

Supplementary Materials

Supplementary Table S1: Antigens included in *H. pylori* multiplex serology

Bacterium	Antigen Name	Putative function	Antigen specific cut off (MFI) for IgG	Antigen specific cut off (MFI) for IgA
<i>H. pylori</i>	GroEl	Chaperonin GroEL	500	100
	UreA	Urease alpha subunit	800	600
	Hp0231	Hypothetical protein	750	150
	NapA	Neutrophil-activating protein	500	250
	Hp0305	Hypothetical protein	300	100
	HpaA	Neuraminylactose binding hemagglutinin homolog	700	100
	CagA	Cytotoxin associated antigen A	4000	2000
	HyuA	Hydantoin utilization protein A	1000	200
	Catalase	Catalase	1000	500
	VacA	Vacuolating cytotoxin A	1200	600
	HcpC	Conserved hypothetical secreted protein	750	150
	Cad	Cinnamyl alcohol dehydrogenase ELI3-2	500	100
	HP1564 (Omp)	Hypothetical protein	1000	350

H. pylori: *Helicobacter pylori*; MFI: median fluorescence intensity. References: [21]

Supplementary Table S2: Seropositivity to individual *H. pylori* proteins and association with CRC development. Controls were restricted to individuals with no abnormalities detected after colonoscopy.

Secondary antibody	Antigen		NAD (n=37)	CRC (n=25)	Adjusted by age and sex			
			n (%)	n (%)	OR	95%CI	p-value	q-value
α-IgA	>3 proteins	-	33(89)	20(80)				
		+	4(11)	5(20)	0.83	0.56-1.22	0.33	0.33
		-	33(90)	19(76)				
	GroEl	+	4(10)	6(24)	3.09	0.69-13.8	0.13	0.83
		-	35(95)	23(92)				
	UreA	+	2(5)	2(8)	1.42	0.19-10.3	0.72	0.92
		-	34(92)	19(76)				
	Hp0231	+	3(8)	6(24)	2.95	0.62-14.5	0.17	0.83
		-	36(98)	23(92)				
	NapA	+	1(2)	2(8)	3.83	0.28-52.4	0.31	0.87
		-	36(98)	100(100)				
	HP0305	+	1(2)	0(0)				
		-	34(92)	24(96)				
	HpaA	+	3(8)	1(4)	0.43	0.04-4.52	0.48	0.91
		-	25(68)	17(68)				
	CagA	+	12(32)	8(32)	0.96	0.30-3.03	0.95	0.96
		-	33(90)	21(84)				
	HyuA	+	4(10)	4(16)	1.49	0.31-7.16	0.61	0.91
		-	29(79)	23(92)				
	Catalase	+	8(21)	2(8)	0.30	0.05-1.71	0.17	0.83
		-	32(87)	22(88)				
	VacA	+	5(13)	3(12)	0.84	0.17-4.07	0.39	0.91
		-	32(87)	24(96)				
	HcpC	+	5(13)	1(4)	0.28	0.02-2.81	0.28	0.87
		-	100(100)	23(92)				
	Cad	+	0	2(8)				
		-	33(90)	22(88)				
	HP1564	-	33(90)	22(88)				
		+	4(10)	3(12)	0.96	0.17-5.32	0.96	0.96

α-IgG	>3 proteins	-	21(57)	10(40)				
		+	16(43)	15(60)	0.78	0.57-1.04	0.33	0.33
	GroEl	-	19(52)	10(40)				
		+	18(48)	15(60)	2.10	0.67-6.59	0.20	0.57
	UreA	-	30(81)	17(68)				
		+	7(19)	8(32)	2.14	0.58-7.84	0.24	0.57
	Hp0231	-	32(87)	17(68)				
		+	5(13)	8(32)	2.26	0.58-8.73	0.23	0.57
	NapA	-	27(73)	18(64)				
		+	10(27)	9(36)	1.88	0.58-6.06	0.28	0.57
	HP0305	-	29(79)	20(80)				
		+	8(21)	5(20)	1.59	0.39-6.45	0.51	0.62
	HpaA	-	34(92)	22(88)				
		+	3(8)	3(12)	2.80	0.43-17.9	0.27	0.57
	CagA	-	20(55)	12(48)				
		+	17(45)	13(52)	1.48	0.49-4.44	0.47	0.62
	HyuA	-	31(84)	15(60)				
		+	6(16)	10(40)	3.02	0.87-10.4	0.08	0.57
	Catalase	-	31(84)	22(88)				
		+	6(16)	3(12)	0.92	0.19-4.43	0.92	0.92
	VacA	-	33(90)	23(92)				
		+	4(10)	2(8)	0.70	0.12-4.14	0.70	0.80
	HcpC	-	32(87)	18(72)				
		+	5(13)	7(28)	2.51	0.67-9.44	0.17	0.57
	Cad	-	35(95)	22(88)				
		+	2(5)	3(12)	3.03	0.43-21.0	0.26	0.57
	HP1564	-	20(55)	12(48)				
		+	17(45)	13(52)	1.73	0.56-5.37	0.33	0.59

logistic regression analysis adjusted by age and sex. q-value: p-value adjusted after False Discovery Rate. Ab: antibody; OR: Odd Ratio; CI: Confidence Interval; NAD: no abnormalities detected; *H. pylori*: *Helicobacter pylori*; +: Positive; -: Negative; Statistically significant p and q-values are indicated in bold.

Supplementary Table S3: Seropositivity to individual *H. pylori* proteins and association with advanced adenoma development. Controls were restricted to individuals with no abnormalities detected after colonoscopy.

Secondary antibody	Antigen		NAD (n=37)	AA (n=82)	Adjusted by age and sex			
			n (%)	n (%)	OR	95%CI	p-value	q-value
α-IgA	>3 proteins	-	33(89)	69(84)				
		+	4(10)	13(16)	0.82	0.48-1.33	0.42	0.42
	GroEl	-	33(90)	69(84)				
		+	4(10)	13(16)	1.63	0.47-5.63	0.43	0.72
	UreA	-	35(95)	76(92)				
		+	2(5)	6(8)	0.95	0.16-5.46	0.96	0.96
	Hp0231	-	34(92)	77(93)				
		+	3(8)	5(7)	0.58	0.12-2.70	0.49	0.73
	NapA	-	36(98)	73(89)				
		+	1(2)	9(11)	5.29	0.62-44.6	0.12	0.46
	HP0305	-	36(98)	81(98)				
		+	1(2)	1(2)	0.35	0.02-6.08	0.35	0.72
	HpaA	-	34(92)	76(92)				
		+	3(8)	6(8)	0.82	0.18-3.70	0.80	0.88
	CagA	-	25(68)	65(79)				
		+	12(32)	17(21)	0.46	0.18-1.16	0.10	0.46
	HyuA	-	33(90)	72(87)				
		+	4(10)	10(13)	1.15	0.32-4.13	0.82	0.88
	Catalase	-	29(79)	74(90)				
		+	8(21)	8(10)	0.30	0.09-1.00	0.50	0.46
	VacA	-	32(87)	73(89)				
		+	5(13)	9(11)	0.82	0.24-2.75	0.75	0.88
	HcpC	-	32(87)	77(93)				
		+	5(13)	5(7)	0.44	0.11-1.74	0.24	0.61
	Cad	-	100(100)	76(92)				
		+	0	6(8)				
	HP1564	-	33(90)	75(91)				
		+	4(10)	7(9)	0.79	0.20-3.06	0.73	0.88
α-IgG	>3 proteins	-	21(57)	30(37)				

	+	16(43)	52(63)	0.78	0.55-1.09	0.30	0.30
GroEl	-	19(52)	37(45)				
	+	18(48)	45(55)	1.06	0.47-2.41	0.87	0.87
UreA	-	30(81)	55(65)				
	+	7(19)	27(35)	2.11	0.79-5.62	0.22	0.76
Hp0231	-	32(87)	67(81)				
	+	5(13)	15(19)	1.25	0.40-3.90	0.69	0.78
NapA	-	27(73)	53(63)				
	+	10(27)	29(37)	1.50	0.62-3.63	0.36	0.76
HP0305	-	29(79)	60(78)				
	+	8(21)	22(27)	1.47	0.56-3.86	0.42	0.76
HpaA	-	34(92)	67(81)				
	+	3(8)	15(19)	3.11	0.81-11.8	0.09	0.76
CagA	-	20(55)	39(47)				
	+	17(45)	43(53)	1.21	0.53-2.74	0.63	0.78
HyuA	-	31(84)	58(70)				
	+	6(16)	24(30)	1.97	0.70-5.51	0.19	0.76
Catalase	-	31(84)	57(69)				
	+	6(16)	25(31)	1.57	0.55-4.52	0.39	0.76
VacA	-	33(90)	78(95)				
	+	4(10)	14(5)	1.65	0.53-5.14	0.38	0.76
HcpC	-	32(87)	65(79)				
	+	5(13)	17(21)	1.36	0.47-3.94	0.56	0.78
Cad	-	35(95)	72(87)				
	+	2(5)	10(13)	2.46	0.50-12.0	0.26	0.76
HP1564	-	20(55)	42(51)				
	+	17(45)	40(49)	1.15	0.51-2.60	0.73	0.78

logistic regression analysis adjusted by age and sex. q-value: p-value adjusted after False Discovery Rate. Ab: antibody; OR: Odd Ratio; CI: Confidence Interval; NAD: no abnormalities detected. *H. pylori*: *Helicobacter pylori*; +: Positive; -: Negative; Statistically significant p and q-values are indicated in bold.

Supplementary Table S4: Seropositivity to individual *H. pylori* proteins and association with polyp development. Controls were restricted to individuals with no abnormalities detected after colonoscopy.

Secondary antibody	Antigen		NAD (n=37)	Polyp (n=85)	Adjusted by age and sex			
			n (%)	n (%)	OR	95%CI	p-value	q-value
α -IgA	>3 proteins	-	33(89)	71(84)				
		+	4(11)	14(16)	0.91	0.23-3.00	0.48	0.48
	GroEl	-	33(90)	73(85)				
		+	4(10)	12(15)	1.71	0.33-4.10	0.80	0.90
	UreA	-	35(95)	78(91)				
		+	2(5)	7(9)	1.19	0.22-6.25	0.83	0.90
	Hp0231	-	34(92)	75(88)				
		+	3(8)	10(12)	1.40	0.35-5.60	0.62	0.90
	NapA	-	36(98)	83(97)				
		+	1(2)	2(3)	1.29	0.10-15.3	0.83	0.90
	HP0305	-	36(98)	100(100)				
		+	1(2)	0(0)				
	HpaA	-	34(92)	79(92)				
		+	3(8)	6(8)	0.71	0.16-3.16	0.65	0.90
	CagA	-	25(68)	58(68)				
		+	12(32)	27(32)	0.93	0.39-2.23	0.88	0.90
	HyuA	-	33(90)	76(89)				
		+	4(10)	9(11)	0.82	0.22-2.99	0.76	0.90
	Catalase	-	29(79)	76(89)				
		+	8(21)	9(11)	0.36	0.12-1.13	0.08	0.56
	VacA	-	32(87)	75(88)				
		+	5(13)	10(12)	0.70	0.21-2.35	0.57	0.90
	HcpC	-	32(87)	75(88)				
		+	5(13)	10(12)	0.70	0.20-2.40	0.57	0.90
	Cad	-	100(100)	84(98)				
		+	0	1(2)				
	HP1564	-	33(90)	80(97)				
		+	4(10)	5(3)	0.09	0.009-0.98	0.049	0.56
α -IgG	>3 proteins	-	21(57)	37(44)				

	+	16(43)	48(56)	0.72	0.32-1.62	0.34	0.34
GroEl	-	19(52)	27(31)				
	+	18(48)	58(69)	2.23	0.97-5.12	0.05	0.69
UreA	-	30(81)	55(64)				
	+	7(19)	30(36)	1.61	0.59-4.37	0.34	0.69
HP0231	-	32(87)	67(81)				
	+	5(13)	18(19)	1.11	0.35-3.44	0.85	0.91
NapA	-	27(73)	63(74)				
	+	10(27)	22(26)	0.71	0.27-1.82	0.48	0.69
HP0305	-	29(79)	56(65)				
	+	8(21)	29(35)	1.78	0.70-4.51	0.22	0.69
HpaA	-	34(92)	80(94)				
	+	3(8)	5(6)	0.85	0.18-3.88	0.83	0.91
CagA	-	20(55)	34(40)				
	+	17(45)	51(60)	1.54	0.68-3.48	0.29	0.69
HyuA	-	31(84)	62(75)				
	+	6(16)	23(25)	1.55	0.55-4.38	0.40	0.69
Catalase	-	31(84)	54(63)				
	+	6(16)	31(37)	2.32	0.84-6.39	0.10	0.69
VacA	-	33(90)	72(84)				
	+	4(10)	13(16)	1.19	0.37-3.79	0.76	0.91
HcpC	-	32(87)	68(80)				
	+	5(13)	17(20)	1.22	0.41-3.57	0.71	0.91
Cad	-	35(95)	83(97)				
	+	2(5)	3(3)	0.500	0.07-3.30	0.47	0.69
HP1564	-	20(55)	46(54)				
	+	17(45)	39(46)	0.94	0.42-2.12	0.94	0.94

logistic regression analysis adjusted by age and sex. q-value: p-value adjusted after False Discovery Rate. Ab: antibody; OR: Odd Ratio; CI: Confidence Interval; NAD: no abnormalities detected; *H. pylori*: *Helicobacter pylori*; +: Positive; -: Negative; Statistically significant p and q-values are indicated in bold.

Supplementary Table S5: Spearman's correlation analysis between *H. pylori* abundance in tissue and antibody response.

Antigen		RQ in colorectal disease tissue ¹ (n=34)		RQ in normal mucosa(n=29)	
		r*	p-value	r*	p-value
IgA	GroEl	0.09	0.60	0.11	0.56
	UreA	-0.00	0.96	-	-
	HP0231	-0.06	0.71	0.15	0.42
	NapA	-0.00	0.97	-0.02	0.90
	HP305	-	-	-	-
	HpaA	-0.34	0.046	-0.24	0.19
	CagA	0.00	1.00	-0.18	0.33
	HyuA	0.02	0.87	0.36	0.05
	Catalase	-0.16	0.35	0.48	0.007
	VacA	-0.02	0.88	0.37	0.046
	HcpC	-0.01	0.92	-0.03	0.86
	Cad	-0.28	0.10	0.34	0.07
	HP1564	-0.09	0.60	0.23	0.23
IgG	GroEl	-0.21	0.27	0.22	0.24
	UreA	-0.07	0.69	0.34	0.06
	HP0231	-0.41	0.016	0.29	0.12
	NapA	-0.30	0.07	0.30	0.11
	HP305	0.19	0.27	0.19	0.30
	HpaA	0.03	0.86	-0.07	0.65
	CagA	-0.13	0.43	0.23	0.22
	HyuA	-0.06	0.73	0.26	0.15
	Catalase	-0.07	0.65	0.51	0.005
	VacA	-0.09	0.58	0.37	0.048
	HcpC	-0.28	0.10	-0.02	0.91
	Cad	-0.02	0.87	0.29	0.11
	HP1564	-0.09	0.59	0.24	0.19

Spearman correlation test. *Correlation coefficient. RQ: relative quantification. ¹Colorectal disease tissue includes colorectal cancer and colorectal adenomas; Statistically significant r* and p-values are indicated in bold.