***IL1RN* Polymorphism and *cagA*-Positive *Helicobacter pylori* Strains Increase the Risk of Duodenal Ulcer in Children**

DULCIENE MARIA MAGALHÃES QUEIROZ, PAULO BITTENCOURT,
JULIANA BECATTINI GUERRA, ANDREIA MARIA CAMARGOS ROCHA,
GIFONE AGUIAR ROCHA, AND ANFRISINA SALES TELES CARVALHO

Laboratory of Research in Bacteriology, Faculdade de Medicina [D.M.M.Q., J.B.G., A.M.C.R., G.A.R., A.S.T.C.], and Hospital das Clínicas [P.B.], UFMG, Belo Horizonte 30130-100, Brazil.

ABSTRACT

Duodenal ulcers in children are associated with *Helicobacter pylori* gastric infection with *cagA*-positive strains, but factors linked to the host are poorly known. The authors evaluated the role of proinflammatory interleukin-1 gene cluster polymorphisms in the pathogenesis of duodenal ulcer. They studied prospectively 437 children 1 to years old, 209 of whom were *H. pylori* positive and 228 of whom were *H. pylori* negative. *IL1B*-511-C/T, -31T/C, and *IL1RN* Variable number of tandem repeats were genotyped by polymerase chain reaction (PCR) restriction fragment length polymorphism, PCR with confronting two-pair primers, and PCR, respectively. *cagA* status was evaluated by PCR. The role of the proinflammatory cytokine genotypes in the genesis of duodenal ulcer was evaluated before and after stratification of *H. pylori* status on logistic regression models. In the group of children without duodenal ulcer, no association was observed between *H. pylori* status and proinflammatory polymorphisms. Furthermore, no association between *IL1* cluster genotypes and *cagA* status was seen in the *H. pylori*-positive children. However, increasing age, male sex, and *IL1RN**2 were independently associated with duodenal ulcer.

After stratification, in the *H. pylori*-positive children, increasing age, male sex, the presence of *IL1RN**2 allele, and *cagA*-positive status were independently associated with duodenal ulcer. The risk for the development of duodenal ulcer increased when a combined association of the presence of *IL1RN**2 allele and infection by a *cagA*-positive *H. pylori* strain was the variable. This study provides evidence supporting independent roles of *IL1RN**2 allele and *cagA*-positive status in the genesis of duodenal ulcer in children. (*Pediatr Res* 58: 892–896, 2005)

Abbreviations

cagA, cytotoxin-associated gene
CI, confidence interval
DU, duodenal ulcer
IL, interleukin
IL1RN, interleukin-1 receptor antagonist gene
OR, odds ratio
PAI, pathogenicity island
PCR, polymerase chain reaction
VacA, vacuolating cytotoxin

Helicobacter pylori infection is predominantly acquired in childhood and usually persists for life unless treated. In most persons, the natural history of infection is without complications, but peptic ulcer disease, distal gastric carcinoma, or mucosa-associated lymphoid tissue gastric lymphoma will occur in a small percentage of infected individuals (1–3).

In children, DU, a severe complication of the infection is much less common than in adults. The pathobiology of the uncommon childhood-onset DU is uncharacterized, but this adverse outcome may be linked to a more marked gastric

inflammatory response seen in children with DU than in infected children without DU (4–6). Contributing factors include host genetics and bacterial virulence markers. In fact, the *cagA*-PAI and VacA, two of the major bacterial virulence factors involved in host cell modulation, are associated with the disease in childhood (6–8). Several genes of the *cag*-PAI code proteins with similarities to components of type IV secretion systems induce an increased inflammation in the gastric mucosa through release of cytokines such as IL-8 and IL-1 β (9,10). In addition to the VacA properties linked to the gastric mucosa damage, the toxin may increase the inflammatory response of the gastric mucosa by different pathways, such as a recently demonstrated action in increasing the expression of the proinflammatory enzyme cyclooxygenase-2, not only in T cells but also in neutrophils and macrophages (11).

Received November 10, 2004; accepted March 1, 2005.

Correspondence: Dulciene Maria Magalhães Queiroz, M.D., Laboratory of Research in Bacteriology, Faculdade de Medicina, UFMG, Av. Alfredo Balena, 190, S/4026, 30130–100, Belo Horizonte, Brazil; e-mail: dqueiroz@medicina.ufmg.br

Supported by CNPq and FAPEMIG, Brazil

DOI: 10.1203/01.PDR.0000181380.14230.8B

Although infection with virulent *H. pylori* strains may contribute to the difference in the inflammatory response observed in children with DU, it also may depend on the genetic background of the host.

Among the genetic factors, polymorphisms in gene promoter regions encoding for cytokines are attractive candidates as host-related risk factors. They may affect cytokine transcription and then the host immune response. Indeed, *IL1B* (-31C, -511T) and *IL1RN**2 alleles were recently associated with an increased risk for severe gastric lesions, such as atrophy and intestinal metaplasia, and the development of gastric carcinoma (12–16). Individuals harboring these genotypes are thought to overexpress gastric IL-1 β in response to *H. pylori* infection that amplifies the inflammation and increases the gastric lesion.

Our hypothesis is that *IL1* polymorphisms in children may contribute to a more marked gastritis (both antral and oxyntic) than is seen in those with DU, without, however, leading to atrophic changes that need a more prolonged period of infection to be established (4–6).

Therefore, our aim was to investigate whether *IL1* gene cluster polymorphisms are associated with DU in children, controlling for confounding factors. Because VacA high producer *H. pylori* strains are also *cagA*-positive and VacA/*cagA*-positive strain is associated with DU in children in certain populations, *cagA* was included in the logistic model as the virulence *H. pylori* marker (6).

METHODS

This study was approved by the Ethics Committee of the Universidade Federal de Minas Gerais, Brazil. Informed consent to participate was obtained from children (whenever possible) and their parents.

We studied 437 children (241 girls, mean age 9.37 ± 3.82 years, range 1–18 years) who underwent endoscopy for investigation of upper abdominal pain. Seventy-two (16.5%) *H. pylori*-positive children had DU. Among them, 52 were included in other studies (6,7).

All the included patients were from the same socioeconomic level with similar cultural habits, and all were natives of Minas Gerais state with the same ethnic background: approximately 33% of Portuguese ancestry, 33% of Amerindian ancestry, and 33% of African ancestry (17).

The patients were considered to be *H. pylori* positive if the culture was positive or if they were positive by two of the following tests: the preformed urease test, carbolfuchsin stained smear examination, or ^{13}C -urea breath test. The children were considered *H. pylori* negative if the results of all tests were negative.

At endoscopy, biopsy specimens were obtained from the antral and oxyntic gastric mucosa of all patients for microbiologic analyses.

Tissue samples for culture were maintained in sodium thioglycolate broth (Difco, Detroit, MI, USA) at 4°C for a maximum of 1 h, ground separately in a tissue homogenizer (Kontes, Vineland, NJ, USA), and plated onto Petri dishes containing freshly Belo Horizonte medium (18). *H. pylori* was identified as previously described (6,9). One antral fragment was placed in a tube containing Christensen's 2% urea agar and examined within 24 h of incubation at 37°C for urea hydrolysis.

One antral biopsy specimen was immediately rubbed on a glass slide and subsequently heat-fixed and stained with 40% carbolfuchsin for the presence of spiral-shaped bacteria.

All *H. pylori* strains, as well as gastric fragments from *H. Pylori*-positive patients from whom the microorganism was not isolated, were evaluated for the presence of *cagA* and *ureA*.

The ^{13}C -Urea breath test was performed according to our previous study (19).

Tissue and bacterial culture DNA was extracted with the QIAamp DNA mini kit (QIAGEN GmbH, Hilden, Germany) (20).

Genomic DNA from bacterial and tissue samples were PCR amplified for *cagA* by the use of two sets of synthetic oligonucleotides primers as described

elsewhere by Kelly *et al.* (21) and Peek *et al.* (22). The strains were considered to be *cagA* positive when at least one of the reactions was positive. The presence of *H. pylori* DNA was confirmed by the detection of *ureA* (23).

To analyze gene host polymorphisms, the DNA was extracted from one antral gastric fragment.

The *IL1B*-31 T/C biallelic polymorphism was genotyped by PCR with confronting two-pair primers (24).

The PCR-RFLP (restriction fragment length polymorphism) method was used for the *IL1B*-511C/T bi-allelic polymorphism genotyping (25).

IL1RN penta-allelic variable number tandem repeats was genotyped according to Mansfield *et al.* (25). The polymorphism was based on the number of repeats of an 86 bp, and the alleles were coded conventionally as follows: allele 1, 4 repeats (410 bp); allele 2, 2 repeats (210 bp); allele 3, 5 repeats (500 bp); allele 4, 3 repeats (325 bp), and allele 5, 6 repeats (595 bp). For the statistical analysis, and because of the rarity of alleles 3, 4 and 5, this polymorphism was treated as bi-allelic by classifying the alleles in short (allele 2) and long (alleles 1, 3, 4 and 5) categories (13).

Statistical analysis. The data were analyzed with SPSS (SPSS Inc., Chicago, IL, USA) statistical software package version 10.0.

Linkage disequilibrium between the loci was estimated by using the program GENEPOP (available from wbiomed.curtin.edu.au/cgi-bin/genepop.cgi) in the control group without DU.

Associations between *H. pylori* and *cagA* status and gene polymorphisms as well as *cagA* status and DU were evaluated by the two-tailed χ^2 test with Yate's correction or Fisher's exact test. The level of significance was set at $p < 0.05$.

Variables such as sex, mean age, *cagA* status (negative or positive), and the cytokine genotypes (*IL1B*-31T/C, -511C/T, and *IL1RN* VNTR) were evaluated in the risk of DU in a logistic model. For this analysis, the presence of proinflammatory polymorphic alleles was scored as 0 (absence of allele 2), 1 (presence of 1 allele 2) and 2 (presence of 2 alleles 2). The association of each variable with peptic ulcer (dependent variable) was tested in univariate analysis. All the variables with a p value of 0.25 or less were included in the full model of logistic regression. The OR and 95% CI were used as an estimate of the risk. The Hosmer-Lemeshow goodness-of-fit test was used to evaluate the fit of the model (26).

RESULTS

The characteristics of the children are shown in Table 1. Two hundred and twenty-eight children were *H. pylori* negative and 209 were *H. pylori* positive. In the last group, the *cagA* status was positive in 61 (84.7%) children and 75 (54.7%) children with and without DU, respectively ($p < 0.001$, OR = 4.58, 95% CI = 2.11–10.13).

To evaluate whether *H. pylori* status was associated with cytokine polymorphisms, *H. pylori*-positive and -negative children carrying or not carrying polymorphic alleles were compared. No association was observed between the groups ($p = 0.87$, OR = 0.93, 95% CI = 0.55–1.57 for *IL1B*-31 and $p = 0.12$; OR = 0.72, 95% CI = 0.48–1.08 for *IL1RN*). No association between *cagA* status and the presence or absence of polymorphic genes was observed ($p = 0.49$, OR = 0.84, 95% CI = 0.54–1.32 for *IL1B*-31 and $p = 0.80$; OR = 1.10, 95% CI = 0.65–1.88 for *IL1RN*).

When we analyzed the linkage disequilibrium among different loci, only the results of *IL1B*-31 were included in the logistic model because there was a strong association between

Table 1. Characteristics of the children ($n = 437$)

Patients	n	Girls/boys	Age (y)	
			Mean	Range
<i>H. pylori</i> -positive	209	107/102	9.4	1–18
Duodenal ulcer	72	18/54	12.2	4–18
Gastritis	137	89/48	10.0	1–17
<i>H. pylori</i> -negative	228	134/94	8.1	1–17

n, number.

IL1B-511T and *IL1B-31C* in all groups ($p < 10^{-6}$). All the alleles at the other loci were segregated independently (*IL1B-31* and *IL1RN*, $p = 0.46$; *IL1B-511* and *IL1RN*, $p = 0.11$).

When the cytokine genotypes were analyzed as a dichotomous variable, a trend toward higher frequency of *IL1RN2/2* ($p = 0.09$) was seen in patients with DU, but no difference was observed in the frequency of *IL1RN1/2*, *IL1B-31T/C* and *IL1B-31C/C* genotypes between patients with and without DU (Table 2).

Analysis according to haplotype frequencies showed a statistically significant association between *IL1RN* allele 2 and DU ($p = 0.04$), but no other association was seen (Table 2).

When we analyzed the group of *H. pylori*-positive children in dichotomous analysis, a trend toward higher frequency of the *IL1RN2/2* allele ($p = 0.08$) and a statistically significant higher frequency of the *IL1RN1/2* allele ($p = 0.01$) were seen in patients with DU, but no difference was observed in the frequency of *IL1B-31* genotypes between patients with and without the disease (Table 3).

Again, when the cytokine haplotype frequency was analyzed, the *IL1RN* allele 2 was significantly more frequent in children with DU ($p = 0.002$) (Table 3).

After, we treated every cytokine genotype as variable having three values each, according to the amount of inflammatory alleles in logistic models. In the univariate analysis, DU was also associated with increasing age, male sex, *cagA*-positive status, and *ILRN*2* allele, but not with *IL1B-31* polymorphisms (Table 4). All the included variables remained positively associated with DU in the multivariate analysis (Table 4). The Hosmer-Lemeshow test showed good fit of the model ($\chi^2 = 8.22$, 8 df, $p = 0.42$, with 10 steps).

To assess the combined effect of infection with *cagA*-positive strain and the presence of the *ILRN*2* allele on the development of DU, we compared the frequencies of these risk factors in children with peptic ulcer disease and in those without the disease. The patients were classified as follows: 0, without the factors; 1, with one of the risk factors; and 2, with

the two risk factors. Individuals carrying the *ILRN*2* allele or infected by a *cagA*-positive strain had an increased risk for DU with an OR of 5.42 (95% CI = 1.45–23.84), and individuals who had the two high-risk factors had an OR of 19.20 (95% CI = 4.48–95.03).

DISCUSSION

H. pylori infection is mainly acquired in childhood, very early in certain developing countries. Although most infected children remain asymptomatic without complications, DU will develop in a few (6,27,28). Possible explanations for these facts include the characteristics of the colonizing *H. pylori* strains, the genotype of the host, exposure to environmental cofactors, or interactions among two or more of these factors.

Among the putative bacterial factors, *cagA*, which is part of the *cag*-PAI, is now accepted as a risk factor for the development of DU in both adults and children (6,27,29). Products of the genes of the *cag*-PAI are associated with increased production of cytokines such as IL-1 β , tumor necrosis factor- α , and IL-8 that cause more marked gastric inflammation (9). The results of this study demonstrated that infection with *cagA*-positive *H. pylori* strains is independently associated with duodenal peptic ulcer in children, with an OR of 4.1. The *cagA*-positive strains are frequently cytotoxin producers (16). We have previously demonstrated the presence of antibodies to cytotoxin more often in children with DU (90.5%) than in those without DU (44.4%), showing that VacA is expressed *in vivo* in the majority of children with DU (8). In addition to the properties of VacA linked to damage of the gastric mucosa by multiple mechanisms, the toxin emerges as a potent immunomodulatory protein with proinflammatory activities and immunosuppressive effects that would be very useful to promote persistent bacterial mucosal colonization (11).

Together with the virulent bacterial strains, the adverse outcome of *H. pylori* infection also depends strongly on the host response. Because IL-1 β induces the transcription of other proinflammatory cytokines initiating and amplifying the im-

Table 2. *IL1B-31*, *IL1B-511* and *IL1RN* genotypic frequencies in children with ($n = 72$) and without ($n = 365$) duodenal ulcer

Genotype	Control n (%)	Duodenal ulcer n (%)	OR	95% CI	<i>p</i>
<i>IL1B-31</i>					
T/T	117 (32.0)	26 (36.1)	1.0	–	–
C/T	185 (50.7)	33 (45.8)	0.80	0.44–1.46	0.53
C/C	63 (17.3)	13 (18.1)	0.93	0.42–2.04	0.98
Allele T	419 (57.4)	85 (59.0)	1.0	–	–
Allele C	311 (42.6)	59 (41.0)	0.94	0.69–1.37	0.79
<i>IL1B-511</i>					
C/C	126 (34.5)	27 (37.5)	1.0	–	–
C/T	184 (50.4)	33 (45.8)	0.84	0.46–1.52	0.62
T/T	55 (15.1)	12 (16.7)	1.02	0.45–2.28	0.88
Allele C	436 (59.7)	87 (60.4)	1.0	–	–
Allele T	294 (40.3)	57 (39.6)	0.97	0.66–1.92	0.95
<i>IL1RN</i>					
1/1	229 (62.7)	37 (51.4)	1.0	–	–
1/2	111 (30.4)	26 (36.1)	1.45	0.81–2.60	0.23
2/2	25 (6.8)	09 (12.5)	2.23	0.89–5.50	0.09
Allele 1*	569 (77.9)	100 (69.4)	1.0	–	–
Allele 2**	161 (22.1)	44 (30.6)	1.56	1.03–2.35	0.04

n, number; OR, odds-ratio; CI confidence interval; *, all the long alleles; **, the short allele.

Table 3. *IL1B-31, IL1B-511 and IL1RN genotypic frequencies in H. pylori-positive children with (n = 72) and without (n = 137) duodenal ulcer (DU)*

Genotype	Without DU n (%)	With DU n (%)	OR	95% CI	p
<i>IL1B-31</i>					
T/T	42 (30.6)	26 (36.1)	1.0	—	—
C/T	69 (50.4)	33 (45.8)	0.77	0.39–1.54	0.53
C/C	26 (19.0)	13 (18.1)	0.81	0.33–1.99	0.76
Allele T	153 (55.8)	85 (59.0)	1.0	—	—
Allele C	121 (44.2)	59 (41.0)	0.88	0.57–1.35	0.60
<i>IL1B-511</i>					
C/C	47 (34.3)	27 (37.5)	1.0	—	—
C/T	68 (49.6)	33 (45.8)	0.84	0.43–1.66	0.71
T/T	22 (16.1)	12 (16.7)	0.95	0.37–2.40	0.92
Allele C	162 (59.1)	87 (60.4)	0.95	0.61–1.46	0.88
Allele T	112 (40.9)	57 (39.6)	1.0	—	—
<i>IL1RN</i>					
1/1	99 (72.3)	37 (51.4)	1.0	—	—
1/2	29 (21.2)	26 (36.1)	2.40	1.19–4.84	0.01
2/2	09 (6.6)	09 (12.5)	2.68	0.89–8.07	0.08
Allele 1*	227 (82.8)	100 (69.4)	1.0	—	—
Allele 2**	47 (17.2)	44 (30.6)	2.13	1.29–3.51	0.002

n, number; OR, odds-ratio; CI, confidence intervals; *, all the long alleles; **, the short allele.

Table 4. *Variables associated with duodenal ulcer in H. pylori-positive children**

Covariate	Univariate analysis	Multivariate analysis		
	p value	OR	95% CI	p value
Male gender	<0.001	5.17	2.46–10.88	<0.001
Increasing age	<0.001	1.17	1.04–1.31	0.009
<i>ILRN</i> *2 allele	0.01	2.51	1.43–4.41	0.001
<i>CagA</i> + status	<0.001	4.12	1.72–9.87	0.002
<i>IL1B-31</i> *C	0.45	—	—	—

* 72 with and 137 without duodenal ulcer; OR, odds-ratio; CI confidence intervals.

mune response, polymorphisms on the *IL1* cluster that may affect cytokine transcription are natural host risk candidates. The genes encoding for IL-1 cytokines are clustered on chromosomes 2q12–22 and are polymorphic at several loci (30). A bi-allelic polymorphism at positions -31 and -511 of *IL1B* has been described with the allele C and T, respectively, representing IL-1 β high secretory phenotypes (31). Similarly, it has been shown that the polymorphic *ILRN* allele 2 enhances IL-1 β production, although the mechanism of this association is unknown (15,31,32).

In the present study, we demonstrated that *IL1RN* heterozygote and polymorphic homozygote were associated with an increased and independent risk of the development of DU in children, whereas *IL1B* polymorphic genes were not associated with the disease. This result may be explained by the fact that *IL1RN* allele 2 enhances the production of IL-1 β to a much greater extent than do the *IL1B* promoter region polymorphisms, as demonstrated by different authors in mononuclear cell culture and in the gastric mucosa of *H. pylori*-infected patients (15,31,32). Thus, high IL-1 β production may contribute to an increased antral inflammation that may lead to a decreased somatostatin antral concentration and an increased gastrin releasing that may elicit, in a healthy corpus mucosa,

the high gastric acid output necessary to the development of DU (33–35).

The results of this study are different from those in the adult population. Furuta *et al.* (36) observed that carriage of *IL1RN* allele 2 significantly protected Japanese individuals against DU, and Garcia-Gonzales *et al.* (37) and Zamboni *et al.* (38) did not show associations between single *IL-1* polymorphic genes and DU in Caucasian adults. These results allow important conclusions to be made. First, there is a significant difference in the distribution of various cytokine genotypes in different regions and ethnic groups, which may partially explain the discrepancies observed when our results were compared with those from other populations. We have already demonstrated that in our population the distribution of the inflammatory alleles at the *IL1* loci was intermediate between the Asians and Caucasians (39). The second point that deserves emphasis is that differences exist between adults and children with DU.

In the context of *H. pylori* infection, the gastric inflammation seems to be downmodulated in infected children in comparison with adults (40). This may explain, at least in part, why the infection is mainly acquired in childhood. However, more marked inflammation seems to be essential to the development of DU in children, there being necessary additional proinflammatory factors to overcome the natural low inflammatory response to *H. pylori* in children. Thus, an interplay between infection with *H. pylori* virulent strains that elicit a high host inflammation and a host who is able to have a more marked inflammatory response seems to be necessary for development of the disease. In other words, children who harbor *IL1RN* polymorphic genes associated with high IL-1 β production, when they are colonized by VacA/cagA-positive virulent strains that are high cytotoxin producers and have inflammatory properties, are under increased risk for the development of DU. In fact, the risk for the development of DU was enhanced substantially (OR = 19.20) when carriers of the allele 2 of

IL1RN were infected with a *cagA*-positive strain, showing that both host genetic constitution and *H. pylori* virulence markers should be taken into account in defining disease risk.

Otherwise, increased risk to DU depends on an imbalance among factors that were not evaluated in this study, such as those associated with high acid output, among them an increased parietal cell mass (35). Thus, factors associated with increased inflammation, as is the case with virulence bacterial markers and host *IL1RN* polymorphisms, in conjunction with host factors associated with the capacity to secrete large amounts of acid that may neutralize the gastric acid inhibitory activity of IL-1 β , may work together, predisposing to the genesis of ulcers.

In conclusion, this study provides evidence supporting an independent role of virulence marker of *H. pylori* strains and *IL1RN* polymorphisms in the genesis of DU in children. The combined association of these factors significantly increases the risk of the disease in this age group. Further studies are necessary to identify additional host and bacterial factors as well as environmental influences on the bacteria-host interactions that predispose to DU in childhood.

REFERENCES

- Mégraud F, Lamouliatte H 1992 *Helicobacter pylori* and duodenal ulcer: evidence suggesting causation. *Dig Dis Sci* 37:769–772
- Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelstein JH, Orentreich N, Sibley RK 1992 *Helicobacter pylori* infection and the risk of gastric carcinoma. *N Engl J Med* 325:1127–1131
- Wotherspoon AC, Ortiz-Hidalgo O, Falzon MR, Isaacson PG 1991 *Helicobacter pylori*-associated gastritis and primary B-cell gastric lymphoma. *Lancet* 338:1175–1176
- Drumm B 1993 *Helicobacter pylori* in the pediatric patient. *Gastroenterol Clin North Am* 169:162–182
- Nogueira AM, Cabral MM, Carvalho AS, Oliveira CA, Queiroz DM, Oliveira AM, Rocha GA, Mendes EN, Magalhães PP, Castro AM, Paturle P 2000 Gastrite associada ao *Helicobacter pylori* em adultos e crianças: estudo comparativo. *J Bras Patol* 36:110–117
- Queiroz DM, Mendes EM, Carvalho AS, Rocha GA, Oliveira AM, Soares TF, Santos A, Cabral MM, Nogueira AM 2000 Factors Associated with *Helicobacter pylori* infection by a *cagA*-positive strain in children. *J Infect Dis* 181:626–630
- De Gusmão VR, Nogueira Mendes E, De Magalhães Queiroz DM, Aguiar Rocha G, Camargos Rocha AM, Ramadan Ashour AA, Carvalho AS 2000 *vacA* genotypes in *Helicobacter pylori* strains isolated from children with and without duodenal ulcer in Brazil. *J Clin Microbiol* 38:2853–2857
- Rocha GA, Rocha AM, de Magalhães Queiroz D, Nogueira Mendes E, Nogueira AM, Teles de Carvalho AS 2001 Validation of a commercial enzyme-linked immunosorbent assay to detect anti-*cagA* antibodies in children with *Helicobacter pylori* infection. *J Pediatr Gastroenterol* 33:515–518
- Crabtree JE, Covacci A, Farmery SM, Xiang Z, Tompkins DS, Perry S, Lindley IJ, Rappuoli R 1995 *Helicobacter pylori* induced interleukin-8 expression in gastric epithelial cells is associated with *CagA* positive phenotype. *J Clin Pathol* 48:41–45
- Censini S, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, Rappuoli R, Covacci A 1996 *Cag*, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. *Proc Natl Acad Sci U S A* 93:14648–14653
- Montecucco C, de Bernard M 2003 Immunosuppressive and proinflammatory activities of the *VacA* toxin of *Helicobacter pylori*. *J Exp Med* 198:1767–1771
- El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, Herrera J, Lissowska J, Yuan CC, Rothman N, Lanyon G, Martin M, Fraumeni JF Jr., Rabkin CS 2000 Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 404:398–402
- Machado JC, Pharoah P, Sousa S, Carvalho R, Oliveira C, Figueiredo C, Amorim A, Seruca R, Caldas C, Carneiro F, Sobrinho-Simões M 2001 Interleukin 1B and interleukin 1RN polymorphisms are associated with increased risk of gastric carcinoma. *Gastroenterology* 121:823–829
- Figueiredo C, Machado JC, Pharoah P, Seruca R, Sousa S, Carvalho R, Capelinha AF, Quint W, Caldas C, van Doorn LJ, Carneiro F, Sobrinho-Simões M 2002 *Helicobacter pylori* and interleukin 1 genotyping: an opportunity to identify high-risk individuals for gastric carcinoma. *J Natl Cancer Inst* 94:1680–1687
- Rad R, Dossunbekova A, Neu B, Lang R, Bauer S, Saur D, Gerhard M, Prinz C 2004 Cytokine gene polymorphisms influence mucosal cytokine expression, gastric inflammation, and host specific colonisation during *Helicobacter pylori* infection. *Gut* 53:1082–1090
- El-Omar EM, Rabkin CS, Gammon MD, Vaughan TL, Risch HA, Schoenberg JB, Stanford JL, Mayne ST, Goedert J, Blot WJ, Fraumeni JF Jr., Chow WH 2003 Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. *Gastroenterology* 124:1193–1201
- Parra FC, Amado RC, Lambertucci JR, Rocha J, Antunes CM, Pena SD 2003 Color and genomic ancestry in Brazilians. *Proc Natl Acad Sci U S A* 100:177–182
- Queiroz DM, Mendes EN, Rocha GA 1987 Indicator medium for isolation of *Campylobacter pylori*. *J Clin Microbiol* 25:2378–2379
- de Carvalho Costa Cardinali L, Rocha GA, Rocha AM, de Moura SB, de Figueiredo Soares T, Esteves AM, Nogueira AM, Cabral MM, de Carvalho AS, Bittencourt P, Ferreira A, Queiroz DM 2003 Evaluation of [¹³C] urea breath test and *Helicobacter pylori* stool antigen test for diagnosis of *H. pylori* infection in children from a developing country. *J Clin Microbiol* 41:3334–3335
- Monteiro L, Gras N, Vidal R, Cabrita J, Mégraud F 2001 Detection of *Helicobacter pylori* DNA in human feces by PCR: DNA stability and removal of inhibitors. *J Microbiol Methods* 45:89–94
- Kelly SM, Pitcher MC, Farmery SM, Gibson GR 1994 Isolation of *Helicobacter pylori* from feces of patients with dyspepsia in the United Kingdom. *Gastroenterology* 107:1671–1674
- Peek RM Jr., Miller GG, Tham K, Pérez-Pérez GI, Cover TL, Atherton JC, Dunn GD, Blaser MJ 1995 Detection of *Helicobacter pylori* gene expression in human gastric mucosa. *J Clin Microbiol* 33:28–32
- Clayton CL, Kleanthous H, Coates PJ, Morgan DD, Tabaqchali S 1992 Sensitive detection of *Helicobacter pylori* by using polymerase chain reaction. *J Clin Microbiol* 30:192–200
- Hamajima N, Matsuo K, Saito T, Tajima K, Okuma K, Yamao K, Tominaga S 2001 Interleukin 1 polymorphisms, lifestyle factors, and *Helicobacter pylori* infection. *Jpn J Cancer Res* 92:383–389
- Mansfield JC, Holden H, Tarlow JK, Di Giovine FS, McDowell TL, Wilson AG, Holdsworth CD, Duff GW 1994 Novel genetic association between ulcerative colitis and the anti-inflammatory cytokine interleukin-1 receptor antagonist. *Gastroenterology* 106:637–642
- Hosmer DW, Lemeshow S 2000 Applied Logistic Regression. JH Wiley Interscience Publication, New York, pp 143–202
- Oderda G, Figura N, Bayeli PF, Bassagni C, Bugnule M, Arnellini D, Altare F, Ansaldi N 1993 Serologic IgG recognition of *Helicobacter pylori* cytotoxin-associated protein, peptic ulcer and gastroduodenal pathology in childhood. *Eur J Gastroenterol Hepatol* 5:695–699
- Blanchard SS, Bauman L, Czinn SJ 2004 Treatment of *Helicobacter pylori* in pediatrics. *Curr Treat Options Gastroenterol* 7:407–412
- Evans DG, Queiroz DM, Mendes EN, Evans Jr DJ 1998 *Helicobacter pylori cagA* status and s and m alleles of *vacA* in isolates from individuals with a variety of *H. pylori*-associated gastric diseases. *J Clin Microbiol* 36:3435–3437
- Dinarello CA 1996 Biologic basis for interleukin-1 in disease. *Blood* 87:2095–2147
- Hwang JR, Kodoma T, Kikuchi S, Sakai K, Peterson LE, Graham DY, Yamaoka Y 2002 Effect of Interleukin 1 polymorphisms on gastric mucosal Interleukin 1 β production in *Helicobacter pylori* infection. *Gastroenterology* 123:1793–1803
- Santtila S, Savinainen K, Hurme M 1998 Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1 β production in vitro. *Scand J Immunol* 47:195–198
- Queiroz DM, Moura SB, Mendes EN, Rocha GA, Barbosa AJ, de Carvalho AS 1994 Effect of *Helicobacter pylori* eradication on G-cell and D-cell density in children. *Lancet* 343:1191–1194
- Queiroz DM, Mendes EN, Rocha GA, Moura SB, Resende LM, Barbosa AJ, Coelho LG, Passos MC, Castro LP, Oliveira CA, Lima GF Jr 1993 Effect of *Helicobacter pylori* eradication on antral gastrin- and somatostatin-immunoreactive cell density and gastrin and somatostatin concentrations. *Scand J Gastroenterol* 28:858–864
- El-Omar EM 2001 The importance of interleukin 1[beta] in *Helicobacter pylori* associated disease. *Gut* 48:743–747
- Furuta T, El-Omar EM, Xiao F, Shirai N, Takashima M, Sugimura H, Sugimura H 2002 Interleukin 1beta polymorphisms increase risk of hypochlorhydria and atrophic gastritis and reduce risk of duodenal ulcer recurrence in Japan. *Gastroenterology* 123:92–105
- Garcia-Gonzales MA, Lanas A, Santolaria, Crusius BA, Serrano MT, Pena AS 2001 The polymorphic IL-1B and IL-1RN gene in the aetiopathogenesis of peptic ulcer. *Clin Exp Immunol* 125:368–375
- Zambon CF, Basso D, Navaglia G, Germano G, Gallo N, Milazzo M, Greco E, Fogar P, Mazza S, Di Mario F, Basso G, Rugge M, Plebani M 2002 *Helicobacter pylori* virulence genes and host IL-1RN and IL-1beta genes interplay in favouring the development of peptic ulcer and intestinal metaplasia. *Cytokine* 18:242–251
- Queiroz DM, Guerra JB, Rocha GA, Rocha AM, Santos A, de Oliveira AG, Cabral MM, Nogueira AM, de Oliveira CA 2004 IL1B and IL1RN polymorphic genes and *Helicobacter pylori cagA* strains decrease the risk of reflux esophagitis. *Gastroenterology* 127:73–79
- Queiroz DM, Rocha GA, Mendes EN, Carvalho AS, Barbosa AJ, Oliveira CA, Lima GF Jr 1991 Differences in distribution and severity of *Helicobacter pylori* gastritis in children and adults with duodenal ulcer disease. *J Pediatr Gastroenterol Nut* 12:178–181