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RESEARCH ARTICLE

GENETIC POLYMORPHISM OF INTERLEUKIN-4 GENE IN INFLAMMATORY BOWEL DISEASE OF IRAQI PATIENTS

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ABSTRACT

The association between a single nucleotide polymorphism of interleukin-4 gene (*IL4*) at position-590 of the promoter region ($IL4_{-590}$ SNP) and inflammatory bowel disease (IBD) was determined in 34 Crohn's disease (CD) and 66 ulcerative colitis (UC) Iraqi patients, as well as 43 controls. The results revealed thatcomparing $IL4_{-590}$ genotypes and alleles between IBD patients and controls revealed some significant variations. Among CD patients, it was observed that frequencies of TT genotype (52.9 *vs*. 11.6%; P = $1.2*10^{-4}$) and *T* allele (70.6 *vs*. 24.4%; P = $1.6*10^{-8}$) were significantly increased in patients compared to controls, and the associated RR values were 8.55 and 7.43, respectively. In contrast, CC genotype (11.8 *vs*. 62.8%, P = $5.6*10^{-6}$) and *C* allele (29.4 *vs*. 75.6%; P = $1.6*10^{-8}$) frequencies were significantly decreased in CD patients, and the associated PF values were 0.58 and 0.65, respectively. In the case of UC, frequencies of TC genotype (69.7 *vs*. 25.6%; RR = 6.69; P = $1.1*10^{-5}$) and *T* allele (62.1 *vs*. 24.4%; RR = 5.08; P = $4.6*10^{-8}$) were significantly increased in patients compared to controls, while CC genotype (3.0 *vs*. 62.8%; PF = 0.62; P = $2.5*10^{-12}$) and *C* allele (37.9 vs. 75.6%; PF = 0.61; P = $1.6*10^{-8}$) frequencies were significantly decreased. However, no such variation was observed between CD and UC patients. These findings suggest that $IL4_{-590}$ SNP might have a role in the etiopathogenicmechanism of CD and UC.

INTRODUCTION

Inflammatory bowel disease (IBD) has been regarded as a world healthcare problem that is presented with a global sustained increasing incidence. Two major forms are recognized as IBD; Crohn's disease (CD)and ulcerative colitis (UC), which represent distinct chronic bowel inflammatory disorders (Mansour-Ghanaei *et al*., 2015). The former can cause inflammation of a transmural type and affect any part of the gastrointestinal tract in a non-continuous type, but most commonly it affects the terminal ileum or the perianal region, and commonly associated with clinical complications like abscesses, fistulas and strictures. By contrast, UC inflammatory lesions are continuous and restricted to the mucosa of large intestine, involving rectum and a variable portion of colon (Abraham *et al*., 2009). The etiology of IBD is largely unknown, but recent investigations highlighted that host genetic susceptibility, external environment, intestinal microbiota and immune responses are functionally involved and integrated in IBD pathogenesis (Zhang and Li, 2014; Trifunović *et al*., 2015). Studies comparing the prevalence of IBD among different ethnic groups suggest a genetic tendency.However, there have been great technological advances in the understanding of genetic factors that contribute to IBD etiology, which include DNA analysis and sequencing and the employment of multinational databases. These advances allowed for the achievement of genome-wide association studies (GWAS) that identified different single nucleotide polymorphisms (SNPs) and defined their role in IBD. Based on these studies, the number of IBD-associated gene loci has been brought to163, which are distributed as 110 shared loci for both diseases, 30 loci as CD specific and 23 loci as UC associated. An understanding of gene loci that are shared by UC and CD may provide a pathway to elucidate the common pathogenesis involved in both forms of IBD (Jostins *et al*., 2012).However a wealth of data have indicated the importance of genetic background in regulating the cytokine network in IBD, and in fact, polymorphisms of cytokine/cytokine receptor genes have been shown to be associated with the development of IBD; implicating their role in determining the risk or protection from the disease expression, and one of these cytokines is IL-4 (Andersen *et al*., 2010; Palmieri *et al*., 2010; Kim *et al*., 2011).

Interleukin-4 is a pleiotropic cytokine that acts on T and B lymphocytes, monocytes, polymorphonuclear cells, fibroblasts and endothelial cells (Maes *et al*., 2012),and executes

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pleiotropic functions, including induction of T helper 2 (Th2) differentiation, immunoglobulin class switching, B cell proliferation, and suppression of Th1 differentiation and macrophage activation (Gilmour and Lavender, 2008). It is also termed anti-inflammatory because of its ability to suppress TNF-α, IL-1, IL-6, and prostaglandin E2 (PGE2) production by activated monocytes (Rai *et al*., 2011; Karimi*et al*., 2013).

Such cytokine is encoded by a gene located on the long arm of chromosome 5 (5q31.1). Three polymorphisms in *IL4* gene (*IL4*$_{-1089}$ T/G, *IL4*$_{-590}$ T/C and *IL4*$_{+33}$ T/C) have been described (Karimi *et al*., 2013). Several studies have examined the *IL4SNPs* in IBD patients, and although different associations were reported, the investigations agreed that *IL4* polymorphisms are involved in the etiopathogenesis of CD and UC (Aithal *et al*., 2001; Peng *et al*., 2002; Hong *et al*., 2008; Ahirwar *et al*., 2012; Connelly *et al*., 2014). The protective effect of these SNPs has also been highlighted; for instance, Gao *et al*. (2014) reported that *IL4*$_{-590}$*C* allele is a protective allele against CD and UC in Turkish patients. Therefore, the present investigation contributed further to understand the association between one of *IL4SNPs*(*IL4*$_{-590}$) and IBD(CD and UC) in samples of Iraqi patients.

MATERIALS AND METHODS

Subjects

One hundred Iraqi Arab IBD patients were enrolled in this study. The patients attended the Gastrointestinal Tract Units at hospitals in Baghdad for diagnosis and treatment during the period August 2013 - October 2014. The disease was clinically diagnosed by the consultant medical staff, which was based on clinical, radiological, endoscopic, and histopathological findings according to the criteria of Lennard - Jones (1989). According to diagnosis, the patients were distributed into two clinical groups: 34 CD cases (12 males and 22 females; 40.65 ± 3.89 years) and 66 UC cases (22 males and 42 females; 40.19 ± 8.05 years), and their age mean ± S.E. was 40.19 ± 8.05 years. For the purpose of a comparison, 43 apparently healthy controls of blood donors (12 males and 31 females) matched patients for age (38.67 ± 4.93 years) and ethnicity (Iraqi Arabs) were also enrolled in the study.

Detection of IL4Polymorphism

Genomic DNA was extracted from EDTA blood using Wizard Genomic DNA Purification Kit (Promega, USA). The polymorphism was detected at -590 position of the promoter region (*IL4*$_{-590}$) by polymerase chain reaction-specific sequence primer (PCR-SSP) assay, followed by electrophoresis on 2% agarose-gel, by using CTS-PCRSSP Tray Kit (Heidelberg, Germany). The thermocycling conditions were: initial denaturation at 94˚C for 2 minutes, followed by denaturation at 94˚C for 15 seconds, and then 10 cycles of annealing and extension at 65˚C for 60 seconds. This was followed by denaturation at 94˚C for 15 seconds, and then 20 cycles of annealing 61˚C at 50 seconds and extension at 72˚C for 30 seconds.

Statistical Analysis

Genotypes of *IL4*$_{-590}$ SNP were presented as percentage frequencies, and significant differences between their distributions in IBD patients and controls were assessed by two-tailed Fisher's exact probability (P). In addition, relative risk (RR), etiological fraction (EF) and preventive fraction (PF) were also estimated to define the association between a genotype with the disease. These estimations were calculated by using the WINPEPI computer programs for epidemiologists. The latest version of the WINPEPI package is available free online at http://www.brixtonhealth.com.

RESULTS

Genetic polymorphism of *IL4* gene was determined at position -590 of the promoter region (*IL4*$_{-590}$ SNP), which was presented with three genotypes (TT, TC and CC). These genotypes were significantly departed from HWE in UC patients and controls (P ≤ 0.001 and 0.05, respectively), while CD patients were in a good agreement with such equilibrium (Table 1).

Table 1 Observed numbers and percentage frequencies and Hardy-Weinberg equilibrium (HWE) of *IL4*$_{-590}$ genotypes and alleles in inflammatory bowel disease (Crohn's disease and ulcerative colitis) patients and controls.

Groups			*IL4*$_{-590}$ Genotype or Allele					H-W
			TT	TC	CC	*T*	*C*	P ≤
Crohn's Disease (No. = 34)	Observed	No.	18	12	4	48	20	N.S.
		%	52.9	35.3	11.8	70.6	29.4	
	Expected	No.	16.9	14.1	2.9	Not Estimated		
		%	49.8	41.5	8.7			
Ulcerative Colitis (No. = 66)	Observed	No.	18	46	2	82	50	0.001
		%	27.3	69.7	3.0	62.1	37.9	
	Expected	No.	25.5	31.1	9.5	Not Estimated		
		%	38.6	47.1	14.4			
Controls (No. = 43)	Observed	No.	5	11	27	21	65	0.05
		%	11.6	25.6	62.8	24.4	75.6	
	Expected	No.	2.6	15.9	24.4	Not Estimated		
		%	5.9	36.9	57.1			

Comparing *IL4*$_{-590}$ genotypes and alleles between IBD patients (CD and UC) and controls also revealed some significant variations.

Among CD patients, it was observed that frequencies of TT genotype (52.9 *vs*. 11.6%; P = $1.2*10^{-4}$) and *T* allele (70.6 *vs*. 24.4%; P = $1.6*10^{-8}$) were significantly increased in patients compared to controls, and the associated RR values were 8.55 and 7.43, respectively. In contrast, CC genotype (11.8 *vs*. 62.8%, P = $5.6*10^{-6}$) and *C* allele (29.4 *vs*. 75.6%; P = $1.6*10^{-8}$) frequencies were significantly decreased in CD patients, and the associated PF values were 0.58 and 0.65, respectively. In the case of UC, frequencies of TC genotype (69.7 *vs*. 25.6%; RR = 6.69; P = $1.1*10^{-5}$) and *T* allele (62.1 *vs*. 24.4%; RR = 5.08; P = $4.6*10^{-8}$) were significantly increased in patients compared to controls, while CC genotype (3.0 *vs*. 62.8%; PF = 0.62; P = $2.5*10^{-12}$) and *C* allele (37.9 vs. 75.6%; PF = 0.61; P = $1.6*10^{-8}$) frequencies were significantly decreased. However, no such variation was observed between CD and UC patients (Table 2).

Table 2 Statistical analysis of associations between *IL4*$_{-590}$ genotypes or alleles and inflammatory bowel disease (Crohn's disease and ulcerative colitis).

Type of Comparison	*IL4*$_{-590}$ Genotype or Allele	Statistical Evaluation			
		Relative Risk	Etiological or Preventive Fraction	Fisher's Exact Probability	95% Confidence Intervals
Crohn's Disease *Versus* Controls	**TT**	8.55	0.47	**$1.2*10^{-4}$**	2.75 - 26.61
	TC	1.59	0.13	0.453	0.60 - 4.18
	CC	0.08	0.58	**$5.6*10^{-6}$**	0.02 - 0.26
	T	7.43	0.61	**$1.6*10^{-8}$**	3.64 - 15.14
	C	0.13	0.65	**$1.6*10^{-8}$**	0.07 - 0.27
Ulcerative Colitis *Versus* Controls	**TT**	2.85	0.18	0.058	0.98 - 8.28
	TC	6.69	0.59	**$1.1*10^{-5}$**	2.85 - 15.72
	CC	0.02	0.62	**$2.5*10^{-12}$**	0.00 - 0.09
	T	5.08	0.50	**$4.6*10^{-8}$**	2.78 - 9.26
	C	0.20	0.61	**$4.6*10^{-8}$**	0.11 - 0.36
Crohn's Disease *Versus* Ulcerative Colitis	**TT**	3.0	0.35	0.016	1.28 - 7.05
	TC	0.24	0.53	0.001	0.10 - 0.56
	CC	4.27	0.01	0.176	0.75 - 24.17
	T	1.46	0.22	0.274	0.78 - 2.73
	C	0.68	0.12	0.274	0.37 - 1.28

DISCUSSION

According to the presented results, *IL4*$_{-590}$ SNP can be highlighted as an important genetic marker in the pathogenesis of CD and UC; especially if we consider RR values of 8.55 and 7.43 for TT genotype and *T* allele, respectively in CD, and 6.69 and 5.08 for TC and *T* allele, respectively in UC. Therefore, the genetic predisposition conferred by these genotypes cannot be ignored, and the estimated EF values are in favor of such generalization (EF range: 0.47 – 0.61). The protective effects, as estimated by PF values (PF range: 0.58 – 0.62), of CC genotype and *C* allele are also a further feature of *IL4*$_{-590}$ SNP. However, only one study reported the protective effect of *IL4*$_{-590}$*C* allele against IBD of both types (CD and UC) in Turkish patients (Gao *et al*., 2014), and no further investigation has reported such SNP in IBD patients. However, other studies investigated other polymorphisms in intron and promoter regions of *IL4* gene and the results were almost conflicting due to ethnic variations, but they agreed that that IL-4 is an important cytokine involved in mucosal immunity and its polymorphisms play a critical role in IBD development (Aithal *et al*., 2001; Peng *et al*., 2002; Hong *et al*., 2008; Ahirwar *et al*., 2012; Connelly *et al*., 2014).These findings suggest that *IL4*$_{-590}$ SNP might have a role in the etiopathogenic mechanism of CD and UC, but further investigations are certainly required.

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