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ORIGINAL ARTICLE

Association of inflammatory cytokine gene polymorphisms with inflammatory bowel disease in a Moroccan cohort

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The purpose of this study was to investigate whether common variants in inflammatory and immune response genes influence inflammatory bowel disease (IBD) risk among Moroccan patients. Using a candidate gene approach, 10 single-nucleotide polymorphisms mapping on six genes (*MIF*_rs755622, *TNFA*_rs1800629, *IL6*_rs2069840, *IL6R*_rs2228145, *IL6ST*_rs2228044, *IL17A* (rs2275913, rs4711998, rs7747909, rs8193036, rs3819024)) were assessed in 510 subjects grouped in 199 IBD and 311 healthy controls. Genotyping was performed with the TaqMan allelic discrimination technology. The results were analyzed using PLINK software. The frequency of allele A for *TNFA* rs1800629 was significantly higher in ulcerative colitis (UC) patients compared with controls (30.16 vs 16.72%; $P = 0.0005$; odds ratio (OR) = 2.15; 95% confidence interval (CI) = 1.39–3.32). Statistically significant association to UC was also found under dominant AA+AG vs GG (OR = 1.85, 95% CI = 1.07–3.21; $P = 0.02$) and recessive models (OR = 8.38; 95% CI = 2.86–24.53; $P = 0.0001$). In the same way, an association of *TNFA* rs1800629 variant was observed with IBD under recessive model AA vs AG+GG (OR = 4.10; 95% CI = 1.56–10.76; $P = 0.004$). No evidence of significant associations was found for the remaining investigated polymorphisms. Our data suggest that *TNFA* gene promoter polymorphism participates in determining IBD susceptibility in Moroccan patients.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a group of chronic disorders that includes two main disease forms, Crohn's disease (CD; MIM 266600) and ulcerative colitis (UC; MIM 191390). It represents an important and worldwide common health problem with a continually increasing incidence.[1] The underlying etiology and pathogenesis mechanisms have not yet been fully determined, but remain a field of intensive research. Current knowledge defines IBD as a complex disease with interplay of multiple factors such as environment, intestinal microbial flora, aberrant immune response mechanisms and individual's genetic predisposition.[2]

Both genome-wide association studies and candidate-gene analysis have revealed a major contribution of genetic susceptibility factors involved in different components of the immune system (cytokines, cytokine receptors, innate and adaptive immune response) in IBD pathogenesis.[3] Analysis of candidate genes with known immunologic function include single-nucleotide polymorphisms (SNPs) in the Interleukin 6 (IL6), Interleukin 6 receptor (IL6R), Interleukin 6 signal transducer Glycoprotein 130 (IL6ST GP130), Macrophage migration inhibitory factor (MIF), Tumor necrosis factor-α (TNFA) and Interleukin 17A (IL17A) genes, as well as a number of other genes involved in pathways that have been reported to have a critical regulatory role in IBD, and that are essential to the regulation of intestinal immune responses.[4]

The overall balance of pro-inflammatory and anti-inflammatory cytokines production is believed to trigger disease onset and appears to be likely a contributor to the clinical outcome of IBD, implying mucosal inflammation and loss of intestinal function.[5,6]

Increased spontaneous production of TNF-α, IL-6 as well as IL-1 by lamina propria mononuclear cells have been shown to partially induce functional changes in the intestinal mucosa of IBD patients.[7] Analysis of cytokine profile provided evidence of a high level of the pro-inflammatory cytokine IL-17 in patients with IBD.[8] Colonic MIF mRNA expression was increased during DSS-induced colitis.[9] In addition, increased formation of IL-6/sIL-6R complexes, which interact with membrane-bound gp130 on T cells via trans-signaling, supported an essential role in the development and perpetuation of IBD.[10]

Therefore, the aim of the present study was to ascertain whether polymorphisms of genes encoding mediators of inflammation known to be involved in determining the level of the immune response in the inflammatory pathway are associated with the occurrence of IBD, CD and UC subsets among Moroccan patients.

RESULTS

Baseline demographic and clinical characteristics of patients are reported in Table 1.

The success rates of genotyping assays were (93–99%) for controls and (91–99.5%) for patients. The statistical power for the different studied polymorphisms ranged between 56 and 88% to detect genotypic relative risk of Odds ratio (OR) = 1.5. In both patients and controls, the genotype frequencies for all SNPs agree with those predicted under Hardy–Weinberg equilibrium and any deviation from the expected was not statistically significant.

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Table 1. Basic characteristics of IBD patients

Patient characteristics	*CD (n = 136)*	*UC (n = 63)*
Gender		
Male	96	34
Female	40	29
Age at diagnosis (years)		
< 16	16	1
17–40	79	34
⩾ 40	22	19
Data not available	19	9
Location of CD (%)		
L1 ± L4	38 (28)	–
L2 ± L4	30 (22)	–
L3 ± L4	53 (39)	–
L4	4 (3)	–
Data not available	11 (8)	–
Behavior of CD (%)		
B1 ± p	35 (26)	–
B2 ± p	47 (34)	–
B3 ± p	39 (29)	–
Data not available	15 (11)	–
Location of UC (%)		
E1	–	5 (8)
E2	–	24 (38)
E3	–	8 (12)
E4	–	13 (21)
Data not available	–	13 (21)
Smoking habits		
Yes	35	11
No	67	32
Data not available	34	20

Abbreviations: CD, Crohn's disease; IBD, inflammatory bowel disease; UC, ulcerative colitis.

TNFA rs1800629

Frequency distribution of *TNFA* rs1800629 genotypes and variant allele in patients and controls are shown in Tables 2 and 3. In the present study, the frequency of allele A for *TNFA* rs1800629 was significantly higher in UC patients compared with the controls (30.16 vs 16.72%; $P = 0.0005$; OR = 2.15; 95% confidence interval (CI) = 1.39–3.32). When including all patients (IBD), a slightly increased rate of minor allele frequencies was encountered without being statistically significant (20.35 vs 16.72%; $P = 0.14$; OR = 1.27; 95% CI = 0.92–1.76).

With regard to genotype frequencies, the homozygous variant genotype distribution was increased in all disease subgroups UC (14.29%), IBD (7.54%) and CD (4.41%), compared with controls (1.95%). Genotypic test disclosed a statistically significant difference for IBD ($X^2 = 9.83$, $P = 0.007$), UC ($X^2 = 21.45$, $P = 2.20E\text{-}05$), but not for CD patients ($X^2 = 3.93$, $P = 0.14$; Table 3).

When Genetic models were assessed for the effect of *TNFA* SNP on disease risk, the significant associations were observed under recessive genetic model AA vs AG+GG (OR = 4.10; 95% CI = 1.56–10.76; $P = 0.004$) for IBD, and under dominant AA+AG vs GG (OR = 1.85; 95% CI = 1.07–3.21; $P = 0.02$) and recessive genetic models (OR = 8.38; 95% CI = 2.86–24.53; $P = 0.0001$) for UC.

IL17A, MIF, IL6, IL6R and IL6ST genes

All the studied polymorphisms in *IL17A* showed a lack of significant associations for CD, UC as well as IBD when considering minor allele frequencies distribution. A trend of a protective effect for the SNP rs3819024 was observed for UC patients ($OR_{unadj} = 0.60$, 95% CI = 0.35–1.05, $P = 0.07$; Table 2). Lower frequencies of both the homozygous and heterozygous variant genotypes of the latter polymorphism were observed in UC patients compared with controls (GG: 1.72% vs 6.92%; GA: 25.86% vs 30.45%). Taking into account these results, we performed genetic model analysis to account for the variation of risk effect, a non-significant reduced risk was observed for both dominant (OR = 0.63; 95% CI = 0.34–1.19; $P = 0.15$) and recessive models (OR = 0.23; 95% CI = 0.03–1.79; $P = 0.16$). The genetic model analysis for the remaining SNPs disclosed a significant association with CD under dominant genetic model for rs7747909 (OR = 1.57; 95% CI = 0.99–2.47; $P = 0.05$), but this was not observed after FDR-BH correction.

The distribution of alleles and genotypes of *MIF* rs755622, *IL6* rs2069840, *IL6R* rs2228145 and *IL6ST* (GP130) rs2228044 in the patients and control groups is shown in Tables 2 and 3. According to the obtained results, there was no statistically significant difference between the different groups when comparing allele and genotype frequencies of the investigated polymorphisms.

Regarding the possible effects of genetic models on disease risk, no association was seen with any of the studied polymorphisms, either for the IBD group as a whole or when dividing into CD and UC groups. Noteworthy that, the decreased frequency of mutant allele C and heterozygous variant genotype CG for *IL6ST* (GP130) rs2228044 in CD patients compared with controls, was suggestive of a protective effect. However, the difference was not statistically significant.

DISCUSSION

This is the first study to examine the association of polymorphisms in genes encoding cytokines/cytokine receptors: *MIF*, *TNFA*, *IL6*, *IL6R*, *IL6ST* (GP130) and *IL17A* with the pathogenesis of IBD (CD and UC) in the Moroccan population. Our findings support a strong evidence of association between the functional *TNFA* rs1800629 polymorphism and the risk of UC and IBD. Genetic model testing demonstrated recessive inheritance (genotype AA vs AG+GG), to be the best-fit genetic model in the reported associations: 4.10 (95% CI, 1.56–10.76) for IBD and 8.38 (95% CI = 2.86–24.53) for UC. The high relative risk of the TNF-α -308 polymorphism obtained in our study drives us to confirm the previously observed associations in other populations.

In line with our results, Song *et al.* reported significantly higher *TNFA* -308 genotypic and allelic frequencies (15.5% and 8.7% vs 4.1% and 2.0%, respectively; $P < 0.001$), in patients with UC compared with controls. Similarly to our study, no significant difference was observed between patients with CD and the healthy controls.[11]

In the same way, variant allele A frequency of the *TNFA* rs1800629 was not different in CD compared with that in the control group in the study reported by Sykora *et al.*,[12] whereas significant differences were found between the IBD group ($P < 0.05$), the UC group ($P < 0.001$) and controls. In addition, differences in the haplotype AG (−308A, − 238 G) carrier frequency were noted between UC patients and the control group (OR = 4.76; 95% CI = 1.53–14.74; $P < 0.01$).[13] In contrast to our finding, no statistical differences in TNFA genotypes and allele distributions between the IBD groups and healthy controls were found in the Spanish population.[14] In addition, the homozygous and the heterozygous variant genotypes of TNFA -308 G > A (rs1800629) ($OR_{unadj} = 0.75$; 95% CI = 0.58–0.98; $P = 0.04$) were associated with reduced risk of UC but not with CD in a Danish Cohort.[15] Furthermore, Bouma *et al.*,[16] observed a decreased -308A allele frequency in patients with UC compared with healthy controls (0.15 in UC vs 0.25 in HC, $P = 0.044$). The same results were observed by Vatay *et al.*[17] In the meta-analysis conducted by Fan W *et al.*, the AA genotype significantly increased the risk of UC (OR = 2.041; 95% CI = 1.261–3.301) and CD (OR = 1.730; 95% CI = 1.168–2.564) in

Table 2. Minor allele frequencies of *MIF*, *TNFA*, *IL6*, *IL6R*, *IL6ST* and *IL17A* genetic variants in IBD patients and healthy controls from Morocco

SNP ID	*Subgroup*	*Number of Alleles*	*MAF %*	*Allele test*	
				OR (95% CI)	P-*value*
*TNFA*rs1800629	Controls ($n=308$)	103/513	16.72		
	IBD ($n=199$)	81/317	20.35	1.27 (0.92–1.76)	0.14
	CD ($n=136$)	43/229	15.81	0.94 (0.63–1.38)	0.73
	UC ($n=63$)	38/88	30.16	2.15 (1.39–3.32)	**0.0005**
*MIF*rs755622	Controls ($n=308$)	160/456	25.97		
	IBD ($n=199$)	103/295	25.88	1.00 (0.75–1.33)	0.97
	CD ($n=136$)	72/200	26.47	1.03 (0.74–1.42)	0.87
	UC ($n=63$)	31/95	24.60	0.93 (0.60–1.45)	0.74
*IL6*rs2069840	Controls ($n=298$)	151/445	25.34		
	IBD ($n=195$)	89/301	22.82	0.87 (0.65–1.18)	0.36
	CD ($n=133$)	59/207	22.18	0.84 (0.60–1.18)	0.31
	UC ($n=62$)	30/94	24.19	0.94 (0.60–1.48)	0.78
*IL6R*rs2228145	Controls ($n=303$)	191/415	31.52		
	IBD ($n=197$)	125/269	31.73	1.01 (0.77–1.33)	0.94
	CD ($n=135$)	82/188	30.37	0.95 (0.69–1.29)	0.73
	UC ($n=62$)	43/81	34.68	1.15 (0.77–1.73)	0.49
IL6ST(GP130) rs2228044	Controls ($n=311$)	139/483	22.35		
	IBD ($n=194$)	76/312	19.59	0.85 (0.62–1.16)	0.29
	CD ($n=132$)	48/216	18.18	0.77 (0.54–1.11)	0.16
	UC ($n=62$)	28/96	22.58	1.01 (0.64–1.61)	0.95
*IL17A*rs4711998	Controls; $n=289$	220/358	38.06		
	IBD ($n=182$)	141/223	38.74	1.03 (0.79–1.35)	0.83
	CD ($n=124$)	103/145	41.53	1.16 (0.85–1.57)	0.34
	UC ($n=58$)	38/78	32.76	0.79 (0.52–1.21)	0.28
*IL17A*rs8193036	Controls; $n=289$	141/437	24.39		
	IBD ($n=182$)	75/289	20.60	0.80 (0.59–1.10)	0.17
	CD ($n=124$)	51/197	20.56	0.80 (0.56–1.15)	0.23
	UC ($n=58$)	24/92	20.69	0.81 (0.50–1.32)	0.39
*IL17A*rs3819024	Controls; $n=289$	128/450	22.15		
	IBD ($n=182$)	72/292	19.78	0.87 (0.63–1.20)	0.38
	CD ($n=124$)	55/193	22.18	1.00 (0.70–1.43)	0.99
	UC ($n=58$)	17/99	14.66	0.60 (0.35–1.05)	**0.07**
*IL17A*rs2275913	Controls; $n=289$	94/484	16.26		
	IBD ($n=182$)	58/306	15.93	0.98 (0.68–1.40)	0.89
	CD ($n=124$)	46/202	18.55	1.17 (0.79–1.73)	0.42
	UC ($n=58$)	12/104	10.34	0.59 (0.31–1.12)	0.10
*IL17A*rs7747909	Controls; $n=289$	79/499	13.67		
	IBD ($n=182$)	54/310	14.84	1.10 (0.76–1.60)	0.61
	CD ($n=124$)	44/204	17.74	1.36 (0.91–2.04)	0.13
	UC ($n=58$)	10/106	8.62	0.60 (0.30–1.19)	0.13

Abbreviations: CD, Crohn's disease; IBD, inflammatory bowel disease; SNP, single-nucleotide polymorphism; UC, ulcerative colitis. Bold: significant *P*-values.

Europeans. Meanwhile in Asians, the GA genotype increased the risk of UC (OR = 2.360; 95% CI = 1.269 4.390).[18]

Several studies have reported the involvement of *TNFA* genetic variations on the enhancement of susceptibility to a large number of inflammatory and auto-immune diseases.[19] Human *TNF-α* gene, spanning ~3 kb,[20] has been mapped to the IBD3 region on chromosome 6p21.3, which is a good functional candidate for involvement in predisposition to IBD.[21] SNPs in the promoter region of the *TNFA*, including the rs1800629 G > A located at position − 308, have been associated with enhanced TNF-α expression both *in vivo* and *in vitro*.[22,23] The encoded protein is a key proinflammatory cytokine considered to have an essential role as a potent immunomediator in driving a wide variety of effector functions that are of major importance in IBD pathogenesis. This was evidenced by increased TNF-α levels found in the serum, mucosa, intestinal tissues, peripheral phagocytes and stool of patients with IBD.[24–26] In addition, it should be noted that the infusion of monoclonal anti-TNF antibody is a highly efficacious IBD therapy.[27]

As for TNFA, an accumulating body of evidence has demonstrated the important pathological role played by genetic variations of IL-17 in the development of inflammatory and auto-immune diseases. The functional relevance of T helper 17 cells (Th17) and their signature cytokines (IL-17) have contributed to advance our understanding of mechanisms that regulate mucosal homeostasis and inflammation in the gut. Highly differentiated Th17 cells were abundantly found in the inflamed intestinal mucosa.[28] Proinflammatory qualities of IL-17 were evidenced and are involved in several chronic inflammatory disorders, including IBD. IL-17A was abundantly found in inflamed IBD mucosa,[29] its characterization represented a hallmark in IBD immunobiology by providing a new distinctive pathway for the communication between adaptive and innate immunity. Upregulation of the pleiotropic proinflammatory IL-17A cytokine has also been reported in active UC and CD.[30] IL-17 contribute to perpetuation of intestinal inflammation through several mechanisms, including the proinflammatory effects mediated by the induction of TNF-*α*, IL-1b, IL-6, chemokines and metalloproteases,[31] on intestinal epithelial cells, epithelial barrier, and other effectors (macrophages, lymphocytes and dendritic cells). We investigated the relevance of *IL17A* genetic variations in

Table 3. Genetic models and genotypes distribution in patients and controls

Gene SNP	*Group*	*1/2*	*Genotype, N (%)*			*Genotypic test*		*Dominant model Genotype 11+12 vs 22*		*Recessive model Genotype 11 vs 12+22*	
			AA	AG	GG	X^2	P-*value*	*OR (95% CI)*	P-*value*	*OR (95% CI)*	P-*value*
*TNFA*rs1800629	Controls	**A/G**	6 (1.95)	91 (29.55)	211 (68.51)						
	IBD		15 (7.54)	51 (25.63)	133 (66.83)	9.83	**0.007**	1.07 (0.73–1.57)	0.69	4.10 (1.56–10.76)	**0.004**
	CD		6 (4.41)	31 (22.79)	99 (72.79)	3.93	0.14	0.81 (0.51–1.27)	0.36	2.32 (0.73–7.33)	0.15
	UC		9 (14.29)	20 (31.75)	34 (53.97)	21.45	**2.20E-05**	1.85 (1.07–3.21)	**0.02**	8.38 (2.86–24.53)	**0.0001**
			CC	CG	GG						
*MIF*rs755622	Controls	**C/G**	22 (7.14)	116 (37.66)	170 (55.19)						
	IBD		9 (4.52)	85 (42.71)	105 (52.76)	2.26	0.32	1.10 (0.77–1.57)	0.59	0.61 (0.27–1.36)	0.23
	CD		7 (5.15)	58 (42.65)	71 (52.21)	1.32	0.51	1.12 (0.75–1.69)	0.56	0.70 (0.29–1.69)	0.43
	UC		2 (3.17)	27 (42.86)	34 (53.97)	NA	NA	1.05 (0.60–1.81)	0.85	0.42 (0.09–1.86)	0.25
			GG	GC	CC						
*IL6*rs2069840	Controls	**G/C**	19 (6.38)	113 (37.92)	166 (55.70)						
	IBD		11 (5.64)	67 (34.36)	117 (60.00)	0.89	0.64	1.83 (0.58–1.21)	0.34	0.87 (0.40–1.88)	0.73
	CD		7 (5.26)	45 (33.83)	81 (60.90)	1.04	0.59	0.80 (0.53–1.22)	0.31	0.81 (0.33–1.99)	0.65
	UC		4 (6.45)	22 (35.48)	36 (58.06)	NA	NA	0.90 (0.52–1.58)	0.73	1.01 (0.33–3.08)	0.98
			CC	CA	AA						
*IL6R*rs2228145	Controls	**C/A**	33 (10.89)	125 (41.25)	145 (47.85)						
	IBD		20 (10.15)	85 (43.15)	92 (46.70)	0.19	0.90	1.04 ((0.73–1.5)	0.80	0.92 (0.51–1.66)	0.79
	CD		13 (9.63)	56 (41.48)	66 (48.89)	0.16	0.92	0.95 (0.63–1.44)	0.84	0.87 (0.44–1.71)	0.69
	UC		7 (11.29)	29 (46.77)	26 (41.94)	0.76	0.68	1.27 (0.73–2.20)	0.39	1.04 (0.43–2.47)	0.92
			CC	CG	GG						
*IL6ST*rs2228044	Controls	**C/G**	15 (4.82)	109 (35.05)	187 (60.13)						
	IBD		9 (4.64)	58 (29.90)	127 (65.46)	1.51	0.46	0.79 (0.54–1.15)	0.22	0.96 (0.41–2.23)	0.92
	CD		6 (4.55)	36 (27.27)	90 (68.18)	2.68	0.26	0.70 (0.45–1.08)	0.11	0.93 (0.35–2.47)	0.89
	UC		3 (4.84)	22 (35.48)	37 (59.68)	NA	NA	1.01 (0.58–1.77)	0.94	1.00 (0.28–3.57)	0.99
			GG	GA	AA						
*IL17A*rs4711998	Controls	**G/A**	44 (15.22)	132 (45.67)	113 (39.10)						
	IBD		32 (17.58)	77 (42.31)	73 (40.11)	0.69	0.70	0.95 (0.65–1.40)	0.82	1.18 (0.72–1.95)	0.49
	CD		24 (19.35)	55 (44.35)	45 (36.29)	1.11	0.57	1.12 (0.72–1.74)	0.59	1.33 (0.77–2.31)	0.30
	UC		8 (13.79)	22 (37.93)	28 (48.28)	1.72	0.42	0.68 (0.39–1.21)	0.19	0.89 (0.39–2.00)	0.78
			CC	CT	TT						
*IL17A*rs8193036	Controls	**C/T**	15 (5.19)	111 (38.41)	163 (56.40)						
	IBD		6 (3.30)	63 (34.62)	113 (62.09)	1.94	0.37	0.78 (0.54–1.15)	0.22	0.62 (0.23–1.63)	0.33
	CD		4 (3.23)	43 (34.68)	77 (62.10)	NA	NA	0.78 (0.51–1.21)	0.28	0.60 (0.19–1.87)	0.38
	UC		2 (3.45)	20 (34.48)	36 (62.07)	NA	NA	0.79 (0.44–1.41)	0.42	0.65 (0.14–2.93)	0.57
			GG	GA	AA						
*IL17A*rs3819024	Controls	**G/A**	20 (6.92)	88 (30.45)	181 (62.63)						
	IBD		6 (3.30)	60 (32.97)	116 (63.74)	2.90	0.23	0.95 (0.64–1.40)	0.80	0.45 (0.18–1.16)	0.1
	CD		5 (4.03)	45 (36.29)	74 (59.68)	2.23	0.32	1.13 (0.73–1.74)	0.57	0.56 (0.20–1.54)	0.26
	UC		1 (1.72)	15 (25.86)	42 (72.41)	NA	NA	0.63 (0.34–1.19)	0.15	0.23 (0.03–1.79)	0.16
			GG	GA	AA						
*IL17A*rs2275913	Controls	**G/A**	10 (3.46)	74 (25.61)	205 (70.93)						
	IBD		5 (2.75)	48 (26.37)	129 (70.88)	0.20	0.90	1.00 (0.66–1.50)	0.98	0.78 (0.26–2.34)	0.66
	CD		4 (3.23)	38 (30.65)	82 (66.13)	NA	NA	1.25 (0.79–1.96)	0.33	0.93 (0.28–3.02)	0.90
	UC		1 (1.72)	10 (17.24)	47 (81.03)	NA	NA	0.57 (0.28–1.15)	0.11	0.48 (0.06–3.9)	0.49
			AA	AG	GG						
*IL17A*rs7747909	Controls	**A/G**	6 (2.08)	67 (23.18)	216 (74.74)						
	IBD		2 (1.10)	50 (27.47)	130 (71.43)	NA	NA	1.18 (0.78–1.79)	0.42	0.52 (0.1–2.62)	0.43
	CD		1 (0.81)	42 (33.87)	81 (65.32)	NA	NA	1.57 (0.99–2.47)	**0.05**[a]	0.38 (0.04–3.21)	0.37
	UC		1 (1.72)	8 (13.79)	49 (84.48)	NA	NA	0.54 (0.25–1.16)	0.11	0.82 (0.09–7.00)	0.86

Abbreviations: CD, Crohn's disease; FDR_BH, Benjamini and Hochberg step-up false discovery rate control; IBD, inflammatory bowel disease; SNP, single-nucleotide polymorphism; UC, ulcerative colitis; UNADJ, unadjusted. [a]$P_{UNADJ} = 0.05$; $P_{FDR\_BH} = 0.25$. Bold: significant *P*-values.

IBD pathogenesis. Our results showed that the genotype AA+AG vs GG of *IL17A* rs7747909 was associated with CD before correcting for multiple testing. However, no significant associations were found with regards to minor allele frequencies distributions. Several studies have indicated that the *IL-17A* G197A (rs2275913) is significantly linked to the development of UC,[32–34] an association that was not confirmed in the present study.

In the other hand, studies on *MIF*, *IL6*, *IL6R* and *IL6ST* gene polymorphisms distribution in relation to IBD generated conflicting results in different populations. Data on association of these genes with IBD in the North African population are currently lacking. No significant difference was found at both allelic and genotypic levels between IBD, CD, UC patients and healthy controls in our population, suggesting that genetic variants of these genes have no effect on IBD pathogenesis.

We suggest that *TNFA* gene promoter polymorphism -308G/A participates in determining IBD susceptibility in Moroccan patients. The possible mechanism by which genetic variation in *TNFA* promoter region contribute to the development of IBD could be through alterations in the function of inflammatory pathways leading to enhancement of disease risk. Our finding requires further replication on larger study groups with additional functional investigations, evaluating *TNFA* transcriptional and cytokine production levels.

MATERIAL AND METHODS

Study population

A total of 199 patients diagnosed with IBD (136 CD; 63 UC) at the CHU Ibn Rochd Hospital (Casablanca, Morocco) were included. Samples from 311 blood donors were used as ethnically matched controls. The diagnosis of CD or UC was established according to the conventional clinical, endoscopic, radiological and histological criteria as previously described.[35,36] CD phenotype was classified according to the Montreal classification.[36] UC anatomic location was subgrouped using Paris classification.[37] Patient's clinical and demographic characteristics were collected in a case report form including questions on toxic behavior, disease location and phenotype, age at diagnosis and other clinical features. Informed written consent was obtained from all participants and approval from the local ethics committee was obtained.

Genotyping

Genomic DNA was isolated from peripheral blood using standard procedures and from Formalin Fixed Paraffin Embedded Tissues using the QIAamp DNA FFPE Tissue Kit (Qiagen, Valencia, CA, USA). Ten SNPs located within the following genes were analyzed: *MIF* rs755622, *TNFA* rs1800629, *IL6* rs2069840, *IL6R* rs2228145, *IL6ST* (GP130) rs2228044and *IL17A* (rs4711998; rs8193036; rs3819024; rs2275913; rs7747909). All the SNPs were genotyped using TaqMan allelic discrimination assays on the Light Cycler 480 System (Roche, Barcelona, Spain).

Statistical analysis

The overall statistical power of the present study was calculated using Power Calculator of Genetic Studies 2006 software (http://www.sph.umich.edu/csg/abecasis/CaTS/). Data analysis has been carried out using Plink software V1.07 (http://pngu.mgh.harvard.edu/purcell/plink/). The genotypes and alleles frequencies distribution were compared between patients and controls using the χ^2 test or Fisher's test. ORs with a CI of 95% were calculated to measure the strength of association. The Hardy–Weinberg equilibrium was tested for all SNPs by χ^2 analysis. Benjamini and Hochberg step-up false discovery rate control correction for multiple testing was applied to significant *P*-values of IL17A polymorphisms. A *P*-value < 0.05 was considered statistically significant.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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