Abstract. **Background:** PAR-1 has been involved in inflammation of the gastrointestinal tract, tumour cell growth and invasion of gastric carcinoma cells. Thus, we aimed at determining, for the first time, the association between PAR-1 -506 ins/del and -IVSn-14 A/T and risk of *Helicobacter pylori*-related gastric cancer (GC) in an ethnic Chinese population. **Materials and Methods:** A case-control study comprising of 225 ethnic Chinese individuals (77 non-cardia GC cases and 148 controls with functional dyspepsia) was conducted. PAR-1 IVSn-14 A/T and 506 ins/del were genotyped by means of real-time PCR and MALDI-TOF mass spectrometry, respectively. **Results:** *H. pylori* infection, male gender and the PAR-1 IVSn-14 TT genotype increased GC risk (OR: 3.15, 95% CI: 1.54-6.45, OR: 2.44, 95% CI: 1.35-4.42 and OR: 2.58, 95% CI: 1.09-6.13, respectively). PAR-1 -506 ins/del did not provide significant results. **Conclusion:** PAR-1 IVSn-14 T allele is a risk factor for *H. pylori*-related GC in ethnic Chinese subjects. PAR-1 -506 ins/del polymorphism is not involved in gastric carcinogenesis.

Gastric cancer (GC) is the fourth most common cancer and the second most common cause of cancer-related death worldwide. According to the latest global estimation GLOBOCAN, the age-standardised incidence rate of GC in China is 41.3 per 100,000 men and 18.5 per 100,000 women (1). In multiracial countries such as Singapore and Malaysia, Chinese have the highest (25.7 per 100,000 men) age-standardised incidence rates as compared with the Malays and Indians who have incidence rates of 6.6 per 100,000 men and 8.4 per 100,000 men, respectively (2). Furthermore, Chinese ethnicity has been identified as an independent risk factor for the development of GC (3, 4).

Although *Helicobacter pylori* has been established as the most important aetiological risk factor for GC, the pathogenesis of GC involves the combined effects of bacterial, host and environmental factors (5-9). Currently, it is well-established that cancer arises in chronically inflammed tissue, and this is predominantly notable in the gastrointestinal tract, *H. pylori*-related GC being a classic example (10-11). Therefore, polymorphisms within genes involved in innate and adaptive immunity might directly influence inter-individual variation in the magnitude of the inflammatory response against *H. pylori* infection, which could explain the high variation in the incidence of GC around the world.

Protease-activated receptors (PARs) are part of the G protein-coupled receptors superfamily. To date, four different receptors have been identified: PAR-1, PAR-2, PAR-3 and PAR-4. Among these, PAR-1 has been involved in several pathophysiological processes such as growth and development, mitogenesis and inflammation (12). The inflammatory effects mediated by PAR-1 include vasodilatation, vasoconstriction, increased vascular permeability, cellular adhesion, chemotaxis and activation of a T-helper type 1 cytokine profile in the gastrointestinal tract (12, 13).

In the last decade, several studies have determined the association between PAR-1 and gastrointestinal disorders (14-17). For example, Toyoda et al. showed that thrombin through PAR-1 stimulates the secretion of mucin, as well as...
prostaglandin E and its receptors do in rat gastric epithelial cell lines (15). Additionally in mouse models, PAR-1 has been implicated in the protection of the gastric mucosa against ethanol-induced damage (14). Furthermore, PAR-1 expression in the human gastric mucosa is reported to be increased in gastritis and H. pylori infection (15, 17).

In the context of GC, PAR-1 has been associated with cell proliferation, cell motility and cell invasion (18-19). A recent study showed that galectin-3, a protein known to be involved in tumorigenesis and metastasis, increased migration and invasion in a GC cell line through PAR-1 up-regulation (19). In addition, Fujimoto et al. (18) showed that PAR-1 activation involves the activation of NF-κB and transactivation of the epidermal growth factor receptor, which results in an increase in GC cell proliferation and invasion. Further, PAR-1 and -2 have been associated with other gastrointestinal malignancies. For example, most colon cancer cell lines express both PAR-1 and PAR-2, activation of which leads to cell proliferation via the MAPK pathway (20-21). Further, Darmoul et al. showed that PAR-1 is aberrantly expressed in human colon cancer cells but not in normal colonic epithelial cells, and that it is involved in cellular motility in colon cancer, a finding that suggests that PAR-1 might be engaged in metastatic events (20).

PAR-1 is encoded by a gene located on chromosome 5q11.2-q13.3, which is approximately 27-kilobase (kb) long and comprises of 2 exons separated by a large intron of approximately 22 kb in length. Three main polymorphisms have been identified in PAR-1, known as PAR-1 -506 ins/del, PAR-1 IVSn -14 A/T and PAR-1 -1426 C/T (22). According to previous studies, PAR-1 -506 ins/del and PAR-1 IVSn -14 A/T are DNA sequence variations that have the potential to modulate PAR-1 production and activity, which might affect a wide range of physiological systems including the gastrointestinal tract (23-24). To date, there is no evidence of any functional relevance of the polymorphism PAR-1 -1426 C/T.

In general, countries with a high incidence of GC have a high prevalence of H. pylori infection and thus, antibiotic eradication of H. pylori in such populations is not feasible. However, H. pylori, infected subjects with a genetic-susceptibility profile associated with an increased risk of developing GC could be identified and a targeted approach to treatment could potentially be introduced. Thus, the aim of the current study was to determine the prevalence of PAR-1 -506 ins/del and PAR-1 IVSn -14 A/T, polymorphisms that have been shown to influence the production and activity of the receptor, in ethnic Chinese subjects, and to establish the association between these polymorphisms and GC in this high-risk population.

Materials and Methods

Subjects. Subjects were ethnic Chinese individuals presenting for upper gastrointestinal endoscopy at The Changi General Hospital (Singapore) and the Department of Medicine at the University Hospital of Malaysia (Kuala Lumpur). Individuals prescribed non-steroidal anti-inflammatory drugs (NSAIDs), anti-microbial agents or acid suppressants (H2 receptor antagonists and proton pump inhibitors) in the two-month period prior to recruitment were excluded. In addition, subjects known to be infected with the human immunodeficiency virus or affected by any other immunosuppressive condition were excluded.

The study sample comprised of 225 subjects. Seventy-seven patients, newly diagnosed with a primary non-cardia GC (International Classification of Diseases, 9th revision, code 151) using histological confirmation, were recruited from January 2004 to April 2007. One hundred and forty-eight individuals diagnosed with functional dyspepsia (FD) over the same period, composed the control group. FD was defined as persistent or recurrent symptoms (pain or discomfort centered in the upper abdomen) in the absence of organic disease (including at upper endoscopy), in accordance with the Rome II classification system (25).

This study was approved by the Human Ethics Committee (HREC) of the University of New South Wales (UNSW) (HREC 08115 and HREC 02144), and written informed consent was obtained from each individual recruited to the study.

Helicobacter pylori detection. The presence of H. pylori IgG antibodies in GC patients and FD controls was determined using an in-house enzyme-linked immunosorbent assay (ELISA), which has previously been shown to have high sensitivity and specificity in a Chinese population (26). Serum samples from GC patients shown to be H. pylori-negative by ELISA were further investigated by Immunoblot (MPD Helico Blot 2.1, MP Biomedicals, Australia) according to the manufacturer’s instructions. These results have been partially published elsewhere (27).

Genotyping of PAR-1 IVSn -14 A/T by real-time PCR. Genomic DNA was extracted from EDTA-anticoagulated peripheral blood obtained from each subject by means of the QIAamp® Blood DNA Mini Kit as described by the manufacturer (Qiagen; Hilden, Germany). Genotyping of PAR-1 IVSn -14 A/T by real-time PCR. All experiments were performed using the Rotor Gene 6000 cycler (Corbett Life Sciences; Doncaster, Australia). This polymorphism was amplified by the following primers: 5’-GCC TTG TTG ATG CGT TCA C- 3’ (forward), 5’-TGC TTT TGA TTC TGA AAA ATA AAA TA-3’ (reverse 1) and 5’-TGC TTT TGA TTC TGA AAA ATA AAA TT-3’ (reverse 2), as previously described by Martinelli et al. (28). Each 10 μl reaction contained a mixture of 3 μl 2x SensiMix SYBR Kit (Bioline; Alexandria, Australia), 10 pmol of each primer (Sigma-Aldrich; Castle Hill, Australia), approximately 30 ng of DNA template and sterile water to complete the volume. Thermal cycling conditions are described in Table I.

As recommended by Hazbon et al. (29), only reactions with a ΔCt greater than 5 were considered as consistent results. The data were collected at the annealing step of stage 3 (60°C) and were analysed using the Rotor Gene 6000 software version 1.7 (Corbett Life Sciences).

As a validation of this method, genotyping of PAR-1 IVSn -14 A/T was repeated in ten percent of the study samples, which were randomly chosen.

Genotyping of PAR-1 -506 ins/del. From each individual, genomic DNA was extracted from peripheral whole blood samples, as described above. DNA was rehydrated in sterile water and normalised to 10 ng/μl for customised SNP genotyping through the application of matrix-assisted laser desorption ionisation time-of-flight (MALDI-
Table I. **PCR thermal conditions used for genotyping of PAR-1 polymorphisms in gastric cancer patients and functional dyspepsia controls.**

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Molecular technique</th>
<th>Product (bp)</th>
<th>Tm&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Tm&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Tm&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAR-1 IVSn-14 A/T</td>
<td>Real-time PCR&lt;sup&gt;d&lt;/sup&gt;</td>
<td>67</td>
<td>Stage 2: 95°C for 15 s</td>
<td>Stage 2: 69°C for 20 s</td>
<td>Stage 2: 72°C for 20 s</td>
<td>10</td>
</tr>
<tr>
<td>(rs168753)</td>
<td></td>
<td></td>
<td>Stage 3: 95°C for 15 s</td>
<td>Stage 3: 60°C for 30 s</td>
<td>Stage 3: 72°C for 10 s</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>95°C for 30 s</td>
<td>57°C for 30 s</td>
<td>72°C for 1 min</td>
<td>40</td>
</tr>
<tr>
<td>PAR-1 -506 ins/del</td>
<td>Standard PCR</td>
<td>insertion:118</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(rs11267092)</td>
<td></td>
<td>deletion:105</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PCR: Polymerase chain reaction. <sup>a</sup>Denaturation temperature, <sup>b</sup>annealing temperature, <sup>c</sup>extension temperature. <sup>d</sup>Stage 1 is 95°C for 10 min.

TOF) mass spectrometry, the Sequenom MassARRAY iPLEX™ assay (San Diego, USA) (30, 31), at the Australian Genome Research Facility Ltd, St Lucia, University of Queensland, Australia.

Validation of the results using DNA fragment analysis was performed in 10% of the study samples, which were randomly selected. The following primers, of which the reverse primer was previously described by Martinelli et al. (28), were used: 5′- [6FAM] CTG TCG AGC TCT CCA CAT CCC AGG A-3′ (fluorescent labelled forward primer) and 5′-CGA AGC TGT CAG TGA CTC ACA CTG G-3′ (reverse primer). The PCR reaction consisted of 30 ng of DNA, 1× PCR buffer (Fisher Biotech; Subiaco, Australia), 200 nM of each deoxynucleotide-triphosphate (Fisher Biotech), 2.0 mM MgCl₂ (Fisher Biotech), 1 U of Taq polymerase (Fisher Biotech), and sterile water to complete the volume. Thermal cycling conditions are described in Table I. Finally, 1-3 μl aliquot of each PCR reaction was loaded in a 96-well AB3730 compatible plate for further fragment analysis using an Applied Biosystems 3730 DNA Analyzer (Applied Biosystems; Foster City, USA) at the Ramaciotti Centre, UNSW (Sydney, Australia). DNA fragment data were visualised and analysed using the software Peak Scanner version 1.0 (Applied Biosystems).

**Statistical analysis.** The clinical characteristics of the study subjects were compared using the unpaired Student’s t-test. Deviation from Hardy-Weinberg equilibrium (HWE) was tested using the chi-square goodness-of-fit test (χ²). Estimation of the genotype and allele frequencies was done by the direct count method. The odds ratios (OR) and 95% confidence intervals (CI) were calculated by means of frequencies was done by the direct count method. The odds ratios (OR) and 95% CI were calculated by means of the Fisher’s exact probability test (two-tailed p-values). A multivariate analysis was performed using a binary logistic regression (LR) adjusted for H. pylori status and gender. p-Values less than 0.05 were considered statistically significant. Statistical analyses were done using the programs Arlequin version 3.1 (32), GraphPad Prism version 5.02 (GraphPad Software Inc; San Diego, USA) and SPSS version 19.0.0 (SPSS Inc; Chicago, USA).

**Results.**

**Clinical characteristics.** The clinical characteristics of the study subjects including gender, *H. pylori* infection status and median age are shown in Table II. In this ethnic Chinese population, male gender was associated with increased risk of GC (OR: 1.74, 95% CI: 1.18-2.57, p-Value: 0.005 on the univariate analysis). Overall, 69.8% of individuals in this study were infected with *H. pylori*. The prevalence of *H. pylori* was significantly higher in GC patients (84.4%), as compared with FD controls (62.2%), indicating a positive association with GC development (2.35, 95% CI: 1.36-4.05, p-value: 0.001 on the univariate analysis). Although subjects in this study were matched according to 5-year age groups, the median age of the GC patients (68.7 yrs) was significantly higher than that of FD controls (61.3 yrs) (p-value: <0.0001).

**Prevalence of PAR-1 IVSn -14 A/T and PAR-1 -506 ins/del in gastric cancer patients and functional dyspepsia controls.** The PAR-1 -506 ins/del genotyping call rate was 95% in this study. DNA fragment analysis confirmed the results of PAR-1 -506 ins/del obtained by the Sequenom MassARRAY iPLEX™ assay showing 100% concordance (Figure 1). Both PAR-1 IVSn 14 A/T and PAR-1 -506 ins/del were found to be in HWE in the control group showing non-significant χ² values.

The prevalence of the PAR-1 IVSn -14 T allele in GC patients was 59.1% (91/154) and in FD controls it was 48.0% (142/296) (Table III). Analysis of PAR-1 -506 ins/del showed the prevalence of the insertion allele to be 4.6% (7/152) in GC patients and 3.1% (9/294) in FD controls (Table III).

**Association between PAR-1 IVSn -14 A/T and PAR-1 -506 ins/del polymorphisms and gastric cancer in an ethnic Chinese population.** Univariate statistical analysis of PAR-1
IVSn -14 A/T showed significant results inferring an association between this polymorphism and risk of GC development in this ethnic Chinese population. The allele analysis showed an OR of 1.57 (95% CI: 1.06-2.32) for the T allele while the TT genotype showed an OR of 2.56 (95% CI: 1.12-5.85) (Table III).

In contrast to PAR-1 IVSn -14 A/T, comparison of PAR-1 -506 ins/del allele and genotype frequencies between GC patients and FD controls showed no statistically significant results (Table III).

The PAR-1 IVSn -14 A/T results were further included in a multivariate analysis using a binary LR adjusted for H. pylori infection and gender. The multivariate analysis provided consistent significant data (adjusted OR: 2.58, 95% CI: 1.09-6.13 and adjusted OR: 1.71, 95% CI: 0.78-3.75 for the TT and AT genotype, respectively) (Table IV). H. pylori infection and male gender remained significant risk factors for GC development in this population (adjusted OR: 3.15, 95% CI: 1.54-6.45 and adjusted OR: 2.44, 95% CI: 1.35-4.42, respectively) (Table IV).

Association between PAR-1 IVSn -14 A/T and PAR-1 -506 ins/del polymorphisms and risk of Helicobacter pylori infection in an ethnic Chinese population. To determine if an association existed between PAR-1 IVSn -14 A/T and PAR-1 -506 ins/del and risk of H. pylori infection, univariate statistical analyses were conducted, however no significant association was observed (Table V).

**Discussion**

Intestinal-type GC is considered a progressive process initiated by inflammation of the gastric mucosa induced by H. pylori (33). In addition to a central role in coagulation, PAR-1 has been implicated in numerous biological functions that are related to inflammation including vasodilatation and vasoconstriction, increased vascular permeability, cellular adhesion and infiltration by chemotaxis, and release of inflammatory mediators such as histamine (12, 34).

Furthermore, there is evidence that this receptor also enhances the production of IL-1 by macrophages exposed to LPS (35). Studies both on PAR-1 IVSn -14 A/T and -506 ins/del have shown that these polymorphisms influence the production and activity of the receptor (23-24). Given this, in the current study, PAR-1 IVSn -14 A/T and -506 ins/del were investigated in an attempt to establish an association with GC in ethnic Chinese individuals, a high risk ethnic group for intestinal-type GC.

Gender and H. pylori infection were two clinical characteristics analysed in this study. Male gender was found to be associated with an increased risk of GC, a finding that remained statistically significant after adjustment for H. pylori infection and PAR-1 IVSn 14 A/T. The male predominance in GC is a global phenomenon irrespective of the difference of GC incidence among populations (36). The presence of sex hormones, in particular estrogens, and the dissimilar age in the appearance of new GC cases between males and females, have been postulated as explanations for the observed pattern in the male:female ratio (36-38). Assessment of the association between H. pylori and GC using multivariate analysis showed no statistically significant results.

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**Table III. Association between PAR-1 polymorphisms and risk of gastric cancer in ethnic Chinese individuals (univariate analysis).**

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Genotype/allele</th>
<th>Cases (N=77)</th>
<th>Controls (N=148)</th>
<th>OR (95%CI)</th>
<th>p-Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAR-1 IVSn -14 A/T</td>
<td>AA</td>
<td>12</td>
<td>39</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AT</td>
<td>39</td>
<td>76</td>
<td>1.67 (0.78-3.54)</td>
<td>0.205</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>26</td>
<td>33</td>
<td>2.56 (1.12-5.85)</td>
<td>0.028</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>63</td>
<td>154</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>91</td>
<td>142</td>
<td>1.57 (1.06-2.32)</td>
<td>0.029</td>
</tr>
<tr>
<td>PAR-1 -506 ins/del</td>
<td>del/del</td>
<td>69</td>
<td>138</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ins/del</td>
<td>7</td>
<td>9</td>
<td>1.56 (0.56-4.36)</td>
<td>0.420</td>
</tr>
<tr>
<td></td>
<td>ins/ins</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>del</td>
<td>145</td>
<td>285</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ins</td>
<td>7</td>
<td>9</td>
<td>1.53 (0.56-4.19)</td>
<td>0.428</td>
</tr>
</tbody>
</table>

OR: Odds ratio, CI: confidence intervals, ins: insertion, del: deletion, NA: not applicable. aFisher’s exact test two-tailed p-value.

**Table IV. Associations between risk/protective factors and Helicobacter pylori-related gastric cancer in Chinese individuals (binary logistic regression).**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adjusted OR</th>
<th>95%CI</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. pylori infection</td>
<td>3.15</td>
<td>1.54-6.45</td>
<td>0.003</td>
</tr>
<tr>
<td>Male gender</td>
<td>2.44</td>
<td>1.35-4.42</td>
<td>0.002</td>
</tr>
<tr>
<td>PAR-1 IVSn -14 TT genotype</td>
<td>2.58</td>
<td>1.09-6.13</td>
<td>0.032</td>
</tr>
<tr>
<td>PAR-1 IVSn -14 AT genotype</td>
<td>1.71</td>
<td>0.78-3.75</td>
<td>0.179</td>
</tr>
</tbody>
</table>

OR: Odds ratio, CI: confidence intervals.
H. pylori to be associated with an increased risk of GC in this ethnic Chinese population, with the prevalence (69.8%) and effect size (OR: 3.15, 95% CI: 1.54-6.45, \(p\)-value: 0.003) being comparable with results from previous studies among similar populations (4, 39-41). Therefore, our findings are part of the extensive evidence supporting H. pylori as the most consistent risk factor associated with the development of GC.

The prevalence of the PAR-1 IVSn -14 T allele in our FD controls (48.0%, 142/296) was comparable to the reference allele frequency (47.7%) in Chinese individuals available from the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP). Allele and genotype frequency analyses, by means of a univariate approach, provided significant results (OR: 1.37, 95% CI: 0.91-2.05 and OR: 2.56, 95% CI: 1.12-5.85 for the T allele and TT genotype, respectively). Further multivariate analyses adjusted for H. pylori infection and gender also provided significant results (adjusted OR: 2.58, 95% CI: 1.09-6.13, \(p\)-value: 0.032 for the TT genotype), inferring that the PAR-1 IVSn -14 T allele is a risk factor for the development of GC in Chinese subjects.

The presence of PAR-1 506 ins/del heterozygosity in this population was found to be very low (9.2% (7/76) and 6.1% (9/147) in GC patients and FD controls, respectively). Since this is the only study that has assessed this polymorphism in Chinese subjects, apart from a study by Lurje et al. (24), which only included 28 Asian individuals, reference allele frequencies of this polymorphism in Chinese are not available. Reference allele frequencies in other populations available from the NCBI dbSNP database (22.2%, 55.6% and 22.2% in Africans and 60%, 30% and 10% in Caucasians for the ins/ins,

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**Table V. Association between PAR-1 polymorphisms and risk of Helicobacter pylori infection in ethnic Chinese individuals (univariate analysis).**

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Genotype/allele</th>
<th>H. pylori-positive (N)</th>
<th>H. pylori-negative (N)</th>
<th>OR (95%CI)</th>
<th>(p)-Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAR-1 IVSn -14 A/T</td>
<td>AA</td>
<td>33</td>
<td>18</td>
<td>1.15 (0.57-2.30)</td>
<td>0.723</td>
</tr>
<tr>
<td></td>
<td>AT</td>
<td>78</td>
<td>37</td>
<td>1.93 (0.83-4.48)</td>
<td>0.141</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>46</td>
<td>13</td>
<td>1.94 (0.54-6.93)</td>
<td>0.411</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>144</td>
<td>73</td>
<td>1.37 (0.91-2.05)</td>
<td>0.150</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>170</td>
<td>63</td>
<td>1.98 (0.55-7.20)</td>
<td>0.402</td>
</tr>
<tr>
<td>PAR-1 -506 ins/del</td>
<td>del/del</td>
<td>142</td>
<td>65</td>
<td>1.96 (0.55-7.20)</td>
<td>0.402</td>
</tr>
<tr>
<td></td>
<td>ins/del</td>
<td>13</td>
<td>3</td>
<td>1.96 (0.55-7.20)</td>
<td>0.402</td>
</tr>
<tr>
<td></td>
<td>ins/ins</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>del</td>
<td>297</td>
<td>133</td>
<td>1.94 (0.54-6.93)</td>
<td>0.411</td>
</tr>
<tr>
<td></td>
<td>ins</td>
<td>13</td>
<td>3</td>
<td>1.94 (0.54-6.93)</td>
<td>0.411</td>
</tr>
</tbody>
</table>

OR: Odds ratio, CI: confidence intervals, ins: insertion, del: deletion, NA: not applicable. aFisher’s exact test two-tailed \(p\)-value.
ins/del and del/del genotype, respectively) differed from the observed allele frequencies in our study sample, which is not surprising due to the peculiarities previously found in other genetic polymorphisms associated with GC in the Chinese population (42). Statistically non-significant results were obtained when comparison of PAR-1 -506 ins/del allele and genotype frequencies between GC patients and FD controls was performed.

In the current study PAR-1 IVSn -14 A/T and PAR-1 506 ins/del polymorphisms were not associated with an increased risk of H. pylori infection (OR: 1.37, 95% CI: 0.91-2.05 and OR: 1.94, 95% CI: 0.54-6.93 for PAR-1 IVSn -14 A/T and PAR-1 506 ins/del, respectively). These results, in conjunction with the multivariate analysis results, lead to the conclusion that PAR-1 IVSn -14 A/T is a novel independent factor that increases the risk of GC, a known multifactorial disease.

Currently, the exact role of PAR-1, in gastric pathology associated and non-associated with H. pylori infection, remains unclear. In a recent study, Wee and collaborators investigated the role of PAR-1 in inflammation and the humoral response to H. pylori in mice, and demonstrated that expression of PAR-1 down-regulated the inflammatory response to H. pylori infection in this murine model (16). As a result of their study, Wee et al. hypothesised that initial detection of H. pylori by the host immune system through activation of PAR-2 by a bacterial protease, is followed by limitation of excessive inflammation and tissue damage mediated by PAR-1 activation. Thus, the authors concluded that PAR-1 may contribute to host susceptibility or resistance to H. pylori-associated diseases including peptic ulcer disease and GC (16).

Interestingly, a study by Dupont et al. (23) has shown a relationship between platelet PAR-1 phenotype and the PAR-1 IVSn -14 T allele, as this variant was associated with lower PAR-1 expression on the platelet surface and a weaker aggregation and secretion response to the thrombin receptor activation peptide SFLLRN. Therefore, the PAR-1 IVSn -14 T allele might decrease the expression of PAR-1 in the gastric mucosa and this could lead to excessive inflammation and tissue damage mediated by PAR-2.

Only one study, conducted by Lurje et al. (24), has addressed the influence of PAR-1 polymorphisms in Caucasian, African-American, Asian and Hispanic GC patients. In this study the authors attempted to determine the association between PAR-1 IVSn -14 A/T and PAR-1 -506 ins/del polymorphisms and two variables: 1) time-to-tumour recurrence (TTR) and 2) overall survival (OS) (24). While PAR-1 -506 ins/del was found to be a prognostic marker in patients with localised GC, no association was found between the PAR-1 IVSn -14 A/T polymorphism and TTR or OS (24). This study reinforced the theory that PARs play a pivotal role in the regulation of local and early-onset angiogenesis and in turn might influence the process of tumour growth and disease progression in GC patients (24).

In conclusion, our study represents the first attempt to assess the influence of PAR-1 polymorphisms on the risk of developing GC. A novel association was observed between the PAR-1 IVSn -14 T allele and risk of GC. Further studies are required to confirm this association in other populations. In addition, it is possible that PAR-1 polymorphisms might influence tumour progression and invasion, thus, it would be interesting to perform functional studies assessing the influence of PAR-1 IVSn 14 A/T on cell motility.

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