Overview on Gastric Cancer

Chapter 6

Synergistic Effect of *Helicobacter pylori*, Epstein-Barr virus and Host susceptibility for the development of gastric cancer

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Abstract

Recently, advancement in surgical techniques and preoperative care conditions have had positive effects on the clinical course of gastric cancer. However, gastric cancer still constitutes a significant public health problem because of its high prevalence, poor prognosis. Therefore, gastric cancer screening strategy has received widespread attention because of its significantly increased cancer detection rate. In some developed countries such as Japan, due to the well-established strategy for gastric cancer prevention screening, most of the new cases are now diagnosed at early stage and the patient's prognosis is extremely good with more than 90% could survive for 5 years or more. Globally *Helicobacter pylori (H. pylori)* has been classified as a Class I carcinogen and the major cause of gastric cancer. *H. pylori*-specific genetic diversity has been proposed to

play an important role in determining gastric cancer risk. Additionally, with acceptance of *H. pylori* as a causative agent of gastric cancer, Epstain-Barr virus (EBV) has also been regarded to be a gastric cancer causing infective agent. Furthermore, host factors have been identified that influence the propensity toward gastric cancer development. Many studies recently indicated that it is better to discuss the synergistic effect of these factors with each for the gastric cancer development than to discuss which of these factors is the most virulent. Since gastric cancer is a multifactorial disease, and both infectious agents and host factors have an essential role in its etiology, thus early identification of these factors will positively impact gastric cancer screening strategy. This article is conducted to clarify risk of gastric cancer associated with *H. pylori* genetic diversity, EBV and host polymorphisms (IL-1β) and pepsinogen expression.

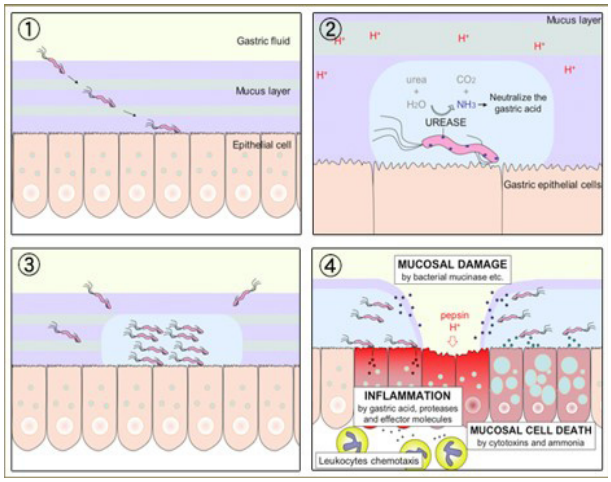
Abbreviations:*H. pylori: Helicobacter pylori*; GC: gastric cancer; PUD: peptic ulcer disease; CG: chronic gastritis; MALT: mucosa-associated lymphoid tissue; *cagA* gene: cytotoxin-associated gene A; *cag* PAI: *cag* pathogenicity island; PAI: pathogenicity island; *vacA:* vacuolating cytotoxin A; EBV: Epstein-Barr virus; EBVaGC, EBV: associated gastric cancer; IL-1: interleukin 1; IL-8: interleukin-8; IL-10: interleukin 10, IL-17: interleukin-17

1. *Helicobacter Pylori* with Gastric Disease

1.1. *Helicobacter pylori* as a causative agent of gastric cancer

Gastric cancer (GC) is the fourth most common cancer in the world and the third most common cancer in Asia (GLOBOCAN 2012). In Vietnam, gastric cancer remains the fourth most common type of cancer; the third leading cause of cancer-related death in both genders (globocan.iarc.fr). Thus, gastric cancer screening strategy has received widespread attention because of its significantly increased cancer detection rate [1-3]. Preventative measures for gastric cancer have been conducted with the focus on H. pylori and this has succeeded in decreasing the mortality.

H. pylori is gram-negative bacterium, colonizes the stomach of half of the global human population [4]. *H. pylori* secretes urease, which converts the chemical urea to ammonia. The production of ammonia around *H. pylori* neutralizes stomach acid in the vicinity of the organism, favoring bacterial multiplication. The ammonia may also both cause injury and potentiate the effects of a cytotoxin produced by *H. pylori* [5]. Colonization of the stomach by *H. pylori* can result in variety of upper gastrointestinal disorders, such as chronic gastritis (CG), peptic ulcer disease (PUD), gastric mucosa-associated lymphoid tissue (MALT) and gastric cancer (GC) [4-15].



Figue 1: Diagram of *H. pylori* infection

1) *H. pylori* invading mucous layer.
2) *H. pylori* neutralizing surroundings using the enzymic activity of urease.
3) *H. pylori* colonizing mucous layer.
4) *H. pylori* causing inflammation, mucosal degredation, and cell death

1.2. The association of H. pylori genetic diversity and gastric cancer

H. pylori has emerged as the most important causal factor for gastric cancer. However, *H. pylori* infection only is insufficient to cause gastric cancer [16]. Indeed, *H. pylori* infection is common in all most Asian countries but gastric cancer incidence is significantly different between countries, being extremely higher in some countries such as Mongolia, South Korea, Japan, moderate in some countries such as Vietnam and being low in some countries like Thailand, Cambodia... This has led to the hypothesis that not all *H. pylori* strains are equal in virulence; some strains might be more virulent and better adapted to causing gastric cancer than others. Therefore, in order to evaluate *H. pylori* pathogenic, the emphasis is now shifting towards determining virulence factors.

H. pylori exhibits a high level of interspecies genetic diversity and many studies have endeavored to identify strain-specific features of *H. pylori* that are linked to development of gastric cancer. One of the most prominent differences among *H. pylori* strains is the presence or absence of *cag* Pathogenicity Island (*cag* PAI). Current evidence suggests that the risk of gastric cancer is very low among persons harboring *H. pylori* strains that lack the *cag* PAI. Among persons harboring strains that contain the *cag* PAI, the risk of gastric cancer is shaped by the diversity of *cag* PAI or a complex interplay among multiple strain-specific bacterial factors such as *cagA, vacA* genotypes. Numerous studies have been reported regarding the correlation of putative virulence factors such as *cagA* to gastric cancer development [17-19].

The *cagA* gene (cytotoxin-associated gene A) has been classified into Western-type *cagA* and East-Asian-type *cagA* based on the sequences of repeat regions of the *cagA* containing Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs [20,21]. Individuals infected with East-Asian-type *cagA H.*

pylori have been reported to have an increased risk of peptic ulcer disease (PUD) and/or gastric cancer compared to those infected with Western-type *cagA* strains [22-24]. However, most strains isolated from East Asian countries are positive for East Asian *cagA*, thus the presence of *cagA* is insufficient to predict the risk of gastric cacer, consequently, the prevalence of East Asian *cagA* is insufficient to explain the difference in gastric cancer incidence between East Asian countries. Our previous studies also indicated that there was no significant difference in *cagA* prevalence between peptic ulcer (PU) and chronic gastritis (CG) in Vietnam [25].

The *cagA* gene was found to be part of a pathogenicity island (PAI), a horizontally transferred 40-kb gene fragment containing 27 genes. Although the *cagA* gene has served as a marker for the PAI, the presence of this single gene does not necessarily indicate the presence of a complete set of cag PAI genes. Upon contact with host cells, *H. pylori* induces a signaling cascade involving Ca2+-calmodulin and extracellular signal-regulated kinase (ERK) that leads to the activation of the transcriptional regulator NF-κB, which activates IL-8 production [26]. Several of the genes but not *cagA* within the cag PAI, have been shown to be required for the stimulation of IL-8 production in host epithelial cell lines. Furthermore, genetic diversity within the *cag* PAI has been determined to involve in the development of atrophic gastritis and may increase the risk for gastric cancer [24,27-29]. *cagA* show considerable genetic diversity, but the diversity of the *cag* PAI, which transports the bacterial oncogene *cagA* into host cells, has not been systematically investigated. Comparative analysis of the nucleotide sequences and functional diversity of the *cag* PAI of the *H. pylori* strains isolated from patients presenting with the different clinical situations provides an important resource that can guide future research on the biological roles and host interactions of *cag* PAI proteins, including several whose function is still unknown.

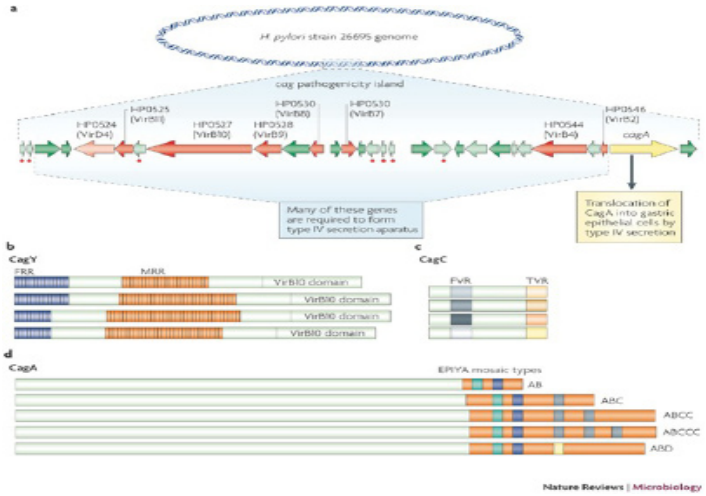


Figure 2: The *cag* pathogenicity island contains genes that show marked sequence variation [29]

A | Arrangement of cag PAI genes in H. pylori strain 26695. Most of the cag genes are probably involved in the assembly of the type IV secretion system that translocates the protein CagA into the cytoplasm of gastric epithelial cells. Seven genes (marked in red) show similarity to components of the type IV secretion system of the plant pathogen Agrobacterium

tumefaciens. Proteins encoded by the island are involved in two major processes, the induction of interleukin-8 (IL-8) production by gastric epithelial cells and the translocation of CagA from the bacterium into host cells. All genes depicted by arrows in dark shades of red and green are essential for IL-8 induction, whereas lighter shades of red and green indicate genes that are not involved in this process. The arrows marked with a red dot indicate genes that are not required for translocation of CagA, the non-marked genes are essential for translocation.

b–d | Exposure of cag proteins to the host presumably places them under strong positive selection in vivo. Extensive sequence variation, possibly linked to host adaptation, has so far been documented for three cag PAI-encoded proteins, CagY (HP0527)

b), A protein that probably forms a sheath covering the type IV pilus, CagC (HP0546)

c), The putative cag pilin, and the translocated effector CagA (HP0547)

d). CagA shows striking ethnic and individual variation in its C-terminal repetitive phosphorylation (EPIYA) motifs; the upper four combinations of EPIYA types depicted are characteristic for Western strains, and the lower combination (ABD), including the unique Asian D-type EPIYA motif, is associated with East Asian strains. FRR, 5'-repeat region; FVR, 5'-variable region; MRR, middle repeat region; TVR, 3'-variable region [29]

Vacuolating cytotoxin A (*vacA*) is another extensively studied *H. pylori* virulence factor [30-33]. As an intracellular-acting protein exotoxin, *vacA* affects multiple cellular pathways in different host cell types, induces host cell vacuolation and finally cell death. Furthermore, specific *vacA* genotype shave been reported to be useful for predicting different clinical outcomes [34-36]. Individuals infected with *vacA* s1, i1 or m1 *H. pylori* strains have an increased risk of peptic ulcer disease and/or gastric cancer compared to those with s2, i1 or m2 strains. The prevalence of *vacA* genotypes contribute to incidence differences between countries and also between regions in a country. Our previous study showed that the prevalence of strains with the *vacA* m1 type was predominant in Hanoi (northern region), but not in Ho Chi Minh (southern region) (58% vs 36.2%, $p<0.05$) [25,37]. We suggested that *vacA* m1 type might contribute to the difference in the incidence of gastric cancer between Hanoi city and Ho Chi Minh city; the incidence is approximately 1.5 times higher in Hanoi. Recently, two additional regions of variation were found in *vacA*: the deletion (d)-region, located between the i- and the m-region exhibiting either d1 genotype without the 69 to 81 base pair (bp) deletion or d2 genotype with the deletion; and c-region [38,39]. The last includes a deletion of 15bp located at the 3'-end region sequences of the *vacA* and divided into c1 (with deletion) and c1 (without deletion). Even though the knowledge of the structure function relationships of the *vacA* d1 region have been limited, the presence of the *vacA* d1 strains have been proposed as new determinant of gastric cancer and potential for atrophy rather than the s-, m-, and i-region [38,40].

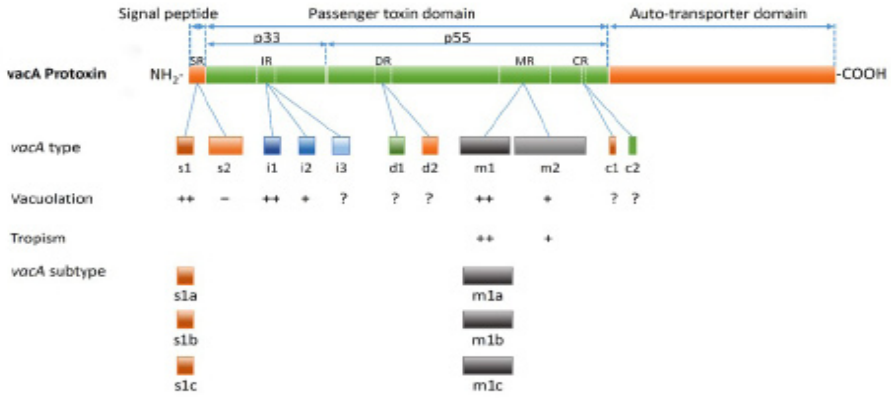


Figure 3: Diversity of vacA genes [39]

Sequence diversity regions of the vacA closely associated with vacuolating activity of H. pylori and clinical outcomes are localized to the signal region (SR); the intermediate region (IR) on p33 domain; the d-region (DR), middle region (MR) and c-region (CR) on p55 domain. The different types of these regions are associated with differences in vacuolation, specificity and clinical outcome. The s1, m1, i1 type have been classified as fully active vacA and are associated with a higher risk of development of gastric cancer than the s2, m2, or i2. In contrast to the s1 type, the s2 forms of vacA consistently lack detectable vacrolation activity in most in vitro assays. In comparison to the m1/i1 types , the m2/i2 types are considerably less active and virtually nontoxic. The function of the i3 remains undefined. The d-region has been considered to be related with vacA binding to the host gastric cells and vacuolating activity, however, compelling evidence to support this is still lacking. The function of the c-region remains a mystery; however, the c1 genotype has been strongly associated with the risk of gastric cancer. The s1 and m1 genotype have been further classified into three subtypes s1a, s1b, s1c and m1a, m1b, m1c, respectively

In addition, whole-genome sequencing allows further analysis the genetic differences between gastric cancer strains and non-gastric cancer strains. The genome comparison of strains isolated from gastric cancer and no gastric cancer (ulcer gastric, chronic gastritis) cases provides comprehensive insight of contribution between gastric cancer and bacterial genetic diversity.

2. The association between Epstain-Barr virus and gastric cancer

Epstein-Barr virus (EBV), also known as human herpes virus 4, is a gamma-herpes virus that consists of double-stranded DNA of ~170 kb in length. It is one of the most common human herpes viruses and infects > 90% of the world's population by adulthood and establishes lifelong, latent infections.

EBV was the first virus to be associated with human malignancy, which was discovered from a Burkitt's lymphoma cell line in 1964 [41]. EBV has been associated with a variety of lymphoid and epithelial malignancies, such as Hodgkin's disease [42], nasopharyngeal carcinoma (NPC) [43], T-cell lymphomas [44], AIDS-related lymphoma [45] and lymphoepithelioma-

like carcinomas (LELC) of several organs including salivary glands, thymus and lung [46].

In 1990, Burke *et al* [47], first reported the association between EBV and gastric carcinoma with characteristic lymphoepithelioma-like histology based on polymerase chain reaction (PCR) techniques. Subsequent development of *in situ* hybridization (ISH) techniques to detect EBV-encoded smal RNAs (EBERs) facilitated the detection of EBV in cancer tissues [48],[49]. Among EBV-associated neoplasms, EBV - associated gastric carcinoma (EBVaGC) is most common and distributed worldwide, while Burkitt's lymphoma and nasopharyngeal carcinoma are endemic to equatorial Africa and southeast China, respectively.

The frequency of EBV infection in gastric carcinoma ranges from 2 to 20%, with a worldwide average of nearly 10%. EBV associated gastric cancer varies in different countries, for example 19.5 % in German [50], 13% in Colombia [51], 12% in United States [52], 11.3% in Brazil [53], 10.2% among Japanese Americans in Hawaii [54], 9.0% in Iran [55], 8.5% in France [56], 7.3 % in Mexico [57], 6.4 % in China [58], and 5.6% in Korea [59].

These differences in reported frequencies may be because of geographical and environmental factors, although this remains controversial. In a meta-analysis done by Murphy *et al* [60], the pooled estimates of EBV-associated gastric cancer (EBVaGC) frequency in American, European and Asian were 9.9, 9.2 and 8.3%, respectively, with an overall frequency of 8.7%. A recent meta-analysis done by Camargo *et al* [61] revealed a similar overall frequency (8.2%), although the frequencies they found were slightly higher in American (12.5%) and European (13.9%) cases and lower in Asian cases (7.5%). Yanagi *et al* [62], screened for EBV infection in 1067 gastric cancer lesions of 1132 patients who underwent surgical resection from 2007 to 2017 in Japan and examined clinicopathological features of EBVaGC. Research results indicate that EBV was infected in 80 gastric cancer lesions (7.1%). Based on the annual incidence of gastric carcinoma (934.000 cases per year), nearly 70.000-80.000 people per year are estimated to develop EBVaGC [63].

Several constant clinical pathological features were seen in EBV associated gastric cancer such as moderately to poorly differentiated type of gastric cancer [64],[65] and predisposition to upper stomach [66],[67].

By endoscopy, EBVaGC appears as superficial depressed (or ulcerated) lesions in the upper part of the stomach. Tumor locates predominantly in the non-antrum part of the stomach [68]. EBV-associated gastric cancer often takes the form of an ulcerated or saucer-like tumor accompanied by marked thickening of the gastric wall. These features are well discernible on endoscopic ultrasonography and computed tomography scans of the stomach [69].

Because gastric cancer related to *H. pylori* a causative agent of chronic gastritis, intestinal metaplasia, and cancer, locates predominantly in the antrum, these pathogens have

been thought to cause gastric cancer by independent mechanisms [68]. Gastritis related to *H. pylori* frequently starts in the antrum. However, Yanai *et al.* reported that EBVaGC are frequently located near the mucosal atrophic border, where mild to moderate chronic atrophic gastritis is common [70]. They also showed frequent detection of both EBV and *H. pylori* the mucosa with moderate chronic atrophic gastritis, where inflammatory cell infiltration is abundant, and not at the mucosa with marked chronic atrophic gastritis, where inflammatory cell infiltration is scarce [71].

To be oncogenic, after pervading to host cell, EBV must maintain its genome inside its own to avoid from recognition of the immune system. Atrophic gastritis has been believed to facilitate the infiltration of EBV-carrying lymphocytes and increase the chance contacting with the gastric epithelial cells of EBV. Additionally, atrophy leads to hypochlorhydria, which is permits overgrowth of more pH-sensitive competing bacteria following produces a cytokine-rich microenvironment to support clonal growth of EBV infected epithelial cells. On the other hand, atrophic gastritis is well known as the morphological phenotype of *H. pylori* gastritis. Moreover, both *H. pylori* and EBV present in the gut, each of them have been associated to gastric cancer, so the interaction between these pathogens might enhance the risk of gastric cancer development. Based on these evidences, detection of EBV in gastric cancer cases, especially in *H. pylori* related gastric cancer cases has a positive impact on gastric cancer prognosis. [71]

3. Host factors and gastric cancer susceptibility

Even though the mortality of gastric cancer has shown a decreasing trend in Western countries, it still remains high in such Eastern countries as Mongolia, Korea, Japan and China. Despite an overall decrease in gastric cancer incidence in recent years, this disease is still responsible for over 700000 deaths per year [72],[73], and represents a significant medical burden in many countries. Carcinogenesis of gastric is caused by various risk factors, including genetic predisposition, environment, and microbial infections.

The Gram negative bacterium, *Helicobacter pylori (H. pylori)*, has been classified as the definite etiological factor for gastric adenocarcinoma [74]. However, of infected patients only 15-20% and <1% will develop ulcers (gastric or duodenal) or gastric adenocarcinoma, respectively [75]. It is believed that bacterial and host factors such as the *H. pylori* strain virulence, environmental factors and genetic predisposition are all responsible for the different pathological outcomes [76]. Therefore, some genetic factors may contribute to the development of gastric cancer. Many single-nucleotide polymorphisms (SNPs) have been implicated in gastric carcinogenesis [77],[78]. Here are some of Interleukin studied and related to gastric cancer

3.1. Interleukin 1 Family

The interleukin (IL)-1 gene cluster on chromosome 2q contains three related genes within a 430 kb region, IL-1A, IL-1B, and IL-1RN, which encode the pro-inflammatory cytokines IL-1α and IL-1β, as well as the endogenous anti-inflammatory cytokine IL-1ra, respectively [79].

IL-1β is a proinflammatory cytokine induced by *H. pylori* infection and is a powerful inhibitor of gastric acid secretion. Its effects promote hypochlorhydria, favoring further colonization of *H. pylori* and a more severe gastritis. IL-1β, upregulated in the gastric mucosa infected with *H. pylori*, plays a crucial role in initiating and amplifying the inflammatory response to *H. pylori* infection and is simultaneously a potent inhibitor of gastric acid secretion [80],[81]. With espect to IL-1ra, it competitively binds IL-1β receptors, thus modulating the presumptively deleterious effects of IL-1β.

Three biallelic single nucleotide polymorphisms (SNP) of the IL-1B gene at positions -511, -31, and +3954 base pairs (bp) from the transcriptional start site have been most commonly described for potential association with gastric cancer: both C-T base transitions at positions -511 and +3954, and a T-C base transition at position -31 [81]. The SNP at -31 and -511 are in near-complete linkage disequilibrium [82]. The IL-RN gene has a variable number of tandem repeats (VNTR) of 86 bp polymorphism in intron2, generating a short allele with two repeats (IL-1RN*2) and long alleles with three to six repeats (IL-1RN), respectively [83].

Numerous studies have found associations between *IL1B* polymorphisms and gastric cancer in populations of European and African origin: *IL1B*-31, *IL1B*-511 and *IL1RN**2* were independently associated with hypochlorhydria and increased frequency of atrophic gastritis, intestinal metaplasia and gastric cancer in *H. pylori* infected Scottish, Polish and German patients [80],[84].

While an increased risk of *H. pylori* associated gastric cancer has been noted in American Caucasians [84] with the *IL1B* –511 or IL1RN*2 polymorphism, a study of African American and Caucasian patients in the USA found that the IL1B +3954 polymorphism, but not *IL1B* –31, *IL1B* –511 and IL1RN*2 polymorphisms, was associated with increased risk of *H. pylori* dependent multi atrophic gastritis [85].

Mexicans with the *IL1B* –31 polymorphism alone or in combination with IL1RN*2 appear to have an increased risk of *H. pylori* associated gastric cancer [86], while no association between *IL1B* polymorphisms and gastric cancer was found in Spanish Caucasian patients [87].

Two studies investigated *IL1B* polymorphisms in the Portuguese, who have a high

incidence of *H. pylori* infection and gastric cancer. One found that *IL1B* –511 and *IL1RN*2* polymorphisms were independently associated with an increased risk of gastric cancer, with a substantial increase in gastric cancer risk in individuals carrying both polymorphisms [88]. A second found an increased risk of gastric cancer in patients with *IL1B* –511 or both *IL1B* –511 and *IL1RN*2* polymorphisms, but not *IL1RN*2* alone [89]. While studies in central Italy, Costa Rica and Oman (areas of high gastric cancer prevalence) found no association between *IL1B* –31 and *IL1B* –511 polymorphisms and gastric cancer, carriers of the *IL1RN*2* allele had an increased risk of gastric cancer [90-92].

The studies in Asia are different from those in Europe. Increasing evidence suggests that *IL1B* polymorphisms are less important to gastric cancer development in Japanese populations, with no correlation between *IL1B* polymorphisms and expression of the *IL-1β* cytokine in the stomach, the severity of *H. pylori* induced inflammation, or atrophy [93-96]. It has even been suggested that the *IL1B-511* polymorphism may indicate less risk for gastric cancer in the Japanese [97], although a different study found *H. pylori* infected Japanese patients with the *IL1B –511* polymorphism had higher gastric pH, associated with more widespread infection and more severe inflammation [98].

Similarly in Korea, studies have found no association between *IL-1B* polymorphisms and *H. pylori* induced pathologies, including gastric cancer [99-101], with the exception of a possible link with IL1RN*2 [102].

In contrast, however, many studies in China reflect the Caucasian findings, with *IL1B* –511 and *IL1RN*2* polymorphisms associated with increased risk of *H. pylori*-induced pathologies, including gastric cancer [103-107].

Overall, these observations indicate a strong ethnic effect on the relative importance of *IL-1β* and *H. pylori* pathogenesis, with associations between *IL1B* polymorphisms and gastric cancer depending upon the country and/or ethnic origin of the infected population.

3.2. Interleukin-8

Interleukin 8 (IL-8) seems to have significant potential as a prognostic and predictive cancer biomarker. IL-8 was originally identified as a chemoattractant for neutrophils that release angiogenic growth factors, stimulating angiogenesis as a part of cancer progression.

IL8 is up-regulated after *H. pylori* infection and is potentially the most important cytokine produced by the host in response to *H. pylori* infection [108].. The infiltration of neutrophils into the stomach mucosa in response to *H. pylori* infection (termed ‘active’ gastritis) is associated with more severe disease outcomes

IL-8 polymorphisms may increase the risk of gastric cancer. Taguchi et al [109] reported

the association of the IL-8-251 A/T polymorphism with higher expression of IL-8 protein, severe neutrophil infiltration and increased risk of atrophic gastritis and gastric cancer. IL-8-251 T/A and IL-8-251 A/A polymorphisms may be associated with angiogenesis in gastric carcinogenesis in *H. pylori*-infected Koreans [110]. In the study, there were significant correlations between MMP-9, angiopoietin-1 concentrations and disease progression in IL-8-251 A/A and IL-8-251 A/T genotypes. Felipe *et al* [111] reported that patients with the heterozygous IL-8-251 A/T genotype, high fat intake and smokers or ex-smokers presented an increased risk of gastric cancer in a Brazilian population. However, the association of IL-8 polymorphisms and gastric cancer is controversial. The IL-8 polymorphism was not consistently associated with gastric cancer risk in a Polish population [112]. Furthermore, a meta-analysis of epidemiological studies revealed an overall lack of association between IL-8-251 gene polymorphisms and risk of gastric cancer; any association is likely to be variable depending on histological type, tumor location, *H. pylori* infection, and ethnicity/country [113].

3.3. Interleukin-10.

Interleukin-10 (IL-10) is produced by a wide range of cells including monocytes, macrophages, mast cells, T and B lymphocytes, regulatory T cells and dendritic cells. IL-10 is a potent inhibitor of antigen presentation as well as dendritic cell activation and maturation, thereby suppressing production of a range of important inflammatory cytokines including IL-1, IL-6, IL-12 and TNF-α [114].

Three main polymorphisms in the IL-10 promoter have been identified (–1082 (G/A), –819 (C/T) and –592 (C/A)), which combine to form three main haplotypes: GCC (associated with increased IL-10 production), ACC and ATA (associated with reduced IL10 production) [115],[116].

However, there is no consensus on the results of different studies regarding the association between *H. pylori* infection and IL-10. One study found that American Caucasians with the IL-10 ATA haplotype had an increased risk of *H. pylori* induced gastric cancer [117]. It is hypothesized that people carrying this low IL-10 producing haplotype are at increased risk of gastric cancer due to the increased inflammatory response resulting from reduced levels of this protective cytokine. IL-10 polymorphisms have also been associated with increased risk of gastric cancer and intestinal metaplasia in Mexican and Korean patients, gastritis in Indian patients and gastric cancer in a Chinese population [118-121].

However, an equal number of studies have failed to find any association between IL-10 polymorphisms and increased risk of *H. pylori* induced pathology, including atrophic gastritis and gastric cancer in European [116],[122],[123], Chinese [124],[125] and Japanese patients [126],[127].

3.4. Interleukin-17

Recently there has been heightened interest in the potential significance of interleukin 17 (IL-17) in the development/progression of human malignancies. IL-17A, the original member of this family, was first identified in 1951 and was initially recognized for its similarity to a sequence belonging to open reading frame 13 of Herpesvirus saimiri. IL-17 is a relatively newly described family of pro-inflammatory cytokines that consists of six family members (IL-17A–F) [128]. IL-17 is produced by CD4+ memory T cells, and it is involved in both innate and adaptive immune responses [129]. It has been reported that IL-17A, a pro-inflammatory cytokine, is associated with the pathogenesis of chronic inflammatory diseases, autoimmune diseases [130] and cancer progression [131].

There are many studies that focus on the relationship between IL-17A G197A polymorphism and gastric cancer [132-146]. These studies have been done a lot in China, both experimentally and clinically. However, their results are always inconsistent. Since 2015, only one meta-analysis has been conducted, and 11 case-control studies were included in this meta-analysis. Today, more than seven studies that assessed the association between IL-17A G197A polymorphism and the risk of gastric cancer have been published.

4. The synergistic effect of infectious agents and host for GC development.

Many studies recently indicated that it is better to discuss the synergistic effect of multiple factors for the development of GC than to discuss which factors is the most virulent. The *cagA*-positive strains were defined to be correlated with severer histopathological modifications and this gene was commonly associated with the *vacAs1* genotype, and such isolates are frequently found in patients with peptic ulcer disease [147]. The observations of combination of the *vacA* s-, m-, i-, region genotypes among *H. pylori* strains have provided better insight into determination of the difference of vacuolating activity between strains and clinical outcomes. The variations in the s- and m-regions give rise to four different *H. pylori* genotypes; *s1m1, s1m2, s2m1* and *s2m2* with different abilities in inducing the formation of acidic vacuole in the infected cell. In general, *s1m1* strains were characterized a large amount of toxin and caused the vacuolization of epithelial cell to a greater extent; *s1m2* strains were indicated that may or not induce cell vacuolation depending on the infected cell line, *s2m2* strains showed indeterminate levels of produced cytotoxin and *s2m1* strains were reported to be rare and non-vacuolating [148-152]. All *s1m1i1* strains arevacuolating, whereas all *s2m2i2* are non-vacuolating, the *s1m2* strains containing the *i1* genotype induce cell were recognized inducing cellular vacuolation while those containing the *i2* genotype were not. Thus, *s1m1i1* and *s1m2i1* strains showed more virulent and more likely associated with gastric cancer than the *s2m2i2* and *s1m2i2,* respectively [34],[149],[153]. Taking genotypes of d- and c-region into combination with genotypes of other variant regions, d1/c1 and d2/c2 strains almost

exclusively showed the types producing vacuolatingcytotoxin (*s1m1i1*) and non-vacuolating types (*s2 m2 i2*), respectively [38],[39].

H. pylori and EBV have been associated with cancer development, Sanket *et al* found that the dual prevalence of *H. pylori* infection and EBV was significantly higher in patients with gastric cancer and peptic ulcer disease than in those with non-ulcer dyspepsia (NUD). Median copy number of EBV-DNA was considerably higher in gastric cancer and peptic ulcer disease than NUD. There was a trend for higher EBV-DNA load in *H. pylori* positive individuals suggesting a probable role of *H. pylori* in modulating the conversion of EBV to its lytic phase [154]. Evidently, *H. pylori* factors and the host inflammatory response confer oxidative stress to the gastric epithelium during *H. pylori* infection that may lead to apoptosis [155]. Jun-Bo Hong *et al* found that *H. pylori* infection has a synergistic effect on the development of gastric cancer with *IL-1β* gene polymorphisms, and the highest prevalence of severe gastric abnormalities are found in patients with both host and bacterial high-risk genotypes (*cagA*(+)/*vacA*s1(+)/*IL-1β*-511T) [156]. Infection with *H. pylori* strains harboring more than one *CagA* EPIYA C motif was clearly associated with gastric cancer and higher number of EPIYA C segments was also associated decreased serum levels of pepsinogen I [157].

5. Conclusion

Gastric is a highly lethal disease and one of the most common cancer. The establishment of *H. pylori* as a risk factor for this malignancy permits an approach to identify persons at increased risk; however, infection with this organism is extremely common (around 50% in worldwide), and most colonized persons never develop cancer. Thus, techniques to identify high-risk subpopulations must utilize other additional biological markers. It is apparent from recent studies that cancer risk is the summation of the polymorphic nature of the bacterial population in the host, the host genotype and susceptibility, and environmental exposures including EBV infection, each affecting the level of long term interactions between *H. pylori* and humans. Analytical tools including sequencing *H. pylori* genome, genotyping virulence factors (*cag*PAI, *cag*A, *vac*A), detecting EBV co-infection, host susceptibility analysis comprising host polymorphism (e.g *IL1β*) may be used to discern the fundamental biological basis of *H. pylori*-associated gastric cancer, which should have direct clinical applications.,.

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