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The heterogeneic distribution of *Helicobacter pylori cag* pathogenicity island reflects different pathologies in multiracial Malaysian population

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ABSTRACT

Background: *Helicobacter pylori* harbouring *cag*-pathogenicity island (*cag*PAI) which encodes type IV secretion system (T4SS) and *cagA* virulence gene are involved in inflammation of the gastric mucosa. We examined all the 27 *cag*PAI genes in 88 *H. pylori* isolates from patients of different ethnicities and examined the association of the intactness of *cag*PAI region with histopathological scores of the gastric mucosa.

Results: 96.6% (n = 85) of *H. pylori* isolates were *cag*PAI-positive with 22.4% (19/85) having an intact *cag*PAI, whereas 77.6% (66/85) had a partial/rearranged *cag*PAI. The frequency of *cag2* and *cag14* were found to be significantly higher in *H. pylori* isolated from Malays, whereas *cag4* was predominantly found in Chinese isolates. The *cag24* was significantly found in higher proportions in Malay and Indian isolates than in Chinese isolates. The intactness of *cag*PAI region showed an association with histopathological scores of the gastric mucosa. Significant association was observed between *H. pylori* harbouring partial *cag*PAI with higher density of bacteria and neutrophil activity, whereas strains lacking *cag*PAI were associated with higher inflammatory score.

Conclusions: The genotypes of *H. pylori* strains with various *cag*PAI rearrangement associated with patients' ethnicities and histopathological scores might contribute to the pathogenesis of *H. pylori* infection in a multi-ethnic population.

Background

Helicobacter pylori is a Gram-negative, microaerophilic, curved-shaped and flagellated bacterium frequently found in the

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stomach of humans.[1] It is an important pathogen that causes gastrointestinal diseases such as chronic gastritis, peptic ulcer, gastric cancer and gastric mucosa-associated lymphoid tissue (MALT) lymphoma,[2,3] and most infected patients appear asymptomatic. Although *H. pylori* is designated as type I carcinogen, other factors including environment (lifestyle and diet), host genetics and host immune responses contribute to the disease sequelae in infected patients.[4–6]

Cytotoxin-associated gene pathogenicity island (*cag*PAI) is one of the major virulence factors associated with disease outcome in infected hosts. It is approximately 40 kb in size consisting of around 28 genes,[7] encoding mainly CagA protein, type IV secretion system (T4SS) and other genes for induction of host's interleukin-8 (IL-8).[7,8] Studies show that intactness of *cag*PAI has a significant correlation with disease severity, whereas *H. pylori* strains with partial deletions within *cag*PAI region are significantly less pathogenic in nature.[9,10] However, the rates of severe disease development vary between human populations, and differences in *H. pylori* genotypes may partially explain these differences.[11,12]

Integrity of *cag*PAI seems to have an important role in the progress of the gastroduodenal disorders, so that intact *cag*PAI could be seen in *H. pylori* strains from countries with higher rate of gastric cancer.[13–15] This integrity also has important effect on eliciting inflammatory response in the gastric mucosa.[16] Several studies have investigated the association of *H. pylori cag*PAI and gastroduodenal diseases.[13,17] However, knowledge about the relationship between *H. pylori cag*PAI intactness and changes of the infected gastric tissue is sparse. More than 90% of *H. pylori* strains in Malaysia are *cag*PAI-positive[18] and Malaysia being a multi-ethnic country, the interaction of *H. pylori* strains with different genotype combined with various host genetics may have an impact on associated disease outcomes.

There is a lack of comprehensive information with regarding intact *versus* rearranged *cag*PAI among *H. pylori* strains in the Malaysian population. Hence, in this study, we characterized the genes within *cag*PAI to determine the association of various *cag*PAI structure in *H. pylori* isolates with histopathological changes of the infected gastric mucosa. The outcome of this study provides valuable conclusions regarding association between presence of *cag*PAI genes and disease sequelae in strains from multi-ethnic population and associations with different histopathological states.

Methods

Bacterial isolates

A total of 88 non-repetitive *H. pylori* clinical isolates were obtained from patients (47 females and 41 males) recruited in previous studies (research no. ETP-2013-042 and GUP-2011-307) between 2011–2015. Patients (15 Malays, 52 Chinese and 21 Indians) had a mean age of 54.68 ± 16.87 years ranging from 17 to 83 years. In these projects, gastric biopsies were taken from the antrum and/or corpus of the patients' stomach for *H. pylori* culture and used as one of the diagnotic methods for determination of the *H. pylori* infection status in patients. *H. pylori* isolates grew from the culture method during the studies were stored at −70 °C in Brucella broth containing 15% glycerol. Histopathological examination was performed on gastric biopsies in both studies to detect the presence of *H. pylori* along with grading for gastritis. *H. pylori* were subcultured from frozen stock onto Columbia blood agar (Oxoid, Basingstoke, England) supplemented with 7% sheep blood and Dent's supplement (Oxoid, Basingstoke, England) and incubated at 37 °C for five to seven days under microaerophilic environment.

Histopathological examination

Gastric biopsies fixed in 10% formalin and paraffin embedded section were cut and stained with hematoxylin-eosin and, when necessary, sections were also stained with Warthin-Starry for better visualization of *H. pylori*. All patients had gastritis graded according to Updated Sydney Classification[31] except in two patients where the histopathological examination (HPE) results were not available. Severity of gastritis was graded from 0 to 3 (none, mild, moderate, and marked).

DNA extraction

H. pylori colonies were scraped from the agar surface of Columbia blood agar plate and subjected to DNA extraction using FavorPrep™ Tissue Genomic DNA Extraction Mini kit according to the manufacturer's instructions (Favorgen Biotech Corporation, Ping-Tung 908, Taiwan). DNA samples were diluted with ultrapure water to a concentration of 25 ng/μl and stored at -20 °C until further processing.

Determination of cagPAI genes

The presence or absence of *cag*PAI in *H. pylori* strains was determined by PCR using primers for detection of the 5′ and 3′ flanking region of the *cag*PAI as described by Olbermann et al.[28] The amplifications were carried out in 25 μL volume, each containing 12.5 μL mastermix (Lucigen, USA), 10 μL of each primers, 1 μL (25 ng) DNA and 10 μL DNAse and RNAse free sterile distilled water. PCR amplification for detection of *cag*PAI region consisted of initial denaturation at 95 °C for 3 min, followed by 30 cycles of 95 °C for 30 s, 50 °C for 60 s, and 72 °C for 45 s, ending with final extension at 72 °C for 5 min. The amplifications were performed in a PCR thermal cycler T100 Series (Bio-Rad, USA). The products were run on 1.5% agarose gel and stained with FloroSafe DNA stain (1st BASE Pte. Ltd, Singapore) and visualized with gel documentation (AlphaImager, Biosciences, CA). The *cag*PAI-positive isolates (n = 91) were then subjected to subsequent PCRs for identification of all *cag*PAI genes using primers as described previously.[28,32] The absence of *cag2* was confirmed with 690 or 1100 bp amplicon using empty-site PCR.[24] *cag14* was detected using four sets of primer pair as described earlier.[32] PCR amplification for *cag*PAI genes consisted of initial denaturation at 95 °C for 3 min, followed by 30 cycles of 95 °C for 30 s, annealing temperature for 60 s (48 °C for *cag11*, 48.8 °C for *cag3* and 55 °C for *cag1*, *cag2*, *cag4*, *cag5*, *cagα*, *cag6* to *cag10*, *cag12* to *cag26*), and extension at 72 °C for 45 s. A final extension at 72 °C for 5 min was performed for each PCR run. Representative positive PCR products (n = 28)

Table 1 – Histopathological scores of the gastric mucosa in different ethnic groups.

Histopathological examination	Score	Ethnic group, n (%)		
		Malay (n = 15)	Chinese (n = 52)	Indian (n = 19)
H. pylori density	0	1 (6.7)	16 (30.8)	3 (15.8)
	1	6 (40)	14 (26.9)	11 (57.9)
	2	8 (53.3)	12 (23.1)	2 (10.5)
	3	0	10 (19.2)	3 (15.8)
Total score	Mean ± SE	1.47 ± 0.17	1.31 ± 0.15	1.26 ± 0.21
Mononuclear cell infiltration	0	0	1 (1.9)	0
	1	5 (33.3)	16 (30.8)	5 (26.3)
	2	9 (60)	27 (51.9)	14 (73.7)
	3	1 (6.7)	8 (15.4)	0
Total score	Mean ± SE	1.73 ± 0.15	1.81 ± 0.10	1.74 ± 0.10
Neutrophil activity	0	2 (13.3)	19 (36.5)	1 (5.3)
	1	9 (60)	16 (30.8)	15 (78.9)
	2	3 (20)	13 (25)	2 (10.5)
	3	1 (6.7)	4 (7.7)	1 (5.3)
Total score	Mean ± SE	1.20 ± 0.20	1.04 ± 0.13	1.16 ± 0.14
Intestinal metaplasia	0	12 (80)	45 (86.5)	16 (84.2)
	1	3 (20)	6 (11.5)	1 (5.3)
	2	0	1 (1.9)	1 (5.3)
	3	0	0	1 (5.3)
Total score	Mean ± SE	0.2 ± 0.11	0.15 ± 0.06	0.32 ± 0.19
Atrophy	0	11 (73.3)	41 (78.8)	14 (73.7)
	1	4 (26.7)	5 (9.6)	5 (26.3)
	2	0	3 (5.8)	0
	3	0	3 (5.8)	0
Total score	Mean ± SE	0.27 ± 0.12	0.38 ± 0.12	0.26 ± 0.10

were sent for sequencing and the nucleotide sequences were blasted against NCBI databases to confirm the gene identity.

Statistical analysis

Statistical analysis was performed using SPSS software version 23 (SPSS Inc, Chicago, IL, USA). Differences between groups were evaluated using Chi-square (χ^2) test with Yate's continuity correction and Fisher's exact test. Independent *t*-test was used to compare means between different groups of histopathological scores. Score was represented with mean ± standard error of mean (SE). Differences were considered significant when p value was <0.05.

Results

Histopathological characteristics of the gastric mucosa in the studied ethnic groups

Histopathological scores of the gastric mucosa among different ethnic groups showed that the Malays had higher mean scores for *H. pylori* density and neutrophil activity whereas the Chinese showed higher grade of inflammation (Table 1). Higher mean score for intestinal metaplasia was observed among the Indians, while atrophy of higher grade was observed in the Chinese. Patients of different ethnicities were grouped into different types of disease conditions based on the histopathological changes (Table 2), *i.e.* chronic gastritis (CG) (n = 18), chronic active gastritis (CAG) (n = 41) and intestinal metaplasia/atrophy (IM/Atr) (n = 26). There was a significant difference in the proportion of CG and CAG between Chinese and non-Chinese patients. CG was more diagnosed in Chinese patients compared to non-Chinese ($p = 0.03$), whereas

Table 2 – Disease groups in different ethnicities.

Ethnic	Disease, n (%)		
	CG	CAG	IM/Atr
Malay (n = 15)	2 (13.3)	8 (53.3)	5 (33.3)
Chinese (n = 51)	15 (29.4)	22 (43.1)	14 (27.5)
*Indian (n = 19)	1 (5.3)	11 (57.9)	7 (36.8)

*HPE (histopathogical examination) results for two patients were not available, and one patient had normal HPE scores. CG; chronic gastritis, CAG; chronic active gastritis, IM/Atr; Intestinal metaplasia/Atrophy.

Statistical analysis:

Ethnic	Disease, n (%)	
	CG	CAG
Non-Chinese (n = 22)	3(13.3)	19 (86.4)
Chinese (n = 37)	15(40.5)	22 (59.5)

*Non-Chinese (Malay and Indian), $\chi^2 = 4.710$, df = 1, $p = 0.03$.

Ethnic	Disease, n (%)	
	CG	IM/Atr
Non-Chinese (n = 15)	3 (20)	12 (80)
Chinese (n = 29)	15 (51.7)	14 (45.3)

$\chi^2 = 4.116$, df = 1, $p = 0.042$.

Table 3 – Association of *H. pylori cag*PAI intactness with histopathological changes of gastric mucosa.

Histopathological changes	Score	*cag*PAI, n (%)		
		Intact	Partial	Deleted
H. pylori density[a]	0	6 (31.6)	13 (20.3)	1 (33.3)
	1	9 (47.4)	21 (32.8)	1 (33.3)
	2	3 (15.8)	18 (28.1)	1 (33.3)
	3	1 (5.3)	12 (18.8)	0
Total score	Mean ± SE	0.95 ± 0.19	1.45 ± 0.13	1.0 ± 0.58
MNC infiltration[b]	0	0	1 (1.6)	0
	1	5 (26.3)	21 (32.8)	0
	2	12 (63.2)	35 (54.7)	3 (100)
	3	2 (10.5)	7 (10.9)	0
Total score	Mean ± SE	1.84 ± 0.14	1.75 ± 0.08	2.0 ± 0
Neutrophil activity[c]	0	9 (47.4)	12 (20.9)	1 (33.3)
	1	7 (36.8)	32 (50)	1 (33.3)
	2	2 (10.5)	15 (23.4)	1 (33.3)
	3	1 (5.3)	5 (7.8)	0
Total score	Mean ± SE	0.74 ± 0.2	1.20 ± 0.11	1.0 ± 0.58
Intestinal metaplasia	0	14 (73.7)	56 (87.5)	3 (100)
	1	4 (21.1)	6 (9.4)	0
	2	1 (5.3)	1 (1.6)	0
	3	0	1 (1.6)	0
Total score	Mean ± SE	0.32 ± 0.13	0.17 ± 0.07	0
Atrophy	0	14 (73.7)	50 (78.1)	2 (66.7)
	1	3 (15.8)	10 (15.6)	1 (33.3)
	2	1 (5.3)	2 (3.1)	0
	3	1 (5.3)	2 (3.1)	0
Total score	Mean ± SE	0.42 ± 0.19	0.31 ± 0.09	0.33 ± 0.33

Statistical analysis (Independent *t*-test):
[a]Partial *vs* Intact; t = 2.173, $p = 0.037$, 95% CI (0.033–0.978).
Partial *vs* Deleted; $p = 0.46$.
Deleted *vs* Intact; $p = 0.92$.
[b]Deleted *vs* Partial; t = 3.000, $p = 0.004$, 95% CI (0.083–0.417).
Deleted *vs* Intact; $p = 0.661$.
Intact *vs* Partial; $p = 0.591$.
[c]Partial *vs* Intact; t = 2.108, $p = 0.038$, 95% CI (0.026–0.906).
Deleted *vs* Intact; $p = 0.701$.
Deleted *vs* Partial; $p = 0.760$.

CAG and IM/Atr were more observed in non-Chinese than in Chinese ($p = 0.042$) patients.

Distribution of the cagPAI genes in H. pylori isolates

A total of 96.6% (n = 85) of the isolates were *cag*PAI-positive. Five genes in the *cag*PAI region (*cag1*, *cag5*, *cag6*, *cag8* and *cag21*) were detected in all isolates whereas *cag2* was detected in 34.1% (n = 29) and *cag14* in 51.7% (n = 44) of the isolates (Table S1). Detection of other genes ranged from 69.4 to 98.8%.

Six genes (*cag1, cag5, cag6, cag8, cag21* and *cag26*) in the *cag*PAI region were detected in all Indian isolates, whereas 12 and 19 genes were detected in all Chinese and Malay isolates, respectively (Table S1). The twelve genes detected in Chinese isolates were *cag1*, *cag3*, *cag5*, *cag6, cag8, cag9, cag12, cag13, cag15*, *cag20-22*. The 19 genes detected in Malay isolates were *cag1*, *cag3*, *cag5-9, cag11-13, cag15-19, cag21-23* and *cag26*. A significant difference in detection of *cag2*, *cag4*, *cag14* and *cag24* were observed among *H. pylori* from patients with different ethnicities. Detection of *cag2* was significantly higher in isolates from Malays (86.7%), followed by Indians (57.9%) and was least in Chinese isolates (9.8%) ($\chi^2 = 36.62$, df = 2, $p < 0.0001$). The presence of *cag4* was more frequent in isolates from Chinese (80.4%) compared to the Malays (46.7%) and Indians (63.2%) ($\chi^2 = 7.001$, df = 2, $p = 0.03$). Significant difference was observed in the detection of *cag14* in Malay isolates (93.3%) compared to Chinese (39.2%) and Indian (52.6%) isolates ($\chi^2 = 13.603$, df = 2, $p = 0.001$). Also, the frequency of *cag24* was significantly higher in isolates from Malays (93.3%) and Indians (89.5%) compared to isolates from Chinese patients (54.9%) ($\chi^2 = 12.701$, df = 2, $p = 0.002$).

We did further analyses to look for the distribution of individuals *cag*PAI genes in different disease conditions. All *cag*PAI genes showed a similar distribution in CG, CAG and IM/Atr (data not shown) except for the *cag2*. *cag2* was detected in 11.8% (2/17) of CG, 35% (14/40) of CAG and 44% (11/25) of IM/Atr. However, no significant difference was observed for the detection of *H. pylori* carrying *cag2* in different groups of diseases ($p = 0.086$).

Analysis of cagPAI intactness in H. pylori isolates

The *cag*PAI was defined as intact if all gene sets of the *cag*PAI were present including strains lacking only the *cag2* (HP0521). A previous systematic mutagenesis study showed that the HP0521 gene was not involved in the process of CagA translocation and IL-8 induction.[7] In addition, NCBI database defined the HP0521 as a pseudogene (NCBI-Gene ID: 900040) (DBGET/LinkBD: an integrated database retrieval system, last accessed Oct 8, 2018). Partial *cag*PAI was defined when an isolate lacked one or more *cag*PAI genes other than *cag2* (HP0521), while negative/deleted *cag*PAI was defined if none of the genes was present and a product of approximately 650 bp with primers from the flanking regions was obtained. Among the 85 *cag*PAI-positive *H. pylori* strains, 22.4% (n = 19) had intact *cag*PAI and 77.6% (n = 66) exhibited partial (rearranged) *cag*PAI. Strains harbouring intact or partial *cag*PAI were not associated with patients' ethnicities ($p > 0.05$).

Association between *cag*PAI intactness and histopathological scores of the gastric mucosa are shown in Table 3. The presence of partial *cag*PAI was significantly related to higher total score of *H. pylori* density ($p = 0.037$) and neutrophil activity ($p = 0.038$) compared to intact *cag*PAI. *H. pylori* harbouring deleted *cag*PAI was significantly correlated with higher inflammatory score (mononuclear infiltration) compared to *H. pylori* with partial *cag*PAI ($p = 0.004$). The distribution of *H. pylori* with intact *cag*PAI was more detected in the gastric mucosa with IM/Atr, whereas partial *cag*PAI *H. pylori* was more detected in CAG, however the difference was not significant. Moderate and severe scores of *H. pylori* density, mononuclear infiltration, neutrophil activity, and atrophy were observed more in gastric mucosa from Chinese patients infected with H. pylori strains harbouring *cag*PAI than other ethnicities (Malays and Indians) (Table S2).

Discussion

Major differences in the prevalence of *H. pylori* infection and disease-related severity were observed among patients from multiracial ethnicities.[19,20] Bacterial virulence factor is one of the contributing factors for the development of severe *H. pylori*-related diseases. The diversity of *cag*PAI organization in the *H. pylori* genome may have a modifying effect on the pathogenic potential of the infecting strain.[21]

In this study, we comprehensively determined the presence of *cag*PAI region in 88 *H. pylori* isolates from Malaysian population which were isolated from patients of different ethnic groups. The results showed that more than 95% of *H. pylori* strains were *cag*PAI-positive where 22.4% of the isolates carried all *cag*PAI genes and 77.6% exhibited partial or rearrangement in the *cag*PAI genes. Our previous study had showed that 3.2% of the isolates had all the *cag*PAI genes.[18] Low percentage of *H. pylori* isolates harbouring intact *cag*PAI genes was observed in our previous study because only a subset of the *cag*PAI genes (*cag67*, *cag10*, *cag13*, *cagT*, *cagM* and *cagE*) was analysed as these genes were shown to have linkage with certain *cag*PAI genes and severe disease outcome as described in earlier studies.[22,23] In contrast, high frequency of intact *cag*PAI and low frequency of partial *cag*PAI in *H. pylori* strains isolated from similar ethnic populations was reported by Schmidt et al.[24] In their study, *cagE*, *cagL*, *cagT* and HP521 were examined to assess the intactness of *cag*PAI region. Discordance in the frequency of *cag*PAI intactness in many reports was due to the different *cag*PAI genes being examined.[13,25,26] Thus, results of the present study indicate that deletions can occur in all parts of the *cag*PAI and screening the entire genes in the *cag*PAI is essential for an accurate determination of the organization of the *cag*PAI region. For comparison with our results, we reviewed only studies that screened all the *cag*PAI genes. A previous study observed complete *cag*PAI present in 82.6% of the strains, while a partially deleted *cag*PAI in 9.6% of the strains and 7.7% lacked the entire *cag*PAI in Indian population.[10] In Swedish population, 76% of the strains carried an intact *cag*PAI, 15% had partially deleted *cag*PAI and 9% of the strains lacked the *cag*PAI.[9] A study by Azuma et al.[27] showed that the complete *cag*PAI was identified in all 11 Japanese isolates. Variation in the *cag*PAI positivity in different population of *H. pylori* isolates might be related to different geographical origin of *H. pylori* subpopulations. Presence of the *cag*PAI region is almost universal in *H. pylori* hpEastAsia and hpAfrica1 populations, intermediate presence in hpEurope, and complete absence in hpAfrica2.[28] Malaysian isolates showed a mixed subpopulation of hpEastAsia, hpAsia2, and hpEurope as indicated by multiracial communities living in the country.[29,30]

Analysis of the entire 27 *cag*PAI genes in the present study revealed that *cag1*, *cag5*, *cag6*, *cag8* and *cag21* were present in all isolates, which might represent core genes of the *cag*PAI region. However, function of the *cag1*, *cag6* and *cag21* are still unknown.[28] *cag5* (HP0524, *cagβ*) and *cag8* (HP0528, *cagX*) is a component of T4SS (VirB9 and VirD4).[28] One strain lacked *cagA* gene but had other *cag*PAI genes indicating that *cagA*-positive isolates do not necessarily harbour intact *cag*PAI region. Indian isolates showed more rearrangement in the *cag*PAI region compared to Malay and Chinese isolates. Studies show that the subpopulations of *H. pylori* Indian isolates in Malaysia consist of mixed populations *i.e.*, hpEurope, hpAsia2, and hpEAsia reflecting on the diversity of *cag*PAI genes rearrangement in the Indian isolates.[29,30]

The presence of specific genes in *H. pylori* isolates associated with different ethnicities (*cag4* in the Chinese isolates and *cag2*, *cag14* and *cag24* in the non-Chinese isolates) might represent strains associated disease outcomes. The *cag4* (VirB1) is a component of T4SS, whereas the function is still unknown for *cag2*, *cag14* and *cag24*.[28] Although the difference was not statistically significant, high frequency of *cag2* was detected in gastric mucosa with CAG and IM/Atr and reflects the presence of this gene in non-Chinese isolates. These observations require further investigation to decipher the role of these genes.

We found an association of *cag*PAI intactness with histopathological scores of the gastric mucosa. *H. pylori* harbouring partial *cag*PAI were associated with higher density of *H. pylori* and neutrophil activity, whereas *H. pylori* with deleted *cag*PAI caused increased in inflammatory score. The presence of neutrophil activity in the gastric mucosa was associated with CAG. In addition, in our study partial *cag*PAI *H. pylori* strains was more often detected in patients with CAG. As strains with deleted *cag*PAI only cause inflammation of the gastric mucosa, the presence of *cag*PAI proteins encoded by *H.*

pylori strains is needed to cause more severe disease such as active gastritis and intestinal metaplasia. However, no specific gene causing severe conditions could be identified. A group of genes that encodes T4SS and induction of IL-8 secretion has been shown to be involved in disease development process.[7,24]

Conclusions

This study shows the diversity in the arrangement of *cag*PAI in *H. pylori* isolates obtained from different ethnic groups in Malaysia. Comprehensive screening of the entire *cag*PAI genes provided a more accurate overview of the *H. pylori cag*PAI genotype allowing for better identification of virulence traits of *H. pylori* in a multiracial population. This has implications for assessing different treatment options for treating *H. pylori* infections and can affect various disease outcomes. *H. pylori* strains harbouring partial/rearrangement of the *cag*PAI genes were associated with increased colonization and recruitment of neutrophils at the site of infection and further contribute to various disease outcomes caused by different *H. pylori* genotypes. Further studies in this area not only provide more information about the disease prevalence in various ethnic groups in Malaysia but also inform healthcare practitioners if a person from an ethnic group may be at risk of developing severe disease leading to the development of a customized treatment plan. The data obtained can be used to monitor national trends and develop further appropriate intervention strategies.

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Availability of data and materials

Data will be shared upon request to the corresponding author alfizah@ppukm.ukm.edu.my.

Authors' contribution

SAR performed all experiments and data analysis. HMN and NMZ participated in the study design and data analysis. AH involved in the design of the study, data analysis and manuscript writing. BSL participated in data analysis and manuscript writing. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The research protocol was approved by the Medical Research Ethic Committee of the University (UKM1.5.3.5/244/JEP-2016-095). The present study used *H. pylori* stock cultures where the informed consent was not applicable. However, these isolates were obtained from patients in previous studies (research no. ETP-2013-042 and GUP-2011-307) where informed consent was obtained from all individuals included in the studies.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.bjid.2020.10.005.

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