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Correlations of miR-146a and IRAK1 gene polymorphisms with ankylosing spondylitis

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Abstract. – OBJECTIVE: The aim of this study was to explore the correlations of micro ribonucleic acid (miR)-146a and interleukin 1 receptor associated kinase 1 (IRAK1) gene polymorphisms with ankylosing spondylitis.

PATIENTS AND METHODS: A total of 200 patients with ankylosing spondylitis in our hospital were enrolled in the disease group. The diagnosis of ankylosing spondylitis was in accordance with the 1984 New York Revised Criteria for Ankylosing Spondylitis. Meanwhile, 200 healthy people were taken as the control group. Peripheral blood was collected from patients in both disease group and control group. Subsequently, polymorphic regions of rs2910164 and rs7702165 in miR-146a and those of rs763737 and rs3027898 in IRAK1 were amplified by polymerase chain reaction (PCR). The polymorphisms were analyzed by sequencing based on gene expression and clinical data of patients.

RESULTS: The allele distribution of miR-146a rs7702165 (p=0.008) and that of IRAK1 rs3027898 (p=0.004) in disease group were significantly different from those in control group. Besides, the allele T frequency of miR-146a rs7702165 and the allele A frequency of IRAK1 rs3027898 were relatively higher in disease group. Statistically significant differences in the genotype distribution of miR-146a rs2910164 (p=0.032) and rs7702165 (p=0.000) and that of IRAK1 rs3027898 (p=0.001) were observed between disease group and control group. In addition, the frequencies of genotypes CG and TT of miR-146a rs7702165 and the frequency of genotype AA of IRAK1 rs3027898 were higher in disease group. Moreover, the distribution in the dominant model of IRAK1 rs3027898 (p=0.011) and that in the recessive model of miR-146a rs7702165 (p=0.015) showed remarkable differences between disease group and control group. The frequency of CC+CA in the dominant model of IRAK1 rs3027898 and that of TG+GG in the recessive model of miR-146a rs7702165 in disease group were lower than those in control group. Additionally, the haplotype CG distribution of miR-146a rs2910164 and rs7702165 (p<0.043) and the haplotype GA distribution of IRAK1 rs763737 and rs3027898 (p=0.035) in disease group displayed significant differences compared with those in control group. It was discovered that the genotype of miR-146a rs2910164 was correlated with the expressions of miR-146a (p=0.024) and IRAK1 (p=0.002). Similarly, IRAK1 gene polymorphism rs763737 was related to the expression of IRAK1 (p=0.023), Furthermore, miR-146a gene polymorphism rs7702165 was associated with the level of human leukocyte antigen B27 (HLA-B27) (p<0.05), and patients with genotype GG exhibited a lower level of HLA-B27. In addition, it was found that IRAK1 gene polymorphism rs3027898 was correlated with the C-reactive protein (CRP) level of patients (p<0.05), and CRP level was relatively high in patients with genotype CC.

CONCLUSIONS: MiR-146a and IRAK1 gene polymorphisms are prominently associated with ankylosing spondylitis.

Key Words:
MiR-146a, IRAK1, Gene polymorphism, Ankylosing spondylitis.

Introduction

Ankylosing spondylitis is a rheumatic disease that can damage the spine, sacroiliac joints and other parts, seriously affecting the life quality of patients[1,2]. It frequently occurs in males aged 20-30 years old, and the number of male patients is 4 times that of female patients[3,4]. The onset of ankylosing spondylitis is associated with inheritance, infection and other factors, which often leads to dysregulated immune level *in vivo*. Meanwhile, diverse cytokines may participate in the development of the disease[5,6]. Therefore, it is of great significance to find the pathogeny and susceptible factors of ankylosing spondylitis for the prevention of this disease.

Toll-like receptor pathway activates signal transduction *in vivo* by recognizing pathogens, thus inducing immune responses. It has been found related to the onset of a variety of disea-

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ses[7]. Meanwhile, the activation of toll-like receptor pathway is associated with ankylosing spondylitis[8]. IRAK1 (a key molecule in this pathway) and miR-146a (a miRNA regulating IRAK1) gene polymorphisms are evidently correlated with the susceptibility to ankylosing spondylitis[9].

In this study, therefore, 200 patients with ankylosing spondylitis and 200 healthy people were selected as research subjects. Peripheral blood was collected to detect miR-146a gene polymorphisms (rs2910164 and rs7702165) and IRAK1 gene polymorphisms (rs763737 and rs3027898). The expression levels of miR-146a and IRAK1 genes and the levels of human leukocyte antigen-B27 (HLA-B27) and C-reactive protein (CRP) *in vivo* were detected based on haplotype analysis. The aim of this study was to explore the effects of miR-146a and IRAK1 gene polymorphisms on ankylosing spondylitis.

Patients and Methods

General Data

A total of 200 patients with ankylosing spondylitis and 200 healthy people treated in our hospital were taken as research subjects. General data and clinical data, such as name, gender, age, past medical history, family history and drug allergy history, were collected in both disease group and control group. In disease group, there 142 males and 58 females, with an average age of (28.41±2.31) years old. In control group, there were 144 males and 56 females, with an average age of (26.98±3.21) years old. There were no significant differences in general data such as gender and age distribution between disease group and control group (p>0.05). The diagnosis of ankylosing spondylitis was in accordance with the 1984 New York Revised Criteria for Ankylosing Spondylitis. This invesitgation was approved by the Ethics Committee of Zaozhuang Municipal Hospital. Signed written informed consents were obtained from all participants before the study.

Collection and Treatment of Clinical Samples

5-7 mL of peripheral blood was first collected from the elbow vein of research subjects using anticoagulant tubes in both disease group and control group. After centrifugation at 3000 rpm/min for 5 min within 30 min, the middle lamella (nucleated cell layer) was carefully transferred to a new centrifuge tube. Next, genomic deoxyribonucleic acid (DNA) extraction was performed.

DNA Extraction

DNAs were extracted in disease group and control group in strict accordance with the TIANGEN blood genomic extraction kit. In brief, protease K solution was added to the centrifuge tube according to the sample volume solution. Subsequently, peripheral blood samples and buffer were added. After mixing uniformly using a vortex oscillator for 1 min, the mixture was incubated at 65°C for 10 min. DNA solution was then obtained, and the quality of extracted DNAs was detected using a spectrophotometer. The optical density $(OD)_{260}/OD_{280}$ value should be 1.8-2.0.

Polymerase Chain Reaction (PCR) Amplification and Gene Polymorphism Analysis of MiR-146a and IRAK1

The polymorphic regions of miR-146a rs2910164 and rs7702165 as well as IRAK1 rs763737 and rs3027898 were amplified using PCR apparatus. The total PCR system (25 μL) contained 1 μL of each primer, 1 μL of DNA template, 12.5 μL of Taq enzymes and 9.5 μL of ddH_2O. PCR products were sent to Hunan Biotechnology Co., Ltd. (Changsha, China). For sequencing. MiR-146a and IRAK1 gene polymorphisms in disease group and control group were observed through analysis. Primer sequences in the polymorphic regions of miR-146a rs2910164 and rs7702165, as well as IRAK1 rs763737 and rs3027898 were shown in Table I.

Detection of the Expressions of MiR-146a and IRAK1

Real-time fluorescence quantitative PCR was adopted to detect the expressions of miR-146a and IRAK1. GAPDH was used as an internal reference. Primers of each gene were designed *via* Primer Premier 5.0 and synthesized by Shanghai Bioengineering Co., Ltd. (Shanghai, China). Primer sequences of miR-146a and IRAK1 genes were shown in Table I.

Determination of the Clinical Indicators of Patients in Disease Group

The levels of clinical indicators, including HLA-B27 and CRP, in patients in disease group were determined with the assistance of the Immunology Department of the Clinical Laboratory of our hospital. Peripheral blood was extracted from patients in disease group, followed by centrifugation at 3000 rpm/min for 10 min. The supernatant was removed for detection on machine.

Table I. Primer sequences of each locus and each gene.

	Forward/reverse primer	Primer sequence
rs2910164	Forward primer	GCACCCACAACTTCTCGGAG
	Reverse primer	CACCGTGTTCCTCATCACCG
rs7702165	Forward primer	TGAGGAACACGGTGTATGCTG
	Reverse primer	GTTTGGGTGACGAAACCTGGA
rs763737	Forward primer	AGGTTTCGTCACCCAAACATT
	Reverse primer	CGGGCTGTACCCAGAAGGA
rs3027898	Forward primer	CCACCCTGGGTTATGTGCC
	Reverse primer	GAGGATGTGAACGAGGTCAGC
MiR-146a	Forward primer	ACTCCAGAGAAGTCCCAACCA
	Reverse primer	CAGGAATGCAGGGTAGCAGAG
IRAK1	Forward primer	TCCTCCACCAAGCAGTCAAG
	Reverse primer	AAAACCACCCTCTCCAATCCT
GAPDH	Forward primer	CCTGGGTTATGTGCCGCTT
	Reverse primer	GAGGATGTGAACGAGGTCAGC
U6	Forward primer	CTCGCTTCGGCAGCACATAT
	Reverse primer	TTGCGTGTCATCCTTGCG

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 (IBM Corp., Armonk, NY, USA) was adopted for statistical analysis. Measurement data were compared *via* *t*-test and Hardy-Weinberg equilibrium test, while count data were compared *via* chi-square test. The haplotype analysis was conducted on-line through the SHEsis website. $p<0.05$ was considered statistically significant.

Results

Allele Distributions of MiR-146a rs2910164 and rs7702165 and IRAK1 rs763737 and rs3027898 in Disease Group and Control Group

The allele distributions of miR-146a rs2910164 and rs7702165 and IRAK1 rs763737 and rs3027898 in disease group and control group were shown in Table II. It was found that the allele distribution of miR-146a rs7702165 ($p=0.008$) and that of IRAK1 rs3027898 ($p=0.004$) in disease group were significantly different from those in control group. Besides, the allele T frequency of miR-146a rs7702165 and the allele A frequency of IRAK1 rs3027898 were higher in disease group.

Genotype Distributions of MiR-146a rs2910164 and rs7702165 and IRAK1 rs763737 and rs3027898 in Disease Group and Control Group

The genotype distributions of miR-146a rs2910164 and rs7702165 and IRAK1 rs763737 and rs3027898 in disease group and control group were displayed in Table III. Statistically significant differences in the genotype distribution of miR-146a rs2910164 ($p=0.032$) and rs7702165 ($p=0.000$) and that of IRAK1 rs3027898 ($p=0.001$) were observed between disease group and control

Table II. Allele distributions of miR-146a rs2910164 and rs7702165 and IRAK1 rs763737 and rs3027898.

Gene	Locus	Allele	Control group	Disease group	Odds ratio (OR)	95% confidence interval (95% CI)	c^2	*p*
MiR-146a	rs2910164	C	213 (0.532)	207 (0.517)	0.94	0.71-1.24	0.18	0.671
		G	187 (0.468)	193 (0.482)				
	rs7702165	T	202 (0.505)	239 (0.598)	0.68	0.51-0.91	6.91	0.008
		G	198 (0.495)	161 (0.403)				
IRAK1	rs763737	G	211 (0.527)	217 (0.542)	0.94	0.71-1.24	0.18	0.671
		A	189 (0.472)	183 (0.458)				
	rs3027898	C	197 (0.492)	157 (0.393)	1.51	8.11	1.13-1.98	0.004
		A	203 (0.507)	243 (0.608)				

Table III. Genotype distributions of miR-146a rs2910164 and rs7702165 and IRAK1 rs763737 and rs3027898.

Gene	Locus	Genotype	Control group	Disease group	c^2	*p*
MiR-146a	rs2910164	CC	60 (0.300)	44 (0.220)	6.84	0.032
		CG	93 (0.465)	119 (0.595)		
		GG	47 (0.235)	37 (0.185)		
	rs7702165	TT	46 (0.230)	81 (0.405)	15.51	0.000
		TG	110 (0.550)	77 (0.385)		
		GG	44 (0.220)	42 (0.210)		
IRAK1	rs763737	GG	54 (0.270)	56 (0.280)	0.25	0.882
		GA	103 (0.515)	105 (0.525)		
		AA	43 (0.215)	39 (0.195)		
	rs3027898	CC	42 (0.210)	36 (0.180)	13.74	0.001
		CA	113 (0.565)	85 (0.425)		
		AA	45 (0.225)	79 (0.395)		

group. In addition, the frequencies of genotypes CG and TT of miR-146a rs7702165 and the frequency of genotype AA of IRAK1 rs3027898 were higher in disease group.

Analysis of MiR-146a Gene Polymorphisms rs2910164 and rs7702165 and IRAK1 Gene Polymorphisms rs763737 and rs3027898 in Disease Group and Control Group

The analysis results of miR-146a gene polymorphisms rs2910164 and rs7702165 and IRAK1 gene polymorphisms rs763737 and rs3027898 in disease group and control group were shown in Table IV. It was indicated that the distribution in the dominant model of IRAK1 rs3027898 (p=0.011) and that in the recessive model of miR-146a rs7702165 (p=0.015) showed remarkable differences between disease group and control group. Besides, the frequency of CC+CA in the dominant model of IRAK1 rs3027898 and that of TG+GG in the recessive model of miR-146a rs7702165 in disease group were lower than those in control group.

Haplotype Analysis of MiR-146a rs2910164 and rs7702165 and IRAK1 rs763737 and rs3027898

As shown in Table V, the haplotype analysis results of miR-146a rs2910164 and rs7702165 as well as IRAK1 rs763737 and rs3027898 manifested that the haplotype CG distribution of miR-146a rs2910164 and rs7702165 (p<0.043) and the haplotype GA distribution of IRAK1 rs763737 and rs3027898 (p=0.035) in disease group displayed significant differences compared with those in control group.

Correlations of MiR-146a Gene Polymorphisms rs2910164 and rs7702165 and IRAK1 Gene Polymorphisms rs763737 and rs3027898 with Gene Expression

The results of the haplotype analysis of miR-146a gene polymorphisms rs2910164 and rs7702165 and IRAK1 gene polymorphisms rs763737 and rs3027898 (Table VI) manifested that the genotype of miR-146a gene polymorphism rs2910164 was correlated with the expressions of miR-146a (p=0.024) and IRAK1 (p=0.002). Meanwhile, IRAK1 gene polymorphism rs763737 was associated with IRAK1 gene expression (p=0.023).

Correlations of MiR-146a Gene Polymorphisms rs2910164 and rs7702165 and IRAK1 Gene Polymorphisms rs763737 and rs3027898 with the Clinical Indicators of Patients

Associations of miR-146a gene polymorphisms rs2910164 and rs7702165 and IRAK1 gene polymorphisms rs763737 and rs3027898 with the levels of HLA-B27 and CRP were displayed in Figures 1 and 2. It was discovered that miR-146a gene polymorphism rs7702165 was associated with the level of HLA-B27 in patients (p<0.05). Patients with genotype GG exhibited a lower level of HLA-B27. Additionally, IRAK1 gene polymorphism rs3027898 was correlated with the level of CRP in patients (p<0.05), and CRP level was relatively higher in patients with genotype CC.

Table IV. Analysis of miR-146a gene polymorphisms rs2910164 and rs7702165 and IRAK1 gene polymorphisms rs763737 and rs3027898.

	Gene	Locus	Genotype group	Control group	Disease	c^2	p
Dominant model	MiR-146a	rs2910164	CC+CG	153 (0.765)	163 (0.815)	4.93	0.085
			GG	47 (0.235)	37 (0.185)		
		rs7702165	TT+TG	156 (0.780)	158 (0.790)	5.57	0.062
			GG	44 (0.220)	42 (0.210)		
	IRAK1	rs763737	GG+GA	157 (0.785)	161 (0.805)	4.74	0.093
			AA	43 (0.215)	39 (0.195)		
		rs3027898	CC+CA	155 (0.775)	121 (0.605)	9.01	0.011
			AA	45 (0.225)	79 (0.395)		
Recessive model	MiR-146a	rs2910164	CC	60 (0.300)	44 (0.220)	4.67	0.097
			CG+GG	140 (0.700)	156 (0.780)		
		rs7702165	TT	46 (0.230)	81 (0.405)	8.46	0.015
			TG+GG	154 (0.770)	119 (0.595)		
	IRAK1	rs763737	GG	54 (0.270)	56 (0.280)	0.74	0.691
			GA+AA	146 (0.730)	144 (0.720)		
		rs3027898	CC	42 (0.210)	36 (0.180)	4.02	0.134
			CA+AA	158 (0.790)	164 (0.810)		
Hybrid model	MiR-146a	rs2910164	CC	60 (0.300)	44 (0.220)	4.56	0.102
			CG	93 (0.465)	119 (0.595)		
		rs7702165	TT	46 (0.230)	81 (0.405)	4.89	0.087
			TG	110 (0.550)	77 (0.385)		
	IRAK1	rs763737	GG	54 (0.270)	56 (0.280)	5.16	0.076
			GA	103 (0.515)	105 (0.525)		
		rs3027898	CC	42 (0.210)	36 (0.180)	5.17	0.075
			CA	113 (0.565)	85 (0.425)		
Homozygous model	MiR-146a	rs2910164	CC	60 (0.300)	44 (0.220)	4.36	0.113
			GG	47 (0.235)	37 (0.185)		
		rs7702165	TT	46 (0.230)	81 (0.405)	4.27	0.118
			GG	44 (0.220)	42 (0.210)		
	IRAK1	rs763737	GG	54 (0.270)	56 (0.280)	3.98	0.137
			AA	43 (0.215)	39 (0.195)		
		rs3027898	CC	42 (0.210)	36 (0.180)	3.6	0.165
			AA	45 (0.225)	79 (0.395)		

Discussion

Ankylosing spondylitis is a chronic rheumatic immune disease that damages peripheral joints and visceral organs including the lung and intestine[10,11]. Different factors, such as urinary tract and gastrointestinal tract infections, changes in the expression and content of cytokines including IL-2, may trigger ankylosing spondylitis[12]. Hereditary factors are also considered as vital factors influencing the disease. The prevalence rate of the disease in the patients' family members is notably higher than that in the general population. Meanwhile, there is a significant relationship between HLA-B27 gene expression and disease development[13]. As a crucial hereditary factor, gene polymorphisms have been proved to be associated with the onset of ankylosing spondylitis, such as ERAP1[14] and TNF-α[15] gene polymorphisms. Hence, it is of great significance to search for susceptible genes and corresponding loci for early screening and prevention of ankylosing spondylitis.

IRAK1 is a pivotal molecule in the toll-like receptor-related signal transduction pathway, and the latter plays a key role in regulating the immune homeostasis in patients[16]. IRAK1 has endotoxin tolerance, induces inflammatory cascade, and plays an irreplaceable role in the process of bacterial infection[17]. Meanwhile, IRAK1 can regulate the key checkpoint of autoimmune diseases[18]. IRAK1 gene polymorphism has also been confirmed to be related to the onset of various diseases[19,20]. MiR-146a regulates its target gene IRAK1[21], and miR-146a gene polymorphisms are correlated with the progression of gastric cancer[22] and colorectal cancer[23]. In the present study, miR-146a gene polymorphisms (rs2910164 and rs7702165) and IRAK1 gene

Table V. Haplotype analysis of miR-146a rs2910164 and rs7702165 and IRAK1 rs763737 and rs3027898.

Gene	Haplotype	Control group	Disease group	OR	95% CI	c^2	p
MiR-146a	CG	108.56 (0.271)	87.46 (0.219)	0.751	0.543-1.038	3.01	0.043
	CT	104.44 (0.261)	119.54 (0.299)	1.206	0.885-1.643	1.415	0.234
	GG	89.44 (0.224)	73.54 (0.184)	0.782	0.554-1.105	1.947	0.163
	GT	97.56 (0.244)	119.46 (0.299)	1.32	0.965-1.805	3.031	0.082
IRAK1	AA	86.59 (0.216)	98.70 (0.247)	1.186	0.853-1.648	1.03	0.310
	AC	102.41 (0.256)	84.30 (0.211)	0.776	0.558-1.078	2.292	0.130
	GA	116.41 (0.291)	144.30 (0.361)	1.375	1.022-1.850	4.426	0.035
	GC	94.59 (0.236)	72.70 (0.182)	0.717	0.509~1.011	3.621	0.057

Table VI. Correlations of miR-146a gene polymorphisms rs2910164 and rs7702165 and IRAK1 gene polymorphisms rs763737 and rs3027898 with gene expression.

Gene	Locus	Genotype	MiR-146a expression level	p	IRAK1 expression level	p
MiR-146a	rs2910164	CC	2.34±0.13	0.024	23.14±2.14	0.002
		CG	1.01±0.11		37.43±3.74	
		GG	2.55±0.15		23.16±1.65	
	rs7702165	TT	2.24±0.16	0.123	25.23±2.84	0.245
		TG	2.28±0.17		24.58±2.41	
		GG	2.19±0.22		24.88±2.37	
IRAK1	rs763737	GG	2.27±0.14	0.184	21.42±1.03	0.023
		GA	2.23±0.13		20.84±2.22	
		AA	2.20±0.21		31.74±2.87	
	rs3027898	CC	2.21±0.18	0.113	24.45±2.31	0.212
		CA	2.27±0.14		25.74±1.54	
		AA	2.23±0.13		24.89±2.15	

polymorphisms (rs763737 and rs3027898) of peripheral blood were detected in 200 patients with ankylosing spondylitis and 200 healthy people. It was found that there were statistically significant differences in the allele distributions of miR-146a gene polymorphism rs7702165 (p=0.008) and IRAK1 gene polymorphism rs3027898 (p=0.004) between disease group and control group. Disease group exhibited higher frequencies of allele T of miR-146a gene polymorphism rs7702165 and allele A of IRAK1 gene polymorphism rs3027898 than control group. Additionally, the genotype distribu-

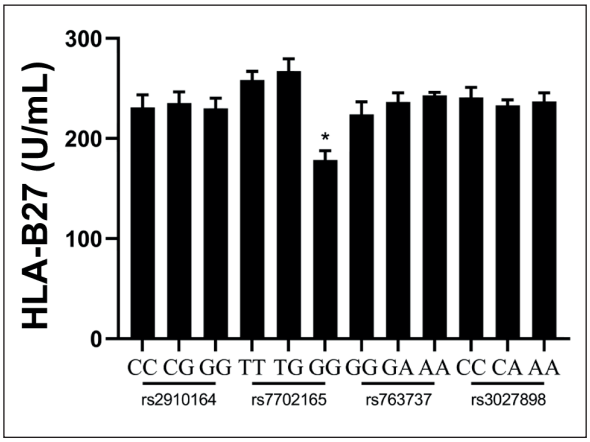

Figure 1. Associations of miR-146a gene polymorphisms rs2910164 and rs7702165 and IRAK1 gene polymorphisms rs763737 and rs3027898 with HLA-B27 level in patients (*p<0.05 *vs.* patients with other genotypes at the same locus).

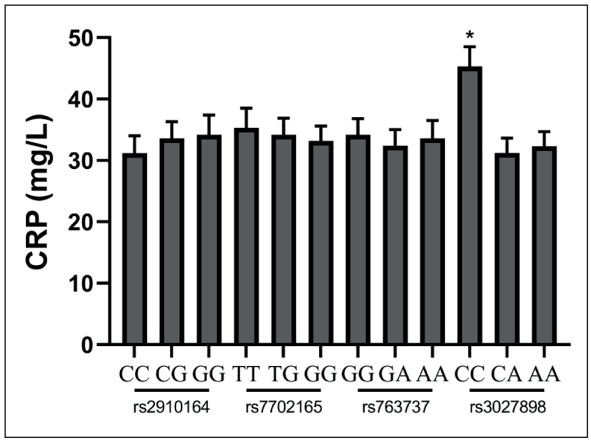

Figure 2. Associations of miR-146a gene polymorphisms rs2910164 and rs7702165 and IRAK1 gene polymorphisms rs763737 and rs3027898 with CRP level in patients (*p<0.05 *vs.* patients with other genotypes at the same locus).

tions of miR-146a gene polymorphisms rs2910164 (p=0.032) and rs7702165 (p=0.000), as well as IRAK1 gene polymorphism rs3027898 (p=0.001) in disease group were different from those in control group. The frequencies of genotype CG of miR-146a gene polymorphism rs2910164, genotype TT of miR-146a gene polymorphism rs7702165 and genotype AA of IRAK1 gene polymorphism rs3027898 were higher in disease group. The above results indicate that the gene polymorphisms of miR-146a and its target gene IRAK1 are closely associated with the occurrence and development of ankylosing spondylitis. In addition, they can be used as important indicators for early screening of high-risk population.

Polymorphism analysis showed that there were statistically significant differences in the distribution in the dominant model of IRAK1 rs3027898 (p=0.011) and that in the recessive model of miR-146a rs7702165 (p=0.015) between disease group and control group. Disease group exhibited lower frequencies of CC+CA in the dominant model of IRAK1 gene polymorphism rs3027898 and TG+GG in the recessive model of miR-146a gene polymorphism rs7702165 than control group. Meanwhile, differences in the distributions of haplotype CG (p=0.043) of miR-146a rs2910164 and rs7702165 as well as haplotype GA (p=0.035) of IRAK1 rs763737 and rs3027898 were observed between disease group and control group. These results suggest that the effects of the gene polymorphisms of miR-146a and its target gene IRAK1 on ankylosing spondylitis may be complicated. In addition, more polymorphic loci are still needed for further research.

MiR-146a plays its role by affecting the expression level of IRAK1. Therefore, the correlations of miR-146a gene polymorphisms (rs2910164 and rs7702165) and IRAK1 gene polymorphisms (rs763737 and rs3027898) with their gene expressions were detected in this study. The results revealed that the genotype of miR-146a gene polymorphism rs2910164 was correlated with the gene expressions of both miR-146a (p=0.024) and IRAK1 (p=0.002). Moreover, the IRAK1 gene polymorphism rs763737 was associated with the expression of IRAK1 (p=0.023). Most previous researches have mainly focused on the relationship between functional gene polymorphism and disease development. However, the regulatory role of miRNAs is ignored. Up to date, increasingly more studies have confirmed that miRNA gene polymorphisms may also affect the expression of their target genes. Based on the results of this study, it is speculated that miR-146a gene polymorphism may affect the expression level of miR-146a to change IRAK1 expression, thus influencing the progression of ankylosing spondylitis.

Finally, the analysis of clinical indicators demonstrated that miR-146a gene polymorphism rs7702165 was correlated with the level of HLA-B27 in patients (p<0.05), and patients with genotype GG had a lower level of HLA-B27. Moreover, IRAK1 gene polymorphism rs3027898 was correlated with the level of CRP in patients (p<0.05), and the level of CRP was higher in patients with genotype CC.

Conclusions

We demonstrated that miR-146a and IRAK1 gene polymorphisms may influence the progression of ankylosing spondylitis by affecting the levels of clinical indicators, such as HLA-B27 and CRP. All our findings suggest that gene polymorphism detection can probably indirectly predict the development of this disease.

Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Hong C, Kwan YH, Leung YY, Lui NL, Fong W. Comparison of ankylosing spondylitis and non-radiographic axial spondyloarthritis in a multi-ethnic Asian population of Singapore. Int J Rheum Dis 2019; 22: 1506-1511.
2) Wang T, Song D, Zheng G, Wang Y. Staged cervical osteotomy:a new strategy for correcting ankylosing spondylitis thoracolumbar kyphotic deformity with fused cervical spine. J Orthop Surg Res 2019; 14: 108.
3) Liang L, Pan Y, Wu D, Pang Y, Xie Y, Fang H. Effects of multidisciplinary team-based nurse-led transitional care on clinical outcomes and quality of life in patients with ankylosing spondylitis. Asian Nurs Res (Korean Soc Nurs Sci) 2019; 13: 107-114.
4) Law L, Beckman RJ, Deminger A, Klingberg E, Jacobsson L, Forsblad-d'Elia H. Factors related to health-related quality of life in ankylosing spondylitis, overall and stratified by sex. Arthritis Res Ther 2018; 20: 284.
5) Aykurt KI, Dulger S, Kasapoglu AM, Guzelsoy M, Turkoglu AR, Altan L, Yildiz T. Effect of cigarette smok-

ing on sexual functions, psychological factors, and disease activity in male patients with ankylosing spondylitis. Aging Male 2019; 22: 109-115.
6) Kuo FC, Chiang KL, Kao YS. Structural damage and motion rhythm of the spine and hip during trunk lateral bending in ankylosing spondylitis patients with mild to moderate radiographic signs. Clin Biomech (Bristol, Avon) 2019; 63: 112-118.
7) Raby AC, Gonzalez-Mateo GT, Williams A, Topley N, Fraser D, Lopez-Cabrera M, Labeta MO. Targeting Toll-like receptors with soluble Toll-like receptor 2 prevents peritoneal dialysis solution-induced fibrosis. Kidney Int 2018; 94: 346-362.
8) Almasi S, Aslani S, Poormoghim H, Jamshidi AR, Poursani S, Mahmoudi M. Gene expression profiling of toll-like receptor 4 and 5 in peripheral blood mononuclear cells in rheumatic disorders: ankylosing spondylitis and rheumatoid arthritis. Iran J Allergy Asthma Immunol 2016; 15: 87-92.
9) Zhang X, Guo Y, Xu X, Tang T, Sun L, Wang H, Zhou W, Fang L, Li Q, Xie P. MiR-146a promotes Borna disease virus 1 replication through IRAK1/TRAF6/NF-kappaB signaling pathway. Virus Res 2019; 271: 197671.
10) Chiowchanwisawakit P, Thaweeratthakul P, Wattanamongkolsil L, Srinonprasert V, Koolvisoot A, Muangchan C, Nilganuwong S, Arromdee E, Katchamart W. relationship between health-related quality of life and patient acceptable symptom state with disease activity and functional status in patients with ankylosing spondylitis in Thailand. J Clin Rheumatol 2019; 25: 16-23.
11) Sag S, Nas K, Sag MS, Tekeoglu I, Kamanli A. Relationship of work disability between the disease activity, depression and quality of life in patients with ankylosing spondylitis. J Back Musculoskelet Rehabil 2018; 31: 499-505.
12) Jamalyaria F, Ward MM, Assassi S, Learch TJ, Lee M, Gensler LS, Brown MA, Diekman L, Tahanan A, Rahbar MH, Weisman MH, Reveille JD. Ethnicity and disease severity in ankylosing spondylitis a cross-sectional analysis of three ethnic groups. Clin Rheumatol 2017; 36: 2359-2364.
13) Qiao M, Qian BP, Zhao SZ, Qiu Y, Wang B, Jiang J. Clinical and radiographic results after posterior wedge osteotomy for thoracolumbar kyphosis secondary to ankylosing spondylitis: comparison of long and short segment. World Neurosurg 2018; 117: e475-e482.
14) Hemmatzadeh M, Babaie F, Ezzatifar F, Mohammadi FS, Ebrazeh M, Golabi AS, Hajaliloo M, Azizi G, Gowhari SA, Shekari N, Sehati N, Hosseinzadeh R, Mohammadi H, Babaloo Z. Susceptibility to ERAP1 gene single nucleotide polymorphism modulates the inflammatory cytokine setting in ankylosing spondylitis. Int J Rheum Dis 2019; 22: 715-724.
15) Li Y, Tang HB, Bian J, Li BB, Gong TF. Genetic association between TNF-alpha -857 C/T polymorphism and ankylosing spondylitis susceptibility: evidence from a meta-analysis. Springerplus 2016; 5: 1930.
16) Vollmer S, Strickson S, Zhang T, Gray N, Lee KL, Rao VR, Cohen P. The mechanism of activation of IRAK1 and IRAK4 by interleukin-1 and Toll-like receptor agonists. Biochem J 2017; 474: 2027-2038.
17) Degirmenci I, Ozbayer C, Kebapci MN, Kurt H, Colak E, Gunes HV. Common variants of genes encoding TLR4 and TLR4 pathway members TIRAP and IRAK1 are effective on MCP1, IL6, IL1beta, and TNFalpha levels in type 2 diabetes and insulin resistance. Inflamm Res 2019; 68: 801-814.
18) Sun M, Yang P, Yang Y, Ye J. Upregulated IRAK1 and IRAK4 promoting the production of IFN-gamma and IL-17 in Behcet's disease. Int Ophthalmol 2018; 38: 1947-1953.
19) Zhang H, Pu J, Wang X, Shen L, Zhao G, Zhuang C, Liu R. IRAK1 rs3027898 C/A polymorphism is associated with risk of rheumatoid arthritis. Rheumatol Int 2013; 33: 369-375.
20) Chatzikyriakidou A, Chorti A, Papavramidis T. Association of IRAK1 gene polymorphism rs3027898 with papillary cancer restricted to the thyroid gland: a pilot study. In Vivo 2019; 33: 2281-2285.
21) Jiang S, Hu Y, Deng S, Deng J, Yu X, Huang G, Kawai T, Han X. MiR-146a regulates inflammatory cytokine production in Porphyromonas gingivalis lipopolysaccharide-stimulated B cells by targeting IRAK1 but not TRAF6. Biochim Biophys Acta Mol Basis Dis 2018; 1864: 925-933.
22) Sun Y, Li M. Genetic polymorphism of miR-146a is associated with gastric cancer risk: a meta-analysis. Eur J Cancer Care (Engl) 2017; 26.
23) Gao X, Zhu Z, Zhang S. MiR-146a rs2910164 polymorphism and the risk of colorectal cancer in Chinese population. J Cancer Res Ther 2018; 14: S97-S99.