

# Association of CYP2C19, TNF- $\alpha$ , NOD1, NOD2, and PPAR $\gamma$ polymorphisms with peptic ulcer disease enhanced by *Helicobacter pylori* infection

Laith N. AL-Eitan, MSc, PhD, Fouad A. Almomani, MSc, PhD, Sohaib M. Al-Khatib, MD.

## ABSTRACT

**الأهداف:** كشف الارتباط بين عدد من الاعتلالات الوراثية في عدد من الجينات مثل CYP2C19، TNF- $\alpha$ ، NOD1، NOD2، PPAR $\gamma$  وخطر الإصابة بجرثومة المعدة في المرضى الأردنيين.

**المنهجية:** تم استخراج وتحليل عينة الحمض النووي من الأنسجة المحفوظة بالبارافين من المرضى المراجعين لعيادة التنظير في مستشفى الملك المؤسس باستخدام الأدوات المتخصصة ووفقاً للطرق القياسية.

**النتائج:** هذه الدراسة مكونة من 251 مريضاً بقرحة المعدة (متوسط العمر = 42.12 ± 16.09 عام) و مائتين وسبعة عشر شخصاً سليماً غير مصابين بالقرحة (متوسط العمر = 52.76 ± 19.45 سنة). أظهرت هذه الدراسة عدم وجود أي ارتباط بين الأماط الجينية المختلفة المدروسة في هذا البحث مع خطر الإصابة بجرثومة المعدة والاستجابة للعلاج ماعداً نمطاً جينياً واحداً، حيث أن هذا النمط يقلل من خطر الإصابة بالمرض.

**الخلاصة:** قدمت هذه الدراسة على وجود ارتباط بين الاعتلالات الجينية وخطر الإصابة بجرثومة المعدة ولكن لا بد من تكرار هذه النوع من الدراسات بصورة أشمل حتى يتمكن من وضع دليل خطة علاجية خاصة لكل مريض بناءً على خارطة الجينية.

**Objectives:** To assess the correlation between a number of genetic variations of CYP2C19, TNF- $\alpha$ , NOD1, NOD2, and PPAR $\gamma$  genes with the severity of *Helicobacter pylori* (*H. pylori*) infections and peptic ulcers (PU).

**Methods:** A retrospective cross-sectional design was used in this study. Formalin-fixed paraffin-embedded (FFPE) tissue was used to extract genomic DNA that was collected from Jordanian patients who visited endoscopy clinics between 2014 to 2018 at the King Abdullah University Hospital (KAUH), Irbid, Jordan. Genotyping of the studied single nucleotide polymorphisms (SNPs) were applied using the sequencing protocol.

**Results:** A total of 251 patients (mean age: 42.12 ± 16.09 years) and healthy controls (mean age: 52.76 ± 19.45 years) were enrolled in this study. This study showed no significant association between patients and the studied polymorphisms except for rs2075820 of the NOD1 ( $p=0.0046$ ). It is hypothesized that the heterozygous

genotype (TC); 44.8% in patients versus 61.3% in controls has a decreased risk of peptic ulcers (OR: 0.49). The alleles frequency association was insignificant in all studied SNPs with a  $p$ -value more than 0.05.

**Conclusion:** This study provided evidence regarding the association of the rs2075820 with *H. pylori* infections. The other studied SNPs were not statistically significant.

**Keywords:** *Helicobacter pylori*; peptic ulcers; cytochrome P-450; peroxisome proliferator-activated receptors; endoscopy

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From the Department of Biotechnology and Genetic Engineering (AL-Eitan, Almomani) Jordan University of Science and Technology and from the Department of Pathology and Microbiology (Al-Khatib), Jordan University of Science and Technology, Irbid, Jordan.

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Address correspondence and reprint request to: Dr. Laith N. AL-Eitan, Associate Professor, Department of Biotechnology & Genetic Engineering, Faculty of Science and Arts, Jordan University of Science and Technology, Irbid, Jordan. E-mail: lneitan@just.edu.jo  
ORCID: <https://orcid.org/0000-0003-0064-0190>

*Helicobacter pylori* (*H. pylori*) is one of the major causes of gastric and duodenal ulcers, chronic gastritis, and gastric carcinomas.<sup>1,2</sup> The clinical diagnosis of an infection with *H. pylori* is extremely complicated and affected by host and microbial influences.<sup>3</sup> The diagnosis outcome of *H. pylori* is influenced by many factors including the genetic composition of the infectious strains, the genetic profile of the patients, and particularly the differences in the patients immune system.<sup>1,3,4</sup> Various cytokine responses to the gastric mucosal inflammation induced by *H. pylori* seem to play an important role in the patient diagnosis outcome, such as the gastric cancer (GC) and different gastric diseases.<sup>2</sup>

However, the association between the variations in the host cytokines genes and the vulnerability to *H. pylori* infections and the extent of the clinical outcome has not been analyzed perfectly.<sup>5,6</sup> It is recognized that the ethnic diversity of the population is a major element correlated with the frequency variability of many markers and thus influences the vulnerability and intensity of *H. pylori* infection.<sup>7,8</sup> Several studies have shown that IL-1 $\beta$  is a crucial factor that induces and amplifies inflammatory responses by influencing the levels of gastric mucosal cytokines.<sup>9</sup>

The function of the host genetic factors involved in *H. pylori* infections has been proved.<sup>5,6</sup> The nucleotide-binding oligomerization domain (NOD)-like receptors (namely, NOD1 and NOD2) have been identified as key factors of chronic inflammation caused by *H. pylori* infections and are important to the clinical outcome of *H. pylori* infections.<sup>6,7</sup> Several studies have demonstrated that the genetic variations found in the NOD1 and NOD2 genes among different ethnicities are attributed to different diagnosis manifestations of *H. pylori* infections and are associated with risk of the GC.<sup>7,8</sup> Reports have also revealed that there is a relationship between the up-regulation of several inflammatory response genes (for example, TNF- $\alpha$ , IL-8, IL-10, IL-1 $\beta$ , and IL-1RN) and *H. pylori* infection.<sup>9</sup> To date, triple therapy involving a mixture of 2 antibiotics (for example, imidazole, amoxicillin clarithromycin, or metronidazole) and one proton pump inhibitor (PPI) (for example, lansoprazole, pantoprazole, omeprazole, or esomeprazole) has shown the best outcome in the treatment of *H. pylori* infection.<sup>5,10,11</sup> However, the failure rate of this therapy has increased up to 30% due to several factors, including weak compliance, high bacterial load, antibiotic resistance, high gastric acidity, cytochrome P450 2C19 (CYP2C19) genetic polymorphisms, leading to a reduction in eradication rate of *H. pylori*.<sup>10,12</sup> The metabolism of PPI is mediated by CYP2C19 in the liver.<sup>13</sup> Proton pump inhibitors (PPIs) also have a key role in improving the therapeutic efficiency of antibiotics for treatment of *H. pylori* infections and possess direct anti-*H. pylori* activity.<sup>10</sup> Additionally, PPIs can increase the efficacy of the antibiotic.<sup>10-12</sup> The genetic polymorphisms of

the CYP2C19 gene that are considered as a main factor influencing antibiotic resistance among *H. pylori* strains, found to affect both the pharmacokinetic and pharmacodynamic characteristics of PPIs.<sup>10,13</sup> Therefore, the current research aimed to study the genetic correlation between *H. pylori* infections and peptic ulcers in the Jordanian Arab population and different candidate gene polymorphisms within CYP2C19, TNF- $\alpha$ , NOD1, NOD2, and PPAR $\gamma$  genes.

**Methods.** This retrospective cross-sectional study was conducted to examine the association between a number of genetic polymorphisms of several candidate genes (CYP2C19, TNF- $\alpha$ , NOD1, NOD2, and PPAR $\gamma$ ) and the severity of *H. pylori* infections and peptic ulcers (PU). Human ethics approval was obtained from Jordan University of Science and Technology, Irbid, Jordan. All individuals participated in this study signed a written informed consent before sample collection. This study was also performed in accordance with the principles of the Declaration of Helsinki.

A total of 217 formalin fixed paraffin impeded (FFPE) tissues from controls with negative *H. pylori* infections and 251 from Jordanian Arab peptic ulcer patients with *H. pylori* infections were used. All participants visited the endoscopy clinic during 2014 to 2018 in the King Abdullah University Hospital (KAUH), Irbid, Jordan. Clinical and demographic data were collected using the computerized systems in the KAUH. Patients were included if they are of Arab descent and have a confirmed *H. pylori* infection by a consultant based on symptoms and clinical data. Patients were classified according to the Updated Sydney Classification on *H. pylori* chronic gastritis into 2 groups (active/acute and inactive/chronic) based on the presence of mononuclear cells (lymphocytes) and neutrophils (multinuclear cells) in the microscopic histopathological analysis. In addition, the severity of the *H. pylori* infection was based on the number of multinuclear cells and classified into 3 groups: mild, moderate, and severe.

**DNA analysis.** The DNeasy Blood & Tissue Kit (Qiagen Ltd., West Sussex, UK) was used for genomic DNA extraction from FFPE tissue that was taken from the patients who visited the endoscopy clinics. The single nucleotide polymorphisms (SNPs) were genotyped using the Sequenom MassARRAY<sup>®</sup> system (iPLEX GOLD) (Sequenom, San Diego, CA, USA) at the Australian Genome Research Facility (AGRF, Melbourne Node, Melbourne, Australia) for CYP2C19 (rs17882687, rs6413438, rs4986893, and rs17879685), TNF- $\alpha$  (rs1799964, rs1800629, and rs361525), NOD1 (rs2075820, rs2907749, and rs7789045), NOD2

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(rs1861759 and rs3135500), IL-1 $\beta$  (rs16944 and rs1143627), and PPAR $\gamma$  (rs10865710, rs1801282, rs3856806, and rs13306747) genes.

**Statistical analysis.** The genotypic distribution and the correlation between the CYP2C19 (rs17882687, rs6413438, rs4986893, and rs17879685), TNF- $\alpha$  (rs1799964, rs1800629, and rs361525), NOD1 (rs2075820, rs2907749, and rs7789045), NOD2 (rs1861759 and rs3135500), IL-1 $\beta$  (rs16944 and rs1143627), and PPAR $\gamma$  (rs10865710, rs1801282, rs3856806, and rs13306747) polymorphisms and clinical variables (independent variables) were analyzed using the Chi-square test and estimated the risk as an odds ratio with a 95% confidence interval. The Hardy-Weinberg equilibrium (HWE) values and the genetic haplotype association were assessed using the SNPStats Web Tool (<https://www.snpstats.net/start.htm>). Finally, the Statistical Package for Social Sciences IBM SPSS Statistics for Windows Version 25 (IBM Corp., Armonk, N.Y., USA) was used to identify the genotypic, allelic, and clinical data associations with *H. pylori* infection.

**Results.** Demographic and clinical characteristics of 251 peptic ulcer patients caused by *H. pylori* are presented in Table 1. The average age of the patients was 42.78  $\pm$  17.3 years for males and 41.57  $\pm$  16.4 for females. A significant difference was found between gender and the activity of *H. pylori* with a  $p=0.032$ , where the percentage of inactive *H. pylori* in female patients was 4 times that of male patients. These results confirmed previous studies that there were gender differences in the response to *H. pylori* infections that may lead to variances in inflammation scores, activity, intestinal metaplasia, and different incidences of gastric cancer between men and women. Many scientists have hypothesized that this variation in response is due to basic biological differences between men and women, including hormones-particularly estrogen-and differences in lifestyles, such as diet and smoking. Table 2 reveals the MAF of the studied polymorphisms (rs17882687, rs6413438, rs4986893, and rs17879685 within the CYP2C19 gene, rs1799964, rs1800629, and rs361525 within the TNF- $\alpha$  gene, rs2075820, rs2907749, and rs7789045 within the NOD1 gene, rs1861759 and rs3135500 within the NOD2 gene, rs16944 and rs1143627 within the IL-1 $\beta$  gene, and rs10865710, rs1801282, rs3856806, and rs13306747 within the PPAR $\gamma$  gene) and their location on the genomic DNA as well as the HWE  $p$ -value.

All studied genotypes were in HWE ( $p>0.05$ ), so it is suggested that the population was derived from the same as the Mendelian population, except for 6 SNPs.

Four polymorphisms were found as monomorphic (rs6413438, rs4986893, and rs17879685 in the CYP2C19 gene and rs13306747 in the PPAR $\gamma$  gene). Moreover, genotypic and allelic distribution of these SNPs are shown in Table 3 and revealed no significant differences in the SNP distribution frequencies of the CYP2C19, TNF- $\alpha$ , NOD2, and PPAR $\gamma$  genes between patients and controls.

For the NOD1 polymorphisms, the only SNP that showed a significant difference in genotype frequencies was rs2075820 (C>T) between healthy subjects and peptic ulcer patients ( $p<0.05$ ); 43.5% (TT), 44.8% (CT), and 11.7% (CC) in patients compared to 29.2%

**Table 1 -** Demographic and clinical details of PU patients (251) in comparison with gender.

Clinical data	Gender		<i>P</i> -value*
	Male	Female	
Age	42.78 $\pm$ 17.3	41.57 $\pm$ 16.4	0.57
<b>Marital status</b>			
Single	38 (15.1)	30 (12.0)	0.03
Married	101 (40.2)	80 (31.9)	
Divorced	1 (0.4)	0 (0.0)	
Widowed	0 (0)	1 (0.4)	
<b>Helicobacter pylori activity</b>			
Yes	104 (41.1)	120 (47.8)	0.032
No	7 (2.8)	20 (8.0)	
<b>Other diseases</b>			
No	61 (24.3)	83 (33.1)	0.58
Yes	47 (18.7)	58 (23.1)	
<b>Duodenal ulcer</b>			
No	92 (36.7)	122 (48.6)	0.22
Yes	19 (7.6)	18 (7.2)	
<b>Esophagitis</b>			
No	89 (35.5)	115 (45.8)	0.23
Yes	22 (8.8)	25 (10.0)	
<b>Chronicity</b>			
Yes	110 (43.8)	139 (55.4)	0.35
No	1 (0.4)	1 (0.4)	
<b>Proton pump inhibitors</b>			
No	1 (0.4)	7 (2.8)	0.488
Lansoprazole	86 (34.3)	97 (38.3)	
Esomeprazole	0 (0.0)	3 (1.2)	
Omeprazole	1 (0.4)	1 (0.4)	
Two or three	43 (9.2)	32 (12.8)	
<b>Antibiotics</b>			
No	27 (10.8)	33 (13.1)	0.65
Amoxicillin	5 (2.0)	2 (0.8)	
Clarithromycin	1 (0.4)	4 (1.6)	
Amoxicillin + Clarithromycin	76 (30.3)	97 (38.6)	
Others	2 (0.8)	4 (1.6)	

Values are presented as number and percentage,  
\* $p<0.05$  is considered significant

**Table 2** - Details of the candidate genes polymorphisms of interest and the minor allele frequency.

Gene	Chromosome #	rs numbers	Minor allele	Patients		Control	
				MAF	HWE <i>p</i> -value	MAF	HWE <i>p</i> -value
CYP2C19	10q23.33	rs17882687	C	0.01	1	0.01	1
		rs6413438	T	0	NA	0	NA
		rs4986893	C	0	NA	0	NA
		rs17879685	T	0	NA	0	NA
TNF- $\alpha$	6p21.33	rs1799964	C	0.19	0.68	0.17	1
		rs1800629	A	0.11	1	0.12	1
		rs361525	A	0.07	0.27	0.06	0.58
NOD1	7p14.3	rs2075820	T	0.34	1	0.41	0.00042
		rs2907749	G	0.33	0.24	0.32	0.046
		rs7789045	T	0.45	0.44	0.4	0.28
NOD2	16q12.1	rs1861759	G	0.42	0.5	0.42	T0.17
		rs3135500	A	0.46	0.88	0.44	0.26
IL-1 $\beta$	2q14.1	rs16944	A	0.36	1	0.43	1
		rs1143627	G	0.38	0.48	0.31	0.87
PPAR $\gamma$	3p25.2	rs10865710	G	0.17	0.35	0.18	0.62
		rs1801282	G	0.03	1	0.041	0.23
		rs3856806	T	0.04	1	0.05	0.098
		rs13306747	C	0	NA	0	NA

MAF: minor allele frequency, HWE: hardy weinberg equilibrium  $p > 0.05$ , NA: not available

(TT), 61.3% (TC), and 9.5 % (CC) in controls. The homozygous (CC) genotype frequency was notably increased in peptic patients compared to the control group and the opposite for the heterozygous (TC) frequency, so the homozygous genotype (CC) could have an increased risk of peptic ulcer (odd ratio: 0.49,  $p=0.0046$ ), but this polymorphism and the remaining studied SNPs showed an inconsiderable association of allele frequency between patients and healthy individuals.

**Table 4** illustrates the correlation between the activity of *H. pylori* and the candidate polymorphisms in peptic ulcer patients. The data showed no significant association between the activity of *H. pylori* (active/acute and inactive/chronic) and the polymorphisms of the studied genes with a  $p > 0.05$ . Moreover, the relationship between the studied polymorphisms and the severity of the *H. pylori* infection (mild, moderate, or severe) in peptic ulcer patients are shown in **Table 5**. The statistical analysis of the data revealed there was no important association between the severity of the *H. pylori* infection and these studied SNPs with a  $p > 0.05$ .

**Discussion.** *Helicobacter pylori* is one of the predominant human pathogens with a worldwide infection rate of more than 50%.<sup>14-16</sup> The treatments for *H. pylori* elimination consist of PPIs and antimicrobial drugs (namely, amoxicillin, clarithromycin, and metronidazole).<sup>17,18</sup> Proton pump inhibitors are mainly metabolized by CYP2C19, which is highly polymorphic.<sup>10,11,19</sup> Many recent studies have reported that the CYP2C19 genotypes have variable effectiveness of PPIs and imperative roles in peptic ulcer healing.<sup>20,21</sup> The distributions of the CYP2C19 polymorphisms vary among different populations.<sup>22,23</sup> This study was conducted to explore the impacts of genetic polymorphisms of different candidate genes (CYP2C19 [rs17882687, rs6413438, rs4986893, and rs17879685], TNF- $\alpha$  [rs1799964, rs1800629, and rs361525], NOD1 [rs2075820, rs2907749, and rs7789045], NOD2 [rs1861759 and rs3135500], IL-1 $\beta$  [rs16944 and rs1143627], and PPAR $\gamma$  [rs10865710, rs1801282, rs3856806, and rs13306747]) on the risk of gastric lesions in a Jordanian Arab descent patients.

**Table 3** - Distribution of the genotypes of the studied genes among cases and controls.

Gene/rs numbers/models	Control (%)	PUD patients (%)	Odd ratio (95%CI)	P-value
<i>TNF-α</i>				
<i>rs1799964</i>				
TT / TC / CC	69 / 28.4 / 2.5	65.7 / 30.2 / 4	1 / 1.12 / 1.67	0.59
TT / TC + CC	69 / 31	65.7 / 34.3	1 / 1.16	0.46
TT + TC / CC	97.5 / 2.5	96 / 4	1 / 1.61	0.38
TT + CC / TC	71.6 / 28.4	69.8 / 30.2	1 / 1.09	0.68
<i>rs1800629</i>				
GG / GA / AA	78 / 20.6 / 1.4	79.5 / 19.3 / 1.2	1 / .92 / .84	0.92
GG / GA + AA	78 / 22	79.5 / 20.5	1 / 0.91	0.96
GG + GA / AA	98.6 / 1.4	98.8 / 1.2	1 / .85	0.85
GG + AA / GA	79.4 / 20.6	80.7 / 19.3	1 / 0.92	0.73
<i>rs361525</i>				
GG / GA / AA	87.6 / 11.9 / 1.5	87.8 / 11.4 / 0.8	1 / 0.96 / 1.64	0.91
GG / GA + AA	87.6 / 12.4	87.8 / 12.2	1 / 0.98	0.95
GG + GA / AA	99.5 / 0.5	99.2 / 0.8	1 / 1.65	0.68
GG + AA / GA	99.2 / 0.8	88.6 / 11.4	1 / 0.95	0.87
<i>NOD1</i>				
<i>rs2075820</i>				
CC / CT / TT	29.2 / 61.3 / 9.5	43.5 / 44.8 / 11.7	1 / 0.49 / 0.82	0.0046
CC / TC + TT	29.2 / 70.8	43.5 / 56.5	1 / 0.53	0.0035
CC + TC / TT	90.5 / 9.5	88.3 / 11.7	1 / 1.25	0.5
CC + TT / TC	38.7 / 61.3	55.2 / 44.8	1 / 0.51	0.0012
<i>rs2907749</i>				
GG / GA / AA	43.5 / 49.7 / 6.8	42.9 / 47.9 / 9.2	1 / 0.98 / 1.36	0.66
GG / GA + AA	43.5 / 56.5	42.9 / 57.1	1 / 1.02	0.91
GG + GA / AA	93.2 / 6.8	90.8 / 9.2	1 / 1.38	0.37
GG + AA / GA	50.3 / 49.7	52.1 / 47.9	1 / 0.93	0.71
<i>rs7789045</i>				
TT / AT / AA	34.2 / 51.9 / 13.8	29.2 / 52.1 / 18.8	1 / 1.18 / 1.59	0.3
TT / AT + AA	34.2 / 65.8	29.2 / 70.8	1 / 1.27	0.27
TT + AT / AA	86.2 / 13.8	81.2 / 18.8	1 / 1.44	0.17
TT + AA / AT	48.1 / 51.9	47.9 / 52.1	1 / 1.01	0.98
<i>NOD2</i>				
<i>rs1861759</i>				
TT / GT / GG	30.6 / 54.4 / 15	35.1 / 46.5 / 18.4	1 / 0.74 / 1.07	0.27
TT / GT + GG	30.6 / 69.4	35.1 / 64.9	1 / 0.81	0.33
TT + GT / GG	85 / 15	81.6 / 18.4	1 / 1.28	0.36
TT + GG / GA	45.6 / 54.4	53.5 / 46.5	1 / 0.73	0.11
<i>rs3135500</i>				
GG / GA / AA	28.5 / 54.4 / 17.1	29.2 / 48.9 / 21.7	1 / 0.87 / 1.23	0.49
GG / GA + AA	28.5 / 71.5	29.4 / 70.6	1 / 0.95	0.85
GG + GA / AA	82.9 / 17.1	78.3 / 21.7	1 / 1.34	0.31
GG + AA / GA	45.6 / 54.4	51.1 / 48.9	1 / 0.8	0.63
<i>IL-1β</i>				
<i>rs16944</i>				
GG / GA / AA	32.2 / 49.4 / 18.4	41.1 / 46.1 / 12.8	1 / 0.73 / 0.54	0.12
GG / GA + AA	32.2 / 67.8	41.1 / 58.9	1 / 0.68	0.069
GG + GA / AA	81.6 / 18.4	87.2 / 12.8	1 / 0.65	0.13
GG + AA / GA	50.6 / 49.4	53.9 / 46.1	1 / 0.88	0.51
<i>rs1143627</i>				
GG / GA / AA	31.1 / 50.3 / 18.6	37.6 / 49.6 / 12.8	1 / 0.81 / 0.57	0.18
GG / GA + AA	31.1 / 68.9	37.6 / 62.4	1 / 0.75	0.11
GG + GA / AA	81.4 / 18.6	87.2 / 12.8	1 / 0.64	0.89
GG + AA / GA	49.7 / 50.3	50.4 / 49.6	1 / 0.97	0.071
<i>PPARγ</i>				
<i>rs16944</i>				
GG / GC / CC	32.2 / 49.4 / 18.4	41.1 / 46.1 / 12.8	1 / 0.73 / 0.54	0.12
GG / GC + CC	32.2 / 67.8	41.1 / 58.9	1 / 0.68	0.069
GG + GC / CC	81.6 / 18.4	87.2 / 12.8	1 / 0.65	0.13
GG + CC / GC	50.6 / 49.4	53.9 / 46.1	1 / 0.88	0.51
<i>rs1801282</i>				
CC / CG / GG	31.1 / 50.3 / 18.6	37.6 / 49.6 / 12.8	1 / 0.81 / 0.57	0.18
CC / CG + GG	31.1 / 68.9	37.6 / 62.4	1 / 0.75	0.11
CC + CG / GG	81.4 / 18.6	87.2 / 12.8	1 / 0.64	0.89
CC + GG / CG	49.7 / 50.3	50.4 / 49.6	1 / 0.97	0.071
<i>rs3856806</i>				
CC / CT / TT	90.5 / 8.6 / 1	92 / 8 / 0.0	1 / 0.91 / 0.0	0.2
CC / CT + TT	90.5 / 9.5	92 / 8	1 / 0.82	NA**
CC + CT / TT	99 / 1	100 / 0.0	1 / 0.0	0.076
CC + TT / CT	91.4 / 8.6	92 / 8	1 / 0.92	0.81

MAF: minor allele frequency, HWE: hardy weinberg equilibrium  $p > 0.05$ , NA: not available, PUD: peptic ulcer disease

**Table 4 -** The effect of the genotype distribution of studied SNPs on the activity of *Helicobacter pylori* infected Jordanian patients.

Gene/rs numbers/models	Activity		Odd ratio (95%CI)	P-value
	Yes	No		
<i>TNF-α</i>				
<i>rs1799964</i>				
TT / TC / CC	64.4 / 31.1 / 4.6	74.1 / 25.1 / 0	1 / 1.38 / NA	0.24
TT / TC + CC	64.4 / 35.6	74.1 / 25.9	1 / 1.58	0.31
TT + TC / CC	95.4 / 4.6	100 / 0	1 / NA	0.12
TT + CC / TC	69 / 31	74.1 / 25.9	1 / 1.29	0.2
<i>rs1800629</i>				
GG / GA / AA	80 / 18.6 / 1.4	74.1 / 25.9	1 / 0.66 / NA	0.49
GG / GA + AA	80 / 20	74.1 / 25.9	1 / 0.71	0.48
GG + GA / AA	98.6 / 1.4	100 / 0	1 / NA	0.4
GG + AA / GA	81.4 / 18.6	74.1 / 25.9	1 / 0.65	0.38
<i>rs361525</i>				
GG / GA / AA	86.4 / 12.4 / 0.9	96.3 / 3.7 / 0	1 / 3.73 / NA	0.24
GG / GA + AA	86.6 / 13.4	96.3 / 3.7	1 / 4.01	0.1
GG + GA / AA	99.1 / 0.9	100 / 0	1 / NA	0.49
GG + AA / GA	87.6 / 12.4	96.3 / 3.7	1 / 3.69	0.13
<i>NOD1</i>				
<i>rs2075820</i>				
CC / CT / TT	42.4 / 46 / 11.6	52.2 / 34.8 / 13	1 / 1.62 / 1.1	
CC / TC + TT	42.4 / 57.6	52.2 / 47.8	1.0 / 1.48	0.37
CC + TC / TT	88.4 / 11.6	87 / 13	1.0 / 0.88	0.84
CC + TT / TC	54 / 46	65.2 / 34.8	1.0 / 1.59	0.3
<i>rs2907749</i>				
GG / GA / AA	43.4 / 49.1 / 7.5	42.3 / 38.5 / 19.2	1.0 / 1.24 / 0.38	0.18
GG / GA + AA	43.4 / 56.4	42.3 / 57.7	1.0 / 0.96	0.92
GG + GA / AA	92.5 / 7.5	80.8 / 19.2	1.0 / 0.34	0.076
GG + AA / GA	50.9 / 49.1	61.5 / 38.5	1.0 / 1.54	0.3
<i>rs7789045</i>				
TT / AT / AA	28.9 / 52.6 / 18.5	25.9 / 51.9 / 22.2	1.0 / 0.91 / 0.75	0.88
TT / AT + AA	28.9 / 71.1	25.9 / 74.1	1.0 / 0.86	0.74
TT + AT / AA	81.5 / 18.5	77.8 / 22.2	1.0 / 0.79	0.65
TT + AA / AT	47.7 / 52.6	48.1 / 51.9	1.0 / 1.03	0.94
<i>NOD2</i>				
<i>rs1861759</i>				
TT / GT / GG	34.2 / 47.5 / 18.3	41.7 / 41.7 / 16.7	1.0 / 1.39 / 1.34	0.77
TT / GT + GG	34.2 / 65.8	41.7 / 58.3	1.0 / 1.38	0.47
TT + GT / GG	81.7 / 18.3	83.3 / 16.7	1.0 / 1.12	0.84
TT + GG / GA	52.5 / 47.5	58.3 / 41.5	1.0 / 1.27	0.59
<i>rs3135500</i>				
GG / GA / AA	29.4 / 47.9 / 22.7	29.4 / 58.8 / 11.8	1.0 / 0.81 / 1.93	0.51
GG / GA + AA	29.4 / 70.5	29.4 / 70.6	1.0 / 1.0	1
GG + GA / AA	77.3 / 22.7	88.2 / 11.8	1.0 / 2.0	0.27
GG + AA / GA	52.1 / 47.9	41.2 / 58.8	1.0 / 0.64	0.39
<i>IL-1β</i>				
<i>rs16944</i>				
GG / GA / AA	41.8 / 45.4 / 12.9	33.3 / 54.2 / 12.5	1.0 / 0.67 / 0.82	0.69
GG / GA + AA	41.8 / 58.2	33.3 / 66.7	1.0 / 0.7	0.42
GG + GA / AA	87.1 / 12.9	87.5 / 12.5	1.0 / 1.04	0.96
GG + AA / GA	54.6 / 45.4	45.8 / 54.2	1.0 / 0.7	0.42
<i>rs1143627</i>				
GG / GA / AA	38.2 / 49.2 / 12.6	30.8 / 53.9 / 15.4	1.0 / 0.74 / 0.66	0.74
GG / GA + AA	38.2 / 61.8	30.8 / 69.2	1 / 0.72	0.46
GG + GA / AA	87.4 / 12.6	84.6 / 15.4	1.0 / 0.79	0.69
GG + AA / GA	50.8 / 49.2	46.1 / 53.9	1.0 / 0.83	0.66
<i>PPARγ</i>				
<i>rs10865710</i>				
GG / GC / CC	68.2 / 29.9 / 1.9	70.4 / 29.6 / 0	1 / 104 / NA	
GG / GC + CC	68.2 / 31.8	70.4 / 29.6	1 / 1.11	0.82
GG + GC / CC	98.1 / 1.9	100 / 0	1 / NA	0.32
GG + CC / GC	70.1 / 29.9	70.4 / 29.6	1 / 1.01	0.98

\*P-value &lt;0.05 is considered significant. NA: not available

**Table 5** - The effect of the genotype distribution of studied SNPs on the severity of *Helicobacter pylori* infected Jordanian patients.

Gene/rs numbers/ genotype	Activity			P-value
	Mild	Moderate	Severe	
<b>TNF-<math>\alpha</math></b>				
<i>rs1799964</i>				
TT	44 (20.0)	73 (33.1)	25 (11.3)	0.46
TC	19 (8.5)	33 (15.0)	16 (7.3)	
CC	2 (0.0)	6 (0.3)	2 (0.0)	
<i>rs1800629</i>				
GG	53 (2.0)	86 (39.8)	34 (15.7)	0.74
GA	9 (4.2)	24 (11.1)	7 (3.2)	
AA	1 (0.5)	1 (0.5)	1 (0.5)	
<i>rs361525</i>				
GG	53 (24.3)	96 (44.0)	40 (18.3)	0.75
GA	9 (4.1)	15 (6.9)	3 (1.4)	
AA	1 (0.5)	1 (0.5)	0 (0.0)	
<b>NOD1</b>				
<i>rs2075820</i>				
TT	7 (3.5)	12 (6.0)	4 (2.0)	0.83
TC	31 (15.6)	31 (15.0)	17 (8.5)	
CC	23 (11.6)	45 (22.2)	16 (8.0)	
<i>rs2907749</i>				
GG	3 (1.4)	10 (4.7)	3 (1.4)	0.86
GA	34 (16.0)	53 (24.6)	48 (22.5)	
AA	17 (8.0)	18 (8.5)	2 (1.4)	
<i>rs7789045</i>				
TT	14 (6.6)	20 (9.4)	5 (2.4)	0.68
AT	34 (16.0)	55 (25.9)	22 (10.4)	
AA	15 (7.1)	35 (16.5)	12 (5.7)	
<b>NOD2</b>				
<i>rs1861759</i>				
TT	17 (8.4)	38 (18.7)	14 (6.9)	0.22
TG	34 (16.7)	41 (20.2)	21 (10.3)	
GG	9 (4.4)	24 (11.8)	5 (2.5)	
<i>rs3135500</i>				
G	52 (2.8)	89 (21.6)	31 (7.6)	0.78
T	68 (16.7)	117 (28.8)	49 (12.1)	
AA	10 (6.1)	20 (12.3)	7 (4.3)	
<b>IL-1<math>\beta</math></b>				
<i>rs16944</i>				
GG	24 (12.3)	44 (22.6)	14 (7.2)	0.76
GA	21 (10.8)	48 (24.6)	19 (9.7)	
AA	9 (4.6)	12 (6.2)	4 (2.1)	
<i>rs1143627</i>				
GG	8 (4.0)	13 (6.5)	4 (2.0)	0.94
GA	27 (13.5)	50 (25.0)	21 (10.5)	
AA	22 (11.0)	42 (21.0)	13 (6.5)	
<i>rs10865710</i>				
GG	2 (0.9)	2 (0.9)	0 (0.0)	0.33
GC	23 (10.8)	30 (14.2)	10 (4.7)	
CC	36 (17.0)	77 (36.3)	32 (15.1)	
<b>PPAR<math>\gamma</math></b>				
<i>rs1801282</i>				
GC	6 (2.7)	6 (2.7)	1 (0.5)	0.32
CC	60 (27.4)	105 (47.9)	41 (18.7)	
CC	58 (26.0)	107 (48.0)	41 (18.4)	
<i>rs3856806</i>				
CC	58 (26.0)	107 (48.0)	41 (18.4)	0.08
CT	9 (4.0)	7 (3.1)	1 (0.4)	
TT	0 (0.0)	0 (0.0)	0 (0.0)	

\*P&lt;0.05 is considered significant, NA: not available

A previous meta-analysis in Asian patients reported that omeprazole-based therapies were influenced by the different genotypes of the CYP2C19, while lansoprazole-based treatments were not.<sup>11,23,24</sup> Nevertheless, this analysis revealed that the studied SNPs of the CYP2C19 gene in the Jordanian Arab descent population did not show any significant correlation with the type of drugs used and the responsiveness to the treatment.

The genotypes of IL-1 $\beta$  rs16944 potentiate the synthesis of cytokines and are significantly correlated with the clinical development of *H. pylori* infection.<sup>9,25,26</sup> The presence of the A allele of the IL-1 $\beta$  rs1143627 showing a higher chance of developing the genotype AG in a German population.<sup>27</sup> The same result was obtained from the Caucasian and Brazilian populations that IL-1 $\beta$  (AA) and (AG) genotypes of rs1143627 SNP variant were correlated with chronic gastritis and gastric tumor progression in individuals infected with *H. pylori*,<sup>8,27,28</sup> but the current research showed no association between the genotypes IL-1 $\beta$  rs16944 and rs1143627 and the distribution between healthy individuals and peptic ulcer patients, in addition to the severity of the *H. pylori* infection. These findings are consistent to other research on the European and Mexican populations.<sup>29</sup>

Additionally, several researchers have found that modifications in the TNF- $\alpha$  gene promoter region are correlated with a greater risk of promoting gastritis.<sup>9,20</sup> The TNF- $\alpha$  produced in the gastric submucosa by macrophages plays a major role in the regulation of gastric acid secretion, which is one of the main factors of duodenal diseases development due to infection with *H. pylori*.<sup>30,31</sup> The environment of low acid secretion and aggressive inflammatory response can enhance the *H. pylori* colonization in the gastric mucosa.<sup>9</sup> Results of the meta-analysis specify that the TNF- $\alpha$  -308 (G>A) (rs1800629) and -1031 (T>C) (rs1799964) polymorphisms may be associated with a reduced risk of *H. pylori* infection in the Chinese population.<sup>29</sup> It has been found in the Japanese population that the C/C and T/C genotypes of the TNF- $\alpha$  rs1800629 were at the greatest risk from *H. pylori* infections.<sup>31,32</sup> Our results about the TNF- $\alpha$  polymorphisms (rs1799964, rs1800629, and rs361525) matched the results from other studies in Asian, European, and Caucasian populations.<sup>9,29</sup> In this study, the results show no statistical evidence of a considerable correlation between the vulnerability to *H. pylori* infections in gastric ulcer patients, activity, and severity in the Jordanian Arab population.

The NOD1 and NOD2 genes might play a crucial role in *H. pylori*-related processes of inflammation and

carcinogenesis.<sup>33</sup> Therefore, variations in these 2 genes could affect chronic inflammation caused by *H. pylori* and increase the risk of gastric cancer.<sup>7</sup> It was found that rs2907749 and rs3135500 were associated with the risk of gastric ulcers.<sup>33</sup> Many studies suggest the (TT) homozygote genotype of the polymorphism (rs2075820) found in the NOD1 gene raises the potential risk of development of peptic ulceration in the patients diagnosed with *H. pylori*-positive in the Hungarian population.<sup>7,34</sup> Many studies also suggest that if the structure or regulation of the NOD1 protein is altered, this would change the reactivity to *H. pylori* and the nature of downstream inflammatory mechanisms.<sup>7</sup> This study has investigated the relationship of 3 mutations in NOD1 (rs2075820, rs2907749, and rs7789045) gene and 2 mutations (rs1861759 and rs3135500) in NOD2 gene with the risk to *H. pylori* infections in the Jordanian Arab population. This study revealed that the individuals who carry the heterozygous genotype (TC) rs2075820 have a decreased risk of developing peptic ulcers (odd ratio: 0.49,  $p=0.0046$ ). The allele frequency in the patients were 66% (C) and 34% (T), compared to 60% (C) and 40% (T) in healthy individuals with insignificant association. Many studies have shown that having the (A) allele at rs3135500 was significantly connected with a slightly decreased chance of developing a gastric ulcer in the Chinese population, but the NOD2 mutations did not show any significant association either in genotype or allele models with *H. pylori* infection.<sup>6,9</sup> The variation of our results from other reported results could be due to the sample size and the source type of DNA and ethnicity. A small number of studies were conducted in this field concerning *H. pylori* infection in the Arab population. It is approved that the FFPE tissue is an important source of genetic material that can be used for molecular and pharmacogenetic studies. Therefore, the FFPE tissue could represent an alternative source of DNA instead of peripheral blood samples as for retrospective studies. The genetic material quality and quantity that extracted from FFPE is low compared with that extracted from fresh tissues, but this problem could be overcome by the optimization of the methods that used for DNA extraction and amplification. Furthermore, this type of studies is limited by the lack of clinical information for the studied patients.

In conclusion, this study analyzed the genetic association of many genetic variants with the risk of *H. pylori* infection and the response to the treatments in the Jordanian Arab population. TLR-10 rs10004195 SNP was associated with decreasing the risk of *H. pylori* infections; others, such as NOD1 rs2075820 SNP,

TLR-4 rs10759932 SNP, and TNF- $\alpha$  rs361525 and rs1799964 SNPs showed a significant association with the responsiveness to the type of treatment prescribed to the patients by the consultant. However, a high percentage of the types of medications written to the patients did not depend on the response of the patients to the prescribed drugs. Internationally, a phenomenon of *H. pylori* isolates resisting the antibiotics has been proved and should be considered to develop an optimal therapy. Therefore, these types of genetic association analyses can be used for matching patients into different treatment options based on their genetic profiles to improve medical treatment strategy used for treating ulcer patients.

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