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ORIGINAL ARTICLE

IL10 Promoter Polymorphisms are Associated with Rheumatic Heart Disease in Saudi Arabian Patients

Atiyeh M. Abdallah[1] · Aisha Alnuzha[2] · Abdulhadi H. Al-Mazroea[1] · Amr E. Eldardear[1] · Ala Y. AlSamman[1] · Yousef Almohammadi[3] · Khalid M. AL-Harbi[1,2]

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Abstract Rheumatic heart disease (RHD) is an inflammatory disease that develops following streptococcal infections. IL10 helps to balance immune responses to pathogens. *IL10* polymorphisms have been associated with RHD, although results remain inconclusive. Our aim was to investigate the association between *IL10* polymorphisms and RHD in Saudi Arabian patients. *IL10* promoter polymorphisms (-1082A/G, -829C/T, and -592C/A) were genotyped in 118 RHD patients and 200 matched controls using the TaqMan allelic discrimination assay. There was a significant difference in *IL10*-1082 genotype frequency between patients and controls ($p = 0.01$). -1082G allele carriage (GG+GA vs AA) and the (-1082, -819, -592) GCC haplotype carriage were associated with an increased risk of RHD ($p = 0.004$, OR 2.1, 95 % CIs 1.7–3.4 and $p = 0.004$, OR 2, 95 % CIs 1.3–3.4, respectively). The ACC haplotype was associated with a decrease in RHD risk ($p = 0.015$, OR 0.6, 95 % CIs 0.4–0.9). *IL10* promoter polymorphisms may play an important role in the development of RHD and provide an opportunity for therapeutic stratification.

Keywords Interleukin 10 · Polymorphism · Haplotype · Rheumatic heart disease

✉ Khalid M. AL-Harbi
drkharbi@yahoo.com

[1] Cardiogenetic Team, Department of Pediatrics, College of Medicine, Taibah University, Al-Madinah, Saudi Arabia

[2] Children & Maternity Hospital, Al-Madinah, Saudi Arabia

[3] Security Forces Medical Centre, Al-Madinah, Saudi Arabia

Introduction

Rheumatic heart disease (RHD) is an inflammatory disease that can develop following upper respiratory tract infection with *Streptococcus pyogenes*. RHD remains a common cause of acquired heart disease in children in many developing countries, even when antibiotics are available [4, 28]. In one report from Saudi Arabia, rigorous clinical and cardiac examination of 9418 schoolchildren aged between 6 and 15 years in the Western District of the country revealed a RHD prevalence of 2.4 per 1000 children [3], one of the highest reported worldwide. In another hospital-based study of children presenting with acute rheumatic fever (ARF) in the Riyadh Province, cardiac involvement was reported in over half of cases and was especially common in rural areas with a relative lack of medical facilities [21].

Although the exact etiology of RHD is not fully understood, it is thought that a persistent and uncontrolled inflammatory response to *S. pyogenes* infection in genetically susceptible hosts contributes to the development of the disease [2, 7, 8]. Interleukin 10 (IL10; encoded by the *IL10* gene; Gene ID 3586) is a pluripotent anti-inflammatory cytokine and important inflammatory modulator that plays an important role in balancing immune responses to pathogens and maintaining tissue homeostasis [12]. However, impaired IL10 expression or signaling can impair antigen clearance during acute bacterial infections, which may create a favorable environment for persistent inflammation [19].

IL10 is located on chromosome 1q31–32 and is encoded by five exons, with over 30 single nucleotide polymorphisms (SNPs) reported in the promoter region [25]. Three of these SNPs, rs1800896 (-1082A/G), rs1800871 (-829C/T), and rs1800872 (-592C/A), have been widely studied in a number of autoimmune and infectious diseases; rs1800871 and

rs1800872 are in high linkage disequilibrium (LD). In viral infections, the -1082GG genotype is associated with persistent infection [14], and in acquired pneumonia infection, -1082GG increases the risk of septic shock compared to individuals with -1082GA or -1082AA genotypes [27]. An intron two *IL10* polymorphism is in high LD with -592C/A and -829C/T and has been shown to be strongly associated with Behçet's disease (an autoimmune vasculitis) in patients from Middle Eastern populations in a genome-wide association study [23]. All three promoter SNPs have been implicated in the expression and production of IL10 [24].

IL10 has been reported as a biomarker for rheumatic valve disease, especially in patients with chronic rheumatic fever [17]. However, although a few studies have investigated the association between *IL10* promoter polymorphisms and RHD, the results are inconclusive, and no data are available with respect to *IL10* polymorphisms and RHD in the Saudi population. Therefore, the aim of this study was to evaluate the association between *IL10* promoter polymorphisms (-1082A/G, -829C/T, and -592C/A) and RHD susceptibility or severity in Saudi patients.

Methods

Study Population

This is a case–control study carried out at Taibah University, Al-Madinah, Saudi Arabia. The Center for Genetics and Inherited Diseases (CGID) and the Maternity & Children Hospital ethics committees approved the study, and the authors followed the norms of the World Medical Association Declaration of Helsinki. All adult patients and donors or the parents or guardians of child participants (<18 years old) signed a fully informed and written consent form approved by the committees. One hundred and eighteen unrelated RHD patients attended the Pediatric Cardiology Clinic at the Maternity and Children Hospital, Al-Madinah region, Saudi Arabia, between March 2013 and June 2014. Diagnosis was made according to modified Jones' criteria at initial diagnosis and confirmed by echocardiography [10]. Complete clinical and laboratory assessments were performed in all patients. Patients were subgrouped according to echocardiographic findings into either mitral valve lesion (MVL) or multiple valve lesions including the mitral valve, termed a 'combined valve lesion' (CVL). Patients with rheumatic fever but without valvular disease, heart complications, or other inflammatory conditions were excluded from the study. Two hundred age-, gender-, and ethnically matched unrelated healthy volunteers with no history of cardiac diseases or autoimmune disease were included in the study as controls. All participants were of Saudi Arabian ethnicity.

Genotyping of the IL10 Promoter Polymorphisms

Genomic DNA was prepared from 2 ml whole peripheral blood using the QIAamp DNA Mini Kit (Qiagen, Germany). Extracted DNA was quantified by spectrophotometry (MaestroNano; MaestroGen, Las Vegas, USA). Samples were stored at −20 °C until use. Allelic variations were genotyped using the duplex quantitative TaqMan 5′ Allelic Discrimination Assay (Applied Biosystems, Foster City, CA) using established protocols as directed by the manufacturer. The assay IDs were: rs1800896 (C_1747360_10), rs1800871 (C_1747362_10), and rs1800872 (C_1747363_10). Briefly, assays were performed in a final volume of 10 µl (including TaqMan Genotyping Master Mix, 40 × SNP Genotyping Assay Mix, DNase-free water, and 10 ng DNA) in 96-well plates using the following amplification protocol: 95 °C for 10 min followed by 50 cycles at 95 °C for 15 s and 60 °C for 1 min (annealing/extension). Fluorescence detection took place at 60 °C. Non-template controls were included in each run. The genotype call rate for all SNPs was over 99 %. Duplicate genotyping of 10 % of samples selected at random was performed for quality control. Assays were performed using StepOnePlus system, and the automated sequence detection software (SDS) version 2.3 was used for auto-calling (Applied Biosystems, Foster City, CA, USA).

Statistical Analysis

Statistical analysis of the data was performed using Statistical Package for Social Sciences (SPSS) version 17 (IBM, Chicago, USA). All results were confirmed using a freely available online statistical tool, VassarStats (www.vassarstats.net). The unpaired Student's *t* test was used to compare the mean age between groups. Genotype and allele frequencies were determined by direct counting in patients and controls. Haplotypes were constructed by inference and subsequently checked using the haplotype construction program PHASE (version 2.1.1), which employs a statistical haplotype reconstruction method [30]. The carrier frequency of each haplotype was determined by direct counting. The differences in the polymorphism genotype distributions between cases and controls were analyzed using Chi-square contingency table analysis or Fisher's exact test, as appropriate. A p value equal to or less than 0.05 was considered statistically significant. Odds ratios (ORs) and 95 % confidence intervals (CIs) were calculated. Odds ratios (OR) are used to measure the relative odds of the occurrence of the disease. Meanwhile, the 95 % confidence interval (CI) is used to determine the degree of uncertainty of the OR. The narrower the CI, the more precise the point estimate. Chi-square is a statistical test to determine whether results are statistically significant. There would be a greater likelihood

of a significant statistical difference between the groups if there is a higher Chi-square value. All genotyping data were checked for any deviation from Hardy–Weinberg equilibrium (HWE) using Chi-square contingency table analysis.

Results

Patient Characteristics

The baseline characteristics of the patients at disease presentation according to echocardiographic findings are presented in Table 1. Three hundred and eighteen Saudi Arabian individuals (200 controls and 118 patients) were genotyped. There was no statistically significant difference between the groups in terms of age and gender (Table 1). The MVL group contained 56 patients, and the CVL group contained 62 patients. Carditis was present in 61 % of patients, and arthritis was found in 55 % of patients.

Distribution of IL10 Polymorphisms in a Saudi Population

The genotypes, allelic frequencies, and allele carriage distributions are shown in Table 2. No deviations from HWE in the patient and control groups were observed for all three promoter polymorphisms. The haplotype frequencies are shown in Table 3; three haplotypes were identified. The genotype and haplotype frequencies of *IL10* polymorphisms in the control group were consistent with previously published studies from different regions in Saudi Arabian [1, 11] and European populations [20]. Polymorphisms at -819 and -592 positions were found in complete linkage disequilibrium (LD) as it has been reported previously in different populations [12, 15].

Table 1 Demographic characteristics and clinical details of the controls ($N = 200$) and patients ($N = 118$)

Parameter	Value	p value
Average age (mean ± SD years)		
Controls	20.6 (4.5)	0.06
Patients	19.2 (5.2)	
Gender: male/female (%)		
Controls	50/50	0.55
Patients	50.8/49.2	
Clinical manifestations of patient [*N* (%)]		
Valvular lesion		
Mitral valve lesion (MVL)	56 (47.5)	
Combined valve lesion (CVL)	62 (52.5)	
Carditis	72 (61)	
Arthritis	65 (55)	
Chorea	10 (8.5)	
Skin rash	3 (2.5)	
Subcutaneous nodules	2 (1.7)	
Recurrence	*NA*	

N number, *SD* standard deviation, *NA* not available

Disease Susceptibility

There was a significant difference in the distribution of *IL10*-1082 genotype frequency between patients and controls ($\chi^2 = 9.06$, $p = 0.01$) (Table 2). G allele carriage (GG+GA vs AA) of the -1082 polymorphism was associated with an increased risk of developing RHD ($\chi^2 = 8.4$, $p = 0.004$, OR 2.1, 95 % CIs 1.7–3.4). Genotype and allele frequencies of other *IL10* polymorphisms were not associated with RHD in this cohort of Saudi patients.

Haplotype Analysis

According to the genotyping data and using the PHASE algorithm, three common haplotypes were identified in the promoter region of *IL10*. Haplotype frequencies are shown in Table 3. The *IL10* (-1082, -819, -592) genotype GCC/ATA conferred susceptibility to RHD ($\chi^2 = 5.9$, $p = 0.015$, OR 1.9, 95 % CIs 1.1–3.1). Likewise, GCC carriers were susceptible to the disease ($\chi^2 = 8$, $p = 0.004$, OR 2, 95 % CIs 1.3–3.4). However, GCC frequency was not significantly different between patients and controls. In addition, ACC haplotype was associated with a decrease in RHD risk ($\chi^2 = 4.5$, $p = 0.03$, OR 0.62, 95 % CIs 0.41–0.95) (Table 3).

Disease Subgroups and Severity

To clarify whether *IL10* promoter polymorphisms are associated with disease severity, possession of the -1082G allele was investigated with respect to disease subgroups. There was significant difference in the distribution of -1082G genotype between CVL and control groups ($\chi^2 = 10.4$, $p = 0.006$) (Table 4). G allele carriage (GG+AG vs AA) was present at higher frequency in the CVL subgroup compared to controls ($\chi^2 = 7.3$, $p = 0.007$, OR 2.4, 95 % CIs 1.3–4.7) (Table 4).

Discussion

This is the first study to investigate the association of *IL10* promoter polymorphisms with RHD in Saudi Arabian patients. The -1082G allele was more prevalent in RHD patients than in controls, with no association present for *IL10*-829 and *IL10*-592 polymorphisms. In addition, -1082G allele carriage was significantly higher in the CVL subgroup

Table 2 Distribution of IL10 polymorphisms genotypes and allele frequencies in the Saudi patients and controls

IL10 SNP ID	Genotype	Control ($N = 200$)		Patients ($N = 118$)		χ^2	p value	OR (95 % CI)
		Count	Frequency	Count	Frequency			
-1082								
Genotype	AA	83	0.42	30	0.25	**9.06**	**0.01**	–
	AG	86	0.43	69	0.58			
	GG	31	0.16	19	0.16			
Allele frequency	A	252	0.63	129	0.55	**4.3**	**0.04**	**2.1 (1.7–3.4)**
	G	148	0.37	107	0.45	0.02	0.9	0.95 (0.5–1.8)
Allele carriage	(AA+AG) vs GG	169	0.85	99	0.84	0.02	0.9	0.95 (0.5–1.8)
	(GG+AG) vs AA	117	0.59	88	0.75	**8.4**	**0.004**	**2.1 (1.7–3.4)**
-819								
Genotype	CC	100	0.50	57	0.48	0.11	0.95	–
	CT	81	0.41	50	0.42			
	TT	19	0.10	11	0.09			
Allele frequency	C	281	0.7	164	0.69	0.003	0.96	1.1 (0.5–2.2)
	T	119	0.3	72	0.31	0.04	0.8	1.5 (0.7–1.7)
Allele carriage	(CC+CT) vs TT	181	0.91	107	0.91	0.003	0.95	1 (0.5–2.2)
	(TT+CT) vs CC	100	0.5	61	0.62	0.09	0.8	1.1 (0.7–1.7)
-592								
Genotype	CC	100	0.50	57	0.48	0.11	0.95	–
	CA	81	0.41	50	0.42			
	AA	19	0.10	11	0.09			
Allele frequency	C	281	0.7	164	0.69	0.003	0.96	1.1 (0.5–2.2)
	A	119	0.3	72	0.31	0.04	0.8	1.5 (0.7–1.7)
Allele carriage	(CC+CA) vs AA	181	0.91	107	0.91	0.003	0.95	1 (0.5–2.2)
	(AA+CA) vs CC	100	0.5	61	0.62	0.09	0.8	1.1 (0.7–1.7)

Significant findings are shown in bold

N number

compared to controls. Positions at *IL10*-829 and *IL10*-592 are in complete LD [12, 15]; therefore, only three haplotypes were found. Analysis of these haplotypes revealed an association between the GCC haplotype and the presence of RHD. Interestingly, the ACC haplotype was associated with a decrease in RHD risk in this population.

Our results are similar to those of Settin et al. [29], who reported that GCC frequency and GCC carriers were significantly greater in Egyptian RHD patients. In contrast, Rehman et al. [22] could not confirm this finding in a Pakistani cohort of RHD patients. However, patients and controls were not in HWE for *IL10*-1082 in the Rehman et al. study, which may have contributed a source of bias [34]. In Chinese patients, a different polymorphism in the *IL10* promoter, *IL10*-627, was not associated with RHD [5]; this polymorphism is located between *IL10*-829 and *IL10*-592, neither of which were associated with RHD in our population. Another study from Turkey also found no association between these three polymorphisms and acute rheumatic fever without valvular involvement [6]. The association of *IL10*-1082 with RHD and not with ARF suggests that this polymorphism plays an important role in valvular involvement of the disease, which is supported by our finding that the *IL10*-1082G allele is associated with RHD and is significantly greater in the CVL subgroup of patients. We also found that the ACC haplotype (including the -1082A allele) is protective against RHD. In a recent meta-analysis, the GCC haplotype was shown to be a risk factor for another autoimmune disease, primary Sjögren's disease, in which a different haplotype (ATA) was protective [20]. GCC, ACC and ATA haplotypes are associated with high, medium and low levels of IL10 expression. Individuals carrying GCC haplotype secrete two or three times more IL10 that ATA haplotype carriers [9]. This supports our hypothesis that the -1082G allele is a risk factor for different autoimmune diseases and certain haplotypes may be protective against the development of these diseases.

IL10 promoter polymorphisms have been associated with the transcriptional activity of the gene. In twin studies,

Table 3 IL10 haplotype frequencies in Saudi patients and controls

IL10 (-1082, -819, -592)	Control ($N = 200$)		Patients ($N = 118$)		χ^2	p value	OR (95 % CI)
	Count	Frequency	Count	Frequency			
Genotype							
GCC GCC	31	0.15	19	0.16	0.02	0.9	1.1 (0.6–2)
GCC ACC	43	0.21	29	0.25	0.4	0.5	1.2 (0.7–2)
GCC ATA	43	0.22	40	0.34	**5.9**	**0.015**	**1.9 (1.1–3.1)**
ACC ACC	26	0.13	9	0.08	2	0.1	0.6 (0.3–1.2)
ACC ATA	38	0.19	10	0.08	**6.4**	**0.01**	**0.4 (0.2–0.8)**
ATA ATA	19	0.1	11	0.09	0.003	1	1 (0.5–2)
Haplotype carrier							
GCC carrier	117	0.6	88	0.75	**8**	**0.004**	**2 (1.3–3.4)**
ACC carrier	107	0.54	48	0.41	**4.9**	**0.03**	**0.6 (0.4–0.9)**
ATA carrier	100	0.5	61	0.52	0.1	0.8	1.1 (0.7–1.7)
Haplotype frequency							
GCC	148	0.37	107	0.45	**4.3**	**0.04**	**1.4 (1.02–2)**
ACC	133	0.33	57	0.24	**5.9**	**0.015**	**0.6 (0.4–0.9)**
ATA	119	0.3	72	0.31	0.04	0.8	1 (0.7–1.5)

Significant findings are shown in bold

N number

Table 4 Comparison of IL10-1082 genotype, allele frequency, and allele carriage in CVL subgroups and controls

IL10 -1082	Control ($N = 200$)		CVL ($N = 62$)		χ^2	p value	OR (95 % CI)
	Count	Frequency	Count	Frequency			
Genotype							
AA	83	0.42	14	0.23	**10.4**	**0.006**	–
AG	86	0.43	41	0.66			
GG	31	0.16	7	0.11			
Allele frequency							
A	252	0.63	69	0.56	2	0.14	0.74 (0.5–1.1)
G	148	0.37	55	0.44	2	0.4	1.4 (0.9–2)
Allele carriage							
(AA+AG) vs GG	169	0.85	55	0.89	0.7	0.4	1.4 (0.6–3.5)
(GG+AG) vs AA	117	0.59	48	0.77	**7.3**	**0.007**	**2.4 (1.3–4.7)**

Significant findings are shown in bold

CVL combined valve lesion, *N* number

heritability was estimated to be between 50 and 75 % for the production of *IL10* [24, 32]. Of these three promoter polymorphisms, the *IL10*-1082G allele most influenced the regulation of *IL10* mRNA transcription and serum protein levels [31]. However, this polymorphism regulates *IL10* expression in a cell-specific manner. In B cells stimulated with lipopolysaccharide, the -1082G allele has a higher affinity than the -1082A allele for the Sp1 transcription factor, which may contribute to increased *IL10* production and B cell proliferation [16]. In macrophages stimulated by apoptotic cells, the -1802A allele was shown to have a higher affinity for poly(ADP-ribose)polymerase-1 (PARP1), a transcription factor that suppresses cytokine production and has important functions in systemic inflammation [13]. Macrophages activated by apoptotic cells carrying the -1082G allele would be expected to produce more IL10, which may result in immunological suppression and impaired apoptotic body clearance during acute bacterial infections [33]; this may create a favorable environment for persistent inflammation. Interestingly, PARP1 inhibitors, which are potent anti-cancer and anti-ischemia drugs, have been shown to reduce gut inflammation and limit *Pseudomonas aeruginosa* bacterial translocation in a rat model [18]. The use of PARP

inhibitors in acute and chronic inflammatory conditions such as RHD in patients with specific genotypes may have potential therapeutic applications [26].

One of the limitations of this study is the small sample size which limits the statistical power of the study. In addition, only the *IL10* promoter region was investigated. Therefore, studies with larger sample size that includes screening all known functional polymorphisms in *IL10* gene are needed for future meta-analyses.

In conclusion, our data suggest that *IL10* polymorphisms and haplotypes play an important role in RHD, at least in a Saudi population. These polymorphisms may influence the inflammatory stage of the disease and may represent important biomarkers for targeted therapies. Further studies are needed to reconcile the molecular mechanisms that underlie these associations.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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