Analysis of Pro12Ala PPAR gamma polymorphism and Helicobacter pylori infection in gastric adenocarcinoma and peptic ulcer disease

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Background: Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-dependent transcription factor involved in various disease processes including inflammation and carcinogenesis. We investigated the association of Pro12Ala PPARγ polymorphism and Helicobacter pylori infection with gastric cancer and peptic ulcer disease (PUD).

Patients and methods: In total, 348 adult patients [62 gastric adenocarcinoma, 45 PUD and 241 nonulcer dyspepsia (NUD)] who underwent an upper gastrointestinal endoscopy were enrolled. PPARγ polymorphism was analyzed by PCR-based restriction fragment length polymorphism. H. pylori infection was diagnosed by rapid urease test, culture, histopathology and PCR.

Results: PPARγ G carrier had significant association with gastric adenocarcinoma [P = 0.023, odds ratio (OR) = 2.136, 95% CI = 1.112–4.104] and PUD (P = 0.028, OR = 2.165, 95% CI = 1.008–4.306) when compared with NUD. Combination of G carrier and H. pylori infection further increased the risk of gastric adenocarcinoma (OR = 3.064, 95% CI = 1.198–7.807) and PUD (OR = 11.161, 95% CI = 3.495–35.644). PPARγ polymorphism did not increase the risk of gastric adenocarcinoma and PUD in H. pylori-negative subjects.

Conclusions: The study suggests that Pro12Ala PPARγ polymorphism is associated with gastric adenocarcinoma and PUD, and is a potential marker for genetic susceptibility to these two diseases in the presence of H. pylori infection.

Key words: gastric adenocarcinoma, Helicobacter pylori infection, peptic ulcer disease, PPARγ polymorphism

introduction

Helicobacter pylori has been classified as a major cause of peptic ulcer disease (PUD) and a risk factor for gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma [1–3]. On a global scale, gastric cancer is the second commonest cancer in the world. There is a substantial international variation in gastric cancer incidence with the highest rates reported from China, Japan and other Eastern Asian countries. Epidemiological studies have proved that H. pylori infection is considered as risk factor for gastric cancer and World Health Organization International Agency for Research on Cancer has classified this bacterium as a definite carcinogen [2]. However, while the majority of the infected individuals develop no significant clinical disease, others develop two kinds of divergent clinical outcomes—PUD and gastric cancer [4]. The reasons for developing these two extreme phenotypes remain poorly understood and are not explained by bacterial virulence factors alone [4, 5]. This highlights the need to explore potential candidate genes of the host involved in the H. pylori-associated gastric carcinogenesis.

Peroxisome proliferator-activated receptor gamma (PPARγ) is member in the nuclear receptor superfamily that regulates adipocyte differentiation, fatty acid uptake and storage [6]. Human PPARγ expression was first described in hematopoietic cells [7] and later also in spleen, liver, testis, skeletal muscle and brain [8]. PPARγ exists as two isoforms, γ1 and γ2, generated by alternative promoters and differential splicing of at least three different transcripts from the PPARγ gene on chromosome 3p25. PPARγ2 is the most important isofrom in adipose tissue, where it is almost exclusively expressed [8, 9]. Several polymorphisms in PPARγ2 have been identified so far. Only a few, however, seemed to have a functional effect on the transcription. Recently, Yen et al. [10] reported a common C > G polymorphism located in codon 12 of exon B of the PPARγ gene, which codes the aminoterminal polypeptide that defines the PPARγ2 isoform, results in a Pro12Ala substitution. With the strong effects of PPARγ on the growth of cancer cells, the role of Pro12Ala polymorphism has been recently studied in cancer [6]. Individuals with the Ala12 allele were found to have a reduced risk of colorectal cancer [11, 12]. A similar observation, however, could not be established in...
prostate cancer [13], endometrial cancer [14], sporadic colorectal adenoma [15] and gastric cancer [16, 17]. Little information is available regarding interactions of PPARγ polymorphism and risk for gastric cancer. The aim of the present study was to determine the role of Pro12Ala PPARγ polymorphism and *H. pylori* infection in patients with gastric adenocarcinoma and PUD.

**materials and methods**

**study population**

We enrolled 348 adult patients [62 gastric adenocarcinoma, 45 PUD and 241 non-ulcer dyspepsia (NUD)] who underwent upper gastrointestinal endoscopy at two tertiary referral centers in northern India between September 2002 and May 2007. The diagnosis of gastroduodenal diseases was on the basis of clinical, endoscopic and histopathological examinations. Patients with NUD were considered as controls in our study. The ethics committee of the institute granted approval for the study and the consent was obtained from all the patients. Subjects who had received antimicrobial therapy, *H. pylori* receptor blockers, proton pump inhibitors and nonsteroidal anti-inflammatory drugs in the preceeding 30 days before endoscopy or anti-*H. pylori* treatment in the past were excluded from this study.

**detection of *H. pylori* infection**

During each endoscopy, antral biopsies were obtained and subjected to the following tests: rapid urease test, culture, histopathology and *H. pylori*-specific *ureA* PCR following the standard protocol as described earlier [18].

**Pro12Ala PPARγ polymorphism**

Genomic DNA was isolated from gastric tissues using the QIAamp DNA mini kit (Qiagen, Hilden, Germany) as per the manufacturer’s instruction. The analysis of Pro12Ala PPARγ polymorphism was determined by PCR-based restriction fragment length polymorphism (PCR-RFLP) as previously described [17]. The sequences of PCR primers were as follows: forward 5′-GCC AAT TCA AGC CCA GTC-3′ and reverse 5′-GAT ATG TTT GCA GAC AGT GTA TCA GTG AAG GAA TCG CTT TCC G-3′ (Metabion, Martinsried, Deutschland). PCR conditions were as follows: an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturing at 94°C for 30 s, annealing at 51°C for 40 s and extension at 72°C for 40 s. The final extension was continued at 72°C for 10 min and cooling to 4°C. Template-free water was used as negative control. After amplification, the purified PCR products were subjected to restriction digestion by BuiI restriction endonuclease (Fermentas, Vilnius, Lithuania) for 8 h at 60°C. The DNA fragments were then separated on 2% agarose gel electrophoresis. Fragment sizes of 227 and 43 bp indicated the presence of a homozygous GG genotype (Ala12Ala), a single 270-bp fragment indicated the presence of a heterozygous CG genotype, lanes 7–9: homozygous GG genotype, lanes 10–12: homozygous CC genotype.

**results**

**patient characteristics**

A total of 348 patients (mean age 46.78 ± 15.96 years; 216 males) were enrolled in the study and their distributions were as follows: gastric adenocarcinoma 62 (mean age 56.60 ± 15.42 years; 47 males), PUD 45 (mean age 49.47 ± 17.22 years; 31 males) and NUD 241 (mean age 43.75 ± 14.76 years; 138 males) (Table 1).

**detection of *H. pylori* infection**

Prevalence of *H. pylori* infection in our study population was 58.6%. *H. pylori* infection was significantly higher in patients with PUD than with gastric adenocarcinoma (80% versus 56.5%, *P* = 0.01) and NUD (80% versus 55.2%, *P* = 0.002) (Table 1).

**Pro12Ala PPARγ polymorphism**

Genotypic distributions of patients with gastric adenocarcinoma, PUD and NUD were in Hardy–Weinberg equilibrium. The potential association of Pro12Ala PPARγ polymorphism in patients with gastroduodenal diseases is shown in Table 2. Presence of G carrier was almost similar in patients with gastric adenocarcinoma and PUD. The frequency of G carrier was significantly higher in patients with gastric adenocarcinoma and PUD. The frequency of G carrier was significantly higher in patients with gastric adenocarcinoma (37.1% versus 22.8%, *P* = 0.023, odds ratio (OR) = 2.14, 95% CI = 1.11–4.10) and PUD (37.8% versus 22.8%, *P* = 0.028, OR = 2.17, 95% CI = 1.09–4.31) when compared with NUD.

**interaction between *H. pylori* infection and Pro12Ala PPARγ polymorphism**

We also examined the potential interaction between *H. pylori* infection and Pro12Ala PPARγ polymorphism for the development of gastric adenocarcinoma and PUD in our population. Presence of G carrier with *H. pylori* infection significantly increased the risk for the development of gastric adenocarcinoma (*P* = 0.020; OR = 3.05, 95% CI = 1.21–7.81) and PUD (*P* < 0.001; OR = 11.16, 95% CI = 3.49–35.64). Interestingly, the risk was not increased in *H. pylori*-negative individuals with gastric cancer (*P* = 0.682; OR = 1.23, 95% CI = 0.79–1.90) and PUD (95% CI = 1.09–4.31) when compared with NUD.
Table 1. Demography of the study populations and *Helicobacter pylori* infection

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gastric adenocarcinoma (n = 62)</th>
<th>Peptic ulcer disease (n = 45)</th>
<th>Nonulcer dyspepsia (n = 241)</th>
<th>Overall (348)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age ± SD (years)</td>
<td>56.60 ± 15.423</td>
<td>49.47 ± 17.216</td>
<td>43.75 ± 14.764</td>
<td>46.78 ± 15.959</td>
</tr>
<tr>
<td><em>H. pylori</em> infection* (%)</td>
<td>35 (56.5)</td>
<td>36 (80)</td>
<td>133 (55.2)</td>
<td>204 (58.6)</td>
</tr>
</tbody>
</table>

*Peptic ulcer disease versus nonulcer dyspepsia: (80% versus 55.2%, P = 0.002); Gastric adenocarcinoma versus nonulcer dyspepsia: (56.5% versus 55.2%, P = 0.858); Peptic ulcer disease versus gastric adenocarcinoma: (80% versus 56.5%, P = 0.01).

Table 2. Allelic distribution of Pro12Ala peroxisome proliferator-activated receptor gamma (PPARγ) polymorphism in gastric adenocarcinoma, peptic ulcer disease (PUD) and nonulcer dyspepsia (NUD)

<table>
<thead>
<tr>
<th>PPARγ genotype</th>
<th>Gastric adenocarcinoma (n = 62)</th>
<th>PUD (n = 45)</th>
<th>NUD (n = 241)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>39 (62.9)</td>
<td>28 (62.2)</td>
<td>186 (77.2)</td>
</tr>
<tr>
<td>CG</td>
<td>18 (29.0)</td>
<td>15 (33.3)</td>
<td>52 (21.6)</td>
</tr>
<tr>
<td>GG</td>
<td>5 (8.1)</td>
<td>2 (4.4)</td>
<td>3 (1.2)</td>
</tr>
<tr>
<td>G carrier</td>
<td>23 (37.1)</td>
<td>17 (37.8)</td>
<td>55 (22.8)</td>
</tr>
</tbody>
</table>

*Gastric adenocarcinoma versus nonulcer dyspepsia: P = 0.023, odds ratio (OR) = 2.14, 95% confidence interval (CI) = 1.11–4.10; PUD versus NUD: P = 0.028, OR = 2.17, 95% CI = 1.09–4.31.

CI = 0.46–3.32) (Table 3) and PUD (P = 0.944; OR = 1.06, 95% CI = 0.19–5.86) (Table 4).

**discussion**

We investigated the potential association of Pro12Ala PPARγ polymorphism and *H. pylori* infection with gastric adenocarcinoma and PUD. We report that Pro12Ala PPARγ polymorphism in the presence of *H. pylori* infection could be a marker for genetic susceptibility to gastric adenocarcinoma and PUD. PPARs represent a family of nuclear receptors that are closely related to thyroid or retinoid receptors. Three PPAR subtypes (PPARα, PPARδ and PPARγ) have been identified so far [19]. PPARγ, however, is the most extensively studied of the three PPAR subtypes to date. Upon ligand binding, PPAR activates the transcription of many PPAR-responsive genes involved in the regulation of adipocyte differentiation, the enhancement of target tissues to insulin, inflammation, carcinogenesis and cell cycle control [19]. PPARγ was expressed in both cancer and normal gastric epithelium; activation of PPARγ inhibits the growth and induces apoptosis of gastric cancer cells [20]. Lu et al. [21] showed that treatment with the PPARγ ligand troglitazone substantially reduced the development of gastric cancer in mice induced by carcinogens. More interestingly, the chemopreventive effect of troglitazone was absent in PPARγ heterozygous-deficient (+/-) mice; it suggests the importance of functional PPARγ receptors in preventing gastric cancer development. Presence of the Ala12 polymorphism, which is associated with reduced PPARγ activity, may increase the risk of gastric adenocarcinoma [22, 23]. PPARγ may modulate the inflammatory response of the host cells associated with chronic *H. pylori* infection. Activation of the PPARγ pathway attenuates the ability of *H. pylori* to induce NF-kappa B-mediated apoptosis in gastric epithelial cells [24]. It may be speculated that *H. pylori* infection and PPARγ polymorphism further enhance the resistance to apoptosis which ultimately results in cellular proliferation [17]. In our study, G carriers (Ala12) were frequently present in gastric adenocarcinoma when compared with controls (Table 3). We found that patients with G carriers had 2.14-fold (95% CI = 1.11–4.10) increased risk of progression to gastric adenocarcinoma. Tahara et al. [16] described a 2.4-fold increased risk gastric adenocarcinoma due to Ala12 polymorphism. Liao et al. [17] reported nearly 2.5-fold increased risk of progression to gastric adenocarcinoma. It appears that the risk for the development of gastric adenocarcinoma related to Pro12Ala PPARγ polymorphism in our population is similar with the other published studies. When we analyzed the combination of G carrier and *H. pylori* infection in patients with gastric adenocarcinoma, the risk was further increased (P = 0.020, OR = 3.05, 95% CI = 1.21–7.81). The risk, however, was not increased in *H. pylori*-negative individuals (P = 0.682, OR = 1.23, 95% CI = 1.21–7.81). The present study clearly shows that G carriers in the presence of *H. pylori* infection are susceptible to develop gastric adenocarcinoma. So far, only one study from China had analyzed the association between *H. pylori* infection and Pro12Ala PPARγ polymorphism in the development of gastric adenocarcinoma and reported 12.8-fold risk [17]. The increased risk in Chinese study may be related to higher prevalence of *H. pylori* infection in gastric adenocarcinoma (81.7%) as compared with our patients (56.5%). The reason for such discrepancy in *H. pylori* infection between Chinese and present studies may be because of different methods used for detection of *H. pylori* infection. The presence of anti-*H. pylori* immunoglobulin G was the diagnostic criteria in Chinese study, while in the present study *H. pylori* infection was diagnosed on the basis of gastric tissue, which is considered more specific.

We also analyzed the association of Pro12Ala PPARγ polymorphism and *H. pylori* infection with PUD. We found that patients with G carriers had increased risk for PUD (P = 0.028, OR = 2.17, 95% CI = 1.09–4.31). When we analyzed the combination of G carrier and *H. pylori* infection, the risk increased to 11.16-fold in *H. pylori*-positive individuals.
Table 3. *Helicobacter pylori* (HP) infection and Pro12Ala peroxisome proliferator-activated receptor gamma (PPARγ) polymorphism as risk for gastric adenocarcinoma

<table>
<thead>
<tr>
<th>HP status</th>
<th>PPARγ genotype</th>
<th>Gastric adenocarcinoma (n = 62)</th>
<th>Nonulcer dyspepsia (controls, n = 241)</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP−</td>
<td>CC</td>
<td>18</td>
<td>74</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>HP−</td>
<td>G carriers</td>
<td>9</td>
<td>34</td>
<td>1.23 (0.46–3.32)</td>
<td>0.682</td>
</tr>
<tr>
<td>HP+</td>
<td>CC</td>
<td>21</td>
<td>112</td>
<td>0.961 (0.46–2.01)</td>
<td>0.916</td>
</tr>
<tr>
<td>HP+</td>
<td>G carriers</td>
<td>14</td>
<td>21</td>
<td>3.05 (1.21–7.81)</td>
<td>0.020</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval.

Table 4. *Helicobacter pylori* (HP) infection and Pro12Ala peroxisome proliferator-activated receptor gamma (PPARγ) polymorphism as risk for peptic ulcer disease (PUD)

<table>
<thead>
<tr>
<th>HP status</th>
<th>PPARγ genotype</th>
<th>PUD (n = 45)</th>
<th>Nonulcer dyspepsia (controls, n = 241)</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP−</td>
<td>CC</td>
<td>5</td>
<td>74</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>HP−</td>
<td>G carriers</td>
<td>4</td>
<td>34</td>
<td>1.06 (0.19–5.86)</td>
<td>0.944</td>
</tr>
<tr>
<td>HP+</td>
<td>CC</td>
<td>23</td>
<td>112</td>
<td>3.66 (1.31–10.22)</td>
<td>0.013</td>
</tr>
<tr>
<td>HP+</td>
<td>G carriers</td>
<td>13</td>
<td>21</td>
<td>11.16 (3.49–35.64)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval.

(P < 0.001, OR = 11.16, 95% CI = 3.49–35.64); however, the risk did not increase in *H. pylori*-negative individuals (P = 0.944, OR = 1.06, 95% CI = 0.19–5.86) (Table 4). There are no data available in literature to compare our observations with. To the best of our knowledge, this is the first study to show that patients with G carriers of Pro12Ala PPARγ polymorphism are susceptible to develop PUD in the presence of *H. pylori* infection.

In conclusion, the study suggests that Pro12Ala PPARγ polymorphism could be a potential marker for genetic susceptibility to gastric adenocarcinoma in the presence of *H. pylori* infection. We also report for the first time, patients with *H. pylori* infection and Pro12Ala PPARγ polymorphism have increased risk to develop PUD. Thus, Pro12Ala PPARγ polymorphism might be useful in predicting the risk of gastric adenocarcinoma and PUD in the presence of *H. pylori* infection. Further studies on different ethnic groups are needed to confirm the association of this polymorphism with the risk of gastric adenocarcinoma and PUD.

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references


