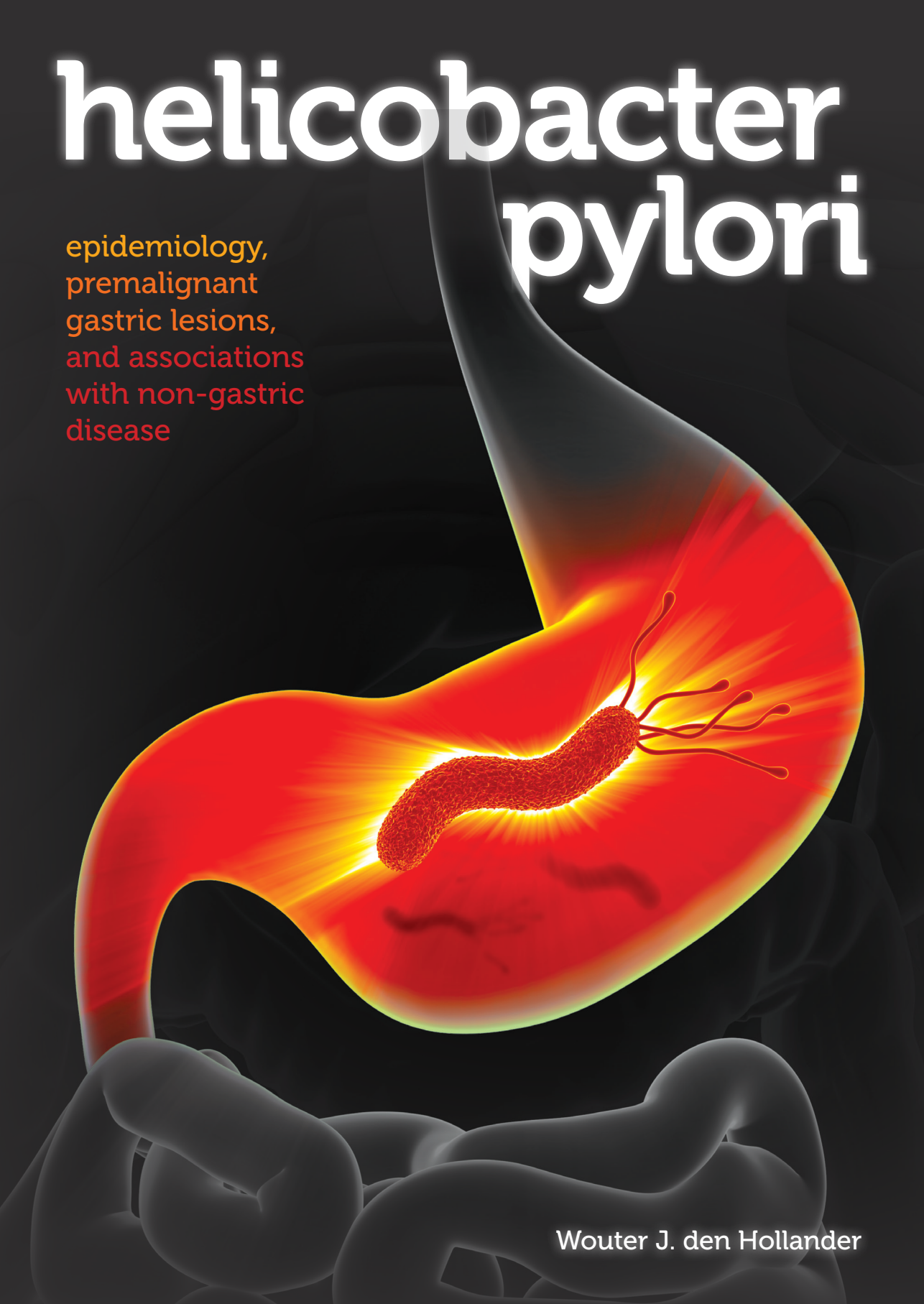
helicobacter pylori

epidemiology, premalignant gastric lesions, and associations with non-gastric disease



Wouter J. den Hollander

Helicobacter pylori – Epidemiology, Premalignant Gastric Lesions, and Associations with Non-gastric Disease

Wouter J. den Hollander

COLOFON

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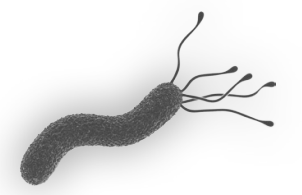
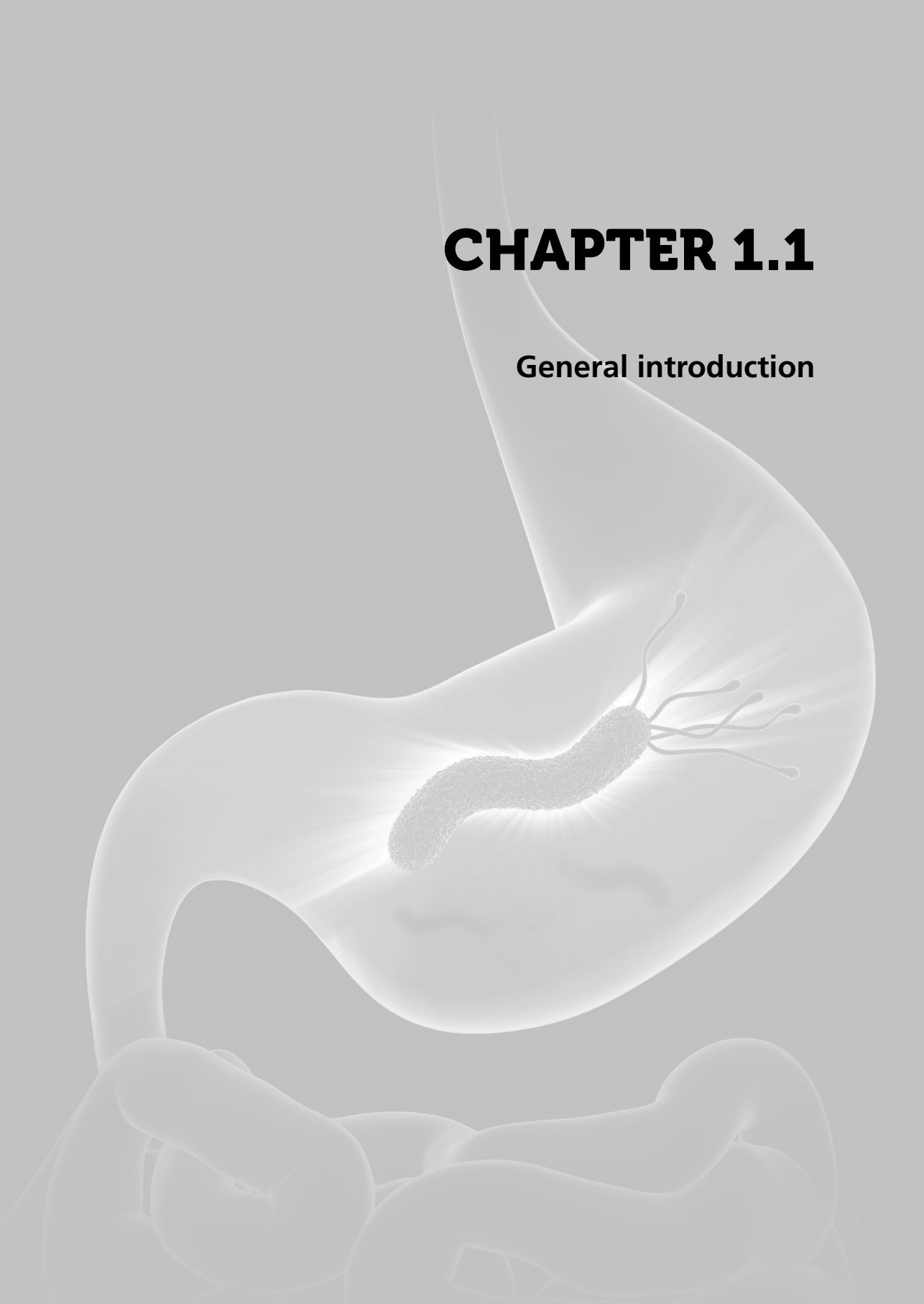
PART I

Introduction and thesis outline



CHAPTER 1.1

General introduction



For a very long time he was ubiquitous, but unknown. Then he became recognized, but forgotten. Now, he is well known, but disappearing.

In short, these three sentences could be the historical description of *Helicobacter pylori (H. pylori)*, the spiral shaped gram-negative bacterium colonizing the stomach in about half of the world's population.

This thesis focuses on its epidemiology in a multi-ethnic Western society, in which subjects from high and low prevalence countries are living together. Although colonizing the stomach, *H. pylori* has been associated with extra-gastric diseases, like asthma and allergy, pregnancy complications and obesity. Because of the adaptive immunological activity of the stomach in terms of both T and B-cell function, it is thought that the strong, lifelong interaction between *H. pylori* and the gastric mucosa influences the maturation of the immune system. In this context, the "disappearing microbiota" hypothesis states that the loss of our ancient indigenous microbiota contributes to the epidemic rise in diseases like asthma, allergy and obesity (1). *H. pylori*, as a member of the ancient indigenous microflora, can be used as a proxy for this modern phenomenon. In this thesis we focus on the epidemiological relationship between *H. pylori* and asthma/allergy, and obesity. We further assess the association between *H. pylori* and pregnancy related complications. Most of above mentioned studies are embedded in a population-based prospective cohort study in Rotterdam, the Netherlands, called the Generation R study. In this study pregnant women and their children have been followed from early pregnancy onwards, until they will reach adulthood. The study is designed to identify environmental and genetic causes of normal and abnormal growth, development and health during fetal life, childhood and adulthood (2). Enrolment was aimed in the first trimester, but was allowed until birth of the child. In total, 9,778 mothers with a delivery date between April 2002 and January 2006 were enrolled in the study. Data collection during each trimester of pregnancy included physical examination, biological samples, fetal ultrasound examinations, and self-administered questionnaires. Information from midwife and hospital registries was obtained. During the pre-school period, which refers to the period from birth to 4 years of age, information was mainly obtained by means of postal questionnaires. At the age of 6 years, blood samples were collected, and information on presence of pulmonary symptoms and disease was obtained by questionnaire. Additional detailed hands-on assessments were performed in a dedicated research centre to measure e.g. length and weight.

The thesis further focuses on the potential long-term consequence of *H. pylori* colonization at higher age. Chronic active gastritis caused by *H. pylori* colonization eventually leads to intestinal type gastric cancer in a small part of infected subjects. Recognition and surveillance of its precursor lesions may prevent patients form invasive cancer. In one study we follow-up a cohort of these patients.

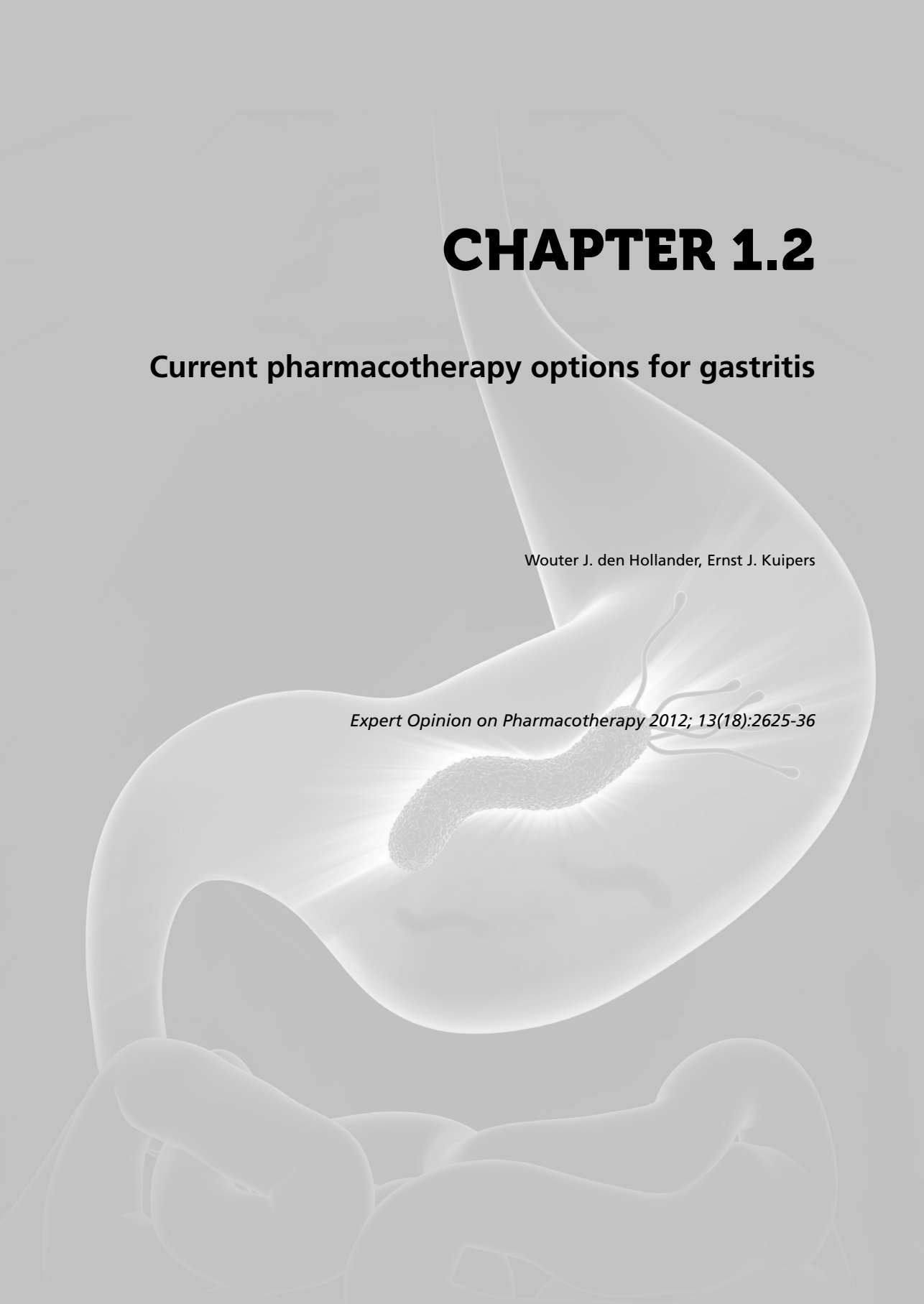
This thesis is divided in four parts. In **part I** most of the main topics are introduced, followed by the aims and outline of this thesis. **Part II** and **part III** enclose the original studies on the epidemiology of *H. pylori*, its association with extra-gastric diseases, and the follow-up of premalignant gastric lesions. The main findings of this thesis are summarized and discussed in **part IV**.

CHAPTER 1.2

Current pharmacotherapy options for gastritis

Wouter J. den Hollander, Ernst J. Kuipers

Expert Opinion on Pharmacotherapy 2012; 13(18):2625-36



ABSTRACT

Introduction: Gastritis is a broad term, which is used for different conditions by clinicians, endoscopists and pathologists. Classification strategies have led to more congruence between specialists. The histological evaluation of the gastric mucosa is mandatory for diagnosing and classifying gastritis. Main etiologic factor is infection with *H. pylori*. The clinical importance of gastritis lays in the fact that it predisposes to more pronounced damage to the gastric mucosa, in particular peptic ulcer disease, and eventually atrophic gastritis, intestinal metaplasia, and gastric malignancy, both adenocarcinoma and MALT lymphoma.

Areas covered: This review covers the current pharmacotherapy options for different forms of gastritis. The main focus is on *H. pylori*-induced gastritis. Thereafter, other forms of gastritis like autoimmune gastritis and NSAID-related gastropathy are covered.

Expert opinion: The emerging problem of antibiotic resistance requires an accurate knowledge of local eradication rates. Standard triple therapy should be abandoned in regions with high clarithromycin resistance. In these areas sequential or quadruple therapy is best initial treatment. Further research should focus on non-invasive and effective techniques of susceptibility testing, making a tailored approached available and cost-effective. Primary prevention of NSAID-related gastropathy can be enhanced by better education for clinicians and patients, so that both right prescription of gastro-protective agents as therapy adherence will improve.

1. INTRODUCTION ON GASTRITIS

Gastritis is in clinical practice a confusing term owing to different definitions used by clinicians, endoscopists, and pathologists. Clinicians often use the term as a descriptor for patients with acute symptoms of vomiting, presumably of infectious or toxic origin, with or without diarrhea ('gastro-enteritis'). Usually, there is no indication for endoscopy and the condition is self-limiting. Acute hemorrhagic or erosive gastritis is caused by acute chemical or irritant injury. Endoscopists however use the term 'gastritis' to describe endoscopic signs of inflammation and irritation of the gastric epithelium, including vascular injection, erythema, and edema. Finally, pathologists reserve the term for histological signs of inflammation of the mucosa, with 'acute' gastritis referring to polynuclear infiltration and 'chronic' gastritis referring to mononuclears, and 'acute and chronic gastritis' to a mixed infiltrate (see **Table 1** for different forms of gastritis).

Table 1 Different forms of gastritis and gastropathy

Acute gastritis	**Uncommon**
Acute *Helicobacter pylori* gastritis Acute hemorrhagic and erosive gastropathy	Eosinophilic gastritis Lymphocytic gastritis Infectious gastritis, other than *H. pylori* - Viruses (CMV, EBV, Herpes virus) - Bacteria (streptococci, *E.coli,* enterobacteria) - Mycobacteria - Fungi (Candida) - Parasites (Cryptosporidiosis) Crohn's disease Sarcoidosis Isolated granulomatous gastritis
Common	
Helicobacter pylori gastritis Chemical gastritis/gastropathy - NSAIDs/ aspirin use - Bile reflux - Alcohol Autoimmune atrophic gastritis	

CMV: Cytomegalovirus; EBV: Epstein-Barr virus; NSAID: Non-steroidal anti-inflammatory drug.

Twenty years ago, the need for conformity in definition increased as it became clear that there was no obvious correlation between dyspepsia, endoscopic signs of gastritis, and histological gastritis was found (3). Often signs of edema, reddening, and swelling of the mucosa were found by endoscopy, suggesting gastritis. However, macroscopic features observed during gastroscopy were poorly related to histological signs of gastritis (4, 5), unless the gastric mucosa either had a typical miniature cobblestone appearance, named nodular gastritis (particularly found in children with *H. pylori* infection), or grossly enlarged folds, termed hypertrophic gastritis (6, 7). Since then, endoscopic imaging has strongly improved in terms of image resolution, magnification, and light techniques such as narrow band imaging (NBI). These improvements have enabled the experienced endoscopists to improve on identification of patients with different conditions of the

gastric mucosa, such as inflammation, atrophy, and metaplasia. Further techniques such as magnification endoscopy are useful in specific situations, such as the evaluation of dysplasia, but are not used in routine daily practice (8). These endoscopic improvements have for now not made histology redundant. The histological evaluation of the gastric mucosa remains mandatory for diagnosing and classifying gastritis. This review will focus on the combination of endoscopic and histological gastritis, not on the acute clinical picture of nausea and vomiting clinically defined as gastritis.

1.1 Definitions and histological classification

In 1990 a new classification of endoscopic and histological signs of gastritis was presented: the Sydney System. Until now, this histologic score system is still important and widely used (9). It comprises an endoscopic and a histological division, the latter being the most important part. Topography of gastritis was considered to be the core of the classification: gastritis limited to the antrum, the corpus, or a pangastritis. If known, the etiology can be added as prefix. Morphology of the gastric mucosa is divided into five graded variables: chronic inflammation (presence of mononuclear cells), activity of gastritis (presence of neutrophils), atrophy defined as loss of specialized glands, intestinal metaplasia, and the presence of *H. pylori* (9). Each of these variables is graded as absent, mild, moderate or severe. In 1997 an update of this Sydney system was published (10). A recommendation to include biopsies from the angulus of the stomach beyond antrum and corpus was added. Moreover, a 'visual analogue scale' was presented to increase the interobserver consistency in grading of the five morphologic variables.

In 2007 a further gastritis classification was introduced, the Operative Link on Gastritis Assessment (OLGA) (11). Rationale for this project was that the histology report till then failed to establish a link between gastritis phenotype and risk of malignancy. The OLGA system stages a patient into different cancer risk groups, based on the combination of antrum and corpus atrophy scores. Stage I comprises the lowest atrophy score and stage IV the highest. The stages III and IV are associated with an increased cancer risk compared with the lower stages (12). From a clinical perspective, this system was a significant step forward. To improve clinical usefulness and reduce interobserver variation as much as possible, a system modification, the OLGIM system, was later proposed based on replacement of antral and corpus atrophy scores by scores of intestinal metaplasia at the same locations (see **Table 2**) (13). OLGA and OLGIM are both staging systems that provide useful information on prognosis and need for follow-up strategies (14). A recent guideline on management of premalignant gastric lesions recommends use of these systems to define surveillance management for subgroups of patients with different risks of progression to gastric cancer (15).

Table 2 OLGIM staging system(13). Replacement of IM by atrophy results in the OLGA frame(110).

		Corpus			
	IM score	**No IM (score 0)**	**Mild IM (1)**	**Moderate IM (2)**	**Severe IM (3)**
Antrum (including incisura angularis)	**No IM (score 0)**	Stage 0	Stage I	Stage II	Stage II
	Mild IM (1)	State I	Stage I	Stage II	Stage III
	Moderate IM (2)	Stage II	Stage II	Stage III	Stage IV
	Severe IM (3)	Stage III	Stage III	Stage IV	Stage IV

OLGIM, operative link on gastric intestinal metaplasia assessment; *IM,* Intestinal metaplasia; *OLGA,* operative link for gastritis assessment

1.2 Etiology

Although gastritis was known for decades by clinicians as a very common condition, the causative agent for the vast majority of gastritis cases was not recognized until 1982 (16). With the almost ubiquitous prevalence at older age, gastritis was for long even considered a normal process of aging. The detection of *H. pylori* as cause of chronic gastritis therefore totally altered the former concepts. This turned the most common type of gastritis into a potentially curable disease.

H. pylori is a gram-negative bacterium, which colonizes more than half of the world's population. Owing to a remarkably high urease activity the bacterium can survive in the acid environment of the stomach (17, 18). Urease converts urea present in the gastric lumen into alkaline ammonia and carbon dioxide. The prevalence of *H. pylori* widely varies geographically, and is highest in developing countries. In Western countries, however, prevalences have been declining over recent decades to below 40% – supposedly as a result of improved hygiene and sanitation and the active elimination by antibiotics (18). In parallel to the declining infection rate, incidences of *H. pylori*-related diseases also have shown a major decline (19, 20). Acquisition of infection usually occurs in early childhood, and is mainly transmitted within families (21). One recent study found that during the last decades the decline in acquisition in children has slowed down, suggesting a stabilization of the previously decreasing trend (22). Colonization will induce gastritis in virtually all infected people, and will persist in adults unless eradication by antibiotics. *H. pylori* is a noninvasive bacterium, but it nevertheless stimulates a robust inflammatory and immune response (23). The bacterium disrupts the mucous layer, making the mucosa more vulnerable to acid peptic damage. In addition, the host immune response in reaction to *H. pylori* further aggravates tissue injury. Level of immune response, tissue damage and development of *H. pylori* related diseases is influenced by bacterial, host, and environmental factors. In *H. pylori*-positive patients the life-time risk for developing ulcer disease is about 10-20% and for developing gastric cancer about 1-2% (18).

This discovery of *H. pylori* has also led to the recognition of other distinctive forms of gastritis (10). In *autoimmune atrophic gastritis* an immune response in the oxyntic mucosa is directed against parietal cells and intrinsic factor. Parietal cell destruction results

in hypochlorhydria and ultimate achlorhydria, hypergastrinemia, and loss of pepsin activity (24). Serum anti-parietal cell and anti-intrinsic factor antibodies can be found. Inflammation is typically found in the fundus-corpus mucosa. This may eventually lead to vitamin B12 deficiency with pernicious anemia. Some studies have shown a possible association between *H. pylori* infection and autoimmune gastritis (25, 26). However, confirmation of this association needs further large cohort studies of *H. pylori*-positive subjects (13).

A *chemical* or *reactive gastritis/gastropathy* can occur due to chemical irritants and drugs. Some people prefer to consider this mucosal injury as a reactive *gastropathy*, since evident signs of an inflammatory process are lacking (27). For example bile reflux and NSAIDs/aspirin may cause mucosal damage (10). NSAIDs inhibit gastrointestinal mucosal cyclooxygenase (COX) activity. As consequence, synthesis of mucosal protective prostaglandins is suppressed, resulting in mucosal damage and eventually in many patients mucosal erosions and ulcera. NSAID/aspirin use is therefore a major risk factor for gastrointestinal bleeding, with serious morbidity and mortality. Bile reflux gastritis results from regurgitation of bile into the stomach owing to an operative stoma, an incompetent pyloric sphincter, cholecystectomy, or abnormal duodenal motility (28, 29).

Rare forms of gastritis include *eosinophilic gastritis* and *lymphocytic gastritis*. In eosinophilic gastritis the esophagus and intestine can also be involved. Although the exact etiology is still unknown, it is thought that there may be involvement of environmental factors in diet that play a critical role (30). Damage to the gastrointestinal wall is caused by eosinophilic infiltration and degranulation (31). Abdominal pain and diarrhea are common complaints. Lymphocytic gastritis is associated with celiac disease and possibly with *H. pylori* infection (10, 32). This rare condition is characterized by a dense lymphocytic infiltration of surface and pit gastric epithelium.

1.3 Symptoms and clinical outcomes

The majority of patients with gastritis do not report any complaints. Dyspepsia, a chronic or recurrent pain centered in the upper abdomen, is often reason for diagnostics to *H. pylori* and related diseases (33). The association between *H. pylori* and non-ulcer dyspepsia however is arbitrary. Benefits of *H. pylori* eradication for relief of dyspepsia symptoms differed in several studies, yet were the highest in regions with a high prevalence of peptic ulcer disease (33-35). In a systematic review of these trials, *H. pylori* eradication was in comparison with placebo associated with a 10% higher chance of cure of dyspeptic symptoms (35).

Although gastritis does not give rise to specific complaints, it may have long-term consequences. *H. pylori* gastritis can lead to mucosal disruption leading to gastric and duodenal ulcers (21). It is estimated that 10-20% of *H. pylori*-positive subjects develop

peptic ulcer disease during their lifetime, with duodenal ulcer being more common at younger age, and gastric ulcer more at older age. Besides *H. pylori*, chemical irritants or drugs also give mucosal erosions, which may lead to ulceration and in addition bleeding or perforation. Eradication of *H. pylori* results in an evident decrease in ulcer recurrence rate (36).

Furthermore, *H. pylori* has since 1994 been classified as type I carcinogen by the WHO, because of convincing evidence that chronic *H. pylori* gastritis increases the risk of development of gastric cancer. Chronic *H. pylori*-induced gastritis eventually leads to atrophic gastritis, because of destruction of gastric glands and replacement by fibrosis and intestinal-type epithelium (18). In this sequence intestinal metaplasia may progresses to dysplasia, which eventually ends in intestinal-type gastric adenocarcinoma in 1-2% of infected patients (37). Epidemiological studies have suggested that the vast majority of gastric adenocarcinomas are thus related to *H. pylori* infection. *H. pylori*-positive subjects also have a significant increased risk for the development of gastric MALT lymphoma (38). Exact prevalence is unknown, but it occurs in less than 1% of *H. pylori*-positive patients and is declining (39). In approximately 75% of the patients with early stage MALT lymphoma eradication induces long-term remission of the lymphoma (40).

Both ulcer disease and gastric cancer can present with widely different features, like dyspepsia, bleeding, anaemia, and weight loss. If 'alarm' symptoms (i.e. weight loss, dysphagia, overt GI bleeding, abdominal mass, and iron deficient anaemia) are present, a prompt endoscopy is required. Without these symptoms a test-and-treat strategy for *H. pylori* is recommended (41).

2. PHARMACOTHERAPY OPTIONS FOR GASTRITIS

2.1 Current treatment of H. pylori*-induced gastritis*

2.1.1 Indications

Testing for *H. pylori* is only justifiable if a positive test will be followed by eradication treatment. The rationale for eradication should be the prevention or treatment of *H. pylori*-related diseases, which can develop as a result of the chronic active gastritis. Ideally, *H. pylori* eradication is only indicated when either the lesions it causes are reversible or progression is stopped. To date, evidence for all possible indications is not available. Generally accepted indications are peptic ulcer disease (active or in past), previous gastric cancer without total gastrectomy, and MALT lymphoma (see **Table 3**). Other reasons for treatment include chronic atrophic gastritis, uninvestigated dyspepsia, functional dyspepsia, and first-degree relatives of patients with cancer (41). Currently accepted indications for eradication vary among countries. Recently, the European Helicobacter

Study Group has published a new consensus report on management of *H. pylori* infection (41). Compared to the American College of Gastroenterology guideline there is a slight difference in their recommendations for testing and treatment of *H. pylori* infection (17). For example, the European in contrast to the American guideline recommends testing for *H. pylori* in case of atrophic gastritis, unexplained iron deficiency anemia, idiopathic thrombocytopenic purpura and vitamin B12 deficiency (41).

Table 3 Indications for *H. pylori* eradication

Generally accepted	Optional
Chronic active gastritis	Chronic atrophic gastritis
Peptic ulcer disease (active or in past)	Uninvestigated dyspepsia
Previous gastric cancer without total gastrectomy	Functional dyspepsia
MALT-lymphoma	First degree relatives of patients with gastric cancer

Chronic active gastritis is completely reversible by *H. pylori* eradication, and the gastric mucosa can be restored to normal (42). However, reversibility is less likely with the appearance of gastric atrophy or intestinal metaplasia (43). These premalignant lesions particularly occur in patients with corpus-predominant and atrophic gastric, rather than in patients with antrum-predominant gastritis. Patients with corpus-predominant gastritis therefore are most at risk for developing gastric cancer (18). *H. pylori* eradication can prevent the progression of premalignant lesions to gastric cancer, but the point of no return beyond which eradication cannot prevent progression is still unclear (43, 44). A meta-analysis presented on this topic found improvement for atrophy of the corpus but not for antrum atrophy or gastric intestinal metaplasia (45). Another meta-analysis however has reported improvement for both antrum and corpus atrophy (46). Intestinal metaplasia is uniformly considered as an irreversible gastric lesion (41). Nevertheless, the evidence that eradication prevents the development of gastric cancer is critically evaluated in several reviews and meta-analyses, and has been confirmed (46-48). The conflicting results regarding the effect of *H. pylori* eradication on premalignant lesions underline the point that *H. pylori* eradication should as much as possible be performed before the appearance of gastric atrophy and in particular intestinal metaplasia (43, 49). A still controversial reason for *H. pylori* testing and treatment is uninvestigated dyspepsia. It is related to peptic ulcer disease, but in the majority of patients the nature is functional (33). Management of patients with uninvestigated dyspepsia is primarily depending on the presence of alarm symptoms (33, 41). If present a prompt endoscopy is required. In patients younger than 45 years and living in an area with an *H. pylori* population prevalence above 20%, a test and treat approach is recommended (33, 41). In populations with lower prevalence, empiric PPI therapy is a valid option (33, 35).

2.1.2 Antibiotic resistance

Treatment regimens for *H. pylori* infection continue to change, particularly owing to declining effectiveness of most commonly recommended antibiotic strategies. Paradoxically, *H. pylori* is sensitive to many antibiotics *in vitro*, but they all fail as monotherapy *in vivo* (18). Several reasons can be mentioned. The niche of *H. pylori* has a low pH and a viscous mucus layer, lowering the effectiveness of the antibiotics. Moreover, at any time, a proportion of the bacteria is either not replicating or is replicating very slowly, making it difficult for antibiotics to affect them (50). In this way persistence of infection usually develops, owing to outgrowth of a small existing population of resistant bacteria (51). Several approaches have been introduced to overcome this problem: combination of antibiotics, increasing the dose and duration, addition of bismuth to reduce bacterial load, and addition of a PPI to increase pH and hence antimicrobial efficacy (50). Effectiveness of eradication therapy is also influenced by compliance, which is often suboptimal because of the frequent occurrence of antimicrobial-related side effects (52).

Resistance to clarithromycin is the major reason of this reduced effectiveness. Increase of clarithromycin dose or duration is not effective, since resistance is associated with failure to bind ribosomes (50, 53, 54). This binding is necessary to kill *H. pylori*. During the past 10 years clarithromycin resistance is almost doubled from 10% to 18% in Europe (55). The highest clarithromycin resistance rates are observed in Southern Europe, Mexico, Japan and the Middle East. In Northern Europe resistance rates generally are <10%, whereas the other regions have a prevalence >20% (41, 55).

Another important cause of decreasing effectiveness is resistance to metronidazole, which also differs geographically. Primary metronidazole resistance was observed in 27% of patients worldwide, with highest rates in Africa (92%) and lowest in Europe (17%) (56). The latter percentage may be an underestimation given the recently found resistance rate of 35% in Europe (55). In contrast to other antibiotics, resistance to metronidazole can be partially overcome by increase of dose and duration of treatment (57). Metronidazole is widely used as substitution of amoxicillin in penicillin-allergic individuals. Use of either metronidazole or amoxicillin is supposed to be equivalent with respect to effectiveness (41).

It had been observed that gene polymorphisms of cytochrome P450 2C19 play an essential role in PPI metabolism (58). This genetic polymorphism determines fast, intermediate, and slow PPI metabolism (resp. 70%, 25-30%, and 2-5% of Caucasian population). Triple therapies containing omeprazole achieve lower eradication rates in extensive metabolisers (59, 60). Therefore, a tailored therapeutic regimen is suggested to improve effectiveness. Prior screening of patients for primary antibiotic resistance and CYP2C19 polymorphisms has shown a higher eradication rate, owing to a tailored antibiotic treatment and PPI choice (61).

2.1.3 H. pylori *eradication therapy*

Initial treatment for *H.* pylori can consist of triple, quadruple or sequential therapy (see **Table 4**). The usually recommended *triple therapy*, consisting of a PPI, clarithromycin and amoxicillin, is failing in most studies to reach 80% eradication intention-to-treat (50). Although antibiotic resistance is increasing to unacceptable levels, this clarithromycin-containing triple therapy is still recommended by American, European, and Asia-Pacific guidelines (41, 62, 63). Choice for this *first-line* therapy should depend on regional clarithromycin resistance. In areas with high clarithromycin resistance guidelines advise to abandon the clarithromycin-containing triple therapy (41). Increase of PPI-dose and extension of therapy duration to 10-14 days has some effect on eradication rates (64). Rationale is that particularly clarithromycin is sensitive for degradation by acid. Increase of gastric pH therefore prevents this degradation. In a meta-analysis of single versus double dose PPI-therapy, cure rates were significant higher with twice daily PPI-therapy (resp. 78% vs. 84% intention-to-treat analysis) (65). Attempts for further improvement of eradication are made by extending the duration of therapy. A meta-analysis of 21 randomized trials showed that treatment for 10 to 14 days had about 5-10% advantage over 7-day therapy (66). Nevertheless, 7-day treatment may still be acceptable in regions where local studies have shown that a particular treatment is very effective.

Table 4 Summary of current treatment regimens available for *H. pylori* eradication

Drug class	Drug	Triple therapy[1]	Quadruple therapy[2]	Sequential therapy[3]
Acid suppression	Proton pump inhibitor[4]	20-40 mg bid	20-40 mg bid	20-40 mg bid
Antimicrobials	Bismuth compound[5]	2 tablets bid	2 tablets bid	
	Amoxicillin	1 g bid		1g bid
	Metronidazole	500 mg bid	500mg tid	500 mg bid
	Clarithromycin	500 mg bid		500 mg bid
	Tetracyclin		500mg qid	
Salvage antimicrobials	Levofoxacin	300 mg bid	300mg bid	
	Rifabutin	150 mg bid		
	Furazolidone	100 mg bid		
	Doxycycline		100 mg bid	
	Nitazoxanide		1 g bid	

1: Triple therapy consists of a PPI or bismuth compound, together with two of the listed antibiotics, given for 7-14 days.
2: Quadruple therapy consists of a PPI plus either the combination of a bismuth compound, metronidazole, and tetracycline for 4-10 days, or the combination of levofloxacin, doxycyclin, and nitazoxanide for 10 days.
3: Sequential therapy consists of 10 days of PPI treatment, plus amoxicillin for 5 days and a combination of clarithromycin and an imidazole (when available, tinidazole; otherwise, metronidazole) during days 6-10.
4. PPI dose equivalent to omeprazole 20 mg bid.
5. When available (in some countries use is restricted).

Quadruple therapy consists of a PPI plus either the combination of a bismuth compound, metronidazole, and tetracycline, or the combination of levofloxacin, doxycycline, and nitazonaxide. A quadruple regimen is recommended both as *first-* and *second-line* treatment (41, 62). Bismuth salts have a local antimicrobial effect on *H. pylori* (67). Until now, resistance has not been described. Bismuth salts are however not generally available due to concerns about toxicity in some countries, particular as a result of their potential neurological consequences (68). These however never have been described with short-term *H. pylori* treatment, but only in the past with high-dose longer treatments for instance long time ago for syphilis. A meta-analysis identified the number of adverse events in patients using bismuth containing *H. pylori* eradication therapy (68). In total 4763 patients were assessed, and no serious adverse events occured with bismuth therapy. Non-bismuth quadruple therapy can be an alternative to triple therapy in countries where bismuth use is restricted. A recently performed meta-analysis of randomized controlled trials compared standard triple therapy with nitroimidazole added to triple therapy (69). The latter showed better eradication rates compared with standard triple therapy (90% vs. 78% ITT). In areas with high clarithromycin resistance quadruple therapy is supposed to be an appropriate alternative (41). Nevertheless, meta-analyses comparing both regimens yielded conflicting results (70, 71). A meta-analysis of 93 studies showed that in populations with either clarithromycin or metronidazole resistance, quadruple therapy containing both antibiotics led to higher eradication rates compared with clarithromycin-containing triple therapy (70). However, another meta-analysis of 9 RCTs showed no significant difference between clarithromycin triple therapy and bismuth quadruple therapy (resp. 77% vs 78%) (71). Hence, both regimens yielded suboptimal eradication rates of <80%. Based on these results it was suggested to base the choice of treatment upon *H. pylori* susceptibility testing. To date, susceptibility testing requires endoscopy and gastric mucosal biopsy, which is invasive and costly. The new European guideline recommends susceptibility testing only after failure of second-line therapy (41).

A superior alternative to the standard triple therapy can be the so-called *sequential therapy*, firstly introduced in Italy in 2000 (72). This therapeutic approach is based on different combinations of the available antibiotics. It starts with a dual therapy including a PPI for 10 days plus amoxicillin 1 g twice daily during day 1-5, followed by a combination of clarithromycin 500 mg and tinidazole or metronidazole (all twice daily) for day 6-10. Initial therapy with amoxicillin during the first 5 days may serve as an induction phase that amplifies the effectiveness of the second phase of treatment including clarithromycin and tinidazole (73). Moreover, regimens containing amoxicillin may prevent the selection of secondary clarithromycin resistance (74). On the other hand, the improved eradication rate could also be due to the larger number of antibiotics

to which the organism is exposed. Several randomized clinical trials and meta-analyses have demonstrated that this sequential therapy is more effective than triple therapy (74-79). A randomized controlled trial found a significant higher eradication with a 10-day sequential therapy containing tinidazole compared with standard triple therapy (89% vs. 77% ITT) (76). By using pooled data a recently published systematic review demonstrated that *H. pylori* infection was cured in 86% of 2,853 patients treated with sequential therapy compared with 75% out of 3,079 patients treated with standard triple therapies (80). In patients with clarithromycin resistance, clarithromycin-containing sequential therapy has also been shown to be more effective than standard triple therapy (75). Moreover, substitution of clarithromycin by levofloxacin further improved results. In an area with >15% prevalence of clarithromycin resistance, a levofloxacin-based regimen led to eradication rate of 97% (ITT) compared to 81% (ITT) with clarithromycin-containing sequential therapy (81). However, use of levofloxacin as first-line treatment prevents to apply it as a second-line treatment in cases of eradication failure (82, 83).

2.1.4 Confirmation of eradication

No treatment regimen is able to achieve a 100% eradication rate. Thus, it is uncertain in a given patient whether treatment successfully eradicated *H. pylori*. In patients with for instance non-ulcer dyspepsia, this is acceptable and the effect of eradication is only assessed by symptoms. However, in cases with complicated peptic ulcer disease, after local treatment for gastric cancer, or with a gastric MALT-lymphoma, the effect of eradication treatment needs to be assessed. This can be done by means of upper endoscopy, making it possible to perform biopsy-based tests. It is recommended to wait at least 4 weeks before testing (41).

2.2 Pharmacotherapy for chemical gastritis/gastropathy

2.2.1 NSAID/aspirin induced gastropathy

The best treatment for chemical gastropathy is withdrawal of the causative agent. However, since many patients need longer duration treatment with NSAIDs or aspirin, *primary prevention* of gastric injury is important, in particular for high risk patients. Risk factors include a history of ulcer disease, dual antiplatelet therapy, age >65 years, and concomitant corticosteroid use (84, 85). At the start of NSAID therapy, an accurate risk and benefit assessment is essential to prevent harmful gastrointestinal events (86). There are several options that reduce the risk for gastric damage; using misoprostol or a PPI together with a nonselective NSAID or using a selective COX-2 inhibitor with or without a PPI. Misoprostol is a prostaglandin E analog, which decreases the risk for NSAID-induced gastric or duodenal ulcer. Misoprostol 200 mcg qd was in comparison with placebo associated with an approximate 3-fold risk reduction for serious upper

gastrointestinal complications in patients with rheumatoid arthritis treated with NSAIDs (87). However, long-term use of misoprostol is often impaired by adverse events, like diarrhea, abdominal pain and nausea (88). H2 receptor antagonists used in standard dose are also effective for the prevention of NSAID-related duodenal ulcers, but not for gastric ulcers (89). Double doses of H2 receptor antagonists were found effective in reducing risk for both gastric and duodenal ulcers.

NSAID-induced mucosal damage is influenced by intragastric acidity (90). Acid suppression therapy is thus effective in the prevention and treatment of NSAID-related diseases. PPIs therefore are useful for the prevention of NSAID-induced ulcers (91). Clinical studies have suggested that PPIs are better tolerated but have slightly lower efficacy compared with misoprostol (92). A multicenter trial compared the efficacy of lansoprazole with misoprostol in long-term NSAID users (88). Misoprostol was found superior to lansoprazole, but due to limited compliance and more frequent adverse effects associated with misoprostol, both lansoprazole and misoprostol were clinically equivalent. Pooled data of two placebo-controlled trials showed that the estimated cumulative risk for development of ulcers over 6 months was 17% with placebo, 5.2% with 20 mg esomeprazole and 4.6% with 40mg esomeprazole (93). Furthermore, no differences were found in ulcer development between selective COX-2 inhibitors and non-selective NSAIDs. In general, success of primary prevention is largely depending on adherence to gastro-protective agents like misoprostol and PPIs. Non-adherence is significantly associated with higher risk of upper gastrointestinal bleeding (94).

Secondary prevention of NSAID-related gastric injury is preferentially achieved by the withdrawal of NSAIDs. In patient who must continue taking NSAIDs it is recommended to prescribe a COX-2 inhibitor with a PPI (95, 96). This combination is associated with lower risk of secondary peptic ulcer complications than treatment with a COX-2 inhibitor alone. Omeprazole is superior to ranitidine and misoprostol in the prevention of recurrent erosions and ulcers (97, 98).

NSAID use and *H. pylori* infection are independent risk factors for peptic ulcer disease. It has been shown that there is an increased risk when these factors are both present (99). Particularly naive NSAID users have clear benefit of *H. pylori* eradication (100). For aspirin, *H. pylori* eradication can prevent gastropathy and is strongly advised in patients with peptic ulcer history (41).

In summary, the focus with respect to NSAID-related chemical gastropathy should be on primary prevention in high risk patients. Proton pump inhibition (at a dose equivalent to omeprazole 20 mg once daily) and misoprostol (at a dose of 800 mg/day) can protect against gastric ulcers during NSAID use.

2.2.2 Bile reflux induced gastritis

Recurrent reflux of bile into the stomach can lead to chemical gastritis. A general approved medical treatment for this bile reflux gastritis is not available. Use of proton pomp inhibitors can provide some relief of symptoms (29). However, it is still arbitrary whether PPIs are as effective at suppressing duodenogastro reflux as they are at inhibiting acid reflux. One study evaluated the combination of rabeprazole and hydrotalcite in patients with bile reflux gastritis after cholecystectomy (29). Combination therapy was associated with a significant improvement of histological activity score compared with mono-therapy of either drug alone or no therapy. However, no differences were found in the histological score of chronic inflammation. Moreover, this study had no placebo group, so that possible placebo effects on symptoms relief not could be identified. Another study compared rabeprazole, sucralfate, and no therapy in patients after cholecystectomy (101). After 12 weeks follow-up treatment with both rabeprazole and sucralfate equally led to significant improvement of symptoms, as well as endoscopic and histological findings. To date, randomized controlled trials confirming these benefits are still lacking.

2.3 Treatment of autoimmune gastritis

There is no curative treatment for autoimmune gastritis at this moment. Pernicious anemia can be alleviated by vitamin B12 replacement therapy. However, this has no impact on the underlying destructive autoimmune disease process (102). The effect of immunosuppression by prednisolone was assessed in a mouse model of autoimmune gastritis. It led to remission of the gastric inflammation during therapy (103). However, withdrawal promptly results in disease recurrence. Patients with pernicious anemia have an increased risk of intestinal-type gastric cancer and gastric carcinoid tumors, the latter as a result of the associated persistent hypergastrinemia in the presence of achlorhydria due to the severe atrophy with pronounced parietal cell loss (104, 105). The benefits of surveillance in patients with pernicious anemia have not been established. A guideline of the American Society for Gastrointestinal Endoscopy recommends a single endoscopy to reveal gastric lesions in patients with pernicious anemia, but there are insufficient data to support routine endoscopic surveillance of these patients (106). The new European guideline recommends surveillance endoscopy at 2-3 years intervals (41). For carcinoid tumors, surveillance is controversial and should be individualized per patient (106).

2.4 Treatment of special forms of gastritis

Treatment of eosinophilic gastritis or gastroenteritis is usually focused on symptoms relief, and medications used are largely based on empiric observation and experience (30). Steroids have an effect on the inflammatory process, yet require prolonged

treatment (107). This is in particular a disadvantage of systemic therapy, but less of topical treatment. Other treatments include proton pump inhibitors, mast cell stabilizers, ketotifen, and leukotriene antagonists (30). However, none of these have been stated as standard treatment. Owing to the rarity of this condition, no controlled clinical trials are performed.

Lymphocytic gastritis can be found in the presence of *H. pylori* infection, which is probably the major etiologic factor. Eradication of *H. pylori* has shown healing of lymphocytic gastritis (108, 109). Hence, testing for *H. pylori* in case of histological diagnosed lymphocytic gastritis is important.

3. CONCLUSION

In this review we focused on the combination of endoscopic and histologic gastritis. Accurate classification is essential for choosing the right therapy. Although upper endoscopy is mandatory in the diagnostic process, diagnosis can only be made by histology. Treatment for *H. pylori*-induced gastritis is best studied and several regimens are available. However, owing to expanding antibiotic resistance eradication rates are declining. In many countries standard triple therapy does no longer achieve acceptable eradication rates. Therefore knowledge of local resistance rates and eradication effectiveness of specific regimens is indispensable. In addition, it is impossible to provide one applicable therapy for *H. pylori* eradication. Prior susceptibility testing is a promising tool for improvement of eradication rates. However, until now costs are too high for general implementation. Regarding NSAID/aspirin-induced gastropathy, focus should be on primary prevention of gastroduodenal damage by gastro-protective agents, especially in high risk patients. NSAID use accompanied with a PPI has shown significant reduction in ulcer development. PPIs can be beneficial in patients with bile reflux gastritis, but further research by randomized controlled trials is required for confirmation of found benefits. To date, curative treatment of autoimmune gastritis is not available and therefore therapy targets on treatment of pernicious anemia. Surveillance of this preneoplastic condition is still arbitrary. Testing for *H. pylori* is important in lymphocytic gastritis. Eradication has shown healing of former gastritis. Therapy for eosinophilic gastritis is focused on symptom relief, since exact etiology is unknown till now.

4. EXPERT OPINION

Gastritis is a condition with different definitions used by clinicians, endoscopists, and pathologists. The condition is generally based on the combination of endoscopy and histology. These reveal that chronic gastritis is a very common condition, in particular associated with *H. pylori* colonization. There is only a weak association between dyspeptic symptoms, and endoscopic plus histologic evidence for gastritis. However, the clinical importance of gastritis lays in the fact that it predisposes to more pronounced damage to the gastric mucosa, in particular peptic ulcer disease, and eventually atrophic gastritis, intestinal metaplasia, and gastric malignancy, both adenocarcinoma and MALT lymphoma.

The management of gastritis aims at prevention and cure of ulcer disease, and prevention of progression towards gastric malignancy. For this purpose, a range of *H. pylori* eradication regimens have been developed. At this moment, greatest challenge in this field is to overcome the problem of growing antibiotic resistance. Eradication treatment for *H. pylori* is close to 20 years old, and hampered by diminishing instead of increasing efficacy. Besides induction of antimicrobial resistance by the extensive use of antibiotics, this may be influenced by changing treatment indications, deficient therapy adherence, and change in bacterial strains.

It is clear that no current therapy for *H. pylori* is able to achieve a 100% eradication rate, however rates below 80% are in clinical practice unacceptable. Triple therapies without prior susceptibility testing should therefore be abandoned in regions with a high clarithromycin resistance (>15-20%). Without susceptibility testing, first-line treatment in these regions should consist of either quadruple or sequential therapy. Exact content of these regimens can be determined per region, according to results of local studies to effectiveness of particular regimens.

Further research should concentrate on new techniques for testing of susceptibility to antibiotics rather than the currently ongoing comparisons of different eradication therapies, as antimicrobial resistance is likely to increase. A tailored approach to *H. pylori* infection leads to high success rates and prevents development of resistance. For the same reason, it is also mandatory to carefully adhere to guidelines in terms of indications for treatment.

Some indications for *H. pylori* testing and treatment remain to be debated, in particular due to conflicting data regarding benefits of eradication. This pertains among others to patients with premalignant gastric lesions. Although *H. pylori* eradication cures gastritis and may to some extent lead to regression of premalignant lesions, prospective studies have so far not shown that this also reduces the risk of cancer.

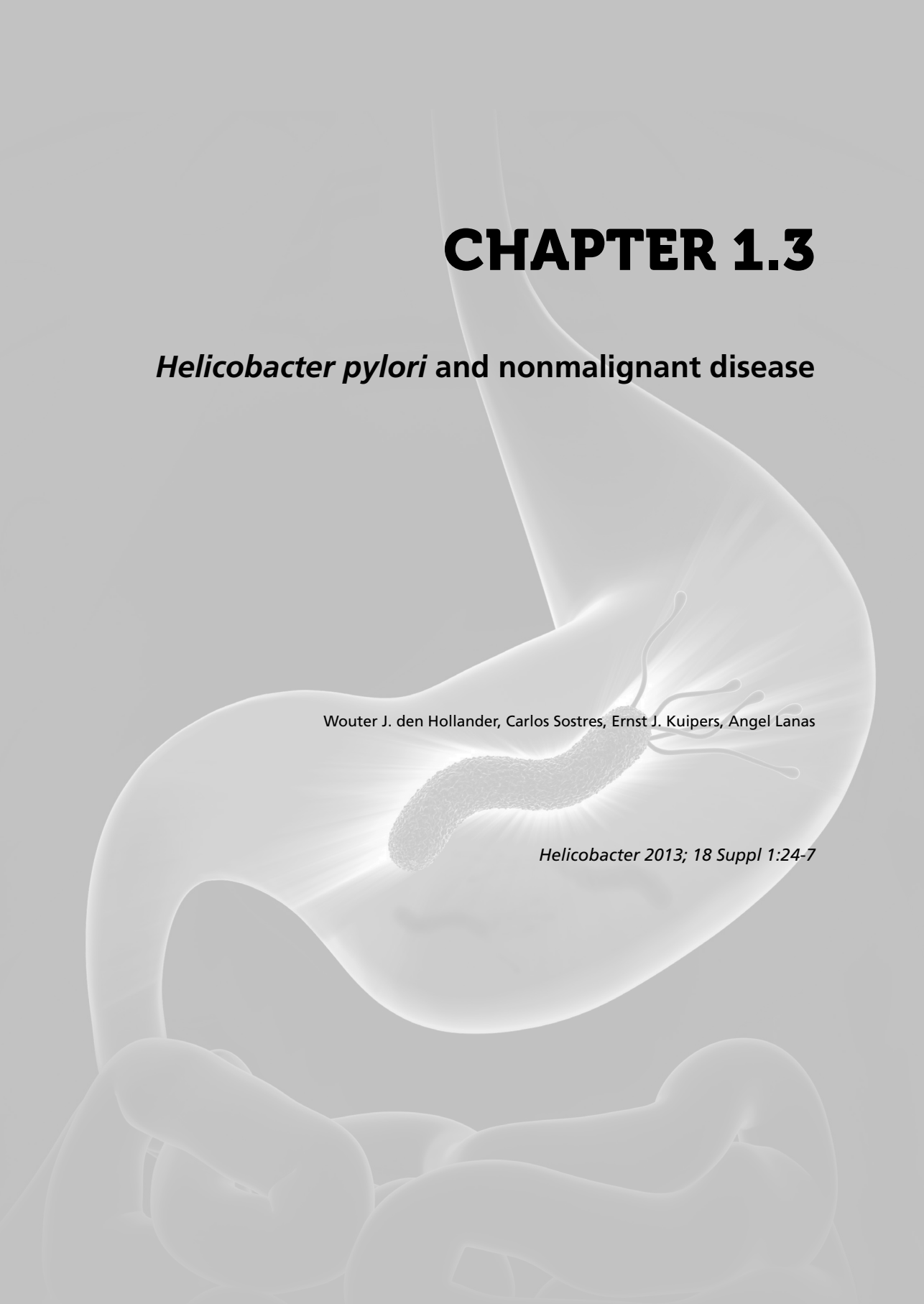
Primary prevention of NSAID/aspirin-induced gastropathy can be improved by restrictive use of these drugs, and by more adequate prescription of gastro-protective agents, and increase of therapy adherence. Until now, remarkably high numbers of patients with at least one risk factor for gastrointestinal bleeding do not receive concomitant gastro-protective agents. Therefore clinicians should be better informed about risk factors and already existing recommendations. In addition, better adherence can be achieved if patients are convinced that non-adherence can have serious effects. We suppose that adequate education is the key for more indicated prescriptions and enlargement of therapy adherence.

5. KEY ASPECTS

- Adequate classification of gastritis is essential for management and choices on pharmacotherapy.
- The main etiologic factor of gastritis is *H. pylori*. Other common causes include autoimmune gastritis and chemical gastritis/gastropathy.
- Owing to raising clarithromycin resistance in many countries, standard triple therapy should be abandoned as first-line treatment in those countries. Quadruple and sequential therapies are proved superior for *H. pylori* eradication.
- Gastro-protective agents are essential in preventing NSAID/aspirin-induced gastropathy, which eventually leads to gastroduodenal ulcer disease. Misoprostol and PPIs are both effective therapies.
- PPIs can be beneficial in patients with bile reflux gastritis, but further research by randomized controlled trials is required for confirmation of found benefits.

CHAPTER 1.3

Helicobacter pylori and nonmalignant disease



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ABSTRACT

Declining *Helicobacter pylori (H. pylori)* prevalence rates have resulted in a decrease of peptic ulcer bleeding incidence. Moreover, eradication reduces peptic ulcer recurrence rate. Newer studies confirm that *H. pylori* eradication lowers the risk of recurrent peptic ulcer bleeding. Guidelines therefore advocate a test-and-treat strategy for *H. pylori*-positive patients with a history of ulcer bleeding and NSAIDs and/or aspirin use. There is mounting evidence that *H. pylori* status has no effect on symptoms and treatment efficacy in patients with gastroesophageal reflux disease (GERD). Some studies observed an improvement of GERD complaints after *H. pylori* eradication, which underlines that *H. pylori* treatment is not contra-indicated in GERD patients. The exact role of *H. pylori* in functional dyspepsia (FD) remains controversial. However, there is growing consensus that *H. pylori*-positive FD should be assessed as a separate entity. In these patients eradication can be beneficial and appropriate. Finally, several studies suggest that *H. pylori* colonization may also be associated with beneficial effects for the host. Epidemiological studies showed an inverse relation between *H. pylori* colonization and asthma and allergy, although data are conflicting and need to be expanded.

Peptic Ulcer Disease

The relationship between *H. pylori* infection and peptic ulcer disease (PUD) and also peptic ulcer bleeding (PUB) has been extensively studied. A meta-analysis reported that the prevalence of PUD ranged worldwide between 0.1% and 4.7%, with an annual incidence ranging from 0.19% to 0.3% (111). The majority of studies have reported a decrease in the incidence and/or prevalence of PUD over time, presumably due to a decrease in *H. pylori*–associated PUD. *H. pylori* was initially responsible for up to 95% of all gastroduodenal ulcers, but more recent studies reported that the prevalence of *H. pylori* in patients with PUD ranged between 36% to 73%, depending on ethnicity, geographic and socioeconomic factors (112).

A compilation of 71 original studies, including 8496 patients, found a mean 72% prevalence of *H. pylori* infection in PUB (113). The association between *H. pylori* infection and PUB was further studied in a meta-analysis which confirmed that *H. pylori* infection increased the risk of ulcer bleeding (OR 1.79) (99). As a consequence of the introduction of potent acid inhibitors and eradication of *H. pylori*, a rapid decrease in both incidence and mortality of PUB was expected. However, although most studies confirm such a decrease, the rate of hospitalization because of PUB only slowly decreases (114).

H. pylori resistance rates to antibiotics vary even in different regions of the same country. Effective *H. pylori* eradication reduces the rate of ulcer recurrence. Therefore, it is plausible that *H. pylori* eradication also prevents recurrence of ulcer bleeding. However, the efficacy of eradication for the prevention of recurrent bleeding from peptic ulcer has not been completely established. A prospective, long-term study included 1000 patients with previous PUB, 41% of them had previously used an NSAID and none received a PPI or NSAID during follow-up (115). Peptic ulcer rebleeding virtually did not occur after *H. pylori* eradication (0.5%). The authors concluded that maintenance antisecretory therapy is not necessary if eradication is achieved. However, NSAID intake or *H. pylori* reinfection may exceptionally cause rebleeding in *H. pylori*-eradicated patients.

In daily clinical practice, concomitant *H. pylori* infection and NSAID and/or aspirin use are common, in particular in elderly. Both *H. pylori* infection and NSAID use are independent risk factors for the development of PUD and associated bleeding. There is a synergistic effect for the development of GI bleeding when these factors are both present (41). Although *H. pylori* is frequently reported as a risk factor for upper GI bleeding in aspirin users, the real effect of *H. pylori* eradication on reducing the risk of bleeding remains unclear. The international Maastricht guideline advocates an *H. pylori* test-and-treat strategy in aspirin users with a history of gastroduodenal ulcer because the long-term incidence of peptic ulcer bleeding is low in these patients after *H. pylori* eradication, even in the absence of gastroprotective treatment (41). Despite these findings, further studies are needed to confirm whether this strategy is a (cost-) effective therapy to reduce ulcer bleeding in high-risk aspirin users.

A prospective 10 years cohort study from Hong Kong assessed whether testing for *H. pylori* in aspirin users with a high ulcer risk would reduce the long-term incidence of ulcer bleeding (116). The investigators divided patients into three different cohorts. The first included *H. pylori*–positive aspirin users with a PUB history in whom *H. pylori* had been eradicated (n=249). The second group consisted of *H. pylori*–negative aspirin users with a PUB history (n=118). The third group assigned as average-risk cohort consists of aspirin users without a history of ulcers (n=537). The incidence of ulcer bleeding (per 100 patient-years) in the *H. pylori*–eradicated cohort (OR 0.97; 95% CI 0.53–1.80) was similar to the average-risk cohort (OR 0.66; 95% CI 0.38-0.99). On the other hand, the *H. pylori* negative cohort had a high incidence of recurrent bleeding (OR 5.22; 95% CI 3.04-8.96). This confirms that the long-term incidence of recurrent ulcer bleeding with aspirin use is low after *H. pylori* eradication despite a history of ulcer bleeding. Aspirin users without current or past *H. pylori* infections who develop ulcer bleeding however have a high risk of recurrent bleeding. Tests for *H. pylori* infection can be used to assign high-risk aspirin users to groups that require different gastroprotective strategies, in particular patients with a positive test for *H. pylori* should receive anti–*H. pylori* therapy followed by confirmation of eradication. Their need for long-term gastroprotective therapy depends on the success of *H. pylori* eradication and concomitant use of drugs that can cause bleeding. However, *H. pylori* negative patients should receive adequate gastroprotective co-therapy if they have a history of ulcer because they are prone to ulcer bleeding with aspirin use.

Gastroesophageal Reflux Disease

Gastroesophageal reflux disease (GERD) is a highly prevalent condition in the general population. Although it has previously been suggested that *H. pylori* eradication may cause both reflux symptoms and erosive esophagitis, the existence of such an association remains largely unsubstantiated.

A meta-analysis of 10 trials in which data of patients treated for *H. pylori* infection were compared to those receiving placebo concluded that the post-treatment incidence of reflux symptoms (17% *vs* 22.6%) and erosive esophagitis (5% *vs* 5.1%) were similar between both groups (117). A further sub-analysis revealed a significantly lower incidence of GERD symptoms in the eradicated versus non-eradicated group (13.8% vs 24.9%) (OR 0.55; 95% CI 0.35-0.87, p=0.01). Overall, these data suggest that *H. pylori* eradication is not significantly associated with either reflux symptoms or erosive esophagitis onset, with some data actually suggesting an advantage in eradication in terms of a negative association with later development of GERD symptoms. One study showed a significant improvement in reflux disease-related quality of life scores one year after *H. pylori* eradication therapy (118). In another study from the United States 1611

cases of an African-American population with esophagitis and/or gastritis and confirmed *H. pylori* status were included between 2004-2007 and compared with controls (119). The frequency of *H. pylori* positivity in gastritis patients was 40%, in esophagitis patients 4% and in normal controls 34%. After adjusting for effects of age and sex, odds ratio of *H. pylori* was 0.06 (95% CI 0.01-0.59; p = 0.01) for the esophagitis group versus the normal group. They concluded that *H. pylori* has a significant negative association with esophagitis in African-Americans, which may point to a protective role of *H. pylori* in the pathogenesis of esophagitis. In addition, another study on 2442 patients referred for upper gastrointestinal endoscopy observed *H. pylori* colonization in 82% of GERD patients. A statistically significant relationship was found between *H. pylori* positivity and the grade of GERD (120). In line with these observations, the updated Maastricht consensus on management of *H. pylori* infection concluded that *H. pylori* status has no effect on symptom severity, symptom recurrence, and treatment efficacy in GERD (41). *H. pylori* eradication does not exacerbate pre-existing GERD nor affect treatment efficacy. Therefore, the presence of GERD should not dissuade *H. pylori* eradication treatment when otherwise indicated. Furthermore, long-term efficacy of PPI maintenance treatment for GERD is not influenced by *H. pylori* status (121).

Functional Dyspepsia

Functional dyspepsia (FD) is currently defined as symptoms of epigastric pain, epigastric burning, postprandial fullness, or early satiation, in the absence of any organic, systemic, or metabolic disease that is more likely to explain the symptoms (122). This chronic, relapsing and remitting disorder is commonly seen in individuals from all around the world. Data from a large population-based study demonstrated no effect on life expectancy and no differences in the numbers of gastrointestinal related deaths between subjects with or without dyspepsia (123).

The exact role of *H. pylori* in FD is still under debate. Some investigators have argued that if *H. pylori* gastritis is considered an organic disease, *H. pylori*-associated FD should not be considered as a functional disorder (124, 125).

Possible mechanisms by which *H. pylori* may elicit dyspeptic symptoms include alterations of gastric motility, as well as endocrine and acid-secretory abnormalities (126). Hunger sensations, acid secretion and gastrointestinal motility are regulated by ghrelin, particularly produced by the gastric enteroendocrine cell compartment (126). Gastric colonization with *H. pylori* is associated with decreased ghrelin secretion (127). A study from China in fifty children with FD showed a significant increase in plasma ghrelin levels and gastric ghrelin mRNA expression after successful *H. pylori* eradication treatment (128). In contrast, no significant differences were found in children who did not achieve successful eradication. A small RCT from Japan demonstrated an improvement of upper

gastrointestinal symptoms in adult patients treated with rikkunshito (i.e. a traditional Japanese medicine) compared to patients treated with domperidone (129). The improvement of symptoms correlated with enhanced plasma ghrelin levels. Apart from the need for more trials on this topic, these findings may give insights in the underlying pathophysiology of FD symptoms.

Most guidelines recommend a test-and-treat strategy for *H. pylori,* especially in populations with a high *H. pylori* prevalence (126). However, the efficacy of this approach is limited, with a number to treat of 14 to achieve complete symptomatic response in one patient (35). It is becoming more clear that the role of *H. pylori* infection in FD differs between Western and Asian populations. *H. pylori* colonization and related diseases are more common in Asia, and therefore considered as the major differential diagnoses of FD (130). Moreover, there is a trend of higher symptom response by *H. pylori* eradication treatment in Asian patients. Hence, particular in these patients exclusion of *H. pylori* infection is necessary before diagnosing FD. As in the past, current studies do not always give support for this statement. Sodhi et al. found no effect of *H. pylori* eradication on FD symptoms (131). In this trial from India *H. pylori* positive patients suffering from FD (Rome II criteria) were randomly allocated to triple therapy (n=259) or PPI and placebo (n=260) for two weeks. After 12 months follow-up no difference in symptom resolution was found between the triple therapy and placebo group (44% vs 37%, p=0.13). It should be taken into account that despite the low eradication rate of 70% all patients allocated to the triple therapy arm were included in the comparison, which may have influenced the outcome.

Asthma and Allergy

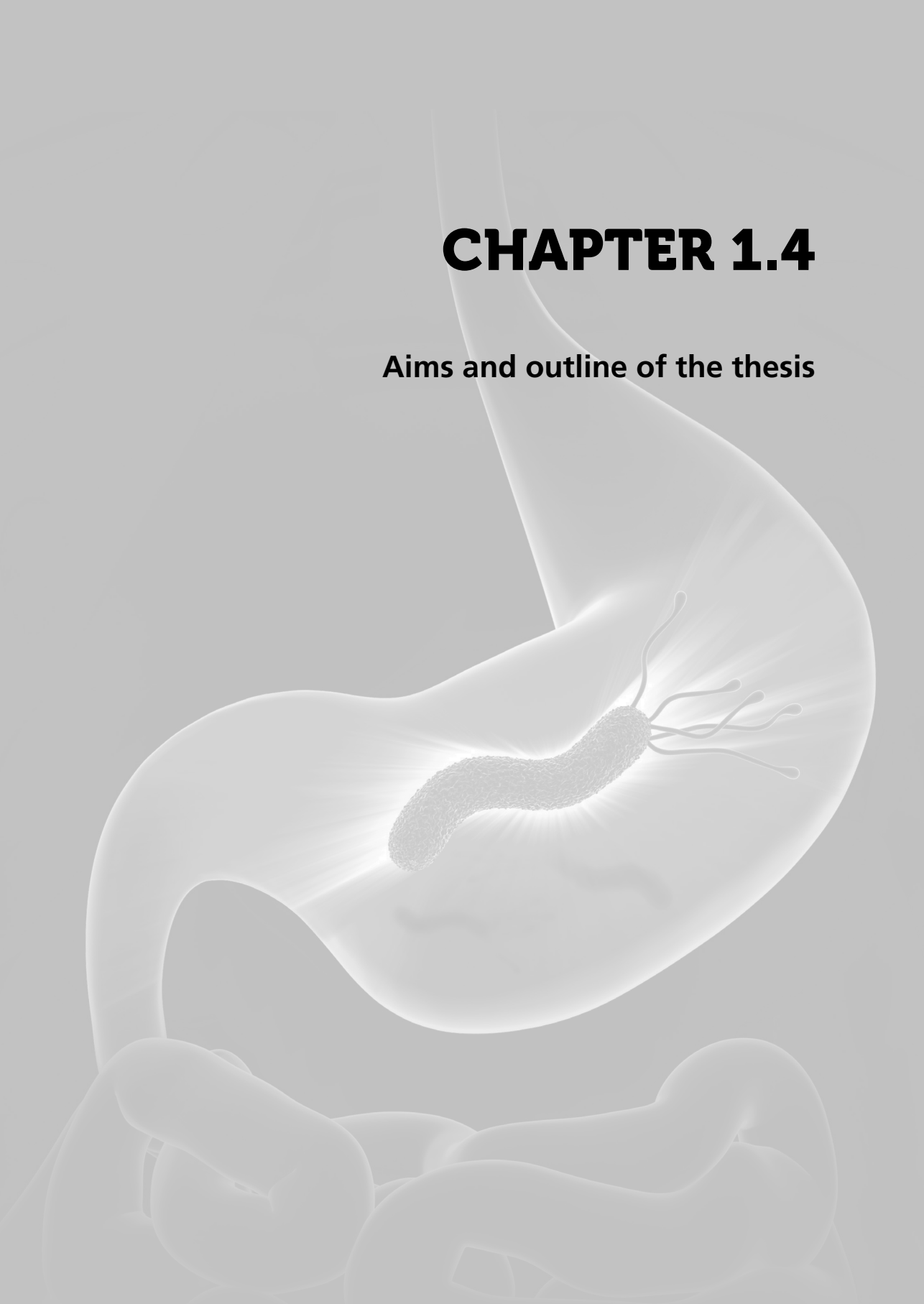
H. pylori is suggested to have not only pathogenic properties. Considered as a human commensal, *H. pylori* is thought to influence the development of the host immune system (1). Changes in our microbiota affected by altered ecological circumstances might explain the increasing prevalence of atopic diseases like asthma and allergy. *H. pylori* is a specific component of the human microbiome. In this context, several epidemiological studies showed an inverse relation between *H. pylori* colonization and asthma occurrence (132), but data are conflicting. Last year, two meta-analyses both found a weak inverse association between asthma and *H. pylori* colonization (133, 134). One study included cross-sectional, case-control, and cohort studies (133). Meta-analyses of pooled data from separate study types revealed a significant inverse association between *H. pylori* colonization and asthma for cross-sectional studies only (OR 0.84; 95% CI 0.74-96). Discrepancies among pooled outcome of the study groups might be explained by differences in heterogeneity of study design, participants, and study quality. Stratification by age did not show a difference in trend between children

and adults. No conclusions for children below age of 10 could be demonstrated, due to their low number in the analysis. The second meta-analysis was based on 14 studies, either with cross-sectional or case-control study design (134). Overall, a significant lower *H. pylori* colonization rate was found in asthmatic participants (OR 0.84; 95% CI 0.73-0.96). Stratification to geographical region revealed that data from the United States determined this outcome, since studies from Asia and Europe did not show a significant inverse association. The prevalence of CagA-positive strains was similar in asthmatics and non-asthmatics. In both children and adults an inverse but non-significant association between asthma and *H. pylori* was found.

Both meta-analyses in particular included adults rather than children. Therefore, more studies in children are needed for validation of the hypothesis that asthma is inversely associated with *H. pylori* colonization.

CHAPTER 1.4

Aims and outline of the thesis



This thesis focuses on the epidemiology of *H. pylori* and its relationship with gastric and extra-gastric diseases. The two main aims of this thesis are: 1) getting insight in the current epidemiology of *H. pylori* colonization in a multi-ethnic Western population, and 2) assessment of the prior suggested involvement of *H. pylori* in extra-gastric diseases in children and their mothers participating in a large prospective cohort study.

Part I provides two review articles in which the main topics of this thesis are discussed.

In **Part II**, we focus on the epidemiology of *H. pylori* colonization in mothers and their children participating in the Generation R study. Besides antibodies against *H. pylori*, we also measure antibodies against an important virulence factor, called cytotoxin-associated gene A (CagA). *H. pylori* strains that express CagA are more pathogenic, causing more severe gastritis, which is associated with an increased risk for ulceration and gastric cancer. **Chapter 2** defines *H. pylori* prevalence as well as risk groups for *H. pylori* in a cohort of young women living in Rotterdam, a city with a multi-ethnic population. The focus in **Chapter 3** is first on the prevalence and risk factors for *H. pylori* colonization in their children, and secondly we look at transmission from mother-to-child and intergenerational differences between these two multi-ethnic cohorts.

Part III comprises the chapters on *H. pylori* and associated extra-gastric diseases, and the follow-up of patients known with premalignant gastric lesions. *H. pylori* infection has been implicated in the aetiology of nausea and vomiting during pregnancy (NVP). Adverse birth outcomes are more common after severe NVP. In **chapter 4**, we investigated the association of *H. pylori* with vomiting severity and its effect on birth outcome. Apart from NVP, *H. pylori* colonization may be associated with pregnancy complications like pre-eclampsia (PE), small for gestational age (SGA), and spontaneous preterm birth (PTB). In **chapter 5**, we address the question whether *H. pylori* is indeed associated with these pregnancy complications. *H. pylori* colonization may also have beneficial effects, in line with the "disappearing microbiota" hypothesis. One of the supposed effects is that carriage of *H. pylori* has a protective effect on the development of asthma and allergy during childhood. We aim to examine the association between *H. pylori* colonization in children and risk of asthma and related conditions at school age (**Chapter 6**). We secondly examine additional effects of maternal *H. pylori* status by pairing with children's status. Epidemiological studies indicated that colonization with *H. pylori* may affect body mass index (BMI), but with inconsistent results. In **chapter 7**, we examine the relationship between *H. pylori* colonization and BMI/obesity in a large population-based cohort setting using a Mendelian Randomization approach. *H. pylori* is a well-defined risk factor for intestinal type gastric cancer. Identification and endoscopic surveillance of precursor lesions may prevent subjects from invasive gastric carcinoma. **Chapter 8** shows the results of a prospective follow-up study including patients with premalignant gastric lesions.

In this study we aim to assess the incidence of neoplastic progression in patients from a low-risk area and to assess discriminative tests for identifying those patients who are at risk.
In **part IV**, we summarize and discuss the main results of this thesis (**chapter 9**).

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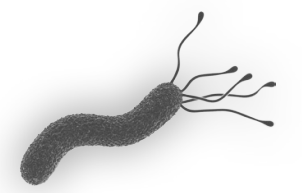
PART II

Epidemiology of *H. pylori* in a multi-ethnic population



CHAPTER 2

Ethnicity is a strong predictor for *Helicobacter pylori* infection in young women in a multi-ethnic European city

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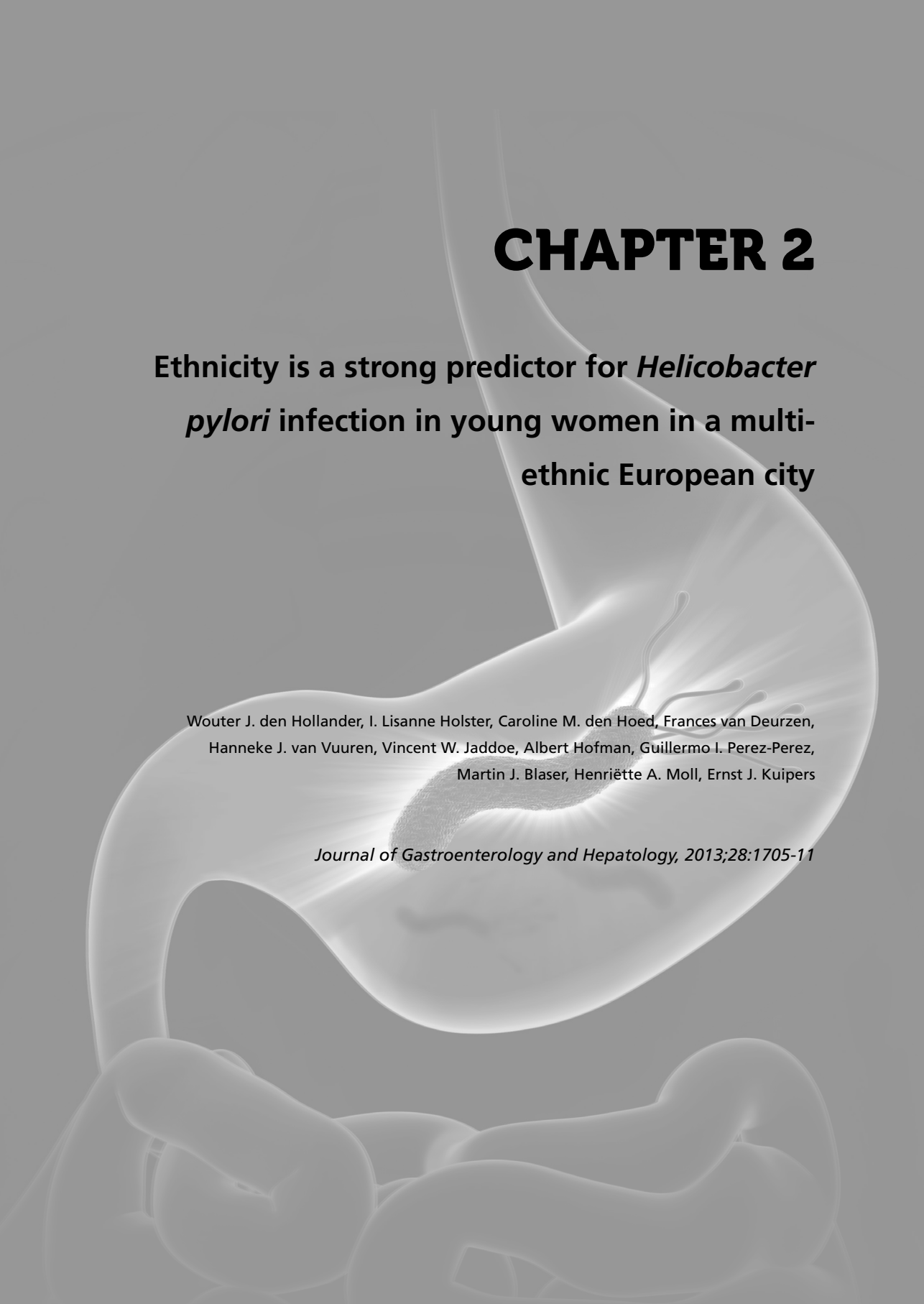

ABSTRACT

Background and aim: At the same time that *H. pylori* prevalence is declining in Western countries, immigrants from developing countries with high *H. pylori* prevalence have settled in Western urban areas. Actual epidemiologic data on *H. pylori* in a migrant community may help in realizing a more selective approach to assess *H. pylori*-related diseases. We aimed to define *H. pylori* prevalence as well as risk groups for *H. pylori* in a cohort of young women living in a multi-ethnic European city.

Methods: We measured IgG anti-*H. pylori* and CagA-antibodies in serum of pregnant women included in a population-based prospective cohort study. Information on demographics, and socio-economic status was collected by questionnaires. Chi-square and logistic regression were used.

Results: In total, 3146 (46%) of the 6837 tested women (mean age 29.7 ± 5.3) were *H. pylori*-positive and 1110 (35%) of them were CagA-positive. The *H. pylori* prevalence in Dutch women was 24%, which was significantly lower than in non-Dutch women (64%; p<0.001). In particular, *H. pylori* positivity was found in 92% of Moroccan (OR 19.2; 95% CI 11.8-32.0), 80% of Cape Verdean (7.6; 5.0-11.5), 81% of Turkish (9.0; 6.7-12.1), 60% of Dutch Antillean (3.3; 2.3-4.7), and 58% of Surinamese women (3.0; 2.3-3.8). Among *H. pylori*-positive Dutch subjects, 19% were CagA-positive compared with 40% of the non-Dutch subjects (p<0.001).

Conclusions: Despite a general trend of declining prevalence in Western countries, *H. pylori* remains highly prevalent in migrant communities, which may constitute target groups for screening and eradication to prevent *H. pylori*-related diseases.

INTRODUCTION

Isolation and identification of *Helicobacter pylori* in 1982 led to better understanding of gastric pathophysiology. This Gram-negative bacterium is an important risk factor for peptic ulcer disease, gastric adenocarcinoma and MALT-lymphoma (1). The prevalence of *H. pylori* widely varies geographically, and is highest in developing countries. In Western countries, however, prevalences have declined over recent decades to below 40%, in part as a result of improved hygiene and sanitation as well as the active elimination by antibiotics (1). In parallel to the declining *H. pylori* prevalence, incidences of certain *H. pylori*-related diseases are decreasing as well (2, 3).

These trends in Western populations call for risk group identification, as test-and-treat strategies for *H. pylori* both on population as well as individual level are unlikely to be cost-effective in low prevalence countries (4). Knowledge about specific risk groups may permit assessment of disease risk, and will offer opportunities for targeted interventions. The *H. pylori* colonization rate is associated with factors such as age, socioeconomic status, childhood crowding, and non-western ethnicity (5). During past decades, many immigrants from developing countries with high *H. pylori* prevalence have settled in Western urban areas. In Rotterdam, a large Western city, more than 50% of the urban population is originating from outside The Netherlands. The largest non-Dutch ethnic groups consist of people from Morocco, Turkey, Suriname, Dutch Antilles, and Cape Verde. Previous studies indicated that *H. pylori* colonization rates in immigrants are higher compared to native western populations (6-8). Some of these migrant communities have a high risk of gastric cancer (9, 10), in which *H. pylori* is involved as the major causative agent. Especially CagA-positive *H. pylori* strains are known to be more interactive with higher risk of peptic ulcer disease, atrophic gastritis, and gastric cancer (11, 12), and lower risk of gastro esophageal reflux disease, Barrett's esophagus, adenocarcinoma of the gastric-esophageal junction, and childhood-onset asthma (13, 14).

In this study, we aimed to obtain actual epidemiologic data on *H. pylori* prevalence in different ethnic groups living in a Western urban area. Moreover, we also measured anti-CagA-antibodies. As mothers are considered to be a source for transmission to their children, this study was performed in a cohort of pregnant women living in Rotterdam, a multi-ethnic European city.

METHODS

Setting and participants

This study was embedded in the Generation R Study, a population-based cohort study from fetal life until young adulthood in Rotterdam, with a multiethnic community, and the second largest city in the Netherlands. The background, design and aims of this study have been reported in detail (15).

Briefly, 8880 pregnant women were enrolled in the study between April 2002 and January 2006. Medical data were collected by physical examination, and by questionnaires, and information on age, ethnicity, educational level, life style, and household income was obtained by questionnaires (15).

The Generation R Study was approved by the Medical Ethical Committee of the Erasmus University Medical Center. All participants gave written informed consent.

Socio-demographic determinants

The cohort comprises various ethnic groups, reflecting the urban population of Rotterdam. The largest ethnic groups consist of Dutch, Surinamese, Turkish, Moroccan, Dutch-Antilles and Cape Verdean mothers. Ethnicity was determined by country of birth of the pregnant mother and her parents. A participating mother was considered of non-Dutch ethnic origin if one of her parents was born abroad (according to the definition of Statistics Netherlands) (16). If both parents were born in different countries other than the Netherlands, the country of the mother prevailed. Subjects of non-Dutch origin and born abroad were defined as first-generation immigrants. A participant of non-Dutch origin but born in the Netherlands was defined as a second-generation immigrant. People of Dutch origin were considered as the native population. According to the definitions of Statistics Netherlands(16) participants with other ethnic background as mentioned above were grouped as 'other western' for European (n=493), North American (n=24), Oceanean (n=9), Japanese (n=7) and Indonesian (n=194), and as 'other non-western' for African (n=123), Asia (n=189) and South- and Central American (n=91). The division into 'western' and 'non-western' is based on differences in socioeconomic and cultural situation. Participants from Indonesian were classified as 'western', since they are originating from former Dutch-East Indies. Educational level was classified into four educational levels on the basis of the highest completed education: high (university or higher vocational training), high-secondary (general secondary school or intermediate vocational training), low-secondary (intermediate general school or lower vocational training), and low (primary school or no education). Educational level served as proxy for the socio-economic status. Household income, defined by the total net monthly income of the family, was categorized as < € 1200, € 1200-2000, and > € 2000. Data on

possible confounders like age, smoking habits, and alcohol use were obtained from the questionnaires.

Serologic determinants

Mid-pregnancy serum samples of 7185 mothers were available for analysis (**Figure 1**). Mothers included more than once (due to more than one pregnancy within inclusion period) were excluded (n=348), which left a total study population of 6837 pregnant women. Procedures for collection and storage of the sera samples have been described in detail previously (17). Samples were examined for *H. pylori* IgG antibody levels by enzyme-linked immunosorbent assay (ELISA), using whole cell antigens (18). A separate ELISA was performed to determine serum IgG antibodies against a specific recombinant truncated CagA protein, as described (19). All samples were measured in duplicate. For each sample, the optical density ratio (ODR) was calculated by dividing the optical density (OD) by the mean OD of the positive controls. *H. pylori* positivity was defined as either an ODR ≥ 1 or CagA positivity. The cut-off for CagA positivity was an ODR value ≥ 0.35. Both ELISAs were validated locally.

Statistical analysis

Chi-square tests (categorical variables) and t-tests (continuous variables) were used to compare different variables in relation to *H. pylori* status. Univariate analysis was performed to assess determinants associated with the presence of *H. pylori*. To study the individual effect of each potential determinant, each was separately tested, followed by a multivariate analysis corrected for all other determinants. In addition, a stratified analysis according to ethnicity was performed. Subjects with missing data on any of above-mentioned covariates were excluded from the multivariate analysis; this left a total of 4605 evaluable subjects.

A two sided p-value <0.05 was considered to be statistically significant. All analyses were performed using PASW Statistics 20.0 (SPSS, IBM, New York, United States).

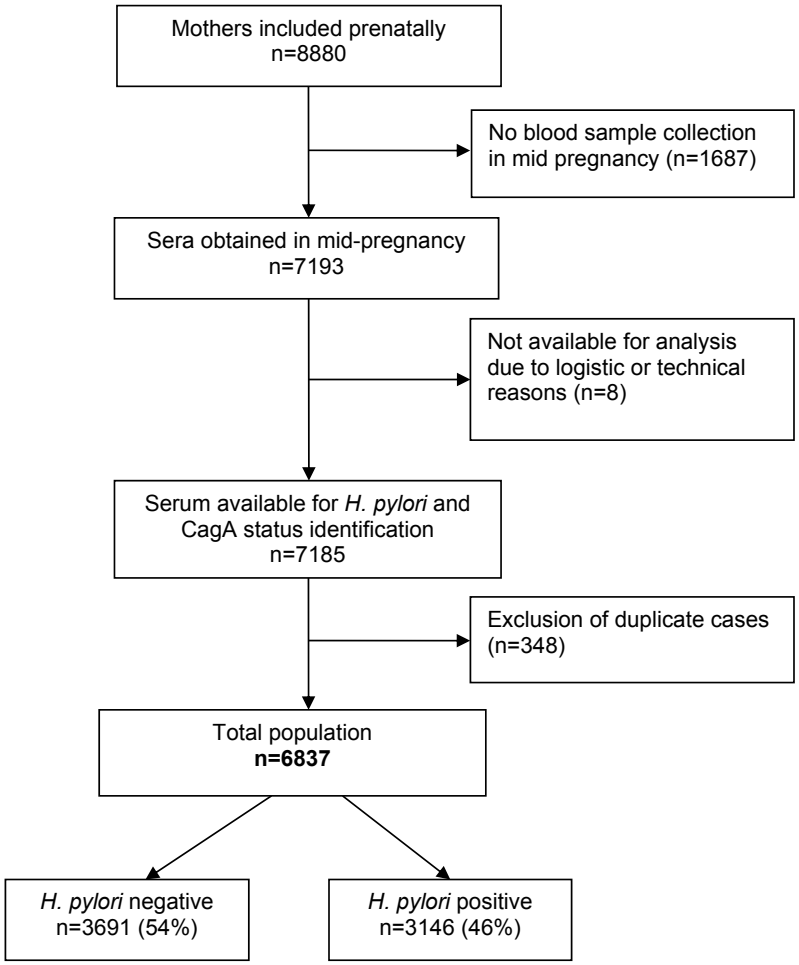

Figure 1. Study design.

RESULTS

***Presence of* H. pylori**

In total, the serum samples of 6837 women were analyzed. Their mean age was 29.7 (± 5.3) years. Subjects of Dutch origin were older (mean age 31.4 years) than non-Dutch participants (mean age 28.4 years) ($p<0.001$). **Table 1** shows the general characteristics and *H. pylori* status: 3146 (46%) subjects were *H. pylori*-positive and 3691 (54%) subjects *H. pylori*-negative.

Table 1. Baseline characteristics of study population, distribution by *H. pylori* status, and univariate analysis

	Study population (n)	*H. pylori* - (n=3691)	*H. pylori* + (n=3146)	OR Univariate	95% CI
Age, mean (SD)	6837 (100)	30.7 (4.9)	28.7 (5.4)	0.93	0.92-0.94
Dutch population (%)	3166 (46)	31.5 (4.4)	31.0 (4.9)	0.98	0.96-1.0
Non-Dutch	3223 (47)	29.2 (5.5)	27.9 (5.4)	0.96	0.94-0.97
Parity (%)					
Nulli parity	3746 (55)	2173 (58)	1573 (42)	1.0	
Multi parity	3025 (45)	1496 (50)	1529 (51)	1.4	1.3-1.6
Missing	*66 (1)*				
Ethnicity (%)					
Dutch	3166 (50)	2415 (76)	751 (24)	1.0	
Other western	726 (11)	467 (64)	259 (36)	1.8	1.5-2.1
Turkish	597 (9)	116 (19)	481 (81)	13.3	10.7-16.6
Surinamese	577 (9)	244 (42)	333 (58)	4.3	3.7-5.3
Moroccan	408 (6)	32 (8)	376 (92)	37.8	26.0-54.7
Cape Verdean	275 (4)	54 (20)	221 (80)	13.2	9.7-17.9
Dutch Antilles	237 (4)	94 (40)	143 (60)	4.9	3.7-6.4
Other non-western	403 (6)	154 (38)	249 (62)	5.2	4.2-6.5
Missing	*448 (7)*				
Dutch + Western	3892 (61)	2882 (74)	1010 (26)	1.0	
Non Western	2497 (39)	694 (28)	1803 (72)	7.4	6.6-8.3
Immigrant generation (%)					
Native	3115 (49)	2376 (76)	739 (24)	1.0	
1st generation	2225 (35)	688 (31)	1537 (69)	7.2	6.4-8.1
2nd generation	1046 (16)	510 (49)	536 (51)	3.4	2.9-3.9
Missing	*451 (7)*				
Education (%)					
Low	719 (11)	199 (28)	520 (72)	6.6	5.5-8.0
Low secondary	980 (16)	451 (46)	529 (54)	3.0	2.6-3.5
High secondary	1971 (31)	1026 (52)	945 (48)	2.3	2.1-2.6
High	2613 (42)	1873 (72)	740 (28)	1.0	
Missing	*554 (8)*				
Household income (%)					
<1200 Euro	1101 (21)	354 (32)	747 (68)	5.2	4.5-6.0
1200-2000 Euro	992 (19)	489 (49)	503 (51)	2.5	2.2-2.9
>2000	3182 (60)	2264 (71)	918 (29)	1.0	
Missing	*1562 (23)*				
Alcohol (%)					
Yes	3005 (51)	2027 (68)	978 (32)	0.4	0.3-0.4
No	2948 (49)	1296 (44)	1652 (56)	1.0	
Missing	*884 (13)*				
Smoking (%)					
Yes	1688 (28)	930 (55)	758 (45)	1.0	0.9-1.2
No	4348 (72)	2430 (56)	1918 (44)	1.0	
Missing	*801 (12)*				

Data on ethnicity were available for 6389 women: 3166 (50%) subjects were of Dutch ethnicity, 597 (9%) of Turkish, 577 (9%) of Surinamese, 408 (6%) of Moroccan, 275 (4%) of Cape Verdean, 237 (4%) of Dutch Antilles, 726 (11%) of other western and 403 (6%) of other non-western origin. The *H. pylori* positivity rate was highest among Moroccan women (92%), followed by Cape Verdian (80%), Turkish (81%), Dutch Antillean (60%), and Surinamese subjects (58%) (**Figure 2**). In contrast, the prevalence in women of Dutch origin was 24% (p<0.001).

The overall CagA-prevalence rate was 16.2%. Among *H. pylori*-positives, 1110 women (35.2%) were colonized with a CagA-positive strain, which however varied widely between ethnicities (**Figure 2**). Only 64 subjects were CagA-positive but *H. pylori*-negative (6%). Colonization with a CagA-positive *H. pylori* strain was most common in Surinamese (56%) mothers, followed by subjects of other non-western (46%), Dutch Antillean (46%), Turkish (39%), Cape Verdean (34%), Moroccan (34%), other western (29%), and Dutch (19%) origin (p<0.001).

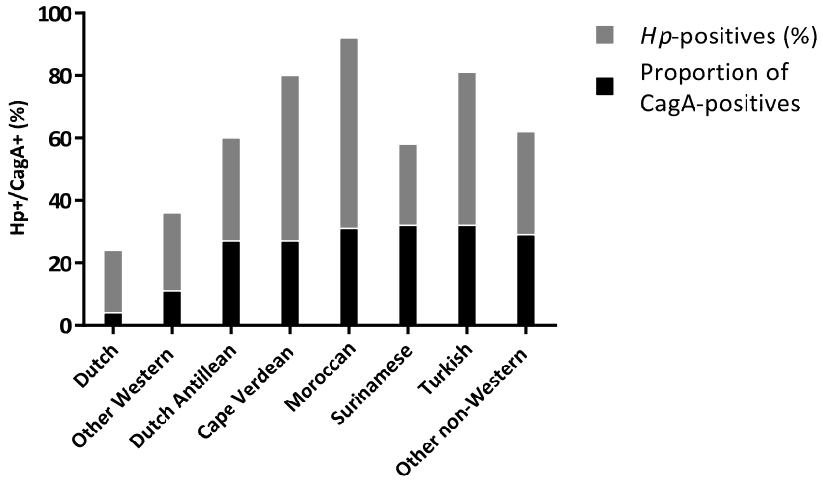

Figure 2. Proportion of CagA-positive and *H. pylori*-positive subjects.

Determinants of* H. pylori *colonization

Univariate logistic regression revealed that non-Dutch ethnicity was an independent risk factor for *H. pylori* positivity (**Table 1**). Furthermore, lower educational level, first generation immigrant, and lower household income were significantly associated with *H. pylori* infection. However, alcohol use was inversely associated with *H. pylori* presence. The following variables were entered into the multivariate logistic regression model: age, ethnicity, education level, household income, smoking, and alcohol use. This analysis continued to reveal ethnicity as the most important independent risk factor for *H. pylori*

(**Figure 3**). Compared to Dutch ethnicity, highest risk was found for Moroccan women (odds ratio 19.4; 95% confidence interval 11.8-32.0), followed by Turkish (9.0; 6.7-12.1), Cape Verdean (7.6; 5.0-11.5), other non-western (3.4; 2.6-4.5), Dutch Antillean (3.3; 2.3-4.7), Surinamese (3.0; 2.3-3.8), and other western (1.7; 1.4-2.0) subjects. In addition to ethnicity, the following independent risk factors were found: low education level compared with high education level (1.9; 1.4-2.5), and low income compared with high income (1.5; 1.2-1.9).

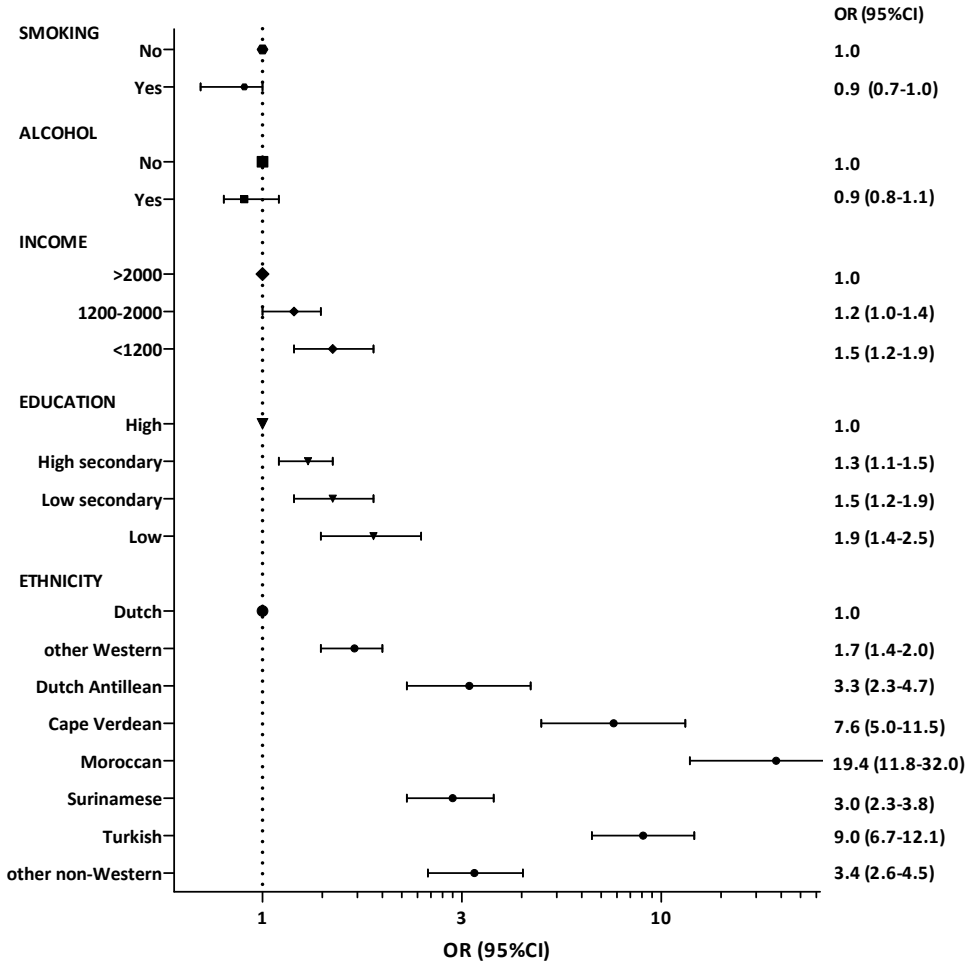

Figure 3. Multivariate analysis of determinants associated with *H. pylori* colonization in one model. Odds ratios (ORs) and 95% confidence intervals are adjusted for age.

Table 2. Multivariate analysis of *H. pylori* stratified to ethnicity

	Dutch n=2545	Other western n=574	Turkish n=376	Surinamese n=355	Moroccan n=196	Cape Verdean n=149	Dutch Antillean n=154	Other non-western n=254
Age	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (0.9-1.0)	1.0 (1.0-1.1)	1.0 (0.9-1.1)	1.0 (0.9-1.1)	1.0 (0.9-1.1)	1.0 (1.0-1.1)
Education								
Low	2.0 (1.1-3.4)	2.2 (0.9-5.3)	0.5 (0.2-1.4)	1.4 (0.6-3.6)	3.7 (0.5-24.7)	--	1.7 (0.4-6.9)	10.3 (2.7-38.6)
Low secondary	1.4 (1.0-1.9)	1.5 (0.7-3.2)	0.8 (0.3-2.3)	1.7 (0.8-3.7)	3.1 (0.5-17.7)	--	3.0 (0.8-10.8)	2.0 (0.7-5.7)
High secondary	1.2 (0.9-1.5)	1.5 (1.0-2.1)	1.0 (0.4-2.4)	1.5 (0.8-2.8)	1.1 (0.3-5.0)	--	1.2 (0.4-3.4)	1.6 (0.8-3.0)
High	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Generation								
Native	1.0	N.A	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
1st generation	N.A.	1.4 (1.0-2.1)	2.9 (1.6-5.3)	1.8 (1.1-3.0)	4.3 (1.4-13.3)	4.6 (1.6-13.4)	4.9 (1.9-12.7)	1.8 (0.8-3.8)
2nd generation	N.A.	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Household income								
<1200 Euro	1.7 (1.2-2.5)	1.6 (0.9-3.1)	1.3 (0.6-2.8)	2.0 (1.1-3.7)	0.8 (0.2-2.7)	0.3 (0.1-1.8)	0.7 (0.2-1.8)	1.6 (0.8-3.3)
1200-2000 Euro	1.0 (0.8-1.4)	1.4 (0.9-2.2)	1.0 (0.5-2.1)	1.6 (0.9-2.8)	3.1 (0.7-15.1)	0.1 (0.0-0.8)	0.4 (0.1-1.3)	1.4 (0.6-3.1)
>2000	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Alcohol								
Yes	0.9 (0.7-1.1)	0.7 (0.5-1.1)	0.7 (0.3-1.5)	1.0 (0.6-1.6)	3.6 (0.3-43.7)	1.2 (0.5-2.9)	0.8 (0.4-1.7)	0.9 (0.5-1.7)
No	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Smoking								
Yes	1.1 (0.9-1.3)	1.0 (0.6-1.5)	0.8 (0.4-1.3)	0.6 (0.4-1.1)	0.6 (0.1-3.0)	0.5 (0.2-1.3)	1.0 (0.5-2.4)	1.0 (0.4-2.1)
No	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Numbers are displayed as Odds Ratio (95% Confidence Interval). Reference categories are put as 1.0.
All listed variables were entered into the multivariate analysis.
N.A. = Not applicable.
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After stratification by ethnicity, multivariate analysis showed within all non-Dutch ethnicities that subjects born abroad (first generation immigrants) had a significantly higher risk to be colonized with *H. pylori* compared with second-generation immigrants ($p<0.05$ for Turkish, Surinamese, Moroccan, Cape Verdean, and Dutch Antillean subjects) (**Table 2**). Within the different ethnicities, age, smoking, and alcohol use were not associated with *H. pylori* colonization. Except for the Turkish and Cape Verdean subjects, low socio-economic status was an independent risk factor for *H. pylori* colonization. Dividing the whole population into either western or non-western showed in both groups first generation immigrants, subjects with low education level, and low household income to be more at risk for *H. pylori* colonization (**Table 3**).

Table 3. Multivariate analysis stratified to ethnicity (western vs non-western)

	All Western n=3119	All non-Western n=1484
Age	1.0 (1.0-1.0)	1.0 (1.0-1.0)
Education		
Low	2.0 (1.3-3.2)	2.1(1.4-3.2)
Low secondary	1.4 (1.1-1.9)	2.0 (1.4-3.0)
High secondary	1.2 (1.0-1.5)	1.4 (1.0-1.9)
High	1.0	1.0
Generation		
Native	1.0	N.A.
1st generation	1.8 (1.4-2.3)	2.0 (1.5-2.6)
2nd generation	1.4 (1.1-1.8)	1.0
Household income		
<1200 Euro	1.7 (1.2-2.4)	1.4 (1.1-2.0)
1200-2000 Euro	1.1 (0.9-1.4)	1.4 (1.0-1.9)
>2000	1.0	1.0
Alcohol		
Yes	0.9 (0.7-1.0)	0.6 (0.5-0.8)
No	1.0	1.0
Smoking		
Yes	1.0 (0.8-1.3)	0.8 (0.6-1.1)
No	1.0	1.0

Numbers are displayed as Odds Ratio (95% Confidence Interval). Reference categories are put as 1.0. All listed variables were entered into the multivariate analysis.
N.A. = Not applicable

DISCUSSION

This population-based study demonstrates that *H. pylori* colonization is still common in a Western society, in particular in migrant communities. Among women of non-Dutch ethnicity, first generation immigrants were more at risk for *H. pylori* colonization then second-generation immigrants. Although others have focused on risk group identification as well, both the cohort size and design of this study are unique, and results reflect the actual epidemiologic data on *H. pylori* colonization in a Western urban area.

The relatively high prevalence of *H. pylori* and CagA-positive strains found in ethnic minority groups indicates that the health risks imposed by *H. pylori* remain a significant concern. Studies comparing first and second-generation immigrants with the native population have demonstrated a higher risk for non-cardia gastric cancer in first-generation immigrants (20, 21). Childhood colonization with *H. pylori* could be a possible cause (22, 23). Our results support this explanation, since *H. pylori* colonization among second-generation immigrants was significantly lower compared to first-generation immigrants, but still higher than in the native population.

Based on estimates of the incidence of gastric cancer in different regions all around the world, study participants originating from Surinam, Caribbean, and Turkey are expected to have a higher gastric cancer risk compared with subjects from Western Europe (24). Indeed, studies from Sweden have indicated a higher gastric cancer incidence ratio in immigrants born in high risk countries and living in Sweden (20). Hence, those migrant communities could be an appropriate focus for considering the health advantages of *H. pylori* eradication. The latter is found effective for gastric cancer prevention in patients without precancerous lesions (25, 26).

We tested for *H. pylori* antibodies in pregnant women, about to give birth. Close family members like mothers and siblings are considered to be the major transmission sources for *H. pylori* acquisition during the first years of life (1). Hence, a high prevalence in mothers may be considered predictive for a high colonization rate in their children. Moreover, via day care attendance these children may also be a source for transmission to other children (27, 28). Our finding indicates that *H. pylori* will remain to be prevalent for the coming decades, even in Western societies.

The observed *H. pylori* colonization rate in the Dutch study subjects was consistent with a previous study in subjects of the same age (29). Studies evaluating *H. pylori* colonization in immigrant groups all showed higher infection prevalence than in native populations (8, 29-33). A previous study of 288 adults in Rotterdam showed nearly similar positivity rates among different immigrant groups, however higher prevalence in subjects of Dutch origin (46%) than we observed (8). This may have been due to both the relative higher age and the small number of Dutch patients in that study.

The geographical variance of *H. pylori* strains was confirmed by this study. *H. pylori* strains colonizing Western subjects were least often CagA-positive (34). In a Finnish population, CagA-positive *H. pylori* strains declined faster than CagA-negative *H. pylori* strains, especially among subjects less than 45 years old (35). The low CagA prevalence among Dutch and other Western subjects is consistent with those observations. Nevertheless, overall prevalence of CagA-positive strains was lower than expected, especially in subjects born abroad.

This study is limited by the inclusion of young women in a limited age range which restricted the possibility of extrapolating our finding to older age cohorts. Owing to the well described birth cohort effect for *H. pylori* (27), we expect *H. pylori* to be more prevalent in older age cohorts. However, whether this is true for all various ethnic groups remain unclear. Second, the population in our study may not have been reflective of the general population of Rotterdam owing to overrepresentation of more highly educated women in this cohort (36).

In conclusion, in a multi-ethnic population, ethnicity is the strongest predictor of *H. pylori* colonization in young women. In particular, migrant communities constitute target groups for screening of *H. pylori* to minimize *H. pylori*-related diseases. Additional knowledge of *H. pylori* biological costs, and details on implementation require further research.

Acknowledgements

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CHAPTER 3

Intergenerational reduction in *Helicobacter pylori* prevalence is similar between different ethnic groups living in a Western city

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ABSTRACT

Objective: *Helicobacter pylori (H. pylori)* colonization rates in childhood have declined in Western populations, but it is unknown whether this trend is similar in children of non-Western ethnic backgrounds, born in a Western country. We aimed to identify *H. pylori* status in children, and determine both mother-to-child transmission and risk factors for colonization.

Design: Antibodies against *H. pylori* and cytotoxin-associated gene A (CagA) were measured in children participating in a population-based prospective cohort study in Rotterdam, the Netherlands. Information on demographics and characteristics was collected using questionnaires.

Results: We analysed the serum of 4,467 children (mean age 6.2 years ±0.5 SD) and compared the results with the *H. pylori* status of their mothers (available for 3,185 children). Overall, 438 (10%) children were *H. pylori*-positive, of whom 142 (32%) were CagA-positive. Independent risk factors for colonization were: maternal *H. pylori* positivity (OR 2.12; 95%CI 1.62-2.77), non-Dutch ethnicity (OR 2.05; 95%CI 1.54-2.73), female gender (OR 1.47; 95%CI 1.20-1.80), and lower maternal education level (OR 1.38; 95%CI 1.06-1.79). Comparing mothers and children, we found an intergenerational decrease of 76% and 77% for $Hp^{+}CagA^{-}$ and $Hp^{+}CagA^{+}$-strains, respectively, consistent across all nine ethnic groups studied. Male gender, higher maternal educational level, and no older siblings, were independently associated with absence of *H. pylori*.

Conclusions: Although the highest *H. pylori* and CagA prevalence was found in children of non-Dutch ethnicities, the decreased colonization rates were uniform across all ethnic groups, implying the importance of environmental factors in *H. pylori* transmission in modern cities, independent of ethnicity.

SUMMARY BOX

1. What is already known about this subject?
    - *H. pylori* prevalence of children living in Western countries is low.
    - Maternal *H. pylori* status is an important transmission source for *H. pylori* colonization in their children.
    - Migrant communities in Western populations constitute risk groups for *H. pylori* colonization.
2. What are the new findings?
    - A high intergenerational reduction in *H. pylori* prevalence was found, comparing mothers with their children, with nearly identical rates (76% and 77%) for $Hp^{+}CagA^{-}$ and $Hp^{+}CagA^{+}$-strains, respectively.
    - The intergenerational drop in *H. pylori* prevalence was uniform in nine separate ethnicities.
    - Risk factors for *H. pylori* positivity are mostly the same among diverse ethnic groups.
    - Our data suggest a continuing acquisition of *H. pylori* at least to age 7.
3. How might it impact on clinical practice in the foreseeable future?
    - The maternal-child linkage is to some degree predictive of *H. pylori* positivity in a child, which affects risk of subsequent diseases.

INTRODUCTION

The gastric bacterium *Helicobacter pylori (H. pylori)* colonizes more than half of the human population. It usually induces the influx of inflammatory cells in the stomach wall, which is a major risk factor for peptic ulcer disease and gastric cancer (1, 2), and may also be associated with diminished risk of oesophageal reflux and childhood-onset asthma (3-5), and possibly more resistance to infectious diseases (6, 7). *H. pylori* colonization is usually acquired during early childhood, and in most cases persists unless eliminated by antibiotic treatment (8). A recent study reported that the risk of *H. pylori* colonization was influenced by host genetics (9).

The prevalence of colonization differs between children and adults (10). Several cross-sectional surveys in Western countries have shown that *H. pylori* prevalence increases with age (11, 12). Since acquisition during adulthood is rare (13, 14), the higher prevalence in the elderly rather reflects a birth cohort effect with higher rates of childhood exposure to the organism in the past (1). The current lower levels of exposure to *H. pylori* and consequent lower prevalence in children are believed to be due to improved hygiene,

and active elimination by antibiotics, together contributing to declining transmission risk (1, 15).

However, a recent study in Dutch children reported similarity in the *H. pylori* prevalence in two subsequent birth cohorts (16), possibly indicating that determinants previously responsible for declining colonization in the past now have stabilized. One factor contributing to this trend is the altered composition of western populations; during recent decades, the populations of western cities have become multi-ethnic as a result of immigration, often from countries where *H. pylori* remains endemic. Recently, we reported large differences in colonization rates among pregnant women of different ethnic origins living in Rotterdam, the Netherlands (17), but whether these differences are reflected in their offspring was not determined. Analysis of *H. pylori* transmission and risk factors would allow better prediction of the future incidence of *H. pylori*-associated illnesses.

In this population-based prospective cohort study, we aimed to measure *H. pylori* status, as well as risk factors for colonization and transmission, in children living in a multi-ethnic Western urban population, and in relation to colonization of their mothers. Unexpectedly, we found a relatively uniform intergenerational decrease in *H. pylori* prevalence in all nine ethnic groups studied. We explore the factors associated with this broad change.

METHODS

Design and setting

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onwards. All participants live in the multi-ethnic Rotterdam, the second largest city in the Netherlands. The children were born in Rotterdam between April 2002 and January 2006. The background, design, and aims of the Generation R study have been reported in detail (18). In total, 8,305 children and their parents participated in the postnatal phase of the study (from birth onwards) (**Figure 1**). From this initial population, 6,690 children visited the research centre at the age of 6 years. During these visits, blood samples were collected from 4,593 (69%) children (see **Table S1** comparing the children, with and without *H. pylori* data). Data on age, ethnicity, breastfeeding, day-care attendance, antibiotic use, and socioeconomic status of the mother were collected using questionnaires. The Generation R Study was approved by the Medical Ethical Committee of the Erasmus University Medical Centre, and parents of the children gave written informed consent.

Covariates

The Generation R cohort comprises a wide range of ethnic groups, reflecting Rotterdam's urban population as a typical Western city; the largest ethnic groups are of Dutch, Surinamese, Turkish, Moroccan, Dutch-Antilles, and Cape Verdean descent. Ethnicity is determined by country of birth of the parents. According to the definition of Statistics Netherlands, a child was considered of non-Dutch ethnic origin if one of its parents was born abroad (19). If both parents were born in two different countries outside the Netherlands, the country of the mother prevailed. Children of Dutch origin are considered as the native population. Participants with an ethnic background other than mentioned above are grouped together as European, Asian, African, or 'rest of the world', which included Central and South America (n = 95), Indonesia (n = 32), North America (n = 21), Asia, western (i.e. western ethnicity but parents had lived in Asia, n = 21), and Oceania (n = 9). Data on type of delivery was obtained by review of midwife and hospital registries. Exclusiveness of breastfeeding was categorized into three breastfeeding groups: never, non-exclusive breastfeeding until 4 months, or exclusive breastfeeding until 4 months. Data on day-care attendance was based on each child's first year of life. Maternal parity served as a proxy for the presence of older siblings in the family, which was categorized as either none or at least one older sibling. Use of antibiotics was assessed by questionnaire at the ages of 12, 24, 36, 48, and 72 months. Parents were asked whether their child had received antibiotics (for example, penicillin) during the past year. Data on antibiotic use were not validated by physician prescriptions or pharmacy records. The socioeconomic status of the children was defined according to the educational level of their mother on the basis of her highest level of completed education. The highest educational level was defined as completion of university or higher vocational training. Mothers were categorized as having a middle/low level of education if they had completed intermediate vocational training, or had completed education below that level.

Serological determinants

Serum samples from 4,467 children, obtained at the mean age of 6.2 (±0.4) years (range 4-8 years), were available for analysis (**Figure 1**). The main reasons for missing blood samples were non-consent of the parents and technical or logistic failure. Procedures for the collection and storage of serum samples have been described (20). Samples were examined for *H. pylori* IgG antibody levels by enzyme-linked immunosorbent assay (ELISA), using whole cell and water-extracted antigens (21). These preparations were previously tested and only cross-reactivity in children or adults infected with *C. jejuni* was observed with flagellin, which is a minor antigen in these preparations. A separate ELISA was performed to determine serum IgG antibodies against a specific recombinant truncated cytotoxin-associated gene A (CagA) protein, as described (22). Both ELISAs have been previously tested against many different ethnic groups, and in all cases the

tests have been validated and had similar sensitivity and specificity (23-26). Furthermore, the antigenic diversity of *H. pylori* strains around the world was studied; the antigens reacted in a similar way against specific antisera (27). Both ELISAs have been validated in children (28), and have been previously used in Dutch children (16). All samples were examined in duplicate; for each, the optical density ratio (ODR) was calculated by dividing the optical density (OD) by the mean OD of the positive controls. *H. pylori*-positive samples were those having either an ODR ≥ 1 or a CagA-positive test result. The cut-off value for CagA positivity was ODR value ≥ 0.35 (22). Only 13 children were found to be CagA-positive but *H.* pylori-negative (0.3% of *H. pylori*-negative children). Based on prior studies, these subjects were considered as truly *H. pylori* positive (29). Data on the maternal *H. pylori* status was available for 3,185 children (71%). The tests on the mothers were done 1 year earlier, using the same assays as for children; however, with specific calibration for adults. *H. pylori* antibody distributions for both children and their mothers are shown in **Figure S1**. Details regarding *H. pylori* colonization in the total cohort of mothers have been described (17).

Statistical analysis

Chi-square tests (categorical variables) and t-tests (continuous variables) were used to compare different variables with *H. pylori* status. Univariate analyses were performed to assess determinants associated with *H. pylori* presence. To study the individual effect of each potential determinant, each was tested separately, followed by a multivariate analysis corrected for all others. For all covariates, the percent of missing values within the population for analysis was lower than 10%, except for caesarean section, breastfeeding, day-care attendance, and antibiotic use. Missing data in the covariates (except maternal *H. pylori* status) were imputed with multiple imputations using chained equations, by which the most likely value for a missing response is selected (30). Ten new datasets were created by imputation based on all covariates and outcomes in the model. Data from each separate imputation was analysed, after which results were combined. Except for breastfeeding and antibiotic exposure, no major differences in the direction or magnitude of the effect estimates were observed between analyses with imputed missing data and complete cases only. Only the results based on the pooled imputed datasets are presented in this manuscript. To identify the potential modifying effect of determinants included in the multivariate analyses, we evaluated statistical interaction by adding to the multivariate model the product term of an independent variable and subgroup (independent variable ^{x} subgroup) as covariate. The interaction was tested between ethnicity and day-care, ethnicity and gender, and ethnicity and maternal education level. We calculated the population attributable fraction (PAF) for *H. pylori* disappearance in children with an *H. pylori*-positive mother, using adjusted odds ratios estimated from logistic regression models (31). All measures of associations

are presented as Odds Ratios (OR) with their 95% Confidence Intervals (CI). Statistical analyses were performed using IBM SPSS Statistics 21.0 for Windows (SPSS IBM, Armonk, New York, USA).

RESULTS

H. pylori *prevalence*

The serum of 4,467 children was analysed (**Figure 1**). **Table 1** summarizes both the observed population characteristics as well as the imputed data, stratified by *H. pylori* status. Overall, 438 (10%; 95% CI 8.9-10.7%) children were *H. pylori*-positive, of whom 142 (32%; 95% CI 28.0-36.8%) also were CagA-positive. In all children of non-Dutch ethnicity, the colonization rate was significantly higher than that in children of Dutch ethnicity (**Figure 2A**).

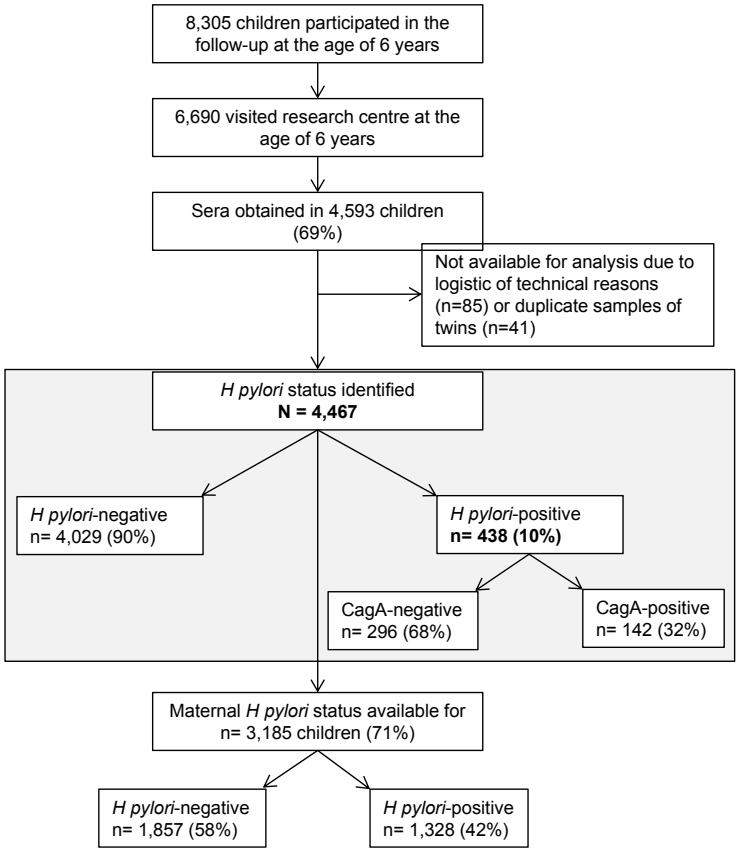


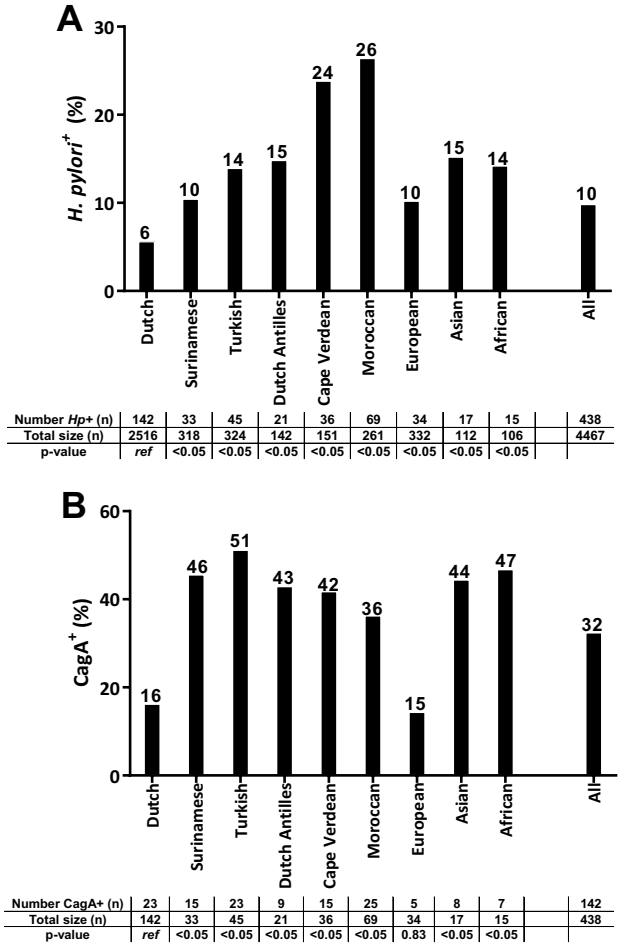
Figure 1. Definition of the study population.

Table 1. Characteristics of the 4,467 children and their mothers, categorized according to *H. pylori* status.

Child characteristics	Observed	Imputed	*H. pylori*$^{-}$ n = 4,029	*H. pylori*$^{+}$ n = 438	Univariate OR (95% CI)
Female sex (%)	2,164 (48.4)	2,164 (48.4)	1,916 (47.6)	248 (56.6)	1.44 (1.18-1.76)*
Mean age sera taken, years (SD)	6.2 (0.4)	6.2 (0.4)	6.2 (0.01)	6.4 (0.03)	1.66 (1.39-1.98)*
Ethnicity (%)					
Dutch	2,505 (56.1)	2,516 (56.3)	2,374 (58.9)	142 (32.4)	1.0
Surinamese	317 (7.1)	318 (7.1)	285 (7.1)	33 (7.5)	1.95 (1.29-2.94)*
Turkish	311 (7.0)	324 (7.3)	279 (6.9)	45 (10.3)	2.64 (1.71-4.07)*
Moroccan	256 (5.7)	261 (5.8)	192 (4.8)	69 (15.8)	6.01 (4.27-8.44)*
Dutch Antilles	141 (3.2)	142 (3.2)	120 (3.0)	21 (4.8)	2.96 (1.78-4.92)*
Cape Verdean	129 (2.9)	151 (3.4)	115 (2.9)	36 (8.2)	5.20 (3.31-8.16)*
Other:					
European	331 (7.4)	332 (7.4)	298 (7.4)	34 (7.8)	1.92 (1.28-2.89)*
Asian	111 (2.5)	112 (2.5)	95 (2.4)	17 (3.9)	3.01 (1.73-5.23)*
African	100 (2.2)	106 (2.4)	91 (2.3)	15 (3.4)	2.63 (1.31-5.30)*
Rest of the world[a]	148 (3.3)	206 (4.6)	180 (4.5)	26 (5.9)	2.11 (0.74-6.00)
Data missing	*118 (2.6)*				
Caesarean Section (%)					
No	3,358 (75.2)	3,878 (86.8)	3,483 (86.4)	395 (90.2)	1.0
Yes	496 (11.1)	589 (13.2)	546 (13.6)	43 (9.8)	0.70 (0.47-1.02)
Data missing	*613 (13.7)*				
Breastfeeding[b] (%)					
Never	264 (5.9)	388 (8.7)	356 (8.8)	32 (7.3)	1.0
Partial	1,857 (41.6)	2,925 (65.5)	2,623 (65.1)	302 (68.9)	1.34 (0.73-2.43)
Exclusive	723 (16.2)	1,154 (25.8)	1,050 (26.1)	104 (23.7)	1.14 (0.64-2.03)
Data missing	*1,623 (36.3)*				
Day-care attendance[c] (%)					
No	1,019 (22.8)	2,110 (47.2)	1,849 (45.9)	261 (59.6)	1.0
Yes	1,548 (34.7)	2,357 (52.8)	2,180 (54.1)	177 (40.4)	0.57 (0.44-0.75)*
Data missing	*1,900 (42.5)*				
Number of older siblings (%)					
0	2,370 (53.1)	2,425 (54.3)	2,219 (55.1)	206 (47.0)	1.0
≥1	1,936 (43.3)	2,042 (45.7)	1,810 (44.9)	232 (53.0)	1.38 (1.13-1.69)*
Data missing	*161 (3.6)*				
Cumulative antibiotic use					
Never	529 (11.8)	940 (21.0)	861 (21.4)	78 (17.8)	1.0
1-2 courses	1,162 (26.0)	1,715 (38.4)	1,564 (38.8)	151 (34.5)	1.07 (0.73-1.56)
≥3 courses	828 (18.5)	1,813 (40.6)	1,604 (39.8)	209 (47.7)	1.44 (1.01-2.06)*
Data missing	*1,948 (43.6)*				
Maternal characteristics					
Maternal education level (%)					
Primary, or secondary	2101 (47.0)	2,438 (54.6)	2,127 (52.8)	311 (71.0)	2.20 (1.76-2.75)*
Higher	1955 (43.8)	2,029 (45.4)	1,902 (47.2)	127 (29.0)	1.0
Data missing	*411 (9.2)*				
Mean age sera taken, years (SD)	30.5 (5.0)	30.5 (5.0)	31.3 (4.6)	29.4 (5.4)	0.92 (0.91-0.94)*

Values are means (and standard deviation), absolute numbers (and percentages) or odds ratio (and 95% confidence interval). Missing data on maternal *H. pylori* and CagA status were not imputed.
[a]Includes subjects from Central and South America (n = 95), Indonesia (n = 32), North America (n = 21), Asia, western (n = 21), and Oceania (n = 9).
[b]Data until 4 months of life
[c]Data completed from the first year of life. * $p < 0.05$

Higher *H. pylori* colonization rates were observed in children of either Dutch or non-Dutch ethnicity of older age (**Figure S2**). The proportion of CagA-positivity amongst *H. pylori*-positive children varied widely between ethnic groups (**Figure 2B**). The lowest proportions were found in children with Dutch or other European ethnicities (16% and 14%, respectively).



Number *Hp*+ (n)	142	33	45	21	36	69	34	17	15		438
Total size (n)	2516	318	324	142	151	261	332	112	106		4467
p-value	*ref*	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05		

Number CagA+ (n)	23	15	23	9	15	25	5	8	7		142
Total size (n)	142	33	45	21	36	69	34	17	15		438
p-value	*ref*	<0.05	<0.05	<0.05	<0.05	<0.05	0.83	<0.05	<0.05		

Figure 2. Prevalence of *H. pylori* and CagA-positivity in 4,467 children according to ethnic group. Panel A: *H. pylori* prevalence, by ethnic group. Panel B: Proportion of CagA-positive strains amongst the *H. pylori*-positive children, by ethnic group.
Numbers are either percent (above bars) or absolute numbers (Tables).
P-values reflect differences between children of Dutch ethnicity (reference group) and any other group, using Chi-square test. Children classified as 'rest of the world' are not shown in this figure. Their *H. pylori*-positivity rate is 12.6% (26 of 206), and proportion of CagA-positive strains is 42% (11 of 26).

Risk factors for* H. pylori *colonization

In univariate analyses, a child's *H. pylori* positivity was associated with an *H.* pylori-positive mother (OR 3.22; 95% CI 2.52-4.12), and non-Dutch ethnicity (OR 2.99; 95% CI 2.32-3.86). Female gender, age, breastfeeding, presence of older siblings, antibiotic exposure (see also **Table S3**), and lower maternal educational level also were positively associated with *H. pylori* status, whereas day-care attendance was negatively associated (**Table 1**).

Using multivariate analysis (**Figure 3**), we identified the following independent risk factors for *H. pylori* positivity in a child: maternal *H. pylori* positivity [(CagA-positive mother OR 2.25; 95% CI 1.61-3.16) (CagA-negative mother OR 2.05; 95% CI 1.53-2.74)], non-Dutch ethnicity (OR 2.04; 95% CI 1.53-2.72), female gender (OR 1.47; 95% CI 1.20-1.81), and lower maternal education level (OR 1.37; 95% CI 1.06-1.78). A separate multivariate analysis to examine risk for CagA-positivity amongst all *H. pylori*-positive children revealed independent associations with lower educational level of mother (OR 2.65; 95% CI 1.33-5.28), and non-Dutch ethnicity (OR 2.48; 95% CI 1.27-4.85) (**Table S4**). Compared with males, we found female gender independently associated with never having had exposure to antibiotics (OR 1.30; 95% CI 1.07-1.60) and lower educational level of the mother (OR 1.17; 95% CI 1.01-1.35) (**Table S5**). Caesarian section was not significantly associated with *H. pylori* colonization. Comparison of C-section with vaginal birth revealed independent associations with no breastfeeding (OR 1.98; 95% CI 1.31-2.98), nulliparity (OR 1.81; 95% CI 1.48-2.20), and day-care attendance (OR 1.34; 95% CI 1.01-1.77) (**Table S6**).

A stratified analysis by ethnicity was performed (Dutch *vs.* non-Dutch), based on the significantly lower *H. pylori* colonization rate in children of Dutch ethnicity compared to all other subjects (**Figure 3**, and **Table S7** for comparison of European *vs.* non-European). Differences in the odds ratios for *H. pylori* colonization were observed for gender, educational level of mother, and day-care attendance. There was no evidence for effect modification by ethnicity for the associations of gender, educational level of mother, and day-care attendance with *H. pylori* colonization (p-value for interactions >0.05).

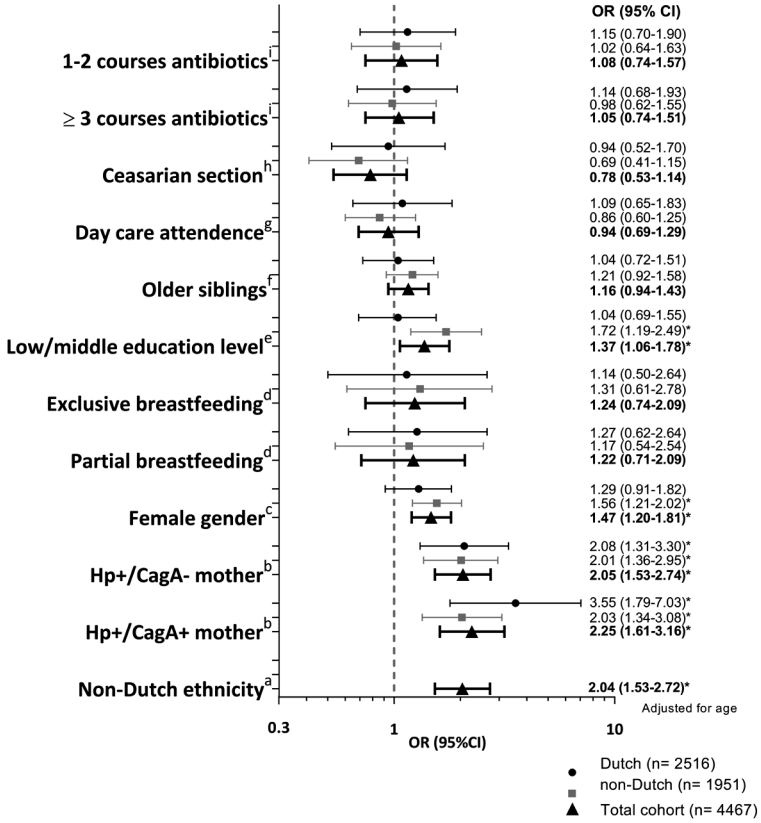


Figure 3. Multivariate analyses of determinants associated with *H. pylori* colonization in the total population, and comparing Dutch and non-Dutch origins. All listed variables are entered into the logistic regression model. Odds ratios (ORs) and 95% confidence intervals (CIs) are adjusted for age and express the association with *H. pylori* positivity. The ORs for the total population are in bold. Reference groups: [a]*H. pylori*-negative mother, [b]Dutch ethnicity, [c]male gender, [d]no breastfeeding, [e]higher educational level, [f]no older siblings, [g]no day-care attendance, [h]vaginal birth, [i]never exposed to antibiotics.
* $p < 0.05$.

Comparison of H. pylori *colonization in mothers and their children*

Data on the *H. pylori* status of mother was available for 3,185 (71%) children (**Table 2**). The *H. pylori* positivity rate in mothers (mean age of 30.5 ±5.0 years) was 42%. An *H. pylori*-positive mother was associated with an *H. pylori*-positive child (OR 3.22; 95% CI 2.52-4.12). Of the 1,328 children with an *H. pylori*-positive mother, 211 (15.9%) were *H. pylori*-positive, compared to 103 (5.5%) of the 1,857 children with an H. pylori-negative mother. As a result, 33% (n=103) of all *H. pylori*-positive children had a mother who tested *H. pylori*-negative. The median antibody titer in these children was significantly lower (1.43; 2.5-97.5th percentile 0.74-6.68) than in children with an *H. pylori*-positive mother

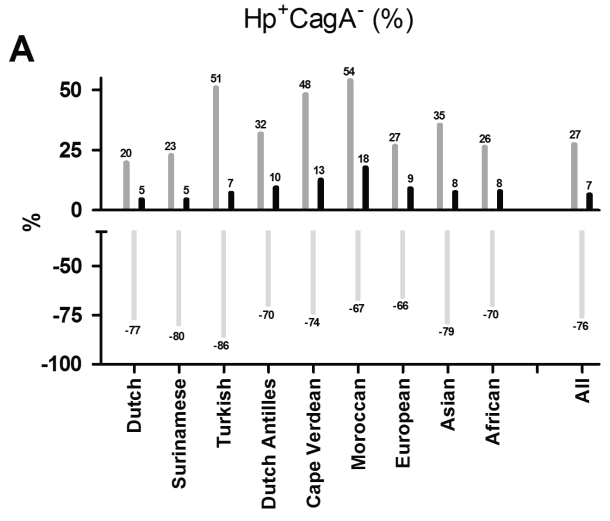
(2.11; 2.5-97.5th percentile 0.56-15.68). In children of non-Dutch ethnicity, the proportion *H. pylori*-positive children with an *H. pylori*-negative mother was 22% compared to 55% of children with Dutch ethnicity (p<0.001). **Table 2** shows the associations between mother and child's *H. pylori* colonization rates by strain type. Children born from *H. pylori*-negative mothers were significantly less likely to be colonized at age 6 with either a CagA-negative or CagA-positive *H. pylori* strain than children born from *H. pylori*-positive mothers. Conversely, children born from *H. pylori*$^{+}$CagA^{-} mothers were more likely to be colonized with the same strain type, and for children with *H. pylori*$^{+}$CagA^{+} mothers, the OR for carrying the same strain type was 6.74 (95% CI 4.52-10.05).

Table 2. *H. pylori* colonization by strain type in 3,185 mother-child pairs[a].

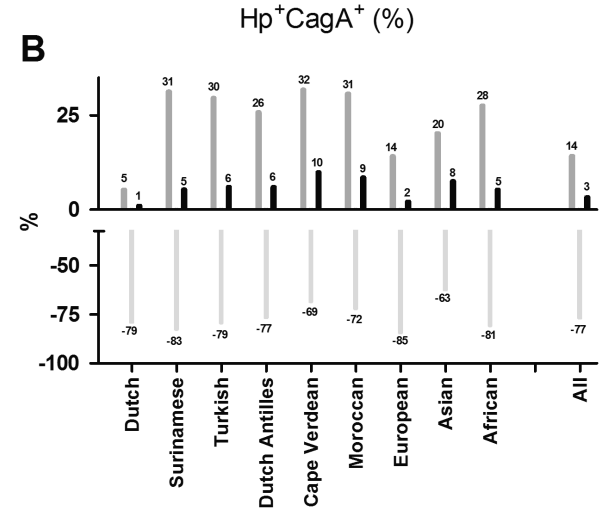
		Child's *H. pylori* status			
		Hp- (n= 2,871)	Hp+ (n= 314)	Hp+CagA- (n=210)	Hp+CagA+ (n=104)
Mother's *H. pylori* status	Hp- [n= 1,857 (%)]	1,754 (94.5)	103 (5.5)	86 (4.6)	17 (0.9)
	OR (95% CI)	*Reference*	**0.31 (0.24-0.40)***	**0.44 (0.33-0.59)***	**0.12 (0.07-0.21)***
	Hp+ [n= 1,328 (%)]	1,117 (84.1)	211 (15.9)	--	--
	OR (95% CI)	*Reference*	**3.22 (2.52-4.12)***		
	Hp+CagA- [n= 873 (%)]	746 (85.5)		92 (10.5)	35 (4.0)
	OR (95% CI)	*Reference*	--	**2.22 (1.67-2.95)***	1.45 (0.95-2.19)
	Hp+CagA+ [n= 455 (%)]	371 (81.5)		32 (7.0)	52 (11.5)
	OR (95% CI)	*Reference*	--	1.21 (0.82-1.79)	**6.74 (4.52-10.05)***

[a]Values shown are absolute numbers and their percentages in relation to maternal *H. pylori* status. The odds ratios and 95% confidence intervals represent the association between the reference group (children without *H. pylori*) and the other groups. * p <0.05

Overall, the *H. pylori* prevalence decreased 76% comparing mothers and their children. A significant reduction in *H. pylori* prevalence was observed across all nine ethnic groups studied (**Figure 4**). This reduction was consistent for both *H. pylori*$^{+}$CagA^{-} and *H. pylori*$^{+}$CagA^{+}-strains (**Figure 4A and 4B**). The overall reduction rate for males (-80%) was slightly higher than for females (-72%), which was consistent across all ethnic groups. Multivariate analysis of the absence of *H. pylori* in children with an *H. pylori*-positive mother (n = 1,328) revealed male gender (OR 1.64; 95% CI 1.21-2.23), higher maternal education level (OR 1.78; 95% CI 1.15-2.76), and no older siblings (OR 1.37; 95% CI 1.01-1.88) independently associated with an *H. pylori*-negative child (**Table S8**). The proportion of the *H. pylori* reduction attributable to male gender (21%), having no older siblings (14%), and higher maternal education level (14%), were all significant (**Figure 5**).



Total size (n)	1780	224	247	116	110	185	232	79	76		3185
Mothers CagA- (n)	353	51	126	37	53	100	62	28	20		873
Children CagA- (n)	82	10	18	11	14	33	21	6	6		210
p value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05		<0.05



Total size (n)	1780	224	247	116	110	185	232	79	76		3185
Mothers CagA+ (n)	95	70	73	30	35	57	33	16	21		455
Children CagA+ (n)	19	12	15	7	11	16	5	6	4		104
p value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05		<0.05

Figure 4. Prevalence and differential rates of *H. pylori* -strains in 3,185 mothers and their children by ethnic group. A: CagA$^-$ strains B: CagA$^+$ strains. Numbers are either percentages (above or below bars) or absolute numbers (Tables). Light grey bars reflect the percent differences between mothers and their children. The p-values reflect differences between the positivity rates in mothers (dark grey bars) compared to their children (black bars). Subjects categorized in 'rest of the world'-group are not shown as a separate group, but included in 'All' category.

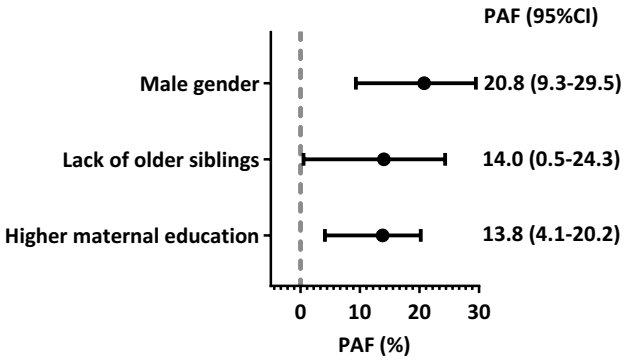


Figure 5. Population-attributable fraction (PAF) for decline in *H. pylori* colonization in 1,328 children with an *H. pylori*-positive mother. PAF was calculated from adjusted ORs (aOR) derived from multivariable logistic regression model comparing *H. pylori*-negative children (n = 1,117) with *H. pylori*-positive children (n = 211), additionally adjusted for caesarean section, breastfeeding, day-care attendance and cumulative antibiotic exposures. PAF, population-attributable fraction; CI, confidence interval.

DISCUSSION

In this multi-ethnic population-based cohort, we found highly variable *H. pylori* colonization rates in six-year old children, with both the prevalence of *H. pylori* and the proportion of CagA-positive strains higher in children of non-Dutch ethnicity. Independent of ethnic background, maternal *H. pylori* colonization was the strongest risk factor for *H. pylori*-positivity in their offspring. Our study design made it possible to compare *H. pylori* colonization in children directly with that of their mothers, showing essentially identical intergenerational reductions for both *H. pylori*$^{+}$CagA^{-} and *H. pylori*$^{+}$CagA^{+}-strains.

The overall colonization rate of 10% differs from that found in a previous study performed in the Netherlands (16). This recent study of 545 Dutch children between 7 and 9 years old, which used the same ELISA as did we, found an *H. pylori* positivity rate of 9% (95% CI 6.6-11.4%) (16), a prevalence slightly higher than that measured in children of Dutch ethnicity in our study (6%). This difference may reflect a continuing decline in colonization, or may be due to the different study designs, or the younger age of children in our study. The latter may contribute, as our data suggest continuing acquisition of *H. pylori* at least until the age of 7, consistent in children of Dutch and non-Dutch ethnicities. The higher *H. pylori* prevalence in children of non-Dutch ethnicity confirms findings of other studies (32, 33). The CagA-prevalence is lower than expected (1). However, recent studies from The Netherlands have found similar CagA prevalence to our results (34). In addition, there is evidence from Finland that CagA^{+}-strains are disappearing faster than CagA^{-}-strains (35).

The intergenerational reduction was of the same magnitude among all different ethnic groups, resulting in the same birth cohort effect in all groups. A recent study from Japan independently confirms the decline in two generations (36). We calculated the reduction in *H. pylori* colonization rate across generations: comparing the *H. pylori* prevalence in grandmothers and mothers showed a reduction of 37%, while the prevalence in mothers and their children showed a reduction of 72%. The latter is essentially the same reduction as we observed. The decline in children with an *H. pylori*-positive mother can be partially attributed to male gender, lack of older siblings, and higher educational level of mother. The lack of older siblings may reduce horizontal transmission of *H. pylori* within a family. Others found the number of siblings within a family rather than birth order independently associated with *H. pylori* colonization (37). Nevertheless, our findings imply that environmental factors and living conditions of the country in which a child is raised have a major impact on transmission, irrespective of ethnicity. The consistent reduction across all ethnic groups support the hypothesis that in contemporary Dutch society, and probably elsewhere as well, there are highly prevalent factors that interfere with the early life acquisition and/or maintenance of *H. pylori*. Besides the involvement of socio-economic status, family size, and other living conditions, possible candidates include the widespread use of antibiotics, particularly in young children. The effect of antibiotic monotherapy on *H. pylori* status is limited (38), but repeated antibiotic exposure may eventually result in eradication. Another possibility could be the run-off of antibiotics from farms where antibiotic-intensive husbandry is being practiced, which may contaminate surface and drinking water (39). In contrast to the use of antibiotics in humans, the Dutch consumption of antibiotics per animal exceeds the consumption of all European countries, and despite the prohibition of antibiotics as growth promoters, the use remained stable (40). However, there is no strong evidence of contaminated drinking water (40). Regardless of its cause, the clinical consequences of this rapid disappearance may have opposite effects: a fall in prevalence of the later life expression of gastric and duodenal ulcer disease, and gastric carcinoma (41, 42), but a rise in earlier life-expressed atopy, asthma, and reflux-related disorders (11), since epidemiological studies have shown inverse associations of these disorders with *H. pylori* colonization (3, 5).

The association of specific *H. pylori* types in mother and child provides further evidence supporting a role for maternal inheritance in early life transmission, shown in molecular typing studies (43). A recent German study that included the *H. pylori* status of parents and siblings in a multivariate model, showed that only maternal infection was associated with *H. pylori* positivity in the children (33). Despite this important maternal role, we found that one third of all positive children had an apparently *H. pylori*-negative mother. This proportion was even higher (>50%) in children of Dutch ethnicity, implying the involvement of other transmission sources, such as fathers and siblings (43), or

grandmothers (36). There also is evidence for transmission from outside the nuclear family, including environmental reservoirs or other children in the community (44, 45), although these seem more likely in developing than in developed countries (46). An alternative hypothesis is that some maternal *H. pylori* colonisations were missed, due to lack of complete sensitivity of the assay, or post-natal acquisition of the organism. Especially in a multi-ethnic population, children may become colonized with *H. pylori* by acquiring the bacterium from persons of ethnicities with higher *H. pylori* prevalence, e.g., in day-care facilities. However, a recently published meta-analysis found no significant effect of day-care attendance on *H. pylori* colonization (summary OR 1.12; 95% CI 0.82-1.52) (47). Nevertheless, a Portuguese study of 1,047 children reported increasing *H. pylori* prevalence with cumulative attendance in day-care centres (48). Our stratified analysis of ethnicity revealed opposite trends for the relation between *H. pylori* colonization and day-care attendance. Such observations suggest that child-to-child transmission in a day-care setting may be more likely for children of Dutch ethnicity where children of non-Dutch ethnicity could serve as transmission sources.

Remarkably, female gender was found to be associated with *H. pylori* colonization. Although most prior studies found a decreased risk for infection, pooled data from studies in children found no difference (49). Specific multivariate analysis of determinants associated with female gender (**Table S5**) showed that both lower socioeconomic status and lack of antibiotic use were associated with female gender. Our findings are consistent with prior studies from The Netherlands, which observed that males from 0 to 4 years of age received more antibiotics than females of the same age (50, 51), while for older ages, the trends were opposite. In most prior studies, breastfeeding is associated with lower *H. pylori* colonization rates: a meta-analysis of pooled data found an OR of 0.78 (95% CI 0.61-0.99) for *H. pylori* infection (52). However, 6 of 14 included studies observed an increased risk for *H. pylori* infection, which is in line with our trend. We speculate that breastfed children had been in closer contact with mother, and were more likely to be born vaginally, which may have increased the risk of transmission. Nevertheless, we did not observe a significant association between breastfeeding and *H. pylori* status, and therefore firm conclusions are not possible. The positive associations of Caesarian section with nulliparity, and no breastfeeding, confirm prior observations (53), but that mothers who underwent Caesarian section were more likely to use day-care suggests that mode of delivery correlates with other lifestyle aspects.

An important strength of this study is that we had a large multi-ethnic study population drawn from the general population of Rotterdam; since immigration is common in many western countries, our findings may be more broadly applicable. An additional strength is the use of maternal data on *H. pylori* colonization, which provides insight into mother-to-child transmission.

This study has some limitations, including missing data for several characteristics and potential risk factors for colonization, which may have biased the outcome. However, we performed the final analyses after a multiple imputation procedure, considered useful to deal with missing data, as it requires the fewest assumptions and reduces potential bias when missing data are not random (30). A second limitation was lack of data on *H. pylori* colonization in fathers and siblings, precluding examination of their potential roles in transmission. A third limitation is that data on antibiotic exposures were not validated by pharmacy records, nor was information available on specific types. Strong conclusions on the effect of antibiotic exposure for the *H. pylori* colonization are not possible. Our data may overestimate prescribed antibiotic courses: a Dutch longitudinal observational study found that 25% of the studied children between 0-4 years of age received at least one oral antibiotic prescription per year (51). Taking these limitations into consideration, we believe that the questionnaire-based antibiotic data are at least worthwhile to report, since prior studies had considered such exposure as a potential confounder for the *H. pylori* colonization rates. A fourth limitation is that since we do not exactly know the maternal *H. pylori* status at age 6, the true intergenerational reduction may be over- or under-estimated. Since we have no indication that the likelihood of new infections or loss of infection after age 6 would be different across the ethnic groups, it should not affect our main finding of uniform rates of reduction across all ethnic groups. Finally, although both ELISAs have been validated in adults and children, including Dutch adults, and have been used in previous studies in Dutch children (16), they have not been separately validated in Dutch children.

In conclusion, we found relatively high *H. pylori* colonization rates in children of non-Dutch ethnicity who were born and raised in a western city. Regardless of ethnicity, maternal *H. pylori* type was an important predictor for a child's *H. pylori* type. The high and consistent intergenerational reduction in *H. pylori* prevalence irrespective of ethnicity and sex points toward very common exposures fuelling this phenomenon.

Acknowledgements

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SUPPLEMENTARY MATERIAL

Intergenerational reduction in *Helicobacter pylori* prevalence is similar between different ethnic groups living in a Western city

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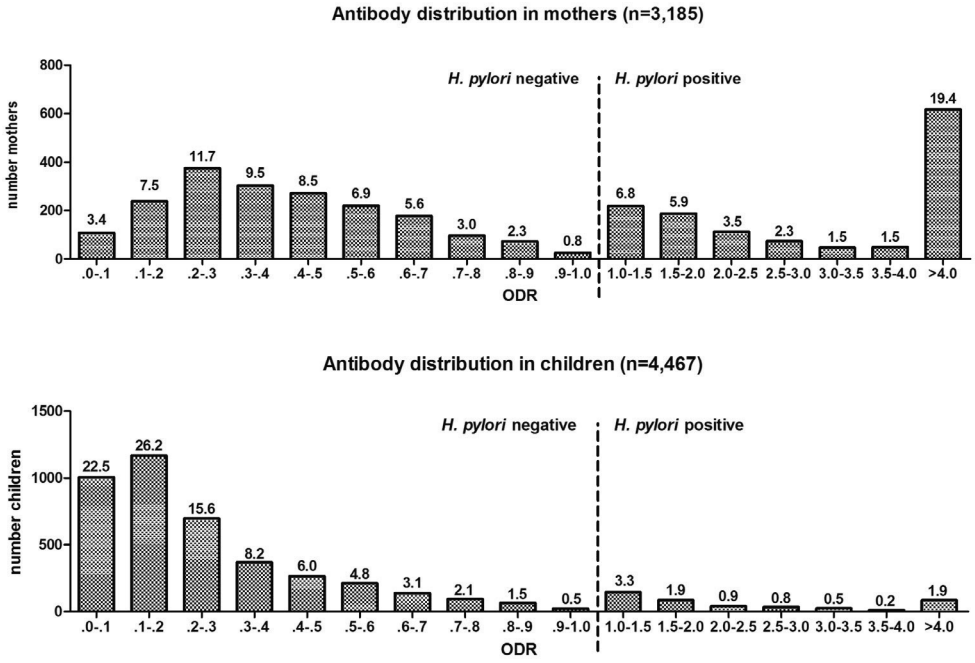


Figure S1. *H. pylori* antibody distribution in mothers and children. Distribution of levels (ODR) of mothers (A) and children (B). The numbers above the bars reflect the percent within the total population.

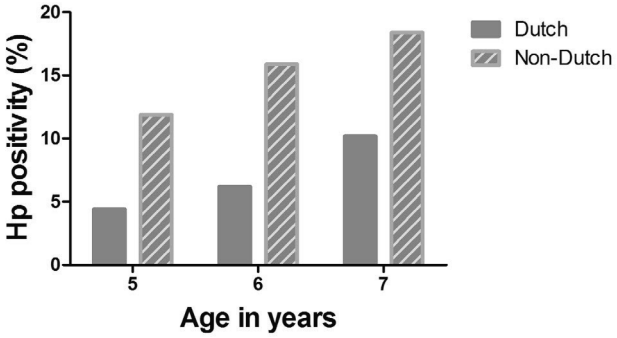


Figure S2. Continuing acquisition of *H. pylori* by age. *H. pylori* positivity rates (%) in 2,516 children of Dutch and 1,951 children non-Dutch ethnicity at ages 5, 6, and 7. The p-value for trend is <0.05 in both groups.

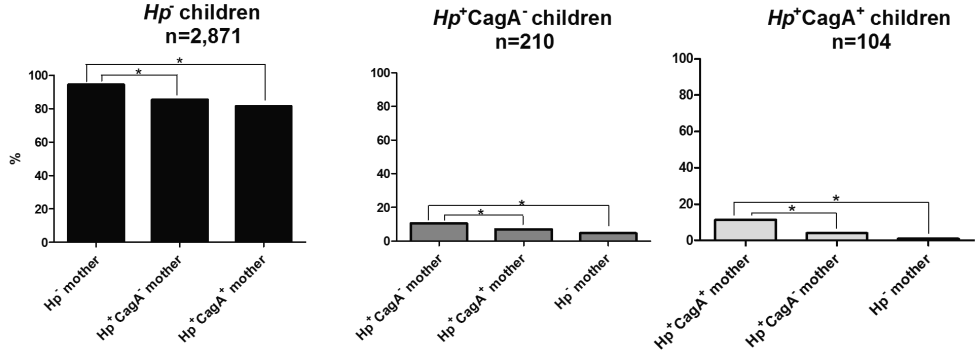


Figure S3. Comparison of *H. pylori* and CagA status in 3,185 mothers and their children. The 3,185 children are grouped by *H. pylori* and CagA status. Each figure shows the percent (%) of children with an *H. pylori*-negative (Hp^-), $CagA^-$ (Hp^+CagA^-), or $CagA^+$ (Hp^+CagA^+) mother. *p < 0.05 by Chi-square test. The p-values reflect the difference between the corresponding *H. pylori* status of mother and child, and the two other groups.

Table S1. Comparison of the maternal and child characteristics between those included and those not included in the study among the 8,305 eligible subjects.

	Population without *H. pylori* data N=3,338 (40.2)	Population with *H. pylori* data N=4,467 (59.8)	p-value
Child characteristics			
Age	6.1 (0.5)	6.2 (0.6)	<0.01
Female sex	1,948 (50.8)	2,164 (48.4)	0.04
Gestational age at birth	39.7 (1.9)	39.8 (1.8)	0.06
Birth weight	3,383 (586)	3,413 (569)	0.26
Ethnicity			
Dutch	1,999 (55.2)	2,505 (57.6)	0.02
Surinamese	287 (7.9)	317 (7.3)	
Turkish	309 (8.5)	311 (7.2)	
Moroccan	241 (6.7)	256 (5.9)	
Dutch Antilles	118 (3.3)	141 (3.2)	
Cape Verdean	99 (2.7)	129 (3.0)	
Other	630 (17.4)	690 (15.9)	
Caesarean section	441 (13.1)	496 (12.9)	0.74
Breastfeeding	2,448 (90.9)	3,248 (92.5)	0.03
Day-care attendance 1st year	1,043 (57.2)	1,548 (60.3)	0.04
Maternal characteristics			
Parity			
Nulliparous	2,077 (55.9)	2,370 (55.0)	0.45
Multipara	1,640 (44.1)	1,936 (45.0)	
Maternal education			
Low/medium	1,989 (58.3)	2,101 (51.8)	<0.01
High	1,420 (41.7)	1,955 (48.2)	

Values shown are absolute numbers (and percentages) for categorical variables, except that age, gestational age at birth, and birth weight are reported as the mean (and standard deviation).

Table S2. Missing data analyses in 4,467 children.

	Population with incomplete data on covariates* N= 3,039 (68.0)	Population with complete data on covariates N= 1,428 (32.0)	P-value	Missing (%)[a]
Child characteristics				
Age (SD)	6.36 (0.54)	6.03 (0.35)	<0.001	0
Female sex (%)	1,469 (48.3)	695 (48.7)	0.84	0
Gestational age at birth (SD)	39.6 (1.9)	40.0 (1.7)	<0.001	0.7
Birth weight (SD)				0.2
Ethnicity (%)			<0.001	2.6
Dutch	1,509 (51.7)	996 (69.7)		
Surinamese	247 (8.5)	70 (4.9)		
Turkish	250 (8.6)	61 (4.3)		
Moroccan	222 (7.6)	34 (2.4)		
Dutch Antilles	118 (4.0)	23 (1.6)		
Cape Verdean	108 (3.7)	21 (1.5)		
Other	467 (16.0)	223 (15.6)		
Caesarean section (%)	299 (12.3)	197 (13.8)	0.19	13.7
Breastfeeding (%)	1,917 (92.0)	1,331 (93.2)	0.18	21.4
Day-care attendance 1st year (%)	637 (55.9)	911 (63.8)	<0.001	42.5
Maternal characteristics				
Parity (%)			<0.001	3.6
Nulliparous	889 (51.2)	866 (60.6)		
Multiparous	846 (48.8)	562 (39.4)		
Maternal education (%)			<0.001	9.2
Low/medium	1,562 (59.4)	539 (37.7)		
High	1,066 (40.6)	889 (62.3)		

*****Data on ≥ 1 covariate is missing. [a]Percentage of missings per category.
Values shown are absolute numbers (and percentages) for categorical variables. Gestational age at birth and birth weight are reported as the mean (and standard deviation).

Table S3. Antibiotic exposure during the first 6 years of life.

	Observed	Imputed	*H. pylori*⁻ n = 4,029	*H. pylori*⁺ n = 438	Univariate OR (95% CI)
Antibiotic exposure (%)					
6-11 months					
No	1,800 (40.3)	2,728 (61.1)	2,467 (61.2)	261 (59.6)	1.0
1-2 courses	879 (19.7)	1,390 (31.1)	1,263 (31.3)	127 (29.0)	0.95 (0.68-1.32)
≥3 courses	105 (2.4)	350 (7.8)	300 (7.4)	50 (11.4)	1.51 (0.82-2.77)
Data missing	*1,683 (37.7)*				
12-23 months					
No	1,638 (36.7)	2,337 (52.3)	2,114 (52.5)	223 (50.9)	1.0
1-2 courses	1,111 (24.9)	1,702 (38.1)	1,541 (38.2)	161 (36.8)	0.99 (0.74-1.32)
≥3 courses	195 (4.4)	428 (9.6)	374 (9.3)	54 (12.3)	1.35 (0.86-2.11)
Data missing	*1,523 (34.1)*				
24-35 months					
No	1,817 (40.7)	2,642 (59.1)	2,404 (59.7)	238 (54.3)	1.0
1-2 courses	886 (19.8)	1,521 (34.0)	1,364 (33.9)	156 (35.6)	1.16 (0.87-1.56)
≥3 courses	105 (2.4)	305 (6.8)	260 (6.5)	44 (10.0)	1.71 (0.99-2.95)
Data missing	*1,659 (37.1)*				
36-47 months					
No	1,977 (44.3)	2,820 (63.1)	2,566 (63.7)	254 (58.0)	1.0
1-2 courses	763 (17.1)	1,253 (28.1)	1,131 (28.1)	122 (27.9)	1.09 (0.79-1.50)
≥3 courses	75 (1.7)	395 (8.8)	333 (8.3)	62 (14.2)	1.80 (0.91-3.57)
Data missing	*1,652 (37.0)*				
60-71 months					
No	2,956 (66.2)	3,303 (73.9)	2,999 (74.4)	304 (69.4)	1.0
1-2 courses	760 (17.0)	921 (20.6)	829 (20.6)	93 (21.2)	1.10 (0.83-1.46)
≥3 courses	73 (1.6)	243 (5.4)	202 (5.0)	41 (9.4)	1.95 (1.03-3.67)*
Data missing	*678 (15.2)*				

Values are absolute numbers (and percentages) or odds ratio (and 95% confidence interval).
* $p < 0.05$

Table S4. Multivariate analysis of determinants for CagA-positivity in 438 *H. pylori*-positive children[a].

Age[b]	0.93 (0.51-1.71)
Gender	
Female	0.87 (0.56-1.35)
Male	*Reference*
Ethnicity	
Dutch	*Reference*
Non-Dutch	2.48 (1.27-4.85)*
Breastfeeding[c]	
Never	*Reference*
Partial	0.84 (0.31-2.25)
Exclusive	0.60 (0.20-1.87)
Educational level	
Primary/secondary	2.65 (1.33-5.28)*
Higher	*Reference*
Number of older siblings	
0	*Reference*
≥1	1.02 (0.66-1.60)
Day-care attendance[d]	
No	*Reference*
Yes	0.62 (0.35-1.12)
Caesarian section	
No	*Reference*
Yes	1.66 (0.72-3.81)
Cumulative antibiotics exposure[e]	
0 courses	*Reference*
1-2 courses	0.76 (0.34-1.73)
≥3 courses	0.76 (0.35-1.67)

[a]Multivariate logistic regression analysis comparing 142 $CagA^+$ with 296 $CagA^-$-chlildren. Numbers are displayed as odds ratio (95% confidence interval) and represent the association with $CagA^+$-children. All listed variables were entered into the multivariate analysis.
[b]for each additional year
[c]Data pertain to the first 4 months of life
[d]Data pertain to the first year of life
[e]Cumulative antibiotic exposure data combined until the age of 72 months.
* $p < 0.05$

Table S5. Multivariate analysis of determinants associated with female gender[a].

Age[b]	0.94 (0.82-1.07)
Ethnicity	
Dutch	*Reference*
Non-Dutch	1.04 (0.91-1.18)
Breastfeedingc	
Never	*Reference*
Partial	1.00 (0.75-1.34)
Exclusive	1.03 (0.78-1.35)
Educational level	
Primary/secondary	1.17 (1.01-1.35)*
Higher	*Reference*
Number of older siblings	
0	*Reference*
≥1	0.90 (0.79-1.01)
Day-care attendance[d]	
No	*Reference*
Yes	1.09 (0.92-1.29)
Caesarian section	
No	*Reference*
Yes	0.89 (0.73-1.08)
Cumulative antibiotic exposure[e]	
0	1.30 (1.07-1.60)*
1-2 courses	1.06 (0.89-1.25)
≥3 courses	*Reference*

[a]Multivariate logistic regression analysis comparing 2,164 females with 2,303 males. Numbers are displayed as odds ratio (and 95% confidence interval) and represent the association with female gender. All listed variables were entered into the multivariate analysis.
[b]for each additional year
[c]Data pertain to the first 4 months of life
[d]Data pertain to the first year of life
[e]Cumulative antibiotic exposure data combined until the age of 72 months.
* $p < 0.05$

Table S6. Multivariate analysis of determinants associated with Ceasarian section[a].

Age[b]	1.01 (0.81-1.27)
Ethnicity (%)	
Dutch	*Reference*
Non-Dutch	0.98 (0.79-1.21)
Breastfeeding[c]	
Never	1.98 (1.31-2.98)*
Partial	1.30 (0.95-1.78)
Exclusive	*Reference*
Educational level	
Primary/secondary	0.98 (0.69-1.30)
Higher	*Reference*
Number of older siblings	
0	1.81 (1.48-2.20)*
≥1	*Reference*
Day-care attendance[d]	
No	*Reference*
Yes	1.34 (1.01-1.77)*
Cumulative antibiotic exposure[e]	
0	*Reference*
1-2 courses	0.95 (0.69-1.30)
≥3 courses	1.00 (0.76-1.31)

[a]Multivariate logistic regression analysis comparing Ceasarian section (n=589) with vaginal births (n=3,878). Numbers are displayed as odds ratio (95% confidence interval) and represent association with Ceasarian section. All listed variables were entered into the multivariate analysis.
[b]for each additional year
[c]Data pertain to the first 4 months of life
[d]Data pertain to the first year of life
[e]Cumulative antibiotic exposure data combined until the age of 72 months.
* $p < 0.05$

Table S7. Multivariate analysis of *H. pylori* status by ethnicity (European vs. non-European)[a].

	European	Non-European
Age[b]	1.55 (1.07-2.24)*	1.09 (0.82-1.44)
Gender		
Female	1.50 (1.10-2.06)*	1.46 (1.12-1.92)*
Male	*Reference*	*Reference*
Breastfeeding[c]		
Never	*Reference*	*Reference*
Partial	1.10 (0.58-2.12)	1.33 (0.57-3.11)
Exclusive	1.08 (0.51-2.27)	1.45 (0.64-3.29)
Educational level		
Primary/secondary	1.10 (0.76-1.60)	1.74 (1.13-2.69)*
Higher	*Reference*	*Reference*
Number of older siblings		
0	*Reference*	*Reference*
≥1	1.13 (0.81-1.56)	1.16 (0.87-1.54)
Day-care attendance[d]		
No	*Reference*	*Reference*
Yes	1.22 (0.71-2.08)	0.77 (0.52-1.14)
Caesarean section		
No	*Reference*	*Reference*
Yes	0.90 (0.53-1.53)	0.69 (0.39-1.22)
Cumulative antibiotic use[e]		
0	*Reference*	*Reference*
1-2 courses	1.24 (0.77-1.99)	0.92 (0.56-1.50)
≥3 courses	1.20 (0.74-1.93)	0.91 (0.56-1.48)
Maternal *H. pylori* status		
Hp-	*Reference*	*Reference*
Hp+CagA-	2.16 (1.42-3.27)*	1.99 (1.31-3.04)*
Hp+CagA+	3.75 (2.14-6.59)*	1.95 (1.24-3.07)*

Multivariate logistic regression analysis comparing 2,848 subjects of European with 1,619 subjects of non-European ethnic background. Numbers are displayed as odds ratio (and 95% confidence interval) and represent association with *H. pylori* positivity. All listed variables were entered into the multivariate analysis.

[b]for each additional year

[c]Data pertain to the first 4 months of life

[d]Data pertain to the first year of life

[e]Cumulative antibiotic exposure data combined until the age of 72 months.

* $p < 0.05$

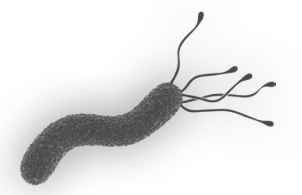
Table S8. Multivariate analysis of risk factors for *H. pylori* loss in 1,328 children with an *H. pylori*-positive mother[a].

Gender	
Female	*Reference*
Male	1.64 (1.21-2.23)*
Breastfeeding[b]	
Never	1.38 (0.55-3.48)
Partial	1.10 (0.65-1.84)
Exclusive	*Reference*
Educational level	
Primary/secondary	*Reference*
Higher	1.78 (1.15-2.76)*
Number of older siblings	
0	1.37 (1.01-1.88)*
≥1	*Reference*
Day-care attendance[c]	
No	*Reference*
Yes	1.30 (0.81-2.09)
Caesarian section	
No	*Reference*
Yes	1.58 (0.74-3.39)
Cumulative antibiotic exposures[d]	
0	*Reference*
1-2 courses	1.08 (0.56-2.06)
≥3 courses	1.03 (0.58-1.83)

[a]Multivariate logistic regression analysis comparing 1,117 *H. pylori*-negative children with 211 *H. pylori*-positive children. Numbers are displayed as odds ratio (and 95% confidence interval) and represent association with *H. pylori*-negative children (loss of *H. pylori*). All listed variables were entered into the multivariate analysis.
[b]Data pertain to the first 4 months of life
[c]Data pertain to the first year of life
[d]Cumulative antibiotic exposure data combined until the age of 72 months.
* $p < 0.05$

PART III

H. pylori, extra-gastric diseases, and premalignant gastric lesions



CHAPTER 4

Helicobacter pylori infection: a predictor of vomiting severity in pregnancy and adverse birth outcome

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Submitted

ABSTRACT

Background and aim: Nausea and occasional vomiting in early pregnancy (NVP) is common. It is unknown why some women experience severe NVP. Causes are multifactorial and only symptomatic treatment options are available, while adverse birth outcomes have been described. *Helicobacter pylori (H. pylori)* infection has been implicated in the etiology of NVP. We aimed to investigate the association of *H. pylori* with vomiting severity in pregnancy and its effect on birth outcome.

Methods: Population-based prospective cohort of pregnant women in the Netherlands. Enrolment took place between 2002 and 2006. *H. pylori* serology was determined in mid gestation. Women reported vomiting symptoms and had their weight measured in early, mid and late gestation. Birth outcomes were obtained from medical records. Main outcome measures were vomiting frequency (no-occasional-daily) and duration (early-mid-late gestation), maternal weight gain, birthweight, small for gestational age (SGA) and prematurity. Data were analyzed using multivariate regression.

Results: 5,549 Women were included, of whom 1,932 (34,8%) reported occasional vomiting and 601 (10.8%) reported daily vomiting. *H. pylori*-positive women (n=2,363) were more likely to report daily vomiting (adjusted odds ratio (OR) 1.44, 95%CI 1.16–1.78). *H. pylori*-positivity was associated with a reduction of total weight gain in women with daily vomiting (adjusted difference -2.1 kg, 95%CI -2.7–-1.5) and infants born to women with *H. pylori* and daily vomiting had an increased risk of being small for gestational age (SGA; adjusted OR 1.49, 95%CI 1.04–2.14). *H. pylori* and daily vomiting did not significantly affect birth weight or prematurity rate.

Conclusion: This study suggests that *H. pylori* is an independent risk factor for vomiting in pregnancy. In women with daily vomiting, *H. pylori* is also associated with low maternal weight gain and SGA. Since effective treatments for severe NVP are currently lacking, the effect of *H. pylori* eradication therapy on NVP symptom severity should be the target of future studies.

INTRODUCTION

Nausea and occasional vomiting in early pregnancy (NVP) affects 50-90% of pregnant women in the first half of gestation,(1) and can greatly impact maternal wellbeing and quality of life.(2) When vomiting is severe or protracted, or is accompanied by weight loss, dehydration, electrolyte disturbances or hospitalization, it is referred to as hyperemesis gravidarum (HG).(3) In the absence of an internationally recognized definition, HG and severe NVP are likely to overlap in studies.(4) In the Western world, severe NVP more often affects socially disadvantaged women and those of non-Western ethnicity.(5) To date, there is no clear explanation for the risk differences between Western and non-Western ethnic groups. In a recent meta-analysis, colonization with the gastric bacterium *Helicobacter pylori (H. pylori)* was positively associated with severe NVP (odds ratio (OR) 3.34, 95% CI 2.92–4.81).(6) Interestingly, the *H. pylori* prevalence in pregnant women of Western ethnicity is much lower than in women of non-Western ethnicity.(7) The association between *H. pylori* and severe NVP has been replicated in several studies, but mainly in non-Western populations in which the prevalence of *H. pylori* is high.(8-10) Three small studies on this topic have been conducted in a Western setting but reported conflicting findings.(11-13) Furthermore, some have suggested that more pathogenic variants of *H. pylori*, such as cytotoxin associated gene A (CagA)-positive strains are more often found among women with severe NVP.(14) Several small studies have suggested that *H. pylori* infection is not only associated with the presence of severe NVP, but also positively associated with symptom severity(8) and persistence.(10)

Severe NVP has repeatedly been associated with adverse birth outcome. This includes low birth weight, small for gestational age (SGA) and prematurity,(15) but the mechanism by which severe NVP may lead to adverse birth outcomes is not well understood. Weight loss or insufficient weight gain during pregnancy has been suggested to play a role,(16, 17) although other factors such as the presence of *H. pylori* on birth outcome has not been investigated.

In the present study, we investigate the hypothesis that *H. pylori* is associated with vomiting severity in pregnancy, and contributes to adverse birth outcomes in women with severe NVP. Furthermore, we investigate whether *H. pylori* explains the marked ethnic differences in maternal daily vomiting incidence. The study was performed in a large prospective multi-ethnic cohort study in the Netherlands, the Generation R study.

METHODS

Study population

This study was embedded in the Generation R study, a population-based, prospective cohort study from early pregnancy until young adulthood. Approval of the Generation R Study was obtained from the Central Committee on Research involving Human Subjects in the Netherlands (CCMO) via the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam (198.782/2001/31). All participants provided written informed consent. The study is still ongoing and conducted in Rotterdam, the second largest city of the Netherlands with a multi-ethnic community. Study design and aims have been described in detail elsewhere.(18) In brief, 8,879 pregnant women were enrolled between 2002 and 2006. Women underwent physical examinations (measurement of height and weight) and filled out questionnaires, in early, mid and late gestation (<18 weeks, 18-25 weeks, >25 weeks respectively). These questionnaires contained information on medical history, socioeconomic background, lifestyle and current pregnancy. The number of physical examinations and questionnaires received was dependent on the gestational age at enrolment. Serum samples were obtained during mid gestation.(19)

In this study, 5,549 women with complete data on *H. pylori* serology and vomiting status at enrolment were included. Women were excluded if they participated multiple times in subsequent pregnancies or delivered before 23 weeks gestation. Because the diagnosis of severe NVP is unlikely when symptoms first appear after 22 weeks gestation, women enrolled from 23 weeks gestation onwards were also excluded. See **figure 1**.

Definition of daily vomiting

There is no internationally recognized definition for severe NVP or HG. We used self-reported daily vomiting during the past three months, present at study enrolment. Answers ranged from never through daily on a one to five scale (never - less than once a week - once a week - few times a week - daily). When vomiting occurred less than once a week, once a week or few times a week, this was defined as occasional vomiting. Since occasional vomiting in pregnancy is considered 'physiological', women with no or occasional vomiting are considered the reference group.

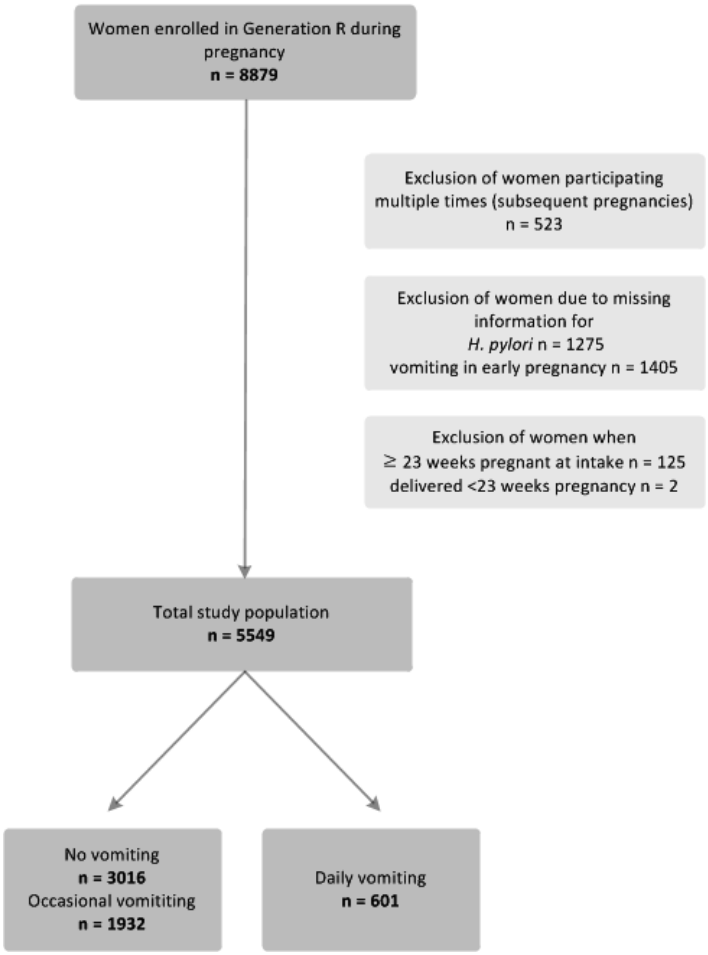

Figure 1. Flow diagram of participant selection

Symptom severity

Symptom severity was explored according to vomiting frequency (no - occasional - daily vomiting) and vomiting duration. When daily vomiting during the past three months was reported in both the first and second questionnaire, women were considered to have daily vomiting persisting into mid gestation. Similarly, when daily vomiting was reported in all three questionnaires, women were considered to have daily vomiting persisting into late gestation. Because inadequate weight gain is often part of severe NVP, the association of *H. pylori* and daily vomiting with total maternal weight gain (kg) was also investigated. Weight gain was based on self-reported pre-pregnancy weight and measured weight in late gestation (adjusted for gestational age difference at measurement).

H. pylori *serology*

Mid pregnancy (18-25 weeks) serum samples were used to determine *H. pylori* serology. *H. pylori* immunoglobulin G (IgG) antibody levels were examined by enzyme-linked immunosorbent assay (ELISA), using whole cell antigens.(20) A separate ELISA was performed to determine serum IgG antibodies against cytotoxin-associated gene A (CagA) protein.(21) Both ELISA's were validated locally, by adapting the ELISA properties based on positive and negative controls.

Pregnancy outcomes

Gestational age at birth, birth weight and neonatal sex were obtained from medical records.(18) Prematurity was defined as birth <37 weeks gestation, small for gestational age (SGA) was defined as gestational age-adjusted birth weight below the 10th percentile in this study's population, based on the reference standard by Niklasson *et al.*(22)

Covariates

General characteristics

Maternal age (y) was assessed at enrolment. Pre-pregnancy BMI (kg/m^2) was calculated using self-reported pre-pregnancy weight and height measured at enrolment. All socio-demographic characteristics were self-reported. Ethnicity was determined according to the definition of Statistics Netherlands(23) by country of birth of the pregnant woman and her parents. Based on the urban population of Rotterdam, the following ethnic groups were identified: Dutch, 'other Western' (women originating from Europe, North America, Oceania, Japan or Indonesia), Surinamese, Turkish, Cape Verdean/Dutch Antilles, Moroccan and 'other non-Western' (women originating from Africa, Asia, South or Central America). Dutch and 'other Western' ethnic groups were classified as Western, all other ethnic groups described were classified as non-Western. Educational level served as a proxy for socioeconomic status and was based on the highest completed education (none or primary school; secondary school; higher education). Smoking during pregnancy was also self-reported.

Pregnancy characteristics

All major pregnancy characteristics and outcomes were obtained from medical records. (18) These included parity, twin pregnancy and information on hypertensive disorders in pregnancy (pregnancy induced hypertension; preeclampsia; HELLP-syndrome: hemolysis, elevated liver enzymes, low platelets).

Data analysis

Logistic regression analysis was performed to study associations between *H. pylori* and vomiting frequency and vomiting persistence. We adjusted for maternal age, parity, ethnicity (ethnic groups), education level and smoking (model 2). Effects of *H. pylori* and daily vomiting on maternal weight gain and birth outcomes were explored using linear regression analysis. These analyses were adjusted for neonatal sex, gestational age at birth, twin pregnancy, maternal age, parity, hypertensive disorders, ethnicity, education level, pre-pregnancy BMI and smoking. We examined potential interactions for these outcomes between *H. pylori* and daily vomiting, ethnicity and education level. Sensitivity analyses were performed by including maternal weight gain based on measured weight in early and late gestation, and excluding twin pregnancies. We also investigated whether *H. pylori* explained ethnic differences in daily vomiting. Using logistic regression analysis we first explored the association of ethnicity and daily vomiting, followed by adjustment for *H. pylori* (model 2). We then further adjusted for maternal age, parity, education level and smoking (model 3). Possible strain specific effects on vomiting frequency were assessed among *H. pylori*-positive (CagA-positive and CagA-negative) women. Due to small numbers, analyses on birth outcomes were not repeated in this subgroup. Lastly, sensitivity analyses were performed to examine whether the associations between *H. pylori*, vomiting severity and birth outcomes were similar for Dutch women only (largest ethnic group). Possible confounders were identified using Directed Acyclic Graphs,(24) based on known risk factors for HG. Missing data of covariates were imputed using multiple imputation (five datasets). The percentages of missing values within the population for analysis were below 2%, except for pre-pregnancy weight (10.8%), total weight gain (14.1%), vomiting in mid pregnancy (14.7%) and vomiting in late pregnancy (18.8%). All analyses were performed using IBM SPSS Statistics for Windows 21.0 (SPSS, IBM, Armonk, NY, USA).

RESULTS

Baseline characteristics of women with and without daily vomiting

The study population consisted of 5,549 pregnant women, of which 601 women experienced daily vomiting at enrolment (10.8%). **Table 1** describes socio-demographic and clinical characteristics of women and infants according to vomiting frequency. Compared to women with no or occasional vomiting, women with daily vomiting were younger, more often of non-Western ethnicity and less highly educated. Women with daily vomiting were more often *H. pylori* positive (64.4%), compared to women with no vomiting (36.5%) and occasional vomiting (45.2%, $p<0.001$).

Table 1 Demographics and clinical characteristics of women and infants according to daily vomiting

Characteristics	Total population	No vomiting	Occasional vomiting	Daily vomiting	p Value
n=	5,549	3,016	1,932	601	
Demographics					
Age (y)	29.7±5.2	30.6±5.0	28.9±5.1	27.6±5.0	<0.001
Pre-pregnancy BMI (kg/m^2)	22.6 (20.8-25.5)	22.4 (20.7-25.1)	22.7 (20.7-25.5)	23.7 (21.1-27.6)	<0.001
Ethnicity					<0.001
Western	62.7	71.6	58.5	31.8	
Non-Western	37.3	28.4	41.5	68.2	
Ethnic groups					<0.001
Dutch	50.8	58.9	46.7	23.5	
Other Western	11.9	12.8	11.7	8.3	
Moroccan	6.0	3.9	6.8	13.7	
Turkish	8.6	6.5	9.0	18.1	
Surinamese	9.2	7.4	10.5	14.6	
Cape Verdean/Dutch Antilles	7.6	5.7	9.0	12.4	
Other non-Western	5.9	5.0	6.3	9.5	
Education level					<0.001
Primary	10.6	8.2	11.2	20.0	
Secondary	47.3	42.5	50.7	59.9	
Higher	42.1	49.2	38.1	20.1	
Smoking	18.1	17.7	18.3	19.3	0.62
IgG anti-*H. pylori* positive*	42.6	36.5	45.2	64.4	<0.001
CagA +	34.4	11.9	15.4	41.1	<0.001
Pregnancy characteristics					
Nulliparous	61.8	63.1	62.0	54.9	<0.05
Twin pregnancy	1.1	0.9	1.4	1.0	0.23
Gestational age at intake (wk)	13.8 (12.4-16.2)	13.6 (12.2-16.1)	13.8 (12.5-16.4)	14.2 (12.5-16.6)	<0.05
Duration of daily vomiting					
Early gestation	7.2	-	-	66.6	
Mid gestation	2.4	-	-	22.1	
Late gestation	1.2	-	-	11.3	
Total weight gain (kg)**	10.5±5.1	10.9±4.9	10.6±5.2	8.5±5.9	<0.001
Infant characteristics					
Gestational age at birth (d)	281 (273-287)	281 (274-287)	281 (274-287)	280 (273-286)	0.18
Prematurity (<37 wk)	5.6	5.3	5.7	6.5	0.51
Birth weight (g)	3,402±569	3,422±563	3,387±572	3,360±577	<0.05
SGA (<p10)	10.1	9.5	10.2	11.9	0.19

Data represent mean±SD, median (IQR) or %. Differences in subject characteristics between groups were evaluated using Chi-square tests for proportions and One-way ANOVA or Kruskal Wallis for continuous variables. Abbreviations: BMI: body mass index; IgG: immune globuline G; CagA: Cytotoxin-associated gene A protein; SGA: small for gestational age. * CagA-negative or positive **Total weight gain based on pre-pregnancy weight and measured weight in late gestation

Does* H. pylori *underlie symptom severity?

After adjustment for confounders including ethnicity and education level, the association between *H. pylori* and daily vomiting was still present (**table 2**). *H. pylori* was also positively associated with symptom duration. 39.9% of women with no or occasional vomiting were *H. pylori* positive, compared to 62.4% of women with daily vomiting in early pregnancy, 66.4% of women with daily vomiting persistent into mid pregnancy, and 72.1% of women with daily vomiting persistent into late pregnancy (p<0.001). Logistic regression for the association of *H. pylori* and daily vomiting duration is shown in **figure 2**. After adjustment for confounders, the association was reduced but a similar trend was seen. Furthermore, women with daily vomiting had reduced weight gain in pregnancy (**table 3**). The presence of *H. pylori* further reduced weight gain in these women (p-value for interaction 0.07).

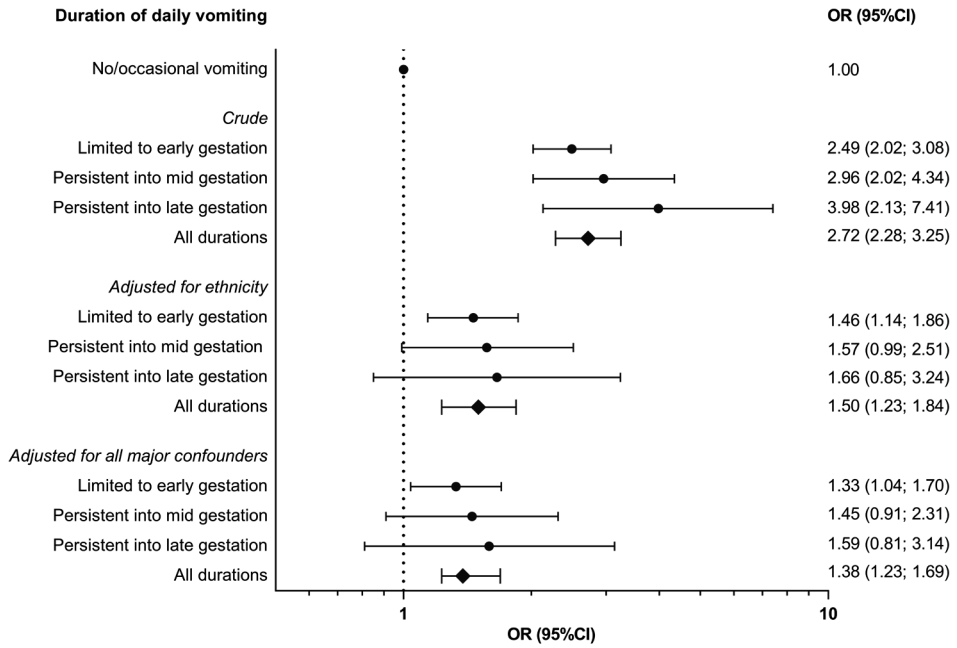

Figure 2. Logistic regression for *H. pylori* and daily vomiting according to symptom duration

Table 2 Logistic regression for *H. pylori* and vomiting frequency

	No vomiting		Occasional vomiting		Daily vomiting	
	OR	95% CI	OR	95% CI	OR	95% CI
Crude						
H. pylori	1.0		1.44**	1.28; 1.61	3.14**	2.62; 3.77
Adjusted						
H. pylori	1.0		1.10	0.96; 1.26	1.44*	1.16; 1.78

'No vomiting' is reference group. Adjusted for maternal age, parity, ethnicity, education level, smoking. OR: odds ratio; CI: confidence interval. * p<0.05, ** p<0.001

Does* H. pylori *underlie the association between daily vomiting and poor pregnancy outcome?

In **table 3**, we also examined the effects of *H. pylori* and daily vomiting on birth outcomes. After adjustment for confounders, there was no significant association between *H. pylori* or daily vomiting and birth weight, SGA or prematurity. However, infants born to women with both *H. pylori* and daily vomiting, had an increased risk for SGA. Interaction of *H. pylori* and daily vomiting for SGA was borderline significant (p=0.07), but not for birth weight or prematurity. There was no significant interaction for *H. pylori* and ethnicity or education level. Maternal weight gain was significantly associated with birth weight (for every kg maternal weight gain adjusted birth weight difference (β) 18g, 95%CI 15–21, p<0.001).

Table 3 Associations of *H. pylori* and daily vomiting with maternal weight gain and birth outcomes

	H. pylori		Daily vomiting		*H. pylori* + daily vomiting	
	β/OR	95% CI	β/OR	95% CI	β/OR	95% CI
Maternal outcome						
Total weight gain (kg)	-0.1	-0.4; 0.2	-1.1*	-1.9; -0.4	-2.1**	-2.7;-1.5
Neonatal outcome						
Birthweight (g)	-24	-57; -9	19	-57; 95	-58	-119; 2
SGA (<p10)	1.08	0.87; 1.34	0.77	0.45; 1.30	1.49*	1.04; 2.14
Prematurity (<37 wk)	1.13	0.85; 1.49	1.21	0.67; 2.18	1.36	0.83; 2.22

'No/occasional vomiting and no H. pylori' is reference group. Adjusted for neonatal sex, gestational age at birth, twin pregnancy, maternal age, parity, hypertensive disorders, ethnicity, education level, pre-pregnancy BMI, smoking, *H.pylori**daily vomiting. β: difference; OR: odds ratio; CI: confidence interval; SGA: small for gestational age. * p<0.05, ** p<0.001

Does* H. pylori *underlie ethnic differences in daily vomiting?

Daily vomiting occurred more often in women of non-Western ethnicity (crude OR 4.25, 95%CI 3.55–5.10). After adjustment for *H. pylori*, the OR diminished only slightly (*model 2,* adjusted OR 3.47, 95%CI 2.84–4.24). Across all ethnicities, the OR for daily vomiting according to the presence of *H. pylori* was similar. After further adjustment for major confounders, non-Western ethnicity remained significantly associated with daily

vomiting (*model 3*, adjusted OR 2.49, 95% CI 1.99–3.10). In fact, this was true for all non-Dutch ethnicities (**supplement table S1**).

Are there* H. pylori *strain specific effects?

Sub-analysis among *H. pylori*-positive women (n=2,363) showed that CagA-positive women were more likely to suffer from daily vomiting compared to women that were *H. pylori*-positive but CagA-negative (crude OR 1.36, 95%CI 1.09–1.70). After adjustment for ethnicity this difference was rendered non-significant (adjusted OR 1.17, 95% CI 0.93–1.48).

DISCUSSION

This study confirms the association between *H. pylori* and daily vomiting and adds to the existing evidence that the presence of *H. pylori* is associated with reduced maternal weight gain. More importantly, we found evidence that *H. pylori* contributes to SGA. This makes *H. pylori* eradication in pregnancy in women with severe NVP an attractive target for future intervention studies.

Previous studies have established that *H. pylori* infection is associated with severe NVP, although the strength and size of these associations varied between different populations and countries.(6, 11, 25, 26) Our findings confirm this association. Similarly, the prevalence of *H. pylori* infection among Dutch (24%) and non-Dutch women (64%) in this cohort(7) is in line with the existing literature. We found that *H. pylori* remained a risk factor for daily vomiting after adjustment for ethnicity and socioeconomic status, lending further support to the hypothesis that *H. pylori* is causally implicated in the pathophysiology of severe NVP.

Several studies evaluating birth outcome in pregnancies complicated by severe NVP have demonstrated modest negative effects on birth weight, SGA and prematurity rates.(15) Both *H. pylori* and severe NVP have been implicated in placental dysfunction disorders.(27, 28) Interestingly, we observed an increased risk for SGA in *H. pylori*-positive women with daily vomiting, but not in *H. pylori*-negative women with daily vomiting. This might explain why not all studies found adverse birth outcomes following pregnancies complicated by severe NVP. SGA may be a result of poor placentation, including failed remodeling of the spiral arteries.(29) Causes of impaired remodeling might include an excessive or atypical maternal immune response to trophoblasts.(30) Franceschi *et al.* have shown that anti-CagA antibodies *in vitro* were able to recognize β-actin on the surface of trophoblast cells in a dose-dependent binding activity.(31) This binding resulted in impaired cytotrophoblast invasiveness, which is crucial for the

development of the placental syndrome. This study may provide a biological explanation for the epidemiological association between *H. pylori* and the placental syndrome. Taken together, it is possible that *H. pylori* has a local gastrointestinal effect leading to NVP symptoms, and a systemic placental effect resulting in an increased risk for SGA. Furthermore, interaction of *H. pylori* and NVP might result in reduced maternal weight gain, which in turn negatively affects birth outcome.

Most likely *H. pylori* is acquired at a young age leading to lifelong colonization unless specifically treated.(32) More pathogenic variants of *H. pylori*, in particular CagA-positive strains, are associated with increased gastric inflammation.(21, 33, 34) Like Xia *et al*,(14) we found that CagA-positive women were more likely to suffer from daily vomiting compared to women that were *H. pylori*-positive but CagA-negative, which might be partly explained by differences in geographic distribution of CagA-strains.

This study was embedded in a large prospective cohort study and data collection was performed by trained research assistants. Questionnaires enquiring the presence of vomiting were collected prospectively, making the risk of recall bias low. If misclassification of vomiting frequency had occurred, the presented effects could be underestimated. Furthermore, we were unable to confirm HG diagnosis based on hospital admission or other more common used criteria(35) and numbers for persistent vomiting in late pregnancy were small. Due to the observational design, residual confounding might still be an issue. Despite these limitations, characteristics of women suffering from daily vomiting largely resembled previous reported data on severe NVP patients.(5, 36) Additionally, we were informed about maternal weight gain and symptom persistence and able to adjust for all previously described major confounders known to be associated with *H. pylori* and severe NVP,(12) with detailed information on ethnic background. Sensitivity analyses on the studied associations including only Dutch women resulted in similar findings (data not shown). We were underpowered to show that the presence of *H. pylori* was associated with daily vomiting persistence, and to study potential effects of *H. pylori* strains on vomiting severity and birth outcome.

There is debate on the accuracy of various diagnostic strategies to establish *H. pylori* infection. Many studies(6) including this study, have investigated the presence of *H. pylori* IgG antibodies in serum using ELISA. Other tests with greater accuracy have replaced serology in clinical practice, but due to low costs, acceptability to patients and practicability, serology is still indicated for epidemiological studies.(37) The declining *H. pylori* prevalence in the Netherlands might affect the positive predictive value of serology(38) but it is unlikely this has influenced our findings. Unlike most infectious diseases, *H. pylori* infection does not result in acquired immunity. Therefore, IgG anti-*H. pylori* and anti-CagA antibodies are indicative of active disease, unless eradication therapy has been prescribed in the previous months.(39) This would have been rare among pregnant women.

To date, no effective treatment options are available for severe NVP, nor do we know how to identify patients at risk for persistent symptoms during the course of pregnancy. Several case studies have reported that *H. pylori* eradication effectively relieved symptoms in women with persistent vomiting, unresponsive to conventional treatment.(40-43) *H. pylori* eradication therapy in the Netherlands normally consists of triple therapy, including a proton pump inhibitor (PPI), amoxicillin and clarithromycin or metronidazole.(38) A meta-analysis performed by Gill *et al.* showed no teratogenic effects of PPI use in early pregnancy.(44) No teratogenic effects were described for amoxicillin, clarithromycin and metronidazole.(45-47) Clarithromycin, but not amoxicillin and PPI, was associated with miscarriage when administered in first trimester.(47) These studies indicate that triple therapy consisting of PPI, amoxicillin and metronidazole might be safely used in pregnancy. Further evidence to confirm the effectiveness of *H. pylori* eradication on NVP symptom reduction and adverse birth outcome is needed from a randomised controlled trial.

In conclusion, our study suggests that *H. pylori* is an independent risk factor for vomiting in pregnancy, leading to low maternal weight gain and increased risk of SGA. Since treatment options for severe NVP are currently lacking, the effect of *H. pylori* eradication therapy on NVP severity should be target of future studies.

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CHAPTER 5

Helicobacter pylori colonization and pregnancy complications: the Generation R study

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Submitted

ABSTRACT

Background and aim: Preeclampsia (PE), small for gestational age (SGA), and spontaneous preterm birth (PTB) each may be complications of impaired placental function in pregnancy. Although their exact pathogenesis is still unknown, certain infectious agents seem to play a role. *Helicobacter pylori (H. pylori)* colonization has been associated with increased risk for PE. Our aim was to assess the association between *H. pylori* colonization and PE, SGA, and PTB.

Methods: We measured IgG anti-*H. pylori* and CagA-antibodies in serum of pregnant women (median 20.5 weeks, range 16.5-29.4) who participated in a population-based prospective cohort study. Delivery and medical records were assessed. Information on demographics, education, and maternal risk factors was collected by questionnaire. We used multivariate logistic regression analyses to assess associations between *H. pylori* colonization and PE, SGA, and PTB.

Results: In total, 6348 pregnant women were assessed. *H. pylori*-positivity was found in 2915 (46%) women, of whom 1023 (35%) also were CagA-positive. Pregnancy was complicated by PE, SGA or PTB in 927 (15%) women. *H. pylori* colonization was associated with PE (aOR 1.51; 95%CI 1.03-2.25). Differentiation according to CagA-status revealed the same risk. *H. pylori* was positively related with SGA, mainly explained by CagA-positive strains (aOR 1.34; 1.04-1.71). No association was observed between *H. pylori* and PTB.

Conclusions: Our data suggest that *H. pylori* colonization may be a risk factor for PE and SGA. If these associations are confirmed by future studies and shown to be causal, *H. pylori* eradication may reduce related perinatal morbidity and mortality.

INTRODUCTION

The involvement of systemic inflammatory responses in pregnancies complicated by pre-eclampsia (PE), small for gestational age (SGA), and spontaneous preterm birth (PTB) has led to the hypothesis that maternal infections may play a role in the etiology and pathogenesis of these pregnancy complications (1, 2). Although the exact causes of these complications are still unknown, one hypothesis for their origin is that they each are related to suboptimal placentation in early pregnancy (3-5). In this respect, colonization with *Helicobacter pylori (H. pylori)* may be of interest as it might be involved in the pathogenesis of impaired remodeling of the spiral arteries (6).

H. pylori is a Gram-negative bacterium that colonizes the stomach of about half of the world's population. After its re-discovery in 1982, extensive research demonstrated that *H. pylori* is an important risk factor for peptic ulcer disease, gastric adenocarcinoma, and mucosa associated lymphoid tissue (MALT)-lymphoma (7). An important host-interaction factor of *H. pylori* is the cytotoxin-associated gene A (cagA). The CagA protein is directly injected by *H. pylori* into the cytoplasm of gastric epithelial cells and subsequently affects cell morphology, proliferation and apoptosis (8). Colonization with CagA-positive strains is associated with higher levels of inflammatory cells and mediators compared to CagA-negative strains, both locally and systemically (9).

As such, recent studies have focused on extra-gastric manifestations of *H. pylori* colonization, including cardiovascular, hematologic, respiratory, and pregnancy-related diseases, including PE, SGA, and PTB (10). However, only few studies, each with a small number of cases, assessed the associations between *H. pylori* colonization and PE (11-14), and SGA (12, 15). These studies yielded conflicting results. Therefore, we examined the association between *H. pylori* colonization and each of these pregnancy-related complications in pregnant women participating in a large population-based prospective cohort study. As colonization with a CagA-positive strain is associated with higher levels of inflammatory mediators (16), we also assessed the effects of CagA-positive *H. pylori* strains on the risk of having these illnesses.

MATERIALS AND METHODS

Design and setting

This study was embedded in The Generation R Study, a population-based prospective cohort study among women and their children in Rotterdam, The Netherlands. In total 8879 pregnant women were included between April 2002 and January 2006. Assessments consisted of physical examinations, fetal ultrasounds, biological samples,

and questionnaires (17, 18). Approval was obtained from the Medical Ethics Committee of the Erasmus Medical Center. All participants provided written informed consent. *H. pylori* status could be measured in 6837 women. For the present study, women with maternal comorbidity known to be associated with an increased risk for the occurrence of these three illnesses (i.e. chronic hypertension, heart disease, diabetes, high cholesterol, thyroid disease and systemic lupus erythematosus) were excluded (n=179). Twin pregnancies, and women without data on PE, SGA, and PTB were also excluded. This left a study population of 6348 pregnant women with available information on both *H. pylori* status and pregnancy complications (**Figure 1**).

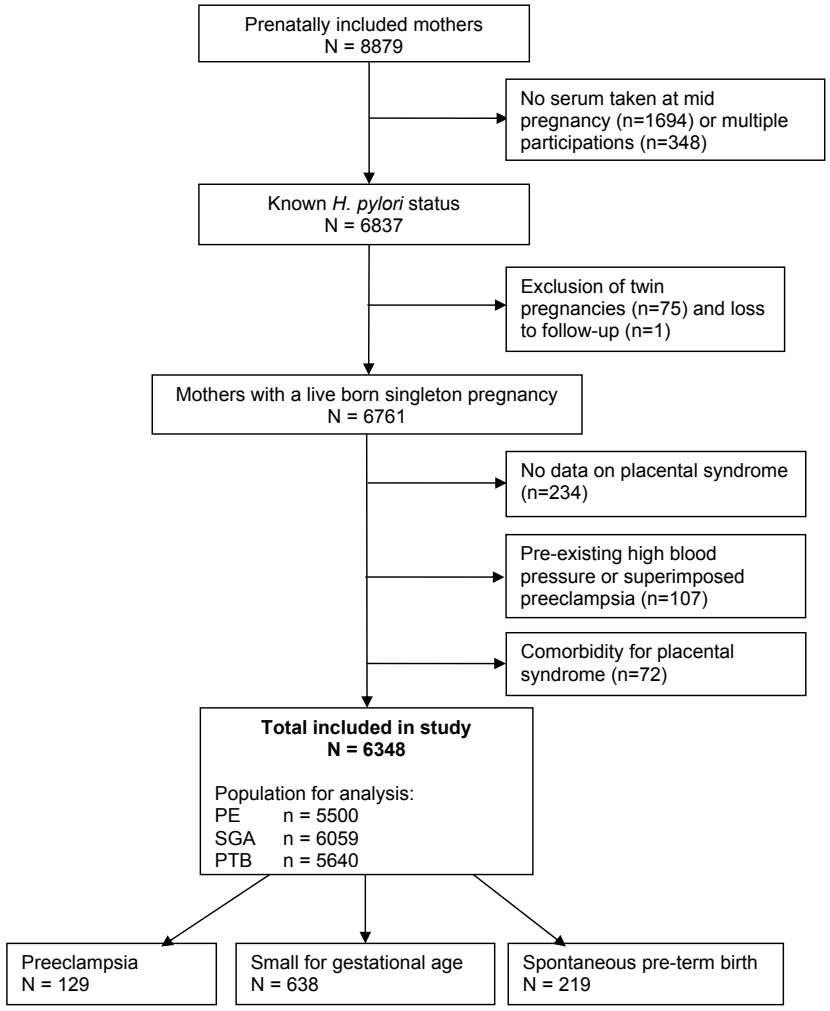

Figure 1. Study design

H. pylori *colonization during pregnancy*

Mid-pregnancy serum samples (median 20.5 weeks, range 16.5-29.4) were examined for IgG antibodies against *H. pylori* and the cytotoxin-associated gene A (CagA) protein using two separate enzyme-linked immunosorbent assays (ELISA), as described (19, 20). All samples were measured at least in duplicate. For each sample, the optical density ratio (ODR) was calculated by dividing the optical density (OD) by the mean OD of the positive controls. *H. pylori* positivity was defined as either an ODR≥1 or CagA positivity. The cut-off for CagA positivity was an ODR value ≥0.35. Details regarding *H. pylori* colonization in this cohort of pregnant women have been described (21). Both ELISAs were validated locally.

Pregnancy complications: PE, SGA, and PTB

Information on the pregnancy complications PE, SGA and PTB was obtained from medical records. For women who had suspected PE, the records were cross-checked with the original hospital charts (22). PE was defined as the development of systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg after 20 weeks of gestation in previously normotensive women, with concurrent proteinuria (≥0.3 g in a 24-hour urine specimen or ≥2+ (1 g/L] from a voided specimen, or ≥1+ [0.3 g/L] from a catheterized specimen) (23). Pregnancies complicated by hemolysis, elevated liver enzymes, and low platelet (HELLP) syndrome (n=22) were defined as cases of preeclampsia. PTB was defined as spontaneous onset of birth <37 weeks of gestation. We defined SGA as birth weight under the 10th percentile based on reference curves from our own cohort (24). Birth weight was adjusted for gestational age and transformed resulting in a normal distribution, which allowed use of means and standard deviations (SD) (25).

Covariates

Data on maternal age, ethnicity, educational level, parity, smoking during pregnancy, and maternal comorbidity (chronic hypertension, heart disease, diabetes, high cholesterol, thyroid disease and systemic lupus erythematosus) were obtained from questionnaires repeatedly applied during pregnancy. Height and weight were measured at enrolment and body mass index (BMI, kg/m^2) was calculated. In Rotterdam, the largest ethnic groups are of Dutch, Surinamese, Turkish, Moroccan, Dutch-Antilles, and Cape Verdean descent. Women with another ethnic background were categorized into 'other Western' (European, North American, Oceanean) or 'other non-Western' (African, Asian, South- and Central American). The highest educational level of the mother was defined as completion of university or higher vocational training. Mothers were categorized as having a middle/low level of education if they had completed intermediate vocational training, or had completed education below that level.

Statistical analysis

Firstly, with respect to missing data on BMI (0.6%), ethnicity (6.5%), educational level (8.1%), parity (0.8%) and smoking (11.9%), values were imputed using multiple imputation (26). In the present study, five draws for each missing value were performed providing five substituted data points, which in turn created five completed data sets. Analyses were performed separately on each completed dataset and thereafter combined into one global result (26). **Supplementary table 1** provides the percentages of missing values per covariate. Secondly, the frequency distributions between risk factors for pregnancy complications and *H. pylori* colonization were examined using the Independent Students' t-test (normally distributed continuous data) and the Chi-square test (categorical data). Then, univariate and multivariate logistic regression analyses were applied to assess the associations between *H. pylori* colonization and each separate pregnancy outcome (i.e. PE, PTB, and SGA). A number of cases was diagnosed with more than one of these pregnancy complications. The inclusion of potential confounders was based on earlier literature and/or if they changed the effect estimates with ≥10%. We used three regression models to explore the effect of potential confounders on the association between *H. pylori* and PE, PTB, and SGA. In model 1, we adjusted for maternal age, ethnicity and parity. Model 2 was additionally adjusted for body mass index and smoking during pregnancy. The third model was also adjusted for maternal education level as a proxy for socio-economic status. Lastly, to examine effect modification we evaluated statistical interaction by multiplying the *H. pylori* colonization status with the covariates maternal educational level, parity, smoking, body mass index, and fetal gender. The latter may be involved with sex specific associations regarding placentation (27). If the p-value for interaction was <0.05, a stratified analysis according to the specific covariate was performed. All measures of associations are presented as Odds Ratios (OR) with their 95% Confidence Intervals (CI). Statistical analyses were performed using IBM SPSS Statistics 21.0 for Windows (SPSS IBM, Armonk, New York, USA).

RESULTS

Population characteristics

In total, 6348 women were included in the study. Their baseline characteristics are shown in **Table 1**. Mean age at enrolment was 29.7 years (SD 5.3), and 51.0% were of non-Dutch ethnic background. Overall, the *H. pylori* colonization rate was 45.9%, of whom 35.1% carried CagA-positive strains. Nine-hundred and twenty-seven (14.6%) developed either PE, SGA or PTB. Compared to women without one of the indicator conditions, women with a complicated pregnancy were younger, had attained a lower level of education,

were more often of non-Dutch ethnicity, and were more often pregnant with their first child. (**Table 1**, p<0.001 for univariate comparisons; see **supplementary table 1** for observed and imputed data). In total, 129 (2.0%) women were diagnosed with PE, 638 (10.1%) with SGA, and 219 (3.4%) with PTB (**Figure 1**). Fifty-eight women (0.9%) had more than one of these complications, and contribute to both complication groups.

Table 1 Characteristics of mothers (n = 6348)

	Imputed	Complicated pregnancy[1]		p-value
		No (n=5421)	Yes (n=927)	
Age, years (SD)	29.7 (5.3)	29.9 (5.2)	28.9 (5.6)	<0.001
Body mass index, kg/m^2 (range)	23.7 (15.2-51.2)	23.8 (15.2-51.2)	23.3 (15.8-50.2)	<0.001
Ethnicity (%)				<0.001
Dutch	3031 (47.7)	2656 (49.0)	375 (40.1)	
Turkish	654 (10.3)	546 (10.1)	88 (9.5)	
Surinamese	569 (9.0)	433 (8.0)	143 (15.4)	
Moroccan	458 (7.2)	421 (7.8)	45 (4.9)	
Cape Verdean	302 (4.8)	235 (4.3)	68 (7.3)	
Dutch Antillean	246 (3.9)	190 (3.5)	59 (6.4)	
Other Western	689 (10.9)	605 (11.2)	86 (9.3)	
Other non-Western	399 (6.3)	335 (6.2)	63 (6.8)	
Education level (%)				<0.001
Low/Middle	3807 (60.0)	3178 (58.6)	628 (67.7)	
High	2541 (40.0)	2243 (41.4)	299 (32.3)	
Parity (%)				<0.001
Nulli parity	3503 (55.2)	2865 (52.9)	638 (68.8)	
Multi parity	2845 (44.8)	2556 (47.1)	289 (31.2)	
Smoking during pregnancy (%)				<0.001
No	5136 (80.9)	4462 (82.3)	675 (72.8)	
Yes	1211 (19.1)	959 (17.7)	252 (27.2)	
Children's gender				0.65
Female	3209 (50.5)	2733 (50.4)	475 (51.2)	
Male	3139 (49.5)	2687 (49.6)	452 (48.8)	
H. pylori and CagA				
H. pylori-negative	3433 (54.1)	2979 (55.0)	454 (49.0)	<0.001
H. pylori-positive	2915 (45.9)	2442 (45.0)	473 (51.0)	
CagA-negative	1892 (64.9)	1604 (65.7)	288 (60.9)	<0.001
CagA-positive	1023 (35.1)	838 (34.3)	185 (39.1)	

Values are means (SD), medians (range) or absolute numbers (percentages).
[1]Pregnancy complicated by PE, SGA, or PTB

H. pylori *colonization and PE, SGA, and PTB*

Women with one of these pregnancy complications were more often *H. pylori* positive (51.0%) than women with an uncomplicated pregnancy (45.0%) (p<0.001). Among those women with *H. pylori*, the CagA-positivity rate was higher in those with a complicated pregnancy (39.1% vs. 34.3%, p<0.001) (**Table 1**).

Supplementary table 2 shows the prevalence of PE, SGA, and PTB according to *H. pylori* and CagA-status. Univariate logistic regression analyses showed an increased risk of PE and SGA in *H. pylori*-positive mothers (OR 1.49; 95% CI 1.05-2.12 and OR 1.32; 1.12-1.56, respectively) (**Supplementary table 3**). Parallel results for *H. pylori* and SGA were observed when using an ethnic-specific 10th percentile definition for SGA. Differentiation into CagA-negative and CagA-positive strains revealed a positive association between *Hp*+CagA+ mothers and SGA (OR 1.59; 95% CI 1.28-1.97). No association was observed between *H. pylori* and PTB. Multivariate analyses revealed an association with PE (final OR 1.51; 95%CI 1.03-2.25), but not with SGA or PTB (**Table 2**). Differentiation into CagA-negative or CagA-positive strains showed an association between CagA-positive strains and SGA (final OR 1.34; 95% CI 1.04-1.71). Increased risk of PE was observed in mothers with a CagA-negative strain (Model 2, OR 1.58; 95% CI 1.03-2.40). The association attenuated slightly after additional adjustment for educational level. We did not observe an interaction between *H. pylori* status and fetal gender, maternal educational level, parity, smoking, and body mass index.

When excluding cases with more than one pregnancy complication from each group, multivariate analyses showed only CagA-positivity independently associated with SGA (OR 1.33; 95% CI 1.03-1.72) (**Supplementary table 4**).

Table 2 Associations of *H. pylori* and CagA status with PE, SGA, and PTB.

	Preeclampsia (n = 5550)			Small for gestational age (n = 6059)			Spontaneous preterm birth (n = 5640)		
Model	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Hp-	*Reference* *n = 58 / 3037*	*Reference*	*Reference*	*Reference* *n = 306 / 3285*	*Reference*	*Reference*	*Reference* *n = 113 / 3092*	*Reference*	*Reference*
Hp+	**1.59** **(1.07, 2.35)*** n = 71 / 2513	**1.57** **(1.06, 2.32)***	**1.51** **(1.03, 2.25)***	1.20 (0.99, 1.46) n = 332 / 2774	1.18 (0.97, 1.42)	1.16 (0.96, 1.40)	1.18 (0.87, 1.61) n = 106 / 2548	1.17 (0.86, 1.60)	1.15 (0.84, 1.57)
Hp+CagA-	**1.58** **(1.04, 2.40)*** n = 46 / 1650	**1.58** **(1.03, 2.40)***	1.53 (1.00, 2.33)	1.12 (0.91, 1.38) n = 195 / 1799	1.09 (0.88, 1.34)	1.08 (0.87, 1.33)	1.21 (0.86, 1.68) n = 70 / 1674	1.19 (0.86, 1.67)	1.18 (0.84, 1.64)
Hp+CagA+	1.61 (0.95, 2.73) n = 25 / 863	1.56 (0.92, 2.64)	1.50 (0.89, 2.55)	**1.38** **(1.08, 1.76)*** n = 137 / 975	**1.36** **(1.06, 1.74)***	**1.34** **(1.04, 1.71)***	1.13 (0.73, 1.73) n = 36 / 874	1.11 (0.72, 1.70)	1.08 (0.71, 1.66)

Values are odds ratios for PE, SGA, and PTB (95% confidence interval) from logistic regression models. n = number of cases per total group.
Model 1 was adjusted for maternal age, parity, ethnicity.
Model 2: model 1 additionally adjusted for body mass index and smoking.
Model 3: model 2 additionally adjusted for educational level.
*p<0.05

DISCUSSION

This large population-based prospective cohort study showed that *H. pylori* colonization is associated with an increased risk on PE and that carriage of a CagA+ *H. pylori* strain is a risk factor for SGA. These findings may be helpful for a better understanding of the pathogenesis of these pregnancy complications and support the link with chronic inflammatory conditions. The potential association between *H. pylori* and these gestational disorders has been studied before. Previous studies however were considerably smaller, thereby limiting power. Furthermore, not all studies included separate analyses of CagA data.

The observed association between *H. pylori* and PE is consistent with prior epidemiological studies (11-14). In a small Italian study of 47 PE cases and 47 controls, Ponzetto et al. found a higher *H. pylori* seropositivity in mothers with PE (51.1%) compared to women with an uncomplicated pregnancy (31.9%) (OR 2.67; 95% CI 1.08-6.57) (11). In contrast to our observation, they observed greater differences between those having CagA-positive and CagA-negative strains (80.9% vs. 14.9%). Another study from the same group investigated the association of several *H. pylori* virulence factors with SGA (SGA, n = 13), PE (n = 17), and both (PE and SGA, n = 32) compared with controls (n = 49) (12). In PE women with or without SGA, the *H. pylori*-positivity rate was higher while there was no difference in prevalence between SGA-only and controls. They observed that CagA-strains were more prevalent in PE pregnant women compared to controls, while there was no difference between SGA cases and controls. An Australian study determined the association between *H. pylori* colonization and SGA in 448 pregnant women (15), of whom 34 (7.5%) had SGA. Multivariate analysis revealed that *H. pylori* seropositivity was associated with SGA (OR 2.59; 95% CI 1.12-5.95; p = 0.025). A similar trend was observed in our study, but only for those with CagA-positive strains. CagA status data were not available in the prior study. We found no association between *H. pylori* and PTB, regardless of CagA-status. One other study, assessing this relation in 416 pregnant women, did not observe a significant association between *H. pylori* colonization and PTB (28).

The overall *H. pylori* prevalence in this cohort may be higher than for other populations in a Western country. This difference is mainly explained by the high colonization rates in women with a non-European ethnic background. Studies evaluating the *H. pylori* colonization in multi-ethnic populations all showed higher prevalence among immigrant groups, compared to the original population (29, 30). Although PE, SGA, and PTB are different clinical entities, all three may be caused by suboptimal deep placentation in early pregnancy (5) (31, 32) (33, 34). Large numbers of non-transformed spiral arteries are frequently observed in PE patients with or without SGA (5), in patients with SGA

without gestational hypertension (31, 32), and in patients with preterm labor with or without preterm pre-labor rupture of membranes (PROM) (33, 34). Impaired remodeling of the spiral arteries may lead to insufficient uteroplacental arterial flow and episodes of irregular placental perfusion (3). Such impaired remodeling might be due to failure of appropriate uterine preconditioning (35), and excessive or atypical maternal immune responses to trophoblasts (36). Franceschi et al. have shown that anti-CagA antibodies were able to recognize β-actin on the surface of trophoblast cells in a dose-dependent binding assay *in vitro* (6). This binding resulted in impaired cytotrophoblast invasiveness, which is characteristic for the development of the placental syndrome; however, we observed no association between CagA-positive strains and PE. The association between *H. pylori* and PE disappeared when excluding women with more than one of the studied pregnancy complications (i.e. with both PE and SGA), which suggests that the significant result was based on those cases. PTB is a syndrome caused by multiple pathologic processes with placental involvement as one of the possibilities (37). In our study, although all spontaneous in onset, we were not able to distinguish between different causes of PTB. Our data add further evidence to the associations between *H. pylori* and PE and SGA. Although association does not imply causation, epidemiological findings should stimulate biological studies. If the association between *H. pylori* and these illnesses is causal, eradication of *H. pylori* may be part of an effective intervention for reducing related perinatal morbidity and mortality. As the overall *H. pylori* prevalence in Western countries is declining, screening for *H. pylori* may be most efficient in pregnant women with increased *H. pylori* prevalence, like a low socioeconomic status, or a non-Western ethnic background (21).

The strengths of this study include the large number of subjects participating in a population-based cohort, with detailed prospectively collected data on socioeconomic, and sociodemographic characteristics, together with other potential confounding factors. However, it cannot be excluded that the findings may partly result from unmeasured confounding. Our study may be limited by the fact that we measured IgG antibodies against *H. pylori* and CagA, indicating present or recent colonization. However, it is not clear whether any effect of *H. pylori* is dependent on active colonization or the presence of circulating anti-*H. pylori* and CagA antibodies. The use of one general definition for SGA in all ethnic groups may limit our results, as a prior study has shown differences in birth weights between ethnic populations of this cohort (38). However, additional analyses, using the ethnic-specific 10th percentile revealed parallel results for *H. pylori* and SGA. Since validated ethnic-specific growth curves are lacking, we continued to use the population-specific 10th percentile. Although this study is population-based, selective participation occurred since participating women were generally higher educated, and were more often from Dutch ethnic background (17). Missing data for several

characteristics and potential confounders may have biased the outcome. Therefore, we performed the final analyses after a multiple imputation procedure. This is considered useful to deal with missing data, since it requires the fewest assumptions and reduces potential bias when missing data are not random (26).
In summary, *H. pylori* colonization is positively associated with PE. In addition, we confirmed the important role of CagA-positive strains in SGA, as the association was determined by colonization with these strains.

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SUPPLEMENTARY MATERIAL

Helicobacter pylori colonization and pregnancy complications: the Generation R study

Table S1. Characteristics of mothers – observed and imputed (n = 6348)

	Observed	Imputed
Age, years (SD)	29.7 (5.3)	29.7 (5.3)
Body mass index, kg/m^2 (range)	23.7 (15.2-51.2)	23.7 (15.2-51.2)
Data missing	*41 (0.6)*	
Ethnicity (%)		
Dutch	2947 (49.6)	3031 (47.7)
Turkish	558 (9.4)	654 (10.3)
Surinamese	531 (8.9)	569 (9.0)
Moroccan	389 (6.6)	458 (7.2)
Cape Verdean	260 (4.4)	302 (4.8)
Dutch Antillean	212 (3.6)	246 (3.9)
Other Western	669 (11.3)	689 (10.9)
Other non-Western	370 (6.2)	399 (6.3)
Data Missing	*412 (6.5)*	
Education level (%)		
Low/Middle	3392 (58.1)	3807 (60.0)
High	2444 (41.9)	2541 (40.0)
Data missing	*512 (8.1)*	
Parity (%)		
Nulli parity	3488 (55.4)	3503 (55.2)
Multi parity	2808 (44.6)	2845 (44.8)
Data missing	*52 (0.8)*	
Smoking during pregnancy (%)		
No	4525 (80.9)	5136 (80.9)
Yes	1066 (19.1)	1211 (19.1)
Data missing	*757 (11.9)*	
Children's gender		
Female	3208 (50.5)	3209 (50.5)
Male	3139 (49.5)	3139 (49.5)
Data missing	*1 (0.0)*	
H. pylori and CagA		
H. pylori-negative	3433 (54.1)	3433 (54.1)
H. pylori-positive	2915 (45.9)	2915 (45.9)
CagA-negative	1892 (64.9)	1892 (64.9)
CagA-positive	1023 (35.1)	1023 (35.1)

Values are means (SD), medians (range) or absolute numbers (percentages).

Table S2. Prevalences of PE, SGA, and PTB according to *H. pylori* and corresponding CagA status

p = 0.08	**PE (n= 5550)**	
	No (n=5421)	**Yes** (n=129)
Hp negative (%) (n=3037)	98.1 (2979)	1.9 (58)
Hp positive CagA negative (n=1650)	97.2 (1604)	2.8 (46)
CagA positive (n=863)	97.1 (838)	2.9 (25)

p<0.001	**SGA (n= 6059)**	
	No (n=5421)	**Yes** (n=638)
Hp negative (%) (n=3285)	90.7 (2979)	9.3 (306)
Hp positive CagA negative (n=1799)	89.2 (1604)	10.8 (195)
CagA positive (n=975)	85.9 (838)	14.1 (137)

P=0.62	**PTB (n= 5640)**	
	No (n=5421)	**Yes** (n=219)
Hp negative (%) (n=3092)	96.3 (2979)	3.7 (113)
Hp positive CagA negative (n=1674)	95.8 (1604)	4.2 (70)
CagA positive (n=874)	95.9 (838)	4.1 (36)

Table S3. Univariate analyses of *H. pylori* and CagA status with PE, SGA, and PTB.

	Preeclampsia (n = 5550)	Small for gestational age (n = 6059)	Spontaneous preterm birth (n = 5640)
Hp-	*Reference* *n = 58 / 3037*	*Reference* *n = 306 / 3285*	*Reference* *n = 113 / 3092*
Hp+	**1.49** **(1.05, 2.12)*** n = 71 / 2513	**1.32** **(1.12, 1.56)**** n = 332 / 2774	1.14 (0.87, 1.50) n = 106 / 2548
Hp+CagA-	1.47 (1.00, 2.18) n = 46 / 1650	1.18 (0.98, 1.43) n = 195 / 1799	1.15 (0.85, 1.56) n = 70 / 1674
Hp+CagA+	1.53 (0.95, 2.46) n = 25 / 863	**1.59** **(1.28, 1.97)**** n = 137 / 975	1.13 (0.77, 1.66) n = 36 / 874

Values are odds ratios for PE, SGA, and PTB (95% confidence interval) from logistic regression models. n = number of cases per total group.
*p<0.05 **p<0.01

Table S4. Associations of *H. pylori* and CagA status with PE, SGA, and PTB

	PE	SGA	PTB
	Odds Ratio (95% CI)	**Odds Ratio (95% CI)**	**Odds Ratio (95% CI)**
	n = 5508	n = 6005	n = 5619
Hp-	*Reference* *n = 42 / 3021*	*Reference* *n = 284 / 3263*	*Reference* *n = 105 / 3084*
Hp+	1.35 (0.84, 2.17) n = 45 / 2487	1.14 (0.93, 1.38) n = 300 / 2742	1.10 (0.79, 1.53) n = 93 / 2535
Hp+CagA-	1.33 (0.80, 2.23) n = 29 / 1633	1.05 (0.84, 1.30) n = 175 / 1779	1.13 (0.79, 1.60) n = 62 / 1666
Hp+CagA+	1.38 (0.73, 2.63) n = 16 / 854	**1.33** **(1.03, 1.72)*** n = 125 / 963	1.03 (0.65, 1.63) n = 31 / 869

Values are odds ratios for pre-eclampsia (PE), small for gestational age (SGA), or preterm birth (PTB) (95% confidence interval) from logistic regression models.
Cases diagnosed with more than one pregnancy outcome were excluded.
n = number of cases per total group.
Adjusted for maternal age, parity, ethnicity, body mass index, smoking, and educational level.

CHAPTER 6

Helicobacter pylori in children with asthmatic conditions at school age, and their mothers

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ABSTRACT

Background: *Helicobacter pylori (H. pylori)* prevalence in Western countries has been declining simultaneously with increases in childhood asthma and allergic diseases; prior studies have linked these phenomena.

Aims: We aimed to examine the association between *H. pylori* colonization in children and risk of asthma and related conditions at school age. We secondly examined additional effects of maternal *H. pylori* status by pairing with children's status.

Methods: This study was embedded in a multi-ethnic population-based cohort in Rotterdam, The Netherlands. We measured anti-*H. pylori* and anti-CagA antibodies in serum of children obtained at age 6 years, and of their mothers obtained during mid-pregnancy. Asthma or related conditions were reported for children at age 6 years. We used multivariate logistic regression analyses among 3,797 subjects.

Results: In children, the *H. pylori* positivity rate was 8.7%, and 29.2% of these were CagA-positive. A child's colonization with a CagA-negative-*H. pylori* strain was associated with an increased risk of asthma (Odds ratio 2.11; 95% CI 1.23-3.60, but this differed for European (3.64; 1.97-6.73) and non-European (0.52; 0.14-1.89) children. When taking into account maternal *H. pylori* status, only *H. pylori* positive children with an *H. pylori* negative mother had increased risk of asthma (2.42; 1.11-5.27), accounting for 3.4% of the asthma risk.

Conclusions: Colonization of a European child with a CagA-negative-*H. pylori* strain at age 6 was associated with an increased prevalence of asthma, but there was no association for non-European children. The underlying mechanisms for the observed risk differences require further research.

INTRODUCTION

Reduced exposure to exogenous microbes and their products have been suggested to be involved in the pathogenesis of asthma and related conditions, such as eczema and allergies (1). An alternate hypothesis is that the epidemic rise in asthma and related conditions may be partially explained by altered composition of our indigenous microbiota due to changes in human ecology (2). The gastric bacterium *Helicobacter pylori (H. pylori)* has been used as a proxy for this modern phenomenon (2). Studies in mice have demonstrated that experimental infection with *H. pylori* prevents allergic asthma through the induction of regulatory T cells (Tregs) (3). Direct contact between *H. pylori* and dendritic cells (DCs) was found to be essential for the induction of tolerogenic DCs. These DCs produce IL-18, important for the conversion of naïve T-cells into Tregs (3). Eventually, these Tregs may suppress asthmatic immune responses in the airways (4). This interaction between *H. pylori* and the immune system may be affected by its genotype: expression of the *cagA* gene *(*cytotoxin-associated gene A) has been associated with a more marked cellular and humoral immune response, with persists throughout human life (5).

During recent decades the prevalence of *H. pylori* colonization has dropped dramatically in Western countries (6). Currently, fewer than 10% of children born in Western countries are *H. pylori* positive (7) (8). Several epidemiologic studies examined the relation between *H. pylori* colonization and asthma and asthma-related conditions in childhood (9-14). However, results from these studies appear contradictory. This may have been due to differences in study design (9, 10, 14), low number of participants (9, 10, 13), differing methods and timing of *H. pylori* status identification (11, 14), and insufficient accounting for potential confounders (12, 14). It also is unclear whether maternal *H. pylori* colonization during pregnancy affects the child's risk for asthma and related conditions. Maternal *H. pylori* colonization during pregnancy may be a risk factor for foetal growth retardation (15, 16), which subsequently might lead to increased risk for asthma (17). Children of an *H. pylori*-positive mother may be more likely to acquire colonization at younger age, but it is not known whether this also may affect risk of asthma and related conditions.

Therefore, we first examined the associations of asthma and related conditions with *H. pylori* colonization in children, and secondly we focused on the effect of paired maternal and child's *H. pylori* status on these outcomes. These analyses were facilitated by a large multi-ethnic population-based prospective cohort study in Rotterdam, The Netherlands.

METHODS

Design and setting

This study was embedded in the Generation R Study, a population-based prospective cohort study of pregnant women and their children in Rotterdam, The Netherlands (18). All children were born between April 2002 and January 2006. The Medical Ethics Committee of the Erasmus University Medical Centre approved the study protocol and parents gave written informed consent for themselves and their children. For the current study, data from 3,797 children with information on *H. pylori* colonization and any asthma or related conditions were available (**Figure 1**).

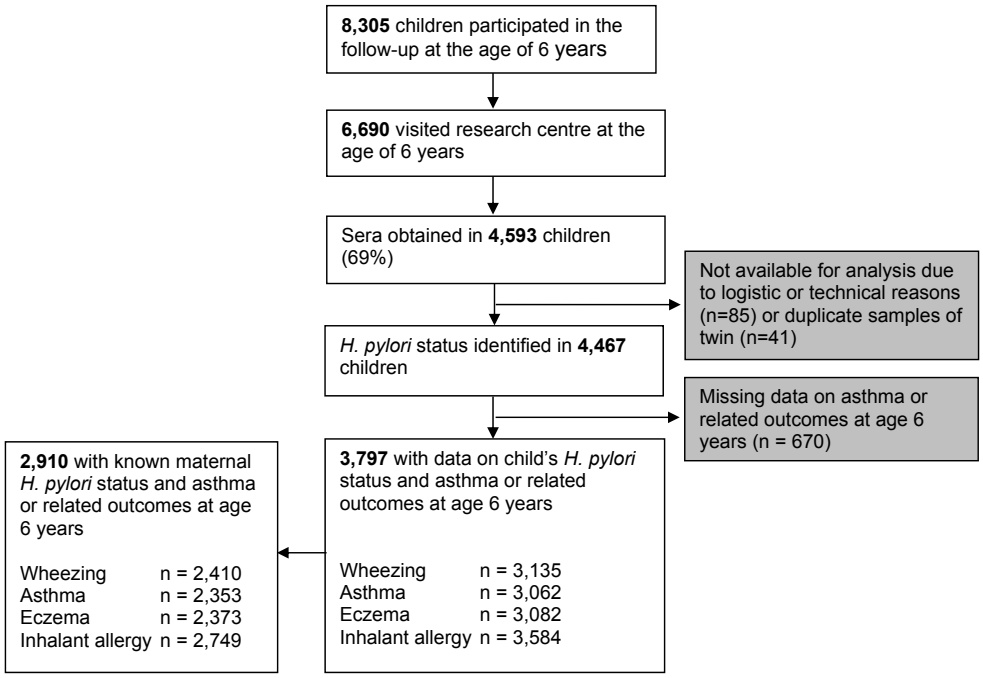

Figure 1. Study design and number of participants

H. pylori *colonization in children and their mothers*

H. pylori colonization of children was defined by measuring IgG antibody levels in serum using an enzyme-linked immunosorbent assay (ELISA) (19). Sera samples were obtained at age 6 years. A separate ELISA was performed to determine serum IgG antibodies against a specific recombinant truncated cytotoxin-associated gene A (CagA) protein (20). Both ELISAs were validated locally, by adapting the ELISA properties based on positive and negative controls. *H. pylori* and CagA colonization in mothers was measured

from serum samples obtained during mid-pregnancy (gestational age 18-25 weeks) (21). Similar ELISAs were used for mothers and children, with specific properties known from use in previous birth cohorts (19, 22).

Asthma and related conditions at school age

Wheezing in the prior 12 months (no, yes), physician-diagnosed asthma ever (no, yes) and physician-diagnosed eczema in the last 12 months (no, yes) were assessed using questions adapted from the International Study on Asthma and Allergy in Childhood (ISAAC) core questionnaires at age 6 years (23). Inhalant allergy was assessed by questionnaire at age 6 years; a positive history was defined as inhalant allergy to pollens, mites, or pets in the prior 12 months (no, yes), diagnosed by a physician.

Covariates

Information on birth weight, gestational age, and sex of the children were obtained from midwife and hospital registries at birth. Postnatal questionnaires at ages of 6 and 12 months supplied information on breastfeeding, and at age of 6 years about lower respiratory tract infections during the prior 12 months. Use of antibiotics in the prior 12 months was assessed by questionnaires yearly at the ages of 1 to 6 years. Information on maternal age, anthropometrics, ethnicity, socio-economic status, history of asthma or atopy, parity, and pet keeping were obtained by questionnaire, completed by the mother at enrolment. Socio-economic status was assessed using the educational level of mother on the basis of her highest level of completed education. Smoking during pregnancy was reported at enrolment. Maternal psychological distress in the second trimester of pregnancy was defined using a global severity index (GSI) (24).

Statistical analyses

The prevalence of asthma and related conditions in relation to child's *H. pylori* and CagA status were examined using Chi-square tests. We used multivariate logistic regression analysis to examine the association of child's *H. pylori* and CagA status with asthma and related conditions, taking potential confounders into account. Missing data in the covariates were imputed with multiple imputations using chained equations (25).

If available, a child's *H. pylori* status was paired with maternal *H. pylori* status, resulting in four different groups: mother and child both *H. pylori* negative, mother *H. pylori* negative and child positive, mother *H. pylori* positive and child negative, both mother and child *H. pylori* positive. We used multivariate logistic regression analysis to examine the association of combined *H, pylori* status of mother and child, with asthma and related conditions. Potential confounders were taken into account. Due to the small numbers of cases we were not able to differentiate with respect to CagA-status. We calculated the

population attributable fraction of *H. pylori* colonization for asthma, using adjusted ORs estimated from logistic regression models (26).
All measures of associations are presented as Odds Ratios (OR) with their 95% Confidence Intervals (CI). Statistical analyses were performed using SPSS version 23.0 for Windows (SPSS Inc., Chicago, IL, USA). An extensive description of the methods is provided in the Supplementary material.

RESULTS

Population characteristics. Characteristics of included children and their mothers are provided in **Table 1** and **Table S1** shows the comparison of the included (n = 3,797) with the excluded (n = 4,508) subjects. At a mean age of 6.1 years (SD 0.5), 8.7% of the children were *H. pylori*-positive. *H. pylori* status was determined in their mothers at a mean age of 31.1 years (SD 4.9), and 38.0% tested positive. The proportion of CagA-positive strains among *H. pylori*-positive mothers and children was 33.0% and 29.2%, respectively. As expected, the colonization rate of children with an *H. pylori*-positive mother was higher than in children with an *H. pylori*-negative mother (14.3% *vs.* 5.4%, p<0.05).

Table 1 Characteristics of the study population

	Population for analysis (n = 3,797)	
	Observed	**Imputed**
Sex (%)		
Female	48.1 (1,828)	48.1 (1,828)
Male	51.9 (1,969)	51.9 (1,969)
Gestational age at birth (weeks)	40.1 (35.7-42.3)	40.1 (35.7-42.3)
Missing	*0.8 (29)*	-
Birth weight (grams)	3,446 (550)	3,445 (551)
Missing	*0.1 (4)*	-
Ethnicity[1] (%)		
European	65.8 (2,492)	65.6 (2,492)
Non-European	34.2 (1,294)	34.4 (1,305)
Missing	*0.3 (11)*	-
Breastfeeding (%)		
Never	9.0 (230)	12.9 (491)
Ever	91.0 (2,331)	87.1 (3,306)
Missing	*32.6 (1,236)*	-
Pet keeping (%)		
No	66.3 (1,977)	64.8 (2,460)
Yes	33.7 (1,007)	35.2 (1,337)
Missing	*21.4 (813)*	-

Lower respiratory tract infections at 6 years (%)		
No	95.5 (3,458)	95.2 (3,616)
Yes	4.5 (162)	4.8 (181)
Missing	*4.7 (177)*	-
Antibiotic use (%)		
Never	17.6 (508)	17.3 (657)
For 1-2 time periods	55.5 (1,599)	36.3 (1,378)
For 3 or more time periods	26.8 (772)	46.4 (1,762)
Missing	*24.2 (918)*	
Maternal education level (%)		
Primary, or secondary	48.8 (1,723)	50.6 (1,922)
Higher	51.2 (1,811)	49.4 (1,875)
Missing	*6.9 (263)*	-
Maternal age[2] (years)	31.1 (4.9)	31.1 (4.9)
Maternal body mass index[2] (kg/m2)	23.7 (18.9-35.5)	23.8 (18.7-35.2)
Missing	*10.5 (398)*	-
Maternal history of asthma or atopy (%)		
No	61.7 (1,904)	61.8 (2,346)
Yes	38.3 (1,183)	38.2 (1,451)
Missing	*18.7 (710)*	-
Parity[2] (%)		
0	56.2 (2,057)	55.3 (2,100)
≥1	43.8 (1,601)	44.7 (1,697)
Missing	*3.7 (139)*	-
Smoking during pregnancy (%)		
No	85.4 (2,859)	85.1 (3,235)
Yes	14.6 (490)	14.9 (562)
Missing	*11.8 (448)*	-
Psychological distress during pregnancy (%)		
No	92.4 (2,646)	91.0 (3,455)
Yes	7.6 (218)	9.0 (342)
Missing	*24.6 (933)*	-
Children's *H. pylori* colonization rate (%)		
Hp-	91.3 (3,465)	-
Hp+CagA-	6.2 (235)	
Hp+CagA+	2.6 (97)	
Maternal *H. pylori* colonization rate[3] (%)		
Hp-	62.0 (1,804)	-
Hp+CagA-	25.5 (741)	
Hp+CagA+	12.5 (365)	
Missing	*23.4 (887)*	
Paired maternal and child's *H. pylori* status (%)		
mHp-cHp-	58.6 (1,706)	-
mHp+cHp-	32.6 (948)	
mHp-cHp+	3.4 (98)	
mHp+cHp+	5.4 (158)	
Missing	*23.4 (887)*	

Values are means (SD), medians (2.5-97.5th percentile) or percentages (absolute numbers).
[1]Ethnic background is based on data of mother
[2]Data measured at moment of enrolment of pregnant mother
[3]Data on *H. pylori* colonization is not imputed

Child's* H. pylori *colonization and asthma and related conditions

We observed a higher prevalence of recent asthma, but not of wheezing, eczema, or inhalant allergy, in *H. pylori*-positive compared with *H. pylori*-negative children (asthma prevalence 9.5% *vs.* 5.2% respectively, p=0.045) (**Figure 2**, **Supplementary Table 2**). Multivariate analyses showed an association between *H. pylori* and increased risk of asthma (OR 1.75; 95% CI 1.07-2.87) (**Table 2**). Compared with *H. pylori*-negative children, those colonized with a CagA-negative strain had an increased risk of asthma (OR 2.11; 95% CI 1.23-3.60), but those colonized with a CagA-positive strains were not (OR 0.94; 95% CI 0.32-2.79). The size of the effect estimates did not change after additional adjustment for maternal *H. pylori* status. Carriage of an $Hp^{+}CagA^{+}$ strain tended to be inversely related to wheezing and inhalant allergy, but effects were not significant (OR 0.24; 95% CI 0.05-1.05 for wheezing, OR 0.26; 95%CI 0.06-1.07 for inhalant allergy).

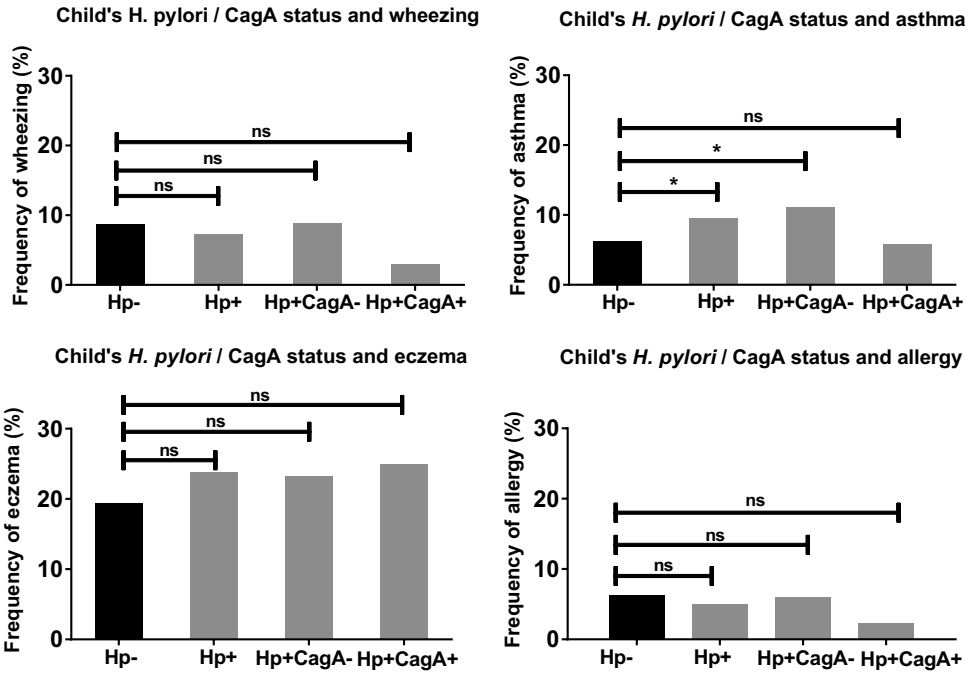

Figure 2 Prevalence of wheezing, asthma, eczema, and inhalant allergy in 6-year old children, according to *H. pylori* and corresponding CagA status of children.
The y-axis reflects the proportion of children with asthma or related outcome at age 6 years. *H. pylori* status is shown on the x-axis, divided by CagA- and CagA+ strains. The reference group consists of *H. pylori*-negative children.
Values are percentages. Mean proportions are compared using Chi-square tests (ns = non-significant).
* p<0.05

Table 2. Associations of children's *H. pylori* and corresponding CagA status with asthma and asthma-related outcomes at age 6 years.

		Wheezing	Asthma	Eczema	Inhalant allergy
		Odds Ratio (95% CI)	**Odds Ratio (95% CI)**	**Odds Ratio (95% CI)**	**Odds Ratio (95% CI)**
		n = 3,135	n = 3,062	n = 3,082	n = 3,584
Hp-		*Reference* *n = 251 / 2,886*	*Reference* *n = 175 / 2,820*	*Reference* *n = 551 / 2,834*	*Reference* *n = 206 / 3,282*
Hp+		0.73 (0.43, 1.27) n = 18 / 249	**1.75 (1.07, 2.87)*** n = 23 / 242	1.04 (0.75, 1.43) n = 59 / 248	0.69 (0.39, 1.20) n = 15 / 302
	Hp+CagA-	0.98 (0.55, 1.75) n = 16 / 181	**2.11 (1.23, 3.60)**** n = 19 / 173	1.12 (0.77, 1.61) n = 42 / 180	0.91 (0.50, 1.66) n = 13 / 216
	Hp+CagA+	0.24 (0.05, 1.05) n = 2 / 68	0.94 (0.32, 2.79) n = 4 / 69	0.87 (0.48, 1.55) n = 17 / 68	0.26 (0.06, 1.07) n = 2 / 86

Values are odds ratios for wheezing, asthma, eczema, and inhalant allergy (95% confidence interval) from logistic regression models.
Models were adjusted for maternal age, ethnicity, pre-pregnancy body mass index, educational level, history of asthma or atopy, parity, pet keeping, smoking during pregnancy, psychological stress during pregnancy, and child's birth weight, gestational age at birth, sex, breastfeeding, lower respiratory tract infections, and antibiotic use.
[1]n = number of cases (i.e. children with asthma or related conditions) per total group (i.e. according to *H. pylori*/CagA status)
*p < 0.05, **p<0.01.

Stratification for ethnicity showed that the association of *H. pylori* colonization with asthma was only present in children of European background (OR 3.11; 95% CI 1.70-5.72), and not in those of non-European background (OR 0.72; 95% CI 0.29-1.74), with a p-value for interaction of 0.009 **(Table 3**, **Supplementary Table 4**). Consistent trends were observed for wheezing, eczema, and inhalant allergy, however, the interaction terms were non-significant (**Supplementary Table 4**). Other tests for interactions were non-significant (p-values >0.05).

Table 3. Associations of child's *H. pylori* status with asthma stratified by ethnicity.

	Asthma Odds Ratio (95% CI)		**Asthma** Odds Ratio (95% CI)
European	n = 2,140	**Non-European**	n = 922
Hp-	*Reference* *n = 111 / 2,026*[1]	*Hp-*	*Reference* *n = 65 / 794*
Hp+	**3.11** **(1.70, 5.72)*** n = 16 / 114	*Hp+*	0.72 (0.29, 1.74) n = 7 / 128
Hp+CagA-	**3.64** **(1.97, 6.73)*** n = 16 / 100	*Hp*+CagA-	0.52 (0.14, 1.89) n = 3 / 73
Hp+CagA+	NA n = 0 / 14	*Hp*+CagA+	0.98 (0.31, 3.11) n = 4 / 55

Values are odds ratios for asthma (95% confidence interval) from logistic regression models.
Models were adjusted for maternal age, pre-pregnancy body mass index, educational level, history of asthma or atopy, parity, psychological stress during pregnancy, and child's sex, gestational age at birth, birth weight, breastfeeding, pet keeping, lower respiratory tract infections, and antibiotic use.
Overall $P_{Interaction}$ ethnicity * *H. pylori:* <0.05.
[1]n = number of cases (i.e. children with asthma) per total group (i.e. according to *H. pylori*/CagA status
*p < 0.01, NA = not applicable.

Paired mother-child* H. pylori *status and asthma and related conditions in children

In 2,910 (77%) of the children, the *H. pylori* status of their mother was known. We observed no differences in prevalence of wheezing, asthma, eczema and inhalant allergy of the children according to the combined *H. pylori* status of mother and child **Supplemental table S5**) (p-values>0.05). Multivariate analysis revealed a positive association with asthma in children who were *H. pylori* positive, but with a negative mother (OR 2.42; 95% CI 1.11- 5.27) (**Table 4**). The proportion of asthma attributable to *H. pylori*-positivity in children with an *H. pylori*-negative mother was 3.4% (95% CI 0.6-4.7) Stratification for ethnicity showed that the association with asthma was only present in children of European background, both for *H. pylori* positive children with a *H. pylori* negative or positive mother (OR 3.25; 95% CI 1.41-7.54 for *mHp-cHp+*, and OR 4.07; 95% CI 1.47-11.31 for *mHp+cHp+*) (**Supplementary Table 6**). Interactions of paired *H. pylori* status with maternal asthma or atopy, and child's sex, gestational age or weight at birth, or antibiotic use were not associated with asthma or related conditions (p-values for interaction >0.05).

Table 4. Associations of combined maternal and children's *H. pylori* status with asthma and related outcomes at the child's age of 6 years.

	Wheezing	**Asthma**	**Eczema**	**Inhalant allergy**
	Odds Ratio (95% CI)	**Odds Ratio (95% CI)**	**Odds Ratio (95% CI)**	**Odds Ratio (95% CI)**
	n = 2,410	n = 2,353	n = 2,373	n = 2,749
mHp-cHp-	*Reference* *n = 122 / 1,476*[1]	*Reference* *n = 85 / 1,441*	*Reference* *n = 268 / 1,451*	*Reference* *n = 97 / 1,626*
mHp+cHp-	0.99 (0.68, 1.43) n = 68 / 740	1.11 (0.73, 1.68) n = 50 / 726	0.85 (0.66, 1.09) n = 163 / 731	0.86 (0.59, 1.24) n = 65 / 888
mHp-cHp+	0.55 (0.18, 1.63) n = 4 / 82	**2.42 (1.11, 5.27)*** n = 9 / 79	1.04 (0.59, 1.84) n = 17 / 82	0.55 (0.17, 1.79) n = 3 / 94
mHp+cHp+	0.91 (0.43, 1.93) n = 11 / 112	1.76 (0.81, 3.79) n = 10 / 107	0.89 (0.55, 1.45) n = 28 / 109	0.62 (0.28, 1.38) n = 8 / 141

Values are odds ratios for wheezing, asthma, eczema, and inhalant allergy (95% confidence interval) from logistic regression models. Models were adjusted for maternal age, ethnicity, pre-pregnancy body mass index, educational level, history of asthma or atopy, parity, pet keeping, smoking during pregnancy, psychological stress during pregnancy, and child's birth weight, gestational age at birth, sex, breastfeeding, lower respiratory tract infections, and antibiotic use.
[1]n = number of cases (i.e. children with asthma or related conditions) per total group (i.e. according to *H. pylori* status). *p < 0.05

DISCUSSION

In this large population-based prospective cohort, we observed that *H. pylori* colonization in children was associated with an increased risk of asthma at the age of 6 years. Differentiation of *H. pylori*-positivity into CagA-negative or CagA-positive strains showed that the effects were explained by CagA-negative-*H. pylori* strains only. This association of CagA-negative *H. pylori* colonization with asthma was present in children of European ethnic background, and not in those of non-European ethnic background. In non-stratified analysis, maternal *H. pylori* colonization seems to have a protective effect on asthma in their children, as an increased risk of asthma was only found in *H. pylori*-positive children with an *H. pylori*-negative mother. Although the relative risk was higher, the attributable risk for this association only explained 3.4% of the asthma in this population.

Comparison with previous studies

The positive association between *H. pylori* and physician-diagnosed asthma is in contrast with most prior studies in adults and children (9-14, 27, 28). A meta-analysis of pooled

data (n= 34,018 subjects) from these studies showed an inverse association between *H. pylori* and asthma in children (OR 0.81; 95% CI 0.72-0.91) (29). However, the pooled OR of included (birth) cohort studies was non-significant (10, 11, 13) (OR 0.82; 95% CI 0.53-1.27) (29). Two of these studies had comparable study designs, but smaller sample size; the Dutch study included 575 children between 7 years and 9 years old, and showed an OR of 0.87 (95% CI 0.37-2.08) for the relation between *H. pylori* and physician-diagnosed asthma (13). The second study, from Ethiopia, assessed the association with current *H. pylori* colonization at age 3 years and allergic disease in a birth cohort of 878 children (11). Due to low asthma prevalence, calculations could not be performed associating *H. pylori* and asthma; however, for *H. pylori* and wheezing, the outcome was a trend towards an inverse association, which is consistent with our study. A second evaluation within the cohort from Ethiopia, revealed an inverse association between *H. pylori* and eczema in children at the age of 5 years, but not for wheezing and rhinitis (30). The contrast with other studies implies that so far no universal conclusion can be made on the relation between *H. pylori* and asthma.

In contrast to asthma outcome, the observed tendency of inverse associations of *H. pylori* status with wheezing and inhalant allergy were consistent with prior studies, and also consistent with the hypothesis that the endemic rise in asthma and allergy may be causally related to compositional changes in our indigenous microbiota (31), probably as a result of a modern lifestyle (2). Since *H. pylori* colonization persists for life, it may be considered as a model to examine the effects of once-common microorganisms on the development of asthma and related disease.

Interpretation of results

Considering this paradigm and the data from prior studies, our finding of CagA-negative *H. pylori* colonization in 6-year old children of European origin as a risk factor for asthma is notable, and requires further explanation. First, the positive association between *H. pylori* and asthma was explained by CagA-negative-strains in children with a European ethnic background only. In this group, none of the asthmatic children were colonized with a CagA-positive strain. This suggests that the asthma risk may be lower in CagA-positive children compared with *H. pylori*-negatives. The low number of cases in this group reflects the overall low *H. pylori*-prevalence in children of European ethnic background, and therefore limits our conclusions based on these findings. Due to lack of CagA-positives in asthmatic children in this subgroup, the overall detected effect of *H. pylori*-positivity might be skewed to an increased risk for asthma. It has been shown that CagA-positive strains have a stronger interaction with their hosts, leading to pronounced immune responses (5). Prior studies have observed more pronounced effects of CagA-positivity in lowering the risk on asthma and wheezing (32). Accordingly, we found

larger effects towards an inverse trend for CagA-positive strains than for CagA-negative strains in relation to all outcomes examined. Second, although an association between *H. pylori* and asthma was only present in children of European ethnic background, consistent trends were observed for wheezing, eczema, and inhalant allergy, either for CagA-negative or CagA-positive strains. Such differences may reflect variation of the gut microbiome by ethnicity, in both richness and composition. Children (33) and adults living in developing countries have significantly greater faecal diversity than those in developed countries (34). Among subjects differing in ethnic background, but migrating to the same country, gut microbiome composition also varies (35). In animal models, there is growing evidence that *H. pylori* colonization not only affects the gastric microbiome (36), but also the composition of the lower gastro-intestinal tract (37). In addition, the composition of the gut microbiome itself also may modulate the development of *H. pylori*-associated disease (38). Within this context, we speculate that the effects of *H. pylori* on asthma or related disease may depend on both the richness and composition of the gut microbiome. Depending on its composition, it might sometimes promote and other times mitigate *H. pylori* disease (39). Taken together, these observations from the literature might explain the differences between ethnic groups in the present study. Third, any potential protective effect of *H. pylori* on asthma may differ for allergic asthma and non-allergic asthma. However, our questionnaires did not distinguish between allergic and non-allergic asthma, and hence we cannot explore this issue further. Fourth, the effect estimates for asthma were in the opposite direction compared with wheezing and inhalant allergy, which are usually closely related with allergic asthma. This can be explained by the difference in definition between asthma, and wheezing and inhalant allergy used in this study. Asthma was diagnosed as having ever occurred during the age period of 1 to 6 years, while data on wheezing and inhalant allergy referred to complaints and diagnosis during the last 12 months at the age of 6 years. Ever asthma could reflect various phenotypes including early wheezing, mostly induced by respiratory tract infections and transient, and multitrigger wheezing. Current wheezing at age 6 years most likely reflects multitrigger wheezing only. The difference in definition means that *H. pylori* acquisition could have occurred before or after the first asthma period, while for wheezing and inhalant allergy it is more likely that *H. pylori* acquisition may have preceded these outcomes.

In the unstratified paired group, we observed no increased risk of asthma in *H. pylori*-positive children with an *H. pylori*-positive mother (**Table 4**). This finding suggests that maternal *H. pylori* carriage affects the risk of asthma, possibly by facilitating early *H. pylori* colonization in their children. Maternal IgG antibodies against *H. pylori* can be transferred to the child via the placenta, but seem not to protect the child against colonization (40). Direct contact between mother and child is most intense during the

first years of life, which may result in early acquisition of *H. pylori* by a child. Children with an *H. pylori*-positive mother were more likely to become colonized with *H. pylori* themselves, confirming a recent analysis of an overlapping subset of mothers and children (41). Whether *H. pylori*-positive children with an *H. pylori*-negative mother received the infection from other family members or children at day care is not known. Moreover, in stratified analysis according to ethnicity, the increased risk of asthma was the same both for European children with an *H. pylori*-negative and positive mother.

Strengths and limitations

The strengths of this study in comparison to prior studies include the large number of subjects within a birth cohort of children within the same age period, all living in one urban region with detailed prospectively collected information on socioeconomic characteristics, and numerous potential confounding factors. Limitations of the study include that missing data on outcomes, determinants, and several covariates might have resulted in biased effect estimates. The population of analysis might reflect a selection towards a more healthy and affluent population (18). Included subjects more often were of higher socio-economic status, from European ethnic background, and less often exposed to adverse lifestyle factors. Of all children participating in the follow-up at age 6 years, 54.4% did not have data on both *H. pylori* colonization and asthma or related conditions. Based on the differences in characteristics between those included and not included in the study and previous literature (41), we speculate that the prevalence of *H. pylori* and asthma could have been higher in the excluded subjects, compared to the population of analysis. We have aimed to reduce the potential selection bias by the use of multiple imputation of covariates. Second, although both ELISAs have been validated in adults and children, and have been previously used in Dutch children (22), they have not been separately validated in Dutch children of different origins. Third, all outcomes largely relied on data obtained from questionnaires completed by the parents. Although the questions were previously validated and commonly used for epidemiological studies (42), we cannot rule out the possibility of misclassification or misinterpretation. Forth, data on antibiotic use were not available about specific types, nor validated by pharmacy records. Fifth, lacking of detailed data of allergic features in mothers made direct comparison with their children in relation to *H. pylori* status impossible. Finally, despite the large sample size of this study, the number of children with *H. pylori* colonization, especially CagA-positive strains, and asthma or related conditions was limited. In total, only a small proportion of the asthma risk could be attributed to *H. pylori*. Therefore, conclusions based on these numbers should be interpreted with caution.

Conclusions

In conclusion, we observed no significant protective association of *H. pylori* status at age 6 with asthma and related conditions. Instead, colonization with a CagA-negative *H. pylori* strain at age 6 was a risk factor for asthma in children, but only in those of European ethnic background. Explanations of underlying mechanisms for the differences between ethnic groups are still speculative, and therefore need further research. Such studies also should include the role of the gut microbiome in relation to *H. pylori* colonization and ethnic background, which may indicate new directions for asthma prevention.

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SUPPLEMENTARY MATERIAL

Helicobacter pylori in children with asthmatic conditions at school age, and their mothers

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SUPPLEMENTARY TEXT

Covariates

Information on maternal age, anthropometrics, ethnicity, socio-economic status, history of asthma or atopy, parity, and pet keeping were obtained by questionnaire, completed by the mother at enrolment. Socio-economic status was assessed using the educational level of mother on the basis of her highest level of completed education. Smoking during pregnancy was reported at enrolment, and reported smoking habits in the second and third trimesters of pregnancy were used to reclassify maternal smoking habits, when appropriate. Maternal psychological distress in the second trimester of pregnancy was defined using a global severity index (GSI), a measure of current level or depth of the symptoms, and denotes overall psychological distress. High scores represent an increased occurrence of overall distress, based on Dutch cut-offs (1). We used parity as a proxy for older siblings (2). Birth weight, gestational age, and sex of the children were obtained from midwife and hospital registries at birth. Postnatal questionnaires at ages of 6 and 12 months supplied information on breastfeeding, and at age of 6 years about lower respiratory tract infections during the prior 12 months. Use of antibiotics in the prior 12 months (never; yes, for 1-2 time periods; yes, for 3-4 time periods; yes, for 5 or more time periods) was assessed by questionnaires at the ages of 1 to 6 years, and was categorized into never, for 1-2 time periods, and for 3 or more time periods.

Statistical analyses

The prevalence of asthma and related conditions in relation to child's *H. pylori* and CagA status were examined using Chi-square tests. We used multivariate logistic regression analysis to examine the association of child's *H. pylori* and CagA status with asthma and related conditions, taking potential confounders into account, including maternal age at enrolment, pre-pregnancy body mass index, ethnicity, socio-economic status, history of asthma or atopy, parity, pet keeping, smoking during pregnancy, psychological stress during pregnancy, and child's birth weight, gestational age at birth, sex, breastfeeding habits, lower respiratory tract infections, and antibiotic use. Confounders were included based on the prior literature, if they were associated with both the determinant and the outcome or if they changed the effect estimates with ≥10%.

If available, child's *H. pylori* status was paired with maternal *H. pylori* status, resulting into four different groups: mother and child both *H. pylori* negative, mother *H. pylori* negative and child positive*,* mother *H. pylori* positive and child negative, both mother and child *H. pylori* positive. We used multivariate logistic regression analysis to examine the association of combined *H, pylori* status of mother and child, with asthma and related conditions. Potential confounders were taken into account, including maternal age at

enrolment, pre-pregnancy body mass index, ethnicity, socio-economic status, history of asthma or atopy, parity, pet keeping, smoking during pregnancy, psychological stress during pregnancy, and child's birth weight, gestational age at birth, sex, breastfeeding habits, lower respiratory tract infections, and antibiotic use. Due to small numbers of cases we were not able to differentiate between CagA-status.

For all covariates, the percentage of missing values within the population for analysis were lower than 10%, except for history of asthma or atopy (18.7%), smoking during pregnancy (11.8%), psychological distress during pregnancy (24.6%), breastfeeding (32.6%), pet keeping (21.4%), and antibiotic use (24.2%). Missing values were mainly due to non-response. Missing data in the covariates were imputed with multiple imputations using chained equations (3). Ten new datasets were created by imputation based on all determinants, covariates and outcomes in the model plus paternal age and educational level, paternal history of asthma and atopy, and child's antibiotic use during first 6 months of life. Data from each separate imputation were analysed, after which results were combined. Only the results based on the adjusted pooled imputed datasets are presented in this manuscript. To identify the potential modifying effect of covariates such as maternal ethnicity, asthma or atopy, psychological distress, and child's sex, gestational age and weight at birth, and antibiotic use, we evaluated statistical interaction by adding the product term of *H pylori* status and the covariate as a variable to the multivariate model. We calculated the population attributable fraction of *H. pylori* colonization for asthma, using adjusted ORs estimated from logistic regression models (4).

All measures of associations are presented as Odds Ratios (OR) with their 95% Confidence Intervals (CI). Statistical analyses were performed using SPSS version 23.0 for Windows (Statistical Package of Socioeconomic Sciences; SPSS Inc., Chicago, IL, USA).

Supplementary Table 1. Comparison of the maternal and child characteristics between those included and those not included in the study among the 8,305 eligible subjects

	Total population (N=8,305)	Included (N=3,797)	Not included (N=4,508)	P-value differences[a]
Maternal characteristics				
Age (years)	30.2 (5.2)	31.1 (4.9)	29.6 (5.4)	<0.001
Pre-pregnancy body mass index (kg/m^2)	23.8 (18.7-36.2)	23.7 (18.9-35.5)	24.0 (18.6-36.6)	<0.001
Ethnicity (European *vs.* non-European)	58.4	65.6	52.3	<0.001
Maternal education (low/medium *vs.* high)	54.8	48.8	60.2	<0.001
History of asthma and atopy (yes *vs.* no)	38.9	38.3	39.4	0.39
Smoking during pregnancy (yes *vs.* no)	17.4	14.6	19.8	<0.001
Psychological distress pregnancy (yes *vs.*no)	9.5	7.6	11.3	<0.001
Parity (nulliparous *vs.* multiparous)	55.4	56.2	54.8	0.18
Child characteristics				
Age (years)	6.2 (0.5)	6.1 (0.5)	6.2 (0.6)	<0.001
Female sex	49.5	48.1	50.7	0.02
Gestational age at birth (weeks)	40.0 (35.3-42.3)	40.1 (35.7-42.3)	40.0 (34.9-42.3)	<0.001
Birth weight (grams)	3,399 (577)	3,446 (550)	3,359 (596)	<0.001
Breastfeeding (yes *vs.* no)	91.8	92.5	91.1	0.04

Values are percentages for categorical variables. Age and birth weight are reported in means (standard deviation). Body mass index and gestational age at birth are reported in medians (2.5-97.5th percentile).

[a]P-value reflects difference between included and not-included subjects.

Supplementary Table 2 (Figure 2). Prevalence of asthma and related outcomes of children according to child's *H. pylori* and corresponding CagA status.

	Wheezing (n= 3,135)		
	No (n=2,866)	**Yes** (n=269)	**Chi-square (p=0.24)**
Hp negative (%) (n=2,886)	91.3 (2,635)	8.7 (251)	
Hp positive CagA negative (n=181)	91.2 (165)	8.8 (16)	
CagA positive (n=68)	88.4 (66)	11.6 (2)	

	Physician diagnosed asthma (n= 3,062)		
	No (n=2,864)	**Yes** (n=198)	**Chi-square (p=0.045)**
Hp negative (%) (n=2,820)	93.8 (2,645)	6.2 (175)	
Hp positive CagA negative (n=173)	89.0 (154)	11.0 (19)	
CagA positive (n=69)	94.2 (65)	5.8 (4)	

	Eczema (n= 3,082)		
	No (n=2,472)	**Yes** (n=610)	**Chi-square (p=0.25)**
Hp negative (%) (n=2,834)	80.6 (2,283)	19.4 (551)	
Hp positive CagA negative (n=180)	76.7 (138)	23.3 (42)	
CagA positive (n=68)	75.0 (51)	25.0 (17)	

	Inhalant allergy (n= 3,584)		
	No (n=3,363)	**Yes** (n=221)	**Chi-square (p=0.32)**
Hp negative (%) (n=3,282)	93.7 (2,639)	6.3 (206)	
Hp positive CagA negative (n=216)	94.0 (1,072)	6.0 (13)	
CagA positive (n=86)	97.7 (493)	2.3 (2)	

Values are percentages (absolute numbers). *H. pylori* and CagA status of children related to childhood wheezing, asthma, eczema, and inhalant allergy.

Supplementary Table 3. Prevalence of asthma according to child's *H. pylori* status stratified for ethnicity.

European (n = 2,140)		**No** (n=2,013)	**Yes** (n=127)	**Chi-square (p<0.001)**
	***Hp* negative (%)** (n=2,026)	94.5 (1,915)	5.5 (111)	
	***Hp* positive** (n=114)	86.0 (98)	14.0 (16)	
Non-European (n = 922)		**No** (n=851)	**Yes** (n=711)	**Chi-square (p=0.32)**
	***Hp* negative (%)** (n=794)	91.9 (730)	8.1 (64)	
	***Hp* positive** (n=128)	94.5 (121)	5.5 (7)	

Values are percentages (absolute numbers).

Supplementary Table 4. Associations of children's *H. pylori* and corresponding CagA status with asthma-related outcomes, stratified by ethnicity.

	Wheezing OR (95% CI)	**Eczema OR (95% CI)**	**Inhalant allergy OR (95% CI)**
European	n = 2,199	n = 2,155	N = 2,388
Hp-	*Reference* *n = 164 / 2,079*	*Reference* *n= 313 / 2,035*	*Reference* *n = 110 / 2,260*
Hp+	1.00 (0.48, 2.08) n = 10 / 120	1.23 (0.76, 2.00) n = 22 / 120	1.10 (0.49, 2.48) n = 7 / 128
Hp+CagA-	0.91 (0.41, 2.05) n = 8 / 105	1.21 (0.72, 2.02) n = 19 / 105	1.32 (0.58, 2.98) n = 7 / 111
Hp+CagA+	1.65 (0.31, 8.72) n = 2 / 15	1.42 (0.39, 5.13) n = 3 / 15	NA n = 0 / 17
Non-European	n = 936	n = 927	n = 1,196
Hp-	*Reference* *n = 87 / 807*	*Reference* *n = 238 / 799*	*Reference* *n = 96 / 1,022*
Hp+	0.49 (0.21, 1.11) n = 8 / 129	0.87 (0.56, 1.35) n = 37 / 128	0.52 (0.24, 1.12) n = 8 / 174
Hp+CagA-	0.95 (0.40, 2.25) n = 8 / 76	0.97 (0.57, 1.66) n = 23 / 75	0.67 (0.28, 1.64) n = 6 / 105
Hp+CagA+	NA n = 0 / 53	0.74 (0.38, 1.43) n = 14 / 53	0.30 (0.07, 1.28) n = 2 / 69

Values are odds ratios for wheezing, eczema, and inhalant allergy (95% confidence interval) from logistic regression models. n = number of cases (i.e. children with wheezing/eczema/inhalant allergy) per total group (i.e. according to *H. pylori*/CagA status). Models were adjusted for maternal age, ethnicity, pre-pregnancy body mass index, educational level, history of asthma or atopy, parity, pet keeping, smoking during pregnancy, psychological stress during pregnancy, and child's birth weight, gestational age at birth, sex, breastfeeding, lower respiratory tract infections, and antibiotic use.

Supplementary Table 5. Prevalence of asthma and related outcomes of children according to paired maternal and children's *H. pylori* status.

	Wheezing (n= 2,410)		
	No % (n=2,205)	**Yes** % (n=205)	**Chi-square (p=0.53)**
mHp- cHp- (n=1,476)	91.7 (1,354)	8.3 (122)	
mHp+ cHp- (n= 740)	90.8 (672)	9.2 (68)	
mHp- cHp+ (n= 82)	95.1 (78)	4.9 (4)	
mHp+ cHp+ (n= 112)	91.5 (101)	9.8 (11)	

	Physician diagnosed asthma (n= 2,353)		
	No % (n=2,199)	**Yes** % (n=154)	**Chi-square (p=0.14)**
mHp- cHp- (n=1,441)	94.1 (1,356)	5.9 (85)	
mHp+ cHp- (n= 726)	93.1 (676)	6.9 (50)	
mHp- cHp+ (n= 79)	88.6 (70)	11.4 (9)	
mHp+ cHp+ (n= 107)	90.7 (97)	9.3 (10)	

	Eczema (n= 2,373)		
	No % (n=1,897)	**Yes** % (n=476)	**Chi-square (p=0.08)**
mHp- cHp- (n= 1,451)	81.5 (1,183)	18.5 (268)	
mHp+ cHp- (n= 731)	77.7 (568)	22.3 (163)	
mHp- cHp+ (n= 82)	79.3 (65)	20.7 (17)	
mHp+ cHp+ (n= 109)	74.3 (81)	25.7 (28)	

	Inhalant allergy (n= 2,749)		
	No % (n=2,576)	**Yes** % (n=173)	**Chi-square (p=0.32)**
mHp- cHp- (n) (n= 1,626)	94.0 (1,529)	6.0 (97)	
mHp+ cHp- (n= 888)	92.7 (823)	7.3 (65)	
mHp- cHp+ (n= 94)	96.8 (91)	3.2 (3)	
mHp+ cHp+ (n= 141)	94.3 (133)	5.7 (8)	

Values are percentages (absolute numbers). *H. pylori* status of mother (mHp) and children (cHp) related to childhood wheezing, asthma, eczema, and inhalant allergy.

Supplementary Table 6. Associations of paired mother-child's *H. pylori* status with asthma stratified by ethnicity.

	Asthma Odds Ratio (95% CI)		**Asthma Odds Ratio (95% CI)**
European	n = 1,642	**Non-European**	n = 711
mHp- cHp-	*Reference* *n = 67 / 1,197*[1]	*mHp- cHp-*	*Reference* *n = 18 / 244*
mHp+ cHp-	1.08 (0.62, 1.87) n = 19 / 353	*mHp+ cHp-*	0.93 (0.46, 1.89) n = 31 / 373
mHp- cHp+	**3.25** **(1.41, 7.54)*** n = 8 / 59	*mHp- cHp+*	0.57 (0.06, 5.92) n = 1 / 20
mHp+ cHp+	**4.07** **(1.47, 11.31)*** n = 6 / 33	*mHp+ cHp+*	0.73 (0.26, 2.59) n = 4 / 74

Values are odds ratios for asthma (95% confidence interval) from logistic regression models. Models were adjusted for maternal age, pre-pregnancy body mass index, educational level, history of asthma or atopy, parity, psychological stress during pregnancy, and child's sex, gestational age at birth, birth weight, breastfeeding, pet keeping, lower respiratory tract infections, and antibiotic use.

Overall $P_{Interaction}$ ethnicity * *H. pylori:* <0.05.

[1]n = number of cases (i.e. children with asthma) per total group (i.e. according to *H. pylori* status

*p < 0.01

Supplementary Table 7. Univariate comparisons of child's and maternal characteristics with *H. pylori* and with asthma.

	H. pylori **N = 3,797**			**Asthma** **N = 3,062**		
	No	**Yes**	**p-value**	**No**	**Yes**	**p-value**
Male sex	1819	150	0.01	1461	120	0.01
Gestational age at birth, weeks	39.9	39.9	0.49	39.9	39.3	<0.001
Birth weight, grams	3445	332	0.89	3461	3353	0.01
Ethnicity			<0.001			0.07
European	2354	138		2013	127	
Non-European	1111	194		851	71	
Breastfeeding			0.55			0.05
Never	461	46		348	33	
Ever	3004	286		2516	165	
Pet keeping			0.01			0.75
No	2222	238		1857	131	
Yes	1243	94		1007	67	
LRTI's at age 6 years			0.96			<0.001
No	3300	316		2771	154	
Yes	165	16		93	44	
Antibiotic use cumulative			0.05			<0.001
Never	479	29		461	4	
1-2 periods	1463	136		1226	82	
3 or more periods	722	50		577	78	
Maternal education level			<0.001			0.05
Primary, or secondary	1702	220		1324	104	
Higher	1763	112		1540	94	
Maternal age, years	31.3	29.5	<0.001	31.5	31.4	0.73
Maternal body mass index	24.6	25.2	0.03	24.5	25.3	0.01
History of asthma or atopy			0.29			<0.001
No	2132	213		1804	95	
Yes	1333	119		1060	103	
Parity			0.01			0.47
0	1936	164		1624	107	
≥1	1529	168		1240	91	
Smoking during pregnancy			0.19			0.01
No	2961	274		2483	161	
Yes	504	58		381	37	
Psychological distress during preganancy			<0.001			0.02
No	3179	276		2661	176	
Yes	285	56		203	22	

Numbers are means or absolute values

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CHAPTER 7

Helicobacter pylori colonization and obesity – a Mendelian randomization study

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ABSTRACT

Introduction: Obesity is associated with substantial morbidity, costs, and decreased life expectancy, and continues to rise worldwide. While etiological understanding is needed for prevention, epidemiological studies indicated that colonization with *Helicobacter pylori (H. pylori)* may affect body mass index (BMI), but with inconsistent results. Here, we examine the relationship between *H. pylori* colonization and BMI/obesity in a large population-based cohort setting using a Mendelian Randomization approach.

Methods: Cross-sectional analyses were performed in two independent population-based cohorts of elderly from the Netherlands and Germany. Genetic risk scores were conducted based on genetic loci associated with either *H. pylori* colonization or BMI/obesity. We performed a bi-directional Mendelian randomization. Results of both cohorts were combined for meta-analysis.

Results: In total, 13,044 subjects were included. Meta-analysis of cross-sectional data revealed no association between anti-*H. pylori* IgG titer and BMI, nor of *H. pylori* positivity and BMI. Anti-*H. pylori* IgG titer was negatively associated with obesity (OR 0.99972; 95% CI 0.99946-0.99997, p=0.03) and with obesity classes (Beta -6.91 $\bullet 10^{-5}$; 95% CI -1.38$\bullet 10^{-4}$, -5.49$\bullet 10^{-7}$, p=0.048), but the magnitude of these effects was very limited. Mendelian randomization showed no causal relation between *H. pylori* genetic risk score and BMI/obesity, nor between BMI or obesity genetic risk scores and *H. pylori* positivity.

Conclusion: This study provides no cross-sectional evidence for a clinically relevant association between *H. pylori* positivity and BMI/obesity. Bi-directional Mendelian randomization revealed no causal relation between *H. pylori* and BMI/obesity.

INTRODUCTION

The prevalence of obesity rises worldwide. This is associated with significant morbidity, costs, and decreased life expectancy. The latter can be reduced with 8-13 years (1), which results in a huge economic burden (2). The causes of obesity are diverse and include excessive energy intake, lack of physical activity, but also culprits such as stress, lack of sleep, or exposure to chemical endocrine disruptors (3). There is increasing evidence from mouse as well as human studies that shows that the gut microbiome may play an important role in energy balance (4). Modern lifestyle, and the widespread use of antibiotics may affect the composition of our microbiome, which may have consequences for our health (5).

In this context, *Helicobacter pylori (H. pylori)*, is of relevance. This Gram-negative, spiral-shaped, gastric bacterium is gradually disappearing in Western populations (5, 6). *H. pylori* colonization is virtually always associated with chronic active gastritis, which can have various effects. This includes interference with gastric hormone regulation, including ghrelin and leptin. Both have multiple roles in energy homeostasis (7). Disturbance of their normal regulation interferes with metabolism and our energy household. *H. pylori* eradication increases serum ghrelin levels (8).

For these reasons, several epidemiological studies have recently focused on the correlation between *H. pylori* colonization and BMI and obesity. They showed contrasting results, which were based on *H. pylori* status and BMI data, but did not include genetic information (9-13). Incorporation of genetic data could give unbiased information on the presumed association. A recent genome wide association study (GWAS) identified two genetic loci associated with anti-*H. pylori* IgG titers (14). Numerous GWAS have identified many genetic loci associated with BMI variation and / or obesity risk (15, 16). Combining these results into risk scores enables a Mendelian randomization study for association between *H. pylori* serology and BMI. Mendelian randomization is a technique that aims at unbiased detection of causal effects (17).

We aimed to assess the relationship between *H. pylori* seroprevalence and obesity using both epidemiological and genetic data of two population-based cohort studies. Results of cross-sectional and genetic analyses were compared. In addition, we performed a meta-analysis of data derived from both cohorts.

METHODS

Study cohorts

The Rotterdam Study is a large, population based prospective study of elderly individuals of European ancestry consisting of three cohorts (RS-I, RS-II, RS-III), who are residing in a suburb of Rotterdam, the Netherlands. The study design has been described in detail previously (18, 19). Baseline recruitment and measurements for the RS-I study were obtained between 1990 and 1993. The second cohort, RS-II, was set-up in 2000-2001. A third cohort, RS-III, started in 2006 and recruitment ended in December 2008.

The SHIP study comprises two independent prospectively recruited population-based cohorts in Northeastern Germany: SHIP and SHIP-TREND. The study design of SHIP has been described in detail previously (20). Participants were recruited between October 1997 and May 2001. SHIP-TREND is an independent cohort from the same region. Individuals were recruited between September 2008 and summer 2012 (20). An important characteristic of SHIP is that it attempts to describe health-related conditions with the widest focus possible.

Data from SHIP, SHIP-TREND, RS-I, and RS-II (RS from now on) were used in this study. Written informed consent was obtained from all participants. Both the medical ethics committee of the Erasmus MC University Medical Center Rotterdam and University Medicine Greifswald approved the study.

Phenotype definition

Serologic *H. pylori* colonization in individuals from SHIP, SHIP-TREND, and RS was defined by measuring IgG antibody levels in serum using commercial enzyme-linked immunosorbent assay (Pyloriset EIA-G III ELISA; Orion). Seropositivity was defined as an anti-*H. pylori* IgG titer of ≥20 U/mL according to the manufacturer's instructions (21). Seropositivity is an indicator for current or past colonization. The sensitivity of the Pyloriset EIA-G III ELISA is reported as 97.8%, with a specificity of 58% (22). To increase specificity and reduce the number of false-positive *H. pylori* infections, we defined subjects with the 25% highest IgG titers as *H. pylori* cases, and those with the 75% lowest IgG titers as controls (14).

We further assessed the presence of *H. pylori* antigen in stool of subjects from SHIP-TREND by using the *H. pylori* antigen ELISA kit (Immunodiagnostics). For this purpose, 100 mg feces was stored at -20°C. An optical density (OD) ≥0.025 at 450 nm was considered evidence of *H. pylori* infection, according to the manufacturer's recommendation. This test has a sensitivity of 97.7% and specificity of 96.3%.

Genetic risk score conduction

For the creation of the genetic risk scores (BMI risk score, obesity risk score, *H. pylori* risk score), we firstly searched the literature for publications of genome-wide association studies (GWAS) for these traits. A list of SNPs that reached genome-wide significance ($P<5x10^{-8}$) with BMI or binary obesity status in populations of European ancestry was established. Three different strategies were used to optimize the SNP selection procedure using a key word search (e.g. BMI) on i) the National Human Genome Research Institute (NHGRI) GWAS Catalog (www.genome.gov/gwastudies/) ii) the HuGE Navigator GWAS Integrator (www.hugenavigator.net/HuGENavigator/gWAHitStartPage.do) iii) the PubMed database (www.ncbi.nlm.nih.gov/pubmed). Using this strategy, 45 independent loci were found to be associated with BMI variation and 48 with binary obesity status. We chose to analyze risk scores for BMI and obesity separately. BMI is a phenotype which results in a relatively clean risk score. In contrast, various different definitions have been used to define obesity, like BMI ≥ 25, or BMI ≥ 30. For this reason we used both phenotypes (e.g. BMI continuous and binary) in our analyses. A genotype score (GS) was calculated by summing the alleles of BMI / obesity / *H. pylori* status-associated SNPs. An unweighted GS was used as previously recommended by Dudbridge et al. (23). Imputed genotypes (1000G PhaseIv3) were used for the creation of the GS. All GWAS published before June 2014 were included. Per genetic locus, 1 SNP was selected based on the following criteria: 1) SNP genotyped with an imputation quality (R2) of 0.3 or higher in all populations; 2) Preference for A/C or G/T variants to avoid strand issues. A list of all studies and variants considered, as well as the variants selected can be found in **Supplementary Table S6, S7 and S8**.

Statistical analysis

In total, we used three different approaches to assess the relationship between *H. pylori* status and BMI/obesity. First, cross-sectional analyses were performed to assess the relationship between *H. pylori* colonization and BMI/obesity at time of inclusion, by using linear and logistic regression. Outcomes were defined as continuous BMI, binary obesity (BMI ≥30 kg/m^2, with BMI <30 kg/m^2 as reference group), and obesity classes (lean BMI <18.5 kg/m^2; normal-weight BMI ≥ 18.5 and <25 kg/m^2; overweight BMI ≥ 25 and <30 kg/m^2; class I obesity BMI ≥30 and < 35 kg/m^2; class II obesity BMI ≥35 and <40 kg/m^2, class III obesity BMI ≥40 kg/m^2). The latter outcome was defined as a continuous variable with value '0' for lean, and value '5' for class III obesity. Unadjusted effects of *H. pylori* titer or antigen (continuous) and *H. pylori* positivity (binary phenotype) were assessed for each outcome. We additionally adjusted for sex and age. All analyses were done separately for RS, SHIP, and SHIP-TREND. A meta-analysis was performed to observe the combined effect of *H. pylori* colonization on BMI/obesity.

Second, a Mendelian randomization approach was carried out to explore a bi-directional link between *H. pylori* colonization and BMI. Linear regression analysis was used to assess the relationship between BMI gene score and *H. pylori* titer and *H. pylori* positivity. Analyses were first adjusted for sex, age, and additionally for BMI at baseline. The same analyses were done regarding the obesity gene score. Linear regression analysis was also used to examine the effect of the *H. pylori* gene score with BMI, binary obesity status, and obesity classes, all defined at baseline. Analyses were adjusted for sex, and age, and additionally for *H. pylori* status (binary phenotype). Data of the three cohorts were combined and examined by using meta-analysis approach.
All measures of associations are presented as Odds Ratios (OR) or Beta's with their 95% confidence intervals (CI). Statistical analyses were performed using IBM SPSS Statistics 21.0 for Windows (SPSS IBM, Armonk, New York, USA). Meta-analyses were done with R library meta (R Core Team (2014), R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Baseline characteristics

In total, 13,044 participants were initially included in this study. **Table 1** summarizes the baseline characteristics of each cohort. In 220 subjects (1.7%) no data on BMI was available. Data on *H. pylori* titer was lacking in 252 individuals (1.9%) of SHIP. The total population included in the cross-sectional analyses consisted of 12,572 (96.4%) subjects. According to the predefined phenotypic seroprevalence, a total of 3,147 (25.0%) subjects were considered as cases, and 9,425 (75%) as controls.
Genotyping data was available for 6,883 (86.3%) subjects of RS, for 3,824 (93.7%) subjects of SHIP, and for 983 (99.7%) of SHIP-TREND, leaving a total population for analysis of 11,690 (89.6%) subjects. **Table 1** summarizes the mean gene risk score for each cohort with respect to the correlation between BMI, obesity, and *H. pylori*. Linear regression analysis within RS focusing on the BMI gene score and BMI revealed a beta of 0.10 kg/m^2 per additional BMI-increasing allele (95% CI 0.07-0.12, p=5.29•10^{-16}). Logistic regression analysis focusing on the obesity gene score and obesity (BMI ≥30 kg/m^2) revealed an OR of 1.04 per additional obesity risk allele (95% CI 1.02-1.05, p=1.09•10^{-4}). The *H. pylori* gene score was significantly associated with *H. pylori* positivity (OR 1.39, 95% CI 1.27-1.51; p=3.46•10^{-13}). The proportion of variance of BMI, obesity, and *H. pylori* explained by the genetic risk scores ranged from 0.3% for obesity, 0.5% for *H. pylori* and 1% for BMI per 1 unit increase in score.

Table 1. Baseline characteristics (total cohort n = 13,044)

Cohort	RS-I and RS-II	SHIP	SHIP-TREND
Total number (n)	7,977	4,081	986
Age (years), mean (sd)	690 (9.3)	49.7 (16.3)	50.1 (13.7)
Female sex (%)	4,391 (55.0)	2,073 (50.8)	554 (56.2)
H. pylori titer distribution, median (range)	24.3 (6.2-5587.4)	30.4 (5-500)	16.1 (5-500)
H. pylori positive (cut-off)[1], %	4,372 (54.8)	2,275 (59.4)	440 (44.8)
H. pylori positive (highest 25% IgG titer)[2], %	1,994 (25.0)	958 (25.0)	246 (25.0)
H. pylori antigen distribution, median (range)	NA	NA	-0.004 (-0.151, 3.983)
H. pylori antigen (cut-off)[1], %	NA	NA	255 (26.9)
BMI (kg/m^2), mean (sd)	26.7 (3.9)	27.3 (4.7)	27.4 (4.6)
Missing, n (%)	*209 (2.6)*	11 (0.3)	0 (0.0)
Obesity (BMI≥30), %	1,343 (17.3)	1,042 (25.6)	252 (25.6)
Overweight (BMI≥25), %	5,027 (64.7)	2,690 (66.1)	660 (66.9)
Obesity classes (BMI), %			
Lean, <18.5	79 (1.0)	42 (1.0)	2 (0.2)
Normal weight, 18.5-24.9	2,662 (34.3)	1,338 (32.9)	324 (32.9)
Overweight, 25.0-29.9	3,684 (47.2)	1,648 (40.5)	408 (41.4)
Class I obesity, 30.0-34.9	1,110 (14.3)	775 (19.0)	200 (20.3)
Class II obesity, 35.0-39.9	203 (2.6)	217 (5.3)	42 (4.3)
Class III obesity, ≥40.0	30 (0.4)	50 (1.2)	10 (1.0)
BMI risk score, mean (sd)	43.22 (3.98)		
Missing, n (%)	*1,094 (13.7)*	*257 (6.3)*	*3 (0.3)*
Obesity risk score, mean (sd)	42.58 (3.94)		
Missing, n (%)	*1,094 (13.7)*	*257 (6.3)*	*3 (0.3)*
H. pylori risk score, mean (sd)	3.34 (0.65)		
Missing, n (%)	*1,094 (13.7)*	*257 (6.3)*	*3 (0.3)*

[1]According to manufacturer's definition
[2]According to phenotype definition
NA, not applicable

Cross-sectional analyses

Cross-sectional analyses revealed an association between *H. pylori* titer and BMI in RS and SHIP (**Table S1**), however with opposite direction. Meta-analysis of all three cohorts showed no association between *H. pylori* titer and BMI, nor between *H. pylori* positivity and BMI (**Table 2**). *H. pylori* titer, adjusted for age and sex, was negatively associated with obesity (OR 1.00; 95% CI 0.99-1.00, p=0.03) and with obesity classes (Beta -6.91 •10^{-5}; 95% CI -1.38•10^{-4}, -5.49•10^{-7}, p=0.048) (**Table 2**).

Cross-sectional analyses regarding fecal *H. pylori* status and BMI/obesity showed no association (**Table S2**).

Table 2. Cross-sectional analyses regarding serologic *H. pylori* status and BMI/obesity – meta-analysis

Model	Cohort			Meta-analysis	
BMI~*H. pylori*	**RS**	**SHIP**	**SHIP-TREND**	**Beta kg/m² (95% CI)**	**p-value**
Hp titer - crude	-	+	+	$1.19 \bullet 10^{-3}$ ($-1.61 \bullet 10^{-3}$; $3.99 \bullet 10^{-3}$)	0.39
Hp titer - adjusted[1]	-	+	-	$-3.09 \bullet 10^{-4}$ ($-6.46 \bullet 10^{-4}$; $2.81 \bullet 10^{-5}$)	0.07
Hp positivity - crude	-	+	+	0.37 (-0.48; 1.22)	0.40
Hp positivity - adjusted[1]	-	+	-	0.04 (-0.34; 0.41)	0.84
Obesity[2]~*H. pylori*				**OR (95% CI)**	
Hp titer - crude	-	+	+	1.00 (0.99; 1.00)	0.72
Hp titer - adjusted[1]	-	+	-	1.00 (0.99; 1.00)	**0.03**
Hp positivity - crude	-	+	+	1.04 (0.78; 1.39)	0.78
Hp positivity - adjusted[1]	-	+	-	0.95 (0.86; 1.06)	0.37
Obesity classes[3]~*H. pylori*				**Beta (95% CI)**	
Hp titer - crude	-	+	+	$2.28 \bullet 10^{-4}$ ($-2.91 \bullet 10^{-4}$; $7.48 \bullet 10^{-4}$)	0.39
Hp titer - adjusted[1]	-	+	-	$-6.91 \bullet 10^{-5}$ ($-1.38 \bullet 10^{-4}$; $-5.49 \bullet 10^{-7}$)	**0.05**
Hp positivity - crude	-	+	+	0.06 (-0.10; 0.22)	0.49
Hp positivity - adjusted[1]	-	+	-	-0.02 (-0.05; 0.02)	0.36

[1]adjusted for sex and age
[2]Obesity defined as BMI ≥ 30 kg/m²
[3] Lean: BMI < 18.5 kg/m²; normal-weight: BMI ≥ 18.5 and < 25 kg/m²; overweight: BMI ≥ 25 and < 30 kg/m²; class I obesity: BMI ≥ 30 and < 35 kg/m²; class II obesity: BMI ≥ 35 and < 40 kg/m², class III obesity: BMI ≥ 40 kg/m²

Table 3. Mendelian randomization *H. pylori* and BMI/obesity gene score – meta-analysis

Model	Cohort			Meta-analysis	
***H. pylori* titer~BMI gene score**	**RS**	**SHIP**	**SHIP-TREND**	**Beta (95% CI)**	**p-value**
BMI gene score – crude	+	+	+	0.12 (-0.63; 0.87)	0.76
BMI gene score – adjusted[1]	+	-	+	0.03 (-0.71; 0.77)	0.95
BMI gene score – adjusted[2]	+	-	+	0.04 (-0.71; 0.78)	0.92
***H. pylori* positivity~BMI gene score**				**OR (95% CI)**	
BMI gene score – crude	+	+	-	1.01 (1.00; 1.02)	0.14
BMI gene score – adjusted[1]	+	+	-	1.01 (1.00; 1,02)	0.20
BMI gene score – adjusted[2]	+	+	-	1.01 (1.00; 1,02)	0.15
***H. pylori* titer~Obesity gene score**				**Beta (95% CI)**	
Obesity gene score – crude	+	+	-	0.76 (0.02; 1.50)	**0.04**
Obesity gene score – adjusted[1]	+	+	-	0.73 (-0.00; 1.47)	0.05
Obesity gene score – adjusted[2]	+	+	-	0.71 (-0.03; 1.45)	0.06
***H. pylori* positivity~Obesity gene score**				**OR (95% CI)**	
Obesity gene score – crude	+	+	-	1.01 (1.00; 1.02)	0.12
Obesity gene score – adjusted[1]	+	+	-	1.01 (1.00; 1.02)	0.14
Obesity gene score – adjusted[2]	+	+	-	1.01 (1.00; 1.02)	0.15

[1]Adjusted for age and sex
[2]Adjusted for age, sex, and BMI

Mendelian randomization

The BMI gene score was not associated with *H. pylori* titer or positivity (**Table 3**). **Table S3** shows the results of each cohort. Crude analysis showed a positive association between obesity gene score and *H. pylori* titer (Beta 0.76; 95% CI 0.02-1.50, p=0.04) (**Table 3**). *H. pylori* gene score was not associated with BMI, neither with obesity nor obesity classes (**Table 4** and **Table S4**). Also, no associations were observed regarding fecal *H. pylori* status and the BMI or obesity gene score (**Table S5**).

Table 4. Mendelian randomization regarding BMI/obesity and *H. pylori* gene score – meta-analysis

Model	Cohort			Meta-analysis	
BMI~*H. pylori* gene score	**RS**	**SHIP**	**SHIP-TREND**	**Beta (95% CI)**	**p-value**
Hp gene score – crude	-	-	-	-0.05 (-0.16; 0.07)	0.43
Hp gene score – adjusted[1]	-	-	-	-0.06 (-0.17; 0.05)	0.31
Hp gene score – adjusted[2]	-	-	-	-0.05 (-0.17; 0.06)	0.37
Obesity[3]~*H. pylori* gene score				**OR (95% CI)**	
Hp gene score – crude	-	+	-	0.98 (0.92; 1.05)	0.60
Hp gene score – adjusted[1]	-	-	-	0.98 (0.91; 1.04)	0.47
Hp gene score – adjusted[2]	+	-	-	0.98 (0.91; 1.05)	0.56
Obesity classes[4]~*H. pylori* gene score				**Beta (95% CI)**	
Hp gene score – crude	+	-	-	-0.01 (-0.03; 0.02)	0.54
Hp gene score – adjusted[1]	+	-	-	-0.01 (-0.03; 0.01)	0.37
Hp gene score – adjusted[2]	+	-	-	-0.03 (-0.06; 0.01)	0.15

[1]Adjusted for age and sex
[2]Adjusted for age, sex, and *H. pylori*
[3]Obesity defined as BMI ≥ 30 kg/m^2
[4]Obesity classes defined as lean: BMI < 18.5 kg/m^2; normal-weight: BMI ≥ 18.5 and < 25 kg/m^2; overweight: BMI ≥ 25 and < 30 kg/m^2; class I obesity: BMI ≥ 30 and < 35 kg/m^2; class II obesity: BMI ≥ 35 and < 40 kg/m^2, class III obesity: BMI ≥ 40 kg/m^2

DISCUSSION

This study included a meta-analysis of 13,044 subjects from two large population-based cohorts. This analysis did not demonstrate an association between *H. pylori* colonization and BMI, neither when examined by means of serology, nor by fecal antigen, or Mendelian randomization. *H. pylori* serology, adjusted for age and sex, was negatively associated with obesity (BMI ≥30 kg/m^2), and obesity classes. However, these effects were small. Active *H. pylori* colonization, determined by a positive fecal antigen test, was also not positively or negatively associated with obesity. While the unadjusted and adjusted effect estimates for the obesity gene score on anti-*H. pylori* IgG titer were positive, this association did not remain statistically significant after adjustment for age and sex. So,

the use of a Mendelian randomization method did not show a causal bi-directional link between *H. pylori* serology and BMI or obesity.

Our meta-analysis of *H. pylori* status as determined by serology showed a small negative association with both obesity and obesity classes. Considering both the small effect estimates, and opposite directions in the individual cohorts, we consider these associations as clinically irrelevant. Prior epidemiological studies have shown either negative (9), or positive (10, 11), or no association (12, 13) between *H. pylori* and BMI or obesity. The latter findings are most in line with our findings. A recent review of studies reporting data on *H. pylori* and obesity prevalence rates in developed countries, showed an inverse correlation ($r=-0.29$, $p<0.001$) between *H. pylori* colonization and obesity and overweight (24). In total, data of 99,463 subjects from 49 studies were pooled. Prevalence rates for *H. pylori,* but also for overweight and obesity were highly variable between included studies. Nevertheless, no additional analyses were performed to examine whether this correlation was related to potential significant confounders such as age. Age is an important confounder as it is positively correlated with *H. pylori* colonization (20), and negatively with obesity (20). This may explain the negative correlation between *H. pylori* and obesity reported in the systematic review. Other studies have observed weight gain following successful *H. pylori* eradication (18, 25-28). A clinical trial from Japan randomized 1,558 *H. pylori*-positive adults to either antibiotic treatment or placebo with a subsequent follow-up of 6 months. *H. pylori* eradication was associated with a mean weight gain of 0.6 kg (95% CI 0.31, 0.88) and an increase in BMI of 0.2 kg/m^2 (25). The simultaneous improvement of dyspepsia symptoms in the eradication group may have stimulated the appetite and subsequently caused the weight gain by increased food intake. Others have suggested that circulating meal-associated leptin and ghrelin levels, which changed after *H. pylori* eradication, gave rise to the increased BMI. US investigators observed an increase in both post-prandial levels of leptin and ghrelin a median seven months following *H. pylori* eradication in a group of 21 patients (29). In addition, BMI significantly increased after over 18 months of follow-up, while no change was observed in those who were *H. pylori*-negative at baseline. Although these studies provided evidence that *H. pylori* eradication may result in weight gain, it does not imply that there is an absolute difference in BMI between *H. pylori*-negative and positive subjects. Both our cross-sectional as well as Mendelian randomization results suggest that *H. pylori*-colonized and *H. pylori*-negative subjects have similar BMI. The congruence between the results of our cross-sectional and Mendelian randomization analyses is important, as the latter is based on an unbiased approach (17).

One of the strengths of this study was the use of different methods to detect *H. pylori* colonization, by means of both serology and stool antigen. The latter is a reliable method to identify active *H. pylori* colonization. While most prior studies reported data

on serology, we were able to show that results for serology and fecal antigen did not differ. In addition, the use of SNP typing data with genetic risk scores for BMI, obesity, and *H. pylori* colonization is unique in this field. The Mendelian randomization method is a powerful control for reverse causation and confounding, which otherwise affects epidemiological studies (17). It is based on the common disease, common variant hypothesis, which argues that common variants with modest effects underlie many complex traits (30).

As any method, the Mendelian randomization has its limitations. Although many genetic variants are discovered, these common variants only explain a small proportion of the estimated trait heritability. Regarding the *H. pylori*-gene risk score, we were only able to include two genetic variants, due to the fact that no others (as far as we know) have been discovered so far, and these explain only 0.5% of the variance. Finally, we did not account for socio-economic status in the cross-sectional analyses. Although both populations are similar regarding ethnicity and age distribution, differences in socio-economic status are associated with both *H. pylori* colonization and BMI, and may therefore have influenced our outcome.

In conclusion, this study provides no evidence for a cross-sectional association between *H. pylori* colonization and BMI or obesity in adults. Mendelian randomization revealed no causal relation between *H. pylori* and BMI or obesity.

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SUPPLEMENTARY MATERIAL

Helicobacter pylori colonization and obesity – a Mendelian randomization study

Table S1 Cross-sectional analyses regarding serologic *H. pylori* status and BMI/obesity

	Cohort								
Model	**RS**			**SHIP**			**SHIP-TREND**		
BMI~*H. pylori*	**Beta**	**se**	**p-value**	**Beta**	**se**	**p-value**	**Beta**	**se**	**p-value**
Hp titer - crude	$-4.93 \cdot 10^{-4}$	$1.83 \cdot 10^{-4}$	**0.01**	$3.30 \cdot 10^{-3}$	$6.31 \cdot 10^{-4}$	**$2.51 \cdot 10^{-7}$**	$8.06 \cdot 10^{-4}$	$1.16 \cdot 10^{-3}$	0.49
Hp titer - adjusted[1]	$-4.27 \cdot 10^{-4}$	$1.82 \cdot 10^{-4}$	**0.02**	$0.95 \cdot 10^{-3}$	$6.11 \cdot 10^{-4}$	0.12	$-4.19 \cdot 10^{-4}$	$1.12 \cdot 10^{-3}$	0.71
Hp positivity - crude	-0.22	0.10	**0.03**	0.96	0.18	**$6.36 \cdot 10^{-8}$**	0.39	0.34	0.24
Hp positivity - adjusted[1]	-0.18	0.10	0.07	0.33	0.17	0.05	$-8.22 \cdot 10^{-3}$	0.33	0.98
Obesity[2]~*H. pylori*									
Hp titer - crude	$-4.09 \cdot 10^{-4}$	$1.47 \cdot 10^{-4}$	**0.01**	$7.20 \cdot 10^{-4}$	$2.95 \cdot 10^{-4}$	**0.01**	$3.16 \cdot 10^{-4}$	$5.70 \cdot 10^{-4}$	0.58
Hp titer - adjusted[1]	$-3.92 \cdot 10^{-4}$	$1.48 \cdot 10^{-4}$	**0.01**	$0.90 \cdot 10^{-4}$	$3.07 \cdot 10^{-4}$	0.77	$-9.55 \cdot 10^{-6}$	$5.83 \cdot 10^{-4}$	0.99
Hp positivity - crude	-0.15	0.07	**0.04**	0.26	0.08	**$2.03 \cdot 10^{-3}$**	$1.25 \cdot 10^{-2}$	0.17	0.94
Hp positivity - adjusted[1]	-0.13	0.07	0.07	0.08	0.09	0.31	$-9.57 \cdot 10^{-2}$	0.17	0.58
Obesity classes[3]~*H. pylori*									
Hp titer - crude	$-1.04 \cdot 10^{-4}$	$3.80 \cdot 10^{-5}$	**0.01**	$5.62 \cdot 10^{-4}$	$1.25 \cdot 10^{-4}$	**$7.32 \cdot 10^{-6}$**	$1.78 \cdot 10^{-4}$	$2.27 \cdot 10^{-4}$	0.43
Hp titer - adjusted[1]	$-9.24 \cdot 10^{-5}$	$3.80 \cdot 10^{-5}$	**0.01**	$1.49 \cdot 10^{-4}$	$1.23 \cdot 10^{-4}$	0.23	$-5.52 \cdot 10^{-5}$	$2.21 \cdot 10^{-4}$	0.80
Hp positivity - crude	-0.05	0.02	**0.02**	0.17	0.04	**$1.13 \cdot 10^{-6}$**	0.05	0.07	0.45
Hp positivity - adjusted[1]	-0.04	0.02	**0.04**	0.06	0.03	0.08	-0.03	0.06	0.69

[1]adjusted for sex and age
[2]Obesity defined as BMI>30
[3] Lean (BMI < 18.5); normal-weight BMI >= 18.5 and < 25; overweight BMI >=25 and < 30; class I obesity BMI >=30 and < 35; class II obesity BMI >= 35 and < 40, class III obesity BMI >= 40

Table S2. Cross-sectional analyses regarding fecal *H. pylori* status and BMI/obesity

Model	**SHIP-TREND**	
BMI~*H. pylori*	**Beta (95% CI)**	**p-value**
Hp titer - crude	0.12 (-0.24, 0.48)	0.51
Hp titer - adjusted[1]	-0.08 (-0.43, 0.27)	0.67
Hp positivity - crude	0.21 (-0.44; 0.86)	0.52
Hp positivity - adjusted[1]	-0.05 (-0.68; 0.58)	0.88
Obesity[2]~*H. pylori*	**OR (95% CI)**	
Hp titer - crude	1.00 (0.83; 1.19)	0.97
Hp titer - adjusted[1]	0.94 (0.78; 1.13)	0.52
Hp positivity - crude	0.95 (0.68; 1.32)	0.76
Hp positivity - adjusted[1]	0.88 (0.63; 1.23)	0.46
Obesity classes[3]~*H. pylori*	**Beta (95% CI)**	
Hp titer - crude	0.01 (-0.04; 0.08)	0.82
Hp titer - adjusted[1]	-0.03 (-0.01; 0.04)	0.39
Hp positivity - crude	0.02 (-0.13; 0.15)	0.81
Hp positivity - adjusted[1]	-0.04 (-0.17; 0.09)	0.59

[1]Adjusted for age and sex
[2]Obesity defined as BMI>30
[3]Lean (BMI < 18.5); normal-weight BMI >= 18.5 and < 25; overweight BMI >=25 and < 30; class I obesity BMI >=30 and < 35; class II obesity BMI >= 35 and < 40, class III obesity BMI >= 40

Table S3. Mendelian randomization regarding serologic *H. pylori* status and BMI/obesity gene score

	Cohort								
Model	**RS**			**SHIP**			**SHIP-TREND**		
***H. pylori* titer~BMI gene score**	**Beta**	**se**	**p-value**	**Beta**	**se**	**p-value**	**Beta**	**se**	**p-value**
BMI gene score – crude	0.18	0.73	0.80	0.03	0.49	0.95	0.41	1.08	0.70
BMI gene score – adjusted[1]	0.16	0.73	0.83	-0.06	0.48	0.90	0.17	1.08	0.87
BMI gene score – adjusted[2]	0.49	0.74	0.51	-0.18	0.48	0.70	0.20	1.08	0.85
***H. pylori* positivity~BMI gene score**									
BMI gene score – crude	0.01	0.01	0.14	0.01	0.01	0.46	$-3.73 \bullet 10^{-3}$	0.02	0.85
BMI gene score – adjusted[1]	0.01	0.01	0.15	0.01	0.01	0.58	-0.01	0.02	0.62
BMI gene score – adjusted[2]	0.01	0.01	0.06	$2.18 \bullet 10^{-3}$	0.01	0.82	-0.01	0.02	0.62
***H. pylori* titer~Obesity gene score**									
Obesity gene score – crude	0.77	0.74	0.30	1.01	0.49	**0.04**	-0.43	1.06	0.69
Obesity gene score – adjusted[1]	0.76	0.74	0.31	0.97	0.48	**0.04**	-0.49	1.05	0.64
Obesity gene score – adjusted[2]	1.05	0.75	0.16	0.81	0.48	0.09	-0.46	1.06	0.66
***H. pylori* positivity~Obesity gene score**									
Obesity gene score – crude	0.01	0.01	0.42	0.02	0.01	0.11	$-4.13 \bullet 10^{-3}$	0.02	0.83
Obesity gene score – adjusted[1]	0.01	0.01	0.43	0.01	0.01	0.12	$-5.64 \bullet 10^{-3}$	0.02	0.77
Obesity gene score – adjusted[2]	0.01	0.01	0.26	0.01	0.01	0.26	$-5.71 \bullet 10^{-3}$	0.02	0.77

[1]Adjusted for age and sex
[2]Adjusted for age, sex, and BMI

Table S4. Mendelian randomization regarding BMI/obesity and *H. pylori* gene score

	Cohort								
Model	**RS**			**SHIP**			**SHIP-TREND**		
BMI~*H. pylori* gene score	**Beta**	**se**	**p-value**	**Beta**	**se**	**p-value**	**Beta**	**se**	**p-value**
Hp gene score – crude	-0.02	0.07	0.74	-0.04	0.11	0.69	-0.24	0.22	0.26
Hp gene score – adjusted[1]	-0.02	0.07	0.76	-0.08	0.11	0.43	-0.28	0.21	0.18
Hp gene score – adjusted[2]	-0.01	0.07	0.92	-0.09	0.11	0.40	-0.28	0.21	0.19
Obesity[3]~*H. pylori* gene score									
Hp gene score – crude	$-1.00 \cdot 10^{-3}$	0.05	0.99	$3.35 \cdot 10^{-3}$	0.05	0.95	-0.18	0.11	0.09
Hp gene score – adjusted[1]	$-3.46 \cdot 10^{-4}$	0.05	1.00	$-9.67 \cdot 10^{-3}$	0.05	0.86	-0.19	0.11	0.07
Hp gene score – adjusted[2]	0.01	0.05	0.87	$-7.23 \cdot 10^{-3}$	0.06	0.90	-0.20	0.11	0.06
Obesity classes[4]~*H. pylori* gene score									
Hp gene score – crude	$2.00 \cdot 10^{-3}$	0.02	0.92	-0.01	0.02	0.53	-0.06	0.04	0.17
Hp gene score – adjusted[1]	$2.00 \cdot 10^{-3}$	0.02	0.89	-0.02	0.02	0.33	-0.06	0.04	0.12
Hp gene score – adjusted[2]	$5.00 \cdot 10^{-3}$	0.05	0.87	-0.02	0.02	0.34	-0.06	0.04	0.12

[1]Adjusted for age and sex
[2]Adjusted for age, sex, and *H. pylori*
[3]Obesity defined as BMI>30
[4]Obesity classes defined as lean (BMI < 18.5); normal-weight BMI >= 18.5 and < 25; overweight BMI >=25 and < 30; class I obesity BMI >=30 and < 35; class II obesity BMI >= 35 and < 40, class III obesity BMI >= 40

Table S5. Cross-sectional analyses regarding fecal *H. pylori* status and BMI/obesity gene score

Model	**Cohort SHIP-TREND**	
***H. pylori* titer~BMI gene score**	**Beta (95% CI)**	**p-value**
BMI gene score – crude	$-1.20 \cdot 10^{-3}$ ($-1.5 \cdot 10^{-2}$; $1.27 \cdot 10^{-2}$)	0.86
BMI gene score – adjusted[1]	$-2.20 \cdot 10^{-3}$ ($-1.59 \cdot 10^{-2}$; $1.59 \cdot 10^{-2}$)	0.75
BMI gene score – adjusted[2]	$-2.10 \cdot 10^{-3}$ ($-1.58 \cdot 10^{-2}$; $1.16 \cdot 10^{-2}$)	0.77
***H. pylori* positivity~BMI gene score**	**OR (95% CI)**	
BMI gene score – crude	1.00 (0.99; 1.01)	1.00
BMI gene score – adjusted[1]	1.00 (0.99; 1.01)	0.88
BMI gene score – adjusted[2]	1.00 (0.99; 1.01)	0.89
***H. pylori* titer~Obesity gene score**	**Beta (95% CI)**	
Obesity gene score – crude	$2.60 \cdot 10^{-3}$ ($-1.11 \cdot 10^{-2}$; $1.61 \cdot 10^{-2}$)	0.70
Obesity gene score – adjusted[1]	$2.20 \cdot 10^{-3}$ ($-1.11 \cdot 10^{-2}$; $1.55 \cdot 10^{-2}$)	0.75
Obesity gene score – adjusted[2]	$2.40 \cdot 10^{-3}$ ($-1.09 \cdot 10^{-2}$; $1.57 \cdot 10^{-2}$)	0.72
***H. pylori* positivity~Obesity gene score**	**OR (95% CI)**	
Obesity gene score – crude	1.00 (0.99; 1.01)	0.99
Obesity gene score – adjusted[1]	1.00 (0.99; 1.01)	0.99
Obesity gene score – adjusted[2]	1.00 (0.99; 1.01)	0.99

[1]Adjusted for age and sex
[2]Adjusted for age, sex, and BMI

Table S6. List of SNPs associated with BMI or obesity

BMI			
Gene	**RS number**	**Trait**	**Reference**
FTO	rs9939609, rs9930506, rs1121980, rs1421085, rs8050136, rs1558902, rs17817449, rs12149832, rs9940128, rs62033400	BMI	Frayling Science 2007, Scuteri PLOS Genet 2007, Loos Nat Genet 2008, Thorleifsson Nat Genet 2009, Willer Nat Genet 2009, Cho Nat Genet 2009, Speliotes Nat Genet 2010, Wen Nat Genet 2012, Okada Nat Genet 2012, Guo HMG 2012, Graff Hum Mol Genet 2013, Pei HMG 2013
MC4R	rs17782313, rs571312, rs12970134, rs2331841, rs6567160, rs8089364, rs7234864, rs723486	BMI	Loos Nat Genet 2008, Thorleifsson Nat Genet 2009, Speliotes Nat Genet 2010, Paternoster PLOS One 2011Okada Nat Genet 2012, Wen Nat Genet 2012, Graff Hum Mol Genet 2013, Pei HMG 2013
MC4R	rs7227255, rs2229616	BMI	Speliotes Nat Genet 2010, Guo HMG 2012
TMEM18	rs6548238, rs7561317, rs2867125, rs12463617	BMI	Willer Nat Genet 2009, Thorleifsson Nat Genet 2009, Speliotes Nat Genet 2010, Guo HMG 2012, Graff Hum Mol Genet 2013
GNPDA2	rs10938397, rs13130484, rs348495	BMI	Willer Nat Genet 2009, Speliotes Nat Genet 2010, Graff Hum Mol Genet 2013
SH2B1	rs7498665, rs4788102, rs7359397, rs4788099	BMI	Willer Nat Genet 2009, Thorleifsson Nat Genet 2009, Speliotes Nat Genet 2010, Guo HMG 2012
KCTD15	rs11084753, rs29941	BMI	Willer Nat Genet 2009, Thorleifsson Nat Genet 2009, Speliotes Nat Genet 2010
MTCH2	rs10838738, rs3817334	BMI	Willer Nat Genet 2009, Speliotes Nat Genet 2010
NEGR1	rs2815752, rs2568958	BMI	Willer Nat Genet 2009, Thorleifsson Nat Genet 2009, Speliotes Nat Genet 2010
SEC16B	rs10913469, rs543874, rs574367, rs516636, rs591120	BMI	Thorleifsson Nat Genet 2009, Speliotes Nat Genet 2010, Graff Hum Mol Genet 2013, Wen Nat Genet 2012, Okada Nat Genet 2012
ETV5	rs7647305, rs9816226	BMI	Thorleifsson Nat Genet 2009, Speliotes Nat Genet 2010
BDNF	rs6265, rs4923461, rs10767664, rs2030323, rs10767664	BMI	Thorleifsson Nat Genet 2009, Speliotes Nat Genet 2010, Wen Nat Genet 2012, Okada Nat Genet 2012, Guo HMG 2012
FAIM2	rs7138803, rs7132908	BMI	Thorleifsson Nat Genet 2009, Speliotes Nat Genet 2010, Paternoster PLOS One 2011
TFAP2B	rs987237, rs734597, rs2272903	BMI	Speliotes Nat Genet 2010, Paternoster PLOS One 2011, Guo HMG 2012
NRXN3	rs10150332	BMI	Speliotes Nat Genet 2010
GPRC5BB	rs12444979	BMI	Speliotes Nat Genet 2010
POMC	rs713586, rs6545814, rs1561288	BMI	Speliotes Nat Genet 2010, Wen Nat Genet 2012, Graff Hum Mol Genet 2013
MAP2K5	rs2241423, rs4776970, rs997295	BMI	Speliotes Nat Genet 2010, Wen Nat Genet 2012, Guo HMG 2012
GIPR	rs2287019, rs11671664	BMI	Speliotes Nat Genet 2010, Wen Nat Genet 2012, Okada Nat Genet 2012
FANCL	rs887912	BMI	Speliotes Nat Genet 2010
TNNI3K	rs1514175, rs12142020	BMI	Speliotes Nat Genet 2010, Graff Hum Mol Genet 2013
LRRN6C	rs10968576	BMI	Speliotes Nat Genet 2010
FLJ35779	rs2112347	BMI	Speliotes Nat Genet 2010

BMI			
Gene	**RS number**	**Trait**	**Reference**
SLC39A8	rs13107325	BMI	Speliotes Nat Genet 2010
TMEM160	rs3810291	BMI	Speliotes Nat Genet 2010
CADM2	rs13078807	BMI	Speliotes Nat Genet 2010
LRP1B	rs2890652	BMI	Speliotes Nat Genet 2010
PRKD1	rs11847697	BMI	Speliotes Nat Genet 2010
MTIF3	rs4771122	BMI	Speliotes Nat Genet 2010
ZNF608	rs48361333	BMI	Speliotes Nat Genet 2010
PTBP2	rs1555543	BMI	Speliotes Nat Genet 2010
TUB	rs4929949	BMI	Speliotes Nat Genet 2010
HMGA1	rs206936	BMI	Speliotes Nat Genet 2010
CDKAL1	rs2206734, rs9356744	BMI	Wen Nat Genet 2012, Okada Nat Genet 2012
PCSK1	rs261967	BMI	Wen Nat Genet 2012
GP2	rs12597579	BMI	Wen Nat Genet 2012
KLF9	rs11142387	BMI	Okada Nat Genet 2012
TOMM40/APOE/APOC1	rs2075650	BMI	Guo HMG 2012
FANCL/FLJ30838	rs12617233	BMI	Guo HMG 2012
NTRK2	rs1211166	BMI	Guo HMG 2012
GALNT10	rs7708584	BMI	Monda NG 2013 african
MIR148A/NFE2L3	rs10261878	BMI	Monda NG 2013 african + european
ADCY3	rs7586879, rs6545814	BMI	Wen Nat Genet 2012 east asian, Monda NG 2013 african
BRE	rs116612809	BMI	Gong AJHG 2013
MAP2K3	rs11652094	BMI	Bian Hum Mol Genet 2013 Pimas + Europeans

Obesity binary status			
Gene	**RS number**	**Trait**	**Reference**
FTO	rs1421085, rs1121980, rs9936385, rs9941349, rs3751812, rs1558902, rs17817449	obesity, childhood obesity, young-onset extreme overweight, overweight	Dina Nat Genet 2007, Hinney PLOS One 2007, Meyre Nat Genet 2009, Costapas HMG 2009, Scherag PLOS Genet 2010, Paternoster PLOS One 2011, Wang PLOS One 2011, Bradfield Nat Genet 2012, Berndt Nat Genet 2013, Wheeler Nat Genet 2013
MC4R	rs17782313, rs17700144, rs663129, rs571312, rs476828	extreme obesity, obesity, overweight	Meyre Nat Genet 2009, Scherag PLOS Genet 2010, Bradfield Nat Genet 2012, Berndt Nat Genet 2013, Wheeler Nat Genet 2013

Obesity binary status			
Gene	**RS number**	**Trait**	**Reference**
PCSK1	rs6232	obesity	Benzinou Nat Genet 208
PCSK1	rs6234/rs6235	obesity	Benzinou Nat Genet 208
FAIM2	rs7132908, rs7138803	young-onset extreme overweight, obesity, overweight	Paternoster PLOS One 2011, Bradfield Nat Genet 2012, Berndt Nat Genet 2013
MAF	rs1424233	extreme obesity	Meyre Nat Genet 2009
NPC1	rs1805081	extreme obesity	Meyre Nat Genet 2009
SDCCAG8	rs12145833	childhood obesity	Scheragh PLOS Genet 2010
TNKS	rs17150703	childhood obesity	Scheragh PLOS Genet 2010
KCNMA1	rs2116830	obesity	Jiao BMC Med Genomics 2011
BDNF	rs988712, rs10767664	obesity, overweight	Jiao BMC Med Genomics 2011
TMEM18	rs4854344, rs2867125, rs6548238, rs12463617	childhood obesity, obesity, overweight	Bradfield Nat Genet 2012, Berndt Nat Genet 2012, Berndt Nat Genet 2013, Wheeler Nat Genet 2013
POMC	rs6752378, rs10182181, rs713586	childhood obesity, obesity, overweight	Bradfield Nat Genet 2012, Berndt Nat Genet 2013
TNNI3K	rs1040070	childhood obesity	Bradfield Nat Genet 2012
SEC16B	rs10913469, rs543874	childhood obesity, obesity, overweight	Bradfield Nat Genet 2012, Berndt Nat Genet 2013
OLFM4	rs9568856, rs9568867	childhood obesity, obesity	Bradfield Nat Genet 2012, Berndt Nat Genet 2013
HOXB5	rs9299	childhood obesity	Bradfield Nat Genet 2012
GPR120	rs116454156	obesity	Ichimura Nature 2012
HS6ST3	rs7989336	obesity	Berndt Nat Genet 2013
ZZZ3	rs17381664	obesity	Berndt Nat Genet 2013
GNAT2	rs17024258	obesity	Berndt Nat Genet 2013
HNF4G	rs4735692	obesity, overweight	Berndt Nat Genet 2013

Obesity binary status			
Gene	**RS number**	**Trait**	**Reference**
MRPS33P4	rs13041126	obesity	Berndt Nat Genet 2013
ADCY9	rs2531995	obesity	Berndt Nat Genet 2013
RPTOR	rs7503807	overweight	Berndt Nat Genet 2013
NEGR1	rs2815752, rs1993709	obesity, overweight	Berndt Nat Genet 2013, Wheeler Nat Genet 2013
GNPDA2	rs10938397	obesity, overweight	Berndt Nat Genet 2013
TFAP2B	rs987237, rs734597	obesity, overweight	Paternoster PLOS One 2011, Berndt Nat Genet 2013
TMEM160	rs3810291	obesity	Berndt Nat Genet 2013
ETV5	rs9816226	obesity, overweight	Berndt Nat Genet 2013
QPCTL	rs2287019	obesity, overweight	Berndt Nat Genet 2013
MTCH2	rs3817334	obesity, overweight	Berndt Nat Genet 2013
SH2B1	rs7359397	obesity, overweight	Berndt Nat Genet 2013
GPRC5B	rs12444979	obesity, overweight	Berndt Nat Genet 2013
MAP2K5	rs2241423	obesity, overweight	Berndt Nat Genet 2013
LRRN6C	rs10968576	obesity	Berndt Nat Genet 2013
TNNI3K	rs1514174, rs1514175	obesity	Berndt Nat Genet 2013
RPL27A	rs11042023	obesity	Berndt Nat Genet 2013
FLJ35779	rs2112347	obesity, overweight	Berndt Nat Genet 2013
FANCL	rs887912	obesity, overweight	Berndt Nat Genet 2013
CADM2	rs13078807	overweight	Berndt Nat Genet 2013
NRXN3	rs10150332	obesity	Berndt Nat Genet 2013
PRKCH	rs1957894	childhood obesity	Wheeler Nat Genet 2013
LEPR	rs11208659	childhood obesity	Wheeler Nat Genet 2013
PACS1	rs564343	childhood obesity	Wheeler Nat Genet 2013
RMST	rs11109072	childhood obesity	Wheeler Nat Genet 2013
NEGR1	rs3101336	childhood obesity	Wheeler Nat Genet 2013
LPIN2	rs643507	adult obesity in asthmatic subjects	Melen Clin Exp Allergy 2013

Table S7. Selected SNPs associated with BMI

		RS1				RS2				SHIP			
Gene	**SNP ID**	**Coded**	**Ref**	**Freq**	**Rsq**	**Coded**	**Ref**	**Freq**	**Rsq**	**Coded**	**Ref**	**Freq**	**Rsq**
FTO	rs9930506	A	G	0,58	0,99	A	G	0,57	0,99	G	A	0,45	1,00
MC4R (1)	rs17782313	T	C	0,75	1,00	T	C	0,74	1,00	C	T	0,25	1,00
MC4R (2)	rs7227255	G	A	0,97	0,96	G	A	0,98	0,96	A	G	0,02	0,94
TMEM18	rs6548238	C	T	0,83	1,00	C	T	0,83	1,00	C	T	0,82	1,00
GNPDA2	rs10938397	A	G	0,59	1,00	A	G	0,58	1,00	G	A	0,45	0,99
SH2B1	rs7498665	A	G	0,59	0,99	A	G	0,58	0,99	G	A	0,42	0,98
KCTD15	rs11084753	G	A	0,67	0,94	G	A	0,68	0,95	G	A	0,68	1,00
MTCH2	rs10838738	A	G	0,66	1,00	A	G	0,67	1,00	G	A	0,35	1,00
NEGR1	rs2815752	A	G	0,59	0,97	A	G	0,57	0,97	A	G	0,63	1,00
SEC16B	rs10913469	T	C	0,80	1,00	T	C	0,82	1,00	C	T	0,18	0,98
ETV5	rs7647305	C	T	0,79	1,00	C	T	0,80	1,00	C	T	0,79	0,98
BDNF	rs6265	C	T	0,81	1,00	C	T	0,80	1,00	T	C	0,20	1,00
FAIM2	rs7138803	G	A	0,63	1,00	G	A	0,63	1,00	A	G	0,42	1,00
TFAP2B	rs987237	A	G	0,83	1,00	A	G	0,83	1,00	G	A	0,20	1,00
NRXN3	rs10150332	T	C	0,79	1,00	T	C	0,78	1,00	C	T	0,22	1,00
GPRC5BB	rs12444979	C	T	0,85	0,99	C	T	0,85	1,00	T	C	0,15	1,00
POMC	rs713586	C	T	0,47	1,00	C	T	0,48	1,00	C	T	0,47	1,00
MAP2K5	rs2241423	G	A	0,77	1,00	G	A	0,76	1,00	A	G	0,22	1,00
GIPR	rs11671664	G	A	0,90	1,00	G	A	0,90	1,00	A	G	0,11	0,81
FANCL	rs887912	C	T	0,70	1,00	C	T	0,71	1,00	C	T	0,73	1,00
TNNI3K	rs1514175	A	G	0,43	1,00	A	G	0,44	1,00	G	A	0,59	1,00
LRRN6C	rs10968576	A	G	0,69	1,00	A	G	0,71	1,00	G	A	0,31	1,00
FLJ35779	rs2112347	T	G	0,63	0,98	T	G	0,64	0,99	G	T	0,37	0,99
SLC39A8	rs13107325	C	T	0,95	0,94	C	T	0,95	0,93	T	C	0,08	1,00
TMEM160	rs3810291	G	A	0,32	0,80	G	A	0,33	0,82	A	G	0,67	0,90
CADM2	rs13078807	A	G	0,80	1,00	A	G	0,79	1,00	G	A	0,20	0,99
LRP1B	rs2890652	T	C	0,82	0,98	T	C	0,82	0,98	C	T	0,19	1,00
PRKD1	rs11847697	C	T	0,96	0,97	C	T	0,95	0,97	T	C	0,04	0,96
MTIF3	rs4771122	A	G	0,74	0,91	A	G	0,74	0,91	A	G	0,72	0,96
PTBP2	rs1555543	C	A	0,58	0,98	C	A	0,56	0,98	C	A	0,57	1,00
TUB	rs4929949	T	C	0,48	0,99	T	C	0,49	0,99	C	T	0,49	1,00
HMGA1	rs206936	A	G	0,79	0,98	A	G	0,80	0,99	G	A	0,19	1,00
CDKAL1	rs2206734	C	T	0,83	1,00	C	T	0,83	1,00	T	C	0,18	1,00
PCSK1	rs261967	A	C	0,60	1,00	A	C	0,59	1,00	C	A	0,44	1,00
GP2	rs12597579	C	T	0,95	1,00	C	T	0,96	1,00	T	C	0,04	1,00
KLF9	rs11142387	A	C	0,47	0,99	A	C	0,46	0,99	C	A	0,54	0,98
TOMM40	rs2075650	A	G	0,85	1,00	A	G	0,86	1,00	G	A	0,16	0,69
FANCL/FLJ30838	rs12617233	C	T	0,61	0,97	C	T	0,60	0,96	C	T	0,59	1,00
NTRK2	rs1211166	A	G	0,79	1,00	A	G	0,79	1,00	A	G	0,79	1,00
GALNT10	rs7708584	A	G	0,43	1,00	A	G	0,42	1,00	G	A	0,58	1,00
MIR148A/NFE2L3	rs10261878	C	A	0,94	0,98	C	A	0,94	0,99	C	A	0,95	0,98
ADCY3	rs7586879	C	T	0,66	1,00	C	T	0,66	1,00	T	C	0,33	1,00
BRE	rs116612809	A	G	1,00	0,82	A	G	1,00	0,91	G	A	0,00	0,94
MAP2K3	rs11652094	C	G	0,71	0,98	C	G	0,71	0,95	C	G	0,71	1,00

Coded, coded allele
Ref, Reference allele
Freq, frequency of coded allele
Rsq, Imputation quality

Table S8. Selected SNPs associated with obesity

		RS1				RS2				SHIP			
Gene	SNP ID	Coded	Ref	Freq	Rsq	Coded	Ref	Freq	Rsq	Coded	Ref	Freq	Rsq
FTO	rs1421085	T	C	0,62	1,00	T	C	0,60	1,00	C	T	0,43	1,00
MC4R	rs17782313	T	C	0,75	1,00	T	C	0,74	1,00	C	T	0,25	1,00
PCSK1 (1)	rs6232	T	C	0,93	1,00	T	C	0,94	1,00	C	T	0,05	0,83
PCSK1 (2)	rs6234	G	C	0,73	0,98	G	C	0,73	0,99	C	G	0,26	1,00
FAIM2	rs7132908	G	A	0,62	0,98	G	A	0,62	0,96	A	G	0,43	0,98
MAF	rs1424233	T	C	0,50	1,00	T	C	0,50	1,00	C	T	0,51	1,00
NPC1	rs1805081	T	C	0,59	1,00	T	C	0,60	1,00	C	T	0,43	0,99
SDCCAG8	rs12145833	T	G	0,83	1,00	T	G	0,83	0,99	G	T	0,16	1,00
TNKS	rs17150703	G	A	0,90	1,00	G	A	0,90	1,00	A	G	0,10	1,00
KCNMA1	rs2116830	G	T	0,83	0,92	G	T	0,83	0,95	T	G	0,18	1,00
BDNF	rs988712	G	T	0,77	0,98	G	T	0,75	0,98	T	G	0,25	1,00
TMEM18	rs4854344	T	G	0,83	1,00	T	G	0,83	1,00	T	G	0,82	1,00
POMC	rs6752378	A	C	0,46	1,00	A	C	0,47	1,00	A	C	0,46	0,99
TNNI3K	rs1040070	G	C	0,44	0,99	G	C	0,44	0,99	C	G	0,58	0,95
SEC16B	rs10913469	T	C	0,80	1,00	T	C	0,82	1,00	C	T	0,18	0,98
OLFM4	rs9568856	G	A	0,86	0,98	G	A	0,86	0,97	A	G	0,12	1,00
HOXB5	rs9299	T	C	0,64	1,00	T	C	0,63	1,00	T	C	0,66	0,98
GPR120	rs116454156	G	A	0,99	0,37	G	A	0,99	0,42	A	G	0,02	0,70
HS6ST3	rs7989336	A	G	0,48	0,99	A	G	0,49	0,99	A	G	0,47	1,00
ZZZ3	rs17381664	T	C	0,59	0,97	T	C	0,59	0,96	C	T	0,40	0,96
GNAT2	rs17024258	C	T	0,97	0,99	C	T	0,97	0,99	T	C	0,04	0,98
HNF4G	rs4735692	G	A	0,41	0,98	G	A	0,42	0,97	G	A	0,41	1,00
MRPS33P4	rs13041126	T	C	0,73	1,00	T	C	0,74	0,99	C	T	0,25	1,00
ADCY9	rs2531995	C	T	0,36	1,00	C	T	0,39	1,00	T	C	0,62	1,00
RPTOR	rs7503807	A	C	0,55	0,99	A	C	0,55	1,00	C	A	0,45	1,00
NEGR1	rs2815752	A	G	0,59	0,97	A	G	0,57	0,97	A	G	0,63	1,00
GNPDA2	rs10938397	A	G	0,59	1,00	A	G	0,58	1,00	G	A	0,45	0,99
TFAP2B	rs987237	A	G	0,83	1,00	A	G	0,83	1,00	G	A	0,20	1,00
TMEM160	rs3810291	G	A	0,32	0,80	G	A	0,33	0,82	A	G	0,67	0,90
ETV5	rs9816226	T	A	0,83	1,00	T	A	0,83	0,99	T	A	0,82	0,98
QPCTL	rs2287019	C	T	0,80	1,00	C	T	0,81	0,94	T	C	0,21	0,74
MTCH2	rs3817334	C	T	0,61	0,99	C	T	0,62	0,99	T	C	0,40	1,00
SH2B1	rs7359397	C	T	0,60	0,95	C	T	0,59	0,96	T	C	0,42	0,98
GPRC5B	rs12444979	C	T	0,85	0,99	C	T	0,85	1,00	T	C	0,15	1,00
MAP2K5	rs2241423	G	A	0,77	1,00	G	A	0,76	1,00	A	G	0,22	1,00
LRRN6C	rs10968576	A	G	0,69	1,00	A	G	0,71	1,00	G	A	0,31	1,00
TNNI3K	rs1514174	C	T	0,44	1,00	C	T	0,45	1,00	T	C	0,58	1,00
RPL27A	rs11042023	C	T	0,66	1,00	C	T	0,65	1,00	C	T	0,65	0,99
FLJ35779	rs2112347	T	G	0,63	0,98	T	G	0,64	0,99	G	T	0,37	0,99
FANCL	rs887912	C	T	0,70	1,00	C	T	0,71	1,00	C	T	0,73	1,00
CADM2	rs13078807	A	G	0,80	1,00	A	G	0,79	1,00	G	A	0,20	0,99
NRXN3	rs10150332	T	C	0,79	1,00	T	C	0,78	1,00	C	T	0,22	1,00
PRKCH	rs1957894	G	T	0,90	0,99	G	T	0,90	0,99	G	T	0,91	0,95
LEPR	rs11208659	T	C	0,92	1,00	T	C	0,91	1,00	C	T	0,10	0,99
PACS1	rs564343	A	G	0,41	0,99	A	G	0,41	0,98	G	A	0,55	1,00
RMST	rs11109072	C	A	0,96	0,89	C	A	0,95	0,55	A	C	0,04	0,99
LPIN2	rs643507	T	C	0,98	0,92	T	C	0,98	0,94	T	C	0,98	1,00

Coded, coded allele
Ref, Reference allele
Freq, frequency of coded allele
Rsq, R-square

CHAPTER 8

Surveillance of premalignant gastric lesions – a multi-center prospective cohort study from low incidence regions

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ABSTRACT

Background and study aims: Patients with atrophy and metaplasia of the gastric mucosa are at risk for progression to gastric cancer. International guidelines therefore recommend endoscopic surveillance of premalignant gastric lesions. However, the diagnostic yield and preventive effect of surveillance require further study. We therefore aimed to assess the incidence of neoplastic progression in patients from a low-risk area and to assess discriminative tests to identify patients most at risk for progression.

Patients and methods: Patients with a previous diagnosis of atrophic gastritis (AG), intestinal metaplasia (IM), or dysplasia from six hospitals throughout the Netherlands and one in Norway were offered endoscopic surveillance according to European guidelines. All histological specimens were assessed according to the updated Sydney classification and the operative link for gastric intestinal metaplasia (OLGIM) system. In addition, we measured serum pepsinogens I and II, and gastrin-17 obtained before surveillance endoscopy.

Results: Two-hundred-and-eighty-four (mean age 57.8 year SD 11.4, M/F 142/142) patients were included. In 279 (98%) patients at least one surveillance endoscopy was performed. The mean follow-up time was 57 months (SD 36) with a total of 1,312 patient-years follow-up. Four subjects (1.4%) were diagnosed with high grade dysplasia or gastric cancer during follow-up. This occurred in 3 (2.2%) of 134 patients with OLGIM stage 0-II disease at baseline, versus 1 (1.9%) of 54 patients with stage III-IV (p=0.87). Two of these patients were successfully treated with endoscopic submucosal dissection, while the other two underwent a total gastrectomy. Patients with normal serology markers and limited disease did not develop gastric cancer during follow-up, (p=0.41) (serology available for n=234).

Conclusions: In a low gastric cancer incidence area, a surveillance program for premalignant gastric lesions can detect gastric cancer at an early curable stage with an overall risk of neoplastic progression of 0.3% per year. Use of serologic markers in endoscopic surveillance programs improves risk stratification. These data strengthens international guidelines in their recommendations on surveillance of premalignant gastric lesion.

INTRODUCTION

Gastric cancer is the third leading cause of cancer-related mortality, and the fifth most common malignancy worldwide (1). More than 70% of gastric cancer cases occur in developing countries, with highest incidences in Eastern Asia, Eastern Europe, and parts of central and South America. Although the global incidence and mortality rates are declining, the number of new cases and fatalities increase annually due to expansion and aging of the world population (2). In Western Europe, the age standardized rates of gastric cancer incidence are relatively low with 8.8 cases/ 100 000/ year in men and 4.3 cases in women (1). Nevertheless, mortality rates remain high, as most of the cases are diagnosed in a late stage, leading to a 5-year survival rate of approximately 21% in the Netherlands, and 24% in Norway (3).

Premalignant gastric lesions predispose to the development of intestinal type gastric cancer. *Helicobacter pylori* gastritis is considered the starting point of the carcinogenic pathway, in which inflammation evolves to gland loss, metaplasia, low-grade dysplasia, high-grade dysplasia and eventually gastric cancer in 1-2% of the infected patients (4). As a result, regular endoscopic follow-up is recommended in patients with premalignant gastric lesions (5). However, most of the patients with *H. pylori* and premalignant gastric lesions do not develop gastric cancer. Moreover, premalignant gastric lesions affect a substantial proportion of the population above 50 years of age (6). Furthermore, a proportion of patients show regression of premalignant lesions during endoscopic follow-up, either reflecting an effect of sampling or true regression after *H. pylori* eradication (7, 8). Therefore, identification and surveillance of subjects with an increased risk of gastric cancer is indicated. This will additionally result in a decrease in burden for patients and use of limited endoscopy resources.

Currently, it remains difficult to identify patients with an increased gastric cancer risk, and to determine optimal surveillance strategies in order to prevent invasive gastric cancer. In 2012, the first international guideline on this topic was published (MAnagement of Precancerous conditions and lesions of the Stomach - MAPS guideline) (5). This guideline recommended endoscopic surveillance in patients with moderate to severe atrophic gastritis, those with marked intestinal metaplasia in both antrum and corpus, and patients with dysplasia. Surveillance was not recommended for patients with atrophic gastritis or intestinal metaplasia limited to antrum. However, prospective cohort studies are needed to evaluate the yield of surveillance of premalignant gastric lesions, and to confirm that the guideline recommendations are also applicable for low-risk areas. Due to a lower incidence of gastric cancer in these areas there is less experience in a comprehensive endoscopic assessment of the gastric mucosa, which may influence the yield of surveillance.

In this multi-center prospective cohort study, we aimed to evaluate the incidence of neoplastic progression during endoscopic follow-up in patients living in low-incidence countries. The yield and value of the operative link on intestinal metaplasia assessment (OLGIM) classification and MAPS guideline recommendations were evaluated. We further aimed to assess the potential additional value of serologic markers like pepsinogens and gastrin on risk stratification.

PATIENTS AND METHODS

Patient selection

This prospective study was performed in six hospitals (one academic, 5 regional) in The Netherlands, and one regional hospital in Norway. Initials results of the study in 140 patients have been published before (7). Patients were eligible for inclusion if they were over 18 years of age and had a previous diagnosis of atrophic gastritis, intestinal metaplasia and/or dysplasia of the gastric mucosa. They were identified in the histopathology database of each hospital or selected from the outpatient clinic. Patients were excluded from participation if they had previously undergone upper gastrointestinal surgery, a previous diagnosis of gastric carcinoma, or any other malignancy not being in remission, if they had severe comorbidity limiting their expected survival to less than 2 years, portal hypertension, or a proven CDH1 mutation.

Eligible patients were included after written informed consent had been obtained. All patients underwent at least one surveillance endoscopy after the index endoscopy. Surveillance endoscopies were performed between April 2006 and January 2015. The institutional review boards of all participating hospitals approved the study.

Baseline data collection

Data on demographic details, including age, sex and ethnicity were registered for each patient. In addition, all patients were asked to complete a structured questionnaire including several items on lifestyle factors, medical history, medication use, and family history of gastric cancer.

Endoscopy procedures at baseline and during follow-up

All endoscopies were done using a standard forward-viewing videogastroscope (Olympus). At index endoscopy, biopsies had been taking using the local protocol, with non-targeted biopsies from the antrum and/or corpus, and targeted biopsies of visible lesions.

At the surveillance endoscopy, random biopsies were taken according to a standardized biopsy protocol from five standardized intragastric locations for histological assessment of the severity and distribution of premalignant gastric lesions (9, 10). Four biopsies were taken from the antrum, two from the angulus, two from the corpus greater curvature, two from the corpus lesser curvature, and two from the cardia. In case of endoscopic abnormalities or visible lesions in the stomach, further targeted biopsies were obtained. The time interval between surveillance endoscopies was 2 years, except for cases with low or high grade dysplasia (LGD / HGD). Those patients underwent an endoscopy within 1 year (LGD) or 6 months (HGD).

Histological assessment of surveillance biopsies

All participating hospitals used the same histopathological protocol. Biopsy samples were fixed in formalin, embedded in paraffin blocks, cut into 5-µm sections, stained with hematoxylin and eosin and processed for routine diagnosis. Pathologists from the participating hospitals assessed the biopsy samples of each intragastric location and scored them for presence of *H. pylori*, atrophic gastritis, intestinal metaplasia, and dysplasia. A representative part of all samples was reassessed by an expert pathologist, who was not aware of patient data, endoscopic findings, and the baseline and follow-up histological diagnoses of the general pathologist. If differences were observed, the expert score was used.

All specimens were scored according to the updated Sydney classification system (11). In general, for every biopsy sample *H. pylori* density, acute inflammation (by means of neutrophil infiltration), chronic inflammation (by means of mononuclear infiltration), atrophic gastritis, and intestinal metaplasia were assessed (0=absent, 1=mild, 2=moderate, 3=marked). Dysplasia was scored according to the Vienna system, which includes low grade dysplasia, high grade dysplasia and neoplasia (12).

The operative link on gastric intestinal metaplasia (OLGIM) system was used to evaluate the severity and distribution of intestinal metaplasia throughout the stomach (13). This system combines the IM score (no IM, mild, moderate or severe IM) of antrum and angulus, with the score of the corpus lesser and greater curvature, in order to classify patients into 5 stages (0 to IV).

Follow-up

The MAPS guideline recommends no follow-up in patients with mild or moderate AG or IM limited to antrum, as these patients are thought to have a low risk for progression to gastric cancer (5). In our cohort all patients were followed, at least until two endoscopies had shown no lesions or progression of histopathology. To evaluate the guideline, we categorized patients as low or high risk for developing gastric cancer. Patients with mild

and moderate AG or IM limited to antrum were considered as low risk, while patients with moderate to severe AG or IM in both antrum and corpus as well as those with dysplasia were considered as high risk.
In general, patients were categorized according to their most advanced lesion. Disease progression was defined as more advanced histology compared to the prior endoscopic evaluation. Regression was defined as the opposite, meaning that histopathology of surveillance biopsies showed less advanced histology. Progression to HGD/carcinoma was defined as neoplastic progression.
To assess the role of serological markers for identifying patients at risk, patients were divided into a low or high risk group, based on both histology and serology results. Patients were defined as low risk if histology showed AG and/or IM limited to the antrum and normal biomarkers and as high risk if histology showed AG and/or IM in both antrum and corpus and/or abnormal biomarkers.

Serological determinants
Fasting blood samples were taken before surveillance endoscopy. Separated serum samples were stored at -80 °C until use for analyses. Serologic testing of pepsinogens I and II, gastrin-17 and *H. pylori* antibodies was performed by commercial enzyme-linked immunosorbent assay (ELISA) tests (GastroPanel, Biohit, Helsinki, Finland). All tests were performed according to the manufacturers guideline.

Statistical analyses
Continuous variables were reported as mean with standard variation (SD) or median with 25th and 75th percentiles or range. Categorical variables were reported as counts and percentages. Chi-square test was applied to compare HGD/gastric cancer development between patients categorized at low and high risk. Analyses of variance (ANOVA) were used to compare means of serology markers between MAPS risk groups and OLGIM stages. Two-sided p-values <0.05 were considered statistically significant. Analyses were performed using SPSS software, version 23.

RESULTS

Baseline characteristics
In total 295 patients consented for surveillance of premalignant gastric lesions (**Figure 1**). After re-assessment of their baseline histology, 11 subjects (4%) had to be excluded because no premalignant lesions were observed, leaving a study population of 284 patients. Main baseline characteristics are summarized in **Table 2**. The median age at

baseline was 58 years (range 14-79 years). At index endoscopy, 4% of patients was diagnosed with AG, 87% with IM, and 9% with dysplasia as most severe lesion. Histology showed *H. pylori* infection in 71 patients (26%). In 22 patients (7%) targeted biopsies from visible lesions were obtained. These lesions were located in antrum (n=12), corpus (n=6), angulus (n=1), cardia (n=1), and data were missing for the remainder (n=2). Histopathology of these visible lesions showed gastritis or no abnormalities in 8 patients, intestinal metaplasia in 10 patients, low grade dysplasia in 3 patients, and high grade dysplasia in 1 patient.

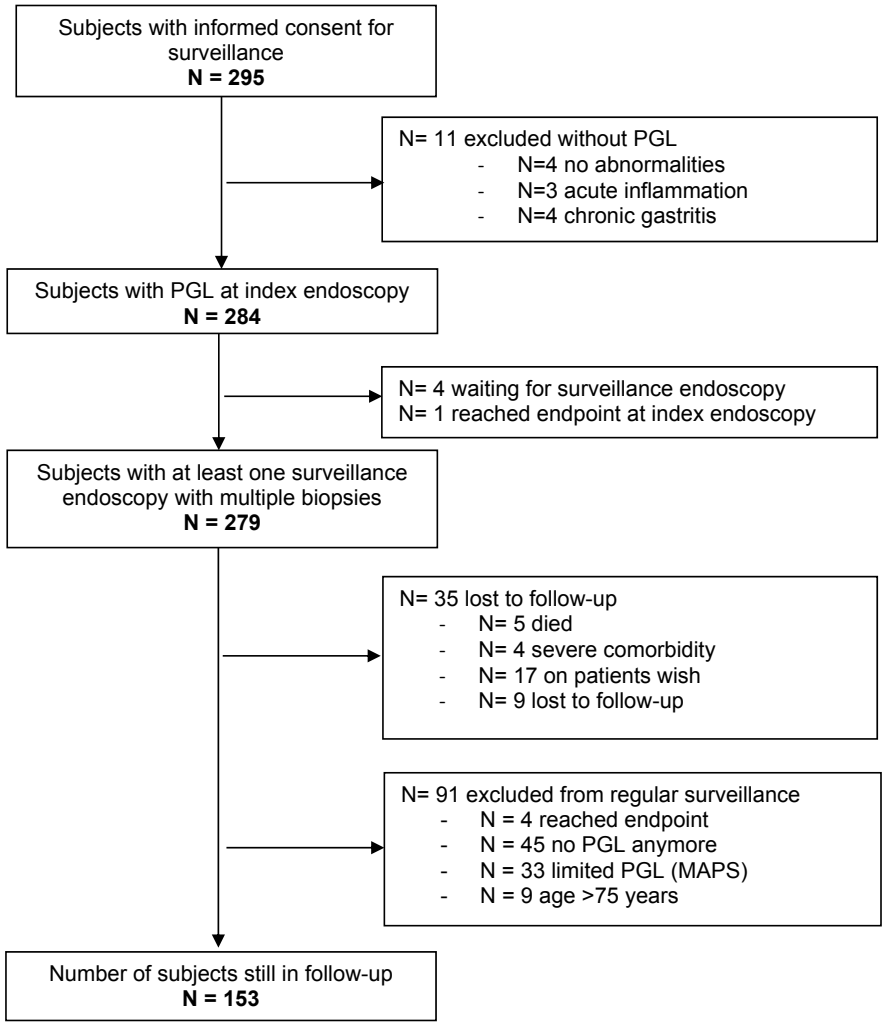

Figure 1. Study flow chart

The patients' OLGIM stage at index endoscopy was available for 190 patients (stage 0: n= 11; stage I: n=65; stage II: n=58; stage III: n=38; stage IV: n=16; **Table 1**).

Table 1. Baseline characteristics of 284 patients with premalignant gastric lesions

Variable	
Gender n (%)	
Male	142 (50)
Female	142 (50)
Mean age (SD)	57.8 (11.4)
Ethnicity (%)	
Caucasian	237 (84)
Non-Caucasian	44 (16)
Missing	*3 (1)*
BMI, kg/m^2 [a], SD	26.1 (4.3)
Missing	*20 (7)*
Smoking[a] (%)	
No	204 (75)
Yes	69 (25)
Missing	*11 (4)*
Alcohol[a] (%)	
No	127 (48)
Yes	136 (52)
Missing	*21 (7)*
Family history of gastric cancer (%)	
No	183 (72)
Yes	72 (28)
Missing	*29 (10)*
***H. pylori* infection[b] (%)**	
Negative	201 (74)
Positive	71 (26)
Missing	*12 (4)*
Most severe lesion (%)	
Atrophic gastritis	11 (4)
Slight IM	81 (29)
Moderate IM	114 (40)
Severe IM	53 (19)
Low grade dysplasia	23 (8)
High grade dysplasia	2 (1)
OLGIM classification (%)[c]	
0	11 (6)
I	66 (35)
II	58 (30)
III	38 (20)
IV	17 (9)
Missing	*93 (33)*

[a]At T1
[b]Based on histology at index endoscopy
[c]Only applicable if biopsies in antrum and corpus were taken at baseline

Progression and regression according to most severe lesion

Two-hundred seventy-nine (99%) patients underwent at least one surveillance endoscopy. The first surveillance endoscopy was performed after a mean time of 35 months (SD 28.8). In total, 814 endoscopies (average 2.9 per patient, range 1-7) were performed within a mean follow-up time of 57.1 months (SD 35.5) and a total of 1,312 patient-years. Persistent *H. pylori* infection based on histological evaluation was present in 14 patients (5%).

Table 2 shows the progression and regression by means of most severe gastric lesions at index endoscopy compared with last performed surveillance endoscopy. Progression occurred in 11 patients (4%). Progression to a visible dysplastic or early cancer lesion was observed in four patients (1.4%). This equals to one case of neoplastic progression per 328 patient years. Three out of these four patients had been diagnosed with IM at index endoscopy, and one with HGD (without visible lesions). Three patients (1.1%) progressed to LGD, which equals to one new case with LGD per 437 patient-years. In 165 patients (59%) no progression nor regression was found. Histology in these patients revealed IM in 98.2% of patients, AG in 1.2%, and LGD in 0.6% of patients. Regression was observed in 103 patients (37%). Twenty-two patients diagnosed with LGD at index endoscopy regressed to IM (73%), AG (5%) or gastritis/normal histopathology (23%). Eight of them (36%) had positive *H. pylori* histology and/or positive serology. Twenty-seven (32%) patients with IM at index endoscopy regressed to AG (13%), gastritis (40%), and normal histopathology (47%). Four (36%) patients with AG regressed to gastritis (25%) and normal histopathology (75%). No regression was observed in cases with HGD at baseline. *H. pylori* was successfully eradicated in 79% of the *H. pylori* positive patients at index endoscopy. In these patients stable disease or regression was observed in 93%, compared to 97% of patients who were *H. pylori* negative at time of index endoscopy (p= 0.14).

Progression and regression according to OLGIM stage

To assess OLGIM stages over time, only patients in whom biopsies from antrum and corpus were available at index endoscopy, were included. The results between index and last surveillance endoscopy according to the OLGIM stages are shown in **table 3**. This comparison revealed regression to a lower OLGIM stage in 48% of the cases after a mean follow-up time of 54 months (SD 35.9). In 32% of the patients the OLGIM did not change over time, whereas progression was observed in 20%. In **table 4** the OLGIM stages of the first and second surveillance endoscopy, with a planned surveillance interval of 24 months, are shown. In all these cases (n = 211) an extensive biopsy protocol was used. The mean follow-up time between first and second surveillance endoscopy was 24.6 months (SD 5.9).

Table 2. Regression and progression of premalignant gastric lesions in terms of most severe lesion

		Follow-up									
		Normal	Gastritis	AG	IM	LGD	HGD	Carcinoma	Regression	Stable	Progression
	N=279	40	36	13	183	4	1	2	103 (37%)	165 (59%)	11 (4%)
Baseline											
Normal	-	-	-	-	-	-	-	-	-		-
Gastritis	-	-	-	-	-	-	-	-	-		-
AG	11	3	1	2	5	0	0	0	4 (36%)	2 (18%)	5 (45%)
IM	244	36	31	10	162	3	1	1	77 (32%)	162 (66%)	5 (2%)
LGD	23	1	4	1	16	1	0	0	22 (96%)	1 (4%)	0 (0%)
HGD	1	0	0	0	0	0	0	1	0 (0%)	0 (0%)	1 (100%)

Squares denote the patients who had the same histology at both baseline and during follow-up (mean follow-up 57 months). Above the diagonal indicates patients in whom histology showed progression to a more severe lesion. Under the diagonal indicates patients in whom histology showed regression to a less severer lesion.
AG, atrophic gastritis; IM, intestinal metaplasia; LGD, low grade dysplasia; HGD, high grade dysplasia.

Table 3. OLGIM stage at index and last surveillance endoscopy in 190 patients (mean follow-up of 54 months)

		Tmax					Regression	Stable	Progression
		0	**I**	**II**	**III**	**IV**			
	N=190	65	39	42	37	6	91 (48%)	61 (32%)	39 (20%)
T0									
0	11	10	1	0	0	0	-	91%	9%
I	66	24	20	13	9	0	36%	30%	33%
II	58	17	11	15	14	1	49%	26%	26%
III	38	11	7	8	11	1	68%	29%	3%
IV	17	2	0	6	5	4	76%	24%	-

Squares denote the patients who had the same OLGIM stage (0, I, II, III, or IV) at both index (T0) and last surveillance endoscopy (Tmax) (mean follow-up 54 months). Above the diagonal indicates patients in whom OLGIM stage became higher during follow-up. Under the diagonal indicates patients in whom OLGIM stage became lower during follow-up.

Table 4. OLGIM stage at first and second surveillance endoscopy in 211 patients (mean interval of 25 months)

		T2					Regression	Stable	Progression
		0	**I**	**II**	**III**	**IV**			
	N=212	68	45	52	38	8	58 (27%)	99 (47%)	54 (25%)
T1									
0	75	48	16	10	1	0	-	64%	36%
I	30	8	12	7	2	1	27%	40%	33%
II	57	9	13	20	13	2	39%	35%	26%
III	32	2	4	8	16	2	44%	50%	6%
IV	17	1	0	7	6	3	82%	18%	-

Squares denote the patients who had the same OLGIM stage (0, I, II, III, or IV) at both first (T1) and second surveillance endoscopy (T2) (mean interval 25 months). Above the diagonal indicates patients in whom OLGIM stage became higher during follow-up. Under the diagonal indicates patients in whom OLGIM stage became lower during follow-up.

Progression and regression according to MAPS guideline

In total 202 patients (72%) were eligible for evaluation of the MAPS guideline, as biopsies were available from both antrum and corpus, or histology showed dysplasia at baseline. In our cohort 71 patients (35%) were categorized as low risk, and 131 (65%) as high risk. Their overall mean follow-up time was 54.9 months (SD 36.4). **Table 5** shows the regression and progression rate of both groups. Progression rate in the low risk group was 15%, compared to 2% in the high risk group ($p<0.001$). One patient (1%) of the low risk group was diagnosed with (early) gastric cancer, compared to 3 patients (2%) of the high-risk group ($p=0.67$), which equals to one neoplastic progression per 307 patients-years *vs.* one case per 205 patient-years.

Table 5. Regression and progression according to MAPS guideline

		Tmax					
		Low	**High**	**HGD/cancer**	**Regression**	**Stable**	**Progression**
	N=202	118	81	3	58 (29%)	131 (65%)	13 (6%)
T0							
Low risk	71	60	10	1	-	85%	15%
High risk	131	59	70	2	45%	53%	2%

Squares denote patients who had the same risk at both index (T0) and last surveillance endoscopy (Tmax) (mean follow-up time 55 months) Above the diagonal indicates patients who progressed to the high risk group or developed (early) gastric cancer during follow-up. Under the diagonal indicates patients who regressed to the low risk group. Low risk means patients with mild and moderate AG or IM limited to antrum, while patients with moderate to severe AG or IM in both antrum and corpus as well as those with dysplasia were considered at high risk.

HGD/gastric cancer

During follow-up, four patients were diagnosed with HGD/gastric cancer within a mean time of 49 months (SD 25.3) after index endoscopy. **Table 6** summarizes these patients. OLGIM stage at index endoscopy varied between stages I and IV. Two patients underwent successful endoscopic submucosal dissection (ESD) of the lesion. Histology of the resected specimens showed high-grade dysplasia without infiltrative growth, two intestinal type gastric cancers, and one diffuse type gastric cancer with signet cells. Histology of surrounding tissue of the lesion (assessed after surgery or ESD) showed IM in all specimens, except for the diffuse type carcinoma. One patient died 8 months after ESD, which appeared not to be related to gastric cancer or ESD. Total gastrectomy was performed in two patients: histology showed signet cells in one patient, and the other had dysplastic lesions in both antrum and cardia. The latter patient died 3 months post-operative due to septic shock based on massive intestinal ischemia and perforation.

Table 7. MAPS guideline, serology markers and detection of HDG/gastric cancer during follow-up

		Tmax
		HDG/gastric cancer
	N=234	3 (2%)
T serum		
Low risk	42	0 (0%)
High risk	192	3 (2%)

Based on both histology and serology results, patients were categorized into a low risk or high risk group. Low risk was defined as low risk according to MAPS guideline and normal biomarkers (no *H. pylori,* normal PgI/PgII and G-17 levels). High risk group was defined as high risk according to MAPS guideline or abnormal biomarkers. Both histology and serology were obtained at the same moment. HGD/gastric cancer was diagnosed at this time point (n = 1) or during follow-up (n = 2). Mean follow-up was 23 months (SD 17.1).

Table 6. Description of the location, OLGIM stage, and management of all cases with HGD/gastric cancer

Case	Age at diagnosis	Sex	Family history (FDR)	*H. pylori* at baseline	Visible lesion at baseline	Location	MAPS baseline	OLGIM baseline	OLGIM Tx	Follow-up time (months)	Treatment	Histology type	Histology surrounding mucosa
1	53	Female	Yes	No	No	NA	High	I	III	44	Total gastrectomy	Diffuse	Gastritis
2	65	Male	Yes	Yes	Yes, IM	Angulus + Cardia	NA	IV	III	80	Total gastrectomy	Intestinal	Severe IM
3	71	Male	No	No	No	Angulus	High	II*	II	43	ESD	Intestinal	Moderate IM
4	73	Male	No	Yes	Yes, IM	Antrum	Low	II	IV	58	ESD	HGD	Severe IM

*At least OLGIM II, as biopsies were taken from limited locations
ESD, endoscopic submucosal dissection; FDR, first degree relative with gastric cancer; hGD, high grade dysplasia; IM, intestinal metaplasia; NA, not applicable; OLIGIM, operative link on gastric intestinal metaplasia; Tx, last endoscopy before treatment

***Serologic markers: pepsinogens, gastrin, and* H. pylori**

During follow-up, serum samples were obtained from 234 patients (84%). In 61 patients (26%) all biomarkers tests were normal. In this group, one patient was diagnosed with HGD/gastric cancer, compared to two patients with abnormal serology results (p = 0.77). *H. pylori* serology was positive in 121 patients (52%). According to the manufacturers' references, antral atrophic gastritis (defined as low G17, normal PgI/II ratio), was observed in 58 patients (25%), while atrophic gastritis limited to the corpus (defined as normal G17, low PgI/II ratio) was measured in 37 (16%) patients. In only one patient an atrophic pangastritis was observed (defined as low PgI/II ratio and low G17). Serologic markers with their corresponding OLGIM stage are depicted in **Figure 2**. Mean serologic levels of PgI, PgI/PgII, and G-17 differed significantly between OLGIM categories, but were not consistently correlated with an increase in OLGIM stage. The mean values PgI, PgII, PgI/PgII, and G-17 differed significantly between low and high MAPS risk groups with serology and histology showing matching low and high risk categories (**Figure 3**). When patients were divided into low and high risk groups based on both histology and serology results, no patients in the low risk group progressed to HGD/gastric cancer, compared to 3 patients in the high risk group (p = 0.41). One out of the four cases who progressed to HGD/gastric cancer could not be included in above descripted comparison due to missing serologic data.

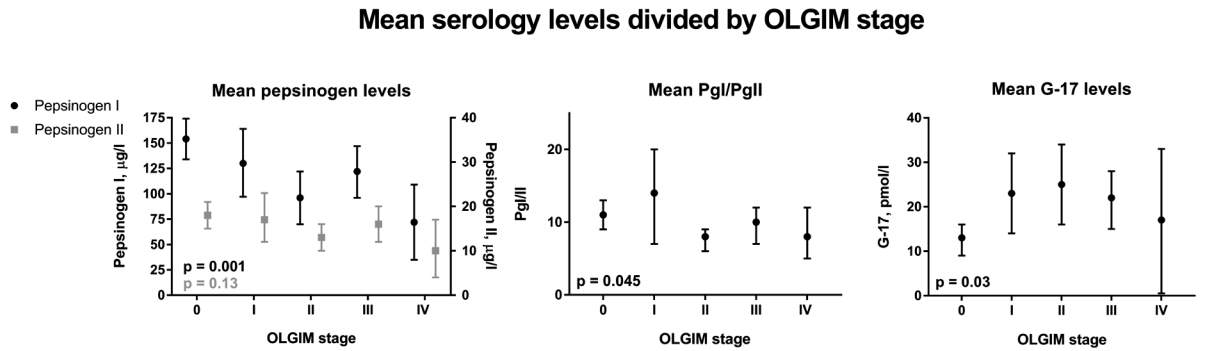

Figure 2. Mean serology markers divided by OLGIM stage
These plots show the mean serology levels with their 95% confidence interval. Data was available for 235 patients, who were divided by OLGIM stage (stage 0: n=85, stage I: n=35, stage II: n=61, stage III: n=42, stage IV: n=12).

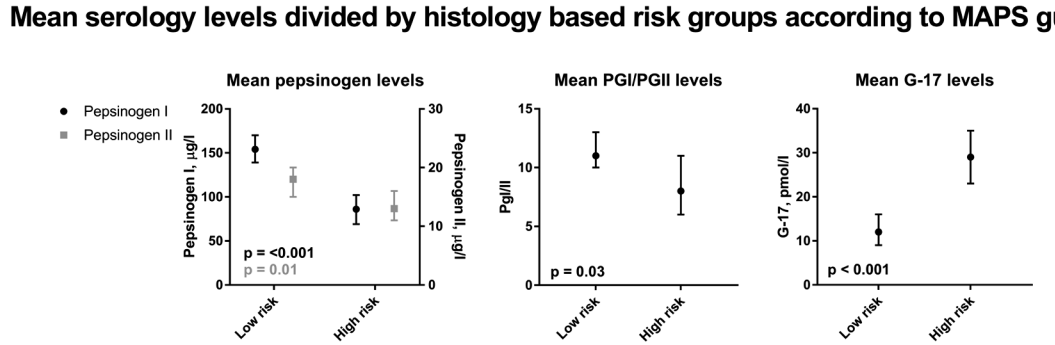

Figure 3.
These plots show the mean serology levels with their 95% confidence interval. Data was available for 235 patients, who were divided according to the MAPS guideline. Low risk was defined as patients with mild and moderate AG or IM limited to antrum, while patients with moderate to severe AG or IM in both antrum and corpus as well as those with dysplasia were considered at high risk (low risk: n=136, high risk: n=99).

DISCUSSION

Although the incidence of gastric cancer has strongly declined in most Western countries over the past 80 years, it remains a relatively common malignancy with marked differences between subgroups in our populations. We have over the years come to understand risk factors for progression. Two recent guidelines thus recommended risk stratification of patients with surveillance of those at highest risk (5, 14). These recommendations need further support from prospective cohort studies that report on the incidence of disease progression, and also assess methods for risk categorization. This multi-center prospective cohort study helps to provide this background. It showed stable disease in the majority of patients during an average 4.7 years follow-up. In total, four cases (1.4%) showed neoplastic progression and were diagnosed with early curable HGD/gastric cancer during follow-up. Neither the OLGIM classification, nor the MAPS guideline were completely sufficient in discriminating low risk from high risk patients as a sole selection tool. Addition of serology markers helped to identify the subgroup without progression to neoplasia during follow-up.

The majority of patients with precursor lesions will never develop gastric cancer. Consequently, the yield of surveillance endoscopy in all patients with premalignant lesions is low. This phenomenon is also known for surveillance of Barrett's esophagus, and colorectal surveillance after adenoma removal. Surveillance of all patients leads therefore to an unnecessary burden for patient and endoscopic resources. It is known that the gastric cancer risk increases with progression of the lesion according to the Correa cascade (15, 16). The international MAPS consensus guideline therefore recommended endoscopic follow-up only for patients with marked AG and IM in both antrum and corpus, and for those with dysplasia (5). We are the first to evaluate this approach in a prospective study.

The rate of neoplastic progression did not significantly differ between high and low risk patients, categorized according to the MAPS guideline. One patient, categorized as not in need for endoscopic surveillance, developed a visible lesion with HGD that was successfully removed by ESD. Nevertheless, the majority of patients (85%) in this low risk group had stable disease during follow-up. Long-term follow-up is necessary to confirm that indeed most patients will not develop gastric cancer in future. The same applies for the OLGIM classification, which has been shown as an accurate staging system for the extent and severity of intestinal metaplasia (13). This staging system is comparable with the operative link on gastritis assessment (OLGA) (17), but interobserver agreement for scoring IM was superior to AG (kappa 0.9 *vs*. 0.6) (13). High stages of OLGA and OLGIM are associated with a higher risk for progression to gastric cancer, and endoscopic surveillance may only be necessary in these patients (18). Nevertheless, three out of four

patients who developed gastric cancer during follow-up had a low OLGIM stage (I-II) at index endoscopy. It indicates that one risk stratification tool based on histopathology only is not sufficient for distinction between low and high risk patients.

In our study, combining histopathology data with serologic markers adequately discriminated between low risk and high risk patients. None of the patients with mild or moderate AG/IM limited to antrum (low risk according to MAPS) and normal serologic parameters developed HGC/gastric cancer during follow-up. Pepsinogen I and II are pro-enzymes produced by the gastric mucosa, which can be used in the assessment of the functional status of gastric mucosa (19). A pepsinogen I/II ratio <3 is well related with corpus atrophy (20). Serum gastrin-17 correlates with the functional status of gastric antral glands, and levels are reduced in case of severe antrum atrophy (19). Normal serology levels, including a negative *H. pylori* test, gives greater confidence in stopping endoscopic surveillance in patients with moderate AG/IM limited to the antrum. Serology tests are non-invasive for patients and its implementation in risk stratification and decision making on endoscopic surveillance could help reducing the burden on patients and endoscopic resources.

Regression of histopathology findings was observed in about one third of the patients. Remarkably, more than 90% of the cases with LDG at index endoscopy showed regression to less advanced lesions during follow-up. This regression occurred after *H. pylori* eradication in some patients, but may in part also have been related to sampling error, especially if lesions had a patchy distribution. Regression may be a reason for ending the endoscopic surveillance. In a large population based cohort no increased incidence for gastric cancer was found in patients with regression to less advanced lesions after 10 years follow-up, except patients with dysplasia at baseline (16).

Our study is limited by the relatively short follow-up time of 4.7 years. Gastric cancer development is usually a slowly ongoing process. This implies that patients at risk should be followed longer. A second limitation is that we used regular white-light endoscopy. The use of narrow band imaging (NBI) is recommended, but still not routine practice in most countries (5). As such, our study reflects daily clinical practice. This study is also limited by the small number of patients with neoplastic progression. Both The Netherlands and Norway represent low incidence regions for gastric cancer. The number of four cases with HGD/gastric carcinoma allowed to use descriptive statistics only.

In conclusion, this multi-centre prospective cohort study in a low gastric cancer area showed that with a surveillance program for premalignant gastric lesions, gastric cancer can be detected at an early curable stage with an overall risk of neoplastic progression of 0.3% per year. Risk stratification only based on histopathology may result in excluding patients at risk for gastric cancer from follow-up. Serology markers as non-invasive tool for differentiation between low and high risk patients may be added.

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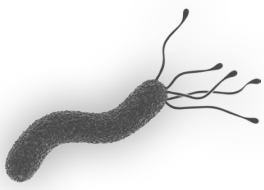
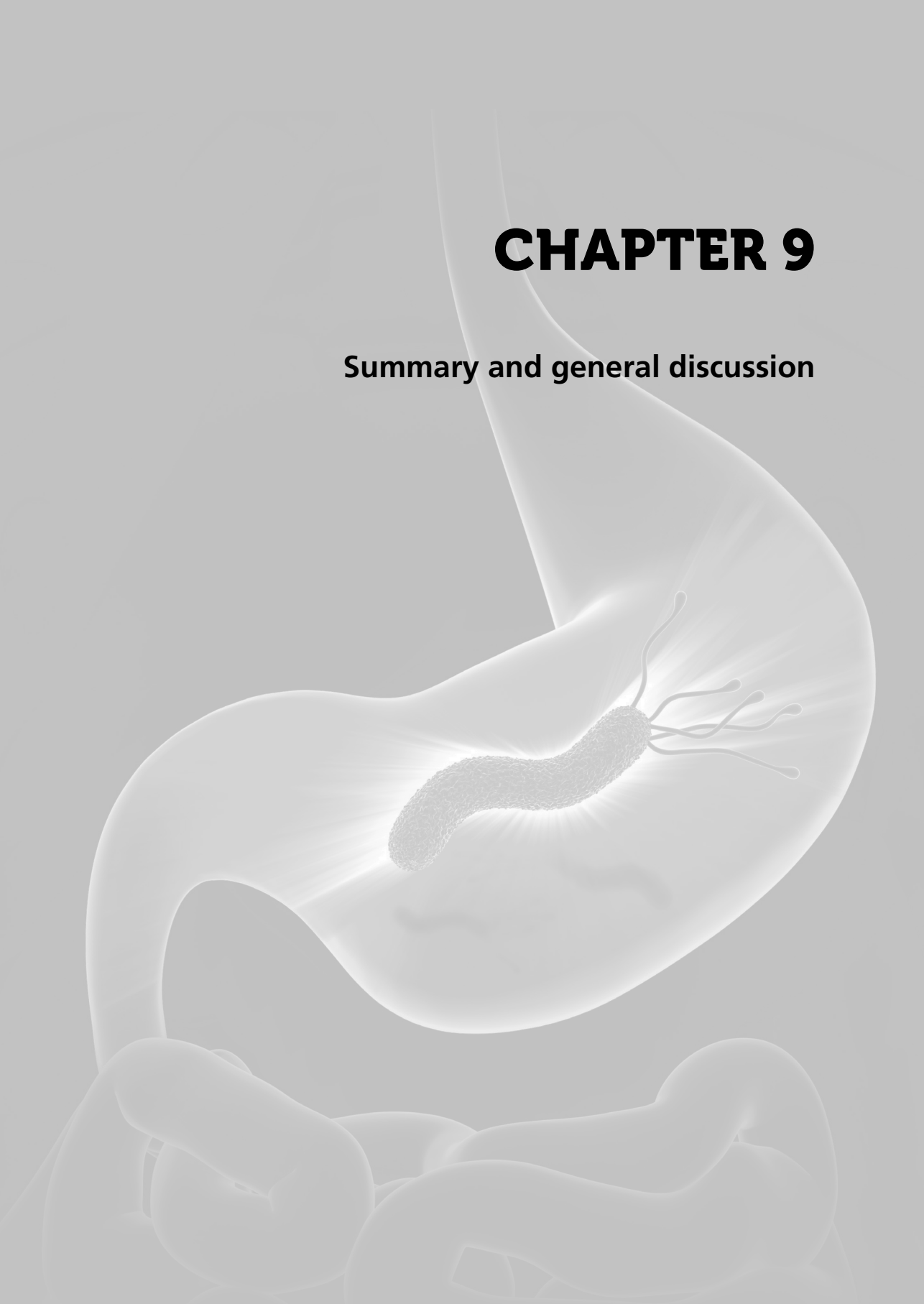
PART IV

Discussion



CHAPTER 9

Summary and general discussion



SUMMARY

This thesis focused on the epidemiology of *Helicobacter pylori*, the association with extra-gastric diseases, and the surveillance of premalignant gastric lesions. It consists of in total four different parts. Part one provides an introductory summary on the main and related topics of this thesis, including gastritis, epidemiology and treatment of *H. pylori*, associated non-malignant diseases, and premalignant gastric lesions. Part one ends with a description of the aims and outline of this thesis.
In part two, we showed that ethnicity is a strong predictor for *H. pylori* colonization in young women. As mothers are important for transmission of *H. pylori* to the next generation, these data show that *H. pylori* will remain prevalent in groups with a non-Western ethnic background. Despite the great variance in *H. pylori* prevalence between ethnic groups, the intergenerational decline was similar for all groups. It indicates the importance of environmental factors in the transmission of *H. pylori*.
In part three we showed that *H. pylori* colonization is an independent risk factor for severe nausea and vomiting during pregnancy, leading to low maternal weight gain and an increased risk for the newborn of small for gestational age. *H. pylori* colonization in pregnant women appeared also to be associated with an increased risk on preeclampsia. This makes eradication of *H. pylori* in women with a complicated pregnancy an attractive target for future intervention studies. We observed no significant protective association of *H. pylori* status in children at age 6 with asthma and related conditions. Instead, colonization with a CagA-negative *H. pylori* strain at age 6 was a risk factor for asthma, but only in those of European ethnic background. The underlying mechanisms for the observed risk differences require further research. The Mendelian randomization study showed no causal bi-directional link between *H. pylori* and obesity or body mass index. Follow-up of patients with premalignant gastric lesions revealed a low incidence of neoplastic progression. Risk stratification may benefit from serology markers, including pepsinogens, for adequate differentiation between low and high risk patients.
The final part of this thesis discusses the main conclusions from our research projects and directions for future research.

EPIDEMIOLOGY OF *H. PYLORI* IN A MULTI-ETHNIC POPULATION (PART II)

H. pylori is a gram-negative bacterium, which colonizes more than half of the world's population. The prevalence of *H. pylori* widely varies geographically, and is highest in developing countries. In Western countries, however, prevalences have been declining over recent decades to below 40% – supposedly as a result of improved hygiene and

sanitation and the active elimination by antibiotics (1). In parallel to the declining infection rate, incidences of *H. pylori*-related diseases also have shown a major decline (2, 3). Acquisition of infection usually occurs in early childhood, and is mainly transmitted within families (4). As test-and-treat strategies for *H. pylori* both on population as well as individual level are unlikely to be cost-effective in low prevalence countries, identification of risk groups is necessary (5). Knowledge about specific risk groups may permit assessment of disease risk, and will offer opportunities for targeted interventions. The *H. pylori* colonization rate is associated with factors such as age, socioeconomic status, childhood crowding, and non-western ethnicity (6). During past decades, many immigrants from developing countries with high *H. pylori* prevalence have settled in Western urban areas. In Rotterdam, a large Western city, more than 50% of the urban population originated from outside The Netherlands. The largest non-Dutch ethnic groups consist of people from Morocco, Turkey, Suriname, Dutch Antilles, and Cape Verde. Previous studies indicated that *H. pylori* colonization rates in immigrants are higher compared to native western populations (7-9). Some of these migrant communities have a high risk of gastric cancer (10, 11), in which *H. pylori* is involved as the major causative agent. We defined the presence of antibodies against *H. pylori* and CagA within pregnant women participating in a large population based cohort, called the Generation R study (**chapter 2**). Overall, 46% of the 6,837 tested women were *H. pylori*-positive, and 35% of them were CagA-positive. Prevalences of *H. pylori* were highly variable between the different ethnic groups, with the lowest prevalence in Dutch women (24%), and the highest in Moroccan women (92%). The proportion of CagA-positive strains was also higher among women of non-Dutch origin. Multivariate logistic regression showed ethnicity as the strongest predictor for *H. pylori* colonization. The relatively high prevalence of *H. pylori* and CagA-positive strains found in ethnic minority groups indicates that the health risks imposed by *H. pylori* remain a significant concern. Studies comparing first and second-generation immigrants with the native population have demonstrated a higher risk for non-cardia gastric cancer in first-generation immigrants (12, 13). Childhood colonization with *H. pylori* could be a possible cause (4, 14). Therefore, these migrant communities constitute target groups for screening of *H. pylori* to minimize *H. pylori*-related diseases. The prevalence of colonization differs between children and adults (15). Several cross-sectional surveys in Western countries have shown that *H. pylori* prevalence increases with age (16, 17). Since acquisition during adulthood is rare (18, 19), the higher prevalence in the elderly rather reflects a birth cohort effect with higher rates of childhood exposure to the organism in the past (1). We analysed the serum of 4,467 children (age 6 year) participating in the Generation R study, and compared the results with the *H. pylori* status of their mothers (available for 3,185 children). These results are described in **chapter 3**. Overall, 438 (10%) children were *H. pylori*-positive, of whom

142 (32%) were CagA-positive. Independent risk factors for colonization were: maternal *H. pylori* positivity, non-Dutch ethnicity, female gender, and lower maternal education level. Unexpectedly, by comparing mothers and children, we found an intergenerational decrease of 76% and 77% for *Hp*$^{+}$CagA^{-} and *Hp*$^{+}$CagA^{+}-strains, respectively, consistent across all nine ethnic groups studied. Male gender, higher maternal educational level, and no older siblings, were independently associated with absence of *H. pylori*. A recent study from Japan independently confirms a similar decline in two generations: the prevalence in mothers and their children showed a reduction of 72% (20). Our findings imply that environmental factors and living conditions of the country in which a child is raised have a major impact on transmission, irrespective of ethnicity. It supports the hypothesis that in contemporary societies there are high prevalent factors that interfere with the early life acquisition and/or maintenance of *H. pylori*. Besides the involvement of socio-economic status, family size, and other living conditions, possible candidates include the widespread use of antibiotics, particularly in young children. The association of specific *H. pylori* types in mother and child provides further evidence supporting a role for maternal inheritance in early life transmission, shown in molecular typing studies (21). However, as one-third of the *H. pylori* positive tested children had an *H. pylori*-negative mother, other transmission sources must also play a role, such as fathers and siblings (21), or grandmothers (20). Unfortunately, we were not able to test them for *H. pylori* positivity. There also is evidence for transmission from outside the nuclear family, including environmental reservoirs or other children in the community (22, 23), although these seem more likely in developing than in developed countries (24). Day-care centres may also act contact environment for child-to-child transmission (25). However, in our study we observed no association between day-care attendance and *H. pylori* status.

Future perspectives

The yield and cost-effectiveness of screening and eradication of *H. pylori* in migrant communities requires further research. In particular, the focus should be on first-generation immigrants, as their children born in a Western country already have a lower risk on being *H. pylori* positive. Due to the importance of environmental factors in transmission, one may expect that differences in prevalence between children of the next generation will be less distinct and closer to the prevalence of children from Dutch origin. Nevertheless, given the actual refugees movements from high endemic regions, this topic will remain relevant in the coming future.

H. PYLORI, EXTRA-GASTRIC DISEASES, AND PREMALIGNANT LESIONS (PART III)

H. pylori *and pregnancy complications*

In the Western world, severe nausea and vomiting during pregnancy (NVP) more often affects socially disadvantaged women and those of non-Western ethnicity (26, 27). To date, there is no clear explanation for the risk differences between Western and non-Western ethnic groups. In a recent meta-analysis, colonization with *H. pylori* was positively associated with severe NVP (28). Interestingly, as observed in chapter 2, the *H. pylori* prevalence in pregnant women of Western ethnicity is much lower than in women of non-Western ethnicity (29).

In **chapter 4**, we investigated the association of *H. pylori* with vomiting severity and single and combined effects of *H. pylori* and daily vomiting on birth outcomes, in pregnant women participating in the Generation R study. Furthermore, we investigated whether *H. pylori* explains the marked ethnic differences in maternal daily vomiting incidence. In total, 5,549 women were included. *H. pylori*-positive women (n=2,363) were more likely to suffer from daily vomiting (adjusted odds ratio 1.44, 95%CI 1.16;1.78). *H. pylori*-positivity reduced total weight gain in women with daily vomiting (adjusted difference -2.1 kg, 95%CI -2.7;-1.5) and infants born to women with *H. pylori* and daily vomiting had an increased risk of being small for gestational age (SGA; *a*OR 1.49, 95%CI 1.04;2.14). To our knowledge, this was the first prospective cohort reporting on the association between *H. pylori* and daily vomiting as well as birth outcomes. The study confirms the association between *H. pylori* and daily vomiting and adds to the existing evidence that the presence of *H. pylori* is associated with low maternal weight gain. More importantly, we found evidence that *H. pylori* contributes to SGA. It is possible that *H. pylori* has a local gastrointestinal effect leading to NVP symptoms, and a systemic placental effect resulting in an increased risk for SGA. The involvement of systemic inflammatory responses in complicated pregnancies has led to the hypothesis that maternal infections may play a role in the etiology and pathogenesis of these pregnancy complications (30, 31). Although the exact causes are still unknown, one hypothesis for their origin is that they each are related to suboptimal placentation in early pregnancy (32-34). In this respect, colonization with *H. pylori* may be of interest as it might be involved in the pathogenesis of impaired remodeling of the spiral arteries (35). It has been shown that anti-CagA antibodies *in vitro* were able to recognize β-actin on the surface of trophoblast cells in a dose-dependent binding activity (35). This binding resulted in impaired cytotrophoblast invasiveness, which is crucial for the development of placental mediated pregnancy complications.

In **chapter 5** we focused on *H. pylori* and three pregnancy related complications separately, including pre-eclampsia (PE), fetal growth restriction (SGA), and spontaneous preterm birth (PTB). In total, 6,348 pregnant women were assessed, all participating in the Generation R study. Multivariate analysis showed *H. pylori* colonization associated with PE (aOR 1.51; 95%CI 1.03-2.25). Differentiation according to CagA-status revealed increased risk for SGA (aOR 1.34; 1.04-1.71) in CagA-positive mothers. No association was observed between *H. pylori* and PTB. Our data suggest that *H. pylori* colonization could be a risk factor for PE and that carriage of a CagA+ *H. pylori* strain is a risk factor for SGA. These findings may be helpful for a better understanding of the pathogenesis of these pregnancy complications and support the link with chronic inflammatory conditions. Although PE, SGA, and PTB are different clinical entities, all three may be caused by suboptimal deep placentation in early pregnancy (34) (36-39). Large numbers of non-transformed spiral arteries are frequently observed in PE patients with or without SGA (34), in patients with SGA without gestational hypertension (36, 37), and in patients with preterm labor with or without preterm pre-labor rupture of membranes (PROM) (38, 39). Impaired remodeling of the spiral arteries may lead to insufficient uteroplacental arterial flow and episodes of irregular placental perfusion (32). Such impaired remodeling might be due to failure of appropriate uterine preconditioning (40), and excessive or atypical maternal immune responses to trophoblasts (41). Although association does not directly imply causation, our findings may have a biological explanation, as observed in the study mentioned above (35).

Future perspectives

If these associations are confirmed by future studies and shown to be causal, screening and intervention (e.g. *H. pylori* eradication treatment) may reduce related perinatal morbidity and mortality. The need for such intervention studies is highlighted by the fact that treatment options for severe NVP are currently lacking. Several case studies have reported that *H. pylori* eradication effectively relieved symptoms in women with persistent vomiting, unresponsive to conventional treatment. No teratogenic effects of PPI use in early pregnancy, or amoxicillin, and metronidazole were described (42-45). These studies indicate that triple therapy consisting of PPI, amoxicillin and metronidazole may be safely used in pregnancy. Further evidence from a randomised controlled trial is needed to confirm the effectiveness of *H. pylori* eradication on NVP symptom reduction, PE, and adverse birth outcomes.

H. pylori *and asthmatic conditions*

H. pylori is suggested to have not only pathogenic properties. Considered as a human commensal, *H. pylori* is thought to influence the development of the host immune

system (16). *H. pylori* has a strong interaction with the gastric mucosa. Because of the adaptive immunological activity of the stomach in terms of both T and B-cell function, it is thought that this interaction influences the maturation of the immune system. The "disappearing microbiota" hypothesis states that changes in our microbiota affected by altered ecological circumstances explain the increasing prevalence of atopic diseases like asthma and allergy obesity (16). *H. pylori* is a specific component of the human microbiome. In this context, several epidemiological studies showed an inverse relation between *H. pylori* colonization and asthma occurrence (46), but data are conflicting.

In **chapter 6**, we examined the association between *H. pylori* colonization in 3,797 children and risk of asthma and related conditions at school age. We secondly examined additional effects of maternal *H. pylori* status by pairing with children's status. A child's colonization with a CagA-negative-*H. pylori* strain was associated with an increased risk of asthma (Odds ratio 2.11; 95% CI 1.23-3.60, but this differed for European (3.64; 1.97-6.73) and non-European (0.52; 0.14-1.89) children. When taking into account maternal *H. pylori* status, only *H. pylori* positive children with an *H. pylori* negative mother had increased risk of asthma (2.42; 1.11-5.27), accounting for 3.4% of the asthma risk. These results do not support the "disappearing microbiota" hypothesis for children at the age of 6. Based on our results only, we are not able to explain the risk difference between children of European and non-European ethnic background. Nevertheless, we have the following considerations. First, in the group of children with an European ethnic background, none of the asthmatic children were colonized with a CagA-positive strain. This suggests that the asthma risk may be lower in CagA-positive children compared with *H. pylori*-negatives. Due to lack of CagA-positives in asthmatic children in this subgroup, the overall detected effect of *H. pylori*-positivity might be skewed to an increased risk for asthma. It has been shown that CagA-positive strains have a stronger interaction with their hosts, leading to pronounced immune responses (47). Second, although an association between *H. pylori* and asthma was only present in children of European ethnic background, consistent trends were observed for wheezing, eczema, and inhalant allergy, either for CagA-negative or CagA-positive strains. Such differences may reflect variation of the gut microbiome by ethnicity, in both richness and composition. Children (48) and adults living in developing countries have significantly greater faecal diversity than those in developed countries (49). Among subjects differing in ethnic background, but migrating to the same country, gut microbiome composition also varies (50). Within this context, we speculate that the effects of *H. pylori* on asthma or related disease may depend on both the richness and composition of the gut microbiome. Depending on its composition, it might sometimes promote and other times mitigate *H. pylori* disease (51).

Future perspectives

A diagnosis of childhood asthma is most reliable if obtained by respiratory function test. In future studies, including this continuing prospective cohort study, assessment of the association between *H. pylori* colonization and respiratory function test based asthma may contribute to the discussion on this topic. It will help physicians to decide whether they have to eradicate or preserve the actual *H. pylori* colonization in children. Explanations of underlying mechanisms for the differences between ethnic groups are still speculative, and therefore need further research. Such studies also should include the role of the gut microbiome in relation to *H. pylori* colonization and ethnic background, which may indicate new directions for asthma prevention.

H. pylori *and BMI/obesity*

H. pylori colonization is virtually always associated with chronic active gastritis, which can have various effects. This includes interference with gastric hormone regulation, including ghrelin and leptin. Both have multiple roles in energy homeostasis (52). Disturbance of their normal regulation interferes with metabolism and our energy household. *H. pylori* eradication increases serum ghrelin levels (53). Several epidemiological studies have recently focused on the correlation between *H. pylori* colonization and BMI and obesity. They showed contrasting results, which were based on *H. pylori* status and BMI data, but did not include genetic information (54-58). Incorporation of genetic data could give unbiased information on the presumed association. In **chapter 7**, we assessed the association between *H. pylori* and BMI/obesity among 13,044 subjects participating in two large population-based cohorts. Cross-sectional analysis did not demonstrate a clinical relevant association between *H. pylori* colonization and BMI, neither when examined by means of serology, nor by fecal antigen. By means of a Mendelian Randomization approach, we were able to assess a causal relationship between *H. pylori* and BMI/obesity. However, bi-directional Mendelian randomization revealed no causal relation between *H. pylori* and BMI/obesity.

Future perspectives

The causes of obesity are diverse. Amongst others, gut microbiome composition and diversity seems to play a role. We only tested one of the members of the ancient indigenous microbiome. Future studies, including a broader spectrum of the microbiome, are needed to further assess any potential causal relationship between the gut microbiome and BMI/obesity.

Premalignant gastric lesions and gastric cancer

Premalignant gastric lesions predisposes to the development of intestinal type gastric cancer, with *H. pylori* considered as the starting point which leads to inflammation, metaplasia, low-grade dysplasia, high-grade dysplasia and eventually carcinoma in 1-2% of the infected patients (59). As a result, regular endoscopic follow-up is recommended in patients with premalignant gastric lesions. However, most of the patients with *H. pylori* and premalignant gastric lesions do not develop gastric cancer. Moreover, a substantial part of patients shows regression during endoscopic follow-up (60). Therefore, identification and screening of only a subpopulation at high risk of gastric cancer is indicated. This will additionally result in a decrease in burden for patients and endoscopy resources.

In **chapter 8** we showed a low incidence of neoplastic progression during follow-up of patients participating in a multi-centre prospective cohort study. Consequently, the yield of surveillance endoscopy in all patients with premalignant lesions is low. Risk stratification according to the MAPS guideline is helpful to exclude low risk patients from surveillance. Nevertheless, we observed that stratification only based on histopathology may result in excluding patients from follow-up, who still are at risk for gastric cancer. We found that addition of serologic markers, including pepsinogens, was of value as non-invasive tool for differentiation between low and high risk patients. Regression of histopathology findings was observed in about one third of the patients, which can be result of sampling error, misclassification or true regression. The latter may be a result of *H. pylori* eradication therapy, in particular in patients with non-atrophic and atrophic gastritis (61). Whether eradication of *H. pylori* has effect on intestinal metaplasia is still under debate, but it may slow progression to neoplasia which is an argument for eradication (62).

Future perspectives

Longer follow-up of patients with premalignant gastric lesions is necessary to confirm our results. The current European guideline does not take into account differences in *H. pylori* and gastric cancer incidence between different parts of Europe. Future studies are needed to answer the question whether widening of the screenings interval is possible in areas with a low gastric cancer incidence. The use of narrow band imaging (NBI) is recommended by guidelines, but not routine practice in most countries. Although a simple validated classification system is still lacking, use of NBI may enlarge the yield of targeted biopsies.

CONCLUSIONS

In this thesis, we aimed first to get insight in the current epidemiology of *H. pylori* colonization in a multi-ethnic Western population. Highly variable prevalences of *H. pylori* were observed between different ethnic groups. Nevertheless, *H. pylori* colonization rate is rapidly decreasing in the next generation, independent of ethnic background. It highlights the importance of common environmental factors in the transmission of *H. pylori*.

We secondly aimed to assess the association of *H. pylori* with extra-gastric diseases. No protective effect of *H. pylori* colonization on asthma was observed for children at the age of 6. In pregnant women, *H. pylori* colonization was associated with an increased risk on severe vomiting during pregnancy, and preeclampsia. We further focused on the long-term effect of *H. pylori* colonization at higher age. We observed a low incidence of neoplastic progression in patients who were followed because of premalignant gastric lesions. At least in adults, above mentioned studies support eradication of *H. pylori* in risk groups in order to prevent associated disease in future.

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Appendices

NEDERLANDSE SAMENVATTING

Dit proefschrift richt zich op het actuele vóórkomen (epidemiologie) van *Helicobacter pylori (H. pylori)* in een multi-etnische populatie, het vóórkomen en vervolgen van voorloperafwijkingen van maagkanker en het richt zich op de associatie tussen *H. pylori* en ziekten buiten de maag. Het proefschrift heeft vier delen. Deel één geeft een introductie en samenvatting van de belangrijkste onderwerpen van dit proefschrift: gastritis, het vóórkomen en de behandeling van *H. pylori*, associaties met niet-kwaadaardige ziekten, en voorloperafwijkingen van maagkanker. Dit deel eindigt met een beschrijving van het doel en de opzet van het proefschrift. Deel twee bevat de studies naar het actuele vóórkomen van *H. pylori*. In deel drie staan de studies die gaan over de associatie van *H. pylori* met ziekten buiten de maag en de follow-up van patiënten met voorloperafwijkingen van maagkanker. Deel vier geeft een samenvatting van het geheel en bevat de belangrijkste conclusies.

HET VÓÓRKOMEN VAN *H. PYLORI* IN EEN MULTI-ETNISCHE POPULATIE (DEEL TWEE)

H. pylori is een gram-negatieve bacterie die bij meer dan de helft van de wereldbevolking wordt aangetroffen. Het vóórkomen van deze bacterie verschilt wereldwijd zeer, waarbij de meeste infecties in ontwikkelingslanden worden gevonden. In westerse landen wordt al jaren een dalende trend gezien, waarbij in de doorsnee bevolking minder dan 40% geïnfecteerd is met *H. pylori.* Redenen van deze dalende trend zijn onder andere: verbeterde hygiëne, kleinere gezinnen waardoor er minder kans is op besmetting en het veelvuldig gebruik van antibiotica. Gelijktijdig met deze dalende trend nemen ook de aan *H. pylori* gerelateerde ziekten af in voorkomen. Dit betekent dat minder mensen lijden aan peptische ulcera (maagzweren) en dat ook het vóórkomen van maagkanker afneemt. *H. pylori* infectie treedt meestal op bij jonge kinderen, waarbij andere gezinsleden dan de bron zijn. Omdat het testen en behandelen van *H. pylori* op populatieniveau niet kosteneffectief is, is het noodzakelijk om risicogroepen te definiëren. Kennis van specifieke risicogroepen is belangrijk omdat dit mogelijkheden biedt voor gerichte interventie. Infectie met *H. pylori* is geassocieerd met leeftijd, socio-economische status, groot kinderaantal en niet-westerse etnische achtergrond. Gedurende de laatste decennia hebben veel immigranten uit landen met een hoog *H. pylori* percentage zich gevestigd in stedelijke gebieden van westerse landen. In Rotterdam, een grote westerse stad, is meer dan 50% van de bevolking oorspronkelijk afkomstig van buiten Nederland. De grootste groepen van niet-Nederlandse komaf

bestaan uit mensen afkomstig uit Marokko, Turkije, Suriname, Nederlandse Antillen en Kaapverdië. Eerder gepubliceerde studies geven aan dat *H. pylori* in immigranten vaker voorkomt dan in de autochtone westerse bevolking. Sommige van deze immigranten groepen hebben ook een verhoogd risico op maagkanker, waarbij *H. pylori* kan worden beschouwd als een belangrijke oorzaak hiervan. In **hoofdstuk 2** beschrijven we de resultaten van ons onderzoek naar *H. pylori* in een grote groep van zwangere vrouwen die deelnamen aan de Generation R studie. Deze studie betreft een groot onderzoek in Rotterdam, waarbij zwangere vrouwen werden geïncludeerd met als doel om hun kinderen te vervolgen van foetale leeftijd tot aan het bereiken van volwassenheid. In dit proefschrift is bij meerdere studies gebruik gemaakt van de gegevens die binnen Generation R van deze moeders en kinderen beschikbaar waren. Wijzelf hebben zowel de moeders als hun kinderen getest op de aanwezigheid van *H. pylori.* Dit is gedaan door middel van het aantonen van antilichamen tegen *H. pylori* en CagA (een virulentie factor) in het serum van deze personen. In totaal hebben we 6837 zwangere vrouwen getest, waarbij 46% *H. pylori* positief bleek, en 35% van hen ook CagA positief. Er bleek een groot verschil in het vóórkomen van *H. pylori* tussen de verschillende bevolkingsgroepen met veel hogere percentages *H. pylori* positieven in mensen van niet-westerse afkomst. Migranten populaties vormen dus risicogroepen voor infectie met *H. pylori* en zijn een goede doelgroep voor gerichte screening en behandeling van *H. pylori*.
De kinderen van bovengenoemde moeders werden getest op 6-jarige leeftijd, ook doormiddel van het aantonen van antistoffen in het serum. Deze resultaten worden beschreven in **hoofdstuk 3**. Onafhankelijke risicofactoren voor infectie met *H. pylori* waren: een *H. pylori*-positieve moeder, niet-westerse etnische achtergrond, vrouwelijk geslacht en lage socio-economische status. Bij de kinderen lag het percentage *H. pylori* positieven lager dan bij hun moeders, met opnieuw de hoogste percentages in kinderen met een niet-westerse etnische achtergrond. Echter, bij vergelijking van het cohort moeders en hun kinderen vonden we dat de daling in alle bevolkingsgroepen even groot is (76%), dus ongeacht hun etnische achtergrond. Dit duidt erop dat omgevingsfactoren en leefomstandigheden van het land waar het kind opgroeit (in dit geval voor alle kinderen Nederland) een grote invloed heeft op de transmissie, onafhankelijk van etniciteit.

H. PYLORI, ASSOCIATIES MET ZIEKTEN BUITEN DE MAAG, EN VOORLOPERAFWIJKINGEN VAN MAAGKANKER (DEEL DRIE)

In **hoofdstuk 4** onderzochten we de associatie tussen *H. pylori* en hyperemesis gravidarum (misselijkheid en braken) tijdens de zwangerschap in vrouwen die participeerden in de

Generation R studie. Een recente meta-analyse toonde een positief verband tussen *H. pylori* en ernstig hyperemesis gravidarum. We vonden een verhoogd risico op dagelijks braken bij *H. pylori*-positieve vrouwen. Verder was er een kleinere toename in gewicht bij *H. pylori*-positieve vrouwen met dagelijks braken en hadden hun kinderen een verhoogd risico op een te laag geboortegewicht. Deze studie bevestigt de relatie tussen *H. pylori* en dagelijks braken. In **hoofdstuk 5** onderzochten we de relatie tussen *H. pylori* en drie zwangerschap gerelateerde complicaties: pre-eclampsie (PE), small for gestational age (SGA) en spontane vroegtijdige geboorte (PTB). Multivariate analyse liet zien dat *H. pylori* was geassocieerd met PE. Onderscheiding naar CagA-status toonde een verhoogd risico voor SGA in CagA-positieve moeders. Er bleek geen associatie tussen *H. pylori* en PTB. Indien bovenstaande associaties worden bevestigd bij toekomstige studies en ook causaal blijken te zijn, kan screening en behandeling van *H. pylori* mogelijk bijdragen aan het verlagen van de perinatale morbiditeit en mortaliteit.

H. pylori heeft mogelijk niet alleen pathogene (ziekmakende) eigenschappen. Infectie met *H. pylori* draagt waarschijnlijk bij aan een betere rijping van het immuunsysteem. Binnen deze hypothese wordt gesteld dat de grote toename van mensen met astma en allergieën verband houdt met de verdwijning van *H. pylori* uit de populatie. In dit kader hebben we gekeken naar het vóórkomen van astma en allergie bij kinderen met en zonder *H. pylori* (**hoofdstuk 6**). Het bleek dat kinderen met *H. pylori* (maar zonder virulentiefactor CagA) juist meer risico hadden op astma dan kinderen zonder *H. pylori.* Deze uitkomst verschilde tussen kinderen van Europese en niet-Europese achtergrond, waarbij het risico alleen verhoogd was voor kinderen met een Europese etnische achtergrond. Mogelijk heeft dit verschil in effect te maken met de samenstelling van het darm microbioom, wat ook wordt beïnvloed door etnische afkomst. Vervolgonderzoek zal echter nodig zijn om onderliggende mechanismen te verduidelijken.

Verschillende voorgaande studies hebben gekeken naar de relatie tussen *H. pylori* en obesitas (overgewicht). De resultaten zijn tegengesteld. In **hoofdstuk 7** beschrijven we de resultaten van een grote studie naar het verband tussen *H. pylori* en obesitas. Hierin zijn zowel gegevens gebruikt over het dragerschap van *H. pylori* (antilichamen verkregen uit serum), alsook genetische informatie. Het voordeel van het gebruik van genetische informatie is dat dit een oorzakelijk verband kan aantonen. Ons onderzoek is gedaan in een grote groep volwassen (ruim 13000) woonachtig in Rotterdam en Pomerania (Duitsland). Er werd echter geen oorzakelijk verband gevonden tussen *H. pylori* dragerschap en obesitas.

Infectie met *H. pylori* leidt tot een chronische ontsteking van het maagslijmvlies, wat een verhoogd risico geeft op het ontstaan van maagkanker. Dit verloopt meestal via een aantal stadia die zichtbaar worden in biopten (stukjes weefsel van het maagslijmvlies) die de patholoog onderzoekt onder de microscoop. Dit worden voorloperafwijkingen

van maagkanker genoemd. Deze worden vaak toevallig ontdekt tijdens een maagonderzoek (gastroscopie). Door het vroegtijdig herkennen en behandelen van deze voorloperafwijkingen kan maagkanker worden voorkomen. Echter, gelukkig niet iedereen met deze voorloperafwijkingen zal in de toekomst maagkanker ontwikkelen. Daarom is het belangrijk om vooraf een risico inschatting te kunnen maken welke patiënten het meeste risico lopen. In **hoofdstuk 8** beschrijven we de resultaten van onze studie waarbij we 284 patiënten met voorloperafwijkingen van maagkanker hebben gevolgd. Er werd bij hen om de 2 jaar een gastroscopie verricht, waarbij biopten van het maagslijmvlies werden genomen. Binnen deze groep heeft 1,4% maagkanker ontwikkeld. Bij de meeste patiënten was er stabiele ziekte of juist vermindering van de afwijkingen te zien. Het bleek verder dat gebruik van serologische markers (bloedbepalingen) behulpzaam is voor het juist inschatten van het risico op ontstaan van maagkanker in de toekomst.

CONCLUSIES (DEEL VIER)

In deel vier worden de belangrijkste conclusies van de voorgaande hoofdstukken samengevat en in bredere context geplaatst. Verder worden per hoofdstuk aanbevelingen gedaan voor toekomstig onderzoek. Dit proefschrift had ten eerste tot doel om inzicht te krijgen in het actuele vóórkomen van *H. pylori* in een multi-etnische westerse populatie. Het blijkt dat *H. pylori* infectie in de nieuwe generatie steeds minder voorkomt, waarbij groepen van niet-westerse etnische achtergrond een verhoogd risico op dragerschap blijven houden. Het tweede doel was het onderzoek naar verbanden tussen *H. pylori* en ziekten buiten de maag. Er bleek geen beschermend effect te zijn van *H. pylori* op de astma en allergie in kinderen op de leeftijd van 6 jaar. In zwangere vrouwen bleek *H. pylori* een risicofactor voor ernstig braken tijdens de zwangerschap, alsook voor pre-eclampsie. Verder richtten we ons in dit proefschrift op de lange termijn effecten van infectie met *H. pylori*. Bij volwassenen werd geen oorzakelijk verband aangetoond tussen *H. pylori* en obesitas. In patiënten met voorloperafwijkingen van maagkanker werd bij een beperkt aantal ook daadwerkelijk maagkanker gevonden. Resultaten van hierboven besproken studies onderstrepen het nut *H. pylori* eradicatie in volwassenen, met als doel om zo *H. pylori* gerelateerde ziekten in de toekomst te kunnen voorkomen.

ABBREVIATIONS

AG	atrophic gastritis
ANOVA	analyses of variance
BMI	body mass index
CagA	cytoxin-associated gene A
CI	confidence interval
CMV	cytomegalovirus
COX	cyclooxygenase
DCs	dendritic cells
EBV	Epstein-Barr virus
ELISA	enzyme-linked immunosorbent assay
ESD	endoscopic submucosal dissection
FD	functional dyspepsia
FGR	foetal growth restriction
GERD	gastroesophageal reflux disease
GS	genotype score
GWAS	genome wide association study
HELLP	haemolysis, elevated liver enzymes, low platelets
HG	hyperemesis gravidarum
HGD	high-grade dysplasia
IM	intestinal metaplasia
ITT	intention to treat
LGD	low-grade dysplasia
MALT	mucosa associated lymphoid tissue
MAPS	management of precancerous conditions and lesions of the stomach
NSAIDs	non-steroidal anti-inflammatory drugs
NVP	nausea and vomiting during pregnancy
OD	optical density
ODR	optical density ratio
OLGA	operative link for gastric assessment
OLGIM	operative link for gastric intestinal metaplasia
OR	odds ratio
PAF	population attributable fraction
PE	preeclampsia
PPI	proton pump inhibitor
PTB	spontaneous preterm birth
PUB	peptic ulcer bleeding

PUD	peptic ulcer disease
RCT	randomized controlled trial
RS	Rotterdam Study
SD	standard deviation
SGA	small for gestational age
SHIP	Study of Health in Pomerania
SNP	single nucleotide polymorphism
Tregs	regulatory T cells

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LIST OF PUBLICATIONS

Publications based on studies described in this thesis

1. **den Hollander WJ**, Kuipers EJ. Current pharmacotherapy options for gastritis. *Expert Opinion Phamacother.* 2012; 13(18):2625-36
2. **den Hollander WJ**, Holster IL, den Hoed CM, van Deurzen F, van Vuuren AJ, Jaddoe VW, Hofman A, Perez Perez GI, Blaser MJ, Moll HA, Kuipers EJ. Ethnicity is a strong predictor for *Helicobacter pylori* infection in young women in a multi-ethnic European city. *J Gastroenterol Hepatol.* 2013; 28(11):1705-11
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ABOUT THE AUTHOR

Wouter Jacobus den Hollander was born on June 23rd 1986 in Hendrik-Ido-Ambacht, The Netherlands. He graduated at the Wartburg College in Rotterdam in 2004, after which he attended Medical School at the Erasmus University in Rotterdam. In 2010, he did a rotation abroad at the Ekwendeni Mission Hospital, Ekwendeni, Malawi. He became qualified as a Medical Doctor in 2010. In the same year, he worked as a resident not in training (ANIOS) at the Internal Medicine department of the Sint Franciscus Hospital, Rotterdam, for one year. In December 2011 he started his PhD trajectory on the epidemiology of *Helicobacter pylori* at the Department of Gastroenterology and Hepatology of the Erasmus MC University Medical Center, supervised by prof. dr. E.J. Kuipers and from 2014 also by dr. V.M.V.C. Spaander. As of February 2015 he started the two-year Internal Medicine part at the Medisch Centrum Haaglanden (program director dr. A.H. Bootsma). Hereafter, he will continue his training in Gastroenterology and Hepatology at the Medisch Centrum Haaglanden (program director drs. L. Perk) and at the Leiden University Medical Center (program director dr. R.A. Veenendaal). Wouter is married with Lianne. They have one son, called Ezra (2014).