

Polymorphism in Toll-Like Receptors and *Helicobacter Pylori* Motility in Autoimmune Atrophic Gastritis and Gastric Cancer

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Table S1. Genotype and allele frequencies of the TLR gene polymorphisms in AAG compared to (A) HD and to (B) GC.

		A						
		AAG (n = 67)		HD (n = 97)				
	Genotype	count	frequency	count	frequency	p	Corrected p-value	Age-and sex-adjusted OR (95% CI) *
TLR1 rs4833095	T/T	27	0.40	33	0.34	0.34	0.51	1.00
	C/T	29	0.43	47	0.48			2.64 (0.43-16.32)
	C/C	11	0.16	17	0.18	0.2		1.21 (0.10-14.12)
	T	83	0.62	113	0.58			
	C	51	0.38	81	0.42			
TLR2 rs3804099	T/T	17	0.25	28	0.29	0.77	0.62	1.00
	C/T	37	0.55	54	0.56			0.90 (0.12-6.62)
	C/C	13	0.19	15	0.15	0.74		0.26 (0.01-6.54)
	T	71	0.53	181	0.55			
	C	63	0.47	147	0.45			
TLR2 196-174del	A/A	49	0.73	68	0.70	0.86	0.64	1.00
	T/A	17	0.25	28	0.29			2.35 (0.38-14.5)
	T/T	1	0.01	1	0.01	0.83		--
	A	115	0.86	279	0.85			
	T	19	0.14	49	0.15			
TLR4 rs4986790	A/A	60	0.9	85	0.88	0.7	0.33	1.00
	A/G	7	0.1	12	0.12			4.44 (0.48-41.0)
	A	127	0.95	182	0.94	0.71		
	G	7	0.05	12	0.06			
TLR4 rs4986791	C/C	60	0.9	85	0.88	0.7	0.33	1.00
	C/T	7	0.1	12	0.12			4.44 (0.48-41.0)
	C	127	0.95	182	0.94	0.71		

	T	7	0.05	12	0.06			
	T/T	23	0.34	39	0.40			1.00
	C/T	37	0.55	38	0.40	0.04	0.06	1.57 (1.0-3.09)
	C/C	7	0.1	20	0.20			0.57 (0.21-1.54)
TLR5	T	83	0.62	116	0.60	0.69		
rs5744174	C	51	0.38	78	0.40			
	<i>Overdominant model #</i>							
	T/T + C/C	30	0.45	59	0.61			1.00
	C/T	37	0.55	38	0.39	0.04	0.021	1.85 (1.00-3.44)
	<i>Female</i>							
	A/A	30	0.58	22	0.53			1.00
	A/G	18	0.35	13	0.30	0.32	0.35	1.99 (0.66-6.02)
	G/G	4	0.08	7	0.16			2.66 (0.48-14.6)
TLR8°	A	78	0.75	57	0.68			
rs3764880	G	26	0.25	27	0.32	0.28		
	<i>Male</i>							
	A	11	0.73	41	0.75	0.89	0.10	1.00
	G	4	0.27	14	0.25			0.94 (0.38-2.34)
	T/T	55	0.82	70	0.72			1.00
TLR9	T/C	12	0.18	25	0.26	0.16	0.69	2.06 (0.40-10.60)
rs5743836	C/C	0	0	2	0.02			--
	T	122	0.91	165	0.88	0.11		
	C	12	0.09	29	0.12			
B								
AAG (n = 67)			GC (n = 114)					
	Genotype	count	frequency	count	frequency	p	Corrected p-value	Age-and sex-adjusted OR (95% CI) *
	T/T	28	0.42	34	0.30			1.00
TLR1	C/T	30	0.44	65	0.57	0.44	0.32	2.02 (0.79-5.14)
rs4833095	C/C	9	0.14	15	0.13			1.30 (0.34-4.94)
	T	86	0.64	133	0.58	0.27		
	C	48	0.36	95	0.42			
	T/T	17	0.25	32	0.28			1.00
TLR2	C/T	37	0.55	62	0.54	0.91	0.82	0.89 (0.43-1.82)
rs3804099	C/C	13	0.19	20	0.18			0.82 (0.32-2.03)
	T	71	0.53	126	0.55	0.67		
	C	63	0.47	102	0.45			
TLR2	A/A	49	0.73	78	0.68			1.00
196 -174del	T/A	17	0.25	31	0.27	0.50	0.79	1.14 (0.28-4.61)

	T/T	1	0.01	5	0.05			NA
	A	115	0.86	187	0.82	0.35		
	T	19	0.14	41	0.18			
TLR4 rs4986790	A/A	60	0.9	102	0.89	0.99	0.34	1.00
	A/G	7	0.1	12	0.11			3.19 (0.27-37.15)
	A	127	0.95	216	0.95	0.99		
	G	7	0.05	12	0.05			
TLR4 rs4986791	C/C	60	0.9	102	0.89	0.99	0.34	1.00
	C/T	7	0.1	12	0.11			3.19 (0.27-37.15)
	C	127	0.95	216	0.95	0.99		
	T	7	0.05	12	0.05			
TLR5 rs5744174	T/T	23	0.34	47	0.41			1.00
	C/T	37	0.55	44	0.39	0.07	0.06	0.68 (0.33-1.41)
	C/C	7	0.1	23	0.2			2.26 (0.77-6.66)
	T	83	0.62	138	0.61	0.22		
	C	51	0.38	111	0.47			
	<i>Recessive# model</i>	T/T + C/T	60	0.90	91	0.80	0.07	0.03
	C/C	7	0.10	23	0.20			2.81 (1.04-7.62)
	<i>Female</i>							
TLR8° rs3764880	A/A	30	0.58	19	0.44	0.37	0.37	1.00
	A/G	18	0.35	21	0.49			1.85 (0.78-4.34)
	G/G	4	0.08	3	0.08			1.19 (0.24-5.95)
	A	78	0.75	59	0.68	0.22		
	G	26	0.25	27	0.32			
	<i>Male</i>							
TLR9 rs5743836	A	11	0.73	50	0.70	0.73	0.77	1.00
	G	4	0.27	21	0.30			1.20 (0.34-4.27)
	T/T	55	0.82	80	0.70			1.00
	T/C	12	0.18	30	0.26	0.28	0.31	2.42 (0.62-9.45)
	C/C	0	0	4	0.04			--
	T	122	0.91	190	0.83	0.04		
	C	12	0.09	38	0.17			

* Analysis of association are performed by regression analysis; Corrected p -value for age and sex. # five inheritance models are fitted which correspond to different grouping of the genotypes: codominant, dominant, recessive, co-dominant and additive. Table reports the best model for a specific polymorphism based on akaike's information criterion (AIC). ° TLR8 stratified by gender.

Table S2. List of flagellins (A and/or B) identified in *Helicobacter pylori* isolates.

Gel portion nr. ^{a)}	Database	Accession	Mass	Protein annotation	Score	Matches	Seq. ^{b)}	Seq. cov. % ^{c)}
1	No detection.							
2	SwissProt	2::FLAA_HELPJ	53252	Flagellin A OS = <i>Helicobacter pylori</i> (strain J99 / ATCC 700824)	303	13	10	17
3	SwissProt	2::FLAA_HELPJ	53252	Flagellin A OS = <i>Helicobacter pylori</i> (strain J99 / ATCC 700824)	143	13	8	14
	SwissProt	2::FLAB_HELPJ	53937	Flagellin B OS = <i>Helicobacter pylori</i> (strain J99 / ATCC 700824)	88	9	7	15
4	NCBIInr	1::gi 261839570	53253	Flagellin A [<i>Helicobacter pylori</i> 52]	1086	60	18	34
	NCBIInr	1::gi 317180372	53266	Flagellin A [<i>Helicobacter pylori</i> F32]	1059	55	19	39
	NCBIInr	1::gi 188527549	53278	Flagellin A [<i>Helicobacter pylori</i> Shi470]	781	48	17	29
	NCBIInr	1::gi 108562540	53951	Flagellin B [<i>Helicobacter pylori</i> HPAG1]	540	39	17	33
5	SwissProt	2::FLAB_HELPJ	53937	Flagellin A OS = <i>Helicobacter pylori</i> (strain J99 / ATCC 700824)	272	14	8	30
6	SwissProt	2::FLAB_HELPJ	53937	Flagellin A OS = <i>Helicobacter pylori</i> (strain J99 / ATCC 700824)	549	28	16	30
	NCBIInr	1::gi 261839570	53253	Flagellin A [<i>Helicobacter pylori</i> 52]	508	30	16	30

^{a)} gel portion nr., gel portion numbers refer to Figure 3; ^{b)} Seq., sequences; ^{c)} Seq. cov. %, percentage of sequence coverage.

Table 3. Primers used in the amplification and sequencing PCR of *H. pylori* *flaA* gene.

Primer	Coordinates†	Primer sequences (5'→3')	Application
<i>FlaA F</i>	597956-597981	TGGCTTTTCAGGTCAATACAAATATC	Amplification and cycle sequencing
<i>FlaA R</i>	599451-599476	GCCTTAAGATATTTTGRGTAACGGTA *	Amplification and cycle sequencing
<i>FlaA 451 F</i>	598437-598456	CGGCTCTACCACTTCCGATA	Cycle sequencing
<i>FlaA 716 F</i>	598650-598669	TTATGCGAGCGTTATCACCA	Cycle sequencing
<i>FlaA 1226 F</i>	599160-599179	GATCGCTAGCGGTAACCAA	Cycle sequencing
<i>FlaA 289 R</i>	598256-598275	TTCCGTAGTTTGCCCATCTT	Cycle sequencing
<i>FlaA 551 R</i>	598466-598485	GCCTGTAGCGATACGAACCT	Cycle sequencing
<i>FlaA 802 R</i>	598769-598788	GATCGCTGCGACTAACCTTC	Cycle sequencing

†, coordinates are referred to *H. pylori* J99 strain genome; *, R = A or G, degenerate base.

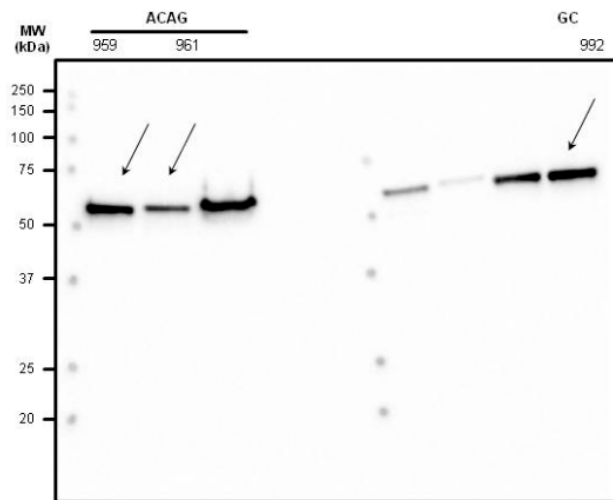


Figure S1. Proteomic characterization of flagellin A. The whole immunoblotting from which figure 2C comes is shown, and arrows indicate signal of proteins cross-reacting with the anti-flagellin antibody at around 54 kDa in *H. pylori* isolated from either AAG (samples 959 and 961) and on GC (sample 992).



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