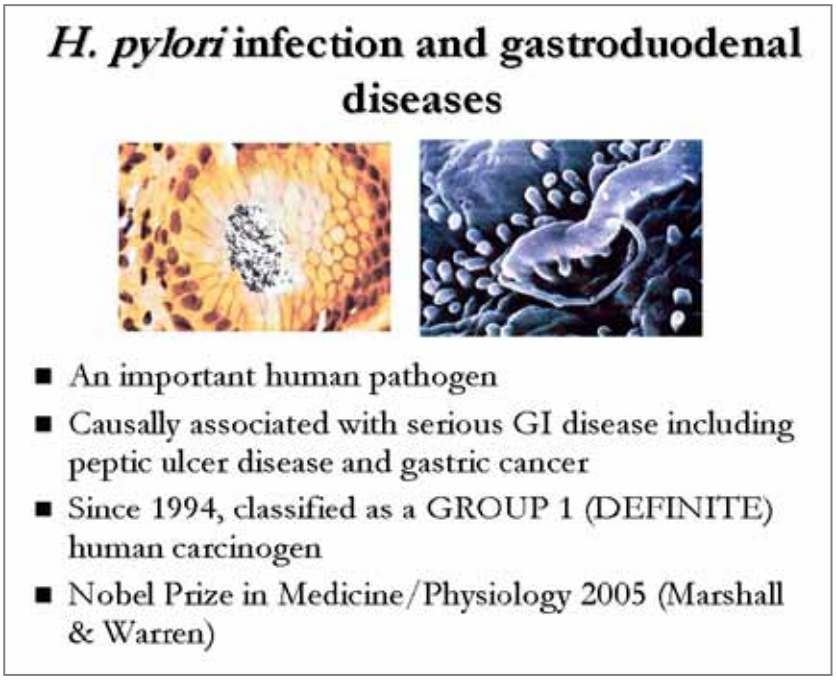
The importance of host genetic factors in *Helicobacter pylori* associated disease

Emad M. El-Omar



Professor, Department of Medicine and Therapeutics Institute of Medical Sciences, Aberdeen University, Aberdeen - Scotland

H. pylori infection & gastroduodenal disease

Chronic *H. pylori* infection

Antral predominant Gastritis
Acid, little or no atrophy
low risk of gastric ca

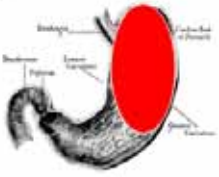
DU disease

Mild Mixed Gastritis

Normal Acid

No significant disease

Corpus predominant Gastritis
MAG + Hypochlorhydria
high risk of gastric ca



Gastric Ca

? Bacterial ? Environment ? Host

The gastric cancer phenotype

- Severe inflammation
- corpus predominant pattern
- gastric atrophy (MAG)
- Hypo/achlorhydria
- Bacterial overgrowth

H. pylori and risk of gastric cancer

TABLE 2. THE DEVELOPMENT OF GASTRIC CANCER IN *H. PYLORI*–POSITIVE PATIENTS ACCORDING TO ABNORMALITIES AT BASE LINE.

ABNORMALITIES AT BASE LINE	ALL *H. PYLORI*–POSITIVE PATIENTS (N=1246)	*H. PYLORI*–POSITIVE PATIENTS WITH GASTRIC CANCER (N=36)	RELATIVE RISK (95% CI)*	*H. PYLORI*–POSITIVE PATIENTS WITH INTESTINAL-TYPE CANCER (N=23)	*H. PYLORI*–POSITIVE PATIENTS WITH DIFFUSE-TYPE CANCER (N=13)
	no.	no. (%)		no.	
Grade of atrophy					
None or mild†	381	3 (0.8)	1.0	0	3
Moderate	657	18 (2.7)	1.7 (0.8–3.7)	9	9
Severe	208	15 (7.2)	4.9 (2.8–19.2)	14	1
Distribution of gastritis					
Antrum predominant†	699	2 (0.3)	1.0	0	2
Pangastritis	337	14 (4.2)	15.6 (6.5–36.8)	4	10
Corpus predominant	210	20 (9.5)	34.5 (7.1–166.7)	19	1
Intestinal metaplasia					
Absent†	782	6 (0.8)	1.0	1	5
Present	464	30 (6.5)	6.4 (2.6–16.1)	22	8

*CI denotes confidence interval.

†Patients in this category served as the reference group.

Uemura et al, *N Engl J Med,* 2001; 345:784-789

Role of host genetic factors

Host factors: Role of acid inhibition

DU phenotype GC phenotype

What endogenous substance could have a similar effect?

Candidate genes

- Pro-inflammatory
- Relevant to *H. pylori* infection
- Central role in gastric acid physiology
- Polymorphic gene with functionally relevant genetic variants that are frequent in the population
- *IL-1B* (encoding IL-1β)
- *IL-1RN* (encoding the naturally occurring antagonist)
- *TNFA* (encoding TNF-α)
- *IL-10*

IL-1/TNF-A genotypes & risk of hypochlorhydria/atrophy

LOCUS	ODDS RATIO	95% CI
IL-1B-511*T+ *IL-1B*-31*C+	**9.1**	2.2-37
*IL-1RN**2/*2	**5.6**	1.8-17
TNF-A-308*A+	**3.2**	1.3-8.0

El-Omar et al, *Nature*, 2000; 404: 398-402

Role of composite pro-inflammatory polymorphisms on risk of non-cardia gastric cancer

Polymorphisms	Non-cardia gastric cancer (N=188)			Controls (N=210)
	N	Odds ratio	95% CI	n
0	22	1.0	(ref)	75
1	74	2.9	(1.6-5.1)	85
2	62	5.4	(2.7-10.6)	46
3-4	30	27.3	(7.4-99.8)	4

Pro-inflammatory polymorphisms: *IL-1B*-31/-511*2, *IL-1RN**2/*2 *TNF-A*-308*2, *IL-10* ATA/ATA

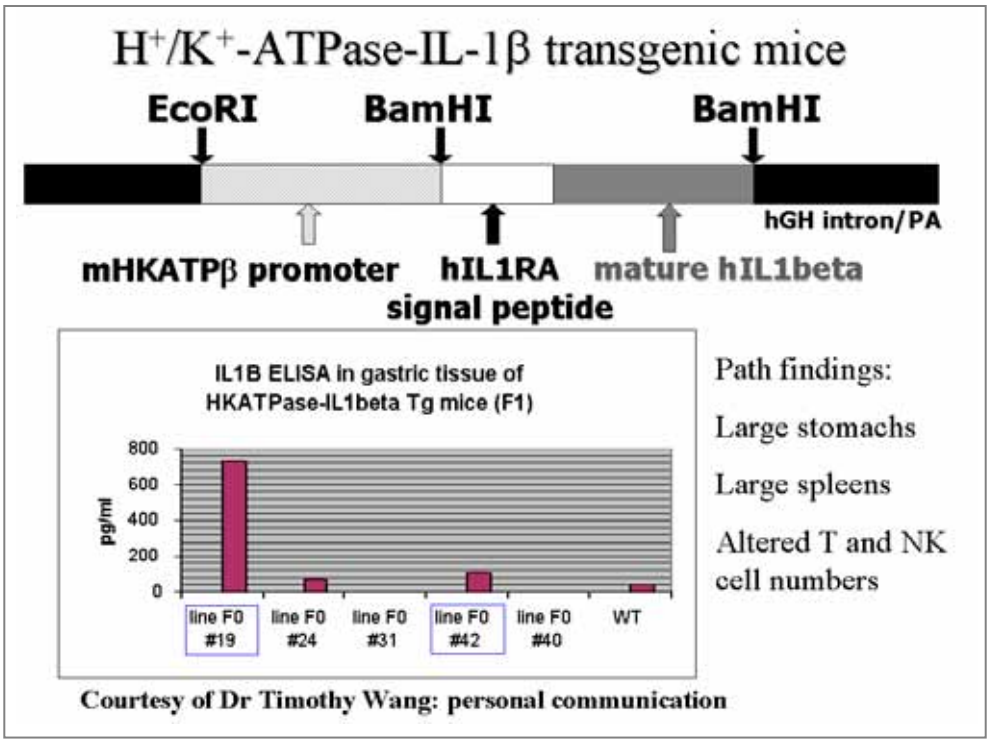
El-Omar et al, *Gastroenterology*, 2003; 124: 1193-1201

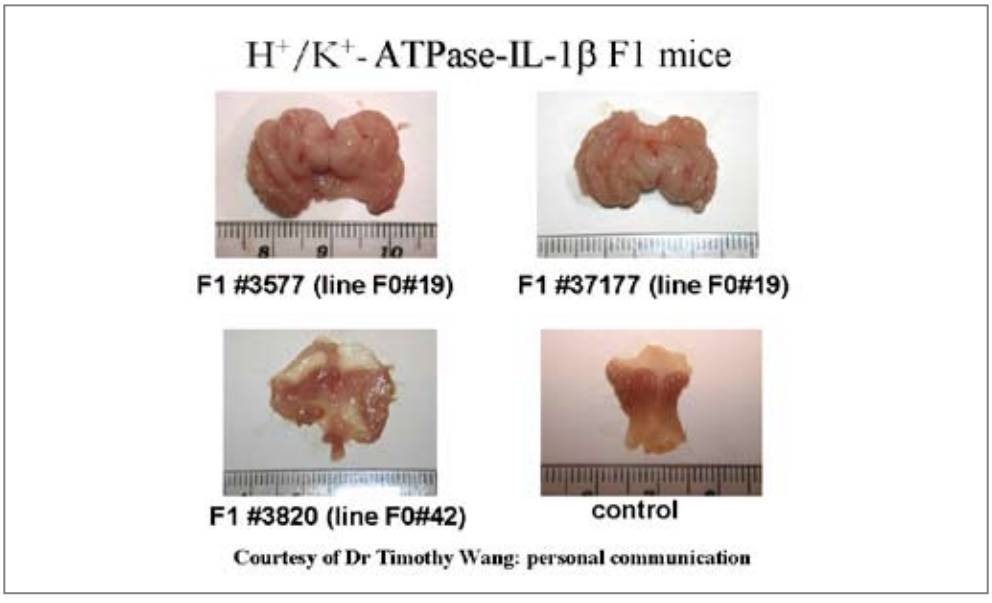
Synergistic effect of *H. pylori* virulence factors & host genetic factors

- Figueiredo et al, JNCI 2002; 94: 1680-1687
- 222 GC cases vs. 221 controls
- *cagA*, *vacA* s & m genotypes
- *IL-1B*-511C>T & *IL-1RN*
- For each combination of bacterial/host genotype, the odds of having gastric carcinoma were greatest in those with both bacterial and host high-risk genotypes

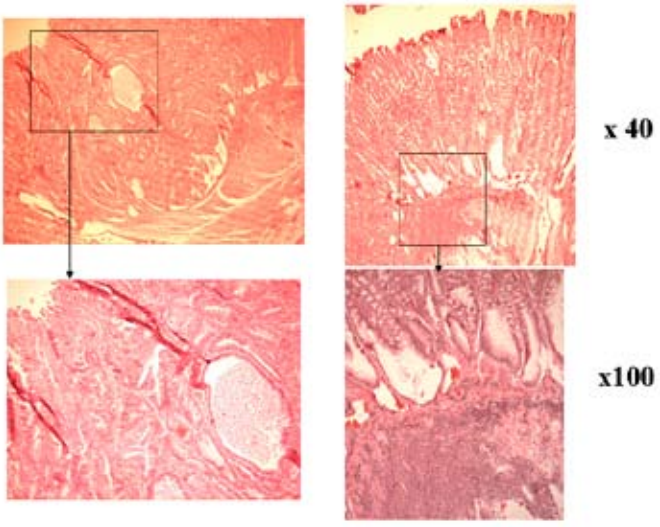
Synergistic effect of *H. pylori* virulence factors & host genetic factors

- *vacAs1*/*IL-1B*-511*T: OR = 87 (11 to 679)
- *vacAm1*/*IL-1B*-511*T: OR = 7.4 (3.2 to 17)
- *cagA*$^+$/*IL-1B*-511*T: OR = 25 (8.2 to 77)
- *vacAs1*/*IL-1RN**2/*2: OR = 32 (7.8 to 134)
- *vacAm1*/*IL-1RN**2/*2: OR = 8.8 (2.2 to 35)
- *cagA*$^+$/*IL-1RN**2/*2: OR = 23 (7.0 to 72)


H+/K+-ATPase-IL-1β transgenic mice
EcoRI
BamHI
BamHI
hGH intron/PA
mHKATPβ promoter
hIL1RA signal peptide
mature hIL1beta
IL1B ELISA in gastric tissue of HKATPase-IL1beta Tg mice (F1)
pg/ml
800
600
400
200
0
line F0 #19
line F0 #24
line F0 #31
line F0 #42
line F0 #40
WT
Path findings:
Large stomachs
Large spleens
Altered T and NK cell numbers
Courtesy of Dr Timothy Wang: personal communication


H+/K+- ATPase-IL-1β F1 mice
F1 #3577 (line F0#19)
F1 #37177 (line F0#19)
F1 #3820 (line F0#42)
control
Courtesy of Dr Timothy Wang: personal communication

H^+/K^+-ATPase-IL-1β transgenic mice progress to atrophy, severe dysplasia and even cancer



H+/K+-ATPase-IL-1β transgenic mice

- Produce high IL-1 β in gastric tissue
- Achlorhydric
- Progress to atrophy, severe dysplasia and cancer
- Process accelerated by *H. felis* infection
- First example of a single cytokine transgenic gastric cancer model

Important host genetic factors

- Cytokine gene polymorphisms
  - *IL-1B*-31/-511 C/T
  - *TNF-A*-308 G/A
  - *IL-10* ATA/ATA haplotype
  - ***IL-8*-251**
  - ***SHP-2***
- Innate immune response
  - ***TLR4*** (TLR4+896 A>G = Asp299Gly)
  - **Mannose binding lectin** (*MBL2* HYD haplotype)

Important host genetic factors

- Genes of the innate immune response

Toll-like receptor 4

TLR4 mutations

- Functional polymorphisms: hyporesponsiveness to LPS
- 2 common SNP polymorphisms in coding region
  - Asp299Gly & Thr399Ile
- Asp299Gly: missense mutation in 4th exon of *TLR4*, alters extracellular domain of the receptor
- LPS hyporesponsive = prone to sepsis by other gram –ve bacteria

Effect of *TLR4*+896 A>G on risk of non-cardia gastric cancer in Caucasians

TLR4+896 Genotype	Polish Study N= 354/419 OR (95%CI)	EGA Study N= 184/211 OR (95%CI)	Combined OR (95% CI)
A/A	1	1	1
A/G + G/G	2.5 (1.6-4.2)	2.0 (1.04-4.1)	2.4 (1.6-3.4)

IL-1 markers in non-Caucasian populations ??

Effect of *IL-1* markers on risk of gastric cancer in non-Caucasian populations

- Effect is variable
- May depend on background prevalence of gastric cancer
- Definite association with pre-cancerous abnormalities and the gastric cancer phenotype but association with cancer itself more difficult to demonstrate
- Role of "haplotype context"

IL-1B markers and haplotype context

- Chen et al Human Molecular Genetics, 2006
- Sequenced *IL-1B* gene from genomic DNA of Caucasian & African-American subjects
- Identified four promoter SNPs active in transient transfection reporter gene assays:
  -3737, -1464, -511, -31
- Examined nuclear protein binding to promoter sequence oligonucleotides containing different alleles of the SNPs
- Discovered functionally significant interactions between SNPs according to haplotype context

IL-1B markers and haplotype context

- Variant alleles at -31(C) and -511(T) enhance transcriptional activity
- -1464 and -3737 polymorphism both exert modifying effects on transcription by interacting with specific alleles at -31 and -511
- The co-inheritance of these alleles is variable in different ethnic groups and should be fully assessed in the context of risk of disease outcome

Role of diet as a modifier of *IL-1B* genetic risk

- Shen et al studied the effect of *IL-1B* genetic variants on the risk of metabolic syndrome (J Nutrition 2007)
- *IL-1B* genetic variants were associated with measures of both chronic inflammation and risk of developing metabolic syndrome
- Genetic influence was more evident among subjects with low (n-3) polyunsaturated fatty acids (PUFA) intake
- Varied diet, excessive or deficient in certain nutrients, may potentially modulate genetic risk

Genetic predictors: are they useful?

- The genetic markers are polymorphisms that are relatively frequent in the general population
- They often have subtle functional effects that have not been fully elucidated
- Their frequency varies across different ethnic backgrounds and their impact on cancer risk is modest (**they are not like inborn errors of metabolism**)
- Are they useful for screening? **Not yet**
- So, what are they useful for?

Genetic predictors: are they useful?

- A pro-inflammatory genetic makeup is a risk factor for serious complications of *H. pylori* infection, including gastric cancer
- The genetic markers highlight the important role of chronic inflammation in this disease
- The aetiology of this inflammation is obvious:
  ***H. pylori* infection**
- The only way to prevent the cancer is to remove the infection or reduce the inflammation

Host genetics and oesophageal disease

- Most of the work has focused on squamous cancer
- Very little data on adenocarcinoma
- Recent interest in the inflammation pathways and their genetic determinants

Effect of *IL-1* markers on risk of GERD

- Queiroz et al: Gastroenterology, 2004; 127: 73-79
- 383 subjects: 285 patients without GERD & 98 with
- GERD patients: 52% Grade 1, 35.7% Grade 2, 8.2% Grade 3, and 4.1% Barrett's
- Examined *IL-1B-31/-511*, *IL-1RN* & *TNF-A-308*
- Also examined the contribution of CagA status

Variables associated with GERD in *H. pylori* +ve patients

Covariate	OR	95% CI	P
Corpus gastritis	0.72	0.55-0.95	0.02
cagA+ve	0.35	0.17-0.58	0.002
*IL-1B-31*C*	0.63	0.38-0.99	0.05
*IL-1RN*2*2*	0.53	0.29-0.92	0.004

Queiroz et al, *Gastroenterology*, 2004; 127: 73-79

Adjusted OR's for GERD in *H. pylori* +ve & -ve patients

	H. pylori negative			*H. pylori* positive		
	OR	P	95%CI	OR	P	95%CI
IL-1B C-511T						
C/C	1	-	-	1	-	-
C/T	1.94	0.30	0.55-6.81	1.24	0.52	0.64-2.40
T/T	0.35	0.39	0.03-3.84	0.05	<0.001	0.01-0.26

Ando et al, *GUT*, 2006

IL-1/TNF-A genotypes & risk of different types of UGI cancer

	OAC	GCAC	NCGAC
IL-1B-511*T+/ *IL-1B*-31*C+	1.1 (0.7-1.8)	1.1 (0.7-1.8)	**2.8 (1.8-4.3)**
*IL-1RN**2*2	1.3 (0.5-3.3)	1.7 (0.7-3.7)	**4.8 (2.5-9.3)**
TNF-A-308*A+	0.9 (0.5-1.5)	1.0 (0.6-1.7)	**1.9 (1.2-2.8)**

El-Omar et al, *Gastroenterology*, 2003; 124: 1193-1201

Conclusions

- Study of host genetic factors is very important for understanding pathogenesis of disease
- The markers we have at present are not sensitive/specific enough to form the basis of a screening strategy
- New genotyping technologies may allow the definition of better predictive haplotypes
- Ultimately, our aim should be to prevent gastric cancer and this could only be achieved by removing *H. pylori* from the equation