Investigation of -308G>A and -1031T>C Polymorphisms in the TNFA Promoter Region in Polish Peptic Ulcer Patients

Aleksandra Sałagacka, Marta Żebrowska, Agnieszka Jeleń, Marek Mirowski, and Ewa Balcerczak

Laboratory of Molecular Diagnostics and Pharmacogenomics, Department of Pharmaceutical Biochemistry, Medical University of Lodz, Lodz, Poland

Background/Aims: Tumor necrosis factor α (TNF-α) encoded by TNFA is a key mediator in inflammation, a precursor condition for peptic ulceration. Promoter polymorphisms of TNFA that influence its transcriptional activity and TNF-α production are known. TNFA-308G>A (rs1800629) and TNFA-1031T>C (rs1799964), which are responsible for increased TNFA transcription, could influence the risk of peptic ulceration. This study aimed to investigate these polymorphisms and to evaluate their association with peptic ulcer disease and Helicobacter pylori infection in the Polish population.

Methods: Gastric mucosa specimens obtained from 177 Polish peptic ulcer patients were used to conduct rapid urease tests and to assess the investigated polymorphisms by polymerase chain reaction-restriction fragment length polymorphism. Genotyping data were compared with the results obtained from healthy individuals of Polish origin.

Results: There were no significant differences in genotype and allele frequency of the investigated polymorphisms between peptic ulcer patients and healthy individuals. No associations between the frequencies of particular genotypes and alleles for both single-nucleotide polymorphisms (SNPs) and the presence of H. pylori infection in peptic ulcer patients and in subgroups of men and women with peptic ulcer disease were found. Conclusions: The investigated SNPs are not risk factors for either peptic ulcer or H. pylori infection development in the Polish population. The results require verification in a larger cohort.

Key Words: Tumor necrosis factor-alpha; Genetic polymorphism; Peptic ulcer; Restriction fragment length polymorphism

INTRODUCTION

Helicobacter pylori infection is the main etiological factor of peptic ulcer disease (PUD). Also, as a cause of the gastroduodenal ulceration, an excessive use of nonsteroidal anti-inflammatory drugs (NSAIDs) is of growing importance. Peptic ulcer formation is inevitably connected with mucosal inflammation, a process modulated by several cytokines. One of them is tumor necrosis factor α (TNF-α) encoded by TNFA gene. This multifunctional cytokine was originally identified in mouse serum after injection with Mycobacterium bovis strain bacillus Calmette-Guerin and endotoxin. TNF-α is secreted mainly by activated macrophages, and its production is stimulated by bacterial lipopolysaccharide. The elevated level of this cytokine was found in the gastric mucosa of patients with H. pylori infection. Since agents reducing TNF-α concentration in gastric mucosa prevented indomethacin-induced gastric damage, TNF-α could also play a role in NSAID-triggered peptic ulcer formation.

Considering the vital role of TNF-α in the pathogenesis of PUD, it is reasonable to expect that genetic polymorphisms influencing the TNFA expression and the cytokine production could affect individual susceptibility to the disease. Single nucleotide polymorphisms at positions -238, -308, -857, -863, -1031 of TNFA have been investigated as potential risk factors of PUD and other H. pylori-induced disorders like gastritis, intestinal metaplasia, or gastric cancer. Unfortunately, there is no such report from Polish population available, and since there is no such report from Polish population available, the purpose of this study was to investigate whether polymorphisms -308G>A (rs1800629) and -1031T>C (rs1799964) of TNFA promoter region are associated with PUD and H. pylori
infection in the Polish population.

**MATERIALS AND METHODS**

The investigation was in accordance with the principles of the Declaration of Helsinki and was approved by the Ethical Committee of Medical University of Lodz. One hundred seventy-seven unrelated outpatients (111 females: median age 53 years, range 14 to 85 years; 66 males: median age 55 years, range 20 to 84 years) who visited the Department of Surgery, District Hospital, Łęczyca, Poland for an gastroduodenoscopy because of dyspepsia and diagnosed as peptic ulcer were enrolled in the study. Presence of *H. pylori* was evaluated at the time of gastroduodenoscopy by rapid urease test (Instytut Żywności i Żywienia, Warszawa, Poland). Patients who were treated with NSAIDs were excluded. Control group was 248 healthy individuals, geographically and ethnically matched to the patients with no symptoms of active gastroduodenal diseases. Genotyping data for the control group was published earlier by Bednarczuk et al. Data concerning exposure to carcinogens in the patients and controls were not available. All the subjects included in the study gave informed consent.

Genotyping of -308G>A and -1031T>C TNFA single-nucleotide polymorphisms (SNPs) was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. DNA was isolated according to Genomic DNA Prep Plus protocol (A&A Biotechnology, Gdynia, Poland) from endoscopic biopsy specimens of gastric mucosa. PCR mixture consisted of DNA template, 0.2 mM of each primer, 10 mM of each primer, 10 bp bands for mutant -1031CC homozygote, and 249, 180, 69, and 126, and 21 bp fragments for wild type -1031TT homozygote, 180, 69, and 15 126, and 21 bp fragments for wild type -308GG homozygote, 147 bp fragment for mutant -308AA homozygote, and three bands 147, 126, and 21 bp fragments for -308GA heterozygote. 249 and 15 bp bands for wild type -1031TT homozygote, 180, 69, and 15 bp bands for mutant -1031CC homozygote, and 249, 180, 69, and 15 bp bands for -1031CT heterozygote were present.

Statistical analysis were performed using the STATISTICA version 10 (StatSoft Inc., Tulsa, OK, USA) software package. The chi-square test was applied to evaluate conformity between the observed and expected genotype frequencies according to the Hardy-Weinberg rule and to determine the significance of differences in allele and genotype frequencies between the patients and controls. An odds ratio with a 95% confidence interval was estimated by logistic regression. A p-value <0.05 was assumed as significant in all the conducted tests.

**RESULTS**

All 177 gastric mucosa biopsy specimens were successfully analysed for polymorphisms at positions -308 and -1031 of TNFA promoter. All the data obtained and results of its statistical analysis were summarized in Table 1 for -308G>A and in Table 2 for -1031T>C.

All the genotypes for both polymorphisms were distributed in accordance with Hardy-Weinberg equilibrium within both patient and control cohorts which confirmed the cohorts as suitable. Genotype and allele frequencies occurred with similar frequencies in peptic ulcer and control groups for both -308G>A and -1031T>C SNPs. No statistically significant differences between investigated and control group were found (Tables 1 and 2).

According to the results of rapid urease tests, peptic ulcer patients were divided into two groups: *H. pylori*-infected and -uninfected individuals. Because of low occurrence of mutated alleles -308A and -1031C in the investigated cohorts, carriers of genotype -308GA and AA and carriers of -1031CC and CT were combined for analysis. There was no statistical difference between genotype and allele frequencies of the investigated polymorphism and the presence of *H. pylori* infection (Tables 1 and 2). Analogous associations were also examined in subgroups of female and male peptic ulcer cases. Although some dissimilarities in genotype and allele distribution between these subgroups and the whole peptic ulcer cohort for both investigated polymorphisms were demonstrated, no statistically significant association was found (Tables 1 and 2).

**DISCUSSION**

Host response to *H. pylori*-induced gastric mucosal inflammation is visible as an increased level of cytokines production. This appears to play a significant role in clinical outcome of the infection. The increased level of cytokines can be connected with ethnic diversity and relationship between expression and gene encoding cytokine polymorphisms.

The present study evaluated the effect of TNFA gene polymorphisms in patients with peptic ulcer in the Polish population. Frequencies of particular genotypes and alleles observed in the present study were similar to those obtained earlier in other Polish healthy control cohorts and in patients suffered from multiple sclerosis or colorectal cancer.

There was no association between occurrence of peptic ulcer and any of investigated polymorphisms. This finding is in agreement with some of previously published results for peptic ulcer patients. No association was stated between PUD incidence and -308G>A TNFA polymorphism in Koreans, duodenal ul-
cers and -308G>A or -1031T>C in eastern Indians,\textsuperscript{9} or duodenal ulcer disease and -308G>A variation in Chinese Han population.\textsuperscript{23} On the other hand, Lu et al.\textsuperscript{7} showed that -1031T>C and -863C>A are host factors determining the risk of peptic ulceration among Taiwanese, which implies the presence of some, not fully known ethnicity-related factors influencing the risk of PUD. In addition, it should be noticed that there are differences among studies in choosing control group (healthy volunteers\textsuperscript{9,13,23} or dyspeptic patients\textsuperscript{7}) which may produce discrepancies in the results. As the sample size used in the research is relatively small, the results should be considered as preliminary and verified in larger groups.

Also, there was no connection between any of investigated SNPs and \textit{H. pylori} infection in peptic ulcer patients. This is in contradiction with data published elsewhere. Lu et al.\textsuperscript{7} found significantly elevated risk of peptic ulcer after \textit{H. pylori} infection in carriers of either -1031C or -863A allele from the Taiwanese population. Observed discrepancy could partly be explained by difference in the prevalence of highly virulent strains of \textit{H. pylori}. While almost 100\% of the \textit{H. pylori} strains possess virulence factor-encoding cytotoxin-associated gene A (cagA+ strains) in Taiwan,\textsuperscript{7} their incidence in Poland is estimated to be approximately 60\%.\textsuperscript{24} There is some evidence that -308A polymorphism was significantly related to infection with the \textit{H. pylori} cagA+ subtype.\textsuperscript{25} On the other hand, TNFA gene expression was stated to be independent of \textit{H. pylori} cagA or vacA (vacuolating cytotoxin A) genotype.\textsuperscript{24} Taking into account the research findings described above, a lack of data about the

### Table 1. Comparison of the TNFA-308G>A Allele and Genotype Frequencies between Peptic Ulcer Patients and Healthy Individuals and between Helicobacter pylori-Infected and -Uninfected Peptic Ulcer Patients

|                         | Peptic ulcer case (n=177) | Healthy individual (n=248) | p-value | OR     | 95\% CI |
|-------------------------|---------------------------|----------------------------|---------|--------|---------|
| GG                      | 121 (68.4)                | 172 (69.4)                 | 0.8775  | 1.00   | -       |
| GA                      | 54 (30.5)                 | 72 (29.0)                  | 1.02    | 0.98–1.53 |
| AA                      | 2 (1.1)                   | 4 (1.6)                    | 1.04    | 0.46–2.35 |
| G                       | 296 (83.6)                | 416 (83.9)                 | 0.9208  | -      | -       |
| A                       | 58 (16.4)                 | 80 (16.1)                  | -       | -      | -       |
| HWE p-value             |                           | 0.3200                     |         | 0.5878 |

|                         | Infected (n=86) | Uninfected (n=91) | p-value | OR     | 95\% CI |
|-------------------------|-----------------|-------------------|---------|--------|---------|
| GG                      | 58 (67.4)       | 63 (69.2)         | 0.7981  | 1.00   | -       |
| GA or AA                | 28 (32.6)       | 28 (30.8)         | 1.09    | 0.57–2.06 |
| G                       | 144 (83.7)      | 152 (83.5)        | 0.9586  | -      | -       |
| A                       | 28 (16.3)       | 30 (16.7)         | -       | -      | -       |
| HWE p-value             | 0.2066          | 0.9802            |         |        |

|                         | Infected (n=54) | Uninfected (n=57) | p-value | OR     | 95\% CI |
|-------------------------|-----------------|-------------------|---------|--------|---------|
| GG                      | 33 (61.1)       | 42 (73.7)        | 0.1573  | 1.00   | -       |
| GA or AA                | 21 (38.9)       | 15 (25.3)        | 1.78    | 0.59–5.39 |
| G                       | 87 (80.6)       | 98 (86.0)        | 0.2797  | -      | -       |
| A                       | 21 (19.4)       | 16 (14.0)        | -       | -      | -       |
| HWE p-value             | 0.2171          | 1.000            |         |        |

|                         | Infected (n=32) | Uninfected (n=34) | p-value | OR     | 95\% CI |
|-------------------------|-----------------|-------------------|---------|--------|---------|
| GG                      | 25 (78.1)       | 21 (61.8)         | 0.1515  | 1.00   | -       |
| GA or AA                | 7 (21.9)        | 13 (38.2)        | 0.45    | 0.02–10.22 |
| G                       | 57 (89.1)       | 54 (79.4)        | 0.1312  | -      | -       |
| A                       | 7 (10.9)        | 14 (20.6)        | -       | -      | -       |
| HWE p-value             | 0.9200          | 0.9556           |         |        |

Values are presented as number (%).

OR, odds ratio; CI, confidence interval; HWE, Hardy-Weinberg equilibrium.
Sałągacka A, et al: -308G>A and -1031T>C of TNFA in Polish Peptic Ulcer Patients

Recently, we showed that 3435C>T polymorphism of the ABCB1 gene is a risk factor for H. pylori infection development in male peptic ulcer patients but not in female peptic ulcer patients. Due to a significant overrepresentation of female in the investigated cohort of patients (women to men ratio, 1.68), analysis was performed in subgroups of women and men. Obtained results indicated that there was no association between investigated SNPs and risk of H. pylori infection in both female and male peptic ulcer cases.

Some researchers postulate the TNFA promoter polymorphisms act synergistically, so it is only possible to observe some their effects when many of these polymorphisms are analysed together. For example, Chakravorty et al. did not found any association between single polymorphism locus of TNFA (-308, -857, -863, -1031) and H. pylori-mediated duodenal ulcer, but observed significantly higher percentage of TNFA haplotype -308G_-857C_-863A_-1031T in H. pylori-infected duodenal ulcer patients than in individuals with infection but without ulceration. Presence of such an effect could not be excluded in the present study. Further study is needes in peptic ulcer patients including other TNFA loci and haplotype analysis.

In conclusion, neither -308G>T nor -1031T>C SNP is a factor for genetic susceptibility to peptic ulcer in the population. Moreover, none of the investigated SNPs are the risk factors for H. pylori infection development in this group.

Table 2. Comparison of the TNFA-1031T>C Allele and Genotype Frequencies between Peptic Ulcer Patients and Healthy Individuals and between Helicobacter pylori-Infected and -Uninfected Peptic Ulcer Patients

|                | Peptic ulcer case (n=177) | Healthy individual (n=248) | p-value | OR | 95% CI |
|----------------|---------------------------|---------------------------|---------|----|--------|
| TT             | 113 (63.8)                | 167 (67.3)                | 0.9663  | 1.00 | -      |
| CT             | 61 (34.5)                 | 76 (30.6)                 | 1.02    | 0.98–1.53 | |
| CC             | 3 (1.7)                   | 5 (2.0)                   | 1.04    | 0.46–2.35 | |
| T              | 287 (81.1)                | 410 (82.7)                | 0.5525  | -   | -      |
| C              | 67 (18.9)                 | 86 (17.3)                 | -       | -   | -      |
| HWE p-value    | 0.2622                    | 0.6228                    |         |     |        |

All peptic ulcer case

|                | Infected (n=86) | Uninfected (n=91) | p-value | OR | 95% CI |
|----------------|---------------|-------------------|---------|----|--------|
| TT             | 54 (62.8)     | 59 (64.8)         | 0.7772  | 1.00 | -      |
| CT or CC       | 32 (37.2)     | 32 (35.2)         | 1.09    | 0.59–2.03 | |
| T              | 139 (80.8)    | 148 (81.3)        | 0.9036  | -   | -      |
| C              | 33 (19.2)     | 34 (18.7)         | -       | -   | -      |
| HWE p-value    | 0.3684        | 0.7816            |         |     |        |

Female peptic ulcer case

|                | Infected (n=54) | Uninfected (n=57) | p-value | OR | 95% CI |
|----------------|---------------|-------------------|---------|----|--------|
| TT             | 33 (61.1)     | 35 (61.4)         | 0.9748  | 1.00 | -      |
| CT or CC       | 21 (38.9)     | 22 (38.6)         | 1.09    | 0.59–2.03 | |
| T              | 86 (79.6)     | 96 (79.8)         | 0.2797  | -   | -      |
| C              | 22 (20.4)     | 23 (20.2)         | -       | -   | -      |
| HWE p-value    | 0.6867        | 0.5982            |         |     |        |

Male peptic ulcer case

|                | Infected (n=32) | Uninfected (n=34) | p-value | OR | 95% CI |
|----------------|---------------|-------------------|---------|----|--------|
| TT             | 21 (65.6)     | 24 (70.6)         | 0.6653  | 1.00 | -      |
| CT or CC       | 11 (34.4)     | 10 (29.4)         | 1.26    | 0.42–3.77 | |
| T              | 53 (82.8)     | 57 (83.8)         | 0.8762  | -   | -      |
| C              | 11 (17.2)     | 11 (16.2)         | -       | -   | -      |
| HWE p-value    | 0.4748        | 1.000             |         |     |        |

Values are presented as number (%).
OR, odds ratio; CI, confidence interval; HWE, Hardy-Weinberg equilibrium.

cagA status in the investigated H. pylori-infected cases could be considered as a limitation of our research. It should be specified in the future.
CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGEMENTS

Supported by grants from Ministry of Science and Higher Education/National Science Centre, Warszawa/Kraków, Poland (N-N405-161339) and Statutory Funds of Department of Pharmaceutical Biochemistry, Medical University of Lodz, Poland (503/3-015-02/503-01); and from Faculty of Pharmacy, Medical University of Lodz, Poland (2012 502-03/3-015-02/502-34-021).

REFERENCES

1. Malfertheiner P, Chan FK, McColl KE. Peptic ulcer disease. Lancet 2009;374:1449-1461.
2. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci U S A 1975;72:3666-3670.
3. Crabtree JE, Shallcross TM, Heatley RV, Wyatt JL. Mucosal tumour necrosis factor alpha and interleukin-6 in patients with Helicobacter pylori pylori-associated gastritis. Gut 1991;32:1473-1477.
4. Hüseyinov A, Kütükçüler N, Aydogdu S, et al. Increased gastric production of platelet-activating factor, leukotriene-B4, and tumour necrosis factor-alpha in children with Helicobacter pylori pylori infection. Dig Dis Sci 1999;44:675-679.
5. Ding SZ, Lam SK, Yuen ST, et al. Prostaglandin, tumor necrosis factor alpha and neutrophils: causative relationship in indomethacin-induced stomach injuries. Eur J Pharmacol 1998;348:257-263.
6. Machado JC, Figueiredo C, Caneo P, et al. A proinflammatory genetic profile increases the risk for chronic atrophic gastritis and gastric carcinoma. Gastroenterology 2003;125:364-371.
7. Lu CC, Sheu BS, Chen TW, et al. Host TNF-alpha-1031 and -863 promoter single nucleotide polymorphisms determine the risk of benign ulceration after H. pylori infection. Am J Gastroenterol 2005;100:1274-1282.
8. Sugimoto M, Furuta T, Shirai N, et al. Different effects of polymorphisms of tumor necrosis factor-alpha and interleukin-1 beta on development of peptic ulcer and gastric cancer. J Gastroenterol Hepatol 2007;22:51-59.
9. Chakravorty M, Datta De D, Choudhury A, Santra A, Roychoudhury S. Association of specific haplotype of TNFalpha with Helicobacter pylori- mediated duodenal ulcer in eastern Indian population. J Genet 2008;87:299-304.
10. Lee SG, Kim B, Yook JH, Oh ST, Lee I, Song K. TNF/LTA polymorphisms and risk for gastric cancer/duodenal ulcer in the Korean population. Cytokine 2004;28:75-82.
11. Wilchanski M, Schlesinger Y, Faber J, et al. Combination of Helicobacter pylori strain and tumor necrosis factor-alpha polymorphism of the host increases the risk of peptic ulcer disease in children. J Pediatr Gastroenterol Nutr 2007;45:199-203.
12. Zambon CF, Basso D, Navaglia F, et al. Pro- and anti-inflammator cytokines gene polymorphisms and Helicobacter pylori infection: interactions influence outcome. Cytokine 2005;29:141-152.
13. Lee JY, Kim HY, Kim KH, et al. Association of polymorphism of IL-10 and TNF-A genes with gastric cancer in Korea. Cancer Lett 2005;225:207-214.
14. Kim N, Cho SI, Yim JY, et al. The effects of genetic polymorphisms of IL-1 and TNF-A on Helicobacter pylori-induced gastroduodenal diseases in Korea. Helicobacter 2006;11:105-112.
15. Canedo F, Durães C, Pereira F, et al. Tumor necrosis factor alpha extended haplotypes and risk of gastric carcinoma. Cancer Epidemiol Biomarkers Prev 2008;17:2416-2420.
16. Bednarzuk T, Hromatso Y, Seki N, et al. Association of tumor necrosis factor and human leucocyte antigen DRB1 alleles with Graves’ ophthalmopathy. Hum Immunol 2004;65:632-639.
17. Skoog T, van’t Hooft FM, Kallin B, et al. A common functional polymorphism (C→A substitution at position -863) in the promoter region of the tumour necrosis factor-alpha (TNF-alpha) gene associated with reduced circulating levels of TNF-alpha. Hum Mol Genet 1999;8:1443-1449.
18. Mirowska-Guzel D, Gromadzka G, Mach A, Czonkowski A, Czonkowski A. Association of IL1A, IL1B, ILRN, IL6, IL-10 and TNF-alpha polymorphisms with risk and clinical course of multiple sclerosis in a Polish population. J Neuroimmunol 2011;236:87-92.
19. Kieszko R, Krawczyk P, Chocholska S, Dmoszyńska A, Milanowski J. TNF-alpha and TNF-beta gene polymorphisms in Polish patients with sarcoidosis: connection with the susceptibility and prognosis. Sarcoïdosis Vascul Disseminated Lung Dis 2010;27:131-137.
20. Talar-Wojnarowska R, Gasiorowska A, Smolarz B, Romanowicz-Makowska H, Kulig A, Malecka-Panas E. Tumor necrosis factor alpha and interferon gamma genes polymorphisms and serum levels in pancreatic adenocarcinoma. Neoplasma 2009;56:56-62.
21. Hou L, El-Omar EM, Chen J, et al. Polymorphisms in Th1-type cell-mediated response genes and risk of gastric cancer. Carcinogenesis 2007;28:118-123.
22. Suchy J, Klusjso-Grabowska E, Kladny J, et al. Inflammatory response gene polymorphisms and their relationship with colorectal cancer risk. BMC Cancer 2008;8:112.
23. Mei Q, Xu JM, Cao HL, et al. Associations of the II-1 and TNF gene polymorphisms in the susceptibility to duodenal ulcer disease in Chinese Han population. Int J Immunogenet 2010;37:9-12.
24. Zambon CF, Basso D, Navaglia F, et al. Pro- and anti-inflammator cytokines gene polymorphisms and Helicobacter pylori infection: interactions influence outcome. Cytokine 2005;29:141-152.
25. Lee JY, Kim HY, Kim KH, et al. Association of polymorphism of IL-10 and TNF-A genes with gastric cancer in Korea. Cancer Lett 2005;225:207-214.
26. Sałagacka A, Bartczak M, Zebrowska M, et al. C3435T polymorphism of the ABCB1 gene: impact on genetic susceptibility to peptic ulcers. Pharmacol Rep 2011;63:992-998.