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GENETIC DETERMINANTS AND CLINICO-PATHOLOGICAL OUTCOMES OF *HELICOBACTER PYLORI* INFECTION

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SUMMARY

***Helicobacter pylori* is a spiral Gram-negative bacterium with a relatively small genome and is known to be the most common human bacterial infection worldwide, infecting about half of the world's population. The bacterium represents one of the most successful human pathogens, inducing severe clinical symptoms only in a small subset of individuals, thus signifying a highly balanced degree of co-evolution of *H. pylori* and humans. The prevalence of *Helicobacter pylori* infection varies greatly among countries and among population groups within the same country, but is falling in most developed countries. The clinical course of *H. pylori* infection is highly variable and is influenced by both microbial and host factors including genetic susceptibility while the pattern and distribution of inflammation correlate strongly with the risk of clinical sequelae, namely duodenal or gastric ulcers, mucosal atrophy, gastric carcinoma, or gastric lymphoma. Cytokine gene polymorphisms directly influence inter-individual variation in the magnitude of cytokine response, and this clearly contributes to an individual's ultimate clinical outcome. Polymorphisms in genes coding for innate immune factors have also been incriminated in the pathogenesis of *H. pylori* related disease, while promoter hypermethylation of tumor suppressor genes is considered an important factor in carcinogenesis and known to be present in *H. pylori* associated gastric tumors. Functional genomics may fill many of the gaps in our understanding of the pathogenesis of *H. pylori* infection and accelerate the development of novel therapies, including *H. pylori* specific antimicrobial agents.**

Keywords: *Helicobacter pylori,* Genetic Factors,Clinic-Pathological Outcomes

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a spiral Gram-negative bacterium with a relatively small genome (~1.65 Mb), and is known to be the most common human bacterial infection worldwide, infecting about half of the world's population. There is now much evidence that *H. pylori* and related *Helicobacter* species have been part of the normal microbiota of humans and our ancestors for millions, if not tens of millions, of years or longer.[1,2]

The prevalence of *Helicobacter pylori* infection varies greatly among countries and among population groups within the same country, but is falling in most developed countries[3]. In the last 30 years, since *H. pylori* was first described and cultured, a complete paradigm shift has occurred in the clinical approach to the diagnosis and treatment of upper gastro-duodenal disease. Peptic ulcer disease is now approached as an infectious disease, in which elimination of the causative agent cures the condition. The role of *H. pylori* infection in gastric cancers is increasingly recognized, and its role in other diseases of the upper gastrointestinal tract is being evaluated. Effective antimicrobial therapy is available, although there is still no ideal treatment, and indications for therapy continue to evolve.

Helicobacter pylori infection is usually acquired early in childhood but infection persists lifelong in the absence of treatment, and the majority (80%–90%) of those infected will carry and transmit *H. pylori* without any symptoms of disease. All strains cause gastric inflammation, however, only 15% of infections result in peptic ulceration and only 0.5%–2% in gastric adenocarcinoma.[4] In some subjects, *H. pylori i*s considered as a commensal and not a pathogen, making it difficult to determine who should be treated to prevent serious sequelae.[5] Many clinical and basic research studies have been undertaken to define what makes *H. pylori* a pathogen. The clinical course of *H. pylori* infection is highly variable and is influenced by both microbial and host factors including genetic susceptibility. The pattern and distribution of

inflammation correlate strongly with the risk of clinical sequelae, namely duodenal or gastric ulcers, mucosal atrophy, gastric carcinoma, or gastric lymphoma.

There has been recent interest in whether *H. pylori* may cause or be a risk factor for human diseases outside the upper gastrointestinal tract. These include idiopathic thrombocytopenic purpura, various skin diseases, liver diseases**,** cardiovascular and cerebrovascular disease.[6] This review highlights recent advances in the understanding of genetic determinants of *Helicobacter pylori* infection as well as the interplay of clinical and morphological patterns in disease manifestation.

Natural History of *Helicobacter Pylori* Infection

H. pylori is a gram-negative bacterium, measuring 2 to 4 μm in length and 0.5 to 1 μm in width. Although usually spiral-shaped, the bacterium can appear as a rod, while coccoid shapes appear after prolonged in vitro culture or antibiotic treatment. Colonization typically occurs during childhood and persists for the lifetime of the host in the absence of treatment, and such persistence within the context of ongoing gastric inflammation is the signature feature of *H. pylori* infection[8].

Whether childhood colonization causes symptoms or changes in gastric acidity is unknown, however, *H. pylori* persistence is central to pathogenesis. Primary acquisition in adults, or reinfection after successful eradication, does occur but is less common, with an annual incidence of 0.3-0.7% in developed countries and 6-14% in developing countries.[9] It has been suggested that the age when *H. pylori* is acquired is an important determinant of the ultimate risk for diseases, such as PUD and noncardia gastric cancer.[10] This hypothesis is also based on changes in the equilibria reached between microbial populations and hosts and is consistent with acquisition age outcome differences observed for other microbial agents (e.g., varicella, hepatitis B, and Epstein-Barr viruses).[11] In the rare cases where colonization first occurs in adults, it can cause a profound gastritis with hypochlohydria, epigastric discomfort, and nausea.[12,13] Ulcers occur mainly in mid- or late adulthood after many years of infection and inflammation, and gastric adenocarcinoma occurs in late adulthood after an even longer period of chronic inflammation and epithelial damage.

Transmission

The very large number of people colonized throughout the world suggest that *H. pylori* evolved a very robust strategy of transmission. Unlike many organisms that have free-living forms in environmental reservoirs and unlike those that can infect many different animal and plant species, natural *H. pylori* infection is restricted to humans and closely related primates. Epidemiologic studies of *H. pylori* transmission show that the majority of infections tend to occur within families through close person-to-person contact.[14]

Most *H. pylori* transmission occurs in childhood, and, in some countries, up to 90% of children become infected by age 10 years, with reports of infection as early as the first months of life.[15] Maternal-to-child and sibling-sibling transmission seem most likely because longitudinal studies have shown that the risk of acquiring the infection is highly correlated to the infection status of the mother[16] and siblings[17] and related to overcrowding conditions in the home.[18]

It seems likely that in industrialized countries direct transmission from person to person by vomitus, saliva, or feces predominates; additional transmission routes, such as water, may be important in developing countries.[19,20] There is currently no evidence for zoonotic transmission, although *H. pylori* is found in some non-human primates and occasionally in other animals[21,22]

Some investigators propose that *H. pylori* may exist in the environment in a dormant, spore-like state that can be viable but non-cultivable. This hypothesis partially comes from the observation that, under stress and nutrient deprivation, *H. pylori* undergoes a morphologic transformation from actively dividing and swimming spiral bacilli to inactive cocci. However, until now, no definitive experimental evidence exists that *H. pylori* can revert from a coccoid form into the infectious spiral bacilli. In contrast, there is ample experimental evidence that culturable bacilli are infectious in animal models, in volunteer studies,[23] and in reports of inadvertent transmission through contaminated endoscopes.[24]

It seems more likely that transmission occurs when *H. pylori* is present outside of the stomach in a culturable form. However, it is known that *H. pylori* is fragile outside of the human stomach because it is rapidly killed by higher oxygen tension and even by light.[25] The most likely modes of transmission seem to be situations in which gastric contents can be transferred quickly from person to person. Gastric-oral transmission is suggested in association with gastroenteritis with vomiting. Oral-oral transmission is also possible if *H. pylori* can survive for short periods after gastric contents have refluxed into the oral cavity. Fecal-oral transmission may be possible under conditions in which *H. pylori* survive transit through the lower gastrointestinal tract, which is uncommon in healthy people.[26–29] An aspect of transmission specific *H. pylori* factors that has received attention and debate

is whether the coccoid form of *H. pylori* is viable or simply a form of bacterial death. A recent report found that the *H. pylori* cell wall enzyme AmiA, a peptidoglycan hydrolase, is involved in the morphologic transition from spiral to coccoid, suggesting that the coccoid transition might be a regulated process rather than a degenerate form of *H. pylori*.[30] Interestingly, this study[30] showed that the peptidoglycan of the coccoid form is a poor activator of the innate immune response, and the authors suggest that the transition into coccoid may represent remodeling of the cell wall for the purpose of immune modulation. Nagai *et al* recently observed that the coccoid form of *H. pylori* is phagocytosed by dendritic cells in Peyer's patches.[31] They proposed that *H. pylori* converts to the coccoid form in the anaerobic small intestine and stimulates the host immune system through Peyer's patches. Although the speculation that the coccoid form of *H. pylori* may act to protect the rest of the population from immune attack remains to be tested, the notion that *H. pylori* exists in many forms that are dynamic and adapt to changing conditions in the gastric mucosa is a recurrent theme in *H. pylori* biology.[14]

Host Genetic Factors in *H. pylori*-Induced Disease

The interactions between *H. pylori* virulence determinants and host epithelial cells induce genetic, epigenetic and chromosomal alterations in the host genetic material. It results in a continuous patching of the genetic information of the host cells, which favours the development of gastric carcinoma.

Cytokine gene polymorphisms directly influence inter-individual variation in the magnitude of cytokine response, and this clearly contributes to an individual's ultimate clinical outcome (Table 1). Specific polymorphisms in the *IL-1B* gene and the IL-1 receptor-antagonist gene (*IL-1RN*) lead to increased gastric mucosal levels of IL-1â in individuals infected with *H. pylori*[32] and increased levels of inflammation.[33] The polymorphisms also increase the risk of gastric atrophy, achlorhydria, intestinal metaplasia,[34] and distal gastric adenocarcinoma.[35] Individuals with the *IL-1B*-31*C or -511*T and *IL-1RN**2/*2 genotypes have been found to be at increased risk of developing hypochlorhydria and gastric atrophy in response to *H. pylori* infection as well as a 2- to 3-fold increased risk of malignancy compared with subjects who have the less pro-inflammatory genotypes**.[36,37] IL-1β is also the most powerful acid inhibitor known.[38] The effect of these polymorphisms on duodenal ulcer risk is uncertain; one report showed an increased risk with pro-inflammatory polymorphisms,[39] but another showed a reduced risk.[34] Furthermore, the pro-inflammatory *IL-1* genotypes increased the risk of both intestinal and diffuse types of gastric cancer, but the risk was restricted to the non-cardia subsite.

Figueiredo *et al*[40] reported on the combined effects of pro-inflammatory *IL-1* genotypes and *H. pylori* bacterial virulence factors (*cagA* positive, *VacA s1*, and *VacA m1*). They showed that, for each combination of bacterial/host genotype, the odds of having gastric carcinoma were greatest in those with both bacterial and host high-risk genotypes, having up to fifty-fold increased risk of gastric cancer as compared with individuals lacking these factors. This highlights the important interaction between host and bacterium in the pathogenesis of gastric cancer. Although polymorphisms affecting IL-1β levels are best studied, others also appear important.

Tumor necrosis factor α (*TNFA*) polymorphisms have not been demonstrated to affect gastric mucosal levels of TNFα, but they have been shown to affect gastric inflammation and cancer risk in some[39] but not all studies (Table 1).[41] Carriage of the pro-inflammatory A allele of *TNF-A*-308 G→A polymorphism increased the odds ratio for non-cardia gastric cancer to 2.2 (95% CI: 1.4 –3.7). IL-10 is an anti-inflammatory cytokine with opposing actions to IL-1β, TNFα, interferon-γ, and other pro-inflammatory cytokines. Relative deficiency of IL-10 may result in a Th-1-driven hyper-inflammatory response to *H. pylori* with greater damage to the gastric mucosa. Homozygosity for the low-IL-10 *A-T-A* haplotype (based on 3 promoter polymorphisms at positions -592, -819, and -1082) increased the risk of non-cardia gastric cancer with an odds ratio of 2.5 (95% CI: 1.1–5.7)[37]. Another important cytokine that plays a central role in the pathogenesis of *H. pylori*-induced diseases is IL-8. This chemokine belongs to the CXC family and is a potent chemoattractant for neutrophils and lymphocytes. It also has effects on cell proliferation, migration, and tumor angiogenesis. The gene has a well-established promoter polymorphism at position -251 (*IL-8*-251 T→A). The A allele is associated with increased production of IL-8 in *H. pylori*-infected gastric mucosa. It was also found to increase the risk of severe inflammation and precancerous gastric abnormalities in white[42] and Asian populations.[43]

Polymorphisms in genes coding for innate immune factors have also been incriminated in the pathogenesis of *H. pylori* related disease. An example is the functional polymorphism at position -896 in exon 4 of the Toll-like receptor-4 (*TLR4)* gene. TLR4, the lipopolysaccharide (LPS) receptor, was initially identified as the potential signaling receptor for *H. pylori* on gastric epithelial cells. ***Amieva & El-Omar[14] proposed that

subjects with an overall pro-inflammatory genetic makeup based on a combination of markers from the adaptive and innate immune systems (eg, IL-1, TNFα, IL-10, IL-8, TLR4, mannose-binding lectin170) respond to *H. pylori* infection by creating an environment within the stomach that is chronically inflamed and with reduced acidity.

Several studies have also examined the role of HLA class I and II alleles in gastric cancer, in white and non-white populations. Lee *et al* found that the HLA class II allele *DQB1*0301* was more common in white patients with gastric adenocarcinoma than non-cancer controls.[44]

Epigenetic changes: Promoter hypermethylation of tumor suppressor genes is considered an important factor in carcinogenesis and known to be present in *H. pylori* associated gastric tumors.

Shin *et al* [45] showed that this aberrant methylation is already present in the premalignant stages of gastric cancer. Work by Schneider *et al*[46] showed that the degree of aberrant methylation is associated with the presence/absence of known virulence factors in the infecting *H. pylori* strain. Of particular interest are the results regarding *LOX, HAND1, THBD, p41ARC, and APC* promoter methylation, which were described by Shin *et al*.[45] In active *H. pylori* infection and intestinal metaplasia, there was hypermethylation involving the *LOX, HAND1, THBD, and APC* genes. And this hypermethylation state persisted in *LOX, HAND1, THBD, p41ARC* in cases with past *H pylori* infection. Among the epigenetic alterations following *H. pylori* infection, deregulation of microRNAs (miRs) expression might also be relevant for pathogenesis. miRs are non-coding small RNAs which control mRNA translation and they frequently are deregulated in human cancers. Ando *et al*[47] studied the methylation status of a series of miRs in a series of gastric cancer cell lines, in primary gastric cancers, and in gastric mucosa from patients with or without *H. pylori* infection, and provided evidence that *H. pylori* infection is associated with higher methylation of miR-124. In vitro experiments with overexpression or silencing of miR-218 allowed Gao *et al*[48] to demonstrate that miR-218 induces apoptosis and decreases cell proliferation by promoting ECOP (epidermal growth factor receptor coamplified and overexpressed protein) degradation, which decreases NF-kB activation. Interference with these miR methylations might provide novel options for fighting gastric cancer development in *H. pylori*-infected patients.

Table 1: Genetic polymorphisms and *H. pylori infection*

Polymorphism site	Effect	Association with *H. pylori* infection
IL-1B gene cluster	IL-1B*-511T, IL-1B*-31C, & IL-1RN*2/*2 result in higher IL-1B expression	Pro-inflammatory response, Hypochlorhydria, pan-gastritis, ↑atrophic gastritis & gastric cancer
IL-8 (-251A/T) *IL-10* (ATA/GCC haplotypes)	↑ expression of IL-8 GCC haplotype results in ↑ expression of IL-10	↑ risk of gastric cancer & peptic ulcer GCC assoc. with Colonization by more-virulent *strains (cagA, vacA s1, and babA2); ATA* ↑ risk of gastric cancer
*TNF-A**-308A allele	↑TNF- expression and ↑ gastrin expression	higher levels of *H. pylori infection* & ↑ risk of gastric cancer

The Clinical Outcomes of Infection

The clinical course of *H. pylori* infection is highly variable and is influenced by both microbial and host factors. The basic process that mediates *H. pylori*-induced damage is gastritis with its associated humoral and cell mediated immune mechanisms. The pattern and distribution of gastritis correlate strongly with the risk of clinical sequelae, namely duodenal or gastric ulcers, mucosal atrophy, gastric carcinoma, or primary gastric lymphoma (Figure 1).

In 2008, Amieva & El-Omar [14] proposed that three main gastric phenotypes have been identified, and each is associated with a set of pathophysiologic abnormalities that could explain why a certain outcome occurs. The commonest phenotype termed the *"simple or benign gastritis"* phenotype, is characterized by mild pan-gastritis with little disruption of gastric acid secretion. This phenotype is commonly seen in subjects who are asymptomatic and who on the whole develop no serious gastrointestinal (GI) disease. The second phenotype is the so-called *duodenal ulcer* (DU) phenotype

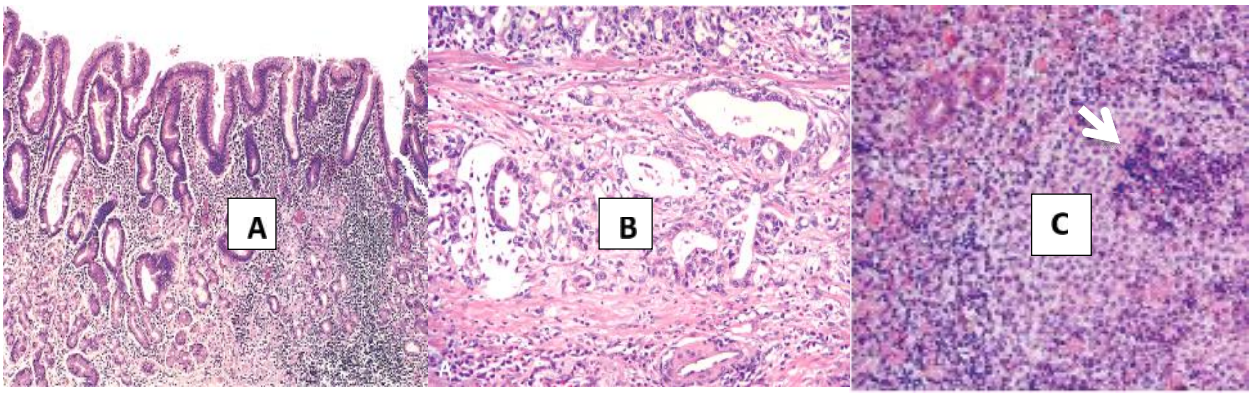

Fig. 1: (A) Chronic gastritis with florid mucosal infiltrates of chronic inflammatory cells, **(B)** Gastric adenocarcinoma-intestinal type, **(C)** Gastric MALT lymphoma with characteristic lymphoepithelial lesions (arrow).

and accounts for 10- 15% of infected subjects, particularly in Western countries where peptic ulcers were common. This phenotype is characterized by an antral-predominant pattern of gastritis with relative sparing of the acid producing corpus mucosa. Subjects with this phenotype have high antral inflammatory scores, high gastrin, relatively healthy corpus mucosa, and very high acid output.[49] *H.pylori*–induced hyper-gastrinemia leads to an increased maximal acid output, which indicates an increased parietal cell mass.These subjects also have defective inhibitory control of gastric acid secretion. Gastric hyperchlorhydria leads to an increased acid load in the duodenum which subsequently develops protective gastric metaplasia. *H. pylori* colonizes this gastric metaplastic tissue, and the resulting local inflammation and damage further predispose to DU. The epidemiology of pre-pyloric and pyloric ulceration is similar to that of DU, but that of *H. pylori*–induced ulceration elsewhere in the stomach is more similar to that of gastric adenocarcinoma. The third and most serious phenotype is the "gastric cancer phenotype," which is characterized by a pan-gastritis or a corpus-predominant pattern of gastritis, multifocal gastric atrophy, and hypo- or achlorhydria despite hyper gastrinaemia.[50] These abnormalities, which affect approximately 1% of infected subjects, develop as a direct result of the chronic inflammation induced by the infection and increase the risk of gastric cancer.[51, 52] However, *H. pylori* treatment increases acid production rapidly in some people with pan-gastritis and hypochlorhydria (although not usually to normal levels) [53]. This result implies a direct suppressive effect of *H. pylori*–induced inflammatory mediators or products such as IL-1β, TNFα, and, in a recent report, the Th-1 cytokines IFN-γ and IL-12[54] which all suppress acid production. The gastric cancer phenotype is particularly prevalent in certain parts of Asia, where this cancer is common.[55] Physiologically, the phenotype is characterized by low acid secretion, high gastrin, and low pepsinogen I and pepsinogen I/II ratio. Carcinogenesis in the environment of gastric atrophy is likely owing to reactive oxygen and nitrogen species secondary to the accompanying inflammation, and possibly partly caused by overgrowth of other bacteria in the now achlorhydric stomach. Other mechanisms of carcinogenesis have also been either demonstrated or implied in the atrophic stomach. Parietal cells express the Sonic hedgehog protein, which is the main organizer of cell polarity in gastric pits. Sonic hedgehog expression is lost in atrophy and intestinal metaplasia.[56] The resultant disruption of parietal cell mass potentially exposes stem cells to mutagenic agents.

Recent reports suggest that *H. pylori* may survive and express virulence factors intracellularly in metaplastic, dysplastic, and neoplastic epithelial cells in vivo, although these reports need further confirmation. However, several potential mechanisms exist for the proposed direct oncogenic effects of *H. pylori*. CagA signaling is strongly pro-proliferative. Also the pro-apoptotic effects of *H. pylori* exhibited through NFχB activation, reduction in p27 levels, and down-regulation of the TNF-related apoptosis-inducing ligand (TRAIL) system contribute to the development of atrophic gastritis a precancerous change. Other direct effects of *H. pylori* on gastric epithelial cells that have potential carcinogenic importance are its stimulation of reactive oxygen species (ROS) and oxidative damage and its impairment of DNA mismatch repair.

The most intriguing aspect of this story is that subjects who develop duodenal ulcers are actually protected from developing gastric cancer, suggesting that the 2 outcomes are mutually exclusive.

Gastric Ulceration (GU), unlike DU, is associated with a pan-gastritic inflammation pattern and reduced or normal acid production. GU's arise most commonly at the transitional zone between antrum

and corpus on the lesser gastric curve. It is likely that the heavy colonization and consequent marked inflammation and epithelial damage at this site lead directly to ulceration. Why *H. pylori*–induced inflammation has a pan-gastritic or corpus-predominant pattern in some people when it is antral-predominant in others is unknown. One possibility is that, like autoimmune gastritis, it may be caused by immune effectors with specificity for the gastric proton pump ATPase.[4]

Primary Gastric Lymphoma

The acquisition of Mucosa-associated lymphoid tissue (MALT), depends upon infection with helicobacter species, usually *H. pylori*. Low-grade MALT lymphomas do usually arise in the context of a pan-gastritic inflammation pattern. In vitro, proliferation of clonal B cells is driven by the presence of non-neoplastic T cells, whose effects in turn are dependent upon the presence of *H. pylori*. *H. pylori* also appears to act through direct antigen stimulation of tumor cells. In humans, eradication of *H. pylori* frequently leads to regression of these low-grade lymphomas.[57, 58] Maltomas are usually very slowly progressive, but they sometimes undergo high-grade transformation to diffuse large-cell B cell lymphomas. They have mutations consistent with oxidative damage and are more commonly associated with *cag*+ *H. pylori* strain infection and a more inflamed stomach. High-grade lymphomas rarely regress following *H. pylori* treatment. Recently, a subgroup of low-grade B cell MALT lymphomas (approximately 25%) have been found to have a translocation from chromosome 11 to 18[59] This causes a specific fusion between the activator protein-12 (*AP-12*) and *MALT-1* genes that creates a new gene[60] whose product stimulates NFêB signaling[61]. This is known to be anti-apoptotic and so promotes cell survival. T(11;18)+ lymphomas are relatively locally invasive but rarely undergo high-grade transformation; most importantly, they are unresponsive to *H.pylori* treatment.

Gastro-esophageal Reflux and its Complications

Epidemiological observations support a link between *H. pylori* and protection against severe GERD and attendant complications- Barrett's esophagus (intestinal metaplasia in the esophagus), and esophageal adenocarcinoma. If the negative association between *H. pylori* and GERD complications is causal, the most likely mechanism is through effects of *H. pylori* on gastric acid secretion. Although some *H. pylori*–infected individuals have increased acid secretion, a reduction is more common, and thus, at a population level, absence of *H. pylori* should lead to higher mean acid secretion. A case control study nested in a cohort established in 1964 has shown that *H. pylori*–positive subjects are less likely than *H. pylori*–negative subjects to develop esophageal adenocarcinoma (odds ratio 0.37)[62]

***H. pylori* and extragastric diseases.**

H. pylori has also been implicated in the pathogenesis of many extra-gastric diseases, ranging from atherosclerosis**,** ischemic heart disease (IHD), liver diseases, skin diseases, blood disorders, and others, but documentation is poor and the associations are controversial. The most convincing data arise in the field of idiopathic thrombocytopenic purpura (ITP) and sideropenic anemia. Long-term follow-up studies have shown that 50% of subjects with ITP maintain a hematological response after *H. pylori* eradication.[63]

CONCLUSION

H. pylori represents one of the most successful human pathogens, inducing severe clinical symptoms only in a small subset of individuals, thus signifying a highly balanced degree of co-evolution of *H. pylori* and humans. Three main gastric phenotypes have been identified as possible outcomes of this co-habitation namely: simple gastritis; duodenal ulcer and the gastric cancer phenotypes.

H. pylori has also been implicated in the pathogenesis of many extragastric diseases which, although interesting and potentially important, their causality are yet unproven.

Cytokine gene polymorphisms directly influence inter-individual variation in the magnitude of cytokine response, and this clearly contributes to an individual's ultimate clinical outcome

Functional genomics may fill many of the gaps in our understanding of the pathogenesis of *H. pylori* infection and accelerate the development of novel therapies, including *H. pylori*–specific antimicrobial agents.

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