Genetic polymorphism of IL-40 in male patients with ankylosing spondylitis

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ABSTRACT
Background: Ankylosing spondylitis (AS) is a kind of arthritis marked by persistent inflammation in the spine's joints, usually at the point where the spine and pelvis meet. Such a disease has different causes, including immunological causes. Therefore, it is necessary to investigate the role of the immune system in this disease to improve a potential treatment strategy. In this context, the present study examined the single nucleotide polymorphisms (SNPs) of the IL-40 gene of IL-40 in a group of Iraqi males with AS to determine their associations in AS pathogenesis. Method: The study included a total of 200 Iraqi male participants (100 with AS and 100 healthy subjects). The detection of SNPs of IL-40 was performed using polymerase chain reaction (PCR) techniques followed by the Sanger sequencing method. Multinomial logistic regression analysis was used to analyze the SNPs under five genetic models (allele, recessive, dominant, and co-dominant). Results: Detected that the heterozygous genotype (GA and AA) and alleles (G and A) of rs72896265, the heterozygous genotype (AG and GG) and alleles (A and G) of rs60316778 and both heterozygous genotype (CC) and alleles (C and T) of rs9915090 were significantly associated with AS risk ($p < 0.001$).

1. Introduction

Ankylosing spondylitis (AS) is an immune-mediated inflammatory arthritis that belongs to the wider family of spondyloarthropathies (SpA), which also includes reactive arthritis, enteropathic arthritis, and psoriasis. Typically, the axial skeleton specifically, the sacroiliac and spinal joints is the target of AS, which results in considerable disability and persistent discomfort. Ankylosis, the formation of new bone, characterizes severe AS disease, leading to vertebral fusion, impaired mobility, and long-term disability [1]. This illness is rather prevalent among inflammatory arthritides, accounting for up to 1.40% [2]. The illness often begins in the sacroiliac (SI) joints, although it can affect any portion of the spine, including the peripheral joints and entheses [3]. AS is more common in men (2-4:1), with the majority of symptoms appearing between the ages of 20 and 35. On average, it takes up to eight years from the start of back discomfort to a clear diagnosis of AS [4]. The processes underlying the disease are not entirely understood. Understanding this mechanism is essential for treating the disease. A complex network of cytokines is implicated in the pathogenesis of AS [5].

Glycoproteins called cytokines help regulate inflammatory responses by acting as both pro- and anti-inflammatory messengers between cells. They are also involved in immune activity coordination [6]. Recently, IL-40 has been one of the cytokines that have garnered interest in AS. It seems that IL-40 is implicated in a lot of inflammatory and autoimmune diseases, including rheumatoid arthritis, primary Sjogren's syndrome, ankylosing spondylitis, type 2 diabetes, Graves' disease, liver cell carcinoma, and systemic lupus erythematosus [7]. In October of 2017, a novel cytokine called IL-40 was discovered. Immunoglobulin A (IgA) levels were significantly lower in IL-40-deficient mice, which was associated with a decreased number of IgA+ B cells. As a result, its role in regulating inflammatory events and the immune response, especially concerning B cells, has been postulated [8]. The C17orf99 gene has around 65 SNPs, with a minor allele frequency of > 10% (http://www.ensembl.org).

To present, none of these SNPs have been studied for their relationship with the risk of any human illness. Several cytokine SNPs have been proposed to modulate serum cytokine levels [9]. An IL-40-expressing cell type includes activated B cells, BM cells, and fetal hepatocytes. This protein has a low molecular weight of 27 kDa. This gene, which may be found on human chromosome 17

(17q25.3), is responsible for encoding it. Due to its lack of structural homology with other well-known cytokines, IL-40 is considered an orphan cytokine [10].

Genome-wide association studies (GWASs) have linked cytokine gene polymorphisms (SNPs) to susceptibility to different diseases, including AS in various ethnic groups [11], [12], [13]. Some SNPs increase the risk of AS, while others decrease it [14], [15], [16].

In this context, three intergenic SNPs (134 rs72896265 G/A and 138 rs60316778 A/G, 198 rs9915090 T/C) of the IL-40 gene were analyzed for the first time in a group of Iraqi males with AS to determine their associations in AS pathogenesis.

Objectives

In this context, three intergenic SNPs (134 rs72896265 G/A and 138 rs60316778 A/G, 198 rs9915090 T/C) of the IL-40 gene were analyzed for the first time in a group of Iraqi males with AS to determine their associations in AS pathogenesis.

2. Methods

The study included a total of 200 Iraqi male participants. The participants' ages ranged from 18 to 73 years. Individuals were divided into 100 patients with ankylosing spondylitis and 100 healthy individuals. Blood samples were obtained from each participant from January 2021 to October 2023 at Al-Yarmouk Teaching Hospital in Baghdad, Iraq. The questionnaire format included various information about each individual, patient and control, such as age, marital status, smoking, family history, disease duration, was reported. Both disease activity and functional impairment were evaluated using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and the Bath Ankylosing Spondylitis Functional Index (BASFI), respectively. The patients were divided into two groups in both instances: those with a score below 4.0 (low) and those with a score over 4.0 (high) [17]. The current study was accomplished in the Department of Biotechnology, College of Science, University of Baghdad.

Three ml of blood was obtained from each participant in this study and placed in EDTA. EasyPure® Blood Genomic DNA Kit (TransGen, biotech. EE121-01) was used to isolate DNA from blood samples in EDTA tube, following the recommendations of the manufacturer. The 2000c Nanodrop spectrophotometer (Thermo Fisher Scientific, USA) was used to evaluate the concentration and purity of extracted DNA to determine the quality of samples for subsequent analysis. The concentration of DNA ranged from (43-86) ng/µl and the absorbance of the samples was measured at two distinct wavelengths to determine DNA purity (260 and 280nm). The presence of an A260/A280 ratio of 1.8-2.0 suggested that the DNA sample was pure.

The complete sequence of the gene (C17orf99) was downloaded from the NCBI site (Gene ID: 100141515) and used to identify the interested SNPs. The results were analyzed under UV light using a 1.5% agarose gel containing 0.5 g/mL ethidium bromide after successful PCR amplification was confirmed with the QIAGEN Rotor gene Q System (Germany). After PCR amplification, the fragment was sequenced using Sanger sequencing. Geneious software was used to assess the data after alignment with a gene bank reference sequence. Primers were designed and listed in table (1).

Table 1: Primer sequence used in PCR

SNP	Sub.	Primer	Sequence (5'-3')	Primer size	Replicon size	Ref.
***IL-40* Gene**	G/A	F	CTTACTGAGGAGGAGGGGTTT	21	517	In this study
		R	CTGGGTTGGGCTTCCTTTAG	20		

The PCR mix contains 2xEasyTaq® PCR SuperMix (12.5 µl), Nuclease free water (6.5µl), Forward primer (1µl), Reverse primer (1µl) and DNA (4µl) to obtain the final volume (25µl). Optimal conditions for amplification were as follows: one 10-minute cycle at 94°C, followed by 40 cycles at 94°C (30 s), 56°C (15 min), and 72°C (20 s) in a thermal cycler device. The PCR mix tube was then

transferred to the device. The QIAGEN Rotor gene Q Real-time PCR System (Germany) was used to measure allele-specific fluorescence, which allowed for the determination of SNP genotypes.

The genotypes and SNP alleles were described using frequency (%) and absolute amount. A two-tailed Fisher's exact test was used to see if there were significant differences. We evaluated the Hardy-Weinberg equilibrium (HWE) in HC using Pearson's chi-square goodness-of-fit test. To determine if SNPs were associated with AS susceptibility, multinomial logistic regression was employed. An odds ratio (OR) and a 95% confidence interval (CI) were used to present the results. Allele, recessive, dominant, over-dominant, and co-dominant models of inheritance were all utilized within this framework. To analyze statistically significant differences among normally distributed variables, analysis of variance (ANOVA) with post hoc least significant difference (LSD) was used. We used the Mann-Whitney U test to determine if there were statistically significant differences, and we presented skewed variables as median and interquartile range (IQR: 25-75%). Statistical significance was granted to results where the p-values were less than 0.05. We used IBM SPSS Statistics 25.0 (Armonk, NY: IBM Corp.) to do the statistical analyses.

3. Results and Dsicussion

The current study included 100 AS patients with a mean duration of disease of 8.87 ±6.38 years and it ranged from 3 months to 26 years. The study also included 100 health control subjects (HC). Comparison of mean age between AS and HC revealed a significant difference ($p < 0.001$); 41.10 ±13.00 years versus 33.54 ±7.92, respectively. Concerning marital status, the proportions of married and not married patients were 74 (74.0 %) and 26 (26.0 %); while those of HC were 62 (62.0 %) and 38 (38.0 %); the difference was not significant ($p = 0.069$). Positive family history was reported in 24 (24.0 %) cases of AS group which was significantly higher ($p < 0.001$) than that reported in HC; 4 (4.0 %). Smoking was reported in 36 (36.0 %) AS and 34 (34.0 %) HC; therefore, there was no significant variation ($p = 0.767$), as displayed in table (4).

Table 4: Baseline characteristics of men with ankylosing spondylitis and healthy subjects.

Characteristic	AS n = 100	HC n = 100	P-value
Age (years)			
Mean ±SD	41.10 ±13.00	33.54 ±7.92	< 0.001 I ***
Range	18 -73	18 -52	
Marital status			
Married	74 (74.0 %)	62 (62.0 %)	0.069 C NS
Not married	26 (26.0 %)	38 (38.0 %)	
Family history			
Positive	24 (24.0 %)	4 (4.0 %)	< 0.001 C ***
Negative	76 (76.0 %)	96 (96.0 %)	
Smoking			
Smoking	36 (36.0 %)	34 (34.0 %)	0.767 C NS
Not smoker	64 (64.0 %)	66 (66.0 %)	

SD: standard deviation; **AS**: Ankylosing spondylitis; **HC**: Healthy controls; **n**: number of cases; **I**: independent samples t-test; **C**: chi-square test; **NS**: not significant; ***: significant at $p \leq 0.01$; **p**: probability (significance was determined using one-way analysis of variance (ANOVA) post-hoc least significant difference (LSD) test; significant p-value is indicated in bold).

Analysis of IL-40 gene SNPs

The analysis of each SNP was conducted using allele, recessive, dominant, over-dominant, and co-dominant genetic models, as shown in Table (5). For 134 rs72896265, genotype GG, in co-dominance mode, was regarded as the reference genotype. The heterozygous genotype GA was significantly more frequent in the AS group in comparison with the HC group, 53.0 % versus 25.0 %,

respectively ($p < 0.001$), for that reason this genotype was considered a risk factor with an odds ratio of 4.31. In addition, the homozygous genotype AA was significantly more frequent in the AS group in comparison with the HC group, 15 % versus 10 % ($p = 0.013$), thus it was regarded as a risk factor with an odds ratio of 3.05. Regarding dominant mode, the homozygous GG genotype was contrasted to the other 2 genotypes (GA+AA) and the results revealed a significantly lower rate in the AS group when compared to the HC group, 32 % versus 65 %, making this genotype a protective factor against the disease with an odds ratio of 0.25 which is an indication of 75 % protection. Regarding recessive mode, the homozygous AA genotype was contrasted to the other 2 genotypes (GA+GG) and the results revealed no significant difference in its rate between the AS group and HC group ($p = 0.285$), therefore, it is neither a risk factor nor a protective factor. Allele analysis revealed that allele G was significantly less frequent and that allele A was significantly more frequent in the AS group in comparison with the HC group, 57.5% versus 77.5 % and 42.5 % versus 22.5 %, respectively ($p < 0.001$), thus, allele G is a protective factor with an odds ratio of 0.41 (69 % protection against the disease) and allele A is a risk factor with an odds ratio of 2.44.

In the co-dominance mode of 138 rs60316778, genotype AA was regarded as the reference genotype. The heterozygous genotype AG was significantly less frequent in the AS group in comparison with the HC group, 26.0 % versus 50.0 %, respectively ($p < 0.001$), for that reason this genotype was considered a protective factor with an odds ratio of 0.29 (protection of 71 % against the disease). In addition, the homozygous genotype GG was significantly less frequent in the AS group in comparison with the HC group, 3 % versus 10 % ($p < 0.001$), thus it was regarded as a protective factor with an odds ratio of 0.17 (protection of 83 % against the disease). Regarding dominant mode, the homozygous AA genotype was contrasted to the other 2 genotypes (AG+GG) and the results revealed a significantly higher rate in the AS group when compared to the HC group, 71 % versus 40 % ($p = 0.004$), making this genotype as a risk factor with an odds ratio of 3.67. Regarding recessive mode, the homozygous GG genotype was contrasted to the other 2 genotypes (AG+AA) and the results revealed a significantly lower rate in the AS group when compared to the HC group, 3 % versus 10 % ($p = 0.045$), making this genotype as a protective factor with an odds ratio of 0.28 (protection against the disease of 72 %). Allele analysis revealed that allele A was significantly more frequent and that allele G was significantly less frequent in the AS group in comparison with the HC group, 84.0 % versus 65.0 % and 16.0 % versus 35.0 %, respectively ($p < 0.001$), thus, allele A is a risk factor with an odds ratio of 2.83 and allele G is a protective factor with an odds ratio of 0.35 (65 % protection against the disease).

In the codominance mode of 198 rs9915090, genotype CC was regarded as the reference genotype. The heterozygous genotype CT was not significantly different in the AS group in comparison with HC, 42.0 % versus 40.0 %, respectively ($p = 0.326$), for that reason this genotype was neither a risk factor nor a protective factor. In addition, the homozygous genotype TT was significantly more frequent in the AS group in comparison with the HC group, 15 % versus 5 % ($p = 0.011$), thus it was regarded as a risk factor with an odds ratio of 2.32. Regarding dominant mode, the homozygous CC genotype was contrasted to the other 2 genotypes (CT+TT) and the results revealed no significant difference between the AS group and HC, 43 % versus 55 % ($p = 0.090$), for that reason, this genotype was neither a risk factor nor a protective factor. Regarding recessive mode, the homozygous TT genotype was contrasted to the other 2 genotypes (CT+CC) and the results revealed a significantly higher rate in the AS group when compared to HC, 15 % versus 5 % ($p = 0.018$), making this genotype as a risk factor with an odds ratio of 3.35. Allele analysis revealed that allele C was significantly less frequent and that allele T was significantly more frequent in the AS group in comparison with HC, 64.0 % versus 75.0 % and 36.0 % versus 25.0 %, respectively ($p < 0.017$), thus, allele C is a protective factor with an odds ratio of 0.59 (41 % protection against the disease) and allele T is a risk factor with an odds ratio of 1.69.

Table 5: Multinomial logistic regression of C17orf99 single nucleotide polymorphisms (134 rs72896265 G/A and 138 rs60316778 A/G, 198 rs9915090 T/C) in males with ankylosing spondylitis and healthy control.

C17orf99 SNP/ genetic model	Allele/ genotype/ haplotype	AS; n=100		HC; n=100		OR (95% CI)	p-value
		n	%	n	%		
134 rs72896265 G/A							
Allele	G	117	57.5	155	77.5	0.41 (0.26 -0.63)	<0.001 C ***
	A	83	42.5	45	22.5	2.44 (1.58 -3.78)	
Co-dominance	GG	32	32.0	65	65.0	Reference	Reference
	GA	53	53.0	25	25.0	4.31 (2.28-8.14)	<0.001 C ***
	AA	15	15.0	10	10.0	3.05 (1.23 -7.53)	0.013 C *
Dominant	GG	32	32.0	65	65.0	0.25 (0.14 -0.46)	<0.001 C ***
	GA+AA	68	68.0	35	35.0	Reference	Reference
Recessive	AA	15	15.0	10	10.0	1.59 (0.68 -3.73)	0.285 C NS
	GG+GA	85	85.0	90	90.0	Reference	Reference
138 rs60316778 A/G							
Allele	A	168	84.0	130	65.0	2.83 (1.75 -4.55)	<0.001 C ***
	G	32	16.0	70	35.0	0.35 (0.22 -0.57)	
Co-dominance	AA	71	71.0	40	40.0	Reference	Reference
	AG	26	26.0	50	50.0	0.29 (0.16 -0.54)	<0.001 C ***
	GG	3	3.0	10	10.0	0.17 (0.04 -0.65)	0.004 C **
Dominant	AA	71	71.0	40	40.0	3.67 (2.04 -6.62)	0.004 C **
	GG+AG	29	29.0	60	60.0	Reference	Reference
Recessive	GG	3	3.0	10	10.0	0.28 (0.07 -1.04)	0.045 C *
	AA+AG	97	97.0	90	90.0	Reference	Reference
198 rs9915090 T/C							
Allele	C	128	64	150	75	0.59 (0.39 -0.91)	0.017 C *
	T	72	36	50	25	1.69 (1.10 -2.60)	
Co-dominance	CC	43	43	55	55	Reference	Reference
	CT	42	42	40	40	0.81 (0.50 -1.33)	0.326 C NS

	TT	15	15	5	5	2.32 (0.82 -6.55)	0.011 C *
Dominant	CC	43	43	55	55	0.62 (0.35 -1.08)	0.090 C NS
	TT+CT	57	57	45	45	Reference	Reference
Recessive	TT	15	15	5	5	3.35 (1.17 -9.62)	0.018 C *
	CC+CT	85	85	95	95	Reference	Reference

C: chi-square test; **AS**: Ankylosing spondylitis; **HC**: Healthy controls; **n**: number of cases**; OR**: odds ratio; **CI:** 95 % confidence interval; **NS:** not significant; *: significant at **p** ≤ 0.05; ***: significant at p ≤ 0.001.

The frequency of each SNP based on Hardy Weinberg equilibrium is shown in Table 6. The observed counts of genotypes GG, GA and AA of rs72896265 G/A SNPs were reported in 97, 78 and 25 cases, respectively of the total sample included in this study and they showed no significant difference in comparison with expected counts (p = 0.142). The observed counts of genotypes GG, GA and AA were reported in 32, 53 and 15 cases, respectively of the AS group and they showed no significant difference in comparison with expected counts (p = 0.360). The observed counts of genotypes GG, GA and AA were reported in 65, 25 and 10 cases, respectively of HC and they showed no significant difference in comparison with expected counts (p = 0.005).

The observed counts of genotypes AA, AG and GG of rs60316778 A/G SNP were reported in 111, 76 and 13 cases, respectively of the total sample included in this study and they showed no significant difference in comparison with expected counts (p = 0.999). The observed counts of genotypes AA, AG and GG were reported in 71, 26 and 3 cases, respectively of the AS group and they showed no significant difference in comparison with expected counts (p = 0.743). The observed counts of genotypes AA, AG and GG were reported in 40, 50 and 10 cases, respectively of HC and they showed no significant difference in comparison with expected counts (p = 0.323). The observed counts of genotypes CC, CT and TT were reported in 98, 82 and 20 cases, respectively of the total sample included in this study and they showed no significant difference in comparison with expected counts (p = 0.642).

The observed counts of genotypes CC, CT and TT of rs9915090 T/C SNP were reported in 43, 42 and 15 cases, respectively of the AS group and they showed no significant difference in comparison with expected counts (p = 0.784). The observed counts of genotypes CC, CT and TT were reported in 55, 40 and 5 cases, respectively of HC and they showed no significant difference in comparison with expected counts (p = 0.505), as shown in table 7.

Table 6: The frequency of IL-40 SNPs based on Hardy Weinberg equilibrium

SNP	Total n = 200	Patients group n = 100	Control group n = 100
IL-40 (134 rs72896265) G/A			
GG	97	32	65
GA	78	53	25
AA	25	15	10
χ^2	2.157	0.838	8.018
p	0.142 NS	0.360 NS	0.005 **
IL-40 (138 rs60316778)A/G			
AA	111	71	40
AG	76	26	50
GG	13	3	10
χ^2	0.000	0.107	0.978
p	0.999 NS	0.743 NS	0.323 NS
IL-40 (198 rs9915090)T/C			
CC	98	43	55

CT	82	42	40
TT	20	15	5
χ^2	0.217	0.784	0.444
p	0.642 NS	0.376 NS	0.505 NS

NS: not significant; **: significant at $p \leq 0.005$

Table 7: Comparison of genotypes and alleles of IL-40 SNPs between patients group and control group.

C17orf99 SNP	**genotypes**	**Total n = 200**		**AS n = 100**		**HC n = 100**		**p-value**
		N	**%**	**n**	**%**	**n**	**%**	
134 rs72896265 G/A	GG	97	48.5	32	32	65	65	0.005 **
	GA	78	39	53	53	25	25	
	AA	25	12.5	15	15	10	10	
138 rs60316778 A/G	AA	111	55.5	71	71	40	40	0.323 NS
	AG	76	38	26	26	50	50	
	GG	13	6.5	3	3	10	10	
198 rs9915090 T/C	CC	98	49	43	43	55	55	0.505 NS
	CT	82	41	42	42	40	40	
	TT	20	10	15	15	5	5	

C17orf99: Chromosome 17 open reading frame 99; SNP: Single nucleotide polymorphism; AS: Ankylosing spondylitis; HC: Healthy controls; p: Two-tailed probability.

The average age of male AS patients was 41.10 years, according to this study. A recent study found that out of 2579 patients with axial spondyloarthritis (axSpA), the vast majority (92%) had an age at onset of less than 45 years, and this was true across all geographic regions evaluated. There are 94% in Asia, 92% in Europe and North America, 89% in Latin America, and 91% in the Middle East [18]. In the study of [19], the age at which patients first experienced AS was a factor in the hereditary risk. The risk was higher for patients whose illnesses started before the age of 25 compared to those whose illnesses started after that age. According to this study, 100 AS patients had an average age of 41.96±9.11 [20]. The median age of the patients and controls was 40.7 and 40.8 years old, respectively, which is not significantly different from the results of the study by [21]. Additionally, it has been stated that 132 patients with AS had an average age of 37.61 ± 10.0 years, which is lower than the results of this study. However, these results were lower than those of a study by [22], which found an average age of 59.3 ± 12.1 years for 974 patients with AS. One possible explanation for these findings is that different research used different sample sizes. Although female patients can also experience AS it is more common in males and typically affects those less than 45 years old [23]. Different people with AS may experience different symptoms and how the disease develops at different ages.

Furthermore, the average ± standard deviation of the time it took for the disease to progress was 8.87 ±6.38 years. This value was similar to that of a study published by [24], which indicated that the median time it took for AS to be diagnosed was 8.0 years; however, it was lower than the value reported by [25], which indicated that the duration of AS was 20.5±11.8 years. Environmental and genetic factors, as well as delays in diagnosis and treatment, as well as the frequency and duration of illness monitoring, all contribute to the fact that AS will last varying amounts of time in various individuals [26], [27], [28]. Regular assessments of disease activity and functional impairment in AS patients are recommended by clinical guidelines. This was achieved by utilizing the BASFI, which

may be evaluated on a scale from 0 to 10. According to previous research, an AS score of 4 or above indicates active disease, while a score below 4.0 indicates clinical improvement of AS [29], [30]. The condition was determined to be clinically developing, as the Mean ±SD of BSAFI was 3.41±1.68 according to this study's data. Consistent with the results of the study cited in [24], the average BSAFI for AS patients was 2.4. According to this study, there was no significant difference between married mothers with AS (67%; n=1322) and healthy mothers (67%; n=8377) [31]. Another finding was that there was a statistically significant difference in family history between the research groups. Assuming that risks between siblings were entirely attributable to genetics, a study described by [32] found a heritability of 77% (95% CI 73, 80). Genomic factors account for more than 90% of the risk of AS, the prototypic seronegative arthropathy. It is recognized that the illness has a strong genetic component. Finding the genes involved in this syndrome via candidate gene or family-based approaches has been slower than with most prevalent heritable disorders [32]. The degree to which variations in a trait's genes explain the observed variation in that trait is called its heritability. Twin studies have demonstrated that AS has a heritability of 90-99% [33], which is significantly higher than other traits including RA (40%), inflammatory bowel disease (65-75%), and adult height (80-90%). Because of this, AS would be considered a highly heritable trait [34]. On the other hand, only 40 sets of twins were included in the research on AS heredity factors [33].

Medical professionals around the world are worried about the correlation between cigarette smoking and other diseases. It can lead to dysregulation by influencing adaptive and innate immunity, and it can enhance pathogenic immunological responses or decrease defence immunity. Smoking and spondyloarthropathy do, however, have a complicated association. Smoking may exacerbate the illness, according to research, but the exact reason is yet unclear [35]. The chronic inflammatory response is thought to play a causal role in AS. Evidence suggests that several cytokine pathways, including pro-inflammatory and anti-inflammatory interactions, contribute to disease etiology [36], [37]. There is no available data or information on the role of SNPs under study in the pathogenesis of AS. To date, there is a dearth of studies investigating the genotypes in the IL-40 promoter region. This study indicated that SNPs of IL-40 may have a significant role in the incidence of AS.

According to a distinct study by Abed et al. [13], TT, CT, and CC are the three different genotypes that were found inside the IL-40 promoter region. The research findings indicate that individuals diagnosed with autoimmune thyroid disease, encompassing autoimmune hypothyroidism (AIH), have a higher prevalence of the C allele within the promoter region of the IL-40 gene, which has a substantial correlation with AIH ($p = 0.000$). Moreover, a significant association between CC genotypes and AIH ($p = 0.000$) was observed. The findings suggest that the homozygotes CC of the IL-40 gene, serve as a potential predisposing factor in AIH development in the Iraqi population. In addition, other Iraqi studies have reported that an intergenic SNP located in a non-coding region, rs2004339 A/G, of the gene encoding interleukin-40 is associated with a risk of rheumatoid arthritis (RA) in Iraqi women. Results revealed that the mutant A allele and the homozygous AA genotype of rs2004339 were significantly associated with an increased risk of RA (odds ratio [OR] = 3.37 and 7.44, respectively; $p < 0.001$) [42].

4. Conclusion

Age is considered a risk factor for AS. Different alleles and genotypes may play a significant role in the incidence of AS.

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Authors' contributions

The student, Rehab Murad Kadhum, purchased the materials and materials, conducted the experiments and wrote the research, and Dr. Reema Muhammad supervised and conducted the scientific evaluation.

Ethics approval and consent to participate

The manuscript does not report on or involve any animals, humans, human data, human tissue or plants, “Not applicable”.

Patient consent for publication

The manuscript does not contain data from any individual person, “Not applicable”.

Competing interests

The authors declare that they have no competing interests.

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