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ORIGINAL ARTICLE

The association between the *CASP5* rs7939842 polymorphism and the risk of rheumatoid arthritis in Chinese Han individuals

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KEYWORDS

Caspase-5;
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Abstract *Background:* The association between inflammatory cysteinyl aspartate protease-5 (CASP5) and the susceptibility to rheumatoid arthritis (RA) remains unclear. This study examined whether the *CASP5* rs7939842 polymorphism affects RA risk in Chinese Han individuals.

Methods: This study recruited 805 RA patients and 1095 healthy controls to investigate the association between the *CASP5* rs7939842 polymorphism and RA risk. Genotype was examined using the 48-Plex SNPscan™ Kit. Plasma CASP5 levels were determined using enzyme-linked immunosorbent assays, and *CASP5* gene expression was detected by quantitative polymerase chain reaction in 40 RA patients and 40 healthy controls.

Result: The *CASP5* rs7939842 polymorphism G allele is a putative risk factor for RA. After stratified analyses, this polymorphism increased the risk of RA among CRP-, ACPA-, RF-, and ESR-positive individuals, as well as individuals with DAS28 $\geq$ 3.20 and functional class III + IV. Furthermore, the plasma CASP5 levels and *CASP5* mRNA expression were higher in RA patients than in healthy controls.

Abbreviations: RA, rheumatoid arthritis; SNP, single nucleotide polymorphism; caspases, cysteine-aspartic proteases; CASP5, cysteinyl aspartate protease-5; 95%CIs, 95% confidence intervals; ORs, odds ratios

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Conclusion: The *CASP5* rs7939842 polymorphism appears to be associated with an elevated risk of RA in Chinese Han individuals. Blood CASP5 protein and mRNA levels were significantly higher in RA patients than in healthy controls.

1. Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease characterized by chronic synovial inflammation, which causes joint destruction with irreversible cartilage degradation (Spel and Martinon 2020). Musculoskeletal defects attributed to RA gradually reduce physical function, affecting the quality of life (Smolen et al. 2016). The estimated prevalence of RA in the Chinese population was approximately 0.42% (*i.e.*, more than 5 million RA patients) (Jin et al. 2017); Worldwide, nearly 5 in 1,000 adults have RA (Aletaha and Smolen 2018). Genetic and environmental factors (particularly smoking) are risk factors for RA (Sparks 2019). Many recent genome-wide association studies have revealed several novel loci associated with RA risk (Eyre et al. 2012; Leng et al. 2020; Stahl et al. 2010; Zhu et al. 2016).

Cysteine-aspartic proteases (caspases), encoded by the *CASP* gene family, are ancient intracellular proteases with ubiquitous distribution in multicellular organisms (Hong et al. 2020). The *CASP* gene family affects cell growth, differentiation, cytokine maturation and apoptosis (Zhang et al. 2013); Its members have three distinct effects as initiator (CASP 2/8/9/10), effector (CASP3/6/7), and inflammatory (CASP 1/4/5/11/12) caspases (Hong et al. 2020). With the aid of CASP1, CASP4 and CASP5 regulate inflammasome activation in monocytes (Vigano et al. 2015). Furthermore, animal studies and samples from RA patients have demonstrated that inflammasomes are involved in the pathogenesis of RA (Spel and Martinon 2020).

The *CASP5* gene is located on chromosome 11q22.2. *CASP5* polymorphisms are associated with the risks of various cancers (e.g., lung (Hosomi et al. 2003; Ulybina et al. 2009), ovarian (Quaye et al. 2009), renal (Dong et al. 2009), prostate (Mittal et al. 2012) and bladder (Mittal et al. 2011)). *CASP5* gene polymorphisms are also presumably associated with susceptibility to autoimmune diseases (e.g., psoriasis vulgaris (He et al. 2015) and RA (Rui et al. 2018)). Of note, Rui et al. (2018) did not investigate the *CASP5* rs7939842 polymorphism in RA (Rui et al. 2018). This polymorphism is located in the intervening region, which may affect gene expression and gene-selective splicing. However, no associations between the *CASP5* rs7939842 polymorphisms and RA susceptibility have been reported. To our knowledge, this is the first study to investigate whether the *CASP5* rs7939842 polymorphism is a risk factor for RA in Chinese Han individuals. This case-control study was conducted to examine the association between the *CASP5* rs7939842 polymorphism and risk of RA in Chinese Han individuals; it examined CASP5 protein and gene expression in the blood of patients with RA and healthy controls.

2. Patients and methods

2.1. Participants

This study recruited 805 RA patients and 1095 healthy controls from the Affiliated Changzhou No.2 People's Hospital of Nanjing Medical University and the Changzhou First Hospital. The 2010 American College of Rheumatology/European League Against Rheumatism diagnostic criteria were used to identify RA patients (Aletaha et al. 2010). Healthy controls matched for age and sex were enrolled from Chinese Han individuals without RA during the study period, at each hospital. All RA patients were required to complete a demographic questionnaire. This study was approved by the Ethics Committee of Nanjing Medical University. All participants provided informed consent and this study adhered to the 1964 Declaration of Helsinki.

An additional 40 RA patients and 40 healthy controls were recruited based on the above inclusion and exclusion criteria. Plasma CASP5 levels were determined by enzyme-linked immunosorbent assays (Tongwei, Shanghai, China). *CASP5* gene expression was quantified using the quantitative polymerase chain reaction method. Relative *CASP5* gene expression was determined using the ΔΔCt method. All protocols were conducted in strict accordance with standard procedures.

2.2. Genotyping

QIAamp DNA Blood Mini Kits (Qiagen, Hilden, Germany) were used to extract DNA from 2 mL of peripheral blood from each participant. Subsequently, the *CASP5* gene rs7939842 polymorphism was assessed with SsoAdvanced™ Universal® SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) using a MX4000 Stratagene detection mechanism, in accordance with the manufacturer's instructions (Zheng et al. 2013).

2.3. Statistical analysis

All statistical analyses were conducted using SPSS (ver. 21, SPSS, USA). Continuous demographic variables were tested to determine their adherence to a normal distribution. Normally distributed parameters were compared using t-tests. The genotype distribution and allelic frequencies of the *CASP5* rs7939842 polymorphism were assessed using the chi-squared (χ^2) test and logistic regression adjusted for age and sex. Crude 95% confidence intervals (95 %CIs) and odds ratios (ORs) were used to assess the correlations of the *CASP5* rs7939842 polymorphism and RA susceptibility. In multiple comparison analyses, *P*-values were corrected using the Bonferroni method. Hardy–Weinberg equilibrium for healthy controls was examined using goodness-of-fit χ^2 tests to verify that the

individuals were representative of a healthy population. Stratified analyses were estimated using χ^2 tests. Graphs were drawn using GraphPad Prism 8.0 (GraphPad, La Jolla, CA, USA).

2.4. CASP5 gene expression and protein levels

TRIzol reagent (Takara Biomedical Technology, Beijing, China) was used to extract RNA; the extracted RNA was stored at –80 °C. In combination with reverse transcription primers (Sangon Biotech, Shanghai, China) and reverse transcriptase (Vazyme Biotech, Nanjing, China), 1–5 μg RNA template was used to synthesize cDNA. Real-time polymerase chain reactions were performed using the Real-Time PCR System (Vazyme Biotech). Relative *CASP5* expression was determined using the ΔΔCt method. GAPDH mRNA was used as a control to normalize *CASP5* gene expression. The *CASP5* primer sequences were 5′–CTTCACAGTCATCTGAGAACCT–3′ (forward) and 5′–GCCTGTGGAACTTCAAATGATT–3′ (reverse).

Serum CASP5 protein levels were measured using the human CASP5 ELISA Kit (Shanghai Tongwei Biological, Shanghai, China), in accordance with the manufacturer's instructions. Serum CASP5 protein levels were calculated using a standard curve through the optical density value of the sample to be tested.

3. Results

3.1. Participant characteristics

This study recruited 805 RA patients and 1095 healthy controls. Table 1 summarizes the participant demographics and clinical characteristics. The RA patients and healthy controls were matched according to sex and age ($P > 0.05$). The mean ages of the cases and healthy controls were 55.92 and 55.01 years, respectively. Other clinical characteristics are listed in Table 1.

3.2. Association between the CASP5 rs7939842 polymorphism and RA risk

Table 2 lists the distributions of various genotypes and allelic frequencies of the rs7939842 polymorphisms in two groups. Hardy–Weinberg equilibrium analysis of the genotype distribution did not identify any difference from the healthy controls ($P = 0.589$). The AG + GG and GG genotypes significantly increased RA susceptibility (GG vs. AA, OR = 8.37, 95% CI = 1.01–69.68, $P = 0.049$; AG + GG vs. AA, OR = 1.56, 95% CI = 1.06–2.31, $P = 0.026$). However, this difference did not remain statistically significant after Bonferroni correction. The presence of the G allele was associated with a high risk of RA compared with the presence of the A allele (G vs. A, OR = 1.68, 95% CI = 1.15–2.45, $P = 0.006$).

3.3. Stratified analyses of the CASP5 rs7939842 polymorphism and RA susceptibility

Stratified analysis suggested that the *CASP5* rs7939842 polymorphism increases the risk of RA in CRP-, ACPA-, RF-, and ESR-positive individuals, as well as individuals with DAS28 ≥ 3.20 and functional class III + IV (Table 3).

3.4. CASP5 protein levels and mRNA expression in RA patients and healthy individuals

Next, we measured CASP5 protein and mRNA levels in 40 RA patients and 40 healthy controls. Statistical analysis revealed that *CASP5* mRNA expression was significantly higher in RA patients than in healthy controls ($P < 0.001$; Fig. 1). The means ± standard deviations of *CASP5* mRNA levels in the cases and controls were 3.21 ± 2.89 and 1.00 ± 1.52, respectively. The serum CASP5 levels were significantly higher in RA patients than in healthy controls ($P < 0.001$; Fig. 2). The means ± standard deviations of CASP5 protein levels in cases and controls were 36.53 ± 22.28 and 15.18 ± 11.77

Table 1 Patient Demographics and Risk Factors in Rheumatoid Arthritis.

Variable*	Cases (n = 805)	Controls (n = 1095)	*P*
Age (years)	55.92 (±15.10)	55.01 (±13.01)	0.169
Female/male	603/202	859/236	0.070
Age at onset, years, mean ± SD	45.97(±12.31)	—	—
Disease duration, years, mean ± SD	10.04(±9.66)	—	—
Treatment duration, years, mean ± SD	9.22(±9.10)	—	—
RF-positive, no. (%)	643(79.86%)	—	—
ACPA positive, no. (%)	451(56.02%)	—	—
CRP-positive, no. (%)	470(58.39%)	—	—
ESR, mm/h	35.90(±28.87)	—	—
DAS28	4.49(±1.55)	—	—
Functional class, no. (%)		—	—
I	79(9.81%)	—	—
II	353(43.85%)	—	—
III	314(39.01%)	—	—
IV	59(7.33%)	—	—

* RF: Rheumatoid factor; ACPA: Anti-cyclic citrullinated peptide antibodies; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; DAS28: RA disease activity score.

Table 2 Correlation between the *CASP5* rs7939842 Polymorphisms and the Risk of Rheumatoid Arthritis.

Genotype	Cases (n = 805)		Controls (n = 1095)	OR (95% CI)	*P*	
	n	%		n	%	
CASP5 rs7939842						
AA	749	93.0	1045	98.4	1.00	–
AG	50	6.2	49	1.6	1.42(0.95–2.13)	0.087*
GG	6	0.7	1	0.1	**8.37(1.01–69.68)**	**0.049***
AG + GG	56	7.0	50	4.6	**1.56(1.06–2.31)**	**0.026***
AG + AA	799	99.3	1094	99.9	1.00	–
GG	6	1.4	1	0.1	8.22(0.99–68.37)	0.051
G allele	62	3.9	51	2.3	**1.68(1.15–2.45)**	**0.006**

Bold values are statistically significant ($P < 0.05$).
*After Bonferroni correction, the $P < 0.017$ indicates a statistically significant difference.

Table 3 Stratified Analyses between the *CASP5* rs7939842 Polymorphisms and the Risk of Rheumatoid Arthritis.

Variable	AA	AG	GG	AG vs. AA	GG vs. AA	GG vs. AG + AA	GG + AG vs. AA
	(Case/Control)			OR(95% CI); *P*	OR(95% CI); *P*	OR(95% CI); *P*	OR(95% CI); *P*
Sex							
Male	189/224	11/12	2/0	1.09(0.47–2.52); 0.847*	NA	NA	1.28(0.52–2.88); 0. 445*
Female	560/821	39/37	4/1	1.55(0.97–2.45); 0.063*	5.86(0.65–52.61); 0.181*	5.73(0.64–51.39); 0.191	**1.66(1.06–2.60); 0.026***
Age (years)							
< 55	335/504	18/19	2/0	1.43(0.74–2.76); 0.290*	NA	NA	1.75(0.93–3.29); 0.077*
≥55	414/541	32/30	4/1	1.39(0.83–2.33); 0.204*	5.23(0.58–46.94); 0.232*	5.12(0.57–45.98); 0.241	1.52(0.92–2.49); 0.098*
CRP status							
Positive	429/1045	35/49	6/1	**1.74(1.11–2.72); 0.014***	**14.62(1.75–121.77); 0.004***	**14.15(1.70–117.84); 0.005**	**2.00(1.30–3.06); 0.001***
Negative	320/1045	15/49	0/1	1.00(0.55–1.81); 0.999*	NA	NA	0.98(0.54–1.77); 0.946*
ACPA status							
Positive	413/1045	33/49	5/1	**1.70(1.08–2.69); 0.021***	**12.65(1.47–108.62); 0.012***	**12.26(1.43–105.28); 0.013**	**1.92(1.24–2.98); 0.003***
Negative	336/1045	17/49	1/1	1.08(0.61–1.90); 0.792*	3.11(0.19–49.86); 0.428*	3.10(0.19–49.67); 0.429	1.12(0.64–1.95); 0.688*
RF status							
Positive	594/1045	43/49	6/1	1.54(1.01–2.35); 0.042*	**10.56(1.27–87.89); 0.020***	**10.30(1.24–85.79); 0.022**	**1.72(1.15–2.59); 0.008***
Negative	155/1045	7/49	0/1	0.96(0.43–2.16); 0.928*	NA	NA	0.94(0.42–2.12); 0.889*
ESR status							
Positive	423/1045	36/49	4/1	**1.82(1.16–2.83); 0.008***	9.88(1.10–88.67); 0.043*	**9.53(1.06–85.53); 0.048**	**1.98(1.28–3.04); 0.002***
Negative	326/1045	14/49	2/1	0.92(0.50–1.68); 0.777*	6.41(0.58–70.93); 0.117*	6.44(0.58–71.19); 0.116	1.03(0.58–1.83); 0.931*
DAS28							
< 3.20	170/1045	9/49	1/1	1.13(0.54–2.34); 0.744*	6.15(0.38–98.75); 0.227*	6.11(0.38–98.16); 0.228	1.23(0.61–2.47); 0.561*
≥3.20	579/1045	41/49	5/1	1.51(0.99–2.31); 0.057*	9.02(1.05–77.43); 0.045*	**8.82(1.03–75.69); 0.049**	**1.66(1.10–2.51); 0.015***
Functional class							
I + II	413/1045	17/49	2/1	0.88(0.50–1.54); 0.650	5.06(0.46–55.96); 0.169	5.09(0.46–56.26); 0.168	0.96(0.56–1.65); 0.887*
III + IV	336/1045	33/49	4/1	**2.09(1.32–3.31); 0.001**	**12.44(1.39–111.69); 0.018**	**11.86(1.32–106.44); 0.022**	**2.30(1.48–3.58); < 0.001***

Bold values are statistically significant ($P < 0.05$); OR: odds ratios; CI: confidence intervals.
*After Bonferroni correction, the $P < 0.017$ indicates a statistically significant difference.

ng/mL, respectively. Plasma CASP5 protein and mRNA levels were significantly higher in CRP-positive individuals (Table 4). Stratified analyses revealed that the CASP5 protein and mRNA levels in RA patients remained significantly elevated in men, women, and individuals aged ≥ 55 or < 55 years (Table 5).

4. Discussion

To our knowledge, this is the first study to explore the association between the *CASP5* rs7939842 polymorphism and RA susceptibility in Chinese Han individuals. It revealed that the *CASP5* rs7939842 polymorphism might elevate RA risk among CRP-, ACPA-, RF-, and ESR-positive individuals, as well as individuals with DAS28 ≥ 3.20 and functional class III + IV. The *CASP5* gene expression and plasma CASP5 levels were significantly higher in RA patients than in healthy controls.

CASP5 is involved in the inflammatory response in inflammatory diseases via caspase-1 independent pyroptosis (Man et al. 2017). Initially, CASP5 recognizes cytosolic lipopolysaccharide, leading to gasdermin D cleavage and pyroptosis; Subsequently, the NLRP3 inflammasome and caspase-1-dependent maturation of IL-1β and IL-18 are

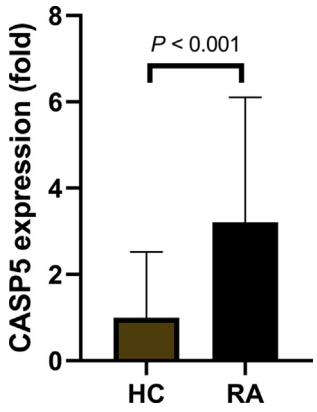

Fig. 1 Comparison of relative *CASP5* mRNA expression between RA patients and healthy controls.

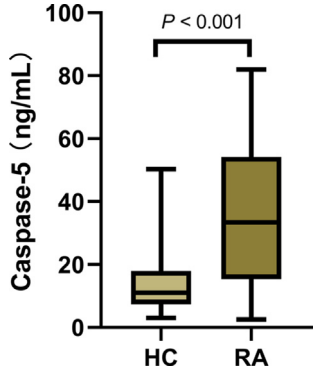

Fig. 2 Comparison of CASP5 plasma levels between RA patients and healthy controls.

activated by the N-terminal fragment after CASP5 binds to lipopolysaccharide (Man et al. 2017; Matikainen et al. 2020). CASP5 is associated with the release of inflammatory markers, including IL-1β and IL-18, which are linked to inflammasome activation (Chadha et al. 2020). The NLRP3 inflammasome is critical to RA (Guo et al. 2018; Shen et al. 2018). In addition, the maturation and release of IL-18 and IL-1β are involved in the pathology of RA (Spel and Martinon 2020). To our knowledge, *CASP5* expression and the plasma CASP5 protein level have rarely been studied in RA patients. In this study, the *CASP5* expression and plasma CASP5 levels appeared to be higher in RA patients and significantly higher in CRP-positive individuals, than in healthy controls. Therefore, CASP5 may have a role in the pathogenesis of RA.

Associations have been identified between *CASP5* polymorphisms and autoimmune diseases. According to He et al., the rs507879, rs518604, and rs523104 *CASP5* gene polymorphisms were not associated with the risk of psoriasis vulgaris in Chinese populations (He et al. 2015). Rui et al. investigated the associations between some CASP5 polymorphisms and RA; Only one locus (rs9651713) was associated with the risk of RA—it was associated with an elevated risk of RA in Chinese Han individuals (Rui et al. 2018). Here, we found a new locus associated with RA risk: the *CASP5* rs7939842 polymorphism was linked to RA risk in Chinese Han individuals such that the AG + GG and GG genotypes significantly increased RA susceptibility. However, this difference did not remain statistically significant after Bonferroni correction. The G allele of the rs7939842 polymorphism was associated with a higher risk of RA. Subgroup analysis indicated that the *CASP5* rs7939842 polymorphism was associated with an elevated risk of RA in CRP-, ACPA-, RF-, and ESR-positive individuals, as well as individuals with DAS28 $\geq$ 3.20 and functional class III + IV. Further analysis indicated that the *CASP5* expression and plasma CASP5 levels were increased in RA patients, particularly in CRP-positive individuals (Table 4). The levels of both *CASP5* expression and plasma CASP5 increased in parallel. These levels remained significantly elevated in stratified analyses of men, women, and

Table 4 Stratification of Associations between Plasma CASP5 protein levels and *CASP5* mRNA expression with other Biomarkers.

Variable	Case	CASP5 protein levels (Mean ± SD) (ng/mL)	*P*	*CASP5* expression (Mean ± SD)	*P*
RF status			0.212		0.069
Negative	9	28.30 ± 22.04		1.68 ± 1.90	
Positive	31	38.92 ± 22.13		3.66 ± 3.00	
ACPA			0.735		0.727
Negative	23	37.57 ± 22.07		3.07 ± 2.88	
Positive	17	35.12 ± 23.16		3.40 ± 2.98	
CRP status			**0.013**		**0.004**
Negative	16	26.04 ± 18.67		1.78 ± 1.82	
Positive	24	43.52 ± 22.07		4.17 ± 3.10	
ESR (mm/h)			0.066		0.056
< 25.00	17	29.01 ± 19.19		2.20 ± 2.43	
≥ 25.00	23	42.09 ± 23.16		3.96 ± 3.02	
DAS28			0.204		0.416
< 3.20	13	30.03 ± 24.76		2.67 ± 3.45	
≥ 3.20	27	39.66 ± 20.75		3.47 ± 2.61	
Functional class			0.731		0.887
I + II	23	35.47 ± 23.81		3.16 ± 3.14	
III + IV	17	37.96 ± 20.66		3.29 ± 2.61	

RF: rheumatoid factor; ACPA: anti-cyclic citrullinated peptide antibodies; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; DAS28: disease activity score in 28 joints. Bold values are statistically significant ($P < 0.05$).

Table 5 Stratification of CASP5 Expression Levels in different Age and Sex between RA and HC.

		Case	CASP5 protein level (Mean ± SD) (ng/mL)	*P*	*CASP5* mRNA level (Mean ± SD)	*P*
Female	RA	31	36.66 ± 23.08	**< 0.001**	3.19 ± 2.90	**0.002**
	HC	30	16.86 ± 11.60		1.17 ± 1.67	
Male	RA	9	36.08 ± 20.55	**0.006**	3.30 ± 3.03	**0.025**
	HC	10	10.13 ± 11.34		0.50 ± 0.83	
Age ≥ 55	RA	24	35.51 ± 21.76	**< 0.001**	3.01 ± 2.55	**0.003**
	HC	24	15.04 ± 11.82		1.03 ± 1.75	
Age < 55	RA	16	38.07 ± 23.68	**0.002**	3.52 ± 3.40	**0.010**
	HC	16	15.38 ± 12.08		0.95 ± 1.15	

RA: rheumatoid arthritis; HC: healthy control. Bold values are statistically significant ($P < 0.05$).

individuals aged ≥ 55 or < 55 years. Therefore, the rs7939842 locus may be involved in the pathogenesis of RA.

There were several limitations in this study. First, unavoidable selection bias among the cases and healthy controls might have caused the sample to be under-representative of the general population, although participants were recruited from two hospitals. Second, gene–gene and gene–environment interactions were not considered. Third, this study investigated only a single nucleotide polymorphism (SNP), thus ignoring synergy among SNPs. Fourth, this study did not explore the association between RA treatment and the *CASP5* rs7939842 polymorphism. Fifth, this study did not analyze the effect of the *CASP5* rs7939842 polymorphism on CASP5 mRNA expression and protein levels because all 80 individuals carried the AA genotype (Supplementary Material), which hindered further effective analysis.

In conclusion, the *CASP5* rs7939842 polymorphism appears to be associated with an increased risk of RA in Chinese Han individuals. Compared with healthy controls, CASP5 protein and *CASP5* gene expression levels were increased significantly in blood. The findings should be validated in larger studies involving multiple populations and functional analyses.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2021.103667.

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