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Aspects of *Helicobacter pylori* transmission

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SUMMARY

The bacterium *Helicobacter pylori* infects the gastric mucosa of about half of the world's population. The infection causes gastritis and contributes to the development of peptic ulcer disease and gastric cancer. *H. pylori* infection is associated with low socioeconomic status, is typically acquired in early childhood and once established can persist throughout life unless treated. Person-to-person transmission appears to predominate and the family stands out as the primary framework for transmission.

In this thesis, an initial cross-sectional study aimed to disentangle the independent contributions of *H. pylori* infections in family members to the risk for the infection in 11- to 13-year old index children from Stockholm schools. *H. pylori* infections in mothers and in siblings, but not in fathers, were notable risk factors for infection in the index children. Furthermore, birth of the index child in a country with high *H. pylori* prevalence was an independent risk factor for infection. In addition to the initial standard analysis, a weighted logistic regression method was applied to accommodate additional non-randomly sampled cases. This exemplified how appropriate analysis of epidemiological data from complex sampling schemes can improve precision and maintain validity, while enabling a more complete investigation of risk factors already identified.

A subset of the infected family members underwent gastroscopy and contributed gastric biopsies from which *H. pylori* was isolated and typed by molecular methods. The same bacterial strains were frequently detected among siblings and between mothers and offspring. No strain concordance was detected between fathers and offspring, but parents sometimes harbored the same strains. The bacterial isolates were also examined with regard to the presence or absence of the *cag* pathogenicity island (PAI), a bacterial virulence factor. In a comparison with serological data, serology was supported as a suitable method to determine *cag* PAI status of *H. pylori* infections in clinical and epidemiological studies. Moreover, clonal and non-clonal bacterial isolates from members of a family were analyzed in more detail, which included microarray-based genome comparisons. Non-clonal *H. pylori* isolates exhibited extensive genetic variability, where certain characteristics could be discerned. However, transmission and host adaptation did not appear to be associated with substantial sequence diversity in the bacterial genome.

The present data support a predominantly mother-child and sib-sib transmission of *H. pylori*, consistent with an important role of intimate contact in the transmission. Furthermore, methodological and microbiological aspects that could aid future research are described. In summary, the findings of this thesis and the discussions thereof shed some light on the characteristics and mechanisms of transmission and persistence of *H. pylori* infection.

SAMMANFATTNING

Bakterien *Helicobacter pylori* infekterar magslemhinnan hos omkring hälften av världens befolkning. Infektionen orsakar gastrit och bidrar till utvecklandet av magsår samt magcancer. *H. pylori*-infektion är associerad med låg socioekonomisk status, inleds vanligen i tidig barndom och när den etablerats kan den utan behandling förbli livslång. Smittspridning tycks huvudsakligen ske från person till person och familjen synes utgöra den miljö där transmission framförallt äger rum.

I denna avhandling syftade en inledande tvärsnittsstudie till att särskilja oberoende effekter av *H. pylori*-infektion hos familjemedlemmar på infektionsrisken hos 11 till 13 år gamla indexbarn från skolor i Stockholmsområdet. *H. pylori*-infektion hos mödrar och syskon, men inte hos fäder, var betydande riskfaktorer för infektion hos indexbarnen. Dessutom var det en oberoende riskfaktor om indexbarnet var fött i ett land med hög prevalens av *H. pylori*. Utöver den inledande standardanalysen tillämpades en viktad logistisk regressionsmetod för att kunna inkludera ytterligare icke slumpmässigt utvalda fall. Detta exemplifierade hur en passande analys av epidemiologiska data från komplexa urvalsprocesser kan förbättra precision, bibehålla validitet samt möjliggöra en mer komplett utredning av redan identifierade riskfaktorer.

En del av de infekterade familjemedlemmarna genomgick gastroskopi och bidrog med biopsier från magslemhinnan, från vilka *H. pylori* isolerades och typbestämdes med molekylära metoder. Samma bakteriestammar identifierades ofta hos syskon samt mellan mödrar och barn. Ingen konkordans av stammar kunde detekteras mellan fäder och barn, men föräldrar var i vissa fall infekterade med samma stammar. De bakteriella isolaten undersöktes också med avseende på om de innehöll *cag* patogenicitetsön (PAI), som är en bakteriell virulensfaktor. I en jämförelse med serologiska data fann serologin stöd som en passande metod för att bestämma *cag* PAI-status hos *H. pylori*-infektioner i kliniska och epidemiologiska studier. Klonala och icke klonala bakteriella isolat från medlemmar i en familj studerades vidare i mer detalj, vilket innefattade microarray-baserade genomjämförelser. Icke klonala *H. pylori*-isolat uppvisade omfattande genetisk variabilitet, där vissa karakteristika kunde urskiljas. Däremot tedde sig transmission och värdanpassning inte vara associerade med betydande sekvensvariation i det bakteriella genomet.

Dessa data ger stöd för att *H. pylori* företrädesvis smittar från mödrar till barn samt mellan syskon, vilket är förenligt med att intim kontakt spelar en viktig roll i transmissionen. Dessutom beskrivs metodologiska och mikrobiologiska aspekter, vilka kan vara av relevans för framtida forskning. Sammanfattningsvis ger fynden i denna avhandling och diskussionen därav viss vägledning vad det gäller karakteristika och mekanismer för transmission och kronicitet av *H. pylori*-infektion.

LIST OF PUBLICATIONS

The thesis is based on the following papers, which will be referred to by their Roman numerals.

I. Kivi M., Johansson A.L.V., Reilly M., Tindberg Y.
Helicobacter pylori status in family members as risk factors for infection in children.
Epidemiology and Infection. 2005(133): 645–652.
© Cambridge University Press

II. Kivi M., Johansson A.L.V., Salim A., Tindberg Y., Reilly M.
Accommodation of additional non-randomly sampled cases in a study of *Helicobacter pylori* infection in families.
Statistics in Medicine. In press.
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III. Kivi M., Tindberg Y., Sörberg M., Casswall T.H., Befrits R., Hellström P.M., Bengtsson C., Engstrand L., Granström M.
Concordance of *Helicobacter pylori* strains within families.
Journal of Clinical Microbiology. 2003(41): 5604–5608.
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IV. Kivi M., Tindberg Y., Bengtsson C., Engstrand L., Granström M.
Assessment of the *cag* pathogenicity island status of *Helicobacter pylori* infections with serology and PCR.
Clinical Microbiology and Infection. 2005 (11): 66–68.
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V. Kivi M., Hjalmarsson S., Kupershmidt I., Lundin A., Tindberg Y., Granström M., Engstrand L.
Helicobacter pylori genome variability in a framework of familial transmission.
In manuscript.

CONTENTS

Sections 7, 8, 9 and 13 are omitted from the electronic version of the thesis due to copyright reasons. Please refer to the original papers.

LIST OF ABBREVIATIONS

CI	Confidence interval
DI	Discriminatory index
ELISA	Enzyme-linked immunosorbent assay
GERD	Gastroesophageal reflux disease
IL	Interleukin
LPS	Lipopolysaccharide
MALT	Mucosa-associated lymphoid tissue
MAR	Missing-at-random
NSAID	Non-steroidal anti-inflammatory drug
OMP	Outer membrane protein
OR	Odds ratio
PAI	Pathogenicity island
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
SE	Standard error
SES	Socioeconomic status
TLR	Toll-like receptor
UBT	Urea breath test

1. INTRODUCTION

Signs of *Helicobacter pylori* infection such as gram-negative gastric bacilli, gastric urease and epidemics of hypochlorhydria have been described since the late nineteenth century (Marshall, 2001). These observations could be better explained after Warren and Marshall in the early 1980's managed to culture a bacterium that was to be designated *Campylobacter pyloridis* (Marshall and Warren, 1984). In 1989, the genus *Helicobacter* was created and the bacterium received the name *Helicobacter pylori* (Goodwin *et al.*, 1989).

Half of the world's population is estimated to be infected with *H. pylori*, which makes it one of the most common bacterial pathogens in humans (Torres *et al.*, 2000). The infection is associated with low socioeconomic status (SES), is typically acquired in early childhood and once established can persist throughout life unless treated (Torres *et al.*, 2000). The association between *H. pylori* and gastritis was recognized early (Marshall and Warren, 1984) and virtually all infected individuals have later been confirmed to have a usually asymptomatic gastritis. A crucial role of the infection in peptic ulcer disease has been firmly established, which has enabled a paradigm shift in the treatment of ulcer patients (NIH Consensus Conference, 1994). A link between *H. pylori* and gastric cancer has also been demonstrated (IARC, 1994) and has been corroborated in subsequent studies (Ekström *et al.*, 2001; Uemura *et al.*, 2001). However, only 10–20% of infected individuals manifest severe complications and this selectivity in disease progression is inadequately understood (Blaser and Atherton, 2004; Suerbaum and Michetti, 2002).

The high *H. pylori* prevalence in many parts of the world, in conjunction with the pertinent links to disease, render the understanding of this infection an important public health issue.

2. OCCURRENCE AND TRANSMISSION

There are substantial differences in *H. pylori* prevalence between populations, originating in different probabilities of acquisition and persistence. The disentanglement of effects on acquisition from those on persistence requires longitudinal studies and thus, the common cross-sectional studies in this field do not separate these effects. Furthermore, risk factors may in some instances reflect both exposure and susceptibility to the infection. Epidemiological data of *H. pylori* infection are sometimes inconclusive or even conflicting, which may be a result of real variations between settings or methodological imperfections. Nonetheless, a number of features of more general character can be discerned in the epidemiology of *H. pylori* infection.

2.1. Prevalence and incidence

There are considerable differences in *H. pylori* prevalence between high-income and low-income countries. The prevalence in child populations ranges from below 10% to over 80% in high-income and low-income countries, respectively (Torres *et al.*, 2000). The infection is also associated with low SES within countries (Graham *et al.*, 1991; Malaty *et al.*, 1992; Malaty *et al.*, 1996). In the United States, for instance, a significantly lower prevalence was found in Caucasians (26%) compared to Hispanics (65%) and Afro-Americans (66%) (Malaty *et al.*, 1992). This dissimilarity was interpreted to reflect the different socioeconomic backgrounds of the groups. In a follow-up study, it was found that the difference in prevalence between Afro-Americans and Caucasians resulted from different seroconversion rates, although the rate of seroreversion could also have played a role (Malaty *et al.*, 2002).

H. pylori infection is usually acquired before the age of five (Granström *et al.*, 1997; Malaty *et al.*, 2002; Mitchell *et al.*, 1992). Annual incidence rates over 20% have been reported in early childhood in low-income countries (Glynn *et al.*, 2002; Goodman *et al.*, 2005). This is consistent with the rapidly increasing prevalence seen in children under the age of five years in many parts of the world (Klein *et al.*, 1994; Torres *et al.*, 2000). In high-income countries, the incidence in early childhood is typically between 1% and 10% (Granström *et al.*, 1997; Kumagai *et al.*, 1998; Malaty *et al.*, 2002), but can also reach as high as 20% (Goodman *et al.*, 2005). Furthermore, infections that apparently clear spontaneously have been reported particularly in childhood (Goodman *et al.*, 2005; Granström *et al.*, 1997; Klein *et al.*, 1994; Malaty *et al.*, 2002). As many as 80% of childhood infections were eliminated by 11 years of age in Sweden (Granström *et al.*, 1997) and by two years of age in Mexico and the United States (Goodman *et al.*, 2005). In adults in high-income countries, the seroconversion rates tend to be about 0.5–1% per annum with slightly higher rates of seroreversion (Kumagai *et al.*, 1998; Parsonnet, 1995; Veldhuyzen van Zanten *et al.*, 1994). In low-income countries, the annual incidence in adults can be higher (Parsonnet, 1995) and reported rates of reinfection after *H. pylori*

eradication have approached 20% in some studies (Hildebrand *et al.*, 2001; Soto *et al.*, 2003; Wheeldon *et al.*, 2005).

H. pylori prevalence is generally found to increase with age, reaching 20–50% in adult populations in Europe and North America (Bergenzaun *et al.*, 1996; Malaty *et al.*, 1992; Veldhuyzen van Zanten *et al.*, 1994). This pattern has been interpreted to partly reflect a birth-cohort phenomenon caused by a higher incidence in the past due to poorer living conditions and sanitation (Banatvala *et al.*, 1993; Parsonnet, 1995). Indeed, mathematical modeling has suggested that the infection will eventually disappear in high-income countries even without intervention (Rupnow *et al.*, 2000). But today, and in the foreseeable future, the infection remains highly prevalent in low-income regions of the world as well as in considerable portions of the populations in high-income countries.

2.2. Risk factors for infection

An obvious necessary cause for acquisition of *H. pylori* infection is exposure to the bacterium. The probability of exposure depends on the characteristics of the infective source and contact, but factors of the recipient host and the bacterium may also influence the probabilities of acquisition and persistence (Table 1). The bacterium has to overcome numerous barriers to successfully establish an infection in a new individual:

- Exit from an infected individual
- Transient survival outside the gastric niche
- Introduction into a new host
- Colonization of the new gastric mucosa
- Maintenance of the colonization

A predominantly person-to-person transmission has been postulated. This notion is based on the clustering of the infection in families (Drumm *et al.*, 1990; Goodman and Correa, 2000; Rocha *et al.*, 2003; Rothenbacher *et al.*, 2002b) and in institutionalized individuals (Lambert *et al.*, 1995), while consistent and verified environmental reservoirs are absent (see "Environmental and behavioral factors" and "Transmission routes").

The family

The family stands out as the most important framework for transmission and a child's risk of being infected is associated with having infected family members (Goodman and Correa, 2000; Rocha *et al.*, 2003; Rothenbacher *et al.*, 2002b). Family size and residential crowding (persons per room or m^2) are frequently described as risk factors for *H. pylori* infection and may be regarded as proxies for the number of infected family members (Goodman *et al.*, 1996; Goodman and Correa, 2000; McCallion *et al.*, 1996; Mendall *et al.*, 1992; Tindberg *et al.*, 2001b; Webb *et al.*, 1994). Similarly, having familial connections to high-prevalence regions

Table 1. Overview of possible determinants of *H. pylori* infection

Necessary cause: Exposure to the bacterium	**References**
Living in or originating from high-prevalence areas	(Rothenbacher *et al.*, 1998b; Tindberg *et al.*, 2001b; Torres *et al.*, 2000; Tsai *et al.*, 2005)
Large family size, infected family members	(Goodman *et al.*, 1996; Goodman and Correa, 2000; McCallion *et al.*, 1996; Mendall *et al.*, 1992; Rocha *et al.*, 2003; Rothenbacher *et al.*, 2002b; Tindberg *et al.*, 2001b; Webb *et al.*, 1994)
Infected contacts in the community: Daycare centers	(Dore *et al.*, 2002)
Environmental reservoirs: Contaminated water	(Brown *et al.*, 2002; Goodman *et al.*, 1996; Klein *et al.*, 1991; Nurgalieva *et al.*, 2002)
Behavior and other factors increasing the exposure: Intimate contact, gastroenteritis, poor sanitary practices	(Brown *et al.*, 2002; Laporte *et al.*, 2004; Luzza *et al.*, 2000; McCallion *et al.*, 1996; Nurgalieva *et al.*, 2002; Rocha *et al.*, 2003; Rothenbacher *et al.*, 2002b; Tindberg *et al.*, 2001b; Webb *et al.*, 1994)
Component cause: Host factors	**References**
Expression of receptors	(Aspholm-Hurtig *et al.*, 2004; Borén *et al.*, 1993; Rothenbacher *et al.*, 2004)
Host defenses: Gastric acid secretion, immune responses	(Björkholm *et al.*, 2004; Hartland *et al.*, 2004; Magnusson *et al.*, 2001; Mohammadi *et al.*, 1997)
Other factors affecting the gastric milieu: Young age, diet	(Granström *et al.*, 1997; Kuepper-Nybelen *et al.*, 2005; Malaty *et al.*, 2002; Mitchell *et al.*, 1992)
Component cause: Bacterial factors	**References**
Protected localization: Motility, adhesion, internalization	(Aspholm-Hurtig *et al.*, 2004; Björkholm *et al.*, 2000; Eaton *et al.*, 1992; Ilver *et al.*, 1998; Mahdavi *et al.*, 2002; Terry *et al.*, 2005)
Withstanding host defenses: Urease activity, immune evasion	(Allen, 2001; Andersen-Nissen *et al.*, 2005; Bergman *et al.*, 2004; Boncristiano *et al.*, 2003; Bäckhed *et al.*, 2003; Eaton and Krakowka, 1994; Gebert *et al.*, 2003; Molinari *et al.*, 1998)
Adaptive evolution	(Aspholm-Hurtig *et al.*, 2004; Salaün *et al.*, 2005; Yamaoka *et al.*, 2002)

is associated with infection in children living in low-prevalence areas (Rothenbacher *et al.*, 1998b; Tindberg *et al.*, 2001b) and this effect decreases in successive generations (Tsai *et al.*, 2005). The living conditions during childhood can be predictive of infection in adulthood (Mendall *et al.*, 1992; Webb *et al.*, 1994; Woodward *et al.*, 2000), being in accordance with *H. pylori* acquisition in childhood

from household members. Furthermore, the possibility of child-child transmission outside the family was not supported in a Swedish study, where the *H. pylori* prevalence in classmates was not a risk factor for infection (Tindberg *et al.*, 2001b). However, daycare attendance was associated with *H. pylori* infection in urban Sardinian children (Dore *et al.*, 2002).

Having an infected mother has been found to be a more prominent risk factor for childhood infection than having an infected father, supporting primarily mother-child transmission (Rocha *et al.*, 2003; Rothenbacher *et al.*, 2002b; Tindberg *et al.*, 2001b). Transmission among siblings has also been indicated by clustering of the infection in sibships (Goodman and Correa, 2000; Rocha *et al.*, 2003). Data from a high-prevalence area in Colombia suggested that having older infected siblings were more predictive of infection in children than having younger infected siblings (Goodman and Correa, 2000). A narrower age gap to the next older infected sibling also seemed to increase the risk for infection. The importance of both infected mothers and siblings has thereafter been corroborated in a high-prevalence community in Brazil (Rocha *et al.*, 2003). The suggested central roles of infected mothers and siblings, and the lesser role played by infected fathers, probably reflect how intimate contact potentiates the effect of having infected family members.

Even though the infection is usually initiated in early childhood, some epidemiological data point to the possibility of acquisition in adulthood from infected family members. Having an infected spouse has been described as a risk factor for infection (Brenner *et al.*, 1999). This study controlled for the country of origin, which could otherwise have explained the association (Perez-Perez *et al.*, 1991). Furthermore, having more children has been identified as a risk factor for infection in adults (Mendall *et al.*, 1992), possibly indicating that children may serve as mediators of transmission within families. Some additional, albeit weak, evidence in this direction may be that having an infected spouse was not a risk factor for infection in childless couples (Perez-Perez *et al.*, 1991).

Environmental and behavioral factors

Reasons for the association between *H. pylori* infection and low SES have been sought among environmental and behavioral factors. A shared environmental source of the infection could theoretically contribute to the observed intrafamilial clustering. Possibly contaminated water has been suggested as an infection source since using particular water sources, such as wells, has been correlated to the infection (Brown *et al.*, 2002; Goodman *et al.*, 1996; Klein *et al.*, 1991; Nurgalieva *et al.*, 2002). However, other studies have not found the water source to be associated with infection (Clemens *et al.*, 1996; Glynn *et al.*, 2002; Malaty *et al.*, 1996). More indirect environmental transmission has also been proposed following the identification of the consumption of raw vegetables as a risk factor for infection (Goodman *et al.*, 1996; Hopkins *et al.*, 1993). Furthermore, *H. pylori* has been proposed to possess zoonotic potential and suggested reservoirs include cats (Handt *et al.*, 1994), houseflies (Grübel *et al.*, 1997) and sheep (Dore *et al.*, 1999), but these theories are controversial.

Behavioral factors may influence the risk of *H. pylori* acquisition and persistence. Residence in a high-prevalence country can facilitate acquisition, as frequent close contact with infected individuals and poor sanitary practices may enhance bacterial exposure (Brown *et al.*, 2002; Goodman *et al.*, 1996; Hopkins *et al.*, 1993; Nurgalieva *et al.*, 2002). The importance of intimate contact for acquisition was mentioned above as a possible explanation for infected mothers and siblings, and not infected fathers, being primary determinants for the infection in children. Intimate contact has likewise been suggested to explain other observed risk factors, such as bed sharing (McCallion *et al.*, 1996; Webb *et al.*, 1994) and breastfeeding (Rothenbacher *et al.*, 2002a). Breastfeeding has also been speculated to possibly provide protection against early infection by passive immunization (Blecker *et al.*, 1994; Thomas *et al.*, 1993). However, such protection, if any, should be of limited relevance after weaning, as supported by negative findings (Dore *et al.*, 2002; Glynn *et al.*, 2002; McCallion *et al.*, 1996). Moreover, *H. pylori* infection has been negatively correlated with antibiotic consumption (Mitchell *et al.*, 1992; Rothenbacher *et al.*, 1998a). In another study, however, a similar negative association disappeared when the country of origin was taken into account, which could be explained by the higher antibiotic consumption in low-prevalence countries (Tindberg *et al.*, 2001b). Some behavioral factors have been assessed as determinants for *H. pylori* infection specifically in adults. Examples include a possible negative association with alcohol intake (Kuepper-Nybelen *et al.*, 2005) and perhaps a positive relationship with smoking (Woodward *et al.*, 2000), but data are discrepant (Malaty *et al.*, 1996; Mitchell *et al.*, 1992; Woodward *et al.*, 2000).

As illustrated above, data regarding environmental and behavioral factors as determinants for *H. pylori* infection are often inconclusive. The independent effects may be modest and confounding by other socioeconomic factors or household characteristics is a major concern. The importance of different risk factors may also differ between populations. Thus, the data on environmental and behavioral exposures are often complicated to interpret, but they may contain important information.

Host and bacterial factors

H. pylori strains differ in their ability to establish and maintain an infection in a given host, which can be attributed to host and bacterial factors and their compatibility (Aspholm-Hurtig *et al.*, 2004; Dubois *et al.*, 1999; Salaün *et al.*, 2005; Suto *et al.*, 2005; Yamaoka *et al.*, 2002). The transient infections in childhood may reflect instances where the bacterium is not optimally suited for the new host and adaptation is not feasible or rapid enough, leading to the host succeeding in clearing the infection (Goodman *et al.*, 2005; Granström *et al.*, 1997; Klein *et al.*, 1994; Malaty *et al.*, 2002).

Host genetics have been indicated to be involved in susceptibility to *H. pylori* infection, based on a higher concordance of infection in monozygotic (81%) than in dizygotic (63%) twin pairs (Malaty *et al.*, 1994). The specific genetic components of this suggested predisposition are unknown, but some host factors that may contribute

susceptibility to the infection have been proposed. Expression of blood group antigens that mediate bacterial adherence to the gastric mucosa has been suggested to be important for susceptibility to *H. pylori* infection (Borén *et al.*, 1993). Some research indicates that *H. pylori* strains have adapted their binding affinities in accordance with the blood group antigen expression of different human populations (Aspholm-Hurtig *et al.*, 2004). Furthermore, individuals that excrete receptors in body fluids, offering removable binding sites that can compete with the tissue-bound receptors, have been reported to have a lower risk of being infected (Rothenbacher *et al.*, 2004). However, some studies have shed doubt on the theory that blood group antigen-mediated adhesion contributes to susceptibility to infection (Clyne and Drumm, 1997; Umlauft *et al.*, 1996; Yamaoka *et al.*, 2002). These discrepancies may reflect multifaceted mechanisms of bacterial adhesion and a need for sophisticated measurements when assessing the binding potential.

The immune system may also be involved in determining predisposition to *H. pylori* infection. This notion is supported by studies that describe alleles within the human leukocyte antigen locus HLA-DQA1 to be correlated with the infection (Azuma *et al.*, 1995; Magnusson *et al.*, 2001). However, there are discordant data showing no association (Karhukorpi *et al.*, 1999). An interleukin (IL)-1β receptor polymorphism of unknown functional consequence has also been correlated with the infection (Hartland *et al.*, 2004). Furthermore, the spread of the bacterium may be promoted by low gastric acid secretion, which may be especially relevant in young children and during infective gastroenteritis (Björkholm *et al.*, 2004; Cook, 1985). Some studies have found a slightly higher prevalence of *H. pylori* infection in males (Goodman *et al.*, 1996; Woodward *et al.*, 2000). The reasons for this tendency are unclear and other studies have not been able to confirm this correlation (Hopkins *et al.*, 1993; Malaty *et al.*, 2002; Mitchell *et al.*, 1992; Tindberg *et al.*, 2001b).

H. pylori has developed a repertoire of functions for survival in the harsh gastric niche, including acid tolerance, motility, adherence, immune evasion and mechanisms for adaptive evolution (see "The microorganism"). These features are all involved in the interplay between the host and the bacterium and may influence acquisition and persistence of infection, as is typically studied in animal models. Bacterial acid tolerance and motility were among the first factors found to play a role in colonization (Eaton *et al.*, 1992; Eaton and Krakowka, 1994). Global mutagenesis approaches have verified these findings and have expanded the collection of putative essential genes (Kavermann *et al.*, 2003; Salama *et al.*, 2004). However, the relevance of these observations in human populations is largely unknown. In a Finnish population, strains with a bacterial virulence factor, the *cag* pathogenicity island (PAI), have been indicated to disappear more rapidly than strains without the *cag* PAI (Perez-Perez *et al.*, 2002). This could speculatively be explained by reduced transmissibility or persistence of *cag* PAI+ strains in this population. However, *cag* PAI+ infections constitute the majority of *H. pylori* infections worldwide (Nilsson *et al.*, 2003; Park *et al.*, 2002; Parsonnet *et al.*, 1997; Simán *et al.*, 2005) and murine studies speak against a significant role of the *cag* PAI in colonization, at least after

the initial phase of the infection (Marchetti and Rappuoli, 2002; Yamaoka *et al.*, 2002).

2.3. Molecular typing

Molecular typing has shown that unrelated individuals harbor distinct *H. pylori* strains (Akopyanz *et al.*, 1992; Taylor *et al.*, 1995). Nevertheless, *H. pylori* genetic variation has been correlated to different human populations and to human migrations such as European colonization and the slave trade (Achtman *et al.*, 1999; Falush *et al.*, 2003; Kersulyte *et al.*, 2000; Wirth *et al.*, 2004). Molecular typing can further exploit the bacterial genome diversity to study the spread of *H. pylori* within shorter time frames. The presence of genetically related bacteria in different individuals indicates person-to-person transmission or acquisition from a common source. Accordingly, molecular typing can corroborate and further characterize the transmission pathways suggested by epidemiological data based on infection status. In this respect, microbial typing techniques should always be evaluated for their ability to cluster related strains and discriminate between unrelated strains (Hunter and Gaston, 1988).

The terminology of bacterial samples and their relatedness is sometimes inconsistent, which justifies a clarification of the terminology that will be used here. An "isolate" is a collection of cells derived from a single cell (van Belkum *et al.*, 2001). A "strain" represents an isolate or a group of isolates displaying specific characteristics that set it apart from other isolates belonging to the same species. The term strain is commonly used interchangeably with "clone", that is, organisms descending from a common ancestor through a direct chain of replication.

H. pylori clonality can be discerned for isolates from different family members, occasionally in combination with clonal variants (Han *et al.*, 2000; Raymond *et al.*, 2004; van der Ende *et al.*, 1996). These observations support intrafamilial transmission and bear analogy to an epidemic clonal population structure of microorganisms (van Belkum *et al.*, 2001), although *H. pylori* is generally regarded as panmictic (Achtman *et al.*, 1999; Suerbaum *et al.*, 1998). It is difficult to obtain gastric biopsies from children and asymptomatic individuals and thus, familial molecular typing data are sparse and often based on small samples. Nevertheless, clustering of strains in sibships has been reported (Han *et al.*, 2000; Miehlke *et al.*, 1999; van der Ende *et al.*, 1996) and it has been suggested that children are colonized more frequently with the mother's strain than the father's (Han *et al.*, 2000). However, both father-offspring and mother-offspring strain concordance have been described (Bamford *et al.*, 1993; Gibson *et al.*, 1998; van der Ende *et al.*, 1996). Some acquisition of the infection in adulthood is indicated by findings of shared strains among spouses (Bamford *et al.*, 1993; Gibson *et al.*, 1998; van der Ende *et al.*, 1996) and reinfections with apparently new strains after *H. pylori* eradication (Hildebrand *et al.*, 2001; Soto *et al.*, 2003). Hence, the molecular typing data seem to

support the transmission patterns outlined from other types of epidemiological studies.

H. pylori infection in an individual appears to be comprised of mainly one strain (Berg *et al.*, 1997; Raymond *et al.*, 2004; Taylor *et al.*, 1995). More than one strain can, however, be present and such mixed infections have been hypothesized to be more common in high-prevalence settings due to repeated acquisition opportunities (Berg *et al.*, 1997). Furthermore, the extensive genetic diversification of *H. pylori* renders each isolate derived from a single cell more or less unique and has lead to descriptions of the infection as being composed of a multitude of quasispecies (Blaser and Berg, 2001; Kuipers *et al.*, 2000). The presence of different strains and strain variants constitutes a methodological limitation in typing studies and has to be considered in the corresponding interpretations. A vast area of the gastric mucosa usually remains unsampled and some isolates may be overlooked. Moreover, quasispecies could perhaps mutate beyond the recognition of clonality by some molecular typing techniques.

2.4. Transmission routes

Possible *H. pylori* transmission routes are gastro-oral, fecal-oral or oral-oral, but firm evidence is scarce. *H. pylori* has been cultured from vomitus, diarrheal stools and saliva, demonstrating that the bacterium is potentially transmissible through these routes (Leung *et al.*, 1999; Parsonnet *et al.*, 1999; Thomas *et al.*, 1992). In contrast, *H. pylori* cultures of environmental water samples have only very rarely been successful (Lu *et al.*, 2002). Bacterial DNA can be detected in the environment by PCR (Hultén *et al.*, 1996), but the DNA may very well represent remnants of dead bacteria and does not constitute strong evidence for the possibility of environmental transmission.

Vomitus in particular appears to harbor viable bacteria and even air in the vicinity of a vomiting study subject can be *H. pylori* positive by culture (Parsonnet *et al.*, 1999). In line with this observation is a study which found a weak association between vomiting in siblings and childhood infection (Luzza *et al.*, 2000). An elevated incidence of *H. pylori* infection after outbreaks of gastroenteritis has also been reported in a French institution (Laporte *et al.*, 2004). Hepatitis A is spread through the fecal-oral route and correlations between antibodies against hepatitis A and *H. pylori* could possibly indicate a common mode of transmission. However, a number of studies have not been able to confirm this hypothesis (Furuta *et al.*, 1997; Hazell *et al.*, 1994) and more conclusive evidence has to be sought elsewhere. Oral-oral transmission of *H. pylori* may be interpreted to be of limited importance, based on data showing that the prevalence of the infection was not higher in dentists compared to non-clinical colleagues (Matsuda *et al.*, 2002), while it was higher in gastroenterologists compared to internists, nurses and population controls (Lin *et al.*, 1994). Furthermore, episodes of diarrhea have been proposed to be more common in children during the months after *H. pylori* acquisition relative to uninfected and

persistently infected children, which may promote dissemination of the infection (Passaro *et al.*, 2001).

It is plausible that close contact within families facilitates exposure to bacteria through contaminated body excretions, being in agreement with familial transmission. Regurgitation, vomiting and diarrhea are common in childhood and children may boost familial *H. pylori* transmission when the bacterium is introduced into a child. Following this line of reasoning, it is appealing to envision a model in which societal development involves a decreasing frequency of gastrointestinal illnesses and improved sanitation, thereby contributing to the declining *H. pylori* prevalence in high-income parts of the world.

3. THE MICROORGANISM

"From our anthropocentric point of view, we say a person with the agent is infected. From the point of view of the infectious agent, however, humans are simply home and lunch, their ecologic niche." (Halloran, 1998)

3.1. General aspects of *H. pylori* microbiology and infection

H. pylori is the best known member of the *Helicobacter* genus, which includes dozens of species that primarily colonize the gastrointestinal tract of a variety of animals (Fox, 2002). *H. pylori* is a curved gram-negative bacillus with a bundle of unipolar flagella (Figure 1). Biochemical identification of *H. pylori* relies on the activities of the urease, catalase and oxidase enzymes. The bacterium is slow-growing and requires a rich medium and a microaerophilic atmosphere for *in vitro* culture. After starvation through prolonged culturing, a coccoid form can be found in the cultures and it has been debated whether this form represents dormant or degenerated, non-viable bacteria (Andersen *et al.*, 2000).

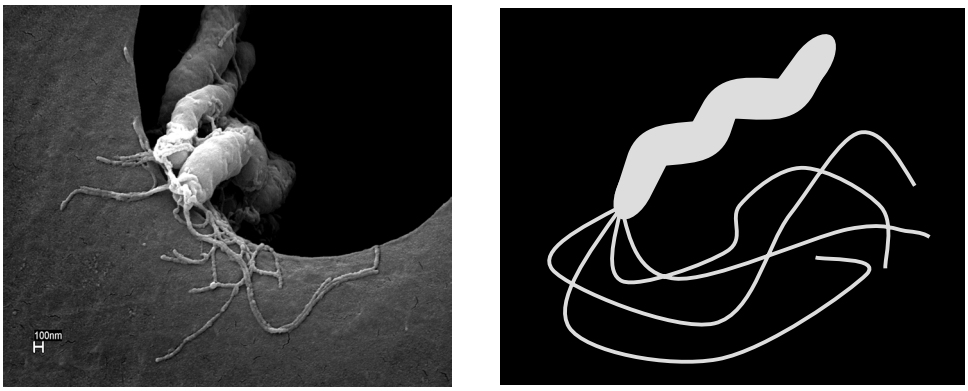

Figure 1. *H. pylori.* The curved bacillus with unipolar flagella is visualized by a scanning electron microscope (left) and depicted in a schematic drawing (right). The microscopic image was kindly provided by Christina Nilsson.

The human stomach is an inhospitable milieu and a fasting stomach is normally devoid of bacterial species other than *H. pylori* and some *Lactobacilli*. *H. pylori* is well adapted to its gastric niche and has developed a broad spectrum of functions that enable colonization. For example, bacterial urease hydrolyzes urea with the formation of carbon dioxide and ammonia, providing protection against the highly acidic gastric environment. The capability of *H. pylori* to maintain a chronic infection is of particular interest and can be facilitated by i) protected localization and adherence, ii) evasion and regulation of the immune response and iii) adaptation to changing conditions.

The infection can be patchy and is primarily localized to the distal parts of the stomach, but can spread proximally, especially in persons with low gastric acid secretion (Bayerdörffer *et al.*, 1989; Testerman *et al.*, 2001). The majority of the bacteria are regarded to be free-living in the gastric mucus layer, which can provide some protection against the harsh environment (Testerman *et al.*, 2001). Part of the bacterial population adheres to the gastric epithelial cells, which may benefit interactions with the host and maintenance of the colonization. A significant role of adherence is indicated by the array of products that contribute to this purpose in *H. pylori* (Edwards *et al.*, 2000; Ilver *et al.*, 1998; Mahdavi *et al.*, 2002; Testerman *et al.*, 2001; Yamaoka *et al.*, 2002). The bacterium is generally extracellular, but may invade host cells, although the significance of internalization is uncertain (Björkholm *et al.*, 2000; Testerman *et al.*, 2001).

Long-term persistence of the infection is likely to require evasion or modulation of the immune response. However, the inflammation may benefit the bacteria to some extent by disrupting the tissue integrity, thereby making nutrients available. Thus, bacterial interactions with the immune system may need to somewhat balance the risk of eradication against a more favorable environment (Blaser and Berg, 2001). The innate immune response towards *H. pylori* may be impaired by relatively inert interactions with the Toll-like receptors (TLR) (Andersen-Nissen *et al.*, 2005; Blaser and Atherton, 2004; Bäckhed *et al.*, 2003) and by resistance to phagocytic killing (Allen, 2001). The adaptive immune response is shifted towards a Th1 response, which is unusual for extracellular pathogens and may contribute to the inflammation (Blaser and Atherton, 2004; Mohammadi *et al.*, 1997). The bacterium can interact with cells of the adaptive immune system directly or through effector molecules to balance the Th1/Th2 responses (Bergman *et al.*, 2004), interfere with antigen presentation (Molinari *et al.*, 1998) and to inhibit activation or induce apoptosis of T lymphocytes (Boncristiano *et al.*, 2003; Gebert *et al.*, 2003; Wang *et al.*, 2001).

H. pylori is a genetically diverse bacterial species (Achtman *et al.*, 1999; Akopyanz *et al.*, 1992; Björkholm *et al.*, 2001b; Israel *et al.*, 2001; Salama *et al.*, 2000; Suerbaum *et al.*, 1998). Genetic diversification may facilitate immune evasion and adaptation to different gastric niches, new hosts or a changing gastric environment over the years. Furthermore, strain variants in the same stomach could constitute a reservoir of better adapted strains if the living requirements would change.

3.2. The genome

H. pylori is the first bacterial species for which two genomes, those of strains 26695 (Tomb *et al.*, 1997) and J99 (Alm *et al.*, 1999), were completely sequenced. A third sequenced genome, AG7:8, is currently in the final stages of annotation and analysis (H. Kling Bäckhed, personal communication). The *H. pylori* genome is small and compact and the 1.65 million base pairs accommodate about 1,500 genes. The limited metabolic potential and the low number of regulatory networks have been interpreted to reflect a restricted gastric niche of the bacterium (Doig *et al.*, 1999;

Tomb *et al.*, 1997). After a revision of the annotation, 77% of the genes have been assigned a functional category (Boneca *et al.*, 2003). Comparison of the two sequenced genomes revealed some larger genomic rearrangements, but the gene order and metabolic potential were relatively conserved (Alm *et al.*, 1999; Doig *et al.*, 1999). Strain-specific genes, 6–7% of the genes, were concentrated in two genomic regions that hence were designated "plasticity zones". These zones have thereafter also been discernible by microarray-based comparative genomics of other strains (Björkholm *et al.*, 2001a; Salama *et al.*, 2000). Some gene functional classes have been found to be especially variable, including genes related to DNA metabolism and the cell envelope (Boneca *et al.*, 2003; Salama *et al.*, 2000; Salaün *et al.*, 2005). Possible explanations for this variability are that DNA diversification and its regulation have important roles in *H. pylori* and that surface structures exposed to the host undergo antigenic variation. As described below, *H. pylori* has developed strategies to facilitate and regulate its extensive genetic diversification, probably to attain optimal instruments for adaptive evolution.

Generation of genome diversity

Recombination has been postulated to be a primary source of *H. pylori* genome diversity and panmixis (Achtman *et al.*, 1999; Falush *et al.*, 2001; Suerbaum *et al.*, 1998). One study estimated characteristics related to recombination by sequencing of DNA fragments from paired isolates (Falush *et al.*, 2001). The resulting estimates give a picture of frequent recombination of comparably small fragments (60 imports spanning 25,000 base pairs per genome per year, that is, 1.5% of the genome). However, the estimated rate of recombination may be inflated since only one isolate from each time point was considered and thus, the diversity could have been present initially. The genetic divergence may be slower in some instances, as discussed after the finding of limited differences in sequential isolates separated by nine years (Lundin *et al.*, 2005).

Horizontal genetic transfer is common in *H. pylori* and transformation, uptake of naked DNA from the environment, is facilitated by the widespread natural competence of *H. pylori* (Nedenskov-Sörensen *et al.*, 1990). Other means of horizontal genetic transfer are conjugation and phage-mediated transduction, of which the former DNase-resistant mechanism has been described in *H. pylori* (Kuipers *et al.*, 1998). The possibility of generating genome diversity by DNA transfer has been hypothesized to decrease in high-income countries due to the reduced circulation of different *H. pylori* strains (Blaser and Atherton, 2004). If this leads to less genetic diversity it may be a contributing factor to the declining *H. pylori* prevalence in high-income countries. Deletion of DNA segments also adds to the genetic diversity of *H. pylori*. In 15 unrelated isolates, 12–18% of the genes of 26695 and J99 were found to be dispensable in each isolate (Salama *et al.*, 2000), while 0.3–1.5% of the J99 genes were deemed to be absent in each of 13 J99 variants (Israel *et al.*, 2001).

Restriction-modification systems can control horizontal genetic transfer and a distinctive feature of the *H. pylori* genome is the presence of a high number of these

systems (Alm *et al.*, 1999; Lin *et al.*, 2001; Tomb *et al.*, 1997; Xu *et al.*, 2000). Functional restriction-modification systems consist of a methyltransferase that methylates a specific target DNA sequence and an endonuclease that cleaves the same sequence when it is unmethylated. Accordingly, invading DNA will be unmethylated and digested, while endogenous DNA will be methylated and protected. The *H. pylori* restriction-modification systems are variable and each strain contains its unique setup, which can sustain genome integrity and allow the existence of separate gene pools (Alm *et al.*, 1999; Lin *et al.*, 2001; Tomb *et al.*, 1997; Xu *et al.*, 2000). Furthermore, these systems may be conceived of as selfish DNA and may provide repetitive DNA and DNA breakage that could facilitate recombination or they may be involved in gene regulation by methylation of promotor sequences (Blaser and Berg, 2001).

H. pylori is prone to point mutation and a considerable proportion of strains have exceptionally high mutation rates, comparable to mutator strains in other species (Björkholm *et al.*, 2001b). A mutator phenotype indicates that the strain is deficient in DNA repair and *H. pylori* appears to lack components of the mismatch and SOS repair systems (Björkholm *et al.*, 2001b; Tomb *et al.*, 1997). Most point mutations do, however, occur at the third base of the codon, where they do not affect the amino acid sequence of the protein (Achtman *et al.*, 1999; Alm *et al.*, 1999; Suerbaum *et al.*, 1998). About 30 genes have been found to be associated with stretches of mono- or dinucleotide repeats, which are liable to slipped strand mispairing that can result in translational frameshifts and stop codons, offering means for regulation of gene expression (Alm *et al.*, 1999; Salaün *et al.*, 2005; Tomb *et al.*, 1997). These phase-variable genes commonly encode proteins involved in restriction-modification, lipopolysaccharide (LPS) biosynthesis and cell surface-associated proteins.

3.3. Virulence factors

A virulence factor contributes some function that renders the microorganism more pathogenic, that is, increases the likelihood for disease development. *H. pylori* infection is usually lifelong and asymptomatic and disease may be attributed to the host response towards the colonization. Thus, some of the factors commonly designated as virulence factors in *H. pylori*, for instance the flagella, may rather be regarded as "colonization factors" (Testerman *et al.*, 2001). These factors primarily facilitate establishment and persistence of the infection, which however, naturally also increases the risk for disease, blurring a distinction from virulence factors. Studies of the contributions of individual bacterial factors to infectivity and pathogenicity have to be cautiously interpreted. Associations between different factors may give rise to confounding (Atherton *et al.*, 1995; Gerhard *et al.*, 1999; Xiang *et al.*, 1995; Zambon *et al.*, 2003) and the influences of unrecognized subtle mutations may result in spurious findings (Salaün *et al.*, 2005; Yamaoka *et al.*, 2002).

The cag PAI and VacA

The *cag* PAI is one of the most studied loci in the *H. pylori* genome and is present in the majority of strains worldwide (Nilsson *et al.*, 2003; Park *et al.*, 2002; Parsonnet *et al.*, 1997; Simán *et al.*, 2005). The locus is associated with a more vigorous host response characterized by IL-8 induction (Akopyants *et al.*, 1998; Censini *et al.*, 1996; Nilsson *et al.*, 2003; Yamaoka *et al.*, 1996) and an increased risk for ulceration and cancer (Gerhard *et al.*, 1999; Nilsson *et al.*, 2003; Nomura *et al.*, 2002; Parsonnet *et al.*, 1997; Zambon *et al.*, 2003). The *cag* PAI is an almost 40 kb stretch of DNA that encodes nearly 30 genes, many of which are homologous to type IV secretion system components (Akopyants *et al.*, 1998; Censini *et al.*, 1996; Odenbreit *et al.*, 2000). Type IV secretion systems assemble into a syringe-like structure that mediates secretion of molecules extracellularly or into the cytosol of host cells. The secretion system of *H. pylori* delivers the *cag* PAI-encoded and immunodominant CagA protein into the gastric epithelial cells (Odenbreit *et al.*, 2000; Segal *et al.*, 1999). Upon translocation, CagA is phosphorylated and initiates signal transduction that results in cytoskeletal rearrangements and an inflammatory response (Brandt *et al.*, 2005; Higashi *et al.*, 2002; Segal *et al.*, 1999). The secretion system may also mediate transfer of *H. pylori* peptidoglycan into the epithelial cells where Nod1, an intracellular pathogen-recognition molecule, can initiate an immune response (Viala *et al.*, 2004). PAIs are typically prone to horizontal genetic transfer. The *cag* PAI exhibits signs of such mobility by the differing GC content compared to the rest of the genome and the presence of flanking direct repeats and insertion sequences (Akopyants *et al.*, 1998; Censini *et al.*, 1996). Accordingly, excision and insertion of the *cag* PAI can result in mixed infections with regard to *cag* PAI status (Björkholm *et al.*, 2001a; Kersulyte *et al.*, 1999; Tomasini *et al.*, 2003). Intermediate strains that lack some of the *cag* PAI genes have also been described (Nilsson *et al.*, 2003; Tomasini *et al.*, 2003).

Early on, *H. pylori* was found to possess a cytotoxic ability involving formation of vacuoles in epithelial cells, which could be attributed to the bacterial exotoxin VacA (Cover and Blaser, 1992). The *vacA* gene appears to be universally present, but there are alleles with different signal (s1/s2) and mid regions (m1/m2) (Atherton *et al.*, 1995). The s1/m1 variant is most cytotoxic and the s1 and m1 genotypes have been proposed to be correlated with the pathogenic potential of the infection (Atherton *et al.*, 1995; Gerhard *et al.*, 1999; Zambon *et al.*, 2003). VacA can induce apoptosis of gastric cells, which may provide the bacteria with nutrients or reduce the acid output through the killing of parietal cells (Boquet *et al.*, 2003; Cover *et al.*, 2003). Furthermore, VacA-mediated inhibition of antigen presentation (Molinari *et al.*, 1998) and activation of T lymphocytes could play a role in immune evasion (Boncristiano *et al.*, 2003; Gebert *et al.*, 2003).

The cytotoxic variant of *vacA* is in linkage disequilibrium with the *cag* PAI (Atherton *et al.*, 1995; Xiang *et al.*, 1995), hence the gene name *cytotoxin-associated gene A* (*cagA*). The *vacA* and *cag* PAI loci are situated at distant sites on the chromosome and their linkage is inadequately understood. Nevertheless, the loci form the basis for a classification of virulence of *H. pylori* strains. The more virulent

type I strains express CagA and a cytotoxic variant of VacA, while the less virulent type II strains do not express CagA and harbor a non-toxic form of VacA (Xiang *et al.*, 1995). The serological response against CagA has been used as a marker of more virulent strains, but serological methods have been questioned due to limited sensitivity (0.71–0.90) and specificity (0.80–0.90) (Figueiredo *et al.*, 2001; Park *et al.*, 2002; Yamaoka *et al.*, 1998).

3.4. Surface structures

Flagella

The unipolar flagella of *H. pylori* enable motility, which is an important bacterial feature (Eaton *et al.*, 1992; Terry *et al.*, 2005). Two different flagellin proteins constitute the flagellar filament, but about 40 additional genes are involved in the secretion and assembly of the whole flagellar apparatus (Tomb *et al.*, 1997). Chemotactic systems offer means for spatial orientation, for example towards the mucosal cell lining where the pH is higher, nutrients can be more abundant and closer interactions with host cells are possible (Terry *et al.*, 2005).

Outer membrane proteins

A relatively large proportion (4%) of the coding capacity of the *H. pylori* genome is devoted to outer membrane proteins (OMP) (Doig *et al.*, 1999). Several of these proteins have been suggested to possess adhesive properties. The receptors include glycoconjugates expressed on host cells, such as the Lewis carbohydrate blood group antigens, extracellular matrix components and unknowns (Testerman *et al.*, 2001). Two OMPs have received particular attention for their ability to bind to host receptors. First, the BabA adhesin binds to Lewis b that is expressed by gastric epithelial cells (Ilver *et al.*, 1998) and the presence of this adhesin has been suggested to be associated with more severe disease (Gerhard *et al.*, 1999; Zambon *et al.*, 2003). Second, the SabA adhesin mediates a weaker and more intimate adherence by binding to sialyl-Lewis x, which is upregulated by the inflammation (Mahdavi *et al.*, 2002). Accordingly, a model was proposed where initial binding is mediated by Lewis b and BabA, which results in inflammation, induction of sialyl-Lewis x and binding through SabA. Altered adhesive properties may provide mechanisms for *H. pylori* to regulate its interactions with the host if, for example, the immune response would necessitate less tight adherence (Mahdavi *et al.*, 2002) or when the availability of receptors differ between human populations (Aspholm-Hurtig *et al.*, 2004). Such modulation of the binding properties may occur by changed expression or evolution of functional variants through frameshift mutation or recombination between homologous loci (Ilver *et al.*, 1998; Mahdavi *et al.*, 2002). For instance, the BabA-encoding gene *babA2* is similar to the *babA1* and *babB* genes and recombination events between these loci have been described (Bäckström *et al.*, 2004; Solnick *et al.*, 2004).

Lipopolysaccharide

LPS covers the surface of *H. pylori* and other gram-negative bacteria and sustains membrane integrity and can mediate interaction with the host. An unusual feature of *H. pylori* LPS is the expression of Lewis antigens on the polymeric carbohydrate O-antigen constituent, resembling blood group antigens expressed on various host tissues. About 80–90% of *H. pylori* strains express Lewis antigens, whereof Lewis x and Lewis y are most common (Simoons-Smit *et al.*, 1996; Taylor *et al.*, 1998). Fucosyltransferases involved in the synthesis of Lewis antigens can undergo slipped strand mispairing, generating variability of Lewis antigen expression, which may aid adaptive evolution (Appelmelk *et al.*, 1999; Salaün *et al.*, 2005; Wirth *et al.*, 1999). The molecular mimicry of *H. pylori* and human Lewis antigen expression has been suggested to facilitate bacterial immune evasion (Wirth *et al.*, 1997) and give rise to autoimmunity (Heneghan *et al.*, 2001). *H. pylori* Lewis antigens could further interact with dendritic cells to balance the Th1/Th2 responses (Bergman *et al.*, 2004) and have been described to mediate adhesion (Edwards *et al.*, 2000). However, much of the evidence regarding the biological roles of *H. pylori* Lewis antigen expression is inconclusive and awaits confirmatory data (Appelmelk *et al.*, 2000; Mahdavi *et al.*, 2003; Taylor *et al.*, 1998).

4. *H. PYLORI*-ASSOCIATED DISEASE

The ability of *H. pylori* to maintain persistent colonization is of key importance for disease development. The infection is accompanied by a usually asymptomatic chronic gastritis, but 10–20% of infected individuals manifest more severe complications, such as peptic ulcer disease and gastric cancer (Suerbaum and Michetti, 2002). This selectivity in disease progression is inadequately understood and explanations are sought among factors of the host (Correa *et al.*, 2004; El-Omar *et al.*, 2000), environment (Correa *et al.*, 2004) and bacterium (Gerhard *et al.*, 1999; Nomura *et al.*, 2002; Parsonnet *et al.*, 1997; Zambon *et al.*, 2003).

4.1. Gastritis

Acute *H. pylori* infection causes gastritis and hypochlorhydria and symptoms such as vomiting and dyspepsia have been associated with acquisition (Graham *et al.*, 2004; Marshall, 2001). Persistent infection causes chronic gastritis in virtually all infected individuals. The gastric inflammation involves infiltration of immune cells, such as neutrophils, lymphocytes, plasma cells and macrophages, and secretion of a multitude of cytokines, of which IL-8 seems to have a central role (Blaser and Atherton, 2004; Suerbaum and Michetti, 2002; Yamaoka *et al.*, 1996). The chronic gastritis is usually asymptomatic, but eradication of *H. pylori* in non-ulcer dyspeptic patients alleviates symptoms in a fraction of the patients (Moayyedi *et al.*, 2005).

The chronic gastritis leads to peptic ulcer disease and gastric cancer in 10–20% of infected individuals (Suerbaum and Michetti, 2002). Duodenal ulcer disease and gastric ulcer/cancer represent different pathways of the infection and correlate strongly with the pattern of colonization and gastritis. Duodenal ulceration is characterized by higher acid secretion and antrum-predominant gastritis, while gastric ulceration/carcinogenesis is associated with lower acid secretion and corpus-predominant gastritis or pangastritis (Blaser and Atherton, 2004; Suerbaum and Michetti, 2002; Uemura *et al.*, 2001).

4.2. Peptic ulcer disease

The discovery of the role of *H. pylori* in the development of peptic ulcer disease has lead to a paradigm shift in the treatment of ulcer patients (NIH Consensus Conference, 1994). The lifetime risk for peptic ulcer in infected individuals ranges from 3% in the United States to 25% in Japan (Suerbaum and Michetti, 2002). It has been estimated that 95% of duodenal ulcers and 70% of gastric ulcers can be attributed to *H. pylori* (Rothenbacher and Brenner, 2003).

Duodenal ulcers are associated with *H. pylori*-induced antrum-predominant gastritis, decreased somatostatin levels and augmented gastrin and acid secretion (Blaser and

Atherton, 2004; Suerbaum and Michetti, 2002). Development of gastric metaplasia in the duodenum can allow further bacterial colonization, leading to duodenitis and epithelial damage. Gastric ulcers are associated with corpus gastritis, which is believed to damage the epithelium (Blaser and Atherton, 2004). Eradication of the infection heals peptic ulcer disease, restores normal acid secretion and prevents ulcer relapse (Ford *et al.*, 2004).

4.3. Gastric cancer

Gastric cancer ranks as the second most frequent cause of cancer deaths worldwide despite a decreasing incidence in high-income countries (Correa *et al.*, 2004). The World Health Organization classified *H. pylori* as a class I carcinogen in 1994 due to its definite carcinogenic potential in humans (IARC, 1994). Subsequent studies have corroborated the association to gastric cancer and about 70% of non-cardia adenocarcinomas have been attributed to *H. pylori* infection (Ekström *et al.*, 2001; Uemura *et al.*, 2001). However, only a few percent of infected individuals develop gastric cancer (Blaser and Atherton, 2004; Uemura *et al.*, 2001). *H. pylori* acquisition in younger ages has been suggested to contribute a greater cancer risk (Blaser *et al.*, 1995), but this study is limited by its sparse adjustments and its use of sibship size and birth order as proxies for early acquisition.

Persons with low gastric acid secretion and corpus-predominant gastritis, leading to atrophic gastritis, loss of parietal cells and further hypochlorhydria, are at increased risk for gastric cancer (Blaser and Atherton, 2004; Suerbaum and Michetti, 2002; Uemura *et al.*, 2001). Postulated carcinogenic mechanisms include the increased epithelial turnover caused by the inflammation. Furthermore, the development of gastric atrophy and hypochlorhydria can result in impaired antioxidant absorption, infection with other carcinogenic microorganisms and formation of carcinogenic compounds (Blaser and Atherton, 2004). *H. pylori* infection upregulates the pro-inflammatory cytokine IL-1β, which is also a potent inhibitor of acid secretion. Polymorphisms considered to increase the activity of IL-1β have been proposed to be associated with hypochlorhydria and cancer, supporting the central role of gastric acidity in cancer development (El-Omar *et al.*, 2000). The atrophic stomach appears to constitute an inhospitable environment for *H. pylori* and cleared infections can lead to misclassification of exposure and underestimation of the gastric cancer risk associated with the infection (Blaser and Atherton, 2004; Ekström *et al.*, 2001).

4.4. Other *H. pylori*-associated conditions

H. pylori has been assessed for its involvement in various additional conditions. An association between the infection and gastric mucosa-associated lymphoid tissue (MALT) lymphoma belongs among the generally accepted findings and antibiotic treatment alone leads to regression of the cancer in many cases (Farinha and Gascoyne, 2005). Moreover, it has been debated whether there is a causal

relationship between the parallel decline of *H. pylori* prevalence and the increase of gastroesophageal reflux disease (GERD) and esophageal adenocarcinoma in high-income countries. There is accumulating evidence in favor of a protective role of *H. pylori* infection against these conditions, but there are a number of additional contributing factors (Lagergren, 2005; Malfertheiner and Peitz, 2005).

The high *H. pylori* prevalence in many parts of the world accentuates the public health importance of any associations between *H. pylori* and disease. This may be particularly noteworthy for associations that are of modest strength or involve relatively benign conditions, as these associations may otherwise tend to be neglected. In this context, a possible role of the infection in malnutrition and iron deficiency anemia may be of special interest (Milman *et al.*, 1998; Salgueiro *et al.*, 2004), given the high *H. pylori* prevalence in low-income countries where malnutrition can be common for other reasons. *H. pylori* infection has, however, also been suggested to provide protection against childhood diarrheal disease (Rothenbacher *et al.*, 2000) and *H. pylori*-induced anemia has been hypothesized to protect against malaria (Dominguez-Bello and Blaser, 2005).

5. MANAGEMENT OF *H. PYLORI* INFECTION

Clinical management of *H. pylori* infection and its associated morbidity, as well as related research, rely on the availability of practical and well-evaluated diagnostic tests and treatments. Disease prevention may be possible by targeting the infection, either by eradication treatment or by preventing the establishment of the infection. Furthermore, it should be kept in mind that *H. pylori* infection is most prevalent in low-income countries and any tools for the management of the infection should thus preferably be accessible also in these countries.

5.1. Diagnosis

H. pylori can be detected through endoscopy by culture, histology or urease test of biopsies (Malfertheiner *et al.*, 2002; Suerbaum and Michetti, 2002). These methods are all liable to false negative results due to patchy bacterial colonization. Culture is the theoretical gold standard for identifying bacterial infections, but can have low sensitivity for *H. pylori*.

The infection elicits a systemic IgG antibody response that can be detected for diagnostic purposes (Malfertheiner *et al.*, 2002; Suerbaum and Michetti, 2002). Anti-*H. pylori* antibodies can be assessed with enzyme-linked immunosorbent assays (ELISA) or Western Blot (also designated immunoblot). The latter method has the advantage of characterizing the immune response towards different bacterial antigens. Serology can have limited sensitivity in young children and the antigenic preparation may influence the results (Malfertheiner *et al.*, 2002; Suerbaum and Michetti, 2002; Tindberg *et al.*, 2001a).

The ^{13}C-urea breath test (UBT) relies on the principle that ^{13}C-labeled urea is hydrolyzed by the bacterial urease with formation of $^{13}CO_2$, which is detected in the expired breath (Malfertheiner *et al.*, 2002; Suerbaum and Michetti, 2002). An advantage of the UBT is its ability to detect current infection, but the performance of the test in the youngest children has been questioned. There are also reliable ELISA-based stool antigen tests that detect *H. pylori* antigens shed in the feces. Current guidelines recommend the UBT or the stool antigen test for diagnosing the infection in primary care because serological methods require local validation (Malfertheiner *et al.*, 2002). The UBT or, if the UBT is not available, the stool antigen test are recommended to confirm eradication of infection after treatment.

5.2. Treatment

The relatively benign nature of *H. pylori* infection in the majority of infected individuals has elicited debate about how a positive diagnostic test should be handled. The argumentation has been further fueled by the suggested protective

effect of the infection on esophageal adenocarcinoma. Nevertheless, general guidelines for treatment of the infection have been developed and continue to evolve (Malfertheiner *et al.*, 2002; Suerbaum and Michetti, 2002). *H. pylori* eradication is strongly recommended for all patients with peptic ulcer disease, MALT lymphoma, atrophic gastritis, after gastric cancer resection and for first-degree relatives of gastric cancer patients. Furthermore, eradication treatment is advised for patients taking non-steroidal anti-inflammatory drugs (NSAID), which is an independent risk factor for peptic ulcer disease, and for GERD patients on long-term profound acid suppression, who can develop corpus atrophic gastritis. Patients presenting with persistent dyspepsia may also be offered eradication treatment, as it may lead to symptom improvement in a subset of the cases.

H. pylori eradication treatment lasts for one to two weeks and usually includes two antimicrobials and an antisecretory agent because acid impairs the efficiency of some antibiotics (Malfertheiner *et al.*, 2002; Suerbaum and Michetti, 2002). The principal antimicrobials are clarithromycin, amoxicillin, metronidazole and tetracycline and the acid suppressant is usually a proton pump inhibitor. Ranitidine bismuth citrate combines antibacterial and antisecretory activities and can also be used. The cure rate is 80% or above, but antibiotic resistance to particularly metronidazole and clarithromycin is an increasing concern (Malfertheiner *et al.*, 2002; Suerbaum and Michetti, 2002).

Reinfection rates are generally considered to be low after successful eradication (Farrell *et al.*, 2004; Mitchell *et al.*, 1998; Rowland *et al.*, 1999; Suerbaum and Michetti, 2002). However, reinfection may be more common in young children (Magistà *et al.*, 2005; Rowland *et al.*, 1999) and in high-prevalence settings (Hildebrand *et al.*, 2001; Soto *et al.*, 2003; Wheeldon *et al.*, 2005). Post-eradication reinfection rates of about 20% have been reported in adults in high-prevalence communities (Soto *et al.*, 2003; Wheeldon *et al.*, 2005), thus being comparable to the incidence in childhood. These reported high reinfection rates speak against a significant role of protective immunity after therapeutic eradication and indicate that prevention of acquisition is needed to attain long-term absence of infection in some high-prevalence settings.

5.3. Prevention of *H. pylori*-associated disease

Prevention of *H. pylori*-associated disease benefits from predictions of who will become clinically ill. Accordingly, current treatment guidelines advise prophylactic *H. pylori* eradication for some individuals at higher risk for disease, for example patients with atrophic gastritis or taking NSAIDs (Malfertheiner *et al.*, 2002; Suerbaum and Michetti, 2002). Some studies have also targeted high-risk population groups to study the effect of *H. pylori* eradication. Anti-*H. pylori* treatment has been reported to increase regression of cancer precursor lesions (Correa *et al.*, 2000; Kuipers *et al.*, 2004) and, despite low power and a lack of studies, there is some evidence that *H. pylori* eradication may protect against gastric cancer (Uemura *et al.*,

2001; Wong *et al.*, 2004). Future prevention approaches may possibly benefit from a deeper knowledge of the pathogenic mechanisms by allowing more precise identification of individuals at high risk for disease.

Indiscriminate treatment of *H. pylori* infections has been proposed as an approach to limit the burden of *H. pylori*-associated disease. The appropriateness of such a large-scale and crude intervention has been questioned due to the uncertain full spectrum of possible harmful consequences, for example the development of antibiotic resistance (Malfertheiner *et al.*, 2002; Sjölund *et al.*, 2003; Suerbaum and Michetti, 2002). Testing and treating large numbers of persons would also imply significant costs and would therefore be unrealistic in many parts of the world.

An alternative approach could be to target the acquisition or persistence of the infection, while limiting the use of antibiotics. The role of an *H. pylori* vaccine is uncertain given the common failure of the immune system to clear the infection and the apparently inadequate protective immunity against reinfection (Hildebrand *et al.*, 2001; Magistà *et al.*, 2005; Soto *et al.*, 2003; Wheeldon *et al.*, 2005). A protective vaccine would also have to be administered at an early age before the infection is acquired. At this age, an immature immune system may not respond sufficiently to immunization. Another approach could perhaps be a therapeutic vaccine that would circumvent problems with antibiotic resistance. There have been considerable efforts to develop vaccines against *H. pylori*, but despite some encouraging results further work is needed to bring about effective and safe candidates for humans (Ruggiero *et al.*, 2003). Moreover, probiotics have been suggested to be capable of contributing to control of *H. pylori* infection, but this area of research is in its infancy (Hamilton-Miller, 2003).

Preventing establishment of infection by interfering with transmission is a strategy that has been used in public health interventions against a variety of infections. Only a few smaller trials have considered preventing the establishment of *H. pylori* infection by limiting the transmission. This can partly be explained by the fact that there is no apparent prevention strategy at present. The lack of thinkable interventions may be attributed to the seemingly multifaceted nature of *H. pylori* acquisition, intertwined with activities of everyday life. One study reported that the introduction of a lidded, narrow-mouthed water vessel into households was protective against seroconversion (Glynn *et al.*, 2002). In the same study, however, fecal contamination of the water source and using a water disinfectant were not related to becoming infected. There has also been an attempt to detect a difference in the reinfection rates in children depending on whether the whole family unit received eradication therapy or not (Farrell *et al.*, 2004). No such difference was detected, but the authors acknowledged that the study was likely underpowered due to an overall low reinfection rate.

Any future prevention of *H. pylori*-associated disease should likely be primarily aimed at high-risk populations and target both the infection and other known risk factors. Antibiotic treatment is likely to play a central role in efforts to eliminate the

infection. However, understanding and interfering with the acquisition or persistence of the infection by other means may become useful supplemental strategies. This is likely to be especially true in some (low-income) populations, where effective antibiotic regimens may be impaired by high cost, poor compliance, antibiotic resistance and high reinfection rates.

6. AIMS OF THE PRESENT INVESTIGATION

The general aim of this thesis was to advance our understanding of some aspects of *H. pylori* infection, specifically related to its transmission, and highlight some methodological issues.

Specific aims were to:

- Disentangle the independent contributions of *H. pylori* infections in mothers, fathers and siblings to the risk for the infection in 11- to 13-year-old index children (Paper I).
- Investigate if appropriate analysis of all available data, including additional non-randomly sampled cases, would reveal further insights into the familial clustering of *H. pylori* infection, while possibly improving the precision of the estimates of risk factors already identified (Paper II).
- Explore patterns of *H. pylori* strain concordance in bacterial isolates from family members by using PCR-based molecular typing (Paper III).
- Assess the suitability of serology for determining the *cag* PAI status of *H. pylori* infections, by investigating the agreement between the occurrence of CagA-reactive antibodies, as assessed by immunoblot, and bacterial *cag* PAI status, as determined by PCR (Paper IV).
- Explore the genetic diversity of clonal *H. pylori* isolates within and between members of a family by sequencing and comparative genomic microarray hybridizations (Paper V).

The following sections summarize the methods and results of Papers I–V and bring the findings together in a context of the current literature. More detailed information regarding the individual studies can be retrieved from the corresponding papers that are provided as appendices.

7. SUBJECTS AND METHODS

Sections 7, 8, 9 and 13 are omitted from the electronic version of the thesis due to copyright reasons. Please refer to the original papers.

8. RESULTS

Sections 7, 8, 9 and 13 are omitted from the electronic version of the thesis due to copyright reasons. Please refer to the original papers.

9. DISCUSSION

Sections 7, 8, 9 and 13 are omitted from the electronic version of the thesis due to copyright reasons. Please refer to the original papers.

10. CONCLUDING REMARKS

Infectious diseases and their sequelae are by definition preventable. In this respect, understanding and interfering with the transmission, colonization or pathogenesis of the infectious agent are fundamental. There are some promising data on prevention of *H. pylori*-associated morbidity by treating the infection in high-risk populations, but attempts to limit the transmission are lacking.

The identification of the family and early childhood as the primary place and time for *H. pylori* acquisition are important pieces of epidemiological knowledge. The present data support a predominantly mother-child and sib-sib transmission of *H. pylori*. This finding is consistent with an important role of intimate contact in the transmission and a relatively low infectiousness of the bacterium. The extensive *H. pylori* genome variability is considered to be important for adaptive evolution and we discerned characteristics of the variability likely to be important for the bacterium. This diversity could, however, not be related to either transmission or host adaptation. Further epidemiological and microbiological insights into *H. pylori* acquisition, persistence and pathogenesis may improve clinical and public health management of the infection. Thus, these issues deserve sustained attention and longitudinal studies of human populations coupled with collection of bacterial samples and experimental investigations could be valuable undertakings. The findings presented in this thesis and the discussions thereof may aid in directing such future endeavors.

For any public health intervention to be ethically defendable, benefits and possible detrimental side effects must be carefully evaluated beforehand. A parallel is found in the study of hazardous exposures in humans, which does not allow randomized experimental approaches. Epidemiology has solved this predicament by providing tools for appropriate analyses of data from the "natural experiment", that is, a population where the exposure is already present. Social and economic development results in a declining *H. pylori* prevalence, not to mention the many other more tangible benefits. However, the specific factors that cause this decreasing prevalence, as well as its consequences for humans, remain elusive. Part of the solution to a better understanding of the relationship between humans and *H. pylori* perhaps lies within clever exploration of this already operative "social vaccine" against *H. pylori* infection.

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