SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) GENOTYPIC PROFILING OF MALAY PATIENTS WITH AND WITHOUT *Helicobacter pylori* INFECTION IN KELANTAN

By

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Thesis Submitted in fulfilment of the requirements for the degree of

Master of Science

APRIL 2012

DEDICATIONS

To My Family

For their never ending love and unrestricted support

To Dr Noorizan, Prof Dr Zilfalil and Dr Lee Yeong Yeh

For their unconditional supports and sincerity

To Siti Nur Fatimah & Rani,

"Live as if you were to die tomorrow. Learn as if you were to live forever"~**Mahatma Gandhi**

To Genomians of Human Genome Center, USM

"Success is sweet: the sweeter if long delayed and attained through manifold struggles and defeats" ~**A. Branson Alcott**

ACKNOWLEDGEMENTS

"Keep away from people who try to belittle your ambitions. Small people always do that, but the really great make you feel that you, too, can become great."

~Mark Twain

I would like to take this golden opportunity to acknowledge all the 'great' people that I have met along in this voyage called MSc.

First and foremost, I would like to thank my greatest teacher of all: God. I will do my very best and will never forget what a great fortune I have had by just being here, even though it comes with lessons and responsibilities.

My deepest appreciation goes to my supervisor, Dr Noorizan HA Majid, for giving me this opportunity and for believing in me. My heartfelt gratitude goes to Prof Dr Zilfalil bin Alwi for putting me in the idea of shooting for MSc and showing me the 'beauty' of research. Despite being in University of Glassgow, Scotland thank you Dr Lee Yeong Yeh for all the help that you have handed over in thesis and manuscript writing.

I am very much grateful to Prof Xu Shuhua for giving me the internship opportunity at Max Planck's Partner Institute for Computational Biology (PICB), Shanghai Institute for Biological Sciences, Shanghai China. Without this opportunity Microarray data analysis would have been almost impossible. I am also most

indebted to Dongshen, Wenfei, Shimeng and Pengfei from PICB, Shanghai for helping me learn the nooks and crooks of microarray data analysis.

My sincere appreciation goes to Assoc Prof Dr Gan Siew Hua, Prof Ravindran Ankhatil, Assoc Prof Dr T.P Kannan, Dr Teguh Haryo Sasongko and Dr Sarina Sulong from Human Genome Center for being very supportive.

I would like to thank the Fundamental Research Grant Scheme (FRGS) and USM Student Fellowship Scheme. Without their grant and Scholarship respectively this MSc would not have been possible.

Not forgetting my dear friends and colleagues that I have met along this journey, especially Siti, Rani, Ina, Huda, Iman, Sha and Hatin. Your friendship throughout these last couple of years has provided me with an impressively beautiful site to see. I also would like to thank you for creating an environment of humour around this whole ordeal.

Finally, my mother- for giving me the life I have now. She is the reason I did this; she is the reason I thrive to be better. Her pride for me is my main goal in life. Thanking you always.

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LIST OF ABBREVIATIONS

e.g.	for example
%	Percentage
***.CEL file**	Files containing intensity calculations
***.CHP file**	Files containing probe set analysis
***.DAT file**	Files containing pixel intensity value of a scanned array
<	Less than
>	More than
°C	Degree Celsius
µl	Microlitre
α	alpha
AE buffer	Elution Buffer
AGCC	GeneChip Command Console® Software
AW1	Wash Buffer 1
AW2	Wash Buffer 2
bp	basepairs
BRLMM	Bayesian Robust Linear Model with Mahalanobis distance classifier

CD-CV	Common disease- common variant
CNV	Copy Number Variation
CYP2C19	Cytochrome P450 2C19
C7orf10	Chromosome 7 open reading frame 10.
DAVID	Database for Annotation, Visualization and Integrated Discovery
DCC	Deleted in Colorectal Cancer
dH_2O	Distilled water
DM Algorithm	Dynamic Model Algorithm
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
EB Buffer	Elution Buffer
EDTA	Ethylenediaminetetraacetic acid
EDTA-TE	Ethylenediaminetetraacetic acid-Tris
EHPSG	The European *H. pylori* Study Group
ELISA	Enzyme Linked Immunoabsorbent Assay
F_{ST}	Fixation Index
GTC	Genotyping Console Software

GWAS	Genome Wide Association Study
H&E	Hematoxylin and Eosin
Hg	mercury
H. pylori	*Helicobacter pylori*
HC-SI	Han Chinese- South Indians
IgG	Immunoglobulin
LOH	Loss of Heterozygosity
MK-HC	Malay Kelantanese-Han Chinese
MK-SI	Malay Kelantane-South Indians
HSDNA	Herring Sperm- deoxyribonucleic acid
HUGO	Human Genome Project
HWE	Hardy Weinberg Equilibrium
IM	Intestinal metaplasia
LCD	Liquid Crystal Density
MAF	Minor Allele Frequency
MALT	Mucosa Associated Lymphoid Tissue
MES	4-Morpholineethanesulfonic acid sodium salt
mg	milligram

MgSO4	Magnesium sulphate
MGST1 gene	Microsomal glutathione S-transferase 1
min	minute
mL	millilitre
Mm	millimetre
MM	Mismatch
mRNA	Messenger RNA
n	number
NaCl	Sodium Chloride
NE buffer	Restriction endonucleus buffer
NER	Nucleotide Excision Repair
NetAffx	Affymetrix probesets and analysis
NIAID	National Institute of Allergy and Infectious Diseases
NMD	Nonsense-mediated decay
ng	Nanogram
OGDS	oesophago-gastric-duodeno-scopy
PEAS	Package for Elementary Analysis of SNP data
PCR	Polymerase Chain Reaction

PPI	Proton Pump Inhibitor
PM	Perfect Match
QC	Quality Control
QTL	quantitative trait loci
ROS	reactive oxygen species
RAS	Relative Allele Signals
RFLP	Restriction Fragment Length Polymorphism
RNS	reactive nitrogen species
rpm	Rotation per minute
SAPE	Streptavidin Phycoerythin
SMG7	Smg-7 homolog, nonsense mediated mRNA decay factor
SNP	Single Nucleotide Polymorphism
SSPE	Saline Sodium Phosphate EDTA
TBE	Tris-Borate-EDTA buffer
TdT Buffer	Terminal Deoxynucleotidyl Transferase
TE Buffer	Tris-EDTA buffer
THBS4	Thrombospondin-4
TMACL	Tetramethyl Ammonium Chloride

TSTD2 gene	Thiosulfate sulphur transferase
U	Unit
UPP	Ubiquitin Proteasome Pathway
UFC1	ufm-1 conjugating enzyme
UFM1	Ubiquitin-fold modifier 1
V	voltage
WHO	World Health Organization
x^2	Chi-squared
XPA	xeroderma pigmentosum
***Xba* 1**	Restriction enzyme of an *E. coli* strain that carries the *xba*I gene from *Xanthomonas badrii*
3'-UTR	Three prime untranslated region

LIST OF WEB RESOURCES

AFFYMETRIX	http://www.affymetrix.com/estore/
DAVID	http://david.abcc.ncifcrf.gov/
dbSNP	http://www.ncbi.nlm.nih.gov/SNP/
Entrez Gene	http://www.ncbi.nlm.nih.gov/gene
F-SNP	http://compbio.cs.queensu.ca/F-SNP/
FAMHAP	http://famhap.meb.uni-bonn.de/
Geneatlas	http://www.geneatlas.org/
GeneCards	http://www.genecards.org/
NCBI Gene	http://www.ncbi.nlm.nih.gov/sites/entrez?db¼ gene
NCBI Genome Browser	http://www.ncbi.nlm.nih.gov/projects/mapview/map_search. cgi? taxid¼9606
Online Mendelian Inheritance in Man (OMIM)	http://www.ncbi. nlm.nih.gov/sites/entrez?db¼omim
SVS Golden Helix Bioinformatics Software	http://www.goldenhelix.com/Company/about. html
UCSC Genome Browser	http://genome.ucsc.edu/cgi-bin/hgGateway

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PENGENOTIPAN PROFIL NUKLEOTIDA POLIMORFISME TUNGGAL DI KALANGAN PESAKIT MELAYU DENGAN DAN TANPA JANGKITAN *Helicobacter Pylori* DI KELANTAN

ABSTRAK

Perbezaan terhadap jangkitan *Helicobacter pylori* (*H. pylori*) dikalangan tiga etnik utama di Malaysia telah dikenal pasti sejak pertama kali ianya dilaporkan pada tahun 1986 oleh Persatuan Patalogi Malaysia. Negeri Kelantan, yang terletak di timur laut semenanjung Malaysia adalah unik dengan 90% daripada keseluruhan penduduknya terdiri daripada etnik Melayu. Kadar kelaziman *H. pylori* yang secara luar biasanya rendah telah dilaporkan di negeri ini dengan peratusan hanya sebanyak 4-5%. Faktor penyebab tepat bagi kadar kelaziman rendah terhadap jangkitan *H. pylori* ini tidak diketahui. Faktor-faktor persekitaraan dan faktor-faktor genetik atau gabungan kedua-duanya mungkin dapat menjelaskan keadaan ini. Kajian untuk menentukan variasi genetik yang memainkan peranan penting dalam melindungi orang Melayu terhadap jangkitan *H. pylori* telah dijalankan dengan menggunakan nukleotida polimorfisme tunggal (SNP). Sejumlah 23 kes (*H. pylori* positif) dan 37 kawalan (*H. pylori* negatif) telah digenotip dalam kajian kes-kawalan. Analisis data telah dilaksanakan dengan Affymetrix Genotyping Console (GTC), perisisan SVS Golden Helix Bioinformatics, perisian DAVID Bioinformatics, program FAMHAP dan F-SNP. Bagi menentukan variasi genetik yang menjadi penyebab dalam perubahan fenotip yang dimanifestasikan semasa infeksi *H. pylori*, analisis genotip-fenotip telah dilaksanakan. Kami telah menemui kaitan yang signifikan antara SNP rs678264, rs10505799, rs9315542 masing-masing dengan metaplasia usus, dysplasia, metaplasia usus serta dysplasia dan gastritis atropik. Disamping itu, kami juga telah

menemui SNP rs10502974 yang terletak di dalam gen DCC sebagai variasi genetik penyebab risisko jangkitan *H. pylori* di kalangan orang Melayu Kelantan. Akhir sekali, kami mencadangkan bahawa rs29886, rs3750370, rs3768591, rs3176670 dan rs3176673 sebagai variasi genetik yang berpotensi untuk melindungi orang Melayu Kelantan terhadap jangkitan *H. pylori*. Sebagai kesimpulan, dengan menggunakn pendekatan genom, kajian ini telah berjaya mengenal pasti variasi-variasi genetik yang boleh dikaitkan dengan jangkitan *H. pylori* di kalangan populasi Melayu Kelantan.

SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) GENOTYPIC PROFILING OF MALAY PATIENTS WITH AND WITHOUT *Helicobacter pylori* INFECTION IN KELANTAN

ABSTRACT

Since first reported in 1986 in Malaysia by the Malaysian Pathology Society, differences in the *Helicobacter pylori* (*H. pylori*) prevalence among the three major ethnics has been much characterized. The state of Kelantan, situated at the north-eastern region of Peninsular Malaysia is unique with Malays comprising 90% of its population. There is an exceptionally low prevalence rate of *H. pylori* reported from this region, in the range of 4-5%. The exact reasons for the low prevalence rate of *H. pylori* infection are unknown. Environmental factors, genetic factors or a combination of both are possible explanation. The current study sets out to determine which of the genetic variant in the form of Single Nucleotide Polymorphisms (SNPs) using the genome wide association, may play a role in protecting the Malays against *H. pylori* infection. A total of 23 cases (*H. pylori* positive) and 37 controls (*H. pylori* negative) were genotyped in this case-control study. High-throughput downstream analysis was conducted using the Affymetrix Genotyping Console (GTC), SVS Golden Helix Bioinformatics Software, DAVID Bioinformatics software, FAMHAP programme and F-SNP tool. Phenotype-Genotype analysis was done in resolving the causative genetic variants causing phenotypic alterations that are manifested during *H. pylori* infection. We found a significant association between SNPs rs678264, rs10505799 and rs9315542 and intestinal metaplasia, dysplasia, and intestinal metaplasia with atrophic gastritis respectively. In addition we also found that the

SNPs rs10502974 located within the DCC gene was associated with an increased risk of *H. pylori* infection among Malays in Kelantan. Finally, our study also suggested that the SNPs rs29886, rs3750370, rs3768591, rs3176670 and rs3176673 as potential genetic variants which may protect the Malays against *H. pylori* infection. Hence, this study concludes the determination of candidate genetic variants associated with *H. pylori* infection in Malays in Kelantan for the first time using the genome wide association approach.

CHAPTER 1

INTRODUCTION

Approximately half of the world's population is infected with *Helicobacter pylori* (*H. pylori*), a gram negative microaerophilic bacterium found in the human gastric epithelium (Volk and Parsonnet 2009). Prevalence of *H. pylori* varies differently across different geographical regions with higher prevalence seen mainly in Asia. The infection tends to be lifelong unless treated. The great importance of *H. pylori* lies in its disease association with peptic ulcers, atrophic gastritis, and gastric adenocarcinoma (Goh, 2009).

H. pylori was first reported in Malaysia in 1986 by the Malaysian Pathology Society and subsequently the existence of differences in the infection rate between the three major ethnic groups in Malaysia; Malay, Chinese and Indian patients was highlighted - a low prevalence amongst Malays and a significantly higher prevalence among the Chinese and Indians (Goh, 2009).

In 2001, Goh and Parasakhti proposed the "racial cohort" theory to explain the differences in *H. pylori* infection rate among the three different races in Malaysia. The authors reported that even though there was a low level of intermarriages between races in Malaysia, *H. pylori* remained confined to a particular racial group. The Malays who were believed to have a low reservoir of infection to begin with, continue to have a low prevalence of infection.

The high prevalence of *H. pylori* infection amongst the Chinese and Indians in Malaysia reflected the high prevalence in Southern China and Southern India respectively from where these races had originally come from (Goh and Parasakthi, 2001).

Table 1.1 summarises a study conducted by Sasidharan *et al*. (2008) at Hospital Seberang Jaya, Penang, Northern Peninsular Malaysia. This table further supports the findings by Goh (2007) and Gurjeet,K. & Naing,N.N. (2003) that *H. pylori* infection rate is low among the Malays (Goh, 2007).

Table 1.1: *Helicobacter pylori* infection rate in relation to ethnic groups (Sasidharan *et al.*)

Ethnicity	**Total**	***H. pylori* present**	***H. pylori* absent**
		n (%)	*n* (%)
Malay	276	16(5.8)	260(94.2)
Chinese	229	44(19.2)	185(80.8)
Indians	166	36(21.3)	130(78.3)

Kelantan situated in the north-eastern region of Peninsular Malaysia is unique with 90% of its population consisting of Malays. It was reported that Malays from this region had an exceptionally low seroprevalence rate of *H. pylori* in the range of 4-5% (Uyub *et al.*, 1994). Goh and Parasakhti (2000) also reported that the Malays in Kelantan had been reported to have a low *H. pylori* infection rate compared to the other two ethnic groups mentioned.

Seroepidemiological studies and endoscopic surveys from the north-eastern region of peninsular Malaysia reported an unexpectedly low prevalence rate of about 5% in the general population (Uyub *et al.*, 1994) whereas a 26-60% overall prevalence rate was reported in Malaysia (Goh and Parasakthi, 2001).

The prevalence of *H. pylori* infection can vary greatly across different geographical areas and this variation was observed most evidently in Malaysia. The speculation to what extent; of different ethnicity portraying different socio-economic and socio-cultural practices are correlated with *H. pylori* infection has always been questioned (Sasidharan *et al.*, 2008).

A low socioeconomic status associated with high density living and inferior hygienic condition has been reported by Uyub *et al*. (1994) to play a major role in the transmission of *H. pylori.*

The population from the north-eastern region of peninsular Malaysia is poorer and more rural compared to the west coast (Gurjeet,K. and Naing,N.N, 2003).Therefore if as reported, urbanization and overcrowding is suppose to contribute to the

transmission and perpetuation of *H. pylori* infection, a uniform infection rate among the various ethnic groups should be observed. However this was not depicted in the observed racial differences. The Chinese and Indians reported a higher infection rate compared to the Malays.

The exact reasons for the low prevalence rate of *H. pylori* infection are unknown. Environmental factors, genetic factors or a combination of both can possibly explain this observation. Current study sets out to determine which of the genetic variants in the form of Single Nucleotide Polymorphisms (SNPs) using the genome wide association may play a role in protecting the Malays against *H. pylori*.

Strategy for eliciting the genetic influence on disease relies on examining a large numbers of SNPs in affected individuals and controls, and this is only possible due to recently devised high throughput technologies (Xiao *et al.*, 2007) .

SNPs are sites in the genome where individuals differ in DNA sequence by a single base pair. There are around 10 million common SNPs that constitute 90% of the variation in the current human population (The International HapMap Consortium) (2003). Although most SNPs have no characterized role in cell functions, selected SNPs associated with altered proteins or phenotypic traits have been found (Xiao *et al.*, 2007).

The sensitivity and high-throughput nature of hybridization-based DNA microarray technology provide an ideal platform for such applications by interrogating up to hundreds of thousands of single nucleotide polymorphisms (SNPs) in a single assay (Xiao *et al.*, 2007).

1.1 OBJECTIVES

The causes for the exceptionally low prevalence of infection rate among the Malay Kelantanese have always been a mystery among researchers. The availability of recent high-throughput screening methods such as microarray have led to tremendous improvements in genome wide screenings in identifying possible mechanism of infection and protective genes that causes the low *H. pylori* infection rate. Because of microarray's ability to screen the whole genome, Affymetrix *xba1* 50k SNP Genotyping was employed in this study to investigate the influence of genetic factors in *H. pylori* infection among the Malay Kelantanese.

The specific objectives of this study were as below:

i. To determine the SNP profiles between patients infected with *Helicobacter pylori* and non-infected Malay Kelantanese controls

ii. To determine the genotype and phenotype association between the SNP profiles of patients infected with *Helicobacter pylori* and harbouring intestinal metaplasia, dysplasia and atrophic gastritis

iii. To determine the SNPs that predispose the Malay Kelantanese to *Helicobacter pylori* infection

iv. To determine the SNPs that confers "protection" against *Helicobacter pylori* infection among Malay Kelantanese

CHAPTER 2

LITERATURE REVIEW

2.1 *Helicobacter pylori*

In 1979, the relationship between *Helicobacter pylori* (*H. pylori*) (Figure 2.1), gastritis and ulceration was first discovered by Robin Warren and the organism was subsequently cultured by Barry Marshall in 1982. Since then *H. pylori* has been the subject of intense investigations and have provoked the interests of bacteriologists, gastroenterologists, infectious disease specialists, cancer biologists, epidemiologists, pathologists, and pharmaceutical scientists.

H. pylori is a helix-shaped Gram-negative bacterium and is microaerophilic, thus requiring oxygen at a low concentration. *H. pylori* is capable of forming biofilms which aid its conversion from a spiral shape to a possibly viable but nonculturable coccoid form, which favors its survival (Stark *et al.*, 1999). The coccoid form is important for adherence to gastric epithelial cells *in-vitro* (Liu *et al.*, 2006).

H. pylori is highly motile by means of one or more polar sheathed flagella, each containing a terminal bulb. Its shape and motility permits the microbe to manoeuvre easily through the gastric mucous layer (Segal *et al.*, 1996).

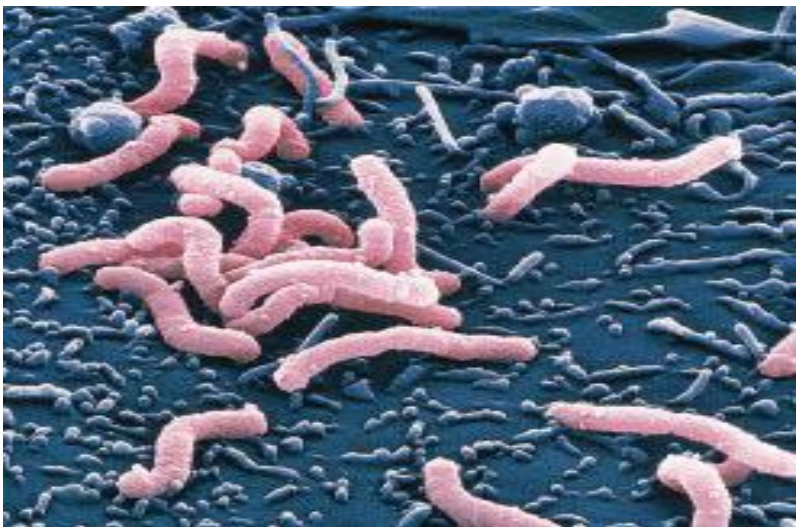

Figure 2.1: Coloured scanning electron micrograph of *H. pylori* on surface of gastric cell (Logan and Walker, 2001)

2.2 Pathophysiology of *Helicobacter pylori* Infection.

To colonize the stomach, *H. pylori* must survive the acidic pH of the lumen and burrows into the mucus layers to reach its niche, which is close to the stomach's epithelial cell layer. *H. pylori* has flagella which aids its movement through the stomach lumen and into the mucous lining of the stomach.

The bacterium is usually found deep in the mucous layer, which is then continuously secreted by the mucous cells to the luminal side. *H. pylori* detects the changes in pH gradient by chemotaxis mechanism and thus swims away from the acidic contents of the lumen towards the more neutral pH environment of the epithelial cell surface (Ottemann and Lowenthal, 2002).

H. pylori is also found on the inner surface of the stomach epithelial cells and occasionally inside the epithelial cells (Schreiber *et al.*, 2004). It produces adhesions which binds to the membrane-associated lipids and carbohydrates and helps its adherence to the epithelial cells (Petersen and Krogfelt, 2003).

H. pylori produces large amounts of urease, which breaks down urea in the stomach to carbon dioxide and ammonia. Ammonia is converted into ammonium ion which is then broken down into hydrogen and hydroxyl ions.

Hydroxyl ion reacts with carbon dioxide and produces bicarbonate which neutralizes gastric acid (Ilver *et al.*, 1998). Thus, the primary mechanism for *H. pylori* survival in the acidic stomach is completely dependent on urease, for if not then it will eventually die.

The colonization of stomach by *H. pylori* often results in inflammation of the stomach lining (Figure 2.2). The degree and severity of inflammation is likely to underlie *H. pylori* related diseases. Duodenal and stomach ulcers are consequences of inflammation allowing the acid and pepsin in the stomach lumen to overwhelm the mechanisms that protect the stomach and the duodenal mucosa from these caustic substances (Shiotani and Graham, 2002).

The type of ulcers that develop during infection depends on the location of chronic gastritis, which occurs at the site of *H. pylori* colonization. The acidity within the stomach lumen affects the colonization pattern of *H. pylori* and therefore ultimately determines whether a duodenal or gastric ulcer will form (Dixon, 2000).

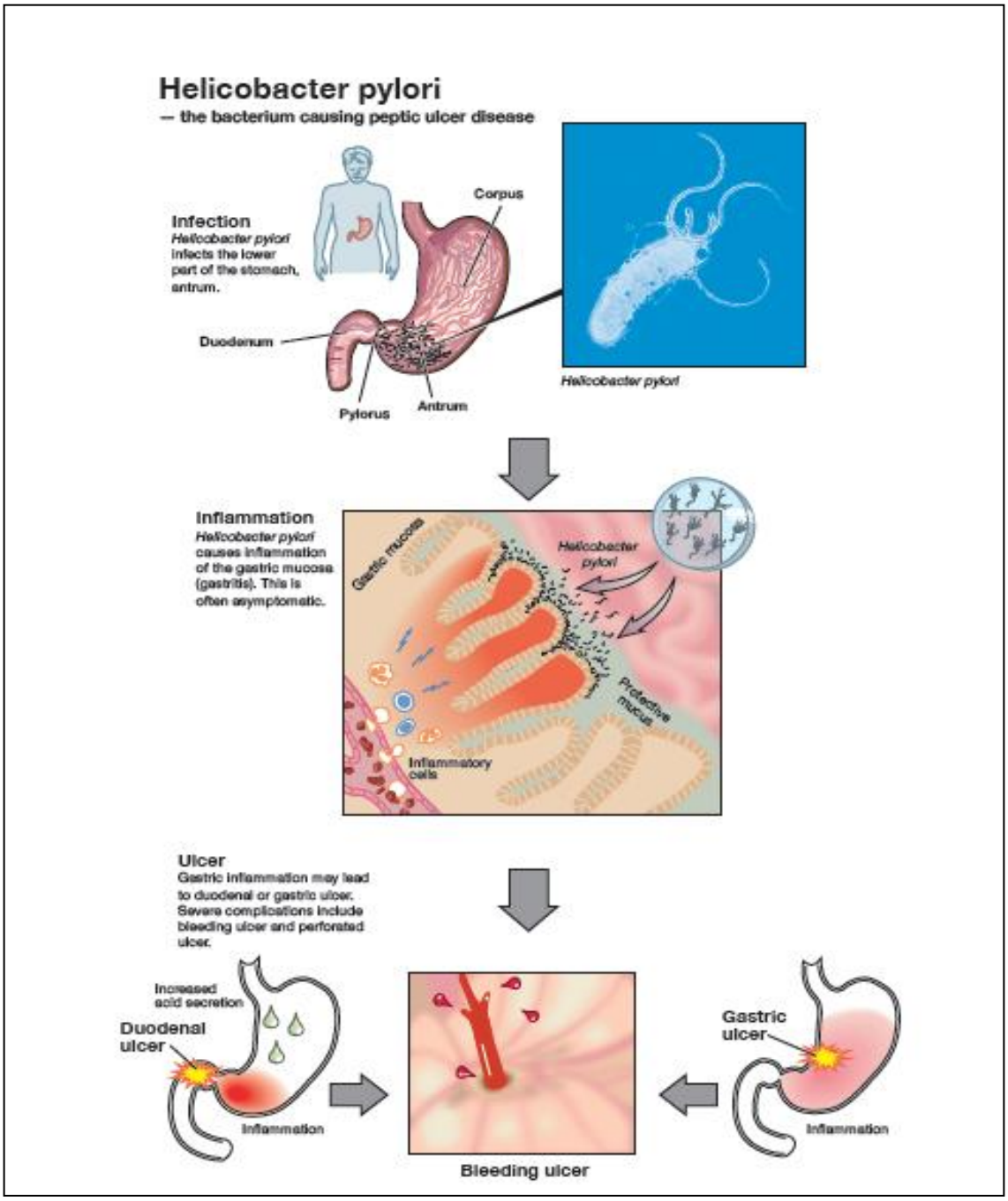

Figure 2.2: Colonization and infection of *Helicobacter pylori*

In individuals producing large amounts of acid, *H. pylori* colonizes the antrum of the stomach to avoid the acid-secreting parietal cells located in the corpus of the stomach (Kusters *et al.*, 2006). The inflammatory response to the bacteria induces G cells in the antrum to secrete the hormone gastrin, which travels through the bloodstream to the corpus.

Gastrin stimulates the parietal cells in the corpus to secrete even more acid into the stomach lumen and the increased gastrin levels causes the number of parietal cells to increase, further escalating the amount of acid secreted (Blaser and Atherton, 2004). The increased acid load damages the duodenum resulting in ulceration.

In contrast, gastric ulcers are often associated with normal or reduced gastric acid production, suggesting that the mechanisms that protect the gastric mucosa are defective (Schubert and Peura, 2008). In these patients *H. pylori* can also colonize the corpus of the stomach, where the acid-secreting parietal cells are located (Suerbaum and Michetti, 2002). However chronic inflammations induced by the bacteria further reduces the production of acid and atrophy of the stomach lining, which may lead to gastric ulcer and increases the risk for stomach cancer (Suerbaum and Michetti, 2002).

2.3 Epidemiology of *Helicobacter pylori* Infection

To date, it is not possible to predict the outcome of *H. pylori* infection in a colonized untreated individual to identify human populations at risk for significant pathology (Bjorkholm *et al.*, 2001). The outcome of infection may depend on the interplay between host, micro-organism and environmental factors (Bjorkholm *et al.*, 2001).

Although *H. pylori* infection is ubiquitous and infects both females and males (Goh *et al.*, 1996), most of the *H. pylori* related diseases are associated with the male gender (de Martel and Parsonnet, 2006). A meta-analysis of a population based study by de Martel and Parsonnet (2006), confirmed that male predominance of *H. pylori* infection in adults was a global and homogeneous phenomenon and was consistent across population from various countries.

Men are on average 16% more often infected with *H. pylori* than women and this finding may partially explain the male predominance of *H. pylori* related adult diseases, including duodenal ulcer and gastric adenocarcinoma.

The prevalence of *H. pylori* infection within and between countries was reported to be significantly different (Goh *et al.*, 1996). In general, *H. pylori* infection has been reported to be lower in developed countries than in developing countries. This difference had been attributed to the rate of acquisition of *H. pylori* during childhood period (Mitchell *et al.*, 1996).

Epidemiological data from developed and developing countries supports this finding, given the prevalence of *H. pylori* infection among children under 10 years in developed countries being approximately 0 % to 5% lower compared to 13% to 60% in developing countries (Graham *et al.*, 1991). It has been proposed that individuals are infected during childhood and the prevalence decreases with age, particularly in developed countries, due to advanced improvement in medical care, sanitation and/or living condition (Banatvala *et al.*, 1993).

2.4 Infection and Transmission of *Helicobacter pylori*

Once *H.pylori* is established within the gastric mucosa, the bacterium persists for life and a number of studies have proposed that acquisition of *H. pylori* occurred via a common environmental source. In particular, animals and water have been implicated as potential sources of infection.

According to Das and Paul (2007), *H.pylori* infection was transmitted mainly through fecal-oral route in developing countries and gastro-oral route in developed countries. The finding of increased *H. pylori* prevalence in institutionalized subjects supported this view and suggested that close personal contact is important for the spread of *H. pylori* (Berkowicz and Lee, 1987).

Transmission of "close-contact infection" was dependent on the degree of mixing between susceptible and infected individuals, and on the degree of crowding and age distribution among those susceptible to infection and those infected (Das and Paul, 2007).

The initial phase of *H. pylori* infection is subclinical which starts with ingestion, organism's penetration through the mucous layer and followed by multiplication in close proximity to the surface epithelial cells. The epithelium then responds to infection by mucin depletion, cellular exfoliation and compensatory regenerative changes.

Vector transmission is also suggested as a mode of infection, and this was biologically plausible because the midgut of housefly has a favourable pH of 3.1 and this has provided an etiological niche for *H. pylori* infection (Das and Paul, 2007).

2.5 Bacterial Virulence and Pathogenic Mechanism.

Racial difference in *H. pylori* seroprevalence was observed in Singapore with Indians having a higher prevalence of *H. pylori* antibodies followed by the Chinese. The increased risk of infection among the Indians and the Chinese maybe due to different sociocultural practices peculiar to each race (Gurjeet,K. and Naing,N.N. 2003).

Communal eating habits which allow close personal contact and the inherent genetic predisposition that plays a role in host bacteria interaction may be a strong plausible explanation as to the wide difference in infection rates among the races (Gurjeet,K. and Naing,N.N. 2003).

Besides environmental and genetic factors, the pathogenicity of certain virulent *H. pylori* strains also plays a role in predisposing certain individuals to infection by the bacterium. The bacterial determinant (pathogenicity) of *H. pylori* infection is influenced by the presence of cytotoxin associated gene A (*cag*A gene) and vaculating cytotoxin gene (*vac*A gene) (Mattar and Laudanna, 2000).

The *cag*A gene product is not itself virulent but is part of a 40kb cluster genes known as cag Pathogenicity Island (cagPAI) that contributes to its pathogenicity. The pathogenicity island consists of about 30 genes, and some of these genes are of particular importance. CagA (Figure 2.3) was inserted into the epithelial cell by the Cag pathogenicity island structure (Marshall, 2001).

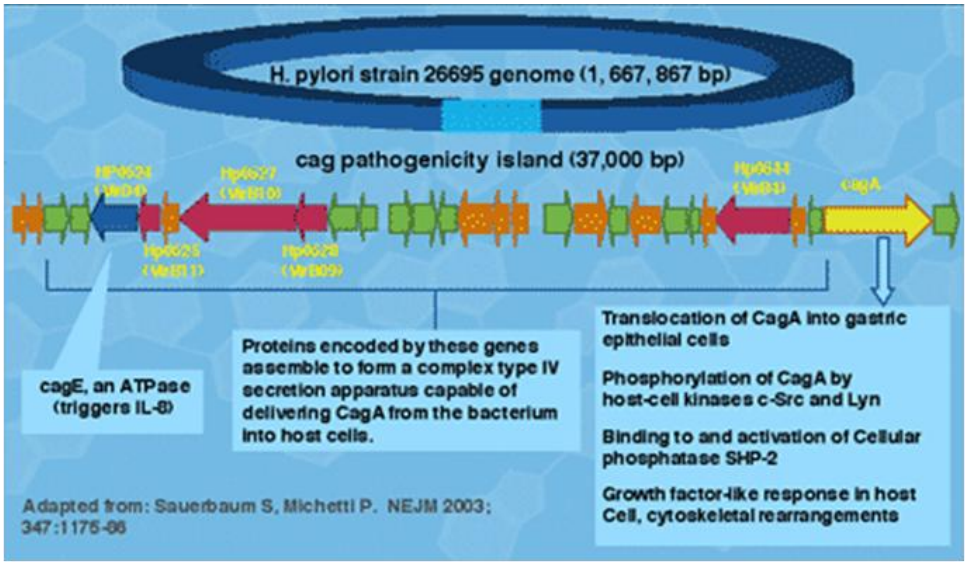

Figure 2.3: Genome of *Helicobacter pylori* (Marshall, 2001)

The CagA pathogenicity island is a secretion system that injects the CagA toxin into the epithelial cells. The CagA toxin is phosphorylated by the host cell kinases, c-Src and Lyn. CagA which is located inside the cell, acts like a growth factor and also plays an important role in cytoskeleton arrangements. Meanwhile, the pseudopodia on the epithelial cells increase the attachment of *H. pylori* to the cell (Marshall, 2001).

On the other end of the CagA island is an interesting component called the CagE, an adenosine triphosphatase (ATPase) that powers the Cag pathogenicity secretion system. CagE also triggers the release of interleukin-8 from the epithelial cells (Marshall, 2001).

A number of studies in western countries have confirmed that infection with *cag*A positive strains was associated with more severe gastritis and a higher prevalence of peptic ulcer and gastric cancer (Umit *et al.*, 2009).

The *vac*A gene is another important virulence factor of *H. pylori* present in all of *H. pylori* strains. This gene comprises two variable parts, which are the *vac*A signal sequence (s) and the mid region (m) sequence. The mosaic combination of (s) and (m) region allelic types determines the production of the cytotoxin and is associated with pathogenicity of the bacteria.

Genotypic alteration of *H. pylori* was thought to be responsible for the various clinical manifestations both in asymptomatic and symptomatic gastric cancer and MALT Lymphoma (Umit *et al.*, 2009).

The variations in clinical consequences are also reported to be due to factors such as duration of infection, inflammatory response of the patient and also virulence of *H. pylori* strain (Ogura *et al.*, 2007). Infection with less virulent strains is associated with milder symptoms whilst more virulent strains are associated with severe gastric inflammation, peptic ulcer, gastric carcinoma and MALT Lymphoma (Umit *et al* 2008).

2.6 Diagnosing *Helicobacter pylori*.

A report by Professor Dr Francis Megraud in the European Pharmacotherapy (2003) stated that there are several methods widely used in diagnosing *H. pylori* infection. The methods are basically divided into two groups: invasive tests for which an endoscopy must be performed and non-invasive tests which do not necessitate an endoscopy. An exception is the stool antigen test which is a direct but non-invasive test.

Biopsies are usually taken from the antrum or body of stomach during endoscopy of which tissues are subjected to a number of methods to detect *H. pylori*. The methods include examination using endoscopy via histology, culture, or urease test methods. These biopsy-based methods for detecting *H. pylori* were liable to sampling errors because the infection is patchy in distribution (Logan and Walker, 2001). Up to 14% of infected patients do not have antral infection but have *H. pylori* elsewhere in the stomach, especially if they have gastric atrophy, intestinal metaplasia, or bile reflux. Current consensus guidelines therefore recommend that multiple biopsies are taken from the antrum and corpus for histology and one other method mentioned above (Logan and Walker, 2001).

Using histological method, *H. pylori* is usually recognised on sections stained with haematoxylin and eosin alone but supplementary stains such as Giemsa, Genta, Gimenez and Warthin-Starry silver are also used to detect low levels of infection and to show the characteristic morphology (Logan and Walker, 2001).

An important advantage of histology is that, in addition to the historical record provided, sections from biopsies can be examined at any time, and that gastritis, atrophy, or intestinal metaplasia can also be assessed (Logan and Walker, 2001).

Urease tests are quick and simple tests for detecting *H. pylori* infection but it can only indicate the presence or absence of infection. However, the sensitivity of urease tests is often higher than that of other biopsy-based methods because the entire biopsy specimen is placed in the media (Logan and Walker, 2001).

Serological tests such as enzyme linked immunoabsorbent assay (ELISA) are also used to detect circulating antibodies to *H. pylori*. These tests are generally simple, reproducible, inexpensive, and can be done on stored samples. ELISA is a widely used technique in epidemiological studies, including retrospective studies to determine the prevalence or incidence of infection (Logan and Walker, 2001).

Individuals can vary considerably in their antibody responses to *H. pylori* antigens, and no single antigen is recognised by sera from all subjects. The accuracy of serological tests is therefore dependent on the antigens used in the test, making it essential that serological tests are locally validated prior to clinical use (Logan and Walker, 2001). Consumption of non-steroidal anti-inflammatory drugs (NSAIDs) has been reported to affect the accuracy of serological tests (Weil *et al.*, 2000).

Non-invasive detection of *H. pylori* using the ^{13}C-urea breath test is based on the principle that a solution of urea labelled with carbon-13 will be rapidly hydrolysed by the urease enzyme of *H. pylori*. The resulting carbon dioxide (CO_2) is absorbed across the gastric mucosa and hence, via the systemic circulation, excreted as $^{13}CO_2$ in the expired breath. The ^{13}C-urea breath test detects current infection and it is not radioactive. It can be used as a screening test for *H. pylori*, to assess eradication and to detect infection in children (Logan and Walker, 2001).

In the stool antigen test a simple sandwich ELISA is used to detect the presence of *H. pylori* antigens shed in the faeces. Studies had reported sensitivities and specificities similar to those of the ^{13}C-urea breath test (> 90%).

2.7 *Helicobacter pylori* and Medical Treatments

Over the past 15 years, the treatment of *H. pylori* infection has evolved from the initial mono and dual therapy to today's effective proton pump inhibitors (PPI) based triple therapy (Cutler and Schubert, 1993).

PPI reduces *H. pylori* density and induces lysis of the bacterium. In addition, PPI induces an increase in gastric pH, thus reducing the degradation of acid-labile antimicrobials. By reducing the volume of gastric acid secretion, PPIs could enhance antibiotic concentration in the gastric juice (Cutler and Achkar, 2000).

Gastric pH affects the degradation of many antibiotics. Amoxicillin, clarithromycin, and metronidazole are the cornerstones of antimicrobials used in *H. pylori* eradication.