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BRIEF ARTICLE

Gastric precancerous lesions are associated with gene variants in *Helicobacter pylori*-susceptible ethnic Malays

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Abstract

AIM: To identify genes associated with gastric precancerous lesions in *Helicobacter pylori* (*H. pylori*)-susceptible ethnic Malays.

METHODS: Twenty-three Malay subjects with *H. pylori* infection and gastric precancerous lesions identified during endoscopy were included as "cases". Thirty-seven Malay subjects who were *H. pylori* negative and had no precancerous lesions were included as "controls". Venous blood was collected for genotyping with Affymetrix 50K Xba1 kit. Genotypes with call rates < 90% for autosomal single nucleotide polymorphisms (SNPs) were excluded. For each precancerous lesion, associated SNPs were identified from Manhattan plots, and only SNPs with a χ^2 *P* value < 0.05 and Hardy Weinberg Equilibrium *P* value > 0.5 was considered as significant markers.

RESULTS: Of the 23 *H. pylori*-positive subjects recruited, one sample was excluded from further analysis due to a low genotyping call rate. Of the 22 *H. pylori*-positive samples, atrophic gastritis only was present in 50.0%, complete intestinal metaplasia was present in 18.25%, both incomplete intestinal metaplasia and dysplasia was present in 22.7%, and dysplasia only was present in 9.1%. SNPs rs9315542 (*UFM1* gene), rs6878265 (*THBS4* gene), rs1042194 (*CYP2C19* gene) and rs10505799 (*MGST1* gene) were significantly associated with atrophic gastritis, complete intestinal metaplasia, incomplete metaplasia with foci of dysplasia and dysplasia, respectively. Allele frequencies in "cases" *vs* "controls" for rs9315542, rs6878265, rs1042194 and rs10505799 were 0.4 *vs* 0.06, 0.6 *vs* 0.01, 0.6 *vs* 0.01 and 0.5 *vs* 0.02, respectively.

CONCLUSION: Genetic variants possibly related to gastric precancerous lesions in ethnic Malays susceptible to *H. pylori* infection were identified for testing in subsequent trials.

Key words: Gastric precancerous lesions; Gene polymorphisms; Genome-wide association; *Helicobacter pylori*; Malays

Core tip: Gastric cancer and its precancerous lesions are exceptionally rare among ethnic Malays. Gene variants may be associated with precancerous lesions in *Helicobacter pylori*-susceptible Malays. Genome-wide association was performed to identify gene variants in Malays with a spectrum of gastric precancerous lesions. Results indicated that at different phases of the Correa cascade, different gene variants were manifest, but they followed a pattern of progression similar to their histological and clinical stages. It is possible that, in addition to histological staging, gene variant markers may serve to identify different phases of gastric cancer progression in the near future.

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INTRODUCTION

Gastric cancers are thought to arise from a cascade of histological changes or precancerous lesions (atrophic gastritis, intestinal metaplasia and dysplasia) before developing into full-blown malignancy[1]. In Japan, studies have shown that surveillance of these precancerous lesions is associated with increased detection of early gastric cancers and improved survival rates[2,3].

These precancerous lesions are associated with *Helicobacter pylori* (*H. pylori*) infection acquired since childhood[4]. In populations with a high prevalence of *H. pylori* infection, including those in China and Japan, precancerous lesions can be detected in up to 80% of adults[5]. Eradication of *H. pylori* infection at this stage has not been shown to be effective in these high risk populations[6].

Ethnic Malays residing in the north-eastern region of Peninsular Malaysia (state of Kelantan) have an exceptionally low prevalence of *H. pylori* infection[7,8]. Exact reasons for this low prevalence are unknown, but it could be a combination of unique environmental, host and strain virulence factors shaped by the population's evolutionary history[9-12]. Due to the extremely low acquisition of *H. pylori* infection, gastric cancer and its precancerous lesions are extremely rare in this population[13-15].

In a survey of 234 subjects undergoing upper endoscopy in a tertiary hospital from the state of Kelantan, the reported rate of atrophic gastritis was 42.3% and intestinal metaplasia was present in 7.7% (14/234) of all biopsies, but was only present in 1.4% (2/146) of the ethnic Malays[15]. This low rate of gastric precancerous lesions observed was a result of a low prevalence of *H. pylori* infection in the studied population of only 6.8%. As shown in a multivariable analysis, the risk of intestinal metaplasia and dysplasia was only significant in the presence of *H. pylori* infection[15].

A minority of this Malay population is genetically susceptible to *H. pylori* infection, and *DCC* gene polymorphism has recently been found to be responsible[16]. An aberrant methylation of this tumor suppressor gene has been observed to occur in the course of gastric carcinogenesis[17]. As such, this population may also be genetically susceptible to the development of gastric precancerous lesions.

The current study aimed to determine the gene polymorphisms associated with gastric precancerous lesions in the Malay population from north-eastern region of Peninsular Malaysia using the genome-wide association approach.

MATERIALS AND METHODS

Study subjects

Only those ethnic Malay subjects (age range 20-80 years) whose gastrointestinal symptoms required upper endoscopy were screened for study eligibility. To avoid ascertainment bias, subjects had upper gastrointestinal symptoms (including dyspepsia and/or abdominal discomfort) and required upper endoscopy to exclude gastro-duodenal diseases before being included into the study.

All Malay subjects included in the study were born in the state of Kelantan, had resided within the region for at least 3 generations and were from different families but had similar socio-economic and socio-cultural backgrounds. Subjects positive for *H. pylori* infection according to a urease test and histology and with gastric precancerous lesions identified during endoscopy were categorized as "cases", while those negative for *H. pylori* infection and precancerous lesions were categorized as "controls". "Cases" and "controls" were matched for age and gender. Subjects satisfying the above inclusion criteria were recruited into the study. Exclusion criteria included an intake of antibiotics 3 mo prior to the upper endoscopy test, upper gastrointestinal bleeding, a positive family history of *H. pylori* infection and gastric cancer, a previous history of *H. pylori* infection and chronic psychiatric and medical conditions, including cancer. Informed consent was obtained from all subjects prior to their enrolment into the study.

Cases with *H. pylori* infection and positive for precancerous lesions were extremely limited in number due to an exceptionally low rate of *H. pylori* infection among ethnic Malays. Only 23 Malay subjects were eventually included as "cases". A larger sample size for the "controls" was sought to compensate for the low sample size in "cases". Furthermore, stringent criteria were set to ensure that only subjects of similar age, socio-economic and socio-cultural backgrounds were included in the study. From a total of 45 screened subjects, 37 Malay subjects

were recruited as "controls" with eight subjects being excluded as they did not meet the inclusion criteria, they did not give consent or blood samples were poor.

The study was approved by the Human Research and Ethics Committee of Universiti Sains Malaysia (USM).

Endoscopic diagnosis and histological definitions of precancerous lesions

All upper endoscopies (model GIF-140 and GIF-160; Olympus Medical Systems, Tokyo, Japan) during this period were performed by one endoscopist with at least 5 years' experience. If needed, patients were sedated accordingly. Subjects who did not stop proton pump inhibitors 2 wk before endoscopy, those who had received antibiotics prior to study, and patients who had upper gastrointestinal bleeding shortly before the study were excluded.

Endoscopic findings of gastritis and atrophy were recorded and classified based on established Sydney criteria[18] and Atrophy Club criteria[19]. Biopsies were taken using standard biopsy forceps at the antrum, incisura and body. A minimum of 2 to 4 biopsies (size between 2 to 4 mm) were taken in each sites and these gastric biopsies, preserved in formalin containers, were transported to the pathology laboratory on the same day.

Only one histopathologist was involved in reviewing all the slides. All biopsies were stained with routine hematoxylin and eosin (HE) stain followed by Alcian blue-periodic acid Schiff stain for the detection of intestinal metaplasia. The Warthin Starry stain would be used in sections where the *H. pylori* bacterium was not detected in the routine HE stain.

Chronic atrophic gastritis was identified based on the updated 1994 Sydney system[20] and Atrophy Club definitions[19]. Intestinal metaplasia was identified by replacing glandular epithelium with goblet cells[21]. Intestinal metaplasia was classified into complete or incomplete types. Complete type resembled the small intestinal phenotype with well-formed goblet cells while incomplete type resembled the colonic phenotype with irregular mucin droplets and absence of a brush border. Dysplasia was identified by epithelium disarray and increased nucleocytoplasmic ratio[22].

For the purpose of the genotyping study, subjects were grouped as follows: atrophic gastritis only, complete intestinal metaplasia, incomplete metaplasia with foci of dysplasia, or dysplasia only.

Genomic DNA preparation

All recruited subjects were called up by one of the investigators (SM) to have 1 mL of venous blood taken during the study day. Unlike conventional methods of DNA extraction, 1 mL of blood was sufficient for the commercially available kits. The blood was collected in an EDTA tube and was transported immediately to a facility (Human Genome Centre, USM, Kubang Kerian, Malaysia) to be stored at 4 ℃. Subsequently, DNA for all recruited cases and controls was isolated using QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany).

Genotyping with Affymetrix 50K Xba1

The isolated DNA from all recruited cases (n = 23) and controls (n = 37) were processed and genotyped using Affymetrix 50k Xba1 array (Affymetrix, United States) following the instructions provided in the Affymetrix GeneChip Human Mapping 100K Assay Manual[23]. Genotypes with call rates < 90% for autosomal single nucleotide polymorphisms (SNPs) were excluded. SNPs that had a minor allele frequency < 5%, that failed to genotype in > 5% of samples, or had a Hardy-Weinberg Equilibrium (HWE) P-value < 0.5 were also excluded from the analysis.

Statistical analysis

Genotype calling to assess the normalization of the SNPs was performed with the Bayesian Robust Linear Model with Mahalanobis distance classifier (BRLMM) algorithm from the Affymetrix® Genotyping Console™ software version 4.0 (Affymetrix, United States). Quality control for genetic markers was assessed using the Genotype filtering tool in the SVS Golden Helix Bioinformatics Tools version 7.4 (Golden Helix Inc., Bozeman, MT, United States).

Association was evaluated for every single SNP in each gene with SVS Golden Helix Bioinformatics Tools. False Discovery Rate, and Bonferroni adjustments were used for multiple-testing corrections. A Manhattan plot for each phenotype was generated to determine SNPs with the highest significant value associated with that phenotype using SVS Golden Helix Bioinformatics Tools (version 7.4). A significant genomic threshold of 3×10^{-7} in Manhattan plots was set in this study and a χ^2 P value for each SNP was calculated based on Fisher's exact χ^2 test. For each type of precancerous lesion studied, of which a group of associated SNPs were identified from Manhattan plots, only a SNP with χ^2 P value < 0.05 and HWE P value > 0.5 was considered as a significant marker.

RESULTS

Of the 23 *H. pylori*-positive subjects recruited, one sample was excluded from further analysis due to a low genotyping call rate (< 90%). The mean age of the remaining 22 "cases" was 56.5 ± 16.5 years, and was 53.2 ± 15.2 years for the 37 "controls". Cases were 54.5% (12/22) male compared with 50% (17/37) in "controls". Of subjects positive for *H. pylori* infection (cases), atrophic gastritis only was present in 50.0% (11/22), complete intestinal metaplasia was present in 18.2% (4/22), both incomplete intestinal metaplasia and dysplasia was present in 22.7% (5/22) and dysplasia only was present in 9.1% (2/22). None of the gastric precancerous lesions were present in subjects negative for *H. pylori* infection (controls).

In 10 "cases" with atrophic gastritis only, compared with controls, 26 SNPs were above the significant ge-

Table 1 Single nucleotide polymorphisms associated with atrophic gastritis among Malays with *Helicobacter pylori*

rs ID	Chromosome	Gene	Minor allele	Allele frequency		χ^2	HWE
				Cases	Control	*P*-value[1]	*P*-value
rs2614074	8 p21.1	*PNOC*	B	0.409	0.062	0.008	0.983
rs10504944	8 q22.1	*GDF6*	A	0.5	0	0.034	0.516
rs9315542	13 q13.3	*UFM1*	B	0.409	0.064	0.007	0.994
rs4943552	13 q13.3	*UFM1*	A	0.318	0.075	0.040	0.815
rs489977	18 q12.3	*KC6*	A	0.181	0	0.064	0.752

rs ID: The unique ID for the identified single nucleotide polymorphisms; HWE: Hardy-Weinberg Equilibrium. [1]All markers were run using the FAMHAP (Haplotype Association Analysis) program. The *P* value represents the simulated overall significance for the particular marker corrected for multiple testing and $P < 0.05$ was considered statistically significant.

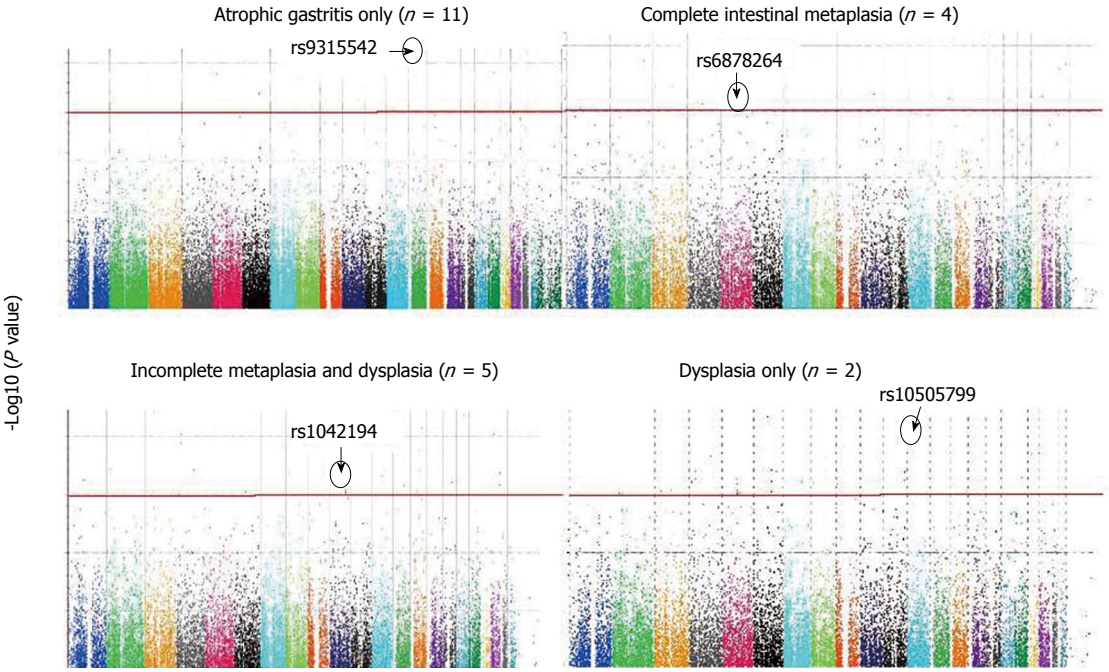

Figure 1 Manhattan plots for different gastric precancerous lesions (phenotype) in susceptible Malays with *Helicobacter pylori*. Red line indicates genomic threshold (3×10^{-7}) set to determine single nucleotide polymorphisms (SNPs) in Hardy-Weinberg Equilibrium associated with the studied phenotype. The most significant SNP, as determined by the χ^2 *P* value, for each phenotype is shown by an arrow.

nomic threshold. Five of the identified 26 SNPs were in HWE, of which rs9315542, located in chromosome 13 q13.3 (*UFM1* gene), was the most significant associated SNP (χ^2 *P* value = 0.007) (Table 1, Figure 1). The allele frequency for rs9315542 in cases *vs* controls was 0.4 *vs* 0.06.

In 4 "cases" with intestinal metaplasia only, compared with controls, 13 SNPs were above the genomic threshold and were in HWE, of which rs6878264, located in intron 4 of the *thrombospondin 4 (THBS4)* gene, was the most significant associated SNP (χ^2 *P* value = 0.01) (Table 2, Figure 1). The allele frequency for rs6878264 in cases *vs* controls was 0.6 *vs* 0.01.

In 6 "cases" with both intestinal metaplasia and dysplasia, compared with controls, 17 SNPs were above the genomic threshold and in HWE, of which rs1042194, located in exon 8 of the *CYP2C19* gene, was the most significant associated SNP (χ^2 *P* value = 0.00536) (Table 3, Figure 1). The allele frequency for rs1042194 in cases *vs* controls was 0.6 *vs* 0.01.

Finally, in 2 "cases" with dysplasia only, compared with controls, 2 SNPs were above the genomic threshold and in HWE, of which rs10505799, located in chromosome 12p12.3 (*MGST1* gene), was the most significant associated SNP (χ^2 *P* value = 0.006) (Table 4, Figure 1). The allele frequency for rs10505799 in cases *vs* controls was 0.5 *vs* 0.02.

DISCUSSION

In this Malay population with an extremely low risk of *H. pylori* infection, gastric cancer and its precancerous lesions are very rare. However, in the current study of subjects susceptible to *H. pylori* infection and those who developed precancerous lesions, certain gene polymorphisms were found to be more commonly associated with precancerous lesions. Notwithstanding the low sample size, resulting from the extremely rare occurrence of gastric

Table 2 Single nucleotide polymorphisms associated with intestinal metaplasia among Malays with *Helicobacter pylori*

rs ID	Chromosome	Gene	Minor allele	Allele frequency		χ^2	HWE
				Cases	Control	*P*-value	*P*-value
rs1166704	1p31.1	*NEXN*	B	0.375	0.031	0.0694	0.654
rs2191508	2q32.3	*SLC39A10*	A	0.081	0.375	0.297	0.591
rs1992736	3p24.3	*TBC1D5*	A	0.750	0.193	0.192	0.78
rs10511297	3q13.13	*CD96*	A	0.375	0.030	0.171	0.659
rs2615485	4q22.1	*DSPP*	A	0.750	0.136	0.190	0.625
rs2434316	5q14.1	*THBS4*	A	0.750	0.257	0.118	0.743
rs6878264	5q14.1	*THBS4*	B	0.625	0.010	0.010	1.000
rs7800141	7p15.3	*DNAH11*	B	0.666	0.096	0.154	0.717
rs4746259	10q22.2	*PPIAL4G*	B	0.750	0.015	0.062	0.794
rs9300471	13q32.2	*FARP1*	A	0.375	0.030	0.145	0.659
rs1881344	16p13.2	*C16orf68*	A	0.375	0.030	0.078	0.659
rs2253429	20p13	*SIRPB1*	B	0.375	0.030	0.0119	0.659
rs2834681	21q22.12	*RUNX1*	B	0.250	0	0.0623	0.859

rs ID: The unique ID for the identified single nucleotide polymorphisms; HWE: Hardy-Weinberg Equilibrium.

Table 3 Single nucleotide polymorphisms associated with intestinal metaplasia and dysplasia among Malays with *Helicobacter pylori*

rs ID	Chromosome	Gene	Minor allele	Allele frequency		χ^2	HWE
				Cases	Control	*P*-value	*P*-value
rs10493872	1p21.3	*ABCD3*	A	0.375	0.062	0.00601	0.518
rs10510792	3p14.3	*DNAH12*	A	0.25	0.030	0.341	0.728
rs2889259	4p14	*KIAA1239*	A	0.375	0.015	0.0623	0.724
rs6837437	4p14	*KIAA1239*	A	0.375	0.015	0.0623	0.728
rs10498879	6q13	*RIMS1*	A	0.75	0.196	0.160	0.629
rs4073894	7q22.1	*LHFPL3*	A	0.375	0.015	0.0623	0.728
rs10487929	7q35	*CNTNAP2*	B	0.25	0.031	0.260	0.724
rs10503727	8p21.2	*SLC25A37*	B	0.75	0.183	0.151	0.908
rs2251417	10q21.3	*ANXA2P1*	A	0.375	0.045	0.579	0.591
rs1042194	10q23.33	*CYP2C19*	B	0.625	0.011	0.00536	0.972
rs10506855	12q21.31	*CCDC59*	B	0.375	0.046	0.0269	0.585
rs10492652	13q22.1	*KLF12*	B	0.25	0.015	0.0623	0.797
rs1565946	14q23.1	*SLC35F4*	A	0.625	0.156	0.0151	0.658
rs10483683	14q23.1	*SLC35F4*	B	0.625	0.181	0.314	0.964
rs10483837	14q24.2	*RGS6*	B	0.5	0.031	0.171	0.585
rs245615	16q21	*CDH8*	A	0.5	0.140	0.183	0.844
rs2058879	19q13.32	*IGFL2*	A	0.375	0.015	0.0623	0.728

rs ID: The unique ID for the identified single nucleotide polymorphisms; HWE: Hardy-Weinberg Equilibrium.

precancerous lesions in this population, the current study, with the use of the genome-wide association approach, allowed identification of genetic markers that can be tested in a larger cohort in the near future[24].

In the 50% of *H. pylori*-infected subjects with atrophic gastritis, the earliest lesion in the Correa cascade, rs9315542, located in chromosome 13 q13.3 (*UFM1* gene), was the identified marker. A recently identified expressed protein, ubiquitin-fold modifier 1 or *UFM1*, is a member of a large family of ubiquitin-like proteins or Ubls[25]. Ubiquitin, a small protein, is associated with the process of "ubiquitination", a target of proteins for degradation by the proteasome. At the moment, the exact cellular functions of proteins modified by *UFM1* remain elusive. A recent report indicated that components of the *UFM1* conjugation pathway are highly expressed in the beta cells of the pancreas and some other protein secretory tissues[26]. In the same report, *UFM1* conjugate prevented endoplasmic reticulum (ER) stress-induced apoptosis. While *UFM1* in gastric tissue has not been investigated, it is known that gastric mucosa secretes a number of peptides and hormones, including pepsinogen and ghrelin, whose levels are reduced in atrophic gastritis[27]. Speculatively, *UFM1* may be a marker of the secretory status of the gastric mucosa, similar to pepsinogen, and remains to be tested and validated.

Complete-type or type I intestinal metaplasia, considered as the benign version compared with the incomplete-type, was present in 18.2% of *H. pylori*-infected subjects. In these subjects, rs6878264 located in intron 4 of the *THBS4* gene, was the identified marker. THBS4 is a member of the THBS protein family, a glycoprotein in the extracellular matrix, which mediates cell-to-cell and cell-to-matrix interactions. Although the exact physiological functions of THBS4 are unknown, the published literature indicate that it promotes neurite outgrowth,

Table 4 Single nucleotide polymorphisms associated with dysplasia among Malays with *Helicobacter pylori*

rs ID	Chromosome	Gene	Minor allele	Allele frequency		χ^2	HWE
				Cases	Control	*P*-value	*P*-value
rs10505799	12p12.3	*MGST1*	B	0.5	0.016	0.006	0.855
rs10498391	14q21.2	*FSCB*	B	0.5	0	0.069	0.928

rs ID: The unique ID for the identified single nucleotide polymorphisms; HWE: Hardy-Weinberg Equilibrium.

stimulates proliferation of erythroid cells, skin fibroblasts and kidney epithelial cells, as well as myoblast adhesion and interaction with other extracellular matrix proteins[28-30]. Recently, THBS4 has been found to be associated with gastric adenocarcinomas especially of the diffuse type[31]. While *H. pylori* infection is associated with atrophic gastritis and intestinal metaplasia, it is not commonly associated with diffuse-type gastric adenocarcinoma[32]. Complete-type intestinal metaplasia represents a reparative process of the epithelium following *H. pylori*-induced gastritis, and in this context, *THBS4* may act as an early proliferative marker but may have a more aggressive pro-oncogenic role in advanced stages. Again, this is speculative in the absence of any published studies, but it is a worthwhile marker for further studies.

Incomplete type intestinal metaplasia is more advanced compared with complete type, and therefore is more closely associated with dysplasia. In 22.7% of cases with both incomplete type intestinal metaplasia and dysplasia, rs1042194 located in exon 8 of *CYP2C19* gene was the identified marker. Cytochrome (CYP) P450 2C19, one of the isoforms of the CYP enzyme (phase I detoxification enzyme), plays an important role in metabolism of drugs and also detoxification of potential carcinogens[33]. Several studies indicated that *CYP2C19* gene polymorphism is associated with increased cancer susceptibility including hepatocellular carcinoma, and lung, esophageal and gastric cancer, especially in patients having a poor metabolizer (PM) genotype[34-36]. A study from Malaysia found that the PM genotype was uncommon among ethnic Malays (5.9%), compared with Chinese (19.1%) and Indians (10%)[37]. This may be one of the reasons for reduced susceptibility to gastric cancer and its precancerous lesions among ethnic Malays. The finding of *CYP2C19* in incomplete type intestinal metaplasia and dysplasia in a group of Malay subjects susceptible to *H. pylori* infection is therefore important and merits further study.

Dysplasia, a histological stage with high risk of malignant transformation, was present in only 9.1% or 2/23 subjects infected with *H. pylori*. Compared with controls, rs10505799 located in chromosome 12p12.3 (*MGST1* gene) was found to be the SNP marker associated with dysplasia. Microsomal glutathione S-transferase 1 (*MGST1*) is one of the glutathione S-transferase (GST) family of enzymes, and GSTs are phase II detoxification enzymes, which, similar to CYP enzymes, are involved in the detoxification of potential carcinogens[38,39]. Recently, *MGST1* gene polymorphism was found to be involved in colorectal carcinogenesis in the Chinese population but there is no data as yet on gastric cancer[40]. However, since *MGST1* and *CYP2C19* are both carcinogen detoxification enzymes, with evidence supporting their involvement in gastrointestinal tract carcinogenesis, the role of *MGST1* in gastric precancerous lesions is likely to be valid. The limited number of cases with dysplasia in the current study means that the results need to be interpreted cautiously, but the potential of *MGST1* as a marker for dysplasia should not be disregarded.

There are a number of studies on gene polymorphisms associated with gastric precancerous lesions in high prevalence populations, but our study covered the entire spectrum of the Correa cascade in a population with an extremely low burden of gastric cancer and *H. pylori* infection. Development of gastric cancer is thought to involve multi-step carcinogenesis and follows a progressive pattern of pathological stages described by Correa. Our results indicated that, at different phases of the Correa cascade, different gene variants are manifest, but they follow a pattern of progression similar to their histological and clinical stages. During the stage of atrophic gastritis, *UFM1* expression reflects the secretory status of epithelium. With early development of intestinal metaplasia, *THBS4* acts as a proliferative marker but at more advanced stages, incomplete intestinal metaplasia and dysplasia involve polymorphisms of detoxification enzymes, *CYP2C19* and *MGST1*. Based on the current study, it is possible that, in addition to histological staging, gene variant markers may also serve to identify different phases of progression of gastric cancer in the near future. Recently, epigenetic silencing of *FOXD3* has been shown to be an early event in gastric carcinogenesis[41] and, together with genomic changes, it would allow a greater understanding of the pathogenesis of gastric cancer.

We acknowledge from the outset that the current study, based upon a genome-wide approach, was extremely limited in sample size, as gastric precancerous lesions are extremely rare among ethnic Malays from the north-eastern region of Peninsular Malaysia. In this respect, bioinformatics and statistical approaches were taken into consideration for a more reliable analysis of the data. To reduce false-positive results, a more stringent significance threshold of 3×10^{-7} was set for Manhattan plots in the current study. Only SNPs in HWE *P*-value > 0.5 were selected to reduce occasionality. In addition to being long-term residents within the studied region, cases and controls were similar in age, socio-cultural and economic backgrounds. The current study only identified

SNPs associated with gastric precancerous lesions, and further validation studies are in progress to confirm their regulatory role in carcinogenesis.

In conclusion, we have shown that, compared with controls, susceptible ethnic Malays with *H. pylori* infection expressed different SNP markers at different spectrums of gastric precancerous lesions. These markers may allow efficient screening of precancerous lesions in larger cohorts of *H. pylori*-infected individuals.

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COMMENTS

Background

Gastric cancer and its precancerous lesions are exceptionally rare among ethnic Malays. Gene variants may be associated with precancerous lesions in *Helicobacter pylori* (*H. pylori*)-susceptible Malays.

Research frontiers

In a case-control study, genome-wide association was performed to identify gene variants in the Malay population with a spectrum of *H. pylori*-associated gastric precancerous lesions.

Innovations and breakthroughs

Results indicated that at different phases of the Correa cascade, different gene variants were manifest, but they followed a pattern of progression similar to the histological and clinical stages.

Applications

It is possible that, in addition to histological staging, gene variant markers may also serve to identify different phases of progression of gastric cancer in the near future.

Terminology

The genome-wide approach utilises microarray technology to identify thousands of single nucleotide polymorphisms (SNPs). Using novel bioinformatics and statistical approaches, the association between SNPs and the studied disease can be determined reliably.

Peer review

Current study indicates that different gene variants exist that reflect different stages of progression during different spectrums of gastric carcinogenesis. These gene variants, appropriately confirmed in later studies, may be useful markers, in addition to histological staging, of gastric precancerous lesions.

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