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Genetic populations and virulence factors of *Helicobacter pylori*

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Abstract

Helicobacter pylori is a bacterium that has infected more than half of the human population worldwide. This bacterium is closely associated with serious human diseases, such as gastric cancer, and identifying and understanding factors that predict bacterial virulence is a priority. In addition, this pathogen shows high genetic diversity and co-evolution with human hosts. *H. pylori* population genetics, therefore, has emerged as a tool to track human demographic history. As the number of genome sequences available is increasing, studies on the evolution and virulence of *H. pylori* are gaining momentum. This review article summarizes the most recent findings on *H. pylori* virulence factors and population genetics.

Keywords

genetic population; *Helicobacter pylori*; virulence factor

1. Introduction

Helicobacter pylori (H. pylori) is a Gram-negative spiral-shaped bacterium found in the gastric epithelium of humans (Suerbaum and Michetti, 2002). Since its first description by Marshall and Warren (1984), this species has gained considerable research attention because of its clinical and evolutionary importance (Marshall and Warren, 1984; Suerbaum and Achtman, 1999; Yamaoka, 2010).

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The bacterium is primarily transmitted within families and acquired during childhood. In the absence of adequate treatment, life-long gastric colonization can result in several diseases such as chronic gastritis, peptic ulcer, and gastric cancer (Yamaoka, 2010). The association between gastric cancer—one of the most common malignancies in the world—and *H. pylori* infection has attracted great interest worldwide, with the International Agency for Research on Cancer (IARC), a subordinate organization of the World Health Organization (WHO), classifying *H. pylori* as a "group 1 (definite carcinogen)" in 1994 (1994). Therefore, many studies have explored bacterial factors to explain the link between gastric cancer and this bacterium. Several bacterial virulence factors have been identified that predict severe clinical outcomes and explain the global geographic distribution of gastric cancer (Yamaoka, 2010). Thus far, the biological function and structure of these virulence factors, as well as the discovery of new candidate virulence factors, has continued to occupy most *H. pylori* -related research.

H. pylori and humans have co-evolved for at least 100,000 years, long before human ancestors left Africa (Moodley et al., 2012). During this long history in its hostile gastric niche in humans, *H. pylori* has developed a wide spectrum of strategies to persist in and adapt to changing conditions in and around its host (Suerbaum and Achtman, 1999). Thus, *H. pylori* is one of the most diverse bacterial species and arguably the most successful human pathogen known to date (Falush et al., 2003b; Suerbaum and Achtman, 1999). However, despite of their high genetic diversity, *H. pylori* strains appear genetically structured, exhibiting phylogeographic patterns that consistently correlate with that of their human hosts. Therefore, the population genetics and phylogenetic relationships among isolates have enabled accurate mapping of the local and global demographic histories of human evolution (Falush et al., 2003b; Linz et al., 2007; Moodley et al., 2012; Moodley et al., 2009). *H. pylori* genetics is promising to shed light on yet unknown dynamics of human evolution. Therefore, an increasing amount of resources are being devoted to detailed analyses of *H. pylori* populations with the aim of describing human history.

In addition, the increasing availability of *H. pylori* whole-genome sequences is enabling more genomic analyses than ever before. Such analyses are empower efforts to detect new virulence factors as well as detailed studies of population genetics. The present literature review addresses the most recent and important findings on bacterial virulence factors and genetic populations of *H. pylori*. Scientific data, mostly that reported in the last three years, are summarized, with the aim of highlighting expected future developments in *H. pylori*-related molecular epidemiological research.

2. New insights on *H. pylori* virulence factors

The pathogenesis of *H. pylori* is driven by several virulence factors that facilitate colonization, induce inflammation, and damage host cells. These virulence factors have been linked to the risk of developing severe gastric diseases and include the *cag* pathogenicity island (PAI), vacuolating cytotoxin (VacA), outer membrane proteins (OMPs), serine protease HtrA, and many others.

2.1. VacA

VacA is an exotoxin that was named for its capacity to induce host cell vacuolation (Cover and Blaser, 1992). At the time of its discovery, no bacterial toxin with similar activity had yet been described, and since this discovery, many studies have been conducted to clarify its function and structure.

VacA have been described as a multi-receptor protein that has pleiotropic effects, including membrane depolarization, mitochondrial dysfunction, autophagy, activation of mitogen-activated protein kinases, inhibition of T cell function, and induction of apoptosis (Foegeding et al., 2016). These functions contribute to the persistent colonization of *H. pylori* and its pathogenesis in several upper digestive tract diseases. Recently, additional VacA-related pathways and functions have been reported. Amilon et al. (2015) described a putative stem-loop structure in the 5′ untranslated region that affects transcription of *vacA* and leads to higher expression and toxicity of VacA (Amilon et al., 2015). An extra-digestive location of functional VacA in lungs has led to the suggestion that VacA plays a role in the pathogenesis of respiratory diseases by inducing IL-8 and IL-6 (Nakashima et al., 2015). In addition, new host factors that interact with or regulate the VacA-induced apoptosis have been reported. Yahiro et al. (2015) described a new signaling pathway for VacA-induced apoptosis that is mediated by cytoplasmic accumulation of connexin 43 (Cx43), a ubiquitous connexin family member that plays a role in gap junction and cell-cell channel formation (Yahiro et al., 2015). In addition, Chang et al. (2016) described the role of cortactin, an actin-binding protein, in the regulation of apoptosis induced by VacA (Chang et al., 2016).

VacA includes a 33-kDa N-terminal domain associated with cytotoxicity and a 55-kDa C-terminal domain involved in binding of the bacterium to cell surface receptors (Yahiro et al., 2015). Almost all *H. pylori* strains harbor the *vacA* gene, and allelic polymorphisms of the protein show clinical significance and toxic activity that are associated with specific combination of its three regions: the signal peptide (s1 and s2 variants), the intermediate region (i1, i2, and i3 variants), and the middle region (m1 and m2 variants). Molecular epidemiological studies have revealed two novel polymorphic sites, the deletion (d1 and d2 variants) and c-region (c1 and c2 variants) located in the 3′-end region of VacA (Fig. 2) (Thi Huyen Trang et al., 2016). Similar to sites described previously, some variants of these two novel regions have been associated with high risk of gastric cancer (Bakhti et al., 2016; Ogiwara et al., 2009). However, the contributions of these regions to different VacA functions such as vacuole formation have not yet been identified.

2.2. *cag* PAI

cag PAI is a chromosomic region of approximately 37 kb that encodes the *cag* type IV secretion system (*cag*-T4SS), including cytotoxin-associated gene A (*cagA*) (Fig. 1A). CagA is a 120–140-kDa cellular effector that is translocated into host cells through the *cag*-T4SS and interacts with a large repertoire of cellular signaling pathways, including those leading to carcinogenesis (Tegtmeyer et al., 2017a). CagA was discovered prior to the *cag* PAI and was named for its presumed link with the vacuolating cytotoxin activity of the VacA protein that had been discovered two years before (Tummuru et al., 1993). Since then, CagA and *cag* PAI have shown no effect on vacuolating cytotoxin production, suggesting the possible

functional independence of *vacA* and *cagA*, two genes located approximately 300 kb apart on the *H. pylori* chromosome (Tummuru et al., 1994).

The *cag* PAI is currently the most extensively studied *H. pylori* virulence factor. Its epidemiological role has been discussed previously (Suzuki et al., 2012; Yamaoka, 2010), and numerous studies have shown that *cag* PAI-positive *H. pylori* strains are associated with an increased risk of gastric cancer compared to strains that lack *cag* PAI (Yamaoka, 2010). The risk of gastric cancer is determined by several *cag* PAI-related features, including *cag* PAI-positivity, sequence variation within CagA (such as the number and type of EPIYA motifs), and the presence or absence of a functional *cag* type IV secretion system (which translocates CagA into host cells) (Suzuki et al., 2012; Tegtmeyer et al., 2017a; Yamaoka, 2010). Host c-Src and c-Abl kinases control hierarchic phosphorylation and CagA function in Western and East Asian *H. pylori* strains (Mueller et al., 2012). Recently, the function and structure of the CagA and *cag*-T4SS have been elucidated further.

First, the crystal structure of the N-terminal segment of the CagA molecule, which harbors a unique structure with no sequence homology to any known proteins, was recently obtained (Hayashi et al., 2012). The structured N-terminal part of CagA consists of several domains and harbors the putative integrin-binding region (Hayashi et al., 2012; Kaplan-Turkoz et al., 2012). The unstructured C-terminal region displays recognized repeated sections, EPIYA (Glu-Pro-Ile-Tyr-Ala), and CM (CagA multimerization) or CRPIA (conserved repeat responsible for phosphorylation-independent activity) motifs, as well as a region that binds to the secretion chaperone CagF, and a C-terminal secretion signal (Fig. 1B) (Schindele et al., 2016).

Second, further insights in the molecular mechanisms regulating *cagA* function through the *cag* PAI have been released. In fact, the *cagA* promoter region, which had been described previously (Loh et al., 2011), was further characterized by Ferreira et al. (Ferreira et al., 2016). This study identified specific sequence motifs located in the promoter region (+59 AATAAGATA and −10 TATAATGA sequence motifs) that are linked to CagA expression levels and interleukin-8 (IL-8) secretion in an infected gastric cell line, as well as to severe clinical outcomes (Ferreira et al., 2016). Because these sequence variations can be used to discriminate between two different levels of gastric cancer risk associated with Colombian strains and those with European and African origins, the discussion should be extended in future studies to strains from other geographical origins. Another important *cagA*-related feature identified recently is the number of copies of this gene found in different strains. Jang et al. (2017) showed that *H. pylori* isolates can carry multiple tandem copies of *cagA* that affect CagA expression and activity and may impact the development of gastric disease (Jang et al., 2017). Consistent with the findings of Jang et al. (2017), Draper et al. (2017) showed, using close strains named PMSS1 and SS1, that the number of *cagA* changes dynamically and modulates CagA activity (Draper et al., 2017). Thus, future epidemiological studies should address not only the sequence variation within CagA (EPIYA and CM/CRPIA motifs) but also the functionality of the whole *cag* PAI/T4SS in determining the biological effects of CagA, as well *cagA* promoter variants and the number of *cagA* copies as useful markers for disease risk. A β-lactamase-dependent reporter system that allows a precise and quantitative determination of CagA translocation into host cells has just

been developed (Schindele et al., 2016). This phosphorylation-independent assay has opened the door to further insights into the in vivo function and epidemiological role played by *H. pylori cag*-T4SS.

Finally, an integrative model of the activity of translocated CagA was recently developed (Tegtmeyer et al., 2017a). This model summarizes the data available on highly complex signaling pathways altered by translocated CagA through multiple receptor kinases (c-Met and EGFR) and non-receptor kinases (Src, Abl, Csk, aPKC, Par1, PI3K, Akt, FAK, GSK-3, JAK, PAK1, PAK2, and MAP kinases) in the human gastric epithelium that manipulate processes ranging from cell adhesion and polarity to apoptosis, inflammation, and cell cycle progression (Tegtmeyer et al., 2017a).

2.3. OMPs

Several OMPs have been predicted, based on the structure of the *H. pylori* genome, to play a role in bacterial adherence to the gastric mucosa (Alm et al., 2000). Though only a few OMPs have been established as adhesins (e.g., BabA/B, SabA, AlpA/B, OipA, and HopQ) with known host receptors (e.g., BabA/B, SabA, AlpA/B, and HopQ), their role in the virulence of *H. pylori* is increasingly evident (Aspholm et al., 2006; Bjornham et al., 2005; Javaheri et al., 2016; Koniger et al., 2016; Senkovich et al., 2011; Yamaoka et al., 2000). Based on epidemiological data, highly virulent *H. pylori* strains are known to express OMPs along with proteins from the *cag* PAI. This is consistent with observations showing that high pathogenic strains, especially those encoding the c*ag* PAI, are also highly adherent strains harboring numerous OMPs with the ability to enhance the expression of OMP ligands on gastric epithelial cells (Marcos et al., 2008). Thus, adhesins likely promote intimate contact between *H. pylori* and gastric epithelial cells, while the *cag*-T4SS, which forms an extracellular pilus-like structure, facilitates translocation of the effector protein CagA to induce pathogenicity leading to severe gastroduodenal diseases such as gastric ulcers and gastric cancer (Koniger et al., 2016). In addition to their adherence function, new roles for OMPs in bacterial virulence have been discovered recently through studies analyzing their interactions with *cag*-T4SS or their direct effects on host cells.

Blood group antigen-binding adhesin (BabA) is the most studied *H. pylori* OMP. It mediates bacterial adherence to ABO/Lewis b (Le^b) blood group antigens in the gastric pit region of the human stomach mucosa (Ansari and Yamaoka, 2017). Evidence for a virulence role for BabA rely on epidemiological data associating the *babA* gene to other virulence genes and to severe gastroduodenal diseases, as well as on the fact that BabA-mediated adherence of *H. pylori* can be a potentiator of *cag*-T4SS activity and inducer of proinflammatory cytokines (e.g., *CCL5* and *IL-8*) and precancer-related factors (e.g., *CDX2* and *MUC2*) (Ansari and Yamaoka, 2017; Ishijima et al., 2011). Recently, additional evidence for this virulence role was provided by an epidemiological study that showed a significant association between the combination of OipA, BabA, and SabA and a diagnosis of *H. pylori*-associated gastric cancer (Su et al., 2016). However, recent investigations have reiterated that *H. pylori* BabA sequences, expression, and corresponding binding phenotypes are highly diverse and dynamic (Bugaytsova et al., 2017; Hansen et al., 2017; Kable et al., 2017; Moonens and Remaut, 2017; Subedi et al., 2014; Sweeney and Guillemin, 2016).

Thus, caution is needed when suggesting an association between *babA* and clinical outcomes based on epidemiological studies. Sweeney and Guillemin (2016) rightly suggested that the discussion sections in some reports should be extended to *babA* sequence and expression variation, host glycans, and disease incidence in different host and *H. pylori* populations (Sweeney and Guillemin, 2016).

Helicobacter outer membrane protein Q (HopQ) is an OMP that was first called *HP1177* or *omp27* after *H. pylori* was completely sequenced but before its presence on the surfaces of bacteria cells, influencing the adherence to human epithelial cells, was demonstrated (Loh et al., 2008). Since two alleles of *hopQ* were described as having an epidemiological association with the *hopQ I* allele and *cagA* gene (Cao et al., 2005; Ohno et al., 2009), this OMP is attracting increasingly interest among researchers. By screening a large number of *H. pylori* mutants, Belogolova et al. (2013) identified HopQ as a non-*cag* PAI-encoded co-factor of T4SS function that is essential for CagA translocation and CagA-mediated host cell responses such as the formation of a hummingbird phenotype and cell scattering. Their work also showed that deletion of *hopQ* reduces the T4SS-dependent activation of NF-κB, induction of MAPK signaling, and secretion of IL-8 in host cells (Belogolova et al., 2013). Moreover, Jiménez-Soto LF et al. (2013) identified HopQ along with other *H. pylori* OMPs such as HopI and AlpAB as factors restricting and controlling subsequent CagA translocation into host cells, independently of β1 integrin receptor availability (Jiménez-Soto et al., 2013). Efforts to clarify the molecular mechanisms underlying OMP interactions and CagA translocation have led to HopQ being implicated in *cag* PAI-related virulence-associated interactions with human receptors from the carcinoembryonic antigen-related cell adhesion molecule (CEACAMs) family (Javaheri et al., 2016; Koniger et al., 2016).

The *H. pylori outer inflammatory protein A* (OipA) adhesion function and its ability to induce a pro-inflammatory response in a gastric epithelial cell line or animal models are controversial. However, based on epidemiological studies, functional OipA correlates with highly virulent strains expressing the *cag* PAI and *vacA*-s1/m-1 (Matsuo et al., 2017). The OipA receptor has not yet been identified, but OipA-related host cell signaling has been reported. This OMP is believed to trigger pathways related to inflammation induction, actin remodeling, and cell apoptosis through epidermal growth factor receptor (EGFR)/focal adhesion kinase (FAK), phosphoinositide-3 kinase (PI3K)-dependent Akt activation, and Forkhead transcription factors of class O (FoxO) (Tabassam et al., 2008, 2012). Recently, new arguments in factor of a virulence role for *H. pylori* OipA have been presented based on the treatment of gastric cell lines with various concentrations of OipA (Teymournejad et al., 2017). While confirming the binding property of OipA, this group showed toxic effects, as well as an apoptosis-triggered cascade via signaling that affected the Bax/Bcl-2 protein ratio and cleaved-caspase 3 level, leading to a mitochondrial apoptotic cascade (Teymournejad et al., 2017).

2.4. *H. pylori* prophages

Bacteriophages (phages) are viruses that infect bacteria (Canchaya et al., 2003). Phage-bacteria interplay includes a step in which the phage is inserted into the host genome, leading to either bacterial lysis or prophage domestication. Thus, the host genome might be

shaped in terms of the evolution of diversity or virulence in conjunction with phage infection (Rodriguez-Valera et al., 2009). Bacteriophages were initially reported in *H. pylori* by Schmid et al. (1990), who hypothesized phage-mediated virulence of this species (Schmid et al., 1990). Subsequent observations of phages in *Helicobacter* spp. have been relatively rare (Arnold et al., 2011; Eppinger et al., 2006; Heintschel von Heinegg et al., 1993; Vale et al., 2008). The putative pathogenic role of *H. pylori* prophages has been revived since the first isolation of an integrated prophage, which was similar to phages of the *Siphoviridae* family, from a patient with gastric mucosa-associated lymphoid tissue lymphoma (Lehours et al., 2011). Recently, Kyrillos et al. (2016), by screening phage-orthologous *H. pylori* sequences from 335 strains, found a correlation between the presence of phage-related sequences, likely acquired by horizontal gene transfer, and that of the two major virulence factors CagA and VacA (Kyrillos et al., 2016). Another *H. pylori* prophage has been reported in the genome of a *cag* PAI-negative strain, which was isolated from a patient suffering from gastric cancer (Mucito-Varela et al., 2016). Because non-pyloric *Helicobacter* prophages have been revealed to encode antibiotic resistance genes and virulence factors (Qumar et al., 2017), we can predict that the genetic content and putative pathogenic role of *H. pylori* prophages will attract increasing attention from researchers.

2.5. *H. pylori* HtrA

High temperature requirement A (HtrA) is a serine protease released in the extracellular environment by *H. pylori* during infection. Extracellular *H. pylori* HtrA had been shown to cleave the cell adhesion protein through proteolysis, and its possible crosstalk with CagA and direct effects on the infection process have been intensively studied (Hoy et al., 2012; Hoy et al., 2010). Identification of tumor suppressor E-cadherin as an HtrA substrate came emphasized the significant role of HtrA activity in *H. pylori*-induced carcinogenesis and disruption of adherens junctions, which allows bacterial transmigration across the epithelium (Hoy et al., 2010). Recently, Schmidt et al. (2016a, 2016b) further elucidated the activity of HtrA on E-cadherin (Schmidt et al., 2016a; Schmidt et al., 2016b). Tegtmeyer et al (2016). showed that the *htrA* gene locus was conserved among 992 *H. pylori* clinical isolates and that the proteolytic activity of HtrA was essential for bacterial survival (Tegtmeyer et al., 2016). Finally, Harrer et al., by successfully introducing a second functional *htrA* gene in *H. pylori* strains P12 and 26695, showed that the overexpression of HtrA enhances cleavage of E-cadherin, bacterial transmigration, and delivery of the *cag*-T4SS effector protein CagA into polarized epithelial cells (Harrer et al., 2017). Taken together, these findings suggests a novel model that relies on *H. pylori* access to the basolateral compartment for deployment of *cag*-T4SS and injection of the oncoprotein CagA into host cells (Harrer et al., 2017; Tegtmeyer et al., 2017b). Therefore, *H. pylori* HtrA-triggered E-cadherin is, without doubt, a bacterial virulence factor. Further studies will elucidate the epidemiological role of such an important virulent factor.

3. Human migration tracked through *H. pylori* genetic diversity

The isolation of genes (e.g., *cagA* and *vacA*), as well as a multilocus sequence typing (MLST) of a set of seven concatenated housekeeping genes (*atpA*, *efp*, *mutY*, *ppa*, *trpC*, *ureI*, and *yphC*) or *cag* PAI genes, have been used as tools to track human migration

throughout history. For details, see our previous review paper (Suzuki et al., 2012). Recently, further interesting insights have been gained by genome-based analyses to identify genetic populations of *H. pylori* genetic. For examples, new potential tools for tracking human migration have been found in *H. pylori* prophage sequences.

3.1. Genome-based inference of *H. pylori* population structure

H. pylori strains from diverse geographical regions have shown a quasi-panmictic structure, with the lack of gene allele clonality resulting from frequent genetic recombination (Suerbaum et al., 1998). However, this species could be split into distinct bacterial populations that exhibit close relationships with the ethno-geographical distribution of its human host (Falush et al., 2003b; Linz et al., 2007). Thus, the identification of genetic populations of *H. pylori* has arisen as a valuable tool for analyzing *H. pylori* population differentiation and selection, as well as for inferring human demographic history (Moodley and Linz, 2009). The software most often used to identify *H. pylori* population structure using genetic data is STRUCTURE, developed by Falush et al. (Falush et al., 2003a; Falush et al., 2003b). Additionally, phylogenetic tree construction has been also used (Moodley et al., 2012). Based on these methods, bacterial populations, including three from Africa (*hpNEAfrica*, *hpAfrica1*, and *hpAfrica2*), one from Europe (*hpEurope*), and three from Asia (*hpEAsia*, *hpAsia2*, and *hpSahul*) had been distinguished (Falush et al., 2003b; Linz et al., 2007; Moodley and Linz, 2009; Moodley et al., 2012). Different subpopulations have been shown in *hpEastAsia* (*hspAmerind*, *hspEAsia*, and *hsp Maori*), *hpNEAfrica* (*hspEastNEAfrica* and *hspCentralNEAfrica*), and *hpAfrica1* (*hspSAfrica, hspWAfrica*, and *hspCAfrica*) (Moodley et al., 2012; Nell et al., 2013). However, the phylogenetic approach is limited to making a rough assessment of populations, and it is not designed to infer the number of populations directly (Yahara et al., 2013). STRUCTURE, even with a linkage model option, is limited by the need for satisfactory convergence that requires prior specification of the K number of populations (Lawson and Falush, 2012; Lawson et al., 2013). This may lead to a violation of the methodological assumptions and to biased results (Lawson et al., 2013).

To circumvent the limitations of these approaches and to improve the accuracy and reliability population and subpopulation identification, new approaches have been developed for use with genome-wide sequence data instead of sequences of isolated genes. A new tool called fineSTRUCTURE, introduced by Lawson et al. and based on the chromosome painting method (Lawson and Falush, 2012), has been used successfully by Yahara et al. (2013) to describe *H. pylori* populations (Yahara et al., 2013). Expectedly, the fineSTRUCTURE approach conducted under linkage and admixture models provided a population structure consistent with those reached using STRUCTURE and phylogenetic approaches. However, it revealed the structure of *H. pylori* populations at a finer scale (Yahara et al., 2013). Thus, novel subgroups, even singletons, could be found within subpopulations defined by previous methods. Moreover, the new approach is able to elucidate the genetic flux between populations, while providing faster and more efficient results based on both small and large sets of genome-wide sequences (Yahara et al., 2013). Data supporting consistency between the different approaches, specifically identification of main genetic populations at a genomic level, have been shown by Montano et al. (Montano

et al., 2015). More recently, by applying the fineSTRUCTURE approach, Thorell et al. (2017) uncovered more dynamic evolution of *H. pylori* in four new subpopulations of *H. pylori*: *hspAfrica1NAmerica, hspAfrica1Nicaragua, hspEuropeColombia, and hspMiscAmerica* (Thorell et al., 2017). Thus, these new subpopulations seen as the result of genetic drift and admixture events occurring over a relatively short period (~500 years), are suggestive of rapid evolution of this bacterium in the Americas (Thorell et al., 2017). More evidence for this rapid evolution was provided by analysis of MLST and phylogenetic data on *cag* PAI and seven housekeeping genes in isolates from Latin America and Portuguese-speakers worldwide (Munoz-Ramirez et al., 2017; Oleastro et al., 2017).

These new data have strengthened the view that *H. pylori* genetic diversity can be used as a marker for historical human migration events (summarized in Table 1 and Table 2). Future studies will be conducted to infer a global demographic history by extending the analysis to isolates from underrepresented regions such as Central Africa, Northeast Africa, Central Asia, and the Siberian region. Moreover, the fine-scale structure of *H. pylori* populations revealed by fineSTRUCTURE would likely be needed to elucidate further details in human evolutionary events, as well as the regional distribution of gastric cancer risk.

3.2. *H. pylori* prophages and the geographical origin of strains

An increasing number of *H. pylori* prophage sequences are being identified and analyzed (Secka et al., 2017; Uchiyama et al., 2016; Vale et al., 2017). Vale et al. (2015) are the first to show that the diversity of two concatenated *H. pylori* prophage genes (integrase and holin) allow strains to be differentiated according to their geographic origins, in agreement with MLST-based classification using seven *H. pylori* housekeeping genes (Vale et al., 2015). More interestingly, analysis of *H. pylori* prophage genes has revealed two European subpopulations of *H. pylori*, *hpNEurope* and *hpSWEurope*, whereas the traditional MLST-based method identified only *hpEurope* (Vale et al., 2015). However, whether these new prophage-based subpopulations of *H. pylori* coincide with those found by genome-based analyses still needs to be analyzed. To gain more insight into the phylogenetic relationships between prophages sequences and bacterial geographic distribution, Vale et al. (2017) just analyzed a complete prophage genome (Vale et al., 2017). This analysis shows similarities with prophage-isolated genes and a refinement of phylogeographic clustering assigned by STRUCTURE (Vale et al., 2017). Additional observations have been made for *H. pylori* strains from Gambia (Secka et al., 2017). Moreover, the insertion sites of prophages suggest ancient acquisition and vertical transfer, with a single ancestral prophage likely responsible for sequences inserted at similar loci in different bacterial genomes (Vale et al., 2017). Therefore, *H. pylori* prophages and their host bacteria appear to share a complex evolutionary history that is shaped by human populations, possibly providing a more detailed approach to population genetic analysis than MLST.

4. Conclusion

Fascinating developments in *H. pylori*-related research, especially regarding bacterial virulence factors and evolution, should be expected. New virulence factors, as well as interconnections between known ones, will be brought to light. Detailed population genetic

studies will indicate previously unknown dynamics of local and more recent demographic processes in human evolution. This research will increasingly be impacted by access to genome-wide data.

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Highlights

- Virulence factors are ever key points for *H. pylori* related pathogenesis
- The genetics *of H. pylori* prophage sequences bring interesting features
- New *H. pylori* populations will enounce the usefulness for tracing human migrations.

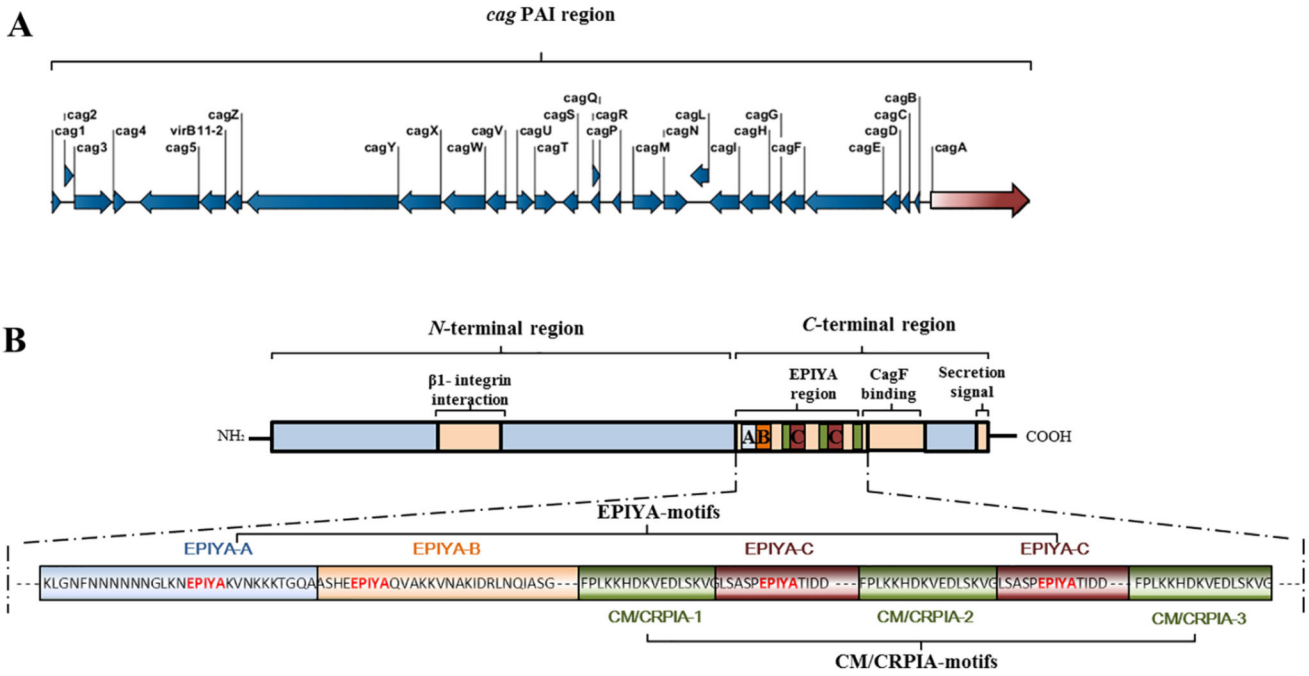

Figure 1. Overall structure of the *cag* PAI (A) and CagA protein (B) in *H. pylori* strain P12

A. Structure of the *cag* PAI region (~37 kb). The region comprises 28 genes that encode components of the *cag*-T4SS, including the CagA protein effector.

B. Structure of the CagA protein (1,214 amino acid residues). The N-terminal part of CagA harbors a putative β-integrin-binding region. The C-terminal region comprises the EPIYA region, which contains EPIYA ABCC motifs and three MKI/CM/CRPIA motifs, regions that bind to the secretion chaperone CagF and contain the C-terminal secretion signal.

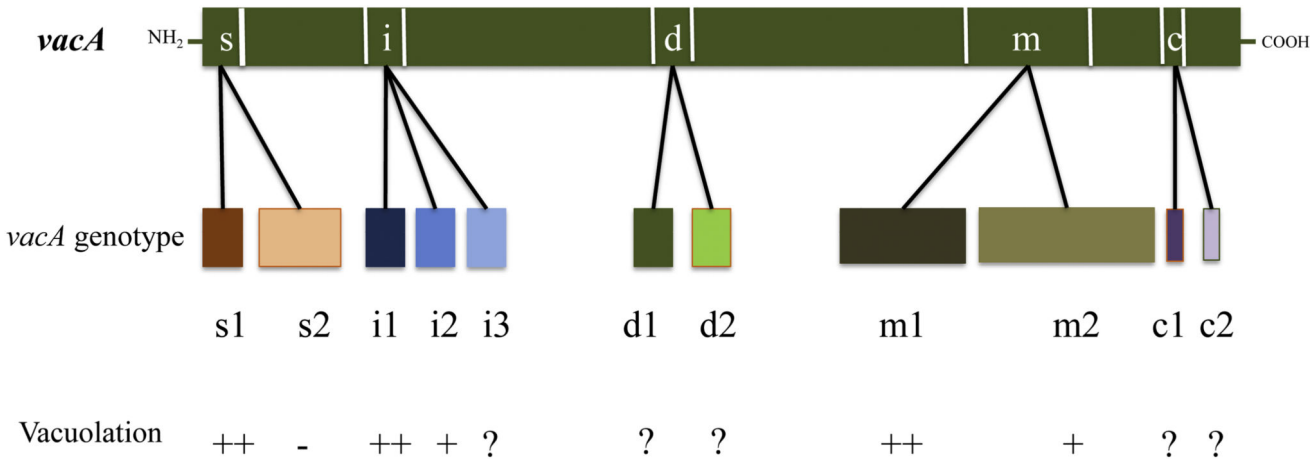

Figure 2. Current allelic diversity inferred from the VacA sequence

The VacA structure comprises five regions of sequence diversity referred to as the signal (s), intermediate (i) middle (m), deletion (d), and c (c) regions. The vacuolating activity of VacA varies with different alleles. In vitro, *vacA* s1/m1/i1 alleles show higher levels of vacuole formation than s2/m2/i2. The functions of novel polymorphic regions c and d, as well as the i3 subtype, have not yet been studied.

Table 1

Geographic range of modern populations and subpopulations of *H. pylori*

Population	Subpopulation	Geographic range/location	Main references
hpAfrica1	hspWAfrica	Africa: West to North, then Eastward to South	(Nell et al., 2013)
	hspCAfrica	Africa: West to center towards Southwest	(Nell et al., 2013)
	hspSAfrica	Africa: South-East (+ Madagascar) to South	(Nell et al., 2013)
hpAfrica1/hpEurope hybrid	hspEuropeColombia	South America	(Thorell et al., 2017)
	hspAfrica1NAmerica	America: North, Center, and South	(Thorell et al., 2017)
	hspAfrica1Nicaragua	America: Center	(Thorell et al., 2017)
	hspMiscAmerica	America: Center and South	(Thorell et al., 2017)
hpAfrica2	hspNorthSan	Africa: South-West	(Linz et al., 2007; Moodley et al., 2012)
	hspSouthSan	Africa: South Africa	(Moodley et al., 2012)
hpNEAfrica	hspENEAfrica	Africa: Horn of Africa, westward to the Nile Valley, and to the Northwest	(Nell et al., 2013)
	hspCNEAfrica	Africa: Nile Valley, toward the West and Northwest	(Nell et al, 2013)
hpSahu1	hspAustralia	Australia	(Moodley et al., 2009)
	hspNGuinea	Papua New Guinea	(Moodley et al., 2009)
hpAsia2	hspLadakh	Asia: Himalayan region of Northern India	(Breurec et al., 2011; Tay et al., 2009)
	hspIndia	India, Bangladesh, Malaysia, Thailand, the Philippines, and even Northern Europe	(Breurec et al., 2011; Tay et al., 2009)
hpEurope	hspEuropeS, hspEuropeN	Europe, as far East as South-East Asia	(Thorell et al., 2017)
	~	Australia, America, South Africa, Australia, and the South Pacific Islands	(Falush et al., 2003b)
hpEastAsia	hspEAsia	Asia: East Asia, toward Japan	(Falush et al., 2003b; Moodley et al., 2009)
	hspAmerind	Asia and America: East Asia, toward North and South America	(Falush et al., 2003b)
	hspMaori	Asia: Taiwan and the Pacific Islands	(Linz et al., 2007; Moodley et al., 2009)

Table 2

Main demographic events inferred from modern populations and subpopulations of *H.pylori*

Population	Subpopulation	Main human demographic events so far inferred	Main references
hpAfrica1	hspWAfrica	Prehistoric expansion of Bantu speakers (Eastern route), ~3,000 to 5,000 BP	(Nell et al., 2013)
	hspCAfrica	Prehistoric expansion of bantu speakers (Western route)	(Nell et al., 2013)
	hspSAfrica	Specific prehistoric Bantu migration of Nguni speakers from central West Africa along East coast	(Nell et al., 2013)
hpAfrica1/hpEurope hybrid	hspEuropeColombia	Post-Colombian migration of Europeans and Africans toward the New World	(Nell et al., 2013)
	hspAfrica1NAmerica,,	Migration of Africans (along with Europeans) toward the New World during the colonial-era slave trade	(Thorell et al., 2017)
	hspAfrica1Nicaragua	Migration of Africans (along with Europeans) toward the New World during the colonial-era slave trade	(Thorell et al., 2017)
	hspMiscAmerica	Migration of Africans (along with Europeans) toward the New World during the colonial-era slave trade	(Thorell et al., 2017)
hpAfrica2	hspNorthSan	Colonization of specific groups of Khoisan peoples (Xun and Khwe)	(Thorell et al., 2017)
	hspSouthSan	Colonization of specific groups of Khoisan peoples (Khomani)	(Linz et al., 2007; Moodley et al., 2012)
hpNEAfrica	hspENEAfrica	Prehistoric expansion of Nilo-Saharan speakers	(Moodley et al., 2012)
	hspCNEAfrica	Prehistoric expansion of Nilo-Saharan speakers during the Holocene humid period	(Nell et al., 2013)
hpSahu1	hspAustralia	Peopling of Sahul and isolation in Australia after the Last Glacial Maximum	(Nell et al., 2013)
	hspNGuinea	Peopling of Sahul and isolation in New Guinea after the Last Glacial Maximum	(Moodley et al., 2009)
hpAsia2	hspLadakh	Past peopling of the eastern Eurasia and isolation in Himalayas	(Breurec et al., 2011; Tay et al., 2009)
	hspIndia	Past peopling of the eastern Eurasia as well as spread of Uralic speakers out of Siberia	(Breurec et al., 2011; Tay et al., 2009)
hpEurope	hspEuropeS, hspEuropeN	Prehistoric mixing of populations (from Africa and Asia) during colonization of Europe	(Thorell et al., 2017)
		Post-Colombian expansion of Europeans during the colonial era, ~500 BP	(Falush et al., 2003b)
hpEastAsia	hspEAsia	Expansion of Chinese speakers (sino-Tibetan), ~3000 years ago during the Zhou Dynasty	(Falush et al., 2003b; Moodley et al., 2009)
	hspAmerind	Prehistoric migration across the Bering strait and colonization of the Americas, >12,000 BP	(Falush et al., 2003b)
	hspMaori	Expansion of Austronesian Speakers into the Pacific	(Linz et al., 2007; Moodley et al.,2009)