

# [H.pylori virulence factors with gastric cancer](https://assignbuster.com/hpylori-virulence-factors-with-gastric-cancer/)

The Relationship between cagH , cagL, cagG, and orf17 Genotypes of Helicobacter pylori cag Pathogenicity Island with Peptic Ulcerations and Gastric Cancer

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### Abstract

Helicobacter pylori ( H. pylori ) specific genotypes have been correlated with an increased risk of gastrointestinal disease in Iran. The aim of this study was finding the relevance of H. pylori virulence factors ( cagH , cagL , cagG , and orf17 ) with gastric cancer (GC) and peptic ulcerations (PU) in Iran. The H. pylori strains were isolated from different ethnic and geographic origin within Iran and genotyped. Overall, the frequency of cagH , cagL , orf17 , and cagG genotypes was 74. 1%, 98%, 79. 9%, and 70. 4% in patients with PU, respectively, while in non-atrophic gastritis (NAG) the frequency was 59. 6% for cagH , 82. 6% for cagL , 61% for orf17 , and 74. 7% for cagG . The frequency of cagH , cagL , cagG , and orf17 was 50%, 83. 3%, 61. 9%, and 54. 8% in GC group, respectively. No association was found between the mentioned genotypes and the risk of GC in Iran ( P = P -value > 0. 05); however, cagL and orf17 genotypes were correlated with an increased risk of PU in Iran ( P = 0. 021 for cagL and P = 0. 015 for orf17 ), cagH and cagG genes showed no consistent relationship with PU in Iran ( P > 0. 05).

## Introduction

Gastric Cancer (GC) is the third common cancer that ends up death in the world,\*\* the incidence rate of this cancer varies across different geographical areas.\*\* The high incidence areas for gastric cancer has geographical distribution in Asia.\*\* Ardabil province that is located in north west of Iran and close to Caspian sea, is reported as area that has high incidence rate of gastric adenocarcinoma in Iran.\*\* Gastric cancer has high rate in male and is the third cancer after breast and colorectal cancer in female in Iran.\*\*The concrete reasons for prevalence of gastric cancer, which varies in different geographical areas, remain unknown; however data show that interaction between host and environmental factors, specially Helicobacter pylori ( H. pylori ) infection may play a remarkable role. This bacterium is found in more than 50 % of the world population and it is a well-known human pathogen. H. pylori is the cause of acute or chronic gastritis, peptic ulcerations (PU) disease, gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue (MALT) lymphoma. In most of cases the infection remains asymptomatic during life, just 20% develops to peptic ulcer or gastric carcinoma.\*\*\* H. pylori results in acute or chronic infection, the acute infection decreases the acidity in the gastric, which promotes the bacterial colonization,\* this hypochlorhydria stimulates gastritis that predisposes to atrophic gastritis, intestinal metaplasia, dysplasia and finally carcinoma.\* H. pylori infection in the gastric triggers a mucosal inflammatory response and is the cause of different clinical outcomes in humans.\* H. pylori harbors a pathogenicity island that is called cag pathogenicity island ( cag PAI). The cag PAI is the main virulence factor that encodes type IV secretion system (T4SS), several genes within cag PAI are associated with an increased interlukin-8 (IL-8) production, which is produced by gastric epithelial cells.\*\* Among the bacterial factors, the adherent ability to gastric epithelial cells is the most important factor in order to initiate the gastric inflammatory response and is crucial for IL-8 induction.\*\*CagL is bacterial factor that is called a pilus protein ( a VirB5 orthologe).\* Integrin α5β1 is a receptor on gastric epithelial cell that CagL binds to; by its arginine-glycine-aspartate (RGD) motif\*\* , this interaction activates α5β1 receptor and facilitates and triggers\*\* the delivery of bacterial CagA oncoprotein through T4SS into the epithelial cell.\* CagL is associated with two other cag PAI proteins ; CagI and CagH.\* All the three factors are highly needed for injection of CagA into the epithelial cell, and it is demonstrated that those factors are responsible for T4SS pili formation. cagG is another factor that is located upstream of cagA, cagG is the gene that shows some homology to adherence-related or motility-related genes of other bacteria in bioinformatics study or gene analysis.\*\*\* orf17 is the gene that shows some homology to the gene of other bacteria in bioinformatics study, the study shows that in Dickeya zeae there is a gene which has homology to orf17 in helicobacter pylori and 36% identity is reported in the pblast studies. Dickeya zeae , is the aerobic/anaerobic phytopathogene bacterium that is the cause of soft rot disease in a broad range plants species, specially many crops that are economically crucial.\*\* this bacterium causes bacterial foot rot and it is reported in many asian countries particularly china which the disease mostly begins at the ligulus.\*\*In this study we aimed to find out the distribution of four target genes ( cagH , cagL , cagG , and orf17 ) in H. pylori genome and their relationship with gastrointestinal disease in Iran.

## Materials and methods:

Collection of tissue specimens

The tissue specimens were collected over 3 years from 2011 to 2014 in Iran; from patients with various gastric diseases.

H. pylori isolation and culture

Antrum and body biopsies were used for H. pylori culture. Biopsies were cultured on selective Brucella agar (Merck, Germany) involving 5% sheep blood, 10 mg/L of vancomycin (Zakaria, Iran), 5 mg/L trimethoprim (MP Biomedicals, France), 2. 5 IU/L polymyxin B (MP Biomedicals, France), and 8 mg/L amphotericin B (Bristol-Myers Squibb, USA). Cultures were incubated at 37 â-¦C under microaerophilic situation for 3-7 days. Bacterial isolates were identified as H. pylori on the basis of Gram-stained morphology and positive urease, catalase, and oxidase tests. Bacterial isolates were harvested in brain heart infusion broth (Merck) enriched with 20% glycerol and 10% inactivated horse serum and stored at −70 â-¦C.

Histological assessment and classification

All biopsies were taken from the gastric body (corpus) and antrum of patients with different gastrointestinal disease, and were used for histopathalogical examination. The biopsy specimens were initially formalin-fixed and paraffin-embedded. Sections of 4μm were obtained and stained with hematoxylin-eosin, Giemsa, and Alcian blue- periodic acid Shiff (pH 2. 5). By the use of Sydney system, GC was classified and graded. 4ms. abdi

DNA extraction

Using protocol DNGTM plus kit (Cinna Gene, Tehran, Iran), DNA was extracted from the urease, oxidase, and catalase positive gastric biopsy specimens, before using the kit, the tissue was completely broken down using the scalpel. Extracted DNA was kept at -20ËšC until polymerase chain reaction (PCR) amplification was carried out.

PCR amplification

The first PCR was conducted in order to detect the H. pylori -specific 16S rDNA gene\*. Using HP1-2 16S Rrna primer PCR amplification was performed (Optimized annealing temperature: 56 °C) in 30-μl reaction mixture and the PCR amplification products (1500 bp in size), which were 16S rDNA, were detected in electrophoreses that proved the specimens were infected by H. pylori ( H. P + ). The second PCR was done in order to detect the target genes which were cagH , cagL , cagG , and orf17 (Optimized annealing temperature (°C): 52, 54, 50, and 50; respectively). The PCR amplification was carried out in 30-μl reaction mixture involving MgCl2 = 1 µl, PCR buffer = 3 µl, dNTPmix = 0. 5 µl, primers (reverse and forward mixture) = 1 µl, enzyme = 0. 2 µl, template DNA = 5 µl, and D. W = 19. 3 µl (total volume = 30 µl); and the PCR amplification products ( cagH = 1113 bp , cagL = 263 bp, cagG = 398 bp, and orf17 = 546 bp in size) were detected.

Statistical analysis

The relationship between the cagH , cagL , cagG and orf17 genotypes of H. pylori cag pathogenicity island and clinical outcomes (including GC, PU, and non-atrophic gastritis or NAG) were analyzed. The SPSS statistical software package version 18. 0 was used for all statistical analyses in this study. With chi-square test and the fisher exact probability the analysis was done. A P -value of < 0. 05 indicated significance. A simple and multiple logistic regression was used to calculate the odd ratio (OR) and 95% confidence interval (CI) of the clinical outcomes by including age, sex and H. pylori genotypes. The OR and CI were used to estimate the risk of PU and GC in the current study.

## Results

A total of 242 strains isolated from H. pylori -positive Iranian patients (59. 5% male and 40. 5 female; 37. 6% age >= 55 and 62% age < 55), were examined in the present study. The strains involving 146 (60. 3%) with NAG, 54 (22. 3%) with PU, and 42 (17. 4%) with GC. The total frequency of cagH , cagL , cagG , and orf17 genotypes was 61. 2%, 86. 4%, 71. 5%, and 64%, respectively.

Association between the virulence factors and PU in Iran: The frequency of the cagH , cagL , orf17 , and cagG genotypes was 74. 1%, 98. 1%, 79. 6%, and 70. 4%in PU group (51. 8% for duodenal ulcer and 37. 03% for gastric ulcer) although in the control group (NAG) the following frequencies were reported: 59. 6% for cagH , 82. 9% for cagL , 61% for orf17, and 74. 7% for cagG . The simple logistic regression analysis illustrated that the cagL and orf17 genotypes were remarkably associated with an increased risk of PU, the OR (95%CI) 10. 95 (1. 446-82. 935) and 2. 504 (1. 193-5. 235) respectively, ( P = 0. 021 for cagL and P = 0. 015 for orf17 ). No significant relationship was found between the cagH and cagG genotypes and the risk of PU ( P > 0. 05). Analysis for combined genotypes showed that cagH / cagL and cagH / orf17 were associated with an increased risk of PU, the OR (95%CI) was 9. 756 (1. 264-75. 303) for cagH / cagL and 2. 861 (1. 167-7. 013) for cagH / orf17 ( P = 0. 029 and 0. 02, respectively). No significant correlation was found between cagH / cagG , cagL / cagG , cagL / orf17 , cagG / orf17 , cagH / cagG / cagL , cagH / cagG / orf17 , cagH / cagL / orf17 , and cagL / cagG / orf17 combined genotypes and the risk of PU ( P > 0. 05).

Multiple logistic regression analysis illustrated that cagL genotype was independently and significantly correlated with the age and sex-adjusted risk (Ø¨Ù‡ Ø¬Ø§ÛŒ Ø§ÛŒÙ† Ø¬Ù…Ù„ Ù‡ Ú†ÛŒ Ù…ÛŒ ØªÙˆÙ†Ù… Ø¨Ù†ÙˆÛŒØ³Ù…ØŸØŸ) for PU, the OR (95%CI) was 9. 557 (1. 219-7. 185) ( P = 0. 032).

Association between the virulence factors and GC in Iran: Of the 42 GC patients, 42. 8% were with cardia cancer (CC), and 57. 1% with non-cardia cancer (NCC) and 38. 1% with diffuse-, 57. 1% with intestinal-, and 2. 3% with mucin producing-type adenocarcinomas, and 2. 3% with invasive squamous cell-type carcinoma. Chi-square test demonstrated no correlation between cagH , cagL , cagG , and orf17 genotypes and risk of GC ( P > 0. 05). statistical analysis for combined genotypes illustrated that cagH / cagG reduced the risk of GC, the OR (95%CI) was 0. 388 (0. 166-0. 908) ( P = 0. 029). However, No remarkable association was found between cagH/cagL , cagH / orf17 , cagL / cagG , cagL / orf17 , cagG / orf17 , cagH / cagG / cagL , cagH / cagG / orf17 , cagH / cagL / orf17 , and cagL / cagG / orf17 combined genotypes and risk of GC ( P > 0. 05).

## Discussion

The presence of H. pylori in the gastric mucosa has been known as an essential risk factor of different gastrointestinal disease including; NAG, PU, and GC.\*\* there are several virulence factors within H. pylori genome that might take part in mucosal damage.\*\* This study investigated the relevance of various virulence factors ( cagH , cagL , cagG , and orf17 ) and their relationship with sever gastrointestinal disease. Studies showed that cagG mutant didn’t result in inflammatory response or increase proliferation, and also demonstrated that lack of cagG gene leads in loss of CagA translocation/phosphorylation.\*\*Recent reports showed that loss of cagG genotype have reduced adherence to epithelial cells.\* An in vivo study in China have also illustrated no relationship between cagG and clinical outcomes. Most reports, suggested that lack of cagG genotype leads in complete removal of H. pylori IL-8 induction.\*\*However, other studies suggested that copmlete deletion of cagG gene resulted in no reduction in IL-8 induction,\*\* according to these data it is impossible to distinguish whether or not lack of inflammation with cagG mutant is associated with reduced colonization, loss of T4SS, or both.\* A study on 145 isolates in china showed that cagG was detected in 91. 7% H. pylori isolates, 100% of isolates from patients with PU (Duodenal Ulcer and Gastric Ulcer) were cagG positive which was high , but not statistically significant ( P > 0. 05). cagG genotype was known as a conservative gene in chines population and there was no significant differences in the frequencies of cagG gene in isolates from patients with various digestive disease.\*\* Hsu et al reported that of the 120 isolates from patients with different gastrointestinal disease in Korea 86. 7% (104/120) were cagG positive.\*\* Mizushamia et al used PCR to investigate the distribution of cagG gene in 236 clinical H. pylori isolates in Japan, and found that 97% of isolates were cagG positive. For both isolates from Korea and Japan ( P > 0. 05), demonstrating no significant association with gastrointestinal disease.\*\*In other study level of gastric mucosal inflammation was compared in the antrum and body (corpus) of both cagG positive and negative group, it was illustrated that the level of inflammation was relatively higher in cagG positive than cagG negative group, both in antrum and body, ( P > 0. 05). resulting cagG genotype has no relationship with intensity of gastritis.\*\*The prior results concordant with our results, we concluded that cagG genotype had no remarkable association with digestive disease in Iran ( P > 0. 05).

A recent study on cagL genotype in India, illustrated that 86. 6% of isolates were cagL positive.\*\*\*other studies in Malaysia, Singapore, and Taiwan demonstrated > 85% of the isolates were cagL positive,\*\* furthermore, the presence of RGD motif in the isolates suggested that the CagA oncoprotein translocation is mediated by an RGD dependent pathway, therefore it is crucial to find out whether or not the cagL amino acid sequence polymorphisms are correlated with clinical outcomes.??? A study on 61 isolates from patients with digestive disease in Iran, showed that 96. 7% were cagL positive. This report was concordant with the results from Taiwan (98. 6%)\*\*\*most of the patients were cagL positive, but no remarkable association was detected between the cagL genotype and clinical outcomes ( P > 0. 05).\*\* these results are in consistent with our results in GC group but not in PU group, demonstrating that cagL genotype is remarkably and independently associated with the risk of PU in Iran ( P = 0. 021), but no association was found with the risk of GC.

cagH and orf17 have not been surveyed on genomics level; however, we found out the cagH genotype had no relationship with gastrointestinal disease in Iran ( P > 0. 05), and we concluded that orf17 genotype had no association with GC group ( P > 0. 05) but there was a remarkable relationship between orf17 gene and an increased risk of PU in Iran ( P = 0. 015).

## Conclusion

We concluded that, if specific H. pylori virulent biomarkers can be known to predict PU or GC risk, patients with NAG can be selected for H. pylori eradication, and also several virulence factors must be studied simultaneously, in order to clarify the correlation between the virulence factors and gastrointestinal disease. It is proposed that the orf17 and specially cagL genotypes of H. pylori cag PAI might be as beneficial factors for the risk prediction of PU, but not GC in Iran. This report is the first one regarding the relevance of H. pylori orf17 genotype to PU in Iran(ØªÙˆ Ø¨Ø®Ø´ Ø¯ÛŒÚ¯Ù‡ Ø§ÛŒ Ø¨ÛŒØ§Ø±Ù… Ø§ÛŒÙ† Ø¬Ù…Ù„ Ù‡ Ø±Ùˆ Ø¨Ù‡ØªØ± Ù†ÛŒØ³ØªØŸØŸØŸØŸ!!). No remarkable correlation was found between the cagH and cagG genotypes and the risk of both the PU and GC in Iran. Genotypes combination analysis showed that cagH / cagG decrease the risk of GC in Iran, but no consistent relationship was observed with PU, and cagH / orf17 and cagH / cagL increased the risk of PU, not GC in Iran.(Ø§ÛŒÙ† Ù‚ Ø³Ù…Øª Ø­Ø°Ù Ø¨Ø´Ù‡ ÛŒØ§ Ù†Ù‡ØŸØŸØŸ!! ØŸ)

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| Virulence factors | Disease type | P -value | OR | CI | Total strains |  |
| PU  N (%) | NAG  N (%) |  | | | |  |
| cagH | 40/54 (74. 1) | 87/146 (59. 6) | 0. 061 | 1. 938 | 0. 969-3. 873 | 127 |
| cagL | 53/54 (98. 1) | 121/146 (82. 9) | 0. 021 | 10. 950 | 1. 446-82. 935 | 174 |
| cagG | 38/54 (70. 4) | 109/146 (74. 7) | 0. 542 | 0. 806 | 0. 403-1. 612 | 147 |
| orf17 | 43/54 (79. 6) | 89/146 (61) | 0. 015 | 2. 504 | 1. 193-5. 253 | 132 |
| cagH/cagL | 40/41 (97. 7) | 82/102 80. 4) | 0. 029 | 9. 756 | 1. 264-75. 303 | 122 |
| cagH / cagG | 32/40 (80) | 70/90 (77. 8) | 0. 776 | 1. 143 | 0. 455-2. 869 | 102 |
| cagH/orf17 | 34/41 (82. 9) | 73/116 (62. 9) | 0. 022 | 2. 861 | 1. 167-7. 013 | 107 |
| cagL/cagG | 36/36 (100) | 96/108 (88. 9) | — | — | — | 132 |
| cagL/orf17 | 41/43 (95. 3) | 81/97 (83. 5) | 0. 071 | 4. 049 | 0. 888-18. 463 | 122 |
| cagG/orf17 | 31/35 (88. 6) | 70/89 (78. 7) | 0. 208 | 2. 104 | 0. 661-6. 698 | 101 |
| cagH/cagG/cagL | 31/31 (100) | 67/77 (87) | — | — | — | 98 |
| cagH/cagG/orf17 | 28/32 (87. 5) | 61/75 (81. 3) | 0. 438 | 1. 607 | 0. 485-5. 323 | 89 |
| cagH/cagL/orf17 | 34/35 (97. 1) | 70/84 (83. 3) | 0. 069 | 6. 800 | 0. 858-53. 873 | 104 |
| cagL/cagG/orf17 | 31/31 (100) | 67/75 (89. 3) | — | — | — | 98 |
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| --- | --- | --- | --- | --- | --- | --- |
| Virulence factors | Disease type | P -value | OR\* | CI‡ | Total strains |  |
| GC  N (%) | NAG  N (%) |  | | | |  |
| cagH | 21/42 (50) | 87/146 (59. 6) | 0. 269 | 0. 678 | 0. 340-1. 351 | 108 |
| cagL | 35/42 (83. 3) | 121/146 (82. 9) | 0. 945 | 1. 033 | 0. 412-2. 589 | 156 |
| cagG | 26/42 (61. 9) | 109/146 (74. 7) | 0. 108 | 0. 552 | 0267-1. 140 | 136 |
| orf17 | 23/42 (54. 8) | 89/146 (61) | 0. 471 | 0. 775 | 0. 388-1. 550 | 112 |
| cagH/cagL | 21/28 (75) | 82/102 (80. 4) | 0. 534 | 0. 732 | 0. 273-1. 960 | 103 |
| cagH/cagG | 19/33 (57. 6) | 70/90 (77. 8) | 0. 029 | 0. 388 | 0. 166-0. 908 | 89 |
| cagH/orf17 | 20/38 (52. 3) | 73/116 (62. 9) | 0. 261 | 0. 654 | 0. 312-1. 372 | 93 |
| cagL/cagG | 24/29 (82. 8) | 96/108 (88. 9) | 0. 378 | 0. 600 | 0. 193-1. 867 | 120 |
| cagL/orf17 | 22/28 (78. 6) | 81/97 (83. 5) | 0. 547 | 0. 724 | 0. 253-2. 070 | 103 |
| cagG/orf17 | 19/31 (61. 3) | 70/89 (78. 7) | 0. 061 | 0. 430 | 0. 178-1. 039 | 89 |
| cagH/cagG/cagL | 19/24 (79. 2) | 67/77 (87) | 0. 350 | 0. 567 | 0. 173-1. 861 | 86 |
| cagH/cagG/orf17 | 19/30 (63. 3) | 61/75 (81. 3) | 0. 054 | 0. 396 | 0. 154-1. 018 | 80 |
| cagH/cagL/orf17 | 20/26 (76. 9) | 70/84 (83. 3) | 0. 461 | 0. 667 | 0. 227-1. 959 | 90 |
| cagL/cagG/orf17 | 19/23 (82. 6) | 67/75 (89. 3) | 0. 394 | 0. 567 | 0. 154-2. 089 | 86 |
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| Characteristics | Total frequency N (%) |
| Age  <55  >= 55 | 150/241 (62)  91/241 (37. 6) |
| Sex  Female = 0  Male = 1 | 98/242 (40. 5)  144/242 (59. 5) |
| Non-atrophic gastritis | 146/242 (60. 3) |
| Peptic ulcer  Duodenal ulcer  Gastric ulcer | 54/242 (22. 3)  28/54 (51. 8)  20/54 (37. 03) |
| Gastric cancer  Cardia cancer  Non-cardia cancer  Intestinal-type adenocarcinoma  Diffuse-type adenocarcinoma  Mucin producing-type adenocarcinoma  Invasive squamous cell-type carcinoma | 42/242 (17. 4)  18/42 (42. 85)  24/42 (57. 14)  24/42 (57. 14)  16/42 (38. 09)  1/42 (2. 38)  1/42 (2. 38) |

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| Gene and Primer | Sequences (5á¿½- 3á¿½) |
| cagH  CagH-F  CagH-R | 5á¿½-ATGGCAGGTACACAAGCTAT-3á¿½  5á¿½-TCACTTCACGATTATTTTAG-3á¿½ |
| cagL  CagL-15  CagL-16 | 5á¿½-AAAACACTCGTGAAAAATACCATATC-3á¿½  5á¿½-TCGCTTCAAAATTGGCTTTC-3á¿½ |
| cagG  CagG-F  CagG-R | 5á¿½-TTATAAAATTAAATTACTATTTGC-3á¿½  5á¿½-GTGGTAAAAACGATGAATCTG-3á¿½ |
| orf17  Orf17-F  Orf17-R | 5á¿½-CTTGATTGATGAAAATTTGGTTG-3á¿½  5á¿½-TTAGTGATATATTCATAATTTTCC-3á¿½ |