Association between common genetic variant of HRH2 and gastric cancer risk

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Abstract. Histamine plays important physiological roles in the upper gastrointestinal tract and acts via the H2 receptor. The -1018 G>A (rs2067474) in an enhancer element of the promoter and non-synonymous rs79385261 (Asn46Thr) were identified in HRH2. We attempted to clarify the associations of these polymorphisms with gastric carcinogenesis. The study was performed in 321 patients with gastric cancer and 599 subjects with no evidence of gastric malignancies on upper gastro-duodenal endoscopy. The genotypes were determined using a one-tube multiplex PCR-SSCP method. The degree of gastritis was assessed in 496 subjects and serum pepsinogen (PG) I/II levels were measured in 124 subjects without gastric cancer. The minor allele of Asn46Thr could not be detected. The frequencies of the -1018 A allele in the non-GC and GC groups were 13.5% and 8.26%, respectively (p=0.00077). Overall, -1018 GG homozygotes had an increased risk for developing gastric cancer (OR 1.68; 95% CI 1.17-2.42; p=0.0052), especially intestinal type cancer (OR 1.94; 95% CI 1.23-3.08; p=0.0047). In subjects aged >60 years, the adjusted risk for gastric cancer among individuals who were -1018 GG homozygotes was 1.87 (range 1.19-2.93; p=0.0065) compared with A carriers. In the gastric cancer cases located in the antrum and at comparative advanced stage, -1018 GG homozygosity was a significantly increased risk factor. In subjects >60 years, the metaplasia score was significantly higher in -1018 GG homozygotes than A carriers. Both atrophy and metaplasia scores were significantly increased with age only in -1018 GG homozygotes. The PG I/II ratio was significantly decreased in H. pylori positive GG homozygotes than negative GG homozygotes and positive A carriers. Our results suggest that -1018 GG homozygosity of HRH2 may be associated with the severity of gastric mucosal atrophy. This genotype has an increased risk for the subsequent development of gastric cancer, especially intestinal type, at advanced age.

Introduction

Gastric cancer remains a considerable public health problem worldwide. The incidence and mortality rates of gastric cancer have decreased gradually. Nevertheless, gastric cancer is second only to lung cancer as the leading cause of cancer death around the world (1,2). Helicobacter pylori (H. pylori) infection is now accepted as a crucial event in the development of gastric cancer, although the etiology of this tumor remains unclear. This infection first induces chronic superficial gastritis, which can progress to chronic atrophic gastritis, intestinal metaplasia, and dysplasia that leads toward gastric carcinoma (3-6). However, only a small number of infected patients actually develop gastric cancer. This suggests that host genetic factors, such as genes associated with inflammatory responses and acid secretion, may also play an important role in gastric carcinogenesis. Therefore, the associations between genetic polymorphisms and gastric carcinogenesis have been investigated in several studies (7-11). We have also revealed the significant association of polymorphisms in TLR2 (12), MIF (13), IL17A (14) and pre-microRNAs (15) with the susceptibility to gastric carcinogenesis.

On the other hand, the stomach is exceedingly rich in the peptide hormone- or active amine-producing cells such as enterochromaffin-like (ECL) cells (histamine), D cells (somatostatin), EC cells (serotonin), and G cells (gastrin) (16). Histamine, one of the active amine released in response to a variety of physiological stimuli, is well known to be involved in the pathogenesis of gastro-duodenal ulceration and gastric inflammation (17). Although this bio-active amine modulates a variety of functions via interacting with specific receptors on the target cells, H1, H2 and H3 receptors (18), H2 receptor has a central role only in the regulation of acid secretion in stomach as confirmed by the widespread use of H2 receptor blockers in the therapy of acid-related disorders (19,20). H. pylori infection as the main cause of gastric and duodenal ulcer heralded a new revolution in our understanding and treatment of acid-peptic disorders (21,22). Evidence was also provided that increased
gastric histamine contributed to the inflammatory changes and tissue damage associated with chronic \textit{H. pylori} infection of the gastric mucosa (23,24). Thus, intra-gastric histamine plays an important role on the gastric inflammation acting via \textit{H\textsubscript{2}} receptor, although \textit{H. pylori} infection is one of the major contributing factors to the development of gastro-duodenal inflammation (25).

The association between genetic polymorphisms of histamine receptor genes and the susceptibility to psychological and neurological disorders has been investigated (26,27). The \textit{rs2607474} (-1018 G>A) focused in these studies is located in an enhancer element of the \textit{HRH2} promoter, encoding histamine \textit{H\textsubscript{2}} receptor (26). It is likely that the \textit{HRH2} variant located in the promoter may induce changes in the expression of receptors. Although the investigators in the gastroenterological field have shown great interest in histamine \textit{H\textsubscript{2}} receptors for a long time, there has been no report whether \textit{-1018 G>A} polymorphism (\textit{rs2607474}) affect on the development of and susceptibility to gastrointestinal disorders, including gastric cancer, or not. Furthermore, non-synonymous SNP (\textit{rs79385261}, 137 A>C, Asn46Thr) was published in dbSNP of NCBI (http://www.ncbi.nlm.nih.gov/snp). The distribution of this genotype in Japanese is still unknown.

This study is aimed to test the hypothesis that genetic alteration in \textit{HRH2}, causing changes in the expression, may cause an increased risk for gastric carcinogenesis. We investigated the association between \textit{HRH2} \textit{-1018 G>A} (\textit{rs2607474}) and gastric carcinogenesis. In addition, the influence of \textit{rs79385261} (Asn46Thr) was also investigated.

Materials and methods

\textbf{Clinical samples.} As a gastric cancer group, 321 patients with gastric cancer (GC group), who were enrolled at the Endoscopy Center of Fujita Health University Hospital or Kanazawa Medical University Hospital from January 2007 to December 2009, were selected. The diagnoses of all gastric cancers were done histologically at the Division of Pathology of our hospitals. As a control, 599 subjects without malignant neoplasm on endoscopic examination were randomly selected from our stock DNA collected during the same period (non-GC group). Finally, the studied population comprised 920 subjects, whose polymorphisms could be clearly analyzed. The patients with severe systemic diseases, malignancies in other organs, and who had received non-steroidal anti-inflammatory drugs, antibiotics, and \textit{H. pylori} eradication treatment were excluded.

All subjects underwent upper gastrointestinal endoscopy and, in some of them, biopsy specimens were taken from non-cancerous mucosa. Parts of each specimen were fixed in 10\% buffered formalin and embedded in paraffin. Later, the degree of cancerous mucosa. Parts of each specimen were fixed in 10\% buffered formalin and embedded in paraffin. Later, the degree of cancerous mucosa. Parts of each specimen were fixed in 10\% buffered formalin and embedded in paraffin. Later, the degree of cancerous mucosa. Parts of each specimen were fixed in 10\% buffered formalin and embedded in paraffin.

Detection of \textit{H. pylori} infection. \textit{H. pylori} infection status was assessed by serology, histological examination, or the urea breath test. Patients were diagnosed as having infection when at least one of the diagnostic tests was positive.

\textbf{Genotyping of polymorphisms.} Sample stock DNA isolated from peripheral blood was used. Polymorphisms were genotyped by the multiplex PCR-SSCP method as reported previously (14,28). To detect \textit{-1018 G>A} and Asn46Thr (A>C) genotypes, using the primer pairs (-1018 forward: 5'-acctggaccc ttgtaaagttgctc-3' and -1018 reverse: 5'-cattccctcatagctg ttgaaacatc-3' and -1018 reverse: 5'-ctctgccggcctgctg-3' for Asn46Thr; respectively), one-tube multiplex PCR was carried out in a volume of 20 µl containing 1 µg of genomic DNA. The DNA was denatured at 95\(^\circ\)C for 3 min, followed by 35 cycles at 96\(^\circ\)C for 15 sec, 60\(^\circ\)C for 30 sec, and 72\(^\circ\)C for 30 sec, with final extension at 72\(^\circ\)C for 5 min. Thereafter, 2 µl of the PCR product was denatured with 10 µl of formamide (Sigma-Aldrich Co., St. Louis, MO, USA) at 95\(^\circ\)C for 5 min. SSCP was carried out at 6\(^\circ\)C using a GenePhor DNA separation system with GeneGel Excel 12.5/24 (GE Healthcare, USA), after which the denatured single strand DNA bands were detected using a DNA Silver Staining Kit (GE Healthcare).

\textbf{Histological evaluation.} In 496 of 599 control subjects, the severity of chronic gastritis was classified according to the updated Sydney system (29) by a pathologist who had no access to any clinical information.

\textbf{Serological evaluation.} The pepsinogen (PG) I/II ratio was calculated based on the data of the serum PG I and PG II levels measured by radioimmunoassay in 124 of 599 control subjects. A PG I/II ratio that showed a decrease in proportion to the severity of gastric mucosal atrophy was used as a marker of atrophic gastritis (30,31).

\textbf{Statistical analysis.} The data were expressed as mean ± SD. Mean ages between 2 groups was compared by Student's t-test. The ratios of \textit{H. pylori} infection status and male/female were also compared by Fisher's exact test. Allele and genotype frequencies were calculated by direct counting. The allele counts were also compared by a Fisher's exact test. The strength of association between allele frequencies and the disease was assessed by calculating the odds ratio (OR) and 95\% confidence intervals (CI) by logistic regression analysis. Adjusted ORs were calculated after adjustment for age, gender and \textit{H. pylori} infection status. Each updated Sydney system scores and PG I/II ratio between 2 groups were compared by Mann-Whitney U-test. The relationship between age and updated Sydney system score was assessed by ANOVA. Concerning the power of study, when setting \(\alpha=0.05\), \(\beta\)-value was calculated. For all analyses, the level of significance was set at \(p<0.05\).

\textbf{Results}

\textbf{Characteristics of subjects and the frequencies of genotypes.} Single strand DNAs of \textit{HRH2} genotypes were clearly separated by SSCP (Fig. 1). The minor allele of \textit{rs79385261} (Asn46Thr) could not be detected in any subject. The distribution of \textit{-1018 G>A} genotype in control subjects was 447GG, 142GA and 10AA (Table I). It was in the Hardy-Weinberg equilibrium (\(p=0.86\)).
The characteristics of subjects in this study are summarized in Table I. The mean age, male/female ratio and *H. pylori* positive ratio were significantly higher in GC group than non-GC group. The distribution of -1018 G>A genotype in GC group was 269GG, 51GA and 1AA (HWE, p=0.71). The -1018 G>A minor allele frequencies in GC and non-GC groups were 8.26% and 13.5%, respectively (p=0.00077 and β=0.93). In addition, the frequency of -1018 GG homozygote was significantly different among GC and non-GC groups (p=0.00078 and β=0.91).

**Association between HRH2 -1018 G>A and gastric carcinogenesis.** Overall, -1018 GG homozygote had a significantly increased risk for gastric carcinogenesis by logistic regression analysis after adjustment for age, gender and *H. pylori* infection status (OR 1.68; 95% CI 1.17-2.42; p=0.0052; Table II). When assessed by subtypes of gastric cancer, -1018 GG homozygote had a more increased risk for the development of intestinal type of cancer (OR 1.94; 95% CI 1.23-3.08; p=0.0047, Table II), whereas no significant association was found between this genotype and diffuse type of cancer.

In the subjects aged <60 years, *HRH2* -1018 G>A was not associated with gastric carcinogenesis (Table III). On the other hand, in the subjects >60 years, -1018 GG homozygote had an increased risk for the development of gastric cancer (OR 1.87; 95% CI 1.19-2.93; p=0.0065).

**Association between HRH2 polymorphism (-1018 G>A) and clinicopathological features of gastric cancer.** We investigated the influences of genetic polymorphisms on the progression of gastric cancer using various parameters of clinicopathological features. The *HRH2* -1018 GG homozygote was significantly associated with the increased risk for the development of gastric cancer located at lower third of stomach (OR 2.26; 95% CI 1.28-3.99; p=0.0050, Table IV). When assessed by tumor stages, -1018 GG homozygote was associated with the increased risk for the cases invaded beyond muscularis propria (OR 1.96; 95% CI 1.19-3.23; p=0.0078). Regarding as lymph node metastasis, this genotype had an increased risk for the cases with both with and without lymph node metastasis (OR 1.99; 95% CI 1.16-3.43; p=0.013 and OR 1.63; 95% CI 1.05-2.54; p=0.031, respectively).

**Histological evaluations of gastritis among genotypes of HRH2 (-1018 G>A).** In 496 control subjects evaluated for histological gastritis, the distribution of genotype was 397GG, 96GA and 3AA. Overall, each updated Sydney system score was not different among -1018 GG homozygote and A carrier (Fig. 2). However, in the subjects aged >60 years, metaplasia score was significantly higher in -1018 GG homozygote than A carrier (p=0.043).

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**Table I. Characteristics of the subjects and frequencies of genotypes.**

<table>
<thead>
<tr>
<th></th>
<th>Non-GC group</th>
<th>GC group</th>
<th>p-value^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>599</td>
<td>321</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean age ± SD</td>
<td>61.7±13.2</td>
<td>65.4±11.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Male:female</td>
<td>345:254</td>
<td>224:97</td>
<td>0.00028</td>
</tr>
<tr>
<td><em>H. pylori</em> positive ratio</td>
<td>61.2%</td>
<td>86.0%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><em>HRH2</em> genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>447</td>
<td>269</td>
<td>0.00078</td>
</tr>
<tr>
<td>G/A</td>
<td>142</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>10</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>A allele frequency</td>
<td>13.5%</td>
<td>8.26%</td>
<td>0.00077</td>
</tr>
</tbody>
</table>

^aNon-GC group vs. GC group; bThe frequency of GG genotype; cThe minor allele frequency.

**Table II. The risk of *HRH2* polymorphism (-1018 G>A) for gastric carcinogenesis.**

<table>
<thead>
<tr>
<th></th>
<th>GG</th>
<th>GA</th>
<th>AA</th>
<th>GG vs. A carrier; OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Non-GC</em></td>
<td>447</td>
<td>142</td>
<td>10</td>
<td>reference</td>
<td>-</td>
</tr>
<tr>
<td>Overall <em>GC</em></td>
<td>269</td>
<td>51</td>
<td>1</td>
<td>1.68 (1.17-2.42)</td>
<td>0.0052</td>
</tr>
<tr>
<td>Intestinal</td>
<td>163</td>
<td>27</td>
<td>0</td>
<td>1.94 (1.23-3.08)</td>
<td>0.0047</td>
</tr>
<tr>
<td>Diffuse</td>
<td>104</td>
<td>23</td>
<td>1</td>
<td>1.36 (0.838-2.20)</td>
<td>0.21</td>
</tr>
<tr>
<td>(Unknown)</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

By logistic regression analysis after adjustment for age, gender and *H. pylori* infection status.

**Table III. The risk of *HRH2* gene polymorphism (-1018 G>A) for gastric carcinogenesis in the subjects aged <60 years or >60 years.**

<table>
<thead>
<tr>
<th></th>
<th>GG</th>
<th>GA</th>
<th>AA</th>
<th>GG vs. A carrier; OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-GC</td>
<td>219</td>
<td>164</td>
<td>51</td>
<td>4 reference</td>
<td>-</td>
</tr>
<tr>
<td>GC</td>
<td>100</td>
<td>81</td>
<td>19</td>
<td>1.60 (0.842-3.04)</td>
<td>0.15</td>
</tr>
<tr>
<td>≥60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-GC</td>
<td>379</td>
<td>282</td>
<td>91</td>
<td>6 reference</td>
<td>-</td>
</tr>
<tr>
<td>GC</td>
<td>221</td>
<td>188</td>
<td>32</td>
<td>1.87 (1.19-2.93)</td>
<td>0.0065</td>
</tr>
</tbody>
</table>

By logistic regression analysis after adjustment for age, gender and *H. pylori* infection status.
In addition, in -1018 GG homozygote, both atrophy and metaplasia scores were significantly increased with age (both p-values by ANOVA: p<0.0001, Fig. 3). In A carrier, however, neither atrophy nor metaplasia score was significantly related to age.

Serum pepsinogen levels between -1018 GG homozygote and A carrier. In 124 control subjects measured for serum PG levels, 79 were H. pylori positive and 45 were negative. The distribution of genotype in H. pylori positive was 64GG, 14GA and 1AA, whereas the distribution in H. pylori negative was 32GG, 12GA and 1AA. In -1018 GG homozygote, PG I/II ratio was significantly decreased under influence of H. pylori infection (p<0.0001), whereas there was no significant difference among H. pylori positive subjects. PG I/II ratio was significantly lower in GG homozygote than A carrier (p=0.036).

Discussion

In the present study, we investigated the association between polymorphisms of HRH2, encoding histamine H2 receptor, and gastric carcinogenesis. A minor allele of Asn46Thr (rs79385261 A>C) could not be detected in our subjects. So, only -1018 G>A (rs2607474), genotype of which was in the Hardy-Weinberg equilibrium, was investigated. We found a strongly increased association between -1018 GG homozygote and gastric carcinogenesis, especially intestinal type of cancer. This strong association was found in the cases with tumor located at antrum and at the comparatively advanced stage. In addition, it was also...
shown that gastric mucosal atrophy was more rapidly progressed with age and under influence of *H. pylori* infection in -1018 GG homozygote than A carrier. The *HRH2*-1018 A allele frequency in non-GC group was 13.5%. This frequency was slightly higher than that reported in Hap-Map JPT and slightly lower than that reported in Japanese by Ito *et al* (32), although it was lower in Caucasians (26,27). One limitation of this study was that our subjects (both cases and controls) came to our hospitals in order to have endoscopic examination for the complaint of abdominal discomfort, or for complete check up of gastric cancer following barium X-ray examination in the health check, not completely healthy subjects. Therefore, minor allele frequency might be comparatively high in our study. Another limitation was that mean age, *H. pylori* infection ratio and male/female ratio were higher in GC group than non-GC group. However, the adjustment for age, gender and *H. pylori* infection status was performed in genotype analysis using logistic regression.

There have been few reports that investigated the influence of polymorphisms of *HRH2* in the risk for human disorders. Most of such studies revealed no association between *HRH2*-1018 G>A polymorphism and psychological or neurological disorder (26,27,32). On the other hand, there is no report on
the association between this polymorphism and gastric carcinogenesis. Our results provided the first evidence that HRH2 -1018 G>A polymorphism was significantly associated with the gastric carcinogenesis.

It has been well known that H. pylori infection has a major role on the progression of gastric mucosal atrophy, subsequently the development of gastric cancer. The factors promoting H. pylori-mediated gastric atrophy have been somewhat more controversial. H. pylori infection results in an elevation in serum gastrin level in the early stage of infection and precedes the development of atrophic gastritis. Gastrin acting on ECL cell leads to increased histamine release, which stimulate acid secretion through histamine H2 receptors on parietal cells. A hypergastrinemic mouse at the age of 5 months later shows a marked decline in acid secretion with the spontaneous development of gastric atrophy, metaplasia, and invasive cancer that can be markedly accelerated by concurrent Helicobacter infection (33,34). In addition, the majority of clinical studies have accepted that proton pump inhibitors (PPIs), which induce achlorhydria and hypergastrinemia, accelerate the onset of atrophic gastritis in H. pylori-positive patients (35-37). The above suggests that hypergastrinemia and/or insufficient acid secretion may promote the gastric mucosal atrophy under influence of H. pylori infection. However, Takaishi et al have demonstrated that the gastrin-histamine axis contributes to the development of gastric atrophy and neoplasia in a mouse model (38). In contrast to the effects of hypergastrinemia seen in gastric transgenic mice, long-term treatment of rats and mice with loxidine, one of potent histamine H2 receptor antagonists and inducing the ECL cells hyperplasia after long treatment as well as omeprazole (39), did not result in loss of parietal cells but instead appeared to result in increased parietal cells (40,41). Histidine decarboxylase knockout (HDC-/-) mice kept on a low-histamine diet showed an expanded parietal cell pool despite exhibiting marked hypergastrinemia (42). In addition, histamine has been shown to be important in modulating parietal cell maturation through H2 receptors (43,44). These observations suggest that not only the influence of hypergastrinemia but up-regulated action of histamine with hypergastrinemia may contribute to the gradual down-regulation of parietal cell number, gastric atrophy.

There is no report whether HRH2 -1018 G>A polymorphism affect on the expression and function of histamine H2 receptors or not, although understanding the effect of this polymorphism on the histamine signal via H2 receptor is informative. It is likely that the HRH2 genome variant located promoter may induce changes in the expression of receptors. In our current study, age-related gastric mucosal atrophy gradually and rapidly progress in HRH2 -1018 GG homozygote than -1018 A carrier. In addition, PG I/II ratio was significantly decreased in only -1018 GG homozygotes under the influence of H. pylori infection. These findings suggested that the action of histamine may be up-regulated in -1018 GG homozygote and A allele may be a loss of function allele.

According to the Lauren classification (45), there are two histologically distinct types of gastric cancer. The intestinal type develops in stomachs affected by chronic inflammation with passing through the intermediate steps of atrophic gastritis or intestinal metaplasia (46). On the other hand, the severity of mucosal inflammation and various host features may directly induce mutagenetic events that ultimately lead to the onset of the diffuse type. Therefore, intestinal type of cancer tends to arise ar antrum, because more severe gastric atrophy and metaplasia develop in the early stage of H. pylori infection and rapidly progress at antrum. In our results, -1018 GG homozygote was associated with intestinal type of gastric cancer, with the cases in comparative older subjects and located at antrum. These findings suggest that -1018 GG homozygote may have an increased risk of which gastric mucosal atrophy progress more rapidly with age and intestinal type of gastric cancer occur as a result.

Our data showed that -1018 GG homozygote was more closely associated with the cases at comparatively advanced stage, invaded beyond muscularis propria and with lymph node metastasis. This result suggests that -1018 GG homozygote may be associated with the gastric cancer progression, as well as development. Previous reports suggested that cimetidine, one of H2 receptor antagonists, might be considered as an anti-cancer agent. In 1988, it was firstly reported that post-operative treatment with cimetidine improved survival in gastric cancer patients of all stages (47). This effect of cimetidine is considered to be mediated by H2 receptor blockade of suppressor T-lymphocytes, leading to their functional inhibition and stimulation of natural killer cell activity (48,49) and antagonism of histamine-stimulated growth (50). Thus, histamine seemed to promote the tumor growth by actions other than stimulation of gastric acid secretion, followed by gastric mucosal atrophy. These actions of histamine may more rapidly progress the gastric cancer in -1018 GG homozygotes.

In conclusion, the current findings indicate that the HRH2 -1018 G>A polymorphism (rs2607474) may be associated with the susceptibility to gastric carcinogenesis in Japanese population. The -1018 GG homozygote may have an increased risk for the rapid progression of severe gastric mucosal atrophy and the subsequent development of intestinal type gastric cancer.

References


