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

Lee YY *et al*. Gene polymorphisms and gastric precancerous lesions in Malays

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**Abstract**

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

**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 absent of 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, of which a group of associated SNPs were identified from Manhattan Plots, only SNP with 2 *P* value < 0.05 and Hardy Weinberg Equilibrium *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%). Of 22 *H. pylori* positive samples, only atrophic gastritis 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 only dysplasia 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 only 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.

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**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 the ethnic Malays. Gene variants may be associated with precancerous lesions in *Helicobacer pylori*-susceptible Malays. Genome wide association was performed to identify gene variants in the Malays having different spectrum of gastric precancerous lesions. Results indicated that at different phases of the Correa cascade, different gene variants were manifested, 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 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].

The ethnic Malays residing in the north-eastern region of Peninsular Malaysia (state of Kelantan) is one population with 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 low *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 of which *DCC* gene polymorphism has been recently 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 aims to determine the gene polymorphisms using genome-wide association approach, which are associated with gastric precancerous lesions in the Malay population from north-eastern region of Peninsular Malaysia.

**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 must present upper gastrointestinal symptoms (including dyspepsia and/or abdominal discomfort) and require upper endoscopy to exclude gastro-duodenal diseases before being included into the study.

All Malay subjects included into the study must be born in the state of Kelantan, reside within the region for at least 3 generations and from different families but have similar socio-economic and socio-cultural backgrounds. Subjects positive for *H. pylori* infection on urease test and histology and gastric precancerous lesions 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 months prior to upper endoscopy test, those who had upper gastrointestinal bleeding, positive family history of *H. pylori* infection and gastric cancer, 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 numbers due to an exceptionally low rate of *H .pylori* infection among the ethnic Malays. Only 23 Malay subjects were eventually included as “cases”. A larger sample size for the “controls” were sought to compensate for the low sample size in “cases”. Furthermore, stringent criteria were set to ensure that only subjects with 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 due to exclusion criteria and or refusal consent and or poor blood samples.

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 of experience. If needed, patients were sedated accordingly. Subjects who did not stop proton pump inhibitors 2 weeks 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 to review all slides. All biopsies were stained with routine haematoxylin and eosin (HE) stain followed by Alcian blue-periodic acid Schiff stain for the detection of intestinal metaplasia. Warthin Starry stain would be used in sections where *H. pylori* bacterium was not detected on 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 type. Complete type resembles small intestinal phenotype with well-formed goblet cells while incomplete type resembles colonic phenotype with irregular mucin droplets and absence of brush border. Dysplasia was identified by epithelium disarray and increased nucleo-cytoplasmic ratio[22].

For the purpose of genotyping study, subjects were grouped into the following: only atrophic gastritis, complete intestinal metaplasia, incomplete metaplasia with foci of dysplasia and only dysplasia.

***Genomic DNA preparation***

All recruited subjects were called up by one of the investigator (SM) to have their 1 ml of venous blood taken during the study day. Unlike conventional method for DNA extraction, 1 ml of blood was sufficient for commercially available kits. The blood was collected in EDTA tube and was transported immediately to a facility (Human Genome Centre, USM, Kubang Kerian, Malaysia) to be stored at 4°C. 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 SNPs were excluded. SNPs that had a minor allele frequency <5%, that failed to genotype in >5% of samples, and that with 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., Montana).

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. 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 2 *P* value for each SNP was calculated based on Fisher’s exact chi-squared test.For each type of precancerous lesion studied, of which a group of associated SNPs were identified from Manhattan Plots, only SNP(s) with 2 *P* value < 0.05 and HWE *P* value > 0.5 was considered as significant marker(s).

**RESULTS**

Of the 23 *H. pylori* positive subjects recruited, one sample was excluded from further analysis due to a low QC call rate (< 90%). The mean age for the remaining 22 “cases” was 56.5 ± 16.5 years while 53.2 ± 15.2 years for the 37 “controls”. Male gender was 54.5% (12/22) in “cases” and 50% (17/37) in “controls”. Of subjects positive for *H. pylori* infection (cases), only atrophic gastritis 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 only dysplasia 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 only atrophic gastritis, compared to controls, 26 SNPs were above significant genomic 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 SNP associated with atrophic gastritis (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 only intestinal metaplasia, compared to controls, 13 SNPs were above genomic threshold and were in HWE, of which rs6878264 located in intron 4 of the *THBS4* gene was the most significant SNP associated with intestinal metaplasia (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 to controls, 17 SNPs were above genomic threshold and in HWE, of which rs1042194 located in exon 8 of *CYP2C19* gene was the SNP significantly associated with both intestinal metaplasia and dysplasia (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 only dysplasia, compared to controls, two SNPs were above genomic threshold and in HWE, of which rs10505799 located in chromosome 12p12.3 (*MGST1* gene) was the SNP significantly associated with dysplasia (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 subjects susceptible to *H. pylori* infection and those who did develop precancerous lesions in the current study, certain gene polymorphisms were found to be more commonly associated. Notwithstanding the low sample size, which no doubt results from the extremely rare occurrence of gastric precancerous lesions in this population, the current study utilizing the genome-wide association approach, allows identification of genetic markers that can be tested in a larger cohort in the near future[24].

In those 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 “ubiquitilation”, 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 indicates 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 prevents 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 which levels are reduced in atrophic gastritis[27]. Speculatively, *UFM1* may be a marker of secretory status of gastric mucosa, similar to pepsinogen, which remains to be tested and validated.

Complete-type or type I intestinal metaplasia, considered as the benign version compared to the incomplete-type, was present in 18.2% of *H. pylori* infected subjects. In these subjects, compared to controls, rs6878264 located in intron 4 of the *THBS4* gene or *Thrombospondin 4* was the identified marker. *Thrombospondin 4* is a member of the thrombospondin protein family, a glycoprotein in the extracellular matrix, which mediate cell-to-cell and cell-to-matrix interactions. Although the exact physiological functions of *thrombospondin 4* are unknown, published literatures indicate that it promotes neurite outgrowth, 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, *thrombospondin 4* has been found to be associated with gastric adenocarcinomas especially that of the diffuse-type[31]. While associated with *H. pylori* infection, atrophic gastritis and intestinal metaplasia are 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, *thrombospondin 4* may act as an early proliferative marker but has a more aggressive pro-oncogenic role in advanced stages. Again, this is speculative in the absence of any published studies, nonetheless, it is a worthwhile marker for further studies.

Incomplete type of intestinal metaplasia is a more advanced version compared to the complete type, and therefore is more closely associated with dysplasia. In 22.7% of cases with both incomplete type of intestinal metaplasia and dysplasia, compared to controls, rs1042194 located in exon 8 of *CYP2C19* gene was the identified marker. Cytochrome (CYP) P450 2C19, one of the isoforms of CYP enzyme (phase I detoxification enzyme), plays an important role in metabolism of drugs and also detoxification of potential carcinogens[33]. Several studies indicate that *CYP2C19* gene polymorphism is associated with increased cancer susceptibility including hepatocellular carcinoma, lung, esophageal and gastric cancer, especially in patients having a poor metabolizer (PM) genotype[34-36]. A study from Malaysia had found that PM genotype was uncommon among the ethnic Malays (5.9%), compared to the 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 the Malays. The finding of *CYP2C19* in incomplete type of 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 for malignant transformation, was present in only 9.1% or 2/23 subjects infected with *H. pylori* infection. Compared to 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, gene polymorphism of *MGST1* was found to be involved in colorectal carcinogenesis in the Chinese population but there is as yet any data on gastric cancer[40]. However, since *MGST1* and *CYP2C19* are both carcinogen detoxification enzymes, with evidence supporting their involvement in gastrointestinal tract cancer genesis, 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 prevalent population, but our study covered all spectrum of the Correa cascade in a population with 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 were manifested, but they followed 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], taking this together with genomic changes, it would allow a greater understanding on the pathogenesis of gastric cancer.

We acknowledge from the outset that the current study, based upon genome-wide approach, was extremely limited in sample size as gastric precancerous lesions are extremely rare among the ethnic Malays from the north-eastern region of Peninsular Malaysia. In this respect, bioinformatics and statistical approaches are taken into consideration for a more reliable analysis of data. To reduce false-positive results, a more stringent significant 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 the 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, with further validation studies being in progress to confirm their regulatory role in carcinogenesis.

Conclusively, we have shown that, compared to controls, susceptible ethnic Malays for *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 the ethnic Malays. Gene variants may be associated with precancerous lesions in *Helicobacter pylori (H. pylori*-*)*-susceptible Malays.

***Research frontiers***

In a case-control fashion, genome wide association was performed to identify gene variants in the Malay population having different 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 manifested, but they followed a pattern of progression similar to their 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***

Genome wide approach utilises microarray technology to identify thousands of single nucleotide polymorphisms (SNPs). Using novel bioinformatics and statistical approaches, 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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**P-Reviewer** Kozarek RA **S-Editor** Wen LL  **L-Editor**  **E-Editor**



**Complete intestinal metaplasia (*n* = 4)**

**-Log10 (*P* value)**

**Atrophic gastritis only (*n* = 11)**



**Incomplete metaplasia and dysplasia (*n* = 5)**

**Dysplasia only (*n* = 2)**

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

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

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **rs ID** | **Chromosome** | **Gene** | **Minor allele** | **Allele frequency** | | 2  ***P*-value1** | **HWE**  ***P*-value** |
| **Cases** | **Control** |
| 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 identified single nucleotide polymorphism; HWE: Hardy-Weinberg Equilibrium. 1All markers are run using the FAMHAP (Haplotype Association Analysis) program. The *P* value represents simulated overall significance for the particular marker corrected for multiple testing and *P* < 0.05 is considered statistically significant.

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

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **rs ID** | **Chromosome** | **Gene** | **Minor allele** | **Allele frequency** | | 2  ***P*-value1** | **HWE**  ***P*-value** |
| **Cases** | **Control** |
| 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 | 0.780 |
| 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 | 0.250 | 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 identified single nucleotide polymorphism; HWE: Hardy-Weinberg Equilibrium. 1All markers are run using the FAMHAP (Haplotype Association Analysis) program. The *P* value represents simulated overall significance for the particular marker corrected for multiple testing and *P* < 0.05 is considered statistically significant.

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

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **rs ID** | **Chromosome** | **Gene** | **Minor Allele** | **Allele Frequency** | | 2  ***P*-value1** | **HWE**  ***P*-value** |
| **Cases** | **Control** |
| 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 identified single nucleotide polymorphism; HWE: Hardy-Weinberg Equilibrium. 1All markers are run using the FAMHAP (Haplotype Association Analysis) program. The *P* value represents simulated overall significance for the particular marker corrected for multiple testing and *P* < 0.05 is considered statistically significant.

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

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **rs ID** | **Chromosome** | **Gene** | **Minor Allele** | **Allele Frequency** | | 2  ***P*-value\*** | **HWE**  ***P*-value** |
| **Cases** | **Control** |
| **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 identified single nucleotide polymorphism; HWE: Hardy-Weinberg Equilibrium. 1All markers are run using the FAMHAP (Haplotype Association Analysis) program. The *P* value represents simulated overall significance for the particular marker corrected for multiple testing and *P* < 0.05 is considered statistically significant.