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Association of PTPN22 single nucleotide polymorphisms (-1123G/C, +788G/A and +1858C/T) with inflammatory bowel disease

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Abstract

Inflammatory bowel disease (IBD) is a class of chronic inflammatory disorders including, Crohn's disease (CD) and ulcerative colitis (UC). The PTPN22 gene is thought to be a T-cell negative regulator, regulates immune cell activation, and an important risk factor for human autoimmunity. This study aimed to investigate the potential association of PTPN22 gene single nucleotide polymorphisms (SNPs) with inflammatory bowel disease in Egyptian patients and their relation to clinical disease characteristics. Three SNPs in the PTPN22 gene (-1123G/C, +788G/A, and +1858C/T) were investigated in 90 IBD patients (19 with CD and 71 with UC) and 81 apparently healthy controls. These 3 polymorphisms were genotyped by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Allele and genotype frequencies were correlated with disease association and with clinical disease characteristics. No statistically significant differences in the genotype and allele frequencies of the PTPN22 gene SNPs (-1123G/C, +788G/A, and +1858C/T) were found between IBD patients and control subjects. In conclusion although the PTPN22 gene is involved in autoimmune diseases, it does not appear to be associated with IBD predisposition or its clinical characteristics in Egyptians.

Keywords: IBD, UC, Crohn's disease, PTPN22, SNPs.

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Introduction

Inflammatory bowel disease (IBD), which includes ulcerative colitis (UC) and Crohn's disease (CD), is a chronic inflammatory disorder of the gastrointestinal tract characterized by significant morbidity and lifelong medication.[1] IBD is characterized by repetitive episodes of inflammation of the gastrointestinal tract, caused by an abnormal immune response to gut microflora.[2] The pathophysiology of IBD is unknown, but an altered innate and adaptive immune response combined with disequilibrium of the gut microbiome and genetic susceptibility is the most likely proposed hypothesis.[3]

IBD is likely caused by a polygenic inheritance process.[4] Genome-wide association studies have allowed a better understanding of IBD; thus, several genetic susceptibility loci for UC and CD were found.[5] Several single nucleotide polymorphisms (SNPs) in immune

system components are associated with either susceptibility or protective effects to IBD development. However, these associations can be contradictory, primarily depending on the onset (pediatric or adult), sample size variations, insufficient statistical power and genetic background that varies depending on ethnicity.[6-9]

The gene for protein tyrosine phosphatase non-receptor 22 (PTPN22) is located on the short arm of Chromosome 1 (1p13.2) and encodes the protein lymphoid tyrosine phosphatase (Lyp) that is expressed in many immune cells, such as T and B lymphocytes, dendritic, and natural killer cells. Lyp maintains intestinal epithelial barrier function and regulates immunological responses to invading microorganisms in intestinal cells.[10] PTPN22 is a potent inhibitor of T-cell activation and adversely affects T and B cell receptor signaling.[11]

The (-1123 G >C) SNP is located in the promoter region of PTPN22 gene.[12] Studies on DNA sequences around the PTPN22 −1123G>C SNP site demonstrate that −1123G matches the binding site of the transcription factors of activating protein 4 (AP-4), but the −1123C allele does not match any of the known transcription factors. Thus, PTPN22 −1123C was assumed to impact Lyp transcription efficiency.[13]

The (788 G>A) SNP in exon 10 of PTPN22 gene results in amino acid change from arginine to glutamine at codon 263, which impacts Lyp interaction with the C-terminal Src Kinase , therefore preventing the formation of the complex and the subsequent suppression of T-cell activation.[14] The (1858 C>T) SNP is located in exon 14 of the PTPN22 gene and results in an amino acid change from arginine to tryptophan at codon 620.[15] The PTPN22 1858T allele was reported to be associated with various autoimmune diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Type I Diabetes (T1D) and other autoimmune diseases. The 1858 T allele of PTPN22 is considered the most significant non-HLA risk allele in autoimmunity.[16] This allele impacts both innate and adaptive immune functions, lymphocyte activation, and cytokine production in various autoimmune situations.[17.]

There were conflicting studies published regarding the association of PTPN22 gene SNPs with IBD.[12, 18-24] To date, there are no reported studies investigating the correlation between PTPN22 gene SNPs and IBD in Egyptians. Therefore, this work aimed to investigate the potential association of PTPN22 gene (-1123G/C, +788G/A, and +1858C/T) SNPs in Egyptian IBD patients and to determine their relation to clinical disease characteristics.

Subjects and Methods

This case-control study included 90 IBD patients (71 UC and 19 CD), and 81 normal control subjects, age and sex matched with IBD patients. Patients were recruited from the Outpatient Department of El-Rajhy Liver Hospital, Assiut University, Egypt.

The diagnosis of UC and CD was determined according to established European Crohn's and Colitis Organization (ECCO) Guidelines.[25, 26] The Montreal classification was used to define the phenotypic characteristics of UC and CD.[27] The clinical disease activity was evaluated using Crohn's Disease Activity Index (CDAI) for CD.[28] and Mayo scoring system for UC disease activity.[29]

Exclusion criteria

Patients with systemic autoimmune disorders, such as systemic lupus erythematosus (SLE), were excluded from our study.

Sample collection

A venous blood sample (about 8 ml) was collected from each subject under aseptic conditions. Each sample was divided into two parts. The first part, 4 ml was collected into 2 tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). Of these, one tube was used for complete blood count (CBC) and erythrocyte sedimentation rate (ESR), and the other was stored at -70°C to be used later for DNA extraction. The remaining 4 ml was allowed to clot in a plain vacutainer tube for serum collection. Serum was obtained by centrifugation, divided into aliquots, and used

either immediately or stored at -70°C until the time of use.

Routine investigations

Complete blood count was done on a hematology analyzer (ADVIA 2120i, Siemens Heathineers, USA), according to the manufacturer's instructions. ESR was performed by a Westergren tube.[30] C-Reactive protein (CRP) was done on an automatic biochemistry analyzer (ADVIA 1800 chemistry auto-Analyzers, Siemens Healthineers, USA), according to the manufacturer's instructions. The antinuclear antibody (ANA) test was done by an indirect immunofluorescence assay using ANA kits (REF 1662, Fluoro kits Diasorin Inc., USA), according to the manufacturer's instructions.

Single nucleotide polymorphism (SNP) genotyping

Genomic DNA was extracted from EDTA anticoagulated blood samples using the Gene JET whole blood genomic DNA purification kit (cat**.** no.; K0781 and K0782, Thermo Fisher Scientific, Baltics), according to manufacturer's instructions. Three SNPs were studied. These included PTPN22 -1123 G/C (rs2488457) SNP, PTPN22 +788 G/A (rs33996649) SNP, and PTPN22 +1858 C/T (rs2476601) SNP. The SNPs were studied using the polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP) method.

The genotyping of the PTPN22 −1123G/C SNP was done using the forward primer: 5'-CCA TTG AGA GGT TAT GCG AGC T-3' and reverse primer: 5'-CGC CAC CTT GCT GAC AAC AT-3'.[31–36] The PCR procedure involved an initial denaturation at 95°C for 5 minutes, followed by 35 cycles each of 95°C for 40 seconds, 58°C for 40 seconds, and 72°C for 40 seconds, followed by a final extension step of 5 minutes at 72°C.[31] The PCR product (205 bp) of the −1123G/C variant was subjected to digestion with the SacI enzyme (cat. no.: ER1131, Thermo Fisher Scientific, Baltics), at 37°C for 16 hours, according to manufacturer's instructions. The digested DNA fragments were separated via electrophoresis on a 3% agarose gel, stained with ethidium bromide, and visualized by UV illumination. The product after digestion with SacI was a 205 bp fragment for a homozygous wild type (G/G), 183 bp and 22 bp for a homozygous mutant type (C/C), and 205 bp, 183 bp and 22 bp for a heterozygous (G/C) genotype (Figure 1.a).

The PTPN22 +788G/A SNP was genotyped using the forward primer 5'-GAT GGA GCA AGA CTC AGA CAC-3' and reverse primer 5'-CCC CAT GTT AGA AGA GCA GAT-3'.[32, 35-38] The PCR procedure involved an initial denaturation at 95°C for 5 minutes, followed by 35 cycles each of 95°C for 40 seconds, 58°C for 40 seconds, and 72°C for 40 seconds, followed by a final extension step of 5 minutes at 72°C. The PCR product (234 bp) was digested with the MspI enzyme (cat. no.; ER0541, Thermo Fisher Scientific, Baltics), at 37°C for 16 hours, according to manufacturer's instructions. The DNA fragments were separated via electrophoresis on a 2.5% agarose gel, stained with ethidium bromide, and visualized by UV illumination. The product after MspI digestion was two fragments, 143 bp and 91 bp for homozygous wild type (G/G), one fragment of 234 bp for homozygous mutant type (A/A), and three fragments of 234 bp, 143 bp, and 91 bp for heterozygous (G/A) (Figure 1.b).

The genotyping of the PTPN22 +1858C/T SNP involved performing PCR using the forward primer 5'-ACT GAT AAT GTT GCT TCA ACG G-3' and reverse primer 5'-TCA CCA GCT TCC TCA ACC AC-3'.[39, 40] The PCR procedure involved an initial denaturation at 95°C for 5 minutes, followed by 35 cycles each of 95°C for 40 seconds, 60°C for 40 seconds, and 72°C for 40 seconds, followed by a final extension step of 5 minutes at 72°C.[39, 40] The 218 bp PCR product of the +1858C/T variant was digested with the RsaI enzyme (cat. no; ER1121, Thermo Fisher Scientific, Baltics), for 16 hours at 37°C, according to manufacturer's instructions. The DNA fragments were separated via electrophoresis on 3% agarose gel, stained with ethidium bromide, and visualized by UV illumination. The product after digestion with the RsaI enzyme was two fragments, 176 bp and 42 bp, representing homozygous wild type (C/C), one fragment of 218 bp representing homozygous mutant type (T/T), and three

fragments (218 bp, 176 bp, and 42 bp) representing heterozygous (C/T) (Figure 1.c).

Statistical Analysis

The statistical analyses were conducted using the RStudio statistical analysis software. Categorical data are expressed as frequencies and percentages, and group comparisons were performed using the Chi-square test or Fisher's exact test. For continuous data, descriptive statistics such as mean ± SD, median (Min-Max) were reported. Normality of continuous data was assessed using the Shapiro-Wilkes test. Student's t-test was employed for group comparisons when data was normally distributed, while the Mann-Whitney test was used for non-normally distributed data. In all statistical tests, significance was set at a *p*-value < 0.05. To estimate risk, odds ratios with 95% confidence intervals (CI) were calculated for both genotypic and allelic frequencies. Allele frequency was determined by direct counting followed by division by the number of chromosomes. Genotype frequency was computed by direct counting divided by the number of participants. Hardy–Weinberg equilibrium (HWE) was assessed to evaluate the study sample's quality. The expected frequency for each genotype was calculated (https://wpcalc.com/en/equilibrium-hardy-weinberg/), and a χ^2-test was employed to compare it with the observed value. A χ^2 value greater than or equal to 3.84 indicated a significant deviation of the genotype distribution from HWE.

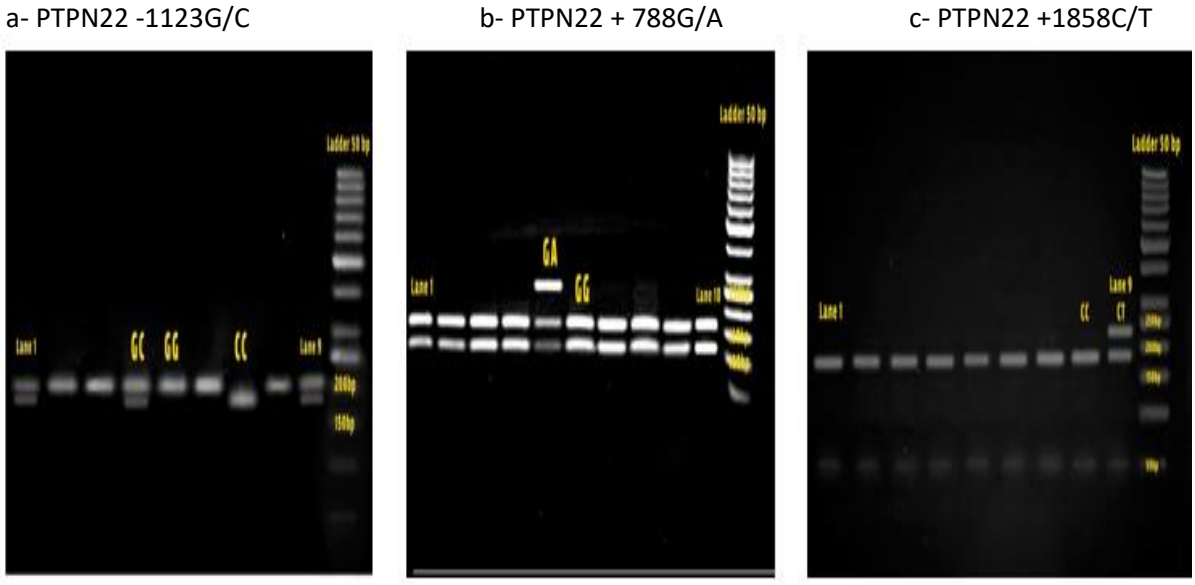

Figure 1. Genotype analysis of PTPN22 (-1123G/C, +788G/A, and +1858C/T) SNPS. (a) PTPN22 -1123G/C genotyping patterns: lanes (1, 4, 9): GC heterozygous type (205bp, 183 bp, ant type (183 bp and 22 bp). 22 bp), Lanes (2, 3, 5, 6, 8): GG homozygous wild type (205bp) and Lane 7: CC homozygous mut. (b) PTPN22 + 788G/A genotyping patterns: Lanes (1-4, 6-10): GG homozygous wild type (143 bp,91 bp), Lane 5: GA heterozygous type (234 bp,143 bp,91 bp). (c) PTPN22 +1858C/T genotyping patterns: Lanes (1-8): C/C homozygous wild type (176 bp, 42 bp), Lane 9: C/T heterozygous type (218 bp, 176 bp, 42 bp).

Results

The study included 90 IBD patients (71 UC and 19 CD) and 81 age- and sex-matched apparently healthy subjects as controls in the study (Table 1). The demographic and clinical data of the UC and CD patients are shown in Table 2. Table 3 shows the results of CBC parameters and inflammatory marker levels (CRP and ESR) in the control group, CD, and UC patients. All study subjects including control group, CD, and UC patients had negative ANA test results.

Table 1. Age and Sex Distribution of Control vs. Inflammatory bowel disease (IBD) Groups.

Characteristic		Control N = 81	IBD N = 90	*p*-value^
Age (years)	Mean ± SD	36.70 ± 11.47	34.49 ± 12.11	NS
	Median (min – max)	34 (18-64)	32.5 (18 -71)	
Sex	Female	43 (53.1%)	55 (61.1%)	NS
	Male	38 (46.9%)	35 (38.9%)	

^Mann-Whitny test and Chi-squared test. N: number; SD: standard deviation; IBD: inflammatory bowel disease
$p > 0.05$ is not significant (NS).

Table 2. Demographic characteristics and clinical features of the patients with ulcerative colitis (UC) and Crohn's disease (CD).

Characteristic		UC N = 71	CD N = 19
Age (years)	Mean ± SD	33.42 ± 10.96	38.47 ± 15.37
	Median (min – max)	32 (18-67)	34 (25-71)
Sex	Female	49 (69.0%)	6 (31.6%)
	Male	22 (31.0%)	13 (68.4%)
Age of disease onset	Early onset (≤ 40)	61 (85.9%)	14 (73.7%)
	Late onset (>40)	10 (14.1%)	5 (26.3%)
Disease duration	≤ 1 year	22 (31.0%)	6 (31.6%)
	1 - 5 years	32 (45.1%)	6 (31.6%)
	> 5 years	17 (23.9%)	7 (36.8%)
Disease location of UC	E1 (Proctitis)	21 (30%)	
	E2 (left sided)	40 (55.7%)	
	E3 (Extensive colitis)	10 (14.3%)	
Disease location of CD	L1 (Terminal ileum)		4 (21.15%)
	L2 (Ileocolon)		6 (31.6%)
	L3 (Colon)		9 (47.4%)
Disease Activity	Remission	3 (4.2%)	5 (26.3%)
	Mild	32 (45.1%)	6 (31.6%)
	Moderate	29 (40.8%)	5 (26.3%)
	Severe	7 (9.9%)	3 (15.8%)
Complications	Intestinal	4 (5.6%)	15 (78.9%)
	Extraintestinal	5 (7%)	3 (15.8%)
Treatment	Conventional therapy only	53 (74.6%)	2 (10.5%)
	Biological therapy	18 (25.4%)	17 (89.5%)
	Operations	0 (0.0%)	6 (31.6%)

N: number; SD: standard deviation; UC: ulcerative colitis; CD: Crohn's disease.

Table 3. Complete blood count (CBC) parameters, inflammatory markers level (C-reactive protein, CRP and erythrocyte sedimentation rate, ESR) in the control group, Crohn's disease (CD) and ulcerative colitis (UC) patients.

Characteristic		Control N = 81	CD N = 19	UC N = 71	*p*1	*p*2	*p*3
CBC:							
Hb F (12-15) g/dl M (13-17) g/dl	Mean± SD	13.57 ± 1.62	12.13 ± 2.53	12.30 ± 1.84	0.021*	<0.001*	NS*
	Median	13.50 (10.3 - 16.5)	12.20 (8.10-16.2)	12.50 (7.4-15.8)			
Hct F (36-46%) M (40-50%)	Mean± SD	38.83 ±4.5	38.29 ±6.84	4.36 ±37.96	NS*	NS*	NS*
	Median	39.0 (30.0-50.0)	38.4 (24.9-49.3)	38.50 (27-46.7)			
WBCs (4.0-11.0)× 10^9/L	Mean± SD	6.33 ± 1.78	9.16 ± 8.25	6.72 ± 2.34	NS^	NS^	NS^
	Median	6.01 (4.0-10.90)	6.46 (2.76-40.05)	6.4 (1.93-15.4)			
Platelets (150-450)× 10^9/L	Mean± SD	271.31 ± 68.75	335.21 ± 169.48	331.49 ± 110.41	NS^	<0.001^	NS^
	Median	271 (146-446)	300 (112-851)	303 (20-705)			
Inflammatory markers:							
ESR M (3-5) mm/hr F (7-12) mm/hr	Mean ± SD	13.59 ± 7.09	79 ± 32.78	40.45 ± 26.03	<0.001^	<0.001^	NS^
	Median	13 (3-31)	25 (2-120)	33 (5-120)			
CRP (0-5) mg/l	Mean ± SD	2.36 ± 1.70	21.85 ± 23.14	11.50 ± 14.63	<0.001^	<0.001^	0.045^
	Median	2.10 (0.10-5.0)	15.20 (0.20-92.5)	5.50 (0-59.30)			

Hb: hemoglobin; WBCs: white blood cells; HCT: hematocrit; *Students t- test; ^ Mann-Whitny test; SD: standard deviation; N: number. *p*1 Control vs. Crohn's & *p2* Control vs. UC & *p*3 CD vs. UC. $p > 0.05$ is not significant (NS).

Hardy-Weinberg equilibrium analyses were conducted for the three polymorphisms (-1123 G/C, +788G/A, and +1858 C/T) within the study population. For -1123G/C, +788G/A, and +1858C/T SNPs, the chi-square ($\chi 2$) values were 0.299 (p= 0.645), 0.074 (p= 0.999), and 0.098 (p= 0.999), respectively. There was no significant departure from Hardy-Weinberg equilibrium, indicating that the observed genotype frequencies align with the expected frequencies for these polymorphisms in the study population.

There was no statistically significant difference in the genotype and allele frequencies of PTPN22 (-1123 G/C, +788 G/A, +1858 C/T) SNPs between CD patients, UC patients, and normal controls. Moreover, there was no significant difference in the genotype and allele frequencies of PTPN22 (-1123 G/C, +788 G/A, +1858 C/T) polymorphisms in IBD patients compared with the control group (Table 4). Regarding disease characteristics, no association was found between PTPN22 (-1123G/C, +788 G/A, +1858 C/T) SNPs and studied disease characteristics including age at onset, location, disease activity, intestinal, and extra-intestinal complications as well as drug therapy, as shown in Tables 5, 6, 7, and 8.

Table 4. Genotype and allele frequencies of the PTPN22 SNPs (-1123G/C, +788G/A and +1858C/T) in inflammatory bowel disease (IBD), ulcerative colitis (UC), Crohn's disease (CD) patients and the control group.

	PTPN22 SNP genotype & allele frequencies	Patients	Control (n=81)	*p* value	Risk Estimates OR (95% CI)
IBD (n=90)	(-1123G/C) SNP				
	Wild genotype GG	52 (57.8%)	55 (67.9%)	--	Ref.
	Heterozygous GC	34 (37.8%)	24 (29.6%)	NS	1.50 (0.79-2.88)
	Homozygous CC	4 (4.4%)	2 (2.5%)	NS	2.12 (0.40-15.72)
	C carrier (GC +CC)	38 (42.2%)	26 (32.1%)	NS	1.55 (0.83-2.89)
	G Allele	138 (76.7%)	134 (82.7%)	--	Ref.
	C Allele	42 (23.3%)	28 (17.3%)	NS	1.46 (0.85-2.48)
	(+788G/A) SNP				
	Wild genotype GG	86 (95.6%)	78 (96.3%)	--	Ref.
	Heterozygous GA (A carrier)	4 (4.4%)	3 (3.7%)	NS	1.21 (0.26-6.30)
	G Allele	176 (97.8%)	159 (98.1%)	--	Ref.
	A Allele	4 (2.2%)	3 (1.9%)	NS	1.20 (0.27-5.47)
	(+1858C/T) SNP				
	Wild genotype CC	86 (95.6%)	77 (95.1%)	--	Ref.
	Heterozygous CT (T carrier)	4 (4.4%)	4 (4.9%)	NS	0.90 (0.21-3.90)
	C Allele	176 (97.8%)	158 (97.5%)	--	Ref.
	T Allele	4 (2.2%)	4 (2.5%)	NS	0.90 (0.22-3.65)
UC (n=71)	(-1123G/C) SNP				
	Wild genotype GG	40 (56.3%)	55 (67.9%)	--	Ref.
	Heterozygous GC	27 (38.0%)	24 (29.6%)	NS	1.55 (0.78-3.08)
	Homozygous CC	4 (5.6%)	2 (2.5%)	NS	2.75 (0.51-20.54)
	C carrier (GC +CC)	31 (43.7%)	26 (32.1%)	NS	1.64 (0.85-3.18)
	G Allele	107 (75.4%)	134 (82.7%)	--	Ref.
	C Allele	35 (24.6%)	28 (17.3%)	NS	1.57 (0.90-2.74)
	(+788G/A) SNP				
	Wild genotype GG	68 (95.8%)	78 (96.3%)	--	Ref.
	Heterozygous GA (A carrier)	3 (4.2%)	3 (3.7%)	NS	1.15 (0.21-6.38)
	G Allele	139 (97.2%)	159 (98.1%)	--	Ref.
	A Allele	3 (2.8%)	3 (1.9%)	NS	1.15 (0.22-5.87)
	(+1858C/T) SNP				
	Wild genotype CC	68 (95.8%)	77 (95.1%)	--	Ref.
	Heterozygous CT (T carrier)	3 (4.2%)	4 (4.9%)	NS	0.85 (0.16-3.98)
	C Allele	139 (97.2%)	158 (97.5%)	--	Ref.
	T Allele	3 (2.8%)	4 (2.5%)	NS	0.85 (0.19-3.88)

Table 4. Continued.

	PTPN22 SNP genotype & allele frequencies	Patients	Control (n=81)	*p* value	Risk Estimates OR (95% CI)
CD (n= 19)	(-1123G/C) SNP				
	Wild genotype GG	12 (63.2%)	55 (67.9%)	--	Ref.
	Heterozygous GC	7 (36.8%)	24 (29.6%)	NS	1.34 (0.45-3.76)
	Homozygous CC	0 (0.0%)	2 (2.5%)	NS	NA
	C carrier (GC +CC)	7 (36.8%)	26 (32.1%)	NS	1.23 (0.44-3.50)
	G Allele	31 (81.6%)	134 (82.7%)	--	Ref.
	C Allele	7 (18.4%)	28 (17.3%)	NS	1.08 (0.43-2.70)
	(+788G/A) SNP				
	Wild genotype GG	18 (94.7%)	78 (96.3%)	--	Ref.
	Heterozygous GA (A carrier)	1 (5.3%)	3 (3.7%)	NS	1.44 (0.07-12.0)
	G Allele	37 (97.4%)	159 (98.1%)	--	Ref.
	A Allele	1 (2.6%)	3 (1.9%)	NS	1.43 (0.14-14.16)
	(+1858C/T) SNP				
	Wild genotype CC	18 (94.7%)	77 (95.1%)	--	Ref.
	Heterozygous CT (T carrier)	1 (5.3%)	4 (4.9%)	NS	1.07 (0.05-7.79)
	C Allele	37 (97.4%)	158 (97.5%)	--	Ref.
	T Allele	1 (2.6%)	4 (2.5%)	NS	1.07 (0.12-9.83)

^Chi-square test was used; OR: odds ratio; CI: confidence interval; N= number. SNP=Single nucleotide polymorphism.
Note: "NA" indicating that the odds ratio (OR) is not applicable due to the presence of zero in one of the cells, preventing the calculation of the OR. $p > 0.05$ is not significant (NS).

Table 5. Association between (-1123G/C) SNP and disease characteristics in Crohn's disease (CD) patients.

Characteristic		GG N = 12	C carrier N = 7	*p*-value^	Risk Estimates OR (95% CI)
Age at onset	Early onset (< 40)	9 (75.0%)	5 (71.4%)	--	Ref.
	Late onset (≥40)	3 (25.0%)	2 (28.6%)	NS	1.20 (0.13-9.91)
Location	L1- Terminal ileum	2 (16.7%)	2 (28.6%)	--	Ref.
	L2- Colon	3 (25%)	3 (42.9%)	NS	1 (0.07-13.85)
	L3- Ileocolon	7 (58.3%)	2 (28.6%)	NS	0.29 (0.02-3.63)
Disease Activity	Mild	3.0 (25.0%)	3.0 (42.9%)	--	Ref.
	Moderate	4.0 (33.3%)	1.0 (14.3%)	NS	0.25 (0.01-3.19)
	Severe	1.0 (8.3%)	2.0 (28.6%)	NS	2.00 (0.12-58.79)
	Remission	4.0 (33.3%)	1.0 (14.3%)	NS	0.25 (0.01-3.19)
Extra intestinal complications	No	10.0 (83.3%)	6.0 (85.7%)	--	Ref.
	Yes	2.0 (16.7%)	1.0 (14.3%)	NS	0.83 (0.03-10.68)
Intestinal complications	No	3 (25.0%)	1 (14.3%)	--	Ref.
	Yes	9 (75%)	6 (85.7%)	NS	2 (0.20 – 45.9)
Drug therapy	Conventional therapy only	1 (8.3%)	1 (14.3%)	--	Ref.
	Biological therapy	11 (91.7%)	6 (85.7%)	NS	0.55 (0.02-15.48)

^Chi-square test was used; OR: odds ratio; CI: confidence interval; N= number. $p > 0.05$ is not significant (NS).

Table 6. Association between (-1123G/C) SNP and disease characteristics in ulcerative colitis (UC) patients.

Characteristic		GG N = 40	C carrier N = 31	*p*-value^	Risk Estimates OR (95% CI)
Age at onset	Early onset (< 40)	34 (85.0%)	27 (87.1%)	--	Ref.
	Late onset (≥40)	6 (15.0%)	4 (12.9%)	NS	0.84 (0.20-3.24)
Location	E1- Proctitis	10 (25.0%)	11 (35.5%)	--	Ref.
	E2 - Left-sided	26 (65.0%)	14 (45.2%)	NS	0.49 (0.16-1.43)
	E3- Extensive Colitis	4 (10.0%)	6 (19.4%)	NS	1.36 (0.30-6.71)
Disease Activity	Mild	18 (45.0%)	14 (45.2%)	--	Ref.
	Moderate	13 (32.5%)	16 (51.6%)	NS	1.58 (0.58-4.42)
	Severe	6 (15.0%)	1 (3.2%)	NS	0.21 (0.01-1.4)
	Remission	3 (7.5%)	0 (0%)	NS	NA
Extra intestinal complications	No	36 (90%)	30 (96.8%)	--	Ref.
	Yes	4 (10%)	1 (3.2%)	NS	0.3 (0.01-2.16)
Intestinal complications	No	37 (92.5%)	30(96.8%)		Ref.
	Yes	3 (7.5%)	1 (3.2%)	NS	0.41 (0.02-3.40)
Drug Therapy	Conventional therapy only	27 (67.5%)	26 (83.9%)	--	Ref.
	Biological therapy	13 (32.5%)	5 (16.1%)	NS	0.40 (0.11-1.22)

^Chi-square test was used; OR: odds ratio; CI: confidence interval; N= number.
Note: "NA" indicating that the odds ratio (OR) is not applicable due to the presence of zero in one of the cells, preventing the calculation of the OR. $p > 0.05$ is not significant (NS).

Table 7. Association between (+788G/A) SNP and disease characteristics in ulcerative colitis (UC) patients.

Characteristic		GG N = 68	A carrier N = 3	*p*-value^	Risk Estimates OR (95% CI)
Age at onset	Early onset (< 40)	59 (86.8%)	2 (66.7%)	--	Ref.
	Late onset (≥40)	9 (13.2%)	1 (33.3%)	NS	3.28 (0.14-37.84)
Location	E1- Proctitis	19 (27.9%)	2 (66.7%)	--	Ref.
	E2 - Left-sided	40 (58.8%)	0 (0%)	NS	NA
	E3- Extensive Colitis	9 (13.2%)	1 (33.3%)	NS	1.06 (0.05-12.50)
Disease Activity	Mild	30.0 (44.1%)	2.0 (66.7%)	--	Ref.
	Moderate	29.0 (42.6%)	0.0 (0.0%)	NS	NA
	Severe	6.0 (8.8%)	1.0 (33.3%)	NS	2.50 (0.11-30.61)
	Remission	3.0 (4.4%)	0.0 (0.0%)	NS	NA
Extra intestinal complications	No	64.0 (94.1%)	2.0 (66.7%)	--	Ref.
	Yes	4.0 (5.9%)	1.0 (33.3%)	NS	8.0 (0.33-104.3)

Table 7. Continued.

Characteristic		GG N = 68	A carrier N = 3	*p*-value^	Risk Estimates OR (95% CI)
Intestinal complications	No	64 (94.1%)	3.0 (100%)	--	Ref.
	yes	4 (5.9%)	0.0 (0.0%)	NS	NA
Drug therapy	Conventional therapy only	51(75.0%)	2 (66.7%)	--	Ref.
	Biological therapy	17(25.0%)	1 (33.3%)	NS	1.50 (0.07-16.63)

^ Chi-square test was used; OR: odds ratio; CI: confidence interval; N= number.
Note: "NA" indicating that the odds ratio (OR) is not applicable due to the presence of zero in one of the cells, preventing the calculation of the OR. $p > 0.05$ is not significant (NS).

Table 8. Association between (+1858C/T) SNP and disease characteristics in ulcerative colitis (UC) patients.

Characteristic		CC N = 68	T carrier N = 3	*p*-value^	Risk Estimates OR (95% CI)
Age at onset	Early onset (<40)	59.0 (86.8%)	2.0 (66.7%)	--	Ref.
	Late onset (≥40)	9.0 (13.2%)	1.0 (33.3%)	NS	3.28 (0.14-37.84)
Location	E1- Proctitis	20 (29.4%)	1 (33.3%)	--	Ref.
	E2 - Left-sided	38 (55.9%)	2 (66.7%)	NS	1.05 (0.10-23.47)
	E3- Extensive Colitis	10 (14.7%)	0 (0%)	NS	NA
Disease Activity	Mild	31.0 (45.6%)	1.0 (33.3%)	--	Ref.
	Moderate	29.0 (42.6%)	0.0 (0.0%)	NS	NA
	Severe	6.0 (8.8%)	1.0 (33.3%)	NS	5.17 (0.19-143.69)
	Remission	2.0 (2.9%)	1.0 (33.3%)	NS	15.50 (0.49-522.26)
Extra intestinal complications	No	63.0 (91.6%)	3.0 (100.0%)	--	Ref.
	Yes	5.0 (7.4%)	0.0 (0.0%)	NS	NA
Intestinal complications	No	64.0(94.1%)	3.0 (100%)	--	Ref.
	Yes	4.0 (5.9%)	0.0 (0.0%)	NS	NA
Drug therapy	Conventional therapy only	51(75.0%)	2 (66.7%)	--	Ref.
	Biological therapy	17(25.0%)	1 (33.3%)	NS	1.50 (0.07-16.63)

^ Chi-square test was used; OR: odds ratio; CI: confidence interval; N= number.
Note: "NA" indicating that the odds ratio (OR) is not applicable due to the presence of zero in one of the cells, preventing the calculation of the OR. $p > 0.05$ is not significant (NS).

Discussion

The present study aimed to investigate the potential association of PTPN22 gene (-1123G/C, +788G/A, and +1858C/T) SNPs in Egyptian IBD patients and to determine their relation to clinical disease characteristics.

The PTPN22 gene is crucial for regulating immune cell activation, playing a vital role in the intestinal immune system.[15] Thus, the presence of IBD-associated variations in PTPN22 may increase pro-inflammatory cytokines, ultimately leading to chronic intestinal inflammation.[41, 42] Variations within the gene locus encoding

PTPN22 are associated with multiple autoimmune disorders, including CD[21,] rheumatoid arthritis,[43] systemic lupus erythematosus[44] and type 1 diabetes.[45]

The present study showed a significant decrease in Hb level in IBD patients (UC, CD) compared to the control group. Iron deficiency in IBD is mainly caused by chronic inflammation and impaired gastrointestinal iron absorption, bowel bleeding, and resection.[46] A combination of iron deficiency anemia and anemia from chronic disease was the most frequent explanation of anemia in the IBD patients.[47.]

C-reactive protein and ESR are the most commonly used inflammatory markers to assess disease activity in patients with IBD.[48] In the current study, we found that patients with UC and CD had significantly higher CRP and ESR levels than the control group. This agreed with findings of the study by Fagan et al., 1982, who found that serum CRP levels were significantly higher in patients with IBD.[49] Also, our results showed significantly higher CRP levels in CD in comparison with UC; this is consistent with findings of a study by Abdelrazeq et al., 2005, who reported a significant variation in the CRP response between patients with CD and UC; whereas CD was associated with a robust CRP response, but UC had only a minor CRP response.[50]

In the present study, the genotype distribution of the PTPN22-1123G/C SNP showed no statistically significant difference between IBD (UC, CD) patients and control subjects and no association with disease characteristics. The frequency of the variant -1123C allele was 18.4% in CD cases, 24.6% in UC cases, and 23.3% in the combined IBD group. Although these were higher than the control group (17.3%), the differences did not reach statistical significance. In agreement with our study, Sadr et al., 2019 found no association between UC in the Iranian population and the PTPN22 −1123G/C SNP,[24] while in the study done by Chen et al., 2013, the presence of the C allele of the -1123G/C polymorphism in the PTPN22 gene was linked to an elevated risk of ulcerative colitis and extensive colitis in the Chinese population.[31] This discrepancy may be due to factors such as disease specificity, ethnicity, and gene-environment interactions that exist in Chinese but not among Egyptian populations. Ethnicity causes genetic variations that affect immune activity, intestinal barrier integrity, and autophagy, which contribute to the pathogenesis of IBD.[51]

The genotype distribution and allele frequency of the PTPN22 (+788G/A) SNP did not show statistically significant differences between patients with IBD (UC, CD) and control subjects and no association with disease characteristics. This agreed with data reported by Sadr et al., 2019, who found no association between PTPN22 (+788G/A) SNP gene polymorphism and UC in the Iranian population.[24.] On the contrary, in 2011, Diaz-Gallo and colleagues found that the +788 A allele of PTPN22 was correlated with a decreased risk of UC in the Spanish population. However, this association was not observed in the New Zealand and Dutch populations; this difference could be attributed to racial differences, while they did not find any association of this polymorphism with CD.[21]

In our study, we found no association of the PTPN22 (+1858C/T) polymorphism with IBD, as there was no statistically significant difference in genotype or allele frequency distribution between patients and the control group. There were conflicting findings published about the relationship between the PTPN22 (+1858C/T) variant with either CD or UC. This could be attributed to sample size differences, patient ethnicity, or allele frequencies.[61] Our findings are in agreement with studies on Iranian,[24] Moroccan,[23] Spanish,[52,53] British,[54] Northern German,[22] Canadian,[55] New Zealand[56] and Czech[57] populations. This finding is further supported by a meta-analysis in 2007 by Latiano et al., which demonstrated no significant association between the PTPN22 1858 C/T SNP and both CD and UC.[20]

On the contrary, our findings are in disagreement with Sfar et al., 2010, who reported an association between the PTPN22 1858T allele and increased risk of IBD among Tunisian patients.[19] Also, in a Canadian study done in 2011 by Waterman and colleges, the PTPN22 1858T allele was associated with colonic CD.[58] Otherwise, in a Danish cohort, the

PTPN22 1858T allele was associated with a decreased risk of CD and UC [59]. Similarly, a meta-analysis that investigated the overall impact of the PTPN22 +1858C/T gene variant found an association with decreased risk of CD but not of UC.[60]

Also, the present study revealed no association between (+1858C/T) SNP and some disease characteristics of CD and UC patients. This agreed with findings of a study by Latiano et al., 2007, who documented no association between PTPN22 gene polymorphism and any of the investigated clinical features in CD patients including age at diagnosis, disease location, extra-intestinal manifestations, and response to medical therapy.[20]

To the best of our knowledge, there are no published studies of PTPN22 (-1123 G/C, +1858C/T and +788 G/A) SNPs in IBD Egyptian patients. In conclusion, our study demonstrated that although PTPN22 gene polymorphisms plays an important role in the pathophysiology of a subgroup of autoimmune diseases, they have no evident effect on predisposition to IBD and no association with disease clinical characteristics in Egyptian population.

Author Contributions

TTHE, AME, ERB; proposed and designed the study. AME, MRA; applied for the research fund. HAE; examined the patients. MRA; collected samples. MRA; performed the laboratory work. TTHE, MRA, ERB; interpreted the laboratory test results and analyzed the data. TTHE, AME, MRA, ERB; participated in writing and reviewing the paper. TTHE; prepared the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the Ethical Committee of the Faculty of Medicine, Assiut University, Egypt (IRB no. 17200446, dated 10/6/2020).

Informed consent

An informed consent was obtained from each subject before being included in the study.

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