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REVIEW

Relatedness of *Helicobacter pylori* populations to gastric carcinogenesis

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Abstract

Helicobacter pylori (*H. pylori*) is a Gram-negative bacterium that infects half of the human population. The infection is associated with chronic inflammation of the gastric mucosa and peptic ulcers. It is also a major risk factor for gastric cancer. Phylogenetic analysis of global strains reveals there are seven populations of *H. pylori*, including hpAfrica1, hpAfrica2, hpEastAsia, hpEurope, hpNEAfrica, hpAsia2 and hpSahul. These populations are consistent with their geographical origins, and possibly result from geographical separation of the bacterium leading to reduced bacterial recombination in some populations. For each population, *H. pylori* has evolved to possess genomic contents distinguishable from others. The hpEurope population is distinct in that it has the largest genome of 1.65 mbp on average, and the highest number of coding sequences. This confers its competitive advantage over other populations but at the cost of a lower infection rate. The large genomic size could be a cause of the frequent occurrence of the deletion of the *cag* pathogenicity island in *H. pylori* strains from hpEurope. The incidence of gastric cancer varies among different geographical regions. This can be attributed in part to different rates of infection of *H. pylori*. Recent studies found that different populations of *H. pylori* vary in their carcinogenic potential and contribute to the variation in incidence of gastric cancer among geographical regions. This could be related to the ancestral origin of *H. pylori*. Further studies are indicated to investigate the bacterial factors contributing to differential virulence and their influence on the clinical features in infected individuals.

Key words: *Helicobacter pylori*; Population genetics; Gastric cancer; Virulence; Genome

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is a Gram-negative bacterium which colonizes the human stomach. As a pathogen, *H. pylori* induces inflammation of the gastric mucosa[1]. It plays a causal role in the ulceration and recurrence of peptic ulcer[2]. Eradication of the bacterium heals ulcers and prevents recurrence of the disease. The infection is also associated with an increased risk of gastric cancer[3,4].

The incidence of gastric cancer shows geographical variation. This is attributed in part to the difference in the prevalence of the *H. pylori* infection among geo-

graphical regions. In Africa and South Asia, however, the incidence of gastric cancer in these areas is much lower than in other countries in spite of the high prevalence of the *H. pylori* infection[5]. Such a disparity has also been found in other local regions[6]. Analysis of global strains reveals seven populations of *H. pylori* that are consistent with their geographical origin[7-10]. These current populations derive from six ancestral populations[7]. It appears that the ancestry, genomic contents and carcinogenic potentials are diversified among *H. pylori* populations. Studies at a population level have improved our understanding of gastric carcinogenesis associated with the *H. pylori* infection.

GENETIC DIVERSITY AND POPULATIONS OF *H. PYLORI*

There are three types of bacterial population structure: clonal, panmictic and endemic[11]. If intra-species or inter-species recombination is rare, the genetic diversity of a bacterial species predominantly comes from evolution of the ancestry. This species has a clonal population structure. In a species with high frequency of recombination, introduction of foreign gene fragments into the genome occurs frequently in the evolution history. As foreign genes have a different evolution history, the evolution speed of individual genes is different. In this case, the species possess a panmictic structure. For a bacterial species with a panmictic structure, a temporal clonal structure may occur if it rapidly spread among naïve hosts. In this situation, a bacterial species has an endemic structure.

H. pylori shows great inter-strain variation in genetic content[12]. None of the individual strains is identical as demonstrated by multiple fingerprinting methods[13,14]. Sequence divergence is the main cause of this variation. Comparison of two sequenced genomes revealed occurrence of substantial silent mutation in the genetic loci[15]. A number of mechanisms are involved in the generation of the sequence variation: *H. pylori* shows a higher mutation rate than *Escheria coli*[16]. Approximately a quarter of strains possess a mutator-like phenotype. This is attributed to the lack of a functional DNA repair system[16,17] and error-prone DNA polymerase in *H. pylori*[18]. Recombination in *H. pylori* is more frequent than in any other organism studied to date[19]. Foreign DNA from the same species or phage has been found in the bacterium[15]. Strand slippage mispairing is another mechanism responsible for genetic diversity. A number of homopolymeric tracts and dinucleotide repeat regions are present in the *H. pylori* genome[20,21], which may cause replication error and subsequently sequence variation. *H. pylori* has a specialized type Ⅳ system for uptake of foreign DNA from the same species or other species[22]. Foreign DNA fragments are subsequently integrated into the genome by recombination. A high frequency of recombination and a high mutation rate in *H. pylori* result in a panmictic structure of the bacterium[23].

Recombination is a rare genetic event in house-keeping genes. Phylogenetic analysis of highly conserved house-keeping genes (*atpA*, *efp*, *mutY*, *ppa*, *trpC*, *ureI* and *yphC*) has then been used to study populations of *H. pylori*[24]. Examination of global strains of *H. pylori* reveals that it has seven populations: hpAfrica1, hpAfrica2, hpEastAsia, hpEurope, hpNEAfrica, hpAsia2 and hpSahul[7-10,25,26]. Some populations could be further divided into subpopulations. For hpEastAsia, there are three subpopulations including hspEAsia, hspAmerind and hspMaori[27], while hpAfrica1 is split into hspWAfrica and hspSAfrica[28]. These populations and subpopulations reflect not only their geographical origin but also ethnic groups of their hosts. Spatial separation reduces bacterial recombination between different geographical regions. A weakly clonal population may thus be generated in strains from a particular geographical region[29]. *H. pylori* spreads mainly through a mode of family transmission[30,31], leading to a reduced chance of recombination between strains from different ethnic groups. Therefore, strains from different ethnic groups could be distinguished in the phylogenetic analysis. This is one of the features that allows phylogenetic analysis of *H. pylori* to be used to trace the history of human migration.

GENOMIC DIVERGENCE BETWEEN *H. PYLORI* POPULATIONS

H. pylori has been accompanying human hosts for more than 58 000 years[10]. The genome of *H. pylori* is thus shaped by its human hosts due to the long co-existence[32]. *H. pylori* strains differ in their affinity to bind blood group antigens expressed in the gastric mucosa[33]. Strains from Europe bind all three blood group antigens, while Amerindian strains have higher affinity for O blood antigen as this antigen is predominant in Amerindians[32]. Therefore, the genomic content of *H. pylori* may vary in different populations.

To date, a number of strains of *H. pylori* have been sequenced[15,34-37]. Of these, the origin and other information of 30 strains are publicly available. These include 14 strains from hpEastAsia (7 from hspEasia and 7 from hspAmerind subpopulations, respectively), 10 from hpEurope, 5 from hpAfrica1 and 1 from hpAfrica2[38-41]. All, except for strain B38 from hpEurope, possess *cagA* and the *cag* pathogenicity island (*cag* PAI). The genomic size of *cagA*-positive *H. pylori* ranges from approximately 1.55 mbp to 1.71 mbp with an average of 1.61 mbp. For *cagA*-negative strains, their genome is generally smaller because of the lack of the *cag* pathogenicity island of about 40 kbp. We analyzed the average genomic size of *cagA*-positive *H. pylori* strains from different populations[42-47]. The average genomic size of strains from hpEurope is approximately 1.65 mbp, which is significantly larger than that from hpEastAsia (1.60 mbp, $P < 0.05$) or hpAfrica1 (1.60 mbp). Consistent with this, strains of hpEurope have the highest number of coding sequences. There was a statistically significant difference between hpEurope and

hpEastAsia in the average genomic size and number of CDS. The size of bacterial genomes is primarily determined by two counteracting processes: the gain of new genes by gene duplication or by horizontal gene transfer; and the decay of non-essential genes[15]. Both of these processes have been observed in *H. pylori*. Recombination, conjugation, insertion elements, mutation and slipped-strain replication lead to gene gain or loss[34]. They may be involved in the variability of genomic size among *H. pylori* of different populations.

For bacteria, a larger genome requires more metabolic activities and consumes more energy[48,49]. Therefore bacteria containing a larger genome may have a lowered capacity of growth, reducing its competitive ability with other bacteria in the same ecological niche. This leads to a decreased spread of bacteria. It is well known that *H. pylori* is less prevalent in Western countries than in other parts of the world[50]. Hygiene, economical incomes and social status have been suggested to contribute to this differential prevalence[51]. It is arguable that a larger genome of *H. pylori* strains in Western countries may also contribute to the low prevalence of the infection in this region.

Comparison of the genomic content of *H. pylori* from different populations revealed differences in the compositions of outer membrane proteins and central metabolism[39]. Compared with hpEurope, strains from hpEastAsia tend to have fewer genes of these two categories. There are a total of 12 genes in *H. pylori* involved in molybdenum metabolism, including those encoding proteins for molybdenum transport and cofactor synthesis and a molybdenum-containing enzyme. A massive decay of molybdenum-related genes occurs in strains from hpEastAsia. At least five genes are fragmented due to mutations. The molybdenum-containing enzyme functions in electron transfer and responses to oxidative and acid stress[52]. It is probable that in hpEastAsia populations, *H. pylori* use alternative pathways for the purpose[39]. Outer membrane proteins consist of several paralog families interacting with the human host[53,54]. In the hpEastAsia population, there is a tendency for a reduced number of these proteins resulting from mutations and recombination. Therefore it appears that *H. pylori* from hpEastAsia have evolved to possess a reduced genome.

The *cag* PAI is a 40-kb DNA fragment which contains 27 to 31 genes flanked by 31-bp direct repeats[55]. It encodes CagA, the major virulence determinant of *H. pylori* and components of a type Ⅳ secretion system[56,57]. The latter translocates CagA into host cells[58]. Once inside the host cells, CagA binds to a number of host cell proteins disrupting intracellular signaling systems *via* tyrosine phosphorylation-dependent or -independent pathways[59]. This causes elongation and loss of polarity of host cells, promoting proliferation and inflammation. The presence of the *cag* PAI in *H. pylori* is associated with increased risk of severe gastritis, atrophic gastritis, and distal gastric cancer compared with strains that lack the *cag* island[60-62].

A marked difference lies between hpEurope and hpEastAsia in the prevalence of strains possessing the *cag* PAI. Approximately 60% to 70% of Western *H. pylori* strains express CagA[61,63], indicating the presence of the *cag* PAI. In East Asia, however, almost 100% of strains possess the *cag* PAI irrespective of pathology[64,65]. It is believed that the *cag* PAI is deleted in Western strains resulted from recombination between the repeats flanking the island[66]. This results in a reduced genomic size by approximately 40 kbp. In addition, it has been demonstrated that the prevalence of strains with an intact cag PAI is the lowest in Western countries[67]. As described above, strains from hpEurope are coincidently 40 kbp larger than the average genomic size of *H. pylori*. Thus, the occurrence of *cagA*-negative strains in hpEurope is probably due to the evolution of the bacterium towards a smaller genome.

VARIATION IN THE CARCINOGENIC POTENTIAL OF *H. PYLORI* POPULATIONS

The incidence of gastric cancer varies in different geographical regions. It is higher in East Asian countries than in any other countries when age-standardized rates are considered[68]. In some countries of West Africa and South America, there is also an increased incidence of gastric cancer[69,70]. The geographic difference in the incidence of gastric cancer can be attributed partially to the difference in the prevalence of *H. pylori* infection[71]. A high prevalence of virulent strains of *H. pylori* is another contributing factor to the high incidence of gastric cancer in East Asia. Virulent strains possess the *cag* PAI and express *Vac*A. There is, however, a disparity between the prevalence of *H. pylori* or virulent strains and the incidence of gastric cancer. In Linqu County, China, the incidence of gastric cancer is extremely high, while in its neighboring county Cangshan the incidence is very low[72,73]. The rate of *H. pylori* infection and the proportion of virulent strains in these two counties, however, show no significant difference[74]. Similar results have been found when comparing two regions in Mexico with contrasting incidence of gastric cancer[75]. These results indicate differential incidence of gastric cancer among different geographical regions is attributable to other bacterial factors.

To explore other bacterial factors related to carcinogenesis, the phylogeographical origin of *H. pylori* has been investigated[76]. In the Andean mountain region of Colombia, habitants have a high incidence of gastric cancer (150 per 100 000 people per year)[77,78], while habitants in the coastal line 200 kilometers away, have a very low incidence of gastric cancer (6/100 000)[77,78]. The prevalence of the infection and virulent strains of *H. pylori* in these two regions are similar[75]. All *H. pylori* strains isolated from the Andean region, however, are from hpEurope, in contrast to strains isolated from the coastal line which are mainly from hpAfrica1[76]. Furthermore, strains from the Andean region caused more severe mucosal inflammation and more DNA damage in epithelial cells. This suggests that strains of hpEurope probably have an

increased carcinogenic potential compared with those of hpAfrica1[76]. Ancestral origin of the bacterium could be an important factor contributing to gastric carcinogenesis. This conclusion is further supported from a study conducted in Malaysia[7]. There are three ethnic groups in the country: Malay, Indian and Chinese. The infection rate of *H. pylori* in Malays is lower than that in Indian and Chinese subjects[79]. The incidence of gastric cancer, however, is similar in Malays and Indians, but is much lower than in the Chinese[80]. Analysis of the ancestral origin of *H. pylori* found that strains isolated from both Malay and Indian subjects belonged to hpAsia2, whereas those isolated from Chinese subjects belonged to hpEastAsia. This suggests a different potential for carcinogenesis between hpAsia2 and hpEastAsia. *H. pylori* populations generally reflect the geographical regions from which they are isolated. Differences in the incidence of gastric cancer among geographical regions could be in part attributed to different populations of *H. pylori*. Further study is required to investigate other bacterial factors involved in the carcinogenesis.

CONCLUSION

In summary, geographical separation reduces the frequency of recombination between *H. pylori* strains from a local area and those from outside. This leads to the formation of a clonal population structure of *H. pylori* in the local area. Thus, populations of *H. pylori* could be identified through examination of global strains. For each population, *H. pylori* have experienced relatively separate evolution processes, resulting in genomic diversity and differential potential for carcinogenesis. Further study to characterize these differences may help elucidate mechanisms involved in the development of gastric cancer induced by *H. pylori*.

REFERENCES

1 **Blaser MJ**, Atherton JC. Helicobacter pylori persistence: biology and disease. *J Clin Invest* 2004; **113**: 321-333
2 **Blaser MJ**. Helicobacter pylori and gastric diseases. *BMJ* 1998; **316**: 1507-1510
3 **Peek RM**, Crabtree JE. Helicobacter infection and gastric neoplasia. *J Pathol* 2006; **208**: 233-248
4 **Suerbaum S**, Michetti P. Helicobacter pylori infection. *N Engl J Med* 2002; **347**: 1175-1186
5 **Lehours P**, Yilmaz O. Epidemiology of Helicobacter pylori infection. *Helicobacter* 2007; **12** Suppl 1: 1-3
6 **Palli D**, Decarli A, Cipriani F, Sitas F, Forman D, Amadori D, Avellini C, Giacosa A, Manca P, Russo A. Helicobacter pylori antibodies in areas of Italy at varying gastric cancer risk. *Cancer Epidemiol Biomarkers Prev* 1993; **2**: 37-40
7 **Tay CY**, Mitchell H, Dong Q, Goh KL, Dawes IW, Lan R. Population structure of Helicobacter pylori among ethnic groups in Malaysia: recent acquisition of the bacterium by the Malay population. *BMC Microbiol* 2009; **9**: 126
8 **Moodley Y**, Linz B, Yamaoka Y, Windsor HM, Breurec S, Wu JY, Maady A, Bernhöft S, Thiberge JM, Phuanukoonnon S, Jobb G, Siba P, Graham DY, Marshall BJ, Achtman M. The peopling of the Pacific from a bacterial perspective. *Science* 2009; **323**: 527-530
9 **Falush D**, Wirth T, Linz B, Pritchard JK, Stephens M, Kidd M, Blaser MJ, Graham DY, Vacher S, Perez-Perez GI, Yamaoka Y, Mégraud F, Otto K, Reichard U, Katzowitsch E, Wang X, Achtman M, Suerbaum S. Traces of human migrations in Helicobacter pylori populations. *Science* 2003; **299**: 1582-1585
10 **Linz B**, Balloux F, Moodley Y, Manica A, Liu H, Roumagnac P, Falush D, Stamer C, Prugnolle F, van der Merwe SW, Yamaoka Y, Graham DY, Perez-Trallero E, Wadstrom T, Suerbaum S, Achtman M. An African origin for the intimate association between humans and Helicobacter pylori. *Nature* 2007; **445**: 915-918
11 **Achtman M**. Population structure of pathogenic bacteria revisited. *Int J Med Microbiol* 2004; **294**: 67-73
12 **Suerbaum S**, Achtman M. Evolution of Helicobacter pylori: the role of recombination. *Trends Microbiol* 1999; **7**: 182-184
13 **Akopyanz N**, Bukanov NO, Westblom TU, Kresovich S, Berg DE. DNA diversity among clinical isolates of Helicobacter pylori detected by PCR-based RAPD fingerprinting. *Nucleic Acids Res* 1992; **20**: 5137-5142
14 **Han FC**, Ng HC, Ho B. Stability of randomly amplified polymorphic DNA fingerprinting in genotyping clinical isolates of Helicobacter pylori. *World J Gastroenterol* 2003; **9**: 2021-2024
15 **Alm RA**, Ling LS, Moir DT, King BL, Brown ED, Doig PC, Smith DR, Noonan B, Guild BC, deJonge BL, Carmel G, Tummino PJ, Caruso A, Uria-Nickelsen M, Mills DM, Ives C, Gibson R, Merberg D, Mills SD, Jiang Q, Taylor DE, Vovis GF, Trust TJ. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen Helicobacter pylori. *Nature* 1999; **397**: 176-180
16 **Björkholm B**, Sjölund M, Falk PG, Berg OG, Engstrand L, Andersson DI. Mutation frequency and biological cost of antibiotic resistance in Helicobacter pylori. *Proc Natl Acad Sci USA* 2001; **98**: 14607-14612
17 **Pinto AV**, Mathieu A, Marsin S, Veaute X, Ielpi L, Labigne A, Radicella JP. Suppression of homologous and homeologous recombination by the bacterial MutS2 protein. *Mol Cell* 2005; **17**: 113-120
18 **García-Ortíz MV**, Marsin S, Arana ME, Gasparutto D, Guérois R, Kunkel TA, Radicella JP. Unexpected role for Helicobacter pylori DNA polymerase I as a source of genetic variability. *PLoS Genet* 2011; **7**: e1002152
19 **Suerbaum S**, Smith JM, Bapumia K, Morelli G, Smith NH, Kunstmann E, Dyrek I, Achtman M. Free recombination within Helicobacter pylori. *Proc Natl Acad Sci USA* 1998; **95**: 12619-12624
20 **Aras RA**, Kang J, Tschumi AI, Harasaki Y, Blaser MJ. Extensive repetitive DNA facilitates prokaryotic genome plasticity. *Proc Natl Acad Sci USA* 2003; **100**: 13579-13584
21 **Gressmann H**, Linz B, Ghai R, Pleissner KP, Schlapbach R, Yamaoka Y, Kraft C, Suerbaum S, Meyer TF, Achtman M. Gain and loss of multiple genes during the evolution of Helicobacter pylori. *PLoS Genet* 2005; **1**: e43
22 **Karnholz A**, Hoefler C, Odenbreit S, Fischer W, Hofreuter D, Haas R. Functional and topological characterization of novel components of the comB DNA transformation competence system in Helicobacter pylori. *J Bacteriol* 2006; **188**: 882-893
23 **Kersulyte D**, Mukhopadhyay AK, Velapatiño B, Su W, Pan Z, Garcia C, Hernandez V, Valdez Y, Mistry RS, Gilman RH, Yuan Y, Gao H, Alarcón T, López-Brea M, Balakrish Nair G, Chowdhury A, Datta S, Shirai M, Nakazawa T, Ally R, Segal I, Wong BC, Lam SK, Olfat FO, Borén T, Engstrand L, Torres O, Schneider R, Thomas JE, Czinn S, Berg DE. Differences in genotypes of Helicobacter pylori from different human populations. *J Bacteriol* 2000; **182**: 3210-3218
24 **Wirth T**, Wang X, Linz B, Novick RP, Lum JK, Blaser M, Morelli G, Falush D, Achtman M. Distinguishing human ethnic groups by means of sequences from Helicobacter pylori: lessons from Ladakh. *Proc Natl Acad Sci USA* 2004; **101**: 4746-4751
25 **Devi SM**, Ahmed I, Francalacci P, Hussain MA, Akhter Y,

Alvi A, Sechi LA, Mégraud F, Ahmed N. Ancestral European roots of Helicobacter pylori in India. *BMC Genomics* 2007; **8**: 184
26 **Breurec S**, Guillard B, Hem S, Brisse S, Dieye FB, Huerre M, Oung C, Raymond J, Tan TS, Thiberge JM, Vong S, Monchy D, Linz B. Evolutionary history of Helicobacter pylori sequences reflect past human migrations in Southeast Asia. *PLoS One* 2011; **6**: e22058
27 **Suzuki R**, Shiota S, Yamaoka Y. Molecular epidemiology, population genetics, and pathogenic role of Helicobacter pylori. *Infect Genet Evol* 2012; **12**: 203-213
28 **Correa P**, Piazuelo MB. Evolutionary History of the Helicobacter pylori Genome: Implications for Gastric Carcinogenesis. *Gut Liver* 2012; **6**: 21-28
29 **Ghose C**, Perez-Perez GI, Dominguez-Bello MG, Pride DT, Bravi CM, Blaser MJ. East Asian genotypes of Helicobacter pylori strains in Amerindians provide evidence for its ancient human carriage. *Proc Natl Acad Sci USA* 2002; **99**: 15107-15111
30 **Ng BL**, Ng HC, Goh KT, Ho B. Helicobacter pylori in familial clusters based on antibody profile. *FEMS Immunol Med Microbiol* 2001; **30**: 139-142
31 **Delport W**, Cunningham M, Olivier B, Preisig O, van der Merwe SW. A population genetics pedigree perspective on the transmission of Helicobacter pylori. *Genetics* 2006; **174**: 2107-2118
32 **Domínguez-Bello MG**, Pérez ME, Bortolini MC, Salzano FM, Pericchi LR, Zambrano-Guzmán O, Linz B. Amerindian Helicobacter pylori strains go extinct, as european strains expand their host range. *PLoS One* 2008; **3**: e3307
33 **Borén T**, Falk P, Roth KA, Larson G, Normark S. Attachment of Helicobacter pylori to human gastric epithelium mediated by blood group antigens. *Science* 1993; **262**: 1892-1895
34 **Tomb JF**, White O, Kerlavage AR, Clayton RA, Sutton GG, Fleischmann RD, Ketchum KA, Klenk HP, Gill S, Dougherty BA, Nelson K, Quackenbush J, Zhou L, Kirkness EF, Peterson S, Loftus B, Richardson D, Dodson R, Khalak HG, Glodek A, McKenney K, Fitzegerald LM, Lee N, Adams MD, Hickey EK, Berg DE, Gocayne JD, Utterback TR, Peterson JD, Kelley JM, Cotton MD, Weidman JM, Fujii C, Bowman C, Watthey L, Wallin E, Hayes WS, Borodovsky M, Karp PD, Smith HO, Fraser CM, Venter JC. The complete genome sequence of the gastric pathogen Helicobacter pylori. *Nature* 1997; **388**: 539-547
35 **Baltrus DA**, Amieva MR, Covacci A, Lowe TM, Merrell DS, Ottemann KM, Stein M, Salama NR, Guillemin K. The complete genome sequence of Helicobacter pylori strain G27. *J Bacteriol* 2009; **191**: 447-448
36 **Clancy CD**, Forde BM, Moore SA, O'Toole PW. Draft genome sequences of Helicobacter pylori strains 17874 and P79. *J Bacteriol* 2012; **194**: 2402
37 **Lehours P**, Vale FF, Bjursell MK, Melefors O, Advani R, Glavas S, Guegueniat J, Gontier E, Lacomme S, Alves Matos A, Menard A, Mégraud F, Engstrand L, Andersson AF. Genome sequencing reveals a phage in Helicobacter pylori. *MBio* 2011; **2**: e00239-11
38 **Schott T**, Kondadi PK, Hänninen ML, Rossi M. Comparative genomics of Helicobacter pylori and the human-derived Helicobacter bizzozeronii CIII-1 strain reveal the molecular basis of the zoonotic nature of non-pylori gastric Helicobacter infections in humans. *BMC Genomics* 2011; **12**: 534
39 **Kawai M**, Furuta Y, Yahara K, Tsuru T, Oshima K, Handa N, Takahashi N, Yoshida M, Azuma T, Hattori M, Uchiyama I, Kobayashi I. Evolution in an oncogenic bacterial species with extreme genome plasticity: Helicobacter pylori East Asian genomes. *BMC Microbiol* 2011; **11**: 104
40 **Avasthi TS**, Devi SH, Taylor TD, Kumar N, Baddam R, Kondo S, Suzuki Y, Lamouliatte H, Mégraud F, Ahmed N. Genomes of two chronological isolates (Helicobacter pylori 2017 and 2018) of the West African Helicobacter pylori strain 908 obtained from a single patient. *J Bacteriol* 2011; **193**: 3385-3386
41 **Kersulyte D**, Kalia A, Gilman RH, Mendez M, Herrera P, Cabrera L, Velapatiño B, Balqui J, Paredes Puente de la Vega F, Rodriguez Ulloa CA, Cok J, Hooper CC, Dailide G, Tamma S, Berg DE. Helicobacter pylori from Peruvian amerindians: traces of human migrations in strains from remote Amazon, and genome sequence of an Amerind strain. *PLoS One* 2010; **5**: e15076
42 **Devi SH**, Taylor TD, Avasthi TS, Kondo S, Suzuki Y, Megraud F, Ahmed N. Genome of Helicobacter pylori strain 908. *J Bacteriol* 2010; **192**: 6488-6489
43 **Thiberge JM**, Boursaux-Eude C, Lehours P, Dillies MA, Creno S, Coppée JY, Rouy Z, Lajus A, Ma L, Burucoa C, Ruskoné-Foumestraux A, Courillon-Mallet A, De Reuse H, Boneca IG, Lamarque D, Mégraud F, Delchier JC, Médigue C, Bouchier C, Labigne A, Raymond J. From array-based hybridization of Helicobacter pylori isolates to the complete genome sequence of an isolate associated with MALT lymphoma. *BMC Genomics* 2010; **11**: 368
44 **Farnbacher M**, Jahns T, Willrodt D, Daniel R, Haas R, Goesmann A, Kurtz S, Rieder G. Sequencing, annotation, and comparative genome analysis of the gerbil-adapted Helicobacter pylori strain B8. *BMC Genomics* 2010; **11**: 335
45 **Dong QJ**, Wang Q, Xin YN, Li N, Xuan SY. Comparative genomics of Helicobacter pylori. *World J Gastroenterol* 2009; **15**: 3984-3991
46 **McClain MS**, Shaffer CL, Israel DA, Peek RM, Cover TL. Genome sequence analysis of Helicobacter pylori strains associated with gastric ulceration and gastric cancer. *BMC Genomics* 2009; **10**: 3
47 **Oh JD**, Kling-Bäckhed H, Giannakis M, Xu J, Fulton RS, Fulton LA, Cordum HS, Wang C, Elliott G, Edwards J, Mardis ER, Engstrand LG, Gordon JI. The complete genome sequence of a chronic atrophic gastritis Helicobacter pylori strain: evolution during disease progression. *Proc Natl Acad Sci USA* 2006; **103**: 9999-10004
48 **McCutcheon JP**, Moran NA. Extreme genome reduction in symbiotic bacteria. *Nat Rev Microbiol* 2012; **10**: 13-26
49 **Kikuchi Y**, Hosokawa T, Nikoh N, Meng XY, Kamagata Y, Fukatsu T. Host-symbiont co-speciation and reductive genome evolution in gut symbiotic bacteria of acanthosomatid stinkbugs. *BMC Biol* 2009; **7**: 2
50 **Bruce MG**, Maaroos HI. Epidemiology of Helicobacter pylori infection. *Helicobacter* 2008; **13** Suppl 1: 1-6
51 **Goh KL**, Chan WK, Shiota S, Yamaoka Y. Epidemiology of Helicobacter pylori infection and public health implications. *Helicobacter* 2011; **16** Suppl **1**: 1-9
52 **Hille R**, Rétey J, Bartlewski-Hof U, Reichenbecher W. Mechanistic aspects of molybdenum-containing enzymes. *FEMS Microbiol Rev* 1998; **22**: 489-501
53 **Yamaoka Y**, Ojo O, Fujimoto S, Odenbreit S, Haas R, Gutierrez O, El-Zimaity HM, Reddy R, Arnqvist A, Graham DY. Helicobacter pylori outer membrane proteins and gastroduodenal disease. *Gut* 2006; **55**: 775-781
54 **Shao SH**, Wang H, Chai SG, Liu LM. Research progress on Helicobacter pylori outer membrane protein. *World J Gastroenterol* 2005; **11**: 3011-3013
55 **Censini S**, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, Rappuoli R, Covacci A. cag, a pathogenicity island of Helicobacter pylori, encodes type I-specific and disease-associated virulence factors. *Proc Natl Acad Sci USA* 1996; **93**: 14648-14653
56 **Covacci A**, Censini S, Bugnoli M, Petracca R, Burroni D, Macchia G, Massone A, Papini E, Xiang Z, Figura N. Molecular characterization of the 128-kDa immunodominant antigen of Helicobacter pylori associated with cytotoxicity and duodenal ulcer. *Proc Natl Acad Sci USA* 1993; **90**: 5791-5795
57 **Kutter S**, Buhrdorf R, Haas J, Schneider-Brachert W, Haas R, Fischer W. Protein subassemblies of the Helicobacter pylori Cag type IV secretion system revealed by localization and interaction studies. *J Bacteriol* 2008; **190**: 2161-2171

58 **Odenbreit S**, Püls J, Sedlmaier B, Gerland E, Fischer W, Haas R. Translocation of Helicobacter pylori CagA into gastric epithelial cells by type IV secretion. *Science* 2000; **287**: 1497-1500

59 **Murata-Kamiya N**. Pathophysiological functions of the CagA oncoprotein during infection by Helicobacter pylori. *Microbes Infect* 2011; **13**: 799-807

60 **Israel DA**, Salama N, Arnold CN, Moss SF, Ando T, Wirth HP, Tham KT, Camorlinga M, Blaser MJ, Falkow S, Peek RM. Helicobacter pylori strain-specific differences in genetic content, identified by microarray, influence host inflammatory responses. *J Clin Invest* 2001; **107**: 611-620

61 **Miehlke S**, Kirsch C, Agha-Amiri K, Günther T, Lehn N, Malfertheiner P, Stolte M, Ehninger G, Bayerdörffer E. The Helicobacter pylori vacA s1, m1 genotype and cagA is associated with gastric carcinoma in Germany. *Int J Cancer* 2000; **87**: 322-327

62 **Plummer M**, Vivas J, Fauchère JL, Del Giudice G, Peña AS, Ponzetto A, Lopez G, Miki K, Oliver W, Muñoz N. Helicobacter pylori and stomach cancer: a case-control study in Venezuela. *Cancer Epidemiol Biomarkers Prev* 2000; **9**: 961-965

63 **Owen RJ**, Peters TM, Varea R, Teare EL, Saverymuttu S. Molecular epidemiology of Helicobacter pylori in England: prevalence of cag pathogenicity island markers and IS605 presence in relation to patient age and severity of gastric disease. *FEMS Immunol Med Microbiol* 2001; **30**: 65-71

64 **van der Ende A**, Pan ZJ, Bart A, van der Hulst RW, Feller M, Xiao SD, Tytgat GN, Dankert J. cagA-positive Helicobacter pylori populations in China and The Netherlands are distinct. *Infect Immun* 1998; **66**: 1822-1826

65 **Watada M**, Shiota S, Matsunari O, Suzuki R, Murakami K, Fujioka T, Yamaoka Y. Association between Helicobacter pylori cagA-related genes and clinical outcomes in Colombia and Japan. *BMC Gastroenterol* 2011; **11**: 141

66 **Salama N**, Guillemin K, McDaniel TK, Sherlock G, Tompkins L, Falkow S. A whole-genome microarray reveals genetic diversity among Helicobacter pylori strains. *Proc Natl Acad Sci USA* 2000; **97**: 14668-14673

67 **Kauser F**, Khan AA, Hussain MA, Carroll IM, Ahmad N, Tiwari S, Shouche Y, Das B, Alam M, Ali SM, Habibullah CM, Sierra R, Megraud F, Sechi LA, Ahmed N. The cag pathogenicity island of Helicobacter pylori is disrupted in the majority of patient isolates from different human populations. *J Clin Microbiol* 2004; **42**: 5302-5308

68 **Bertuccio P**, Chatenoud L, Levi F, Praud D, Ferlay J, Negri E, Malvezzi M, La Vecchia C. Recent patterns in gastric cancer: a global overview. *Int J Cancer* 2009; **125**: 666-673

69 **Malaty HM**. Epidemiology of Helicobacter pylori infection. *Best Pract Res Clin Gastroenterol* 2007; **21**: 205-214

70 **Holcombe C**. Helicobacter pylori: the African enigma. *Gut* 1992; **33**: 429-431

71 **Mbulaiteye SM**, Hisada M, El-Omar EM. Helicobacter Pylori associated global gastric cancer burden. *Front Biosci* 2009; **14**: 1490-1504

72 **You WC**, Zhang L, Gail MH, Li JY, Chang YS, Blot WJ, Zhao CL, Liu WD, Li HQ, Ma JL, Hu YR, Bravo JC, Correa P, Xu GW, Fraumeni JF. Precancerous lesions in two counties of China with contrasting gastric cancer risk. *Int J Epidemiol* 1998; **27**: 945-948

73 **You WC**, Li JY, Blot WJ, Chang YS, Jin ML, Gail MH, Zhang L, Liu WD, Ma JL, Hu YR, Mark SD, Correa P, Fraumeni JF, Xu GW. Evolution of precancerous lesions in a rural Chinese population at high risk of gastric cancer. *Int J Cancer* 1999; **83**: 615-619

74 **Groves FD**, Perez-Perez G, Zhang L, You WC, Lipsitz SR, Gail MH, Fraumeni JF, Blaser MJ. Serum antibodies to Helicobacter pylori and the CagA antigen do not explain differences in the prevalence of precancerous gastric lesions in two Chinese populations with contrasting gastric cancer rates. *Cancer Epidemiol Biomarkers Prev* 2002; **11**: 1091-1094

75 **Torres J**, Lopez L, Lazcano E, Camorlinga M, Flores L, Muñoz O. Trends in Helicobacter pylori infection and gastric cancer in Mexico. *Cancer Epidemiol Biomarkers Prev* 2005; **14**: 1874-1877

76 **de Sablet T**, Piazuelo MB, Shaffer CL, Schneider BG, Asim M, Chaturvedi R, Bravo LE, Sicinschi LA, Delgado AG, Mera RM, Israel DA, Romero-Gallo J, Peek RM, Cover TL, Correa P, Wilson KT. Phylogeographic origin of Helicobacter pylori is a determinant of gastric cancer risk. *Gut* 2011; **60**: 1189-1195

77 **Correa P**, Cuello C, Duque E, Burbano LC, Garcia FT, Bolanos O, Brown C, Haenszel W. Gastric cancer in Colombia. III. Natural history of precursor lesions. *J Natl Cancer Inst* 1976; **57**: 1027-1035

78 **Cuello C**, Correa P, Haenszel W, Gordillo G, Brown C, Archer M, Tannenbaum S. Gastric cancer in Colombia. I. Cancer risk and suspect environmental agents. *J Natl Cancer Inst* 1976; **57**: 1015-1020

79 **Goh KL**, Parasakthi N. The racial cohort phenomenon: sero-epidemiology of Helicobacter pylori infection in a multiracial South-East Asian country. *Eur J Gastroenterol Hepatol* 2001; **13**: 177-183

80 **Goh KL**, Cheah PL, Md N, Quek KF, Parasakthi N. Ethnicity and H. pylori as risk factors for gastric cancer in Malaysia: A prospective case control study. *Am J Gastroenterol* 2007; **102**: 40-45

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