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Variation in human genetic polymorphisms, their association with *Helicobacter pylori* acquisition and gastric cancer in a multi-ethnic country

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Abstract

Background and Aim: The contribution of human genetic polymorphisms to *Helicobacter pylori* infection and gastric cancer (GC) development remains unclear due to geographic variation in the association between specific host genetic polymorphisms and GC. In the current study we investigated the association between polymorphisms related to immune and cancer-related pathways and *H. pylori* infection among the major ethnicities, Chinese, Malay and Indian, resident in Singapore and Malaysia as well as the association between these polymorphisms and GC development in ethnic Chinese patients.

Methods: Thirty-four polymorphisms in 26 genes were typed by mass spectrometry in 422 patients undergoing endoscopy (162 Chinese, 113 Indian and 87 Malay controls and 60 Chinese GC cases). Patients were assessed for evidence of *H. pylori* infection. Odds ratios (OR) and confidence intervals (CI) were obtained using logistic regression models.

Result: The prevalence of 16 polymorphisms varied significantly among the ethnicities. In the Chinese subgroup, nominally significant associations were shown between (i) *EBBR2*+1963G (rs1801200) and *H. pylori* infection (per-allele OR: 0.48, 95% CI 0.23, 0.98, $P = 0.04$), (ii) *PTGS2*-1195G (rs689466) and an increased risk of GC on adjusting for *H. pylori* status (OR: 1.53, 95% CI 0.99, 2.37, $P = 0.05$), and (iii) *IL1B*-1473C (rs1143623) and a decreased risk of GC (OR: 0.64, 95% CI 0.41, 0.99, $P = 0.05$). Borderline significant associations were seen between *IL2*-330G (rs2069762) (OR 1.45, 95% CI 0.95, 2.15, $P = 0.06$) and *IL13*-1111T (rs1800925) (OR 0.65, 95% CI 0.42, 1.01, $P = 0.06$) and *H. pylori* infection.

Conclusion: These findings contribute to the understanding of the genetic variation between ethnicities, which may influence *H. pylori* susceptibility and the outcome of infection.

Introduction

Gastric cancer (GC) is a significant global burden, being the fourth most common cancer and the second most common cause of cancer-related death world wide.[1] While *H. pylori* infection is recognized as a major risk factor for GC development, less than 1% of individuals infected with *H. pylori* will develop GC, suggesting that inter-individual variation in the form of genetic polymorphisms that alter gene expression and functionality may play a significant role.

In 2001 El-Omar *et al*. reported based on a study of patients from Scotland and Poland that carriers of the *IL1B*-31 C allele and homozygous carriers of the *IL1RN*2* allele had an increased risk of GC[2]. The *IL1B*-31 C allele has been reported to enhance IL-1β production resulting in increased inflammation and decreased gastric acid secretion, the latter allowing spread of *H. pylori* from the antrum to the corpus, thus facilitating *H. pylori* induced mucosal damage and GC development.[2] A subsequent study by the same group demonstrated a greater than 20-fold increased risk of GC associated with the presence of three or more pro-inflammatory polymorphisms in the genes of cytokines IL-1β, IL-1RN, IL-10 and tumor necrosis factor-α (TNF-α).[3]

Genetic polymorphisms may not only predict GC development in individuals, but variations in their frequency may explain differences in GC incidence between ethnic groups, such as the three ethnicities resident in Malaysia, where ethnic Chinese have a high rate of GC (age standardized rate of 11.3/100 000 for males), while in ethnic Indians (5.5/100 000) and ethnic Malays (2.1/100 000)

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the rate is much lower.[4] Recently, our group demonstrated significant variation in the frequencies of *IL1B-511, IL1RN, IL10-1082* and *IL10-592* among the three ethnic groups resident in Malaysia and Singapore.[5] Such findings necessitate the validation of putative associations with human genetic polymorphisms in multiple ethnic groups.

Ethnic variation in the frequency of genetic polymorphisms may also explain the considerable difference in *H. pylori* prevalence between ethnic Indians (50–60%), ethnic Chinese (35–55%) and ethnic Malays (10–25%),[6] as yet few studies have investigated whether any such associations exist.[7] *H. pylori* infection initiates an early innate immune response, which is quickly replaced by a vigorous adaptive response which is unable to eliminate *H. pylori* infection.[8] The human genetic factors driving the ineffective response to *H. pylori* infection and carcinogenesis are mostly unknown, although a variety of interconnected pathways have been implicated.[9] To date, few studies have been conducted which not only investigate a panel of polymorphisms from these contributory pathways in relation to GC, but also examine the relationship with *H. pylori* infection independent of GC status, and the variation in prevalence between ethnic groups.

Therefore, this study aimed to describe in the three ethnic groups resident in Malaysia and Singapore, the frequency of a panel of 34 polymorphisms in 26 genes, which may provide a basis for the well characterized differences in *H. pylori* prevalence and GC incidence. We further sought to specifically assess the association between these polymorphisms and *H. pylori* infection status in all three ethnicities, as well as GC in the ethnic Chinese population.

Methods

Study population and samples

Between January 2004 and April 2007, 422 unrelated patients undergoing endoscopy for routine gastrointestinal symptoms were enrolled at the University Hospital of Malaysia, Kuala Lumpur, Malaysia and Changi General Hospital, Singapore. Patients included in this study were diagnosed with functional dyspepsia (FD) or GC based on endoscopy and histological assessment as appropriate. FD was defined as pain or discomfort centered in the upper abdomen without any identifiable organic disease, in accordance with the Rome II classification system.[10] Patients taking anti-microbial agents, non-steroidal anti-inflammatory drugs or acid suppressants in the two months prior to recruitment and patients known to be infected with HIV were excluded. Two 5-mL vials of peripheral blood were collected at the time of endoscopy and serum was immediately separated from one vial. Informed consent was obtained from each subject and a brief sociodemographic questionnaire completed, including information pertaining to gender, age and ethnicity. This study was approved by the Human Research Ethics Committee (HREC) of the University of New South Wales (HREC 08115 and HREC 02144).

Serological evaluation of past/current *H. pylori* infection

An in-house enzyme linked immunosorbent assay (ELISA) to detect antibodies to *H. pylori*, with a sensitivity of 100% and specificity of 94%, was performed on the sera of all 422 patients as described by Mitchell *et al*.[11] Briefly, *H. pylori* strains NTCC16369 and UNSWP10 were used for the solid phase and sera were absorbed with *C. jejuni* strains Cj100 and Cj23. Antibodies to *H. pylori* were visualized using a mouse-antihuman IgG alkaline phosphatase conjugated secondary antibody (Sigma Aldrich, St Louis, MO, USA) (diluted 1:10 000) and the substrate disodium p-nitrophenylphosphate (d-pNPP) (Invitrogen, Carlsbad, CA, USA), prepared according to the manufacturer's instructions. The absorbance of duplicate serum samples was read at 405 nm.

In addition to ELISA, serum samples from 12 GC patients shown to be *H. pylori* negative by ELISA were investigated further by Immunoblot (MPD Helico Blot 2.1, MP Biomedicals, Seven Hills, Australia) according to the manufacturer's instructions.

Selection of polymorphisms for analysis

Thirty-four polymorphisms over 26 genes were selected for analysis from the available literature based on known or potential functionality, and/or previous associations with cancer development, inflammatory diseases or *H. pylori* infection. All selected polymorphisms were reported to have a minor allele frequency > 1% in the National Center for Biotechnology Information (NCBI) dbSNP[12] or in published literature. Polymorphism sequences were identified using dbSNP.

DNA extraction and analysis of polymorphisms by mass spectrometry

DNA was prepared from each subject's whole blood using the DNA Blood Mini kit (Qiagen, Doncaster, Australia) as per the manufacturer's instructions, with the following modification: DNA was rehydrated in sterile water. Samples, normalized to 10 ng/uL, were sent to Australian Genome Research Facility Ltd (AGRF), St Lucia, University of Queensland, Australia, for single nucleotide polymorphism (SNP) analysis by mass spectroscopy.

PCR-RFLP validation of mass spectroscopy results for polymorphism typing

Polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) was conducted to validate the results obtained by Mass Spectroscopy in a subgroup of subjects ($n = 373$) for five polymorphisms over four genes: *IL1B*-511 (RS16944), *IL1B*-1473 (RS1143623), *IL10*-1082 (RS1800896), *TNFA*-308 (RS1800629) and *TLR5*+1174 (RS5744168). PCR primers (Sigma Aldrich), annealing temperatures and restriction enzymes (New England Biolabs, Ipswich, MA, USA) used to detect polymorphisms are presented in Table 1. Briefly, PCR was performed in a 25-μL reaction which included 4 mM $MgCl_2$ (Qiagen), 200 μM dNTP (Qiagen), 1 unit Hot Star Taq polymerase (Qiagen) and 1 μg DNA template. PCR cycling consisted of an initial denaturation at 95°C for 15 min, 35 cycles of 94°C for 30 s, 56–60°C for 30 sand 72°C for 50 s, and a final extension step at 72°C for 5 min. RFLP was conducted on PCR products according to the manufacturer's recommendations.

Statistical analysis

To assess differences in patient demographics and *H. pylori* prevalence between control groups, and controls and cases, the one-way

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Table 1 Oligonucleotide primer pairs and restriction enzymes used for polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) based confirmation of five polymorphisms

dbSNP reference	SNP	Primer sequence	Annealing temperature	Restriction enzyme	Reference
RS1143623	*IL1B*-1473	GAA ACT AAC ATG TTG AAC AG TTG AAT GGG TGA ATG GGA AT	56°C	*Sty*I	[5]
RS1800896	*IL10*-1082	AACACTACTAAGGCT TCTTGAGGA AGCACATAGAATGAAACCTTGG	56°C	*BseR*I	[5]
RS16944	*IL1B*-511	TGG CAT TGA TCT GGT TCA TC GTT TAG GAA TCT TCC CAC TT	56°C	*Ava*I	[13]
RS1800629	*TNFA*-308	AGG CAA TAG GTT TTG AGG GCC AT TCC TCC CTG CTC CGA TTC CG	60°C	*Nco*I	[14]
RS5744168	*TLR5* +1174	GGT AGC CTA CAT TGA TTT GC GAT TCT CTG AAG GGG TTT G	57°C	*Dde*I	[5]

SNP, single nucleotide polymorphism.

Table 2 Details of patients included in the analysis: percentage (%) of males, mean age and standard deviation (SD), and percentage of patients positive for *Helicobacter pylori* (HP)

Ethnicity	Diagnosis	Total no.	% Males	Mean age	SD	%HP positive
Chinese	FD	162	46.3	54.14	14.03	77.8
	GC	60	61.7	64.51	13.43	86.7
Indian	FD	113	40.7	48.67	14.49	83.2
Malay	FD	87	40.2	42.28	14.4	54.0

FD, functional dyspepsia; GC, gastric cancer; SD, standard deviation.

analysis of variance (ANOVA) and unpaired *t*-test available in GraphPad InStat version 3.06 statistical analysis package for Windows (GraphPad Software, San Diego, CA, USA, http://www.graphpad.com) were used.

Hardy–Weinberg equilibrium (HWE) analysis for each polymorphism and pairwise linkage disequilibrium (LD) measures for polymorphisms on the same chromosome were performed using the FD group for each ethnicity. These tests were available in STATA version 10 (STATA Corporation, College Station, TX, USA) using the *genhwi* and *pwld* commands, respectively.

Allele frequencies in the controls (FD) were compared for each polymorphism between the three ethnic groups using 2×3 χ^2 tests in Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA, USA) to determine variation between ethnic groups. Logistic regression analyses were undertaken in STATA version 10 and were used to obtain odds ratios (OR) and 95% confidence intervals (95% CI) as measures of association and precision between polymorphism genotypes, *H. pylori* infection and GC. The polymorphisms were measured as a continuous variable, where if the frequency of allele x was greater than allele y, then xx = 0, xy = 1 and yy = 2. The results presented represent the ORs per increment of the minor allele. Associations were considered statistically significant if $P \leq 0.05$ or if the 95% CI excluded 1.0 in the adjusted models.

Results

Patient demographics and *H. pylori* status

Patient demographics including *H. pylori* status of the 162 Chinese, 113 Indian and 87 Malay FD controls and 60 Chinese GC cases are presented in Table 2.

There was a significant difference in the mean age between the three control groups ($P < 0.001$) and between Chinese cases and controls, despite matching for gender and age ($\pm$ 10 years, mean difference 10 years) ($P < 0.001$).

The prevalence of *H. pylori* was significantly higher in Chinese and Indian controls as compared with Malay controls ($P < 0.0001$), but there was no significant difference between the case (GC) and control (FD) groups in the Chinese sub-population.

Confirmation of mass spectrometry results using PCR-RFLP

The PCR-RFLP analysis showed a high concordance with and confirmed the findings of mass spectrometry; the results for RS1800896, RS1143623, RS16944, RS1800629 and RS5744168 agreeing in 98.93%, 96.78%, 94.91%, 99.2% and 97.84% of cases, respectively. Given this, all polymorphisms were analyzed according to the results of mass spectrometry.

Quality control, call rates, HWE and LD data analysis

Four polymorphisms (RS28720239, RS1800371, RS2069709 and RS5743708) were found to be exceptionally rare (< 1%) or absent and were excluded from further analysis. The polymorphism call rate was found to be acceptable (0–6.45% failure rate). Twenty-eight patients with call-rates below 80% were also excluded from analysis. Four polymorphisms (RS1800566, RS1800871, RS4073 and RS3136674) found to depart significantly from HWE within Chinese controls were also excluded from analysis ($P < 0.05$).

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Based on LD analysis, RS16944 (*IL1B*-511) and RS1143623 (*IL1B*-1473) on chromosome 2 were in LD in the Chinese and Malay populations ($r^2 = 0.76$ and $r^2 = 0.58$, respectively), but not in the Indian population ($r^2 = 0.29$). RS2227307 (*IL8* + 396) and rs2227306 (*IL8* +781), on chromosome 4, were in LD in all three ethnic groups ($r^2 = 0.55$–0.68). All other polymorphism combinations produced r^2 values of ≤ 0.5 (data not shown).

Variation in polymorphisms between ethnic groups

Sixteen of the remaining 26 polymorphisms (62%) were found to vary significantly ($P \leq 0.05$) between the three ethnic groups (Table 3). Further analysis by logistic regression was restricted to ethnicity-adjusted analysis for polymorphisms that varied between the ethnic groups.

Polymorphisms in relation to *H. pylori* status

A borderline non-significant increase (OR 1.45, 95% CI 0.98, 2.15) in the odds of being *H. pylori* positive with carriage of the minor G allele was observed for RS2069762 (*IL2*-330) when adjusting for ethnicity, although this association became non-significant in the stratified analysis of the Chinese. In the stratified analysis of Chinese controls, a significantly decreased risk of being *H. pylori* positive with carriage of the *EBBR2* +1963G allele (RS1801200) (OR 0.48, 95% CI 0.23, 0.98) was observed. No other significant associations were observed. The results of this analysis are presented in Table 4.

Polymorphisms in relation to GC in ethnic Chinese

Carriage of the minor C allele of *IL1B*-1473 (RS1143623) was associated with a significant decrease in the odds ratio for GC (OR 0.64, 95% CI 0.41, 0.99, $P = 0.05$). Carriage of the minor G allele for *PTGS2*-1195 (RS689466) resulted in a borderline significant increase in the odds ratio for GC (OR 1.53, 95% CI 0.99, 2.37, $P = 0.05$). No other significant associations were observed. The results of this analysis are presented in Table 4.

Discussion

The number of host genetic polymorphisms proposed to modify the response to *H. pylori* infection and carcinogenesis has increased exponentially over the last decade; however, none has yet provided a sufficiently reliable marker across all populations. The present study investigated a panel of 34 polymorphisms from 26 genes, for variation between ethnicities, for association with *H. pylori* seropositivity in these ethnic groups and for association with GC development in the ethnic Chinese population. The prevalence of 16 of the 34 (47%) genetic polymorphisms studied varied significantly between the three ethnic groups investigated.

The frequencies of the *IL1B*-511A and +3954T alleles were 63.5% and 8.5% in Malays, 52.5% and 2% in Chinese, and 32.5% and 10.5% in the ethnic Indians. A meta-analysis by Camargo *et al*. similarly reported considerable variation in the frequency of the *IL1B*-511T (corresponding to the A allele in our analysis) and +3954T alleles between Caucasian (33% and 23%, respectively) and Asian populations (50% and 5%, respectively).[15] Our finding that there was no association with either of these polymorphisms and GC development in the Chinese population is consistent with meta-analyses that suggest that such associations are largely confined to Caucasian populations.[15–18] We did, however, find a significantly decreased association between the *IL1B*-1473 C allele and GC development in ethnic Chinese (OR 0.41, 95% CI 0.64, 0.99, $P = 0.05$), an association previously reported in a Korean population.[19] Significantly the presence of the G allele for this polymorphism has been shown to result in decreased promoter activity.[19]

Interestingly the ethnic Indians resident in Malaysia and Singapore exhibited a greater similarity to Caucasians than to East Asians with respect to the *IL1B* polymorphisms, a finding that is consistent with the fact that Malaysian and Singaporean Indians are Tamil migrants from Southern India, a population reported to be genetically half way between Caucasians and East Asians.[20]

The *IL2*-330G allele has been postulated to positively influence the development of a Th1-type immune response through increased production of IFN-γ,[21] which is ineffective at eradicating the initial *H. pylori* infection. In this study we observed this allele to be associated with a borderline significant increase risk of *H. pylori* infection (OR 1.45, 95% CI 0.98, 2.15, $P = 0.06$), contrasting with a recent study by Queiroz *et al*. who found *IL2*-330G to be associated with a decreased risk of *H. pylori* infection in Brazilian adults and with increased serum concentrations of IL-2.[21]

Studies have shown that the *IL13*-1111 T allele results in increased binding of nuclear proteins and expression of IL-13 from Th2 polarized CD4+ T cells, thus suggesting an enhanced predisposition to Th2 responses,[22,23] which may protect against GC development and *H. pylori* infection. A borderline significant decrease in the odds of *H. pylori* seropositivity associated with the T allele was observed in the unadjusted analysis (OR 0.65, 95% CI 0.42, 1.01, $P = 0.06$) which is consistent with a Japanese study, which reported decreased odds of *H. pylori* seropositivity associated with carriers of the T allele.[22]

The *IL10*-1082 promoter polymorphism was found to vary significantly between the ethnic groups ($P < 0.01$). No significant difference in the prevalence of *IL10-592* between ethnic groups was observed, which is in contrast to a recent meta-analysis by Loh *et al*., which demonstrated considerable heterogeneity in Caucasian and East Asian populations.[17] In agreement with recent studies in China and Ireland, we failed to substantiate an association between polymorphisms in the *IL10* gene and GC.[24,25] In contrast several previous studies conducted in Korea, Japan and China have demonstrated associations between one or more of these polymorphisms and GC.[26–28]

PTGS2 encodes the inducible enzyme cyclooxygenase-2, which is involved in the synthesis of prostaglandins and is thus pro-inflammatory. Previous studies have reported the *PTGS2-1195*A polymorphism in *PTGS2* to create a C-MYC binding site, which results in higher transcription levels, thus providing biological relevance for these polymorphisms in GC.[29,30] In the current study carriage of the *PTGS2*-1195 G allele was associated with increased odds of GC in the Chinese (OR 1.53, 95% CI 0.99, 2.37, $P = 0.05$). While a previous study in China reported a significantly increased risk of GC associated with the A allele of *PTGS2*-1195,[30] the authors acknowledged that as the GC cases selected were from a

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Table 3 Details of the 34 polymorphisms (33 single nucleotide polymorphisms [SNPs] and one insertion-deletion polymorphism [INDEL]) and their prevalence in the three major ethnic groups resident in Malaysia and Singapore

Polymorphism details					Polymorphism prevalence															Variation between ethnic groups
dbSNP accession	Gene	Polymorphism location	SNP or INDEL (x/y)	Chromosomal location	Chinese GC			Chinese FD				Indian FD				Malay FD				
					xx	xy	yy	xx	xy	yy	HWE	xx	xy	yy	HWE	xx	xy	yy	HWE	*P*
rs689466	*PTGS2*	–1195	a/g	1q25.2-25.3	0.19	0.51	0.31	0.31	0.46	0.23	0.51	0.68	0.27	0.05	0.74	0.42	0.43	0.15	0.80	**< 0.01**
rs1800896	*IL10*	–1082	a/g	1q31-32	0.95	0.05	0.00	0.92	0.07	0.01	0.23	0.64	0.30	0.06	0.24	0.81	0.16	0.03	0.22	**< 0.01**
rs1800871	*IL10*	–819	t/c	1q31-32	0.41	0.41	0.19	0.52	0.32	0.16	**< 0.01**	0.19	0.39	0.41	0.06	0.44	0.35	0.21	0.09	†
rs1800872	*IL10*	–592	a/c	1q31-32	0.33	0.53	0.14	0.45	0.40	0.16	0.07	0.44	0.41	0.15	0.30	0.28	0.54	0.18	0.64	0.27
rs5744168	*TLR5*	+1174	c/t	1q41-42	0.93	0.07	0.00	0.94	0.06	0.00	1.00	0.78	0.22	0.00	0.61	0.79	0.21	0.00	1.00	**< 0.01**
rs1143623	*IL1B*	–1473	g/c	2q14	0.35	0.57	0.08	0.30	0.46	0.25	0.32	0.43	0.39	0.18	0.15	0.36	0.43	0.21	0.33	0.07
rs16944	*IL1B*	–511	a/g	2q14	0.32	0.52	0.17	0.30	0.45	0.25	0.41	0.07	0.51	0.42	0.26	0.39	0.49	0.12	0.62	**< 0.01**
rs1143634	*IL1B*	+3954	c/t	2q14	0.98	0.02	0.00	0.96	0.04	0.00	1.00	0.80	0.19	0.01	1.00	0.83	0.17	0.00	1.00	**< 0.01**
rs5743836	*TLR9*	–1237	t/c	3p21.3	1.00	0.00	0.00	0.89	0.09	0.02	0.27	0.79	0.19	0.03	0.19	0.90	0.07	0.02	1.00	**< 0.01**
rs4073	*IL8*	–251	t/a	4q13-21	0.27	0.55	0.18	0.38	0.39	0.23	**0.02**	0.44	0.43	0.13	0.51	0.28	0.52	0.20	0.64	†
rs2227307	*IL8*	+396	t/g	4q13-21	0.18	0.47	0.35	0.37	0.45	0.18	0.60	0.19	0.39	0.42	0.14	0.11	0.48	0.41	1.00	0.53
rs2227306	*IL8*	+781	c/t	4q13-21	0.39	0.48	0.13	0.46	0.43	0.11	1.00	0.54	0.34	0.12	0.09	0.55	0.40	0.05	1.00	0.26
rs28720239	*NFkB1*	–94	attg/-	4q24	1.00	0.00		0.97	0.03			1.00	0.00			1.00	0.00			†
rs2069762	*IL2*	–330	t/g	4q26-27	0.37	0.53	0.10	0.48	0.39	0.13	0.20	0.27	0.41	0.32	0.11	0.25	0.54	0.21	0.64	**< 0.01**
rs5743708	*TLR2*	+2408	g/a	4q32	1.00	0.00	0.00	0.99	0.00	0.01	1.00	1.00	0.00	0.00	1.00	0.98	0.02	0.00	1.00	0.18
rs1800925	*IL13*	–1111	c/t	5q31	0.70	0.30	0.00	0.73	0.26	0.01	0.53	0.64	0.31	0.06	0.39	0.61	0.35	0.04	0.75	0.08
rs2070874	*IL4*	–33	t/c	5q31.1	0.54	0.39	0.07	0.68	0.27	0.05	0.19	0.06	0.43	0.51	0.62	0.30	0.40	0.30	0.14	**< 0.01**
rs1800629	*TNFA*	–308	g/a	6p21.3	0.82	0.17	0.02	0.85	0.15	0.00	0.60	0.90	0.10	0.00	1.00	0.91	0.90	0.00	1.00	0.26
rs2234711	*IFNGR1*	–56	c/t	6q23-24	0.20	0.64	0.16	0.37	0.44	0.19	0.31	0.57	0.35	0.08	0.61	0.49	0.42	0.09	1.00	**< 0.01**
rs4880	*SOD2*	+47	t/c	6q25.3	0.72	0.26	0.02	0.76	0.22	0.03	0.29	0.29	0.49	0.22	0.85	0.51	0.42	0.07	1.00	**< 0.01**
rs16906079	*TLR4*	+690	a/g	9q32-33	0.98	0.00	0.02	0.99	0.01	0.00	1.00	0.95	0.04	0.01	0.05	1.00	0.00	0.00	1.00	**0.02**
rs4986790	*TLR4*	+896	a/g	9q32-33	0.98	0.02	0.00	0.91	0.08	0.01	1.00	0.71	0.26	0.03	0.72	0.89	0.09	0.03	1.00	**< 0.01**
rs2069709	*IFNG*	–179	g/t	12q14	1.00	0.00	0.00	1.00	0.00	0.00		1.00	0.00	0.00		1.00	0.00	0.00		†
rs2301756	*PTPN11*	intron 3 +86	c/t	12q24	0.83	0.15	0.02	0.74	0.25	0.01	0.74	0.10	0.47	0.44	0.82	0.44	0.44	0.13	0.80	**< 0.01**
rs696	*NFkBIA*	3′ UNTR (STOP+2)	g/a	14q13	0.32	0.55	0.13	0.27	0.54	0.19	0.33	0.25	0.58	0.17	0.08	0.31	0.49	0.20	1.00	0.95
rs2070600	*AGER*	+242	g/a	14q32	0.55	0.40	0.05	0.63	0.31	0.06	0.46	0.79	0.19	0.02	0.62	0.83	0.15	0.02	0.43	**< 0.01**
rs1801275	*IL4R*	+1902	a/g	16q11.2–12.1	0.69	0.28	0.03	0.56	0.36	0.08	0.83	0.60	0.38	0.02	0.23	0.43	0.43	0.13	1.00	**0.05**
rs1800566	*NQO1*	+609	c/t	16q22.1	0.26	0.55	0.19	0.32	0.39	0.29	**0.01**	0.45	0.49	0.06	0.10	0.42	0.42	0.16	0.46	†
rs1800371	*TP53*	+139	c/t	17p13.1	1.00	0.00	0.00	1.00	0.00	0.00		1.00	0.00	0.00		1.00	0.00	0.00		†
rs1042522	*TP53*	+215	g/c	17p13.1	0.35	0.47	0.18	0.27	0.51	0.23	1.00	0.21	0.54	0.25	0.43	0.24	0.56	0.20	0.82	0.57
rs3136674	*CCL13*	–887	t/c	17q11.2	0.88	0.12	0.00	0.92	0.06	0.03	**< 0.01**	0.95	0.04	0.01	0.07	0.98	0.02	0.00	1.00	†
rs2297518	*NOS2*	+1823	g/a	17q11.2-12	0.69	0.25	0.05	0.73	0.24	0.03	0.36	0.71	0.27	0.02	1.00	0.74	0.25	0.01	1.00	0.78
rs1801200	*ERBB2*	+1963	a/g	17q21.1	0.81	0.19	0.00	0.77	0.20	0.03	0.27	0.68	0.28	0.04	0.73	0.62	0.37	0.01	0.44	0.17
rs1059293	*IFNGR2*	+2083	t/c	21q22.11	0.67	0.33	0.00	0.63	0.33	0.04	0.44	0.48	0.45	0.07	0.33	0.47	0.38	0.16	0.58	**0.01**

†Polymorphism is not in HWE or is monoallelic; therefore variation between ethnic groups cannot be assessed.

FD, functional dyspepsia; GC, gastric cancer; HP, *H. pylori*; HWE, Hardy-Weinberg Equilibrium; x, major allele; y, minor allele.

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Table 4 Association of each polymorphism included in statistical analysis was assessed with (i) *Helicobacter pylori* infection in the functional dyspepsia (FD) control group; and (ii) gastric cancer (GC) in the ethnic Chinese subgroup. The odds ratios (ORs) presented here represent the OR per increment of the minor allele

Polymorphism				Association between polymorphism and HP									Association between polymorphism and GC					
				All ethnicities ($n = 334$)						Chinese subgroup analysis ($n = 153$)			Chinese only ($n = 213$)					
dbSNP accession	Gene	Polymorphism location	SNP or INDEL (x/y)	Unadjusted			Adjusting for ethnicity						Unadjusted			Adjusting for HP		
				OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
rs689466	*PTGS2*	–1195	a/g				0.82	0.55, 1.21	0.31	0.76	0.44, 1.32	0.33	**1.48**	**0.96, 2.26**	**0.07**	**1.53**	**0.99, 2.37**	**0.05**
rs1800896	*IL10*	–1082	a/g				1.02	0.57, 1.82	0.95	2.93	0.39, 22.03	0.30	0.60	0.17, 2.06	0.41	0.56	0.16, 1.95	0.37
rs1800872	*IL10*	–592	a/c	0.89	0.63, 1.27	0.52	0.96	0.66, 1.39	0.81	1.48	0.83, 2.67	0.19	1.18	0.77, 1.81	0.46	1.17	0.76, 1.80	0.48
rs5744168	*TLR5*	+1174	c/t				1.14	0.53, 2.45	0.73	0.57	0.14, 2.34	0.43	1.02	0.31, 3.39	0.97	1.08	0.32, 3.60	0.91
rs1143623	*IL1B*	–1473	g/c	1.01	0.72, 1.43	0.95	1.02	0.71, 1.47	0.90	0.90	0.52, 1.55	0.70	**0.64**	**0.41, 0.99**	**0.04**	**0.64**	**0.41, 0.99**	**0.05**
rs16944	*IL1B*	–511	a/g				1.16	0.77, 1.73	0.49	0.95	0.53, 1.69	0.85	1.30	0.85, 1.99	0.22	1.30	0.85, 1.99	0.22
rs1143634	*IL1B*	+3954	c/t				1.20	0.52, 2.79	0.67	1.32	0.15, 11.69	0.81	0.41	0.05, 3.45	0.41	0.40	0.05, 3.38	0.40
rs5743836	*TLR9*	–1237	t/c				0.85	0.41, 1.77	0.67	0.93	0.27, 3.23	0.91						
rs2227307	*IL8*	+396	t/g	1.05	0.73, 1.50	0.81	0.99	0.68, 1.45	0.96	1.11	0.62, 1.98	0.73	1.07	0.69, 1.66	0.75	1.07	0.69, 1.65	0.77
rs2227306	*IL8*	+781	c/t	1.29	0.86, 1.93	0.22	1.23	0.81, 1.88	0.34	1.31	0.69, 2.49	0.40	1.24	0.78, 1.98	0.36	1.23	0.77, 1.97	0.38
rs2069762	*IL2*	–330	t/g				**1.45**	**0.98, 2.15**	**0.06**	1.62	0.86, 3.06	0.13	1.17	0.76, 1.81	0.48	1.15	0.74, 1.78	0.53
rs1800925	*IL13*	–1111	c/t	**0.65**	**0.42, 1.01**	**0.06**	0.67	0.42, 1.07	0.09	0.76	0.35, 1.65	0.49	1.00	0.53, 1.86	0.99	1.03	0.55, 1.93	0.93
rs2070874	*IL4*	–33	t/c				0.80	0.53, 1.21	0.29	1.10	0.54, 2.25	0.80	1.47	0.90, 2.39	0.13	1.47	0.90, 2.41	0.12
rs1800629	*TNFA*	–308	g/a	1.43	0.63, 3.23	0.39	1.35	0.58, 3.14	0.49	1.34	0.42, 4.24	0.62	1.31	0.62, 2.75	0.48	1.32	0.63, 2.77	0.47
rs2234711	*IFNGR1*	–56	c/t				1.02	0.69, 1.51	0.92	0.73	0.42, 1.26	0.26	1.31	0.84, 2.04	0.23	1.33	0.85, 2.08	0.21
rs4880	*SOD2*	+47	t/c				0.96	0.62, 1.48	0.85	1.47	0.60, 3.58	0.40	1.14	0.62, 2.07	0.68	1.11	0.61, 2.03	0.73
rs16906079	*TLR4*	+690	a/g				0.46	0.10, 2.07	0.31				2.84	0.40, 20.23	0.30	2.65	0.38, 18.74	0.33
rs4986790	*TLR4*	+896	a/g				0.56	0.27, 1.16	0.12	0.42	0.12, 1.55	0.19	0.21	0.03, 1.70	0.14	0.23	0.03, 1.81	0.16
rs2301756	*PTPN11*	intron 3 +86	c/t				1.08	0.69, 1.68	0.74	0.87	0.38, 1.99	0.75	0.64	0.32, 1.30	0.22	0.64	0.31, 1.30	0.21
rs696	*NFkBIA*	3′ UNTR (STOP+2)	g/a	0.89	0.61, 1.30	0.55	0.87	0.59, 1.28	0.49	1.00	0.56, 1.79	0.99	0.77	0.49, 1.20	0.25	0.77	0.49, 1.21	0.26
rs2070600	*AGER*	+242	g/a				1.28	0.75, 2.20	0.37	0.98	0.50, 1.91	0.96	1.27	0.77, 2.08	0.34	1.27	0.77, 2.08	0.35
rs1801275	*IL4R*	+1902	a/g				0.85	0.55, 1.31	0.46	0.95	0.49, 1.81	0.87	0.63	0.37, 1.09	0.10	0.64	0.37, 1.10	0.10
rs1042522	*TP53*	+215	g/c	1.11	0.77, 1.59	0.58	1.07	0.73, 1.56	0.73	1.17	0.67, 2.05	0.57	0.76	0.49, 1.16	0.21	0.76	0.50, 1.17	0.21
rs2297518	*NOS2*	+1823	g/a	1.10	0.67, 1.81	0.72	1.05	0.62, 1.77	0.85	1.10	0.51, 2.36	0.81	1.16	0.67, 1.99	0.59	1.15	0.67, 1.97	0.62
rs1801200	*ERBB2*	+1963	a/g	**0.66**	**0.42, 1.04**	**0.07**	0.66	0.41, 1.07	0.09	**0.48**	**0.23, 0.98**	**0.04**	0.73	0.36, 1.46	0.37	0.75	0.37, 1.52	0.43
rs1059293	*IFNGR2*	+2083	t/c				1.19	0.75, 1.87	0.46	0.83	0.40, 1.72	0.61	0.75	0.41, 1.37	0.36	0.77	0.42, 1.41	0.40

Missing values: polymorphisms not analyzed as varied significantly between the ethnic groups.
CI, confidence interval; HP, *H. pylori*.

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different population to controls there may have been selection bias. Further studies are clearly required.

The proto-oncogene *ERBB2*, encoding HER-2/neu, has been associated with development and poor prognosis in a range cancers.[31] While the *ERBB2*+1963 (Ile655Val) polymorphism has previously been linked to GC development and progression in Japan.[32] However, in the current study we found no association with GC development. Intriguingly, however, the minor G allele (valine) was found to be associated with a significant decrease in the odds of *H. pylori* infection in the Chinese subgroup (OR 0.48, 95% CI 0.23, 0.99, $P = 0.04$).

Our study is typical of many other published genetic association studies, in that it is underpowered to detect associations of the magnitude that might be expected, especially given the observed heterogeneity between the ethnic groups. Despite this, our study adds considerably to the available body of knowledge, although the low power and multiple testing issues mean that our findings may potentially include false positives and negatives, necessitating validation of these results in further studies. Due to the low incidence of GC in ethnic Indian and Malays, as well as the low prevalence of *H. pylori* in the Malays, we were unable to recruit sufficient numbers of these groups for detailed analysis. These populations would certainly be intriguing to evaluate in a subgroup analysis.

In conclusion, this study has examined a large range of polymorphisms in genes specifically associated with the innate and acquired immune responses, oxidative stress, cell cycle regulation and cell signaling. Importantly we have included the association between these polymorphisms and not only GC, but also *H. pylori* seropositivity. This is an important consideration as the strong correlation between *H. pylori* infection and GC may mean an association between a polymorphism and *H. pylori* is mistaken for weak association with GC. Importantly, we identified significant differences in the prevalence of 16 polymorphisms across the ethnic Chinese, Indian and Malay groups, a finding likely to explain the failure to consistently demonstrate significant associations between specific polymorphisms and GC in disparate populations. In the ethnic Chinese, significant associations were identified between *EBBR2*+1963 and *H. pylori* infection, and *PTGS2*-1195 and *IL1B*-1473 and GC. Clearly further studies are required to investigate the importance of these findings in other populations.

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