754558 was conducted using direct sequencing. The results were analyzed with chromas software (version 2.6; Technelysium Pty Ltd) to identify possible mutations. The results are presented in Fig. 1.

Reverse transcription-quantitative PCR (RT-qPCR). PBMCs and neutrophils of 49 patients with RA and 30 healthy controls were separated using density-gradient centrifugation (Beijing Solarbio Science & Technology Co., Ltd.) that was performed at 400 x g for 20 min at 20°C and at 500 x g for 35 min at 20°C, respectively. Total RNA was extracted from PBMCs and neutrophils using Trizol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Subsequently, RNA was reverse transcribed into complementary (c)DNA using the Prime Script RT reagent kit (Takara Biotechnology Co., Ltd.) according to the manufacturer's protocol. The specific primer sequences are presented in Table II. RT-PCR was carried out in a 20 μ l reaction volume comprising of 1.6 μ l primers (0.8 μ l forward primer and reverse primer), 2 μ l cDNA, 0.4 μ l ROX Reference dye II, 6 μ l H₂O and 10 μ l SYBR Premix Ex Taq™ II. The conditions of PCR were as follows: Initial denaturation at 95°C for 15 min, 94°C for 30 sec, 59°C for 60 sec, 72°C for 1 min then 40 cycles and a final extension at 72°C for 7 min. All the samples were amplified at the same site on ABI 7500 real-time polymerase chain reaction system (Applied Biosystems; Thermo Fisher Scientific, Inc). The expression levels of NLRP3, ASC and caspase-1 were calculated using the $2^{-\Delta\Delta C_q}$ method (21).

Statistical analysis. Statistical analyses were performed using SPSS 10.0 software (SPSS Inc.). An exact χ^2 test was applied to assess Hardy-Weinberg equilibrium (HWE). A χ^2 test or a Fisher exact test was used to compare genotype and allele frequencies between patients and controls. The association between NLRP3 SNPs, rs4612666 and rs10754558 and RA were assessed by odds ratio and 95% confidence intervals using logistic regression analyses. Dominant and recessive models were also used for statistical analysis, including the dominant model (for isolated SNPs: Homozygote rare + heterozygote vs. homozygote common) and recessive mode (for isolated SNPs: Homozygote rare vs. heterozygote + homozygote common). A non-parametric Kruskal-Wallis test was performed to compare the expression of NLRP3, ASC, and caspase-1 between groups. Furthermore, Spearman's correlation analysis was applied to analyze correlations between NLRP3 expression and clinical data. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Clinical characteristics of the study population. The clinical and baseline characteristics of patients and controls (n=934) are summarized in Table I. The RA and control groups were matched based on age and sex ($P > 0.05$).

Prevalence of NLRP3 alleles and genotypes in patients with RA and controls. The rs4612666 and rs10754558 SNPs were

Table I. The basic characteristics of the 934 study cases.

Characteristic	RA group (N=402)	Healthy control (N =532)	P-value
Age (years), Mean \pm SD	58.28 \pm 10.119	50.17 \pm 14.862	>0.05
Sex (n)			>0.05
Male	79	128	
Female	323	404	
Disease duration, (years), Mean \pm SD	13 \pm 0.8	-	-
RF (n=402)			-
Positive, n (%)	353 (87.8)	-	
Negative, n (%)	49 (12.2)	-	
A-CCP (n=402)			-
Positive, n (%)	369 (91.8)	-	
Negative, n (%)	33 (8.2)	-	

RF, rheumatoid factor; A-CCP, anti-cyclic citrullinated peptide; RA, rheumatoid arthritis; -, no data.

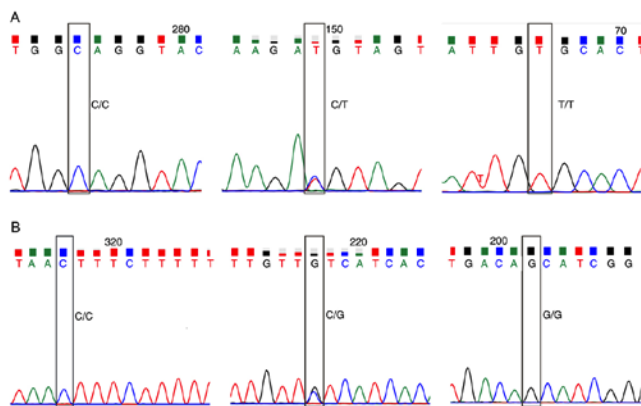


Figure 1. The determined genotyping of NLRP3 rs4612666 and rs10754558. Detection of SNP for (A) rs4612666 and (B) rs10754558 using Sanger sequencing. SNP is indicated in rectangles. SNP, single-nucleotide polymorphisms. Numbers above the sequence indicate the location of the base pairs.

in HWE in controls ($P=0.930$ and 0.086 , respectively), the details are presented in Table III. As presented in Table IV, regarding the NLRP3 rs4612666 polymorphism, individuals carrying the C allele exhibited a significantly increased risk for RA (OR=1.204; 95% CI=1.002-1.447; $P=0.047$). A statistical significance was also revealed in the dominant model (CC + CT vs. TT: OR=1.549; 95% CI=1.120-2.144; $P=0.008$), but not in the recessive model (CC vs. CT + TT: OR=1.119; 95% CI=0.834-1.501; $P=0.454$). Furthermore, significant differences in NLRP3 rs10754558 genotype distribution was observed between RA and control individuals, while the G allele was positively correlated with RA risk compared with the C allele (OR=1.515; 95% CI 1.250-1.835; $P<0.001$). Additionally, significant differences were obtained in the dominant model (GG + GC vs. CC: OR=2.000; 95% CI=1.529-2.616; $P<0.001$), whereas no discrepancy was observed in the recessive model (GG vs. CC + GC: OR=1.244; 95% CI=0.839-1.845; $P=0.277$). The logistic regression analyses demonstrated the rs4612666 and rs10754558 loci were independent risk factors for the development of RA.

Table II. Primers used for reverse-transcription quantitative PCR.

Genes	Primer sequence
β -actin	
Forward	5'-TGGCAGCCAGCACAATGAA-3'
Reverse	5'-CTAAGTCATAGTCCGCCTAGAAGCA-3'
NLRP3	
Forward	5'-CTCTAGCTGTTCTCCTGAGGCTG-3'
Reverse	5'-TTAGGCTTCGGTCCACACAG-3'
ASC	
Forward	5'-TCCTCAGTCGGCAGCCAAG-3'
Reverse	5'-CCCTCCCGTACAGAGCATCC-3'
Caspase-1	
Forward	5'-GCCTGTTCTCTGTGATGTGGA-3'
Reverse	5'-CTTCACTTCCTGCCCCACAGA-3'

NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; ASC, apoptosis-associated speck-like protein.

Association between NLRP3 mRNA expression and SNPs in PBMCs and neutrophils from patients with RA. No significant differences were observed between the rs4612666 and rs10754558 genotypes and NLRP3 mRNA expression in PBMCs and neutrophils ($P>0.05$; Table V).

NLRP3, ASC and caspase-1 mRNA expression in PBMCs and neutrophils from patients with RA and healthy controls. As presented in Figs. 2 and 3, the mRNA expression of NLRP3, ASC and caspase-1 were upregulated in PBMCs [0.93 (0.78-1.18) vs. 0.62 (0.54-0.67); 0.93 (0.82-1.27) vs. 0.61 (0.53-0.64); 0.86 (0.80-1.14) vs. 0.64 (0.54-0.71), respectively] and neutrophils [0.97 (0.81-1.35) vs. 0.60 (0.50, 0.75); 0.95 (0.83-1.24) vs. 0.52 (0.47-0.62); 0.95 (0.84-1.16) vs. 0.62 (0.45-0.69), respectively] of patients with RA compared with

Table III. Primary information of two nucleotide polymorphisms of NLRP3.

SNP	Alleles	MAF		P-value for HWE in controls
		Patients	Control	
rs4612666	C/T	0.450	0.392	0.930
rs10754558	G/C	0.403	0.308	0.086

MAF, minor allele frequency; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; SNP, single-nucleotide polymorphisms; HWE, Hardy-Weinberg equilibrium.

Table IV. Allele and genotype frequencies of rs4612666 and rs10754558 in patients with RA and healthy controls.

SNP and genotype	RA (n=402) n (%)	Controls (n=532) n (%)	χ^2	OR (95% CI)	P-values
rs4612666					
CC	110 (27.36)	134 (25.19)	7.057	N/A	0.029
CT	222 (55.22)	267 (50.19)			
TT	70 (17.41)	131 (24.62)			
C	442 (54.98)	535 (60.78)	3.929	1.204 (1.002-1.447)	0.047
T	362 (45.02)	529 (39.22)		Reference	
Dominant model					
CC + CT	332 (82.6)	401 (75.4)	7.050	1.549 (1.120-2.144)	0.008
TT	70 (17.4)	131 (24.6)		Reference	
Recessive model					
CC	110 (27.4)	134 (25.2)	0.561	1.119 (0.834-1.501)	0.454
CT + TT	292 (72.6)	398 (74.8)		Reference	
rs10754558					
GG	54 (13.43)	59 (11.09)	34.879	N/A	P<0.001
GC	216 (53.73)	210 (39.47)			
CC	132 (32.83)	263 (49.44)			
G	324 (40.30)	328 (30.83)	18.081	1.515 (1.250-1.835)	P<0.001
C	480 (59.70)	736 (69.17)		Reference	
Dominant model					
GG + GC	270 (67.2)	269 (50.6)	25.854	2.000 (1.529-2.616)	P<0.001
CC	132 (32.8)	263 (49.4)		Reference	
Recessive model					
GG	54 (13.4)	59 (11.1)	1.182	1.244 (0.839-1.845)	0.277
CC + GC	348 (86.6)	473 (88.9)		Reference	
rs4612666					P<0.001
CC	110 (27.36)	134 (25.19)	73.341	Reference	
CT	222 (55.22)	267 (50.19)			
TT	70 (17.41)	131 (24.62)			
rs10754558					P<0.001
GG	54 (13.43)	59 (11.09)	83.916	Reference	
GC	216 (53.73)	210 (39.47)			
CC	132 (32.83)	263 (49.44)			

OR, odd ratios; CI, confidence interval; N/A, not available; RA, rheumatoid arthritis.

healthy individuals (all $P<0.001$. Furthermore, the NLRP3, vs. 0.78 (0.68-0.86); 1.03 (0.87-1.38) vs. 0.83 (0.75-0.89); ASC and caspase-1 mRNA levels in PBMCs [1.09 (0.86-1.26) 0.96 (0.84-1.35) vs. 0.82 (0.73-0.88)] and neutrophils

Table V. NLRP3 mRNA expression of PBMCs/neutrophils indifferent genotypes of rs4612666 and rs10754558 in patients with RA.

A, PBMCs			
Genotype	Number	M (P25,P75)	P-value
rs4612666			0.811
CC	12	0.980 (0.665,1.320)	
CT	26	0.930 (0.790,1.115)	
TT	11	0.855 (0.773,1.240)	
rs10754558			0.077
GG	9	1.180 (0.870,1.260)	
CG	21	0.830 (0.720,1.130)	
CC	19	0.930 (0.825,1.183)	
B, Neutrophils			
Genotype	Number	M (P25,P75)	P-value
rs4612666			0.187
CC	12	1.260 (0.945,1.573)	
CT	26	0.945 (0.795,1.280)	
TT	11	0.950 (0.785,1.510)	
rs10754558			0.424
GG	9	1.070 (0.950,2.350)	
CG	21	1.000 (0.820,1.335)	
CC	19	0.850 (0.740,1.430)	

All the mRNA expression levels were showed as median value (interquartile range); NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; PBMC, peripheral blood mononuclear cells; RA, rheumatoid arthritis; M, median value.

[1.12 (0.94-1.47)] vs. 0.87 (0.80-1.14); 0.99 (0.88-1.28) vs. 0.84 (0.75-0.94); 1.00 (0.87-1.18) vs. 0.87 (0.81-0.95)] from patients with active RA were significantly increased compared with those with inactive RA (all $P < 0.05$; Figs. 4 and 5).

Correlation between the expression of NLRP3 and clinical data. The basic clinical and laboratory parameters are listed in Table VI. As presented in Table VII, the NLRP3 expression in PBMCs and neutrophils were significantly correlated with ESR, CRP and DAS28 ($P < 0.05$). No correlation was observed between the expression of NLRP3 and RF, A-CCP and disease course ($P > 0.05$).

Discussion

RA is an autoimmune disease that is characterized by chronic inflammation and joint pain (22). Abundant evidence has shown that inflammasome is activated by its ligands, resulting in abnormalities of the immune system (23). Currently, it is widely considered that the inflammasomes, especially the NLRP3/ASC/caspase-1 pathway, are involved in the pathogenesis of several diseases, including RA (24). It

is widely accepted that the NLRP3 inflammasome is highly expressed in monocytes and macrophages in response to inflammatory stimuli (12,15,16,24,25). A number of studies on gout, ischemia reperfusion injury and renal tubule injury have demonstrated that the mRNA expression of the NLRP3 inflammasome were upregulated; therefore, NLRP3 is thought to exert a vital role in the pathogenesis of these diseases (26-28). A number of studies have also demonstrated that NLRP3 is upregulated in RA and have further suggested that NLRP3 inflammasome may serve an essential role in RA pathogenesis (13,15,16,24,25). It has been previously reported that mutations in the NLRP3 gene serve an indispensable role in a number of diseases, and previous studies have suggested that polymorphisms may affect gene expression (9-11,20).

An increasing number of studies have shown RA symptoms may be improved following suppression of the NLRP3 pathway that might suggest the NLRP3 pathway serves an important role in the severity of RA (29,30). It has also been proposed that NLRP3 may be a promising therapeutic target in autoimmune diseases, including RA (31). The aforementioned studies indicated that the NLRP3 signaling pathway could serve a major role in the pathogenesis of RA and provided a therapeutic approach for its treatment.

Only a few studies have been conducted in PBMCs and neutrophils. Taken together, the results of the present study suggested that SNPs in the NLRP3 inflammasome, and NLRP3 expression maybe involved in the pathogenesis of RA. Therefore, to the best of our knowledge, the current case-control study was the first to reveal the association between NLRP3 rs4612666 and rs10754558 SNPs and RA susceptibility in Chinese Han population. The expression of NLRP3, ASC and caspase-1 in PBMCs and neutrophils were compared between RA cases and controls.

In agreement with previous reports, the current study identified a significant association between the variant genotypes rs4612666 and rs10754558, and RA risk in Chinese Han individuals. Furthermore, the C and the G allele of the rs4612666 and rs10754558 SNPs, respectively, were highly associated with RA, indicating an increased susceptibility to RA. Logistic regression analysis further indicated that the NLRP3 rs10754558 and rs4612666 SNPs were considered to be independent prognostic factors for RA. However, the study failed to reveal any correlation between the rs4612666 and rs10754558 mutations and the NLRP3 mRNA expression levels in both PBMCs and neutrophils from patients with RA. This may be due to other susceptible polymorphisms in the NLRP3 gene, which may affect its expression.

When NLRP3 expression was compared between patients with RA and healthy individuals, NLRP3 was only revealed to be upregulated in PBMCs and neutrophils from patients with RA. In addition, the mRNA levels of other components of the NLRP3 pathway such as ASC and caspase-1 were also upregulated in both cell types. When the mRNA levels were compared between patients with active and inactive RA, ASC and caspase-1 levels were increased in patients with active RA compared with those in patients in remission in both cell types. These findings were consistent with previous reports (13,15,16,24,25), further supporting the importance of the NLRP3/ASC/caspase-1 pathway. Furthermore, a positive correlation between NLRP3 mRNA expression and

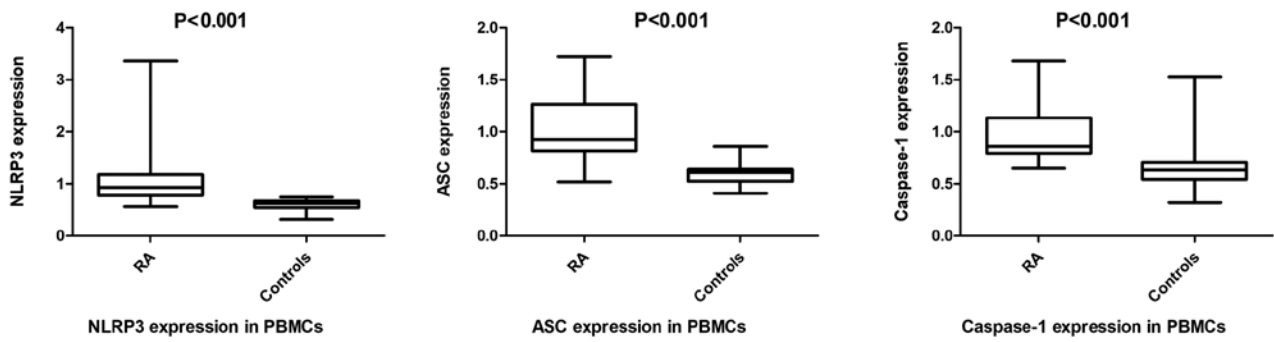


Figure 2. The mRNA expression of NLRP3, ASC and Caspase-1 in PBMCs from patients with RA and healthy controls. NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; ASC, apoptosis-associated speck-like protein; PBMC, peripheral blood mononuclear cells; RA, rheumatoid arthritis.

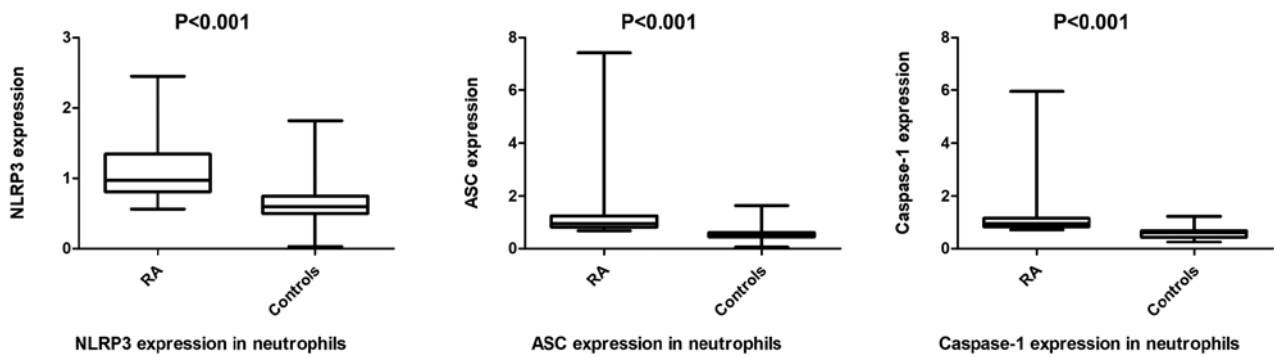


Figure 3. The mRNA expression of NLRP3, ASC and caspase-1 in neutrophils from patients with RA and healthy controls. NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; ASC, apoptosis-associated speck-like protein; PBMC, peripheral blood mononuclear cells; RA, rheumatoid arthritis.

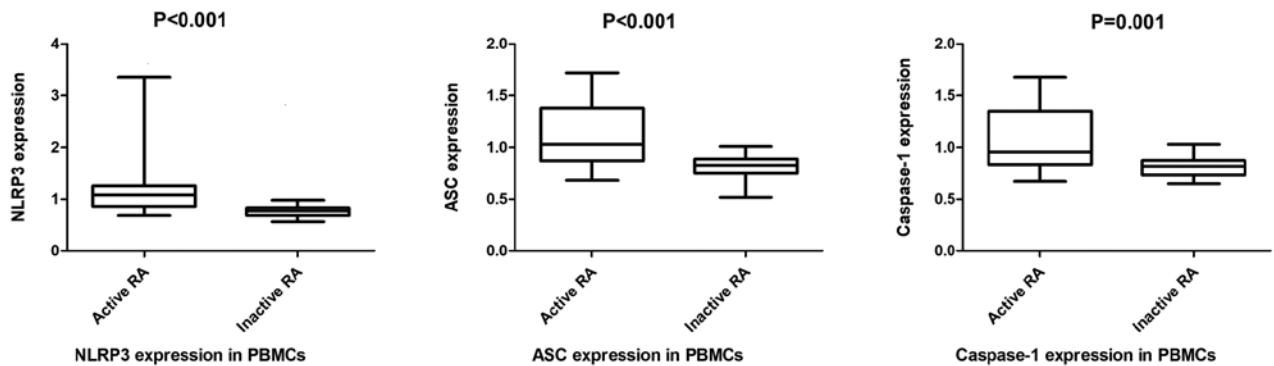


Figure 4. The mRNA expression of NLRP3, ASC and Caspase-1 in PBMCs from patients with active RA and inactive RA. NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; ASC, apoptosis-associated speck-like protein; PBMC, peripheral blood mononuclear cells; RA, rheumatoid arthritis.

DAS28/CRP/ESR was also observed in patients with RA, suggesting that the NLRP3 inflammasome may be closely associated with RA activity. It was suggested that DAS28, CRP and ESR in patients with RA could be decreased by inhibiting the NLRP3 pathway, which was mainly based on the results of previously published articles that demonstrated that RA symptoms might be improved following inhibition of the NLRP3 pathway (29,30). However, further research would be required to confirm the association of the severity of RA and the NLRP3 pathway. Nevertheless, no clear association was observed between NLRP3 expression and autoantibody levels, including RF and A-CCP. Overall the aforementioned findings indicated that the expression of the NLRP3

inflammasome-related genes (NLRP3, ASC and caspase-1) in PBMCs may serve a critical role and be associated with RA pathogenesis and disease activity.

To the best of our knowledge, the current study was the first to demonstrate that the NLRP3 polymorphisms (rs4612666 and rs10754558) were associated with susceptibility to RA in Chinese Han population by investigating the expression of NLRP3 inflammasome in PBMCs and neutrophils from patients with RA and healthy controls. However, the present study has some limitations that should be addressed in future research. Firstly, the selection bias was unavoidable as this was a case-control study. Secondly, the sample size was not large enough, which might be underpowered

Table VI. The basic clinical and laboratory parameters of 49 patients with RA.

Parameter	RA group (n=49)	Healthy control (n=30)
Age (years), mean \pm SD	44.6 \pm 13.2	45.17 \pm 14.86
Sex		
Male	10	8
Female	39	22
Disease duration, years, M (P25, P75)	4.6 (1.2,18.1)	-
ESR (mm/h), mean \pm SD	38.90 \pm 27.50	-
CRP (mg/l), M (P25, P75)	13.0 (4.18,32.0)	-
DAS28, mean \pm SD	4.13 \pm 1.80	-
RF (IU/ml), M (P25, P75)	112.50 (54.18,277.7)	-
A-CCP (RU/ml), mean \pm SD	549.69 \pm 302.10	-

All the mRNA expression levels were displayed as median value (interquartile range); RF, rheumatoid factor; A-CCP, anti-cyclic citrullinated peptide; DAS28, the disease activity score of 28 joint counts; N(n), numbers of cases; RA, rheumatoid arthritis; M, median value; -, no data.

Table VII. Correlations between the expression of NLRP3 at mRNA levels of PBMCs/neutrophils and clinical data.

Expression	CRP	ESR	RF	A-CCP	DAS28	Course
NLRP3 mRNA (PBMCs)						
ρ -value	0.501	0.494	0.206	1.000	0.498	-0.103
P-value	<0.001	<0.001	0.159	0.999	<0.001	0.485
NLRP3 mRNA (neutrophils)						
ρ -value	0.372	0.416	0.068	0.057	0.007	0.378
P-value	0.008	0.003	0.641	0.696	0.007	0.378

RF, rheumatoid factor; A-CCP, anti-cyclic citrullinated peptide; DAS28, the disease activity score of 28 joint counts; N(n), numbers of cases; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; PBMC, peripheral blood mononuclear cells; ESR, erythrocyte sedimentation rate; course, disease course.

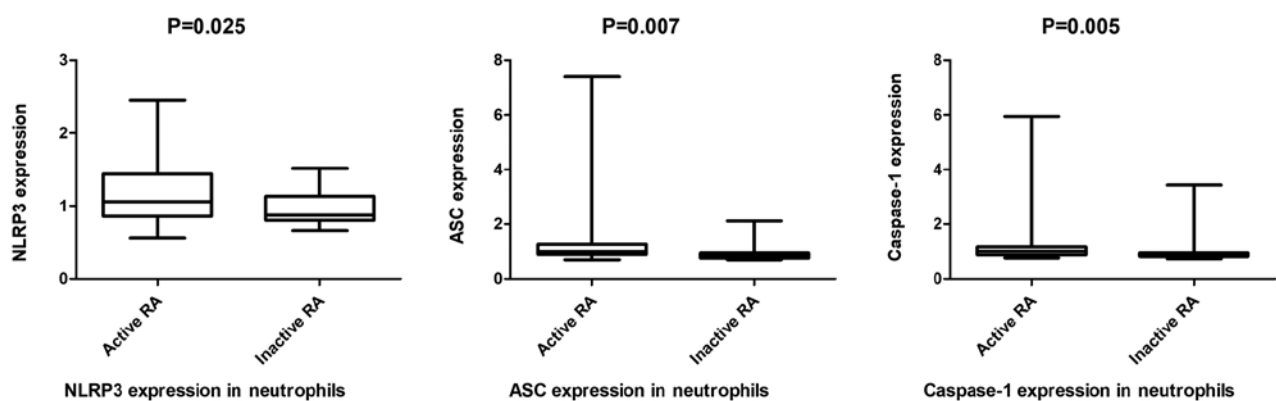


Figure 5. The mRNA expression of NLRP3, ASC and caspase-1 in neutrophils from active RA and inactive RA. NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; ASC, apoptosis-associated speck-like protein; PBMC, peripheral blood mononuclear cells; RA, rheumatoid arthritis.

to detect a weak association, Thirdly, unknown factors, which were not investigated, could be also involved in the NLRP3/ASC/caspase-1 pathway. The aforementioned limitations could explain the relatively small OR value indicated in the NLRP3 rs10754558 C allele and the P value of \sim 0.05. Additionally, only two polymorphisms (rs4612666

and rs10754558) were investigated. Therefore, further clinical and genetic studies on the NLRP3 pathway are required to elaborate the association between NLRP3 and RA.

In summary, the present study revealed a significant association between NLRP3 rs4612666 and rs10754558 SNPs and the risk of RA in a Chinese population. Furthermore,

increased expressions of the NLRP3 inflammasome were detected in PBMCs and neutrophils from patients with RA. These findings provide a novel genetic mechanism that maybe involved in the activation of the NLRP3 inflammasome pathway and may be used as an effective treatment for RA.

Acknowledgements

Not applicable.

Funding

The current study was supported by The Scientific Research Project for Academic and Technical Leaders, Anhui, China (grant no. 2017H143) and The Linkage Projects of Public Welfare Technology Application Research, Anhui, China (grant no. 1704f0804024).

Availability of data and materials

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

Authors' contributions

LQ conceived and designed the experiment(s). LC performed the experiments, contributed to the analysis and drafted the manuscript. XL and DL helped to conduct the experiment(s), CL analyzed the results. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Ethical Committee of the second Affiliated Hospital of Anhui Medical University (Hefei, China) approved the study protocol. All experiments were performed in accordance with relevant guidelines and regulations of the Declaration of Helsinki. Informed consent was obtained from all the participants or their families if the patients were incapable of giving consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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