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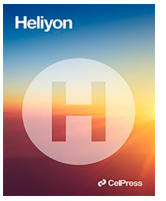

Research article

IL-10 polymorphism genotypes, haplotypes, and diplotypes are associated with colorectal cancer predisposition and outcome in Tunisian population

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ABSTRACT

Background and aim: As the presence of single nucleotide polymorphisms (SNPs) in the interleukin *(IL)-10* gene continues to be a major challenge in the development of effective therapies for digestive cancers, this case-control study was conducted to assess the possible influence of genotype, haplotype and diplotype for two SNPs (−1082A/G (rs1800896) and −592A/C (rs1800872)) located in the promoter region of *IL-10* gene on the incidence, severity and prognosis of colorectal cancer (CRC) in Tunisians.

Methods: IL-10 gene SNPs were analyzed in 130 CRC cases and 165 healthy subjects (HS) using PCR-SSP.

Results: For the *IL-10* -1082A/G SNP, the comparison of genotype frequencies between cases and HS groups showed that the G allele significantly reduced CRC risk under the recessive model (GG vs. AA + AG: OR [95%CI] = 0.44 [0.21–0.93], p = 0.03). Conversely, a positive association was observed between the codominant model (AG vs. AA + GG) and high susceptibility (OR [95%CI] = 1.65 [1.02–2.63], p = 0.04). After stratification by disease site, the recessive model was also found to reduce susceptibility to colon cancer (OR [95%CI] = 0.18 [0.04–0.72], p = 0 0.01), while the homozygote model (AA vs. GG) was suggested as a risk factor (OR [95%CI] = 5.16 [1.31–23.26], p = 0.02). Furthermore, the codominant model (AG vs. AA + GG) doubled the risk of rectum cancer (OR [95%CI] = 1.98 [1.07–3.70], p = 0.03). For the *IL-10* -592A/C SNP, the codominant model (AC vs. AA + CC) has a protective effect against the development of CRC (OR [95%CI] = 0.59 [0.36–0.94], p = 0.03). The *IL-10* gene haplotype was not associated with CRC risk. A stratified analysis by disease site demonstrated that the presence of Hap3 (−1082G and −592C alleles) specifically reduced the risk of developing colon cancer (OR [95%CI] = 0.51 [0.32–0.80], p = 0.003). Moreover, homozygous Hap3/Hap3 diplotype significantly reduced susceptibility to CRC (OR [95%CI] = 0.35 [0.14–0.85], p = 0.02). Interestingly, this diplotype has not been identified in colon cancer patients. Kaplan-Meier analysis showed that the homozygous

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Hap2/Hap2 diplotype was significantly associated with decreased overall survival (Log-rank: p = 0.01). This association was also observed in the colon cancer subgroup (Log-rank: p = 0.001). *Conclusion:* Our findings provide preliminary indications that the −1082A/G and −592/AC SNPs within the *IL-10* gene may exhibit significant associations with the pathogenesis and prognostic outcomes of CRC. However, further investigations are still warranted to validate and establish the veracity of our findings.

1. Introduction

As of 2020, colorectal cancer (CRC) ranked as the third most prevalent condition worldwide in terms of morbidity, and it held the position of the second leading cause of mortality [1]. The 5-year relative survival rate of CRC stands at approximately 90 % when diagnosed at earlier stages, but this rate significantly declines to 32 % once the cancer metastasizes to distant tissues [2]. In Tunisia, CRC is recognized as a critical public health challenge. The incidence rate of CRC is expected to increase from 12.4 registered cases in 2009 to 39.3 new cases per 100,000 population by the year 2024 [3].

Cytokines play an important role in coordinating and regulating inflammation and immune responses. Their deregulation is associated with the development of various diseases including CRC [4]. Interleukin (IL)-10 is a potent anti-inflammatory cytokine produced by myeloid lymphoid, keratinocyte, and epithelial cells [5]. In addition to normal and immune cells, it can also be secreted by various tumor cell lines [5]. Intensive studies have shown that this interleukin may have bimodal functions. Indeed, the balance can be switched from an inflammatory response promoting tumor progression to an immunostimulatory microenvironment that promotes tumor regression [6].

IL-10 is a pleiotropic cytokine that mediates important tumor-inhibiting effects. It suppresses the local release of pro-inflammatory molecules that promote tumor growth, survival, and metastasis. Moreover, IL-10 can create an inhibitory microenvironment that enhances tumor escape and progression by recruiting and activation of cytotoxic $CD8^+$ T cells and NK cells in the tumor site, by disrupting the inflammatory M1 macrophage-Th17 T cells axis, and by reducing the synthesis of pro-angiogenic factors [7].

On the other hand, IL-10 can act as a promoter of tumor growth. Elevated levels of IL-10 stimulate cancer cell proliferation via STAT3 activation and inhibit apoptosis [8,9]. Additionally, IL-10 can reduce or inhibit tumor antigen presentation via downregulation of the expression of MHC class II in antigen-presenting cells (APCs). Furthermore, IL-10 stimulates the expression of immunosuppressive molecules such as the unconventional human leukocyte antigen (HLA) molecules such as HLA-G and HLA-E [10]. These molecules inhibit the functions of various immune cells, including natural killer (NK) cell-mediated lysis, cytotoxicity of $CD8^+$ T cells, and the maturation and proliferation of APCs [11].

IL-10 expression is tightly controlled by genetic regulatory mechanisms. The *IL-10* gene contains five exons separated by four introns, all located on the long arm of chromosome 1 [12]. Several single nucleotide polymorphisms (SNPs), including-1082A/G (rs1800896) and −592A/C (rs1800872), are localized within the promoter region of the *IL-10* gene [12]. These SNPs are considered as prominent regulators of *IL-10* transcription [13,14]. To gain deeper insights into the genetic framework influencing the pathogenesis and progression of CRC, substantial attention has been directed toward investigating the oncogenic implications of *IL-10* -1082A/G and −592A/C SNPs. Numerous studies have provided evidence linking mutations in the *IL-10* gene at loci −1082 and −592 with CRC susceptibility [15–18]. However, contradicting results have been observed in Scottish, Italian or Swedish populations, where no such associations were observed [19–21]. Consequently, these divergent findings have led to conflicting viewpoints. To address this issue and gain further insights, the present study investigates the distribution of genotypes, haplotypes, and diplotypes of the −1082 A/G (rs1800896) and −592 A/C (rs1800872) SNPs within the *IL-10* gene promoter region. By comparing CRC cases and healthy controls, our objective is to assess the potential associations of these genetic variations with disease susceptibility, severity, and outcome in the Tunisian population. Through this investigation, we aim to contribute valuable knowledge and shed light on the role of *IL-10* gene variations in CRC within the Tunisian population.

Table 1
Characteristics of the study population.

Variable		CRC cases (N = 130)	Healthy subjects (N = 165)
Age	Mean, years (SD)	59.51 (12.19)	55.47 (13.32)
Gender, N (%)	Male	67 (52)	79 (48)
	Female	63 (48)	86 (52)
Disease site, N (%)	Colon	69 (53)	
	Rectum	61 (47)	
Stage, N (%)	Early	39 (30)	
	Advanced	91 (70)	
Grade, N (%)	Low	75 (58)	
	High	51 (39)	
	Unknown	4 (3)	

N: Number, SD: standard deviation, %: percentage.

Table 2
Distribution of alleles and genotypes of *IL-10* gene SNPs among CRC, colon cancer, rectum cancer cases, and healthy subjects.

	Genetic Model	CRC cases N (%)	Colon cancer cases N (%)	Rectum cancer cases N(%)	HS N (%)	P_1	OR_1 (95% CI)	P_2	OR_2 (95%CI)	P_3	OR_3 (95% CI)
−1082 A/G (rs1800896)	Allele model					0.67	1.07 (0.77–1.51)	0.17	1.34 (0.88–2.06)	0.44	0.85 (0.56–1.29)
	A	162 (62)	93(67)	69(57)	200 (60)						
	G	98(38)	45(33)	53(43)	130 (40)						
	Dominant model					0.43	0.82 (0.51–1.35)	0.71	1.11 (0.61–1.96)	0.08	0.55 (0.28–1.06)
	AA	40(31)	26(38)	14(23)	58 (35)						
	GG + AG	90(69)	43(62)	47(77)	107 (65)						
	Recessive model					0.03	0.44 (0.21–0.93)	0.01	0.18 (0.04–0.72)	0.41	0.67 (0.27–1.64)
	GG	8(6)	2(3)	6(10)	23 (14)						
	AA + AG	122 (94)	67(97)	55(90)	142 (86)						
	Codominant Model					0.04	1.65 (1.02–2.63)	0.23	1.41 (0.79–2.49)	0.03	1.98 (1.07–3.70)
	AG	82(63)	41(59)	41(67)	84 (51)						
	AA + GG	48(37)	28(41)	20(33)	81 (49)						
	Homozygote model					0.13	1.98 (0.84–4.73)	0.02	5.16 (1.31–23.26)	0.89	0.93 (0.31–2.77)
	AA	40(83)	26(93)	14(70)	58 (72)						
	GG	8(17)	2(7)	6(30)	23 (28)						
	Heterozygote model					0.18	0.71 (0.42–1.16)	0.78	0.92 (0.52–1.65)	0.04	0.49 (0.24–0.99)
	AA	40 (33)	26(39)	14(25)	58 (41)						
	AG	82 (67)	41(61)	41(75)	84 (59)						
592 A C (rs1800872) −592 A/C (rs1800872)	Allele model					0.90	1.02 (0.73–1.43)	0.76	1.07 (0.70–1.63)	0.91	0.98 (0.62–1.52)
	A	88(34)	48(35)	40(33)	110 (33)						
	C	172 (66)	90(65)	82(67)	220 (67)						
	Recessive model					0.09	1.76 (0.88–3.43)	0.10	1.90 (0.88–4.11)	0.27	1.60 (0.70–3.79)
	AA	23(18)	13(19)	10(16)	18 (11)						
	AC + CC	107 (82)	56(81)	51(84)	147 (90)						
	Dominant model					0.33	1.26 (0.79–2.02)	0.48	1.22 (0.70–2.12)	0.38	1.30 (0.72–2.36)
	CC	65(50)	34(49)	31(51)	73 (44)						
	AA + AC	65 (50)	35(51)	30(49)	92 (56)						
	Co-dominant Model					0.03	0.59 (0.36–0.94)	0.07	0.58 (0.32–1.06)	0.10	0.60 (0.32–1.11)
	AC	42(32)	22(32)	20(33)	74 (45)						
	AA + CC	88(68)	47(68)	41(67)	91 (55)						
	Homozygote model					0.31	1.44 (0.71–2.79)	0.29	1.55 (0.67–3.38)	0.55	1.31 (0.53–3.12)
	AA	23(26)	13(28)	10(25)	18 (20)						
	CC	65(74)	34(72)	31(75)	73 (80)						
	Heterozygote model					0.08	0.64 (0.38–1.04)	0.16	0.64 (0.34–1.22)	0.17	0.64 (0.34–1.21)
	AC	42(39)	22(39)	20(39)	74 (50.3)						
	CC	65(61)	34(61)	31(61)	73 (49.7)						

2. Research design and methods

Population enrollment: The study included a cohort of 130 Tunisian patients diagnosed with CRC collected between October 2016 and August 2019. The diagnosis of CRC was substantiated through radiological and histopathological assessments carried out at Salah Azaiez Institute (ISA) of Tunis, Tunisia. Extensive admission record reviews and face-to-face interviews were conducted to obtain comprehensive information on the participants' personal demographics, medical history, family background, and tumor-specific characteristics (Table 1). The control group consisted of 165 healthy subjects (HS) with no history of pathological disease. This study was reviewed and approved by the Ethics Committee at ISA, Tunisia (ISA/03/2016) and informed consent was obtained from all participants.

SNP detection: Genomic DNA was extracted from blood cells using a standard protocol [22,23]. IL-10 genotyping of the −1082A/G (rs1800896) and −592A/C (rs1800872) SNPs was performed using a slightly modified sequence-specific primer--polymorphism-polymerase chain reaction (SSP-PCR) assay. The human growth hormone gene was used as an internal control. The primer sets and their corresponding sizes used in this study have been previously described [24,25]. For IL-10 -1082A/G, we used the following primers: FA (sense) 5′-ACTACTAAGGCTTCTTTGGGAA-3, FG (sense) 5′-CTACTAAGGCTTCTTTGGGAG3, and a common antisense 5′-CAGTGCCAACTGAGAATTTGG-3′. For IL-10 -592A/C, we used the following primers: FA (sense) 5′-GACTGGCTTCCTACAGT-3′, FC (sense) 5′-CTGGCTTCCTACAGG-3′ and a common antisense 5′-GCTCACTATAAAAATAGAG ACGG-3′. Briefly, the reaction begins with a preheating step (94°C/2min) followed by 5 cycles (94°C/25s and 63°C/45s), followed by 26 cycles (94°C/25s, 55°C/55s for −1082AG or 54°C/50s for −592AC and 72°C/50s), and a final extension at 72°C for 3 min. Amplified fragments were separated by electrophoresis on a 2 % agarose gel.

Data interpretation: All statistical analyses were performed using two packages: Graphpad Prism 8.0 and SPSS 25.0. To test for deviations from Hardy-Weinberg equilibrium (HWE), we compared observed and expected genotype frequencies for each SNP in the control group. The 2 × 2 contingency chi-square test (or Fisher's exact test where appropriate) was used to estimate the association between two categorical variables. The degree of association between each allele, genotype, haplotype or diplotype and disease risk and/or severity was determined by calculating odds ratios with 95 % confidence intervals (OR [95% CI]). Overall survival (OS: time from disease onset until death) was estimated and compared using the Kaplan-Meier curve and log-rank test. Distributions of linkage disequilibrium (LD) and haplotype frequencies were analyzed using Haploview 4.2 and PHASE 2.1. A p-value <0.05 was considered statistically significant.

3. Results

3.1. Association between genotypes/alleles of IL-10 gene SNPs with CRC risk and its severity

For the *IL-10* gene SNPs examined in this study, genotype frequencies, in the control group, were found to be consistent with the assumption of HWE (p-values: 0.40 and 0.91 for −1082A/G and −592A/C, respectively). Regarding *IL-10* -1082A/G, when comparing the cases and healthy subjects (HS) groups, the G allele in the recessive model (GG vs. AA + AG) showed a significant reduction in the overall risk of CRC (OR [95%CI] = 0.44 [0.21–0.93], p = 0.03, Table 2). Additionally, the AG genotype was found to be more prevalent among CRC cases (63 %) compared to HS (51 %), and appeared to increase the susceptibility to CRC by 1.65-fold (p = 0.04, Table 2). In the stratified analyses based on the disease site, it was observed that the recessive model (GG vs. AA + AG) significantly decreased the susceptibility to colon cancer (OR [95%CI] = 0.18 [0.04–0.72], p = 0 0.01, Table 2), whereas the homozygote model (AA vs. GG) was associated with a five-fold increase in the risk of this disease (OR [95%CI] = 5.16 [1.31–23.26], p = 0.02, Table 2). Regarding rectum cancer, the codominant model (AG vs. AA + GG) contributed to approximately a 2-fold increased risk of this cancer (OR [95%CI] = 1.98 [1.07–3.70], p = 0.03, Table 2). Similarly, we found a significant association between the heterozygote model and a higher rectal cancer risk (OR [95%CI] = 2.02 [1.01–4.10], p = 0.04).

Regarding *IL-10* -592A/C, a noteworthy reduction in CRC risk was observed under the codominant model (AC vs. AA + CC: OR [95%CI] = 0.59[0.36–0.94], p = 0.03, Table 2). Conversely, the other genetic models did not demonstrate any significant associations

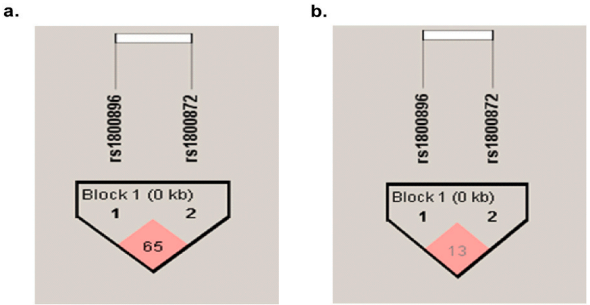

Fig. 1. HaploView linkage disequilibrium (LD) plots of -1082A/G (rs1800896) and -592A/C (rs1800872) SNPs in the promoter region of *IL-10* gene. The LD pattern was derived from the combined study population (both cases and healthy subjects). Values in the LD blocks indicated the D' (a.) and the r^2 (b.) in percentages.

Table 3
Distribution of haplotypes of *IL-10* gene SNPs between CRC, colon cancer, rectum cancer cases, and HS.

Haplotype *IL-10* 1082–592	CRC cases		Colon cancer cases		Rectum cancer cases		HS		P_1	OR_1 (95%CI)	P_2	OR_2(95%CI)	P_3	OR_3(95%CI)
	N = 260	Frequency	N = 138	Frequency	N = 122	Frequency	N = 330	Frequency						
Hap1 ($A^{1082}C^{592}$) vs. others	95	0.364	59	0.430	34	0.277	100	0.303	0.11	1.32 (0.94–1.86)	**0.01**	1.72 (1.15–2.60)	0.61	0.89 (0.56–1.39)
Hap2 ($A^{1082}A^{592}$) vs. others	67	0.259	34	0.244	35	0.290	100	0.303	0.22	0.80 (0.55–1.14)	0.22	0.75 (0.48–1.19)	0.74	0.93 (0.58–1.44)
Hap3 ($G^{1082}C^{592}$) vs. others	77	0.298	31	0.222	48	0.396	120	0.364	0.08	0.74 (0.52–1.04)	**0.003**	0.51 (0.32–0.80)	0.56	1.14 (0.74–1.73)

HS: healthy subjects. N: Number. OR: odds ratio. 95%CI: confidence interval.
P-values are estimated by the chi-square test or the Fisher's exact test when appropriate.
P1 and OR1= CRC cases vs. HS, P2 and OR2= Colon cancer cases vs. HS, P3 and OR3= Rectum cancer cases vs. HS.
Significant P-values are represented in Bold.

with the development of CRC in this study.

Furthermore, stratification analyses did not reveal any significant associations between the studied *IL-10* gene SNPs and CRC clinicopathological characteristics including tumor infiltration depth, lymph node involvement, distant metastasis, TNM staging and grade (data not shown).

3.2. Association between haplotypes of IL-10 gene SNPs with CRC risk and its severity

In this study, *IL-10* -1082A/G exhibited partial linkage disequilibrium with *IL-10* -592A/C (D' = 0.65, $r^2 = 0.13$, Fig. 1a–b). Based on these two SNPs, four possible haplotypes (Hap1, Hap2, Hap3, and Hap4) were identified. Among these haplotypes, only the first three (Hap1, Hap2, and Hap3; Table 3) displayed frequencies above 1 % among both cases and controls, thus warranting further evaluation. No significant disparity in the overall distribution of *IL-10* gene haplotypes was observed between the CRC and HS groups (Table 3). It is essential to note that a trend towards decreased CRC risk was evident in the presence of Hap3 which consisted of *IL-10* -1082G and −592C alleles (OR [95%CI] = 0.74 [0.52–1.04], p = 0.08, Table 3). Subgroup analysis by disease site showed that Hap1, consisting of *IL-10* -1082A and −592 C alleles, was more common among colon cancer cases (43.0 %) than in HS (27.7 %), resulting in increased susceptibility (OR [95%CI] = 1.72 [1.15–2.60], p = 0.01, Table 3). Furthermore, we found that Hap3 was significantly associated with a reduced risk of this type of cancer compared to other combinations (OR [95%CI] = 0.51[0.32–0.80], p = 0.003; Table 3). In rectal cancer, the distribution of haplotypes between cases and HS groups was similar (p > 0.05, Table 3).

Associations between *IL-10* SNPs and the clinicopathological features of CRC at the haplotype level were also evaluated. However, no significant results were found (data not shown).

3.3. Association between diplotypes of IL-10 gene SNPs with CRC risk and its severity

Based on the promising results in the preceding analysis, we proceeded to conduct further evaluations concerning the combined effect of haplotype pairs (diplotypes) within the *IL-10* promoter region on the development and severity of CRC. The homozygous Hap3/Hap3 diplotype demonstrated a significant reduction in susceptibility to CRC (OR [95%CI] = 0.35[0.14–0.85], p = 0.02, Table 4). Nevertheless, consistent with our expectations, there were no distinct associations observed between all diplotypes tested and the clinicopathological characteristics of CRC (data not shown).

3.4. Effect of IL-10 gene SNPs genotype, haplotype, and diplotype on overall survival

Upon analyzing all patients, we found a significant association between *IL-10* -592A/C SNP and worse OS (AA genotype versus other genotypes; Log-rank p = 0.021, Fig. 2c; However, no association at genotype levels Fig. 2b). However, we revealed no significant effect of *IL-10* -1082A/G on OS at genotype level (Log-rank p > 0.05, Fig. 2a). Similarly, no association was observed between the IL-10 haplotype and OS (Log-rank p > 0.05, Fig. 3a–c).

However, examining CRC cases based on disease sites revealed interesting results. Notably, Hap1 exhibited a significant association with improved OS in the colon cancer subgroup (Log-rank p = 0.002, Fig. 4a). No association was found between other analyzed haplotypes and patients' survival (Fig. 4b-c). Furthermore, diplotype-based analysis revealed that patients with homozygous Hap2/Hap2 diplotype had shorter OS (Log-rank p = 0.01, Fig. 5b), and this finding was consistent in the colon cancer subgroup (Log-rank p = 0.001, Fig. 6b). Although the relationship was not statistically significant (Fig. 5a and c), there was a tendency for improved OS among CRC cases with the homozygous Hap3/Hap3 diplotype (Log-rank p = 0.21, Fig. 5c). In addition, it was noteworthy that none of

Table 4
Distribution of diplotypes of *IL-10* gene SNPs between CRC, colon cancer, rectum cancer cases, and HS.

Diplotypes	CRC cases N (%)	Colon cancer cases N (%)	Rectum cancer cases N (%)	HS N (%)	P_1	OR_1 (95%CI)	P_2	OR_2 (95%CI)	P_3	OR_3 (95%CI)
Hap1/Hap1 vs.	16 (12)	12 (17)	4 (7)	15 (9)	0.37	1.40 (0.67–3.00)	0.07	2.10 (0.90–4.60)	0.54*	0.70 (0.25–2.10)
Hap1/others + Others/others	114 (88)	57 (83)	57 (93)	150 (91)						
Hap2/Hap2 vs.	14 (11)	7 (10)	7 (11)	15 (9)	0.63	1.21 (0.59–2.67)	0.80	1.13 (0.44–2.93)	0.59	1.30 (0.51–3.04)
Hap2/others + Others/others	116 (89)	62 (90)	54 (89)	150 (91)						
Hap3/Hap3 vs.	6 (5)	0 (0)	6 (10)	20 (12)	**0.02**	0.35 (0.14–0.85)	–	–	0.63	0.79 (0.31–1.99)
Hap3/others + Others/others	124 (95)	69 (100)	55 (90)	145 (88)						

HS: healthy subjects. N: Number. OR: odds ratio. 95%CI: confidence interval.
P-values are estimated by the chi-square test or the Fisher's exact test when appropriate.
P1 and OR1: CRC cases vs. HS, P2, and OR2: Colon cancer cases vs. HS, P3 and OR3: Rectum cancer cases vs. HS.
Significant P-values are represented in Bold.

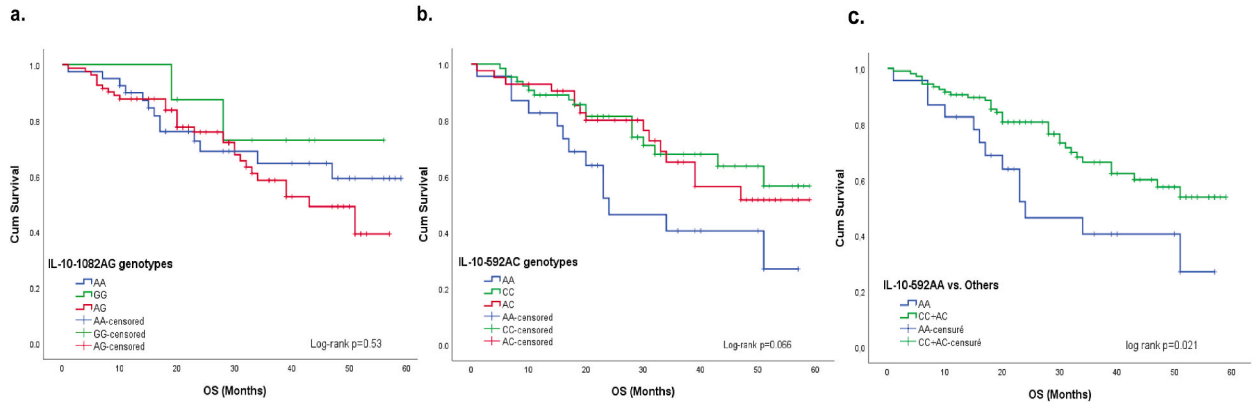

Fig. 2. Effect of *IL-10* gene SNPs on the overall survival in CRC cases Kaplan-Meier estimates showed no association between the genotypes of SNPs −1082A/G (Log-rank p = 0.53) (a.) and −592AC (Log-rank p = 0.066) (b.) in the promoter region of the *IL-10* gene and the overall survival of CRC cases. Kaplan-Meier estimates showed an association between the *IL-10* -592 AA genotype versus other genotypes (CC + AC) (Log-rank p = 0.021) (c.).

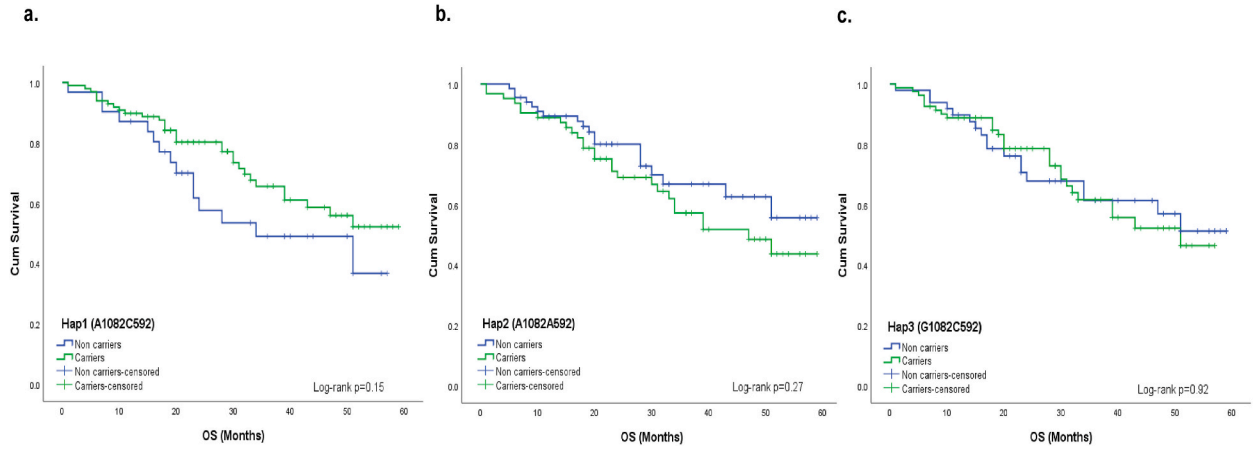

Fig. 3. Effect of *IL-10* gene haplotypes on the overall survival in CRC cases Kaplan-Meier estimates showed no association between all the combinations of haplotypes derived from the −1082A/G and −592A/C SNPs in the promoter region of the *IL-10* gene and the overall survival in CRC cases (Log-rank p value > 0.05) (a. b. and c.).

the colon cancer cases exhibited the homozygous Hap3/Hap3 diplotype. No association was found between homozygous Hap1/Hap1 diplotype and OS among colon cancer patients (Fig. 6a).

4. Discussion

In this study, the frequencies of the *IL-10* -1082G and −592C alleles in the healthy controls were 0.38 and 0.66, respectively. These frequencies were very similar to those previously reported in various studies involving healthy North African subjects [26–28]. Based on the NCBI database of short genetic variation (dbSNP), this consistency was also observed in healthy Caucasian subjects (0.47 and 0.76, respectively). The −1082G allele is very rare (0.06) in Asian populations. However, 592C appears to be less common in Asians than in Caucasians and Africans (0.27, 0.76, and 0.59, respectively).

The genetic SNPs −1082A/G and −592A/C are situated in the proximal promoter region of the *IL-10* gene and are recognized as critical determinants in regulating the levels of IL-10. Studies have demonstrated that the −1082G allele, along with the GCC haplotypes (comprising *IL-10*-1082G, −819C, and −592C), as well as the GCC/GCC diplotype, are strongly associated with higher expression levels of IL-10. Conversely, the ACC haplotype (comprising *IL-10*-1082A, −819C, and −592C) and ATA haplotype (comprising *IL-10*-1082A, −819T, and −592A) are considered as intermediate- and low-producers of IL-10, respectively [13]. To the best of our knowledge, this study represents the first investigation into the influence of genotypes, haplotypes, and diplotypes of-1082A/G (rs1800896) and −592A/C (rs1800872) SNPs within the *IL-10* gene promoter on CRC susceptibility, progression and outcome within the Tunisian population. Our findings revealed that the presence of the genotype AG at position −1082 serves as a risk factor for CRC (OR = 1.65, p = 0.04). Conversely, we observed that −1082G allele in the recessive model is associated with a reduced

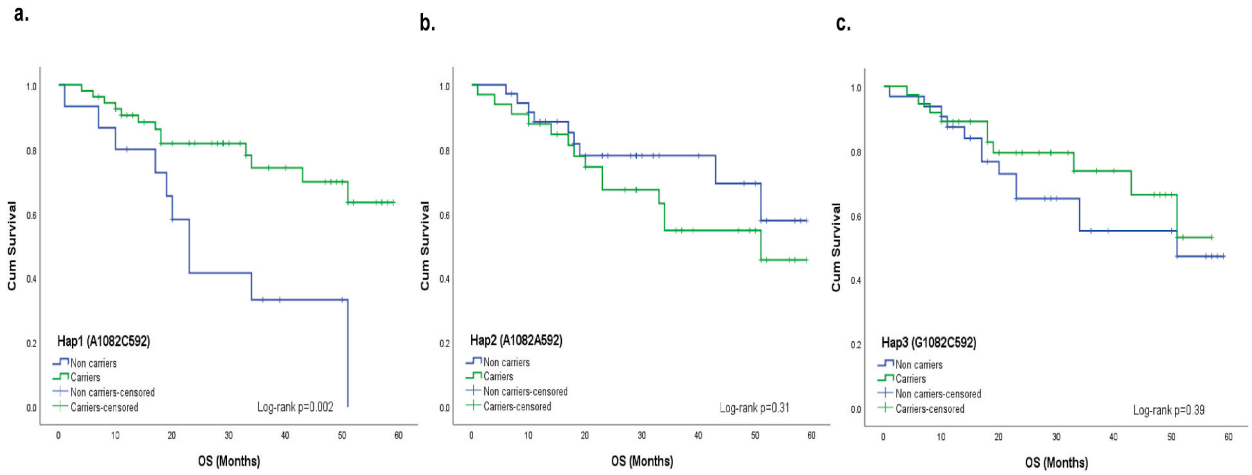

Fig. 4. Effect of *IL-10* gene haplotypes on the overall survival in cases with colon cancer. In cases with colon cancer subgroup, Kaplan-Meier estimates showed that Hap1 carriers (consisting of the 1082A and 592C alleles) had a better overall survival (OS) compared with non-carriers (Log-rank p = 0.002) (a.). No significant results were observed for other haplotype combinations (Log-rank p values > 0.05) (b. and c.).

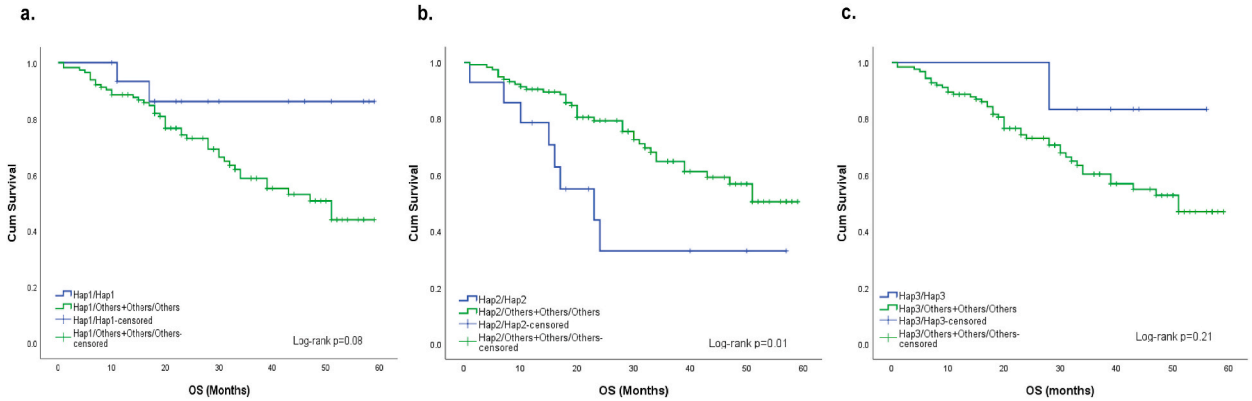

Fig. 5. Effect of *IL-10* gene diplotypes on the overall survival in CRC cases Kaplan-Meier estimates showed that carriers of the homozygous Hap2/Hap2 diplotype had a lower overall survival (OS) compared with Hap2/Others + Others/Others carriers (Log-rank p = 0.01) (b.). No significant association was observed between all the other combinations of diplotype and OS in CRC cases (Log-rank p values > 0.05) (a. and c.).

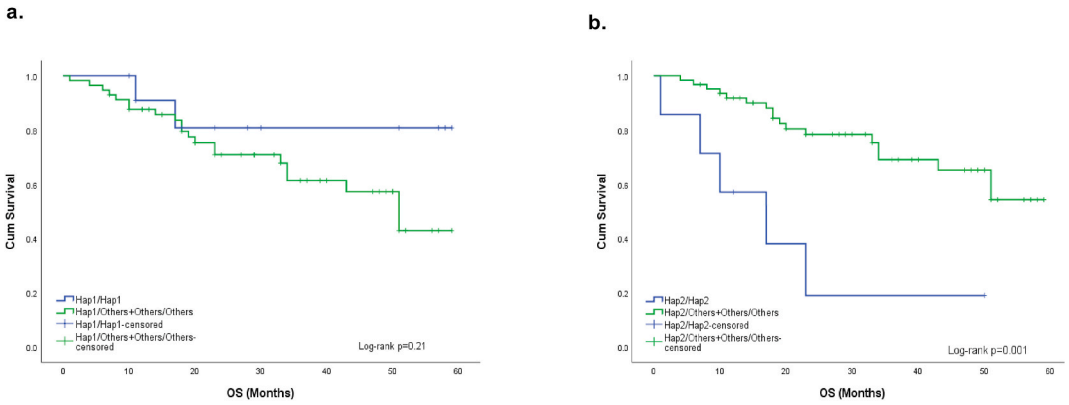

Fig. 6. Effect of *IL-10* gene diplotypes on the overall survival in cases with colon cancer. In cases with colon cancer subgroup, Kaplan-Meier estimates showed that carriers of homozygous Hap2/Hap2 diplotype had a significantly shorter OS compared to Hap2/Others + Others/Others carriers (Log-rank p = 0.001) (b.). No association was observed between homozygous Hap1/Hap1 diplotype and patients' outcome (Log-rank p = 0.21) (a.). None of cases with colon cancer had the homozygous Hap3/Hap3 diplotype.

risk of CRC (OR = 0.44, p = 0.03). After stratification according to the disease site, this association persisted when comparing colon cancer patients with the HS group (OR = 0.18, p = 0.01). These results are consistent with previous studies from the US and Romania supporting a protective role of −1082G allele for the development of CRC [17,29]. However, Miteva et *al.*(2014) reported no genetic association between the *IL-10* -1082A/G SNP and the occurrence of CRC in the Bulgarian population [30]. They suggested that the G allele may be involved in leading the disease to a more aggressive phenotype. In contrast, no discernible association between *IL-10* -1082A/G and CCR risk was observed in various other ethnic groups [19,20].

In this study, a positive association was observed between *IL-10* -592A/C SNP and CRC. Indeed, the codominant model (AC vs. AA + CC) was associated with a low CRC risk (OR = 0.59, p = 0.03). Although these results contradict a previous study [21], they agree with what was reported in a recent Tunisian study [31]. Moreover, it has been shown that Chinese carrying AC or AC/CC genotypes are less prone to CRC compared with the AA genotype [32,33]. There is no consensus on the role of the *IL-10* -592AA variant in CRC. On the contrary, in a study conducted on 142 CRC patients from Kashmir, the authors reported that the −592A variant is a protective factor against the development of CRC at both allelic and genotypic levels [34]. In an attempt to address the inconsistency observed in various investigating the role of *IL-10* -1082A/G and −592A/C SNPs in the development of CRC, Zhang et *al.* (2012) conducted a comprehensive meta-analysis that encompassed a total of 1469 CRC patients and 2566 controls [35]. They reported that none of the aforementioned SNPs had a significant effect on CRC risk. However, through stratified analysis of controls, they found that the −592A allele may potentially increase susceptibility to CRC. More recently, a meta-analysis conducted by Mirjalili et *al.* (2018) involving a larger dataset of 5647 CRC patients and 6908 controls, did not reveal any relevant association between *IL-10* gene SNPs and CRC [36].

Interestingly, haplotype and diplotype-based studies consider the interactions and cumulative effects of different genetic variants and aim to advance our understanding of the multigenic basis underlying disease susceptibility, progression, and response to treatment. These approaches go beyond the analysis of individual genotypes and allow for more comprehensive assessments. In our study, we examined the involvement of haplotypes and diplotypes derived from the two SNPs of the *IL-10* gene in relation to the development of CRC and its severity. Interestingly, we observed that carrying Hap3, formed by the combination of −1082G and −592C alleles, provided significant protection against the onset of CRC compared to other haplotypes. Additionally, individuals who are homozygous for the Hap3/Hap3 diplotype showed a reduced CRC risk (OR = 0.35, p = 0.02). In the subset of patients with colon cancer, we found that Hap1, formed by the combination of *IL-10*-1082A and −592C alleles, increased the susceptibility for this type of cancer compared to other combinations (OR = 1.72, p = 0.01). It was noteworthy that our findings differ from those reported in studies examining gastric and nasopharyngeal cancers, wherein no association was observed between any of the IL-10 haplotypes derived from these two SNPs and cancer susceptibility [27,37]. However, El-Omar et *al.* (2003) showed that the haplotype ATA (consisting of *IL-10*-1082A, −819T and −592A) may increase the risk of noncardia gastric cancer 2.5-fold compared to the GCC haplotype (composed of *IL-10*-1082G, −819C and −592C alleles) [38].

In the overall population with CRC, carriers of the Hap2 haplotype (composed of *IL-10*-1082A and −592A alleles) exhibited a worse OS. Furthermore, when considering only patients with colon cancer, we observed a significant association between the homozygous Hap2/Hap2 diplotype and worse OS. It is important to note that there is limited research investigating the relationship between CRC outcome and these two SNPs at the haplotype level, and as a result, available data in this regard are scarce [19,20,39]. Supporting our findings, a study on Australian CRC patients also reported a shorter OS in patients who were homozygous for the A allele at position −1082, and those who carried at least one copy of the A allele at position −592 in the promoter region of the *IL-10* gene compared to individuals with −1082G and −592C alleles, respectively [40]. Additionally, this study found that the −592A variant is associated with a higher production of IL-10. In another study conducted in Spain, a borderline association was observed between the −1082GG genotype and a favorable OS among patients with lymphoid cancers (p = 0.05) [41].

Martinez-Escribano et *al.* revealed that low-producer genotypes of the *IL-10* gene, particularly the ACC/ATA genotype (representing genotypic variations at positions −1082, −819, and −592 in the *IL-10* gene promoter), were linked to poorer survival in Spanish patients with melanoma patients [42]. Similarly, in Hodgkin's lymphoma, the −592AA genotype and IL-10 haplotype (−3575T/-2849G/-2763C/-1082A/-592A) were associated with a worse prognosis [43]. IL-10 plays a critical role as a regulatory cytokine in the immune system and its differential expression patterns may influence the occurrence or protection against diseases, although this role remains controversial. Some studies have reported a positive association between low IL-10 expression and the development of CRC [44]. However, adenomas and serrated adenomas have been found to exhibit increased concentrations of IL-10 compared to normal cells [45]. and metastatic colon cancer has shown higher IL-10 expression than primary tumors [46]. Furthermore, IL-10 deficiency has been linked to intestinal mucosal lesions, leading to chronic inflammation and potentially paving the way for CRC development [6]. In intestinal epithelial cells, loss of IL-10 has been associated with decreased expression of the immunosuppressive gene Bcl3, and increased expression of IL-17, IFN-γ, and TNF-α expression, which can cause damage to the intestinal mucosal surface [47]. Notably, IL-10 high expression or treatment with pegylated IL-10 has been found to induce cancer regression and confer a persistent and effective immune response in murine models [6]. However, contrasting relationships have been observed in other studies between IL-10 expression and CRC onset and outcome. It has been shown a significant association between low IL-10 levels and disease susceptibility, while elevated levels were correlated with an unfavorable prognosis [44]. These findings further emphasize the duality of IL-10 function in the pathogenesis of CRC, demanding extensive attention to fully understand its complex role in cancer development and progression.

Taken together, our results indicated that the −1082A/G and −592A/C SNPs in the promoter region of the *IL-10* gene are associated with CRC and may be valuable factors in disease susceptibility and prognosis. Further studies in a larger population are needed to advance our understanding of the pathogenesis of CRC, which may lead to improved diagnosis and treatment strategies.

CRediT authorship contribution statement

Sabrine Dhouioui: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Sana Baroudi:** Investigation, Formal analysis. **Ines Zemni:** Writing – review & editing, Validation, Resources, Formal analysis. **Fadia Mahdhi:** Investigation, Formal analysis. **Afef Najjari:** Writing – review & editing. **Hanen Chelbi:** Writing – review & editing, Methodology. **Houyem Khiari:** Validation, Methodology. **Nadia Boujelbene:** Writing – review & editing, Validation, Resources, Methodology, Conceptualization. **Ines Zidi:** Writing – review & editing, Validation, Supervision, Resources, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Ines Zidi is currently serving as an Associate Editor for Heliyon Immunology. Although she was not involved in the review of this specific manuscript, she is disclosing this position to ensure transparency and uphold the integrity of the review process for this submission.

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